From: Staples, Rose
Sent: Wednesday, January 18, 2012 5:35 PM
To: 'Alves, Jim - City of Modesto'; 'Anderson, Craig - USFWS'; 'Asay, Lynette - N-R'; 'Aud, John - SCERD'; 'Barnes, James - BLM'; 'Barnes, Peter - SWRCB'; 'Beuttler, John - CSPA'; 'Blake, Martin'; 'Bond, Jack - City of Modesto'; 'Boucher, Allison - TRC'; 'Boucher, Dave - Allison - TRC'; 'Bowes, Stephen - NPS'; 'Bowman, Art - CWRMP'; 'Brenneman, Beth - BLM'; 'Brewer, Doug - TetraTech'; 'Brochini, Anthony - SSMN'; 'Brochini, Tony - NPS'; 'Buckley, John - CSERC'; 'Buckley, Mark'; 'Burley, Silvia-CVMT'; 'Burt, Charles - CalPoly'; 'Cadagan, Jerry'; 'Carlin, Michael - SFPUC'; 'Catlett, Kelly - FOR'; 'Charles, Cindy - GWWF'; 'Cismowski, Gail - SWRCB'; 'Costa, Jan - Chicken Ranch'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob - TBMWI'; 'Cranston, Peggy - BLM'; 'Cremeen, Rebecca - CSERC'; 'Day, Kevin - TBMI'; 'Day, P - MF'; 'Denean - BVR'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne - OHP'; 'Dowd, Maggie-SNF'; 'Drekmeier, Peter - TRT'; 'Edmondson, Steve - NOAA'; 'Eicher, James - BLM'; 'Fety, Lauren - BLM'; 'Findley, Timothy - Hanson Bridgett'; 'Freeman, Beau - CalPoly'; 'Fuller, Reba - TMTC'; 'Furman, Donn W - SFPUC'; 'Ganteinbein, Julie - Water-Power Law Grp'; 'Giglio, Deborah - USFWS'; 'Gorman, Elaine - YSC'; 'Grader, Zeke'; 'Gutierrez, Monica - NOAA-NMFS'; 'Hackamack, Robert'; 'Hastreiter, James L - FERC'; 'Hatch, Jenny - CT'; 'Hayat, Zahra - MF'; 'Hayden, Ann'; 'Hellam, Anita - HH'; 'Heyne, Tim - CDFG'; 'Holden, James '; 'Holm, Lisa'; 'Horn, Jeff - BLM'; 'Horn, Tini'; 'Hudelson, Bill - StanislausFoodProducts'; 'Hughes, Noah'; 'Hughes, Robert - CDFG'; 'Hume, Noah - Stillwater'; 'Jackman, Jerry '; 'Jackson, Zac - USFWS'; 'Jennings, William - CSPA'; 'Jensen, Art - BAWSCA'; 'Jensen, Laura - TNC'; 'Johannis, Mary'; 'Johnson, Brian - CalTrout'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn - NOAA-MNFS'; 'Kinney, Teresa'; 'Koepele, Patrick - TRT'; 'Kordella, Lesley - FERC'; 'Lein, Joseph'; 'Levin, Ellen - SFPUC'; 'Lewis-Reggie-PRCI'; 'Linkard, David - TRT /RH'; 'Looker, Mark - LCC'; Loy, Carin; 'Lwenya, Roselynn, BVR'; 'Lyons, Bill - MR'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul '; 'Marshall, Mike - RHH'; 'Martin, Michael - MFFC'; 'Martin, Ramon - USFWS'; 'Mathiesen, Lloyd - CRRMW'; 'McDaniel, Dan -CDWA'; 'McDevitt, Ray - BAWSCA'; 'McDonnell, Marty - SMRT'; 'McLain, Jeffrey - NOAA-NMFS'; 'Means, Julie - CDFG'; 'Mills, John - TUD'; 'Morningstar Pope, Rhonda - BVR'; 'Motola, Mary - PRCI'; 'O'Brien, Jennifer - CDFG'; 'Orvis, Tom - SCFB'; 'Ott, Bob'; 'Ott, Chris'; 'Paul, Duane - Cardno'; 'Pavich, Steve-Cardno'; 'Pinhey, Nick - City of Modesto'; 'Pool, Richard'; 'Porter, Ruth - RHH'; 'Powell, Melissa - CRRMW'; 'Puccini, Stephen - CDFG'; 'Raeder, Jessie - TRT'; 'Ramirez, Tim - SFPUC'; 'Rea, Maria - NOAA-NMFS'; 'Reed, Rhonda - NOAA-NMFS'; 'Richardson, Kevin - USACE'; 'Ridenour, Jim'; 'Robbins, Royal'; 'Romano, David O - N-R'; 'Roos-Collins, Richard - Water-Power Law Grp for NHI'; 'Roseman, Jesse'; 'Rothert, Steve - AR'; 'Sander, Max - TNC'; 'Sandkulla, Nicole - BAWSCA'; 'Saunders, Jenan'; 'Schutte, Allison - HB'; 'Sears, William - SFPUC'; 'Shipley, Robert'; 'Shumway, Vern - SNF'; 'Shutes, Chris - CSPA'; 'Sill, Todd'; 'Slay, Ronn - CNRF/AIC'; 'Smith, Jim - MPM'; Staples, Rose; 'Steindorf, Dave - AW'; 'Steiner, Dan'; 'Stone, Vicki -TBMI'; 'Stork, Ron - FOR'; 'Stratton, Susan - CA SHPO'; 'Taylor, Mary Jane - CDFG'; 'Terpstra, Thomas'; 'TeVelde, George A '; 'Thompson, Larry - NOAA-MNFS'; 'Vasquez, Sandy '; 'Verkuil, Colette - TRT/MF'; 'Vierra, Chris'; 'Villalabos, Ruben'; 'Walters, Eric - MF'; 'Wantuck,

Rick - NOAA-NMFS'; 'Welch, Steve - ARTA'; 'Wesselman, Eric - TRT'; 'Wheeler, Dan'; 'Wheeler, Dave'; 'Wheeler, Douglas - RHH'; 'Wilcox, Scott - Stillwater'; 'Williamson, Harry (NPS)'; 'Willy, Alison - FWS'; 'Wilson, Bryan - MF'; 'Winchell, Frank - FERC'; 'Wood, Dave - FR'; 'Wooster, John -NOAA'; 'Workman, Michelle - USFWS'; 'Yoshiyama, Ron'; 'Zipser, Wayne - SCFB'

Subject: Don Pedro Relicensing: Study W&AR-5 Workshop 1 Materials Available on CD

During the November 4, 2011 Resource Work Group Meeting discussion of *Study W&AR-5 – Salmonid Populations Information Integration*, the Districts indicated they would provide the Relicensing Participants with an initial set of relevant information prior to the Study's first planned Workshop in April, 2012.

Materials for Study W&AR-5's Workshop 1 are now available. Due to the volume of information, materials for this first workshop are available on CD. Please contact me at rose.staples@hdrinc.com (or call 207.239.3857) if you would like a copy mailed to you.

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

WATER & POWER
Serving Central California since 1887

Modesto Irrigation District

January 17, 2012

TO: Don Pedro Project Relicensing Participants

FROM: Turlock Irrigation District/Modesto Irrigation District

SUBJECT: FERC Project No. 2299
Study W&AR-5 – Salmonid Populations Information Integration
Preliminary Workshop 1 Materials

During the November 4, 2011, Resource Work Group Meeting discussion of *Study W&AR-5 – Salmonid Populations Information Integration*, the Districts indicated they would provide the Relicensing Participants with an initial set of relevant information prior to the Study's first planned Workshop in April 2012.

Enclosed please find a CD containing the references that were cited in the Pre-Application Document (PAD). Also included is a list of the references provided on the CD. These references provide information on factors affecting salmonid populations in the lower Tuolumne River. General salmonid life history references, as well as Tuolumne River specific information are included. The Districts would like to emphasize that the attached reference set is intended as an initial background survey of available information and some, or portions of some, of these references may not ultimately be required to be included in the final study report. In the course of this study, the reference list will be revised and/or supplemented as required in advance of the initial or subsequent workshops. Also, additional data resources will be reviewed and incorporated, if needed.

Enclosures

STUDY W&AR-5
SALMONID POPULATIONS INFORMATION INTEGRATION
PRELIMINARY WORKSHOP 1 MATERIALS
REFERENCES CITED IN THE DON PEDRO PROJECT
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Appendix 1: Population model documentation. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 2: Stock recruitment analysis of the population dynamics of San Joaquin River system Chinook salmon. Prepared by EA Engineering, Science and Technology. February 1992

Appendix 3: Tuolumne River salmon spawning surveys 1971-1988. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 4: Instream flow data processing Tuolumne River, California. Prepared by Robert E. Meyer Consultants, Inc. August 1984

Appendix 5: Analysis of 1981 lower Tuolumne River IFIM data. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 6: Lower Tuolumne River spawning gravel availability and superimposition report. Prepared by EA Engineering, Science and Technology. February 1992

Appendix 7: Lower Tuolumne River Chinook salmon redd excavation report. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 8: Lower Tuolumne River spawning gravel studies report. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 9: Spawning gravel cleaning methodologies. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 10: 1987 juvenile Chinook salmon mark-recapture study. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 11: An evaluation of the effect of gravel ripping on redd distribution in the Lower Tuolumne River. Prepared by EA Engineering, Science and Technology. November 1991

Appendix 12: Data results: Seining of juvenile Chinook salmon in the Tuolumne, San Joaquin and Stanislaus Rivers, 1986-1989. Prepared by EA Engineering, Science and Technology. August 1991

Appendix 13: Preliminary juvenile salmon study: Report on sampling of Chinook salmon fry and smolts by fyke net and seine in the Lower Tuolumne River 1973-1986. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 14: Tuolumne River fluctuation flow study report. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 15: Tuolumne River fluctuation flow study plan: Draft. Prepared by EA Engineering, Science and Technology. February 1992
Appendix 16: Aquatic invertebrate studies report. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 17: Preliminary Tuolumne River water temperature report. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 18: Lower Tuolumne River instream temperature model documentation: Description and calibration. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 19: Modeled effects of La Grange releases on instream temperatures in the Lower Tuolumne River. Prepared by EA Engineering, Science and Technology. September 1991
Appendix 20: Juvenile salmon pilot temperature observation experiments. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 21: Possible effects of high water temperature on migrating Chinook salmon (Oncorhynchus tshawytscha) smolts in the San Joaquin River System. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 22: Lower Tuolumne River predation study report. Prepared by EA Engineering, Science and Technology. February 1992
Appendix 23: Effects of turbidity on bass predation efficiency. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 24: Effects of introduced species of fish in the San Joaquin River system. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 25: Preliminary summary smolt survival index study. Prepared by Loudermilk, Fjelstad, Neillands, Wingett, Della Valle, Presher and Traylor, California Department of Fish and Game. July 1987
Appendix 26: Export mortality fraction submodel. Prepared by EA Engineering, Science and Technology. February 1992
Appendix 27: Tuolumne River summer flow study report 1988-1990. Prepared by EA Engineering, Science and Technology. November 1991
Appendix 28: Tuolumne River summer flow invertebrate study. Prepared by EA Engineering, Science and Technology. November 1991

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Report 2006-7: Survival to emergence study report. Prepared by Peter Baker, Noah Hume, A.J. Keith, Neil Lassetre, and Frank Ligon, Stillwater Ecosystem, Watershed & Riverine Sciences, Berkeley, CA. March 2007
Report 2006-8: Special run pool 9 and 7/11 reach: Post-project monitoring synthesis report. Prepared by Jennifer Vick, McBain and Trush, Arcata, CA and A.J. Keith, Stillwater Ecosystem, Watershed & Riverine Sciences, Berkeley, CA. March 2007
Report 2006-10: Tuolumne River La Grange gravel addition, phase II annual report. Prepared by Doug Ridgeway Fish Habitat Supervisor, California Department of Fish and Game, Central Region (Region 4). March 2007

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From: Staples, Rose
Sent: Friday, January 20, 2012 2:46 PM
To: Alves, Jim - City of Modesto; Anderson, Craig - USFWS; Asay, Lynette - N-R; Aud, John - SCERD; Barnes, James - BLM; Barnes, Peter - SWRCB; Beuttler, John - CSPA; Blake, Martin; Bond, Jack - City of Modesto; Boucher, Allison - TRC; Boucher, Dave - Allison - TRC; Bowes, Stephen - NPS; Bowman, Art - CWRMP; Brenneman, Beth - BLM; Brewer, Doug - TetraTech; Brochini, Anthony - SSMN; Brochini, Tony - NPS; Buckley, John - CSERC; Buckley, Mark; Burley, Silvia-CVMT; Burt, Charles - CalPoly; Cadagan, Jerry; Carlin, Michael - SFPUC; Catlett, Kelly - FOR; Charles, Cindy - GWWF; Cismowski, Gail - SWRCB; Costa, Jan - Chicken Ranch; Cowan, Jeffrey; Cox, Stanley Rob - TBMWI; Cranston, Peggy - BLM; Cremeen, Rebecca - CSERC; Day, Kevin - TBMI; Day, P - MF; Denean - BVR; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne - OHP; Dowd, Maggie-SNF; Drekmeier, Peter - TRT; Edmondson, Steve - NOAA; Eicher, James - BLM; Fety, Lauren - BLM; Findley, Timothy - Hanson Bridgett; Freeman, Beau - CalPoly; Fuller, Reba - TMTC; Furman, Donn W - SFPUC; Ganteinbein, Julie - Water-Power Law Grp; Giglio, Deborah - USFWS; Gorman, Elaine - YSC; Grader, Zeke; Gutierrez, Monica - NOAA-NMFS; Hackamack, Robert; Hastreiter, James L - FERC; Hatch, Jenny - CT; Hayat, Zahra - MF; Hayden, Ann; Hellam, Anita - HH; Heyne, Tim - CDFG; Holden, James ; Holm, Lisa; Horn, Jeff - BLM; Horn, Tini; Hudelson, Bill - StanislausFoodProducts; Hughes, Noah; Hughes, Robert - CDFG; Hume, Noah - Stillwater; Jackman, Jerry ; Jackson, Zac - USFWS; Jennings, William - CSPA; Jensen, Art - BAWSCA; Jensen, Laura - TNC; Johannis, Mary; Johnson, Brian - CalTrout; Justin; Keating, Janice; Kempton, Kathryn - NOAA-MNFS; Kinney, Teresa; Koepele, Patrick - TRT; Kordella, Lesley - FERC; Lein, Joseph; Levin, Ellen - SFPUC; Lewis-Reggie-PRCI; Linkard, David - TRT /RH; Looker, Mark - LCC; Loy, Carin; Lwenya, Roselynn, BVR; Lyons, Bill - MR; Madden, Dan; Manji, Annie; Marko, Paul ; Marshall, Mike - RHH; Martin, Michael - MFFC; Martin, Ramon - USFWS; Mathiesen, Lloyd - CRRMW; McDaniel, Dan -CDWA; McDevitt, Ray - BAWSCA; McDonnell, Marty - SMRT; McLain, Jeffrey - NOAA-NMFS; Means, Julie - CDFG; Mills, John - TUD; Morningstar Pope, Rhonda - BVR; Motola, Mary - PRCI; O'Brien, Jennifer - CDFG; Orvis, Tom - SCFB; Ott, Bob; Ott, Chris; Paul, Duane - Cardno; Pavich, Steve-Cardno; Pinhey, Nick - City of Modesto; Pool, Richard; Porter, Ruth - RHH; Powell, Melissa - CRRMW; Puccini, Stephen - CDFG; Raeder, Jessie - TRT; Ramirez, Tim - SFPUC; Rea, Maria - NOAA-NMFS; Reed, Rhonda - NOAA-NMFS; Richardson, Kevin - USACE; Ridenour, Jim; Robbins, Royal; Romano, David O - N-R; Roos-Collins, Richard - Water-Power Law Grp for NHI; Roseman, Jesse; Rothert, Steve - AR; Sander, Max - TNC; Sandkulla, Nicole - BAWSCA; Saunders, Jenan; Schutte, Allison - HB; Sears, William - SFPUC; Shipley, Robert; Shumway, Vern - SNF; Shutes, Chris - CSPA; Sill, Todd; Slay, Ronn - CNRF/AIC; Smith, Jim - MPM; Staples, Rose; Steindorf, Dave - AW; Steiner, Dan; Stone, Vicki -TBMI; Stork, Ron - FOR; Stratton, Susan - CA SHPO; Taylor, Mary Jane - CDFG; Terpstra, Thomas; TeVelde, George A ; Thompson, Larry - NOAA-MNFS; Vasquez, Sandy ; Verkuil, Colette - TRT/MF; Vierra, Chris; Villalabos, Ruben; Walters, Eric - MF; Wantuck, Rick - NOAA-NMFS; Welch, Steve - ARTA; Wesselman, Eric - TRT; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas - RHH; Wilcox, Scott -

Stillwater; Williamson, Harry (NPS); Willy, Alison - FWS; Wilson, Bryan - MF; Winchell, Frank - FERC; Wood, Dave - FR; Wooster, John -NOAA; Workman, Michelle - USFWS; Yoshiyama, Ron; Zipser, Wayne - SCFB

Subject: : Don Pedro Draft Study Plans – Sturgeon, Riparian, and O.myskiss Scale Studies

Don Pedro Relicensing Participants,

Following discussions of the Revised Study Plan (RSP) and in response to relicensing participant requests, the Districts agreed to develop three additional study plans:

W&AR 18 – Sturgeon Study
W&AR 19 – Lower Tuolumne Riparian Information and Synthesis Study
W&AR 20 – Oncorhynchus mykiss Scale Collection and Age Determination Study

Pursuant to the Study Plan Determination issued by FERC on December 22, 2011, the Districts are providing drafts of these three study plans for your review. These three studies can be downloaded from the Don Pedro Relicensing Website at donpedro-relicensing.com. In the row of banner headings across the top, please click on DOCUMENTS, then scroll down and select STUDIES under "Documents Now Available." Then you will need to scroll down again, under STUDIES, until you reach WATER-AQUATIC RWG (3). Click on that and you should see the three study plan drafts. Any problems accessing, please let me know.

Following the 30-day review period, the Districts will respond to comments received and file the study plans with FERC within 60 days of the Study Determination.

Please provide comments directly to the Districts via email to Rose.Staples@hdrinc.com (or Fax 207-775-1742) no later than February 20, 2012.

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

TURLOCK IRRIGATION DISTRICT & MODESTO IRRIGATION DISTRICT
DON PEDRO PROJECT
FERC NO. 2299
WATER AND AQUATIC RESOURCES WORK GROUP

Study Plan W&AR-18
Sturgeon Study Plan
January 2012

Related Study Request: SWRCB-11

1.0 Project Nexus

The continued operation and maintenance (O&M) of the Don Pedro Project (Project) may contribute to cumulative effects on habitat availability for in-river life stages of the southern Distinct Population Segment (DPS) green sturgeon (*Acipenser medirostris*) and the potential for green sturgeon to occur in the lower Tuolumne River.

2.0 Resource Agency Management Goals

The Districts believe that four agencies have resource management goals related to the southern DPS green sturgeon and/or their habitat: (1) U.S. Department of Interior, Fish and Wildlife Service (USFWS); (2) United States Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NMFS); (3) California Department of Fish and Game (CDFG); and 4) State Water Resources Control Board, Division of Water Rights (SWRCB).

Green sturgeon was considered in the 1995 Sacramento-San Joaquin Delta Native Fishes Recovery Plan (USFWS 1995). This plan identifies a primary restoration (recovery) objective of a minimum population of 1,000 fish over 1 meter (39 inches) total length each year, including 500 females over 1.3 meters (51 inches) total length (minimum size at maturity), during the spawning period (presumably March-July) when spawners are present in the estuary and the Sacramento River. A broad goal of the USFWS (2001) Anadromous Fish Restoration Program (AFRP), as stated in Section 3406(b)(1) of the Central Valley Project Improvement Act, is to double the long-term production of anadromous fish in California's Central Valley rivers and streams. Although no specific objectives have been established for the Tuolumne River, broader objectives relating to green sturgeon to support the long-term goal for the Central Valley include: (1) improve habitat for all life stages of anadromous fish through provision of flows of suitable quality, quantity, and timing, and improved physical habitat; (2) improve survival rates by reducing or eliminating entrainment of juveniles at diversions; (3) improve the opportunity for adult fish to reach spawning habitats in a timely manner; (4) collect fish population, health, and habitat data to facilitate evaluation of restoration actions; (5) integrate habitat restoration efforts with harvest and hatchery management; and (6) involve partners in the implementation and evaluation of restoration actions.

NMFS has developed Resource Management Goals and Objectives for species listed under the Endangered Species Act (ESA) (16 U.S.C. §1531 et seq.), as well as anadromous species that are not currently listed but may require listing in the future. The southern DPS green sturgeon was

federally listed as threatened under the Endangered Species Act in 2006. Critical habitat was designated for the southern DPS in 2008. Although the Tuolumne River is not currently designated as critical habitat for the southern DPS green sturgeon, critical habitat is designated to include the Sacramento-San Joaquin Delta including all waterways up to the elevation of mean higher high water within the area defined in California Water Code Section 12220, except for specific excluded areas as described in (NMFS 2009).

CDFG's mission is to manage California's diverse fish, wildlife, and plant resources, and the habitats upon which they depend, for their ecological values and for their use and enjoyment by the public. CDFG's resource management goals, as summarized in restoration planning documents such as "Restoring Central Valley Streams: A Plan for Action" (Reynolds et al. 1993), are to restore and protect California's aquatic ecosystems that support fish and wildlife, and to protect threatened and endangered species under California Fish and Game Code (Sections 6920–6924).

SWRCB has responsibility under the federal Clean Water Act (33 U.S.C. §11251–1357) to preserve and maintain the chemical, physical and biological integrity of the State's waters and to protect water quality and the beneficial uses of stream reaches consistent with Section 401 of the federal Clean Water Act, the Regional Water Quality Control Board Basin Plans, State Water Board regulations, the California Environmental Quality Act, and any other applicable state law.

3.0 Study Goals

The goal of this study is to conduct a literature review and synthesize applicable studies and reports on green sturgeon life history and habitat requirements in the Central Valley and San Joaquin Basin, and evaluate the potential for green sturgeon to be affected by Project operations. Study objectives are to:

- collect and summarize available information on green sturgeon distribution in order to characterize green sturgeon habitat requirements,
- evaluate potential habitat availability for in river life stages of green sturgeon in the lower Tuolumne River, and
- identify if there are Project-related factors that are potentially limiting the availability of green sturgeon habitat in the Tuolumne River.

4.0 Existing Information and Need for Additional Information

Green sturgeon life history requirements, distribution, and abundance information in the Sacramento-San Joaquin basin has been reported in several publications (e.g., Beamesderfer et al. 2004, Reclamation 2008, Adams et al. 2002). However, there are no data documenting the presence of green sturgeon in the San Joaquin or Tuolumne rivers. Similarly, there is little information regarding the potential to provide suitable habitat for this species within the San Joaquin River watershed. At the request of Relicensing Participants, a literature review will be conducted to provide a summary and synthesis of available publications and other sources of information on green sturgeon habitat. The study will attempt to identify factors affecting the potential green sturgeon habitat in the Tuolumne River and lower San Joaquin rivers.

5.0 Study Methods

The State Water Board requested the Districts perform a literature review and synthesis of available studies and reports to determine the impacts of the Project upon green sturgeon habitat in the Lower Tuolumne River. The Districts will review and synthesize information on green sturgeon distribution in order to characterize green sturgeon habitat requirements, and evaluate potential habitat availability in the lower Tuolumne River. No field studies will be conducted; the Districts will rely upon previously conducted studies and ongoing fisheries data collection and monitoring activities in the study area.

5.1 Study Area

The study area includes the Tuolumne River from the La Grange Dam (RM 52) downstream to the confluence with the San Joaquin River (RM 0).

5.2 General Concepts

The following general concepts apply to the study:

- The goal of this review is to characterize conditions and identify any Project related effects on those conditions in the study area as they relate to the potential availability of green sturgeon habitat through a focused examination of the available literature.
- The review will synthesize *findings* specific to the study area or green sturgeon habitat characteristics.
- Primary literature sources are preferred and secondary sources are rarely cited. If a secondary or tertiary source is cited, it will be clearly identified as such.

5.3 Study Methods

The study methods will consist of the three steps described below.

Step 1 – Data Compilation. Information from previously conducted studies on green sturgeon habitat, ecological needs and related conditions will be compiled. Attachment A provides a preliminary list of references to be reviewed. Subsequent literature review (Step 2) will focus on studies in Attachment A conducted on green sturgeon habitat and ecological needs in the Sacramento-San Joaquin basin. The highest priority will be given to data and reports specific to the Tuolumne River, then to data and reports related to the San Joaquin and its major tributaries. Information from these studies will be compiled and supplemented with relevant biological, hydrologic, physical habitat, and water quality data information in the study area. Information from broader sources may be used to address specific data or information gaps identified as part of this process. Relicensing participants will be encouraged to provide additional relevant information for the study.

Step 2 – Perform Analysis. The proposed study will compare information on habitat conditions in the study area with green sturgeon ecological and habitat requirements to identify potential Project related effects on green sturgeon habitat. Physical habitat attributes (e.g., temperature, substrate, depth, and velocity) observed in the lower Tuolumne and San Joaquin Rivers will be compared with green sturgeon habitat requirements to identify relative suitability. For example,

water temperature criteria summarized by Van Eenennaam et al. (2005) will be compared to observed temperatures in the lower Tuolumne and San Joaquin rivers to assess habitat suitability for spawning and rearing life stages of green sturgeon. Analyses will be conducted for each in-river life history stage to gain an understanding of the potential for the Tuolumne River in its current condition to provide suitable habitat for green sturgeon. The study will provide an assessment of factors affecting habitat suitability for each life stage and an indication of the level of certainty associated with these conclusions.

Step 3 – Prepare Report. The Districts will prepare a report that includes the following sections: (1) Study Goals and Objectives; (2) Methods and Analysis; (3) Results; (4) Discussion; and (5) Conclusions. The report for this study will be a synthesis of existing information, and will provide an assessment of habitat suitability for green sturgeon in the Tuolumne River.

6.0 Schedule

The Districts anticipate the schedule to complete the study proposal as follows:

- Existing Data Compilation (Step 1).....February – March 2012
- Analysis and Synthesis (Step 2) .....March – May 2012
- Report Preparation (Step 3) .....May – June 2012
- Report Issuance .....July 2012

7.0 Consistency of Methodology with Generally Accepted Scientific Practices

Review and analysis of existing literature and other information sources is an important and well accepted step in scientific practices.

8.0 Deliverables

The Districts will prepare a final study report, which will document the methodology and results of the study.

9.0 Level of Effort and Cost

The Districts estimate that the cost to complete this study is $39,000.

10.0 References

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Beamesderfer, R., M. Simpson, G. Kopp, J. Inman, A. Fuller, and D. Demko. 2004. Historical and current information on green sturgeon occurrence in the Sacramento and San Joaquin rivers and tributaries. Prepared for State Water Contractors. S.P. Cramer and Associates, Oakdale, California.

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U.S. Department of Interior, Fish and Wildlife Service (USFWS). 1995. Recovery plan for the Sacramento San Joaquin Delta native fishes. U.S. Dept. of the Interior, Fish and Wildlife Service, Region 1, Portland, OR. 195 p.

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Attachment A

Study W&AR-18 Sturgeon Study
Preliminary Literature Sources for Review

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Beamesderfer, R. C. P., and M. A. H. Webb. 2002. Green sturgeon status review information. S. P. Cramer and Associates, Gresham, Oregon.

Beamis, W. E., and B. Kynard. 1997. Sturgeon rivers: An introduction to acipensiform biogeography and life history. Environmental Biology of Fishes 48: 167–183.

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Biological Review Team (BRT). 2005. Green sturgeon (*Acipenser medirostris*) status review update. Prepared for the National Marine Fisheries Service. 36 pp.

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Chadwick, H. K. 1959. California sturgeon tagging studies. California Fish and Game 45:297-301.

Heublein, J. C., J. T. Kelly, C. E. Crocker, A. P. Klimley, and S. T. Lindley. 2008. Migration of green sturgeon, *Acipenser medirostris*, in the Sacramento River. Environmental Biology of Fishes Online.

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Mayfield, R. B. and J. J. Cech. 2004. Temperature effects on green sturgeon bioenergetics. Transactions of the American Fisheries Society 133:961-970.

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TURLOCK IRRIGATION DISTRICT & MODESTO IRRIGATION DISTRICT
DON PEDRO PROJECT
FERC NO. 2299
WATER AND AQUATIC RESOURCES WORK GROUP

Study Plan W&AR-19
Lower Tuolumne River Riparian Information and Synthesis Study
January 2012

Related Study Requests: AR-15, BLM-09, SWRCB-03

1.0 Project Nexus

The continued operation and maintenance (O&M) of the Don Pedro Project (Project) may contribute to cumulative effects on the distribution, extent, composition, and structure of riparian vegetation downstream of La Grange Dam.

2.0 Resource Agency Management Goals

Turlock Irrigation District (TID) and Modesto Irrigation District (MID) (collectively, the Districts) believe that four agencies may have resource management goals related to riparian vegetation along the Lower Tuolumne River: (1) U.S. Department of Interior, Fish and Wildlife Service (USFWS); (2) California Department of Fish and Game (CDFG); (3) the California Department of Water Resources (DWR); and (4) State Water Resources Control Board, Division of Water Rights (SWRCB).

The USFWS (2001) identified restoration and protection of riparian habitat as a high priority action for the Tuolumne River in the final restoration plan for the anadromous fish restoration program (Action 2; page 84).

CDFG's mission is to manage California's diverse fish, wildlife, and plant resources, and the habitats upon which they depend, for their ecological values and for their use and enjoyment by the public. There are two management documents published by CDFG which include goals to protect and improve riparian vegetation within the Central Valley (CDFG 1993, 2007). In the California wildlife action plan, CDFG (1993) places a high priority on development of a comprehensive plan that addresses habitat improvements, including riparian habitat, along the San Joaquin River in order to re-establish anadromous fisheries below Friant Dam. Additionally, the California Advisory Committee on Salmon and Steelhead Trout (CACSST 1988), created in consultation with and directed to report to CDFG and the state legislature, recommended state-wide improved enforcement of Streambed Alteration Agreements to better protect riparian habitat and recommended that the State Legislature develop an incentive program to support protection and restoration of the riparian zone.

Outside of the lower Tuolumne River corridor, restoration and protection of riparian habitat in the Bay-Delta watershed is consistent with stated goals of the California Department of Water Resources, the lead agency for the CALFED program. The Ecological Restoration Program of CALFED includes multiple goals for restoration and protection of riparian vegetation and habitat, and for supporting ecological processes in the Central Valley (DWR 2000).

SWRCB has responsibility under the federal Clean Water Act (33 U.S.C. §11251–1357) to preserve and maintain the chemical, physical and biological integrity of the State's waters and to protect water quality and the beneficial uses of stream reaches consistent with Section 401 of the federal Clean Water Act, the Regional Water Quality Control Board Basin Plans, State Water Board regulations, the California Environmental Quality Act, and any other applicable state law.

3.0 Study Goals

The goal of this study is to review, summarize and report information describing the condition of the riparian resources and habitats associated with the Tuolumne River downstream of La Grange dam. Objectives in meeting this goal include:

- collect and summarize available existing information to characterize potential cumulative effects of the Project upon riparian vegetation along the lower Tuolumne River,
- provide a summary and synthesis of literature and other sources used in this study to characterize riparian habitat condition in the study area, and
- identify factors potentially affecting riparian resources and habitats in the study area.

4.0 Existing Information and Need for Review and Synthesis

As stated in the PAD (Section 6.1.6 Riparian, Wetlands, and Littoral Habitats), the Project may contribute to cumulative effects on riparian resources downstream of La Grange Dam by modifying the hydrologic and fluvial processes that influence the establishment, survival, and succession of riparian vegetation. For example, McBain & Trush (2000) suggest opportunities might exist to revise U.S. Army Corps of Engineers flood control operations of Don Pedro Reservoir in order to partially restore fluvial processes that support a more dynamic riparian system and improved habitat. Studies of the Tuolumne River (e.g., McBain & Trush 2000, Stella et al. 2006, Stillwater Sciences 2006) as well as broader studies (Anderson et al. 2006, Arthington et al. 2006, Bendix 1999, Lytle and Merritt 2004, Merritt and Cooper 2000, Rood et al. 1995, Rood et al. 2005, Shafroth et al. 2002) have examined linkages between river hydrographs and riparian vegetation. Other factors affecting riparian landscapes include predicted changes in snowpack and the snowmelt hydrograph (Young et al. 2009; Stromberg et al. 2010) and land use changes. At the request of Relicensing Participants, this study will provide a summary and synthesis of these literature and other sources indentified during this study.

5.0 Study Area and Study Methods

5.1 Study Area

The study area includes the Tuolumne River from the La Grange Dam (RM 52) downstream to its confluence with the San Joaquin (RM 0).

5.2 General Concepts

The following general concepts apply to the study:

- The goal of this review is to summarize factors affecting riparian ecology in the Tuolumne and San Joaquin rivers through a focused examination of the available literature.
- The review will synthesize *findings* specific to the study area.
- Primary sources are preferred and secondary sources are rarely cited. If a secondary or tertiary source is cited, it is clearly identified as such.

5.3 Study Methods

The riparian vegetation study will be accomplished in three steps.

Step 1 – Data Compilation. Source documents will include peer-reviewed literature and reports that address riparian vegetation specific to the study area as well as other sources describing factors (e.g., flow regulation, land use, climate change, and invasive species) that may impact riparian processes and the distribution of riparian vegetation in general. Attachment A provides a preliminary list of references to be reviewed. Subsequent literature review (Step 2) will focus on studies in Attachment A.

Step 2 – Information Review. Documents describing current riparian community structure, composition, and distribution (patch size, connectivity, and floodplain lateral extent) will be reviewed. Linkages between Tuolumne River riparian vegetation dynamics (including creation of fluvial/riparian surfaces, riparian vegetation recruitment, survival, and succession), hydrology (including the frequency and intensity of scouring flows, the spring snow melt hydrograph, and summer low flow conditions), and geomorphology (including fine and coarse sediment supplies and transport) will be assessed. Factors (e.g., hydrologic and geomorphic processes, land use management, invasive species) affecting current riparian conditions in the study area will be reviewed and synthesized. The literature review may include findings of studies in the lower San Joaquin as well as other lowland Central Valley rivers.

Step 3 – Prepare Report. The report will summarize points relevant to evaluating potential cumulative effects of the Project on riparian vegetation in the study area. The report will include the following sections: (1) Study Goals and Objectives; (2) Information sources, assessment methods and analysis; (3) Findings and Discussion; and (5) Conclusions.

6.0 Schedule

The Districts anticipate the schedule to complete the study as follows.

- Data Compilation (Step 1) ..... February 2012
- Information Review (Step 2) ..... February 2012–March 2012
- Report Preparation (Step 3) ..... March 2012–May 2012
- Report Issuance (Step 3) ..... July 2012

7.0 Consistency of Methodology With Generally Accepted Scientific Practices

Review and analysis of existing literature and other information sources is an important and well accepted step in scientific practices.

8.0 Deliverables

The Districts will prepare a report which will document the methodology, literature sources, and findings of the study.

9.0 Level of Effort and Cost

The Districts estimate that the cost to complete this study is $38,000.

10.0 Literature Cited

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Attachment A

Study W&AR-19 Lower Tuolumne River Riparian Information and Synthesis Study Preliminary Literature Sources for Review

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TURLOCK IRRIGATION DISTRICT & MODESTO IRRIGATION DISTRICT
DON PEDRO PROJECT
FERC NO. 2299
WATER AND AQUATIC RESOURCES WORK GROUP

Study Plan W&AR-20
Oncorhynchus mykiss Scale Collection and Age Determination Study Plan
January 2012

Related Study Request: USFWS-10

1.0 Project Nexus

The continued operation and maintenance (O&M) of the Don Pedro Project (Project) may contribute to cumulative effects on salmonid fish habitat in the Tuolumne River downstream of La Grange Dam. These environmental effects include changes in the quality and quantity of physical habitat available for *Oncorhynchus mykiss (O. mykiss*), thereby potentially affecting populations in the lower Tuolumne River.

2.0 Resource Agency Management Goals

The Districts believe that four agencies have resource management goals related to the *O. mykiss* and/or their habitat: (1) U.S. Department of Interior, Fish and Wildlife Service (USFWS); (2) California Department of Fish and Game (CDFG), (3) National Marine Fisheries Service (NMFS), and (4) State Water Resources Control Board, Division of Water Rights (SWRCB).

A broad goal of the USFWS (2001) Anadromous Fish Restoration Program (AFRP), as stated in Section 3406(b)(1) of the Central Valley Project Improvement Act, is to double the long-term production of anadromous fish in California's Central Valley rivers and streams. Although no specific objectives have been established for the Tuolumne River, broader objectives of this long-term goal for the Central Valley include: (1) improve habitat for all life stages of anadromous fish through provision of flows of suitable quality, quantity, and timing, and improved physical habitat; (2) improve survival rates by reducing or eliminating entrainment of juveniles at diversions; (3) improve the opportunity for adult fish to reach spawning habitats in a timely manner; (4) collect fish population, health, and habitat data to facilitate evaluation of restoration actions; (5) integrate habitat restoration efforts with harvest and hatchery management; and (6) involve partners in the implementation and evaluation of restoration actions.

NMFS has developed Resource Management Goals and Objectives for species listed under the Magnuson-Stevens Fishery Conservation and Management Act (16 U.S.C. §1801 et seq.) and the Endangered Species Act (ESA) (16 U.S.C. §1531 et seq.), as well as anadromous species that are not currently listed but may require listing in the future. Although NMFS' (2009) Public Draft Recovery Plan for Sacramento River Winter-run Chinook salmon, Central Valley Spring-run Chinook salmon, and Central Valley steelhead (Draft Recovery Plan) outlines the framework for the recovery of ESA-listed species and populations in California's Central Valley, including the Tuolumne River, this plan has not been adopted by FERC as a comprehensive plan (FERC 2011).

CDFG's mission is to manage California's diverse fish, wildlife, and plant resources, and the habitats upon which they depend, for their ecological values and for their use and enjoyment by the public. CDFG's resource management goals, as summarized in restoration planning documents such as "Restoring Central Valley Streams: A Plan for Action" (Reynolds et al. 1993), are to restore and protect California's aquatic ecosystems that support fish and wildlife, and to protect threatened and endangered species under California Fish and Game Code (Sections 6920–6924).

SWRCB has responsibility under the federal Clean Water Act (33 U.S.C. §11251–1357) to preserve and maintain the chemical, physical and biological integrity of the State's waters and to protect water quality and the beneficial uses of stream reaches consistent with Section 401 of the federal Clean Water Act, the Regional Water Quality Control Board Basin Plans, State Water Board regulations, the California Environmental Quality Act, and any other applicable state law.

3.0 Study Goals

The goal of this study is to determine the age-length relationship of *O. mykiss* in the Tuolumne River. Objectives in meeting this goal include:

- Collect, preserve, and analyze *O. mykiss* scales to determine ages of individual fish, and
- Develop an age to the length relationship for the sampled *O. mykiss*.

4.0 Existing Information and Need for Additional Information

As part of the interrelated *Oncorhynchus mykiss* Population Study (Study Plan W&AR-10), the Districts have agreed to incorporate fish age and growth analyses into the development of population models, relying primarily upon length frequency analysis (e.g., MacDonald and Pitcher 1979) of *O. mykiss* observed in recent snorkel surveys collected in the past several years (e.g., Stillwater Sciences 2011). At the request of Relicensing Participants, the Districts also agreed to collect scales from *O. mykiss* in the lower Tuolumne River, downstream of La Grange Dam to refine age composition and growth estimates. The age-length relationship results of this study will allow *O. mykiss* length data collected from the Tuolumne River during the past several years to be applied in developing representative age structure as part of population modeling in the interrelated *Oncorhynchus mykiss* Population Study (Study Plan W&AR-10).

5.0 Study Methods

5.1 Study Area

The study area includes the Tuolumne River from the La Grange Dam (RM 52) downstream to Robert's Ferry Bridge (RM 39.5), which is the section of the lower Tuolumne River typically inhabited by *O. mykiss*.

5.2 General Concepts

The following general concepts apply to the study:

- Personal safety is an important consideration of each fieldwork team. The Districts and their consultants will perform the study in a safe manner; areas considered unsafe in the judgment of field teams will not be surveyed.
- The Districts will make a good faith effort to obtain permission in advance of performance of the study to access private property where needed. Field crews may make minor modifications in the field to adjust to and to accommodate actual field conditions and unforeseeable events. Any modifications made will be documented and reported in the draft study reports.

5.3 Study Methods

The study method will consist of the following four steps.

Step 1 – Study Design and Permitting. *O. mykiss* will be collected from pool and riffle-tail habitats by angling or other potentially more efficient methods to be determined as part of the ESA Section 10 and Scientific Collection Permitting processes. Length data and scale samples will be obtained from up to 75 fish using 15 individuals per 100 mm size-group (i.e., 50-50 mm, 150-250 mm, 250–350 mm, etc) encountered during sampling.

Because initiation and completion of the study, as described in this Plan, is contingent on permit approval by NMFS and CDFG, permit inquiries and requests will be made in February 2012, in advance of study initiation. The Districts will make a good faith effort to modify the study design to comply with permit conditions and proceed with the study, if possible. In the event permits are not granted or an insufficient number of individuals are captured, the Districts will reevaluate alternative approaches to developing age at length and age structure information as part of the interrelated salmonid studies (e.g.,W&AR-5, W&AR-6 and W&AR-10).

Step 2 – Field Sampling. Juvenile and adult *O. mykiss* will be captured in the Tuolumne River at selected locations from RM 52 (La Grange Dam) downstream to approximately RM 39.5 (Roberts Ferry Bridge), which is the portion of the river where *O. mykiss* have been historically observed (Stillwater Sciences 2011).

The survey crew will record the location (GPS coordinates), habitat type, and length of each captured *O. mykiss*. Fish will be transferred to a measurement cradle and data recorded for all fish meeting the required length criterion, including fork length (FL, mm), total length (TL, mm), and general condition. If possible, the sex of each fish will be determined, and any marks that would aid in determining hatchery vs. wild origin (e.g., adipose fin clip) will be noted.

Scales will be removed from the region between the posterior end of the dorsal fin and the lateral line on the left side, roughly two scale rows above the lateral line. Prior to scale removal, mucous and debris will be cleaned from the sampling location for ease in scale processing. Scales will be removed by scraping a dull knife from the anterior to posterior of the sample area (Figure 1). Approximately 10 scales will be removed per fish.

All collected scales from individual fish will be placed on a square of "Rite in the Rain" paper. The paper will be folded over the blade and pinched to remove the scales. The folded paper will be immediately inserted into an envelope. Each individual envelope will be clearly labeled with species, site location, fork length, weight, date, condition, and any other applicable information.

All envelopes will be pressed flat to reduce scale curling and increase analytical accuracy. Only one envelope will be used for each fish. Knives will then be thoroughly cleaned with ethanol to prevent cross-contamination of scale samples.

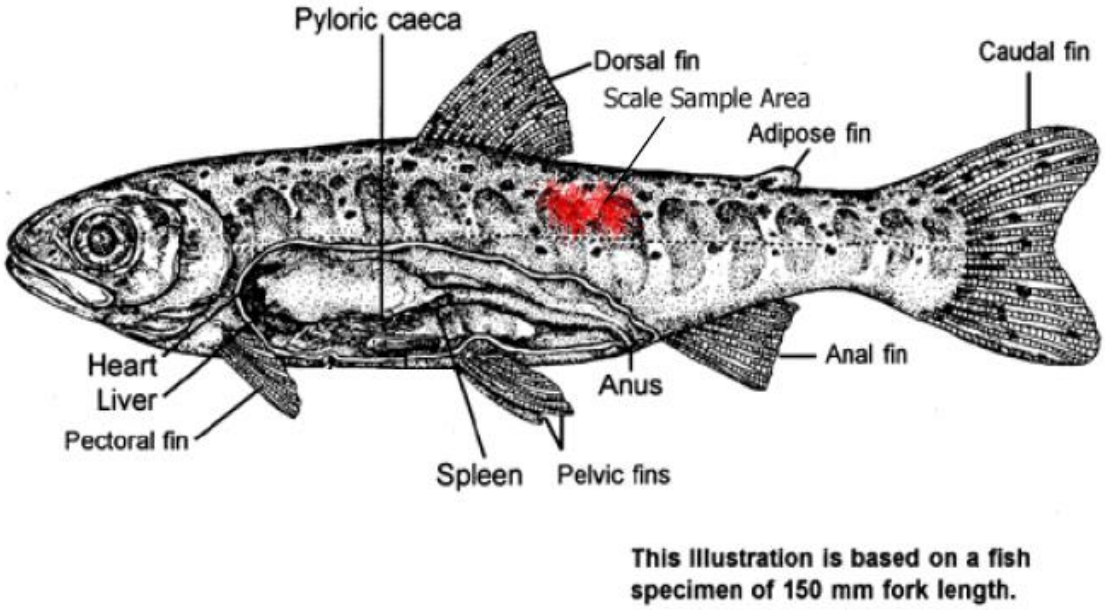

Figure 1. Fish schematic showing area (red) where scale sample will be taken from fish (modified from Columbia Basin Fish and Wildlife Authority. 1999).

Step 3 – Analysis. Scales will be prepared by qualified staff according to standard procedures (Drummond 1966). Scales will be transferred from the envelopes onto a glass slide. The best scales will be arranged towards the top of the slide, and all scales will be oriented the same way. Care will be taken to insure that all scales are laid flat, not curled. Another glass slide will be placed on top and then both slides will be taped together. Each slide will be labeled with the sample identification number and date. Each scale will then be examined under a microscope at both 10x and 40x power so that annuli can be discerned.

Age of fish will be determined using scale analysis following the methods of DeVries and Frie (1996). Results will be recorded in a MS Excel spreadsheet. Scales will be available for independent analysis by USFWS, CDFG and/or NMFS staff. If there is a difference of opinion between analysts or other difficulty reading any scale, all examining staff will convene to review the scales and determine the age.

Step 4 – Prepare Report. The Districts will prepare a report that includes the following sections: (1) Study Goals and Objectives; (2) Methods and Analysis; (3) Results; (4) Discussion; and (5) Conclusions. The report for this study will include an *O. mykiss* length at age relationship and estimated growth rates.

6.0 Schedule

The Districts anticipate the schedule to complete the study proposal as follows:

- Study Design and Permitting (Step 1) .....February – May 2012
- Scale collection (Step 2)…… ... March –July 2012
- Analysis and Synthesis (Step 3) .....August – September 2012
- Report Preparation (Step 4) ..... September – December 2012
- Report Issuance .....January 2013

Initiation of scale collection is dependent on acquisition of the necessary permit modifications from CDFG and/or NMFS. Every effort will be made by the Districts to complete the permit process prior to data collection during the summer of 2012.

7.0 Consistency of Methodology with Generally Accepted Scientific Practices

The methods presented in this study plan are consistent with other generally accepted scientific study methods concerning the ageing and analysis of age-growth relationships for salmonids, including those conducted by the state and federal resource agencies.

8.0 Deliverables

The Districts will prepare a final study report, which will document the methodology and results of the study. The study products will include a description of *O. mykiss* length at age relationships in the lower Tuolumne River.

9.0 Level of Effort and Cost

The Districts estimates that the cost to complete this study is $89,000.

10.0 References

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From: Staples, Rose
Sent: Tuesday, January 24, 2012 12:03 PM
To: Alves, Jim - City of Modesto; Anderson, Craig - USFWS; Asay, Lynette - N-R; Aud, John - SCERD; Barnes, James - BLM; Barnes, Peter - SWRCB; Beuttler, John - CSPA; Blake, Martin; Bond, Jack - City of Modesto; Boucher, Allison - TRC; Boucher, Dave - Allison - TRC; Bowes, Stephen - NPS; Bowman, Art - CWRMP; Brenneman, Beth - BLM; Brewer, Doug - TetraTech; Brochini, Anthony - SSMN; Brochini, Tony - NPS; Buckley, John - CSERC; Buckley, Mark; Burley, Silvia-CVMT; Burt, Charles - CalPoly; Cadagan, Jerry; Carlin, Michael - SFPUC; Catlett, Kelly - FOR; Charles, Cindy - GWWF; Cismowski, Gail - SWRCB; Costa, Jan - Chicken Ranch; Cowan, Jeffrey; Cox, Stanley Rob - TBMWI; Cranston, Peggy - BLM; Cremeen, Rebecca - CSERC; Day, Kevin - TBMI; Day, P - MF; Denean - BVR; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne - OHP; Dowd, Maggie-SNF; Drekmeier, Peter - TRT; Edmondson, Steve - NOAA; Eicher, James - BLM; Fety, Lauren - BLM; Findley, Timothy - Hanson Bridgett; Freeman, Beau - CalPoly; Fuller, Reba - TMTC; Furman, Donn W - SFPUC; Ganteinbein, Julie - Water-Power Law Grp; Giglio, Deborah - USFWS; Gorman, Elaine - YSC; Grader, Zeke; Gutierrez, Monica - NOAA-NMFS; Hackamack, Robert; Hastreiter, James L - FERC; Hatch, Jenny - CT; Hayat, Zahra - MF; Hayden, Ann; Hellam, Anita - HH; Heyne, Tim - CDFG; Holden, James ; Holm, Lisa; Horn, Jeff - BLM; Horn, Tini; Hudelson, Bill - StanislausFoodProducts; Hughes, Noah; Hughes, Robert - CDFG; Hume, Noah - Stillwater; Jackman, Jerry ; Jackson, Zac - USFWS; Jennings, William - CSPA; Jensen, Art - BAWSCA; Jensen, Laura - TNC; Johannis, Mary; Johnson, Brian - CalTrout; Justin; Keating, Janice; Kempton, Kathryn - NOAA-MNFS; Kinney, Teresa; Koepele, Patrick - TRT; Kordella, Lesley - FERC; Lein, Joseph; Levin, Ellen - SFPUC; Lewis-Reggie-PRCI; Linkard, David - TRT /RH; Looker, Mark - LCC; Loy, Carin; Lwenya, Roselynn, BVR; Lyons, Bill - MR; Madden, Dan; Manji, Annie; Marko, Paul ; Marshall, Mike - RHH; Martin, Michael - MFFC; Martin, Ramon - USFWS; Mathiesen, Lloyd - CRRMW; McDaniel, Dan -CDWA; McDevitt, Ray - BAWSCA; McDonnell, Marty - SMRT; McLain, Jeffrey - NOAA-NMFS; Means, Julie - CDFG; Mills, John - TUD; Morningstar Pope, Rhonda - BVR; Motola, Mary - PRCI; O'Brien, Jennifer - CDFG; Orvis, Tom - SCFB; Ott, Bob; Ott, Chris; Paul, Duane - Cardno; Pavich, Steve-Cardno; Pinhey, Nick - City of Modesto; Pool, Richard; Porter, Ruth - RHH; Powell, Melissa - CRRMW; Puccini, Stephen - CDFG; Raeder, Jessie - TRT; Ramirez, Tim - SFPUC; Rea, Maria - NOAA-NMFS; Reed, Rhonda - NOAA-NMFS; Richardson, Kevin - USACE; Ridenour, Jim; Robbins, Royal; Romano, David O - N-R; Roos-Collins, Richard - Water-Power Law Grp for NHI; Roseman, Jesse; Rothert, Steve - AR; Sander, Max - TNC; Sandkulla, Nicole - BAWSCA; Saunders, Jenan; Schutte, Allison - HB; Sears, William - SFPUC; Shipley, Robert; Shumway, Vern - SNF; Shutes, Chris - CSPA; Sill, Todd; Slay, Ronn - CNRF/AIC; Smith, Jim - MPM; Staples, Rose; Steindorf, Dave - AW; Steiner, Dan; Stone, Vicki -TBMI; Stork, Ron - FOR; Stratton, Susan - CA SHPO; Taylor, Mary Jane - CDFG; Terpstra, Thomas; TeVelde, George A ; Thompson, Larry - NOAA-MNFS; Vasquez, Sandy ; Verkuil, Colette - TRT/MF; Vierra, Chris; Villalabos, Ruben; Walters, Eric - MF; Wantuck, Rick - NOAA-NMFS; Welch, Steve - ARTA; Wesselman, Eric - TRT; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas - RHH; Wilcox, Scott - Stillwater; Williamson, Harry (NPS); Willy, Alison - FWS; Wilson, Bryan - MF; Winchell, Frank - FERC; Wood, Dave - FR; Wooster, John -NOAA; Workman, Michelle - USFWS; Yoshiyama, Ron; Zipser, Wayne - SCFB
Subject: TID - MID Motion Filed Today with FERC

Please be aware that the Districts have filed the following motion with FERC:

On 1/24/2012, the following Filing was submitted to the Federal Energy Regulatory Commission (FERC), Washington D.C.:

Filer: Turlock Irrigation District and Modesto Irrigation District
Winston & Strawn LLP (as Agent)

Docket(s): P-2299-075
Filing Type: Procedural Motion
Description: Motion of the Modesto and Turlock Irrigation Districts to Disqualify Agency Dispute Panel Member under P-2299-075.

The filing can be viewed on FERC's E-Library website at www.ferc.gov.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Staples, Rose
Sent: Tuesday, January 24, 2012 12:27 PM
To: 'Alves, Jim - City of Modesto'; 'Anderson, Craig - USFWS'; 'Asay, Lynette - N-R'; 'Aud, John - SCERD'; 'Barnes, James - BLM'; 'Barnes, Peter - SWRCB'; 'Beuttler, John - CSPA'; 'Blake, Martin'; 'Bond, Jack - City of Modesto'; 'Boucher, Allison - TRC'; 'Boucher, Dave - Allison - TRC'; 'Bowes, Stephen - NPS'; 'Bowman, Art - CWRMP'; 'Brenneman, Beth - BLM'; 'Brewer, Doug - TetraTech'; 'Brochini, Anthony - SSMN'; 'Brochini, Tony - NPS'; 'Buckley, John - CSERC'; 'Buckley, Mark'; 'Burley, Silvia-CVMT'; 'Burt, Charles - CalPoly'; 'Cadagan, Jerry'; 'Carlin, Michael - SFPUC'; 'Catlett, Kelly - FOR'; 'Charles, Cindy - GWWF'; 'Cismowski, Gail - SWRCB'; 'Costa, Jan - Chicken Ranch'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob - TBMWI'; 'Cranston, Peggy - BLM'; 'Cremeen, Rebecca - CSERC'; 'Day, Kevin - TBMI'; 'Day, P - MF'; 'Denean - BVR'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne - OHP'; 'Dowd, Maggie-SNF'; 'Drekmeier, Peter - TRT'; 'Edmondson, Steve - NOAA'; 'Eicher, James - BLM'; 'Fety, Lauren - BLM'; 'Findley, Timothy - Hanson Bridgett'; 'Freeman, Beau - CalPoly'; 'Fuller, Reba - TMTC'; 'Furman, Donn W - SFPUC'; 'Ganteinbein, Julie - Water-Power Law Grp'; 'Giglio, Deborah - USFWS'; 'Gorman, Elaine - YSC'; 'Grader, Zeke'; 'Gutierrez, Monica - NOAA-NMFS'; 'Hackamack, Robert'; 'Hastreiter, James L - FERC'; 'Hatch, Jenny - CT'; 'Hayat, Zahra - MF'; 'Hayden, Ann'; 'Hellam, Anita - HH'; 'Heyne, Tim - CDFG'; 'Holden, James '; 'Holm, Lisa'; 'Horn, Jeff - BLM'; 'Horn, Tini'; 'Hudelson, Bill - StanislausFoodProducts'; 'Hughes, Noah'; 'Hughes, Robert - CDFG'; 'Hume, Noah - Stillwater'; 'Jackman, Jerry '; 'Jackson, Zac - USFWS'; 'Jennings, William - CSPA'; 'Jensen, Art - BAWSCA'; 'Jensen, Laura - TNC'; 'Johannis, Mary'; 'Johnson, Brian - CalTrout'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn - NOAA-MNFS'; 'Kinney, Teresa'; 'Koepele, Patrick - TRT'; 'Kordella, Lesley - FERC'; 'Lein, Joseph'; 'Levin, Ellen - SFPUC'; 'Lewis-Reggie-PRCI'; 'Linkard, David - TRT /RH'; 'Looker, Mark - LCC'; Loy, Carin; 'Lwenya, Roselynn, BVR'; 'Lyons, Bill - MR'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul '; 'Marshall, Mike - RHH'; 'Martin, Michael - MFFC'; 'Martin, Ramon - USFWS'; 'Mathiesen, Lloyd - CRRMW'; 'McDaniel, Dan -CDWA'; 'McDevitt, Ray - BAWSCA'; 'McDonnell, Marty - SMRT'; 'McLain, Jeffrey - NOAA-NMFS'; 'Means, Julie - CDFG'; 'Mills, John - TUD'; 'Morningstar Pope, Rhonda - BVR'; 'Motola, Mary - PRCI'; 'O'Brien, Jennifer - CDFG'; 'Orvis, Tom - SCFB'; 'Ott, Bob'; 'Ott, Chris'; 'Paul, Duane - Cardno'; 'Pavich, Steve-Cardno'; 'Pinhey, Nick - City of Modesto'; 'Pool, Richard'; 'Porter, Ruth - RHH'; 'Powell, Melissa - CRRMW'; 'Puccini, Stephen - CDFG'; 'Raeder, Jessie - TRT'; 'Ramirez, Tim - SFPUC'; 'Rea, Maria - NOAA-NMFS'; 'Reed, Rhonda - NOAA-NMFS'; 'Richardson, Kevin - USACE'; 'Ridenour, Jim'; 'Robbins, Royal'; 'Romano, David O - N-R'; 'Roos-Collins, Richard - Water-Power Law Grp for NHI'; 'Roseman, Jesse'; 'Rothert, Steve - AR'; 'Sander, Max - TNC'; 'Sandkulla, Nicole - BAWSCA'; 'Saunders, Jenan'; 'Schutte, Allison - HB'; 'Sears, William - SFPUC'; 'Shipley, Robert'; 'Shumway, Vern - SNF'; 'Shutes, Chris - CSPA'; 'Sill, Todd'; 'Slay, Ronn - CNRF/AIC'; 'Smith, Jim - MPM'; 'Steindorf, Dave - AW'; 'Steiner, Dan'; 'Stone, Vicki -TBMI'; 'Stork, Ron - FOR'; 'Stratton, Susan - CA SHPO'; 'Taylor, Mary Jane - CDFG'; 'Terpstra, Thomas'; 'TeVelde, George A '; 'Thompson, Larry - NOAA-MNFS'; 'Vasquez, Sandy '; 'Verkuil, Colette - TRT/MF'; 'Vierra, Chris'; 'Villalabos, Ruben'; 'Walters, Eric - MF'; 'Wantuck,

Rick - NOAA-NMFS'; 'Welch, Steve - ARTA'; 'Wesselman, Eric - TRT'; 'Wheeler, Dan'; 'Wheeler, Dave'; 'Wheeler, Douglas - RHH'; 'Wilcox, Scott - Stillwater'; 'Williamson, Harry (NPS)'; 'Willy, Alison - FWS'; 'Wilson, Bryan - MF'; 'Winchell, Frank - FERC'; 'Wood, Dave - FR'; 'Wooster, John -NOAA'; 'Workman, Michelle - USFWS'; 'Yoshiyama, Ron'; 'Zipser, Wayne - SCFB'

Subject: RE: TID - MID Motion Filed Today with FERC

I understand that once you access FERC's E-Library website, searching on "P-2299-075" is NOT producing any results. Please try just entering the project number (P-2299), leaving off the sub-docket number 075. I was able to successfully locate the document a few minutes ago by going that route in their search function. Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

From: Staples, Rose
Sent: Tuesday, January 24, 2012 12:01 PM

Please be aware that the Districts have filed the following motion with FERC:

On 1/24/2012, the following Filing was submitted to the Federal Energy Regulatory Commission (FERC), Washington D.C.:

Filer: Turlock Irrigation District and Modesto Irrigation District
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ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

From: Staples, Rose
Sent: Tuesday, February 07, 2012 8:15 PM
To: Alves, Jim - City of Modesto; Anderson, Craig - USFWS; Asay, Lynette - N-R; Aud, John - SCERD; Barnes, James - BLM; Barnes, Peter - SWRCB; Beuttler, John - CSPA; Blake, Martin; Bond, Jack - City of Modesto; Boucher, Allison - TRC; Boucher, Dave - Allison - TRC; Bowes, Stephen - NPS; Bowman, Art - CWRMP; Brenneman, Beth - BLM; Brewer, Doug - TetraTech; Brochini, Anthony - SSMN; Brochini, Tony - NPS; Buckley, John - CSERC; Buckley, Mark; Burley, Silvia-CVMT; Burt, Charles - CalPoly; Cadagan, Jerry; Carlin, Michael - SFPUC; Catlett, Kelly - FOR; Charles, Cindy - GWWF; Cismowski, Gail - SWRCB; Costa, Jan - Chicken Ranch; Cowan, Jeffrey; Cox, Stanley Rob - TBMWI; Cranston, Peggy - BLM; Cremeen, Rebecca - CSERC; Day, Kevin - TBMI; Day, P - MF; Denean - BVR; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne - OHP; Dowd, Maggie-SNF; Drekmeier, Peter - TRT; Edmondson, Steve - NOAA; Eicher, James - BLM; Fety, Lauren - BLM; Findley, Timothy - Hanson Bridgett; Freeman, Beau - CalPoly; Fuller, Reba - TMTC; Furman, Donn W - SFPUC; Ganteinbein, Julie - Water-Power Law Grp; Giglio, Deborah - USFWS; Gorman, Elaine - YSC; Grader, Zeke; Gutierrez, Monica - NOAA-NMFS; Hackamack, Robert; Hastreiter, James L - FERC; Hatch, Jenny - CT; Hayat, Zahra - MF; Hayden, Ann; Hellam, Anita - HH; Heyne, Tim - CDFG; Holden, James ; Holm, Lisa; Horn, Jeff - BLM; Horn, Tini; Hudelson, Bill - StanislausFoodProducts; Hughes, Noah; Hughes, Robert - CDFG; Hume, Noah - Stillwater; Jackman, Jerry ; Jackson, Zac - USFWS; Jennings, William - CSPA; Jensen, Art - BAWSCA; Jensen, Laura - TNC; Johannis, Mary; Johnson, Brian - CalTrout; Justin; Keating, Janice; Kempton, Kathryn - NOAA-MNFS; Kinney, Teresa; Koepele, Patrick - TRT; Kordella, Lesley - FERC; Lein, Joseph; Levin, Ellen - SFPUC; Lewis-Reggie-PRCI; Linkard, David - TRT /RH; Looker, Mark - LCC; Loy, Carin; Lwenya, Roselynn, BVR; Lyons, Bill - MR; Madden, Dan; Manji, Annie; Marko, Paul ; Marshall, Mike - RHH; Martin, Michael - MFFC; Martin, Ramon - USFWS; Mathiesen, Lloyd - CRRMW; McDaniel, Dan -CDWA; McDevitt, Ray - BAWSCA; McDonnell, Marty - SMRT; McLain, Jeffrey - NOAA-NMFS; Means, Julie - CDFG; Mills, John - TUD; Morningstar Pope, Rhonda - BVR; Motola, Mary - PRCI; O'Brien, Jennifer - CDFG; Orvis, Tom - SCFB; Ott, Bob; Ott, Chris; Paul, Duane - Cardno; Pavich, Steve-Cardno; Pinhey, Nick - City of Modesto; Pool, Richard; Porter, Ruth - RHH; Powell, Melissa - CRRMW; Puccini, Stephen - CDFG; Raeder, Jessie - TRT; Ramirez, Tim - SFPUC; Rea, Maria - NOAA-NMFS; Reed, Rhonda - NOAA-NMFS; Richardson, Kevin - USACE; Ridenour, Jim; Robbins, Royal; Romano, David O - N-R; Roos-Collins, Richard - Water-Power Law Grp for NHI; Roseman, Jesse; Rothert, Steve - AR; Sander, Max - TNC; Sandkulla, Nicole - BAWSCA; Saunders, Jenan; Schutte, Allison - HB; Sears, William - SFPUC; Shakal, Sarah - Humboldt State; Shipley, Robert; Shumway, Vern - SNF; Shutes, Chris - CSPA; Sill, Todd; Slay, Ronn - CNRF/AIC; Smith, Jim - MPM; Staples, Rose; Steindorf, Dave - AW; Steiner, Dan; Stone, Vicki -TBMI; Stork, Ron - FOR; Stratton, Susan - CA SHPO; Taylor, Mary Jane - CDFG; Terpstra, Thomas; TeVelde, George A ; Thompson, Larry - NOAA-MNFS; Vasquez, Sandy ; Verkuil, Colette - TRT/MF; Vierra, Chris; Villalabos, Ruben; Walters, Eric - MF; Wantuck, Rick - NOAA-NMFS; Welch, Steve - ARTA; Wesselman, Eric - TRT; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas - RHH; Wilcox, Scott - Stillwater; Williamson, Harry (NPS); Willy, Alison - FWS; Wilson, Bryan - MF; Winchell, Frank - FERC; Wood, Dave - FR; Wooster, John -NOAA; Workman, Michelle - USFWS; Yoshiyama, Ron; Zipser, Wayne - SCFB
Subject: Don Pedro Project Relicensing Water & Aquatic Study Plans Workshop/Meeting Schedule for 2012

In accordance with FERC's Study Plan Determination and the Districts' Water & Aquatic (W&AR) study plans to be underway in 2012, we have developed schedule dates for the various workshops contained within the study plans. Please make note of these below:

April 2012

Apr 09 1:00 pm - 5:00 pm PT Don Pedro Project Relicensing - Hydrology Workshop (W&AR-2) (Modesto Irrigation District Offices, Modesto {MID})

Apr 10 8:00 am – 10:00 am PT Don Pedro Project Relicensing - Reservoir Temperature Modeling Data and Methods (MID)

Apr 10 10:15 am - 5:00 pm PT Don Pedro Project Relicensing - Salmonid Population Information Workshop (W&AR-5) (MID)

June 2012

Jun 26 9:00 am – 4:00 pm PT Don Pedro Project Relicensing - Salmonid Population Information Workshop (W&AR-5) (MID)

September 2012

Sep 18 9:00 am - 4:00 pm PT Don Pedro Project Relicensing - Temperature Model Verification/Calibration Meeting (MID)

November 2012

Nov 15 9:00 am - 4:00 pm PT Don Pedro Project Relicensing - Chinook Population (W&AR-6) and O.mykiss Population

(W&AR-10) Modeling Workshop (MID)

In addition, in accordance with FERC's direction regarding the development and implementation of a more explicit consultation program for those studies with workshops, we are proposing to hold a meeting on March 20th at MID (from 1:30 to 4:30 p.m.) to discuss and finalize such a Workshop Consultation Program. An initial proposal will be forwarded by March 5 to all participants.

March 2012

Mar 20 1:30 pm – 4:30 pm PT Don Pedro Project Relicensing - Workshop on Consultation Process (as per Appendix B of FERC's Study Plan Determination) (MID)

We look forward to continuing to work with all relicensing participants in 2012.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Staples, Rose
Sent: Tuesday, February 07, 2012 2:13 PM
To: Alves, Jim - City of Modesto; Anderson, Craig - USFWS; Asay, Lynette - N-R; Aud, John - SCERD; Barnes, James - BLM; Barnes, Peter - SWRCB; Beuttler, John - CSPA; Blake, Martin; Bond, Jack - City of Modesto; Boucher, Allison - TRC; Boucher, Dave - Allison - TRC; Bowes, Stephen - NPS; Bowman, Art - CWRMP; Brenneman, Beth - BLM; Brewer, Doug - TetraTech; Brochini, Anthony - SSMN; Brochini, Tony - NPS; Buckley, John - CSERC; Buckley, Mark; Burley, Silvia-CVMT; Burt, Charles - CalPoly; Cadagan, Jerry; Carlin, Michael - SFPUC; Catlett, Kelly - FOR; Charles, Cindy - GWWF; Cismowski, Gail - SWRCB; Costa, Jan - Chicken Ranch; Cowan, Jeffrey; Cox, Stanley Rob - TBMWI; Cranston, Peggy - BLM; Cremeen, Rebecca - CSERC; Day, Kevin - TBMI; Day, P - MF; Denean - BVR; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne - OHP; Dowd, Maggie-SNF; Drekmeier, Peter - TRT; Edmondson, Steve - NOAA; Eicher, James - BLM; Fety, Lauren - BLM; Findley, Timothy - Hanson Bridgett; Freeman, Beau - CalPoly; Fuller, Reba - TMTC; Furman, Donn W - SFPUC; Ganteinbein, Julie - Water-Power Law Grp; Giglio, Deborah - USFWS; Gorman, Elaine - YSC; Grader, Zeke; Gutierrez, Monica - NOAA-NMFS; Hackamack, Robert; Hastreiter, James L - FERC; Hatch, Jenny - CT; Hayat, Zahra - MF; Hayden, Ann; Hellam, Anita - HH; Heyne, Tim - CDFG; Holden, James ; Holm, Lisa; Horn, Jeff - BLM; Horn, Tini; Hudelson, Bill - StanislausFoodProducts; Hughes, Noah; Hughes, Robert - CDFG; Hume, Noah - Stillwater; Jackman, Jerry ; Jackson, Zac - USFWS; Jennings, William - CSPA; Jensen, Art - BAWSCA; Jensen, Laura - TNC; Johannis, Mary; Johnson, Brian - CalTrout; Justin; Keating, Janice; Kempton, Kathryn - NOAA-MNFS; Kinney, Teresa; Koepele, Patrick - TRT; Kordella, Lesley - FERC; Lein, Joseph; Levin, Ellen - SFPUC; Lewis-Reggie-PRCI; Linkard, David - TRT /RH; Looker, Mark - LCC; Loy, Carin; Lwenya, Roselynn, BVR; Lyons, Bill - MR; Madden, Dan; Manji, Annie; Marko, Paul ; Marshall, Mike - RHH; Martin, Michael - MFFC; Martin, Ramon - USFWS; Mathiesen, Lloyd - CRRMW; McDaniel, Dan -CDWA; McDevitt, Ray - BAWSCA; McDonnell, Marty - SMRT; McLain, Jeffrey - NOAA-NMFS; Means, Julie - CDFG; Mills, John - TUD; Morningstar Pope, Rhonda - BVR; Motola, Mary - PRCI; O'Brien, Jennifer - CDFG; Orvis, Tom - SCFB; Ott, Bob; Ott, Chris; Paul, Duane - Cardno; Pavich, Steve-Cardno; Pinhey, Nick - City of Modesto; Pool, Richard; Porter, Ruth - RHH; Powell, Melissa - CRRMW; Puccini, Stephen - CDFG; Raeder, Jessie - TRT; Ramirez, Tim - SFPUC; Rea, Maria - NOAA-NMFS; Reed, Rhonda - NOAA-NMFS; Richardson, Kevin - USACE; Ridenour, Jim; Robbins, Royal; Romano, David O - N-R; Roos-Collins, Richard - Water-Power Law Grp for NHI; Roseman, Jesse; Rothert, Steve - AR; Sander, Max - TNC; Sandkulla, Nicole - BAWSCA; Saunders, Jenan; Schutte, Allison - HB; Sears, William - SFPUC; Shakal, Sarah - Humboldt State; Shipley, Robert; Shumway, Vern - SNF; Shutes, Chris - CSPA; Sill, Todd; Slay, Ronn - CNRF/AIC; Smith, Jim - MPM; Staples, Rose; Steindorf, Dave - AW; Steiner, Dan; Stone, Vicki - TBMI; Stork, Ron - FOR; Stratton, Susan - CA SHPO; Taylor, Mary Jane - CDFG; Terpstra, Thomas; TeVelde, George A ; Thompson, Larry - NOAA-MNFS; Vasquez, Sandy ; Verkuil, Colette - TRT/MF; Vierra, Chris; Villalabos, Ruben; Walters, Eric - MF; Wantuck, Rick - NOAA-NMFS; Welch, Steve -

ARTA; Wesselman, Eric - TRT; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas - RHH; Wilcox, Scott - Stillwater; Williamson, Harry (NPS); Willy, Alison - FWS; Wilson, Bryan - MF; Winchell, Frank - FERC; Wood, Dave - FR; Wooster, John -NOAA; Workman, Michelle - USFWS; Yoshiyama, Ron; Zipser, Wayne - SCFB
Subject:FW: FERC Acceptance for Filing in P-2299-075

Please be advised that the TID and MID Districts have filed a request with FERC to extend the deadline to February 28, 2012 to submit Water & Aquatic Study Plans 18, 19, and 20 (the drafts of which you are currently reviewing) for Commission approval.

As stated in the letter:

The Commission's Study Plan Determination for the Don Pedro Project, which was issued on December 21, 2011, directed Turlock Irrigation District and Modesto Irrigation District (collectively, the Districts) to submit for Commission approval three study plans as follows:

W&AR-18	Sturgeon Study
W&AR-19	Lower Tuolumne Riparian Information and Synthesis Study
W&AR-20	O.mykiss Scale and Age Determination

The Commission directed the Districts to file these three study plans within 60 days after the issuance date of the Study Plan Determination, or February 19, 2012. Since February 19 falls on a Sunday and February 20 is a holiday (Presidents' Day), the study plans must be filed by Tuesday, February 21. The Districts issued the three study plans to Relicensing Participants for review and comment on January 20, 2012, and requested that all comments be provided no later than February 20, 2012.

As mentioned above, February 20 is a holiday, so comments would be due from the Relicensing Participants by February 21. To give the Districts adequate time to review and address all of the Relicensing Participants' comments, the Districts respectfully request an extension of time for filing the three study plans with the Commission until February 28, 2012.

A copy of the letter is available on FERC's E-Library at www.FERC.gov under docket P-2299-075 -- and it is also available on the www.donpedro-relicensing.com website under the Introduction/Announcement banner.

ROSE STAPLES CAP-OM
HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103

From: Staples, Rose
Sent: Friday, February 17, 2012 5:04 PM
To: Staples, Rose; Alves, Jim - City of Modesto; Anderson, Craig - USFWS; Asay, Lynette - N-R; Aud, John - SCERD; Barnes, James - BLM; Barnes, Peter - SWRCB; Beuttler, John - CSPA; Blake, Martin; Bond, Jack - City of Modesto; Boucher, Allison - TRC; Boucher, Dave - Allison - TRC; Bowes, Stephen - NPS; Bowman, Art - CWRMP; Brenneman, Beth - BLM; Brewer, Doug - TetraTech; Brochini, Anthony - SSMN; Brochini, Tony - NPS; Buckley, John - CSERC; Buckley, Mark; Burley, Silvia-CVMT; Burt, Charles - CalPoly; Cadagan, Jerry; Carlin, Michael - SFPUC; Catlett, Kelly - FOR; Charles, Cindy - GWWF; Cismowski, Gail - SWRCB; Costa, Jan - Chicken Ranch; Cowan, Jeffrey; Cox, Stanley Rob - TBMWI; Cranston, Peggy - BLM; Cremeen, Rebecca - CSERC; Day, Kevin - TBMI; Day, P - MF; Denean - BVR; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne - OHP; Dowd, Maggie-SNF; Drekmeier, Peter - TRT; Edmondson, Steve - NOAA; Eicher, James - BLM; Fety, Lauren - BLM; Findley, Timothy - Hanson Bridgett; Freeman, Beau - CalPoly; Fuller, Reba - TMTC; Furman, Donn W - SFPUC; Ganteinbein, Julie - Water-Power Law Grp; Giglio, Deborah - USFWS; Gorman, Elaine - YSC; Grader, Zeke; Gutierrez, Monica - NOAA-NMFS; Hackamack, Robert; Hastreiter, James L - FERC; Hatch, Jenny - CT; Hayat, Zahra - MF; Hayden, Ann; Hellam, Anita - HH; Heyne, Tim - CDFG; Holden, James ; Holm, Lisa; Horn, Jeff - BLM; Horn, Tini; Hudelson, Bill - StanislausFoodProducts; Hughes, Noah; Hughes, Robert - CDFG; Hume, Noah - Stillwater; Jackman, Jerry ; Jackson, Zac - USFWS; Jennings, William - CSPA; Jensen, Art - BAWSCA; Jensen, Laura - TNC; Johannis, Mary; Johnson, Brian - CalTrout; Justin; Keating, Janice; Kempton, Kathryn - NOAA-MNFS; Kinney, Teresa; Koepele, Patrick - TRT; Kordella, Lesley - FERC; Lein, Joseph; Levin, Ellen - SFPUC; Lewis-Reggie-PRCI; Linkard, David - TRT /RH; Looker, Mark - LCC; Loy, Carin; Lwenya, Roselynn, BVR; Lyons, Bill - MR; Madden, Dan; Manji, Annie; Marko, Paul ; Marshall, Mike - RHH; Martin, Michael - MFFC; Martin, Ramon - USFWS; Mathiesen, Lloyd - CRRMW; McDaniel, Dan -CDWA; McDevitt, Ray - BAWSCA; McDonnell, Marty - SMRT; McLain, Jeffrey - NOAA-NMFS; Means, Julie - CDFG; Mills, John - TUD; Morningstar Pope, Rhonda - BVR; Motola, Mary - PRCI; O'Brien, Jennifer - CDFG; Orvis, Tom - SCFB; Ott, Bob; Ott, Chris; Paul, Duane - Cardno; Pavich, Steve-Cardno; Pinhey, Nick - City of Modesto; Pool, Richard; Porter, Ruth - RHH; Powell, Melissa - CRRMW; Puccini, Stephen - CDFG; Raeder, Jessie - TRT; Ramirez, Tim - SFPUC; Rea, Maria - NOAA-NMFS; Reed, Rhonda - NOAA-NMFS; Richardson, Kevin - USACE; Ridenour, Jim; Robbins, Royal; Romano, David O - N-R; Roos-Collins, Richard - Water-Power Law Grp for NHI; Roseman, Jesse; Rothert, Steve - AR; Sander, Max - TNC; Sandkulla, Nicole - BAWSCA; Saunders, Jenan; Schutte, Allison - HB; Sears, William - SFPUC; Shipley, Robert; Shumway, Vern - SNF; Shutes, Chris - CSPA; Sill, Todd; Slay, Ronn - CNRF/AIC; Smith, Jim - MPM; Steindorf, Dave - AW; Steiner, Dan; Stone, Vicki -TBMI; Stork, Ron - FOR; Stratton, Susan - CA SHPO; Taylor, Mary Jane - CDFG; Terpstra, Thomas; TeVelde, George A ; Thompson, Larry - NOAA-MNFS; Vasquez, Sandy ; Verkuil, Colette - TRT/MF; Vierra, Chris; Villalabos, Ruben; Walters, Eric - MF; Wantuck, Rick - NOAA-NMFS; Welch, Steve - ARTA; Wesselman, Eric - TRT; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas -

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Subject: RE: : Don Pedro Draft Study Plans – Sturgeon, Riparian, and O.myskiss Scale Studies

On January 20th I sent an email (copy below) advising that comments on the following three study plan were due to the DISTRICTS, via email to me (rose.staples@hdrinc.com) or via fax (207-775-1742), no later than February 20, 2012.

W&AR 18 – Sturgeon Study
W&AR 19 – Lower Tuolumne Riparian Information and Synthesis Study
W&AR 20 – Oncorhynchus mykiss Scale Collection and Age Determination Study

As Monday, February 20th is a holiday, the due date for comments to be received by me is now: No later than Tuesday, February 21st.

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Staples, Rose
Sent: Friday, January 20, 2012 2:46 PM
To: 'Alves, Jim - City of Modesto'; 'Anderson, Craig - USFWS'; 'Asay, Lynette - N-R'; 'Aud, John - SCERD'; 'Barnes, James - BLM'; 'Barnes, Peter - SWRCB'; 'Beuttler, John - CSPA'; 'Blake, Martin'; 'Bond, Jack - City of Modesto'; 'Boucher, Allison - TRC'; 'Boucher, Dave - Allison - TRC'; 'Bowes, Stephen - NPS'; 'Bowman, Art - CWRMP'; 'Brenneman, Beth - BLM'; 'Brewer, Doug - TetraTech'; 'Brochini, Anthony - SSMN'; 'Brochini, Tony - NPS'; 'Buckley, John - CSERC'; 'Buckley, Mark'; 'Burley, Silvia-CVMT'; 'Burt, Charles - CalPoly'; 'Cadagan, Jerry'; 'Carlin, Michael - SFPUC'; 'Catlett, Kelly - FOR'; 'Charles, Cindy - GWWF'; 'Cismowski, Gail - SWRCB'; 'Costa, Jan - Chicken Ranch'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob - TBMWI'; 'Cranston, Peggy - BLM'; 'Cremeen, Rebecca - CSERC'; 'Day, Kevin - TBMI'; 'Day, P - MF'; 'Denean - BVR'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne - OHP'; 'Dowd, Maggie-SNF'; 'Drekmeier, Peter - TRT'; 'Edmondson, Steve - NOAA'; 'Eicher, James - BLM'; 'Fety, Lauren - BLM'; 'Findley, Timothy - Hanson Bridgett'; 'Freeman, Beau - CalPoly'; 'Fuller, Reba - TMTC'; 'Furman, Donn W - SFPUC'; 'Ganteinbein, Julie - Water-Power Law Grp'; 'Giglio, Deborah - USFWS'; 'Gorman, Elaine - YSC'; 'Grader, Zeke'; 'Gutierrez, Monica - NOAA-NMFS'; 'Hackamack, Robert'; 'Hastreiter, James L - FERC'; 'Hatch, Jenny - CT'; 'Hayat, Zahra - MF'; 'Hayden, Ann'; 'Hellam, Anita - HH'; 'Heyne, Tim - CDFG'; 'Holden, James '; 'Holm, Lisa'; 'Horn, Jeff - BLM'; 'Horn, Tini'; 'Hudelson, Bill - StanislausFoodProducts'; 'Hughes, Noah'; 'Hughes, Robert - CDFG'; 'Hume, Noah - Stillwater'; 'Jackman, Jerry '; 'Jackson, Zac - USFWS'; 'Jennings, William - CSPA'; 'Jensen, Art - BAWSCA'; 'Jensen, Laura - TNC'; 'Johannis, Mary'; 'Johnson, Brian - CalTrout'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn - NOAA-MNFS'; 'Kinney, Teresa'; 'Koepele, Patrick - TRT'; 'Kordella, Lesley - FERC'; 'Lein, Joseph'; 'Levin, Ellen - SFPUC'; 'Lewis-Reggie-PRCI'; 'Linkard, David - TRT /RH'; 'Looker, Mark - LCC'; Loy, Carin; 'Lwenya, Roselynn, BVR'; 'Lyons, Bill - MR'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul '; 'Marshall, Mike - RHH'; 'Martin, Michael - MFFC'; 'Martin, Ramon - USFWS'; 'Mathiesen, Lloyd - CRRMW'; 'McDaniel, Dan -CDWA'; 'McDevitt, Ray -

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Subject: : Don Pedro Draft Study Plans – Sturgeon, Riparian, and O.myskiss Scale Studies

Don Pedro Relicensing Participants,

Following discussions of the Revised Study Plan (RSP) and in response to relicensing participant requests, the Districts agreed to develop three additional study plans:

W&AR 18 – Sturgeon Study
W&AR 19 – Lower Tuolumne Riparian Information and Synthesis Study
W&AR 20 – Oncorhynchus mykiss Scale Collection and Age Determination Study

Pursuant to the Study Plan Determination issued by FERC on December 22, 2011, the Districts are providing drafts of these three study plans for your review. These three studies can be downloaded from the Don Pedro Relicensing Website at donpedro-relicensing.com. In the row of banner headings across the top, please click on DOCUMENTS, then scroll down and select STUDIES under "Documents Now Available." Then you will need to scroll down again, under STUDIES, until you reach WATER-AQUATIC RWG (3). Click on that and you should see the three study plan drafts. Any problems accessing, please let me know.

Following the 30-day review period, the Districts will respond to comments received and file the study plans with FERC within 60 days of the Study Determination.

Please provide comments directly to the Districts via email to Rose.Staples@hdrinc.com (or Fax 207-775-1742) no later than February 20, 2012.

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

From: Staples, Rose
Sent: Tuesday, February 21, 2012 8:11 PM
To: Alves, Jim - City of Modesto; Anderson, Craig - USFWS; Asay, Lynette - N-R; Aud, John - SCERD; Barnes, James - BLM; Barnes, Peter - SWRCB; Beuttler, John - CSPA; Blake, Martin; Bond, Jack - City of Modesto; Boucher, Allison - TRC; Boucher, Dave - Allison - TRC; Bowes, Stephen - NPS; Bowman, Art - CWRMP; Brenneman, Beth - BLM; Brewer, Doug - TetraTech; Brochini, Anthony - SSMN; Brochini, Tony - NPS; Buckley, John - CSERC; Buckley, Mark; Burley, Silvia-CVMT; Burt, Charles - CalPoly; Cadagan, Jerry; Carlin, Michael - SFPUC; Catlett, Kelly - FOR; Charles, Cindy - GWWF; Cismowski, Gail - SWRCB; Costa, Jan - Chicken Ranch; Cowan, Jeffrey; Cox, Stanley Rob - TBMWI; Cranston, Peggy - BLM; Cremeen, Rebecca - CSERC; Day, Kevin - TBMI; Day, P - MF; Denean - BVR; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne - OHP; Dowd, Maggie-SNF; Drekmeier, Peter - TRT; Edmondson, Steve - NOAA; Eicher, James - BLM; Fety, Lauren - BLM; Findley, Timothy - Hanson Bridgett; Freeman, Beau - CalPoly; Fuller, Reba - TMTC; Furman, Donn W - SFPUC; Ganteinbein, Julie - Water-Power Law Grp; Giglio, Deborah - USFWS; Gorman, Elaine - YSC; Grader, Zeke; Gutierrez, Monica - NOAA-NMFS; Hackamack, Robert; Hastreiter, James L - FERC; Hatch, Jenny - CT; Hayat, Zahra - MF; Hayden, Ann; Hellam, Anita - HH; Heyne, Tim - CDFG; Holden, James ; Holm, Lisa; Horn, Jeff - BLM; Horn, Tini; Hudelson, Bill - StanislausFoodProducts; Hughes, Noah; Hughes, Robert - CDFG; Hume, Noah - Stillwater; Jackman, Jerry ; Jackson, Zac - USFWS; Jennings, William - CSPA; Jensen, Art - BAWSCA; Jensen, Laura - TNC; Johannis, Mary; Johnson, Brian - CalTrout; Justin; Keating, Janice; Kempton, Kathryn - NOAA-MNFS; Kinney, Teresa; Koepele, Patrick - TRT; Kordella, Lesley - FERC; Lein, Joseph; Levin, Ellen - SFPUC; Lewis-Reggie-PRCI; Linkard, David - TRT /RH; Looker, Mark - LCC; Loy, Carin; Lwenya, Roselynn, BVR; Lyons, Bill - MR; Madden, Dan; Manji, Annie; Marko, Paul ; Marshall, Mike - RHH; Martin, Michael - MFFC; Martin, Ramon - USFWS; Mathiesen, Lloyd - CRRMW; McDaniel, Dan -CDWA; McDevitt, Ray - BAWSCA; McDonnell, Marty - SMRT; McLain, Jeffrey - NOAA-NMFS; Means, Julie - CDFG; Mills, John - TUD; Morningstar Pope, Rhonda - BVR; Motola, Mary - PRCI; O'Brien, Jennifer - CDFG; Orvis, Tom - SCFB; Ott, Bob; Ott, Chris; Paul, Duane - Cardno; Pavich, Steve-Cardno; Pinhey, Nick - City of Modesto; Pool, Richard; Porter, Ruth - RHH; Powell, Melissa - CRRMW; Puccini, Stephen - CDFG; Raeder, Jessie - TRT; Ramirez, Tim - SFPUC; Rea, Maria - NOAA-NMFS; Reed, Rhonda - NOAA-NMFS; Richardson, Kevin - USACE; Ridenour, Jim; Robbins, Royal; Romano, David O - N-R; Roos-Collins, Richard - Water-Power Law Grp for NHI; Roseman, Jesse; Rothert, Steve - AR; Sander, Max - TNC; Sandkulla, Nicole - BAWSCA; Saunders, Jenan; Schutte, Allison - HB; Sears, William - SFPUC; Shakal, Sarah - Humboldt State; Shipley, Robert; Shumway, Vern - SNF; Shutes, Chris - CSPA; Sill, Todd; Slay, Ronn - CNRF/AIC; Smith, Jim - MPM; Staples, Rose; Steindorf, Dave - AW; Steiner, Dan; Stone, Vicki -TBMI; Stork, Ron - FOR; Stratton, Susan - CA SHPO; Taylor, Mary Jane - CDFG; Terpstra, Thomas; TeVelde, George A ; Thompson, Larry - NOAA-MNFS; Vasquez, Sandy ; Verkuil, Colette - TRT/MF; Vierra, Chris; Villalabos, Ruben; Walters, Eric - MF; Wantuck, Rick - NOAA-NMFS; Welch, Steve - ARTA; Wesselman, Eric - TRT; Wheeler, Dan; Wheeler, Dave; Wheeler,

	Douglas - RHH; Wilcox, Scott - Stillwater; Williamson, Harry (NPS); Willy, Alison - FWS; Wilson, Bryan - MF; Winchell, Frank - FERC; Wood, Dave - FR; Wooster, John -NOAA; Workman, Michelle - USFWS; Yoshiyama, Ron; Zipser, Wayne - SCFB
Subject:	Don Pedro Study Plan W&AR-12 O mykiss Habitat Survey DRAFT for your review and comments
Attachments:	Study W AR-12 O mykiss Habitat Survey-DRAFT_02-20-12.doc

Attached please find a modified study plan draft for **W&AR-12 - *Oncorhynchus mykiss* Habitat Survey Study Plan**. Changes were made to the study plan to incorporate NMFS and other agency comments, pursuant to FERC's Study Plan Determination issued December 22, 2011. In the Study Plan Determination, FERC requested that the Districts re-file the study plan, incorporating comments from the resource agencies, within 90-days of the Study Plan Determination. There have been a number of changes throughout the study plan in order to fully incorporate comments received, as discussed in the Study Plan Determination (pages 49-52).

Please provide comments to the Districts on the attached study plan draft no later than March 20, 2012, via email to rose.staples@hdrinc.com. Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

TURLOCK IRRIGATION DISTRICT & MODESTO IRRIGATION DISTRICT
DON PEDRO PROJECT
FERC NO. 2299
WATER & AQUATIC RESOURCE WORK GROUP

Study Plan W&AR-12
Oncorhynchus mykiss Habitat Survey Study Plan
February 2012

1.0 Project Nexus

The continued project operation and maintenance of the Don Pedro Project (Project) may contribute to cumulative effects on anadromous fish habitat in the lower Tuolumne River. These potential environmental effects include changes in the type of physical habitat available for juvenile *Oncorhynchus mykiss (O. mykiss*). Changes to habitat may include reduction in habitat complexity and structure due to reduced availability of large woody debris (LWD). Lack of habitat complexity may affect fish populations in the lower Tuolumne River.

2.0 Resource Agency Management Goals

The Districts believe that four agencies have resource management goals related to salmonid species and/or their habitat: (1) U.S. Department of Interior, Fish and Wildlife Service (USFWS); (2) U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NMFS); (3) California Department of Fish and Game (CDFG); and (4) State Water Resources Control Board, Division of Water Rights (SWRCB).

A goal of the USFWS (2001) Anadromous Fish Restoration Program, as stated in Section 3406(b)(1) of the Central Valley Project Improvement Act, is to double the long-term production of anadromous fish in California's Central Valley rivers and streams. Objectives in meeting this long-term goal include: (1) improve habitat for all life stages of anadromous fish through provision of flows of suitable quality, quantity, and timing, and improved physical habitat; (2) improve survival rates by reducing or eliminating entrainment of juveniles at diversions; (3) improve the opportunity for adult fish to reach spawning habitats in a timely manner; (4) collect fish population, health, and habitat data to facilitate evaluation of restoration actions; (5) integrate habitat restoration efforts with harvest and hatchery management; and (6) involve partners in the implementation and evaluation of restoration actions.

NMFS has developed Resource Management Goals and Objectives for species listed under the Magnuson-Stevens Fishery Conservation and Management Act (16 U.S.C. §1801 et seq.) and the Endangered Species Act (ESA) (16 U.S.C. §1531 et seq.), as well as anadromous species that are not currently listed but may require listing in the future. NMFS' (2009) Public *Draft* Recovery Plan for Sacramento River Winter-run Chinook salmon, Central Valley Spring-run Chinook salmon, and Central Valley steelhead outlines NMFS' framework for the recovery of ESA-listed species and populations in California's Central Valley. For Central Valley steelhead, the

recovery actions identified for the Tuolumne River are to: (1) conduct habitat evaluations; and (2) manage cold water pools behind La Grange and Don Pedro dams to provide suitable water temperatures for all downstream life stages. For Central Valley fall/late fall-run Chinook, the relevant goals are to enhance the essential fish habitat downstream of the Project and achieve a viable population of Central Valley fall/late fall-run Chinook salmon in the Tuolumne River.

CDFG's mission is to manage California's diverse fish, wildlife, and plant resources, and the habitats upon which they depend, for their ecological values and for their use and enjoyment by the public. CDFG's resource management goals, as summarized in restoration planning documents such as "Restoring Central Valley Streams: A Plan for Action" (Reynolds et al. 1993), are to restore and protect California's aquatic ecosystems that support fish and wildlife, and to protect threatened and endangered species under California Fish and Game Code (Sections 6920-6924).

SWRCB has responsibility under the federal Clean Water Act (33 U.S.C. §11251-1357) to preserve and maintain the chemical, physical and biological integrity of the State's waters and to protect water quality and the beneficial uses of stream reaches consistent with Section 401 of the federal Clean Water Act, the Regional Water Quality Control Board Basin Plans, State Water Board regulations, the California Environmental Quality Act, and any other applicable state law.

3.0 Study Goals

The primary goal of this study is to provide information on habitat distribution, abundance and quality in the lower Tuolumne River with a focus on *O. mykiss* habitat related to LWD. An inventory of LWD and associated habitat quality, availability and use by salmonids will inform the evaluation of in-river factors that may affect the juvenile *O. mykiss* life stage. As recommended by FERC staff in its Study Plan Determination of December 27, 2012, several modifications have been made to this study at the request of Relicensing Participants (Elements No. 5 and 6 in Study Request NMFS-5, dated June 10, 2011) in an effort to provide more detailed characterization of LWD distribution in the lower Tuolumne River. In addition, the study will provide a rough estimate of the quantities of LWD removed from Don Pedro on an annual basis.

4.0 Existing Information and Need for Additional Information

Juvenile habitat quality and use has been found to be directly related to habitat complexity (Bustard and Narver 1971; Bisson et al. 1987). Instream habitat complexity is typically associated with large woody debris, pools, and off channel habitat. Cederholm (1997) and others observed a direct relationship between increased steelhead smolt production and increased habitat complexity in the form of LWD. Increases in numbers of anadromous (Ward and Slaney 1981; House and Boehne 1995) and non-anadromous (Gowan and Fausch 1995) fishes after addition of LWD to a stream have been demonstrated.

Instream LWD recruitment is generally from the adjacent riparian forest or allochthonous, originating from the upstream watershed. Large dams, that rarely spill, like Don Pedro Dam, can reduce recruitment from upstream sources. Reduction or elimination of large riparian trees will also reduce LWD recruitment.

The quality and condition of habitat in the lower Tuolumne River has been investigated for Chinook salmon since the 1996 FERC Order (76 FERC 61, 117). The order required that the condition of spawning habitat be assessed along with other monitoring requirements, specific to Chinook salmon. As a result, information is available for other salmonids in the river. For example, McBain and Trush (2000) identified that the uppermost reach of the lower Tuolumne River (River Mile [RM] 52–46.6) was primarily used for spawning salmon where they found gravel bed and banks, along with little valley confinement within the bluffs. Surveys of the channel downstream of La Grange Dam showed the occurrence of channel downcutting and widening, armoring, and depletion of sediment storage features (e.g., lateral bars and riffles) due to sediment trapping in upstream reservoirs, gold and gravel mining, and other land use changes since the 1850s (DWR 1994; McBain & Trush 2004).

Previous riparian investigations found large scale removal of riparian vegetation that was a direct result of mining activities and urban/agricultural encroachment. Clearing of riparian forests decreased large woody debris recruitment, allowed exotic plants to invade the riparian corridor, reduced shading of the water's surface, and contributed to increased water and air temperatures in the Tuolumne River corridor (McBain & Trush 2000). Grazing and other land uses have also resulted in direct impacts on riparian vegetation.

LWD plays an important role in habitat forming events within low-order streams. Where LWD dimensions are large relative to the channel width, LWD readily collects within the channel forming areas of velocity gradation, encouraging localized sediment deposition and scour (McBroom 2010). In higher order streams, such as the lower Tuolumne River, the role of LWD in habitat formation decreases with the stream width. However, LWD becomes more ecologically significant in high order streams where it can provide the majority of stable, firm substrate that supports a substantial portion of invertebrate productivity (McBroom 2010).

Salmonid habitat quality and quantity, including characterization of habitat limitations and relative salmonid production potential is routinely assessed through surveys of instream habitat composition and structure, such as those surveys described by CDFG (2010). Results of such surveys can help identify land use and other related effects on habitat quality, thus the relative potential of the anadromous fish population. Such surveys also can identify opportunities to restore or enhance habitat conditions and salmonid and other aquatic production. In July 2008, Stillwater Sciences conducted a focused assessment of *O. mykiss* in the Tuolumne River that incorporated a habitat mapping component. The assessment identified general habitat units (e.g., pool, riffles) and then discussed the relationship between habitat type and observed *O. mykiss* use (Table 4.0-1). Habitat maps were also created displaying general habitat type from approximately RM 52 to RM 39.5. The results of recent *O. mykiss* monitoring surveys (e.g. Stillwater Sciences 2008) provide a foundation for the focused *O. mykiss* habitat evaluations in this proposed study.

While existing historical data provide a broader characterization of the existing habitat, a more detailed investigation into habitat conditions is proposed. A more detailed assessment of *O. mykiss* habitat availability would include the level and kind of complexity, factors associated with complexity (such as bars, backwater pools, scour pools, etc.), and the amount of habitat available as a function of complexity and use.

Table 4.0-1 Example habitat use by habitat type for two *O. mykiss* size classes during summer (adapted from Stillwater Sciences 2008**).**

Habitat	*O. mykiss* < 150 mm				*O. mykiss* ≥ 150 mm				Total			
	Seen [1]	Est.	Std dev	95% [2] Interval	Seen [1]	Est.	Std dev	95% [2] Interval	Seen	Est.	Std dev	95% Interval
Pool Head	12	20	10.1	12–40	17	45	13.2	19–71	29	65	16.7	33–98
Pool Body	0				3	24	18.0	3–59	3	24	18.0	3–59
Pool Tail	1	2	2.6	1–7	0				1	2	2.6	1–7
Run Head	46	166	179.0	46–517	1	6	8.8	1–23	47	172	179.2	47–523
Run Body	5	860	115.6	634–1,087	6	319	77.5	167–471	11	1,179	139.2	906–1,452
Run Tail	0				0				0			
Riffle	65	1,428	198.2	1,039–1,816	13	226	126.7	13–474	78	1,653	235.2	1,192–2,114
Total	**129**	**2,476**	**291.2**	**1,905–3,047**	**40**	**619**	**150.4**	**325–914**	**169**	**3,096**	**327.7**	**2,453–3,738**

[1] Largest numbers seen in any single dive pass for each unit, summed over units. Note that summation of the largest numbers seen within individual (50 millimeter [mm]) size bins yields higher estimates of total fish smaller and larger than 150 mm.

[2] Nominal confidence intervals calculated as +/- 1.96 standard deviations. When this yielded lower bounds less than the numbers seen, the lower bound was truncated accordingly and the interval shaded.

In addition to a focused survey and assessment of the associations of LWD and other contributors to habitat complexity, and the relationships among complexity and *O. mykiss* utilization, a general accounting of LWD within the study reach will be conducted to identify location, general condition, density and abundance of LWD.

5.0 Study Methods

The study methods described below will be implemented to meet the study objectives.

5.1 Study Area

A one-year habitat assessment will be conducted in the salmonid spawning and rearing reach of the lower Tuolumne River from La Grange to Roberts Ferry Bridge (approximately RM 52–39). The LWD survey area will also extend from approximately RM 52 downstream to RM 24. A separate investigation of LWD removed from Don Pedro reservoir will also be conducted.

5.2 General Concepts

The following general concepts apply to the study:

- Personal safety is an important consideration of each fieldwork team. The Districts and their consultants will perform the study in a safe manner.
- Field crews may make minor modifications in the field to adjust to and to accommodate actual field conditions and unforeseeable events. Any modifications made will be documented and reported in the draft study report.

5.3 Study Methods

The study will consist of two separate components: 1) a semi-quantitative inventory of instream habitat types and physical habitat characteristics, and 2) an appraisal of distribution, abundance, and function of LWD in the lower Tuolumne River. The first component will rely on available aerial photography and habitat mapping, and a reconnaissance-level survey of the lower

Tuolumne River, between approximately RM 52 and RM 39. This study component will rely upon existing broader habitat mapping conducted by Stillwater Sciences (2008) to identify focal research areas where *O. mykiss* occur and then utilize an adaptation of the high-resolution CDFG habitat typing methodology (CDFG 2010), to further characterize and evaluate these areas. CDFG identified four levels of typing, ranging from general broad habitat identification (Level I) to more detailed characterizations entailing 24 different potential habitat descriptors at Level IV. This study will utilize the highest level of detail that is appropriate for a river of this size and which will allow for a strongly supported assessment of habitat for *O. mykiss* and other fish species. In addition, a detailed description of LWD will be made at each focal study location using standard methods (e.g., Moore et al. 2006, Montgomery 2008), as described further below.

The second study component, an LWD inventory, will consist of a detailed survey of large wood and an assessment of its influence on *O. mykiss* habitat quality and quantity. The LWD inventory will be conducted between RM 52 and RM 24. In addition, as recommended by FERC Staff in the December 22, 2011 Study Determination, an evaluation of the frequency and volume of LWD trapped and removed from Don Pedro reservoir on an annual basis will be made (as described by NMFS in their June 10, 2011 study request Element No. 2).

Step 1 – Site Selection, Field Reconnaissance, and Planning. Habitat typing conducted for this study includes a 13 mile reach of the lower Tuolumne River (RM 52–39), with LWD surveys from RM 52–24. Field planning will begin by reviewing reports of existing habitat mapping conducted by Stillwater Sciences (2008), McBain & Trush (2004), and others. Field staff will coordinate with CDFG staff and others knowledgeable of access and navigability of the river to determine proper timing and related survey conditions that would optimize conducting the survey. As recommended by FERC Staff in the December 22, 2011 Study Determination, ortho-rectified digital aerial photographs of the study reach will be prepared for use with habitat typing and in developing a spatial inventory of mapped LWD. A subset of representative sampling units in the study reach will be selected for detailed habitat measurements using CDFG (2010). As recommended by CDFG (2010), sampling units selected for detailed habitat measurements will encompass 10–20 percent of the study reach and will be preferentially located where *O. mykiss* observations have been documented (e.g., Stillwater Sciences 2008).

As recommended by FERC Staff in the December 22, 2011 Study Determination, sampling units for inventorying LWD will be up to 20 channel widths long, consistent with guidelines used in California and the Pacific Northwest (e.g., Leopold 1994). The average bankfull width of the lower Tuolumne is 150 ft; therefore, the average length of a sample site will be around 3,000 ft long. Seven to ten sampling units that are 20 bankfull widths long will be selected for detailed characterization of LWD, encompassing approximately 4 to 6 miles (i.e., 10 to 20 percent) of the study reach by the estimates above.

Step 2 – Field Data Collection. Field surveys will be implemented using multiple teams of two field technicians. Each team will have a map and aerial photos delineating the portions of reach that will be surveyed. Upon accessing these survey areas, each team will collect the suite of measurements detailed in Table 5.3-1. These measurements are representative of the required data collection for Level III and IV CDFG habitat typing. Data will be documented on template datasheets to ensure that all data are collected and in a consistent manner between teams. Each habitat unit will have its upstream and downstream boundaries delineated on an aerial photograph and have an identification number that is the same as that on the datasheet. Field

measurements will be made with standard field equipment: a handheld thermometer will be used to collect water temperature data, a stadia rod will be used to measure water depth, a steel meter tape or optical range finder will be used to measure site dimensions, and a spherical densitometer will measure percent overhead canopy cover. Each team will also be equipped with a handheld GPS and camera with habitat unit dimensions estimated in the field as well as by GIS.

Table 5.3-1 A summary of data collected as part of the Level IV CDFG habitat mapping.

Gathered Data	Description
Form Number	Sequential numbering
Date	Date of survey
Stream Name	As identified on USGS quadrangle
Legal	Township, Range, and Section
Surveyors	Names of surveyors
Latitude/Longitude	Degrees, Minutes, Seconds from a handheld GPS
Quadrant	7.5 USGS quadrangle where survey occurred
Reach	Reach name or rivermile range
Habitat Unit #	The habitat unit ID # that the bankfull width was measured
Time	Recorded for each new data sheet start time
Water Temperature	Recorded to nearest degree Celsius
Air Temperature	Recorded to nearest degree Celsius
Flow Measurement	Can be obtained from USGS monitoring stations
Mean Length	Measurement in meters of habitat unit
Mean Width	Measurement in meters of habitat unit
Mean Depth	Measurement in meters of habitat unit
Maximum Depth	Measurement in meters of habitat unit
Depth Pool Tail Crest	Maximum thalweg depth at pool tail crest in meters
Pool Tail Embeddedness	Percentage in 25% bucket ranges
Pool Tail Substrate	Dominant substrate: silt, sand, gravel, small cobble, large cobble, boulder, bedrock
Large Woody Debris Count	Detailed inventory criteria are listed below
Shelter Value	Assigned categorical value: no shelter, minimal shelter (small debris, bubble curtain etc.), significant shelter (large woody debris, root wads, vegetative cover, etc.)
Percent Unit Covered	Percent of the unit occupied
Substrate Composition	Composed of dominant and subdominant substrate: silt, sand, gravel, small cobble, large cobble, boulder, bedrock
Percent Exposed Substrate	Percent of substrate above water
Percent Total Canopy	Percent of canopy covering the stream
Percent Hardwood Trees	Percent of canopy composed of hardwood trees
Percent Coniferous Trees	Percent of canopy composed of coniferous trees
Right and Left Bank Composition	Identify dominant substrate: sand/silt, cobble, boulder, bedrock
Right and Left Bank Dominant Vegetation	Identify dominant vegetation: grass, brush, hardwood trees, coniferous trees, no vegetation
Right and Left Bank Percent Vegetation	Percent of vegetation covering the bank
Comments	Additional notes as needed

USGS = U.S. Geological Survey

The LWD distribution survey will use the Montgomery (2008) wood size classes, adapted to the Tuolumne River as follows. Information to be collected will include location (e.g., GPS coordinates), LWD size category, type, orientation, associated CDFG (2010) habitat type, and likely source. As recommended by FERC Staff in the December 22, 2011 Study Determination, within each LWD sample site, GPS locations and characteristics of each piece of LWD greater

than 3 ft (1 m) long within the active channel will be recorded and binned within six length classes [3–6.5 ft (1–2 m), 6.5–13 ft (2–4 m), 13–26 ft (4–8 m), 26–52 ft (8–16 m), 52–105 ft (16–32 m), and >105 ft (>32 m)] and four diameter classes [4-8 in (0.1–0.2 m), 8–16 in (0.2–0.4 m), 16–31 in (0.4–0.8 m), 31–63 in (0.8–1.6 m)]. More detailed measurements will be taken for key LWD, which are defined as pieces either longer than 1/2 times the bankfull width, or of sufficient size and/or are deposited in a manner that alters channel morphology and aquatic habitat (e.g., trapping sediment or altering flow patterns). In addition to recording the GPS locations for mapping on ortho-rectified aerial photographs, detailed information to be collected on key LWD pieces includes:

- Piece location, mapped on aerial photos/GPS documentation
- Piece length
- Piece diameter
- Piece orientation to bank
- Position relative to channel
- Rootwad present
- Tree type (hardwood or conifer)
- Associated with log jam
- Jam size (estimated dimensions/number of pieces)
- Source (imported/riparian/unknown)
- Channel dynamic function
- Habitat function (cover, sediment collection, hard substrate)

Lastly, although no detailed records of the quantities of LWD removed from Don Pedro Reservoir or other Project facilities exist, the Don Pedro Recreation Agency (DPRA) conducts an annual program to remove floating LWD at various locations in Don Pedro reservoir as it presents a boating hazard. This material is then placed in piles within suitable landing areas around the reservoir for burning, as conditions permit. To provide an order of magnitude estimate of LWD currently trapped in the reservoir, a team of two field technicians will travel by boat to each landing area and measure the quantity of LWD at each stockpile location in May 2012. Understanding that no meaningful relationship between this annual storage estimate and a LWD budget or loading rate to the lower Tuolumne River is possible, a discussion of the relative sizes and characteristics of LWD in Don Pedro reservoir and at locations in the lower Tuolumne River will be made.

Step 3 – Data Processing and Analyses. Collected data will be stored and managed using a digital spreadsheet database. All data sheets will be physically copied after each week of survey. All data will then be entered into a spreadsheet database. Entered data will be QA/QC'd by two independent technicians reading and confirming each line of data together. Data will be summarized in tables and figures depicting overall habitat characteristics and conditions by reach. The quality and suitability of the habitat will be assessed in light of existing resources that include *O. mykiss* life history needs. This assessment will also discuss patterns of habitat use as found in recent O. mykiss snorkel survey efforts (e.g., Stillwater Sciences 2008). Final data will be made available to Relicensing Participants in digital spreadsheet form (Step 4). Maps depicting the location of the surveys, habitat types and LWD locations with each survey reach, and images of the surveyed habitat will also be provided within the report.

Data collected during the LWD distribution survey will be summarized relative to size class, reach, habitat association, density, and complexity. LWD trapped and removed from Don Pedro Reservoir in 2012 by the Don Pedro Recreation Agency will be quantified and a comparison of size characteristics of trapped LWD with those observed in the lower Tuolumne River will be made. These data summaries will be analyzed to determine the functioning of LWD in the lower Tuolumne River in the context of its channel and habitat type, and ecological role.

The quantity, quality, and use of the lower Tuolumne River by *O. mykiss* will be discussed in the context of other anadromous salmonid streams. The comparison will identify the occurrence and role of LWD and other habitat attributes in the lower Tuolumne River, and provide a basis for assessing the potential implications on *O. mykiss* abundance. Comparisons with other Central Valley streams and similar stream systems outside the Central Valley will be made to place LWD function in the lower Tuolumne River in context with other streams of similar stream order, recruitment potential, and sources.

Step 4 – Prepare Report**.** The Districts will prepare a report that includes the following sections: (1) Study Goals, (2) Methods and Analysis, (3) Results, (4) Discussion, and (5) Conclusions. The quality and suitability of the habitat will be assessed and reported in light of existing resources that include steelhead life history needs. The report will discuss the findings from the Stillwater (2008) report and compare current conditions to population and habitat data collected in 2008.

The report will also contain GIS maps of sampled areas with delineated habitat and LWD features, organized and labeled photos of select habitat, and relevant summary tables and graphs. The reported data will be organized by reach site to allow for a spatial presentation of the findings. Final data will be made available to Relicensing Participants (Section 8.0).

6.0 Schedule

The Districts anticipate the schedule to complete the study as follows:

Project Preparation.....April – May 2012
Field Mapping.....June – August 2012
Data QA/QC..... September 2012
Prepare Report .....October – November 2012
Report Issuance..... January 2013

7.0 Consistency of Methodology with Generally Accepted Scientific Practices

The habitat mapping methodology was developed by CDFG based upon notable prior researchers. The methods described are standards that have been reviewed and used by numerous researchers since 1991. The study will follow the latest survey approach that has been refined into the current 4th edition (CDFG 2010).

8.0 Deliverables

The Districts will prepare a report, which will document the methodology and results of the study. In addition, at the request of relicensing participants, the Districts will provide GIS-based

maps of survey locations documented as part of this study, as well as all LWD survey data (both focused and distribution survey) and all other habitat unit data in tabular (spreadsheet) and geo-spatial (e.g., ArcGIS shapefiles) formats.

9.0 Level of Effort and Cost

The Districts estimate the cost to complete this study to be $110,000

10.0 References

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DRAFT
WORKSHOP CONSULTATION PROCESS
ON INTERIM STUDY PLAN DECISIONS

As part of certain studies to be undertaken in the Don Pedro Project relicensing, the Districts had proposed a series of workshops to share and discuss relevant data with Relicensing Participants (RPs). FERC has recommended that the Workshop Consultation process be formalized. In accordance with Appendix B of FERC's December 22, 2011 Study Plan Determination, the draft workshop consultation process outlined below has been developed to provide guidance for the decision-making process involved within the following study plans:

- W&AR-2 (Project Operations Model): Hydrology Workshop
- W&AR-5 (Salmonid Population Information Synthesis): Literature/Data Review Workshop and Conceptual Model Review Workshop
- W&AR-6 (Chinook Population Model): Conceptual Model Review Workshop and Modeling Approach Workshop
- W&AR-10 (*O.Mykiss* Population Model): Conceptual Model Review Workshop and Modeling Approach Workshop
- W&AR-14 (Temperature Criteria Assessment): Water Temperature Evaluation Criteria Workshop

The purpose of the eight workshops is to provide opportunity for RPs and the Districts to discuss relevant data sources, methods of data use and development, and modeling parameters at key points in the execution of these study plans. The goal of the workshops is for RPs and the Districts to reach agreement where possible after thorough discussion of data, methods and parameters. Consensus on decisions dealing with data acceptability, or study approaches or methods can only be achieved by the active and consistent in-person attendance and participation of interested Relicensing Participants. Additional workshops beyond those already specified above may be held as agreed to between the RPs and the Districts.

FERC has also directed the Districts to formalize the workshop process to define how interim decisions on model inputs and parameters will be made. To promote clear communication and informed participation, the Districts will make a good-faith effort to provide two (2) weeks before each workshop, in electronic format, information and presentation materials to be discussed at the workshops. For studies that involve resource modeling, presentation materials will be tailored to the audience at a level that assumes familiarity with the resource issues being addressed. To promote a common understanding of terms, a glossary of definitions will be prepared prior to each initial workshop, updated and expanded upon periodically, and included in the final study report. Prior to the initial workshops, the Districts will also prepare a logic diagram of the study steps from data selection through model development and numerical procedures to model scenario evaluation. This study "process diagram" will aid in promoting a common understanding of the step-wise approach being used in model development.

Following each workshop, draft meeting notes of the consultation workshop will be distributed to participants within approximately eight (8) working days. The notes will identify areas where participants reached agreement on data, methods and/or parameters, areas where there is disagreement among participants, and action items for any future meetings. Following a 30-day

comment period, the Districts will file with FERC a revised version of the consultation workshop notes describing areas of agreement, areas where agreement was not reached, copies of comments received, a discussion of how the Relicensing Participant comments and recommendations have been considered by the Districts, as well as the rationale for the Districts not adopting any Relicensing Participants recommendations.

The proposed schedule for workshops is included below. All meetings will be held at MID offices in Modesto.

March 2012
Mar 20 - 1:30 pm – 4:30 pm
Don Pedro Project Relicensing - Workshop on Consultation Process (as per Appendix B of FERC's Study Plan Determination)

April 2012
Apr 09 - 1:00 pm - 5:00 pm
Don Pedro Project Relicensing - Hydrology Workshop (W&AR-2)

Apr 10* - 10:30 am - 5:00 pm Don Pedro Project Relicensing - Salmonid Population Information Workshop (W&AR-5)

Apr 11 - 9 am – 12:00 pm Don Pedro Project Relicensing – Temperature Criteria Workshop (W&AR-14)

June 2012
Jun 26 - 9:00 am - 4:00 pm Don Pedro Project Relicensing - Salmonid Population Information Workshop (W&AR-5)

November 2012
Nov 15 - 9:00 am - 4:00 pm Don Pedro Project Relicensing - Chinook Population (W&AR-6) and O.mykiss Population (W&AR-10) Modeling Workshop

2013 (Dates to be determined)

March 2013 (preliminary) - 9 am to 4 pm Don Pedro Project Relicensing - 2nd Workshop Chinook Population (W&AR-6) and O.mykiss Population (W&AR-10) Modeling

***NOTE:** From 8:30 am to 10:15 am, the Districts will conduct an introduction to the MIKE3 reservoir temperature model for use in W&AR-3. The goal is to introduce the model platform, computation methods, model development, and data sources. This is not considered a formal workshop. The Districts are also planning to conduct a discussion and presentation of the reservoir temperature model validation results at a Relicensing Participant Meeting on September 18, 2012 from 9 am to 4 pm at MID. Please add this meeting to your calendars.

DRAFT
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311 California Street
San Francisco CA 94104

415.956.2828
415.956.6457 fax
www.rjo.com

ROGERS JOSEPH O'DONNELL

March 19, 2012

Carin Loy
Jenna Borovansky
HDR Engineering Inc.
2379 Gateway Oaks Drive, Suite 300
Sacramento, CA 95833

Re: *Don Pedro Relicensing Project*

Dear Carin and Jenna:

In connection with the TID/MID effort to relicense the Don Pedro Hydroelectric Project please consider the following:

We have property on Shawmut Road that leads to Lake Don Pedro. Periodically we report to TID/MID and to the Tuolumne County Public Works Department that garbage, refrigerators, car parts, tires and other trash has accumulated along Shawmut Road near the lake. We have several times suggested that additional barriers or fencing should be installed in a handful of locations to prevent people from pulling off of Shawmut Road in order to prevent illegal dumping.

On every occasion that we or our neighbors have reported this, both TID/MID and the County blame the other and tell us the clean up is not their responsibility. This is unacceptable. TID/MID need to protect the watershed and the lake.

Please confirm with me that this will be addressed in the relicensing process.

Very truly yours,

Alan J. Wilhelmy

From: Staples, Rose
Sent: Thursday, March 29, 2012 11:02 AM
To: 'Alves, Jim'; 'Anderson, Craig'; 'Asay, Lynette'; 'Aud, John'; 'Barnes, James'; 'Barnes, Peter'; 'Blake, Martin'; 'Bond, Jack'; Borovansky, Jenna; 'Boucher, Allison'; 'Bowes, Stephen'; 'Bowman, Art'; 'Brenneman, Beth'; 'Brewer, Doug'; 'Buckley, John'; 'Buckley, Mark'; 'Burley, Silvia'; 'Burt, Charles'; 'Byrd, Tim'; 'Cadagan, Jerry'; 'Carlin, Michael'; 'Charles, Cindy'; 'Cismowski, Gail'; 'Colvin, Tim'; 'Costa, Jan'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob'; 'Cranston, Peggy'; 'Cremeen, Rebecca'; 'Day, Kevin'; 'Day, P'; 'Denean'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne'; 'Dowd, Maggie'; 'Drekmeier, Peter'; 'Edmondson, Steve'; 'Eicher, James'; 'Fety, Lauren'; 'Findley, Timothy'; 'Fuller, Reba'; 'Furman, Donn W'; 'Ganteinbein, Julie'; 'Giglio, Deborah'; 'Gorman, Elaine'; 'Grader, Zeke'; 'Gutierrez, Monica'; 'Hackamack, Robert'; 'Hastreiter, James'; 'Hatch, Jenny'; 'Hayat, Zahra'; 'Hayden, Ann'; 'Hellam, Anita'; 'Heyne, Tim'; 'Holley, Thomas'; 'Holm, Lisa'; 'Horn, Jeff'; 'Horn, Timi'; 'Hudelson, Bill'; 'Hughes, Noah'; 'Hughes, Robert'; 'Hume, Noah'; 'Jackman, Jerry'; 'Jackson, Zac'; 'Jennings, William'; 'Jensen, Art'; 'Jensen, Laura'; 'Johannis, Mary'; 'Johnson, Brian'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn'; 'Kinney, Teresa'; 'Koepele, Patrick'; 'Kordella, Lesley'; 'Lein, Joseph'; 'Levin, Ellen'; 'Lewis, Reggie'; 'Linkard, David'; 'Looker, Mark'; 'Lwenya, Roselynn'; 'Lyons, Bill'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul'; 'Marshall, Mike'; 'Martin, Michael'; 'Martin, Ramon'; 'Mathiesen, Lloyd'; 'McDaniel, Dan'; 'McDevitt, Ray'; 'McDonnell, Marty'; 'McLain, Jeffrey'; 'Means, Julie'; 'Mills, John'; 'Morningstar Pope, Rhonda'; 'Motola, Mary'; 'O'Brien, Jennifer'; 'Orvis, Tom'; 'Ott, Bob'; 'Ott, Chris'; 'Paul, Duane'; 'Pavich, Steve'; 'Pinhey, Nick'; 'Pool, Richard'; 'Porter, Ruth'; 'Powell, Melissa'; 'Puccini, Stephen'; 'Raeder, Jessie'; 'Ramirez, Tim'; 'Rea, Maria'; 'Reed, Rhonda'; 'Richardson, Kevin'; 'Ridenour, Jim'; 'Robbins, Royal'; 'Romano, David O'; 'Roos-Collins, Richard'; 'Roseman, Jesse'; 'Rothert, Steve'; 'Sandkulla, Nicole'; 'Saunders, Jenan'; 'Schutte, Allison'; 'Sears, William'; 'Shakal, Sarah'; 'Shipley, Robert'; 'Shumway, Vern'; 'Shutes, Chris'; 'Sill, Todd'; 'Slay, Ron'; 'Smith, Jim'; Staples, Rose; 'Steindorf, Dave'; 'Steiner, Dan'; 'Stone, Vicki'; 'Stork, Ron'; 'Stratton, Susan'; 'Taylor, Mary Jane'; 'Terpstra, Thomas'; 'TeVelde, George'; 'Thompson, Larry'; 'Vasquez, Sandy'; 'Verkuil, Colette'; 'Vierra, Chris'; 'Walters, Eric'; 'Wantuck, Richard'; 'Welch, Steve'; 'Wesselman, Eric'; 'Wheeler, Dan'; 'Wheeler, Dave'; 'Wheeler, Douglas'; 'Wilcox, Scott'; 'Williamson, Harry'; 'Willy, Allison'; 'Wilson, Bryan'; 'Winchell, Frank'; 'Wooster, John'; 'Workman, Michelle'; 'Yoshiyama, Ron'; 'Zipser, Wayne'
Subject: Don Pedro Relicensing Newsletter - New Issue Just Published on Website

The Districts have just published Volume 2 – Issue 1 of the ***Don Pedro Relicensing Newsletter*** and I have uploaded a copy for you onto the www.donpedro-relicensing.com website, in the Announcement section under the INTRODUCTION tab. If you cannot access and/or download the document, please advise me (rose.staples@hdrinc.com or 207-239-3857) and we can mail you a copy. Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

Volume 2 | Issue 1

Don Pedro

A newsletter about the relicensing of the Don Pedro Project

Year one of DP relicensing in the books

The year 2011 marked the Modesto and Turlock irrigation districts' first year of the Don Pedro relicensing process. The first year of the Integrated Licensing Process (ILP) was essentially devoted to working closely with Relicensing Participants (RPs) and the Federal Energy Regulatory Commission (FERC) to develop detailed studies to be conducted by the Districts to support the license application, which will be filed in April 2014. The year ended with FERC issuing its formal Study Plan Determination (SPD) on December 22.

The Districts will be conducting 35 different studies to investigate the project's potential to affect resources in the lower Tuolumne River and at and adjacent to the Don Pedro Reservoir. The Districts have retained the services of a number of experts in their respective fields to assist in the performance of these studies. By the end of 2012, the Districts will have completed most of these studies, but some will continue into 2013.

More Inside: FERC issues Study Plan Determination, including 35 studies in several resource fields.

Further details regarding the Revised Study Plan (RSP), the SPD, upcoming meetings and more are available inside this newsletter and on the DP relicensing website located at **www.donpedro-relicensing.com**.

FERC held two Scoping Meetings in 2011.

The Relicensing Process

The relicensing of the Don Pedro Project formally began in 2011. Below are some of the major stages of the process.

1. Districts filed PAD and Notice of Intent in 2/10/2011.
2. FERC conducts scoping in Spring '11.
3. Interested parties discuss issues and develop study requests.
4. Districts file Proposed Study Plan (PSP) on 7/25/11 and undertake a series of meetings with relicensing participants to review and discuss the PSP.
5. FERC issues Study Plan Determination on 12/22/11.
6. Studies are conducted and Study Report issued for review and comment.
7. Applicant files draft and final license applications.
8. FERC issues new license with new terms and conditions in 2016.

Important dates

April 9, 2012
Hydrology Workshop (W&AR-2)

April 10, 2012
Reservoir Temperature Modeling Data & Methods

April 10, 2012
Salmonid Population Information Workshop (W&AR-5)

April 11, 2012
Temperature Criteria Workshop (W&AR-14)

June 26, 2012
Salmonid Population Information Workshop (W&AR-5)

September 18, 2012
Temperature Model Validation/Calibration Meeting

What's inside

Revised Study Plan filed by Districts

On November 22, 2011, the Districts filed their 900-plus page Revised Study Plan (RSP) with FERC and RPs. The RSP contained the Districts detailed plans and schedules for conducting 35 studies. In all, the RPs had previously requested that the Districts perform more than 140 studies. The RSP also included the Districts' explanation of why they believed that many of the studies requested of the Districts were unnecessary, or were outside the proper scope of the Don Pedro relicensing.

Many RPs filed comments on the Districts RSP by the December 11 deadline. These filings contained comments on the RSP and any points of disagreement a relicensing participant might have with the Districts' reasoning for not undertaking studies that the RPs had requested.

Near the marina at Lake Don Pedro.

A screenshot of the Meetings page located at http://www.donpedro-relicensing.com.

Website offers information

Interested parties can obtain meeting schedules and much more

The Districts have updated the Don Pedro Project Relicensing website with the 2012 schedule of meetings. People interesting in viewing a list of these meetings along with other meeting information can visit the **Meetings** tab located on the relicensing website's main page. The public is welcome to attend and participate in these meetings.

In addition to being regularly updated with the aforementioned meeting times, the site is also updated with agendas, documents, filings and other information. The website also provides a good overall primer and describes the relicensing process, provides useful links and offers contact information.

The website serves as one of the primary communication outlets informing stakeholders of events and meetings that are part of the relicensing process.

The most robust section of the website is the **Documents** page, which has nearly 150 downloadable documents ranging from more recent documents such as FERC's Study Plan Determination all the way back to the Districts' Pre-Application Document (PAD) filings.

FERC issues 140-page Study Plan Determination

On December 22, 2011, FERC issued its 140-page Study Plan Determination (SPD). FERC approved 17 of the Districts studies without modification and 16 with modifications, most of which were minor modifications. FERC also said that two of the studies were not required to be conducted. FERC added one additional study that had been requested by the United States Bureau of Land Management – a bald eagle survey along the reservoir area.

With FERC's SPD issued, the Districts immediately planned to undertake the approved studies in accordance with FERC's directive. Most of the studies involve extensive field work; considerable coordination and logistics need to be worked out to execute the studies efficiently and consistent with the study plans approved by FERC. Some field work began as early as January.

The Studies

The more than 30 studies being undertaken by the Districts can be subdivided into resource areas as follows:

- Two large studies are devoted to Cultural Resources;
- Four studies focus on recreational resources, including a significant study of reservoir recreation;
- Ten studies dealing with resources investigating botanical, wildlife and wetland species and habitats.
- Nineteen studies deal with water resources and aquatic/fish resources. Many of these studies deal with salmon and O. mykiss (rainbow trout/steelhead) in the lower river. One of the studies that FERC did not require the Districts to undertake (temperature preferences for life stages of anadromous fish) will still be completed by the Districts because, although FERC did not find it essential for its own purposes, the Districts continue to feel it is important to the relicensing process.

The studies themselves, as currently proposed, are expected to cost over $7 million by the time they are completed.

Notices of Dispute

Three of the resource agencies filed a formal Notice of Dispute on FERC's SPD as described by the regulations of the Integrated Licensing Process (ILP). The agencies – the National Marine Fisheries Service (NMFS), the United States Fish and Wildlife Service, and the State Water Resources Control Board – are disputing FERC's decisions about certain studies the Districts did not adopt and FERC did not require. The dispute process is detailed in the ILP regulations and involves the convening of a three-member advisory Technical Panel to consider the areas of dispute, and provide an opinion to FERC's Director of the Office of Energy Projects (OEP). The dispute, which is a normal aspect of the relicensing process, will likely be decided sometime this spring. The FERC Director makes the final decision giving due consideration to the opinion of the three-member Technical Panel.

Land Access

Some of the studies required by FERC will require the Districts' consultants to access private lands adjacent to or near the Don Pedro Reservoir, though no one will enter private land without the landowner's consent. The Districts have mailed requests to landowners to allow access. Consultants have been instructed in the need for the utmost care of and respect to private property near the Project. The Districts and consultants will make every effort to contact landowners about the approximate timing of the need for such access. The Districts understand and respect that the final decision to allow, or not allow, such access is to be decided to each landowner.

MID Modesto Irrigation District
Water and Power

TID WATER & POWER
Serving Central California since 1887

333 E. Canal Drive
PO Box 949
Turlock, CA 95381
209.883.8300

From: Staples, Rose
Sent: Monday, April 02, 2012 8:15 PM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Aud, John; Barnes, James; Barnes, Peter; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burley, Silvia; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Cismowski, Gail; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Looker, Mark; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Means, Julie; Mills, John; Morningstar Pope, Rhonda; Motola, Mary; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Steindorf, Dave; Steiner, Dan; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Walters, Eric; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: AGENDA and MATERIAL for Don Pedro W&AR-14 Temp Criteria Evaluation Meeting April 11 at 9:00 am
Attachments: WAR14 meeting materials list_04-11-12.docx; Temperature Criteria Mtg No 1 AGENDA_120402.pdf; W&AR14_Potential_Empirical_Studies.doc

Please find attached the AGENDA and MATERIAL for the Don Pedro W&AR-14 Temperature Criteria Evaluation Consultation Meeting on Wednesday, April 11th at 9:00 am:

1. AGENDA
2. Preliminary Reference List
3. Draft List of Potential Empirical Studies

These documents will also be uploaded to the www.donpedro-relicensing.com website later today.

If you have any problems accessing this information, please let me know. Thank you.

ROSE STAPLES
CAP-OM
HDR Engineering, Inc.
Executive Assistant, Hydropower Services

WATER & POWER
Serving Central California since 1887

Modesto Irrigation District
The Power to Grow

Don Pedro Project - FERC Relicensing
Agenda for Temperature Criteria Assessment Study (W&AR 14)
Meeting with Relicensing Participants
April 11, 2012 – 9:00 a.m. to Noon – MID Offices

Conference Line Call-In Number 866-994-6437; Conference Code 5424697994

1. **Introductions**

   a. **Overview of Study Plan W&AR 14 Temperature Criteria Assessment**

   b. **FERC Determination (December 2011)**

      Reliance on EPA (2003)

      While the Districts' temperature criteria assessment may have the potential to inform W&AR-5 Salmonid Populations Information Integration and Synthesis Study, we will continue to rely upon the temperature criteria in EPA (2003) for our evaluation of project effects,

      Empirical evidence would be considered

      …unless empirical evidence from the lower Tuolumne River is provided that suggests different criteria are appropriate for salmonids in the lower Tuolumne.

2. **Purpose and Scope**

   Develop and implement an approach to identify appropriate temperature criteria for evaluating project effects on anadromous salmonids in the lower Tuolumne River

   Evaluate the appropriateness of site specific temperature evaluation criteria for Chinook salmon and *O. mykiss* relative to the proposed threshold temperatures described in EPA (2003) and identified by FERC and Participants as temperature criteria to be met in the lower Tuolumne River

   Two general questions are to be addressed:

   i. **Do the local populations of Chinook salmon and *O. mykiss* in the lower Tuolumne River have temperature tolerances that allow performance similar to that associated with the EPA (2003) threshold metrics at higher temperatures, and, if so, what are those temperatures; and**

ii. **If the temperature regime that can be achieved in the Tuolumne River exceeds those threshold criteria, what is the associated effect on the Chinook salmon and *O. mykiss* population in reference to agency goals and objectives?**

3. **Study Approach**

The Districts have identified several investigations that are intended to reduce uncertainties and potentially confirm or adjust temperature evaluation criteria to be based on empirical information that has been previously obtained from the lower Tuolumne River or is being contemplated for future acquisition. Consistent with the FERC determination (December 22, 2011), the Districts have determined that evaluation of empirical evidence describing the relationships among temperature conditions and the anadromous fish populations in the lower Tuolumne River should be considered when evaluating project-related, temperature effects on these populations. Such an approach would employ the best available science, which may not necessarily be the direct application of findings from other regions or populations. In developing the approach, the Districts will be guided by methods and procedures that have demonstrated utility in addressing development of temperature criteria for evaluation of anadromous salmonids, with a focus on such approaches conducted in other FERC processes involving Central Valley resources.

In general, each investigation will:

- **Identify issue being addressed (How does it relate to the question)**
- **Develop hypothesis/question**
- **Develop approach and methods to be based on best available science**
- **Consider refinement of evaluation based on input from interested parties, as appropriate**
- **Prepare detailed evaluation protocol and analytical procedures**
- **Apply, implement, analyze, report, integrate into overall assessment of temperature evaluation criteria (W&AR 14)**

Results of the evaluations should serve to identify appropriate modifications in temperature evaluation criteria by revising the temperature thresholds and/or by defining a level of effect associated with threshold exceedance.

Attached is a preliminary list of potential evaluations the Districts have identified as meeting the above guidance.

4. **Next Meeting**

Preliminary list of materials to support discussion with Relicensing Participants regarding Temperature Criteria Assessment Study

(Don Pedro Project, W&AR 14)

1. Teo, L.H., P.L. Sandstrom, E.D. Chapman, R.E.Null, K. Brown, P. Klimley, and B.A. Block. 2011. Archival and acoustic tags reveal the post-spawning migrations, diving behavior, and thermal habitat of hatchery-origin Sacramento River steelhead kelts (Oncorhynchus mykiss). Environ Biol Fish DOI 10.1007/s10641-011-9938-4 http://biotelemetry.ucdavis.edu/publications/EBF_Teo%20et%20al_%20Steelhead%20migration.pdf

2. Strange, Joshua S.(2010) 'Upper Thermal Limits to Migration in Adult Chinook Salmon: Evidence from the Klamath River Basin', Transactions of the American Fisheries Society, 139: 4, 1091 — 1108, First published on: 09 January 2011 http://dx.doi.org/10.1577/T09-171.1

3. Parsons, E.J.E. 2011. Carduirespitory physiology and temperature tolerance among populations of sockeye salmon (Onchorhynchus nerka) Ph D Thesis. University of British Columbia, Vancouver, August 2011.

4. Myrick, C.A. and J.J. Cech. 2001. Temperature Effects on Chinook Salmon and Steelhead: A Review Focusing on California's Central Valley Populations. Bay-Delta Modeling Forum Technical Publication 01-1. Available at http://www.sfei.org/modelingforum/.

5. Deas, M., J. Bartholow, C. Hanson, and C. Myrick. 2004. Peer Review of Water Temperature Objectives Used as Evaluation Criteria for the Stanislaus – Lower San Joaquin River Water Temperature Modeling and Analysis Task 9 - BDA Project No.: ERP-02-P28.

6. Essig, D.A. 1998. The Dilemma of Applying Uniform Temperature Criteria in a Diverse Environment: An Issue Analysis Idaho Division of Environmental Quality Water Quality Assessment and Standards Bureau Boise, Idaho

7. CDFG. 2010. Effects of Water Temperature on Anadromous Salmonids in the San Joaquin River Basin Prepared for the Informational Proceeding to Develop Flow Criteria for the Delta Ecosystem Necessary to Protect Public Trust Resources Before the State Water Resources Control Board Beginning March 22, 2010. CDFG Central Region Fresno, CA. February 2010

8. EPA. 2003. EPA Region 10 Guidance For Pacific Northwest State and Tribal Temperature Water Quality Standards http://www.epa.gov/region10/pdf/water/final_temperature_guidance_2003.pdf

9. Lower Yuba River Accord River Management Team Planning Group. 2011. Lower Yuba River water temperature objectives Technical Memorandum.

10. Sullivan, K., D. J. Martin, R. D. Cardwell, J. E. Toll, and S. Duke. 2000. An analysis of the effects of temperature on salmonids of the Pacific Northwest with implications for selecting temperature criteria. Sustainable Ecosystems Institute, Portland, Oregon, Draft report.

11. Pagliughi, S.W. 2008. Lower Mokelumne River reach specific thermal tolerance criteria by life stage for fall-run Chinook salmon and winter-run steelhead. East Bay Municipal Utility District, Lodi, CA 95240

12. Jaeger, H.K., H.E. Cardwell, M.J. Sale, M.S. Bevelhimer, C.C. Coutant, W. Van Winkle.1997. Modeling the linkages between flow management and salmon recruitment in rivers. Ecological Modeling 103(1977)171-191

13. Stillwater Sciences. 2002. Stream Temperature Indices, Thresholds, and Standards Used to Protect Coho Salmon Habitat: a Review. Prepared for Campbell Timberland Management, Fort Bragg, CA. March 2002.

List of Potential Empirical Evaluations Addressing Temperature Requirements for Tuolumne River Chinook salmon and *O mykiss*

1. Local adaptation of temperature tolerance of *O mykiss* juveniles

Question: Are *O mykiss* that occur in Tuolumne River locally adapted to higher temperature tolerances that may define site-specific temperature performance metrics.

General Approach – Evaluate capabilities of local *O mykiss* to accommodate warmer temperatures, both physiological and anatomical, following methods described by Parsons (2011) and others.

a. Potential local adaptation to higher range of temperature regimes experienced by O. mykiss may be expressed in physiological and related anatomical performance capabilities. A comprehensive discussion and related investigations conducted by Parsons (2011) strongly suggests that there is good reason to expect that temperature tolerance can vary among anadromous salmonid populations/stocks and provide a good, scientific approach to investigating that tolerance in the Tuolumne River O. mykiss population relative to the extant temperature conditions and criteria and thresholds currently used to assess temperature induced effects to condition, performance, and survival.
b. Identify performance at range of temperatures using methods similar to
c. Evaluate physiological performance vs. temperature per UBC study
d. Collect mortalities from RST or other sources to examine organs that can indicate performance adaptation

2. Spatial distribution response to temperature.

Question: Is *O mykiss* distribution influenced by changes in longitudinal temperature distribution between winter and late-summer/ early-fall.
General Approach: Evaluate distribution of O. mykiss during winter and following late summer/early fall to determine potential response related to temperature change

a. Temperature gradient along Tuolumne River changes from winter to summer as temperatures changes increase with distance downstream from La Grange Dam.
b. Spatial distribution, observed as presence/absence and potentially as density, along the Tuolumne River should change between winter and summer corresponding to change in temperature. Temperature tolerances and potentially evaluation criteria would be identified based on temperatures where O. mykiss continue to occur versus where they disappear between winter and late-summer, early-fall.

c. Data are available under various annual temperature regimes that range from substantial change in temperature from winter to fall, to temperature conditions meeting identified thresholds (EPA 2003) throughout a substantially great portion of the river.

3. Influence of temperature on growth of *O. mykiss* and *Chinook salmon*

Question: Is growth of *O mykiss* and Chinook salmon in Tuolumne River being adversely affected by temperature. (Direct observation of individual growth is not likely to be allowed due to permitting issues (ESA), but may not be necessary to address question.)

General Approach: Evaluate growth of *O mykiss* and Chinook salmon based on size distribution within and among years with varying temperature regimes and compare with a reference growth expectation based on literature and observations of other *O mykiss* populations.

a. Growth can be determined for Chinook salmon and *O mykiss* based on size at time. An age and growth evaluation of *O mykiss* to be performed per W&AR 20, will determine if size at time can be used to distinguish age composition of observed *O mykiss* and allow characterization of growth. Observed growth will be evaluated relative to expected results, based on growth rates reported in literature and observed in other waters where conditions are considered suitable for *O mykiss*(and *Chinook salmon*)
b. Growth rates determined to be comparable or expected would indicate that conditions for growth, including temperature, are sufficient so support *O mykiss* in good condition.

4. Effect of temperature observed as changes in condition/health of Chinook salmon

Question: Is the temperature regime of the Tuolumne River affecting Chinook salmon survival potential, measured as specific temperature-related affects to health and condition

General Approach: Evaluate quality of Chinook salmon smolt in reared in the Tuolumne River as temperatures change during the rearing/emigration phase.

Fish health/condition can be influenced by temperature conditions. Significant stages of concern include smolting of Chinook salmon. Methods used previously to evaluate smolt condition in the San Joaquin River and other locals will be used to assess juvenile Chinook salmon collected during emigration surveys. Fish will be collected during the period when larger fish begin to emigrate typically when temperatures and growth conditions increase, after mid-March, through the end of the emigration (June).

5. Influence of temperature on location, movement, survival potential of O. mykiss.

Question: How do *O mykiss* respond to (excessive) summer temperature conditions in Tuolumne River?
Do *O mykiss* relocate of remain in areas as temperature increase during summer and how does temperature exposure affect behavior and ultimately survival potential.

General Approach: Acoustic tagging *O mykiss* during early summer in various locals with various temperature expectations and monitor movement and survival to emigration.

6. Influence of excessive temperatures early in the Chinook salmon spawning period on egg survival as expressed in production v temperature conditions during spawning and incubation

Question: Does exposure to warmer (than EPA thresholds) temperature conditions during spawning significantly affect Chinook salmon survival (to emergence).

General Approach: Evaluate previous emergence trapping studies conducted by Stillwater Science on Tuolumne River.

7. Timing of spawning v temperature

Question: Does temperature during the early spawning period influence timing of spawning? Do Chinook salmon avoid spawning when temperatures exceed "suitable"/ threshold temperatures?

General Approach: Evaluate spawning distribution versus temperature using CDFG redd surveys.

8. Chinook salmon production related to precedent temperature conditions

Question: Does early temperature regime influence Chinook salmon production
Emigration population v temperature (conditioned by escapement)
In combination with 7, above, can early spawning temperature evaluation criteria be defined based on when Chinook salmon spawn versus temperature, in combination with survival (based on the corresponding year's production estimates (per emigration monitoring)?

General Approach: Evaluate estimated Chinook salmon production relative to temperature conditions during spawning.

From: Staples, Rose
Sent: Friday, April 06, 2012 7:43 PM
To: 'Alves, Jim'; 'Anderson, Craig'; 'Asay, Lynette'; 'Aud, John'; 'Barnes, James'; 'Barnes, Peter'; 'Blake, Martin'; 'Bond, Jack'; Borovansky, Jenna; 'Boucher, Allison'; 'Bowes, Stephen'; 'Bowman, Art'; 'Brenneman, Beth'; 'Brewer, Doug'; 'Buckley, John'; 'Buckley, Mark'; 'Burt, Charles'; 'Byrd, Tim'; 'Cadagan, Jerry'; 'Carlin, Michael'; 'Charles, Cindy'; 'Cismowski, Gail'; 'Colvin, Tim'; 'Costa, Jan'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob'; 'Cranston, Peggy'; 'Cremeen, Rebecca'; 'Day, Kevin'; 'Day, P'; 'Denean'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne'; 'Dowd, Maggie'; 'Drekmeier, Peter'; 'Edmondson, Steve'; 'Eicher, James'; 'Ferrari, Chandra'; 'Fety, Lauren'; 'Findley, Timothy'; 'Fuller, Reba'; 'Furman, Donn W'; 'Ganteinbein, Julie'; 'Giglio, Deborah'; 'Gorman, Elaine'; 'Grader, Zeke'; 'Gutierrez, Monica'; 'Hackamack, Robert'; 'Hastreiter, James'; 'Hatch, Jenny'; 'Hayat, Zahra'; 'Hayden, Ann'; 'Hellam, Anita'; 'Heyne, Tim'; 'Holley, Thomas'; 'Holm, Lisa'; 'Horn, Jeff'; 'Horn, Timi'; 'Hudelson, Bill'; 'Hughes, Noah'; 'Hughes, Robert'; 'Hume, Noah'; 'Jackman, Jerry'; 'Jackson, Zac'; 'Jennings, William'; 'Jensen, Art'; 'Jensen, Laura'; 'Johannis, Mary'; 'Johnson, Brian'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn'; 'Kinney, Teresa'; 'Koepele, Patrick'; 'Kordella, Lesley'; 'Lein, Joseph'; 'Levin, Ellen'; 'Lewis, Reggie'; 'Linkard, David'; 'Looker, Mark'; 'Lwenya, Roselynn'; 'Lyons, Bill'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul'; 'Marshall, Mike'; 'Martin, Michael'; 'Martin, Ramon'; 'Mathiesen, Lloyd'; 'McDaniel, Dan'; 'McDevitt, Ray'; 'McDonnell, Marty'; 'McLain, Jeffrey'; 'Means, Julie'; 'Mills, John'; 'Morningstar Pope, Rhonda'; 'Motola, Mary'; 'O'Brien, Jennifer'; 'Orvis, Tom'; 'Ott, Bob'; 'Ott, Chris'; 'Paul, Duane'; 'Pavich, Steve'; 'Pinhey, Nick'; 'Pool, Richard'; 'Porter, Ruth'; 'Powell, Melissa'; 'Puccini, Stephen'; 'Raeder, Jessie'; 'Ramirez, Tim'; 'Rea, Maria'; 'Reed, Rhonda'; 'Richardson, Kevin'; 'Ridenour, Jim'; 'Robbins, Royal'; 'Romano, David O'; 'Roos-Collins, Richard'; 'Roseman, Jesse'; 'Rothert, Steve'; 'Sandkulla, Nicole'; 'Saunders, Jenan'; 'Schutte, Allison'; 'Sears, William'; 'Shakal, Sarah'; 'Shipley, Robert'; 'Shumway, Vern'; 'Shutes, Chris'; 'Sill, Todd'; 'Slay, Ron'; 'Smith, Jim'; Staples, Rose; 'Steindorf, Dave'; 'Steiner, Dan'; 'Stone, Vicki'; 'Stork, Ron'; 'Stratton, Susan'; 'Taylor, Mary Jane'; 'Terpstra, Thomas'; 'TeVelde, George'; 'Thompson, Larry'; 'Vasquez, Sandy'; 'Verkuil, Colette'; 'Vierra, Chris'; 'Walters, Eric'; 'Wantuck, Richard'; 'Welch, Steve'; 'Wesselman, Eric'; 'Wheeler, Dan'; 'Wheeler, Dave'; 'Wheeler, Douglas'; 'Wilcox, Scott'; 'Williamson, Harry'; 'Willy, Allison'; 'Wilson, Bryan'; 'Winchell, Frank'; 'Wooster, John'; 'Workman, Michelle'; 'Yoshiyama, Ron'; 'Zipser, Wayne'
Subject: Revised Don Pedro W-AR-12 O.mykiss Habitat Survey Study Plan filed with FERC today
Attachments: Bose_P-2299-075_RevisedW-AR-12_120406 efiling.pdf

The revised Don Pedro W&AR-12 O.mykiss Habitat Survey Study Plan has been filed with FERC today—and a copy of the filing is attached. It will also be available shortly on FERC's E-Library and on the Don Pedro relicensing website at www.donpedro-relicensing.com--in the Announcement Section accessible via the INTRODUCTION tab.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

April 6, 2012
VIA Electronic Filing

The Honorable Kimberly D. Bose, Secretary
Federal Energy Regulatory Commission
888 First Street NE
Washington DC 20426

Re: **Don Pedro Project Relicensing**
FERC Project No. 2299-075
Revised Study Plan for W&AR-12 *O. mykiss* Habitat Survey Study

Dear Secretary Bose,

This filing is being made on behalf of the Turlock Irrigation District and Modesto Irrigation District (collectively, the Districts).

On November 22, 2011, pursuant to Section 5.11 of 18 CFR, the Districts filed with the Federal Energy Regulatory Commission (FERC) their revised study plan (RSP) for the Don Pedro Project (P-2299). In FERC's study plan determination issued December 22, 2011, FERC recommended modifications to the Water and Aquatic Resources (W&AR) Study 12 - *Oncorhynchus mykiss* Habitat Survey Study Plan. FERC also recommended that after incorporating the recommended modifications, the Districts file a revised W&AR-12 study plan within 90-days of FERC's study plan determination. Subsequently, the Districts requested, and FERC granted an extension of the filing deadline to April 6, 2012.

The Districts revised W&AR-12 to address FERC's recommendations, and provided the revised study plan to relicensing participants on February 21, 2012. Comments were received from the United States Fish and Wildlife Service (USFWS) on March 21, 2012. The attached revised W&AR-12 study plan incorporates clarifications to the habitat typing methodology and large woody debris definitions to be used as requested by the UWFWS. Additional information on references to scientific literature that will be used to support habitat quality and suitability analysis and identification of comparison streams will be incorporated in the study reports after the data collection and analysis under this study plan is conducted.

Respectfully Submitted,

John Devine

John Devine P.E.
Project Manager

cc: Turlock Irrigation District, Modesto Irrigation District, Relicensing Participants

HDR Engineering, Inc.
970 Baxter Boulevard
Suite 301
Portland, ME 04103-5346
Phone: (207) 775-4495
Fax: (207) 775-1742
www.hdrinc.com

TURLOCK IRRIGATION DISTRICT & MODESTO IRRIGATION DISTRICT
DON PEDRO PROJECT
FERC NO. 2299
WATER & AQUATIC RESOURCE WORK GROUP

Study Plan W&AR-12
Oncorhynchus mykiss Habitat Survey Study Plan
April 2012

1.0 Project Nexus

The continued project operation and maintenance of the Don Pedro Project (Project) may contribute to cumulative effects on anadromous fish habitat in the lower Tuolumne River. These potential environmental effects include changes in the type of physical habitat available for juvenile *Oncorhynchus mykiss (O. mykiss*). Changes to habitat may include reduction in habitat complexity and structure due to reduced availability of large woody debris (LWD). Lack of habitat complexity may affect fish populations in the lower Tuolumne River.

2.0 Resource Agency Management Goals

The Districts believe that four agencies have resource management goals related to salmonid species and/or their habitat: (1) U.S. Department of Interior, Fish and Wildlife Service (USFWS); (2) U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NMFS); (3) California Department of Fish and Game (CDFG); and (4) State Water Resources Control Board, Division of Water Rights (SWRCB).

A goal of the USFWS (2001) Anadromous Fish Restoration Program, as stated in Section 3406(b)(1) of the Central Valley Project Improvement Act, is to double the long-term production of anadromous fish in California's Central Valley rivers and streams. Objectives in meeting this long-term goal include: (1) improve habitat for all life stages of anadromous fish through provision of flows of suitable quality, quantity, and timing, and improved physical habitat; (2) improve survival rates by reducing or eliminating entrainment of juveniles at diversions; (3) improve the opportunity for adult fish to reach spawning habitats in a timely manner; (4) collect fish population, health, and habitat data to facilitate evaluation of restoration actions; (5) integrate habitat restoration efforts with harvest and hatchery management; and (6) involve partners in the implementation and evaluation of restoration actions.

NMFS has developed Resource Management Goals and Objectives for species listed under the Magnuson-Stevens Fishery Conservation and Management Act (16 U.S.C. §1801 et seq.) and the Endangered Species Act (ESA) (16 U.S.C. §1531 et seq.), as well as anadromous species that are not currently listed but may require listing in the future. NMFS' (2009) Public *Draft* Recovery Plan for Sacramento River Winter-run Chinook salmon, Central Valley Spring-run Chinook salmon, and Central Valley steelhead outlines NMFS' framework for the recovery of ESA-listed species and populations in California's Central Valley. For Central Valley steelhead, the recovery actions identified for the Tuolumne River are to: (1) conduct habitat evaluations; and

(2) manage cold water pools behind La Grange and Don Pedro dams to provide suitable water temperatures for all downstream life stages. For Central Valley fall/late fall-run Chinook, the relevant goals are to enhance the essential fish habitat downstream of the Project and achieve a viable population of Central Valley fall/late fall-run Chinook salmon in the Tuolumne River.

CDFG's mission is to manage California's diverse fish, wildlife, and plant resources, and the habitats upon which they depend, for their ecological values and for their use and enjoyment by the public. CDFG's resource management goals, as summarized in restoration planning documents such as "Restoring Central Valley Streams: A Plan for Action" (Reynolds et al. 1993), are to restore and protect California's aquatic ecosystems that support fish and wildlife, and to protect threatened and endangered species under California Fish and Game Code (Sections 6920-6924).

SWRCB has responsibility under the federal Clean Water Act (33 U.S.C. §11251-1357) to preserve and maintain the chemical, physical and biological integrity of the State's waters and to protect water quality and the beneficial uses of stream reaches consistent with Section 401 of the federal Clean Water Act, the Regional Water Quality Control Board Basin Plans, State Water Board regulations, the California Environmental Quality Act, and any other applicable state law.

3.0 Study Goals

The primary goal of this study is to provide information on habitat distribution, abundance and quality in the lower Tuolumne River with a focus on *O. mykiss* habitat related to LWD. An inventory of LWD and associated habitat quality, availability and use by salmonids will inform the evaluation of in-river factors that may affect the juvenile *O. mykiss* life stage. As recommended by FERC staff in its Study Plan Determination of December 27, 2012, several modifications have been made to this study at the request of Relicensing Participants (Elements No. 5 and 6 in Study Request NMFS-5, dated June 10, 2011) in an effort to provide more detailed characterization of LWD distribution in the lower Tuolumne River. In addition, the study will provide a rough estimate of the quantities of LWD removed from Don Pedro on an annual basis.

4.0 Existing Information and Need for Additional Information

Juvenile habitat quality and use has been found to be directly related to habitat complexity (Bustard and Narver 1971; Bisson et al. 1987). Instream habitat complexity is typically associated with large woody debris, pools, and off channel habitat. Cederholm (1997) and others observed a direct relationship between increased steelhead smolt production and increased habitat complexity in the form of LWD. Increases in numbers of anadromous (Ward and Slaney 1981; House and Boehne 1995) and non-anadromous (Gowan and Fausch 1995) fishes after addition of LWD to a stream have been demonstrated.

Instream LWD recruitment is generally from the adjacent riparian forest or allochthonous, originating from the upstream watershed. Large dams, that rarely spill, like Don Pedro Dam, can reduce recruitment from upstream sources. Reduction or elimination of large riparian trees will also reduce LWD recruitment.

The quality and condition of habitat in the lower Tuolumne River has been investigated for Chinook salmon since the 1996 FERC Order (76 FERC 61, 117). The order required that the condition of spawning habitat be assessed along with other monitoring requirements, specific to Chinook salmon. As a result, information is available for other salmonids in the river. For example, McBain and Trush (2000) identified that the uppermost reach of the lower Tuolumne River (River Mile [RM] 52–46.6) was primarily used for spawning salmon where they found gravel bed and banks, along with little valley confinement within the bluffs. Surveys of the channel downstream of La Grange Dam showed the occurrence of channel downcutting and widening, armoring, and depletion of sediment storage features (e.g., lateral bars and riffles) due to sediment trapping in upstream reservoirs, gold and gravel mining, and other land use changes since the 1850s (DWR 1994; McBain & Trush 2004).

Previous riparian investigations found large scale removal of riparian vegetation that was a direct result of mining activities and urban/agricultural encroachment. Clearing of riparian forests decreased large woody debris recruitment, allowed exotic plants to invade the riparian corridor, reduced shading of the water's surface, and contributed to increased water and air temperatures in the Tuolumne River corridor (McBain & Trush 2000). Grazing and other land uses have also resulted in direct impacts on riparian vegetation.

LWD plays an important role in habitat forming events within low-order streams. Where LWD dimensions are large relative to the channel width, LWD readily collects within the channel forming areas of velocity gradation, encouraging localized sediment deposition and scour (McBroom 2010). In higher order streams, such as the lower Tuolumne River, the role of LWD in habitat formation decreases with the stream width. However, LWD becomes more ecologically significant in high order streams, where it can provide the majority of stable, firm substrate that supports a substantial portion of invertebrate productivity (McBroom 2010).

Salmonid habitat quality and quantity, including characterization of habitat limitations and relative salmonid production potential is routinely assessed through surveys of instream habitat composition and structure, such as those surveys described by CDFG (2010). Results of such surveys can help identify land use and other related effects on habitat quality, thus the relative potential of the anadromous fish population. Such surveys also can identify opportunities to restore or enhance habitat conditions and salmonid and other aquatic production. In July 2008, Stillwater Sciences conducted a focused assessment of *O. mykiss* in the Tuolumne River that incorporated a habitat mapping component. The assessment identified general habitat units (e.g., pool, riffles) and then discussed the relationship between habitat type and observed *O. mykiss* use (Table 4.0-1). Habitat maps were also created displaying general habitat type from RM 52 to RM 39.5. The results of recent *O. mykiss* monitoring surveys (e.g. Stillwater Sciences 2008) provide a foundation for the focused *O. mykiss* habitat evaluations in this proposed study.

While existing historical data provide a broader characterization of the existing habitat, a more detailed investigation into habitat conditions is proposed. A more detailed assessment of *O. mykiss* habitat availability would include the level and kind of complexity, factors associated with complexity (such as bars, backwater pools, scour pools, etc.), and the amount of habitat available as a function of complexity and use.

Table 4.0-1 Example habitat use by habitat type for two *O. mykiss* size classes during summer (adapted from Stillwater Sciences 2008**).**

Habitat	*O. mykiss* < 150 mm				*O. mykiss* ≥ 150 mm				Total			
	Seen [1]	Est.	Std dev	95% Interval [2]	Seen [1]	Est.	Std dev	95% Interval [2]	Seen	Est.	Std dev	95% Interval
Pool Head	12	20	10.1	12–40	17	45	13.2	19–71	29	65	16.7	33–98
Pool Body	0				3	24	18.0	3–59	3	24	18.0	3–59
Pool Tail	1	2	2.6	1–7	0				1	2	2.6	1–7
Run Head	46	166	179.0	46–517	1	6	8.8	1–23	47	172	179.2	47–523
Run Body	5	860	115.6	634–1,087	6	319	77.5	167–471	11	1,179	139.2	906–1,452
Run Tail	0				0				0			
Riffle	65	1,428	198.2	1,039–1,816	13	226	126.7	13–474	78	1,653	235.2	1,192–2,114
Total	**129**	**2,476**	**291.2**	**1,905–3,047**	**40**	**619**	**150.4**	**325–914**	**169**	**3,096**	**327.7**	**2,453–3,738**

[1] Largest numbers seen in any single dive pass for each unit, summed over units. Note that summation of the largest numbers seen within individual (50 millimeter [mm]) size bins yields higher estimates of total fish smaller and larger than 150 mm.

[2] Nominal confidence intervals calculated as +/- 1.96 standard deviations. When this yielded lower bounds less than the numbers seen, the lower bound was truncated accordingly and the interval shaded.

In addition to a focused survey and assessment of the associations of LWD and other contributors to habitat complexity, and the relationships among complexity and *O. mykiss* utilization, a general accounting of LWD within the study reach will be conducted to identify location, general condition, density and abundance of LWD.

5.0 Study Methods

The study methods described below will be implemented to meet the study objectives.

5.1 Study Area

A one-year habitat assessment will be conducted in the salmonid spawning and rearing reach of the lower Tuolumne River from La Grange to Roberts Ferry Bridge (approx. RM 52–39). The LWD survey area will extend from RM 52 downstream to RM 24. A separate investigation of LWD removed from Don Pedro reservoir will also be conducted.

5.2 General Concepts

The following general concepts apply to the study:

- Personal safety is an important consideration of each fieldwork team. The Districts and their consultants will perform the study in a safe manner.
- Field crews may make minor modifications in the field to adjust to and to accommodate actual field conditions and unforeseeable events. (e.g., In the field it may not be possible to use tape to measure the length or average width of a very long habitat type as some pools are over 1,500 ft long; therefore GIS would be used to measure the habitat length rather than using a tape.) Any modifications made will be documented and reported in the draft study report.

5.3 Study Methods

The study will consist of two separate components: 1) a semi-quantitative inventory of instream habitat types and physical habitat characteristics, and 2) an appraisal of distribution, abundance, and function of LWD in the lower Tuolumne River. The first component will rely on available aerial photography and habitat mapping, and a reconnaissance-level survey of the lower Tuolumne River, between RM 52–39.5. This study component will rely upon existing broader habitat mapping conducted by Stillwater Sciences (2008) to identify focal research areas where *O. mykiss* occur and then utilize an adaptation of the high-resolution CDFG habitat typing methodology (CDFG 2010), to further characterize and evaluate these areas. CDFG identified four levels of typing, ranging from general broad habitat identification (Level I) to more detailed characterizations entailing 24 different potential habitat descriptors at Level IV. This study will utilize the Level III protocol, which differentiates six habitat types (main channel pool, scour pool, backwater pool, riffle, cascade, and flatwater) that can be further compressed into pool, riffle, and flatwater. The Level III will facilitate comparison with the pool, riffle, and run habitat types that were delineated during the 2010 IFIM Mesohabitat typing survey and other earlier efforts. In addition, a detailed description of LWD will be made at each focal study location using standard methods (Moore et al. 2006, Montgomery 2008), as described further below.

The second study component, an LWD inventory, will consist of a detailed survey of large wood and an assessment of its influence on *O. mykiss* habitat quality and quantity. The LWD inventory will be conducted between RM 52 and RM 24. In addition, as recommended by FERC Staff in the December 22, 2011 Study Determination, an evaluation of the frequency and volume of LWD trapped and removed from Don Pedro reservoir on an annual basis will be made (as described by NMFS in their June 10, 2011 study request Element No. 2).

Step 1 – Site Selection, Field Reconnaissance, and Planning. Habitat typing conducted for this study includes a 12.5 mile reach of the lower Tuolumne River (RM 52–39.5), with LWD surveys from RM 52–24. Field planning will begin by reviewing reports of existing habitat mapping conducted by Stillwater Sciences (2008), McBain & Trush (2004), and others. Field staff will coordinate with CDFG staff and others knowledgeable of access and navigability of the river to determine proper timing and related survey conditions that would optimize conducting the survey. As recommended by FERC Staff in the December 22, 2011 Study Determination, ortho-rectified digital aerial photographs of the study reach will be prepared for use with habitat typing and in developing a spatial inventory of mapped LWD. A subset of representative sampling units in the study reach will be selected for detailed habitat measurements using CDFG (2010). As recommended by CDFG (2010), sampling units selected for detailed habitat measurements will encompass 10–20 percent of the study reach and will be preferentially located where *O. mykiss* observations have been documented (e.g., Stillwater Sciences 2008).

As recommended by FERC Staff in the December 22, 2011 Study Determination, sampling units for inventorying LWD will be up to 20 channel widths long, consistent with guidelines used in California and the Pacific Northwest (e.g., Leopold 1994). The average bankfull width of the lower Tuolumne is 150 ft; therefore, the average length of a sample site will be around 3,000 ft long. Seven to ten sampling units that are 20 bankfull widths long will be selected for detailed characterization of LWD, encompassing approximately 4 to 6 miles (i.e., 10 to 20 percent) of the study reach by the estimates above.

Step 2 – Field Data Collection. Field surveys will be implemented using multiple teams of two field technicians. Each team will have a map and aerial photos delineating the portions of reach that will be surveyed. Upon accessing these survey areas, each team will collect the suite of measurements detailed in Table 5.3-1. These measurements are representative of the required data collection for Level III and IV CDFG habitat typing. Data will be documented on template datasheets to ensure that all data are collected and in a consistent manner between teams. Each habitat unit will have its upstream and downstream boundaries delineated on an aerial photograph and have an identification number that is the same as that on the datasheet. Field measurements will be made with standard field equipment: a handheld thermometer will be used to collect water temperature data, a stadia rod will be used to measure water depth, a steel meter tape or optical range finder will be used to measure site dimensions, and a spherical densitometer will measure percent overhead canopy cover. Each team will also be equipped with a handheld GPS and camera with habitat unit dimensions estimated in the field as well as by GIS.

Table 5.3-1 A summary of data collected as part of the Level IV CDFG habitat mapping.

Gathered Data	Description
Form Number	Sequential numbering
Date	Date of survey
Stream Name	As identified on USGS quadrangle
Legal	Township, Range, and Section
Surveyors	Names of surveyors
Latitude/Longitude	Degrees, Minutes, Seconds from a handheld GPS
Quadrant	7.5 USGS quadrangle where survey occurred
Reach	Reach name or rivermile range
Habitat Unit #	The habitat unit ID # that the bankfull width was measured
Time	Recorded for each new data sheet start time
Water Temperature	Recorded to nearest degree Celsius
Air Temperature	Recorded to nearest degree Celsius
Flow Measurement	Can be obtained from USGS monitoring stations
Mean Length	Measurement in meters of habitat unit
Mean Width	Measurement in meters of habitat unit
Mean Depth	Measurement in meters of habitat unit
Maximum Depth	Measurement in meters of habitat unit
Depth Pool Tail Crest	Maximum thalweg depth at pool tail crest in meters
Pool Tail Embeddedness	Percentage in 25% bucket ranges
Pool Tail Substrate	Dominant substrate: silt, sand, gravel, small cobble, large cobble, boulder, bedrock
Large Woody Debris Count	Detailed inventory criteria are listed below
Shelter Value	Assigned categorical value: no shelter, minimal shelter (small debris, bubble curtain etc.), significant shelter (large woody debris, root wads, vegetative cover, etc.)
Percent Unit Covered	Percent of the unit occupied
Substrate Composition	Composed of dominant and subdominant substrate: silt, sand, gravel, small cobble, large cobble, boulder, bedrock
Percent Exposed Substrate	Percent of substrate above water
Percent Total Canopy	Percent of canopy covering the stream
Percent Hardwood Trees	Percent of canopy composed of hardwood trees
Percent Coniferous Trees	Percent of canopy composed of coniferous trees
Right and Left Bank Composition	Identify dominant substrate: sand/silt, cobble, boulder, bedrock
Right and Left Bank Dominant Vegetation	Identify dominant vegetation: grass, brush, hardwood trees, coniferous trees, no vegetation
Right and Left Bank Percent Vegetation	Percent of vegetation covering the bank
Comments	Additional notes as needed

USGS = U.S. Geological Survey

The LWD distribution survey will use the Montgomery (2008) wood size classes, adapted to the Tuolumne River as follows. Information to be collected will include location (e.g., GPS coordinates), LWD size category, type, orientation, associated CDFG (2010) habitat type, and likely source. As recommended by FERC Staff in the December 22, 2011 Study Determination, within each LWD sample site, GPS locations and characteristics of each piece of LWD greater than 3 ft (1 m) long within the active channel will be recorded and binned within six length classes [3–6.5 ft (1–2 m), 6.5–13 ft (2–4 m), 13–26 ft (4–8 m), 26–52 ft (8–16 m), 52–105 ft (16–32 m), and >105 ft (>32 m)] and four diameter classes [4-8 in (0.1–0.2 m), 8–16 in (0.2–0.4 m), 16–31 in (0.4–0.8 m), 31–63 in (0.8–1.6 m)].

More detailed measurements will be taken for key pieces of LWD. Key LWD piece definitions are generally based upon channel widths that are found in lower order timberland channels. For example, Fox (1994) determined that the lengths of key pieces are between 1.4 and 1.5 times the active channel width. No key pieces of LWD would be present in the lower Tuolumne River given that the channel averages 150 ft in width and trees in the area are not 225 ft tall. The focus of this study component is to assess LWD availability as it relates to *O. mykiss* habitat in the Tuolumne River. Therefore, the Districts will use a definition related to the habitat value of the LWD that is appropriate for a river of this size. For this study, a key piece of LWD is defined as a piece that is either longer than 1/2 times the bankfull width, or of sufficient size and/or deposited in a manner that alters channel morphology and aquatic habitat (e.g., trapping sediment or altering flow patterns). The detailed information collected on each piece of LWD will be comparable with other definitions of LWD. In addition to recording the GPS locations for mapping on ortho-rectified aerial photographs, detailed information to be collected on key LWD pieces includes:

- Piece location, mapped on aerial photos/GPS documentation
- Piece length
- Piece diameter
- Piece orientation to bank
- Position relative to channel
- Rootwad present
- Tree type (hardwood or conifer)
- Associated with log jam
- Jam size (estimated dimensions/number of pieces)
- Source (imported/riparian/unknown)
- Channel dynamic function
- Habitat function (cover, sediment collection, hard substrate)

Lastly, although no detailed records of the quantities of LWD removed from Don Pedro Reservoir or other Project facilities exist, the Don Pedro Recreation Agency (DPRA) conducts an annual program to remove floating LWD at various locations in Don Pedro reservoir as it presents a boating hazard. This material is then placed in piles within suitable landing areas around the reservoir for burning, as conditions permit. To provide an order of magnitude estimate of LWD currently trapped in the reservoir, a team of two field technicians will travel by boat to each landing area and measure the quantity of LWD at each stockpile location in May 2012. Understanding that no meaningful relationship between this annual storage estimate and a LWD budget or loading rate to the lower Tuolumne River is possible, a discussion of the relative

sizes and characteristics of LWD in Don Pedro reservoir and at locations in the lower Tuolumne River will be made.

Step 3 – Data Processing and Analyses. Collected data will be stored and managed using a digital spreadsheet database. All data sheets will be physically copied after each week of survey. All data will then be entered into a spreadsheet database. Entered data will be QA/QC'd by two independent technicians reading and confirming each line of data together. Data will be summarized in tables and figures depicting overall habitat characteristics and conditions by reach. The quality and suitability of the habitat will be assessed using existing literature resources that include documented *O. mykiss* life history needs. Literature resources used to define characteristics of habitat quality and suitability will be described in the study report. This assessment will also discuss patterns of habitat use as found in recent O. mykiss snorkel survey efforts (e.g., Stillwater Sciences 2008). Final data will be made available to Relicensing Participants in digital spreadsheet form (Step 4). Maps depicting the location of the surveys, habitat types and LWD locations with each survey reach, and images of the surveyed habitat will also be provided within the report.

Data collected during the LWD distribution survey will be summarized relative to size class, reach, habitat association, density, and complexity. LWD trapped and removed from Don Pedro Reservoir in 2012 by the Don Pedro Recreation Agency will be quantified and a comparison of size characteristics of trapped LWD with those observed in the lower Tuolumne River will be made. These data summaries will be analyzed to determine the functioning of LWD in the lower Tuolumne River in the context of its channel and habitat type, and ecological role.

The quantity, quality, and use of the lower Tuolumne River by *O. mykiss* will be discussed in the context of other anadromous salmonid streams. The comparison will identify the occurrence and role of LWD and other habitat attributes in the lower Tuolumne River, and provide a basis for assessing the potential implications on *O. mykiss* abundance. Comparisons with other Central Valley streams and similar stream systems outside the Central Valley will be made to place LWD function in the lower Tuolumne River in context with other streams of similar stream order, recruitment potential, and sources. The rationale for selection of and documentation of comparability of stream characteristics will be included in the study report.

Step 4 – Prepare Report**.** The Districts will prepare a report that includes the following sections: (1) Study Goals, (2) Methods and Analysis, (3) Results, (4) Discussion, and (5) Conclusions. The quality and suitability of the habitat will be assessed and reported in light of existing resources that include steelhead life history needs. The report will discuss the findings from the Stillwater (2008) report and compare current conditions to population and habitat data collected in 2008.

The report will also contain GIS maps of sampled areas with delineated habitat and LWD features, organized and labeled photos of select habitat, and relevant summary tables and graphs. The reported data will be organized by reach site to allow for a spatial presentation of the findings. Final data will be made available to Relicensing Participants (Section 8.0).

6.0 Schedule

The Districts anticipate the schedule to complete the study as follows:

Project Preparation.....April – May 2012
Field Mapping.....June – August 2012
Data QA/QC..... September 2012
Prepare Report .....October – November 2012
Report Issuance.....January 2013

7.0 Consistency of Methodology with Generally Accepted Scientific Practices

The habitat mapping methodology was developed by CDFG based upon notable prior researchers. The methods described are standards that have been reviewed and used by numerous researchers since 1991. The study will follow the latest survey approach that has been refined into the current 4th edition (CDFG 2010).

8.0 Deliverables

The Districts will prepare a report, which will document the methodology and results of the study. In addition, at the request of relicensing participants, the Districts will provide GIS-based maps of survey locations documented as part of this study, as well as all LWD survey data (both focused and distribution survey) and all other habitat unit data in tabular (spreadsheet) and geo-spatial (e.g., ArcGIS shapefiles) formats.

9.0 Level of Effort and Cost

The Districts estimate the cost to complete this study to be $120,000.

10.0 References

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From: Staples, Rose
Sent: Monday, April 09, 2012 1:25 PM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Aud, John; Barnes, James; Barnes, Peter; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Cismowski, Gail; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Looker, Mark; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Means, Julie; Mills, John; Morningstar Pope, Rhonda; Motola, Mary; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Steindorf, Dave; Steiner, Dan; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Walters, Eric; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Don Pedro-NEW LIVE MEETING LINK--BUT USE HDR CALL-IN NUMBER

Importance: High

It is now my understanding that the HDR Call-In Number previously announced will STILL be used for the audio portion of the meeting—but the "live meeting" link will be GOTOMEETING link shown below. Thank you.

1. Please join my meeting.
https://www3.gotomeeting.com/join/480822654
2. Use your microphone and speakers (VoIP) - a headset is recommended. Or, call in using your telephone.
~~Dial +1 (213) 493-0605~~
~~Access Code: 480-822-654~~
Audio PIN: Shown after joining the meeting
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ROSE STAPLES
CAP-OM
HDR Engineering, Inc.
Executive Assistant, Hydropower Services

From: Staples, Rose
Sent: Tuesday, April 10, 2012 10:51 AM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Aud, John; Barnes, James; Barnes, Peter; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Cismowski, Gail; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Looker, Mark; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Means, Julie; Mills, John; Morningstar Pope, Rhonda; Motola, Mary; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Steindorf, Dave; Steiner, Dan; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Walters, Eric; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Use Links Below for Don Pedro Apr 10 - Apr 11 Meetings-Workshop

Importance: High

For today's meeting / workshop and tomorrow's meeting, we will be using the "live meeting" links previously announced last week—and I have copied them below for your ease of use. If you are unable to connect, please send me an email or call me at 207-239-3857! Thank you.

AUDIO INFORMATION
Use call-in number: **866-994-6437**, Conference Code **5424697994**

ON-LINE MEETING INFORMATION
(On-Line Meetings will be open approximately half hour prior to the meeting start time to allow for any technical issues to be resolved. If you have not used On-Line Meeting in the past, please allow a few extra minutes for your first log-on.)

Tuesday, April 10 (8:30 – 10:15am): Reservoir Temperature Modeling Data and

Methods Consultation Meeting (W&AR-3)

Join online meeting
https://meet.hdrinc.com/jenna.borovansky/QC5C5HN1

First online meeting?

Tuesday, April 10 (10:30am – 5:00pm) Salmonid Information Synthesis Workshop (W&AR-5)

Join online meeting
https://meet.hdrinc.com/jenna.borovansky/37NNBCDP

First online meeting?

Wednesday, April 11 (9:00 am – Noon): Temperature Criteria Evaluation Consultation Meeting (W&AR-14)

Join online meeting
https://meet.hdrinc.com/jenna.borovansky/MHVKDJYZ

First online meeting?

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Staples, Rose
Sent: Friday, April 13, 2012 7:14 PM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Aud, John; Barnes, James; Barnes, Peter; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Cismowski, Gail; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Looker, Mark; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Means, Julie; Mills, John; Morningstar Pope, Rhonda; Motola, Mary; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Steindorf, Dave; Steiner, Dan; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Walters, Eric; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Don Pedro April 9-11 Workshops-Meetings Presentations-Materials on Relicensing Website

In addition to materials distributed and posted prior to the workshops and meetings, the following meeting materials from the April 9-11 relicensing workshops and meetings have been posted to the Don Pedro Relicensing Website at www.donpedro-relicensing.com in the INTRODUCTION / Announcement section:

April 9 – Hydrology Workshop
Schematic of Tuolumne River Storage and Flow Locations

April 10 – Reservoir Temperature Model Overview Meeting
Presentation for Reservoir Temperature Modeling (W&AR-3)

April 10 – Salmonid Information Synthesis Study Workshop
Presentation for Salmonid Information Synthesis Study Workshop (W&AR-5)

April 11 Temperature Criteria Consultation Meeting
Presentation for Temperature Criteria Study (W&AR-14)

If you are unable to view and/or download any of this material, please let me know at rose.staples@hdrinc.com.

Temperature Criteria Assessment Study (W&AR 14)

April 11, 2012

Overview of Study Plan W&AR 14

The overall objective is to develop information on the influence of temperature on the in-river life-stages of Chinook salmon and *O. mykiss*.

Specific study objectives include the following:

- Identify life stage-specific fisheries population effects related to water temperature
- Identify life stage-specific water temperature parameters and compare to current temperature regimes.
- Assess and select an acceptable, informative approach to analyzing temperature regimes and their influences on Chinook salmon and *O. mykiss* in the lower Tuolumne River.

Study Methods

1. Review Relevant Literature

Focus: temperatures beyond optimum thresholds/ benchmarks (e.g., EPA 2003)

Emphasize San Joaquin River Watershed and Central Valley Specific

- Include Other Relevant FERC proceedings (YCWA, Merced River)
- Other Relevant Temperature Reviews (e.g., Upper San Joaquin River, Yuba River Management Team, Mokelumne River, Upper Yuba River Reintroduction)

The review and subsequent tasks to be conducted during this study will involve RP participation to be facilitated by convening bimonthly coordination meetings once the study begins.

Study Methods

2. Develop Water Temperature Evaluation Parameters

- What in-river temperatures would be protective of Chinook salmon and steelhead at each identified in-river life-stage?
- What indices, or metrics, should be used to assess individual and population-level effects of a specific water temperature regime on Chinook salmon and steelhead in the Tuolumne River?
- What are the appropriate water temperature evaluation criteria for the Tuolumne River

Study Methods

3. Relate Baseline Water Temperature Conditions to Population

Results will identify:

- In the lower Tuolumne River, how often was each of the life stage-specific water temperature evaluation parameters met under baseline conditions?
- Based on how often water temperature evaluation parameters were met, what were the likely sub-lethal and population-level effects on Tuolumne River salmonids?

FERC Determination (December 2011)

Reliance on EPA (2003)

While the Districts' temperature criteria assessment may have the potential to inform W&AR-5 Salmonid Populations Information Integration and Synthesis Study, we will continue to rely upon the temperature criteria in EPA (2003) for our evaluation of project effects,

Empirical evidence would be considered

…unless empirical evidence from the lower Tuolumne River is provided that suggests different criteria are appropriate for salmonids in the lower Tuolumne.

“What if the Temperature Criteria are Unattainable or Inappropriate?”

EPA recognizes that because of the inherent variability of Pacific Northwest rivers and streams there are likely to be situations where the recommended temperature criteria will be unattainable or inappropriate. The guidance offers several approaches a State or Tribe can take to address these situations. For example, where the natural background temperature (i.e., the temperature absent human impacts) is estimated to be higher than the recommended criteria, the natural background temperature can be adopted as criteria. Further, if human impacts cannot be remedied, alternative criteria can be established based on the water temperature that is attainable.”

(EPA 2003 Temperature Fact Sheet)

Response to FERC Determination

Updated Study-

Purpose and Scope :

Develop and implement an approach to identify appropriate temperature criteria for evaluating project effects on anadromous salmonids in the lower Tuolumne River

Evaluate the appropriateness of site specific temperature criteria for Chinook salmon and *O. mykiss* relative to the proposed threshold temperatures described in EPA (2003) and identified by FERC and Participants as temperature criteria to be met in the lower Tuolumne River

Updated Study

Two general questions are to be addressed:

- Do the local populations of Chinook salmon and *O. mykiss* in the lower Tuolumne River have temperature tolerances that allow performance similar to that associated with the EPA 2003 metric at higher temperatures, and, if so, what are those temperatures; and

- If the temperature regime that can be achieved in the TR exceeds those criteria, what is the associated effect on the Chinook salmon and *O. mykiss* population in reference to agency goals and objectives

Updated Study

Study Approach :

- Conduct investigations that are intended to reduce uncertainties and potentially confirm or adjust temperature criteria to be based on empirical information

- Results of the evaluations should serve to identify appropriate modifications in temperature evaluation criteria by revising the temperature thresholds and/or by defining a level of effect associated with threshold exceedance.

Discussion of Potential Evaluations

- The following are potential evaluations the Districts have identified as meeting the above guidance.

1. Local adaptation of temperature tolerance of *O mykiss* juveniles

Question: Are *O mykiss* that occur in Tuolumne River locally adapted to higher temperature tolerances that may define site-specific temperature performance metrics.

General Approach – Evaluate capabilities of local *O mykiss* to accommodate warmer temperatures, both physiological and anatomical, following methods described by Parsons (2011) and others.

- Potential local adaptation to higher range of temperature regimes experienced by O. mykiss may be expressed in physiological and related anatomical performance capabilities. A comprehensive discussion and related investigations conducted by Parsons (2011) strongly suggests that there is good reason to expect that temperature tolerance can vary among anadromous salmonid populations/stocks and provide a good, scientific approach to investigating that tolerance in the Tuolumne River O. mykiss population relative to the extant temperature conditions and criteria and benchmarks currently used to assess temperature induced effects to condition, performance, and survival.

2. Spatial distribution response to temperature.

Question: Is *O. mykiss* distribution influenced by changes in longitudinal temperature distribution between winter and late-summer/ early-fall.

General Approach: Evaluate distribution of *O. mykiss* during winter and following late summer/earl y fall to determine potential response related to temperature change.

- Temperature gradient along Tuolumne River changes from winter to summer as temperatures changes increase with distance downstream from La Grange Dam.
- Spatial distribution, observed as presence/absence and potentially as density, along the Tuolumne River should change between winter and summer corresponding to change in temperature. *O. mykiss* should continue to occur in those areas where temperature meets criteria and should disappear where temperatures exceed criteria, between winter and late-summer, early-fall.
- Data are available under various annual temperature regimes that range from substantial change in temperature from winter to fall, to temperature conditions meeting identified thresholds (EPA 2003) throughout a substantially great portion of the river.

3. Influence of temperature on growth of *O. mykiss* and *Chinook salmon*

Question: Is growth of *O. mykiss* and Chinook salmon in Tuolumne River being adversely affected by temperature. (Direct observation of individual growth is not likely to be allowed due to permitting issues (ESA), but may not be necessary to address question.)

General Approach: Evaluate growth of *O. mykiss* and Chinook salmon based on size distribution within and among years with varying temperature regimes and compare with a reference growth expectation based on literature and observations of other *O. mykiss* populations.

- Growth can be determined for Chinook salmon and *O. mykiss* based on size at time. An age and growth evaluation of *O. mykiss* to be performed per W&AR 20, will determine if size at time can be used to distinguish age composition of observed *O. mykiss* and allow characterization of growth. Observed growth will be evaluated relative to expected results, based on growth rates reported in literature and observed in other waters where conditions are considered suitable for *O. mykiss* (and Chinook salmon)
- Growth rates determined to be comparable or expected would indicate that conditions for growth, including temperature, are sufficient so support *O. mykiss* in good condition.

4. Effect of temperature observed as changes in condition/health of Chinook salmon

Question: Is the temperature regime of the Tuolumne River affecting Chinook salmon survival potential, measured as specific temperature-related affects to health and condition.

General Approach: Evaluate quality of Chinook salmon smolt in reared in the Tuolumne River as temperatures change during the rearing/emigration phase.

- Fish health/condition can be influenced by temperature conditions. Significant stages of concern include smolting of Chinook salmon. Methods used previously to evaluate smolt condition in the San Joaquin River and other locals will be used to assess juvenile Chinook salmon collected during emigration surveys when temperatures and growth conditions increase, after mid-March, through the end of the emigration (June).

5. Influence of temperature on location, movement, survival potential of *O. mykiss*.

Question: How do *O. mykiss* respond to (excessive) summer temperature conditions in Tuolumne River?

- Do *O. mykiss* relocate or remain in areas as temperature increase during summer and how does temperature exposure affect behavior and ultimately survival potential.

General Approach: Acoustic tagging *O. mykiss* during early summer in various locals with various temperature expectations and monitor movement and survival to emigration.

6. Influence of excessive temperatures early in the Chinook salmon spawning period on egg survival as expressed in production v temperature conditions during spawning and incubation.

Question: Does exposure to warmer (than criteria) temperature conditions during spawning significantly affect Chinook salmon survival (to emergence).

General Approach: Evaluate previous emergence trapping studies conducted by Stillwater Science on Tuolumne River.

7. Timing of spawning v temperature

Question: Does temperature during the early spawning period influence timing of spawning? Do Chinook salmon avoid spawning when temperatures exceed “suitable”/ threshold temperatures?

General Approach: Evaluate spawning distribution versus temperature using CDFG redd surveys.

8. Chinook salmon production related to precedent temperature conditions

Question: Does early temperature regime influence Chinook salmon production

- Emigration population v temperature (conditioned by escapement)
- In combination with 7, above, can early spawning temperature criteria be defined based on when Chinook salmon spawn versus temperature, in combination with survival (based on the corresponding year's production estimates (per emigration monitoring)?

General Approach: Evaluate estimated Chinook salmon production relative to temperature conditions during spawning.

Additional Discussions?

- Wrap Up

THANK YOU FOR COMING TODAY

- Set date for next discussion in about 2 months

(Mid June)

From: Staples, Rose
Sent: Monday, April 23, 2012 7:33 PM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Aud, John; Barnes, James; Barnes, Peter; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Cismowski, Gail; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lara, Marco; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Looker, Mark; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Means, Julie; Mills, John; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Steindorf, Dave; Steiner, Dan; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Walters, Eric; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: TID - MID Response to NMFS Additional Information Filing Regarding Don Pedro Project
Attachments: 2012-4-23 Response to NMFS.PDF

The Districts filed with FERC today their response (copy attached) to the National Marine Fisheries Service's Additional Information Filing of April 13th. It is also available for viewing/download from FERC's E-Library at www.FERC.gov or from the Don Pedro relicensing website at www.donpedro-relicensing.com (Introduction tab/Announcements).

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

WINSTON & STRAWN LLP

BEIJING
CHARLOTTE
CHICAGO
GENEVA
HONG KONG
LONDON
LOS ANGELES

1700 K STREET, N.W.
WASHINGTON, D.C. 20006-3817

+1 (202) 282-5000

FACSIMILE +1 (202) 282-5100

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JOHN A. WHITTAKER, IV
(202) 282-5766
jwhittaker@winston.com

April 23, 2012

VIA ELECTRONIC FILING

Kimberly D. Bose, Secretary
Federal Energy Regulatory Commission
888 First Street, N.E.
Washington, DC 20426

Re: Modesto and Turlock Irrigation Districts' Response to the National Marine Fisheries Service's Additional Information Filing; Docket No. P-2299-075

Dear Secretary Bose:

This letter is in response to the National Marine Fisheries Service's (NMFS) filing entitled "Additional Information for Consideration by the Study Dispute Resolution Panel, Don Pedro Hydroelectric Project (P-2299-075)," filed with the Secretary on April 13, 2012. The Modesto and Turlock Irrigation Districts (Districts) will be providing separate comments on the NMFS filing entitled "Additional Information for the Commission's Use in its Jurisdictional Review, La Grange Hydroelectric Project, UL11-1-000," that was dated April 12, 2012, and filed with the Secretary on April 13, 2012, in Docket No. UL11-1-000. This latter document, along with two other documents addressing the La Grange Project, were included with the first NMFS filing referenced above.

As a preliminary matter, the Districts object to NMFS' last-minute attempt to interject into the study dispute materials and information regarding the Commission's jurisdictional review of the La Grange Project. These materials and information have nothing to do with the Study Dispute Resolution Panel's (Panel) review of the pending study disputes. The jurisdictional review of the Districts' La Grange Project is a separate proceeding and is not connected with the relicensing proceeding for the Don Pedro Project. As the Panel is well aware, the Commission has already determined that the La Grange Project is not part of the Don Pedro Project.

The Districts concur with the Panel's initial conclusion during the April 17, 2012 technical conference that the jurisdictional issues regarding the La Grange Project are outside the

scope of the Panel's review, which was to address the study disputes filed by NMFS and to make recommendations on those disputes.

Furthermore, the filing by NMFS at the last minute deprived the parties to the relicensing proceeding an opportunity to review the materials prior to the hearing by the Panel.

The Districts also object to NMFS' request to add to the record before the Panel the administrative record for the "Proceeding on Interim Conditions before an Administrative Law Judge" (Interim Proceeding). While it is true that the Interim Proceeding dealt with many of the same issues involved in the current relicensing proceeding, these materials have nothing to do with the Panel's review of the NMFS study disputes. NMFS purposefully confuses the issue of Ms. Strange's dismissal from the Study Dispute Panel because of her involvement as a material witness in the Interim Proceeding with information that is relative to this study dispute. Furthermore, there is simply not enough time during the study dispute process for the Panel to review the voluminous testimony submitted in the Interim Proceeding.

Finally, the recently released *A Guide to Understanding and Applying the Integrated Licensing Process Study Criteria* (March 2012) (Guide) that NMFS also included with its April 13, 2012 filing in P-2299-075 is certainly helpful to participants in the relicensing process. In fact, its purpose is "to help stakeholders craft study requests (18 CFR § 5.9(b)) that clearly identify and explain the basis of their information needs and recommended study methods." (Guide, p. 3.) However, NMFS' assertion that the "rationale [the Guide] contains is at issue in the Study Dispute" is erroneous The reference to the Guide in the April 11, 2012 response from the OEP Director is nothing more than a recitation of the current ability of staff to request additional information, if needed, to conduct its cumulative effects analysis. (See, Guide, p. 11, "Commission staff will consider cumulative effects in its environmental document, when appropriate, based on existing information. If the project contributes to cumulative effects, staff may require additional information from the applicant on the project to assess the issues appropriately.") (Footnote omitted.)

Respectfully submitted,

/s/ John A. Whittaker, IV

John A. Whittaker, IV
ATTORNEY FOR MODESTO AND TURLOCK
IRRIGATION DISTRICTS

From: Staples, Rose
Sent: Tuesday, July 31, 2012 5:58 PM
To: 'Alves, Jim'; 'Anderson, Craig'; 'Asay, Lynette'; 'Aud, John'; 'Barnes, James'; 'Barnes, Peter'; 'Beniamine Beronia'; 'Blake, Martin'; 'Bond, Jack'; Borovansky, Jenna; 'Boucher, Allison'; 'Bowes, Stephen'; 'Bowman, Art'; 'Brenneman, Beth'; 'Brewer, Doug'; 'Buckley, John'; 'Buckley, Mark'; 'Burt, Charles'; 'Byrd, Tim'; 'Cadagan, Jerry'; 'Carlin, Michael'; 'Charles, Cindy'; 'Colvin, Tim'; 'Costa, Jan'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob'; 'Cranston, Peggy'; 'Cremeen, Rebecca'; 'Damin Nicole'; 'Day, Kevin'; 'Day, P'; 'Denean'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne'; 'Dowd, Maggie'; 'Drekmeier, Peter'; 'Edmondson, Steve'; 'Eicher, James'; 'Fargo, James'; 'Ferranti, Annee'; 'Ferrari, Chandra'; 'Fety, Lauren'; 'Findley, Timothy'; 'Fuller, Reba'; 'Furman, Donn W'; 'Ganteinbein, Julie'; 'Giglio, Deborah'; 'Gorman, Elaine'; 'Grader, Zeke'; 'Gutierrez, Monica'; 'Hackamack, Robert'; 'Hastreiter, James'; 'Hatch, Jenny'; 'Hayat, Zahra'; 'Hayden, Ann'; 'Hellam, Anita'; 'Heyne, Tim'; 'Holley, Thomas'; 'Holm, Lisa'; 'Horn, Jeff'; 'Horn, Timi'; 'Hudelson, Bill'; 'Hughes, Noah'; 'Hughes, Robert'; 'Hume, Noah'; 'Jackman, Jerry'; 'Jackson, Zac'; 'Jennings, William'; 'Jensen, Art'; 'Jensen, Laura'; 'Johannis, Mary'; 'Johnson, Brian'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn'; 'Kinney, Teresa'; 'Koepele, Patrick'; 'Kordella, Lesley'; 'Lara, Marco'; 'Lein, Joseph'; 'Levin, Ellen'; 'Lewis, Reggie'; 'Linkard, David'; 'Looker, Mark'; Loy, Carin; 'Lwenya, Roselynn'; 'Lyons, Bill'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul'; 'Marshall, Mike'; 'Martin, Michael'; 'Martin, Ramon'; 'Mathiesen, Lloyd'; 'McDaniel, Dan'; 'McDevitt, Ray'; 'McDonnell, Marty'; 'McLain, Jeffrey'; 'Mein Janis'; 'Mills, John'; 'Minami Amber'; 'Monheit, Susan'; 'Morningstar Pope, Rhonda'; 'Motola, Mary'; 'Murphey, Gretchen'; 'O'Brien, Jennifer'; 'Orvis, Tom'; 'Ott, Bob'; 'Ott, Chris'; 'Paul, Duane'; 'Pavich, Steve'; 'Pinhey, Nick'; 'Pool, Richard'; 'Porter, Ruth'; 'Powell, Melissa'; 'Puccini, Stephen'; 'Raeder, Jessie'; 'Ramirez, Tim'; 'Rea, Maria'; 'Reed, Rhonda'; 'Richardson, Kevin'; 'Ridenour, Jim'; 'Robbins, Royal'; 'Romano, David O'; 'Roos-Collins, Richard'; 'Roseman, Jesse'; 'Rothert, Steve'; 'Sandkulla, Nicole'; 'Saunders, Jenan'; 'Schutte, Allison'; 'Sears, William'; 'Shakal, Sarah'; 'Shipley, Robert'; 'Shumway, Vern'; 'Shutes, Chris'; 'Sill, Todd'; 'Slay, Ron'; 'Smith, Jim'; Staples, Rose; 'Steindorf, Dave'; 'Steiner, Dan'; 'Stone, Vicki'; 'Stork, Ron'; 'Stratton, Susan'; 'Taylor, Mary Jane'; 'Terpstra, Thomas'; 'TeVelde, George'; 'Thompson, Larry'; 'Vasquez, Sandy'; 'Verkuil, Colette'; 'Vierra, Chris'; 'Wantuck, Richard'; 'Welch, Steve'; 'Wesselman, Eric'; 'Wheeler, Dan'; 'Wheeler, Dave'; 'Wheeler, Douglas'; 'Wilcox, Scott'; 'Williamson, Harry'; 'Willy, Allison'; 'Wilson, Bryan'; 'Winchell, Frank'; 'Wooster, John'; 'Workman, Michelle'; 'Yoshiyama, Ron'; 'Zipser, Wayne'
Subject: Don Pedro FERC Filing - Request for Clarification of Director's Formal Study Dispute Resolution of May 24 2012

Today we have filed, on behalf of the Districts, a request for clarification of certain elements in the FERC Director's Formal Study Dispute Resolution of May 24, 2012. A copy of today's filing has been uploaded to the www.donpedro-relicensing.com website (Introduction/Announcement)—and it is also available on FERC's e-library www.ferc.gov (P-2299-075).

From: Staples, Rose
Sent: Monday, August 06, 2012 3:46 PM
To: 'Karl Morton'
Subject: RE: Don Pedro re-licensing

Thank you for your interest in the Don Pedro relicensing. Let me direct you to two websites for background information on the relicensing:

> www.donpedro-relicensing.com (especially the INTRODUCTION/Announcements section for the most recent notifications and the DOCUMENTS section (for major documents as noted in the paragraph below)
>
> www.ferc.gov (Documents and Filings/E-Library for docket P-2299).

The major documents that have been released so far in the relicensing process are the Districts' PAD (Pre-Application document-February 10, 2011), the RSP (Revised Study Plan-November 22, 2011), and FERC's Scoping Document (April 8, 2011) & Study Plan Determination (December 22, 2011). You can find copies of these documents on both the FERC E-Library site under the P-2299 and/or P-2299-075 docket number using the date range of February 10, 2011 to the present date) and on the relicensing website under DOCUMENTS (the PAD and Scoping Documents are under the PAD\NOI\SCOPING subheading and the Revised Study Plan and FERC Study Plan Determination is under the STUDIES sub-heading).

Currently the Districts are working on the first-year studies, and are in the midst of the Workshop Consultation Process (see FERC E-Library filing of May 18, 2012). Dates of the upcoming workshops are posted via announcements on the relicensing website (INTRODUCTION/Announcements Section).

If you would like your email address added to the general email distribution (for relicensing participants' meeting notices and other announcements), please advise.

P.S.: Also note the three relicensing newsletters that have been published by the Districts, copies of which are on the relicensing website/INTRODUCTION/Announcements (by date):

Volume 1 – Issue 1 – May 2, 2011
Volume 1 – Issue 2 – October 1, 2011
Volume 2 – Issue 1 – March 29, 2012

P.S.: If you have any difficulty locating and/or downloading any of these documents, please let me know. Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Karl Morton [mailto:farmerkarl.karl[redacted]
Sent: Monday, August 06, 2012 10:05 AM
To: Staples, Rose
Subject: Don Pedro re-licensing

Please provide all available information. Thank you.

Karl Morton

From: Staples, Rose
Sent: Wednesday, September 26, 2012 7:02 PM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Barnes, James; Barnes, Peter; Beniamine Beronia; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Damin Nicole; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fargo, James; Ferranti, Annee; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jauregui, Julia; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Looker, Mark; Loy, Carin; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Mein Janis; Mills, John; Minami Amber; Monheit, Susan; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Steindorf, Dave; Steiner, Dan; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Districts File Request for Schedule Extension for Don Pedro Initial Study Reports and Initial Study Report Meeting Dates

The Districts have filed with FERC today a request for a minor extension to the schedule for the Initial Study Reports and the Initial Study Report Meetings to January 17, 2013 and January 30, 2013 respectively. A copy of this filing has been posted to the www.donpedro-relicensing.com website (INTRODUCTION Tab/Announcements) and will also be available, most probably by tomorrow, on FERC's E-Library (under docket P-2299-075). If you have any difficulty locating and/or downloading this document, please let me know.

From: Devine, John
Sent: Thursday, October 18, 2012 8:30 AM
To: Jim Alves
Subject: FW: Don Pedro Operations Modeling Training - Validation Meeting October 23 2012
Importance: High

Jim,

Thank you for your recent email to Rose Staples, and letting us know of your concerns . First, let me answer that HDR has, with Districts' approval, retained Cardno Entrix to perform the socioeconomic study for the Don Pedro FERC relicensing. HDR is overseeing the work. The meeting this Friday is just between Cardno and the City of Modesto, with MID attending too. The purpose is to gather relevant information specific to the City for inclusion into the Socioeconomic Study. My understanding is that this meeting date was scheduled a couple of weeks ago in consideration of Rich Ulm's calendar, and that the agenda was sent out yesterday. Meetings with other individual agencies and organizations have been scheduled in a similar manner.

We are also currently trying to schedule a broader Socioeconomic Study Meeting with all interested parties to provide an status report on the overall Socioeconomic study. I hope to have that announcement out today, in fact, and I know that you are included in that invite list. We do appreciate your involvement, and the City's, in the Don Pedro relicensing and I am sorry for any confusion the Friday meeting may have caused.

Please do not hesitate to contact me directly to share any further concerns or discuss the Don Pedro relicensing.

JOHN DEVINE P.E.
HDR Engineering, Inc.
Senior Vice President, Hydropower Services

970 Baxter Boulevard Suite 301 | Portland, ME 04103
207.775.4495 | c: 207.776.2206 | f: 207.775.1742
john.devine@hdrinc.com | hdrinc.com

From: Staples, Rose
Sent: Wednesday, October 17, 2012 9:47 AM
To: Devine, John

Subject: FW: Don Pedro Operations Modeling Training - Validation Meeting October 23 2012
Importance: High

Please see Jim's comments below.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Jim Alves [mailto:jalves@modestogov.com]
Sent: Tuesday, October 16, 2012 9:08 PM
To: Staples, Rose
Subject: RE: Don Pedro Operations Modeling Training - Validation Meeting October 23 2012

Rose,

Is HDR overseeing the Socioeconomic Study Plan effort for the MID/TID relicensing? I know a firm was hired to conduct this effort, but I thought HDR was overseeing all the Study Plans efforts under the relicensing program for MID/TID. The reason I ask is that I was just informed of a meeting this Friday at MID offices on the Socio-economic Study, supposedly for a discussion on various documents, relevant information, etc. And that is all I know. I had to give a blank look in reply regarding any knowledge of it. This information came through our Department's Director, Rich Ulm, via another set of meetings outside of the FERC effort. I was wondering if other stakeholders are aware of this if I was not, since so far I have been notified of such FERC related meetings or if this particular meeting is somehow a separate item from the actual Socio-economic Study Plan being conducted as part of the FERC effort.

My boss, Jack Bond, will attend for Modesto but I am a bit concerned since a longer heads up would have been beneficial in preparation for this meeting. Jack Bond is on the same FERC notification list as I. I had not heard anything on this Study Plan since attending the Study Plan development meetings before it was finalized and was about to inquire of its status.

Regards,

Jim Alves
City of Modesto
Associate Civil Engineer
Utility Planning & Projects Dept.
Ph: 209-571-5557
Fx: 209-522-1780

From: Staples, Rose
Sent: Friday, October 19, 2012 6:15 PM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Barnes, James; Barnes, Peter; Beniamine Beronia; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Damin Nicole; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fargo, James; Ferranti, Annee; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jauregui, Julia; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Loy, Carin; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Mein Janis; Mills, John; Minami Amber; Monheit, Susan; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Steindorf, Dave; Steiner, Dan; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Don Pedro Relicensing Nov 9 2012 Socioeconomics Study Progress Update Meeting AGENDA
Attachments: SocioeconomicsStudyProgressUpdateMeeting_Nov 9 2012_Agenda.pdf

Please find attached the AGENDA for the Don Pedro Relicensing Socioeconomics Study Progress Update Meeting scheduled for November 9, 2012 from 9:00 a.m. to 11:30 a.m. at the MID Offices in Modesto. Thank you.

ROSE STAPLES
CAP-OM
HDR Engineering, Inc.
Executive Assistant, Hydropower Services

Socioeconomics Study Progress Update Meeting
Don Pedro Project Relicensing Water &Aquatic Resources Study Plan #15
November 9, 2012 - 9:00 a.m. – 11:30 a.m. – MID Offices
Call-In #866-994-6437, Conference Code 5424697994

1. **Provide an Overview of Socioeconomics Study Plan**

   The primary goals of the study on socioeconomic resources are to quantify the baseline economic conditions and resources in the region affected by the Don Pedro Project's water supply, flood control, and power benefits. In addition, the study will quantify the socioeconomic effects of the current Project operations and develop methodologies and a framework that can be used to evaluate the potential socioeconomic effects of any proposed changes to Project operations that may be considered as part of the relicensing process, including scenarios affecting the availability of agricultural and urban water supplies. Generally, the objectives of the study plan are to:

   - qualitatively and quantitatively describe local economic conditions in the regions that are affected by the existing Project operations,
   - assess the key factors influenced by Project operations that generate economic activity in affected regions,
   - estimate the economic value generated by the Project's water storage in various uses, both consumptive (agriculture and urban) and non-consumptive (recreation),
   - measure the role and significance of the Project in the economies of the regions, and
   - use these findings to assess the socioeconomic impacts on affected groups and industries resulting from potential changes in Project operations.

2. **Provide a Status Report on Resource Areas**

   A summary of information sources identified and the approach for analysis in each of the resource areas below will be presented and discussed. The Districts are seeking further input from those potentially affected by changes in Project operations. The primary focus of first year activities has been to gather relevant information on the baseline economic values and socioeconomic effects of current Project operations in the following areas:

   - Agriculture
   - Municipal & Industrial
   - Recreation
   - Hydropower Generation
   - Land Values
   - Regional Economics

3. **Request Additional Information and Data**

   The Districts continue their search for additional information that may assist in the development of baseline economic values and socioeconomic effects, as well as sources of information that may be used in the development of future use alternatives.

4. **Review Study Schedule and Discuss Next Steps**

   Review upcoming schedule and opportunity for relicensing participants to provide any further information.

From: Staples, Rose
Sent: Tuesday, October 30, 2012 2:19 PM
To: Alves, Jim; Anderson, Craig; Asay, Lynette; Barnes, James; Barnes, Peter; Beniamine Beronia; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Damin Nicole; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fargo, James; Ferranti, Annee; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackman, Jerry; Jackson, Zac; Jauregui, Julia; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Loy, Carin; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Mein Janis; Mills, John; Minami Amber; Monheit, Susan; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Stapley, Garth; Steindorf, Dave; Steiner, Dan; Stender, John; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Don Pedro Relicensing Dispersed Recreation Use Site Assessments November 8, 2012

Recreation Facility Condition, Public Accessibility, and Recreation Use Assessment Study (Study RR-1)
Dispersed Recreation Use Site Assessment Fieldwork
Thursday, November 8, 2012

As you know, HDR, on behalf of the Districts, is continuing fieldwork for the Recreation Facility Condition, Public Accessibility, and Recreation Use Assessment Study (Study RR-1), which includes dispersed recreation use site assessments.

The FERC-approved study plan states the Districts will schedule the field survey in advance so that Relicensing Participants may attend. Field work is scheduled to occur Thursday, November 8, 2012. On this date, we will be conducting assessments at dispersed recreation sites accessible by vehicle on public roads.

If you plan to attend November 8, 2012, please contact Nancy Craig by November 6th (at Nancy.Craig@hdrinc.com) for details and logistics—or email me at rose.staples@hdrinc.com.

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Staples, Rose
Sent: Thursday, November 01, 2012 8:23 PM
To: Asay, Lynette; Barnes, James; Barnes, Peter; Beniamine Beronia; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Damin Nicole; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fargo, James; Ferranti, Annee; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackson, Zac; Jauregui, Julia; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Loy, Carin; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Mein Janis; Mills, John; Minami Amber; Monheit, Susan; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Stapley, Garth; Steindorf, Dave; Steiner, Dan; Stender, John; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Don Pedro Relicensing Studies WAR14 Meeting and WAR06-10 Workshop - November 15-16 2012
Attachments: DP_TempCriteriaAssessmtMtg_Nov16_AGENDA_121101.doc

W&AR-06 Tuolumne River Chinook Salmon and W&AR-10 O. mykiss Population Model Workshops

November 15 (9:00 am – 4:30 pm) and November 16 (9 am – Noon): The agenda for these workshops will be forthcoming soon; please continue to hold these dates.

W&AR-14 Temperature Criteria Study Meeting

November 16 (1:00 pm – 4:00 pm): AGENDA attached. This Temperature Criteria Study Meeting is to update relicensing participants on the status of empirical studies proposed at the April 2012 meeting. Written background materials on proposed studies will be provided next week.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

Temperature Criteria Assessment Meeting
Don Pedro Relicensing Study W&AR-14
November 16, 2012 - 1:00 p.m. to 4:00 p.m. – MID Offices

AGENDA

1. **Introductions**

2. **Purpose of Meeting: Report and Discuss Status of Study Plan Implementation**

   - Original study plan
   - FERC Study Determination
   - Additional empirical evaluations proposed during April 2012 meeting

3. **W&AR 14 Study Plan**

   -Review relevant literature
   -Develop water temperature evaluation parameters
   -Relate baseline water temperature conditions to population

4. **Additional Studies and Evaluations**

   -Empirical studies proposed by Districts for implementation

   Study 1: Local Adaptation of Temperature Tolerance of O. mykiss Juveniles in the Lower Tuolumne River
   Study 2: Spatial distribution juvenile O. mykiss in response to temperature
   Study 3(b): Influence of temperature on growth of Chinook salmon
   Study 7: Influence of temperature on pre-spawning of Chinook salmon

   -Empirical studies proposed by Districts at April meeting, but not proposed for implementation at this time

   Study 3(a): Influence of temperature on growth of O. mykiss
   Study 4: Effect of temperature on condition/health of Chinook salmon
   Study 5: Influence of temperature on location, movement, survival potential of O. mykiss.
   Study 6: Influence of temperatures during the early Chinook salmon spawning period on egg survival.
   Study 8: Chinook salmon production related to precedent temperature conditions

5. **Next Steps and Study Schedule**

From: Staples, Rose
Sent: Thursday, November 08, 2012 7:35 PM
To: Asay, Lynette; Barnes, James; Barnes, Peter; Beniamine Beronia; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Damin Nicole; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fargo, James; Ferranti, Annee; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackson, Zac; Jauregui, Julia; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Le, Bao; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Loy, Carin; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; Mein Janis; Mills, John; Minami Amber; Monheit, Susan; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; Murray, Shana; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Stapley, Garth; Steindorf, Dave; Steiner, Dan; Stender, John; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; White, David K; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Don Pedro Socioeconomics Study and Temp Criteria Assessment Meetings Materials Uploaded to Relicensing Website

I have uploaded to the Don Pedro relicensing website (www.donpedro-relicensing.com) today, ***as attachments to the Meeting calendar for their respective meeting dates***, the following meeting materials:

Socioeconomics Study (W&AR-15) Progress Update Meeting, Friday, November 9
PowerPoint Presentation being used at the meeting

Temperature Criteria Assessment (W&AR-14) Meeting, Friday afternoon, November 16

Agenda updated with the LIVE MEETING link
Study Update

If you are not able to access or download these documents, please do contact me at rose.staples@hdrinc.com.

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
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Socioeconomics Study Progress Update Meeting
Don Pedro Project Relicensing Water &Aquatic Resources Study Plan #15
November 9, 2012 - 9:00 a.m. – 11:30 a.m. – MID Offices
Call-In #866-994-6437, Conference Code 5424697994

1. **Provide an Overview of Socioeconomics Study Plan**

   The primary goals of the study on socioeconomic resources are to quantify the baseline economic conditions and resources in the region affected by the Don Pedro Project's water supply, flood control, and power benefits. In addition, the study will quantify the socioeconomic effects of the current Project operations and develop methodologies and a framework that can be used to evaluate the potential socioeconomic effects of any proposed changes to Project operations that may be considered as part of the relicensing process, including scenarios affecting the availability of agricultural and urban water supplies. Generally, the objectives of the study plan are to:

   - qualitatively and quantitatively describe local economic conditions in the regions that are affected by the existing Project operations,
   - assess the key factors influenced by Project operations that generate economic activity in affected regions,
   - estimate the economic value generated by the Project's water storage in various uses, both consumptive (agriculture and urban) and non-consumptive (recreation),
   - measure the role and significance of the Project in the economies of the regions, and
   - use these findings to assess the socioeconomic impacts on affected groups and industries resulting from potential changes in Project operations.

2. **Provide a Status Report on Resource Areas**

   A summary of information sources identified and the approach for analysis in each of the resource areas below will be presented and discussed. The Districts are seeking further input from those potentially affected by changes in Project operations. The primary focus of first year activities has been to gather relevant information on the baseline economic values and socioeconomic effects of current Project operations in the following areas:

   - Agriculture
   - Municipal & Industrial
   - Recreation
   - Hydropower Generation
   - Land Values
   - Regional Economics

3. **Request Additional Information and Data**

   The Districts continue their search for additional information that may assist in the development of baseline economic values and socioeconomic effects, as well as sources of information that may be used in the development of future use alternatives.

4. **Review Study Schedule and Discuss Next Steps**

   Review upcoming schedule and opportunity for relicensing participants to provide any further information.

Don Pedro Project Relicensing

Socioeconomics Study Plan – Progress Meeting

November 9, 2012

MODESTO IRRIGATION DISTRICT | TURLOCK IRRIGATION DISTRICT

FERC PROJECT NO. 2299

Agenda

- Overview of Socioeconomics Study Plan
- Status Report on Study Plan Analysis
- Request Additional Information and Data
- Review Study Schedule and Discuss Next Steps

Meeting Purposes

- Provide progress update on socioeconomic study plan
- Describe data sources and economic models
- Continue to seek relevant information from Relicensing Participants
- Describe next steps and schedule

Background & Process

- Pre-Application Document – Filed with FERC in Feb 2011
- FERC Scoping Documents – April 8 and July 25, 2011
- FERC Scoping Meeting – May 11, 2011
- Study Plan Development – July to Nov 2011
  - Meetings: August 23 and September 13, 2011
- FERC Study Plan Determination – Dec. 21, 2011
- First-year studies – 2012 (ongoing)

Research Team

- Cardno ENTRIX
  - Environmental & Natural Resource Management Consulting Firm
  - National Economics Practice
- Lead Researchers
  - Duane Paul, Ph.D., Senior Consultant
  - Steve Pavich, Senior Economist

Socioeconomics Study Plan - Overview

- Study Objectives
  - Characterize local economic conditions
  - Identify key drivers of economic activity related to the Project
  - Estimate economic values of Project water supplies
  - Measure the role of the Project on the local and statewide economies
  - Assess economic effects in context of affected groups and industries
- Study Process
  - Baseline study (2012/13)
  - Scenario analysis (2013) – evaluate future operating scenarios
  - Draft License Application (Nov 2013)
  - Final License Application (April 2014)

Scope of Economic Analysis

- Agriculture water supplies
- Municipal and industrial (M&I) water supplies
- Reservoir recreation
- Hydropower generation
- Land values
- Regional economics
- Environmental justice

Agricultural Water Supplies

- Purpose: Analyze value of irrigation water in agriculture use in MID and TID service areas
- Background
  - Irrigated agriculture about 200,000 acres between MID and TID
  - Broad mix of permanent and annual crops, skewed toward permanent crops
  - Major dairy industry
- Objective: Develop detailed quantitative baseline of agricultural value in the region

Agricultural Water Supplies (cont.)

- Based on Statewide Agricultural Production Model (SWAP)
  - Developed jointly by UC Davis and DWR
  - Based on Central Valley Production Model (CVPM), allows greater detail
  - Emulates farm-level decision making under various constraints, e.g. on water availability, costs
  - Provides estimate of marginal value of water in producing various crops
  - Model outputs will be used in regional economic model to assess role of agriculture to economy
- Data Collection and Sources
  - County, State, Federal government; University of California (crop budgets)
  - Crop prices and yields, costs of production, water requirements (ET), water application rates
  - Districts: crop acreages, water deliveries, irrigation techniques (AG Water Management Plans)
- Status
  - Calibration runs with SWAP model (match existing conditions as precursor for impact analysis)
  - Gross value of crop production in two districts: $413.6 million ($1,820/acre) - preliminary
  - Value of dairy and other agricultural products (under development)

M&I Water Supplies

- Value based on water as input to production/utility of domestic users
- Average and marginal value of water in M&I use
- Literature review
  - Multiple studies related to CA drought (1987-1992)
  - Jenkins, Lund, and Howitt (2003): urban water scarcity costs - $1,766/AF in 2020
  - PPIC (2007): urban scarcity costs, reduced Delta exports- $1,172/AF in 2050 in SJV
- Avoided costs – cost of replacement water for affected municipalities
  - Groundwater pumping costs (City of Modesto)
- Meetings and outreach
  - City of Modesto (October 19, 2012)
  - Other municipalities seeking surface water supplies

Reservoir Recreation

- Focus on value of flat-water recreation at reservoir
  - Value to recreation user (consumer surplus)
- Data sources
  - Interface with recreation surveys (Study RR-1) - identify recreation uses, quantify visitation levels, visitor characteristics (origin)
  - Don Pedro Recreation Agency (DPRA) - Davis-Grunsky reports
- Benefits-Transfer Methodology
  - Apply economic values from other/similar contexts to project
  - Representative values from Loomis (2005)
- Status
  - Awaiting final recreation estimates and RR-1 study report

Hydropower Generation

- Purpose: Quantify value of power produced by Project operations and related socioeconomic effects
- Background
  - Facility has authorized capacity of 168 Megawatts / average generation is 532,500 MWh
  - 2011 electric service revenues: TID revenues $267 million; MID $341 million
  - Customers: TID 100,000 accounts / MID 111,000 accounts
- Data
  - Quantity of hydropower generated
  - Unit values of power (ISO)
  - Replacement energy sources and costs
  - Facility operation costs, incl. O&M, payroll, other
- Coordination with hydrology model (power module)
  - Seasonal generation

Land Values

- Role of water availability on regional land values
- Land value data
  - Source: California Chapter, American Society of Farm Managers and Rural Appraisers (CASFMRA) - Trends in Agricultural Land & Lease Values (2012)
- Preliminary findings
  - MID/TID cropland ($18,500/acre) in 2011
  - Comparatively higher land values in MID/TID service areas relative to other regions in Merced and Stanislaus counties
  - Comparison to rangeland: land values 5x to 20x higher
  - Land values within MID/TID higher than other irrigation districts in region
- Coordination with local real estate appraisers / lenders
  - Identified appraisers/lenders from CASFMRA

Regional Economics

- Regional economic benefits associated with uses of Project water
  - Agriculture, M&I, recreation, and power generation
- IMPLAN economic model
  - Input-output framework
  - Direct, indirect, and induced effects (multiplier or "ripple" effects)
  - Metrics: output (production), labor income, and employment (jobs)
- Models have been constructed
  - Regional model (Merced, Stanislaus, and Tuolumne counties); Statewide model
  - 2010 dataset
- Documented existing demographic and economic conditions
  - Sources: U.S. Census, Bureau of Economic Analysis (BEA), CA EDD
- Awaiting on outputs from other resource analyses – model inputs

Environmental Justice

- Purpose: Provide demographic and social information of affected population in Districts' service areas
  - Used to assess whether minority or low-income populations are disproportionately represented in study area
  - Consistent with Federal guidelines (Executive Order 12898, CEQ guidance)
- Approach
  - Collect pertinent demographic, cultural, related data
  - Assess whether minority or low-income populations are disproportionately affected from changes Project operations
- Data
  - Race, income, housing, poverty measures
  - Sources: 2010 Census data (census tract level)
- Relicensing alternative-based analysis

Request for Additional Information

- Seeking input from relicensing participants
- Relevant data and/or studies
- Information contacts

Schedule & Next Steps

- Baseline conditions: research/analysis through Q1 2013
- Study report to be completed Q2 2013
  - Baseline economic values and benefits
- Analysis of potential future operating scenarios
  - Input for draft and final license application

Temperature Criteria Assessment Meeting
Don Pedro Relicensing Study W&AR-14
November 16, 2012 - 1:00 p.m. to 4:00 p.m. – MID Offices
Call-In Number 866-994-6437 – Conference Code 5424697994

Live Meeting Link:

Join online meeting
https://meet.hdrinc.com/jenna.borovansky/3D64F0F5

First online meeting?

AGENDA

1. **Introductions**

2. **Purpose of Meeting: Report and Discuss Status of Study Plan Implementation**
   - **Original study plan**
   - **FERC Study Determination**
   - **Additional empirical evaluations proposed during April 2012 meeting**

3. **W&AR 14 Study Plan**
   - **Review relevant literature**
   - **Develop water temperature evaluation parameters**
   - **Relate baseline water temperature conditions to population**

4. **Additional Studies and Evaluations**
   - **Empirical studies proposed by Districts for implementation**
     - **Study 1: Local Adaptation of Temperature Tolerance of *O. mykiss* Juveniles in the Lower Tuolumne River**
     - **Study 2: Spatial distribution juvenile *O. mykiss* in response to temperature**
     - **Study 3(b): Influence of temperature on growth of Chinook salmon**
     - **Study 7: Influence of temperature on pre-spawning of Chinook salmon**
   - **Empirical studies proposed by Districts at April meeting, but not proposed for implementation at this time**
     - **Study 3(a): Influence of temperature on growth of O. mykiss**
     - **Study 4: Effect of temperature on condition/health of Chinook salmon**
     - **Study 5: Influence of temperature on location, movement, survival potential of *O. mykiss*.**
     - **Study 6: Influence of temperatures during the early Chinook salmon spawning period on egg survival.**
     - **Study 8: Chinook salmon production related to precedent temperature conditions**

5. **Next Steps and Study Schedule**

TURLOCK IRRIGATION DISTRICT & MODESTO IRRIGATION DISTRICT
DON PEDRO PROJECT FERC NO. 2299

Temperature Criteria Assessment
Water & Aquatic Resources Study #14 Update

November 2012

Eight studies identified by the Districts as potentially yielding empirical evidence on temperature effects in the Tuolumne River were discussed during the April 11, 2012 workshop on *W&AR 14 - Temperature Criteria Assessment Study*. During the workshop, questions were raised concerning data availability and utility to conduct the five studies considered "desktop" studies (Studies 2, 3, 6, 7 and 8) since they would involve evaluation of "existing" data. The studies and their status are briefly described in the following sections. Three additional studies also discussed during the workshop would require additional data collection and potentially Section 10 research permits from NMFS (Studies 1, 4 and 5). Subsequent to the workshop, the Districts have further evaluated the availability and utility of data to conduct the five "desktop" studies and have determined that data are sufficient to conduct all or part of three studies (2, 3 and 7), but are insufficient to conduct all or part of three studies (3, 6 and 8). Additionally, the Districts will proceed with proposed Study 1, but have determined that Studies 4 and 5 will not be pursued further. Further detail on each study is summarized below.

Study 1 - Local Adaptation of Temperature Tolerance of O. mykiss Juveniles in the Lower Tuolumne River

Objective
Determine the temperature tolerance of juvenile and subadult *O. mykiss* captured from the lower Tuolumne River (LTR) to assess any local adaptation to warmer temperatures occurring in the southern extent of *O. mykiss* range.

Status
The Districts have initiated discussions with the National Marine Fisheries Service ("NMFS") in order to obtain a Section 10 permit required to conduct this study. Supporting references for this study are available on the relicensing website with meeting materials.

Approach
Determine optimum and critical temperatures by challenging *O. mykiss* juveniles and subadults to increasing temperature conditions and observing oxygen consumption, following the methods of Parsons (2011)[1].

Speculation on the adaptability of anadromous salmonids to the various, potentially extreme temperature regimes encountered throughout their range suggests that *O. mykiss* in the southern

[1] Parsons, E.J.E. 2011. Cardiorespiratory physiology and temperature tolerance among populations of sockeye salmon (Oncorhynchus nerka). A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of graduate studies (Zoology) The University of British Columbia (Vancouver) August 2011.

extent of their range may be innately more tolerant of warmer temperature regimes than reported in the literature. The local adaptability of LTR *O. mykiss* would allow better performance at warmer temperatures than would be predicted based on studies of *O. mykiss* populations in the northern extent of the range. A determination that LTR *O. mykiss* are locally adapted to warmer temperatures would support discussions of reassessing optimum temperature thresholds (i.e., relative to EPA 2003)[2] that may be more appropriate for *O. mykiss* in the Central Valley stream system.

This study will evaluate if *O. mykiss* that occur in LTR are locally adapted to higher temperature tolerances that may better define site-specific temperature performance metrics. A case study of temperature tolerance among fishes is likely to prove extremely fruitful in addressing the more general and important question of animal resilience and adaptability to environmental change (Farrell 2009)[3]. Fishes generally have evolved around species-specific niches, living in almost every conceivable aquatic habitat and representing almost half of the earth's vertebrate species (Farrell 2009). Thus, it is expected that *O. mykiss* populations in different parts of the species range would show differences in physiological performance and in other biological traits that reflect adaptations to regional or more localized environmental conditions.

Methods

The Districts would follow methods described by Parsons (2011) and others to evaluate the capabilities of local *O. mykiss* to accommodate warmer temperatures. Specifically, Parsons (2011) studied the respiratory physiological basis for temperature tolerance in sockeye salmon and examined the overall hypothesis that each sockeye salmon population has adapted to meet their specific upriver migration conditions. Swimming respiratory performance was compared over a range of temperatures across wild, migrating adult sockeye salmon populations.

Fish evaluated per Parsons (2011) were tested in Brett-type swim tunnels. The first day (24-hour duration) of the Parsons (2011) study required placement of the fish into the swim tunnel to acclimate the fish to its new environment. The Districts have determined that swim tunnels can be used to measure the optimal temperature ("T_{opt}") and critical temperature ("T_{crit}") for fish 100 to 200 mm fork length ("FL"). The T_{opt} window, as defined by Parsons (2011) is *"the range in temperatures between the upper and lower T_p when maximum aerobic scope is maintained*". Aerobic scope--which is measured at a given temperature--is the observed difference or range between the maximum respiratory performance (i.e., maximum oxygen consumption) and resting respiratory performance (i.e., resting oxygen consumption) at that temperature. The T_p points are the pejus temperatures (pejus means getting worse); therefore, the T_p points are the temperatures where aerobic scope is getting worse (i.e., becomes smaller in width) (**Figure 1**). If a respiratory limitation exists for exercising salmonids during warming, increases in aerobic scope should cease once T_{opt} is reached (Farrell 2009). Ultimately, as warming approaches T_{opt}

[2] EPA. 2003. EPA Region 10 Guidance for Pacific Northwest State and Tribal Temperature Water Quality Standards. Available online at: http://www.epa.gov/region10/pdf/water/final_temperature_guidance_2003.pdf

[3] Farrell, A.P., Commentary – Environmental, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. The Journal of Experimental Biology 212, 3771-3780 Published by The Company of Biologists 2009 doi:10.1242/jeb.023671. Available online at: http://jeb.biologists.org/content/212/23/3771.full.pdf

the potential to increase maximum respiratory performance (oxygen consumption by exercising fish) fails to keep up with the required increase in respiratory rate in a resting fish (Farrell 2009). As a result, because aerobic scope does not increase above T_{opt} (Fig. 5), swimming effort either declines or stops (Farrell 2009).

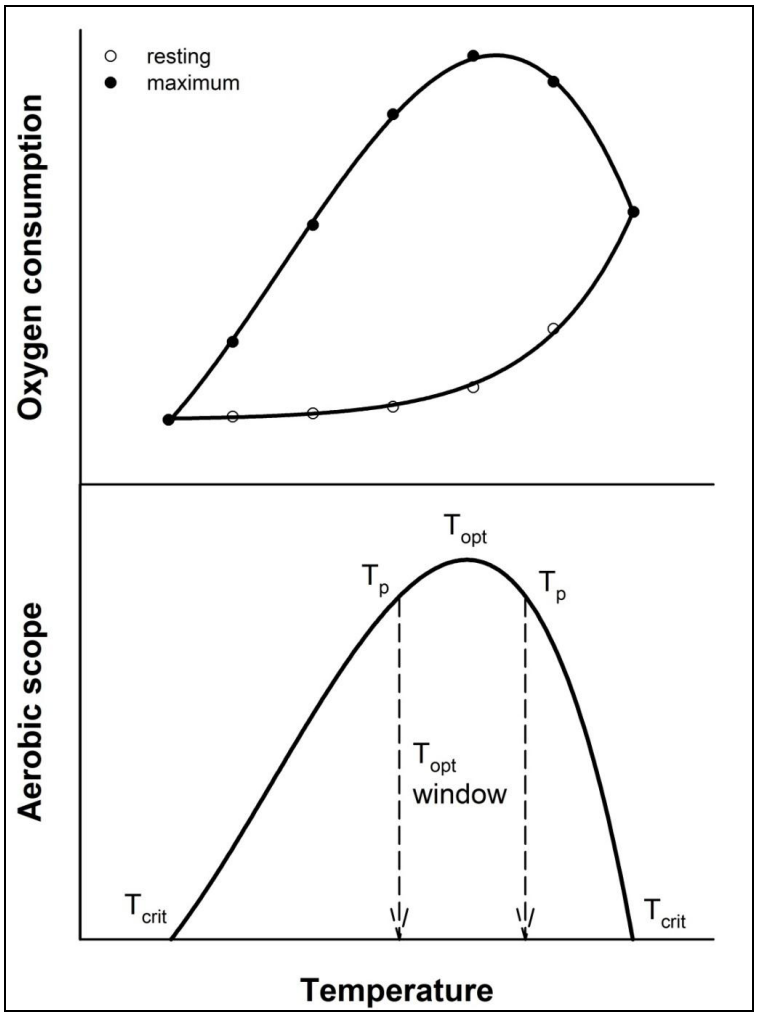

Figure 1. Schematic of resting and maximum oxygen consumption and aerobic scope. See text for details. T_{opt} = optimum temperature, T_p = pejus temperatures, T_{crit} = critical temperatures. The T_{opt} window corresponds to the range of temperatures between the upper and lower T_p (Source – Parsons 2011).

The primary goal of the swim tunnel experiment would be to determine the temperatures that bound the T_{opt} window for LTR *O. mykiss*, and how rapidly aerobic scope declines between the upper T_p and T_{crit}. These temperatures and the shape of the aerobic scope curve could then be compared with those of other *O. mykiss* populations to determine if there is evidence for local temperature adaption for LTR fish compared to more northern populations. These results could also be applied to assess relative responses to temperatures including potential variation in observed T_{opt} compared to EPA (2003) criteria, and relative performance between T_{opt} and Tcrit.

This assessment could help define more accurate criteria for evaluation of temperature tolerance for juvenile *O. mykiss* rearing.

Juvenile *O. mykiss* would be collected from the LTR during spring 2013, using seining or similar methods that would need to be approved by CDFG and NMFS in a 4d or Section 10 permit. Parsons (2011) indicates that between 25 and 30 individuals would be needed for the study. After collection in the field, individual fish would be placed into the Brett-type swim tube for a period of 24 hours to acclimate to the equipment. The experiment would be conducted during the second day once the fish have acclimated to the tube. Following completion of the experiment, fish would be held until they recover. Once recovered, fish would be released further downstream of the initial capture location. One fish per swim tube per use-day would be needed. Results of previous, similar tests conducted by the investigators indicate that the risk of mortality resulting from the test is extremely low.

The study will comprise of four tasks:

Task 1. - Planning and Logistics

- Apply for a 4d Permit or Section 10 Research Permit from NMFS to collect and evaluate up to 30 *O. mykiss* from the LTR
- Secure laboratory equipment and personnel to conduct field evaluations
- Identify source (method) and personnel to collect fish
- Finalize schedule based on permit process and personnel and equipment availability Set up stream-side facilities for tests

Various questions will need to be resolved per this task, including the method to be used to collect the test fish. Based on previous year's RST trapping results on the LTR, the likelihood is that sufficient numbers of *O. mykiss* will not be available from RST captures in a timely manner. Seining surveys of the lower Tuolumne River being conducted by FISHBIO for the Districts have shown seining can most likely be used to successfully capture juvenile *O. mykiss* during the spring to support this study. The abundance of seine-caught *O. mykiss* has been low, less than would be required for the study. However, the abundance of fish required, up to 30 over a 30 day period, would likely be accommodated with an increase in seining effort and an expansion in sampling locations. Other methods of acquiring test fish need to be considered, potentially in conjunction with RST trapped fish, to be used in an opportunistic manner. Ultimately, a Section 10 permit would dictate the allowable capture method. Additionally, the potential effect of the capture method on the ability to acclimate the fish and to conduct the study would need to be evaluated prior to requesting the NMFS permit.

The required test equipment would be available for lease from the University Of British Columbia ("UBC"). Alternative sources of equipment may be available locally and will be explored.

The permit application process has been started which will include informal discussions with NMFS staff to identify specific study details necessary to determine the potential utility of the study and associated take, as determined by NMFS. The application process includes confirmation of options for collecting fish, the details of holding, acclimating, testing, and post-

testing and how the tests are to be conducted at "streamside". Logistical requirements would be identified and accommodated based on the permit.

Task 2. - Fish collection and testing
The conduct of the testing is proposed to occur during spring of 2013 . The targeted species will be *O. mykiss*, ranging in size from ~ 100 mm to 200 mm (FL). Based on current studies being conducted by the Districts to collect *O. mykiss* for an age structure evaluation, collection of *O. mykiss* via angling has successfully yielded fish in this range (primarily between 150 and 200 mm FL. The results of the age structure survey should be included in an assessment of the timing of the study (e.g., if the targeted size can be obtained by angling earlier or later), of if the targeted size should be increased.

Task 3. - Data analysis and QA/QC
Data analysis and QA/QC would be conducted by UBC with and UCD personnel

Task 4. - Report
A report will be prepared and submitted to agencies and FERC.

Schedule
The Districts anticipate the schedule to complete the study proposal as follows, assuming appropriate permits are obtained from NMFS and CDFG by spring 2013:

Prepare implementation plan and other information necessary to prepare and submit Section 10 Permit to NMFS (Task 1) …….……October-November 2012

Schedule
Prepare for field survey……..…..… February-March 2013
Collect test fish and conduct field evaluations (Task 2)…… March-April 2013
Conduct QA/QC and data analysis (Task 3).....May-June 2013
Prepare and deliver final report (Task 4) ..... July-September 2013

Study 2. Spatial distribution juvenile O. mykiss in response to temperature

Objective
Identify temperature thresholds that define rearing temperature tolerances for juvenile *O. mykiss* rearing.

Status
Data availability and utility have been determined to be sufficient to support conduct of this study.

Approach
Compare intra and inter annual distribution of juvenile *O. mykiss* rearing relative to precedent temperature conditions in the LTR.

This study is intended to provide empirical evidence of the influence of temperature on juvenile *O. mykiss* rearing. The expectation is that *O. mykiss* will occupy areas as long as temperatures are tolerable for their survival. This study will compare occupancy with precedent temperature conditions to potentially bracket a threshold for rearing temperature tolerance. Inter-annual variations in longitudinal distribution of *O. mykiss* would be related to differences among temperature gradations. For example, when O. *mykiss* are present within a particular reach of the river subjected to one temperature regime but not there during a different (assume warmer) temperature regime, occupancy versus precedent temperature conditions would be considered an indicator of temperature tolerances. As such, temperature tolerances would be reflected in the response (occupied or vacated) to temperature longitudinally within and among years using existing information on spatial distribution of juvenile rearing and concurrent temperatures.

Existing data have been identified that includes survey results showing longitudinal distributions of *O. mykiss* and data will be subject of a QA/QC assessment to determine if they meet the needs of this study. Some of the results include fish density and some of the surveys occurred seasonally (during both the cool and warm seasons). An example of the data that could support this study is summarized by Stillwater Sciences (2012)[4] and is provided below as Table 1.

Table 1. Example of distribution data available to conduct this study (Stillwater 2012).

Table 3. Tuolumne River reference count snorkel survey locations (2001-2011) with number of *O. mykiss* observed.

Location	River Mile	2001		2002		2003		2004			2005	2006	2007		2008	2009	2010		2011	
		June	September	June	September	June	September	June	August	September	September	September	June	September	June	June	August	November	September	November
Riffle A3/A4	51.6								5											
Riffle A7	50.7	7	3	5	1	66	16	12	6	11	10	115	106	75	76	80	35	33	249	6
Riffle 1A	50.4								4											
Riffle 2	49.9	3	3	1	4	8	2	23	2	7	7	15	34	16	9	12	58	67	203	27
Riffle 3B	49.1	8	1	11	1	5	21	22	5	7	6	66	45	12	78	27	73	67	261	8
Riffle 4B	48.4								8											
Riffle 5B	48.0	4	2	3	X	6	10	11	15	6	36	54	92	10	21	11	26	16	149	41
Riffle 7	46.9	4	X	5	2	14	9	13	5	2	2	106	22	7	13	6	25	6	88	9
Riffle 9	46.4								3											
Riffle 13A–B	45.6	3	X	2	4	1	6	5	13	X	46	103	15	57	24	4	33	14	129	8
Riffle 21	42.9	2	3	1	X	X	6	5	9	7	15	32	10	10	11	X	8	2	33	8
Riffle 23B–C	42.3	X	X	X	X	1	1	X	1	X	14	27	5	7	X	2	9	10	52	32
Riffle 30B	38.5			X	X															
Riffle 31	38.1	X	X			X	X	X	X	X	1	21	12	4	X	X	1	X	10	2
Riffle 35A	37.0			X	X	X	X	X	X	X	2		X	X	X	X	X	X	3	X
Riffle 36A	36.7											4								
Riffle 37	36.2	X	X																	
Riffle 41A	35.3	X	X	X	X	X	X	X	X	X	X	X	2	X	X	X	X	3	2	6
Riffle 57–58	31.5	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	1
Total *O. mykiss*		**31**	**12**	**28**	**12**	**101**	**71**	**91**	**76**	**40**	**139**	**543**	**343**	**198**	**232**	**142**	**268**	**218**	**1179**	**148**

X = Locations that were sampled with no *O. mykiss* observed.

We would evaluate the spatial distribution of rearing *O. mykiss* relative to temperature precedent conditions to identify temperatures where occupancy continued and occupancy ended. The temperature regime where occupancy continued would be considered tolerable and the regime where occupancy ended would be considered intolerable.

[4] Stillwater Sciences (2012). Tuolumne River 2011 Oncorhynchus mykiss monitoring summary report. Final Report. Prepared for Turlock Irrigation District and Modesto Irrigation District.

Response to temperature in the form of occupancy will be identified within years as seasonal temperatures increase and occupancy either continues or ends, and inter annually where sites known to be occupied during the later, warmer period at least once during the 10 year period would be evaluated to determine if and under what precedent temperature conditions occupancy either continued or ended.

Where occupancy continued, the temperature regime would be considered tolerable, where occupancy was not observed, precedent temperature conditions would be considered intolerable. Temperature conditions would be characterized by several, acceptable metrics (used by other investigators to describe temperature conditions relative to fish tolerance), including 7DADM, daily max, mean daily, etc.

For example, if mean daily temperatures increased from May to September, from 15 to 20 ^{o}C and fish continue to occupy the site, the mean daily temperature of 20 ^{o}C would be considered tolerable (for the lifestage/age of fish size etc). If site A is occupied in year 1 when September temperatures are 19 ^{o}C but not in year 2 when September temperatures were 25 ^{o}C, 25 ^{o}C would be considered intolerable, 19 ^{o}C tolerable. The expectation is that the variation in temperature conditions within the 10 year period would be sufficient to broaden understanding of temperature tolerances within the LTR.

This study assumes that distribution is influenced by changes in longitudinal temperature distribution between winter and late-summer/ early-fall.

The following are hypotheses for this specific empirical study:

- Longitudinal temperature gradient along LTR changes from winter to summer as temperatures changes increase with distance downstream from La Grange Dam.
- Spatial distribution, observed as presence/absence and potentially as density, along the Tuolumne River should change between winter and summer corresponding to change in temperature. *O. mykiss* should occur in those areas where temperature meets criteria and should not where temperatures exceed criteria, between winter and late-summer, early-fall.
- Spatial distribution, observed as presence/absence and potentially as density, along the LTR should change between years corresponding to different temperature regimes. Observed relationships between temperature distribution and O mykiss distribution should allow identification of temperature tolerance or intolerance.
- Data are available under various annual temperature regimes that range from substantial change in temperature from winter to fall, to temperature conditions meeting identified thresholds (EPA 2003) throughout a substantially great portion of the Tuolumne River.

The data to be used in the evaluation has been identified and evaluated for applicability and quality and will be used to conduct an evaluation to determine the potential level of confidence that can be obtained from the evaluation.

Study 3. Influence of temperature on growth of O. mykiss and Chinook salmon

Objective
Evaluate influence of temperature on growth of O. mykiss and Chinook salmon juveniles in the LTR by comparing growth observed in the LTR with that reported in the literature and, in particular, observed in other Central Valley streams supporting *O. mykiss* and Chinook salmon populations.

Status
Evaluation of the availability and utility of data to support this study has shown that data are not available to conduct an evaluation of the observed influence of temperature on growth of *O. mykiss*. Data are available and suitable for conducting an evaluation of observed temperature influences on growth of fall-run Chinook salmon.

Approach
Compare observed size at time/age, interpreted as growth, of *O. mykiss* and Chinook salmon in the LTR with expected growth based on literature and growth rates observed/reported in other, similar waters. Relate temperature regime associated with observed growth in the LTR to identify those temperature conditions that either support or do not support expected growth.

This study would evaluate growth of *O. mykiss* and fall-run Chinook salmon in the LTR as a function of precedent temperature conditions. Growth would be evaluated by comparing observed growth in the LTR with expected growth to be defined based on the literature or observations from other similar watersheds, including the Merced, Stanislaus, Mokelumne, and American rivers. The size at time, to be estimated based on timing of spawning and emergence, (as data are available), would be contrasted with reported, acceptable or expected size at time.

Concern has been expressed that Chinook salmon growth in the LTR is too slow, potentially delaying Chinook salmon from reaching a larger, smolt-sized fish in time to successfully emigrate. For example, growth would be considered as expected if the majority of fall-run Chinook salmon achieve 70-90 mm FL by end of April and essentially all fall-run Chinook salmon have the opportunity to achieve smolt size by the end of May. By tracking RST size composition from the earliest migrating juvenile Chinook salmon, a trend in growth can be identified and the timing and cumulative composition of emigrating smolt-sized fish can be determined and contrasted with the precedent temperature regime to evaluate the effects on Chinook salmon growth.

Data on size of *O. mykiss* at time was to be derived from snorkel surveys conducted in the LTR during the previous 10 years or so, and was to be compared with an expected size at time (defined from literature or observations made in similar waters). However, the data that were to be used were not of sufficient detail to allow such an evaluation. Snorkel-derived data were recorded in size bins that did not adequately identify the size of *O. mykiss* to allow comparisons of growth among streams or even over time in the LTR. Size bins were at least 50 mm wide and routinely the data were reported as fish less than or greater than 150 mm in length. As such, the Districts determined that the data would not support the *O. mykiss* growth evaluation component of this study.

This study will evaluate growth of Chinook salmon as described above.

Study 4. Effect of temperature observed as changes in condition/health of Chinook salmon

Objective
This study would evaluate the influence of the temperature regime of the Tuolumne River on Chinook salmon survival potential, measured as specific temperature-related affects to health and condition of smolt or smolt-sized Chinook salmon. The study would evaluate quality of Chinook salmon smolt rearing in the Tuolumne River using methods previously applied by CDFG (Rich and Loudermilk 1991) and USFWS (Nichols and Foote 2002) to assess Chinook salmon condition in the San Joaquin River system
Status: The Districts do not plan to pursue this study. The Districts study lead discussed proposed study goals with Scott Foote, Director of the California-Nevada Fish Health Laboratory. Dr. Foote provided information regarding his experience with Chinook salmon smolt development in the Central Valley, including San Joaquin River tributaries relative to influences of temperature on smolt quality. Dr. Foote stated that although temperature can affect smoltification and in some instances reverse the smolting process, the ability to separate a variety of other factors that can influence smolt quality from those resulting from temperature would not be possible in the wild. Parameters of smolt quality, including hormone levels, lipid content, etc can vary within an individual within short periods, potentially varying simply due to ontogenetic factors as well as exogenous factors, and these variations are normal in smolting Chinook salmon, and as such are not necessarily indicative of stressors or adverse effects. Based on these discussions, the Districts are not planning on pursuing the study.

Study 5. Influence of temperature on location, movement, survival potential of O. mykiss.

Objective
Acoustic tagging *O. mykiss* during early summer in various locations within the LTR with various temperature expectations and monitor movement and survival to emigration.

Status
The Districts will not pursue implementation of this study. Although the Districts believe that the study would provide important information on LTR *O. mykiss* life history including potential responses of *O. mykiss* to various temperature conditions during rearing and migration within the LTR, the study would not be expected to provide empirical information informative to FERC for three years or more. Additionally, the scope of the study is well beyond temperature, and its greatest value would likely be informing the larger fish management community on a variety of currently unknown aspects of steelhead ecology in the San Joaquin River and Central Valley, which ultimately would require substantial logistic and technical support, and is beyond the scope of this relicensing.

Study 6. Influence of temperatures during the early Chinook salmon spawning period on egg survival.

Objective
Identify the relationship between temperature and egg-fry survival in the LTR. Study would evaluate the influence of observed temperature conditions during spawning on Chinook salmon spawning (egg to emergence survival).

Status
The Districts will not to pursue implementation of this study. The Districts determined that data required to conduct this study are not available. Data on emergence of Chinook salmon fry from redds within the LTR are available, but those data are not associated with temperature conditions, were not complete, or were too few to allow evaluation of influences of temperature on redd survival.

Study 7. Influence of temperature on pre-spawning mortality of Chinook salmon

Objective
Identify adult Chinook salmon response to typically warmer temperatures occurring in the LTR in the early portion of the spawning period. Evaluation of inner inter-annual timing of spawning will be compared with temperatures during early spawning period using redd surveys or carcass survey results to identify temporal distribution of early spawning, and pre-spawning mortality, potentially measured as egg retention during carcass surveys.

Status
The Districts have reviewed the data available for this study and have determined that there are data available of sufficient quality to conduct the study.

Study 8 - Chinook salmon production related to precedent temperature conditions

Objective
Identify influence of cumulative temperature regime, from spawning through emigration, on Chinook salmon emigrating population (e.g., abundance, composition, timing, survival-per-spawner). Evaluate estimated Chinook salmon production relative to temperature conditions during spawning.

Status
The Districts have decided not to pursue implementation of this study. The Districts gathered and reviewed data on estimated production of juvenile Chinook salmon obtained from RST monitoring during recent years. These data included estimates of production of emigrating juvenile Chinook salmon per female spawner. Additionally, precedent temperature conditions were characterized as accumulated temperature units ("ATU") from spawning through emigration to determine if ATU was related to estimated production. The Districts determined that the estimated production, a key variable in the evaluation, did not have the level of confidence that would allow distinction among the years when emigration was estimated.

From: Borovansky, Jenna
Sent: Thursday, November 15, 2012 7:04 PM
To: Staples, Rose; Asay, Lynette; Barnes, James; Barnes, Peter; Beniamine Beronia; Blake, Martin; Bond, Jack; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug; Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Damin Nicole; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fargo, James; Ferranti, Annee; Ferrari, Chandra; Fety, Lauren; Findley, Timothy; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackson, Zac; Jauregui, Julia; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Loy, Carin; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; McDevitt, Ray; McDonnell, Marty; McLain, Jeffrey; Mein Janis; Mills, John; Minami Amber; Monheit, Susan; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Schutte, Allison; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Stapley, Garth; Steindorf, Dave; Steiner, Dan; Stender, John; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne; ulibarri@standard.edu
Subject: MEETING TIME CHANGE: Don Pedro Relicensing WAR14 Meeting - Start at 9am
Attachments: DP_TempCriteriaAssessmtMtg_Nov16_AGENDA_Revised.doc

Importance: High

The WAR-14 Temperature Criteria Study Meeting scheduled for 1-4pm on Friday (11/15) will be moved to **begin at 9am on Friday (11/15).**

The same phone number and live meeting link will be used for the meeting.

Mape's Ranch and Lyons' Investments
10555 Maze Road Modesto, CA 95358
Office: (209) 522-1762 FAX: (209) 522-7871

November 16, 2012

John J. Devine, PE
HDR
970 Baxter Blvd., Suite 301
Portland, ME 04103-5346

Duane Paul
Cardno Entrix
701 University Ave., Suite 200
Sacramento, CA 95825

Steve Pavich
Cardno Entrix
701 University Ave., Suite 200
Sacramento, CA 95825

Re: Socioeconomics Study Meeting

Dear Steve, Duane, and John:

I wanted to personally thank you for the opportunity to discuss and provide input regarding the Socioeconomic Study at the FERC workshop held in Modesto at MID on 11-09-12.

I would again encourage you from an agricultural perspective to contact organizations such as; **A) Financial** – Yosemite Farm Credit, Joe Mauzy and Steve Mizuno, 1213 – 13th Street, Modesto, CA 95354, (209) 527-1900; American Ag Credit, Ted Reimers, 3201 W. Monte Vista Ave., Turlock, CA 95380, (209) 667-5101. **B) Farm Appraisal Values** – Way & Associates, Tina & Bruce Way, 4248 Tully Road, Hughson, CA 95326, (209) 883-2796; Edwards, Lein & Toso, Randy Edwards, 8408 N. Lander Ave., Hilmar, CA 95324, (209) 634-9484. **C) Sales** – Lane Menezes, PMZ Real Estate, 1200 E. Orangeburg Ave., Suite 201, Modesto, CA 95350, (209) 527-5640. **D) Farmers** – Farm Bureau, Wayne Zipser, (209) 522-7278; Western United Dairymen, Mike Marsh, (209) 527-6453 to line up some on the ground dairymen, farmers, and ranchers to expand your input and validate your information.

I was personally surprised and disappointed by the lack of attendance by organizations and groups such as the Chamber of Commerce (Modesto, Turlock, Ceres), Manufacturers Council, Stanislaus Economic Development & Workshop Alliance, Stanislaus County Taxpayers Association, Latino Community Roundtable, Senior and Low Income advocates, organized labor, BIA, Stanislaus County, cities of Ceres, Hughson, Turlock, Denair, Waterford, Hilmar, etc. The only elected leader in attendance was Tom Van Groningen from MID, along with Tom Orvis from Farm Bureau, several farmers, several ratepayers, and various staff from MID, TID, City of Modesto, and one representative from Tuolumne River Trust.

It is very apparent that the outreach to the community has seriously missed the mark as demonstrated by such low attendance at this workshop/meeting. Statements from MID and TID staff that they sent out emails, put a notice in the paper, etc. appears to have failed in conveying to the local organizations, the public, and ratepayers how important this workshop was.

Suggestions

1. Personal Contact - Staff needs to make personal contact with the groups that represent these large blocks of ratepayers and personally encourage their engagement and input. Also, staff should request and follow up with senior leadership and board members to personally reach out to the community and encourage engagement and input.

2. Workshop – Hold one evening meeting so that ratepayers and interested parties can attend.

3. Retention – Perhaps contract with a local professional outreach firm to inform, encourage and follow up with the community and its many organizations. Base a contract on performance.

I would hope that both MID and TID's staff were disappointed by the low turnout. As a former MID board member, I would certainly question the outreach plan/effort and the need to change the outreach effort/plan.

The FERC workshop process is very important and the Socioeconomic Study workshop is an area that would benefit greatly from input from many of these organizations.

Sincerely,

William (Bill) J. Lyons, Jr.

Cc: MID Board
TID Board
MID Staff
TID Staff
Modesto Chamber of Commerce
Turlock Chamber of Commerce
Ceres Chamber of Commerce
Manufacturers Council
Latino Community Roundtable
Stanislaus Alliance, Bill Bassitt
Wayne Zipser, Farm Bureau
Ted Reimers, American Ag Credit
Tina Way, Way & Associates
Randy Edwards, Edwards, Lein & Tosso
Mike Marsh, Western United Dairymen
LTF Members
Chris Vierra, Ceres Mayor
Ramon Bawanan, Hughson Mayor
John Lazar, Turlock Mayor
Garrad Marsh, Modesto Mayor

WJL/crc

From: Staples, Rose
Sent: Friday, November 30, 2012 5:20 PM
To: 'Timothy D. Findley'
Subject: RE: Don Pedro Socioeconomics Study and Temp Criteria Assessment Meetings Materials Uploaded to Relicensing Website

A list of the Action Items from the meeting is being prepared—along with information on significant areas of discussion--and will eventually be forwarded by email and posted on the relicensing website; but I don't have a date yet when this will be available.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

From: Timothy D. Findley [mailto:TFindley@hansonbridgett.com]
Sent: Thursday, November 29, 2012 4:49 PM
To: Staples, Rose
Subject: RE: Don Pedro Socioeconomics Study and Temp Criteria Assessment Meetings Materials Uploaded to Relicensing Website

Great. Thanks, Rose. I appreciate it.

From: Staples, Rose [mailto:Rose.Staples@hdrinc.com]
Sent: Thursday, November 29, 2012 1:48 PM
To: Timothy D. Findley
Subject: RE: Don Pedro Socioeconomics Study and Temp Criteria Assessment Meetings Materials Uploaded to Relicensing Website

Let me check further into this and get back to you hopefully tomorrow, if not sooner. Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

From: Timothy D. Findley [mailto:TFindley@hansonbridgett.com]
Sent: Thursday, November 29, 2012 1:32 PM
To: Staples, Rose
Subject: RE: Don Pedro Socioeconomics Study and Temp Criteria Assessment Meetings Materials Uploaded to Relicensing Website

Hi Rose.

I hadn't heard back from you, so I'm just following up again regarding whether there were any official meeting notes for the 11/9 socioeconomic meeting.

Thanks,

-Tim

From: Timothy D. Findley
Sent: Friday, November 09, 2012 11:55 AM
To: 'Staples, Rose'
Subject: RE: Don Pedro Socioeconomics Study and Temp Criteria Assessment Meetings Materials Uploaded to Relicensing Website

Hi Rose.

Unfortunately, I wasn't available to attend or call in to today's socioeconomic study update meeting. Will there be official meeting notes released for the meeting?

Best,

-Tim

Timothy D. Findley
Attorney
Hanson Bridgett LLP
(415) 995-5879 Direct
(415) 995-3585 Fax
tfindley@hansonbridgett.com

From: Staples, Rose [mailto:Rose.Staples@hdrinc.com]
Sent: Thursday, November 08, 2012 4:35 PM
To: Asay, Lynette; Barnes, James; Barnes, Peter; Beniamine Beronia; Blake, Martin; Bond, Jack; Borovansky, Jenna; Boucher, Allison; Bowes, Stephen; Bowman, Art; Brenneman, Beth; Brewer, Doug;

Buckley, John; Buckley, Mark; Burt, Charles; Byrd, Tim; Cadagan, Jerry; Carlin, Michael; Charles, Cindy; Colvin, Tim; Costa, Jan; Cowan, Jeffrey; Cox, Stanley Rob; Cranston, Peggy; Cremeen, Rebecca; Damin Nicole; Day, Kevin; Day, P; Denean; Derwin, Maryann Moise; Devine, John; Donaldson, Milford Wayne; Dowd, Maggie; Drekmeier, Peter; Edmondson, Steve; Eicher, James; Fargo, James; Ferranti, Annee; Ferrari, Chandra; Fety, Lauren; Timothy D. Findley; Fuller, Reba; Furman, Donn W; Ganteinbein, Julie; Giglio, Deborah; Gorman, Elaine; Grader, Zeke; Gutierrez, Monica; Hackamack, Robert; Hastreiter, James; Hatch, Jenny; Hayat, Zahra; Hayden, Ann; Hellam, Anita; Heyne, Tim; Holley, Thomas; Holm, Lisa; Horn, Jeff; Horn, Timi; Hudelson, Bill; Hughes, Noah; Hughes, Robert; Hume, Noah; Jackson, Zac; Jauregui, Julia; Jennings, William; Jensen, Art; Jensen, Laura; Johannis, Mary; Johnson, Brian; Justin; Keating, Janice; Kempton, Kathryn; Kinney, Teresa; Koepele, Patrick; Kordella, Lesley; Le, Bao; Lein, Joseph; Levin, Ellen; Lewis, Reggie; Linkard, David; Loy, Carin; Lwenya, Roselynn; Lyons, Bill; Madden, Dan; Manji, Annie; Marko, Paul; Marshall, Mike; Martin, Michael; Martin, Ramon; Mathiesen, Lloyd; McDaniel, Dan; Ray E. McDevitt; McDonnell, Marty; Mein Janis; Mills, John; Minami Amber; Monheit, Susan; Morningstar Pope, Rhonda; Motola, Mary; Murphey, Gretchen; Murray, Shana; O'Brien, Jennifer; Orvis, Tom; Ott, Bob; Ott, Chris; Paul, Duane; Pavich, Steve; Pinhey, Nick; Pool, Richard; Porter, Ruth; Powell, Melissa; Puccini, Stephen; Raeder, Jessie; Ramirez, Tim; Rea, Maria; Reed, Rhonda; Richardson, Kevin; Ridenour, Jim; Robbins, Royal; Romano, David O; Roos-Collins, Richard; Roseman, Jesse; Rothert, Steve; Sandkulla, Nicole; Saunders, Jenan; Allison C. Schutte; Sears, William; Shakal, Sarah; Shipley, Robert; Shumway, Vern; Shutes, Chris; Sill, Todd; Slay, Ron; Smith, Jim; Staples, Rose; Stapley, Garth; Steindorf, Dave; Steiner, Dan; Stender, John; Stone, Vicki; Stork, Ron; Stratton, Susan; Taylor, Mary Jane; Terpstra, Thomas; TeVelde, George; Thompson, Larry; Vasquez, Sandy; Verkuil, Colette; Vierra, Chris; Wantuck, Richard; Welch, Steve; Wesselman, Eric; Wheeler, Dan; Wheeler, Dave; Wheeler, Douglas; White, David K; Wilcox, Scott; Williamson, Harry; Willy, Allison; Wilson, Bryan; Winchell, Frank; Wooster, John; Workman, Michelle; Yoshiyama, Ron; Zipser, Wayne
Subject: Don Pedro Socioeconomics Study and Temp Criteria Assessment Meetings Materials Uploaded to Relicensing Website

I have uploaded to the Don Pedro relicensing website (www.donpedro-relicensing.com) today, ***as attachments to the Meeting calendar for their respective meeting dates***, the following meeting materials:

Socioeconomics Study (W&AR-15) Progress Update Meeting, Friday, November 9
PowerPoint Presentation being used at the meeting

Temperature Criteria Assessment (W&AR-14) Meeting, Friday afternoon, November 16
Agenda updated with the LIVE MEETING link
Study Update

If you are not able to access or download these documents, please do contact me at rose.staples@hdrinc.com.

Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com | hdrinc.com

From: Staples, Rose
Sent: Thursday, December 06, 2012 5:26 PM
To: 'Amerine, Bill'; 'Asay, Lynette'; 'Barnes, James'; 'Barnes, Peter'; 'Beniamine Beronia'; 'Blake, Martin'; 'Bond, Jack'; Borovansky, Jenna; 'Boucher, Allison'; 'Bowes, Stephen'; 'Bowman, Art'; 'Brenneman, Beth'; 'Brewer, Doug'; 'Buckley, John'; 'Buckley, Mark'; 'Burt, Charles'; 'Byrd, Tim'; 'Cadagan, Jerry'; 'Carlin, Michael'; 'Charles, Cindy'; 'Colvin, Tim'; 'Costa, Jan'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob'; 'Cranston, Peggy'; 'Cremeen, Rebecca'; 'Damin Nicole'; 'Day, Kevin'; 'Day, P'; 'Denean'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne'; 'Dowd, Maggie'; 'Drekmeier, Peter'; 'Edmondson, Steve'; 'Eicher, James'; 'Fargo, James'; 'Ferranti, Annee'; 'Ferrari, Chandra'; 'Fety, Lauren'; 'Findley, Timothy'; 'Fleming, Mike'; 'Fuller, Reba'; 'Furman, Donn W'; 'Ganteinbein, Julie'; 'Giglio, Deborah'; 'Gorman, Elaine'; 'Grader, Zeke'; 'Gutierrez, Monica'; 'Hackamack, Robert'; 'Hastreiter, James'; 'Hatch, Jenny'; 'Hayat, Zahra'; 'Hayden, Ann'; 'Hellam, Anita'; 'Heyne, Tim'; 'Holley, Thomas'; 'Holm, Lisa'; 'Horn, Jeff'; 'Horn, Timi'; 'Hudelson, Bill'; 'Hughes, Noah'; 'Hughes, Robert'; 'Hume, Noah'; 'Jackson, Zac'; 'Jauregui, Julia'; 'Jennings, William'; 'Jensen, Art'; 'Jensen, Laura'; 'Johannis, Mary'; 'Johnson, Brian'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn'; 'Kinney, Teresa'; 'Koepele, Patrick'; 'Kordella, Lesley'; Le, Bao; 'Lein, Joseph'; 'Levin, Ellen'; 'Lewis, Reggie'; 'Linkard, David'; Loy, Carin; 'Lwenya, Roselynn'; 'Lyons, Bill'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul'; 'Marshall, Mike'; 'Martin, Michael'; 'Martin, Ramon'; 'Mathiesen, Lloyd'; 'McDaniel, Dan'; 'McDevitt, Ray'; 'McDonnell, Marty'; 'Mein Janis'; 'Mills, John'; 'Minami Amber'; 'Monheit, Susan'; 'Morningstar Pope, Rhonda'; 'Motola, Mary'; 'Murphey, Gretchen'; 'Murray, Shana'; 'O'Brien, Jennifer'; 'Orvis, Tom'; 'Ott, Bob'; 'Ott, Chris'; 'Paul, Duane'; 'Pavich, Steve'; 'Pinhey, Nick'; 'Pool, Richard'; 'Porter, Ruth'; 'Powell, Melissa'; 'Puccini, Stephen'; 'Raeder, Jessie'; 'Ramirez, Tim'; 'Rea, Maria'; 'Reed, Rhonda'; 'Richardson, Kevin'; 'Ridenour, Jim'; 'Riggs T'; 'Robbins, Royal'; 'Romano, David O'; 'Roos-Collins, Richard'; 'Roseman, Jesse'; 'Rothert, Steve'; 'Sandkulla, Nicole'; 'Saunders, Jenan'; 'Schutte, Allison'; 'Sears, William'; 'Shakal, Sarah'; 'Shipley, Robert'; 'Shumway, Vern'; 'Shutes, Chris'; 'Sill, Todd'; 'Slay, Ron'; 'Smith, Jim'; Staples, Rose; 'Stapley, Garth'; 'Steindorf, Dave'; 'Steiner, Dan'; 'Stender, John'; 'Stone, Vicki'; 'Stork, Ron'; 'Stratton, Susan'; 'Taylor, Mary Jane'; 'Terpstra, Thomas'; 'TeVelde, George'; 'Thompson, Larry'; 'Ulibarri, Nicola'; 'Vasquez, Sandy'; 'Verkuil, Colette'; 'Vierra, Chris'; 'Wantuck, Richard'; 'Welch, Steve'; 'Wesselman, Eric'; 'Wheeler, Dan'; 'Wheeler, Dave'; 'Wheeler, Douglas'; 'White, David K'; 'Wilcox, Scott'; 'Williamson, Harry'; 'Willy, Allison'; 'Wilson, Bryan'; 'Winchell, Frank'; 'Wooster, John'; 'Workman, Michelle'; 'Yoshiyama, Ron'; 'Zipser, Wayne'
Subject: Don Pedro Initial Study Report 2-Day Meeting AGENDA January 30-31, 2013

We have filed with FERC today, on behalf of the Districts, the AGENDA for the upcoming January 30-31, 2013 Initial Study Report Meeting, to be held at the MID Offices in Modesto. A copy of this AGENDA will also be uploaded to the Don Pedro relicensing website www.donpedro-relicensing.com, both as an attachment to the MEETING DATE and as an Announcement under the INTRODUCTION tab.

DON PEDRO PROJECT RELICENSING
FERC PROJECT NO. 2299

DAY 1

Initial Study Report Meeting (Day 1)
Wednesday January 30, 2013 8:00 am – 5:15 pm
Meeting Location: MID Offices, Modesto

Time	Topic		Lead By
Water & Aquatic Resources Study Plans			
8:00	**Opening** – Agenda Review, Purpose of Meeting		
8:15	W&AR-15	Socioeconomics Study	S. Pavich/D. Paul
8:40	W&AR-01	Water Quality Assessment	C.Loy
9:05	W&AR-02	Project Operations/Water Balance Model	D. Steiner
9:30	W&AR-03	Reservoir Temperature Model	S. Lowe
9:55	W&AR-04	Spawning Gravel Study	J. Stillman
Break – 10:20			
10:35	W&AR-05	Salmonid Populations Information Integration	N. Hume/S. Wilcox
11:00	W&AR-06	Tuolumne River Chinook Salmon Population	N. Hume
11:25	W&AR-10	*Onchorhynchus mykiss* Population Study	N. Hume
Lunch Break – 11:50			
12:50	W&AR-07	Predation Study	A. Fuller
1:15	W&AR-08	Salmonid Redd Mapping	J. Guigard
1:40	W&AR-11	Chinook Salmon Otolith Study	M. Singer
2:05	W&AR-12	*Onchorhynchus mykiss* Habitat Assessment	D. Halligan
2:30	W&AR-13	La Grange Reservoir Fish Assemblage and	B.Snider
Break – 2:55			
3:10	W&AR-14	Temperature Criteria Assessment	B. Snider
3:35	W&AR-17	Don Pedro Reservoir Fish Population Study	A. Fuller/B.Snider
4:00	W&AR-18	Sturgeon Study	D. Haligan
4:25	W&AR-19	Riparian Information Study	A. Merrill
4:50	W&AR-20	*O.mykiss* Scale & Age	D. Halligan

Time	Topic		Lead By
	Water & Aquatic Resources Study Plans		
5:15		**Adjournment**	

DAY 2

Initial Study Report Meeting (Day 2)
Thursday January 31, 2013 8:00 am – 4:25 pm
Meeting Location: MID Offices, Modesto

Time	Topic		Lead By
	Cultural Resources Studies		
8:00	**Opening** – Agenda Review, Purpose of Meeting		
8:15	CR-01	Historic Properties Study	D. Risse
8:40	CR-02	Native American Traditional Cultural Properties	D. Risse
	Terrestrial Resources Studies		
9:05	TR-01	Special-Status Plants	R. Kent/D. Malkin
9:30	TR-02	ESA- and CESA-Listed Plants Study	R. Kent/D. Malkin
9:55	TR-03	Wetland Habitats Associated with Don Pedro	G. Bailey/D. Malkin
Break – 10:20			
10:35	TR-04	Noxious Weed Survey	R. Kent/D. Malkin
11:00	TR-05	ESA-Listed Wildlife - Valley Elderberry Longhorn	R. Kent/D. Malkin
11:25	TR-06	Special-Status Amphibians-Aquatic Reptiles	S. Imholt/D. Malkin
11:50	TR-07	ESA-Listed Amphibians - California Red-Legged	S. Imholt/D. Malkin
Lunch Break – 12:15			
1:15	TR-08	ESA-List Amphibians - California Tiger Salamander	S. Imholt/D. Malkin
1:40	TR-09	Special-Status Bats	J. Tortosa/D. Malkin
2:05	TR-10	Bald Eagle Study	J. Tortosa/D. Malkin
	Recreation Resources Studies		
2:30	RR-01	Recreation Facility and Public Accessibility	N. Craig

Time	Topic		Lead By
	Cultural Resources Studies		
Break – 2:55			
3:10	RR-02	Whitewater Boating Take Out Improvement	N. Craig
3:35	RR-03	Lower Tuolumne River Boatable Flow Study	N. Craig
4:00	RR-04	Visual Quality Study	N. Craig
4:25		**Adjournment**	

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

From: Staples, Rose
Sent: Wednesday, December 12, 2012 5:44 PM
To: 'Alves, Jim'; 'Amerine, Bill'; 'Anderson, Craig'; 'Asay, Lynette'; 'Barnes, James'; 'Barnes, Peter'; 'Beniamine Beronia'; 'Blake, Martin'; 'Bond, Jack'; Borovansky, Jenna; 'Boucher, Allison'; 'Bowes, Stephen'; 'Bowman, Art'; 'Brenneman, Beth'; 'Brewer, Doug'; 'Buckley, John'; 'Buckley, Mark'; 'Burt, Charles'; 'Byrd, Tim'; 'Cadagan, Jerry'; 'Carlin, Michael'; 'Charles, Cindy'; 'Colvin, Tim'; 'Costa, Jan'; 'Cowan, Jeffrey'; 'Cox, Stanley Rob'; 'Cranston, Peggy'; 'Cremeen, Rebecca'; 'Damin Nicole'; 'Day, Kevin'; 'Day, P'; 'Denean'; 'Derwin, Maryann Moise'; Devine, John; 'Donaldson, Milford Wayne'; 'Dowd, Maggie'; 'Drekmeier, Peter'; 'Edmondson, Steve'; 'Eicher, James'; 'Fargo, James'; 'Ferranti, Annee'; 'Ferrari, Chandra'; 'Fety, Lauren'; 'Findley, Timothy'; 'Fleming, Mike'; 'Fuller, Reba'; 'Furman, Donn W'; 'Ganteinbein, Julie'; 'Giglio, Deborah'; 'Gorman, Elaine'; 'Grader, Zeke'; 'Gutierrez, Monica'; 'Hackamack, Robert'; 'Hastreiter, James'; 'Hatch, Jenny'; 'Hayat, Zahra'; 'Hayden, Ann'; 'Hellam, Anita'; 'Heyne, Tim'; 'Holley, Thomas'; 'Holm, Lisa'; 'Horn, Jeff'; 'Horn, Timi'; 'Hudelson, Bill'; 'Hughes, Noah'; 'Hughes, Robert'; 'Hume, Noah'; 'Jackson, Zac'; 'Jauregui, Julia'; 'Jennings, William'; 'Jensen, Art'; 'Jensen, Laura'; 'Johannis, Mary'; 'Johnson, Brian'; 'Justin'; 'Keating, Janice'; 'Kempton, Kathryn'; 'Kinney, Teresa'; 'Koepele, Patrick'; 'Kordella, Lesley'; Le, Bao; 'Lein, Joseph'; 'Levin, Ellen'; 'Lewis, Reggie'; 'Linkard, David'; Loy, Carin; 'Lwenya, Roselynn'; 'Lyons, Bill'; 'Madden, Dan'; 'Manji, Annie'; 'Marko, Paul'; 'Marshall, Mike'; 'Martin, Michael'; 'Martin, Ramon'; 'Mathiesen, Lloyd'; 'McDaniel, Dan'; 'McDevitt, Ray'; 'McDonnell, Marty'; 'Mein Janis'; 'Mills, John'; 'Minami Amber'; 'Monheit, Susan'; 'Morningstar Pope, Rhonda'; 'Motola, Mary'; 'Murphey, Gretchen'; 'Murray, Shana'; 'O'Brien, Jennifer'; 'Orvis, Tom'; 'Ott, Bob'; 'Ott, Chris'; 'Paul, Duane'; 'Pavich, Steve'; 'Pinhey, Nick'; 'Pool, Richard'; 'Porter, Ruth'; 'Powell, Melissa'; 'Puccini, Stephen'; 'Raeder, Jessie'; 'Ramirez, Tim'; 'Rea, Maria'; 'Reed, Rhonda'; 'Richardson, Kevin'; 'Ridenour, Jim'; 'Riggs T'; 'Robbins, Royal'; 'Romano, David O'; 'Roos-Collins, Richard'; 'Roseman, Jesse'; 'Rothert, Steve'; 'Sandkulla, Nicole'; 'Saunders, Jenan'; 'Schutte, Allison'; 'Sears, William'; 'Shakal, Sarah'; 'Shipley, Robert'; 'Shumway, Vern'; 'Shutes, Chris'; 'Sill, Todd'; 'Slay, Ron'; 'Smith, Jim'; Staples, Rose; 'Stapley, Garth'; 'Steindorf, Dave'; 'Steiner, Dan'; 'Stender, John'; 'Stone, Vicki'; 'Stork, Ron'; 'Stratton, Susan'; 'Taylor, Mary Jane'; 'Terpstra, Thomas'; 'TeVelde, George'; 'Thompson, Larry'; 'Ulibarri, Nicola'; 'Vasquez, Sandy'; 'Verkuil, Colette'; 'Vierra, Chris'; 'Wantuck, Richard'; 'Welch, Steve'; 'Wesselman, Eric'; 'Wheeler, Dan'; 'Wheeler, Dave'; 'Wheeler, Douglas'; 'White, David K'; 'Wilcox, Scott'; 'Williamson, Harry'; 'Willy, Allison'; 'Wilson, Bryan'; 'Winchell, Frank'; 'Wooster, John'; 'Workman, Michelle'; 'Yoshiyama, Ron'; 'Zipser, Wayne'
Subject: Don Pedro W-AR-14 Temp Criteria Assessment Draft Nov 16 Update Meeting Notes for Review
Attachments: P-2299_W-AR-14_Nov 16_UpdateMtgDrftNotes_121212.docx

Attached please find the DRAFT Meeting Notes from the Don Pedro Project Relicensing Water & Aquatic Resources ("W&AR") Study No. 14: Temperature Criteria Assessment update meeting held on

November 16, 2012. The materials listed under ***Action*** Items for uploading to the relicensing website www.donpedro-relicensing.com are in the process of being uploaded this week.

While Study No. 14 and the notes resulting from its update meetings do not fall under the Workshop Consultation Protocol, we wanted to give relicensing participants the opportunity to review the notes before we file them with FERC. Could you please provide any comments on the draft notes to me at rose.staples@hdrinc.com by no later than Wednesday, December 19. Thank you.

ROSE STAPLES
CAP-OM

HDR Engineering, Inc.
Executive Assistant, Hydropower Services

970 Baxter Boulevard, Suite 301 | Portland, ME 04103
207.239.3857 | f: 207.775.1742
rose.staples@hdrinc.com| hdrinc.com

Don Pedro Project Relicensing
W&AR-14 Temperature Criteria Assessment
(Chinook salmon and *O.mykiss*)
DRAFT Summary Meeting Notes

Friday, November 16, 2012
MID Offices, Modesto CA

Attendees

Bill Sears (CCSF)	Bill Johnston (MID)
Noah Hume (Stillwater Sciences)	Greg Dias (MID)
Karl English (LGL)	Bob Nees (TID)
Ron Yoshiyama (Consultant)	Art Goodwin (TID)
John Devine (HDR)	Allyson Boucher (TRC)
Bao Le (HDR)	Ramon Martin (USFS)
Allison Willy (USFWS)	Mike Maher (SWRCB)
Peter Barnes (SWRCB)	

Attended via phone:	
Bill Snider (HDR)	
Jim Hastreiter (FERC)	
Patrick Koepele (TRT)	
Ellen Levin (CCSF)	
Tim Findley (HB)	
Donn Furman (CCSF)	

Meeting Summary

Following introductions, John Devine (HDR) reiterated that the purpose of this meeting was to provide relicensing participants with an update on the status and progress of W&AR-14, the study that is evaluating temperature criteria for Tuolumne River Chinook salmon and *O.mykiss*. Mr. Devine indicated that as this study was not included under the Workshop Consultation Protocols, only brief meeting notes would be provided.

Mr. Devine then provided background information on W&AR-14, referencing the Districts' Revised Study Plan filed in November 2011 and FERC's Study Plan Determination in December 2011. In the Study Plan Determination, FERC staff indicated they would use the temperature guidelines of EPA 2003 and therefore recommended that the study not be undertaken, unless the study developed empirical data that was site-specific to the Lower Tuolumne River salmonids. An initial study meeting was held on April 11, 2012 where the Districts indicated that they would continue to proceed, but would concentrate

on studies that would develop site-specific empirical information, consistent with FERC staff's guidance/determination. Eight possible studies were then presented at the April 11, 2012 meeting.

Mr. Devine advised that the Districts, upon further evaluation, have now reduced this number to four proposed studies, one being a new investigation not previously discussed. Thus, the purpose of this meeting was to review with relicensing participants these four proposed studies.

A participant asked Jim Hastreiter (FERC) how FERC would deal with a study that FERC staff did not recommend be undertaken. Mr. Hastreiter indicated that FERC staff would consider studies that provided Tuolumne River empirical data. Mr. Devine indicated that it was the Districts' understanding that the purpose of the FERC Study Plan Determination was to identify the studies **required** to be undertaken by the Districts, but not to limit the studies. The Districts were free to undertake other studies, just as are the relicensing participants. Mr. Hastreiter agreed.

Mr. Devine also explained that with the re-scoping of these planned studies, they will now become 2013 studies and a detailed schedule will be included in the Initial Study Report ("ISR") document scheduled to be issued on January 17, 2013.

Patrick Koepele (TRT) asked if there were meeting notes for the April 11, 2012 meeting. Mr. Devine indicated that the pre-meeting package contained a large amount of information and a number of handouts, but noted that as this is not a study to which the Workshop Consultation protocols applied, there were no subsequent meeting notes.

Mr. Devine introduced Karl English of LGL Consultants who presented the proposed Swim Tunnel Study. Mr. English pointed out that this study is being pursued by the Districts to investigate effects on Tuolumne River salmonids of being exposed to temperatures different than EPA 2003 guidelines and to investigate the potential for site-specific adaptations to Tuolumne River temperatures. EPA 2003 identifies optimum temperatures, but this study will investigate effects at sub-optimal temperatures. Mr. English referenced findings from studies done on the Fraser River in British Columbia on sockeye that showed considerable local adaptation to temperatures even within the same river, which are indicative of localized life history strategies. Mr. English described the study methods, basically consisting of measurements of oxygen uptake.

Mr. Hastreiter asked if there would be actual measurements of blood gas levels. Mr. English indicated no, but that there are known relationships between blood gas levels and O2 concentrations. Mr. English indicated that oxygen consumption level in the swim tube is what will be measured. There would be no blood or bodily fluid samples. During exercise trials, the oxygen is fixed and will deplete over time so as to act as the control measurement.

Ramon Martin (USFWS) asked if the data presented to show local adaptations are statistically significant. Mr. English agreed to check this and see how the curves were compared in the Eliason et al. 2012 paper and provide feedback. It appeared that the Parsons paper dealt with this. The Districts will upload the Parsons paper to the website. The Districts also agreed to upload the Farrell paper discussed at the meeting, along with Mr. English's presentation.

Bill Snider (HDR) then provided an update on NMFS consultation related to the necessary Section 10 permitting for the Swim Tube Study. The Districts are currently working through the lengthy Section 10 permitting application online. There have been discussions with NMFS staff and the study appears to be doable in 2013. Once the application is completed, detailed consultations will ensue with NMFS, followed by a public notice in the Federal Register. The scope is currently being refined per NMFS comments and the Districts are hoping to file the application soon. It looks as though the earliest for permit issuance would be April of next year. NMFS' primary questions concerned fish acquisition and study area security.

Mr. Martin noted that in the Districts' permitting conversations with NMFS, they need to make sure to convey that this work is in consultation with agencies and to make sure that it is consistent with the number of research permits allowed in the basin. Mr. Martin indicated that USFWS has a Section 10 permit.

Mr. Martin asked if a detailed study plan will be prepared. Mr. Devine agreed to provide an updated W&AR-14 Study Plan for relicensing participants to review. He made it clear that the Districts were not going to be asking FERC to change its December 2011 Determination; therefore, it would be for information only. The ISR would seem to be a reasonable opportunity to provide the revised study plan.

Mr. Snider then briefly described the updates to the other three site-specific investigations to be undertaken as part of W&AR-14. These were largely reiterations of the April 11 discussions.

Mr. Hastreiter asked when the full description of the studies was sent out. Bill Snider said they were uploaded to the website on November 8, 2012 and participants notified. Mr. Hastreiter also asked if the other three studies could be characterized as desktop studies. Mr. Snider responded yes, but they are all based on Tuolumne River specific data.

Mr. Snider pointed out that CDFG redd surveys and adult weir data will be needed to look at timing of spawning vs. temperature.

Art Godwin noted that the study information write-up does not distinguish between Chinook and O.mykiss evaluations as different studies. This will be clarified as appropriate.

Mr. Martin asked if study W&AR-20 could be discussed briefly. He asked how many fish would be used. Noah Hume (Stillwater Sciences) said 70-75 fish, as limited by permits. Mr. Hume also noted that the Zimmerman report has data that can be used as well. This should result in a fairly robust data set. Mr. Martin wondered if the Districts could back calculate the Zimmerman work to temperature data for those years. This would require a number of additional assumptions. The Districts will look into this.

Action Items:

(1) Upload the Fry, Farrell, and Parsons papers to the website.
(2) Upload Karl English's presentation to the website.
(3) The Districts will develop study plans for the four temperature criteria studies to be provided in the Initial Study Report (ISR) to further facilitate discussions/review/comment.

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Commentary

Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids

A. P. Farrell

Zoology Department, 6270 University Boulevard, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

farrellt@interchange.ubc.ca

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Summary

Animal distributions are shaped by the environment and antecedents. Here I show how the temperature dependence of aerobic scope (the difference between maximum and minimum rates of oxygen uptake) is a useful tool to examine the fundamental temperature niches of salmonids and perhaps other fishes. Although the concept of aerobic scope has been recognized for over half a century, only recently has sufficient evidence accumulated to provide a mechanistic explanation for the optimal temperature of salmonids. Evidence suggests that heart rate is the primary driver in supplying more oxygen to tissues as demand increases exponentially with temperature. By contrast, capacity functions (i.e. cardiac stroke volume, tissue oxygen extraction and haemoglobin concentration) are exploited only secondarily if at all, with increasing temperature, and then perhaps only at a temperature nearing that which is lethal to resting fish. Ultimately, however, heart rate apparently becomes a weak partner for the cardiorespiratory oxygen cascade when temperature increases above the optimum for aerobic scope. Thus, the upper limit for heart rate may emerge as a valuable, but simple predictor of optimal temperature in active animals, opening the possibility of using biotelemetry of heart rate in field situations to explore properly the full interplay of environmental factors on aerobic scope. An example of an ecological application of these physiological discoveries is provided using the upriver migration of adult sockeye salmon, which have a remarkable fidelity to their spawning areas and appear to have an optimum temperature for aerobic scope that corresponds to the river temperatures experienced by their antecedents. Unfortunately, there is evidence that this potential adaptation is incompatible with the rapid increase in river temperature presently experienced by salmon as a result of climate change. By limiting aerobic scope, river temperatures in excess of the optimum for aerobic scope directly impact upriver spawning migration and hence lifetime fecundity. Thus, use of aerobic scope holds promise for scientists who wish to make predictions on how climate change may influence animal distributions.

Key words: thermal niches, optimal temperature, aerobic scope, oxygen uptake, metabolic rate, cardiac output, heart rate, tissue oxygen extraction, oxygen partial pressure, biotelemetry, lifetime fecundity, climate change.

Introduction

The study of the physiological and biochemical mechanisms that set the limits for environmental tolerance, and which in many ways distinguish species, is an active area of investigation that has gained importance in the current era of climate change. This article is focused on the physiological mechanisms that become critical when fishes, particularly salmonids, approach their upper temperature limits. Furthermore, to address the need for examples of how large-scale environmental records of climate are translated at the scale of the organism (Helmuth, 2009), this mechanistic understanding is applied to the river migration of an adult Pacific salmon species.

My focus on predominantly one group of fishes (the salmonids) and on one environmental variable (temperature) is for two reasons. First, this is where data are most abundant. Second, a case study of temperature tolerance among fishes is likely to prove extremely fruitful in addressing the more general and important question of animal resilience and adaptability to environmental change. This is because fishes have evolved around species-specific niches, living in almost every conceivable aquatic habitat and representing almost half of the earth's vertebrate species. However, no single fish species tolerates the entire temperature range exploited by fishes (from –2°C in Antarctica to +42°C in Lake Magadi, Kenya). Similarly, ~43% of all fish species live in freshwater rather than the vastly more abundant saline habitats [>99% of the available aquatic habitat (Nelson, 2006)]. Although the foundation for the thermal distributions that we see today may seem to reflect an absence of the requisite genomic machinery, a more circumspect view may be need. For example, Antarctic fishes, which have lived in a thermally stable environment for many thousands of years, are now known to be able to thermally acclimate to temperatures previously thought to be lethal and well above those found in their present ecological niche (Franklin et al., 2007). Thus, observing a stenothermal existence does not necessarily mean insufficient phenotypic plasticity to tolerate a broader temperature range.

Temperature and aerobic scope

Temperature has a central role in shaping the distribution of animals. In explaining latitudinal and longitudinal limits of biomes, Shelford's law of tolerances envisaged a centre of animal abundance bounded by 'toleration' of environmental 'controlling factors' (Fig. 1A). Clearly, the poleward shift in fish distributions with the progressive warming of aquatic habitats (Brander et al., 2003; Brander, 2007; Pörtner and Knust, 2007; Dulvy et al., 2008)

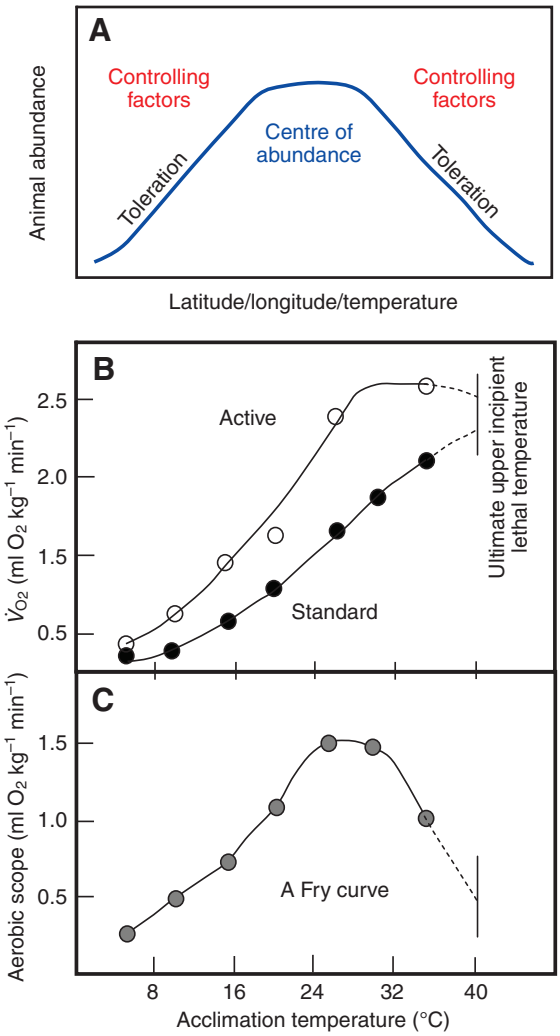

Fig. 1. The controlling and limiting effects of temperature on animal distributions, metabolic rate and scope for activity. (A) A schematic representation of Shelford's law of tolerances (Shelford, 1931). (B) Measurements of standard and active metabolic rates for goldfish as a function of temperature approaching their upper incipient lethal temperature. (C) Aerobic scope (or scope for activity) as a function of temperature, which is the difference between the measurements of standard and active metabolic rates shown in B (Fry, 1947).

represents a more insidious manifestation of the anthropogenic-driven change in animal distribution that Shelford characterised nearly 80 years ago (Shelford, 1931).

Temperature tolerance at the whole animal level was first given a mechanistic explanation for fishes by Fry (Fry, 1947), who showed that temperature both controlled *and* limited their metabolic rate. To illustrate his ideas, he used scope for activity, which is now termed aerobic or metabolic scope, i.e. the difference between standard and active metabolic rates (Fig. 1B,C). In doing so, Fry recognized that the predictive value of knowing the temperature dependence of aerobic scope was considerably greater than that of knowing a temperature tolerance range (e.g. critical maximum and minimum temperatures; CT_{max} and CT_{min}). Indeed, the aerobic scope concept is now being used broadly to examine the impacts of the aquatic warming trends and other environmental climate changes on marine ectotherms (Pörtner, 2001; Pörtner, 2002; Mark et al., 2002; Pörtner and Knust, 2007; Pörtner and Farrell, 2008), illustrating an importance well beyond fishes. Even so, and as shown in the following, our understanding of the proximate causes that limit a fish's aerobic scope beyond its optimal temperature range remains formative.

The Fry curve for aerobic scope

Aerobic scope is derived from measurements of a fish's minimum and maximum rates of oxygen uptake ($\dot{V}_{O_2}$) as a function of temperature (Fig. 1B). The difference between these two rates is aerobic scope, which takes the form of a bell-shaped curve as a function of temperature – a 'Fry curve' for aerobic scope (Fig. 1C). Simplistically, a Fry curve represents an animal's capacity for activity as a function of temperature.

Minimum $\dot{V}_{O_2}$ (standard or basal metabolic rate) represents the metabolic cost to support an animal's existence in a non-feeding, non-reproducing and non-motile state. Minimum $\dot{V}_{O_2}$ is directly affected by body temperature [thermodynamics (Krogh, 1914)], typically doubling or tripling with a 10°C acute increase in temperature (termed a Q_{10} effect; Fig. 1B). Minimum $\dot{V}_{O_2}$ also varies among species (a genetic basis) and with body size [scaling (Schmidt-Nielsen, 1984)].

Clearly, life beyond short-term existence requires a capacity to increase $\dot{V}_{O_2}$ above this minimum level. Energy expenditure for feeding, growth, reproduction and locomotion (used for foraging as well as escape from predators and unfavourable environments) needs an active $\dot{V}_{O_2}$. In terms of the temperature dependence of active $\dot{V}_{O_2}$, Fry (Fry, 1947; Fry and Hart, 1948) made the crucial observation that maximum $\dot{V}_{O_2}$ of exercising goldfish (*Carassius auratus*) failed to continue increasing with temperature beyond an optimal temperature (T_{opt}). By contrast, standard $\dot{V}_{O_2}$ of resting fish continued its exponential increase until temperature approached a lethal level (Fig. 1B). Thus, the T_{opt} for aerobic scope is created by the failure of maximum $\dot{V}_{O_2}$ to continue increasing with temperature. Consequently, because activities such as growth depend on aerobic scope, it is not surprisingly that growth rate as a function of temperature has a similar bell-shaped, species-specific curve for fishes (Fig. 2B) (Brett, 1971). In fact, fish must eat more just to deal with the exponential increase in standard $\dot{V}_{O_2}$. Like minimum $\dot{V}_{O_2}$, active $\dot{V}_{O_2}$ is also species-specific and varies with body size.

At a critical temperature (T_{crit}), aerobic scope is zero and aerobic activity becomes impossible. Thus, a thermal niche for existence in a resting state is bounded by the upper and lower T_{crit} values (which correspond closely to the CT_{max} and CT_{min} values determined using other methods). However, existence without an aerobic scope is necessarily short-lived in nature because, besides being an easy target for predators, starvation is just a matter of time. Consequently, an animal's functional thermal niche is narrower than that bounded by T_{crit}.

Fry curves are species specific. Differences result from their position on the temperature scale (temperature niches), being centred near 27°C for goldfish and at cooler temperatures (<20°C) for most salmonids (Fig. 2A). There are also species differences in standard and active $\dot{V}_{O_2}$. Athletic species such as salmonids have a high aerobic scope, but this does not necessarily translate into a larger thermal niche. For example, generalists such as goldfish (Fig. 2A) and *Fundulus heteroclitus* (Fangue et al., 2006) have a low aerobic scope and a broader thermal niche (eurythermal) compared with salmonids.

Scaling up of laboratory-derived aerobic scope data to ecology and biogeography will not necessarily be a simple task because other environmental factors reduce aerobic scope and narrow an

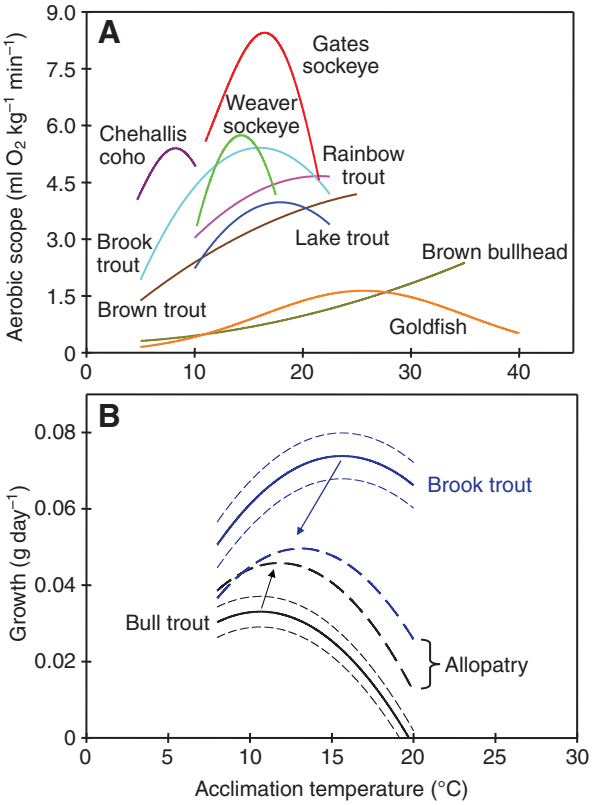

Fig. 2. The influence of temperature on aerobic scope and growth rate. (A) Fry curves for a range of salmonids and other species (Fry, 1947; Fry, 1948; Fry and Hart, 1948; Lee et al., 2003). (B) Growth rates of brook trout and bull trout grown either separately (solid lines with the accompanying dashed lines showing the 95% confidence limits) or together (long dashed lines grouped by allopatry) (McMahon et al., 2007).

animal's functional thermal niche (Fry, 1947; Fry, 1971; Brett, 1971; Pörtner and Farrell, 2008; Munday et al., 2009). For example, aquatic hypoxia, independent of temperature, can reduce aerobic scope (Graham, 1949; Gibson and Fry, 1954; Fry, 1971; Brett, 1971) to the extent that feeding and growth are halted, and development and reproduction are delayed (see Richards et al., 2009). Therefore, both hypoxia and hypercapnia are likely to constrain the breadth and height of a Fry curve (Pörtner and Farrell, 2008). Furthermore, the aerobic scope for a developing fish may not reach its full potential until the cardiorespiratory system is fully developed. Therefore, a family of Fry curves may exist for different life stages. Behaviour adds further complexity. For example, inter-specific competition can shift the T_{opt} for growth (Fig. 2B), as seen in brook trout (*Salvelinus fontinalis*) when growth was suppressed while competing with bull trout (*Salvelinus confluentus*), but not *vice versa* (McMahon et al., 2007).

An important index that can be derived from a Fry curve is the thermal window, the temperature difference between T_{opt} and T_{crit}. This thermal window is an index of a species' resilience to temperature change. In salmonids, the thermal window for the collapse of aerobic scope with warming is just 6–7°C (Fry, 1947; Farrell et al., 2008), which is a relatively small safety margin in the context of global warming scenarios. Tropical species apparently have narrow thermal windows too (Hoegh-Guldberg et al., 2007; Tewksbury et al., 2008) and live close to their T_{crit}. For example, cardinalfishes (*Ostorhinchus doederleini* and *O. cyanosoma*) were found to lose nearly 50% of their aerobic scope with only a 2°C increase above the average summer temperature (Nilsson et al., 2009), and an increase of 3°C compromised growth of spiny-damselfish (*Acanthochromis polyacanthus*) (Munday et al., 2008). However, the collapse of aerobic scope at warm temperatures was less evident (Fig. 2A) for the bullhead (*Ameiurus nebulasa*) and brown trout (*Salmo trutta*), suggesting that other factors may set thermal tolerance.

The rise and fall of aerobic scope in salmonids

As temperature increases, exponentially more oxygen must be delivered to tissues, which is the task of the cardiorespiratory system. Since maximum $\dot{V}_{O_2}$ fails to increase beyond T_{opt}, the decline in aerobic scope beyond T_{opt} (i.e. the downward trend of a Fry curve) therefore reflects the inability of the *maximum* cardiorespiratory capability to keep pace with these increasing tissue oxygen demands. By contrast, T_{crit} corresponds with a failure of the *resting* cardiorespiratory capability to keep pace with increasing tissue oxygen demands. The resultant mismatch between oxygen supply and oxygen demand forces animals to progressively switch to anaerobic metabolism to survive (Pörtner, 2001; Frederich and Pörtner, 2000), perhaps causing an acceleration of cardiorespiratory collapse (Farrell et al., 2008) and the rightward skew often seen in Fry curves.

At present, cardiorespiratory information pertaining to the collapse of aerobic scope during warming is most abundant for salmonids. The data are examined below within the context of the cardiorespiratory oxygen cascade in order to explore why active $\dot{V}_{O_2}$ does not increase beyond T_{opt} and why minimum $\dot{V}_{O_2}$ collapses at T_{crit}.

Active $\dot{V}_{O_2}$ and the cardiorespiratory oxygen cascade

The cardiorespiratory oxygen cascade conceptualizes the movement of oxygen down its partial pressure gradient from a respiratory medium to tissues. Hence, $\dot{V}_{O_2}$ corresponds to the oxygen flux per unit time through this cascade and oxygen diffusion rates are proportional to the relevant oxygen partial pressure (P_{O_2}) gradients. For fish, oxygen diffuses from water across gill secondary lamellae and binds to haemoglobin (Hb) in red blood cells, which are transported by the circulatory system to tissues where oxygen diffuses across the capillary wall and into the cell to be used in mitochondrial respiration (Fig. 3).

A countercurrent arrangement of blood and water flow at the secondary lamellae ensures that the arterial blood leaving the gills has a P_{O_2} (Pa_{O_2}) close to ambient water, and its Hb is almost fully saturated, i.e. the oxygen content of arterial blood (Ca_{O_2}) is near maximal. Convection of oxygen to tissues by the arterial system is quantified as the product of Ca_{O_2} and cardiac output. Thus, increasing cardiac output is the only means to internally transport more oxygen to the tissues, unless stored red blood cells are released from the spleen to increase Hb concentration [Hb] and hence Ca_{O_2} (see Gallaugher and Farrell, 1998). Once in tissue capillaries, factors such as the architecture of the capillaries, the presence of myoglobin and lipid droplets in the cytoplasm and the actual location of mitochondria within the cell significantly influence the rate of diffusion of oxygen from the red blood cell to the mitochondria.

In a resting fish, increasing tissue oxygen delivery with increasing temperature could simply recruit mechanisms that are normally used during exercise. When salmonids exercise at a constant temperature, there are increases in gill ventilation (to deliver more water), cardiac output (to transport more oxygen to

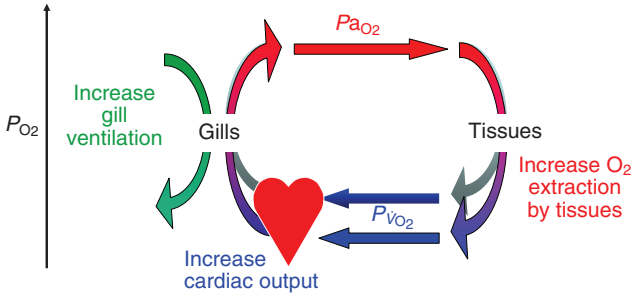

Fig. 3. A schematic diagram representing the oxygen cascade for a fish during rest (shaded lines and arrows) and swimming (dark lines and arrows). The oxygen partial pressure is an arbitrary scale (see text for details).

the tissues) and tissue oxygen extraction from blood (Stevens et al., 1967; Kiceniuk and Jones, 1977). Increased tissue oxygen extraction can contribute almost as much to the increased $\dot{V}_{O_2}$ as cardiac output because resting fish remove only about one third of the arterial oxygen and so venous oxygen content (Cv_{O_2}) and venous blood P_{O_2} (Pv_{O_2}) can decrease considerably during exercise (Fig. 3). While all of these exercise-induced cardiorespiratory changes are possible during warming, as shown below, not all of them occur when resting fish are warmed up to T_{crit}.

When an exercising fish is warmed, it is more a matter of how much the warming increases the rate and force of muscle contraction to enhance maximum cardiorespiratory capacity. In addition, oxygen diffuses at a faster rate, potentially allowing a lower Pv_{O_2}. Furthermore, the temperature sensitivity of the Hb–oxygen binding curve (e.g. Clark et al., 2008a) is such that a rightward shift with warming increases the Pa_{O_2} of fully saturated arterial blood. This also promotes a faster unloading of oxygen at the tissues. In fact, Cv_{O_2} could decrease during warming without a decrease in Pv_{O_2} (this direct temperature effect is in addition to a similar benefit from the Root- or Bohr-shifts as tissues release more carbon dioxide and H^+ during exercise).

Some fairly simple theoretical predictions can be made using this conceptual framework, against which existing cardiorespiratory data on warming in fishes can be compared. The analysis is further simplified by asking where the potential limitation might exist (gills, circulatory system or tissues), and by focusing on underlying mechanisms (at near T_{crit} for resting fish and at T_{opt} for exercising fish).

Changes in cardiorespiratory variables with acute warming in association with T_{opt} in exercising salmonids and T_{crit} in resting salmonids

A limitation at the gills?

Oxygen is poorly soluble in water. Compounding this, its solubility in water decreases ~2% per degree centigrade. Therefore, gill ventilation must compensate for the decreased oxygen availability and the lower Hb–oxygen affinity, as well as increased tissue oxygen demand as temperature increases. Therefore, a decrease in Pa_{O_2} during warming would indicate a clear problem associated with gill oxygen delivery and transfer. However, the data for salmonids are inconsistent on this matter.

When exercising adult sockeye salmon (*Oncorhynchus nerka*) were warmed to a temperature well above T_{opt}, Pa_{O_2} was maintained (Steinhausen et al., 2008). Similar results were found in resting Chinook salmon (*O. tshawytscha*) warmed up to T_{crit} (Clark et al., 2008a). In fact, Pa_{O_2} actually increased in resting sockeye salmon warm to T_{crit} (Steinhausen et al., 2008).

Interpreting Ca_{O_2} data during warming is more complex because of potential pH and temperature effects on the Hb–oxygen affinity curve, and because warming has variable effects on blood [Hb] (Taylor et al., 1997; Farrell, 1997; Sandblom and Axelsson, 2007). Even so, Ca_{O_2} was maintained in resting sockeye salmon warmed to T_{crit} as well as in exercising sockeye salmon warmed above T_{opt} (Steinhausen et al., 2008). By contrast, Ca_{O_2} decreased at T_{crit} in resting rainbow trout (*O. mykiss*) (Heath and Hughes, 1973) and in resting Chinook salmon (Clark et al., 2008a). The modest decrease in Ca_{O_2}, in the absence of an effect on Pa_{O_2}, in resting Chinook salmon probably reflects a decrease in Hb–oxygen affinity rather than a limitation on oxygen diffusion at the gills.

A limitation in the circulatory system?

If a circulatory limitation exists for exercising salmonids during warming, increases in cardiac output should cease once T_{opt} is reached. Indeed, maximum cardiac output in exercising sockeye salmon (Brett, 1971; Steinhausen et al., 2008) and rainbow trout (Taylor et al., 1996) reached a maximum value at a temperature well below T_{crit}, as did $\dot{V}_{O_2}$. Thus, ultimately as warming approaches T_{opt} the potential to increase maximum cardiac output (as revealed by exercising fish) fails to keep up with the required increase in cardiac output in a resting fish (Fig. 4). As a result, because scope for cardiac output does not increase above T_{opt} (Fig. 5), swimming effort either declines or stops.

For resting salmonids, the cardiac limitation at T_{crit} is even more obvious. Cardiac arrhythmias and bradycardia often develop at T_{crit} (Heath and Hughes, 1973; Clark et al., 2008a), although their physiological basis has not been studied. Thus, experimental evidence points unequivocally towards a cardiac limitation both at T_{opt} in exercising salmonids and at T_{crit} in resting salmonids. Further insight into the mechanistic basis of the cardiac response to warming and its limitations comes from an analysis of heart rate (the rate function) and cardiac stroke volume (the capacity function).

The importance of increased heart rate during acute warming is extremely clear. Warming increases cardiac output solely by increasing heart rate. This is true for both resting and exercising salmonids (Sandblom and Axelsson, 2007; Clark et al., 2008a; Steinhausen et al., 2008), presumably through a direct temperature effect on the cardiac pacemaker rate (Randall, 1970). However, because fish have a maximum heart rate (Farrell, 1991) and heart rate is already elevated by the exercise, the maximum heart rate must be reached at a temperature well below that for resting fish (Steinhausen et al., 2008). In fact, the scope for heart rate plummets from its maximum at T_{opt} to zero near T_{crit} (Fig. 5). Fred Fry made a similar observation for heart rate in *Salvelinus fontinalis* alevins (Fig. 6A) (Fry, 1947) and commented that this might reflect the T_{opt} for the activity of an organ (i.e. the heart)! We now know that Fry's assertion was correct because the T_{opt} for the maximum performance of isolated rainbow trout hearts is well below T_{crit} (Fig. 6B).

In contrast to heart rate, cardiac stroke volume appears to be thermally insensitive to warming. This is true for resting and exercising salmonids (Sandblom and Axelsson, 2007; Clark et al., 2008a; Steinhausen et al., 2008), but it is an especially surprising result for resting fish. In fact, it seems paradoxical, given that cardiac stroke volume can triple during swimming at constant temperature (Stevens et al., 1967; Brett, 1971; Kiceniuk and Jones, 1977; Farrell and Jones, 1992; Thorarensen et al., 1996; Gallaugher

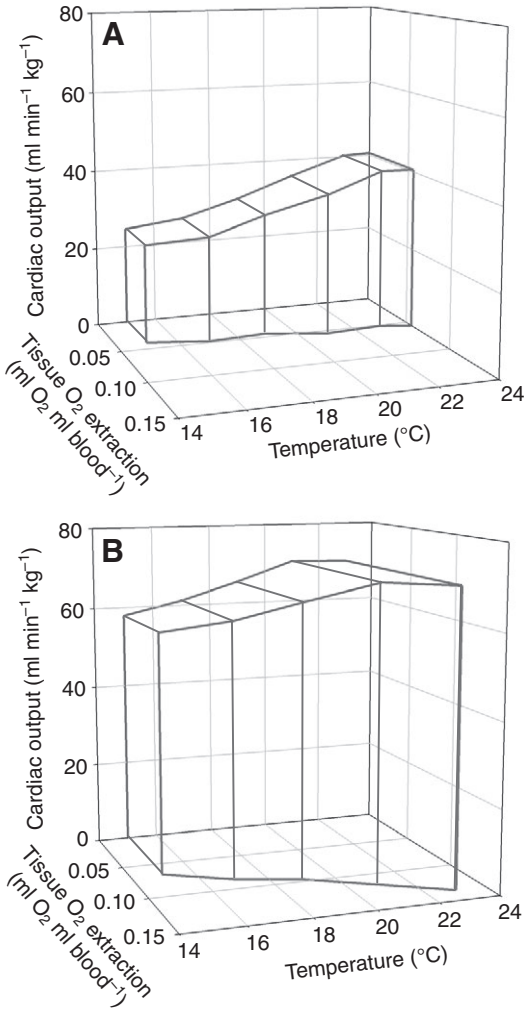

Fig. 4. Cardiac output and tissue oxygen extraction (Ca_{O_2}–Cv_{O_2}) for 12°C-acclimated sockeye salmon either (A) at rest, or (B) swimming continuously at about 70% of maximum swimming speed, while the temperature was acutely increased at 2°C h^{-1} and held at the temperature for 1 h while cardiorespiratory measurements were made. All resting fish completed the temperature challenge and recovered, but above 19°C swimming fish began to stop swimming and so progressively fewer are represented at higher temperatures. The *x*–*y* surface at each temperature represents oxygen uptake (i.e. the product of cardiac output and tissue oxygen extraction), which clearly increases with temperature in resting but not swimming fish above their optimum temperature of around 15°C. Changes in cardiac output with temperature are a result of increased heart rate (see text) (Steinhausen et al., 2008).

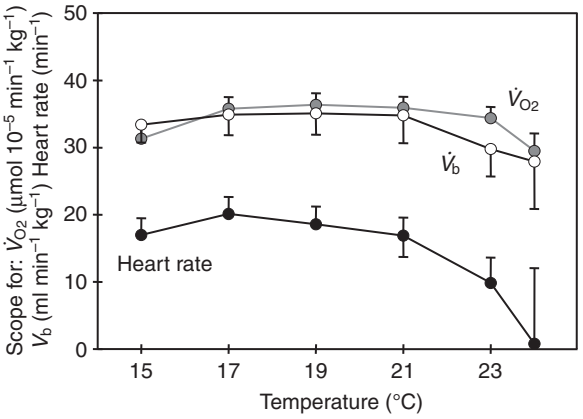

Fig. 5. Changes in scope for oxygen uptake ($\dot{V}_{O_2}$), cardiac output ($\dot{V}_b$) and heart rate (f_H) in swimming sockeye salmon during acute warming. Note that although all fish continued swimming in temperatures up to and including 19°C, some fish stopped swimming at higher temperatures and so the data are only for those that continued to swim (Steinhausen et al., 2008).

et al., 2001), that this additional capacity for increasing cardiac stroke volume is not exploited by resting fish when they are warmed to T_{crit} (Fig. 4). So why is this?

The difficulty may revolve around the fact that cardiac end-systolic volume is essentially zero in salmonids (Franklin and Davie, 1992). This means that, unless venous return and end-diastolic volume are increased first, an increase in cardiac contractility cannot increase cardiac stroke volume appreciably (Sandblom and Axelsson, 2007). Furthermore, there are indications that during warming inadequate venous return may limit cardiac stroke volume in the first instance. In resting rainbow trout warmed from 10 to 13°C, cardiac stroke volume was maintained when heart rate increased because venous blood pressure and mean circulatory filling pressure also increased (Sandblom and Axelsson, 2007). However, with further warming to 16°C, which is near T_{opt}, venous blood pressure was unchanged and cardiac stroke volume decreased when heart rate increased further. Although a complete systolic emptying of the ventricle may be a disadvantage with regard to the capacity to increase cardiac stroke volume during warming, it may be more important in ensuring a completely 'fresh' supply of oxygen enters the lumen of the heart with each heart beat given oxygen diffusion to the myocardium is driven by a low $P_{v_{O_2}}$ (see Farrell, 2002).

The increase in cardiac stroke volume when salmonids swim at a constant temperature is supported by an increase in venous blood pressure (Kiceniuk and Jones, 1977) and by contraction of locomotory muscles aiding venous return (Farrell et al., 1988). There are several potential reasons why warming does not increase cardiac stroke volume any further. There could be physical upper limits to venous return and end-diastolic volume. Also, increasing heart rate during warming reduces cardiac filling time and creates a negative frequency effect on cardiac contraction, both of which could constrain cardiac stroke volume (Farrell, 2007). In addition, at a time when the heart is working maximally, its extracellular environment (the venous blood) becomes acidemic and hyperkalemic, and has a low $P_{v_{O_2}}$ (Steinhausen et al., 2008). Although the negative inotropic effects of these extracellular changes were prevented by adrenergic stimulation of the heart (Driedzic and Gesser, 1994; Nielsen and Gesser, 2001; Hanson et al., 2006), this adrenergic protection was greatly reduced at 18°C compared with 10°C in rainbow trout (Hanson and Farrell, 2007).

A limitation at the tissues?

The rate and degree of oxygen diffusion from capillaries to tissues is influenced by several factors besides the P_{O_2} gradient. These include tissue capillary density, the intracellular mitochondrial location, regional blood flow and red blood cell capillary contact time. Taylor et al. (Taylor et al., 1997) suggested that regional oxygen delivery by convective transport in exercising rainbow trout is determined mainly by changes in cardiac output as temperature changes, i.e. active peripheral redistribution of blood flow is modest. Even so, red muscle blood flow during aerobic swimming

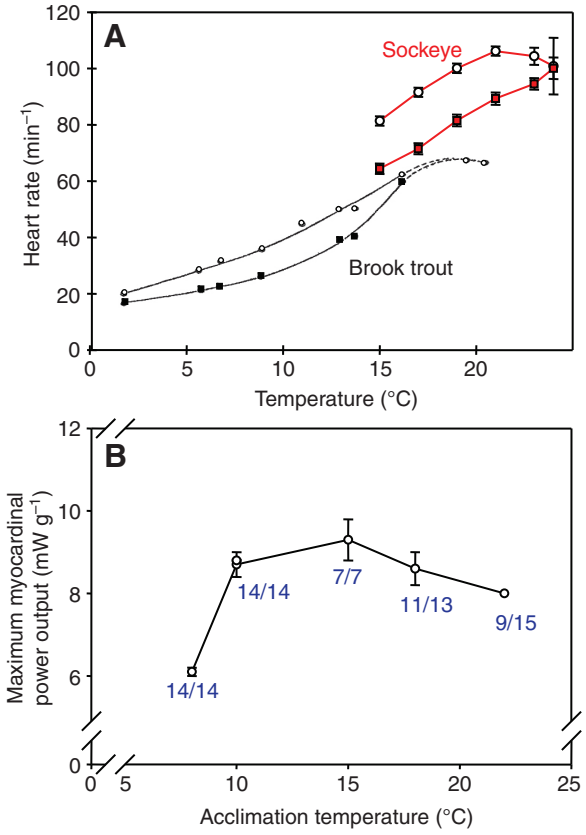

Fig. 6. (A) A comparison of heart rates measured in brook trout alevins (Fry, 1947) and adult sockeye salmon (Steinhausen et al., 2008) to illustrate the convergence of heart rate in resting (lower lines) and active (upper lines) fish such that there is no scope for heart rate at T_{crit}. (B) A composite of the maximum cardiac performance for isolated perfused rainbow trout hearts acclimated to different temperature to illustrate that there is a peak performance around 15°C for the heart. Beyond this temperature, an increasing number of preparations would fail as indicated by the ratio of successful/attempted preparations besides each data point (Farrell et al., 1988; Keen and Farrell, 1994; Farrell et al., 1996).

was lower at 18°C than at 11°C (Taylor et al., 1997). In addition, the basal oxygen requirement of white (fast glycolytic) muscle in fish increases during warming because it accounts for >50% of body mass and receives 28–50% of routine cardiac output in resting rainbow trout (Randall and Daxboeck, 1982; Bushnell et al., 1992). Indeed, the finding that blood flow to white muscle increased from 40% to 75% of cardiac output at 6°C *versus* 18°C in resting rainbow trout (Barron et al., 1987) clearly reflects a significant elevation of white muscle oxygen demand relative to whole animal $\dot{V}_{O_2}$. White muscle also has a low capillary density (Egginton, 2000), which increases the likelihood of a diffusion limitation developing for oxygen diffusion.

Further insight into potential limitations on tissue oxygen removal during warming is evident from measurements of Cv_{O_2} and Pv_{O_2}. For example, Pv_{O_2} and Cv_{O_2} could not decrease if there was a diffusion limitation. In fact, a decrease in Cv_{O_2} is a very important mechanism for increasing tissue oxygen extraction during swimming at constant temperature (Fig. 4). However, for resting sockeye salmon, warming actually increased Pv_{O_2} and Cv_{O_2}, and tissue oxygen extraction (Fig. 4) remained unchanged (Steinhausen et al., 2008). Similarly, Pv_{O_2} was temperature insensitive in resting Chinook salmon, except at 25°C when there was acidemia and Cv_{O_2} decreased (Clark et al., 2008a). When exercising sockeye salmon were warmed, Pv_{O_2} again remained temperature insensitive, albeit it at a lower level compared with resting fish (Steinhausen et al., 2008). This consistent temperature insensitivity of Pv_{O_2} points to a diffusion limitation for oxygen unloading (see Farrell, 2002; Farrell and Clutterham, 2003). Why in resting fish warming does not decrease Pv_{O_2} to the level seen with swimming at a constant temperature is unclear.

In resting salmonids, the decrease in Cv_{O_2} just prior to T_{crit} may reflect a desperate situation created by inadequate tissue perfusion. The ability of fish to recover from warming may be informative in this regard. For example, when sockeye salmon and Chinook were incrementally warmed at 2–4°C h^{-1} and kept at a constant temperature for 1 h between temperature steps, the fish recovered well at the control temperature and within 1–2 h, especially if the heat stress was terminated before cardiac arrhythmias developed (Steinhausen et al., 2008; Clark et al., 2008a). In these experiments, sockeye salmon maintained Cv_{O_2} and Chinook salmon decreased Cv_{O_2} only in association with acidemia at 24°C. By contrast, when 'opportunistic' blood samples were taken from resting rainbow trout during continuous warming (1.5°C h^{-1}), all but one fish died and venous blood became depleted of oxygen (Heath and Hughes, 1973).

What emerges from the above is that the heart becomes a weak link for the cardiorespiratory oxygen cascade when exercising salmonids are warmed above T_{opt}. Although a direct temperature effect on the cardiac pacemaker rate appears to be the predominant mechanism for improving tissue oxygen transport, a crucial limitation is reached when this rate function reaches its maximum. This apparently occurs at T_{opt} for exercising fish and at T_{crit} for resting fish. What follows during warming is a sequela of events: a decrease in scope for heart rate preceding that for cardiac output, which precedes that for aerobic scope (Fig. 5). It is also evident that during warming the contributions of several capacity functions ([Hb], tissue oxygen extraction and cardiac stroke volume) are only small and variable. Why this excess capacity is not exploited when resting fish are warmed is particularly perplexing and warrants further study.

Beyond salmon

The details provided above for salmonids apparently apply more broadly to other fishes. For example, warming of three species showed that like rainbow trout: (1) cardiac output increases predominantly through increased heart rate, (2) routine heart rate shows a plateau or collapse before T_{crit} that is species specific, and (3) cardiac stroke volume is temperature insensitive (Fig. 7) (Sandblom and Axelsson, 2007 and references therein). In addition, the temperature dependence of Hb–oxygen affinity and the variable effects of warming on [Hb] are well known among fishes (Cech et al., 1976; Gallaugher and Farrell, 1998; Gollock et al., 2006), and a direct temperature effect on the spontaneous pacemaker rate is recognised for plaice (*Pleuronectes platessa*) (Harper et al., 1995). Furthermore, in resting Atlantic cod (*Gadus morhua*), although heart rate and cardiac output both collapsed before CT_{max}, heart rate reached a plateau before cardiac output and $\dot{V}_{O_2}$ (at 18°C *versus* at 20°C) (Gollock et al., 2006).

The effects of acute warming have been thoroughly studied in winter flounder (*Pseudopleuronectes americanus*) seasonally acclimated between 5°C and 18°C (Cech et al., 1975; Cech et al., 1976). After a 5°C warming at each acclimation temperature, an increase in $\dot{V}_{O_2}$ (67–83% per 5°C increment) was always accompanied by a nearly equivalent increase heart rate (54–77%

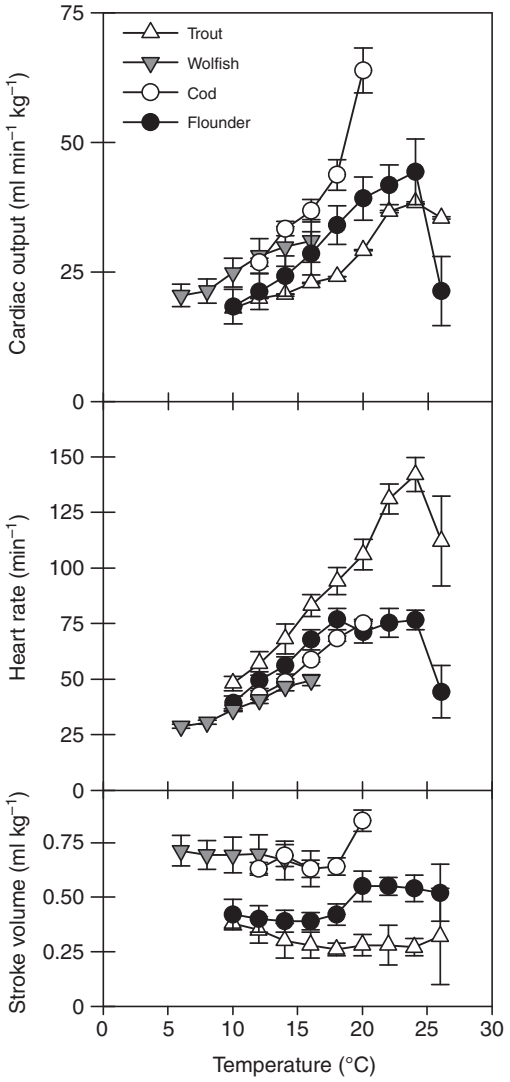

Fig. 7. Changes in cardiorespiratory variables in resting fishes during acute warming: a comparison of wolffish, winter flounder and Atlantic cod with rainbow trout. (Data kindly supplied by Dr Kurt Gamperl: wolffish – N. Joaquim and A. K. Gamperl, unpublished; trout – A. K. Gamperl, unpublished; Atlantic cod – L. H. Petersen and A. K. Gamperl, unpublished; flounder – P. C. Mendonca and A. K. Gamperl, unpublished.)

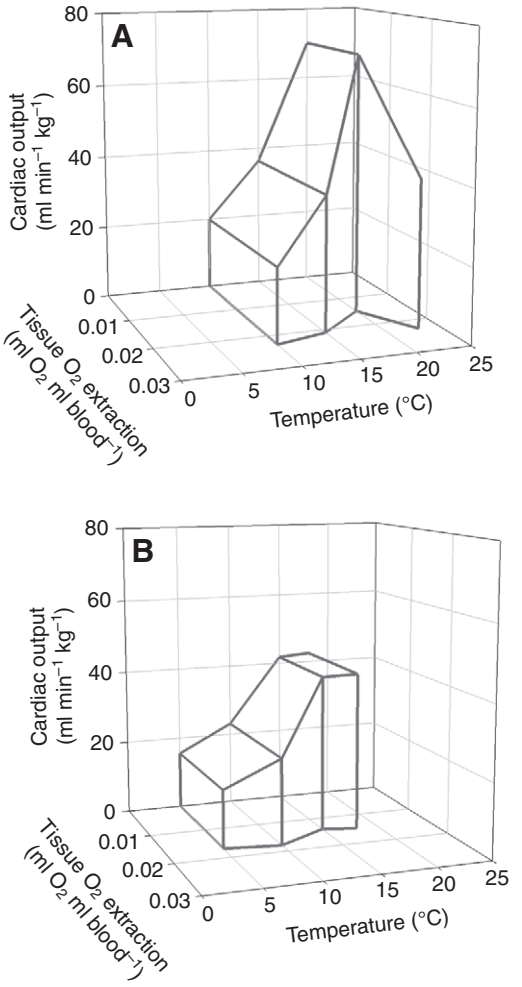

Fig. 8. Cardiac output ($\dot{V}_b$) and tissue oxygen extraction (Ca_{O_2}–Cv_{O_2}) for winter flounder either (A) seasonally acclimated to a temperature, or (B) acutely warmed by 5°C increments from the acclimation temperature. The x-y surface at each temperature represents oxygen uptake (the product of $\dot{V}_b$ and Ca_{O_2}), which clearly increases with temperature and either reaches a plateau between acclimation temperatures of 15 and 18°C, or collapses with an acute increase to 23°C. The greatest contributor to increases in $\dot{V}_{O_2}$ is almost always $\dot{V}_b$, which is a result of increased heart rate (see text) (Cech et al., 1975; Cech et al., 1976).

per 5°C increment). However, with warming from 18°C to a near-lethal temperature, cardiac output and cardiac stroke volume collapsed even though heart rate increased (Fig. 8). Ca_{O_2}, Pa_{O_2}, Cv_{O_2} and Pv_{O_2} were all maintained, except for 5°C- and 18°C-acclimated fish when tissue oxygen extraction increased (Fig. 8).

Heart rate may be a limiting factor during warming in decapod crustaceans as well. Heart rate is reported to reach a plateau near T_{crit} in various crab species: the spider crab [*Maja squinado* (Frederich and Pörtner, 2000)], the rock crab [*Cancer irroratus* (Frederich et al., 2009)] and the kelp crab [*Taliepus dentatus* (Storch et al., 2009)]. Cardiac stroke volume was also temperature insensitive in the kelp crab. Therefore, the upper limit for heart rate may emerge as a valuable, yet simple predictor of T_{opt} in active animals and T_{crit} in resting animals. If this is the case, biotelemetry of heart rate could easily extend this work to field situations (Clark et al., 2008b; Clark et al., 2009), allowing the full interplay of environmental factors on aerobic scope to be properly explored. Accompanying such fieldwork is the need to better understand the control of heart rate at high temperature and to determine if the heart is operating at its maximum pacemaker rate.

Temperature and the river migration of sockeye salmon

Beyond direct temperature reactions (i.e. acute effects occurring in minutes to hours considered above), two other time scales can be applied to temperature effects. Thermal adaptation spans generations and occurs at the population level through natural selection acting on individual variability. The study of heritable factors related to thermal tolerance is in its infancy. Thermal acclimation (or thermal compensation), however, occurs when an individual undertakes physiological and biochemical adjustments over days to weeks [or perhaps months for Antarctic fishes at near freezing temperatures (Franklin et al., 2007)]. Here, a new

phenotype emerges from an existing genome as an animal acclimates to a new thermal environment. Given the potential for thermal acclimation and adaptation, the obvious question becomes: Do the acute responses to temperature in fishes have any ecological or evolutionary relevance? In the specific case of adult sockeye salmon that return to the Fraser River, BC, Canada to spawn, the answer is categorically yes. During this return migration, sockeye salmon can experience large and rapid temperature changes when they make daily vertical ocean movements prior to river entry and exploit deeper, cool water in lakes (Fig. 9).

Adult sockeye salmon return migrations also provide a fascinating insight into something that is normally difficult to witness, an ecological significance for T_{opt} and T_{crit}. The linkage between aerobic scope and lifetime fecundity is obvious for sockeye salmon because their entire lifetime fitness hinges on a single, precise spawning date that is preceded by an energetic upstream migration lasting up to several weeks. Therefore, to spawn, they are committed to an upriver migration that periodically may require their full aerobic scope, with only a sensory imprint for navigation, while developing gonads, without feeding and without prior experience of the temperature conditions en route (Hinch et al., 2006). Consequently, if a warm river temperature reduces aerobic scope, sockeye salmon do not have an option of postponing reproduction as other fishes might do. In fact, with just 4–6 weeks to live after entering the river, even a slower migration could reduce lifetime fecundity.

Using Weaver Creek sockeye salmon as an example and considering only aerobic swimming, upstream migration should be favoured at 14.3°C (their T_{opt} for aerobic scope) but impossible at 20.4°C (their T_{crit}) (Lee et al., 2003). As predicted, when adult Weaver Creek sockeye salmon were intercepted in 2004, implanted with biotelemetry devices and released back to the river to follow their subsequent progress, migration success was inversely related to river temperature above T_{opt}. In fact, migration success was only 0–11% when river temperature was near T_{crit} (at 18–21°C), but increased to 77% when the river seasonally cooled to 14°C and near their T_{opt} (Farrell et al., 2008). This result suggests that a warm river temperature limited aerobic scope, and impaired upriver migration and lifetime fecundity. These warm river temperatures experienced by Weaver Creek sockeye salmon in 2004, which turned out to be record highs, contributed to a catastrophic 70% loss of the migrating population!

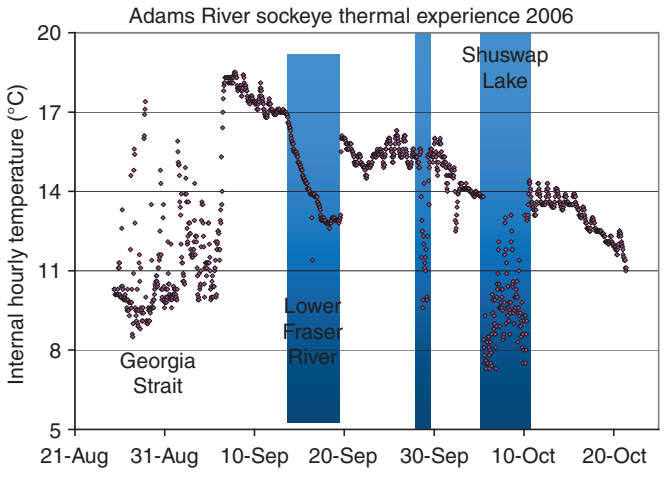

Fig. 9. Hourly temperature recordings from an I-button temperature logger that was recovered from an Adams River sockeye salmon after implantation in the peritoneal cavity in the Georgia Strait (ocean conditions) and a 40-day migration through the Fraser River watershed to its spawning area near the Shuswap Lake, BC, Canada. The highlighted areas represent periods where the fish behaviourally sought out water that was cooler than either the mainstem river or at the surface of lakes. The general downward trend over time represents seasonal cooling of the watershed, and daily oscillations in temperature can be resolved in the shallow spawning streams towards the end of the trace. (Data kindly supplied by David Patterson.)

Thermal acclimation

Warm acclimation alters thermal tolerance (Fry et al., 1942), increasing T_{opt}, T_{crit} and maximum aerobic scope (Fry and Hart, 1948). Warm acclimation, in addition to permitting a higher maximum heart rate, also decreases routine heart rate at the level of the pacemaker. This acclimatory change then provides compensation for the limitation that maximum heart rate imposes on aerobic scope by restoring the scope for heart rate either fully (Harper et al., 1995) or partially (Farrell, 1997). However, the benefits of temperature acclimation for specialists like salmon are small compared with temperature generalist. For example, CT_{max} for salmon increases by only 2°C over a 15°C acclimation temperature range *versus* an increase in CT_{max} of 10°C for goldfish over a 30°C acclimation range (Brett, 1956). In fact, routine and maximum heart rate in 22°C-acclimated sockeye salmon [86 beats min^{-1} and 106 beats min^{-1}, respectively (Brett, 1971)] are barely different for a 14°C-acclimated fish acutely warmed to 22°C [90 beats min^{-1} and 106 beats min^{-1} (Steinhausen et al., 2008)]. Other documented responses to warm acclimation, such as the decrease in cardiac mass (Gamperl and Farrell, 2004) and decrease in capillary density the red (slow aerobic) muscle of rainbow trout (Taylor et al., 1996; Egginton, 2000), even seem counterproductive. Conversely, compensatory decreases in gill epithelial thickness, as seen for other species (Taylor et al., 1997), would be beneficial.

Antecedents and concluding remarks

Like a salmon down on the Fraser, swimmin' with their battered fins,
Searchin' for their childhood home,
A patch of gravel they knew as their own.
Excerpt from 'The Ballad of Old Tom Jones' *by Barney Bentall*

The genomic information passed down by antecedents determines an individual's potential for survival, growth and reproduction. The antecedents of present day Fraser River salmon have passed on their environmental experiences through natural selection for over ~10,000 years since their post-glacial invasion. However, we have only ~60 years of reliable archival records of the river temperatures experienced during recent salmon migrations (Farrell et al., 2008). Nevertheless, remarkably the historic mean and median river migration temperature for Weaver Creek sockeye salmon is 14.5°C (their T_{opt} is 14.3°C). This observation, combined with the fact that the thermal window between T_{opt} and T_{crit} is only 7.3°C and that thermal acclimation provides little benefit to CT_{max}, suggests that their T_{opt} is potentially a product of natural selection. If this is the case, one has to question whether or not natural selection among sockeye salmon can accommodate the rapid warming trend already evident for the Fraser River (peak summer temperature has increased 1.8°C in the past 60 years).

If the salmonid genome is too inflexible to adapt to a new T_{opt}, perhaps the genetic determinants of the spawning date are more flexible. Dangerously high temperatures could then be avoided by

migrating when the river is seasonally cooler (see Keefer et al., 2008), but this may result in a fish encountering other unfavourable conditions such as faster river flows earlier in the year and an inevitable run-on-effect on the timing of larval emergence. Alternatively, warm water could be avoided behaviourally if opportunities exist. Behavioural temperature preferences are certainly shown by adult salmon during migration, which include seeking water cooler than their T_{opt} (Fig. 9) to lower $\dot{V}_{O_2}$ and perhaps slow energy depletion, suggest they likely know which temperature conditions are best for them. However, opportunities to seek cool refuges are very limited in the Fraser River (Donaldson et al., 2009). Without such behavioural responses, the warmer than normal river temperatures may force Pacific salmon near the southern limit of their geographic distribution to follow the fate of other species, a heart-breaking (Wang and Overgaard, 2006) northward shift in their distribution. The response of tropical coral reef fish species to climate change could be equally dramatic.

In closing, the best, albeit limited data set for a single animal group appears to provide a mechanistic understanding for the Fry curve. Heart rate, which is the main driver for the increase in $\dot{V}_{O_2}$ during warming, reaches its maximum rate at T_{opt} and becomes a weak link for the cardiorespiratory oxygen cascade. Shelford (Shelford, 1931) recognized that 'Animals are better short-period indicators (of environmental change) than plants' because animals can potentially move away from unfavourable environments. However, this behavioural response requires an aerobic scope, which is both controlled and limited by temperature. Future study on aerobic scope will continue to inform us of an animal's fundamental thermal niche. By contrast, a continued focus on temperature tolerances for resting animals will only inform us of thermal niche for existence and perhaps create needless worry about the precise techniques for such measurements (Chown et al., 2009).

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Glossary

Aerobic scope	the difference between maximum and minimum (standard or basal) oxygen uptake under a given set of test conditions
Ca_{O_2}	concentration of oxygen in arterial blood
Cv_{O_2}	concentration of oxygen in venous blood
CT_{max}	the critical thermal maximum that a fish can tolerate
CT_{min}	the critical thermal minimum that a fish can tolerate
Fry curve	the relationship between aerobic scope and temperature
Hb	haemoglobin
Pa_{O_2}	partial pressure of oxygen in arterial blood
P_{O_2}	partial pressure of oxygen
Pv_{O_2}	partial pressure of oxygen in venous blood
T_{crit}	the temperature at which a fish has no aerobic scope
T_{opt}	the temperature at which a fish has maximum aerobic scope
$\dot{V}_{O_2}$	rate of oxygen uptake

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MAXIMUM CARDIAC PERFORMANCE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AT TEMPERATURES APPROACHING THEIR UPPER LETHAL LIMIT

A. P. FARRELL, A. K. GAMPERL, J. M. T. HICKS, H. A. SHIELS AND K. E. JAIN
Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6

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Summary

Numerous studies have examined the effect of temperature on *in vivo* and *in situ* cardiovascular function in trout. However, little information exists on cardiac function at temperatures near the trout's upper lethal limit. This study measured routine and maximum *in situ* cardiac performance in rainbow trout (*Oncorhynchus mykiss*) following acclimation to 15, 18 and 22 °C, under conditions of tonic (30 nmol l^{-1}), intermediate (60 nmol l^{-1}) and maximal (200 nmol l^{-1}) adrenergic stimulation. Heart rate increased significantly with both temperature and adrenaline concentration. The Q_{10} values for heart rate ranged from 1.28 at 30 nmol l^{-1} adrenaline to 1.36 at 200 nmol l^{-1} adrenaline. In contrast to heart rate, maximum stroke volume declined by approximately 20 % (from 1.0 to 0.8 ml kg^{-1}) as temperature increased from 15 to 22 °C. This decrease was not alleviated by maximally stimulating the heart with 200 nmol l^{-1} adrenaline. Because of the equal and opposite effects of increasing temperature on heart rate and stroke volume, maximum cardiac output did not increase between 15 and 22 °C. Maximum power output decreased (by approximately 10–15 %) at all adrenaline concentrations as temperature increased. This reduction reflected a poorer pressure-generating ability at temperatures above 15 °C. These results, in combination with earlier work, suggest (1) that peak cardiac performance occurs around the trout's preferred temperature and well below its upper lethal limit; (2) that the diminished cardiac function concomitant with acclimation to high temperatures was associated with inotropic failure; (3) that Q_{10} values for cardiac rate functions, other than heart rate *per se* , have a limited predictive value at temperatures above the trout's preferred temperature; and (4) that heart rate is a poor indicator of cardiac function at temperatures above 15 °C.

Key words: heart, stroke volume, heart rate, cardiac output, temperature, adrenaline, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Much of our knowledge on fish cardiovascular responses to temperature change is limited to information on heart rate. There are two reasons for this. First, heart rate is the easiest cardiac variable to measure. Second, in mammals at least, heart rate is a reliable predictor of cardiac performance. If heart rate were equally reliable as a predictor of integrated cardiac function in fish, this large data base would have a tremendous predictive potential for fish cardiac performance. Unfortunately, there are many indications that heart rate alone is a poor predictor of integrated cardiac function in fish. Among the concerns are the following: (1) stroke volume can change by as much as, and even more than, heart rate when cardiac output increases (Farrell, 1991; Farrell and Jones, 1992); (2) the relative contributions of stroke volume and heart rate to changes in cardiac output vary between species and as a function of temperature (see Kolok and Farrell, 1994); (3) maximum stroke volume can decrease at high heart rates (Farrell *et al.* 1989); and (4) maximum isometric tension developed by cardiac muscle decreases at high contraction frequencies (i.e. a negative staircase effect) (Ask *et al.* 1981; Ask, 1983; Driedzic and Gesser, 1985, 1988). In view of these observations, it would be unwise to predict changes in cardiac performance from temperature-induced changes in heart rate alone. Indeed, a temperature-induced increase in heart rate does not necessarily produce a proportional increase in cardiac output (Brett, 1971; Yamamitsu and Itazawa, 1990; Kolok and Farrell, 1994).

The present study, which reports the first measurements of maximum cardiac performance in rainbow trout at temperatures near their upper lethal limit (23–25 °C; Black, 1953), extends our knowledge of how numerous variables (heart rate, stroke volume, cardiac output, power output) affect cardiac function in fish. The results support our contention that heart rate is a poor indicator of integrated cardiac performance in fish. In addition, this novel information should prove valuable in predicting the effects of increased environmental temperature on fish performance. Our approach was to use an *in situ* perfused heart to measure routine and maximum cardiac performance at 15, 18 and 22 °C after the fish had acclimated to these temperatures. The *in situ* perfused rainbow trout heart

is an appropriate model for investigating the relationship between high environmental temperature and cardiac performance since it is capable of performing at work levels equal to maximum *in vivo* levels (Farrell *et al.* 1989).

Materials and methods

Experimental animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (weighing 403–727 g) were obtained from a local supplier (West Creek Trout Farms, Aldergrove, BC, Canada) and maintained in a 2000 l fibreglass tank receiving dechlorinated Vancouver tapwater. Throughout the experiment only one stock of fish was used. All fish were initially maintained at 15 °C, before subsequent exposure to 18 and then 22 °C. Fish were acclimated at each temperature for at least 2 weeks prior to use. Water temperature was regulated to within 1 °C of the desired test temperature by a Min-O-Cool cooling unit (Frigid Units, Blissfield, MI, USA) and two countercurrent heat exchangers of local construction. Photoperiod was 12 h:12 h L:D. Fish were fed commercially prepared trout pellets daily.

Perfused heart preparations

Fish were anaesthetized in a buffered solution of tricaine methane sulphonate (0.1 g l^{-1} MS 222, with 0.1 g l^{-1} sodium bicarbonate) and transferred to an operating table where their gills were irrigated with aerated buffered anaesthetic at 4 °C (0.05 g l^{-1} MS 222 in 0.1 g l^{-1} sodium bicarbonate). Fish were injected with 0.6 ml of heparinized (100 i.u. ml^{-1}) saline *via* the caudal vessels, and an *in situ* heart preparation was obtained, as detailed in Farrell *et al.* (1986) and modified by Farrell *et al.* (1989). Briefly, an input cannula was secured into the sinus venosus through a hepatic vein and perfusion with saline containing 30 nmol l^{-1} adrenaline was begun immediately. Silk thread was used to secure the input cannula and to occlude any remaining hepatic veins. The output cannula was inserted into the ventral aorta at a point confluent with the bulbus arteriosus and tied firmly in place. Finally, silk ligatures were tied around each ductus Cuvier to occlude these veins and to crush the cardiac branches of the vagus nerve. This procedure left the pericardium intact, while isolating the heart in terms of saline input and output.

Once the surgery had been completed (15–20 min), the fish was immersed in a temperature-controlled saline bath at 15, 18 or 22 °C. The input cannula was attached to a constant-pressure reservoir and the output cannula was connected to a constant pressure head. Output pressure was initially set at 5 kPa to simulate resting *in vivo* ventral aortic blood pressure (Stevens and Randall, 1967), and filling (input) pressure was adjusted to give a cardiac output of 20 ml min^{-1} kg^{-1} body mass for the 15 and 18 °C groups. Cardiac output was set at 25 ml min^{-1} kg^{-1} body mass for the 22 °C group to account for temperature effects on *in vivo* resting cardiac output (Farrell and Jones, 1992). At all temperatures, the heart maintained this initial control level of performance for a period of 20 min to allow for recovery from surgery and for equilibration to the organ bath.

The saline in the organ bath and the perfusion reservoirs was maintained at the desired acclimation temperature by a Lauda cooling unit (Brinkmann Instruments, Rexdale, Ontario, Canada). The saline (pH 7.8 at 15 °C) contained (in mmol l^{-1}): NaCl, 124; KCl, 3.1; $MgSO_4 \cdot 7H_2O$, 0.93; $CaCl_2 \cdot 2H_2O$, 2.52; glucose, 5.6; Tes salt, 6.4; and Tes acid, 3.6 (Keen *et al.* 1994). The Tes buffer system was selected to simulate the buffering capacity of trout plasma and the normal change in blood pH with temperature ($\Delta pKa/dT = 0.016$ pH units $°C^{-1}$). The saline was equilibrated with 100 % O_2 for at least 30 min prior to experimentation. The coronary artery, which supplies the outer compact myocardium of the ventricle, was not perfused and so oxygenated saline was used to ensure that a sufficient amount of oxygen diffused from the ventricular lumen to the compact myocardium. The oxygen gradient from the lumen to the mycardium of our perfused heart was at least 20 times greater than that *in vivo*. The control saline contained 30 nmol l^{-1} adrenaline bitartrate because Graham and Farrell (1989) have established that tonic adrenergic stimulation with 10 nmol l^{-1} adrenaline is essential for long-term viability of perfused hearts at 5 °C. In addition, Keen *et al.* (1994) showed that trout acclimated to high temperatures (18 °C) have a decreased cardiac sensitivity to adrenaline.

Experimental protocols

The maximum pumping ability of the heart was assessed by measuring the following: (1) the ability of the heart to maintain stroke volume when exposed to increases in output pressure (i.e. homeometric regulation); (2) maximum cardiac output; (3) maximum power output; and (4) output pressure at maximum power output. Homeometric regulation was investigated by increasing diastolic output pressure from 4 to 8 kPa in increments of 1 kPa, or until cardiac output fell by 40 %. During homeometric regulation, the input pressure was maintained at control levels. Output pressure was not increased further to ensure that the heart was not damaged prior to the measurement of maximum cardiac output and maximum power output. In fish swimming maximally, or exposed to high adrenaline doses, diastolic ventral aortic pressure is unlikely to exceed 8 kPa (Kiceniuk and Jones, 1977; Gamperl *et al.* 1994*a*). Maximum cardiac output was determined under control conditions by increasing filling pressure in 8–12 steps (in increments of 0.005–0.01 kPa) until cardiac output reached a maximum value. Once maximum cardiac output had been attained, output diastolic pressure was raised in steps of 0.5–1 kPa until the maximum power output was reached. The output pressure at this point was noted. Each step in filling and output pressure was maintained for approximately 1–2 min. The heart was returned to the control work load for a recovery period of 15 min after the determination of homeometric regulation and following the determination of maximum power output. This allowed the heart to recover fully between tests and/or to equilibrate to new adrenaline concentrations. This series of experimental procedures required approximately 1 h to complete.

All cardiovascular measurements were repeated at two additional adrenaline concentrations (60 and 200 nmol l^{-1}) to

cover the range for circulating catecholamine levels observed in stressed rainbow trout (Milligan *et al.* 1989; Gamperl *et al.* 1994*b*; Randall and Perry, 1992). In addition, preliminary experiments at 15 °C (Fig. 1) showed that 200 nmol l^{-1} adrenaline achieved maximum adrenergic stimulation of the *in situ* preparation and that 60 nmol l^{-1} adrenaline was near the EC_{50} for maximum stimulation.

Instrumentation and analysis

An in-line electromagnetic flow probe (Zepeda instruments, Seattle, Washington, USA) was used to record mean cardiac output, and pressure transducers (Narco Life Sciences, Houston, TX, USA) were used to measure input and output pressures through saline-filled side-arms. Prior to experimentation, pressure changes due to cannula resistance were calculated at known flow rates. These values were then used to adjust input and output pressure to the levels experienced by the sinus venosus and bulbus arteriosus, respectively. Calibration of the pressure transducers was performed daily against a static water column. Pressure and flow signals were amplified and displayed on a four-channel chart recorder (Gould, Cleveland, OH, USA) in conjunction with a microcomputer running Labtech Notebook (Laboratory Technologies Corporation, Wilmington, MA). Data were collected at 5 Hz, and block averages were calculated every 15 s. Heart rate was measured by counting the number of systolic peaks recorded during a 10 s period.

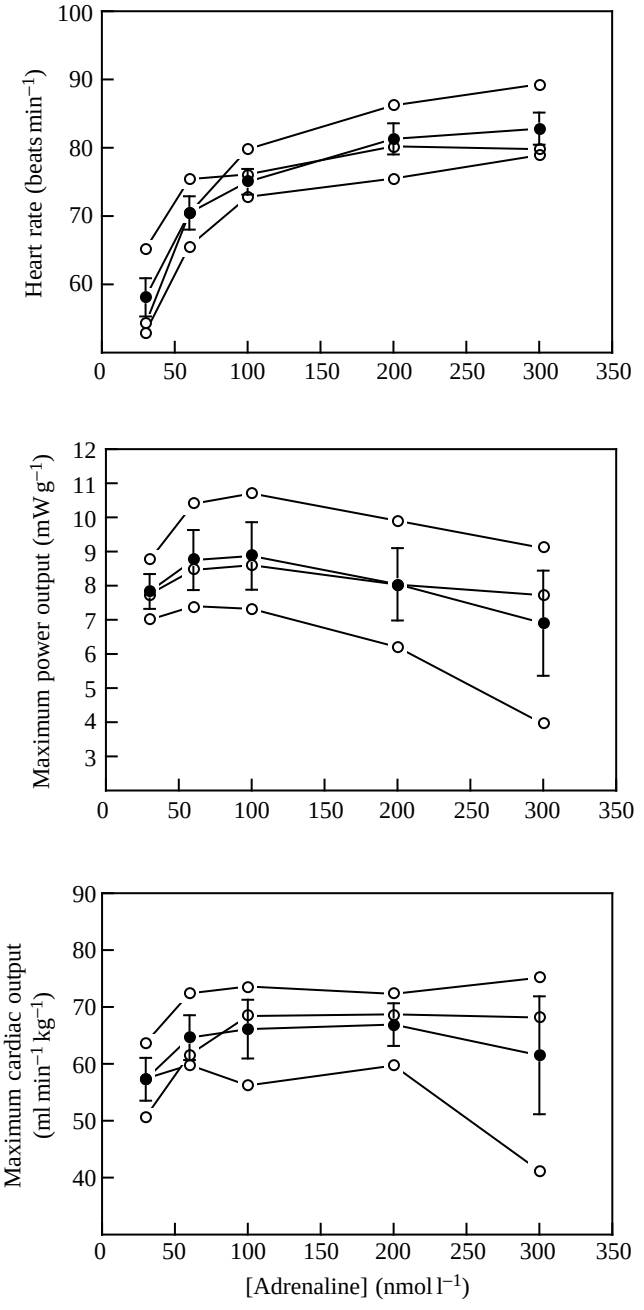

Fig. 1. Relationship between adrenaline concentration and cardiovascular variables for *in situ* perfused trout hearts at 15 °C. Open circles represent individual hearts and filled circles represent group means (N=3). Vertical bars represent ± 1 S.E.M.

Stroke volume and power output were calculated as follows:

$$V_S = (\dot{Q}/f_H)/M_b, \quad (1)$$

$$p = [\dot{Q} \times (P_o - P_i) \times \alpha]/M_v, \quad (2)$$

where $\dot{Q}$ (ml min^{-1}) is cardiac output, P_o is mean output pressure (kPa), P_i is mean filling pressure (kPa), V_S is stroke volume (ml kg^{-1} body mass), f_H is heart rate (beats min^{-1}), M_b is body mass (kg), p is power output (mW g^{-1} ventricle mass), M_v is ventricular mass (g) and α is 0.00162 mW min ml^{-1} kPa^{-1}.

Within each temperature group, paired t-tests were used to identify statistical differences between cardiovascular variables recorded at 30, 60 and 200 nmol l^{-1}. The effect of temperature within a particular adrenaline concentration was assessed using a one-way analysis of variance (ANOVA). A covariant analysis of variance (ANCOVA) was applied to the stroke volume–heart rate relationship to isolate the interactive effects of temperature and adrenaline. A general linear model (Zar, 1974) was used to examine whether temperature and adrenaline affected the relationship between filling pressure and stroke volume (i.e. the Starling curve) (Proc GLM, SAS Institute). For all statistical analyses, the fiducial limit of significance was chosen as 5 %. Values throughout the text are expressed as means ± S.E.M.

Results

Homeometric regulation

In our experience, cardiac failure is not normally a problem once the surgery has been completed. This fact is illustrated by the success of all seven of the preparations attempted at 15 °C. However, at temperatures above 15 °C, increases in output pressure either during the initial elevation to control conditions (5 kPa final pressure) or during the first homeometric regulation test caused cardiac failure (indicated by sustained cardiac arrhythmia) in some hearts. At 18 °C, two of the 13 attempts failed, and at 22 °C the failure rate reached 40 % (six out of 15 preparations). In these failing preparations, increasing the adrenaline concentration to 60 nmol l^{-1} occasionally

restored the normal beat frequency, but only temporarily. These observations suggest that above 15 °C there was a particularly strong negative effect of temperature on the pressure-generating ability of certain hearts. As a result, it should be remembered that the mean values we report for cardiac variables do not take into account the fact that a proportion of heart preparations failed at 18 °C and 22 °C.

At all temperatures, an increase in diastolic output pressure significantly decreased resting stroke volume (Fig. 2). There was also a significant effect of temperature on the relationship between resting stroke volume and temperature. At 15 °C, stroke volume was maintained above 90 % of the resting value even at an output pressure of 8 kPa. In contrast, at 18 °C stroke volume was reduced to less than 80 % of the resting value at an output pressure of only 7 kPa (Fig. 2). There was no significant difference between the curves for 15 °C and 22 °C.

Adrenergic stimulation had no marked effect on the general shape of the homeometric relationships (Fig. 2). However, adrenaline consistently displaced the relationship downwards because adrenergically mediated increases in heart rate meant that the set point for resting stroke volume was lower (see below).

Heart rate

Heart rate increased significantly with both temperature and adrenaline concentration (Fig. 3A). Increasing the temperature from 15 to 22 °C increased heart rate by 13.9 beats min^{-1} (from 69.9 to 83.8 beats min^{-1}) with 30 nmol l^{-1} adrenaline, and by 20.8 beats min^{-1} (from 81.5 to 102.3 beats min^{-1}) with 200 nmol l^{-1} adrenaline. The Q_{10} values for 30 nmol l^{-1} and 200 nmol l^{-1} adrenaline were calculated as 1.28 and 1.36, respectively.

Increasing the adrenaline concentration from 30 to 200 nmol l^{-1} significantly increased heart rate at all temperatures. This increase was 18.5 beats min^{-1} (22 %) at 22 °C and approximately 10 beats min^{-1} at 15 °C (17 %) and 18 °C (13 %).

Stroke volume

In almost all cases, the filling pressures at the routine work loads were subambient and there were no significant effects of temperature on the filling pressure required to generate routine cardiac output (Table 1). Increasing the filling pressure generated a typical Starling curve for stroke volume at all temperatures. However, acclimation temperature significantly altered the shape of the Starling curve (Fig. 4). Acclimation to higher temperatures (18 and 22 °C) caused a significant downward shift in the upper arm of the Starling curve (Fig. 4) and significantly decreased maximum stroke volume (Figs 3B, 4). Maximum stroke volumes were approximately 1 ml kg^{-1} at 15 °C and 0.8 ml kg^{-1} at 22 °C. Adrenaline had no significant effect on maximum stroke volume regardless of the acclimation temperature (Fig. 3B).

Maximum cardiac output

Although there was some variability in cardiac output between temperature groups, there were no significant differences between values. Furthermore, it is clear that maximum cardiac output with 60 and 200 nmol l^{-1} adrenaline was unchanged by acclimation temperature (Fig. 3C). This indicates that the temperature-induced increases in heart rate

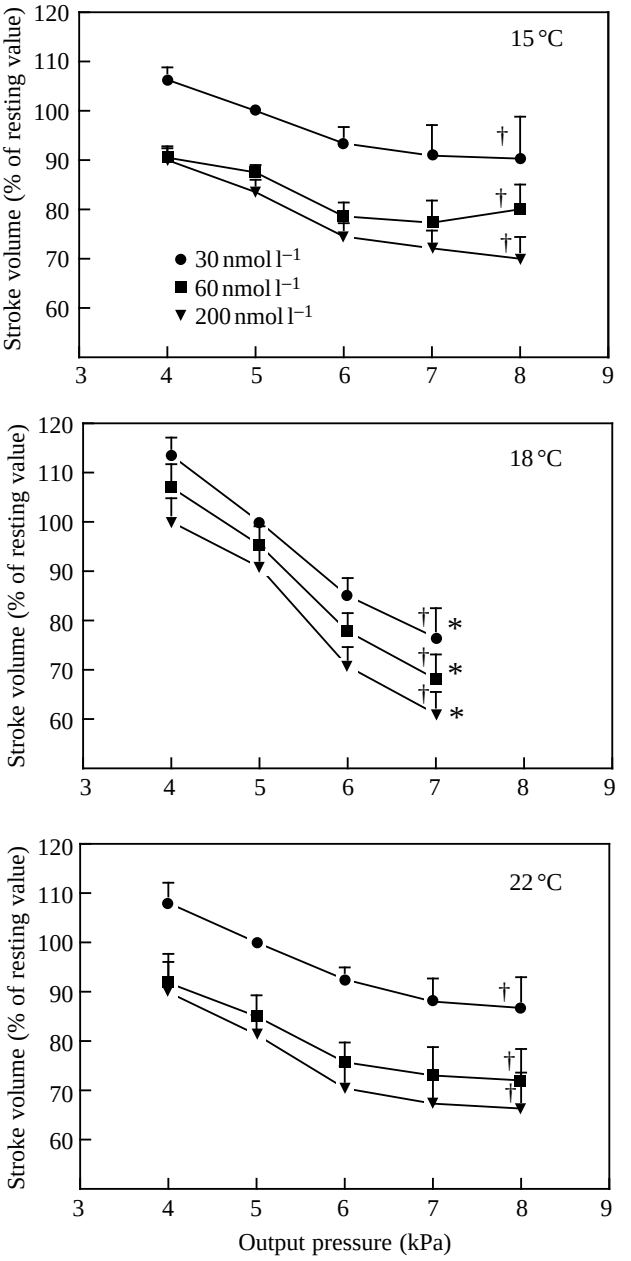

Fig. 2. Relationship between stroke volume and output pressure for *in situ* hearts exposed to various temperatures (15 °C, *N*=7; 18 °C, *N*=11; 18 °C, *N*=8) and adrenaline concentrations. Measurements for hearts at 18 °C and 8 kPa of output pressure are not shown because further increases in output pressure greatly reduced cardiac output (see Materials and methods). A dagger denotes a significant ($P<0.05$) decrease in the stroke volume at the highest output pressure tested when compared with the resting value. An asterisk indicates a significant difference from the value for stroke volume of the 15 °C fish tested at the highest output pressure. Vertical bars represent ± 1 S.E.M.

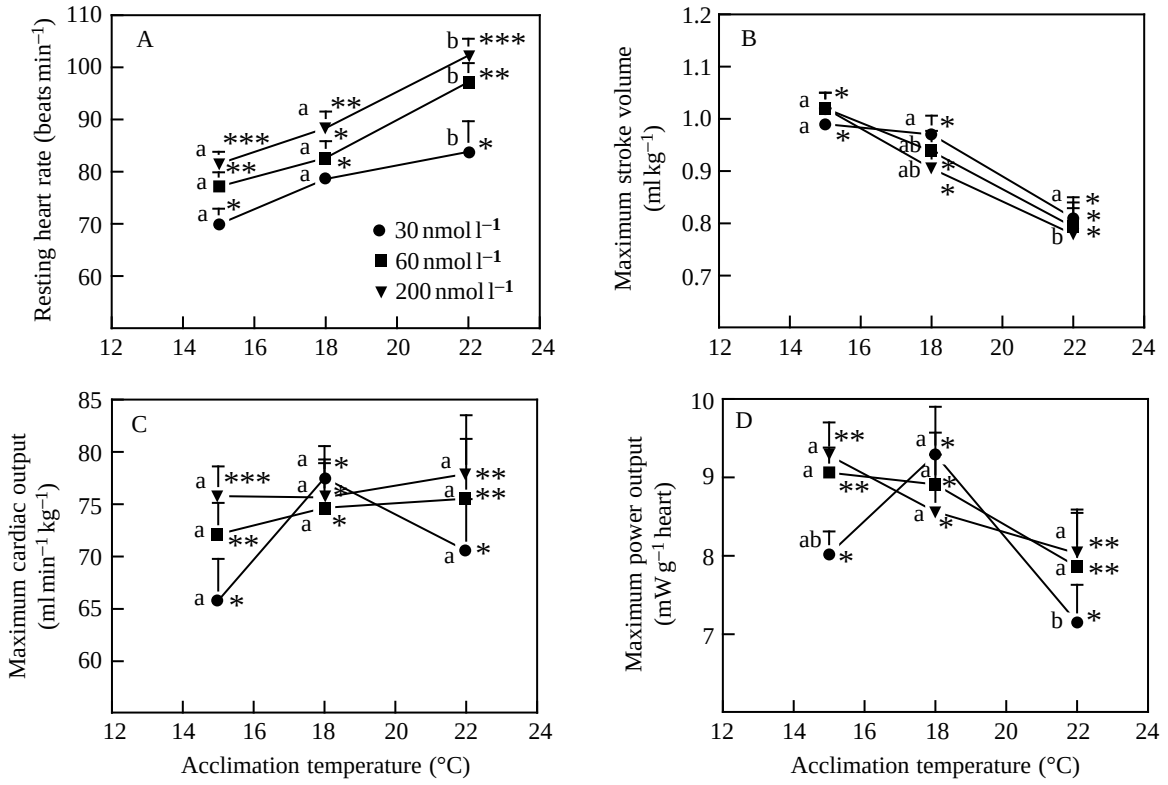

Fig. 3. Relationship between acclimation temperature and (A) resting heart rate, (B) maximum stroke volume, (C) maximum cardiac output and (D) maximum power output for *in situ* perfused trout hearts at 15 (*N*=7), 18 (*N*=11) and 22 °C (*N*=8). Vertical bars represent 1 S.E.M. Dissimilar letters indicate values that are significantly different ($P<0.05$) between acclimation temperatures within each adrenaline concentration. Within each temperature, means with an unequal number of asterisks indicate significant differences between adrenaline concentrations ($P<0.05$).

were offset by equal and opposite changes in maximum stroke volume. This point is illustrated in Fig. 5, where the 29% increase in heart rate between 15 and 22 °C was associated with a 23% reduction in stroke volume.

Within the 15 and 22 °C temperature groups, there were statistically significant effects of adrenaline (Fig. 3C). Increasing the adrenaline concentration from 30 to 200 nmol l^{-1} increased maximum cardiac output by 10 ml min^{-1} kg^{-1} (15%) at 15 °C and by 7.5 ml min^{-1} kg^{-1} (10%) at 22 °C. There was no significant effect of adrenaline on cardiac output at 18 °C.

Maximum power output

While the homeometric regulation test and the failure of a high proportion of hearts hinted at poorer inotropic performance under resting conditions at 22 °C, a reduced maximum power output was a clear indicator of inotropic failure at this acclimation temperature. Maximum power

Table 1. *Morphometric and cardiovascular variables for rainbow trout (*Oncorhynchus mykiss*) acclimated to 15, 18 and 22 °C*

Test temperature (°C)	Body mass (g)	Heart mass (g)	RVM (%)	Resting P_i (kPa)			P_o at maximum power (kPa)		
				30 nmol l^{-1}	60 nmol l^{-1}	200 nmol l^{-1}	30 nmol l^{-1}	60 nmol l^{-1}	200 nmol l^{-1}
15 (*N*=7)	493.1±29.7[a]	0.40±0.1[a]	0.081±0.01	–0.08±0.02	–0.08±0.02	–0.08±0.03	7.14±0.28[a]	7.38±0.23[a]	7.20±0.33[a]
18 (*N*=11)	515.1±28.9[a,b]	0.40±0.0[a]	0.078±0.00	–0.07±0.04	0.00±0.03	–0.01±0.04	6.84±0.15[a,b]	6.80±0.16[a,b]	6.14±0.19[b]
22 (*N*=9)	606.5±41.7[b]	0.53±0.0[b]	0.088±0.00	–0.07±0.03	–0.06±0.02	–0.07±0.03	6.26±0.21[b]	6.60±0.27[b]	6.13±0.24[b]

Resting input pressure, and output pressure at maximum power output, were recorded at three different adrenaline concentrations (30, 60 and 200 nmol l^{-1}) using an *in situ* heart preparation.

Values are expressed as means ± 1 S.E.M.

RVM, relative ventricular mass.

Dissimilar letters indicate significantly different values ($P<0.05$) within a column.

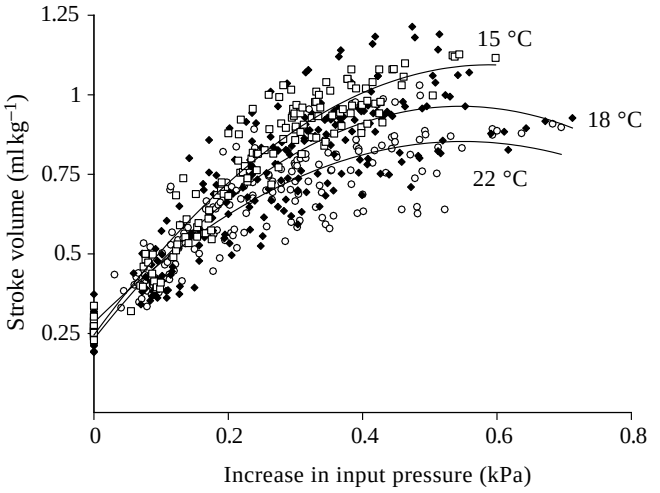

Fig. 4. Starling curves for *in situ* perfused trout hearts at acclimation temperatures of 15 (N=7, □), 18 (N=11, ◆) and 22 °C (N=9, ○). Within each temperature, each point represents data for an individual heart at adrenaline concentrations of 30, 60 and 200 nmol l^{-1}. Best-fitting equations for each acclimation temperature were: 15 °C, $y=-2.439x^2+2.878x+0.245$ (r^2=0.942); 18 °C, $y=-2.454x^2+2.678x+0.233$ (r^2=0.829); 22 °C, $y=-1.887x^2+2.072x+0.284$ (r^2=0.824). Analysis of covariance showed that the stroke volume–input pressure relationships at all temperatures were significantly different from each other (P<0.05).

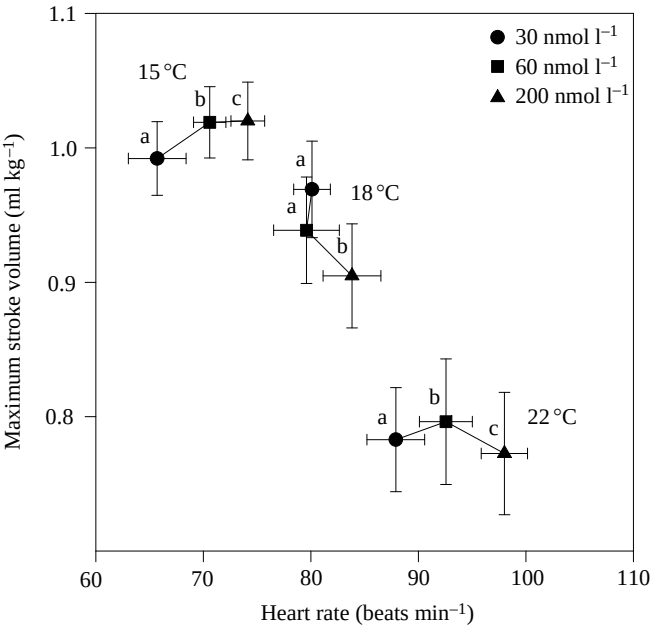

Fig. 5. The relationship between maximum stroke volume and heart rate for *in situ* perfused trout hearts at various temperatures and adrenaline concentrations. Values are expressed as means ± 1 S.E.M. Dissimilar letters within temperature groups indicate significant differences (P<0.05) between adrenaline concentrations. Means for each adrenaline concentration were significantly different between temperatures (P<0.05).

output was significantly lower at 22 °C that at 15 °C, irrespective of the adrenaline concentration (Fig. 3D). This reduction in maximum power output occurred primarily because the maximum pressure-generating ability of the heart was significantly lower (Table 1), since maximum cardiac output was unaffected (Fig. 3C).

Discussion

The present study, which is the first to measure the maximum performance of a perfused salmonid heart at temperatures near the upper lethal temperature, clearly shows that maximum cardiac output reaches a plateau at approximately 15 °C and that temperatures above 18 °C are associated with a reduced pressure-generating ability. This conclusion is consistent with *in vivo* measurements made on another salmonid, the sockeye salmon (*Oncorhynchus nerka*). Davis (1968) and Brett (1971) found that cardiac output in swimming sockeye salmon was essentially unchanged between 15 and 22 °C. In addition, they estimated that cardiac work during activity had a peak at 15 °C because ventral aortic blood pressure was lower at 22 °C than at 15 °C. If it is assumed that maximal prolonged swimming activity elicits a maximal

Table 2. *A comparison of maximum cardiac performance variables at various temperatures using* in situ *heart preparations with intact pericardia and tonic (5–30 nmol l^{-1} adrenaline) or maximal levels of adrenergic stimulation (values in parentheses)*

Temperature (°C)	Heart rate (beats min^{-1})	Stroke volume (ml kg^{-1})	Cardiac output (ml min^{-1} kg^{-1})	Power output (mW g^{-1})	Reference
8	52	0.96	50	6.1	Keen and Farrell (1994)
10	50	0.97	53	5.9	Farrell *et al.* (1988)
10	66	0.69	46	6.5	Milligan and Farrell (1991)
10	62 (73)	0.89 (0.86)	56 (63)	5.2 (6.9)	Farrell *et al.* (1991)
10 (TR)	66 (75)	1.05 (1.05)	67 (75)	6.7 (8.7)	Farrell *et al.* (1991)
15	70 (82)	0.99 (1.02)	66 (76)	8.00 (9.3)	Present study
18	78	0.79	62	8.81	Keen and Farrell (1994)
18	79 (88)	0.97 (0.91)	78 (76)	9.3 (8.6)	Present study
22	84 (102)	0.81 (0.77)	71 (78)	7.2 (8.0)	Present study

TR indicates that this group of fish was exercise-trained for 1 month.

cardiac response in sockeye salmon, then the sockeye salmon's maximal *in vivo* cardiac performance peaks at its preferred temperature (12–14 °C; Brett, 1971) and well below its upper lethal limit (24 °C). We believe that a similar conclusion can be drawn for the rainbow trout, whose preferred temperature and upper lethal temperature are almost identical to those of the sockeye salmon (Black, 1953; Garside and Tait, 1958).

When using a perfused trout heart preparation without a coronary circulation, the exchange of gases and solutes between the myocardium and the perfusate will be affected. This is of particular concern in the present study for two reasons. First, the experimental conditions promoted cardiac failure and, second, any problems with diffusion may have been exacerbated in the hearts of the 22 °C fish because their ventricles were 30 % larger than those of the 15 °C fish. Nonetheless, there are several important reasons why the absence of a coronary circulation was unlikely to bias the experiments towards the poorer cardiac performance observed at 22 °C. Foremost, there is good agreement between the cardiac performance measures for our perfused *in situ* hearts and reported *in vivo* values for swimming sockeye salmon (Davis, 1968; Brett, 1971). In both studies, heart rate increased at 22 °C while stroke volume, cardiac output, pressure generation and cardiac power output decreased. Second, by limiting fish size to less than 750 g and oxygenating the perfusate, we believe that any problem with oxygen diffusion into the myocardium was largely eliminated. The oxygen partial pressure gradient was at least 20 times that normally found in venous blood, and the thickness of the compact myocardium in our fish (<1 mm) was no more than that used in examining cardiac performance with electrically paced, isolated strips. Third, Davie and Farrell (1991) were unable to improve the performance of normoxic dogfish hearts by perfusing the coronary circulation with air-saturated saline. Although we may have eliminated the possibility of an oxygen limitation, there is an additional concern regarding solute transfer, particularly the removal of H^+ and K^+, which in themselves could reduce heart contractility. We know from previous studies that lactate and H^+ are released into the lumen and can be measured in the perfusate leaving the trout heart (Farrell and Milligan, 1986). Therefore, transfers of solute from the trout myocardium are far from completely inhibited. If the larger hearts of the 22 °C group did lead to a poorer cardiac performance, we would predict a negative correlation between heart size and cardiac power output. However, no significant relationship exists between these two variables (r^2=0.30). In view of the above discussion, we feel confident in extrapolating our observations to the *in vivo* situation and in providing mechanistic explanations.

To illustrate the point that peak cardiac performance occurs at approximately 15 °C in rainbow trout, Table 2 summarizes the available data on maximum cardiac performance of perfused rainbow trout hearts at various temperatures. The data in Table 2 are comparable because they were collected in the same laboratory using the same type of heart preparation (i.e. an *in situ* heart with an intact pericardium) and an initial tonic adrenergic stimulation (5–30 nmol l^{-1} adrenaline). Table 2 clearly shows that the maximum stroke volume under conditions of tonic adrenergic stimulation occurs between 10 and 15 °C, whereas maximum cardiac output and maximum power output occur at 18 °C. Although these data suggest that maximum cardiac performance in rainbow trout occurs at 18 °C, it is unlikely that *in vivo* maximal cardiac performance is achieved without significant adrenergic stimulation (humoral and/or sympathetic). Under conditions of 'maximal' adrenergic stimulation, cardiac output remains constant between 10 and 22 °C, an effect which shifts the optimum temperature for maximum power output to 15 °C (Table 2; Fig. 3D). Because power output is the most appropriate index of integrated cardiac performance, it appears that maximum performance of rainbow trout hearts is achieved at 15 °C.

Temperature is generally regarded as having positive chronotropic and negative inotropic effects on the teleost myocardium (Lennard and Huddart, 1992; Matikainen and Vornanen, 1992). The present study supports this generalization with regard to both the chronotropic and inotropic effects of temperature. Matikainen and Vornanen (1992) nicely illustrated the simultaneous and opposing effects of temperature-related negative inotropy and positive chronotropy using isolated carp cardiac muscle. By deriving a maximum tissue pumping capacity term (the product of the spontaneous heart rate and the maximum isometric force; g mg^{-1} tissue min^{-1}), they demonstrated a peak tissue pumping capacity at approximately 20 °C, a temperature well below the upper lethal temperature of carp (approximately 35 °C). The performance curve for isolated carp cardiac muscle strips had an inverted U shape as a function of temperature. Consequently, the decline in tissue pumping capacity of the carp heart at warm temperatures bears a striking resemblance to the decline in maximum power output in *in situ* rainbow trout hearts (see Table 2; see Fig. 6) and *in vivo* in sockeye salmon (Brett, 1971). In all three instances, there was a decrease in inotropic performance and/or decreased maximum stroke volume at higher temperatures.

Inotropic failure in our rainbow trout hearts at temperatures greater than 15 °C was demonstrated by lower values for maximum power output and maximum output pressure at 18 and 22 °C. In addition, the significance of this result is magnified when one considers that the failure of a number of preparations at these temperatures resulted in only the stronger hearts being tested (this bias may in fact explain why the homeometric regulation curves were similar for 15 and 22 °C hearts). The finding that rainbow trout hearts had a poorer pressure-generating ability at temperatures above 15 °C has indirect support from *in vivo* studies. For example, Davis (1968) reported reduced ventral and dorsal aortic blood pressures in swimming sockeye salmon at 22 °C compared with values at 15 °C, even though cardiac output was the same. Also, Wood *et al.* (1979) found a significant attenuation of the increase in dorsal aortic blood pressure in rainbow trout in response to adrenaline injections at 22 °C compared with 12 °C. Thus, in both of these studies, the heart performed less

pressure work at 22 °C. These *in vivo* observations could be related to increased temperature directly affecting the normal neural, hormonal and local control of vasomotor status in the systemic circulation (i.e. either a greater vasodilatory capacity or a weaker vasoconstrictory capacity). However, on the basis of the present observations, we can include another possibility. In response to a poorer cardiac pressure-generating ability at 22 °C, the vasomotor system may produce a vasodilatation to allow for the maintenance of cardiac output. Interestingly, Gamperl *et al.* (1994*a*) reported that adrenaline injection into rainbow trout resulted in significantly lower *in vivo* dorsal aortic pressures if the pericardium was opened. Opening the pericardium is known to cause poorer pressure generation in both the rainbow trout (Farrell *et al.* 1988) and the eel (*Anguilla dieffenbacchi*) (Franklin and Davie, 1991), and reduces maximum power output in the rainbow trout heart by approximately 45 %.

Work on isolated cardiac muscle strips from teleost fish clearly shows that maximum tension decreases with increasing pacing frequencies, a negative staircase effect (Driedzic and Gesser, 1985; Vornanen, 1989; Bailey and Driedzic, 1990). It seems likely that this negative staircase effect would explain the negative inotropic effect of warm temperature in our hearts. Indeed, there is a decrease in force when the spontaneous beat frequency increases with temperature (Ask, 1983; Matikainen and Vornanen, 1992), and indications are that factors associated with either a shortening of the duration of the active state or a reduction in the intensity of the active state may become limiting at high beat frequencies (Vornanen, 1989; Driedzic and Gesser, 1994). The factors could include the inability of the contractile proteins to generate maximal force at shortened active states or impaired calcium delivery to and removal from the contractile proteins (Vornanen, 1989; Matikainen and Vornanen, 1992).

Nevertheless, alternative explanations for the reduction in maximum power output and maximum pressure-generating ability with increasing temperature should not be excluded at this time. For example, decreases in β-receptor number and/or affinity could have diminished the positive inotropic influence of adrenaline. In ventricular strips, Keen *et al.* (1993) showed that it takes approximately 10 times the adrenaline concentration at 18 °C to achieve the same level of tension generation measured at 8 °C, and that this effect was associated with fewer sarcolemmal β-adrenoreceptors. In addition, Ask *et al.* (1981), using atrial tissue, showed that the contractile force elicited by a maximally effective dose of adrenaline ($1.4\,\mu mol\,l^{-1}$) at 14 °C was only 30 % of that produced at 2 °C. Although the observation that heart rate increased with increasing adrenaline concentration at all temperatures (Fig. 3A) is apparently inconsistent with diminished adrenergic influence at high temperatures, it must be remembered that positive chronotropy is mediated primarily through effects on the heart's pacemaker cells (Huang, 1973), whereas inotropic effects occur primarily because of adrenergic stimulation of the ventricle. Thus, there could be differential temperature effects on adrenergic sensitivity for these two regions of the heart.

It is clear from the present study that the application of Q_{10} values to maximum cardiac output has a limited value. At temperatures above the preferred temperature, Q_{10} values could be misleading because of the plateau in maximum cardiac output. Furthermore, because temperatures above 15 °C are associated with increased heart rates but constant cardiac output and falling power outputs, heart rate must be considered to be a very poor predictor of cardiac performance at these temperatures.

Maximum stroke volume decreased with increasing temperature (Fig. 3B). Previous studies with *in situ* trout hearts have also reported that maximum stroke volume decreased with increasing temperature (Graham and Farrell, 1990; Keen and Farrell, 1994). Likewise, Yamamitsu and Itazawa (1990) showed that stroke volume decreased with increasing temperature in the isolated carp heart, although it is unlikely that they measured maximum performance. The data presented in Table 2 suggest that maximum stroke volume of the *in situ* rainbow trout heart (approximately $1\,ml\,kg^{-1}$) occurs at temperatures of 15 °C and below. In a heart preparation with a punctured pericardium, Graham and Farrell (1990) showed that stroke volume decreased from $1\,ml\,kg^{-1}$ at 5 °C to $0.7\,ml\,kg^{-1}$ at 15 °C. Because stroke volume in our *in situ* heart with an intact pericardium was still $1\,ml\,kg^{-1}$ at 15 °C, it is possible that the pericardium plays an important role in maintaining maximum stroke volume at warm temperatures.

Heart rate clearly had an important influence on maximum stroke volume (Fig. 5). This agrees with numerous previous studies where temperature-induced decreases in stroke volume were associated with concomitant increases in heart rate (Graham and Farrell, 1989; Lennard and Huddart, 1992; Keen and Farrell, 1994). There are two possible explanations for this inverse relationship between heart rate and maximum stroke volume: either a limitation on cardiac filling or the negative staircase effect on cardiac contractility referred to above. To what degree these two factors influence stroke volume at higher heart rates can be resolved only by direct measurements of heart chamber volumes during the cardiac cycle. Using echocardiography, Franklin and Davie (1992) showed that ventricular end-systolic volume in rainbow trout is normally near zero. Therefore, if a negative staircase effect is involved in the reduced stroke volume at high heart rates, end-systolic volume would be found to increase. In contrast, a lower end-diastolic volume would account for the decrease in stroke volume if filling time was a problem, as suggested by Farrell *et al.* (1989) to explain a decrease in maximum stroke volume of $0.2\,ml\,kg^{-1}$ when isolated trout hearts were paced at 60 beats min^{-1}. One piece of evidence which suggests that limitations on cardiac filling may contribute to the decrease in stroke volume at high heart rates comes from studies on *in situ* hearts with intact (present study) *versus* punctured pericardia (Graham and Farrell, 1990). Stroke volume decreased by $0.3\,ml\,kg^{-1}$ (from $1\,ml\,kg^{-1}$) when intrinsic heart rate reached 60 beats min^{-1} in hearts with a punctured pericardium. In contrast, hearts with an intact pericardium were able to maintain maximum stroke volume until heart rate exceeded

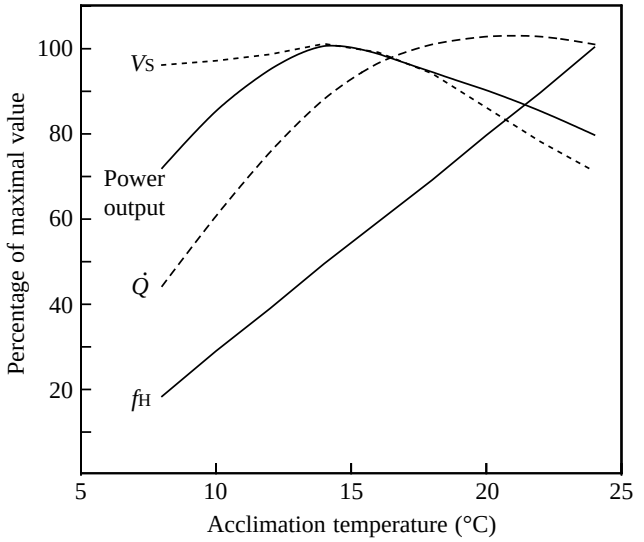

Fig. 6. Proposed relationship between acclimation temperature and the maximal level of cardiovascular variables for the rainbow trout. The 100 % values for stroke volume (V_S), heart rate (f_H), power output and cardiac output ($\dot{Q}$) are $1\,ml\,kg^{-1}$, $120\,beats\,min^{-1}$, $9.5\,mW\,g^{-1}$ and $80\,ml\,min^{-1}\,kg^{-1}$, respectively.

$80\,beats\,min^{-1}$. A mechanistic explanation for the enhanced maintenance of maximum stroke volume in hearts with an intact pericardium is that the pericardium in rainbow trout permits *vis-a-fronte* filling of the heart (Farrell *et al.* 1988) and this type of cardiac filling is likely to be faster than *vis-a-tergo* filling (Farrell and Jones, 1992).

Fig. 6, while somewhat speculative at this time, summarizes our ideas on cardiac performance in rainbow trout as a function of acclimation temperature. We hope that it will provide a useful framework for further research in this area. Heart rate follows a Q_{10} relationship up to the upper lethal temperature, where it reaches its maximum level of approximately $120\,beats\,min^{-1}$. Maximum stroke volume (approximately $1\,ml\,kg^{-1}$) is maintained up to the preferred temperature, above which it decreases. For several degrees above the preferred temperature, the decrease in stroke volume is matched by the increase in heart rate. Consequently, maximum cardiac output (approximately $80\,ml\,min^{-1}\,kg^{-1}$) has a broad plateau extending for several degrees higher than the preferred temperature. In contrast, the pressure-generating ability of the heart decreases at temperatures higher than the preferred temperature such that peak maximum power output (around $9.5\,mW\,g^{-1}$) occurs around the preferred temperature.

Mechanistic explanations for the decline in maximum performance above the preferred temperature require further work at both the organ and tissue level. However, the observation that maximum stroke volume was not maintained at high temperatures suggests that myocardial adaptations are quite limited above the preferred temperature. This is not the case at colder temperatures. For example, cold acclimation results in a larger cardiac mass (Graham and Farrell, 1989) and a greater number of sarcolemmal adrenoceptors that increase the sensitivity of the trout heart to adrenaline (Keen *et al.* 1993). As a result of cold-acclimation, maximum stroke volume and power output tend to be higher than otherwise possible with the accompanying temperature-dependent decrease in heart rate.

Whether any of the above generalizations apply to other teleost species, such as sockeye salmon and carp, remains to be determined. However, it is clear for the rainbow trout (1) that maximum cardiac performance declines at temperatures above the preferred temperature; (2) that the usefulness of Q_{10} relationships for cardiac functions other than heart rate is highly dependent upon the section of the thermal regime of the fish under consideration; and (3) that heart rate is a poor indicator of integrated cardiac function at temperatures above the preferred temperature.

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Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*

Kamini E. Jain and Anthony P. Farrell*

Biological Sciences Department, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada

*Author for correspondence (e-mail: farrell@sfu.ca)

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Summary

While the temperature dependence of exercise performance in fishes is reasonably well documented, information on the temperature dependence of metabolic recovery and reperformance is scant. This study examined the recovery of swimming performance after exhaustive exercise in rainbow trout *Oncorhynchus mykiss* at seasonal temperatures ranging from 5 to 17°C and explored the relationship between performance and preceding metabolic state. The primary objective of the study was to test the hypothesis that increased temperature increases the capability of rainbow trout to repeat a critical swimming speed (U_{crit}), as assessed by two consecutive critical swimming speed tests separated by a 40 min rest interval. An additional expectation was that certain plasma ionic, metabolic and humoral parameters would be correlated with how well fish reperformed and so plasma levels of lactate, potassium, ammonia, osmolality, sodium and cortisol, as well as hematocrit, were monitored before, during and after the swim challenges *via* an indwelling cannula in the dorsal aorta. As expected, performance in the first U_{crit} test (U_{crit1}) was positively related to temperature. However, the relationship between U_{crit1} and reperformance (U_{crit2}) was not dependent on acclimation temperature in a simple manner. Contrary to our expectations, U_{crit2} was less than U_{crit1} for warm-acclimated fish (14.9±1.0°C), whereas U_{crit2} equaled U_{crit1} for cold-acclimated fish (8.4±0.9°C). Cold-acclimated fish also exhibited a lower U_{crit1} and less metabolic disruption compared with warm-acclimated fish. Thus, while warm acclimation conferred a faster U_{crit1}, a similar swimming speed could not be attained on subsequent swim after a 40 min recovery period. This finding does not support the hypothesis that the ability of rainbow trout to reperform on U_{crit} test is improved with temperature. Both plasma lactate and plasma potassium levels were strongly correlated with U_{crit1} performance. Therefore, the higher U_{crit1} of warm-acclimated fish may have been due in part to a greater anaerobic swimming effort compared with cold-acclimated fish. In fact, a significant correlation existed between the plasma lactate concentration prior to the start of the second test and the subsequent U_{crit2} performance, such that U_{crit2} decreased when a threshold plasma lactate level of around 12.2 mmol l^{-1} was surpassed for the initial swim. No other measured plasma variable showed a significant relationship with the U_{crit2} performance. We conclude that warm-acclimated fish, by apparently swimming harder and possibly more anaerobically compared with cold-acclimated fish, were unable to recovery sufficiently well during the fixed recovery period to repeat this initial level of performance, and this poorer repeat performance was correlated with elevations in plasma lactate levels.

Key words: fish, rainbow trout, *Oncorhynchus mykiss*, critical swimming speed, temperature acclimation, repeat swimming, plasma, lactate threshold, ammonium.

Introduction

An extensive literature exists on the recovery of metabolites and ions following exhaustive exercise in fish (see reviews by Driedzic and Hochachka, 1978; Milligan, 1996; Kieffer, 2000). Considerably fewer studies have measured how quickly or how well swimming performance recovers following exhaustive exercise (e.g. Stevens and Black, 1966; Randall et al., 1987; Brauner et al., 1994; Jain et al., 1998; Farrell et al., 1998, 2001, 2003). Given that metabolic recovery in skeletal muscle (muscle lactate, ATP and glycogen, but not PCr) occurs more rapidly at warm than cold temperatures in exhausted rainbow trout *Oncorhynchus mykiss* and Atlantic salmon *Salmo salar* (Kieffer et al., 1994; Wilkie et al., 1997; Kieffer, 2000), the expectation is that swimming performance is restored faster at a higher temperature. This expectation would be consistent with the known increase in both maximum oxygen uptake and maximum cardiac output with temperature (e.g. Butler et al., 1992; Farrell, 1997; Taylor et al., 1997) because an improved oxygen delivery system could support a more rapid recovery of the metabolic debt incurred with exhaustive exercise. However, when Atlantic salmon were angled rather than chased to exhaustion, muscle glycogen, intracellular pH and lactate were restored more rapidly under cold conditions than warm conditions (Wilkie et al.,

1996). Therefore, given this uncertainty and the absence of any study that has directly measured how acclimation temperature affects the recovery of swimming performance, the primary objective of the present study was to test the hypothesis that the ability of rainbow trout to repeat a critical swimming speed (U_{crit}) test is improved with temperature.

A second objective of the present study was to search for correlations between the ability to reperform after an exhaustive U_{crit} swim and the alteration in plasma levels of ions, metabolites and hormones during exercise. In particular, possible linkages were sought between the recovery of swimming performance and the post-exhaustion levels of plasma potassium, lactate and total ammonia concentrations (T_{amm}), all of which have been linked with muscular exhaustion in both mammals and fish. For example, high intensity exercise in mammals produces a potassium loss from the muscle (Sjøgaard et al., 1985; Vøllestad et al., 1994; Hallén, 1996), which could decrease the muscle membrane excitability and compromise tension development (reviewed by Sjøgaard, 1991). Plasma potassium levels increase in rainbow trout just prior to U_{crit} and, moreover, exercise training increased U_{crit} while blunting and delaying the increases in plasma potassium and lactate just prior to exhaustion (Holk and Lykkeboe, 1998). Plasma lactate concentration has long been considered a useful indicator of aerobic limitations and anaerobic capabilities in exercise studies. Indeed, rainbow trout refused to perform repetitive bouts of burst exercise when plasma lactate concentration exceeded 13 mmol l^{-1} (Stevens and Black, 1966) and a poorer repeat U_{crit} was found for sockeye salmon *Oncorhynchus nerka* when plasma lactate concentration was >10 mmol l^{-1} (Farrell et al., 1998). In mammals, elevated plasma T_{amm} has been implicated in exercise fatigue (reviewed by Mutch and Banister, 1983) due to inhibitory influences on anaerobic metabolism (Zaleski and Bryla, 1977; Su and Storey, 1994), aerobic metabolism (McKhann and Tower, 1961; Avillo et al., 1981) and neuromuscular coordination (Binstock and Lecar, 1969; O'Neill and O'Donovan, 1979). Plasma T_{amm} also increases in rainbow trout during exercise (Turner et al., 1983; Wang et al., 1994; but see Beaumont et al., 1995a,b). Furthermore, when routine T_{amm} was elevated in brown trout *Salmo trutta*, as a result of exposure to acidic, copper-containing water, the subsequent U_{crit} performance was inversely related to pre-exercise plasma T_{amm} concentration (Beaumont et al., 1995a). Thus, because plasma levels of potassium, lactate and T_{amm} are good indicators of exhaustion in fish, we anticipated that they are potentially strong indicators of repeat swimming capability in rainbow trout. If this is the case, the expectation is that individual variation in these plasma variables prior to a second swim would be correlated with individual variation in the performance of a second U_{crit} test compared to the initial performance.

Materials and methods

Fish

Rainbow trout *Oncorhynchus mykiss* Walbaum [mass=871.49±43.34 g (mean ± standard error of the mean, S.E.M.); fork length (*FL*)=40.95±0.77 cm, *N*=15] were obtained from a local hatchery (Sun Valley Trout Farm, Mission, British Columbia, Canada). They were held outdoors in a 2000 liter round fiberglass aquarium provided with aerated and dechlorinated Vancouver municipal water, pH 6.7, hardness 5.2–6.0 mg l^{-1} $CaCO_3$, and ambient temperature 5–17°C. Experiments were performed between November 1997 and April 1998, and September–October 1998. All experimental work conformed to the guidelines set out by the Canadian Council on Animal Care, as approved by the Simon Fraser University Animal Care Committee.

Swim tunnel

Fish were swum in a modified Brett-type swim tunnel, similar to that described by Gehrke et al. (1990). The swim chamber was 21 cm diameter and 97 cm length, with a metal grid at each end. The rear grid was equipped with an electrical pulse generator (4 V) that, when contacted by the fish, provided a mild stimulation to encourage the fish to swim forward. Water speed was uniform across the swim tunnel throughout the speed range used in these experiments. The water current in the tunnel was produced by a 3-phase induction motor and a centrifugal pump attached to a tachometer whose readings (Hz) were calibrated with known water velocities, as measured with a Valeport current meter (Valeport Marine Scientific Ltd., Dartmouth, UK).

Protocol for arterial cannulation

The dorsal aorta was cannulated to permit sampling of blood prior to and during the swimming tests, and during the recovery periods. Arterial cannulation was performed under anesthesia (0.1 g l^{-1} buffered MS-222; Syndel Laboratories, Vancouver, BC, Canada), using the method of Smith and Bell (1964). Fish mass, fork length, maximum width and maximum depth were also measured at this time. Cannulated fish were either placed in the swim tunnel to recover or returned to the outdoor tank, where they recovered for up to 3 days before being placed in the swim tunnel. During subsequent transfer from the outdoor tank to the tunnel, fish were lightly and briefly anaesthetized (0.05 g l^{-1} buffered MS-222). There was no significant relationship between post-cannulation recovery time and measured swimming performance (data not presented).

Habituation to the swim tunnel and high water velocities

Fish recovered from anesthesia in the tunnel at a water speed of 10 cm s^{-1} for at least 45 min. After this time, fish performed a 20 min practice swim, as suggested in Jain et al. (1997), during which water speed was increased in 9–10 cm s^{-1} increments every 2 min to a speed of ~41 cm s^{-1}. Water speed was then returned to 10 cm s^{-1} for 2 min and again increased in the same fashion to a speed of either 55 or 59 cm s^{-1}, depending on the fish's swimming capability. The practice swim, which did not exhaust the fish, prevented the training effect often observed with naive fish on a second U_{crit} (Farlinger and Beamish, 1977; Jain et al., 1997). Fish then recovered overnight (14–16 h) at a water speed of 10 cm s^{-1}.

Swimming protocol

All experiments were started between 08:00 h and 10:00 h. Fish performed a ramp-U_{crit} test (Jain et al., 1997). The first U_{crit} test was followed by a 40 min recovery period at a water speed of 10 cm s^{-1} and then a second ramp-U_{crit} test followed by another recovery period. Each ramp-U_{crit} test involved increasing water speed to ~50% of U_{crit} over a 5 min period, after which water speed was increased in 10 cm s^{-1} increments (~15% of U_{crit}) every 20 min until exhaustion. Exhaustion was taken as the point at which the fish failed to swim away from the electrified rear grid after 20 s of contact. The ramp-U_{crit} protocol produces similar values for U_{crit} to the more standard U_{crit} testing protocol in which the longer time intervals are used from the onset of the test (Jain et al., 1997).

U_{crit} values were calculated for the first (U_{crit1}) and second (U_{crit2}) swims, as described by Brett (1964):

$$U_{crit} = u_i + (t_i/t_{ii} \times u_{ii}) \ , \quad (1)$$

where u_i is the highest speed at which the fish swam for the full time period (cm s^{-1}); u_{ii} is the incremental speed increase (cm s^{-1}); t_i is the time the fish swam at the final speed (min), and t_{ii} is the prescribed period of swimming per speed (20 min). As the cross-sectional area of each fish was <20% but sometimes >10% of that of the swimming chamber, the calibrated water speed was corrected for the solid blocking effect according to the calculations described by Bell and Terhune (1970):

$$\text{corrected } U_{crit} = U_{crit} \times \{1 + [0.4FL / 0.5(w+d)] \times (0.25\pi dw/A_t)^{1.5}\} \ , \quad (2)$$

where FL is fork length (cm), w is maximum fish width (cm), d is maximum fish depth (cm) and A_t is tunnel cross-sectional area. Water temperature did not fluctuate by more than 0.5°C from ambient temperature during the period that the fish spent in the tunnel.

Blood sampling

Blood samples (0.9 ml) were taken through the dorsal aorta cannula to measure plasma ion and metabolite levels. Normally, samples were taken immediately prior to the swimming protocol (routine samples), at exhaustion for both swim tests (U_{crit} exhaustion samples), and after a 40 min recovery for both tests (recovery samples; the recovery sample for the first U_{crit} swim also served as the sample taken immediately before the second U_{crit} swim). In 14 of the 16 fish, a blood sample was taken during aerobic swimming, i.e. after 15 min at 45 cm s^{-1} (approx. 69% U_{crit}). (These data are not reported as they simply provided intermediate values between the routine and U_{crit} values.) An equal volume of physiological saline solution was used to replace all blood samples (Gallaugher et al., 1992). Routine hematocrit was never less than 23% and remained elevated throughout the swim tests (see Fig. 2D).

Analytical techniques

Hematocrit was measured in microcapillary tubes after centrifugation at 2000 ***g*** for 3 min. The remainder of the blood was centrifuged at 10 000 ***g*** for 5 min to obtain plasma, which was stored at –80°C. Within 1 week of testing, plasma lactate and glucose concentrations were measured on 25 μl samples using a YSI 2300 lactate/glucose analyzer (Yellow Springs, OH, USA) that calibrated automatically every five samples. Plasma potassium and sodium concentrations were measured using a model 510 Turner flame photometer (Palo Alto, CA, USA). Plasma (5 μl) was diluted 1:200 with a prepared 15 mEq l^{-1} lithium diluent for analysis. The machine was calibrated prior to use and checked against a standard approximately every six samples. The measurement was repeated if there was disagreement between duplicates beyond 2% of absolute value. Osmolality was measured on duplicate 10 μl samples using a calibrated Wescor Vapour Pressure Osmometer, Model 5500 (Wescor, Logan, UT, USA). The measurement was repeated if there was disagreement between duplicates beyond 3% of absolute value. The thermocouple heads were cleaned periodically in order to maintain consistency. Plasma cortisol concentration was measured using a commercial radioammunoassay kit (ICN Biomedicals, Inc., Costa Mesa, CA, USA), with a detection limit of 1.5 ng ml^{-1}. Plasma ammonia concentration (T_{amm}) was measured spectrophotometrically on 0.1 ml plasma samples (Sigma Diagnostics kit no. 171, St Louis, MI, USA) with a calibration every seven samples.

Data analysis

All plasma metabolites and ions were measured in duplicate and averaged for individual data. Fish were subdivided into two temperature acclimation groups based on their swimming performance (see Results) and values (mean ± S.E.M.) are presented for cold- and warm-acclimated fish. One warm-acclimated female fish that was overtly gravid was not included in the statistical analysis to eliminate any confounding effect, because reproductive maturity is known to negatively affect U_{crit} performance in salmon (Williams et al., 1986). Statistical comparisons within temperature groups were made with a one-way repeated measures analysis of variance (ANOVA) followed by a *post hoc* Tukey test. With this test, the values associated with each fish were compared to other levels at other sampling times for the same fish to determine whether either swimming speed or metabolite levels changed throughout testing. Comparisons of swimming performance and metabolite levels between temperature groups were made using *t*-tests. U_{crit1} was compared to U_{crit2} using a Bland–Altman plot. Bland and Altman (1986, 1995) introduced this method of graphical analysis to assess the equivalency of two testing approaches (here U_{crit1} and U_{crit2}), while removing the bias that comes from assuming that one method represents the true value (independent variable). The Bland–Altman plot uses the mean of both methods as the independent variable and the difference between the two testing methods as the dependent variable. If the linear regression of the points is non-significant, then the two testing procedures (i.e. U_{crit1} and U_{crit2} here) can be considered to be

equivalent testing procedures. Sub-groups can be identified within a data set in a Bland–Altman plot by demonstrating different significant linear regressions from each other. In the present study, different regressions would identify sub-groups with different relationships between U_{crit1} and U_{crit2}. Relationships between U_{crit} values and plasma variables were fitted with the best-fitting regressions using the options provided in Sigma-Plot (SPSS Inc.; Chicago, IL, USA). $P<0.05$ was used to establish statistical significance.

Results

Swimming performance

As water speed increased, fish progressed from a steady swimming mode to one that included periods of burst-and-glide swimming. In conjunction with higher speeds, fish ram-ventilated their gills, except during burst-and-glide swimming when active ventilation was observed. Visually, swimming behavior did not appear to be different for the first and second U_{crit} tests.

A Bland–Altman plot revealed that U_{crit1} and U_{crit2} were equivalent testing procedures ($P=0.98$), but visual inspection of the plot revealed that overall the fish could be divided into two sub-groups each with a different and significant linear relationship (Fig. 1A). Each of the two sub-groups corresponded to different acclimation temperatures and hereafter are termed warm- and cold-acclimated fish (14.9±1.0°C and 8.4±0.9°C, respectively; see Table 1).

U_{crit1} performance was temperature dependent (Fig. 1B; $r^2=0.74$, $P<0.05$). U_{crit1} (78.9±1.0 cm s^{-1}) for warm-acclimated fish was significantly greater ($P<0.05$) than that for cold-acclimated fish (59.1±2.5 cm s^{-1}; Table 1). However, U_{crit2} did not show any temperature dependency. Unexpectedly, U_{crit2} performance (65.8±2.70 cm s^{-1}) for warm-acclimated fish was significantly lower than U_{crit1}, whereas U_{crit2} for cold-acclimated fish (58.0±4.2 cm s^{-1}) was

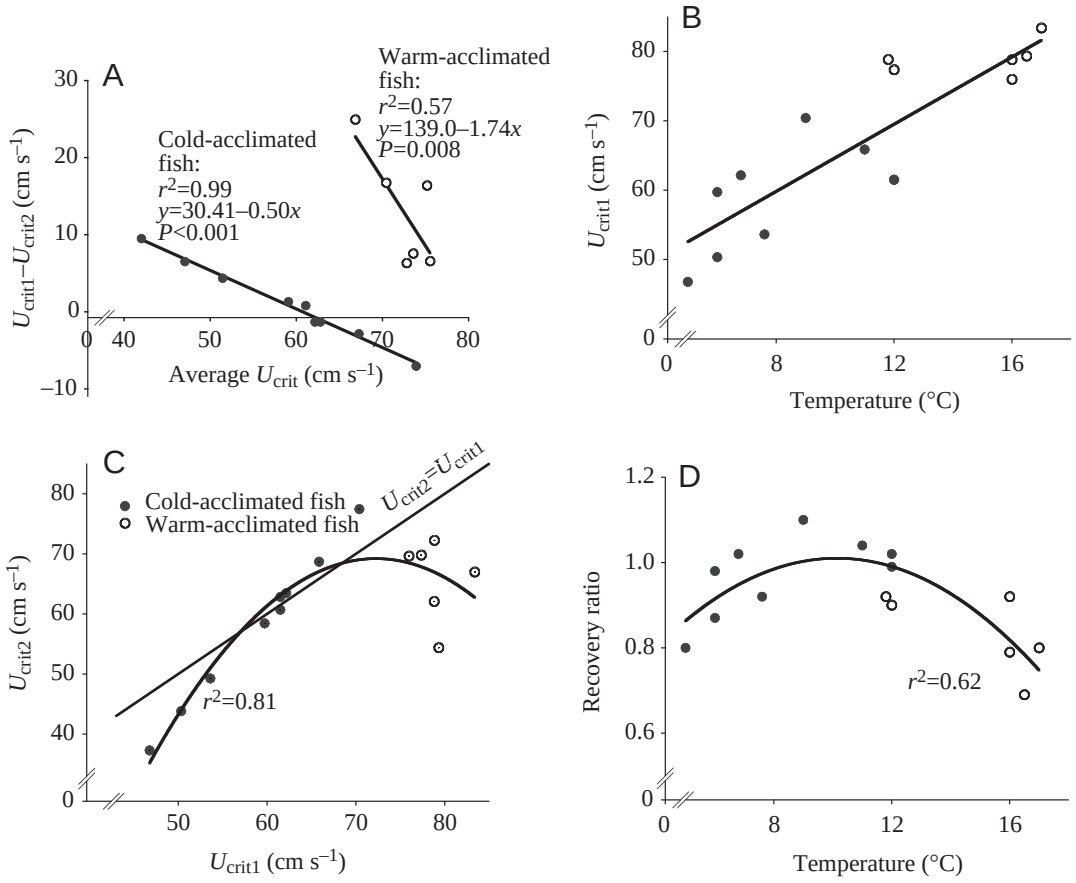

Fig. 1. (A) Bland–Altman plot comparing consecutive U_{crit} tests (U_{crit1} and U_{crit2}) performed by rainbow trout, separated by a 40 min recovery period. Regression lines indicate the existence of two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish, based on the visible groupings in this graph. (B) U_{crit1} *versus* ambient water temperature for rainbow trout. Fish are divided into two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish. Regression: $y=40.44+2.42x$, $r^2=0.74$; $P<0.001$. (C) U_{crit2} *versus* U_{crit1} for individual rainbow trout performing two U_{crit} tests separated by a 40 min recovery period. Fish are divided into cold-acclimated and warm-acclimated groups. The thin line is the line of identity where $x=y$, i.e. the predicted line if $U_{crit1}=U_{crit2}$ independent of temperature, and this was not the case. Regression (thick line): $y=-204.3+7.57x-0.05x^2$, $r^2=0.81$, $P<0.001$. (D) Recovery ratios for individual rainbow trout as a function of acclimation temperature (filled symbols, cold-acclimated group; open symbols, warm-acclimated group). The regression line for these data illustrates that warm-acclimated fish could not attain the same U_{crit} after a 40 min recovery.

Table 1. *Critical swimming speed of the cold- and warm-acclimated groups of rainbow trout for the first and second swim tests*

	Cold-acclimated fish (N=9)	Warm-acclimated fish (N=6)
U_{crit1} (cm s^{-1})	59.1±2.5	78.9±1.0[a]
U_{crit2} (cm s^{-1})	58.9±4.2	65.9±2.7[b]
U_{crit1} (*FL* s^{-1})	1.51±0.10	1.84±0.04[a]
U_{crit1} (*FL* s^{-1})	1.48±0.14	1.54±0.08[b]

Cold-acclimation temperature = 8.4±0.9°C; warm-acclimation temperature = 14.9±1.0°C.

U_{crit1}, first swim test; U_{crit2}, second swim test; *FL*, fork length.

Values are means ± S.E.M.

[a]Statistically significant difference (P<0.05) compared with cold sub-group; [b]statistically significant difference (P<0.05) between comparable U_{crit1} and U_{crit2} values.

the same as their U_{crit1} values (Table 1). As a result, the overall relationship between U_{crit1} and U_{crit2} was best described by a polynomial equation (y=–204.3+7.57x–0.05x^2; P<0.001; Fig. 1C), with cold-acclimated fish lying close to the line of identity and warm-acclimated fish lying below the line of identity. Thus, while warm acclimation conferred a faster U_{crit1}, a similar swimming speed could not be attained after a 40 min recovery period, as shown by recovery ratios that are less than unity for warm-acclimated fish (Fig. 1D).

Plasma status before, during and after U_{crit} *tests*

There were no significant differences between the cold- and warm-acclimated groups of fish in terms of routine values for plasma levels of lactate, potassium, T_{amm}, sodium, glucose, cortisol and osmolality and hematocrit. When cold-acclimated fish were exhausted at U_{crit1}, plasma levels of lactate, potassium and T_{amm}, as well as hematocrit, all increased significantly (Fig. 2A–D). Plasma cortisol (Fig. 2E) and sodium (Fig. 2F) levels were unchanged at exhaustion for U_{crit1}. After a 40 min recovery from U_{crit1}, plasma lactate increased significantly beyond the level observed at exhaustion, plasma T_{amm} decreased to the routine level, and plasma potassium and hematocrit remained elevated at the same level. As a result, plasma lactate and potassium levels, and hematocrit were all significantly elevated at the outset of the U_{crit2} test.

For cold-acclimated fish exhausted at U_{crit2}, plasma levels of lactate, potassium, sodium and T_{amm}, and hematocrit, were again significantly elevated compared with the routine values, but no more so than for U_{crit1}. In fact, compared with the recovery values for U_{crit1}, plasma lactate levels had decreased significantly (Fig. 2A) at exhaustion for U_{crit2}, while T_{amm} had increased significantly (Fig. 2C). Similar to U_{crit1}, plasma lactate increased during the 40 min recovery from U_{crit2} to a level significantly higher than that observed at exhaustion, plasma T_{amm} decreased to the routine level, and plasma potassium and hematocrit remained elevated at the same level. As a result, none of the recovery values for U_{crit2} in cold-acclimated fish were significantly different to those for U_{crit1}. Plasma levels of cortisol, glucose and osmolality remained unchanged throughout both swimming protocols (data not shown). Therefore, the second swim for cold-acclimated fish had no additive effects on any of the measured plasma variables.

When warm-acclimated fish were exhausted at U_{crit1}, plasma T_{amm} and hematocrit increased by the same amount as for cold-acclimated fish (Fig. 2C,D). In contrast, the faster U_{crit1} of the warm-acclimated fish was associated with significantly larger increases in plasma levels of lactate and potassium (Fig. 2A,B) compared with cold-acclimated fish. Furthermore, warm-acclimated fish significantly increased plasma sodium and cortisol levels at exhaustion for U_{crit1} (Fig. 2E,F), unlike cold-acclimated fish. After a 40 min recovery from U_{crit1}, the levels of plasma lactate, potassium, T_{amm}, sodium and cortisol, as well as hematocrit all remained significantly elevated in warm-acclimated fish, whereas only plasma levels of lactate, potassium and hematocrit remained elevated in cold-acclimated fish (Fig. 2). In addition, plasma lactate, potassium, sodium and cortisol remained elevated in warm-acclimated fish at levels that were significantly greater than those observed in cold-acclimated fish during recovery. In fact, the plasma lactate level was about threefold higher and plasma potassium almost twofold higher. These results suggest that the higher U_{crit1} of warm-acclimated fish may have been partly due to a greater anaerobic swimming effort compared with cold-acclimated fish, and (or) lactate and potassium were released from muscle to plasma to a greater extent.

Compared with cold-acclimated fish, warm-acclimated fish clearly began the second U_{crit} test with a greater plasma ionic and metabolic disruption and, as a result in these fish, U_{crit2} was significantly lower than U_{crit1}. In addition, while U_{crit2} for warm-acclimated and cold-acclimated fish was the same, warm-acclimated fish displayed a significant, further increase in plasma potassium levels (Fig. 2B) at exhaustion and a significant, further increase in plasma lactate levels (Fig. 2A) during the recovery from U_{crit2}. However, plasma T_{amm} did not recover to a routine level, as it did in the cold-acclimated fish (Fig. 2C). Therefore, the second U_{crit} swim of warm-acclimated fish produced significant additive effects on some of the plasma variables, unlike in cold-acclimated fish where there were none.

Correlational analysis

The initial swimming performance of individual fish was related to the appearance of lactate in the plasma. Plasma lactate concentrations measured at U_{crit1} and after a 40 min recovery were both linearly related to U_{crit1} (Fig. 3; r^2=0.73, P<0.05 and r^2=0.79, P<0.05, respectively). As might be expected from Fig. 3, plasma lactate concentrations were highly correlated with each other (2nd exhaustion with 1st exhaustion: r^2=0.95, P<0.05; 1st exhaustion with 1st recovery: r^2=0.92, P<0.05; 2nd exhaustion with 1st recovery: r^2=0.94, P<0.05; 2nd recovery with 2nd exhaustion: r^2=0.94, P<0.05).

The difference in swimming performance between U_{crit1} and U_{crit2} was significantly related to the plasma lactate concentration prior to the second U_{crit} test (Fig. 4). This relationship was described by either a polynomial (r^2=0.74), or a 2-parameter power (r^2=0.65) regression. Both types of analysis suggest that the reduction in U_{crit2} relative to U_{crit1} occurred when fish reached a plasma lactate of 12.2 mmol l^{-1} (95% confidence intervals of 7.9 and 16.5 mmol l^{-1}) 40 min after being exhausted by an initial U_{crit} swim test. Only warm-acclimated fish reached this threshold plasma lactate level.

Swimming effort in the initial swim was also related to the appearance of potassium in the blood. Plasma potassium concentration measured at U_{crit1} was linearly related to U_{crit1} (r^2=0.60, P<0.05). However, there was no significant correlation between plasma potassium levels and performance on the second swim. Plasma T_{amm} at exhaustion was not significantly related to U_{crit1}, but T_{amm} values for the 1st

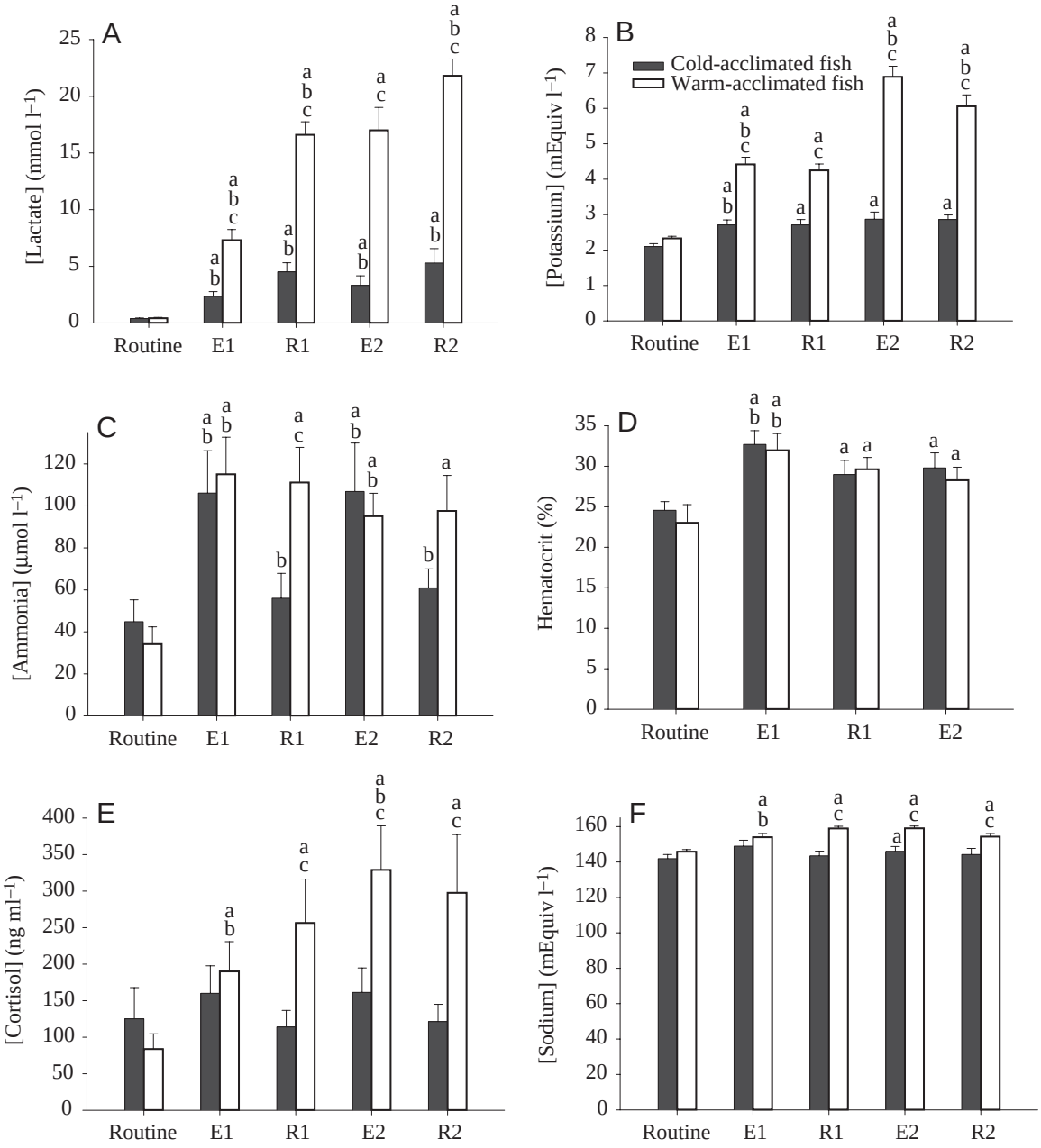

Fig. 2. Blood parameters in cold-acclimated (N=8–9) and warm-acclimated (N=5–6) fish before testing (Routine), at failure in the first U_{crit} test (E1), after a 40 min recovery (R1; this was also immediately before the start of the second U_{crit} test), at failure in a second U_{crit} test (E2), and after another 40 min recovery period (R2). [a]Level different from the routine value; [b]level different from the previous sampling time; [c]value for warm-acclimated fish different from the corresponding value for cold-acclimated fish. (A) Plasma lactate concentration. (B) Plasma potassium concentration. (C) Plasma ammonia concentration. (D) Hematocrit. (E) Plasma cortisol concentration. (F) Plasma sodium concentration.

recovery were related to U_{crit1} (Fig. 5; r^2=0.34, P<0.05). There were no other significant correlations for plasma T_{amm}.

The influence of acclimation temperature on the plasma ionic and metabolic responses to exercise is illustrated by the significant linear correlations that existed between plasma lactate, cortisol and potassium levels and temperature (Table 2). There were no significant correlations with temperature and the other parameters measured (T_{amm}, [sodium] and hematocrit).

One overtly gravid, warm-acclimated female fish was treated as an outlier, based on its slow swimming performance, and was not used for any correlation analysis. However, it is important to note that all the plasma changes observed in this fish were consistent with the slower swimming performance of the cold-acclimated fish.

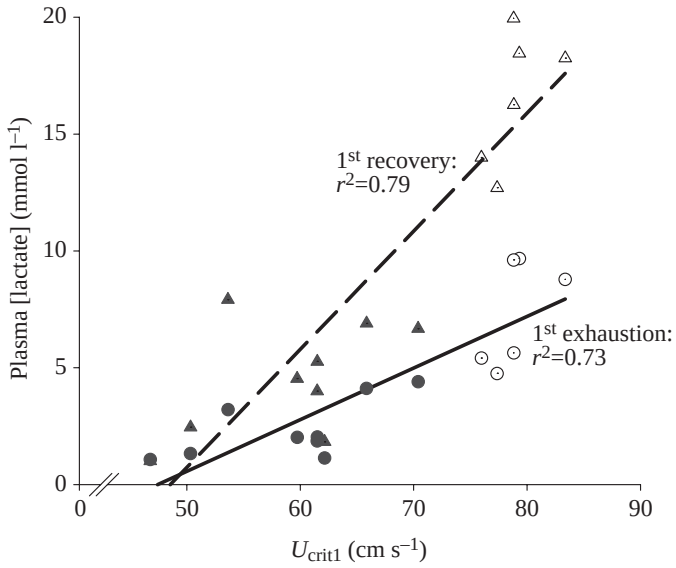

Fig. 3. Plasma lactate concentration at 1st exhaustion (circles) and 1st recovery (triangles) sampling times *versus* U_{crit1} for rainbow trout. Fish are divided into two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish. Regression for 1st exhaustion plasma [lactate] (solid line): y=–10.48+0.22x, r^2=0.73; P<0.001; for 1st recovery plasma [lactate] (broken line): y=–24.52+0.51x, r^2=0.79; P<0.001.

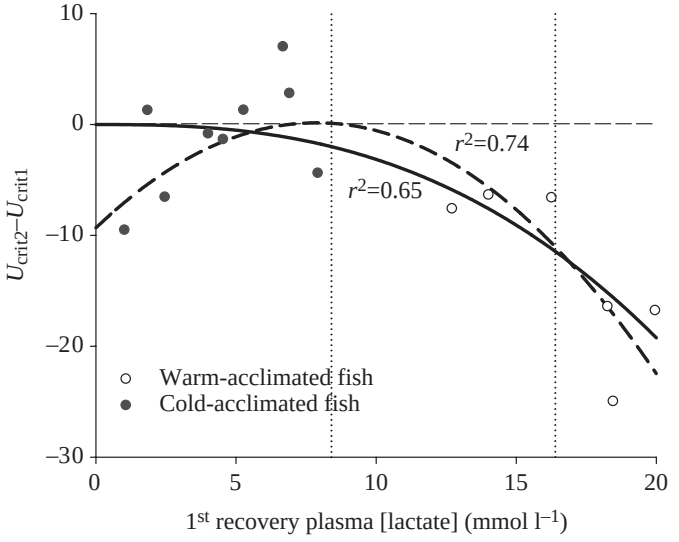

Fig. 4. U_{crit2}–U_{crit1} *versus* the plasma lactate concentration prior to the second U_{crit} test (recovery 1) for individual rainbow trout. Fish were divided into two sub-groups: cold-acclimated (filled symbols) fish and warm-acclimated (open symbols) fish. The data could be described by either a polynomial (broken line; r^2=0.74) or a 2-parameter power (solid line; r^2=0.65) relationship.

Discussion

This study tested the hypothesis that warm-acclimated rainbow trout would perform better in repeated U_{crit} swimming tests than cold-acclimated fish. The present findings, however, do not support this hypothesis because U_{crit2} was significantly lower than U_{crit1} in warm-acclimated fish than in cold-acclimated fish. At U_{crit1}, the warm-acclimated fish showed a greater metabolic disturbance in the plasma compared with cold-acclimated fish and also showed additive effects for the second U_{crit}, unlike the cold-acclimated fish. Therefore, although warm-acclimated fish swam better than cold-acclimated fish for U_{crit1}, as expected, the consequence of this faster U_{crit1} was a reduced performance on the second U_{crit} test. If anything, it appeared that warm-acclimated fish, by apparently swimming harder and possibly more anaerobically, were unable to recover sufficiently well during the fixed recovery period to repeat this initial level of performance. For cold-acclimated fish, however, the 40 min recovery period was sufficient for adequate recovery and allowed swimming performance to be repeated. Therefore, we are left with the

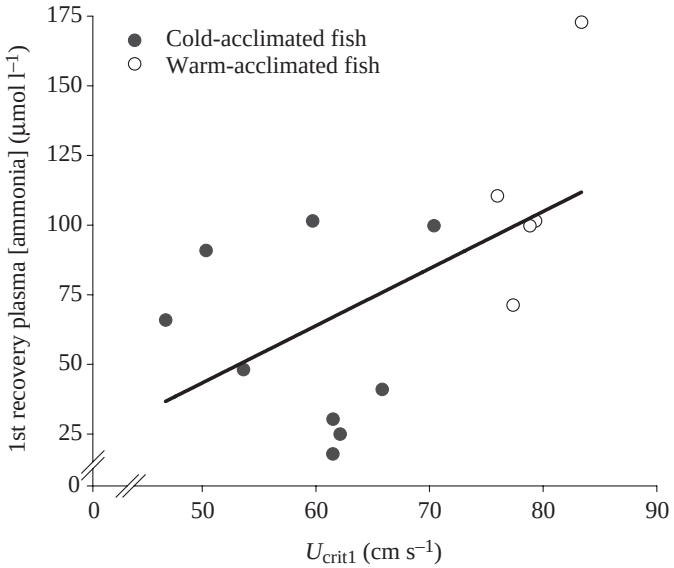

Fig. 5. Plasma ammonia concentration at first recovery *versus* U_{crit1} for rainbow trout. Fish are divided into two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish. Regression: y=–56.51+2.02x, r^2=0.30, P<0.05.

Table 2. *Significant linear regressions between ambient water temperature and individual plasma variables during repetitive swim tests in rainbow trout*

Plasma variable	*P*-value	r^2
[Lactate]		
1st exhaustion	<0.001	0.72
1st recovery	<0.001	0.74
2nd exhaustion	<0.001	0.68
2nd recovery	<0.001	0.69
[Potassium]		
1st exhaustion	<0.001	0.65
1st recovery	<0.001	0.69
2nd exhaustion	<0.001	0.74
2nd recovery	<0.001	0.78
[Cortisol]		
1st exhaustion	<0.01	0.41
1st recovery	<0.05	0.27
2nd exhaustion	<0.05	0.33

conclusion that overall recovery, as it pertains to repeat swimming capabilities and time allowed for recovery, was superior for the cold-acclimated compared with the warm-acclimated group of rainbow trout.

Our original hypothesis, which we now reject, was based on the established temperature dependence of post-exercise metabolic and ionic recovery when salmonids are chased to exhaustion to produce similar levels of intracellular acidosis, lactate accumulation and glycogen depletion in white muscle regardless of temperature (Kieffer et al., 1994; Wilkie et al., 1997). However, when Atlantic salmon were angled to exhaustion at a warmer temperature, essentially the opposite effect of temperature on post-exercise muscle recovery was obtained; they displayed a greater depletion of muscle glycogen, a greater intracellular acidosis and a slower recovery of muscle metabolites at the warmer temperature compared with colder temperatures (Booth et al., 1995; Wilkie et al., 1996). The present findings for U_{crit} swim tests are more in line with data obtained when fish are angled rather than chased to exhaustion because the metabolic disturbances were higher and performance recovery slower at warmer temperatures. We suggest that the disparity among studies could simply reflect differences in the degree of exhaustion and the methods used to exhaust the fish, with fish becoming more exhausted because they perceive the chasing protocol as more of a threat or provocation than either angling or U_{crit} testing. Given this possibility, cold-acclimated fish could opt to stop swimming sooner than warm-acclimated fish to preserve glycogen reserves.

A U_{crit} value, like time-to-exhaustion at a prescribed water speed (e.g. Facey and Grossman, 1990; Mitton and McDonald, 1993), allows quantification of the swimming effort, something that is not easily done when fish are chased or angled to exhaustion. U_{crit} tests also encompass a spectrum of swimming speeds, with the aerobic demands of swimming up to maximum oxygen uptake being met by cardiorespiratory adjustments, while white muscle recruitment and anaerobic metabolism increasingly supports the higher muscular power output near U_{crit} (Burgetz et al., 1998), culminating in exhaustion (Brett, 1964; Beamish, 1978). The simplest explanation for the higher U_{crit1} values obtained for warm-acclimated compared with cold-acclimated fish is a greater involvement of anaerobic swimming, given the significantly larger alterations in plasma metabolites observed for warm-acclimated rainbow trout. Certainly, the warm-acclimated fish were more stressed than the cold-acclimated fish, as judged by the greater elevation in plasma cortisol levels. However, since muscle metabolites were not measured here, we cannot exclude other possibilities. The higher levels of plasma potassium, lactate and T_{amm}, as well as the additive effects of the second swim, could simply reflect a greater release of lactate and potassium into the plasma because the release of lactate and hydrogen ions from white muscle to the blood is known to be temperature dependent (see Kieffer, 2000). Nevertheless, it is unlikely that different muscle glycogen levels were a factor since these are unaffected by acclimation temperature (Kieffer, 2000).

Rome et al. (1985) showed that acutely exposing warm-acclimated carp *Cyprinus carpio* to cold water resulted in white muscle fibres being recruited at a lower swimming speed, and this ‘compression of recruitment order’ led to earlier fatigue and a reduced sustained swimming speed. However, when the carp were cold-acclimated, they recruited white muscle at a higher swimming speed than warm-acclimated fish, presumably because cold temperature acclimation had improved the mechanical performance of the red muscle. The present findings are consistent with this earlier work with carp in that the cold-acclimated rainbow trout appeared to rely less on anaerobic white muscle than warm-acclimated fish, but the two studies differ in that cold-acclimated rainbow trout had a lower U_{crit} than warm-acclimated rainbow trout whereas cold-acclimated carp swam to the same maximum speed as warm-acclimated fish (Rome et al., 1985). Rome et al. (1985) suggested three possible physiological differences in cold-acclimated fish compared with warm-acclimated fish: (1) a higher mechanical power output from aerobic muscle, (2) limitations on the neural control of locomotory muscle and (3) limitations of the respiratory and circulatory systems in supplying oxygen. The present findings suggest a fourth possibility: fish may opt to swim to different states of exhaustion depending on either the temperature or a resulting physiological condition. One benefit of limiting the level of exhaustion under cold conditions appears to be a more reasonable recovery rate, which allows for repeated performance. At warm temperatures, fish benefit from a higher initial level of performance but, by exhausting themselves to a relatively greater degree, have the disadvantage of a more prolonged recovery period. An additional disadvantage, but for unknown reasons, is that exhaustive exercise at warm, but not at cold temperatures, can result in appreciable levels of post-exercise mortality (see Kieffer, 2000).

The present conclusions are also in line with the results of McKenzie et al. (1996) working with Nile tilapia *Oreochromis*

nilotica. They found that warm-acclimated fish had a greater cost of recovery (a higher and more prolonged post-exercise oxygen consumption) after being chased to exhaustion than cold-acclimated fish. Interestingly, white muscle lactate accumulation was similar for 16°C-acclimated and 23°C-acclimated tilapia, suggesting that muscle lactate may not always be a reliable measure of post-exercise recovery. However, 23°C-acclimated tilapia excreted over twice the amount of ammonia post-exercise than 16°C-acclimated fish. Kieffer et al. (1998) similarly found that ammonia excretion at 75% U_{crit} was almost threefold higher for 15°C-acclimated than 5°C-acclimated rainbow trout, while protein utilization at 75% U_{crit} was 30% at 15°C *versus* 15% at 5°C. Likewise, in the present study, we observed a significantly higher plasma T_{amm} in warm-acclimated rainbow trout. As discussed by McKenzie et al. (1996), the elevated ammonia production could be a result of either increased protein metabolism to fuel locomotion or increased protein degradation from tissue damage. Since elevated T_{amm} is thought to have inhibitory actions on neural and muscle activity in fish (Beamount et al., 1995a), the larger elevation in plasma T_{amm} in warm-acclimated fish is perhaps critical to survival post-exhaustion. On the other hand, tissue damage might negatively affect U_{crit2}.

U_{crit} values were comparable to those reported earlier by Jain et al. (1997) for rainbow trout of the same size in the same swim tunnel [1.64–1.66 body lengths (*BL*) s^{-1}] and higher than those reported for 822–1118 g rainbow trout (0.94 *BL* s^{-1} and 0.53 *BL* s^{-1} at 11°C and 18°C, respectively; Taylor et al., 1996). Comparisons also can be made with studies on smaller rainbow trout, which are expected to attain slightly higher U_{crit} values (Brett, 1964) than the 879 g fish used here. U_{crit} values of 1.8 to 2.0 *BL* s^{-1} are reported for 530–730 g rainbow trout at 18–19°C (Gallaugher et al., 1992) and 2.13 *BL* s^{-1} for 431–483 g rainbow trout at 7–11°C (Burgetz et al., 1998). For 320–520 g brown trout, U_{crit} values were 2.2 *BL* s^{-1} at 15°C and 1.85 *BL* s^{-1} at 5°C (Butler and Day, 1993; Butler et al., 1992).

As anticipated, a 40 min recovery period allowed full recovery of swimming performance for cold-acclimated fish. Originally it was suggested that salmonids be given 4 h between U_{crit} tests (Brett, 1964) to ensure a return to routine O_2 consumption but not necessarily to routine glycogen levels. Subsequently, recovery times of 2 h (Brauner et al., 1994), 1 h (Randall et al., 1987), 45 min (Farrell et al., 1998, 2003) and 40 min (Jain et al., 1998) have all been shown to be sufficient for salmonids to repeat U_{crit} tests without any significant decline in performance. Here fish were provided with a low speed water current during recovery and this may have aided their recovery, since recent studies with rainbow trout (Milligan et al., 2000) and coho salmon *Oncorhynchus kisutch* (Farrell et al., 2001) have shown that low to moderate swimming post-exhaustion greatly aids metabolic recovery through a warm-down effect. In contrast, recovery time without a warm-down is >2 h for optimal performance on a time-to-exhaustion test (Mitton and McDonald, 1994). Wang et al. (1994) reported that muscle phosphocreatine and ATP levels were restored within 30 min of rainbow trout being chased to exhaustion, while the post-exercise decline of oxygen consumption lasted 3–3.5 h (Scarabello et al., 1991). However, routine oxygen consumption does not have to be restored before adult sockeye salmon can repeat a second U_{crit} test (Farrell et al., 1998, 2003).

There was generally good agreement between the routine plasma variables reported here and those reported in previous studies (Butler and Day, 1993; Eros and Milligan, 1996; Pagnotta et al., 1994; Thorarensen et al., 1994; Wang et al., 1994). However, the plasma lactate concentrations at U_{crit} in this study, especially those for the warm-acclimated fish (7.3 mmol l^{-1}), were at the high end of literature values for U_{crit} swimming (1.5–5.5 mmol l^{-1}) (Butler and Day, 1993; Gallaugher et al., 1992; Thorarensen et al., 1993; Holk and Lykkeboe, 1998; Farrell et al., 1998). Milligan (1996) cites a range for plasma lactate levels of 2–13 mmol l^{-1} immediately after chasing, increasing to peak values of 12–20 mmol l^{-1} at 2 h post-exercise. The values reported here for cold-acclimated fish of 4.3 mmol l^{-1} at U_{crit} and 8.9 mmol l^{-1} 40 min later are at the low end of this range, whereas those for the warm-acclimated fish (7.3 mmol l^{-1} at U_{crit} and 16.6 mmol l^{-1} 40 min later) are at the upper end of the range and approached the level reached (17.8 mmol l^{-1}) approximately 90 min after a hypoxic U_{crit} test (Farrell et al., 1998).

The second objective of the present study was to determine whether any of the measured metabolites displayed threshold levels that, if surpassed in the first swim challenge, were indicative of a metabolic condition that negatively affected subsequent swimming performance. Plasma lactate level was the only candidate: the plasma lactate level before U_{crit2} was significantly correlated to the subsequent swimming performance (U_{crit2}). The threshold plasma lactate level of approximately 12.2 mmol l^{-1} (95% CI 7.9–16.4) agrees with that of 13 mmol l^{-1} reported by Stevens and Black (1966) for burst exercise with rainbow trout and 10 mmol l^{-1} for sockeye salmon (Farrell et al., 1998). In the earlier studies, fish refused to swim if the lactate threshold was surpassed. However, no fish refused to swim outright in the present study and instead U_{crit} performance was reduced by 8–31%. Thus, because anaerobic metabolism is increasingly required to support swimming speeds greater than 70% U_{crit} (Burgetz et al., 1998), elevated levels of lactate above the lactate threshold is probably indicative of a failure to fully recruit anaerobic metabolism in white muscle (e.g. through decreased muscle pH and glycogen stores). This idea needs further study, however, because plasma lactate dynamics are complex, reflecting rates of production in the muscle, rates of release from the muscle and rates of clearance from the blood. While the present study suggests that production may be greater at warmer temperatures, release rate is dependent on temperature (Keiffer et al., 1994) and clearance rate is inversely related to temperature (Kieffer and Tufts, 1996).

Beaumont et al. (1995a,b) reported that copper-exposed brown trout in water of low pH had poor U_{crit} values and

suggested that the elevated plasma T_{amm} inhibited white muscle activity either directly or through CNS inhibitory mechanisms, because elevated plasma T_{amm} levels were correlated with the reduced U_{crit} values. In the present study, we found no significant correlations between swimming performance and plasma T_{amm}. However, our data are not necessarily at odds with the suggestion of Beaumont et al., (1995a,b) because the plasma T_{amm} levels reported in the present work were half those measured in copper-exposed brown trout and, in the earlier studies, U_{crit} was not reduced appreciably until plasma T_{amm} reached levels >200 μmol l^{-1}. A plasma T_{amm} level >600 μmol l^{-1} resulted in fish refusing to swim. In the present study, T_{amm} reached only 100 μmol l^{-1} and was restored between U_{crit} tests for cold-acclimated fish, although not for warm-acclimated fish (Fig. 2).

Several studies report a temperature optimum for U_{crit}. For sockeye salmon, 15°C was the optimum temperature for U_{crit}, metabolic scope (Brett, 1964) and cardiac performance (Brett, 1971; Davis, 1968). The preferred temperature for sockeye salmon, however, appears to be slightly cooler (10–12°C; Birtwell et al., 1994; Spohn et al., 1996). Garside and Tait (1958) suggested a preferred temperature range for rainbow trout of 11–16°C, which coincides with the optimum temperature range suggested for cardiac performance (Farrell et al., 1996; Taylor et al., 1997; Farrell, 2002). The present experiments show that the shift in responses to repeated swimming for cold- and warm-acclimated fish occurred at around 12°C. Therefore, the fish's preferred temperature may reflect sub-maximal rates for certain activities because of negative consequences in terms of rates of recovery.

In summary, we provide evidence that warm-acclimated rainbow trout have a higher U_{crit} than cold-acclimated fish, but associated with this higher U_{crit} is a greater metabolic and ionic disturbance. A consequence of this elevated disturbance is that warm-acclimated fish do not recover well enough after a 40 min rest to perform a second test at the same level as the first one, whereas cold-acclimated fish do. Elevations in plasma lactate (but not plasma potassium, T_{amm} and cortisol) were significantly correlated with the poorer repeat swimming performance.

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CARDIORESPIRATORY PHYSIOLOGY AND TEMPERATURE TOLERANCE AMONG POPULATIONS OF SOCKEYE SALMON (*ONCORHYNCHUS NERKA*)

by

Erika Jennifer Eliason Parsons

B.Sc., Simon Fraser University, 2003
M.Sc., University of British Columbia, 2006

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ABSTRACT

Elevated summer water temperature has been associated with high mortality in adult sockeye salmon (*Oncorhynchus nerka*) during their once-in-a-lifetime migration up the Fraser River (British Columbia, Canada) to their spawning grounds. There are over 100 genetically distinct populations of sockeye salmon in the Fraser River watershed, varying in migration distance, elevation gain, river temperature and river flow. This thesis studied the physiological basis for temperature tolerance in sockeye salmon and examined the overall hypothesis that each sockeye salmon population has physiologically adapted to meet their specific upriver migration conditions.

Swimming and cardiorespiratory performance were compared over a range of temperatures across six wild, migrating adult sockeye salmon populations. All populations maintained maximum performance across the entire range of temperatures typically encountered during their upriver migration, with Chilko sockeye salmon emerging as the most high temperature-tolerant. In addition, populations with more challenging migrations had greater aerobic scope, larger hearts and improved coronary supply. These results suggest that sockeye salmon populations have physiologically adapted to cope with their local upriver migration conditions, despite never before having performed the upriver migration.

Temperatures exceeding the population-specific thermal optimum resulted in severely impaired aerobic scope and swimming performance. This study suggests that population-specific thermal limits are set by physiological limitations in aerobic performance. Specifically, fish may be unable to swim at warm temperature due to insufficient oxygen supply to meet demand, triggered via a cardiac limitation due to reduced scope for heart rate.

Given the key role of the heart in limiting thermal tolerance, the role of cardiac adrenergic stimulation was examined as a potential mechanism underlying the observed

differences in thermal tolerance across sockeye salmon populations. Chilko sockeye salmon had a greater density of ventricular β-adrenoceptors, which may provide greater cardiac capacity and protection at temperature extremes, thereby expanding their breadth of thermal tolerance compared to other populations.

This thesis suggests that sockeye salmon populations will be differentially affected by warming river temperatures, raising conservation concerns for biodiversity. This work provides important insight into local adaptation in sockeye salmon and identifies a possible cause for in-river mortality associated with warm temperatures in sockeye salmon.

PREFACE

Some of the data presented in Chapters 3, 6 and 7 were previously published in E. J. Eliason, T. D. Clark, M. J. Hague, L. M. Hanson, Z. S. Gallagher, K. M. Jeffries, M. K. Gale, D. A. Patterson, S. G. Hinch, A. P. Farrell. 2011. Differences in thermal tolerance among sockeye salmon populations. *Science* 332: 109-112.

E. J. Eliason was the primary contributor to the experimental design, data collection, data analysis and manuscript preparation. A. P. Farrell and S. G. Hinch provided supervision, assistance with experimental design and helped with manuscript preparation. T. D. Clark, L. M. Hanson, Z. S. Gallagher, K. M. Jeffries and M. K. Gale provided valuable secondary assistance in the field and during data collection. M. J. Hague and D. A. Patterson provided Fraser River temperature information and modeling expertise.

All procedures were approved by the University of British Columbia's Animal Care Committee in accordance with the Canadian Council on Animal Care (A06-0328 and A08-0388).

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% compact	percentage compact myocardium
AIC	Akaike's Information Criterion
ATP	adenosine triphosphate
ATU	accumulated thermal units
A-V_{O2}	tissue oxygen extraction
bl s^{-1}	body lengths per second
B_{max}	β_2-adrenoceptor density
CAER	Centre for Aquaculture and Environmental Research
C_{aO2}	arterial oxygen content
Cl^-	chloride
CLL	Cultus Lake Laboratory
C_{O2}	oxygen content
COT	cost of transport
COT_{net}	net cost of transport
COT-$\dot{Q}$	cardiovascular cost of transport
COT-$\dot{Q}_{net}$	net cardiovascular cost of transport
C_{vO2}	venous oxygen content
DFO	Department of Fisheries and Oceans Canada
D_M	migration distance
E_M	migration elevation
EPOC	excess post oxygen consumption
f_H	heart rate
f_{Hmax}	maximum heart rate

f_{Hrest}	resting heart rate
F_M	migration Fraser River flow
GSI	gonadalsomatic index
Hb	haemaglobin
Hct	hematocrit
HSI	hepatosomatic index
K^+	potassium
K_d	β_2-adrenoceptor binding affinity
M	body mass
MCHC	mean corpuscular haemaglobin concentration
$\dot{M}O_2$	rate of oxygen consumption (measured in mg)
$\dot{M}O_{2max}$	maximum oxygen consumption
$\dot{M}O_{2rest}$	resting oxygen consumption
MS-222	tricaine methanesulfonate
Na^+	sodium
$NaHCO_3$	sodium biocarbonate
OCLTT	oxygen- and capacity-limited thermal tolerance
P_{aO2}	arterial partial pressure of oxygen
P_{O2}	partial pressure of oxygen
POF	post-orbital-fork length
POH	post-orbital-hypural length
P_{vO2}	venous partial pressure of oxygen
$\dot{Q}$	cardiac output
$\dot{Q}_{max}$	maximum cardiac output

$\dot{Q}_{rest}$	resting cardiac output
RDCM	relative dry compact mass
RDVM	relative dry ventricular mass
RR	recovery ratio
RVM	relative wet ventricular mass
SEM	standard error of the mean
SSI	splenosomatic index
T90%	upper temperature experienced by the 90th percentile of fish
T_{aO2}	arterial oxygen transport
T_{crit}	critical temperature
T_M	migration Fraser River temperature
$T_{max0-50}$	group of fish swum at temperatures higher than T_{opt} at which 0-50% of maximum aerobic scope was attained
$T_{max50-90}$	group of fish swum at temperatures higher than T_{opt} at which 50-90% of maximum aerobic scope was attained
$T_{min50-90}$	group of fish swum at temperatures lower than T_{opt} at which 50-90% of maximum aerobic scope was attained
T_{opt}	optimal temperature
T_p	pejus temperature
T_{vO2}	venous oxygen transport
U_{crit}	critical swimming velocity
$\dot{V}_{O2}$	rate of oxygen consumption (measured in ml)
V_s	stroke volume
V_{smax}	maximum stroke volume

V_{srest}	resting stroke volume
w	AIC weight
β-AR	β-adrenoceptor

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DEDICATION

In loving memory to Grandpa Eliason, who taught me the value of gratitude, the power of a smile, to embrace life and most of all, sparked my love and wonder of fish.

CHAPTER 1: INTRODUCTION

Temperature has profound effects on the distribution and physiology of animals. Temperature effects occur over three distinct time scales: acute (direct effects occurring in minutes to hours), acclimation (physiological, morphological and biochemical adjustments occurring over days to weeks) and adaptation (spans generations, due to natural selection acting on individuals). Given that most fish are ectotherms, they are highly susceptible to perturbations in temperature that occur in their aquatic environment. The study of the physiological mechanisms that limit temperature tolerance is a biological problem of fundamental importance.

A central tenet in evolutionary biology is that geographically and reproductively isolated populations are locally adapted to cope with their specific environment (see Schluter, 2000; Taylor, 1991). Heritable traits that enhance survival and reproductive success in a given environment will likely be under strong selection pressure. This thesis examines temperature tolerance and local adaptation using genetically and geographically distinct populations of Fraser River sockeye salmon (*Oncorhynchus nerka*) as a model.

1.1 The Fry Curve for Aerobic Scope

Fry (1947) established that temperature both controlled and limited metabolic rate in fish, making a direct link between thermal tolerance and oxygen consumption. Fry recognized that temperature tolerance tests (e.g. CT_{min} and CT_{max}) were restrictive, only differentiating the temperature limits for short-term survival. Instead, Fry realized that it was essential to characterize and understand the temperature limits for a fish to thrive and interact within its environment – e.g. to escape predators, to interact with other animals, to migrate upstream, to

find, digest and assimilate food. As such, Fry examined the effects of temperature on aerobic scope, or the difference between minimum and maximum oxygen consumption. The 'Fry curve' for aerobic scope is typically bell-shaped as a function of temperature and represents the maximum oxygen available for activities beyond those considered maintenance, such as swimming, reproduction, feeding and growth. Thus, Fry curves can be used to examine the functional temperature limits for performance.

Oxygen consumption ($\dot{M}O_2$) varies as a function of temperature. Minimum or resting $\dot{M}O_2$ ($\dot{M}O_{2rest}$) represents the metabolic cost to simply exist in a resting, thermally acclimated, non-digesting, non-reproducing fish. $\dot{M}O_{2rest}$ typically increases exponentially with increasing temperature until it approaches lethal temperatures, as expected for temperature effects on rate functions (Fig 1.1). Obviously, in order to feed, reproduce, grow and move, fish must be able to increase $\dot{M}O_2$ above minimum levels. As temperatures increases, active or maximum $\dot{M}O_2$ ($\dot{M}O_{2max}$) increases faster than $\dot{M}O_{2rest}$, thus increasing aerobic scope (Fig 1.1). The optimal temperature (T_{opt}) coincides with maximal aerobic scope, as do maximal cardiac and swimming performance (Brett, 1971). Beyond T_{opt}, $\dot{M}O_{2max}$ fails to further increase and rapidly declines, causing a reduction in aerobic scope. The temperatures at which aerobic scope starts to decline are termed the pejus temperatures [T_p, pejus means getting worse (Pörtner, 2001)]. The range of temperatures between the upper and lower T_p when maximum aerobic scope is maintained is termed the T_{opt} window (Fig 1.1). At critical temperatures (T_{crit}), aerobic scope approaches zero (Fig 1.1), resulting in a transition to anaerobic metabolism and only passive, short-term survival (Pörtner, 2002; Pörtner, 2001; Pörtner and Farrell, 2008).

$\dot{M}O_2$ and aerobic scope varies considerably among species. Likewise, Fry curves take on different shapes. For example, eurythermal species such as goldfish (*Carassius auratus*) and

Fundulus heteroclitus have a broader T_{opt} window compared to more stenothermal fish like sockeye salmon (Fangue et al., 2006; Fry, 1947; Fry, 1957; Lee et al., 2003c). In addition, more athletic fish like wild sockeye salmon have a higher maximum aerobic scope compared to goldfish or hatchery-reared rainbow trout (*Oncorhynchus mykiss*) (Farrell, 2009). Moreover, aerobic scope will vary throughout the life cycle of an individual fish, shifting both the T_{opt} window and height of aerobic scope (Brett, 1965; Farrell, 2009). Aerobic scope also varies with environmental conditions (Farrell, 2009). For example, hypoxia (low environmental O_2) and hypercapnia (high environmental CO_2) reduce maximum aerobic scope and constrain the T_{opt} window (Pörtner and Farrell, 2008). Behaviour has also been demonstrated to alter T_{opt}. For example, competition shifted T_{opt} and supressed growth in brook trout (*Salvelinus fontinalis*) (McMahon et al., 2007). Thus, aerobic scope is highly pliable, varying across species, life stages, environmental conditions and with behaviour.

To understand the mechanistic basis of aerobic scope and its dependent relationship with temperature, details of how oxygen is delivered from the water to the mitochondria via the cardiorespiratory system are required. For fish such as salmonids, maximum $\dot{M}O_2$ occurs during maximum aerobic swimming. Therefore, information on cardiorespiratory physiology during swimming is necessary.

1.2 Cardiorespiratory Physiology with Swimming

According to the Fick equation for vascular perfusion, whole-animal $\dot{M}O_2$ is determined by the product of cardiac output ($\dot{Q}$) and the difference between arterial and venous oxygen content (C_{aO2} and C_{vO2}, respectively), which is termed the tissue oxygen extraction ($A\text{-}V_{O2}$ =

$C_{aO2} - C_{vO2}$): $\dot{M}O_2 = \dot{Q} \times A\text{-}V_{O2}$. $\dot{Q}$ is the product of heart rate (f_H) and stroke volume (V_s): $\dot{Q} = f_H \times V_s$. $A\text{-}V_{O2}$ is determined by the partial pressure of oxygen and the capacitance for oxygen in the blood. Accordingly, any change in $\dot{M}O_2$ must be due to alterations in some combination of these factors (i.e. f_H, V_s, C_{aO2} and C_{vO2}).

In order to support aerobic swimming, oxygen must be transported from the gills to the swimming muscles, which is one of the roles of the cardiovascular system. During maximal aerobic swimming, $\dot{Q}$ increases 2-3 fold in salmonids (Kiceniuk and Jones, 1977; Stevens and Randall, 1967; Thorarensen et al., 1996). While increases in both f_H and V_s contribute to the increase in $\dot{Q}$ during swimming, V_s typically increases to a greater extent in salmonids (Kiceniuk and Jones, 1977).

Another factor that can potentially be altered to increase $\dot{M}O_2$ is C_{aO2}. Oxygen transport to the tissues (T_{aO2}) by the circulatory system can be expressed as the product of $\dot{Q}$ and C_{aO2}. Arterial blood leaves the gills close to fully saturated as a consequence of the counter-current arrangement of blood and water flow at the gills. As a result, C_{aO2} is near maximal at rest and during swimming (Gallaugher et al., 2001; Kiceniuk and Jones, 1977; Randall and Daxboeck, 1982; Thorarensen et al., 1996). C_{aO2} could further increase by raising the blood haemoglobin (Hb) concentration either acutely via splenic contraction or chronically via erythropoiesis. However, blood [Hb] appears to be optimized in swimming rainbow trout (Gallaugher et al., 1995; Gallaugher et al., 1998), thus alterations to blood [Hb] may not play a major role during swimming. Therefore, the primary means of increasing T_{aO2} during swimming is via an increase in $\dot{Q}$.

The last means of increasing $\dot{M}O_2$ during swimming is through increased oxygen extraction from the blood by the tissues, which results in decreased C_{vO2} and P_{vO2} and typically a

2-3 fold increase in $A\text{-}V_{O2}$ (Farrell and Clutterham, 2003; Kiceniuk and Jones, 1977; Stevens and Randall, 1967). However, evidence has been presented for a minimum P_{vO2} threshold of around 15 torr in cold and 29 torr in warm, normoxic salmonids (Farrell and Clutterham, 2003, Farrell, 2007), which may serve as a mechanism to ensure sufficient oxygen is supplied to the heart (see section 1.3.2 below). Notably, during maximal swimming, tissue oxygen extraction may continue to increase despite a constant P_{vO2} since a decrease in blood pH (due to anaerobic metabolism) may elicit Root and Bohr effects on haemoglobin (a right and downward shift in the oxyhaemoglobin dissociation curve) thus facilitating oxygen unloading (Rummer, 2010).

As fish approach maximal swimming velocity during critical swimming tests, they switch to anaerobic metabolism (Burgetz et al., 1998). As a result, blood becomes acidic (low pH), hypoxemic (low P_{vO2}) and hyperkalemic (high $[K^+]$) (Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977), inhibiting cardiac contractility (Driedzic and Gesser, 1994). Adrenergic stimulation acts to maintain $\dot{Q}_{max}$ and protect against these noxious venous blood conditions during swimming (Hanson et al., 2006).

During recovery from anaerobic exercise, $\dot{M}O_2$ remains elevated (termed the excess post-exercise oxygen consumption; EPOC) in order to restore oxygen stores and support the metabolic costs associated with restoring high-energy phosphates, biochemical imbalance (e.g. glucose and lactate), ionic and osmotic imbalance and glycogen levels (Scarabello et al., 1992). EPOC represents a cost to the fish and could limit the ability for fish to resume normal activity in a timely manner. Even so, salmonids have been shown to have a remarkable ability to recover rapidly and repeat maximum swim performance after only a brief 30-45 min recovery period (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2006; MacNutt et al., 2004; Wagner et al., 2006).

Two important concepts emerge from this brief overview of cardiorespiratory physiology during swimming in fish. First, temperature could be acting on any or all of the components linking the transport of oxygen from the environment to the mitochondria. Second, in order to understand the effect of temperature on aerobic scope, I need to measure temperature effects on $\dot{M}O_2$, $\dot{Q}$, f_H, V_s, P_{aO2}, C_{aO2}, P_{vO2} and C_{vO2}, which are considered in section 1.3 below.

1.3 Oxygen and Capacity Limited Thermal Tolerance

Oxygen is the final electron acceptor in the suite of mitochondrial reactions that ultimately create ATP, the energy currency of the cell. The oxygen cascade is composed of several convection and diffusion steps during which oxygen travels down a partial pressure gradient from the environment to the mitochondria (Fig 1.2). First, oxygen-rich water is brought into contact with the respiratory surface. Gill ventilation rate and volume determine this step. Next, oxygen diffuses from the water environment, across the secondary lamellae of the gills, and into the blood where it binds to Hb in red blood cells. The partial pressure gradient between the water and the blood as well as gill anatomy set this step. The circulatory system transports the oxygen-bound Hb by convection to the tissues. Cardiac output and [Hb] govern this step. Finally, oxygen diffuses across the capillary wall and into the cell where it is ultimately used during mitochondrial respiration. This final step is controlled by tissue anatomy and the partial pressure gradient between the blood and mitochondria.

The mechanism of the decline in aerobic scope at temperatures above T_{opt} is poorly understood. Oxygen- and capacity-limited thermal tolerance (OCLTT) suggests that thermal tolerance is set by oxygen limitations due to a mismatch between cellular oxygen supply and

demand (Pörtner, 2001). This oxygen limitation is proposed to occur at the whole organism level, due to capacity limitations in ventilation and circulation (Pörtner, 2002; Pörtner, 2001; Pörtner and Knust, 2007). What is unclear is exactly which step(s) in the oxygen cascade limits oxygen flux, thus setting thermal limits. Specifically, is the mismatch in oxygen supply and demand due to a limitation at the gills, at the heart or at the tissues? Evidence for each of these possibilities has been detailed in Farrell (2009), and is outlined below.

The primary reason for the uncertainty is that few studies have studied the effect of temperature on performance limitations while simultaneously and directly measuring sufficient cardiorespiratory and oxygen status variables in order to identify the limiting factor(s) (Wang and Overgaard, 2007). While a small collection of studies have examined acute temperature effects on some of these variables in resting fish (e.g. Clark et al., 2008b; Gollock et al., 2006; Heath and Hughes, 1973; Sartoris et al., 2003), only Steinhausen et al. (2008) has measured all the necessary variables in fish swimming at close to maximum speed. Therefore, comprehensive cardiorespiratory studies in maximally swimming fish are needed.

1.3.1 Is There a Limitation in Oxygen Uptake at the Gill?

There are two primary reasons why an oxygen limitation at the gill has been proposed. First, it is well known that environmental oxygen availability decreases at high temperatures because water oxygen content decreases by around 2% per °C with increasing temperature (Dejours, 1975). Fish must therefore increase gill ventilation or increase oxygen extraction from the water to compensate. Second, there is decrease in blood oxygen affinity (a right-shift in the oxyhaemoglobin dissociation curve) as temperature increases (Jensen et al., 1998; Perry and

Reid, 1994), which hampers oxygen uptake at the gill, though it facilitates tissue oxygen extraction.

The key piece of evidence required to support the hypothesis that there is an oxygen limitation at the gill (via either insufficient oxygen delivery to the gills or an oxygen diffusion limitation across the gills) is a decrease in P_{aO2} and C_{aO2} at temperatures above T_{opt}.

Evidence supporting this hypothesis has been provided by Heath and Hughes (1973) who showed a decrease in C_{aO2} in resting rainbow trout exposed to an acute temperature increase. Notably, hematocrit was not measured to verify whether haemodilution occurred during repeated blood sampling. Similarly, Taylor et al. (1993) found a decrease in C_{aO2} at 18°C in resting and swimming rainbow trout acclimated to seasonal temperatures (4, 11 and 18°C), but hematocrit also decreased by half. A study by Clark et al. (2008b) produced conflicting results. Large resting adult chinook salmon (*Oncorhynchus tshawytscha*) displayed a decrease in C_{aO2} and P_{aO2} during an acute temperature increase while smaller adults did not. However, the holding tubes could have constrained gill movements in the larger chinook salmon, preventing adequate gill ventilation.

In contrast, two studies have found evidence against a limitation in oxygen uptake at the gill. Steinhausen et al. (2008) found that C_{aO2} and hematocrit remained constant in both resting and swimming sockeye salmon exposed to acute warming. Moreover, P_{aO2} actually increased in resting and remained constant in swimming sockeye salmon. Similarly, Sartoris et al. (2003) found that P_{aO2} remained constant during acute temperature increases in resting Atlantic cod (*Gadus morhua*). Therefore, current data are equivocal and further investigation is required.

1.3.2 Is There a Limitation in Oxygen Convection by the Heart?

An oxygen limitation at the level of the heart would be evident by the inability for $\dot{Q}_{max}$ to increase at temperatures above T_{opt}. Since oxygen demand increases with increasing temperature, $\dot{Q}_{max}$ must also increase in order to supply sufficient oxygen to keep pace with the tissue oxygen demand. If $\dot{Q}_{max}$ fails to keep up, insufficient oxygen will reach the muscles and the fish will cease or slow swimming. Indeed, several studies in swimming sockeye salmon and rainbow trout found that both $\dot{M}O_{2max}$ and $\dot{Q}_{max}$ ceased to increase above T_{opt} (Brett, 1971; Steinhausen et al., 2008; Taylor et al., 1996), providing evidence of a cardiac limitation.

The mechanistic basis of cardiac collapse at high temperature has been considered in detail (Farrell, 1997; Farrell, 2002; Farrell, 2007; Farrell, 2009; Farrell et al., 2009; Pörtner, 2002; Taylor et al., 1997). During warming, the increase in $\dot{Q}$ is almost entirely due to an increase in f_H (Clark et al., 2008b; Sandblom and Axelsson, 2007; Steinhausen et al., 2008). This is true in both resting and swimming fish (Farrell, 2009). Conversely, V_s has been demonstrated to be either insensitive to temperature (Cech Jr. et al., 1976; Clark et al., 2008b; Clark and Seymour, 2006; Gollock et al., 2006; Steinhausen et al., 2008) or to decrease at warm temperatures (Axelsson et al., 1992; Brodeur et al., 2001; Sandblom and Axelsson, 2007). The increase in f_H is likely mediated through a direct temperature effect on the pacemaker rate (Randall, 1970), which reaches a maximum of ~120 beats min^{-1} in many active fish (Davie and Farrell, 1991; Farrell, 1991). The prevailing idea is that maximum f_H is reached at T_{opt} (Farrell, 2009). Beyond T_{opt}, maximum f_H can no longer increase while resting f_H continues to increase, resulting in a decreased scope for f_H. Given that scope for f_H approached zero at high temperature for swimming sockeye salmon (Steinhausen et al., 2008), the inability for f_H to further increase

$\dot{Q}_{max}$ has been identified as a possible initiating factor limiting aerobic performance at high temperature (Farrell et al., 2009).

Another potential factor that could limit aerobic performance at high temperature is oxygen delivery to the heart itself. During exercise, oxygen requirements of the heart increase 3-5 fold, which makes up ~1% of total $\dot{M}O_2$ (Farrell and Steffensen, 1987). Salmonid hearts are composed of two types of myocardium; the compact myocardium and spongy myocardium. The outer, compact myocardium receives well-oxygenated arterial blood directly from the gills via the coronary system. As a result, the compact myocardium has a reliable source of oxygen during exercise, just like the skeletal muscles. Certainly, if P_{aO2} declines at warm temperatures (see section 1.3.1 above), then oxygen delivery to the compact myocardium could be impaired. In contrast, the inner spongy myocardium of salmonids lacks capillaries and receives oxygen from whatever is leftover in the venous blood by the other tissues. As such, the spongy myocardium has a much less reliable oxygen supply, especially since P_{vO2} decreases during swimming.

Though the *total* amount of oxygen in the blood (C_{vO2}) is likely sufficient to meet oxygen demand, a limitation may occur in the *rate* in which oxygen can be delivered, which depends on the oxygen tension (P_{vO2}), contact time of the blood (heart rate) and the arrangement of the spongy myocardium. The spongy myocardium is composed of trabeculae which are arranged in meshwork-like sheets that presumably increase the surface area for oxygen exchange (Pieperhoff et al., 2009). Regardless, if P_{vO2} decreases below a threshold level for an adequate rate of oxygen diffusion at high temperature, an oxygen diffusion limitation to the spongy myocardium may occur, resulting in cardiac failure and triggering a limitation in blood oxygen convection to the

swimming muscles. In light of this, individuals possessing a greater percentage of compact myocardium may be able to maintain cardiac performance at higher temperatures.

Cardiac collapse at temperatures above T_{opt} may also relate to the noxious venous blood environment created when exercising at high temperature. Salmonids increase their reliance on anaerobic metabolism when swimming at high temperature (Brett, 1964; Jain and Farrell, 2003; Steinhausen et al., 2008). Anaerobic metabolism leads to the triple threat of acidotic, hypoxemic and hyperkalemic venous blood, which inhibits cardiac contractility (Dridezic and Gesser 1994). Though adrenergic stimulation protected cardiac function and maintained $\dot{Q}_{max}$ in *in situ* perfused rainbow trout hearts exposed to the triple threat at optimal temperatures (Hanson et al., 2006), adrenergic protection was diminished at temperatures above T_{opt} (Hanson and Farrell, 2007). The attenuation of the protective and stimulatory effects of adrenaline at high temperature has been attributed to a decline in adrenaline-binding ventricular cell-surface β-adrenoceptor density (B_{max}) (Keen et al., 1993). As a corollary, individuals possessing an elevated B_{max} may be able to maintain $\dot{Q}_{max}$ at higher temperatures.

In summary, a limitation in oxygen convection by the heart could manifest in a number of ways. $\dot{Q}_{max}$ could fail to increase above T_{opt} due to reduced scope for f_H, due to insufficient oxygen delivery to the cardiac myocardium or due to the negative ionotropic and chronotropic effects of acidotic, hypoxic and hyperkalemic venous blood.

1.3.3 Is There a Limitation in Oxygen Delivery to the Tissue Mitochondria?

Muscle oxygen demand increases at high temperature due to temperature effects on rate functions as well as an increase in mitochondria proton leakage which leads to inefficient ATP

production in skeletal muscle (Barron et al., 1987; Pörtner, 2001). Therefore, the muscle must extract more oxygen from the blood in order to meet the increased demand at high temperature. Either a diffusion limitation or a perfusion limitation could lead to insufficient oxygen reaching the mitochondria to meet demand.

A diffusion limitation could develop due to inadequate driving force (low P_{aO2}), insufficient capillary density [white muscle has particularly low capillary density (Egginton et al., 2000)], or ineffective muscle cell morphology (poor mitochondria density or location). A perfusion limitation could result from inadequate $\dot{Q}$ leading to insufficient muscle capillary perfusion or an issue in blood flow distribution.

Several adjustments can help compensate for the increased oxygen demand at warm temperatures. The right-shift in the oxyhaemoglobin dissociation curve facilitates oxygen extraction (Jensen et al., 1998; Perry and Reid, 1994) as does the similar decrease in oxygen-affinity for myoglobin (Stevens and Carey, 1981). In addition, Krogh's diffusion coefficient for oxygen increases as biological fluid viscosity decreases with warming temperatures (Taylor et al., 1997).

An oxygen diffusion limitation at the tissues would become apparent if P_{vO2} was maintained at temperatures above T_{opt}, as has been reported in several studies. For example, Steinhausen et al. (2008) found no change in P_{vO2} with acute temperature increases in swimming sockeye salmon and P_{vO2} actually increased with temperature in resting fish. Further evidence comes from the observation that when fish quit swimming, venous blood was still partially saturated (Kiceniuk and Jones, 1977), and a venous threshold of ~20 torr (range = 15 to 29 torr in normoxia) has been proposed (Farrell and Clutterham, 2003; Farrell, 2007).

In contrast, other studies provide evidence against a tissue diffusion limitation. Heath and Hughes (1973) observed a decrease in C_{vO2} at high temperatures in resting rainbow trout, but as pointed out earlier, hematocrit was not measured. Likewise, Sartoris et al. (2003) reported a decrease in P_{vO2} in Atlantic cod exposed to an acute temperature increase. Clark et al. (2008b) found a significant decrease in P_{vO2} and C_{vO2} at the highest test temperatures in resting adult chinook salmon. Moreover, McKenzie et al. (2004) used optical fibre sensors in red muscle of rainbow trout at 13-15°C to determine that intramuscular P_{O2} never decreased below 45 torr, suggesting that oxygen supply to red muscle was not a limiting factor in exhaustion from swimming. Altogether, evidence is conflicting and further study is required.

1.4 Can Species Comparisons and Acclimation Studies Help Identify Limitations?

An indirect method of assessing potential limitations for exercise performance or thermal tolerance is to compare cardiorespiratory and morphological variables across a) species and b) with acclimation. Identified variables could represent potential locations where evolutionary adaptation has resulted in improved exercise performance or thermal tolerance.

For example, highly athletic fish such as tuna possess a greater $\dot{M}O_{2max}$, $\dot{Q}_{max}$, and C_{aO2}, enhanced gill surface area, large, pyramidal-shaped hearts with a higher percent compact myocardium, smaller red muscle fibres with greater capillary and mitochondrial density and a higher β-adrenoceptor density compared to less athletic species (Brill and Bushnell, 1991a; Brill and Bushnell, 1991b; Farrell, 1996; Mathieu-Costello et al., 1992; Mathieu-Costello et al., 1996; Olsson et al., 2000). In addition, aerobic exercise training in salmonids has resulted in higher $\dot{M}O_{2max}$, $\dot{Q}_{max}$, Hct, [Hb], C_{aO2}, and A-V_{O2}, increased cross-sectional area of red muscle,

increased red muscle capillarity, and cardiac hypertrophy (Davie et al., 1986; Farrell, 1991; Farrell et al., 1990; Farrell et al., 1991; Gallaugher et al., 2001; Kiessling et al., 1994; Thorarensen et al., 1993).

The same principle can be applied for thermal tolerance. For example, acclimation to warm temperature in teleosts resulted in smaller relative ventricular mass with a higher percent compact myocardium, decreased gill epithelial thickness, decreased red muscle capillarization and decreased β-adrenoceptor density (Egginton et al., 2000; Farrell et al., 1988a; Gamperl and Farrell, 2004; Gamperl et al., 1998; Goolish, 1987; Keen et al., 1993; Pelouch and Vornanen, 1996; Taylor et al., 1997) though many of these findings seem counterintuitive. Notably, stenothermic salmonids appear to have a limited capacity to acclimate in comparison with more eurythermal species such as goldfish. For example, a 10°C difference in acclimation temperature changed the upper incipient lethal temperature by only 0-2°C in juvenile chinook salmon (Brett, 1952) compared to ~5°C in goldfish (Fry, 1947).

Rather than applying this principle by comparing across species, I took advantage of the enormous variety in upriver migration environment among genetically isolated populations of sockeye salmon in the Fraser River watershed in order to make intraspecific comparisons in aerobic performance and temperature tolerance.

1.5 Fraser River Sockeye Salmon

Every year, millions of sockeye salmon return to the Fraser River (BC, Canada) to perform the physically demanding upriver migration. During this highly aerobic feat, sockeye salmon must swim continuously against a fast-flowing river for several weeks, swimming 2 to 4

km h^{-1}, which equates to ground speeds of 20 to 40 km day^{-1} (English et al., 2005; Hinch and Rand, 1998). Sockeye salmon cease feeding in the ocean, prior to entering the river. Therefore, upriver swimming and reproductive maturation (secondary sexual characteristics, gonad growth) are fuelled entirely by endogenous energy stores. Moreover, sockeye salmon are semelparous, meaning that they only spawn once. As a result, individual fish have a single opportunity to complete the journey to their spawning grounds in order to reproduce. Those that don't make it have zero reproductive success and no lifetime fitness. As a corollary, there is likely strong selection pressure for successful upstream migration.

Fraser River sockeye salmon display a remarkable fidelity to return to their natal stream to spawn (Burgner, 1991). This has resulted in over 100 genetically and geographically distinct populations of sockeye salmon within the Fraser River watershed (Beacham et al., 2005). Populations vary in migration distance (100 to 1100 km), elevation gain (10 to 1200 m), river temperature (9° to 22°C), and river flow (2000 to 10,000 m^3 s^{-1}). Moreover, some populations must traverse major hydrological barriers, such as world-famous Hells Gate, located in the Fraser Canyon ~200 km upstream from the mouth of the Fraser River. Swimming through these difficult stretches requires maximum aerobic scope and anaerobic swimming (Hinch and Bratty, 2000; Rand and Hinch, 1998). As such, some populations have a more difficult upstream migration compared to others.

1.5.1 Environmental Adaptation

Local adaptation has been defined as the process that increases the frequency of traits within a population that augments the reproductive success or survival of individuals posessing

such traits (Taylor 1991). For local adaptation to occur, a given trait must be 1) heritable, 2) differentially expressed across individuals, and 3) be associated with differential survival or fitness. Several correlational studies have provided circumstantial evidence of local adaption in salmonids (for a review, see Taylor, 1991). For example, juvenile Atlantic salmon (*Salmo salar*) and coho salmon (*Oncorhynchus kisutch*) from fast-flowing streams were more stream-lined and possessed longer paired-fins compared to fish residing in lower velocity streams (Riddell and Leggett, 1981; Taylor and McPhail, 1985a). Similarly, adult chum salmon (*Oncorhynchus keta*) and adult pink salmon (*Oncorhynchus gorbuscha*) from larger streams (and were thus exposed to faster flows) possessed larger fins relative to salmon in smaller streams (Beacham, 1984; Beacham, 1985; Beacham and Murray, 1987; Beacham et al., 1988a; Beacham et al., 1988b). Steelhead (*Oncorhynchus mykiss*) and coho populations with longer, more difficult upstream migrations had enhanced prolonged swimming performance compared to more coastal populations (Taylor and McPhail, 1985b; Tsuyuki and Williscroft, 1977). In addition, comparisons among 15 anadromous fish populations across 9 species found that populations with more difficult migrations were more energy efficient compared to those with easier migrations (Bernatchez and Dodson, 1985). Furthermore, a trade-off between egg number and migration distance was reported in chinook salmon (Kinnison et al., 2001).

Intraspecific variability in morphological, physiological and behavioural attributes in Fraser River sockeye salmon may be attributed to population-specific local adaptation which facilitates the adult migration and spawning. Indeed, Fraser River sockeye salmon populations with more difficult journeys started their migration with more somatic energy compared to those with shorter, easier migrations (Crossin et al., 2004; Gilhousen, 1980). Moreover, Crossin et al. (2004) demonstrated that Fraser River sockeye salmon populations with more challenging

migrations had fewer eggs and a smaller, more stream-lined body shape. In addition, two Fraser River sockeye salmon populations have been shown to vary in aerobic scope and both possessed a T_{opt} matching their historical river migration temperature (Farrell et al., 2008; Lee et al., 2003c). Finally, one sockeye salmon population (Chilko, which has a particularly arduous migration to spawn at a high elevation in or adjacent to a glacial lake) had more energetically efficient swimming relative to two other populations (Hinch and Rand, 2000). Collectively, these findings suggest that sockeye salmon arrive at the Fraser River prepared for their specific journey ahead, despite never before having performed the upriver challenge. My thesis builds on this theoretical and empirical support for local adaptation of Fraser River sockeye salmon.

1.5.2 Behavioural and Physiological Responses of Salmon to Temperature

The effect of water temperature on Pacific salmon migration has received substantial attention. Water temperature is known to impact a variety of traits: survival (Crossin et al., 2008; Farrell et al., 2008; Gilhousen, 1990; Macdonald, 2000), behaviour (Berman and Quinn, 1991; Cooke et al., 2004; Crossin et al., 2008; Farrell et al., 2008; Goniea et al., 2006; Hodgson and Quinn, 2002; Keefer et al., 2008a; Newell and Quinn, 2005; Patterson et al., 2007), migration speed (Hanson et al., 2008; Keefer et al., 2008a), swimming performance (Lee et al., 2003c; Farrell et al., 2008; Steinhausen et al., 2008), energetics (Crossin et al., 2004; Hinch and Rand, 1998; Rand et al., 2006), physiology (Crossin et al., 2008; Steinhausen et al., 2008; Young et al., 2006) and disease development (Wagner et al., 2005).

Sockeye salmon are exposed to a wide variety of temperatures during their migration period (Hinch et al., 2006), and Fraser River temperatures have been increasing over the last 60

years (Patterson et al., 2007). An individual fish can encounter temperatures ranging from 11-22°C during the short 3-4 week upriver migration. For example, individual fish routinely experience temperature swings of 3-4°C over 8 days while migrating up the mainstem Fraser (Donaldson et al., 2009). Average peak summer water temperature has increased by ~2°C since the 1950s and 8 of the past 10 summers have been the warmest on record (see Patterson et al., 2007). When faced with unfavourably high temperatures, individual sockeye salmon could make some combination of behavioural and physiological modifications.

Behaviourally, Pacific salmon can slow or cease swimming when exposed to temperatures outside their thermal optimum (Goniea et al., 2006; Keefer et al., 2008b; Salinger and Anderson, 2006). Pacific salmon may also alter the timing of their migration in order to avoid peak temperatures (Hodgson and Quinn, 2002; Quinn and Adams, 1996; Quinn et al., 1997; Robards and Quinn, 2002). Finally, Pacific salmon seeking cold-water refuge have been demonstrated to have improved survival and spawning success (Farrell et al., 2008; Mathes et al., 2010; Roscoe et al., 2010). However, given that sockeye salmon have finite energy reserves, it is not a viable long-term option to excessively slow or cease swimming. Spawning date is highly conserved to ensure egg and juvenile survival (Burgner, 1991), so major alterations in entry timing into the Fraser River are not possible. Upriver migration is energetically expensive, typically depleting more than 50% of stored reserves (Brett, 1995; Crossin et al., 2004) and excessive energy use during migration has been demonstrated to cause premature mortality (Rand and Hinch, 1998). Finally, not all populations have cold refugia available to them. For example, Nechako and Early Stuart sockeye salmon spend weeks migrating up the mainstem Fraser River, which has little-to-no cool water relief (Donaldson et al., 2009).

Phenotypic plasticity can play an important role in temperature tolerance in ectothermic vertebrates. However, the role of physiological plasticity in temperature tolerance is poorly understood in adult salmonids. On one hand, beneficial physiological modifications could enable salmon to cope with warm temperatures. For example, modifications could be made in mitochondrial density, membrane composition and the type and kinetic properties of metabolic enzymes (Guderley and St-Pierre, 2002; Pörtner, 2002). In addition, beneficial changes in muscle capillarization, contractility or muscle fibre type could occur (Egginton and Cordiner, 1997; Egginton and Sidell, 1989; Sidell and Moerland, 1989). Cardiac remodelling to alter the size or composition of the ventricle (Farrell et al., 1988a; Graham and Farrell, 1989), adjustments to the number or binding affinity of adrenaline-binding ventricular β-adrenoceptors (Keen et al., 1993) or alteration in respiratory epithelium thickness (Leino and McCormick, 1993) could enhance oxygen delivery. Therefore, phenotypic plasticity may play an important role in allowing sockeye salmon to adjust to the ever-changing environment during their upstream journey.

On the other hand, acclimatory responses to temperature may play a minor role for salmonids. For example, increasing the acclimation temperature of juvenile sockeye salmon from 10 to 23°C only increased the upper lethal temperature by 0.9°C (Brett, 1952). Moreover, f_{Hrest} and f_{Hmax} were similar in sockeye salmon acclimated to 22°C [86 and 106 beats min^{-1}, respectively, (Brett, 1971)] compared to sockeye salmon acutely warmed to 22°C [90 and 106 beats min^{-1}, respectively (Steinhausen et al., 2008)]. In addition, swim performance did not vary between cutthroat trout (*Oncorhynchus clarki*) given 48 h or 3 weeks to acclimate to 7, 14, or 18°C (MacNutt et al., 2004). Given that migrating sockeye salmon are simultaneously senescing, not feeding, undergoing massive morphological modifications as they sexually mature and performing an incredible athletic feat as they swim upstream, normal acclimation mechanisms

may be incomplete in adult sockeye salmon. In addition, temperature swings may be too swift during the short 3-4 week migration to allow a full physiological acclimatory response. As a consequence, swimming performance and cardiorespiratory capacity may be set at a level that is sufficient to meet the demand experienced at the highest and lowest temperatures typically encountered by a given population (Pörtner, 2002) and phenotypic plasticity may play a minor role in responding to temperature in migrating salmon. All told, the role of physiological acclimation in migrating sockeye salmon is poorly understood and warrants further investigation.

1.5.3 Conservation Implications

Peak summer river temperature in the Fraser River has warmed by ~2°C over the last 60 years (Fig 1.3). Elevated river temperatures have been repeatedly associated with adult mortality during the upriver migration, raising conservation concerns for this ecologically, economically and culturally important fish species (Hinch et al., 2006; Hinch and Martins, 2011). Current maximum river temperatures exceed T_{opt} for the two sockeye salmon populations (Gates and Weaver) that have been examined thus far (Farrell et al., 2008; Lee et al., 2003c). En-route mortality for returning adults clearly differs across populations and among years (Hinch and Martins, 2011). For example, in 2004, the Fraser River and its tributaries reached an exceptionally high temperature (>21°C) and an estimated 70-80% of Weaver sockeye salmon died during migration (Farrell et al., 2008). However, the proximate causes of in-river mortality are unknown. Physiological processes are critical in defining temperature-induced mortality (Wang and Overgaard, 2007; Wikelski and Cooke, 2006). Given that the current warming trends are expected to continue (Ferrari et al., 2007; Morrison et al., 2002), it is critical that population-

specific temperature tolerance is defined in order to identify populations most vulnerable to climate change. These discoveries will have important implications for biodiversity and management decisions.

1.6 Thesis Objectives and Hypotheses

The general objective of my thesis was to examine the physiological basis for thermal tolerance in sockeye salmon populations. Specifically, I sought to characterize how cardiorespiratory physiology varies among sockeye salmon populations and determine the mechanism of cardiorespiratory collapse at high temperature. I used an integrative approach, examining several levels of biological organisation, in order to test the overall hypothesis that Fraser River sockeye salmon populations have physiologically adapted to meet their specific upriver migration challenges. I predicted that thermal limits are set at a local level by physiological limitations in aerobic performance due to cardiac collapse. At each level of biological organisation (whole animal, organ and cellular), I made comparisons across populations and temperatures in order to examine this hypothesis.

The form of the thesis is as follows. Chapter 2 provides a detailed description of the Fraser River system, the populations of sockeye salmon I examined and the materials and methods used throughout this thesis. For each experiment, wild, migrating sockeye salmon were collected from the lower Fraser River, very early in the river migration (at the time of capture, the salmon had been in the Fraser River approximately 1-3 days). Therefore, the fish were collected before they encountered the majority of the upriver migration conditions and after they had spent >2 years in a common, cool ocean environment.

The specific research questions are presented in Chapters 3-7. In Chapter 3, I compared cardiorespiratory performance as a function of temperature across four new populations and incorporated data from two previously assessed populations (Lee et al., 2003c). I predicted that populations with more challenging migrations would have greater aerobic, cardiac and heart rate scopes. In addition, I predicted that each population can maintain maximum scope across the entire range of temperatures the adult salmon most frequently encountered during the upriver migration, as has been previously established for two populations (Farrell et al., 2008; Lee et al., 2003c).

Chapter 4 details the swimming physiology for the four upriver populations swum at T_{opt}. Specifically, I compared cardiorespiratory performance as well as arterial and venous blood variables among populations during two consecutive swim challenges. I predicted that each population would demonstrate excellent repeat swim performance, as has been demonstrated in the literature (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006). Moreover, I predicted that these four upriver populations would display similar cardiorespiratory performance since they all encounter similar challenging upriver conditions and demonstrated similar maximum aerobic and cardiac scopes in Chapter 3.

The detailed physiological studies in Chapters 3 and 4 allowed me to examine the mechanism of cardiorespiratory collapse at high temperature in Chapter 5.

In Chapter 5, I pooled the three upriver populations that did not differ in cardiorespiratory performance. I hypothesized that a limitation at the level of the heart, gills or tissue would lead to a mismatch between oxygen supply and demand, and result in impaired aerobic scope and

swim performance. I predicted that aerobic scope is limited at high temperatures due to cardiac collapse.

Since the heart emerged as a key player in temperature tolerance and supporting aerobic swimming in Chapters 3, 4 and 5, I examined cardiac morphology in Chapter 6. I predicted that populations with more challenging migrations would have larger ventricles and a greater proportion of compact myocardium compared to those populations with easier migrations. I also examined how cardiac morphology is affected by temperature exposure.

In Chapter 7, I examined a potential mechanism at the cellular level of the heart for the higher and broader thermal tolerance of one population (Chilko) compared to another co-migrating population (Nechako). I hypothesized that Chilko sockeye salmon would possess an enhanced ability to use adrenaline which would provide greater cardiac capacity and protection at high temperatures, thereby expanding their thermal tolerance.

Chapter 8 concludes my thesis and provides a final synthetic discussion as well as future directions for research.

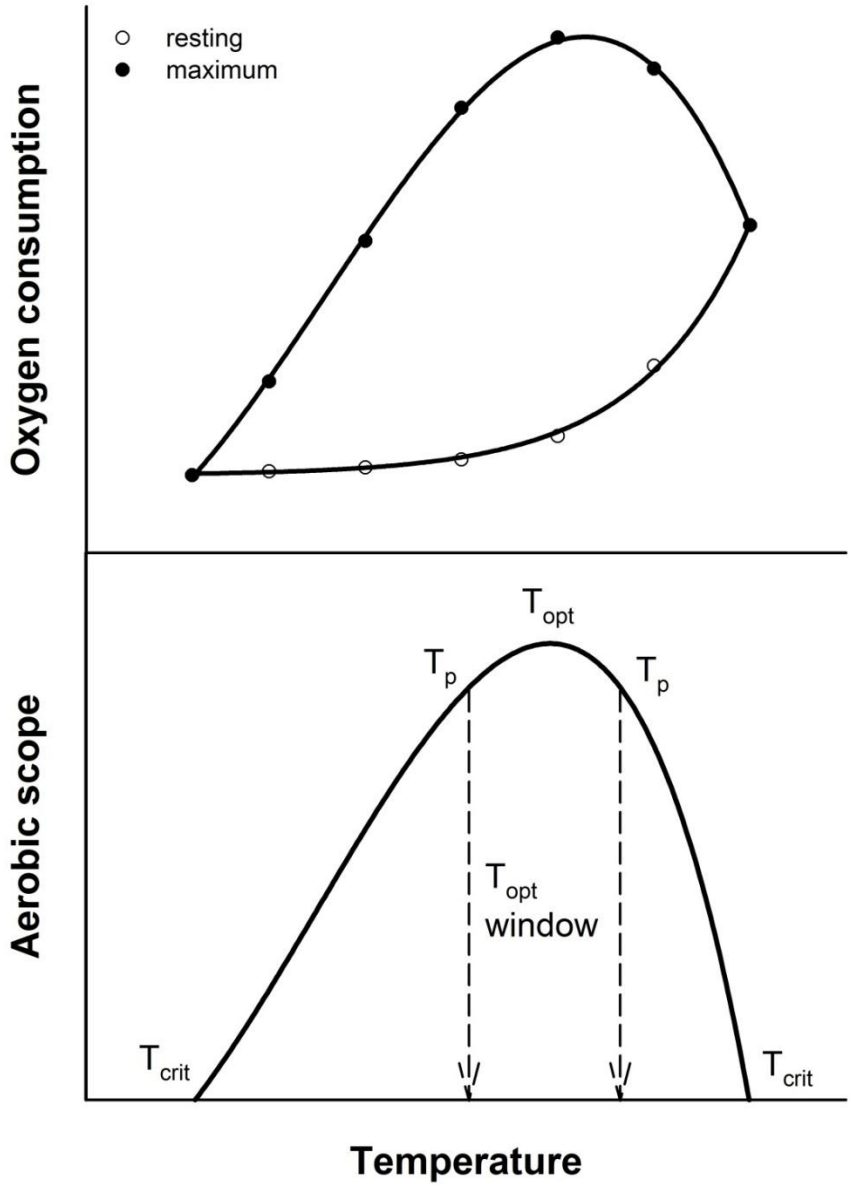

Figure 1.1. Schematic of resting and maximum oxygen consumption and aerobic scope. See text for details. T_{opt} = optimum temperature, T_p = pejus temperatures, T_{crit} = critical temperatures. The T_{opt} window corresponds to the range of temperatures between the upper and lower T_p.

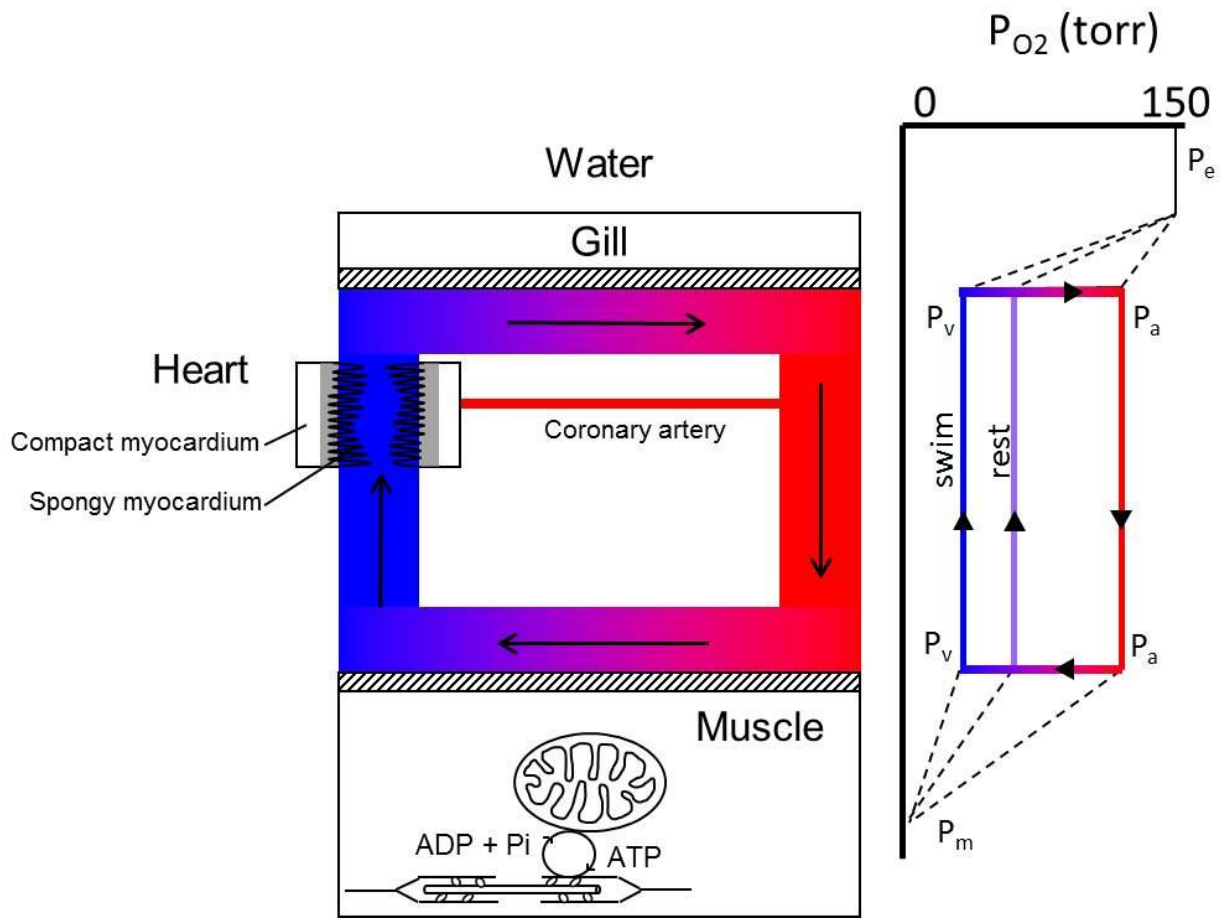

Figure 1.2. Schematic of the oxygen cascade. Step 1: Oxygen-rich water is brought into contact with the gill. This step is determined by gill ventilation and volume. Step 2: Oxygen diffuses from the environment, across the gills and into the blood where it binds to haemoglobin. This step is determined by the partial pressure gradient of oxygen (P_{O2}) between the water (P_e) and the blood (P_v to P_a) as well as gill anatomy (surface area, diffusion distance and the permeability coefficient of oxygen). Step 3: The circulatory system transports the oxygen-bound haemoglobin by convection to the tissues. This step is governed by cardiac output and the quantity of oxygen per unit arterial blood (which in turn is primarily determined by the haemoglobin concentration). Step 4: Oxygen diffuses across the capillary wall and into the cell, where it is ultimately used during mitochondrial respiration. This final step is determined by the partial pressure gradient between the blood (P_a to P_v) and the mitochondria (P_m) as well as tissue anatomy (surface area, diffusion distance, the permeability coefficient of oxygen and the quantity of mitochondria). During swimming, more oxygen is extracted by the swimming muscles, resulting in a lower P_{O2} in the venous blood. See Weibel (1984). Note that the heart is composed of two types of myocardium. The outer compact myocardium has a coronary circulation, so it is perfused with oxygen from the arterial system. The inner spongy myocardium is avascular, so it receives oxygen from the venous system.

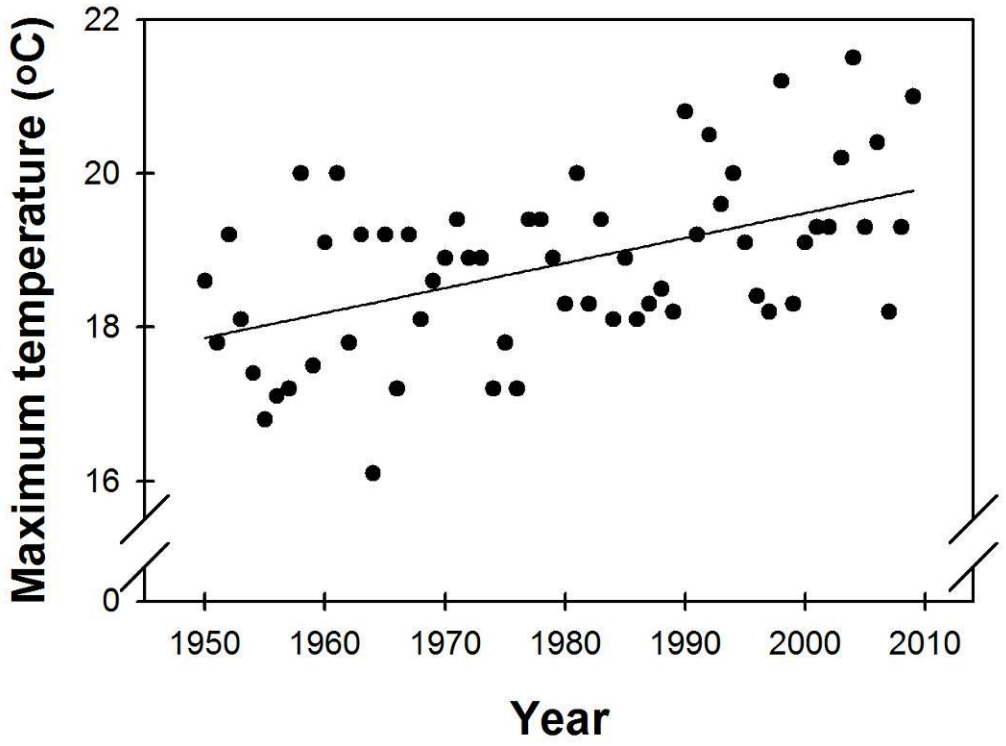

Figure 1.3. Maximum yearly Fraser River water temperature at Hells Gate from 1950-2009 (y = 0.0324x – 45.3776, $p < 0.0001$, $R2 = 0.25$) (see Patterson et al., 2007).

CHAPTER 2: MATERIALS AND METHODS

2.1 Migration Conditions and Migration Difficulty Indices

Spawning grounds differ in their distance and elevation from the mouth of the Fraser River (Table 2.1; Fig 2.1) and populations initiate up-river migrations at different times of the year. Fisheries managers categorize populations into four major run-timing groups (Early Stuart, Early Summer, Summer and Late) based on the historic timing of Fraser River entry. Mainstem Fraser River discharge decreases from June to November, but temperature typically increases until August and declines thereafter. The Early Stuart run populations enter the Fraser River in early July and experience the highest river flows and moderate temperatures early in their migration and increasing temperatures towards the end of their migration. Early Summer and Summer run populations enter in late July and August and experience the warmest temperatures in the mainstem Fraser River early in their migration and moderate flows. Late run populations enter in the fall and experience the lowest flows and coolest average temperatures compared to the other entry runs (Table 2.1). Therefore, the temperature and water velocity experienced also varies among populations.

Clearly, the relative difficulty of the migration varies considerably and complexly among populations. As a result, the difficulty of population-specific migrations was characterized using several indices. The first was based on whether or not populations pass through Hells Gate, a major hydraulic barrier (Hinch and Bratty, 2000) about 200 km upriver from the mouth of the Fraser River (Fig 2.1) and upstream of the location where fish were sampled for all the

experiments in this thesis. Populations were categorized as "coastal" if they did not pass through Hells Gate and "upriver" if they did (Table 2.1).

Migration difficulty was also characterized for each population based on the river migration distance from the Fraser Delta (Steveston, BC) to the spawning grounds (D_M) and the elevation of the main spawning grounds (E_M) (Table 2.1). Three additional indices were calculated according to the concepts of physical work and river slope (Crossin et al., 2004; Gilhausen, 1980). In physics, "work" is defined as the product of force over a given distance. The amount of work a salmon must do to reach the spawning ground can be estimated using E_M or F_M (elevation or river discharge, as a surrogate for force) and D_M (distance). Migratory work was determined as $k_1 \bullet E_M \bullet D_M$ and migratory effort was determined as $k_2 \bullet D_M \bullet F_M$. In addition, while river distance and elevation do co-vary somewhat, a short migration can be steeper than a long migration. Therefore, river slope ($k_3(E_M D_M^{-1})$) was included as an additional index. The correction factors k_1, k_2 and k_3 (0.001, 0.0001 and 500, respectively) simplify presentation.

Historic environmental and migratory data were collected for eight sockeye salmon populations. Lower Fraser River discharge (F_M) data were obtained from the Water Survey of Canada. Lower Fraser River temperature (T_M) data were provided by Fisheries and Oceans Canada (DFO) Environmental Watch Program (see Patterson et al., 2007). For upstream populations, F_M and T_M were measured near Hope (Fig 2.1), centered on the historic date of peak salmon passage through Hells Gate. For coastal populations, F_M and T_M were measured at Mission (Fig 2.1), centered on peak Mission salmon passage. Lower river conditions have been previously used as indices of the total freshwater migratory experience given the generally strong correlation between lower and upper river environmental conditions (Hague et al., 2008). Furthermore, lower river temperature and flow have been correlated to both indirect and direct

estimates of spawning migration mortality (Macdonald et al., 2010; Martins et al., 2011). Median and modal migration temperatures were calculated from the population-level temperature histograms used in the present study (see below, Table 2.1). While river migration speeds vary considerably among individuals and populations, biotelemetry experiments have repeatedly shown that sockeye salmon tend to migrate continuously in freshwater until they reach their natal systems, achieving ground speeds of 15-40 km d^{-1} depending on river section (English et al., 2005; Hanson et al., 2008). Average ground speeds for each population across the total freshwater migration route were determined using data obtained from radio-biotelemetry studies performed by LGL Environment Ltd from 2002-2007 (Hague et al., 2008; Martins et al., 2011). Migration duration was determined by dividing the migration distance by migration rate (Table 2.1).

The average thermal units accumulated during freshwater migration [i.e. Accumulated Thermal Units (ATU)] were calculated for the "active" part of the migration (i.e. the time when fish were actively migrating upstream, which did not include river or lake holding near the spawning ground). Peak Fraser River entry (average date between 1977-2008 at which 50% of the run-timing group passed Mission minus two days for travel time from Fraser Delta to Mission) and peak Hells Gate passage times (average date between 1977-2008 at which 50% of the run-timing group passed Mission plus 4-5 days travel time, depending on average migration rate for each population) were provided by the Pacific Salmon Commission. Peak spawning date was provided by Fisheries and Oceans Canada Stock Assessment Division (Table 2.1).

Population-level temperature distributions (see Chapter 3) were simulated using an individual-level freshwater migration model which integrated across daily average river and lake temperatures experienced over the "active" period of the spawning migration (i.e. did not include

lake or river holding temperatures prior to spawning) from 1995 to 2008 (modified from Farrell et al., 2008).

Since 1995, several populations of late-run sockeye salmon (e.g. Weaver, Harrison, Lower Adams) have entered the Fraser River up to six weeks earlier than previously observed, a phenomenon that is poorly understood (Cooke et al., 2004; Hinch, 2009). As a result of the early river entry, these salmon encounter considerably warmer temperatures and in-river mortality has exceeded 90% in some years (Cooke et al., 2004; Hinch, 2009). Therefore, the environmental data (Table 2.1) and temperature frequency histograms (Chapter 3) are presented for both historical run timing (before 1995) and the current early entry phenomenon (1995-2008) for comparison.

Each population experiences a broad range of temperatures throughout their brief 1-4 week migration, which varies depending on river entry timing, spawning ground location and year-to-year variation. Some Summer run populations routinely experience temperatures as low as 11°C (e.g. Chilko during the final third of their migration when they enter the Chilcotin River and ascend to their spawning location in or downstream of a glacial lake) and as high as 22°C (e.g. near the mouth of the Fraser River during August when river temperatures tend to peak). Studies using radio tags and thermal loggers have shown that individual fish routinely experience temperature swings of 3-4°C over 8 days during their migration up the mainstem Fraser River (Donaldson et al., 2009). Experimental temperatures were selected to span the entire range of temperatures encountered during migration. In addition, brief exposures to temperatures exceeding those typically encountered in the wild were also used to assess high and low temperature tolerance.

For most populations, the highest temperature experienced during river migration occurs in the lower Fraser River. Since the 1940s, the maximum daily water temperature at Qualark (near Hells Gate in the lower Fraser River) has been 21.5°C, which occurred in 2004. The Early Summer and Summer run groups would experience such peak temperatures, as would any Late run population that entered the river early, as they have been doing since the mid-1990s. In fact, Weaver sockeye (which belong to the Late run group) entered the Fraser River early in 2004, experienced temperatures reaching 21.5°C and over 50% of the population died en route to the spawning area (Mathes et al., 2010). The main exception to this generalization is Early Stuart sockeye salmon, which typically experience cool water in the lower Fraser River but warmer temperatures later in their migration when they are closer to their spawning grounds ~1,000 km upstream (Macdonald et al., 2007). A temperature of 21.5°C is a reasonable maximum temperature experienced by Early Stuart sockeye salmon during the final stage of their river migration. Therefore, 21.5°C is indicated in Chapter 3 as the current temperature maxima experienced by Fraser River sockeye salmon.

2.2 Fish Collection

Wild adult sockeye salmon were collected in the lower Fraser River or Harrison River (a lower Fraser tributary) using a beach seine or gill net while fish were en-route to their spawning grounds and shortly after entry into freshwater (Fig 2.1). Notably, the fish were collected very early in their river migration and before they had experienced most of the upriver migration conditions. The sockeye salmon were transported 25-75 km by land to the DFO Cultus Lake Salmon Research Laboratory (CLL, Cultus Lake, BC, Canada). Following capture, all sockeye

salmon were given a unique cinch tag or PIT tag (Passive Integrated Transponder tag, approximately 8.5 mm x 2 mm size, Biomark Inc., Boise, Idaho) for individual identification, a scale was removed and <0.1 g of the adipose fin was clipped for population identification via DNA analysis (Beacham et al., 2005). The DNA analysis compares one major histocompatibility complex (MHC) loci or five single nucleotide polymorphisms (SNPs) in addition to 14 microsatellite loci and assigns a probability of population identification (Beacham et al., 2005). This method has been demonstrated to correctly assign 94% of individuals to the correct population aggregate (as defined below) using simulations run with the program cBAYES (Beacham et al., 2005; Beacham et al., 2004; Beacham et al., 2010). Due to low sample sizes or an inability to definitively assign population identification between co-migrating, adjacent populations, some populations spawning in adjacent rivers or lakes were grouped as a single population. Chilko is composed of two spawning populations, one that spawns in the lake and one that spawns in the lake outlet (Chilko River). Quesnel comprises two main populations which spawn 47 km apart (Mitchell and Horsefly Rivers; inlet tributaries to Quesnel Lake). Nechako is composed of four populations that spawn within 100 km of each other (Stellako, Nadina, Tachie and Middle River). Early Stuart is made up of 40 small populations that spawn within 100 km of each other (Beacham et al., 2005). Lower Adams, Weaver, Harrison and Gates are all genetically distinct, single populations. All procedures were approved by the University of British Columbia's Animal Care Committee in accordance with the Canadian Council on Animal Care (A06-0328 and A08-0388).

2.3 Surgical Procedures

In order to measure cardiorespiratory variables, the fish underwent surgery before the swim tests. Individual fish were anaesthetized with buffered tricaine methanesulfonate in freshwater (0.2 g l^{-1} $NaHCO_3$ and 0.1 g l^{-1} MS-222, Sigma, St. Louis, MO), weighed and transferred onto wet foam on a surgical table where their gills were continually irrigated with aerated, chilled freshwater with a lower dose of anaesthetic (0.15 g l^{-1} $NaHCO_3$ and 0.075 g l^{-1} MS-222). Surgical procedures have been detailed elsewhere (Steinhausen et al., 2008). To sample arterial blood, a PE-50 cannula was inserted into the dorsal aorta (Soivio et al., 1973). To measure cardiac output, a 3 mm SB flowprobe (lateral cable exit, Transonic systems, Ithaca, NY, USA) was positioned around the ventral aorta without opening the pericardium (Steffensen and Farrell, 1998). To sample venous blood, a PE-50 cannula was inserted into the ductus of Cuvier and advanced towards the heart into the sinus venosus (Farrell and Clutterham, 2003). Both cannulae were filled and regularly flushed with heparinized saline solution (150 IU ml^{-1}). The flowprobe lead and cannulae were secured together and sutured to the fish's body using 2-0 silk. The fish were placed in a Brett-type swim tunnel and allowed to recover overnight at low water velocity of ~0.39 bl s^{-1} before starting the swim tests.

2.4 Swimming Experiments

The swimming tests were conducted in 2007, 2008 and 2009 at CLL (N = 97). Fish were held at 11-12°C for 1-4 weeks in outdoor 8,000–12,000 l circular aquaria supplied with filtered and UV sterilized freshwater (~40 l min^{-1}; LS-Permabead Filtration System, Integrated Aqua

Systems Inc., Escondido, California) under seasonal photoperiod. The fish were not fed because they had ceased feeding naturally before entering the Fraser River. Three days before the swimming test, fish were placed in 1,400 l circular aquaria and the temperature was progressively increased to the test temperature (13-22°C) by no more than 5°C day^{-1}. The fish were maintained at this temperature for at least one day before the swim tests were conducted.

Following overnight recovery from surgery at their test temperature, resting oxygen consumption ($\dot{M}O_2$), cardiac output ($\dot{Q}$), arterial and venous blood were measured at a water velocity of ~0.39 bl s^{-1}. Then the fish underwent a ramp-U_{crit} swim protocol (Jain et al., 1997; Lee et al., 2003c). The velocity of the water was increased every 5 min until approximately 50% of U_{crit} was reached (~1.0 bl s^{-1}). Thereafter, the speed was increased by approximately 0.25 bl s^{-1} every 20 min until the fish no longer swam continuously and rested on the back grid for >30 s. The water speed was reduced to the resting velocity and the fish were allowed a 45-min recovery before repeating the same ramp-U_{crit} swim protocol. The fish were allowed to recover for 2 h after the second swim test.

$\dot{Q}$ was measured continuously throughout the swim trial. $\dot{M}O_2$ was measured during the second half of every 20-min speed interval. If the dissolved oxygen levels approached 7.0 mg O_2 l^{-1}, $\dot{M}O_2$ was deliberately not measured to maintain a normoxic environment in the swim tunnel. Blood samples (~0.7 ml per sample) were collected during the second half of the first 20-min swim interval (mean speed = 1.18 bl s^{-1}, or 56% of maximum swim speed) during steady swimming. Blood was sampled again when the fish exhibited burst-and-coast swimming near exhaustion (mean speed = 2.1 bl s^{-1}, or 93% of maximum swim speed). Additional blood samples were occasionally taken at intermediate speeds for some fish. $\dot{M}O_2$ and blood were

sampled immediately after the fish quit swimming (fatigue), and again after 45 min of recovery. Final samples were collected after a 2-h recovery period following the second swim.

A subset of fish did not undergo surgery, but were otherwise treated the same as the fish that were instrumented.

I was unable to hold fish at extremely high or low temperatures; therefore, some fish did not undergo the same three-day temperature exposure prior to surgery. Instead, they were allowed to recover overnight from surgery in the swim tunnel at 12°C and in the morning the water temperature was acutely increased or decreased by 4°C h^{-1} to the test temperature (8-10°C or 22-26°C). After one hour at the test temperature, resting values were recorded as above and then the fish underwent a single ramp-U_{crit} swim protocol, after which the temperature was returned to 12°C over 2 h. Occasionally, some fish at the highest test temperatures displayed cardiac disrhythmias while resting and before the swim test. In the few cases when this occurred, the temperature was immediately decreased and the fish were not used. As such, all fish began their swim test with a regular, rhythmic heart rate.

Upon conclusion of the swim test, the fish was removed from the swim tunnel and sacrificed by a cranial blow. A post-mortem caudal blood sample was collected using a Vacutainer (2-3 ml) and mass (whole body, liver, gonad, spleen, heart), length [standard length, fork length, post-orbital-hypural (POH) length, post-orbital-fork (POF) length], girth and depth were measured for each fish. Gonadosomatic index (GSI), hepatosomatic index (HSI) and splenosomatic index (SSI) were calculated as the mass of the gonad, liver and spleen divided by body mass, respectively (see below for details of heart calculations). In order to estimate the energy status of each fish, proximate constituent analysis was conducted on a ~200 g piece of dorsal muscle, removed from the left side of the fish between the operculum and the dorsal fin.

The concentrations of protein, lipid, moisture and ash were assessed so that gross energy could be estimated (Crossin et al., 2004; Higgs et al., 1979).

2.5 Swim Tunnels

Two Brett-type swim tunnels were used to swim individual fish, which have been fully described elsewhere (Lee et al., 2003c; Steinhausen et al., 2008). Both swim tunnels were equipped with a custom-designed heating system which could maintain the set water temperature ± 0.5°C. The velocity of the water was calibrated (± 1 cm s^{-1}) using an anemometer (Valeport Marine Scientific, Dartmouth, UK).

2.6 Whole Blood and Plasma Analysis

Whole blood samples were used to measure partial pressure of oxygen (P_{O2}), oxygen content (C_{O2}), haemoglobin concentration (Hb) and hematocrit (Hct). The samples were held at 4°C and analyzed shortly after collection. Blood P_{O2} was measured using a blood gas monitor (PHM 73, Radiometer, Copenhagen, Denmark) which was calibrated and maintained at each temperature using a water jacket. Blood C_{O2} was measured according to the method of Tucker (1967). Hb was measured using either a handheld haemoglobin analyzer (Hemacue 201^{+}, Ängelholm, Sweden) calibrated for fish blood (Clark et al., 2008a) or the spectrophotometer method with Drabkin's solution (Clark et al., 2008a; Drabkin and Austin, 1935). Hct was measured in duplicate using microhematocrit capillary tubes spun at 10,000 g. The remaining

blood was centrifuged at 7,000 g and the plasma was flash frozen in liquid nitrogen and stored at -80°C for subsequent analyses.

Plasma cortisol (ELISA kit, Neogen , Lexington, KY, USA), glucose and lactate (YSI 2300 Stat Plus analyzer), sodium and potassium (Cole-Parmer, model 41- single channel flame photometer) and chloride (Haake Buchler digital chloridometer) were measured on all blood samples (see Farrell et al., 2001a). Plasma testosterone and 17β-estradiol (ELISA kit, Neogen , Lexington, KY, USA) were only determined for the final caudal blood sample from Early Stuart and Chilko sockeye salmon in 2008 and 2009.

2.7 Data Analysis and Calculations for Cardiorespiratory Variables

During an $\dot{M}O_2$ measurement, the inflow and outflow water to the tunnel were turned off and the decrease in oxygen content over time was measured. Oxygen content of the water in the swim tunnel (mg O_2 l^{-1}) was measured using an Oxyguard probe (Point Four Systems, Richmond, Canada) attached to a Windaq box (Dataq instruments, Akron, ON, USA) interfaced with Labview software (6.0, National Instruments, Austin, TX, USA). The duration of the measurement was sufficient so that the dissolved oxygen decreased by at least 0.3 mg O_2 l^{-1}, which resulted in a linear regression with r^2 values typically >0.95. $\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1}) was calculated as: $\dot{M}O_2 = \triangle[O_2] \bullet v \bullet M^{-1} \bullet t^{-1}$ where $\triangle[O_2]$ is the change in water content (mg O_2 l^{-1}), v is the volume of the water minus the volume of the fish (l), M is the mass of the fish (kg) and t is the time (min). Background $\dot{M}O_2$ was measured after each swim trial and determined to be negligible.

U_{crit} was calculated as in Brett (1965): $U_{crit} = U_f + (t_f/ti \bullet Ui)$ where U_f is the water velocity of the last fully completed increment, t_f is the time spent in the final water velocity increment, t_i is the time period for each completed increment, and U_i is the water velocity increment. U_{crit} was calculated in both body lengths per second (bl s^{-1}) and cm per second (cm s^{-1}). U_{crit} was corrected for the solid blocking effect according to Bell and Terhune (1970) using the following equation: $U_F = U_T \bullet (1 + \varepsilon_s)$ where U_F is the corrected flow speed, U_T is the speed in the tunnel without the fish, and ε_s is the error due to solid blocking. ε_s is calculated as: $\varepsilon_s = \tau \bullet \lambda \bullet (A_o/A_T)^{1.5}$ where τ is a dimensionless factor depending on the swim chamber cross section (0.8 in this study), λ is the shape factor for the fish (0.5 body length/body thickness), A_o is the cross sectional area of the fish and A_T is the cross sectional area of the swimming chamber. The recovery ratio (RR) was calculated as RR = U_{crit} 2/ U_{crit} 1 to determine how the first U_{crit} compared to the second U_{crit}.

To measure $\dot{Q}$, the flowprobe was connected to a flowmeter (Transonic systems, Ithaca, New York, USA) and blood flow was measured at 200 hz using Biopac hardware and Acknowledge software (Biopac systems, Santa Barbara, CA, USA). $\dot{Q}$ was calculated as the mean of at least three 30 s segments. Heart rate (f_H) was measured from the flow trace during the 30 s segments using the automated software which was confirmed with manual counting. Stroke volume (V_s) was calculated as $\dot{Q} = f_H \bullet V_s$.

Cost of transport (COT) was calculated as: $COT = \dot{M}O_2/U$ where $\dot{M}O_2$ was measured in mg O_2 kg^{-1} min^{-1} and U was the swimming speed in m s^{-1}, corrected for the solid blocking effect. Net cost of transport (COT_{net}) was calculated as: $COT_{net} = (\dot{M}O_2 - \dot{M}O_{2rest})/U$. Similarly, cost of transport for cardiac output (COT-$\dot{Q}$) and net cost of transport for cardiac output (COT-$\dot{Q}_{net}$) were calculated.

Oxygen extraction ($A\text{-}V_{O2}$) was calculated as arterial oxygen content (C_{aO2}) - venous oxygen content (C_{vO2}) and was only assessed in fish that had both cannulae working simultaneously. Arterial oxygen transport (T_{aO2}) to the tissues was calculated as the product of $\dot{Q}$ and C_{aO2}. Venous oxygen transport (T_{vO2}) to the spongy myocardium and gills was calculated as the product of $\dot{Q}$ and C_{vO2}. Mean corpuscular haemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100).

Aerobic scope and cardiac scope were determined as the difference between the resting and maximum values. Scope for heart rate and scope for stroke volume were determined as the difference between the resting values and those measured at maximum cardiac output.

To determine the Fry curves for aerobic scope, a second order polynominal regression was fitted to the aerobic scope data from individual fish of each population swum across a range of temperatures. The same method was used to develop the curves for cardiac scope, scope for heart rate and scope for stroke volume. Optimal temperature (T_{opt}) for each population was determined as the temperature corresponding to the peak of the polynomial regression for aerobic scope. The upper and lower pejus temperatures (T_p) were assigned to 90% of the maximum aerobic scope, with the T_{opt} window being defined as the range of temperatures between the upper and lower T_p. The upper critical temperature (T_{crit}) was defined by extrapolating the polynominal regression for aerobic scope to the upper temperature when aerobic scope reached zero. The value of aerobic scope at T_{opt} was determined as the average of the individual data points within the T_{opt} window for each population. The upper temperature experienced by the 90^{th} percentile of each population (T90%) was determined from the historic temperature distributions and the percentage of maximum aerobic scope available at T90% was determined for each population.

There were insufficient data points across a range of temperatures to plot an aerobic scope curve or determine T_{opt} for Lower Adams sockeye salmon. Similarly, there were insufficient data points at cooler temperatures to define the lower T_p or determine T_{opt} for Quesnel sockeye salmon. Therefore, aerobic scope at T_{opt} was based on the plateau of individual data points from fish swum at temperatures corresponding to those typically encountered during upriver migration. For Lower Adams, this temperature range also corresponds with optimal temperatures previously estimated for this population (Steinhausen et al., 2008)

Data from the swim tests conducted in 2007, 2008 and 2009 are presented over three chapters (Chapters 3-5). Chapter 3 presents the overall highest maximum and scope values obtained over the two swims. All fish were included in this chapter. Chapter 4 compares swim 1 and swim 2 in fish that had undergone surgery from four upriver populations (Early Stuart, Nechako, Chilko and Quesnel). Chapter 5 compares cardiorespiratory performance of swim 1 across four different temperature categories (details of the temperature categories are provided in Chapter 5).

2.8 Gross Heart Morphology

2.8.1 Animal Acquisition

Sockeye salmon heart samples were collected from a variety of experiments. In all cases, the fish were collected early in their migration, prior to encountering any of the major upriver migration challenges (as outlined in section 2.2). The three sections below detail the different experimental conditions.

Population Comparisons

Male and female sockeye salmon hearts from seven populations (N = 194, Early Stuart, Chilko, Quensel, Nechako, Lower Adams, Weaver, Harrison) were collected opportunistically from various experiments conducted at CLL in 2007 and 2008 (e.g. the swimming experiment outlined above). However, I restricted population comparisons of cardiac morphology to female sockeye salmon. It is well known that salmonids can rapidly remodel their hearts in response to biological and environmental cues (Gamperl and Farrell, 2004). For example, male salmonids increase RVM up to 2-fold with sexual maturation. In contrast, female salmonid ventricles do not change size with sexual maturation (Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992). Consistent with this knowledge, cardiac morphology significantly varied with temperature treatment among male but not female sockeye salmon (see Chapter 6). There were insufficient numbers of male fish from a particular temperature treatment and sexual maturation level for all populations in order to make comparisons, so males were excluded from the population analysis.

Temperature Exposure

In 2007, Chilko sockeye salmon (N = 34) were collected from the lower Fraser River and held at CLL in 8,000 – 12,000 l tanks for 4-6 days at 12°C. The fish were then exposed to 14, 16.5 or 19°C (± 0.5°C) for up to 14 days, or until they died. Only fish that were held at their temperature treatment for at least 5 days before dying were included in the analysis.

Swimming Experiment

In 2006, Lower Adams sockeye salmon (N = 16) were collected by purse seine in the Strait of Georgia, held at the DFO & UBC Centre for Aquaculture and Environmental Research (CAER, West Vancouver, BC, Canada) and used in a swimming experiment detailed in Steinhausen et al. (2008). Briefly, the fish were swum at a fixed speed of ~1.35 bl s^{-1}, which is approximately 75% of U_{crit}, in the Brett-type swim tunnels outlined above. The water temperature was incrementally increased at a rate of 2°C h^{-1} from 15°C to 17, 19, 21, 23 and 24°C, or until the fish quit swimming.

2.8.2 Heart Sampling and Analysis

Regardless of the experiment, the heart tissue was processed by the same method. Following death, fish were weighed (M) and the heart was removed and placed in a vial containing 70% ethanol. The compact and spongy myocardial layers of the preserved ventricles were separated according to established methods (Farrell et al., 2007; Poupa and Carlsten, 1973) to provide an index of the proportion of the ventricle composed of compact relative to spongy myocardium. The two layers were dried to a constant mass (at least 3 days at 60°C) and weighed to the nearest 0.1 mg. Percent ventricular compact mass (% compact) was determined using dry compact (M_{CD}) and dry spongy masses (M_{SD}): % compact = $100M_{CD}\ (M_{CD} + M_{SD})^{-1}$. Total dry ventricular mass ($M_{VD} = M_{CD} + M_{SD}$) was used to determine relative dry ventricular mass (RDVM): $RDVM = 100M_{VD}\ M^{-1}$. Since compact myocardium can vary independent of

ventricular mass (i.e. a large ventricle with lower % compact could have the same total compact myocardium as a smaller ventricle with higher % compact), the total compact myocardium was expressed as the relative dry compact mass (RDCM): $RDCM = 100M_{CD}\,M^{-1}$.

To simplify comparisons of our data with the more commonly presented wet ventricular mass (M_{VW}), M_{VW} was measured in a subset of fish (n = 35 from 2 populations, Chilko and Weaver). Immediately after death, the ventricle was blotted dry and weighed to 0.1 g prior to storage in 70% ethanol. Relative wet ventricular mass (RVM) was determined: $RVM = 100M_{VW}\,M^{-1}$. Dry ventricular mass (M_{VD}) was determined to be 14.7 ± 0.3% of M_{VW} (no significant differences existed between Weaver and Chilko fish, data not shown), which corresponds to previous studies on salmonids (12-14%, Simonot and Farrell, 2007). Therefore, we extrapolated from M_{VD} to M_{VW} (using a correction factor of 14.7%) for all populations.

2.9 β-Adrenoceptor Experiment

Chilko and Nechako sockeye salmon were collected from the lower Fraser River on August 11 and 12, 2009 and brought to CLL. The fish were placed in 1,400 l circular aquaria at 13°C and the temperature was either maintained at 13°C or increased to 19 or 21°C over 24 h. After four days at the test temperature, the fish were euthanized by a cranial blow and the ventricle was quickly removed, weighed and freeze-clamped in liquid nitrogen. The hearts were stored at -80°C until analysis. Gross body morphology was measured in each fish (body mass, fork length, gonad mass, condition factor). Condition factor = $(\text{body mass}/\text{length}^3) \times 100$.

Male rainbow trout acclimated to 6°C freshwater at CAER were included as a reference group to validate the assay technique.

Ventricular cell-surface β_2-adrenoceptor density (B_{max}) and binding affinity (K_d) were determined using the tritiated ligand technique [Watson-Wright et al. (1989) as modified for fish hearts (Gamperl et al., 1994; Hanson et al., 2005)]. The frozen ventricles were rinsed in saline to remove any remaining blood and sliced (350 um thickness) using a McIlwain tissue chopper (Brinkman, Rexdale, ON, Canada). Ventricular tissue punches (2 mm diameter) were taken from both the spongy and compact myocardium. Single punches were incubated with various concentrations (0.05 – 3.5 nM) of the hydrophilic β_2-adrenoceptor ligand [^{3}H] CGP-12177 (Amersham Life Science). Separate punches were incubated at each concentration with the competitive β_2-adrenoceptor antagonist timolol (10 μM) to determine non-specific binding.

2.10 Statistics

All data are presented as mean ± SEM, unless otherwise indicated. P-values less than 0.05 were considered statistically significant.

2.10.1 Swimming Experiments in Chapters 3, 4 & 5

All data in Chapters 3 and 4 were compared between sexes and among populations. If there were no statistically significant relationships with sex or population, the data were often pooled for subsequent analysis. All data in Chapter 5 were compared among temperature groups (populations were pooled and sex was not considered).

Independent data were compared using a t-test, one-way ANOVA or two-way ANOVA, as appropriate. Dependent data were compared using a paired t-test, one-way repeated measures ANOVA or a two-way repeated measures ANOVA, as appropriate. When the requirement for

normal distribution and equal variance could not be met after transformation, the data were compared using the appropriate nonparametric test (e.g. Mann-Whitney U test, Kolmogorov-Smirnov test, Kruskal-Wallis test). A post-hoc Holm-Sidak or Dunn's test was used to test for differences among groups.

A Pearson correlation was used to compare aerobic scope with the migration difficulty indices. Three different critical p-values are reported. First, $p < 0.05$ is indicated, with no correction for multiple comparisons. Second, $p < 0.018$ is indicated, which is the critical level using the Benjamini and Yekutieli False Discover Rate correction for multiple comparisons (Benjamini and Yekutieli, 2001; Narum, 2006). Finally, $p < 0.006$ is indicated, which is the critical level using Bonferroni correction for multiple comparisons (Holm, 1979; Rice, 1989).

Linear regression was used to relate maximum aerobic scope with distance to the spawning ground. Linear regression was also used to relate aerobic scope, cardiac scope and scope for heart rate from individual fish.

The goodness of fit of the population-specific aerobic scope curves to the full suite of historic temperature frequency distributions were assessed using AIC (Akaike's Information Criterion, Burnham and Anderson, 2002). In addition, rigorous sensitivity analyses was conducted to test the robustness of the results from the initial AIC analysis (data not shown). For example, the response and predictor variables were reversed in the regression and fit aerobic scope curves to population-specific temperature distributions. Second, the temperatures used to generate the scope data for the linear regression were restricted to match the minimum and maximum temperatures used to fit the population-specific aerobic scope curves. This reduced the uncertainty introduced from extrapolating scope values beyond the ranges of the observed data. Next, the uncertainty in scope was further reduced by re-fitting the linear regressions using

observed values for each population, removing any assumptions about the true shape of the aerobic scope curve. The regression was also fit using raw temperature frequencies, as opposed to logged values. Finally, alternate modelling approaches were attempted, including a comparison of the scope and temperature distributions using single critical values (e.g. regression between T_{opt} and the median of the temperature distribution across all stocks; regression between upper T_p and the 90^{th} percentile of the temperature distribution). While each approach yielded subtly different results, they all demonstrated that aerobic scope is significantly related to the average thermal migratory experience encountered by each population. Notably, the upriver populations encounter very similar average temperatures, which increases the likelihood that the temperature distribution for a given population matches the aerobic scope curve for another co-migrating population. Indeed, all the upriver populations had a similar temperature median (range 16.4-17.6°C) and mode (range 16.8-17.3°C).

2.10.2 Ventricular Morphology in Chapter 6

To examine whether traveling through hydraulically challenging sections of the river (e.g. Hells Gate) imposes strong selection pressure, the cardiac morphology variables were first compared between upriver and coastal populations using a t-test. Comparisons of cardiac variables in female sockeye salmon among seven populations were analyzed using one-way ANOVA. A Pearson correlation matrix was used to relate the various migration difficulty indices to the three cardiac variables in female sockeye from the seven populations, and three critical p-values are reported, as outline above. Linear regression was used to test for relationships between the cardiac variables and the various measures of migration difficulty and with fail temperature

during the swimming experiment performed by Steinhausen et al. (2008). The effect of temperature on the cardiac variables in Chilko sockeye salmon was assessed using a two-way ANOVA (sex × temperature). When appropriate, a Holm-Sidak post-hoc test was used to distinguish between groups.

2.10.3 β-Adrenoceptor Experiment in Chapter 7

Two-way ANOVA was used to test for differences in gross morphology, B_{max} and K_d between populations and temperature treatments.

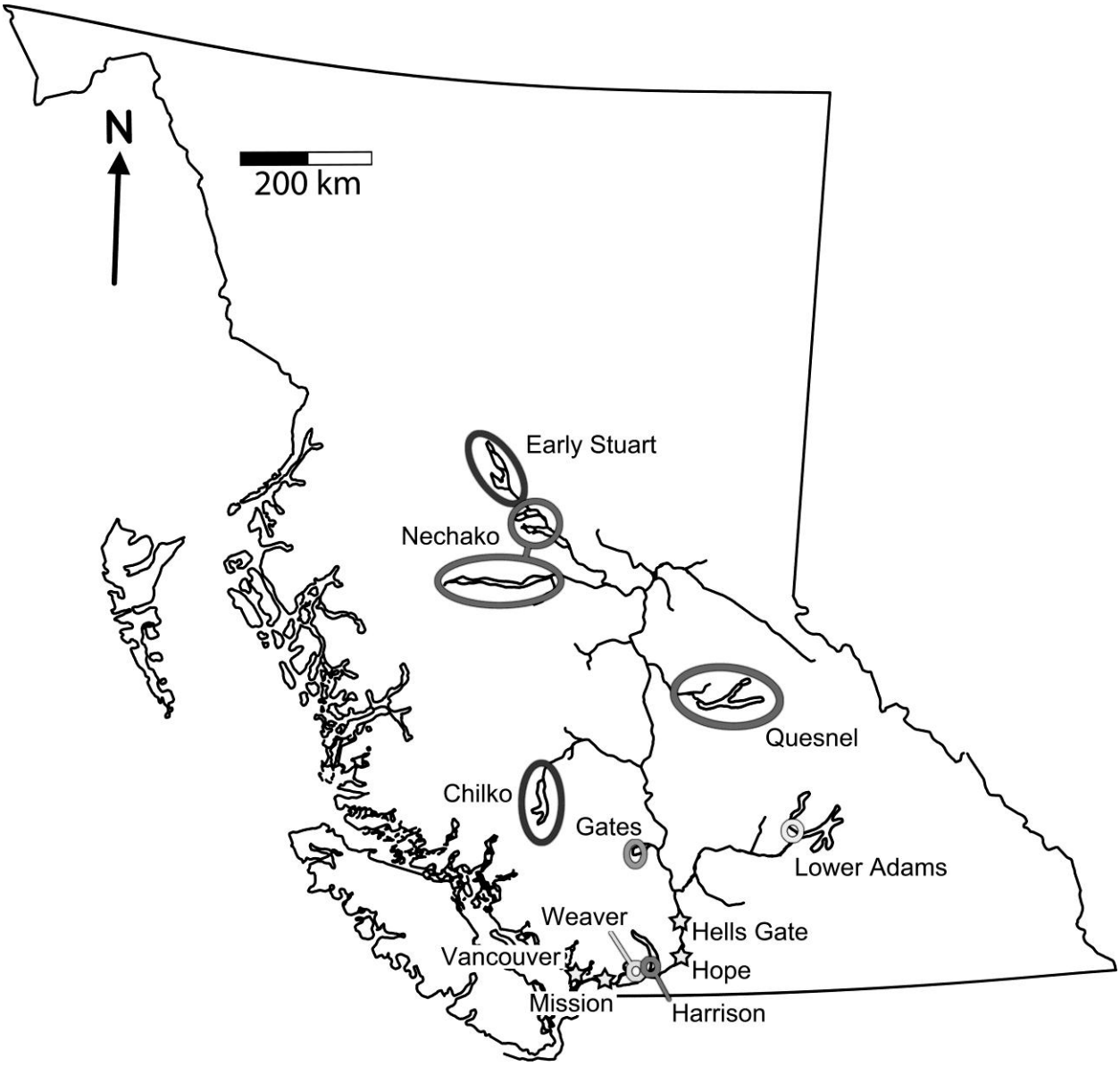

Figure 2.1. Map of the Fraser River, British Columbia, Canada indicating the spawning locations for the eight sockeye salmon populations included in this study.

Table 2.1. Environmental characteristics and migration difficulty indices for eight populations of Fraser River sockeye salmon. Mean ± SEM are presented for T_M, F_M and ATU. Minimum and maximum values for migration rate and migration duration are in parentheses. For late run sockeye salmon populations (Lower Adams, Weaver and Harrison), environmental data corresponding to the current early entry phenomenon are shown in parentheses underneath the historical river entry timing information.

	Early Stuart	**Gates**	**Nechako**	**Quesnel**	**Chilko**	**Lower Adams**	**Weaver**	**Harrison**
Spawning region	upriver	upriver	upriver	upriver	upriver	upriver	coastal	coastal
Run timing group	Early Stuart	Early Summer	Summer	Summer	Summer	Late	Late	Late
Peak Fraser River entry	Jul-07	Jul-31	Aug-11	Aug-11	Aug-11	Sep-27	Sep-27	Sep-27
						(Aug-27)	(Aug-27)	(Aug-27)
Peak Hells Gate passage	Jul-14	Aug-07	Aug-17	Aug-17	Aug-17	Oct-04	n/a	n/a
						(Sep-3)		
Peak spawning ground arrival	Aug-06	Sep-02	Sep-30	Sep-15	Sep-25	Oct-16	Oct-21	Nov-14
Lower Fraser temperature (T_M) (°C)	15.8 ± 1.3	17.7 ± 1.1	17.3 ± 1.0	17.3 ± 1.0	17.3 ± 1.0	11.4 ± 1.4	12.3 ± 1.2	12.3 ± 1.2
						(16.5 ± 0.8)	(17.0 ± 0.8)	(17.0 ± 0.8)
Lower Fraser discharge (F_M) ($m^3\ s^{-1}$)	5686 ± 1331	3860 ± 893	3419 ± 780	3419 ± 780	3419 ± 780	2040 ± 580	2093 ± 577	2093 ± 577
						(2582 ± 537)	(2754 ± 578)	(2754 ± 578)
Migration temperature median (°C)	16.4	17.6	16.2	16.6	16.6	14.2	14.9	14.8
						(16.9)	(17.4)	(17.4)
Migration temperature mode (°C)	17.3	17.3	16.8	16.8	17.3	15.3	15.3	15.3
						(17.8)	(17.3)	(17.3)
Accumulated thermal units (ATU) (°C)	502 ± 36	177 ± 11	492 ± 27	341 ± 15	325 ± 16	281 ± 27	87 ± 11	103 ± 11
						(326 ± 29)	(104 ± 7)	(121 ± 9)
Migration distance (D_M) (km)	1071	364	958	796	642	480	117	121
Migration elevation (E_M) (m)	690	280	716	728	1174	346	32	10
Migration duration (d)	30 (23-42)	11 (8-17)	28 (17-43)	21 (15-31)	19 (14-28)	20 (13-47)	5 (3-10)	7 (4-14)
Migration rate (km d^{-1})	36 (26-46)	35 (21-48)	34 (22-57)	39 (26-51)	34 (23-45)	24 (10-37)	22 (11-44)	18 (9-27)
Work ($0.001 \bullet E_M \bullet D_M$)	739	102	686	579	754	166	4	1
River slope ($500(E_M D_M^{-1})$)	322	385	374	457	914	360	137	41
Migratory effort ($0.0001 \bullet F_M \bullet D_M$)	609	141	328	272	219	98	24	25

CHAPTER 3: DIFFERENCES IN THERMAL TOLERANCE AND MAXIMUM CARDIORESPIRATORY PERFORMANCE AMONG SOCKEYE SALMON POPULATIONS

3.1 Introduction

The Fraser River is home to over 100 genetically and geographically distinct populations of sockeye salmon (Beacham et al., 2005), each of which encounters different upriver migration conditions. For example, populations vary in migration distance (100 to 1100 km), elevation gain (10 to 1200 m), river temperature (9° to 22°C), and river flow (2000 to 10,000 m^3 s^{-1}) (see Chapter 2, Fig 2.1, Table 2.1). The upriver spawning migration is critical for reproductive success since sockeye salmon are semelparous (only spawn once). Consequently, local migratory conditions are expected to exert strong selection pressure. Indeed, morphological and behavioural characteristics (gross somatic energy, body morphology, egg number and swimming behaviour) have been correlated with river migration distance, elevation and/or work (distance × elevation) in Fraser River sockeye salmon populations (Crossin et al., 2004; Gilhousen, 1980; Hinch and Rand, 2000).

The energetic upriver migration is sustained by the cardiorespiratory system, which provides oxygen to the swimming muscles among other valuable functions. Cardiorespiratory performance can be quantified by measuring aerobic scope, which is defined as the difference between maximum oxygen consumption ($\dot{M}O_{2max}$) and resting oxygen consumption ($\dot{M}O_{2rest}$) (Fry, 1947). Aerobic scope represents the maximum amount of oxygen available for any activity beyond routine maintenance, activities such as swimming, reproduction and growth.

Aerobic scope has a strong temperature dependence (Fry, 1947). $\dot{M}O_{2rest}$ typically increases exponentially with temperature until lethal levels are approached, as expected for a temperature effect on a rate function. $\dot{M}O_{2max}$ similarly increases with increasing temperature but reaches a maximum, which may be a plateau. Then $\dot{M}O_{2max}$ sharply declines as temperature increases toward lethal levels. The temperature at which aerobic scope is maximal is termed the optimal temperature (T_{opt}), which in salmonids corresponds to maximal swimming and cardiac performance (Brett, 1971; Lee et al., 2003c; Taylor et al., 1997). The temperatures at which aerobic scope starts to decline from the maximum are termed the pejus temperatures (T_p), which has a lower and upper value. At critical temperatures (T_{crit}), $\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$ intersect and aerobic scope becomes zero. Beyond T_{crit}, there is insufficient oxygen to support the routine needs of the fish and survival becomes passive, time-limited and supported by anaerobic metabolism (Pörtner, 2001; Pörtner and Farrell, 2008).

The central hypothesis of my thesis is that each population has physiologically adapted through natural selection to meet their specific migration challenges. Specifically, I hypothesized that populations with more challenging migrations have greater aerobic, cardiac and heart rate scopes. I predicted that migration distance, elevation gain and work would exert the strongest selection pressure on aerobic scope, given their importance in selecting for morphological traits (Crossin et al., 2004), which has never been tested before. In addition, I hypothesized that each population can maintain maximum scope across the entire range of temperatures most frequently encountered during upriver migration, as has been previously demonstrated for two populations of sockeye salmon (Farrell et al., 2008; Lee et al., 2003c).

Wild, migrating adult sockeye salmon were intercepted in the lower Fraser River, when the fish had only been migrating upstream for 1-3 days and prior to encountering any of the

major selective elements. Individual sockeye salmon were then instrumented to measure cardiovascular variables [cardiac output ($\dot{Q}$), heart rate (f_H), stroke volume (V_s)] and swum at a single temperature (ranging from 8-26°C) in a Brett-type swim tunnel. Detailed materials and methods are found in Chapter 2 (sections 2.2-2.7 & 2.10).

3.2 Results

3.2.1 Gross Morphology and Reproductive Status

Gross body morphology (body mass, fork, standard, POH, and POF lengths, GSI, HSI, SSI) did not differ significantly among the five populations (Table 3.1), although significant differences did exist between sexes. When all populations were pooled, male fish had a significantly greater body mass, fork length, standard length and SSI. Female fish had significantly higher GSI and HSI. None of the swum fish were fully sexually mature (no loose eggs or milt production) but they had begun their sexual maturation process (body colour was starting to turn red, gonads were developing). In addition, plasma cortisol, 17β-estradiol and testosterone did not significantly differ between the two populations tested (Chilko and Early Stuart). Plasma cortisol and 17β-estradiol were significantly higher in females compared to males (cortisol: 619 ± 87 and 380 ± 34 ng ml^{-1}; 17β-estradiol: 0.88 ± 0.18 and 0.07 ± 0.01 ng ml^{-1}, respectively). Plasma testosterone did not significantly differ between sexes (overall mean ± SEM: 2.84 ± 0.52 ng ml^{-1}).

Gross energy density did not significantly differ among populations or between sexes (mean ± SEM: 8.0 ± 0.2 MJ kg^{-1}, range: 5.6-11.3 MJ kg^{-1}).

3.2.2 Cardiorespiratory Performance at T_{opt}

For measurements made at T_{opt}, there were no significant differences in $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ or aerobic scope between Early Stuart sockeye salmon that had undergone surgery and swam with the added drag of leads and those that had no surgery and had no additional drag during swimming (Table 3.2). Sockeye salmon with leads had a significantly higher $\dot{M}O_2$ after the 45-min recovery period between the first and second swim, but not after the 45-min or 2-h recovery periods following the second swim. Sockeye salmon swum without leads had an 18-23% significantly higher U_{crit} compared to those with leads. Notably, both instrumented and un-instrumented fish repeated their swim performance (no significant differences existed between U_{crit} 1 and U_{crit} 2 within a group). Given these comparable results, fish swum without leads were included in the estimates of $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ and aerobic scope presented below. Also, this lack of effect of leads meant that my population-specific $\dot{M}O_2$ data could reliably be compared with previous literature on fish swum without instrumentation (e.g. Lee et al., 2003c).

No significant differences existed between male and female sockeye salmon for any of the cardiorespiratory variables measured at T_{opt} (resting, maximum or scope for $\dot{M}O_2$, $\dot{Q}$, f_H and V_s, $p>0.05$); therefore, data for males and females were pooled within a population to increase statistical power (Table 3.3).

Gates sockeye salmon had a significantly higher $\dot{M}O_{2rest}$ compared to Early Stuart, Nechako, Quesnel, Chilko and Weaver sockeye salmon at their respective T_{opt}. Weaver sockeye salmon had a significantly lower $\dot{M}O_{2max}$ compared to Early Stuart, Nechako, Chilko and Gates sockeye salmon (Table 3.3).

Aerobic scope varied by 69% across populations. Aerobic scope was significantly highest in Early Stuart and Nechako, intermediate in Lower Adams and lowest in Weaver fish. A Pearson correlation matrix revealed that several of the migration difficulty indices correlated significantly with aerobic scope (migration distance, work, duration, rate, ATU; Table 3.4). Among these, aerobic scope had the strongest relationship with migration distance to the spawning ground (Fig 3.1, Table 3.4).

$\dot{Q}$ and f_H did not differ significantly among populations (Table 3.3). Nechako had a significantly higher scope for V_s compared to Early Stuart, Chilko and Lower Adams fish, though V_{srest} and V_{smax} did not significantly differ.

3.2.3 Influence of Temperature on Cardiorespiratory Performance

Resting, Maximum and Scope

$\dot{M}O_{2rest}$ increased exponentially with increasing temperature in each population (Q_{10} ranged from 2.2 to 2.9 across populations, Fig 3.2A). $\dot{M}O_{2max}$ also increased with increasing temperature up to its maximum at T_{opt}, and then declined thereafter. As a result, aerobic scope displayed a clear peak for each population (Fig 3.2B).

$\dot{Q}$ measured in resting and exercising fish showed similar patterns to $\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$ with the result that cardiac scope showed a discernible peak (only Chilko data shown, Fig 3.3A, D).

As expected, f_{Hrest} also increased exponentially with rising temperatures (Q_{10} = 2.0) while f_{Hmax} reached a plateau well below T_{crit} (Fig 3.3B). f_{Hrest} and f_{Hmax} intersected at high temperatures

above T_{opt}. Remarkably, f_{Hmax} decreased below the resting value at the highest temperatures, with the result that scope for f_H became negative at the highest test temperatures (Fig 3.3E). Aerobic scope, cardiac scope and scope for f_H were all positively correlated (Fig 3.4).

In contrast, temperature had no effect on V_{srest} (Fig 3.3C). Furthermore, V_{smax} declined with increasing temperature leading to a decrease in scope for V_s at the highest temperatures (Fig 3.3C, F).

Associations between Aerobic Scope and Historic River Temperatures

The coastal Weaver population experiences the coldest temperatures during upriver migration and had the lowest T_{opt} (14.5°C). Upriver populations experience similar river temperatures during migration and accordingly had a similar T_{opt} (range 16.4-17.2°C, Fig 3.5 and Table 3.5). Notably, Weaver sockeye salmon are currently entering the Fraser River much earlier than normal, which exposes them to considerably warmer temperatures compared with their T_{opt} (see the right-shift for the current Weaver temperature histogram, Fig 3.5).

The width of the T_{opt} window (difference between the upper and lower T_p values) ranged from 4-8°C among populations (Table 3.5). Among the upriver populations (Early Stuart, Nechako, Quesnel, Chilko, Gates), the Chilko population displayed the broadest optimal thermal range (Fig 3.5, Table 3.5). For all upriver populations, between 89-98% of maximum aerobic scope consistently fell within the 90th percentile of historic river temperatures encountered by each population (T90%). In contrast, the Weaver population retained only 81% of maximum aerobic scope for their historical T90% and an alarmingly low 45% for the current T90% (Fig 3.5, Table 3.5).

The maximum river temperature (21.5°C) exceeded the upper T_p of every population examined (Fig 3.6). Extrapolation of the aerobic scope curves to T_{crit} resulted in T_{crit} values that varied between 21 and 29°C among populations (Fig 3.6, Table 3.5).

Aerobic scope curves for each population were significantly related to the historic temperature frequencies they typically experience (Fig 3.5, Table 3.6). While all regressions were significant, the AIC weights indicate that there was typically strong support for a single aerobic scope-temperature frequency relationship. In general, the Early Stuart temperature distribution was the best fit for the aerobic scope data for upriver populations; the Gates and current Weaver temperature distributions provided the poorest fit to upriver populations. The historic Weaver temperature distribution was the best fit for aerobic scope of Weaver sockeye salmon.

3.3 Discussion

This study demonstrates that Fraser River sockeye salmon populations differ in their cardiorespiratory performance and suggests that sockeye salmon populations have physiologically adapted to meet the specific challenges of their local upriver migration conditions. This study greatly extends a previous study which suggested that T_{opt} for aerobic scope varied between two sockeye salmon populations (Lee et al., 2003c) by considering aerobic scope of five additional populations. A novel and strong relationship was found between maximum aerobic scope and the river migration distance to the spawning ground. Populations that travel the furthest had the highest aerobic scope, while those traveling short distances had the lowest aerobic scope. In addition, every population examined could maintain maximum

aerobic, cardiac and heart rate scopes across the entire range of temperatures typically encountered during their migration, although it was clear that the current unusual migratory behaviour of Weaver sockeye salmon exposes them to temperatures well beyond optimal.

3.3.1 Comparing Instrumented and Un-Instrumented Fish

In order to measure cardiorespiratory performance, fish underwent surgery and dragged leads while swimming. Nevertheless, $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ and aerobic scope were the same for instrumented and uninstrumented Early Stuart sockeye salmon, despite the observation that fish swum without leads achieved higher swim speeds compared to those swum with leads. Indeed, values for $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ and aerobic scope (means: 3.0, 14.6 and 11.8 mg O_2 min^{-1} kg^{-1}, respectively) are within the previously observed ranges for Early Stuart sockeye salmon (ranges: 2-6, 11-19 and 9-14 mg O_2 min^{-1} kg^{-1}, respectively, (Lee et al., 2003c; MacNutt et al., 2006). Also, U_{crit} values for uninstrumented Early Stuart sockeye salmon in the current study (mean maximum U_{crit} = 2.44 ± 0.13 bl s^{-1}) compare favourably with two previous studies examining this same population (2.26 – 2.36 bl s^{-1}, Lee et al., 2003c; MacNutt et al., 2006). Both instrumented and un-instrumented groups had excellent repeat swim performance (U_{crit} 1 ≅ U_{crit} 2).

These findings suggest that while the leads did have a substantial drag effect, limiting U_{crit} by ~20%, oxygen delivery was not significantly effected (i.e. the increased drag resulted in the same swimming effort for a lower U_{crit}). Collectively, these results suggest that the data in the current study are consistent with previously published data. Furthermore, I could pool instrumented and uninstrumented fish for the analysis of aerobic scope and I was confident in

using published aerobic scope data for sockeye salmon swum without instrumentation (e.g. Weaver and Gates populations from Lee et al., 2003c) in my population comparisons.

3.3.2 Baseline Morphology and Reproductive Status

The minimum somatic energy density threshold to sustain life for sockeye salmon has been estimated to be 3.5-4.0 MJ kg^{-1}, and the energy required to reach the spawning grounds is estimated to be between 1.5-2.4 MJ kg^{-1}, depending on the migration distance (Clark et al., 2009; Gilhousen, 1980; Hendry and Berg, 1999; Williams et al., 1986). Given that somatic energy ranged from 6-11 MJ kg^{-1}, every fish in the present study likely had sufficient energy to complete its migration and the energetic challenge of the swim test was small by comparison.

Sex-specific differences in mass, length, GSI, and SSI are all consistent with previously published reports (Clark et al., 2010; Gilhousen, 1980; Idler and Clemens, 1959; Patterson et al., 2004; Sandblom et al., 2009). In contrast to previous findings, body size and morphology did not differ among populations (Crossin et al., 2004). However, morphology was only compared across upriver populations and some populations had low sample sizes within a sex (e.g. Lower Adams) which may have limited statistical power. I would expect to find significant differences in body morphology if coastal Weaver sockeye salmon were included in the analysis (e.g. see Crossin et al., 2004, Lee et al., 2003c).

As expected for fish that were collected early in their river migration and several weeks before spawning, sexual maturation was in progress and incomplete. GSI ranged from 0.7-3.1% in males and 4.0-13.4% in females. Given that GSI reaches ~4 and 17% in fully mature males and females, respectively, (Gilhousen, 1980), the fish in the present study were still maturing. In

fact, both 17β-estradiol and testosterone levels were very low relative to values reported in the literature on migrating adult sockeye salmon (Cooperman et al., 2010; Crossin et al., 2008; Hruska et al., 2007; Sandblom et al., 2009; Young et al., 2006). Sex hormones were significantly depressed in association with increased cortisol levels in Early Stuart sockeye salmon navigating through Hells Gate (Hinch et al., 2006). The stress of sequential swim tests performed here may have had the same effect.

Plasma cortisol levels (range: 79-837 ng ml^{-1}) were within the range of published values for adult sockeye salmon (e.g. ~50-800 ng ml^{-1}; Cooke et al., 2006; Cooperman et al., 2010; Sandblom et al., 2009; Young et al., 2006). The present values were likely elevated in part because the final blood sample was taken after the swimming experiment and after extensive handling to remove the fish from the swim tunnel. Even so, it has long been established that salmon normally have very high plasma cortisol levels during this final phase of life (e.g. Hane and Robertson, 1959). Chronically high cortisol levels in migrating salmonids have been hypothesized to be either a consequence of stress during the migration, or due to endogenous mechanisms associated with reproductive maturation, or possibly due to enhance home-stream olfactory memory (Carruth et al., 2002; Kubokawa et al., 1999; Sandblom et al., 2009). Consistent with the literature, females had significantly higher cortisol levels compared to males, which is a phenomenon reported for the entire upriver migration (Carruth et al., 2002; Crossin et al., 2008; Kubokawa et al., 1999; Sandblom et al., 2009; Schmidt and Idler, 1962).

3.3.3 Maximum Cardiorespiratory Performance Among Populations

Aerobic scope at T_{opt} varied considerably (by 69%) across sockeye salmon populations (range: 7.7-13.0 mg O_2 kg^{-1} min^{-1}) and by 3.6 fold among individuals (range: 4.3-15.4 mg O_2 kg^{-1} min^{-1}). Given that the cardiorespiratory system sustains swimming during the upriver migration, I hypothesised that aerobic scope would relate to the migratory environment. The substantial intraspecific variability in aerobic scope and 10-fold variation in migration difficulty across the seven populations examined allowed me to test this hypothesis. Coastal populations only travel ~100 km in cooling fall river temperatures, with little change in river elevation to reach their spawning grounds. In contrast, upriver populations must navigate the difficult passages through the Fraser Canyon, including the notorious Hells Gate, often in mid-summer when river temperatures peak. Some upriver populations must travel over 1000 km to reach their spawning grounds while Chilko sockeye salmon ascend ~1200 m in elevation. Because of this high degree of variability, migration difficulty was quantified using various environmental indicies (see Chapter 2): distance, elevation gain, temperature, migration rate, migration duration, work, river slope and migration effort. Elevation does not appear to have exerted a strong selective pressure since neither elevation gain nor river slope had a significant relationship with aerobic scope. However, aerobic scope was significantly related to numerous indices, including work, migration duration, migration rate and accumulated thermal units, with migration distance emerging as the best predictor. These results suggest population level adaptation of maximum aerobic scope to the selection imposed by certain river conditions encountered during migration (see discussion below, Endler, 1986; Schluter, 2000; Taylor, 1991).

This was the first study to compare cardiovascular variables across populations of sockeye salmon. In contrast to the findings for aerobic scope, neither maximum cardiac scope nor maximum scope for f_H varied among the five populations examined. However, I only examined cardiorespiratory performance in upriver populations that travel through Hells Gate. Due to logistical constraints, cardiorespiratory performance was not measured in the population with the lowest aerobic scope (Weaver). Given the findings for aerobic scope and the good correlation between aerobic and cardiac scope, I would expect coastal populations to exhibit lower cardiac performance compared to upriver populations, a subject that should be considered for future studies. Scope for V_s was significantly higher in Nechako sockeye salmon compared to the Early Stuart, Chilko and Lower Adams populations. This demonstrates that the mechanism of achieving the same $\dot{Q}$ differs among populations, a finding that is explored further in Chapter 4.

3.3.4 Cardiorespiratory Performance with Temperature

Aerobic scope, cardiac scope and scope for heart rate were all postively correlated and varied in parallel with temperature, suggesting that the temperature dependence of cardiac performance is linked to that of aerobic capacity at the population level. The optimal water temperature for cardiorespiratory performance matched the typical water temperatures historically encountered by each population. The upriver populations all experience a similar range, mean and mode for river temperature, and accordingly demonstrated a similar T_{opt}. In contrast, the coastal Weaver population historically experience colder temperatures and had a corresponding colder T_{opt}. All six populations had 81-98% of maximum aerobic scope at the upper 90th percentile of encountered temperatures, clearly demonstrating that each population

could theoretically maintain swimming performance across the majority of river temperatures that they currently encounter. These findings support earlier work demonstrating that aerobic scope matched historic temperatures for two sockeye salmon populations (Gates and Weaver, Farrell et al., 2008; Lee et al., 2003b). Therefore, the present study adds considerably more weight to the idea of intraspecific variability for aerobic scope among Fraser River sockeye populations. This then opens up the possibility that other salmon populations with similar reproductive isolation may also demonstrate local adaptations.

While the overall temperature range may be similar among upriver populations, the timing of river entry and spawning location can create more subtle differences. For example, Early Stuart sockeye salmon, which have a very long river migration, encounter moderate temperatures and the fastest river flow early in their migration, but temperatures escalate (up to ~21.5°C) during the final stages of their migration when they are close to their spawning grounds (Macdonald et al., 2007). Chilko sockeye salmon experience the opposite temperature pattern. They encounter peak summer temperatures (again up to ~21.5°C) early in their migration while traveling through Hells Gate, but the final third of their migration is spent ascending the hydraulically challenging, but up to 10°C cooler, Chilcotin river to reach spawning grounds in or adjacent to a glacier lake. The effect of temporal differences in temperature exposure on salmon physiology and selection pressure is poorly understood. Regardless, the present data suggests that Chilko sockeye salmon possess the broadest and highest thermal tolerance for aerobic scope of all the populations examined due to adaptations to the difficult migration conditions at both warm (Hells Gate) and cold (Chilcotin river) temperatures.

The mechanism of the decline in aerobic scope above T_{opt} will be examined in detail in Chapter 5. Suffice it to say here that scope for f_H collapsed at a lower temperature than aerobic

scope in two populations, suggesting that the reduced scope for f_H above T_{opt} may limit $\dot{Q}_{max}$ and the capacity of the cardiorespiratory system to transport oxygen. This result corroborates earlier work (Steinhausen et al., 2008).

3.3.5 Perspectives and Significance

Collectively, these results suggest that populations have locally adapted to their specific upriver migration environment. Considering that the upriver migration only lasts a few weeks, representing a mere ~2% of a sockeye salmon's lifespan, this finding is remarkable. However, given the semelparous life history of sockeye salmon, successful upriver migration is essential in order to achieve reproductive success and thus is likely under strong selection pressure. In order for local adaptation to occur, three conditions must be met: 1) the trait must have a genetic basis, 2) variability in trait expression must result in differential survival or reproductive capability, and 3) a functional link between variability in the trait and variability in survival or reproductive success. The correlations presented here provide circumstantial, but promising, evidence for local adaption (Endler, 1986; Schluter, 2000; Taylor, 1991). Conclusive evidence for local adaptation would require breeding studies to generate an F1 and F2 generation, which would demand 4 and 8 years, respectively, a timeframe well beyond the scope of my thesis. Given the present results, such experiments would be worthwhile.

It is highly unlikely that the intraspecific differences observed in the present study were due to a plastic response to encountered river conditions prior to capture and experimentation. The fish were collected only 1-3 days into their upriver migration, after spending more than two years in the much cooler Pacific Ocean and prior to encountering any of the upriver migratory

challenges. In addition, it is highly unlikely that conditions prior to ocean entry (during rearing and downstream smolt migration) caused differential expression of the physiological characteristics that distinguished the adult populations. Foremost, downstream migration occurs at a cooler spring temperature (<12°C), goes with rather than against the current, and reduces in vertical elevation. Therefore, adults have never before experienced nor will they ever experience again the warm river migration conditions that they must overcome to successfully reproduce. As a result, the physiological traits that enabled a successful upriver migration are passed on to the offspring and their genetic basis is conserved by the strong reproductive fidelity of sockeye salmon to their natal spawning area (Burgner, 1991), which are geographically isolated. Thus, I conclude that the population-specific differences observed in the present study were most likely due to genetic adaptation, rather than phenotypic plasticity.

Peak summer temperature in the Fraser River has warmed by ~2°C since the 1950s and is expected to continue along the same trajectory (Ferrari et al., 2007; Morrison et al., 2002). The present study supports the hypothesis that further increases in summer river temperatures will result in population-specific responses in sockeye salmon (Farrell et al., 2008). Populations markedly differ in T_{crit} (when aerobic scope is zero), however, the highly aerobic, long upriver migration is clearly impossible at T_{crit}. Thus, T_{crit} is an unreliable management tool, particularly since it also suffers from the inaccuracy of extrapolating from a polynomial curve. It is unknown exactly how much of aerobic scope is required for successful upriver migration. A biotelelmetry study with Weaver sockeye salmon suggests that at least 50% of maximum aerobic scope was needed for their short, low elevation upriver migration [<10% of fish reached their spawning area at 18 to 21°C when aerobic scope is 0 to 68% of maximal (Farrell et al., 2008; Mathes et al., 2010)]. However, for upriver populations experiencing greater migration difficulty, perhaps up to

90% of maximum aerobic scope is needed. This suggestion is based on the observation that all the upriver populations retained 89-97% of maximum aerobic scope at T90%. Future research should incorporate biotelemetry and biologging techniques in the field with lab-derived cardiorespiratory data to determine the population-specific functional aerobic scope requirements.

Temperatures exceeding the population-specific upper T_p must at some point limit upriver swimming due to a functional collapse in aerobic scope. The T_{opt} window is rather narrow across populations (4-8°C). Thus, only 2-4°C separates T_{opt} from the upper T_p, leaving sockeye salmon with a narrow safety margin for temperature change. In fact, the current temperature maximum (21.5°C) already exceeds the upper T_p (set at 90% of aerobic scope) for every population in the current study. As a result, populations are already experiencing temperatures at their upper limit, and given the individual variability in aerobic scope, some individuals may be dying en route because they cannot reach the spawning ground due to insufficient aerobic scope. Given the present data, it is not surprising that no sockeye salmon population has initiated river migration at temperatures exceeding 21°C (Hyatt et al., 2003), nor has a historic mean migration temperature been above 19°C (Hodgson and Quinn, 2002). Nechako and Weaver populations appear especially susceptible to high temperature, which could prove catastrophic under the continued warming scenario. In particular, Weaver sockeye salmon could be considered "dead fish swimming" if they continue to enter the Fraser River up to six weeks earlier than normal, exposing themselves to temperatures higher than their historic norm and suffering high mortality (Cooke et al., 2004; Farrell et al., 2008; Mathes et al., 2010). In contrast, Chilko sockeye salmon appear to be "superfish", and may have greater resilience to climate change by being able to maintain cardiorespiratory performance at a higher temperature

compared with the other populations studied so far. A potential mechanism for Chilko sockeye salmon's exceptionally high and broad thermal tolerance relative to the co-migrating Nechako population is explored in Chapter 6.

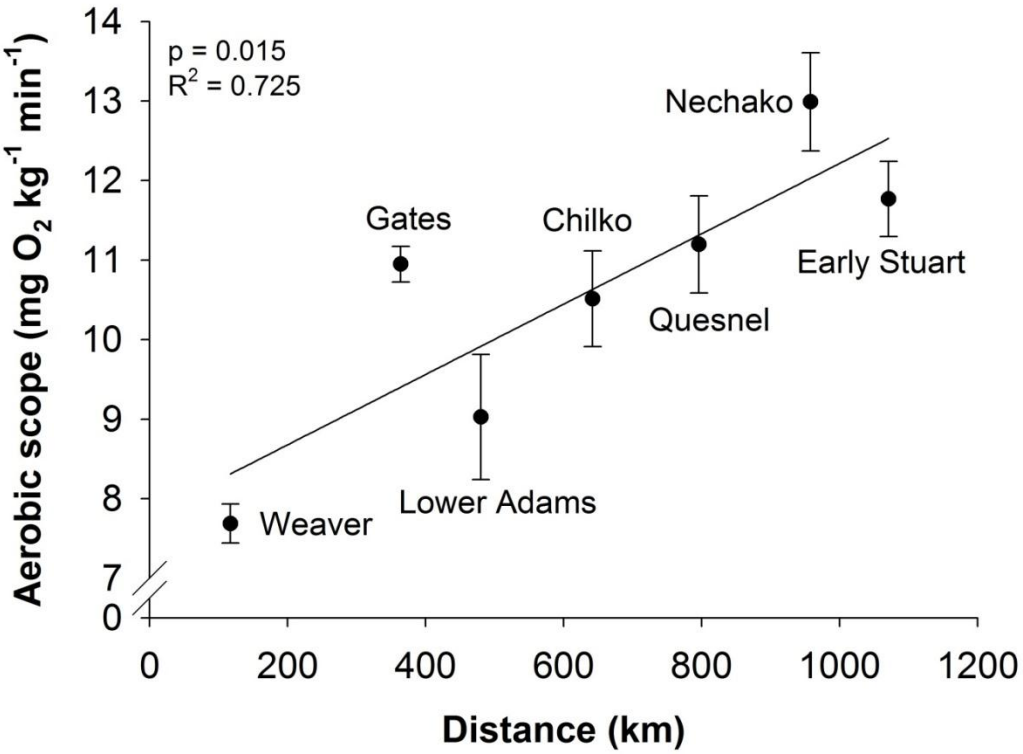

Figure 3.1. Linear regression between migration distance to the spawning ground and population-specific maximum aerobic scope measured at T_{opt}. Means ± SEM are presented.

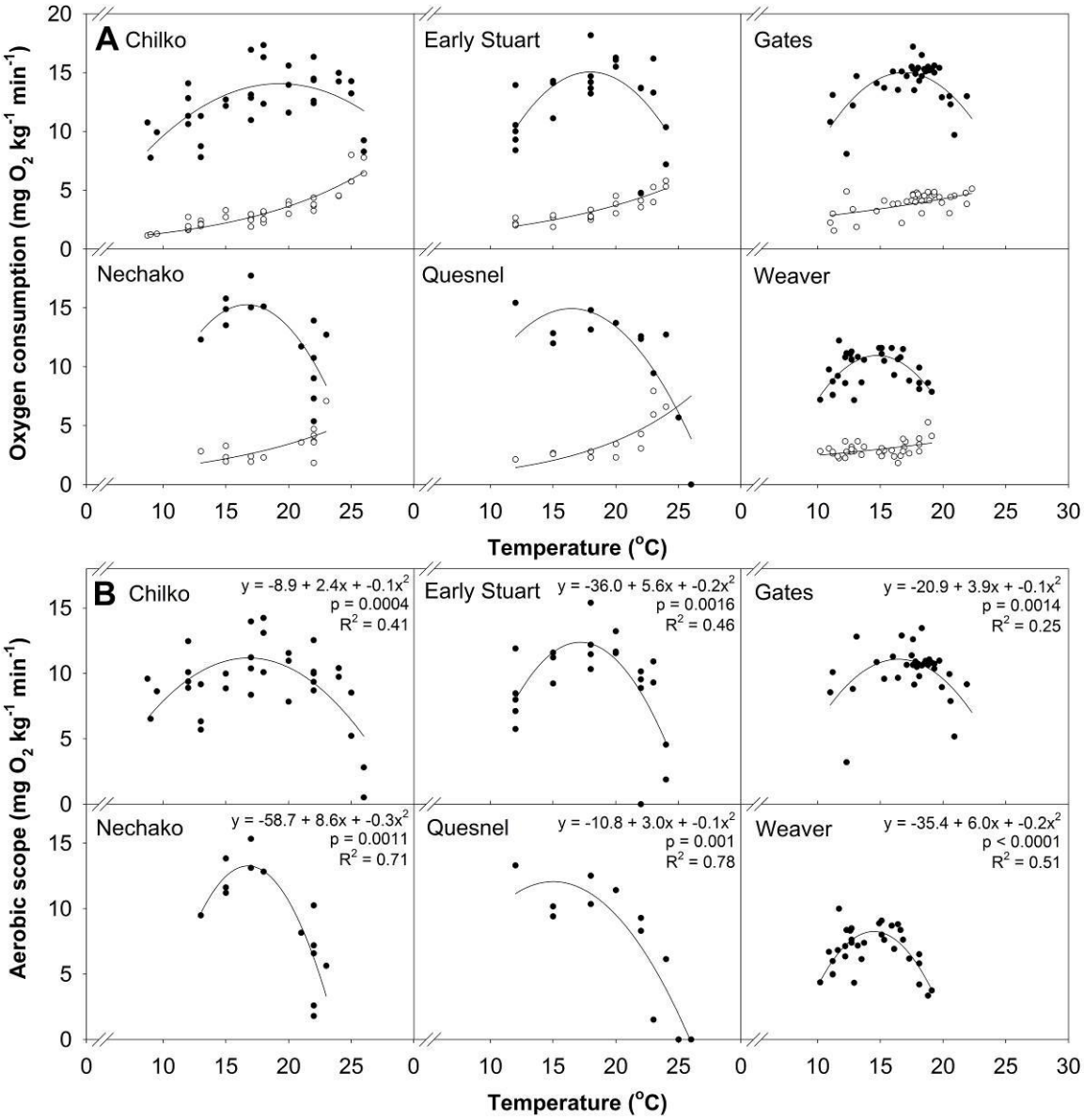

Figure 3.2. (A) Population-specific estimates of resting (open circles) and maximum (closed circles) oxygen consumption rates in relation to water temperature for sockeye salmon. Each point corresponds to a single fish. (B) Population-specific estimates of aerobic scope, the difference between the maximum and resting oxygen consumption data presented in panel A. An exponential equation was fit to the minimum oxygen consumption rate and a polynomial quadratic equation was fit to the maximum oxygen consumption rate and aerobic scope data sets for each population. Data for Gates and Weaver provided by Lee et al. (2003c).

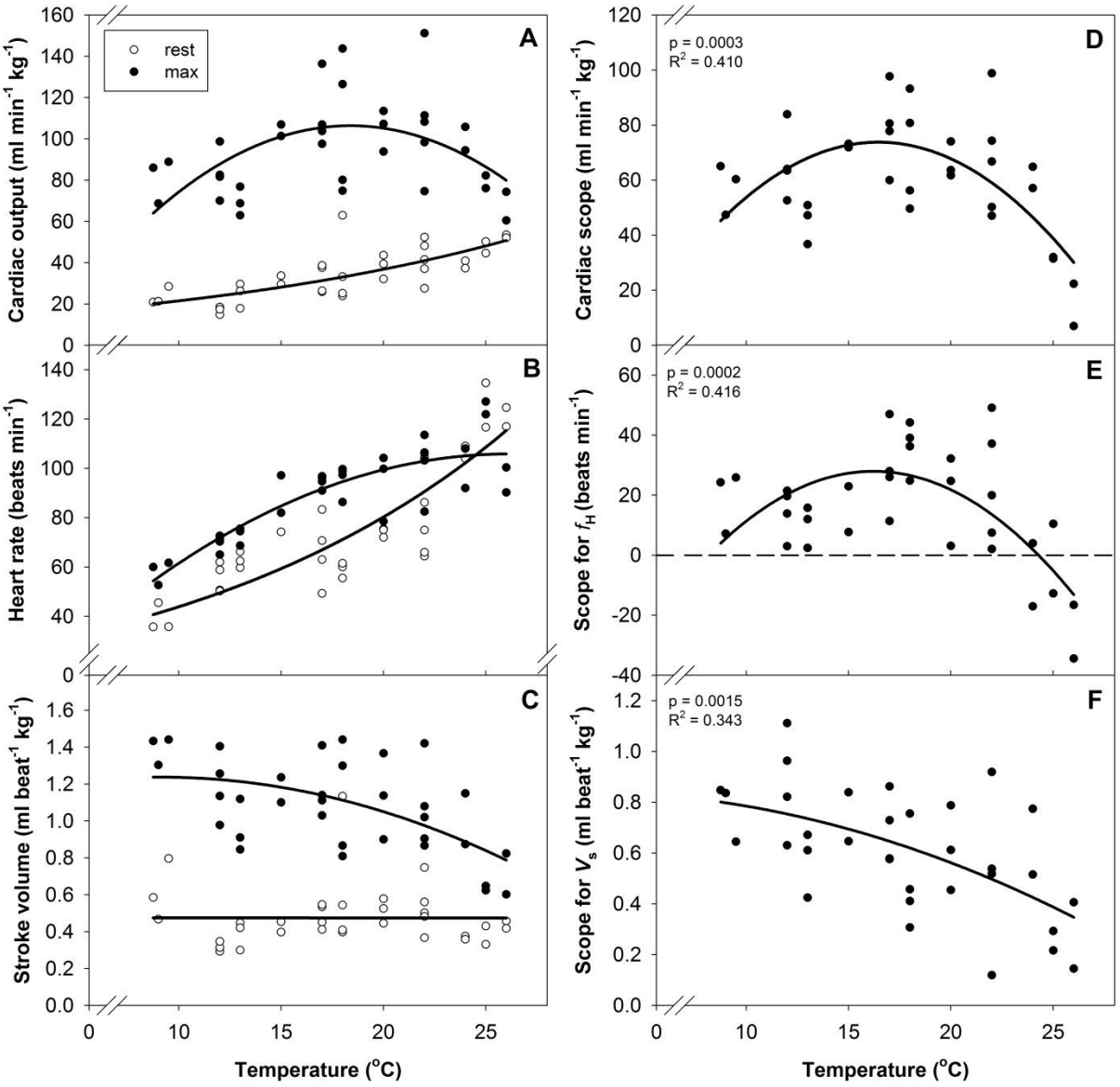

Figure 3.3. Resting (open circles) and maximum (closed circles) values for (A) cardiac output, (B) heart rate and (C) stroke volume in Chilko sockeye salmon. Each point corresponds to a single fish. Scope, the difference between maximum and resting data presented in A, B and C are shown in (D) cardiac scope, (E) scope for heart rate (f_H) and (F) scope for stroke volume (V_s). A polynomial quadratic equation was fit to the maximum and scope data, an exponential equation was fit to the resting data for cardiac output and heart rate and no relationship was found with temperature for resting stroke volume.

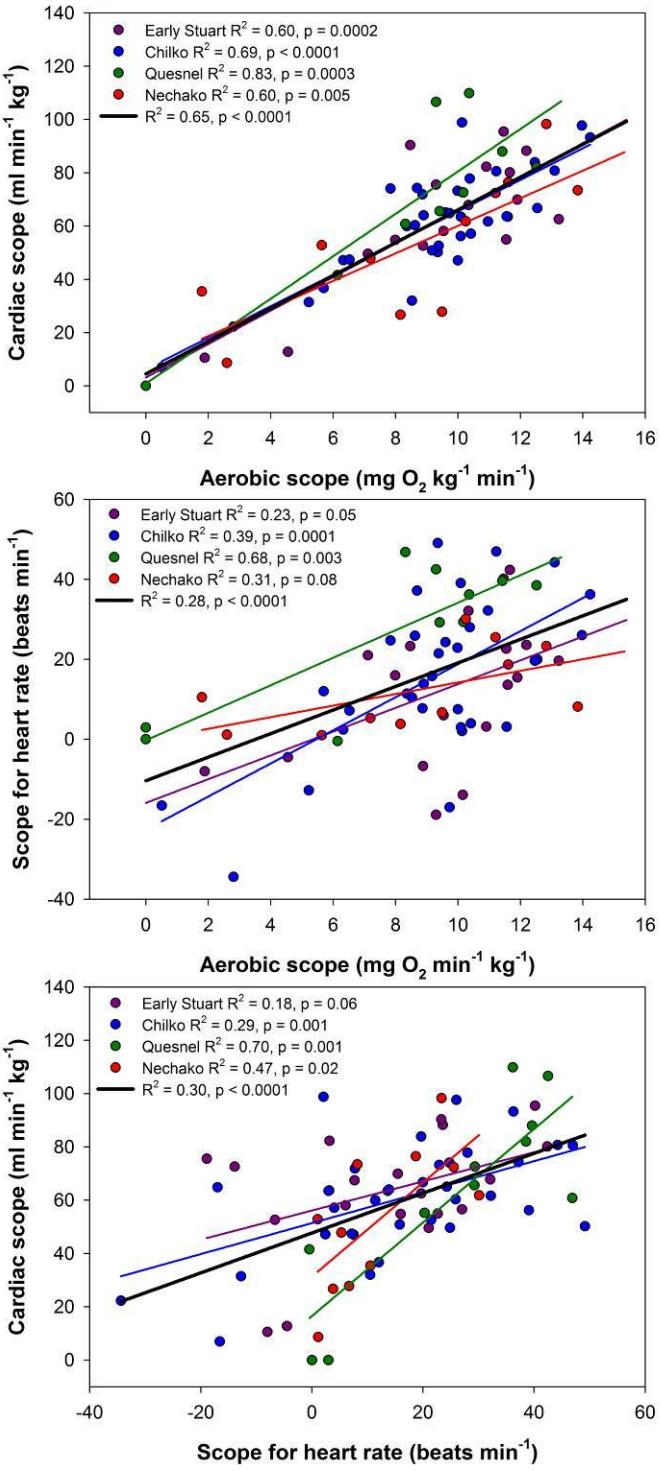

Figure 3.4. Linear regressions between aerobic scope, cardiac scope and scope for heart rate. Each data point corresponds to an individual fish, the overall R^2 and p-value with all populations and temperatures combined is indicated in black.

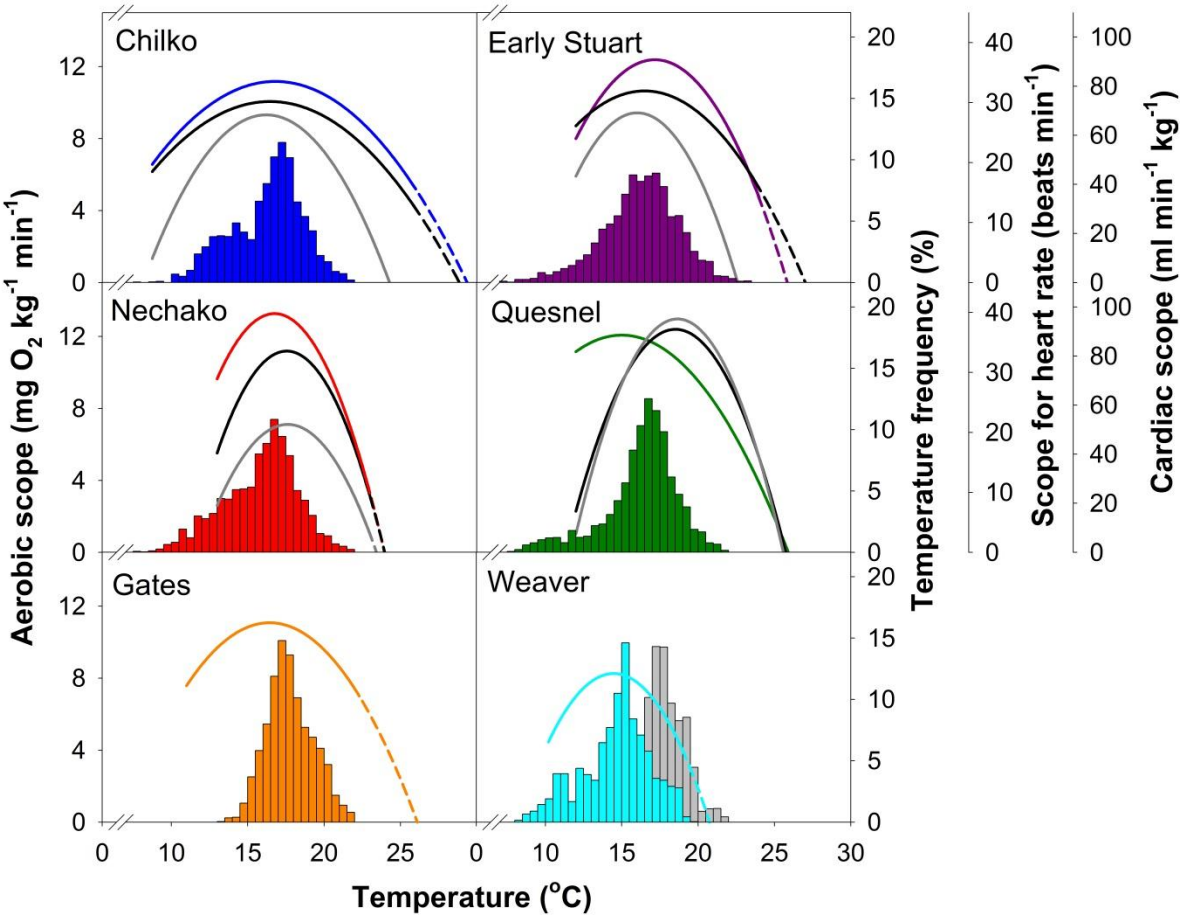

Figure 3.5. Population-specific estimates of aerobic scope (coloured lines) cardiac scope (black lines) and scope for heart rate (grey lines) in relation to water temperature. The frequency histogram shows simulated distributions of average river temperatures encountered by individual modeled fish from each population during their upriver migration from 1995 to 2008. For Weaver fish, two temperature histograms are presented, one for historical river entry (blue), the other for the current early entry phenomenon (grey). Aerobic scope data for Gates and Weaver were provided by Lee et al. (2003c).

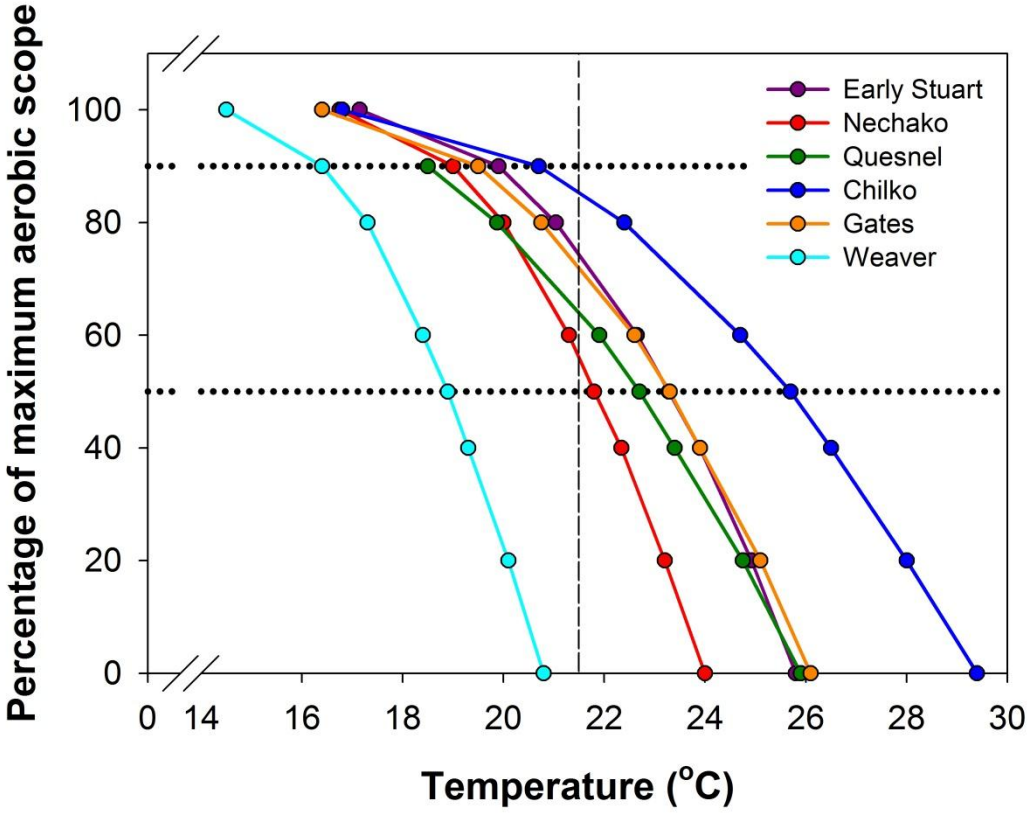

Figure 3.6. Percentage of maximum aerobic scope available for each population in relation to temperature. Dashed line at 21.5°C indicates the maximum Fraser River temperature measured near Hells Gate since the 1940s. Although it is unknown what proportion of aerobic scope is needed to successfully ascend the river, 90% and 50% are indicated as guidelines (dotted lines).

Table 3.1. Gross morphology among populations and between sexes. Post-orbital-hypural (POH) length, post-orbital-fork (POF) length, gonadosomatic index (GSI), hepatosomatic index (HSI), and splenosomatic index (SSI) are indicated.

	Early Stuart		**Nechako**		**Quesnel**		**Chilko**		**Lower Adams**	
	male	*female*	*male*	*female*	*male*	*female*	*male*	*female*	*male*	*female*
N	17	9	6	8	4	9	24	11	4	3
mass (kg)	2.41 ± 0.04	2.39 ± 0.08	2.46 ± 0.20	2.11 ± 0.13	2.86 ± 0.20	2.24 ± 0.07	2.52 ± 0.09	2.12 ± 0.12	2.85 ± 0.17	2.41 ± 0.18
fork length (cm)	59.6 ± 0.4	59.9 ± 0.9	60.6 ± 1.2	57.5 ± 1.0	63.5 ± 2.4	58.4 ± 0.6	60.6 ± 0.6	57.6 ± 0.7	61.8 ± 0.7	59.3 ± 1.2
standard length (cm)	54.4 ± 0.4	53.6 ± 1.0	55.1 ± 2.8	51.6 ± 1.2	57.2 ± 2.2	53.0 ± 0.5	54.8 ± 0.7	52.3 ± 0.7	56.0 ± 0.7	53.7 ± 1.6
POH (cm)	49.5 ± 0.5	50.2 ± 0.6	50.5 ± 1.1	48.4 ± 1.0	51.5 ± 2.3	50.3 ± 0.8	50.3 ± 0.6	48.5 ± 0.6	51.5 ± 0.7	49.0 ± 1.2
POF (cm)	54.6 ± 0.4	55.6 ± 0.7	55.8 ± 1.1	54.2 ± 0.8	57.7 ± 2.4	54.2 ± 0.8	55.8 ± 0.6	53.5 ± 0.6	57.3 ± 0.7	54.8 ± 1.1
GSI (%)	2.01 ± 0.09	5.75 ± 0.49	1.49 ± 0.26	5.49 ± 0.31	1.71 ± 0.22	8.31 ± 0.88	1.88 ± 0.10	6.24 ± 0.80	1.54 ± 0.24	6.95 ± 0.77
HSI (%)	1.42 ± 0.05	1.66 ± 0.06	1.32 ± 0.08	1.53 ± 0.14	1.47 ± 0.19	1.58 ± 0.06	1.46 ± 0.06	1.53 ± 0.10	1.51 ± 0.08	1.44 ± 0.13
SSI (%)	0.14 ± 0.01	0.11 ± 0.01	0.14 ± 0.03	0.11 ± 0.02	0.13 ± 0.02	0.10 ± 0.01	0.16 ± 0.01	0.13 ± 0.02	0.20 ± 0.02	0.15 ± 0.01
energy (MJ kg^{-1})	7.77 ± 0.23	8.08 ± 0.46	8.90 ± 0.25	7.69 ± 0.47	7.02 ± 1.08	7.95 ± 0.39	8.55 ± 0.33	8.45 ± 0.58	6.80 ± 0.71	7.04 ± 0.79

Table 3.2. Measurements of oxygen consumption ($\dot{M}O_2$), critical swimming velocity (U_{crit}) and recovery ratio (RR) in Early Stuart sockeye salmon swum at T_{opt} that had (with leads) and had not (no leads) been instrumented with a flowprobe and catheters to measure cardiovascular variables. Mean ± SEM are presented, an asterisk indicates a statistically significant difference between fish with leads and those without ($p<0.05$).

$\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1})	n	No leads	n	With leads
rest	4	2.6 ± 0.2	8	3.2 ± 0.2
maximum	4	14.4 ± 1.4	8	14.7 ± 0.4
scope	4	11.9 ± 1.3	7	11.7 ± 0.3
fatigue 1	3	8.6 ± 0.2	7	10.0 ± 0.8
fatigue 2	4	9.0 ± 2.3	7	8.9 ± 0.8
45-min recovery 1	4	4.2 ± 0.9	7	6.6 ± 0.3*
45-min recovery 2	4	6.3 ± 1.4	7	5.4 ± 0.8
2-h recovery 2	4	4.0 ± 0.7	7	3.9 ± 0.4
U_{crit} 1 (bl s^{-1})	4	2.41 ± 0.13	9	2.02 ± 0.06*
U_{crit} 2 (bl s^{-1})	4	2.35 ± 0.19	8	1.91 ± 0.06*
U_{crit} 1 (cm s^{-1})	4	144.1 ± 7.6	9	122.1 ± 4.1*
U_{crit} 2 (cm s^{-1})	4	140.6 ± 12.1	8	114.4 ± 3.5*
RR	4	0.97 ± 0.05	8	0.95 ± 0.02

Table 3.3. Oxygen consumption ($\dot{M}O_2$), cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_s) at the optimal temperature (T_{opt}) (mean ± SEM). $\dot{M}O_2$ data for Gates and Weaver are taken from Lee et al. (2003c). No cardiac variables were measured in Lee et al. (2003c). Populations with differing letters are significantly different within each variable ($p<0.05$).

	Early Stuart	**Nechako**	**Quesnel**	**Chilko**	**Lower Adams**	**Gates**	**Weaver**
n	9-12	4-6	6-7	12-13	4-5	27	24-26
$\dot{M}O_{2rest}$ (mg O_2 kg^{-1} min^{-1})	3.0 ± 0.2^{a}	2.4 ± 0.2^{a}	2.6 ± 0.2^{a}	2.9 ± 0.2^{a}	3.4 ± 0.5^{ab}	4.0 ± 0.1^{b}	2.8 ± 0.1^{a}
$\dot{M}O_{2max}$ (mg O_2 kg^{-1} min^{-1})	14.6 ± 0.5^{a}	15.3 ± 0.6^{a}	13.7 ± 0.5^{ab}	13.8 ± 0.6^{a}	12.6 ± 1.4^{ab}	15.0 ± 0.2^{a}	10.5 ± 0.3^{b}
$\dot{M}O_2$ scope (mg O_2 kg^{-1} min^{-1})	11.8 ± 0.5^{a}	13.0 ± 0.6^{a}	11.2 ± 0.6^{ab}	10.9 ± 0.6^{ab}	9.0 ± 0.8^{b}	10.9 ± 0.2^{ab}	7.7 ± 0.2^{c}
$\dot{Q}_{rest}$ (ml min^{-1} kg^{-1})	34.8 ± 2.7	29.9 ± 1.7	34.7 ± 3.9	34.8 ± 2.9	27.8 ± 3.1	-	-
$\dot{Q}_{max}$ (ml min^{-1} kg^{-1})	105.5 ± 5.5	110.0 ± 5.6	113.6 ± 10.7	107.1 ± 5.5	85.4 ± 10.4	-	-
$\dot{Q}$ scope (ml min^{-1} kg^{-1})	70.7 ± 4.7	80.2 ± 6.1	78.9 ± 7.8	72.4 ± 3.9	57.6 ± 7.4	-	-
f_{Hrest} (beats min^{-1})	70.1 ± 2.3	65.8 ± 2.6	60.9 ± 4.7	67.3 ± 2.7	67.7 ± 6.7	-	-
f_{Hmax} (beats min^{-1})	95.5 ± 2.8	84.7 ± 4.0	93.1 ± 3.5	94.1 ± 2.1	91.2 ± 7.4	-	-
f_H scope (beats min^{-1})	25.4 ± 3.8	18.9 ± 3.9	32.2 ± 3.0	26.7 ± 3.7	23.5 ± 9.7	-	-
V_{srest} (ml $beat^{-1}$ kg^{-1})	0.49 ± 0.03	0.46 ± 0.02	0.57 ± 0.06	0.53 ± 0.05	0.43 ± 0.06	-	-
V_{smax} (ml $beat^{-1}$ kg^{-1})	1.10 ± 0.05	1.30 ± 0.05	1.22 ± 0.11	1.14 ± 0.06	0.93 ± 0.06	-	-
V_s scope (ml $beat^{-1}$ kg^{-1})	0.60 ± 0.04^{a}	0.85 ± 0.05^{b}	0.65 ± 0.05^{ab}	0.62 ± 0.05^{a}	0.50 ± 0.02^{a}	-	-

Table 3.4. Pearson correlation matrix relating aerobic scope of fish from seven populations and eight migration difficulty variables (see Table 2.1). ATU = accumulated thermal units, F_M = Fraser River discharge. Three critical values are indicated: $p < 0.05$ (no correction for multiple comparisons), $p < 0.018$ (Benjamini and Yekutieli False Discovery Rate) and $p < 0.006$ (Bonferroni). Bold font indicates the migration difficulty variable with the highest correlation coefficient.

	Aerobic scope
migration distance (D_M)	**0.856**†
migration elevation (E_M)	0.653
work ($0.0001 \bullet E_M \bullet D_M$)	0.785*
river slope ($500(E_M D_M^{-1})$)	0.335
migration effort ($0.0001 \bullet D_M \bullet F_M$)	0.732
migration duration	0.777*
migration rate	0.842†
ATU	0.832*

* $p < 0.05$; † $p < 0.018$, ‡ $p < 0.006$

Table 3.5. Population-specific optimal temperature (T_{opt}), upper and lower pejus temperatures (T_p) and predicted critical temperatures (T_{crit}). T_p range refers to the width of the T_{opt} window (i.e. upper T_p – lower T_p). T90% indicates the upper 90th percentile of historic temperatures encountered by each population (1995-2008). % Scope at T90% indicates the percent of maximum aerobic scope available at T90%. Values for current river entry timing for Weaver are shown in parentheses under the historical timing.

Population	T_{opt} (°C)	Lower T_p (°C)	Upper T_p (°C)	T_p range (°C)	T90% (°C)	% Scope at T90%	Predicted T_{crit} (°C)
Early Stuart	17.2	14.4	19.9	5.5	19.0	96	25.8
Nechako	16.8	14.5	19.0	4.5	18.4	95	24.0
Quesnel	-	-	18.5	-	18.6	89	25.9
Chilko	16.8	12.9	20.7	7.8	18.8	98	29.4
Gates	16.4	13.4	19.5	6.1	19.7	89	26.1
Weaver	14.5	12.5	16.4	3.9	17.2	81	20.8
					(19.1)	(45)	

Table 3.6. Summary of model selection statistics for regressions between population-specific aerobic scope predictions and population-specific temperature frequency distributions. Best-fit relationships correspond to $\Delta AIC \leq 2$ (bold font). Both current (WeaverCurrent) and historical (WeaverHistoric) temperature frequency histograms for Weaver are included.

Population scopes	Temperature frequencies	AIC	ΔAIC	*w*	R^2	p-value	Bonferroni corrected
Early Stuart							
	Early Stuart	182.53	0.00	0.94	0.72	8.35E-12	3.51E-10
	Nechako	200.18	17.65	0.00	0.62	1.08E-09	4.54E-08
	Quesnel	206.71	24.18	0.00	0.55	2.53E-08	1.06E-06
	Chilko	187.98	5.45	0.06	0.72	3.08E-12	1.29E-10
	Gates	194.68	12.15	0.00	0.67	7.68E-11	3.23E-09
	WeaverCurrent	231.99	49.47	0.00	0.16	6.89E-03	2.89E-01
	WeaverHistoric	226.41	43.88	0.00	0.27	3.98E-04	1.67E-02
Nechako							
	Early Stuart	179.13	1.99	0.27	0.81	4.54E-15	1.91E-13
	Nechako	197.43	20.29	0.00	0.73	1.12E-12	4.70E-11
	Quesnel	205.95	28.82	0.00	0.67	6.69E-11	2.81E-09
	Chilko	177.14	0.00	0.73	0.84	2.00E-16	8.40E-15
	Gates	195.18	18.05	0.00	0.75	3.82E-13	1.60E-11
	WeaverCurrent	239.72	62.58	0.00	0.23	9.63E-04	4.04E-02
	WeaverHistoric	232.31	55.17	0.00	0.36	2.39E-05	1.00E-03
Chilko							
	Early Stuart	123.41	0.00	1.00	0.78	5.43E-14	2.28E-12
	Nechako	151.63	28.22	0.00	0.66	1.49E-10	6.26E-09
	Quesnel	155.80	32.38	0.00	0.62	1.11E-09	4.66E-08
	Chilko	148.83	25.41	0.00	0.68	3.87E-11	1.63E-09
	Gates	166.99	43.58	0.00	0.49	2.49E-07	1.05E-05
	WeaverCurrent	183.82	60.40	0.00	0.23	1.00E-03	4.20E-02
	WeaverHistoric	176.99	53.58	0.00	0.35	3.32E-05	1.39E-03
Gates							
	Early Stuart	135.53	0.00	1.00	0.86	2.00E-16	8.40E-15
	Nechako	160.52	24.99	0.00	0.78	2.26E-14	9.49E-13
	Quesnel	169.16	33.63	0.00	0.73	1.41E-12	5.92E-11
	Chilko	157.08	21.55	0.00	0.80	4.35E-15	1.83E-13
	Gates	190.17	54.64	0.00	0.54	3.46E-08	1.45E-06
	WeaverCurrent	206.21	70.68	0.00	0.32	8.87E-05	3.73E-03
	WeaverHistoric	196.87	61.34	0.00	0.46	8.97E-07	3.77E-05
Weaver							
	Early Stuart	169.31	46.00	0.00	0.64	7.58E-10	3.18E-08
	Nechako	158.12	34.81	0.00	0.75	2.61E-13	1.10E-11
	Quesnel	171.22	47.91	0.00	0.66	1.40E-10	5.88E-09
	Chilko	168.74	45.44	0.00	0.68	4.25E-11	1.79E-09
	Gates	207.36	84.05	0.00	0.15	7.16E-03	3.01E-01
	WeaverCurrent	140.68	17.37	0.00	0.84	2.00E-16	8.40E-15
	WeaverHistoric	123.30	0.00	1.00	0.90	2.00E-16	8.40E-15

CHAPTER 4: A COMPARISON OF CARDIORESPIRATORY AND SWIMMING PERFORMANCE AMONG UPRIVER SOCKEYE SALMON POPULATIONS AT T_{opt}

4.1 Introduction

The previous chapter compared resting, maximum and scope for $\dot{M}O_2$, $\dot{Q}$, f_H and V_s among sockeye salmon populations and demonstrated that aerobic scope varies according to the difficulty of the upriver spawning migration. However, detailed analyses of how the various components of the cardiorespiratory oxygen convection system change with swimming were not considered. Therefore, this chapter greatly expands on Chapter 3 through a comprehensive assessment of swimming physiology at T_{opt}.

The upriver spawning migration is physically demanding for sockeye salmon. During this once-in-a-lifetime migration, Fraser River sockeye salmon swim continuously against a fast flowing river for several weeks at swimming speeds of 2 to 4 km h^{-1} and ground speeds of 20 to 40 km day^{-1} (English et al., 2005, Hinch and Rand, 1998). Moreover, because the fish cease feeding in the ocean, upriver swimming is fuelled entirely by endogenous energy stores. Also, sockeye salmon have a finite amount of time to complete their migration in order to successfully spawn. Upriver populations must negotiate hydraulically challenging river sections through the Fraser Canyon, such as Hells Gate, which requires anaerobic swimming (Hinch and Bratty, 2000; Rand and Hinch, 1998). Consequently, it is critical that sockeye salmon are able to recover rapidly from exhaustive exercise in order to continue their upriver migration. Indeed, previous studies on sockeye salmon, pink salmon, coho salmon, cutthroat trout and rainbow trout showed that salmonids have an excellent ability to repeat their swim performance after a short recovery

period of 30-60 min (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006).

This is the first study to compare cardiovascular performance and blood variables across wild sockeye salmon populations. The objective of this study was to examine how the cardiovascular system supports aerobic scope and swim performance and whether the mechanism changes over sequential swim tests or across populations. I compared swimming and cardiorespiratory performance among upriver Fraser River sockeye salmon populations (N = 32, Early Stuart, Chilko, Quesnel and Nechako) performing two sequential U_{crit} swim challenges at their T_{opt}. By performing these comparisons at T_{opt}, I removed temperature as a confounding factor in the population comparison. Only fish that had been instrumented were included in the analysis. Detailed materials and methods are found in Chapter 2 (sections 2.2-2.7 and 2.10).

All four populations must navigate through Hells Gate and travel 650 to 1100 km upstream, reaching an elevation of 700 to 1200 m on their spawning grounds. Furthermore, all four populations encounter a similar migration temperature median and mode (Table 2.1, 16-17°C) and have a similar T_{opt} (Table 3.6, ~17°C). I hypothesized that all four populations would be able to repeat their swim performance following a brief 45-min recovery, since there is likely strong selection pressure on the ability to rapidly recover from exhaustive exercise in adult sockeye salmon. Furthermore, since all four populations experience challenging migrations and did not differ in aerobic scope (Chapter 3), I hypothesized that they would have similar cardiorespiratory and swimming performance.

4.2 Results

4.2.1 Swimming Behaviour and Performance

Most of the sockeye salmon ventilated regularly and remained steady and calm during the rest period, with occasional exploratory movements. Many sockeye salmon exhibited unsteady, erratic swimming behaviour or they tended to rest on the bottom during the initial ramping phase of the U_{crit} swim challenge until they reached swim speeds of ~1 bl s^{-1}. Thereafter, there were typically three clear swimming phases. During the first phase, fish regularly ventilated their gills via opercular pumping while swimming steadily. Throughout phase two, fish continued to swim in a steady manner, but switched to ram ventilation. In the third swim phase, the fish transitioned to burst-and-coast swimming and continued to ram ventilate. Phase three typically started during the penultimate or final swim speed, which corresponded to speeds ~80-90% of maximum or ~2.0 bl s^{-1}. Interestingly, during the first two swim phases, fish occasionally exhibited "side burst" behaviour, where they would slowly fall back in the swim tunnel and then flip onto their sides and burst forward with a few quick tail flicks to regain their position at the front of the swim tunnel. This behaviour differed from the vertical burst-and-coast behaviours exhibited at the fastest swim speeds. Most sockeye salmon spent the duration of the recovery periods ventilating via opercular pumping with occasional light swimming, near the front of the swim tunnel.

U_{crit} 1, U_{crit} 2 and RR did not significantly differ among populations or between sexes (Table 4.1). In addition, all populations were able to repeat their swim performance because U_{crit} 1 did not significantly differ from U_{crit} 2 (overall mean maximum U_{crit} = 2.04 ± 0.04).

4.2.2 Cardiorespiratory Performance

There were no significant differences in any of the cardiorespiratory variables between sexes, therefore, the data were pooled.

$\dot{M}O_{2max}$ and aerobic scope were not compared between swim 1 and swim 2 due to missing paired measurements. Likewise, Nechako sockeye salmon were excluded from the $\dot{M}O_2$ analysis because there were insufficient measurements at each swimming speed.

During swimming, $\dot{M}O_2$ significantly increased ~5-fold from resting values (Fig 4.1). $\dot{M}O_2$ did not significantly differ among the three populations at any swimming speed (Fig 4.1, Table 4.2). $\dot{M}O_2$ remained significantly elevated above resting levels at both 45-min recovery periods; however, it had recovered by the 2-h recovery period (Table 4.2). $\dot{M}O_2$ did not significantly differ between swim 1 and 2 at any swimming speed. Accordingly, COT and COT_{net} did not significantly differ between swims (Fig 4.2). Notably, COT did not display the characteristic U-shape, instead, it plateaued between 1.12 and 2.37 bl s^{-1}.

As expected, $\dot{Q}$ significantly increased ~3-fold above resting levels during swimming (Fig 4.3A). $\dot{Q}$ did not significantly differ among the four populations at any swimming speed (Fig 4.3A, Table 4.2, 4.3). $\dot{Q}$ did not recover back to resting levels during any of the recovery periods, except in Nechako sockeye salmon at 2 h (Table 4.2). In addition, $\dot{Q}$ did not significantly differ between swim 1 and swim 2, except at the very first swim speed (0.62 bl s^{-1}). Consequently, COT-$\dot{Q}$ and COT-$\dot{Q}_{net}$ only significantly differed between swims at 0.62 bl s^{-1}, although there was a general, non-significant trend for COT-$\dot{Q}$ and COT-$\dot{Q}_{net}$ to be higher during swim 2 compared to swim 1 (Fig 4.2).

V_s increased ~2-fold above resting levels during swimming. V_s did not significantly differ among populations at rest, during either swim or during recovery (Fig 4.3B, Table 4.2, 4.3). Even so, scope for V_s during swim 1 was significantly higher in Nechako compared to Quesnel sockeye salmon (Table 4.3). V_s returned back to resting levels by the 45-min recovery time point following both swims (Table 4.2). V_s did not significantly differ between swim 1 and swim 2 at any of the swimming speeds or recovery times, although scope for V_s was significantly higher in swim 2 compared to swim 1 for Quesnel sockeye salmon (Table 4.3).

During the first swim, f_H increased by ~1.5 fold from resting levels. f_H did not significantly differ among populations at any swim speed during swim 1 (Fig 4.3C). Notably, f_H did not recover back to resting levels after swim 1 for Early Stuart, Chilko and Quesnel populations (Table 4.2). Instead, following a brief decrease at fatigue, f_{Hmax} was maintained throughout the recovery period and the entire second swim for these three populations (Fig 4.3). In contrast, f_H recovered back to resting levels after swim 1 in Nechako sockeye salmon (Fig 4.3C, Table 4.2). During swim 2, f_H was significantly lower in Nechako compared to the Early Stuart and Quesnel populations until they reached a velocity of 1.37 bl s^{-1} (Fig 4.3C). Moreover, Nechako had a significantly lower f_H relative to Quesnel sockeye salmon during fatigue following the second swim (Table 4.2). f_H remained elevated above resting levels at the 2-h recovery period for Early Stuart, Chilko and Quesnel sockeye salmon (Table 4.2). Despite differences in the f_H response to swimming and recovery, f_{Hmax} and scope for f_H did not significantly vary among populations or between swims (Table 4.3).

4.2.3 Oxygen Transport and Removal by Tissues

Since $\dot{M}O_2$, $\dot{Q}$, V_s and f_H did not significantly differ at rest or during swimming in Early Stuart, Chilko and Quesnel sockeye salmon, I pooled the results for the blood analyses from these three populations. Nechako sockeye salmon were not included in the analysis because V_s and f_H differed from the other populations at various time points. Again, there were no significant differences in any of the blood variables between male and female sockeye so the data were pooled.

Blood samples were collected at rest, during steady state swimming when most of the fish were still ventilating by opercular pumping ("steady", mean speed = 1.18 ± 0.02 bl s^{-1}, or 55.8 ± 0.9% of maximum swim speed), during burst-and-coast swimming with ram ventilation ("burst", mean speed = 2.05 ± 0.06 bl s^{-1} or 92.6 ± 1.7% of maximum swim speed), immediately following fatigue, following 45 min of recovery and 2 h after the second swim was terminated.

P_{aO2}, P_{vO2}, C_{aO2} and C_{vO2} all significantly decreased from rest during swimming (Fig 4.4). P_{vO2} and C_{vO2} reached a plateau of 17.6-24.0 torr and 2.5-3.3 ml dl^{-1}, respectively, during burst swimming and fatigue. P_{aO2}, P_{vO2}, C_{aO2} and C_{vO2} did not differ between swim 1 and swim 2, despite significant decreases in [Hb] and Hct during swim 2. Both P_{aO2} and P_{vO2} returned to resting levels by the 45-min recovery periods. However, C_{aO2} and C_{vO2} remained depressed below resting levels during the second 45-min and the 2-h recovery period (Fig 4.4).

Hct only significantly varied between arterial and venous blood samples during fatigue 1; however, both [Hb] and Hct were consistently higher in venous compared to arterial blood throughout both swim tests. Moreover, MCHC was consistently lower in venous compared to arterial blood and significantly differed during several time points (Table 4.4). To verify whether

this was an artefact, I compared paired arterial and venous blood samples from fish that had both cannulae working simultaneously (paired samples, Table 4.5). Paired samples revealed that [Hb] was equivalent between arterial and venous blood samples, except at steady 1 (Table 4.5). However, Hct was significantly higher and MCHC was significantly lower in venous compared to arterial blood, but only during burst swimming and at fatigue (Table 4.5).

Hct and [Hb] were significantly lower during swim 2 compared to swim 1, suggesting that hemodilution may have occurred (Table 4.4). To check for this possibility, comparisons were made between fish that had both cannulae working (~20 blood samples collected total, ≅14 ml of blood) and those with only the venous cannula working (~10 blood samples, ≅ 7 ml of blood). Both [Hb] and Hct were significantly lower during swim 2 in fish that had two cannulae compared to those that only had one functioning cannula, confirming that [Hb] and Hct were significantly lower during swim 2 due to hemodilution (Fig 4.6). This hemodilution had no effect on P_{vO2}; however, C_{vO2} was significantly lower during the recovery periods in fish with two cannulae relative to those with one cannula (Fig 4.6). There were insufficient fish with only the arterial cannula functioning to perform a similar analysis for arterial blood.

There was a general trend for $A\text{-}V_{O2}$ to increase during swimming. However, $A\text{-}V_{O2}$ did not significantly differ from rest or between swims (Fig 4.6); probably because comparisons were limited to fish with both cannulae working (N = 9). Notably, $A\text{-}V_{O2}$ decreased by ~50% from resting values during the 2-h recovery period, since both C_{aO2} and C_{vO2} remained depressed below resting levels.

Arterial transfer of oxygen to the tissues (T_{aO2}) integrates changes in $\dot{Q}$ and C_{aO2} ($T_{aO2} = \dot{Q} \times C_{aO2}$). T_{aO2} significantly increased from rest by 2.5-fold during burst swimming and returned back to resting levels by the 45-min recovery period (Fig 4.6). No significant differences were

detected between swim 1 and 2. In contrast, venous transfer of oxygen to the heart and gills ($T_{vO2} = \dot{Q} \times C_{vO2}$) remained constant throughout the entire swimming protocol and did not significantly vary from rest or between swim 1 and 2 (Fig 4.6).

4.2.4 Other Blood Variables

Plasma lactate did not significantly differ between arterial and venous blood samples. Plasma lactate was significantly elevated above resting levels during fatigue and 45 min after swim 1, verifying that the salmon did transition to anaerobic swimming during the swim challenge (Fig 4.4). Plasma lactate levels also remained significantly elevated during swim 2, resulting in a significant difference between swim 1 and swim 2 during steady swimming. Although plasma lactate did tend to recover somewhat by burst 2, it was again significantly elevated above resting levels during fatigue 2 and the second 45 -min recovery. However, plasma lactate did not significantly differ from resting levels at the 2-h recovery period (Fig 4.4).

Plasma glucose, chloride and sodium varied minimally from resting levels and no significant differences were detected between arterial and venous blood samples (Table 4.4). In contrast, plasma potassium was highly variable between swim 1 and 2 and between arterial and venous blood samples. In general, plasma potassium was higher in arterial relative to venous blood (Tables 4.4 and 4.5). In addition, plasma potassium tended to be higher during swim 2 compared to swim 1. Remarkably, plasma potassium actually decreased from rest during burst swimming and at fatigue with swim 1 (Table 4.4).

4.2.5 General Trends with Swimming

To summarize the general trends in cardiovascular physiology and oxygen status associate with swimming, fold changes from the initial resting value were examined for $\dot{V}O_2$, $\dot{Q}$, A-V_{O2}, T_{aO2} and T_{vO2} exclusively from fish with both cannulae working from pooled data from the Early Stuart, Chilko and Quesnel populations (Fig 4.7). $\dot{V}O_2$ increased 5-fold during both swims, primarily due to a 3-fold increase in $\dot{Q}$ (Fig 4.7A). $\dot{V}O_2$ returned to resting levels by the 2-h recovery after swim 2, even though $\dot{Q}$ remained elevated, because A-V_{O2} decreased by ~50% from resting levels (Fig 4.7A, B & C). The 3-fold increase in $\dot{Q}$ during both swims was primarily due to a >2-fold increase in V_s (Fig 4.7B). f_H increased ~1.5 fold above resting levels during the first swim and never decreased below maximum levels throughout the entire second swim and both recovery periods. As such, $\dot{Q}$ remained elevated above rest at both 45-min and the 2-h recovery periods, even though V_s had returned to resting levels. The ~1.5-fold increase in A-V_{O2} was driven by a large decrease in C_{vO2}, though the A-V_{O2} response was attenuated since C_{aO2} also decreased (Fig 4.7C). T_{aO2} increased by 2.5-fold during both swims, which was entirely due to the aforementioned increase in $\dot{Q}$ (Fig 4.7D). T_{vO2} changed very little from rest throughout both swims and the recovery periods because the decrease in C_{vO2} was offset by the increase in $\dot{Q}$ (Fig 4.7E).

4.3 Discussion

The goal of the present study was to compare cardiorespiratory performance and blood variables across upriver Fraser River sockeye salmon populations swimming two sequential

swim tests. As anticipated, all four populations demonstrated similar increases in $\dot{M}O_2$ and $\dot{Q}$ with swimming. However, in comparison to the other three populations, Nechako sockeye salmon relied more on V_s than f_H to increase $\dot{Q}$. This finding suggests that the mechanism of achieving the same $\dot{M}O_2$ and $\dot{Q}$ can vary across populations. In addition, despite incomplete metabolic recovery, all populations showed an exceptional ability to repeat their swim performance following a brief 45-min recovery, supporting previous studies on salmonids (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006). A rapid rate of recovery is clearly beneficial for salmon to ensure a timely migration to reach the spawning grounds.

4.3.1 Swimming Behaviour and Performance

Preliminary, practise swim tests have been demonstrated to improve ramp-U_{crit} swim performance in rainbow trout (Jain et al., 1997), presumably because the fish become habituated to the tunnel and learn how to swim effectively. I was unable to give the sockeye salmon a practise swim due to time and logistical constraints. Although the fish often swam erratically during the initial ramping phase of the U_{crit} swim test, they quickly grew accustomed to the tunnel at faster speeds. Despite swimming with leads, the sockeye salmon demonstrated classic swim behaviours and clearly transitioned to burst-and-coast anaerobic swimming at the highest swim speeds, as confirmed by the appearance of lactate in the plasma at fatigue and during recovery. Advanced sexual maturation has been reported to decrease swim performance in pink salmon (Williams et al., 1986), sockeye salmon (M. Steinhausen, pers. communication) and chinook salmon (E. Eliason, pers. observation). This was not a major concern in the present

study since the sockeye salmon were collected early in their migration, several weeks before their spawning date and none of the fish were fully sexually mature (no loose eggs or milt production).

The U_{crit} values obtained in the present study for adult sockeye salmon swum with leads (mean overall maximum U_{crit} = 2.0 bl s^{-1}) were within the reported range for un-instrumented adult salmonids: sockeye salmon (1.4-2.4 bl s^{-1}, Brett and Glass, 1973; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003c; MacNutt et al., 2006), chinook salmon (2.1 bl s^{-1}, Geist et al., 2003), pink salmon (1.6-3.2 bl s^{-1}, Farrell et al., 2003; MacNutt et al., 2006; Williams et al., 1986), coho salmon (1.4-1.9 bl s^{-1}, Farrell et al., 2003; Lee et al., 2003a; Lee et al., 2003c), Arctic charr *Salvelinus alpinus* (L.) (2.8 bl s^{-1}, Jones et al., 1974), mountain whitefish *Prosopium williamsoni* (1.4 bl s^{-1}, Jones et al., 1974), Arctic cisco *Coregonus autumnalis* (1.9 bl s^{-1}, Jones et al., 1974), least cisco *Coregonus sardinella* Valenciennes (2.0 bl s^{-1}, Jones et al., 1974), wild-caught rainbow trout (2.2 bl s^{-1}, Jones et al., 1974), and hatchery-reared rainbow trout (2.1-2.8 bl s^{-1}, Jain et al., 1997; Jones et al., 1974).

U_{crit} did not differ between swim 1 and 2 or among populations, supporting the observation that migrating, adult Pacific salmon have excellent repeat swim performance (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006). Collectively, these comparisons suggest that the fish in the present study had recovered well from surgery since the repeatability of swim test decreases when sockeye salmon or rainbow trout are diseased or exposed to toxicants (Jain et al., 1998; Tierney and Farrell, 2004; Wagner et al., 2005).

4.3.2 Cardiorespiratory Performance with Comparisons across Populations

$\dot{M}O_{2rest}$ measured at T_{opt} (15-20°C) did not differ among the four upriver sockeye salmon populations and ranged between 2.4 and 3.2 mg O_2 kg^{-1} min^{-1} (also see Chapter 3). This range is comparable to resting values in other adult salmonids: sockeye salmon at 11-21°C (1.6-4.4 mg O_2 kg^{-1} min^{-1}, Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003c; Steinhausen et al., 2008; Wagner et al., 2005; Wagner et al., 2006), chinook salmon at 8-17°C (2.0-3.4 mg O_2 kg^{-1} min^{-1}, Clark et al., 2008b; Geist et al., 2003), pink salmon at 9-22°C (1.1-4.3 mg O_2 kg^{-1} min^{-1}, Farrell et al., 2003; MacNutt et al., 2006; Williams et al., 1986), coho salmon at 8-10°C (2.2-2.9 mg O_2 kg^{-1} min^{-1}, Farrell et al., 2003; Lee et al., 2003a; Lee et al., 2003c).

Studies on sexually immature rainbow trout report considerably lower $\dot{M}O_{2rest}$ values ranging from 0.8-1.3 mg O_2 kg min^{-1} (Claireaux et al., 2005; Eliason et al., 2008; Kiceniuk and Jones, 1977; Taylor et al., 1996; Thorarensen et al., 1996). The finding that adult Pacific salmon have a comparatively higher $\dot{M}O_{2rest}$ relative to immature rainbow trout is not surprising since adult salmon undergo considerable morphological changes (developing gonads and secondary sexual characteristics) which undoubtedly has an oxygen cost. They may have also been more restless in the swim tunnel due to the migratory life stage (Lee et al., 2003c; Wagner et al., 2006). In addition, the fish in the present study were only allowed an overnight recovery due to logistical issues and to time constraints. Farrell et al. (2003) demonstrated that $\dot{M}O_{2rest}$ significantly declined for fish given a 48-h habituation period to the swim tunnel compared to those only given an overnight recovery. Moreover, some of the studies with rainbow trout attempted to measure standard metabolic rate and thus measurements were made over several days under dark conditions (e.g. Eliason et al., 2008). These comparisons emphasize the

importance of considering experimental apparatus and design when comparing across studies, particularly with $\dot{M}O_2$. To what degree aerobic scope was underestimated in these population comparisons will require further study, though the oxygen cost of sexual development and restlessness are likely unavoidable when measuring $\dot{M}O_{2rest}$ in adult salmonids.

All the populations increased $\dot{M}O_2$ by ~5-fold during swimming, attaining $\dot{M}O_{2max}$ values ranging between 13.7-15.3 mg O_2 kg^{-1} min^{-1} and aerobic scope values of 10.9-13.0 mg O_2 kg^{-1} min^{-1} (see Chapter 3). This increase is at the high end of the 3- to 5-fold increase reported for other adult salmonids (Farrell et al., 2003; Geist et al., 2003; Lee et al., 2003c; MacNutt et al., 2006; Williams et al., 1986). Similarly, the present study's $\dot{M}O_{2max}$ values are at the high end relative to previous studies on adult salmonids: sockeye salmon (5.8-15.1 mg O_2 kg^{-1} min^{-1}, Brett and Glass, 1973; Farrell et al., 2003; Hinch et al., 1996; Jain et al., 1998; Lee et al., 2003, MacNutt et al., 2006, Wagner et al., 2005, Wagner et al., 2006), chinook salmon (11.2 mg O_2 kg^{-1} min^{-1}, Geist et al., 2003), pink salmon (12.6-16 mg O_2 kg^{-1} min^{-1}, Farrell et al., 2003; MacNutt et al., 2006; Williams et al., 1986) and coho salmon (8.7-9.8 mg O_2 kg^{-1} min^{-1}, Farrell et al., 2003; Lee et al., 2003a; Lee et al 2003c).

$\dot{Q}$ at rest and during swimming were indistinguishable across populations. Only two previous studies have examined $\dot{Q}$ in sockeye salmon [Davis, 1968 (as reported in Brett, 1971); Steinhausen et al., 2008]. $\dot{Q}_{rest}$ reported here (30-35 ml min^{-1} kg^{-1}) is slightly higher than $\dot{Q}_{rest}$ at 15°C for Lower Adams sockeye salmon (25 ml min^{-1} kg^{-1}, Steinhausen et al., 2008) but comparable with $\dot{Q}_{rest}$ in adult chinook salmon at 13°C (29 ml min^{-1} kg^{-1}, Clark et al., 2008b). $\dot{Q}$ steadily increased with increasing swimming velocity until the fish fatigued. Both $\dot{Q}_{max}$ (100-118 ml min^{-1} kg^{-1}) and $\dot{Q}$ measured at ~75% of U_{crit} (80 ml min^{-1} kg^{-1}) in the present study exceeded $\dot{Q}$ for Lower Adams sockeye salmon swimming at ~75% of U_{crit} (~68 ml min^{-1} kg^{-1}; Steinhausen

et al., 2008). Davis (1968) only reported $\dot{Q}$ in ml min^{-1}($\dot{Q}_{max}$ at 20°C = ~165 ml min^{-1}) and did not report body mass for the adult sockeye salmon of unknown origin. However, if we assume the sockeye salmon were ~2.3 kg, $\dot{Q}_{max}$ is estimated to be ~72 ml min^{-1} kg^{-1}, which is substantially lower than the present study. Moreover, $\dot{Q}_{max}$ greatly exceeded $\dot{Q}_{max}$ values reported for other salmonids from hatchery sources: rainbow trout at 10-18°C (42-69 ml min^{-1} kg^{-1}, Brodeur et al., 2001; Claireaux et al., 2005; Kiceniuk and Jones, 1977; Taylor et al., 1996; Thoraresnsen et al., 1996) and immature chinook salmon at 8-10°C (66 ml min^{-1} kg^{-1}, Gallaugher et al., 2001). Therefore, the wild, upriver sockeye salmon used here have a greater cardiac capacity compared with the limited dataset available for other salmonid species.

V_{srest} was similar across populations (0.46-0.57 ml $beat^{-1}$ kg^{-1}) and within the range reported for other salmonids (e.g. 0.38-0.63 ml $beat^{-1}$ kg^{-1}, in sockeye salmon, chinook salmon and rainbow trout, Claireaux et al., 2006; Clark et al., 2008b; Gallaugher et al., 2001; Kiceniuk and Jones, 1977; Steinhausen et al., 2008). Similar to $\dot{Q}$, V_s steadily increased with increasing swim speed until the fish fatigued. V_{smax} (1.08-1.29 ml $beat^{-1}$ kg^{-1}) also exceeded the range reported for hatchery-reared salmonids (0.66-1.04 ml $beat^{-1}$ kg^{-1} in rainbow trout and immature chinook salmon, Claireaux et al., 2006; Gallaugher et al., 2001; Kiceniuk and Jones, 1977).

f_{Hrest} ranged between 61-70 beats min^{-1} across populations, which is similar to f_{Hrest} reported for Lower Adams adult sockeye salmon at 15°C (65 beats min^{-1}, Steinhausen et al., 2008) but slightly higher than f_{Hrest} reported in adult chinook salmon at a slightly cooler temperature of 13°C (58 beats min^{-1}, Clark et al., 2008b), Stamp River adult sockeye salmon at 13-16°C (49 beats min^{-1}, Smith et al., 1967) and Weaver and Harrison sockeye salmon at 11-13°C (43-52 beats min^{-1}, Sandblom et al., 2009). Free-swimming adult sockeye salmon equipped with biologgers exhibited a lower $f_{Hroutine}$ (35-44 beats min^{-1} at 13°C, Clark et al., 2010) and (50-

59 beats min^{-1} at 10°C, Clark et al., 2009). Differences in f_H among studies may partially be attributed to the $\dot{Q}_{10}$ effect. Also, free-swimming fish were able to swim throughout their environment and measurements were made over several days which would likely result in lower values since f_H appears to lag behind other cardiorespiratory variables during recovery (see Fig 4.3 and 4.7).

f_{Hmax} ranged between 81-95 beats min^{-1} across populations, which is similar to f_{Hmax} in tethered Lower Adams sockeye salmon swimming at ~75% of U_{crit} at 15°C (81 beats min^{-1}, Steinhausen et al., 2008) and tethered Stamp River sockeye salmon at 13-16°C (83 beats min^{-1}, Smith et al., 1967). In free-swimming sockeye salmon on the spawning ground or in a raceway at 10-13°C, f_{Hmax} reached ~75-79 beats min^{-1} (Clark et al., 2009, Clark et al., 2010).

Although $\dot{Q}$ did not differ among populations, f_H was lower and V_s was higher at some of the time points in Nechako sockeye salmon relative to the other populations. During the first swim, f_H steadily increased with swimming speed in all populations. However, f_H remained elevated at maximal levels throughout the first 45-min recovery, the entire second swim and the duration of second recovery period in Early Stuart, Chilko and Quesnel sockeye salmon. In contrast, f_H recovered back to resting levels in Nechako sockeye salmon. Notably, this recovery may be partially attributed to the low scope for f_H in Nechako sockeye salmon since f_H at the first 45-min recovery period did not differ from f_{Hmax} or f_{Hrest}. As such, Nechako sockeye salmon rely more on V_s and less on f_H in order to achieve the same $\dot{Q}$ as the other three populations. A similar phenomenon was reported by Nelson et al. (1994), who found that despite differences in exercise physiology between two populations of Atlantic cod, swimming performance and aerobic scope were identical.

No significant differences were detected in U_{crit}, $\dot{M}O_2$, $\dot{Q}$, V_s or f_H between male and female sockeye salmon. Despite significant differences in relative ventricular mass (RVM) between males and females (male RVM was 2-25% higher than female RVM depending on the temperature, see Chapter 6), this did not translate to significant differences in $\dot{Q}_{max}$ or V_{smax}. Similarly, Gallaugher et al. (2001) found that a 13% increase in RVM in exercise-trained immature chinook salmon did not result in differences in $\dot{Q}$. In contrast, sexually mature male rainbow trout with larger ventricles were demonstrated to have higher $\dot{Q}_{max}$ and V_{smax} compared to sexually mature females in an *in situ* perfused heart preparation (Franklin and Davie, 1992). However, the male trout had ~2-fold larger RVM compared to females, which is a much more dramatic difference then the present study. In addition, Sandblom et al. (2009) reported significantly higher f_{Hrest} in female compared to male sockeye salmon confined in holding tubes, while biologging and telemetry studies on free-swimming fish report that male salmonids spent a greater proportion of time with a high f_H (Altimiras et al., 1996; Clark et al., 2009; Lucas et al., 1993), likely due to increased activity and aggressive behaviour on the spawning grounds. Regardless, no significant differences in f_H were detected between sexes in the present study.

4.3.3 Oxygen Transport and Removal by Tissues

Resting Hct, [Hb] and MCHC were within expected levels (Clark et al., 2009; Sandblom et al., 2009). However, Hct and [Hb] decreased throughout the experiment until Hct reached ~22-24% during the final sample at the 2-h recovery. This was clearly due to haemodilution since fish with both cannulae operational (and thus had twice the amount of blood samples removed) had a significantly lower Hct and [Hb] during the second swim relative to fish with

only one cannula operational. Approximately 20 blood samples or ~14 ml of blood was collected from fish with paired cannulae. Overall mean body mass was 2.3 kg, so assuming a blood volume of 3.5 ml 100 g^{-1} body mass (Olson, 1992), each sockeye salmon had on average ~80.5 ml of blood. Thus, around 17% of the blood volume was removed and replaced with saline throughout the experiment. Notably, reduced [Hb] due to hemodilution during the second swim also decreased C_{vO2}, without affecting P_{vO2}. I could not confirm that that C_{aO2} was also reduced due to insufficient numbers of fish with only the arterial cannula functioning. However, since T_{aO2} was identical between swim1 and swim 2, the decreased Hct and [Hb] did not result in a differential perfusion limitation to the tissues between swims and accordingly, U_{crit} did not differ between swims. Similarly, Gallaugher et al. (1995) previously found that U_{crit} was not impaired in rainbow trout until Hct declined below 22%.

No significant differences in Hct, [Hb] or MCHC were detected between males and females, which is consistent with a previous study on sockeye salmon (Sandblom et al., 2009). In contrast, Clark et al. (2009) reported significantly higher [Hb] in female compared to male sockeye salmon on the spawning ground.

Resting P_{aO2} and C_{aO2} were within the expected range for salmonids (Clark et al., 2008b; Gallaugher et al., 1992; Gallaugher et al., 2001; Farrell et al., 1998; McKenzie et al., 2004; Steinhausen et al., 2008; Thorarensen et al., 1993). Both P_{aO2} and C_{aO2} declined during swimming by 34 and 23%, respectively. Several studies on salmonids report a similar decrease in P_{aO2} with swimming (Farrell et al., 1998; Gallaugher et al., 1992; Gallaugher et al., 2001; McKenzie et al., 2004; Steinhausen et al., 2008; Thorarensen et al., 1993) but C_{aO2} remained constant during swimming in several studies (Kiceniuk and Jones, 1977; Gallaugher et al., 2001; McKenzie et al., 2004; Thorarensen et al., 1993). Conversely, another report found a 21%

decrease in C_{aO2} when sockeye salmon swam ~75% of U_{crit} (Steinhausen et al., 2008). Two possibilities may account for the decrease in C_{aO2} during swimming. A normal problem encountered by salmon migrating upriver is the accumulation of fungus on the gills and body. In addition, the surgery to implant the flowprobe and sinus venosus cannula may have caused some gill damage. As such, the gill surface area for diffusion may have been limited and/or the diffusion distance may have increased, resulting in impaired C_{aO2}. Regardless, T_{aO2} was exceptionally high in the present study, primarily due to the extremely high $\dot{Q}$.

As expected, P_{vO2} and C_{vO2} also significantly declined during swimming by 57 and 70%, respectively, due to increased oxygen uptake to support the increased oxygen demand at the tissues. A threshold value for P_{vO2} during swimming has been proposed, which would ensure adequate oxygen supply to the spongy myocardium (Davie and Farrell, 1991; Farrell, 2002; Farrell, 2007; Farrell and Clutterham, 2003). Notably, the minimum P_{vO2} values measured here were 18-24 torr, which compare well with previous studies which suggest a P_{vO2} threshold of 15-16 and 29 torr in normoxic rainbow trout at 6-10°C and 13-15°C, respectively (Farrell and Clutterham, 2003).

4.3.4 Repeat Swim Performance

U_{crit} swim tests involve aerobic swimming at the lower swim speeds, followed by a transition to anaerobic metabolism as the fish near U_{crit} (Jones, 1982), as is evident by the accumulation of lactate in the blood (Black, 1955). Furthermore, during exhaustive exercise, the blood becomes acidic (low pH due to CO_2 and lactate accumulation), hypoxemic (low P_{vO2} and C_{vO2} due to oxygen extraction by the tissues) and hyperkalemic (high $[K^+]$ due to K^+ loss from

working muscles) (Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977). As expected, plasma lactate levels increased and P_{vO2} and C_{vO2} decreased during swimming. However, plasma K^+ was highly variable, decreasing during swim 1, and increasing during swim 2. Moreover, plasma K^+ was significantly higher in the arterial relative to the venous blood. The difference in [K+] between the arterial and venous blood may be attributed to the effect of pH and haemoglobin-oxygen saturation on K^+ movement across red blood cells. Specifically, red blood cells take up K^+ when blood pH and haemoglobin-oxygen saturation is low and lose K^+ when pH and haemoglobin-oxygen saturation is high (Nielsen and Lykkeboe, 1992b).

The elevated $\dot{M}O_2$ following an anaerobic swim challenge is termed the excess post oxygen consumption (EPOC). EPOC represents the $\dot{M}O_2$ cost to restore oxygen stores, high energy phosphates and glycogen and reverse biochemical, ionic and osmotic imbalances (Gaesser and Brooks, 1984; Scarabello et al., 1992). An extended EPOC and prolonged rate of recovery could be detrimental to migrating sockeye salmon since they must migrate upstream in a timely manner. However, as described above, sockeye salmon from all four upriver populations were able to repeat their swim performance after a 45-min recovery and $\dot{M}O_2$ had returned to resting revels by 2 h after the second swim challenge, which is a comparable timeframe to an earlier study for sockeye salmon (Lee et al., 2003b).

Complete recovery of cardiovascular and metabolic indicators was not required in order for the fish to repeat their U_{crit} swim performance. The same $\dot{Q}_{max}$, V_{smax}, f_{Hmax}, and T_{aO2} were obtained during swim 2 as compared with swim 1, even though $\dot{M}O_2$, $\dot{Q}$, f_H, and plasma lactate remained significantly elevated and C_{vO2} remained significant depressed at the outset of swim 2.

There are several potential explanations for this. Naïve fish may swim inefficiently or prematurely quit swimming during the first swim, but improve during the second attempt. The

increased scope for $\dot{Q}$ and V_s during the second swim in Quesnel sockeye salmon may be attributed to these types of behavioural differences between swims.

The consistent repeat swim performance was not due to a larger anaerobic contribution during swim 2 relative to swim 1 since lactate did not accumulate in the plasma. In fact, plasma lactate was highest during the 45-min recovery period following swim 1. A similar finding was reported for sockeye salmon swum twice and even three times (Farrell et al., 1998, Jain et al., 1998). In the present study, plasma lactate levels were always less than 10-13 mmol l^{-1}, which is the proposed threshold above which sockeye salmon and rainbow trout cannot repeat their swim performance (Farrell et al., 1998; Jain and Farrell, 2003; Stevens and Black, 1966).

Training effects may physiologically allow fish to improve or maintain swim performance during the second swim, despite incomplete metabolic recovery. For example, faster recovery rates for lactate, creatine phosphate and respiratory gases during a second exhaustive burst swim test were suggested to be training effects (Scarabello et al., 1992). Notably, plasma lactate decreased between steady and burst swimming during swim 2, which suggests that lactate may have been used as a fuel or metabolically cleared during the lower speeds of the second swim. Indeed, light swimming has been demonstrated to accelerate recovery ability in rainbow trout (Milligan et al., 2000).

More efficient swimming during the second swim (lower COT) could allow for repeat swim performance (Farrell et al., 1998). However, this was not the case in the current study since COT did not differ between swims. In fact, COT-$\dot{Q}$ was consistently, though not significantly, higher during the second swim, which may have assisted metabolic recovery. Notably, neither COT nor COT-$\dot{Q}$ displayed the classic U-shaped curve with speed (e.g. Hoyt and Taylor, 1981; Lee et al., 2003c; Prange, 1976; Prange and Schmidt-Nielsen, 1970; Wakeman and Wohlschlag,

1982). Instead, both plateaued at speeds higher than ~1 bl s^{-1}. Thus, upriver sockeye salmon maintained their swimming efficiency across the entire range of swim speeds. While COT_{net} and COT-$\dot{Q}_{net}$ steadily increased with higher swim speeds, both plateaued once the fish transitioned to burst swimming, providing further evidence that high velocity swimming was fuelled by anaerobic metabolism.

An important consideration in this study system is that adult sockeye salmon must 'multi-task' during their upriver migration. Namely, while swimming almost continually upstream to their spawning grounds, sockeye salmon must also undergo sexual maturation (grow their gonads and develop secondary sexual characteristics). Thus, sockeye salmon may divert blood away from the gonads and to the muscle when swimming at high speeds, and blood flow distribution may vary across sequential swims. This idea is supported by the observation that gut blood flow in digesting salmon decreases with increased swimming speeds (Thorarensen et al., 1993). These ideas should be tested experimentally.

4.3.5 Summary and General Trends in Oxygen Convection with Swimming

In summary, comprehensive studies that have directly measured all the cardiovascular and oxygen transport variables in the Fick equation for vascular perfusion ($\dot{V}O_2 = \dot{Q} \times A\text{-}V_{O2}$) are rare in swimming fish (e.g. Steinhausen et al., 2008). Most studies have estimated $\dot{Q}$ from the Fick equation (e.g. Kiceniuk and Jones, 1977). All the variables were measured here for four populations of sockeye salmon swimming at T_{opt} to test two hypotheses. As hypothesized, all four populations had similar cardiorespiratory and swimming performance, though Nechako sockeye salmon relied more on V_s than f_H to achieve the same $\dot{Q}$. I similarly found support for the

hypothesis that all four populations can repeat their swim performance following a brief 45-min recovery and it appears that sockeye salmon are able to recover while swimming aerobically at intermediate speeds.

In addition, a clear picture emerged for the quantitative changes in the various cardiorespiratory components during swimming. The 5-fold increase in $\dot{V}O_2$ came about primarily though a 3-fold increase in $\dot{Q}$, which was driven by a 2-fold increase in V_s. f_H and A-V_{O2} both increased ~1.5-fold. Though V_s returned to rest by the final 2-h recovery, f_H remained elevated at maximal levels and as a result, $\dot{Q}$ remained elevated by ~1.5-fold. However, $\dot{V}O_2$ did return back to resting levels since A-V_{O2} actually decreased by ~50% at the 2-h recovery. During swimming, T_{aO2} met the tissue oxygen demand entirely through an increase in $\dot{Q}$. T_{vO2} maintained a constant oxygen delivery to the spongy myocardium and gills throughout the swim tests and recovery since increases in $\dot{Q}$ offset decreases in C_{vO2}.

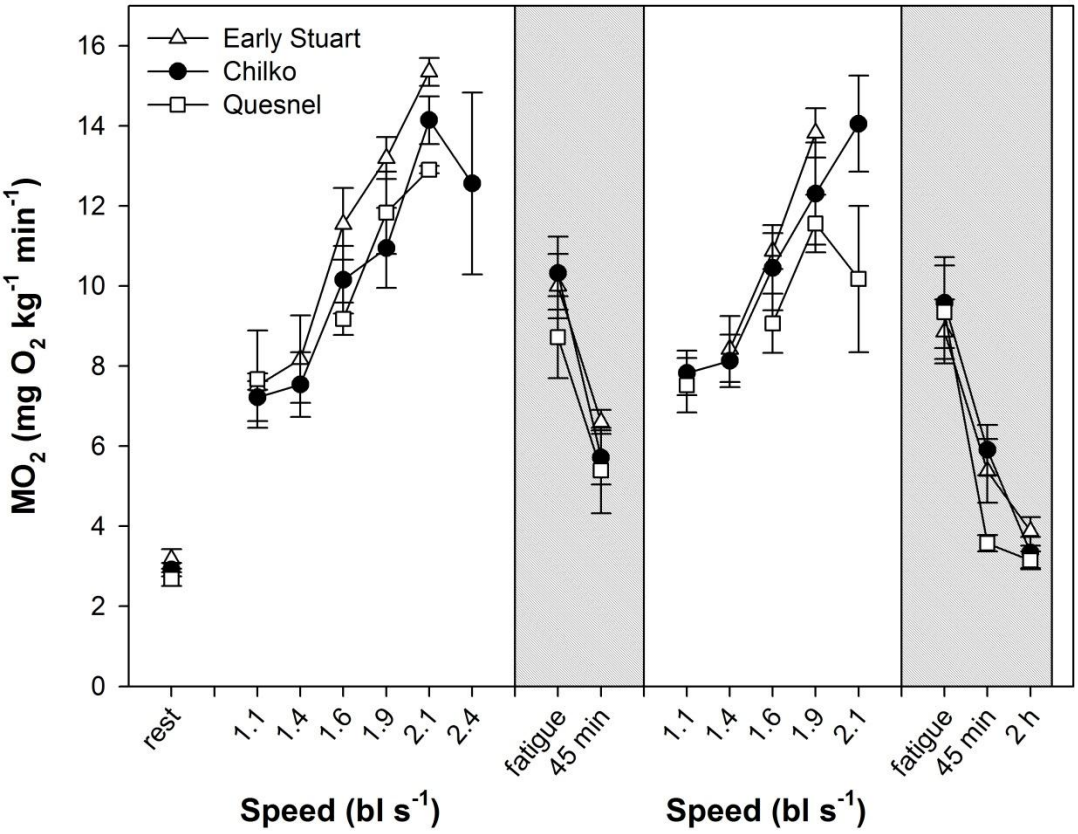

Figure 4.1. Oxygen consumption ($\dot{M}O_2$) with swimming speed over two consecutive swim challenges in three populations of sockeye salmon. There were no significant differences among populations or between sexes. Shaded areas indicate the recovery periods, starting with the fatigue value collected immediately following the U_{crit} test.

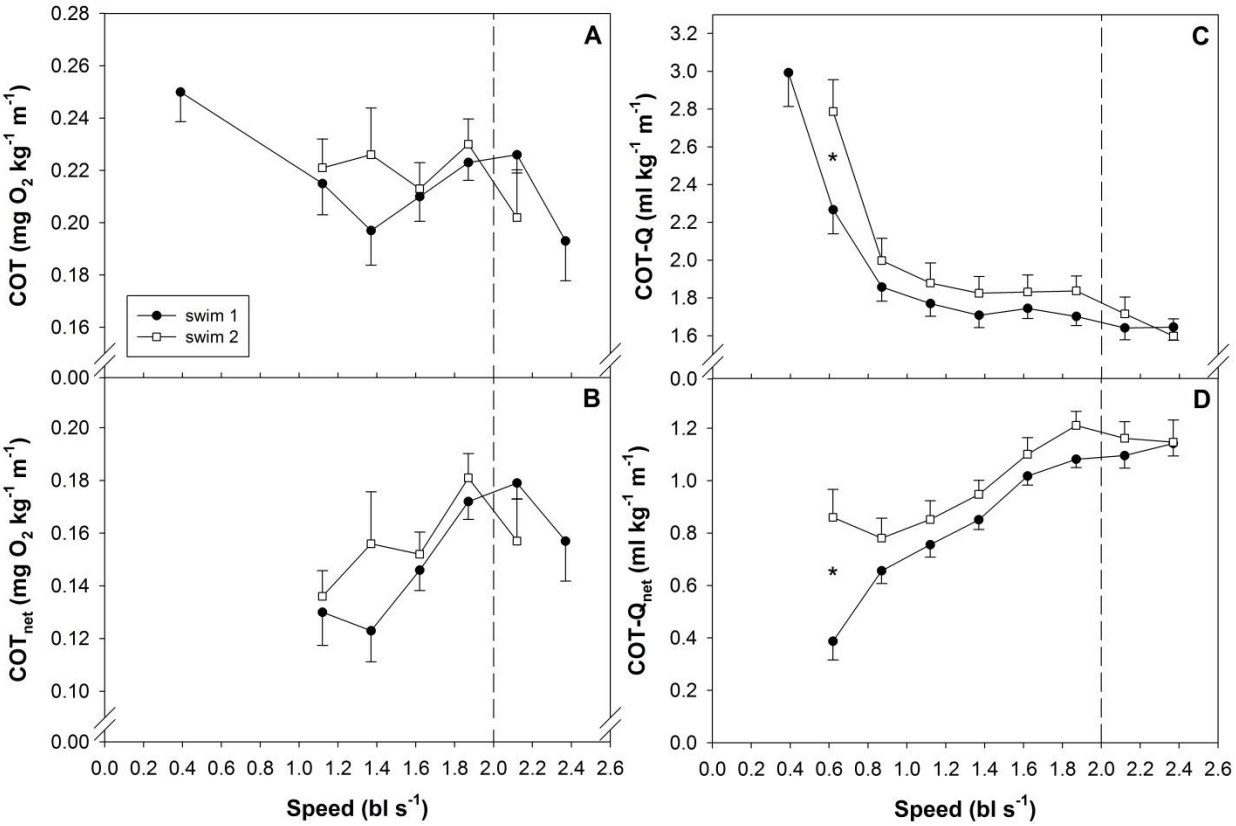

Figure 4.2. (A) Cost of transport (COT), (B) net cost of transport (COT_{net}), (C) cardiovascular cost of transport (COT-$\dot{Q}$) and (D) net cardiovascular cost of transport (COT-$\dot{Q}_{net}$) over two consecutive swim challenges. Since there were no significant differences in $\dot{M}O_2$ or $\dot{Q}$ among populations, all populations were combined. Dashed line indicates typical swim speed at which the fish transitioned from steady swimming to burst swimming. Mean ± SEM are presented. There were no significant differences between swim 1 and swim 2 in COT or COT_{net}. Significant differences between swims in COT-$\dot{Q}$ and COT-$\dot{Q}_{net}$ are indicated by an asterisk.

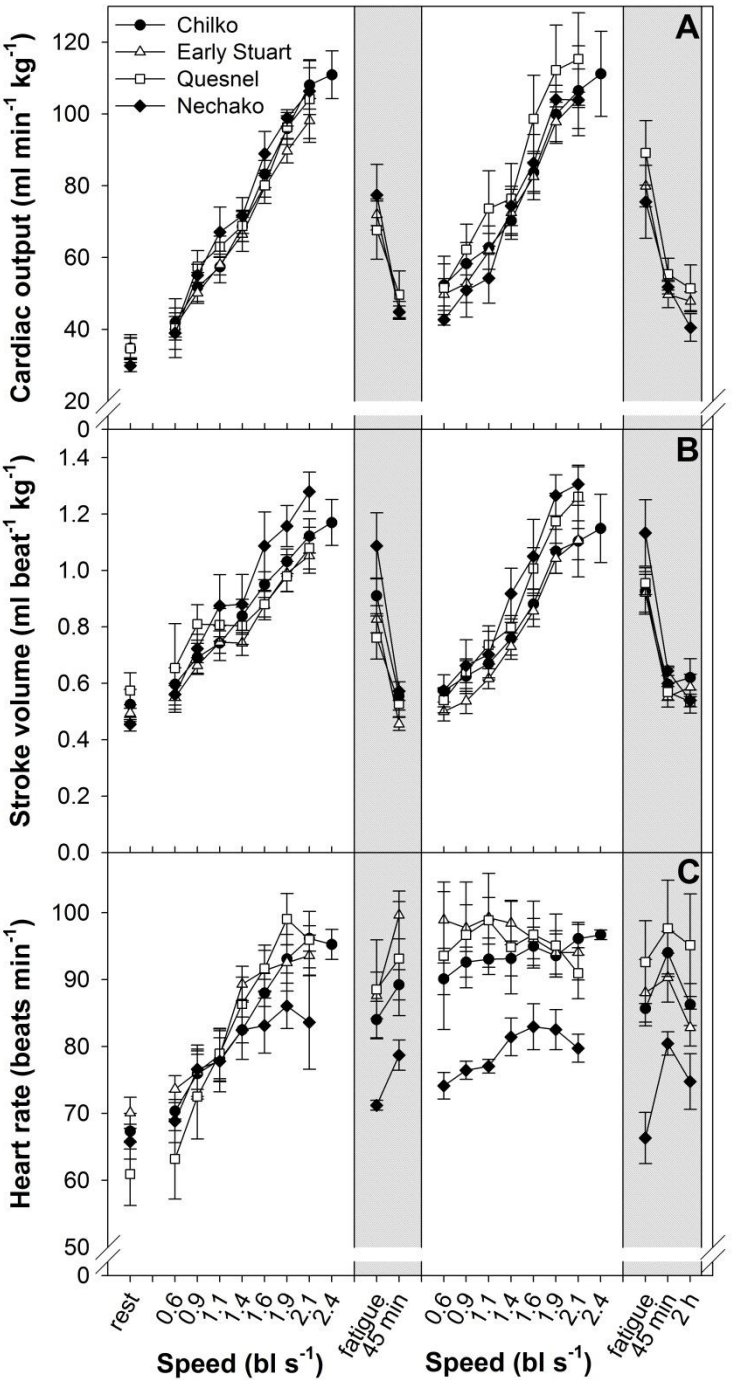

Figure 4.3. (A) Cardiac output, (B) stroke volume and (C) heart rate with swimming speed over two consecutive swim challenges in four populations of sockeye salmon. There were no significant differences in cardiac output or stroke volume among populations or between sexes. Heart rate was significantly lower in Nechako sockeye salmon compared to some of the other populations during the first three speeds of the second swim, see text for details.

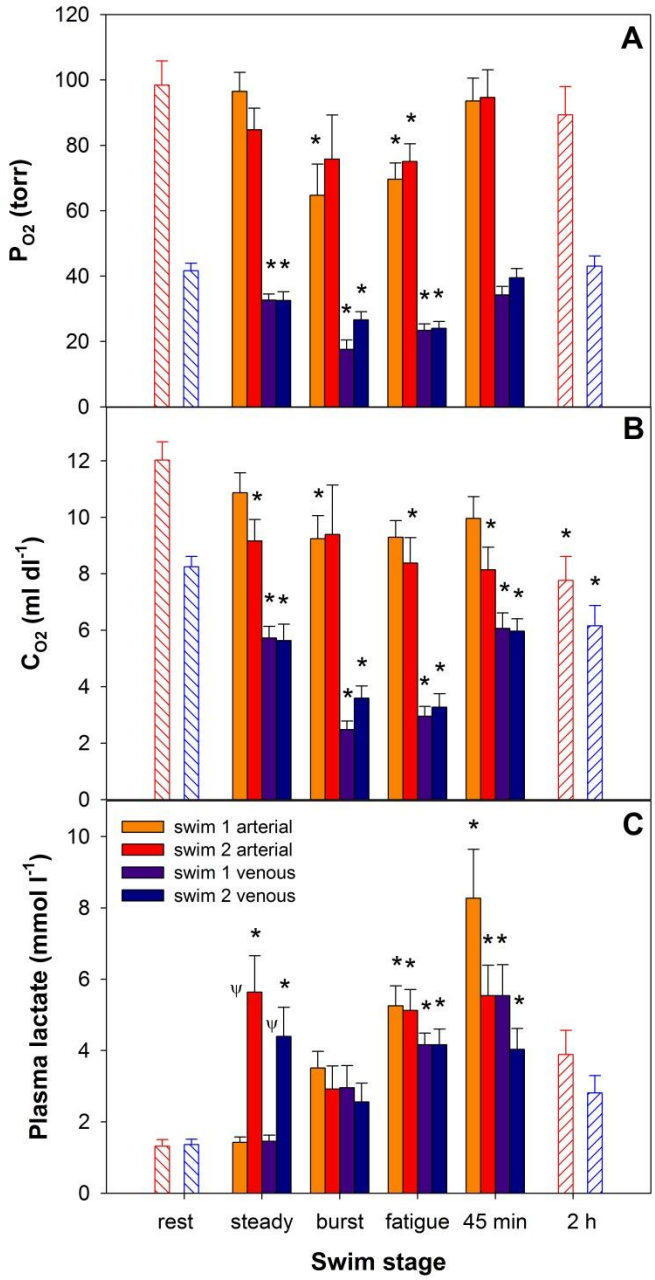

Figure 4.4. Arterial and venous (A) partial pressure of oxygen (P_{O2}), (B) oxygen content (C_{O2}) and (C) plasma lactate levels in Early Stuart, Chilko and Quesnel populations combined, over two consecutive swim challenges. Mean ± SEM are presented, there were no significant differences between sexes. Significant differences from rest are indicated by an asterisk (*), significant differences between swims are indicated by the symbol (ψ). Lactate did not significantly differ between arterial and venous blood samples. P_{O2} and C_{O2} significantly differed between arterial and venous blood samples at every time point, except C_{O2} did not differ at 2 h recovery.

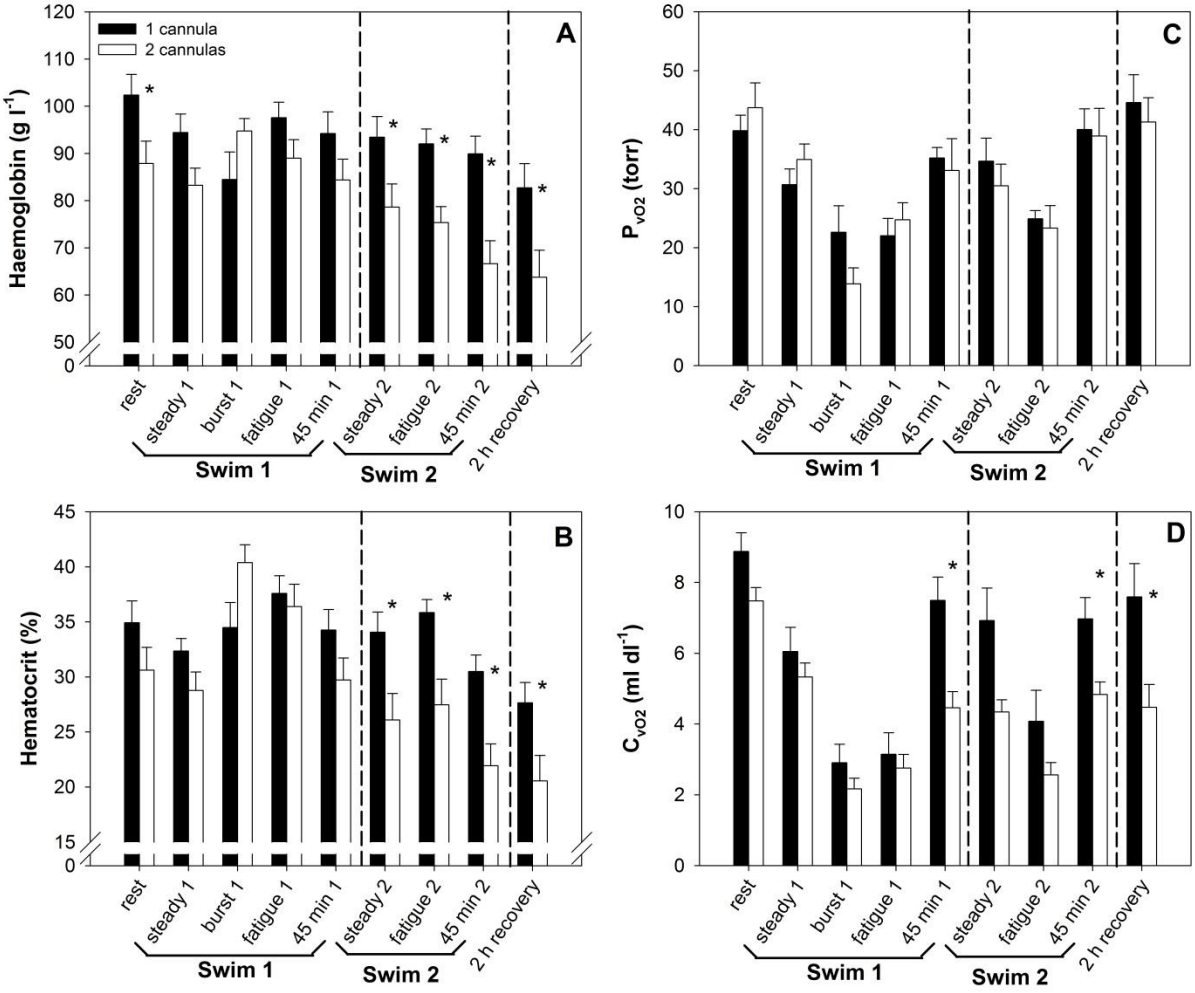

Figure 4.5. Venous (A) haemoglobin, (B) hematocrit, (C) partial pressure of oxygen and (D) oxygen content in fish with both the arterial and venous cannulae operational (2 cannulae, n = 9) and fish with only the venous cannula functioning (1 cannula, n = 11), over two consecutive swim challenges. Mean ± SEM are presented, significant differences between fish with 2 cannulae and those with 1 cannula working are indicated by an asterisk (*). Note that there were insufficient blood samples during burst swimming in the second swim to compare between groups.

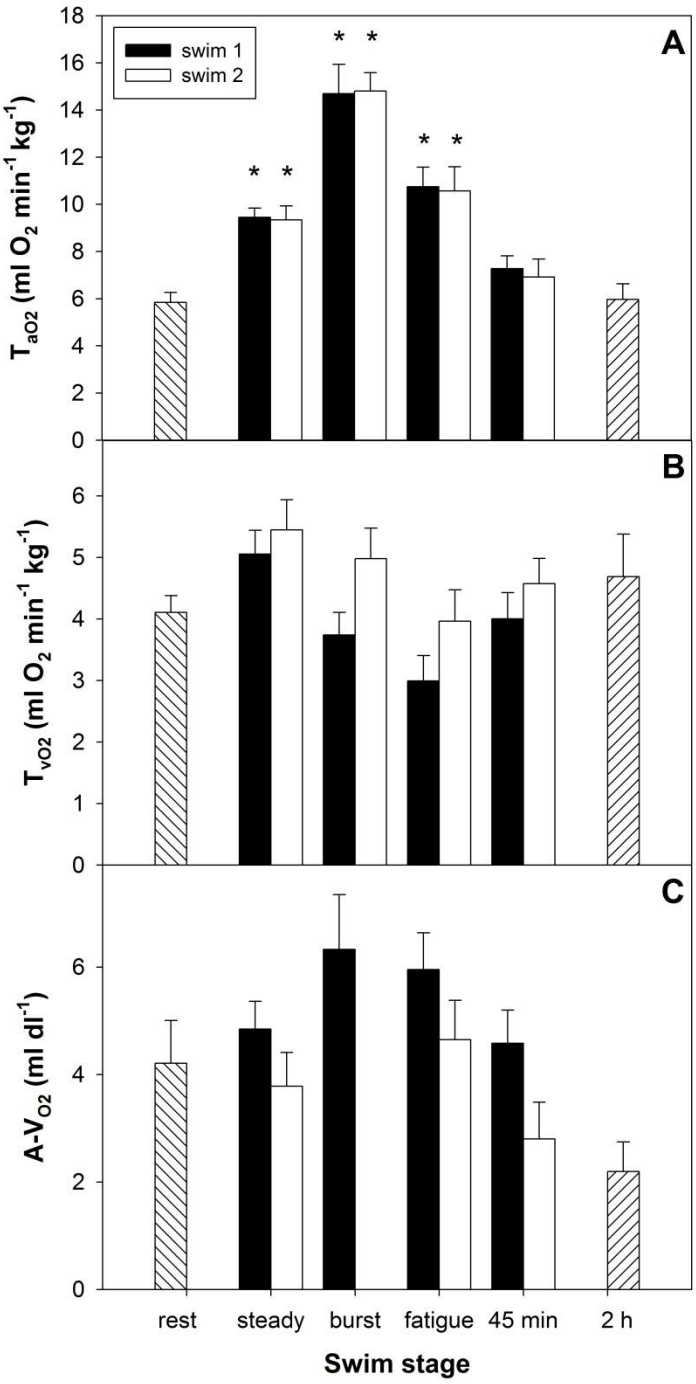

Figure 4.6. (A) Arterial oxygen transport (T_{aO2}), (B) venous oxygen transport (T_{vO2}) and (C) tissue oxygen extraction (A-V_{O2}) in Early Stuart, Chilko and Quesnel populations combined, over two consecutive swim challenges. Measurements were made at rest, during steady swimming (steady), during burst swimming (burst) immediately after the fish quit swimming (fatigue), 45 min after the fatigue (45 min) and 2 h after the conclusion of the second swim test (2 h). Mean ± SEM are presented, significant differences from rest are indicated by an asterisk (*), there were no significant differences between swim 1 and swim 2 or between sexes.

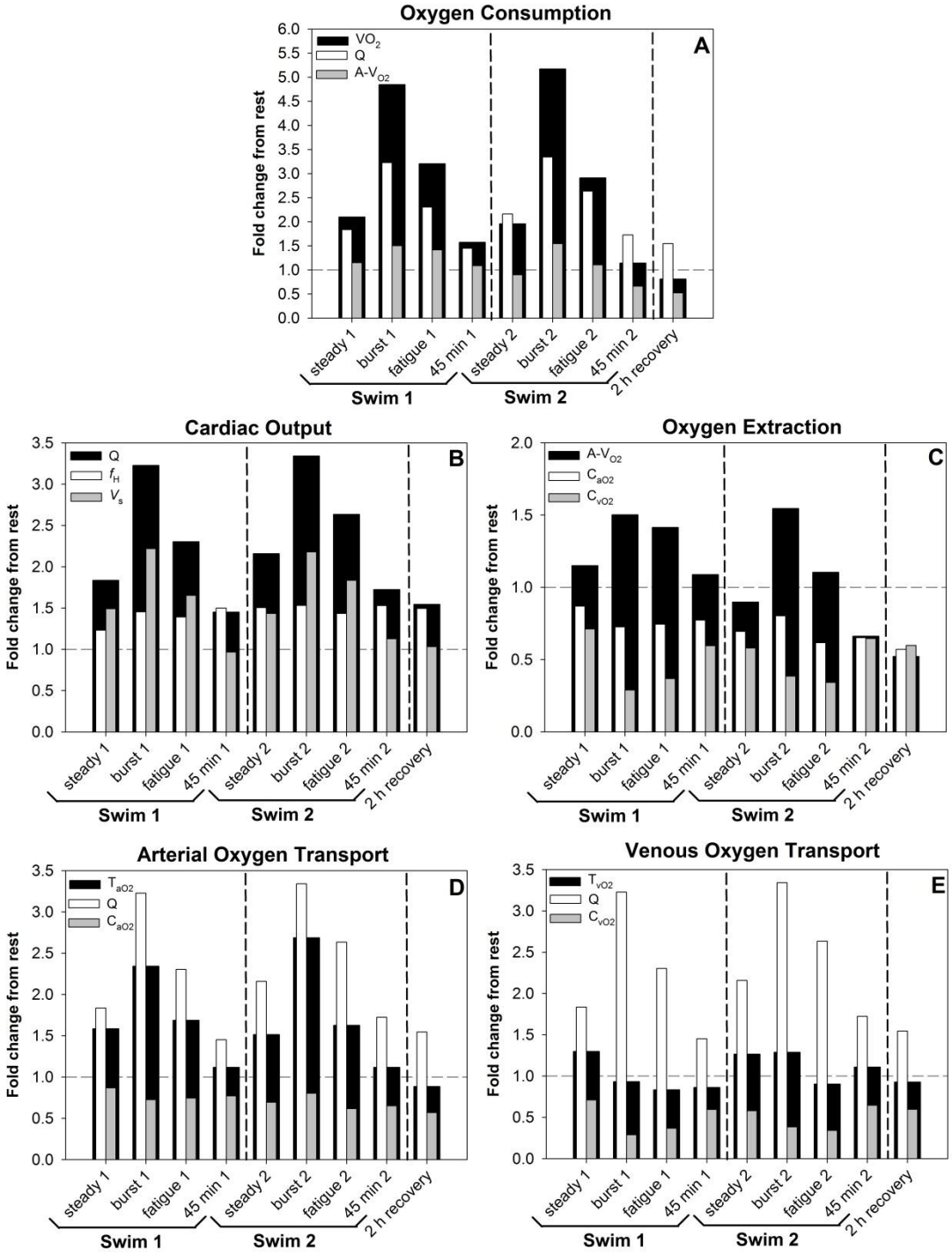

Figure 4.7. Fold changes from rest for (A) oxygen consumption ($VO_2 = \dot{Q} \times A\text{-}V_{O2}$), (B) cardiac output ($\dot{Q} = f_H \times V_s$), (C) tissue oxygen extraction ($A\text{-}V_{O2} = C_{aO2} - C_{vO2}$), (D) arterial oxygen delivery ($T_{aO2} = \dot{Q} \times C_{aO2}$) and (E) venous oxygen transport ($T_{vO2} = \dot{Q} \times C_{vO2}$). f_H = heart rate, V_s = stroke volume, C_{aO2} = arterial oxygen content, C_{vO2} = venous oxygen content. Only fish from Early Stuart, Chilko and Quesnel with both cannulae working were included in this analysis.

Table 4.1. Measurements of critical swimming speed (U_{crit}) and the recovery ratio (RR) in four populations of sockeye salmon at their T_{opt}. Mean ± SEM are presented, there were no significant differences in U_{crit} between sexes, among populations or between swim 1 and swim 2 ($p > 0.05$).

		U_{crit} (bl s^{-1})		U_{crit} (cm s^{-1})		
Population	n	U_{crit} 1	U_{crit} 2	U_{crit} 1	U_{crit} 2	RR
Early Stuart	8-9	2.02 ± 0.06	1.91 ± 0.06	122.1 ± 4.1	114.4 ± 3.5	0.95 ± 0.02
Chilko	9-13	1.99 ± 0.08	1.95 ± 0.09	117.8 ± 3.8	114.2 ± 4.9	0.97 ± 0.03
Quesnel	6	2.02 ± 0.11	1.98 ± 0.04	121.1 ± 6.3	118.7 ± 3.2	0.99 ± 0.05
Nechako	3-4	1.94 ± 0.11	1.96 ± 0.06	111.0 ± 5.6	114.4 ± 2.5	1.07 ± 0.06

Table 4.2. Recovery measurements for oxygen consumption ($\dot{M}O_2$), cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_s) in four sockeye salmon populations after two consecutive U_{crit} swim challenges. Measurements were made at rest, immediately after the fish quit swimming (fatigue), 45 min after fatigue (45-min recovery) and 2 h after the conclusion of the second swim test (2-h recovery). Mean ± SEM are presented. There were no significant differences between sexes. Significant differences from rest are indicated by an asterisk (*), significant differences between swim 1 and swim 2 are indicated by a dagger (‡) and significant differences among populations are indicated by differing letters.

	n	Rest	Fatigue 1	Fatigue 2	45-min recovery 1	45-min recovery 2	2-h recovery 2
$\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1})							
Early Stuart	5-8	3.2 ± 0.2	10.0 ± 0.8*	8.9 ± 0.8*	6.6 ± 0.3*	5.4 ± 0.8*	3.9 ± 0.4
Chilko	8-13	2.9 ± 0.2	10.3 ± 0.9*	9.6 ± 1.1*	5.7 ± 0.7*	5.9 ± 0.6*	3.3 ± 0.4
Quesnel	5-6	2.7 ± 0.2	8.7 ± 1.0*	9.3 ± 1.2*	5.4 ± 1.1*	3.6 ± 0.2*	3.1 ± 0.2
$\dot{Q}$ (ml min^{-1} kg^{-1})							
Early Stuart	7-9	34.8 ± 2.7	72.0 ± 4.3*	79.9 ± 5.8*	45.3 ± 2.5*	49.7 ± 3.7*	47.9 ± 2.7*
Chilko	9-13	34.8 ± 2.9	77.0 ± 6.4*	80.0 ± 8.2*	49.8 ± 4.9*	56.2 ± 5.9*	53.6 ± 6.2*
Quesnel	5-6	34.7 ± 3.9	67.6 ± 8.1*	89.2 ± 9.0*‡	49.6 ± 6.6*	55.4 ± 4.4*	51.4 ± 6.5*
Nechako	3-4	29.9 ± 1.7	77.4 ± 8.5*	75.5 ± 10.2*	44.9 ± 1.6*	51.8 ± 1.9*	40.5 ± 3.8
V_s (ml $beat^{-1}$ kg^{-1})							
Early Stuart	7-9	0.49 ± 0.03	0.83 ± 0.05*	0.92 ± 0.07*	0.46 ± 0.02	0.55 ± 0.04	0.59 ± 0.05
Chilko	9-13	0.53 ± 0.05	0.91 ± 0.06*	0.93 ± 0.08*	0.56 ± 0.05	0.60 ± 0.06	0.62 ± 0.07
Quesnel	5-6	0.57 ± 0.06	0.76 ± 0.08	0.96 ± 0.04*	0.53 ± 0.05	0.57 ± 0.03	0.54 ± 0.04
Nechako	3-4	0.46 ± 0.02	1.09 ± 0.12*	1.13 ± 0.12*	0.57 ± 0.03	0.64 ± 0.02	0.54 ± 0.02
f_H (beats min^{-1})							
Early Stuart	7-9	70.1 ± 2.3	87.6 ± 3.5*	88.0 ± 5.0^{ab}*	99.6 ± 3.6*	90.3 ± 3.7*	82.8 ± 2.8*
Chilko	9-13	67.3 ± 2.7	84.0 ± 2.7*	85.7 ± 2.0^{ab}*	89.2 ± 2.3*	94.0 ± 3.2*	86.3 ± 3.1*
Quesnel	5-6	60.9 ± 4.7	88.5 ± 7.4*	92.6 ± 6.2^{a}*	93.1 ± 8.5*	97.7 ± 7.2*	95.1 ± 7.7*
Nechako	3-4	65.8 ± 2.6	71.2 ± 0.7	66.3 ± 3.8^{b}	78.7 ± 2.3	80.4 ± 1.7	74.8 ± 4.2

Table 4.3. Maximum measurements for cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_s) in four sockeye salmon populations taken over two U_{crit} swim challenges. Scope is the difference between maximum and resting values for each individual fish. Mean ± SEM are presented. There were no significant differences between sexes. Significant differences between swim 1 and swim 2 are indicated by a dagger (‡) and significant differences among populations are indicated by differing letters.

	n	Max 1	Max 2	Scope 1	Scope 2
$\dot{Q}$ *(ml min^{-1} kg^{-1})*					
Early Stuart	8-9	100.3 ± 5.2	104.1 ± 6.4	65.5 ± 4.7	68.2 ± 5.3
Chilko	9-13	105.0 ± 4.9	103.2 ± 9.3	70.2 ± 3.2	68.0 ± 6.5
Quesnel	5-6	101.9 ± 9.2	117.7 ± 12.1‡	67.2 ± 6.6	83.6 ± 7.6‡
Nechako	3-4	107.3 ± 6.7	104.5 ± 1.9	77.4 ± 7.0	74.1 ± 1.2
V_s *(ml beat^{-1} kg^{-1})*					
Early Stuart	8-9	1.08 ± 0.05	1.10 ± 0.06	0.58 ± 0.04^{ab}	0.60 ± 0.04
Chilko	9-13	1.11 ± 0.05	1.12 ± 0.09	0.59 ± 0.04^{ab}	0.57 ± 0.07
Quesnel	5-6	1.09 ± 0.10	1.28 ± 0.11	0.52 ± 0.07^{a}	0.69 ± 0.04‡
Nechako	3-4	1.25 ± 0.07	1.29 ± 0.07	0.80 ± 0.07^{b}	0.82 ± 0.06
f_H *(beats min^{-1})*					
Early Stuart	8-9	93.1 ± 2.2	94.6 ± 2.8	23.0 ± 3.7	23.5 ± 3.3
Chilko	9-13	94.4 ± 1.9	92.0 ± 3.2	27.1 ± 3.5	27.4 ± 5.4
Quesnel	5-6	94.0 ± 3.6	91.3 ± 3.6	33.1 ± 3.6	34.6 ± 2.2
Nechako	3-4	85.9 ± 3.9	81.3 ± 3.0	20.1 ± 4.3	17.5 ± 5.0

Table 4.4. Haematological variables from Early Stuart, Chilko and Quesnel populations combined over two consecutive swim challenges. Arterial and venous blood samples were taken at rest, during steady state swimming (steady) and burst-and-coast swimming (burst), immediately after the fish quit swimming (fatigue), 45 min after the fatigue (45 min) and 2 h after the conclusion of the second swim test (2 h rec). Haemoglobin concentration (Hb), hematocrit (Hct) and mean cell haemoglobin concentration (MCHC) are indicated. Mean ± SEM are presented. There were no significant differences between sexes. Significant differences from rest are indicated by an asterisk (*), significant differences between swim 1 and swim 2 are indicated by a dagger (‡) and significant differences between arterial and venous blood are indicated by differing letters.

		n	Hb (g l^{-1})	Hct (%)	MCHC (g l^{-1})	Glucose (mmol l^{-1})	Chloride (mmol l^{-1})	Sodium (mmol l^{-1})	Potassium (mmol l^{-1})
rest	*arterial*	12	92.4 ± 4.6	31.0 ± 1.7	300.5 ± 6.6	5.6 ± 0.5	127.5 ± 0.9	140.1 ± 1.7	4.9 ± 0.4[a]
	venous	19-20	95.9 ± 3.5	33.0 ± 1.5	293.7 ± 6.0	5.3 ± 0.4	128.2 ± 1.5	142.4 ± 1.7	3.4 ± 0.2[b]
steady 1	*arterial*	13-14	90.3 ± 4.2	30.4 ± 1.6	299.4 ± 6.8	5.1 ± 0.5	129.9 ± 1.4	143.8 ± 1.6	4.6 ± 0.4‡[a]
	venous	18-20	89.4 ± 2.9	30.7 ± 1.0	292.2 ± 5.9	5.1 ± 0.4	130.3 ± 1.3	146.7 ± 1.7	3.2 ± 0.3‡[b]
steady 2	*arterial*	12	81.3 ± 4.7	26.1 ± 1.9	315.7 ± 9.4[a]	5.5 ± 0.5	127.3 ± 1.8	140.5 ± 2.1	8.1 ± 0.6*‡[a]
	venous	15-16	86.0 ± 3.7*	30.1 ± 1.8	291.8 ± 7.7[b]	5.6 ± 0.4	127.4 ± 1.5	144.9 ± 1.9	4.3 ± 0.4‡[b]
burst 1	*arterial*	6	91.8 ± 5.4	33.9 ± 2.5‡	273.1 ± 8.1‡[a]	6.6 ± 0.5	130.2 ± 1.3	145.9 ± 2.1	2.7 ± 0.7*‡
	venous	7	90.4 ± 3.3	37.9 ± 1.7‡	239.4 ± 3.2*‡[b]	6.0 ± 0.7	126.8 ± 1.8	142.1 ± 2.4	2.8 ± 0.9
burst 2	*arterial*	3	86.5 ± 12.9	26.5 ± 4.7‡	329.1 ± 9.7‡[a]	6.5 ± 0.9	129.9 ± 4.9	139.8 ± 4.2	6.1 ± 1.5‡
	venous	8	84.8 ± 4.4*	29.4 ± 1.8‡	290.7 ± 6.0‡[b]	5.1 ± 0.4	126.1 ± 2.4	141.3 ± 2.5	3.5 ± 0.5
fatigue 1	*arterial*	11	87.3 ± 3.0‡	31.1 ± 1.4‡[a]	282.3 ± 4.9[a]	6.2 ± 0.6	133.3 ± 1.2*	152.9 ± 2.0*	2.6 ± 0.3*‡[a]
	venous	15-16	93.3 ± 2.7	37.0 ± 1.3‡[b]	253.8 ± 5.5*[b]	6.3 ± 0.4*	131.7 ± 1.3	152.1 ± 2.1*	1.8 ± 0.3*[b]
fatigue 2	*arterial*	11	79.3 ± 5.0*‡	26.8 ± 2.1‡	301.0 ± 7.7[a]	6.4 ± 0.7	130.0 ± 2.0	146.1 ± 2.2	4.4 ± 0.4‡[a]
	venous	14-15	82.5 ± 3.2*	31.4 ± 1.7‡	272.1 ± 8.8[b]	6.1 ± 0.5	127.9 ± 1.8	147.5 ± 2.4	2.6 ± 0.4[b]
45-min 1	*arterial*	11	89.7 ± 5.5‡	30.4 ± 2.2*‡	297.7 ± 7.0	5.7 ± 0.5	131.0 ± 1.6	148.6 ± 2.2	6.2 ± 0.6[a]
	venous	15-17	89.6 ± 3.3‡	32.1 ± 1.4‡	281.4 ± 6.2	5.4 ± 0.4	130.0 ± 1.3	147.0 ± 2.0	3.5 ± 0.3[b]
45-min 2	*arterial*	10	72.4 ± 5.7*‡	23.7 ± 2.0‡	307.2 ± 4.0	6.3 ± 0.9	129.3 ± 1.9	144.8 ± 1.7	6.4 ± 0.5[a]
	venous	14-17	79.0 ± 4.1*‡	26.5 ± 1.6*‡	302.3 ± 7.2	6.0 ± 0.6	127.6 ± 1.5	144.2 ± 2.3	4.1 ± 0.3[b]
2-h rec	*arterial*	8-9	72.2 ± 6.2*	22.0 ± 2.2*	334.7 ± 12.4*[a]	6.7 ± 0.9	127.3 ± 2.4	144.2 ± 2.2	5.9 ± 1.0[a]
	venous	11-13	74.0 ± 4.6*	24.4 ± 1.7*	306.9 ± 5.4[b]	6.1 ± 0.7	126.5 ± 1.2	143.8 ± 1.9	3.5 ± 0.3[b]

Table 4.5. Haematological variables from Early Stuart, Chilko and Quesnel populations combined over two consecutive swim challenges. Only fish with paired arterial and venous cannulae were included. Haemoglobin concentration (Hb), hematocrit (Hct) and mean cell haemoglobin concentration (MCHC) are indicated. Mean ± SEM are presented. Significant differences between arterial and venous blood are indicated by differing letters.

		n	Hb (g l^{-1})	Hct (%)	MCHC (g l^{-1})	Potassium (mmol l^{-1})
rest	*arterial*	9	89.5 ± 5.1	30.3 ± 2.1	298.0 ± 8.1	4.4 ± 0.4[a]
	venous	9	87.9 ± 4.7	30.6 ± 2.1	290.6 ± 9.6	3.3 ± 0.4[b]
steady 1	*arterial*	9	88.5 ± 4.1[a]	29.9 ± 1.6	297.7 ± 8.1	4.0 ± 0.3[a]
	venous	9	83.3 ± 3.6[b]	28.8 ± 1.7	292.2 ± 8.7	2.7 ± 0.4[b]
steady 2	*arterial*	8	79.0 ± 5.2	25.4 ± 2.3	316.9 ± 13.8	7.6 ± 0.7[a]
	venous	8	78.7 ± 4.9	26.1 ± 2.4	308.3 ± 11.8	4.1 ± 0.2[b]
burst 1	*arterial*	4	95.2 ± 2.2	36.6 ± 1.0[a]	260.4 ± 1.4[a]	1.6 ± 0.2
	venous	4	94.8 ± 2.6	40.4 ± 1.6[b]	235.2 ± 4.8[b]	1.0 ± 0.2
fatigue 1	*arterial*	8	88.9 ± 3.5	32.0 ± 1.6[a]	278.5 ± 4.8[a]	2.4 ± 0.3
	venous	8	89.0 ± 3.9	36.4 ± 2.0[b]	246.3 ± 6.0[b]	1.8 ± 0.5
fatigue 2	*arterial*	8	75.2 ± 5.0	25.1 ± 2.3[a]	304.9 ± 9.7[a]	3.9 ± 0.4[a]
	venous	8	75.4 ± 3.4	27.5 ± 2.3[b]	281.3 ± 12.7[b]	2.0 ± 0.3[b]
45-min 1	*arterial*	8	86.2 ± 5.5	28.9 ± 2.2	300.8 ± 9.3[a]	6.5 ± 0.7[a]
	venous	8	84.4 ± 4.4	29.7 ± 2.0	286.3 ± 7.6[b]	3.2 ± 0.4[b]
45-min 2	*arterial*	8	66.3 ± 4.4	21.6 ± 1.6	308.5 ± 4.9	5.7 ± 0.4[a]
	venous	8	66.7 ± 4.8	21.9 ± 2.0	308.4 ± 11.0	3.9 ± 0.4[b]
2-h rec	*arterial*	6	65.2 ± 7.2	20.1 ± 2.8	333.8 ± 19.0	4.1 ± 0.3[a]
	venous	6	63.8 ± 5.7	20.6 ± 2.3	315.1 ± 9.5	2.9 ± 0.3[b]

CHAPTER 5: CARDIORESPIRATORY COLLAPSE AT HIGH TEMPERATURE IN SOCKEYE SALMON

5.1 Introduction

Chapter 3 provided convincing evidence for a decline in aerobic scope and swim performance outside T_{opt} in every sockeye salmon population examined and confirmed the previously held notion that aerobic and cardiac scopes are closely linked (e.g. Brett, 1971; Farrell et al., 2009). The purpose of this chapter is to examine the mechanism of the decline in aerobic scope above T_{opt} in sockeye salmon.

Temperature has been coined the "ecological master factor" because of its role in biochemistry, physiology, behaviour and ecology (Fry, 1971). As previously discussed, all fish have an optimum temperature (T_{opt}) for performance, outside of which whole animal performance declines until eventually death occurs. The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis attributes the decline in aerobic scope above an animal's optimum temperature to capacity limitations of the organ systems that deliver oxygen to the tissues (Pörtner, 2001; Pörtner and Knust, 2007). Any one of the steps in the oxygen cascade (from the environment to the mitochondria) could become problematic outside the T_{opt} window. Among these steps include the capacity for oxygen delivery to the gills, oxygen diffusion across the gills, oxygen transport via the blood, oxygen extraction by the tissues, and oxygen use by the mitochondria (Weibel, 1984). Thus, a limitation could occur at the organ level of the heart, the gills, or the muscle (Brett, 1971; Farrell, 1997; Farrell, 2002; Farrell, 2007; Farrell, 2009; Farrell

et al., 2009; Heath and Hughes, 1973; Pörtner, 2002; Steinhausen et al., 2008; Taylor et al., 1997), though evidence supporting a limitation at any single site is incomplete.

I used the OCLTT hypothesis as a framework to examine cardiorespiratory collapse at high temperature in sockeye salmon. I hypothesized that the limitation on aerobic performance above T_{opt} is initiated by an oxygen limitation at the level of the heart.

It is possible to examine most of the critical steps in the oxygen cascade by simultaneously measuring $\dot{M}O_2$, $\dot{Q}$, and oxygen status [partial pressure (P_{O2}) and content (C_{O2})] in arterial and venous blood as a function of increasing temperature. For example, if there was a limitation in delivering oxygen to the gill or oxygen diffusion at the gill, I would expect P_{aO2} and C_{aO2} to decrease above T_{opt}. Alternatively, a limitation at the level of the heart would be apparent by a plateau or decrease in $\dot{Q}_{max}$ at temperatures above T_{opt}, Finally, if sufficient blood was delivered to the working muscles, but a limitation in oxygen diffusion to mitochondria was present, P_{vO2} would remain constant above T_{opt}.

Most of the studies to date examining temperature effects on aerobic scope in fish have not directly measured all the required variables outlined above (e.g. Brett, 1971; Fry, 1947; Taylor et al., 1993). Some variables have been measured in resting fish acutely exposed to increasing temperatures (e.g. Clark et al., 2008b; Gollock et al., 2006; Heath and Hughes, 1973; Sartoris et al., 2003), however, only Steinhausen et al. (2008) has directly measured all these variables in fish swimming near $\dot{M}O_{2max}$. Therefore, this is the first comprehensive study to simultaneously and directly measure all the necessary variables in fish swimming at U_{crit} in order to address these mechanistic questions.

To maximize statistical power, my approach was to pool data for Early Stuart, Chilko and Quesnel sockeye salmon since they did not differ in maximum cardiorespiratory performance

(Chapter 4) and had a similar T_{opt} (~17°C, Chapter 3). I pooled the data into four temperature groups based on aerobic scope (Fig 5.1). The T_{opt} grouping (n = 33) combined data for sockeye salmon that attained 90-100% of population-specific maximum aerobic scope. The temperature range for the T_{opt} grouping was 15-20°C for all three populations. The $T_{min50\text{-}90}$ grouping (n = 8) included sockeye salmon at temperatures below T_{opt} attaining only 50-90% of maximum aerobic scope. The $T_{min50\text{-}90}$ grouping corresponded to 12°C and 9-10°C, respectively, for Early Stuart and Chilko populations and no Quesnel sockeye salmon were included. The $T_{max50\text{-}90}$ grouping (n = 11), included fish swum at temperatures above T_{opt}, and attaining 50-90% of maximum aerobic scope. This grouping typically corresponded to 22-23°C for Early Stuart and Quesnel sockeye salmon and 24-25°C for Chilko sockeye salmon. The final $T_{max0\text{-}50}$ grouping (n = 8) included fish above T_{opt} whose aerobic scope was only 0-50% of maximum. This corresponded to 23-26°C for Early Stuart and Quesnel sockeye salmon and 25-26°C for Chilko sockeye salmon. While many fish were swum twice (e.g. all fish in the T_{opt} grouping and several fish in the $T_{min50\text{-}90}$ and $T_{max50\text{-}90}$ groupings), only the first swim was compared across the four groupings. This approach avoided the potentially confounding effect of incomplete recovery from the first swim. Detailed materials and methods are found in Chapter 2 (sections 2.2-2.7 and 2.10).

5.2 Results

5.2.1 Cardiorespiratory and Swimming Performance

Resting, Swimming, Maximum & Scope

The cardiorespiratory response of the four temperature groupings with swimming are shown in Fig 5.2. The corresponding rest, maximum and scope values for each temperature grouping are shown in Fig 5.3.

As defined, aerobic scope was highest at T_{opt}. Also, aerobic scope did not significantly differ between $T_{min50\text{-}90}$ and $T_{max50\text{-}90}$, and was lowest at $T_{max0\text{-}50}$ (Fig 5.3). Therefore, the four temperature groupings created the equivalent of a Fry aerobic scope curve.

The response for aerobic scope reflected the different responses of $\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$ to temperature. As expected, $\dot{M}O_{2rest}$ increased significantly from the lowest to the highest temperature grouping (Fig 5.3). While swimming significantly increased $\dot{M}O_2$ for the $T_{min50\text{-}90}$, T_{opt} and $T_{max50\text{-}90}$ groupings, it did not for the $T_{max0\text{-}50}$ grouping (Fig 5.2). Furthermore, $\dot{M}O_{2max}$ increased significantly between $T_{min50\text{-}90}$ and T_{opt}, did not differ between T_{opt} and $T_{max50\text{-}90}$, and decreased significantly at $T_{max0\text{-}50}$ (Fig 5.3).

$\dot{Q}_{rest}$ and $\dot{Q}_{max}$ varied with temperature with similar patterns as $\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$, respectively (Fig 5.3). $\dot{Q}$ significantly increased with swimming for the $T_{min50\text{-}90}$, T_{opt} and $T_{max50\text{-}90}$ groupings, but not the $T_{max0\text{-}50}$ group (Fig 5.2). Cardiac scope at $T_{max0\text{-}50}$ was significantly lower compared to the other three temperature groupings (Fig 5.3).

The relative contribution of f_H and V_s to $\dot{Q}$ varied with temperature and with swimming. f_{Hrest} significantly increased with each temperature grouping (Fig 5.3). In contrast, V_{srest} did not change substantially among temperature groups, but was significantly lower at $T_{max0-50}$ compared with T_{opt} (Fig 5.3).

At T_{opt}, swimming increased both V_s and f_H (Fig 5.2). Both $T_{min50-90}$ and $T_{max50-90}$ groupings only increased V_s with swimming, f_H did not change significantly from rest at any swimming speed (Fig 5.2). At $T_{max0-50}$, f_H actually decreased during swimming and V_s did not change from rest (Fig 5.2). Notably, at the lowest swim speeds, V_s was similar across temperature groupings. However, differences became apparent at higher swim velocities: V_s was highest in the $T_{min50-90}$ grouping and decreased with increasing temperature groups (Fig 5.2).

f_{Hmax} significantly increased with temperature until $T_{max50-90}$ and plateaued between the two warmest groups (Fig 5.3). Scope for f_H was highest at T_{opt}, approached zero for $T_{max50-90}$ and was actually negative for $T_{max0-50}$. V_{smax} significantly decreased above $T_{min50-90}$. As a result, scope for V_s was maintained between $T_{min50-90}$ and $T_{max50-90}$ but decreased significantly at $T_{max0-50}$.

For comparison among these cardiorespiratory variables with temperature, scope is presented as a percentage of its highest value (Fig 5.4). Scope was highest at T_{opt} for $\dot{M}O_2$, $\dot{Q}$ and f_H and highest at $T_{min50-90}$ for V_s (Fig 5.4). While scope for $\dot{M}O_2$, $\dot{Q}$ and V_s decreased by 13- 25% of maximum at $T_{max50-90}$, scope for f_H plummeted by 80% of maximum at $T_{max50-90}$ and became almost -40% of maximum for f_H at $T_{max0-50}$. At $T_{max0-50}$, scope for $\dot{M}O_2$, $\dot{Q}$ and V_s declined to 16-20% of maximum.

No cardiac disrhythmias or deaths accompanied swimming at either $T_{min50-90}$ or T_{opt}. In contrast, every fish swum at $T_{max0-50}$ exhibited an irregular heart rate immediately after failing the swim test. In addition, 57% of these fish exhibited cardiac disrhythmias during swimming,

shortly before fatigue (Fig 5.5). Despite decreasing the temperature immediately following fatigue, 29% of the $T_{max0-50}$ fish died, even though they had swum at 1.1-1.5 bl s^{-1} before reaching fatigue. While 27% of $T_{max50-90}$ fish exhibited cardiac disrhythmias during swimming or fatigue, none died.

Swimming Performance

$T_{max0-50}$ fish attained a lower maximum swim speed compared to the other three groupings (Fig 5.2, 5.6), which is consistent with $\dot{M}O_2$ and $\dot{Q}$ not changing significantly during the swim test of this group.

Cost of transport (COT) at the slowest swim speed (0.4 bl s^{-1} = "rest") was ~ 2-fold higher in the $T_{max50-90}$ and $T_{max0-50}$ groups relative to the T_{opt} and $T_{min50-90}$ groups (Fig 5.6). COT decreased with increasing swimming speed and was maintained ~0.12-0.26 mg O_2 kg^{-1} m^{-1} across all groups. The $T_{max50-90}$ and $T_{max0-50}$ groups tended to have a higher COT compared to the T_{opt} and $T_{min50-90}$ groups across all speeds.

Similarly, COT-$\dot{Q}$ was significantly lower at $T_{min50-90}$ compared to the $T_{max50-90}$ and $T_{max0-50}$ groups at the slowest speed (Fig 5.6). COT-$\dot{Q}$ decreased with increasing swimming speeds until ~0.87 bl s^{-1}, after which it was maintained at around 0.7-1.9 ml kg^{-1} m^{-1} in $T_{min50-90}$, T_{opt} and $T_{max50-90}$. In contrast, COT-$\dot{Q}$ declined steadily in $T_{max0-50}$ until the fish quit swimming. Again, warmer temperature groups tended to have a higher COT-$\dot{Q}$ relative to the cooler groups across all speeds. The opposite temperature pattern was observed in COT_{net} and COT-$\dot{Q}_{net}$, both of which tended to be higher in colder groups.

5.2.2 Oxygen Transport and Removal by Tissues

Resting P_{aO2} and C_{aO2} were not significantly affected by an acute increase in temperature from 12°C (the starting temperature) to the test temperature (22-26°C) in either the $T_{max50-90}$ or $T_{max0-50}$ groupings (Table 5.1). The tendency for P_{aO2} to increase with temperature can likely be attributed to a decreased affinity for haemoglobin (right-shift in the oxyhaemoglobin dissociation curve).

P_{aO2} and C_{aO2} did not significantly differ across temperature groups at any of the swimming speeds (Table 5.2). There was an overall trend for P_{aO2} and C_{aO2} to decrease with swimming relative to rest (Table 5.2, Fig 5.7).

There were no significant differences in [Hb], Hct, or MCHC across temperature groups or with swimming, except that MCHC was significantly lower at fatigue relative to rest in the $T_{max50-90}$ grouping (Table 5.3). As a result, any changes in C_{aO2} or C_{vO2} reflected changes in Hb saturation. In fact, when C_{aO2} was divided by [Hb], there were no significant differences across temperatures or with swim speed (data not shown).

T_{aO2} was significantly lower during burst swimming and fatigue for the $T_{max0-50}$ grouping when compared with T_{opt} (Fig 5.8, Table 5.2). While the $T_{min50-90}$, T_{opt} and $T_{max50-90}$ groupings increased T_{aO2} by 294%, 153% and 80%, respectively, during burst swimming compared with rest, at $T_{max0-50}$ fish were unable to significantly increase T_{aO2} from resting values.

Unlike arterial blood, both P_{vO2} and C_{vO2} varied significantly among temperature groups (Table 5.2). Resting P_{vO2} declined above T_{opt}. Resting P_{vO2} was only 10 torr at $T_{max0-50}$, or 25% of the T_{opt} value. As a result, resting C_{vO2} at $T_{max0-50}$ was only 16-20% of the C_{vO2} for the other three temperature groupings.

At fatigue, C_{vO2} and P_{vO2} were 114% and 77%, respectively, lower in the $T_{max50-90}$ group compared to T_{opt}. Limited blood samples at $T_{max0-50}$ prevented analysis for swimming and fatigue (Table 5.2), but these limited numbers showed a decreasing trend for both resting and fatigue P_{vO2} and C_{vO2} at temperatures above T_{opt} (Fig 5.7).

The increase in oxygen uptake by swimming muscles was reflected in the significant decline of both P_{vO2} and C_{vO2} with swimming (Table 5.2). Analysis of A-V_{O2} was restricted to paired arterial and venous samples (Table 5.2). With this caveat, the trend of increasing A-V_{O2} with swimming and at warmer temperatures did not reach statistical significance, likely due to low statistical power (Table 5.2).

At rest, T_{vO2} was over 4-fold lower at $T_{max0-50}$ compared to T_{opt} (Table 5.2). In addition, T_{vO2} was maintained at resting levels throughout the swim test at T_{opt}, while it significantly declined at burst and fatigue in $T_{max50-90}$ fish (Fig 5.8).

5.2.3 Other Blood Variables

Plasma glucose did not vary significantly with temperature or swimming except for the $T_{max0-50}$ grouping where plasma glucose significantly declined with swimming (Table 5.3). In contrast, plasma lactate varied significantly with both temperature and swimming. Resting plasma lactate was more than 3-fold significantly higher in $T_{max0-50}$ compared to T_{opt} fish. At fatigue, the T_{opt}, $T_{max50-90}$ and $T_{max0-50}$ groupings all displayed significant elevations in plasma lactate, increasing by 2 to 4-fold relative to resting levels. Moreover, plasma lactate was significantly higher in $T_{max50-90}$ fish at fatigue compared to the other groups (Table 5.3).

Plasma sodium varied significantly with both temperature and swim speed (Table 5.3). It tended to be highest at T_{opt} and increased with swimming speed for T_{opt} and $T_{max50-90}$ fish. Plasma potassium did not vary significantly with temperature (Table 5.3). Plasma potassium tended to decrease with swimming speed and was lowest at fatigue. Plasma chloride varied significantly with temperature, but not with swimming (Table 5.3). In general, plasma chloride was significantly higher at T_{opt} relative to $T_{max0-50}$.

5.3 Discussion

This study is the most comprehensive assessment of the oxygen cascade in fish swimming at temperatures bracketing their T_{opt}. It greatly extends upon the study by Steinhausen et al. (2008), which swam fish at a constant speed (~1.35 bl s^{-1} or ~70% of maximum) while acutely increasing the water temperature, by swimming individual fish to U_{crit} and at discrete temperatures.

Aerobic scope and swim performance collapsed at temperatures above T_{opt}, which is consistent with previous assertions that a limitation in maximum cardiorespiratory performance inhibits exercise at high temperatures in salmonids (Brett, 1971; Farrell, 1997; Farrell, 2002; Farrell, 2009; Farrell et al., 2009; Lannig et al., 2004; Mark et al., 2002; Pörtner et al., 2004; Pörtner and Knust, 2007; Sartoris et al., 2003; Steinhausen et al., 2008; Taylor et al., 1996). In addition, novel findings with respect to venous oxygen status, heart rate and stroke volume were revealed.

The present study provides clear evidence for a cardiac limitation in fish swimming at warm temperatures. At temperatures above T_{opt}, $\dot{Q}_{max}$ failed to increase because f_{Hrest} reached its

maximum and could not further increase during swimming. Moreover, cardiac disrhythmias developed at the highest temperatures grouping. As discussed further below, changes in both P_{aO2} and C_{aO2} were minor at warm temperature, so neither oxygen delivery to or across the gills presented themselves as a major problem in terms of the oxygen cascade, despite a decrease in oxygen content in the water. Instead, a perfusion limitation developed because T_{aO2} failed to increase above T_{opt} given that $\dot{Q}$ did not increase and C_{aO2} was unchanged. A diffusion limitation at the swimming muscles likely followed the cardiac limitation since P_{vO2} and C_{vO2} did decrease significantly at warm temperatures.

5.3.1 Fish Performance during Swim Challenge

Swimming a sufficient number of fish to resolve any subtle changes that occur in the oxygen cascade as a function of warming had many inherent challenges. Arterial and venous cannulae had to remain functional for multiple samples, yet blood sampling was selective to minimize hemodilution. In addition, adult Pacific salmon often acquire a fungal infection on the gills during migration. My fish were no exception, which may have contributed to the individual variability for P_{aO2} (see below). To compensate and increase statistical power, I pooled three populations and created four temperature groupings relative to T_{opt}. Notably, a similar analysis of the temperature responses in Chilko sockeye salmon for $\dot{M}O_2$, $\dot{Q}$, f_H and V_s (see Chapter 3) mimicked the responses observed here for the pooled populations. In addition, the variance was small for $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ and aerobic scope in each of the pooled temperature categories (see Fig 5.3). Furthermore, aerobic, cardiac and heart rate scopes measured over the full range of

temperatures and populations were all positively correlated (see Chapter 3). Therefore, I have confidence that pooling was valid.

Sex-specific differences in cardiorespiratory physiology and blood oxygen status were not apparent at T_{opt} (see Chapters 3 and 4). Sex-specific differences were not considered in the analysis presented here due to low n-values. However, each temperature grouping contained approximately equal numbers of males and females, which offsets concerns regarding sex-differences. Nevertheless, the possibility that temperature tolerance as well as the physiological response to temperature varies between males and females has not been excluded and therefore should be considered in future studies.

As expected, fish swum above T_{opt} and attaining less than 50% of maximum aerobic scope had a much lower maximum swim velocity compared to the other groups. This result provides evidence of the link between aerobic scope and swim performance.

Also, lactate was elevated at temperatures above T_{opt} and at fatigue. It is well known that as fish approach U_{crit}, swimming gait transitions to burst-and-coast behaviours, which activates the white glycolytic muscles relying on anaerobic metabolism, producing lactate and lowering blood pH (Brauner et al., 2000). Moreover, fish are known to increase their reliance on anaerobic swimming at high temperatures (Brett, 1964; Jain and Farrell, 2003; Steinhausen et al., 2008), which was clearly evident here.

There was a clear trend for increasing COT and COT-$\dot{Q}$ with increasing temperature, though the characteristic "U-shaped" pattern of COT with speed (e.g. Hoyt and Taylor, 1981; Lee et al., 2003c; Prange, 1976; Prange and Schmidt-Nielsen, 1970; Wakeman and Wohlschlag, 1982) was not observed in any of the temperature groups. The remaining cardiovascular and oxygen status results are discussed in the sections below.

Arterial [Hb] and Hct did not significantly vary with temperature or swimming, supporting previous findings (Clark et al., 2008b; Steinhausen et al., 2008). A minor decrease in MCHC was observed at warm temperatures during swimming, which was primarily due to a general non-significant trend for increased Hct. Similar observations have previously been made in resting chinook (Clark et al., 2008b). Previous studies have shown a variable response of Hct with temperature. Hct has been shown to increase by up to 27% due to splenic contraction in acutely warmed resting rainbow trout (Sandblom and Axelsson, 2007), to decrease by 50% in warm-acclimated rainbow trout (Taylor et al., 1993) and to have minimal effects with temperature (see Farrell, 1997). Thus, though splenic contraction can be a short-term solution to increase [Hb] during swimming or acute temperature changes, this was not observed in the present study.

Plasma ions were differentially affected by temperature and swimming. Both plasma chloride and sodium were reduced above T_{opt}, in contrast to resting chinook salmon (Clark et al., 2008b). Plasma potassium was insensitive to temperature. The decrease in plasma potassium with swimming sharply contrasts previous results (Holk and Lykkeboe, 1998; Nielsen et al., 1994; Nielsen and Lykkeboe, 1992a; Steinhausen et al., 2008). As discussed in Chapter 4, increased plasma potassium during swimming has been attributed to potassium loss from working muscles and associated with reduced excitability of muscle cells, which is suggested to contribute to muscle fatigue (both cardiac and skeletal) (Bangsbo et al., 1996; Sjøgaard, 1996; Holk and Lykkeboe, 1998; Nielsen and Lykkeboe, 1992a). The decreased potassium levels observed here are interesting and warrant further study.

5.3.2 The Possibility of a Limitation in Gill Oxygen Uptake

Water oxygen content decreases by around 2% $°C^{-1}$ with increasing water temperature (Dejours, 1975), limiting environmental oxygen availability at high temperatures. In addition, haemoglobin oxygen affinity decreases (the oxyhaemoglobin dissociation curve shifts to the right) with high temperature exposure (Jensen et al., 1998; Perry and Reid, 1994), which hampers oxygen uptake at the gill although it facilitates tissue oxygen extraction. Accordingly, a limitation in oxygen uptake at the gills (either water delivery to the gills or diffusion of oxygen across the gills) has been proposed as a mechanism causing decreased cardiorespiratory and swimming performance at elevated temperatures in fish (Brett, 1971; Heath and Hughes, 1973; Taylor et al., 1997). In support of this hypothesis, Heath and Hughes (1973) showed that C_{aO2} decreased with acute increases in water temperature in resting rainbow trout though hematocrit was not measured concurrently to check for haemodilution. Similarly, Taylor et al. (1993) found a decrease in C_{aO2} at 18°C in resting and swimming rainbow trout seasonally acclimated to 4, 11 and 18°C, but hematocrit was halved. Clark et al. (2008b) found that large, but not smaller, resting adult chinook salmon decreased C_{aO2} and P_{aO2} during acute warming, but narrow holding tubes may have constrained gill movements in the larger fish.

In contrast, the present study found that C_{aO2} and P_{aO2} did not significantly differ across the four temperature categories. Similarly, Steinhausen et al. (2008) found that C_{aO2} and hematocrit remained constant in both resting and swimming sockeye salmon exposed to acute increases in temperature. Moreover, P_{aO2} increased in resting and remained constant in swimming sockeye salmon exposed to increasing temperatures. Likewise, Sartoris et al. (2003) found that P_{aO2} remained constant during acute warming in Atlantic cod. Thus, there is

accumulating evidence that neither water delivery to the gills nor oxygen diffusion across the gills become limited at temperatures warmer than T_{opt}.

P_{aO2} and C_{aO2} did tend to decrease with swimming. A similar phenomenon was observed in swimming sockeye salmon exposed to acute temperature increases (Steinhausen et al., 2008). If instead fish had maintained or increased C_{aO2} during swimming, T_{aO2} would have been higher, which would have been particularly beneficial for warm fish.

Notably, there were a couple experimental concerns, which relate to the variation in P_{aO2} among individual fish. P_{aO2} and C_{aO2} varied considerably, ranging from 49-128 torr and 6.7-14.8 ml dl^{-1}, respectively, at rest, and from 42-104 torr and 6.1-16.1 ml dl^{-1}, respectively, at fatigue. As described in Chapter 4, this may be attributed to the progressive accumulation of fungus on the body and gills. Gill fungal infection could increase the diffusion distance and decrease the maximum surface area for oxygen, creating variable P_{aO2}. In addition, gill damage during surgery (implantation of the flowprobe and venous catheter occurs in the opercular cavity adjacent to the gills) may have created similar gill diffusion problems that might account for the tendency for C_{aO2} to decrease with swimming. Even so, arterial oxygen saturation was not substantially hampered during swimming.

5.3.3 The Possibility of a Limitation in Cardiac Performance

The idea that the temperature dependence of aerobic scope is closely linked with that of cardiac scope is clearly supported by the similarity of the temperature-induced changes for $\dot{Q}$ and $\dot{M}O_2$ in resting and swimming fish (also see Chapter 3). A striking observation was that maximum $\dot{M}O_2$, $\dot{Q}$ and T_{aO2} all failed to increase above T_{opt}, and all three decreased at $T_{max0\text{-}50}$.

This finding provides evidence of a cardiac limitation at high temperatures, supporting earlier studies (Brett, 1971; Steinhausen et al., 2008; Taylor et al., 1996).

Warming increased $\dot{Q}$ in resting and swimming fish entirely via an increase in f_H, corroborating previous work (Clark et al., 2008b; Sandblom and Axelsson, 2007; Steinhausen et al., 2008). Such increases in f_H are probably mediated through a direct temperature effect on the pacemaker rate (Randall, 1970). The highest f_H was achieved in resting fish in the highest temperature group (mean: 123.9 beats min^{-1}; range: 117-135 beats min^{-1}), and thus sometimes exceeded the proposed maximum of 120 beat min^{-1} in active fish (Davie and Farrell, 1991; Farrell, 1991). However, at temperatures above T_{opt}, f_H was unable to increase above resting levels during swimming and even decreased below resting levels for the $T_{max0-50}$ grouping. The negative scope for f_H is a novel finding for fish. Because scope for f_H became limiting at a lower temperature compared to scope for V_s or $\dot{Q}$, the present study provides support for the previous proposal (Farrell, 2009; Steinhausen et al., 2008) that reduced scope for f_H may be the mechanism that limits $\dot{Q}_{max}$ above T_{opt}.

Cardiac disrhythmias at the highest test temperatures provide further evidence of a cardiac limitation. Disrhythmias were never present in resting fish at any temperature, nor in swimming or fatigued fish at T_{opt} or $T_{min50-90}$. Yet, every fish in the $T_{max0-50}$ group exhibited cardiac disrhythmias during fatigue and 50% displayed disrhythmias during swimming. What is truly remarkable is that the fish at the highest test temperatures continued to swim, albeit to a lower swim speed, despite severe disrhythmias and dramatic declines in P_{vO2}, C_{vO2}, T_{aO2} and T_{vO2}, demonstrating an impressive tenacity that is obviously fuelled by anaerobic swimming given the high plasma lactate levels. This tenacity, however, could result in delayed mortality. Fish that displayed the most severe disrhythmias (29% of the $T_{max0-50}$ fish) died, despite quickly

decreasing the water temperature following fatigue. In every case, the cardiac disrhythmia was preceded by bradycardia. While irregular heart rates have been reported in resting rainbow trout, Atlantic cod and chinook salmon acutely exposed to high temperatures (Clark et al., 2008b; Gollock et al., 2006; Heath and Hughes, 1973), this is the first known study to report bradycardia followed by disrhythmia in a swimming fish at high temperature.

The mechanism of the bradycardia and subsequent cardiac disrhythmia is unclear. During anaerobic swimming, venous blood becomes acidotic (low pH), hypoxemic (low P_{vO2}) and hyperkalemic (high K^+) (Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977), all of which can inhibit cardiac contractility (Dridezic and Gesser, 1994). High temperatures can exacerbate these conditions as salmonids rely more on anaerobic metabolism (Brett, 1964; Jain and Farrell, 2003; Steinhausen et al., 2008), though hyperkalemia was absent here. Simulated exercise conditions at high temperature with *in situ* rainbow trout hearts severely impaired maximum cardiac performance (Hanson and Farrell, 2007), thus it is possible that the deleterious venous blood environment caused the bradycardia and disrhythmias. However, bradycardia could also be under central nervous system control via the vagus nerve. A reduction in f_H would increase the residence time of blood in the lumen of the heart which may enhance oxygen delivery to the spongy myocardium (see below). The critical experiments to examine these questions require the use of atropine or vagotomy to determine if heart rate increases when the vagal tone is blocked.

Since scope for f_H declined above T_{opt}, $\dot{Q}$ could only increase by an increase in V_s. This was not evident. Resting V_s failed to increase with increasing temperatures, corroborating previous studies (Brodeur et al., 2001; Clark et al., 2008b; Gamperl et al., 2011; Gollock et al., 2006; Mendonça and Gamperl, 2010; Sandblom and Axelsson, 2007; Steinhausen et al., 2008). In addition, a novel negative relationship between temperature and V_s was observed here in

swimming fish. V_{smax} decreased at the highest temperatures, resulting in a decreased scope for V_s. This result contrasts a previous report, which suggested that V_s was insensitive to temperature in salmonids exercising at ~75% of U_{crit} (Steinhausen et al., 2008). However, the present study pushed fish to swim at higher temperatures and higher speeds, resulting in substantially reduced scope relative to previous studies, which may have resulted in the observed decrease in V_{smax}.

Several potential reasons have been suggested for why V_s does not increase in conjunction with f_H at high temperature, especially since it can triple with swimming (Brett, 1971; Kiceniuk and Jones, 1977; Stevens et al., 1967). First, venous return and end-diastolic volume must first increase in order for cardiac contractility to increase V_s (Sandblom and Axelsson, 2007) because cardiac end-systolic volume is essentially zero in salmonids (Franklin and Davie, 1992). In addition, cardiac contractility could be inhibited by the deleterious blood environment at high temperatures (e.g. low pH, high K^+, low P_{vO2},) (Hanson et al., 2006). Finally, the negative force-frequency relationship for fish cardiac muscle dictates that cardiac contractility decreases with increasing contraction frequency (Hove-Madsen, 1992; Shiels et al., 2002). Accordingly, the high f_H at high temperatures decreases both filling time and ventricular contractions which may increase end-systolic volume and reduce V_s. In support of this concept, a recent study by Gamperl et al. (2011) demonstrated that elevated temperature, per se, does not limit the ability of rainbow trout to increase V_s since zatebradine treatment halved f_H at the highest test temperature but V_s doubled to maintain $\dot{Q}$. This result suggests that perhaps the increase in f_H associated with high temperature is an inescapable direct effect of temperature on the pacemaker, which prevents any beneficial changes in V_s.

It is important to note that the heart is a muscle requiring oxygen, and its requirements increase 3 to 5-fold during exercise, equating approximately to 1% of total $\dot{M}O_2$ (Farrell and

Steffensen, 1987). Salmonid hearts have two oxygen supply routes to their two distinct types of myocardium (see Chapter 6). The outer, compact myocardium is perfused with oxygenated blood from the arterial coronary circulation. Given that P_{aO2} and C_{aO2} were maintained with increasing temperature, and that coronary blood flow increases concomitantly with cardiac output during swimming (Axelsson and Farrell, 1993; Gamperl et al., 1995), T_{aO2} to the compact myocardium likely wasn't limited, except perhaps at the highest temperatures when T_{aO2} declined. The inner, spongy myocardium is avascular and relies on the leftover oxygen in venous blood returning to the heart. Since both P_{vO2} and C_{vO2} decrease during exercise due to increased oxygen extraction by the muscles, a diffusion limitation could develop. The temperature-induced increase in f_H could then exacerbate the situation by decreasing the residence time of the blood in the lumen and thereby decreasing diffusion time between heart beats. The bradycardia observed here during swimming at high temperatures could serve to alleviate a diffusion limitation.

Some have suggested that a threshold value for P_{vO2} exists in order to guarantee sufficient oxygen supply to the spongy myocardium (Davie and Farrell, 1991; Farrell, 2002; Farrell, 2007; Farrell and Clutterham, 2003). However, in the present study, P_{vO2}, C_{vO2}, and T_{vO2} declined in salmon swimming above T_{opt}. Thus, a state of cardiac hypoxia could have developed, possibly contributing to the disrhythmias discussed above, cardiac collapse and a concomitant decrease in oxygen delivery to the tissues, ultimately leading to decreased swimming performance.

5.3.4 The Possibility of a Limitation in Tissue Oxygen Extraction

As oxygen demand increases with warming, several situations could limit oxygen delivery to the mitochondria of the locomotory muscles. A diffusion limitation could occur due

to inadequate capillary density, ineffective muscle morphology (e.g. poor mitochondria density or location) or insufficient driving force (low P_{aO2}). A perfusion limitation could result from inadequate $\dot{Q}$ or insufficient capillary perfusion at the muscle. Evidence exists for both possibilities in fish swimming at high temperatures.

Steinhausen et al. (2008) found evidence of a diffusion limitation since P_{vO2} was maintained during acute temperature increases in swimming sockeye salmon. In addition, when fish quit swimming, venous blood is still partially saturated (Farrell and Clutterham, 2003; Farrell, 2007; Kiceniuk and Jones, 1977).

In contrast, P_{vO2} and C_{vO2} decreased above T_{opt} in resting sockeye salmon in the present study. In fact, resting P_{vO2} decreased to 10 torr and resting plasma lactate became elevated in the highest temperature group, signifying insufficient oxygen delivery to tissue mitochondria. Similarly, warming decreased C_{vO2}, P_{vO2} or both in resting rainbow trout, Atlantic cod and adult chinook salmon (Clark et al., 2008b; Heath and Hughes 1973; Sartoris et al., 2003). Thus, a tissue diffusion limitation was likely not manifest at T_{opt}.

Given the dramatic decline in T_{aO2} and the obvious increase in plasma lactate during swimming and at fatigue in the highest temperature group relative to T_{opt}, there was clearly a mismatch between oxygen supply and demand at the tissues, suggesting a perfusion limitation. There were insufficient venous blood samples in $T_{max0-50}$ during swimming and at fatigue to include in the analysis across groups; however, the scatterplot in Fig 5.7 shows a steady decline in C_{vO2} and P_{vO2} at fatigue above T_{opt}. Therefore, I suggest that there may not have been an immediate diffusion limitation at the muscle in swimming fish at high temperature. However, a decrease in C_{vO2} and P_{vO2} does not definitively preclude a diffusion limitation since the decrease may not have been proportional to the oxygen demand (Wagner, 1996). Therefore, these data do

not definitely exclude the possibility that both a diffusion and perfusion limitation may have been occurring.

The role of muscle morphology in limiting oxygen diffusion at high temperature and with exercise is ripe for future research. A diffusion limitation for oxygen uptake due to low capillarity in white muscle (Egginton and Sidell, 1989; Mosse, 1978) has been suggested to be an important mechanism "governing" systemic tissue utilization and thus ensuring an adequate P_{vO2} threshold to supply the spongy myocardium with oxygen (Farrell et al., 2009). It would be particularly interesting to compare cardiac and skeletal muscle morphology (e.g. capillary, mitochondria and lipid density and location) in sockeye salmon populations differing in cardiorespiratory capacity and temperature tolerance.

5.3.5 A Possible Death Spiral for Salmon Swimming above T_{opt}

A "death spiral" for salmon swimming at temperatures above T_{opt} was proposed by Farrell et al. (2009). Here, I provide further evidence for and expand upon the death spiral progression. My results are entirely consistent with the death spiral starting with a plateau in maximum heart rate above T_{opt}, which prevents $\dot{Q}_{max}$ from further increasing to satisfy the increased tissue oxygen demand. With no compensatory increase in C_{aO2}, a perfusion limitation to swimming muscles creates a mismatch between oxygen supply and demand, as evidenced by elevated lactate levels. Low P_{vO2} levels coupled with low pH due to anaerobic swimming likely impair cardiac contraction, further exacerbating the perfusion limitation and causing a positive feedback loop. At sufficiently low P_{vO2} levels, a diffusion limitation to the swimming muscles likely develops as well and eventually swimming ceases. At temperatures well above T_{opt},

corresponding to precipitous declines in aerobic scope that would certainly prevent successful upstream migration, the situation is dire. Even in resting fish, T_{aO2} levels are insufficient to meet the increased oxygen demand, as shown by high resting lactate levels. Swimming actually decreases f_H below resting levels and maximum V_s plummets, leading to a massive collapse of $\dot{Q}$. A perfusion limitation, which is likely followed by a diffusion limitation, develops at the swimming muscles. Dramatic declines in P_{vO2}, C_{vO2} and T_{vO2}, coupled with low pH, create a deleterious venous environment for the spongy myocardium, which weakens cardiac contraction and may be the cause of the bradycardia and cardiac disrhythmias. Eventually, fish quit swimming and at excessively warm temperatures, cardiac function cannot recover and the fish perish.

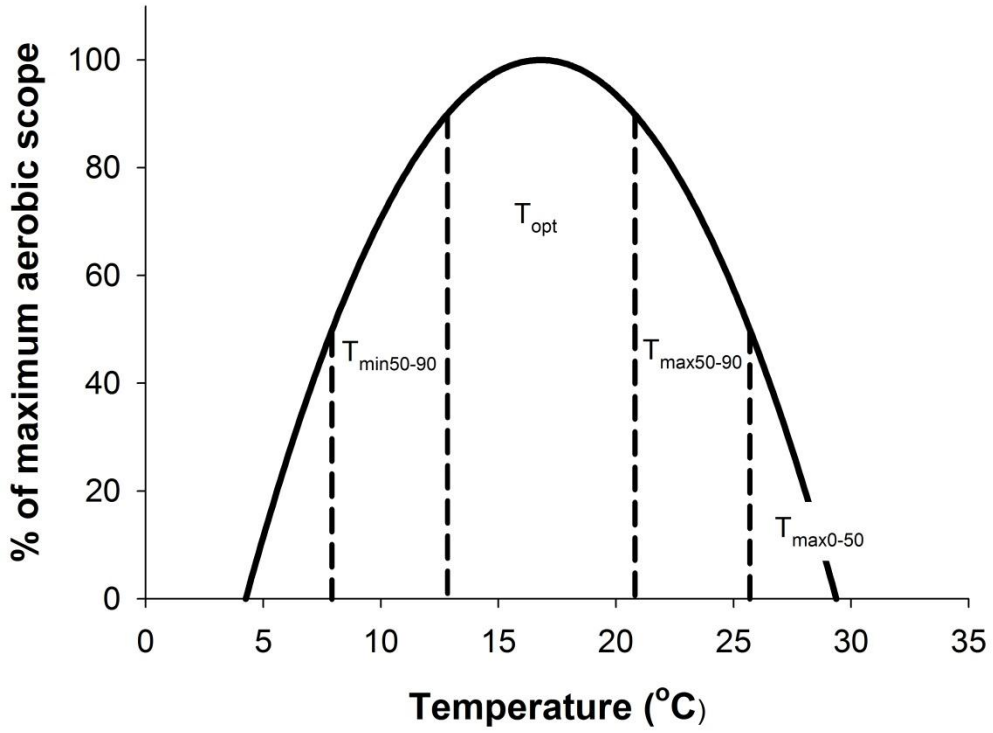

Figure 5.1. Schematic of the four categories of cardiorespiratory performance with temperature. T_{opt} included fish swum at the optimal temperature range, at which 90-100% of maximum aerobic scope was attained. $T_{min50-90}$ included fish that were swum at temperatures lower than T_{opt} at which only 50-90% of maximum aerobic scope was measured. $T_{max50-90}$ included fish swum at temperatures above T_{opt}, when 50-90% of maximum aerobic scope was measured. $T_{max0-50}$ included fish whose aerobic scope was only 0-50% of maximum.

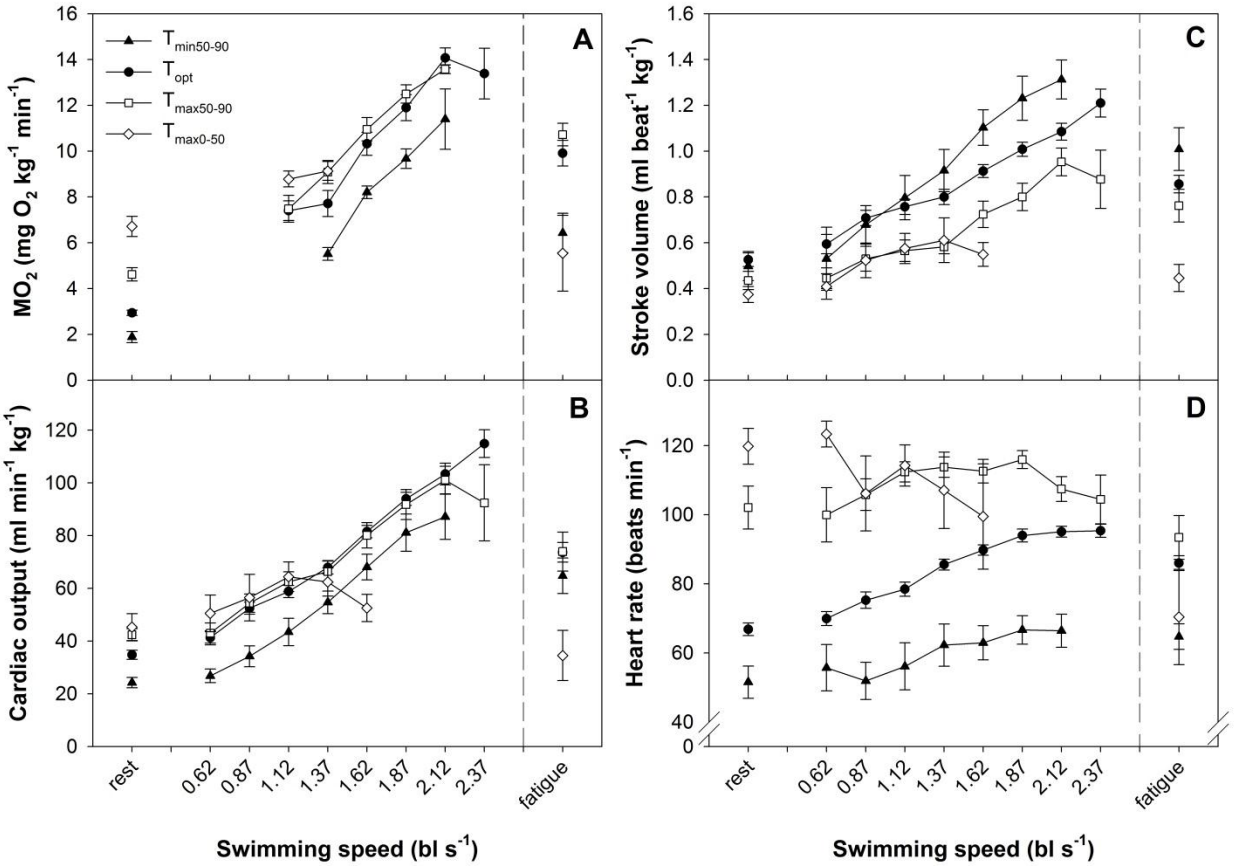

Figure 5.2. (A) Oxygen consumption ($\dot{M}O_2$), (B) cardiac output, (C) stroke volume and (D) heart rate with swimming speed across the four temperature groups. Mean ± SEM are shown.

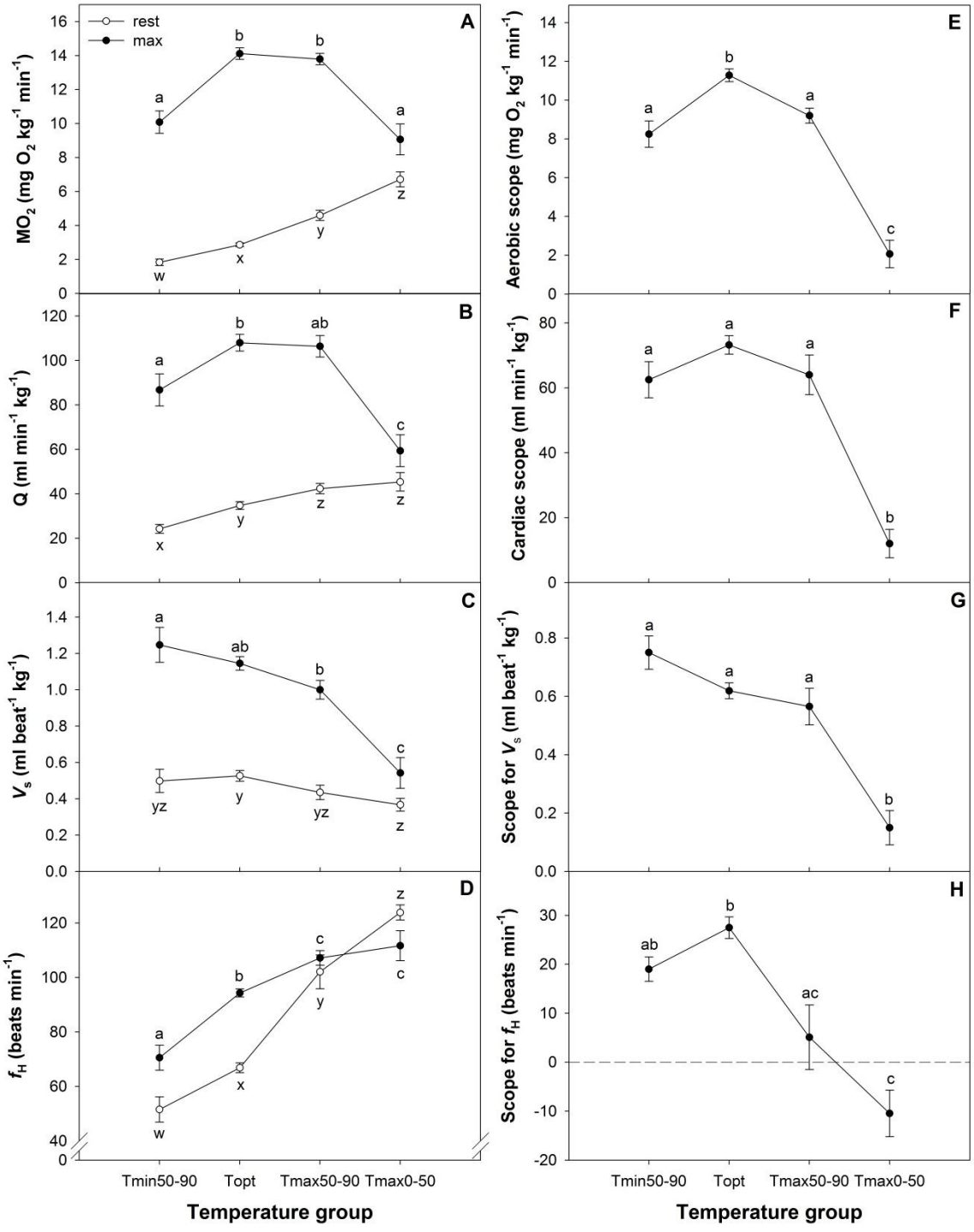

Figure 5.3. Resting and maximum (A) oxygen consumption ($\dot{M}O_2$), (B) cardiac output ($\dot{Q}$), (C) stroke volume (V_s), (D) heart rate (f_H) at the four temperature categories. Scope for $\dot{M}O_2$ (E), $\dot{Q}$ (F), V_s (G) and f_H (H) are shown. All values are presented as mean ± SEM. Significant differences among temperature categories are indicated by differing letters ($p<0.05$).

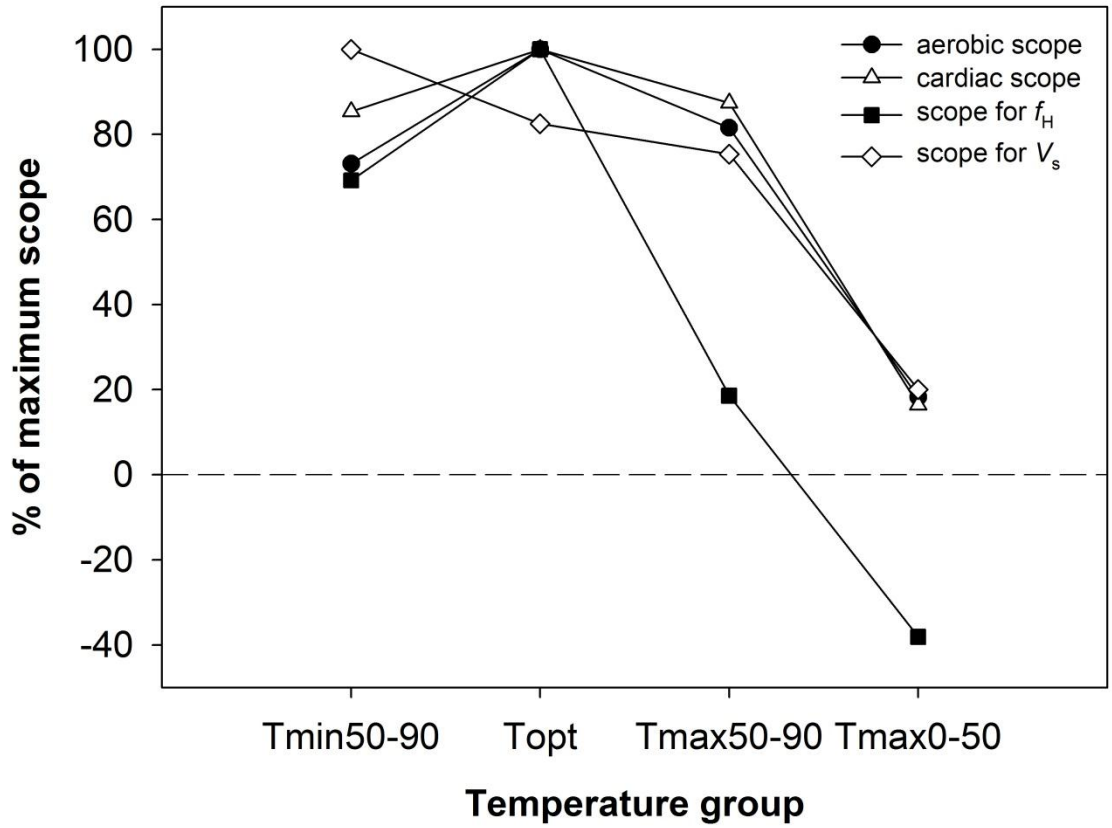

Figure 5.4. Percent of maximum aerobic scope, cardiac scope, scope for heart rate (f_H) and scope for stroke volume (V_s) for each temperature category.

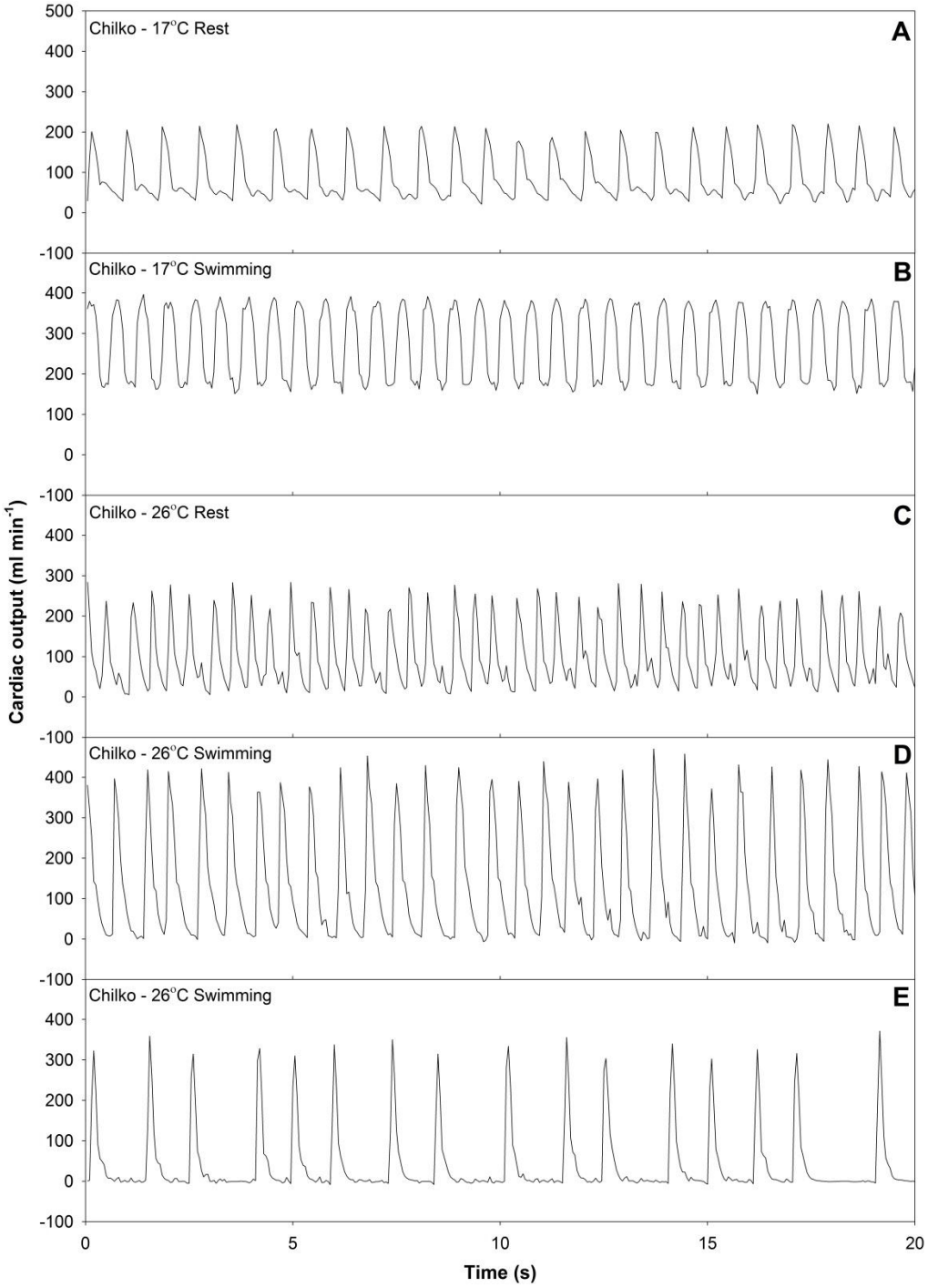

Figure 5.5. Individual blood flow traces for two Chilko sockeye salmon at 17°C (T_{opt}) and 26°C ($T_{max0-50}$) at rest (A, C) and during swimming (B, D, E). Swimming traces were recorded during the final swim speed before each fish fatigued (measured at 2.3 bl s^{-1} and 1.5 bl s^{-1} for the 17 and 26°C fish, respectively). Trace D was recorded 5 min before trace E, at the same swimming speed.

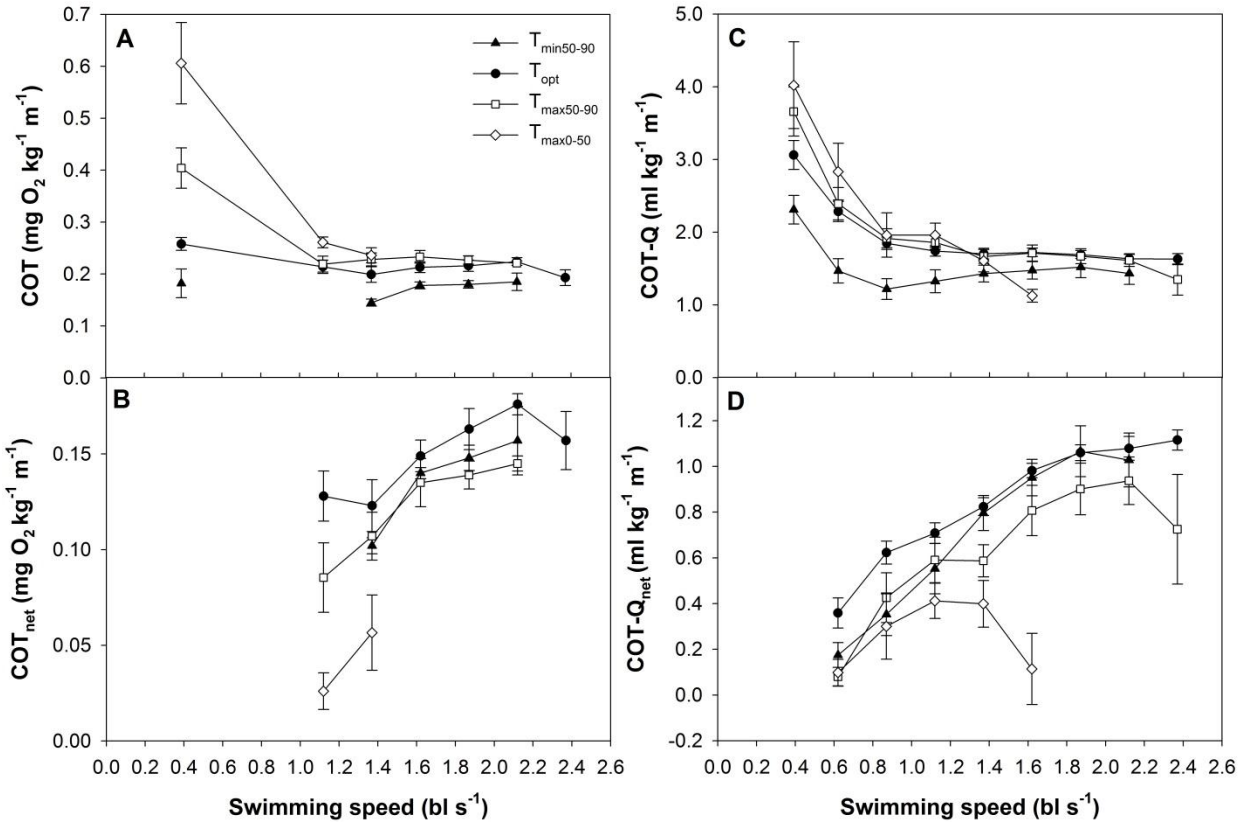

Figure 5.6. (A) Cost of transport (COT), (B) net cost of transport (COT_{net}), (C) cardiovascular cost of transport (COT-$\dot{Q}$) and (D) net cardiovascular cost of transport (COT-$\dot{Q}_{net}$) with swimming speed across the four temperature groups. Mean ± SEM are shown.

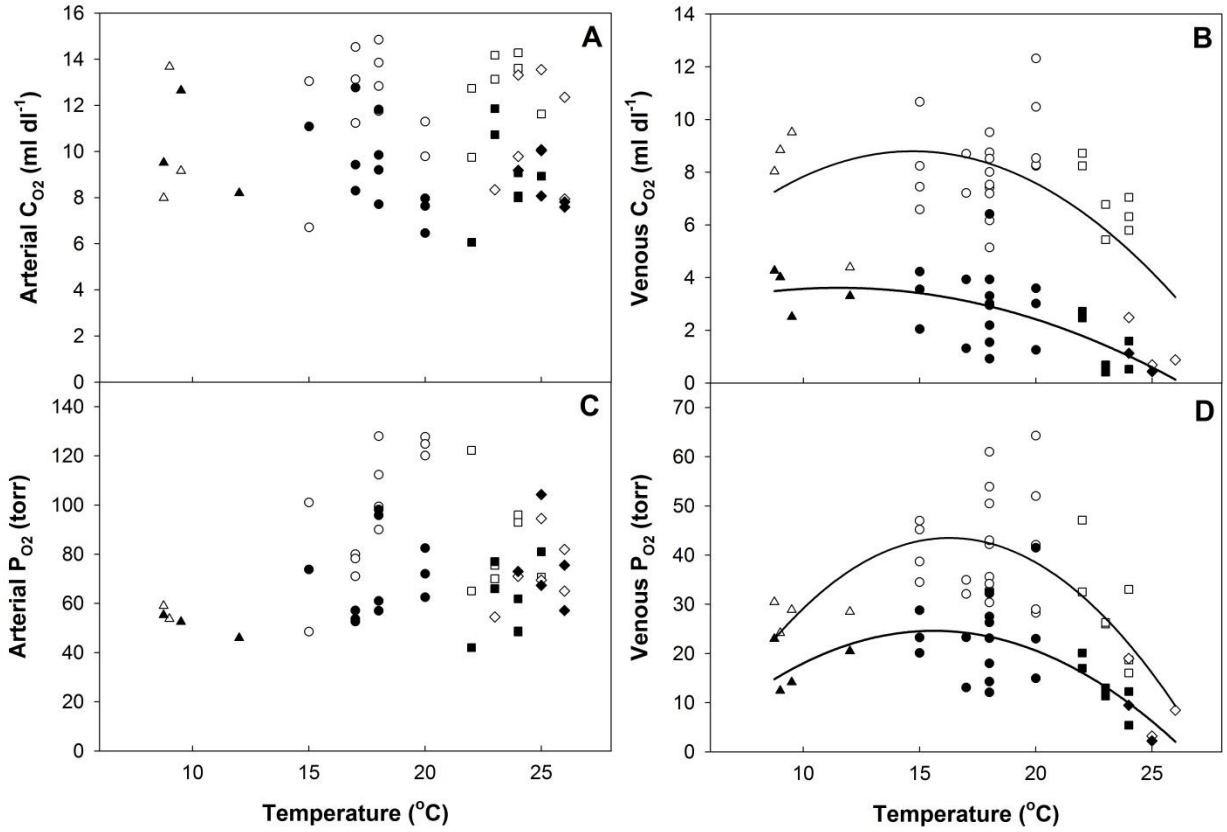

Figure 5.7. Arterial and venous (A, B) oxygen content (C_{O2}) and (C, D) partial pressure of oxygen (P_{O2}) at rest (open symbols) and fatigue (filled symbols) in four temperature groups (△= $T_{min50\text{-}90}$; ○ = T_{opt}; □ = $T_{max50\text{-}90}$; ◇ = $T_{max0\text{-}50}$). Each data point corresponds to an individual fish. A quadratic equation was fit through the venous data. Resting C_{vO2}: $R^2 = 0.37$, $p = 0.0007$; Fatigue C_{vO2}: $R^2 = 0.39$, $p = 0.002$; Resting P_{vO2}: $R^2 = 0.51$, $p < 0.0001$; Fatigue P_{vO2}: $R^2 = 0.41$ $p = 0.001$.

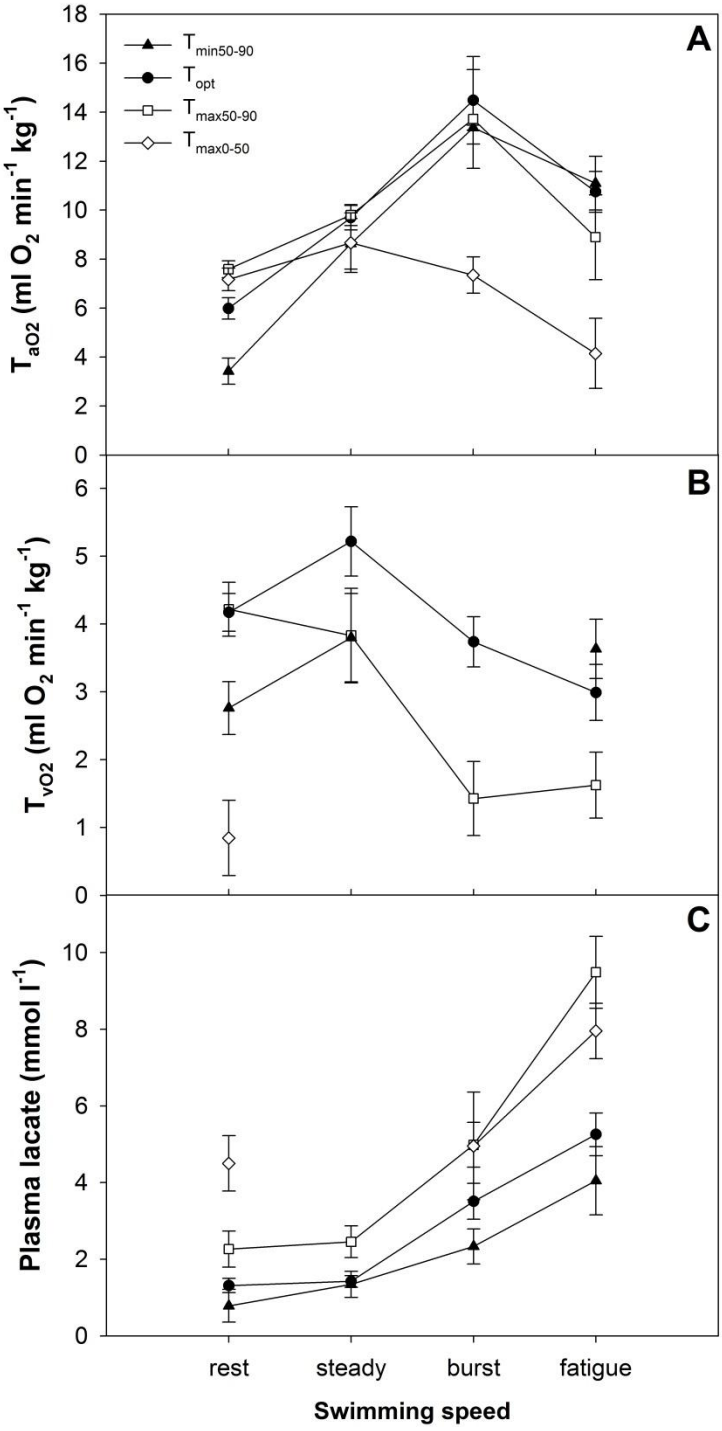

Figure 5.8. (A) Arterial oxygen transport (T_{aO2}), (B) venous oxygen transport (T_{vO2}) and (C) arterial plasma lactate across the four temperature groups and with swimming. Refer to Tables 5.2 and 5.3 for statistical information.

Table 5.1. Arterial partial pressure of oxygen (P_{aO2}) and oxygen content (C_{aO2}) in resting fish, measured at 12°C and at the test temperature. Mean ± SEM are presented, there were no significant differences within a temperature group ($p>0.05$).

		P_{aO2}		C_{aO2}	
	n	12°C	test temp	12°C	test temp
$T_{max50-90}$	5	67.5 ± 3.3	81.0 ± 5.6	14.2 ± 0.6	13.4 ± 0.5
$T_{max\ 0-50}$	7	63.5 ± 6.4	72.8 ± 4.8	12.0 ± 0.4	10.8 ± 0.9

Table 5.2. Oxygen status variables across the four temperature categories and with swimming. Arterial and venous partial pressure of oxygen (P_{aO2} and P_{vO2}), oxygen content (C_{aO2} and C_{vO2}), oxygen extraction ($A\text{-}V_{O2}$), arterial oxygen transport (T_{aO2}) and venous oxygen transport (T_{vO2}) are indicated. Mean ± SEM, temperatures groups with differing letters within a swim speed are statistically different, an asterisk indicates a statistically significant difference from rest within a temperature group ($p<0.05$).

		rest	**steady**	**burst**	**fatigue**
P_{aO2} (torr)	$T_{min50-90}$	-	60.7 ± 9.3	62.9 ± 3.5	49.8 ± 2.4
	T_{opt}	98.4 ± 7.4	96.5 ± 5.8	64.7 ± 9.6*	69.7 ± 4.9*
	$T_{max50-90}$	84.6 ± 7.7	71.9 ± 3.8	58.1 ± 4.3	60.7 ± 5.7
	$T_{max0-50}$	72.8 ± 4.8	70.8 ± 3.7	71.7 ± 7.4	75.4 ± 7.9
P_{vO2} (torr)	$T_{min50-90}$	28.0 ± 1.3^{ab}	24.0 ± 1.1	-	17.5 ± 2.5
	T_{opt}	41.6 ± 2.4^{a}	32.6 ± 1.9*	17.6 ± 2.8*	23.4 ± 2.0*
	$T_{max50-90}$	28.5 ± 3.9^{b}	21.1 ± 3.3	11.6 ± 3.7*	13.2 ± 2.18*
	$T_{max0-50}$	10.3 ± 4.6^{c}	-	-	-
C_{aO2} (ml dl^{-1})	$T_{min50-90}$	10.3 ± 1.7	11.6 ± 1.1	11.9 ± 1.2	11.6 ± 1.8
	T_{opt}	12.0 ± 0.6	10.9 ± 0.7	9.2 ± 0.8	9.3 ± 0.6
	$T_{max50-90}$	12.8 ± 0.6	11.2 ± 0.6	10.2 ± 0.5	9.0 ± 0.7*
	$T_{max0-50}$	10.8 ± 0.9	9.9 ± 1.6	9.2 ± 1.5	8.5 ± 0.5
C_{vO2} (ml dl^{-1})	$T_{min50-90}$	7.7 ± 1.1^{a}	4.9 ± 1.2	-	3.5 ± 0.4^{a}*
	T_{opt}	8.2 ± 0.4^{a}	5.7 ± 0.4*	2.5 ± 0.3 *	3.0 ± 0.4^{a}*
	$T_{max50-90}$	6.9 ± 0.5^{a}	4.3 ± 0.8	1.0 ± 0.4*	1.4 ± 0.4^{b}*
	$T_{max0-50}$	1.4 ± 0.6^{b}	-	-	-
$A\text{-}V_{O2}$ (ml dl^{-1})	$T_{min50-90}$	1.6 ± 1.6	-	-	6.8 ± 1.7*
	T_{opt}	4.2 ± 0.8	4.8 ± 0.5	6.3 ± 1.0	6.0 ± 0.7
	$T_{max50-90}$	6.1 ± 1.2	7.0 ± 0.9	9.2 ± 0.7	8.0 ± 1.4
	$T_{max0-50}$	-	-	-	-
T_{aO2} (ml O_2 min^{-1} kg^{-1})	$T_{min50-90}$	3.4 ± 0.5	8.6 ± 1.0	13.4 ± 0.1^{ab}*	11.1 ± 1.1^{a}*
	T_{opt}	5.8 ± 0.4	9.4 ± 0.4*	14.7 ± 1.2^{a}*	10.7 ± 0.8^{a}*
	$T_{max50-90}$	7.6 ± 0.3	9.8 ± 0.4	13.7 ± 2.0^{a}*	8.9 ± 1.7^{ab}
	$T_{max0-50}$	7.2 ± 0.5	8.7 ± 1.2	7.4 ± 0.7^{b}	4.1 ± 1.4^{b}
T_{vO2} (ml O_2 min^{-1} kg^{-1})	$T_{min50-90}$	2.8 ± 0.4^{b}	3.8 ± 0.7		3.6 ± 0.4
	T_{opt}	4.1 ± 0.3^{a}	5.1 ± 0.4	3.7 ± 0.4	3.0 ± 0.4
	$T_{max50-90}$	4.2 ± 0.4^{ab}	3.8 ± 0.7	1.4 ± 0.5*	1.6 ± 0.5*
	$T_{max0-50}$	0.8 ± 0.6^{b}	-	-	-

Table 5.3. Arterial haematological variables across the four temperature categories and with swimming. Haemoglobin concentration (Hb), hematocrit (Hct), mean cell haemoglobin concentration (MCHC), plama sodium (Na^+), plasma potassium (K^+) and plasma chloride (Cl^-) are indicated. Mean ± SEM, temperatures groups with differing letters within a swim speed are statistically different, an asterisk indicates a statically significant difference from rest within a temperature group ($p<0.05$).

		rest	steady	burst	fatigue
Hb (g l^{-1})	$T_{min50-90}$	92.3 ± 9.0	89.9 ± 10.6	93.5 ± 11.8	81.5 ± 12.6
	T_{opt}	92.4 ± 4.6	90.3 ± 4.2	91.8 ± 5.4	87.3 ± 3.0
	$T_{max50-90}$	100.3 ± 10.9	95.8 ± 2.7	89.3 ± 6.3	89.1 ± 6.3
	$T_{max0-50}$	89.0 ± 5.0	98.3 ± 6.0	91.6 ± 7.5	91.8 ± 2.3
Hct (%)	$T_{min50-90}$	30.7 ± 3.4	29.2 ± 1.6	30.6 ± 3.5	29.6 ± 5.2
	T_{opt}	31.0 ± 1.7	30.4 ± 1.6	33.9 ± 2.5	31.1 ± 1.4
	$T_{max50-90}$	32.9 ± 1.8	31.4 ± 0.8	34.2 ± 2.2	36.1 ± 1.4
	$T_{max0-50}$	32.6 ± 1.5	32.1 ± 0.9	35.8 ± 3.4	37.5 ± 3.3
MCHC (g l^{-1})	$T_{min50-90}$	302.2 ± 9.1	306.4 ± 19.7	305.1 ± 6.3	278.3 ± 10.9
	T_{opt}	300.5 ± 6.6	299.4 ± 6.8	273.1 ± 8.1	282.3 ± 4.9
	$T_{max50-90}$	308.4 ± 32.7	306.7 ± 13.5	267.7 ± 25.1	249.0 ± 22.0*
	$T_{max0-50}$	274.5 ± 15.8	305.7 ± 11.7	259.1 ± 19.4	250.4 ± 16.4
Glucose (mmol l^{-1})	$T_{min50-90}$	-	9.6 ± 0.7	9.0 ± 0.3	9.8 ± 0.7
	T_{opt}	5.6 ± 0.5	5.1 ± 0.5	6.6 ± 0.5	6.2 ± 0.6
	$T_{max50-90}$	7.6 ± 1.9	8.3 ± 1.2	6.9 ± 1.0	7.8 ± 1.1
	$T_{max0-50}$	10.2 ± 2.0	-	5.4 ± 1.9*	7.3 ± 1.6*
Lactate (mmol l^{-1})	$T_{min50-90}$	0.8 ± 0.4^{a}	1.3 ± 0.3	2.3 ± 0.5	4.0 ± 0.9^{a}
	T_{opt}	1.3 ± 0.2^{a}	1.4 ± 0.2	3.5 ± 0.5	5.3 ± 0.6^{ab}*
	$T_{max50-90}$	2.3 ± 0.5^{ab}	2.5 ± 0.4	5.0 ± 0.6	9.5 ± 0.9^{c}*
	$T_{max0-50}$	4.5 ± 0.7^{b}	-	5.0 ± 1.4	8.0 ± 0.7^{b}*
Na^+ (mmol l^{-1})	$T_{min50-90}$	142.3 ± 2.9^{a}	138.1 ± 1.1	133.1 ± 6.0	-
	T_{opt}	140.1 ± 1.7^{a}	143.8 ± 1.6	145.9 ± 2.1	152.9 ± 2.0^{a}*
	$T_{max50-90}$	120.7 ± 6.8^{b}	140.4 ± 5.2*	138.9 ± 3.2*	139.6 ± 4.7^{ab}*
	$T_{max0-50}$	137.7 ± 4.1^{ab}	-	135.9 ± 2.6	137.6 ± 2.6^{b}
K^+ (mmol l^{-1})	$T_{min50-90}$	3.3 ± 0.1	3.9 ± 0.5	4.3 ± 0.8	4.0 ± 1.8
	T_{opt}	4.9 ± 0.4	4.6 ± 0.4	2.7 ± 0.7	2.6 ± 0.3*
	$T_{max50-90}$	6.2 ± 1.0	4.5 ± 0.7	2.8 ± 0.6*	1.7 ± 0.3*
	$T_{max0-50}$	3.8 ± 0.3	-	3.9 ± 0.9	1.7 ± 0.6
Cl^- (mmol l^{-1})	$T_{min50-90}$	121.0 ± 2.0^{ab}	122.0 ± 5.2	117.4 ± 2.3^{ab}	123.6 ± 3.8^{ab}
	T_{opt}	127.5 ± 0.9^{a}	129.9 ± 1.4	130.2 ± 1.3^{a}	133.3 ± 1.2^{a}
	$T_{max50-90}$	119.3 ± 6.0^{ab}	119.8 ± 2.1	118.3 ± 4.5^{ab}	119.9 ± 5.2^{b}
	$T_{max0-50}$	112.0 ± 3.1^{b}	-	113.4 ± 3.9^{b}	111.9 ± 2.7^{b}

CHAPTER 6: DIFFERENCES IN GROSS CARDIAC MORPHOLOGY AMONG SOCKEYE SALMON POPULATIONS AND IN RELATION TO TEMPERATURE TREATMENT

6.1 Introduction

The preceding chapters demonstrated that aerobic scope is correlated with migration difficulty among Fraser River sockeye salmon populations (Chapter 3) and that cardiac and aerobic scope are tightly related (Chapters 3, 4 and 5). Furthermore, I proposed that cardiac collapse precipitates a decrease in aerobic swimming performance at temperatures above T_{opt} (Chapter 5). Given the key role of the heart in temperature tolerance and supporting aerobic swimming, I sought to determine whether there were differences in cardiac morphology across sockeye salmon populations related to migration difficulty and whether cardiac morphology was affected by temperature exposure.

Relative ventricular mass (RVM) varies considerably across fish species [ranging over 10-fold from 0.03 to 0.4% of body mass; (Santer, 1985)]. Some of these interspecific differences can be attributed to diversity of habitat, life history and activity levels. Like all other muscles, cardiac mass is a primary determinant of force development and a larger heart can presumably generate higher cardiac outputs ($\dot{Q}$) and greater arterial blood pressures. Correspondingly, athletic fish tend to have larger, more powerful hearts that generate higher $\dot{Q}$ and arterial blood pressure compared with sedentary species, though Antarctic icefishes are an important exception (Gamperl and Farrell, 2004). Such species distinctions also extend to ventricular composition. Salmonid ventricles are composed of two distinct layers of myocardium. The outer compact

myocardium is perfused with well-oxygenated arterial blood via a coronary circulation. The inner spongy myocardium is avascular, so it relies on a more variable and lower oxygen tension from the venous blood returning to the heart. Some athletic species (e.g. salmonids and tunas) have 30-50% compact myocardium (Farrell et al., 1988a; Poupa and Lindström, 1983), while most sluggish fish (e.g. hagfishes, Atlantic cod) only have spongy myocardium. While the influence of athleticism on interspecific ventricular design is clear across fish species, its influence among populations within a fish species is unknown.

I predicted that cardiac morphology would vary among sockeye salmon populations according to migration difficulty, mimicking the patterns observed in aerobic scope (Chapter 3). Specifically, I hypothesized that sockeye salmon populations with more challenging migrations would have a larger relative ventricular mass (to allow the heart to generate more power output) and a greater percent compact myocardium (to have a more secure supply of oxygen while swimming). Furthermore, since the total amount of compact myocardium depends on both the size of the ventricle (RVM) and proportion of compact myocardium (% compact) (e.g. a large ventricle with a low proportion of compact myocardium can have the same total amount of compact myocardium as a smaller ventricle with a higher percent compact), I also assessed relative dry compact mass (RDCM). Again, I predicted that populations facing more difficult migrations would display a higher RDCM.

In making comparisons among sockeye salmon populations, I first broadly categorized migration difficulty by dividing the populations into those that pass through Hells Gate, a hydraulically challenging river segment (upriver populations) and those that do not [coastal populations (Table 2.1)]. I predicted that Hells Gate may impose a major selection pressure on the cardiovascular system, especially since Chapter 3 revealed that the coastal Weaver

population possessed the lowest $\dot{M}O_{2max}$ and aerobic scope. I also considered that the river environment may impose selection at a finer scale since Chapter 3 showed that migration distance correlated significantly with aerobic scope across sockeye salmon populations. In addition, migration distance, elevation gain and work (distance × elevation) were the best predictors for various energetic, morphological and reproductive attributes among Fraser River sockeye salmon populations (Crossin et al., 2004). Therefore, my primary hypothesis was that migration distance, elevation gain and work correlate with the heart morphology indices. However, I also took into account the possibility that heart morphology may interact with the environment in a more complex manner. For example, warm temperatures may necessitate a greater percent compact myocardium because of the requirement for a more reliable, stable oxygen supply when P_{vO2} is reduced (see Chapter 5). In addition, swimming at a greater rate or against a stronger river current may require a larger heart to supply a greater $\dot{Q}$. Therefore, I included ATU and various new indices that had not been previously considered (e.g. migration rate, migration duration, migration effort) in the analysis.

It is well known that individual fish show remarkable cardiac plasticity and variability. Indeed, salmonids in particular can rapidly remodel their ventricle in response to various environmental and biological cues (Gamperl and Farrell, 2004). For example, ventricle mass and composition are known to change with temperature acclimation, exercise-training, anemia and sexual maturation in fishes (Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992; Gamperl and Farrell, 2004; Goolish, 1987; Graham and Farrell, 1989; Pelouch and Vornanen, 1996; Simonot and Farrell, 2007; West and Driedzic, 1999). Specifically, RVM increased during sexual maturation in male, but not female salmonids (Clark and Rodnick, 1998; Franklin and Davie, 1992; Graham and Farrell, 1992; West and Driedzic, 1999). In addition, cold

acclimation significantly increased RVM, but decreased % compact in rainbow trout (Farrell et al., 1988a, Gamperl and Farrell, 2004; Graham and Farrell, 1989). Therefore, I also examined cardiac morphology in male and female sockeye exposed to different holding temperatures. I hypothesized that male sockeye salmon would have a greater RVM relative to females and that only males would demonstrate cardiac remodelling. I additionally predicted that RVM would decrease and % compact would increase at warmer temperatures in males, supporting previous observations in rainbow trout (Farrell et al., 1988a, Graham and Farrell, 1989). Finally, to further examine the effect of temperature, I compared cardiac morphology in sockeye salmon exposed to an acute temperature increase while swimming at a constant velocity (Steinhausen et al., 2008). I hypothesized that a greater % compact would translate to a higher temperature tolerance.

Detailed materials and methods are provided in Chapter 2 (sections 2.1, 2.2, 2.8 and 2.10.2).

6.2 Results

6.2.1 Population Comparisons with Migration Difficulty

Population comparisons were restricted to females because male cardiac morphology was shown to significantly differ with temperature treatment (see below). Notably, there were no significant relationships between GSI and any of the cardiac variables within a population (data not shown).

Upriver sockeye salmon had significantly higher RVM, % compact and RDCM compared to coastal sockeye salmon ($p<0.01$). In addition, RVM, % compact and RDCM varied

across populations by 40, 27 and 60%, respectively (Table 6.1). Chilko and Quesnel fish had significantly higher RVM than Weaver fish and all populations had significantly higher RVM compared to Harrison fish. Early Stuart and Nechako had significantly higher % compact (~44%) compared to Quesnel, Lower Adams, Weaver and Harrison (~36%). RDCM exhibited more of a gradient across populations. Early Stuart, Nechako, Chilko and Quesnel had the highest RDCM (~0.0090%), Lower Adams and Weaver displayed an intermediate RDCM (~0.0075%) and Harrison exhibited the lowest RDCM of all (0.0060%).

Each cardiac morphology variable was significantly correlated with migration difficulty (Table 6.2). Linear regressions between the migration difficulty indices with the strongest Pearson correlation coefficient and each cardiac variable are shown in Figure 6.1. RVM, % compact and RDCM had the strongest correlation coefficients with migration rate, migration effort (distance × Fraser River discharge) and migration distance, respectively. In addition, RDCM significantly correlated with aerobic scope.

6.2.2 Temperature Effects and Sex Differences

Cardiac morphology varied significantly with holding temperature (>5 days of thermal acclimation to 14, 16.5 and 19°C) for males, but not for females (Figure 6.2). Males had a 17% higher RVM at 19^{o}C compared with 14^{o}C. Males also had a significantly higher RVM compared with females at 16.5^{o}C and 19^{o}C. Male fish held at 16.5^{o}C and 19^{o}C had a RDCM that was 19-21% significantly higher compared with male fish held at 14^{o}C. Again, males had a significantly higher RDCM compared to females at 16.5^{o}C and 19^{o}C. Percent compact myocardium did not vary significantly between sexes, or within males as a function of holding temperature. Notably,

GSI did not significantly differ among temperature treatments within sex ($p>0.05$), thereby reducing the possibility that the temperature effects on ventricular composition were related to differences in the state of maturity.

6.2.3 High Temperature Swimming Experiment

There were no statistically significant differences in any of the cardiac variables between male and female Lower Adams sockeye salmon used in the high temperature swimming experiment performed by Steinhausen et al. (2008). Therefore, male and female fish were pooled in order to assess the relationship between the temperature at which the fish failed to continue swimming at approximately 75% of U_{crit} (fail temperature) and the various cardiac variables (Figure 6.3). No relationship was found between any of the cardiac variables and fail temperature ($p>0.05$).

6.3 Discussion

The present study clearly demonstrates for the first time that cardiac morphology can vary among wild populations of the same fish species. As predicted, the differences in ventricular morphology among Fraser River sockeye salmon populations were related to the difficulty of the upriver migration. These findings add to similar discoveries for population-specific variation in aerobic scope (Chapter 3) and gross morphology [e.g. body mass, egg number and energy content (Crossin et al., 2004; Gilhousen, 1980)] according to migration difficulty. The population differences in ventricular morphology likely represent adaptations to

the upriver environment since all fish were sampled early in the migration, prior to encountering the major selective elements. Individuals from a given sockeye salmon population are therefore prepared for the athletic task that lies ahead during the upriver migration, even though they themselves have never experienced the upstream migration conditions. Consistent with previous studies, ventricular morphology was shown to be sexually dimorphic in sockeye salmon in some respects and plastic with response to environmental temperature in male fish.

6.3.1 Population Differences in Ventricular Morphology

The range for RVM (0.09-0.19%) among individual female sockeye salmon corresponds well with values reported for other sexually mature salmonids: sockeye salmon (0.11-0.13%, Clark et al., 2009; Sandblom et al., 2009), rainbow trout (0.07-0.24%, Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992; Graham and Farrell, 1992) but was lower than in sexually mature male chinook salmon near the spawning ground (0.24%, Clark et al., 2008b).

Substantial individual variation was also observed for % compact, ranging between 25 and 50%. This range corresponds with previously reported values for mature sockeye salmon (43%, Sandblom et al., 2009) and chinook salmon (53%, Clark et al., 2008b).

RDCM is a new metric that integrates the two cardiac measures, RVM and % compact, to illustrate how much total myocardium relative to body mass is independent of the venous circulation and instead has a secure oxygen supply via the coronary circulation. RDCM can increase either by maintaining RVM while increasing % compact, or by maintaining % compact while increasing RVM or by increasing both % compact and RVM. As such, a large ventricle with a smaller percentage compact myocardium could have the same total amount of compact

myocardium as a small ventricle with a large percentage myocardium. In both cases, the same total amount of heart is perfused with stable, oxygenated blood via the coronary circulation. RDCM was observed to vary substantially across individual sockeye, between 0.005% and 0.011%.

Beyond individual variation, cardiac morphology also varied considerably across populations. Indeed, RVM, % compact and RDCM varied by 40, 27 and 60% across the seven Fraser River sockeye salmon populations investigated here. Clear differences in cardiac morphology existed between coastal and upriver populations, suggesting that the difficult journey through the Fraser Canyon and Hells Gate likely imposes strong selection pressure for a greater RVM, % compact and RDCM. In addition to this broad classification, other aspects of the upriver migration also appear to influence cardiac morphology. RVM only correlated with migration rate ($p < 0.05$, no correction for multiple comparisons), suggesting that fast swimming requires large ventricles. Alternatively, the primary selection force for RVM may simply have been Hells Gate. Percent compact myocardium correlated with several of the indices (i.e. migration distance, migration duration and ATU) but migration effort had the strongest correlation ($p < 0.006$, Bonferroni level). As such, long river distances with a strong current may necessitate a higher percentage compact myocardium. Finally, RDCM correlated with every difficulty index examined except river slope, and migration distance had the strongest relationship ($p < 0.006$, Bonferroni level). Additionally, RDCM correlated with aerobic scope. Therefore, RDCM appears to be under strong selection pressure across many levels of migration difficulty. None of the ventricular morphology variables significantly correlated with river slope, supporting an earlier finding by Crossin et al. (2004) which suggested that river slope was not a major selective element for gross morphology among Fraser River sockeye salmon populations.

Collectively, these correlational analyses suggest that migratory difficulty is likely a strong selective force responsible for the population-specific differences in cardiac morphology. As discussed in Chapter 3, correlations only provide circumstantial, though promising, evidence of local adaptation (Endler, 1986; Schluter, 2000; Taylor, 1991) and conclusive evidence would require breeding studies. I cannot reject the possibility that differences may be due to developmental plasticity. However, given that the fish were collected early in their migration, had never before encountered the upstream migration conditions and spent the last >2 years in a common ocean environment, I conclude that the observed differences among populations were most likely due to genetic adaptation rather than environmental acclimation (refer to Chapter 3 for further discussion).

6.3.2 Temperature and Sex Differences

Male sockeye salmon had up to 25% more RVM compared to females, depending on the temperature. These results correspond well to previously reported values for sexually maturing sockeye salmon (males had 11-13% greater RVM compared to females, Clark et al., 2009; Sandblom et al., 2009). In contrast, studies performed on sexually mature rainbow trout found a more dramatic, up to 2-fold difference between male and female fish (Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992).

The much larger RVM in mature male rainbow trout has been demonstrated to increase V_s and cardiac power output, which has been hypothesized to support increased functional demands placed on the hearts of spawning male salmon (Franklin and Davie, 1992, Gamperl and Farrell 2004). However, clear evidence supporting this hypothesis from wild migrating fish is

lacking. In the present study, the smaller difference in RVM resulted in no differences in $\dot{Q}$ or V_s between male and female sockeye salmon (Chapter 4). Recent studies suggest that f_H was similar between sexes in sockeye salmon on the spawning ground, though males spent more time with elevated heart rates and had a higher routine $\dot{M}O_2$, both of which can be attributed to increased activity (Clark et al., 2009). In addition, no differences in arterial blood pressure were observed between mature male and female sockeye salmon (Sandblom et al., 2009). Perhaps, compared to wild conditions, the hatchery environment exacerbates the sexual diochotomy because hatchery-reared rainbow trout had significantly smaller RVM compared to wild, migrating trout (Graham and Farrell, 1989), which could potentially allow for a greater scope for cardiac growth with sex development. Collectively, these observations suggest that the remarkable ventricular growth observed in mature male rainbow trout may not be a general characteristic shared with all salmonids. Clearly, more research is needed to address these ideas.

Temperature clearly remodelled the heart in male, but not female, sockeye salmon. RVM and RDCM were 17-21% significantly higher in warm compared to cool temperature-treated male sockeye salmon, which was not due to differences in sexual maturation since GSI did not differ. Percent compact did not significantly differ with temperature treatment. These findings sharply contrast with my hypotheses and previous reports in the literature for rainbow trout. Rainbow trout acclimated to 5°C had a 20-40% higher RVM but a 15-30% decrease in % compact compared to fish held at 15°C (Farrell et al., 1988a, Graham and Farrell, 1989). A larger RVM at cold temperatures has been postulated to compensate for the decrease in contractility associated with cold, helping to maintain stroke volume, cardiac output and power output (Gamperl and Farrell, 2004). Why such a difference in the cardiac remodelling response to temperature exists within the genus *Oncorhynchus* is unclear. It could possibly be attributed to

the different temperatures chosen for the studies, although this remains to be tested. Regardless, the higher RVM with no change in % compact in warm-temperature treated sockeye resulted in a concomitant higher RDCM, meaning that a larger total amount of myocardium was perfused with blood from the coronary rather than the venous circulation. This could enhance oxygen delivery to the ventricle at warm temperature, matching the increased oxygen demand.

The individual cardiac plasticity in male migrating, adult sockeye salmon is quite remarkable given that when the fish enter the river, they are 4-6 weeks from death, have ceased feeding, are in the midst of tremendous physiological flux as secondary sexual characteristics develop and the gonads grow all while performing the enormous athletic task of returning upstream to their spawning ground. This finding demonstrates that male sockeye salmon retain the ability to adjust morphological features when faced with changing environmental variables, even once they have commenced their upstream migration.

Individual variability in cardiac anatomy has been linked to differences in $\dot{Q}_{max}$, $\dot{M}O_{2max}$ and swim performance in rainbow trout (Claireaux et al., 2005). Specifically, poor swimmers had more rounded hearts compared to good swimmers. I similarly sought to examine the possible role of individual variation in cardiac composition on high temperature tolerance. However, no relationship was found between ventricular morphology and high temperature swim performance in fish subjected to a high temperature challenge while swimming near maximally (Steinhausen et al., 2008). Unfortunately, only three fish were truly classified as "poor" swimmers, resulting in very low statistical power. Therefore, these results do not preclude the possibility that population differences in cardiac composition are related to temperature tolerance, or that the cardiac responses to holding temperature could improve temperature tolerance. Rather, more experimental work is needed to test these ideas.

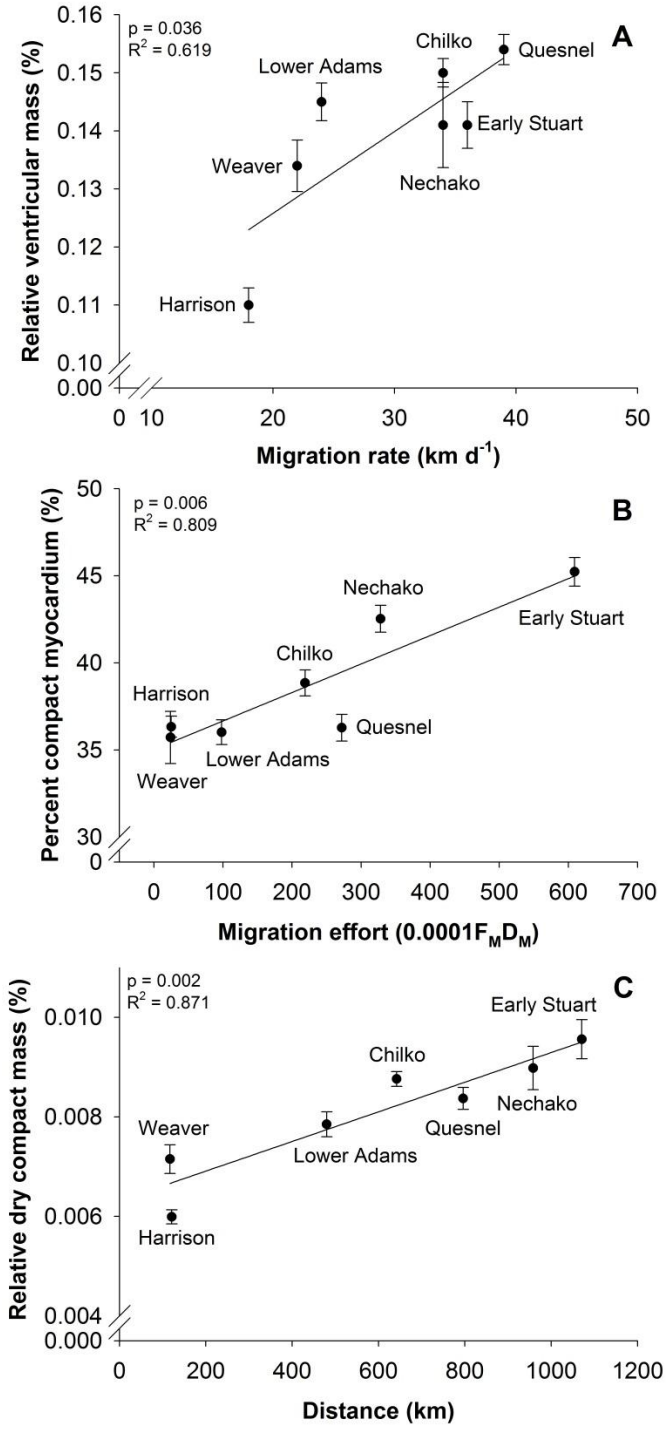

Figure 6.1. Linear regressions between migration difficulty indices and (A) relative ventricular mass (B) percent compact myocardium and (C) relative dry compact mass. Population means ± SEM are presented. The migration difficulty indices with the strongest Pearson correlation coefficient are presented (Table 6.2). F_M, Fraser River Discharge, D_M, distance to spawning grounds. Only female sockeye salmon were compared.

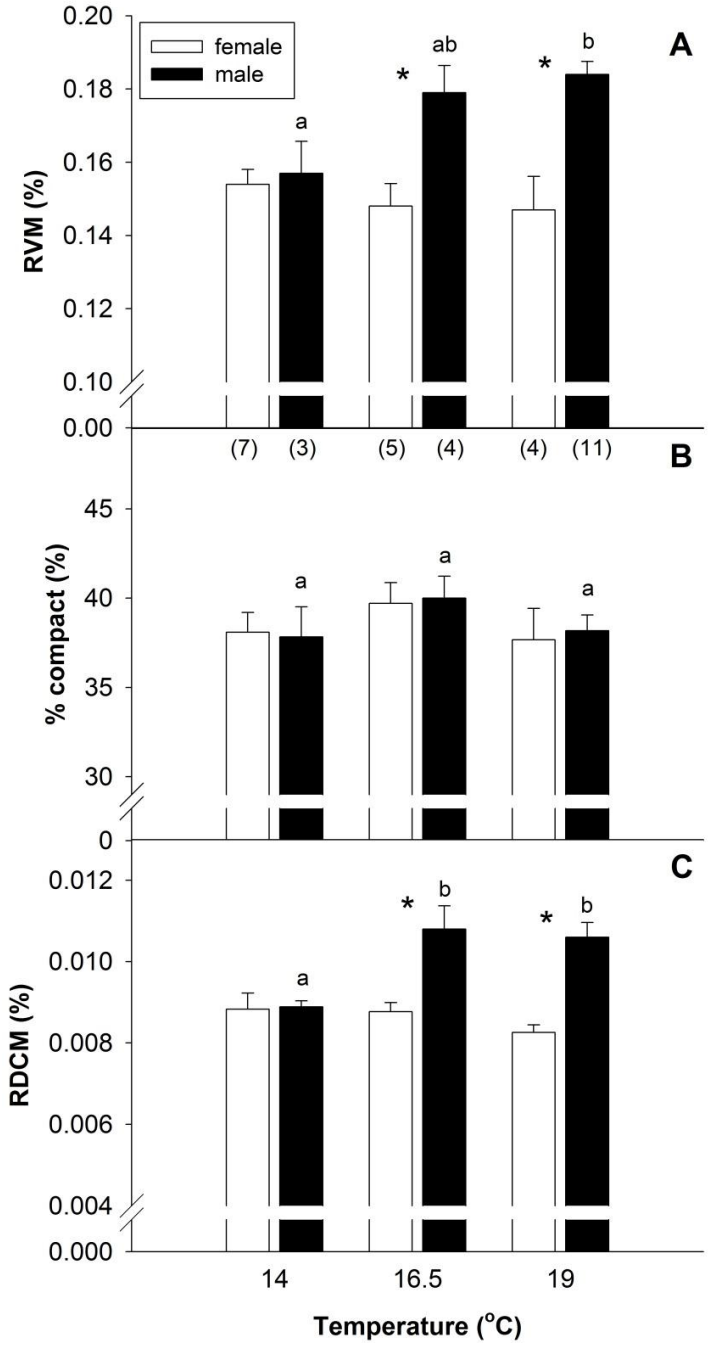

Figure 6.2. (A) Relative ventricular mass (RVM), (B) percentage compact myocardium (% compact), and (C) relative dry compact mass (RDCM) are shown for male and female Chilko sockeye salmon acclimated to 14, 16.5 and 19°C for at least 5 days before death. N values are indicated in parentheses. An asterisk indicates a statistically significant difference between male and female fish. Significant differences with temperature treatment among male sockeye are indicated by differing letters. There were no significant differences with temperature treatment among female sockeye ($p>0.05$).

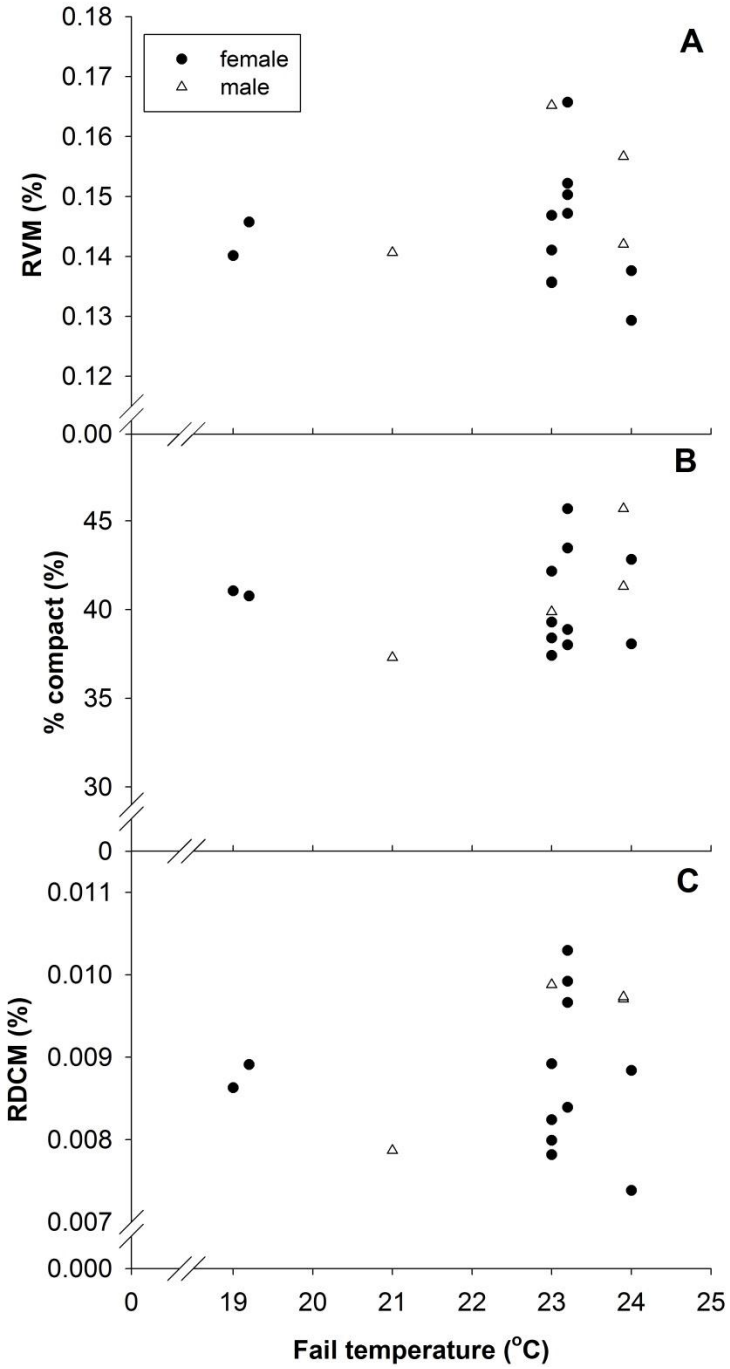

Figure 6.3. (A) Relative ventricular mass (RVM), (B) percentage compact myocardium (% compact), and (C) relative dry compact mass (RDCM) as a function of the temperature at which Lower Adams sockeye salmon failed to continue swimming at approximately 75% of U_{crit} (fail temperature). Each point corresponds to an individual fish. Males and females are indicated (open triangles and closed circles, respectively); however, none of the cardiac variables differed significantly with sex. Therefore, statistical analysis was performed on the entire group. No relationship was found between any of the cardiac variables and fail temperature ($p>0.05$).

Table 6.1. Relative ventricular mass (RVM), percentage compact myocardium (% compact) and relative dry compact mass (RDCM) for each sockeye salmon population (mean ± SEM). Only female sockeye salmon from each population were compared. Populations with differing letters are significantly different within each variable.

Population	Spawning location	n	RVM (%)	% compact	RDCM (%)
Early Stuart	upriver	7	0.141 ± 0.004^{ab}	45.2 ± 0.8^{a}	0.0096 ± 0.0004^{a}
Nechako	upriver	9	0.141 ± 0.007^{ab}	42.5 ± 0.8^{ab}	0.0090 ± 0.0004^{a}
Quesnel	upriver	11	0.154 ± 0.003^{a}	36.3 ± 0.8^{c}	0.0084 ± 0.0002^{ab}
Chilko	upriver	35	0.150 ± 0.002^{a}	38.9 ± 0.7^{bc}	0.0088 ± 0.0001^{a}
Lower Adams	upriver	21	0.145 ± 0.003^{ab}	36.0 ± 0.7^{c}	0.0079 ± 0.0003^{bc}
Weaver	coastal	11	0.134 ± 0.004^{b}	35.7 ± 1.5^{c}	0.0072 ± 0.0003^{c}
Harrison	coastal	13	0.110 ± 0.003^{c}	36.3 ± 0.6^{c}	0.0060 ± 0.0001^{d}

Table 6.2. Pearson correlation matrix relating relative ventricular mass (RVM), percentage compact myocardium (% compact), relative dry compact mass (RDCM) of female fish from seven sockeye salmon populations and eight migration difficulty variables (see Table 2.1). ATU = accumulated thermal units, F_M = Fraser River discharge. Bold font indicates the migration difficulty variable with the highest correlation coefficient for a given physiological variable. Three critical values are indicated: $p < 0.05$ (no correction for multiple comparisons), $p < 0.018$ (Benjamini and Yekutieli False Discovery Rate) and $p < 0.006$ (Bonferroni).

	RVM	**% compact**	**RDCM**
Migration distance (D_M)	0.626	0.801*	**0.933**‡
Migration elevation (E_M)	0.744	0.482	0.812*
Work ($0.0001 \bullet E_M \bullet D_M$)	0.662	0.725	0.913‡
River slope ($500(E_M D_M^{-1})$)	0.736	0.170	0.607
Migration effort ($0.0001 \bullet D_M \bullet F_M$)	0.426	**0.899**‡	0.866†
Migration duration	0.598	0.785*	0.906‡
Migration rate	**0.787***	0.558	0.880†
ATU	0.580	0.833*	0.925‡
Aerobic scope	0.354	0.748	0.893†
RVM	-	0.164	0.764*
% compact	-	-	0.761*
RDCM	-	-	-

* $p < 0.05$; † $p < 0.018$, ‡ $p < 0.006$

CHAPTER 7: THE EFFECT OF TEMPERATURE ACCLIMATION ON MYOCARDIAL β-ADRENOCEPTOR DENSITY AND LIGAND BINDING AFFINITY IN TWO POPULATIONS OF SOCKEYE SALMON

7.1 Introduction

The preceding chapters clearly demonstrated a link between maximum aerobic scope and maximum cardiac performance (Chapters 3, 4 and 5) and provided evidence that the decline in aerobic scope above T_{opt} is driven by cardiac collapse (Chapter 5). Similar to aerobic scope, ventricular composition also differed among populations and is likely under strong selection pressure by the upriver migration conditions (Chapters 3 and 6). Therefore, I again turned to the heart in order to examine whether adrenergic cellular stimulation was an important mechanism for high thermal tolerance in sockeye salmon.

Adrenergic stimulation is critical for increasing cardiac performance during exercise and maintaining cardiac performance at temperature extremes in salmonids (Hanson et al., 2006; Hanson and Farrell, 2007; Keen et al., 1993; Shiels et al., 2002). Adrenergic stimulation has both chronotropic (rate) and ionotropic (force) effects on the teleost heart (Ask, 1983; Axelsson et al., 1987; Cobb and Santer, 1973; Farrell et al., 1986; Farrell et al., 1982; Laurent et al., 1983; Temma et al., 1986; Vornanen, 1989). These effects are mediated through the β-adrenoceptor (β-AR) signalling pathway, which primarily involves a β_2 subtype in salmonids (Ask et al., 1980; Ask et al., 1981; Gamperl et al., 1994; Keen et al., 1993). Cardiac adrenergic stimulation is possible through both sympathetic and humoral (catecholamines are released from chromaffin tissue within the head kidney) routes in salmonids, though many other fish lack sympathetic

cardiac innervation (Donald and Campbell, 1982; Farrell and Jones, 1992; Laurent et al., 1983). Indeed, circulating catecholamines increased 10-fold above resting levels in rainbow trout swum to 2 bl s^{-1} and as much as 92-fold in rainbow trout chased to exhaustion (Butler et al., 1986; Perry et al., 1996).

Cardiac sensitivity to adrenaline changes with temperature acclimation in rainbow trout (Ask et al., 1981; Farrell et al., 1996), which has partly been attributed to changes in cell surface β-AR density (Gamperl et al., 1998; Keen et al., 1993). Specifically, cardiac adrenergic stimulation protects rainbow trout cardiac function at low temperatures (Hanson and Farrell, 2007; Graham and Farrell, 1989; Keen et al., 1993), and β_2-AR density (B_{max}) increased almost 3-fold in cold-acclimated rainbow trout (Keen et al., 1993). However, adrenergic stimulation and protection diminishes at high temperatures in rainbow trout (Farrell et al., 1997; Hanson and Farrell, 2007). In light of these findings, an elevated B_{max} at warm temperatures could improve cardiac performance and protection, leading to enhanced thermal tolerance.

I sought a mechanistic explanation for the observed differences in thermal tolerance among populations of Fraser River sockeye salmon. Chilko and Nechako sockeye salmon are co-migrating populations that enter the river at the same time and encounter similar warm water temperatures and velocity conditions in the lower Fraser River and Hells Gate (Table 2.1, 3.5). Both the Chilko and Nechako populations have difficult migrations, traveling 642 and 958 km upstream and reaching 1174 and 716 m in elevation, respectively. However, Chilko sockeye salmon spend the final third of their migration ascending the steep, cool Chilcotin River and spawn in or near a glacial lake. Chilko sockeye salmon correspondingly displayed an exceptionally high and broad thermal tolerance compared with Nechako sockeye salmon (Chapter 3). I hypothesized that Chilko sockeye salmon would have a greater β_2-AR density in

ventricular tissues compared to Nechako sockeye salmon. To test this hypothesis, I compared cell surface B_{max} and β_2-AR binding affinity (K_d) from Chilko and Nechako sockeye salmon exposed to 13, 19 and 21°C for 4 days. Detailed materials and methods are provided in Chapter 2 (sections 2.2, 2.9 and 2.10.3).

7.2 Results

All Chilko sockeye salmon survived the 4-day treatments at 13, 19 and 21°C, as did all the Nechako sockeye salmon at 13 and 19°C. In contrast, only 4 out of 21 Nechako sockeye salmon (19%) survived the 4-day treatment at 21°C.

There were no significant differences in gross body morphology among temperature treatments or between sexes within a population, except for the significantly higher gonadosomatic index (GSI) of females compared to males in both populations (Table 7.1). Body mass, fork length and condition factor did not significantly differ between the two populations. However, Chilko sockeye salmon had a significantly higher relative ventricular mass (RVM) and a higher GSI compared to Nechako sockeye salmon (Table 7.1).

Independent of the temperature treatment, Chilko sockeye salmon had a 2-fold higher B_{max} compared to Nechako sockeye salmon (Fig 7.1). Furthermore, B_{max} significantly increased when Chilko sockeye salmon were warmed to 19° and 21°C from 13°C (Fig 7.1). In contrast, temperature exposure had no effect on B_{max} in Nechako sockeye salmon. Thus, not only did Chilko sockeye salmon have a greater B_{max} compared to Nechako, they actually increased B_{max} in response to warming.

In contrast, K_d did not significantly differ between populations or with temperature treatment (Fig 7.2).

7.3 Discussion

Nechako and Chilko sockeye salmon populations clearly differ in both ventricular B_{max} and the ability to alter cell surface B_{max} within a short (4-day) thermal acclimation period. The response to warming in Chilko sockeye salmon sharply contrasts with previous studies that showed a decreased in B_{max} with warm acclimation in rainbow trout (Gamperl et al., 1998; Keen et al., 1993). Chilko sockeye salmon clearly have a higher thermal tolerance compared to Nechako sockeye salmon, as is evident from the respective aerobic scope Fry curves (Chapter 3), and the observation that only 19% of Nechako sockeye salmon survived the 4-day temperature treatment at 21°C, while all the Chilko sockeye salmon survived. I propose that the elevated B_{max} for Chilko sockeye salmon may provide greater cardiac performance and protection at temperature extremes and thus may be one of the mechanisms leading to their broader and higher thermal tolerance relative to the Nechako population.

7.3.1 Differences in B_{max} between Populations

Rainbow trout acclimated to 6°C were included as a reference group to confirm the quality of the assay. The present study's results (B_{max} = 36.3 fmol mg protein^{-1} and K_d = 0.23 nM) fall within expected values. Previous studies reported a B_{max} of 23-26 fmol mg protein^{-1} and K_d of 0.13-0.19 nM for rainbow trout acclimated to 12-14°C (Gamperl et al., 1998; Hanson et al.,

2005; Olsson et al., 2000). Gamperl et al. (1994) reported a higher B_{max} and comparable K_d (40 fmol mg protein^{-1} and 0.25 nM) for rainbow trout acclimated to a colder temperature (8°C) in seawater. These results for rainbow trout provide confidence in the assay technique.

B_{max} for Chilko sockeye salmon was at least twice as high as any other salmonid (Gamperl et al., 1994; Gamperl et al., 1998; Hanson et al., 2005; Olsson et al., 2000). Similary, their B_{max} was also more than twice that of non-salmonid athletic fish species: mahimahi (*Coryphaena hippurus*) (46.9 fmol mg^{-1} protein^{-1}), skipjack tuna (*Katsowonus pelamis*) (41.3 fmol mg^{-1} protein^{-1}), yellowfin tuna (*Thunnus albacares*) (25.7 fmol mg^{-1} protein^{-1}), and Pacific mackerel (*Scomber japonicus*) (27.2 fmol mg^{-1} protein^{-1}) (Olsson et al., 2000). Only the winter flounder (*Pleuronectes americanus*) (B_{max} = 252 fmol mg^{-1} protein^{-1}) has a higher B_{max}, however, the binding affinity was extremely low, leading the investigators to propose that flounder hearts may also be populated by β_3-adrenoreceptors (Mendonca and Gamperl, 2009). In contrast, Nechako sockeye salmon displayed B_{max} values that were similar to mahi-mahi, skipjack tuna and previous estimates of sockeye and chinook salmon (Gamperl et al., 1998; Olsson et al., 2000). Both sockeye salmon populations displayed B_{max} values well above those determined for African catfish (*Claris gariepinus*) (14.3-17.8 fmol mg^{-1} protein^{-1}), warm acclimated-rainbow trout (12-14°C, 23-26 fmol mg^{-1} protein^{-1}) and an Antarctic nototheniid (*Trematomus bernacchii*) (10.5 fmol mg^{-1} protein^{-1}) (Hanson et al., 2005; Olsson et al., 2000).

This experiment on wild fish was conducted under a very controlled setting and many of the potential confounding factors for this population comparison were removed or minimized. For example, all the salmon were collected at the same time and over a period of two days. The fish were collected very early, ~1-3 days into the upriver migration; therefore, they had yet to experience the majority of the upriver conditions and had experienced a common ocean and river

migration environment prior to capture. As a result, population differences were unlikely due to a plastic response to differential environmental conditions encountered prior to capture. Furthermore, all fish were held for the same amount of time and in the same tanks according to temperature treatment, thus eliminating the possibility for differential plastic responses after capture. Also, the differences in B_{max} were unlikely due to variation in the level of sexual maturation since previous studies suggest that gonadal steroid hormones do not modulate B_{max} in mature rainbow trout or chinook salmon (Gamperl et al., 1994; Gamperl et al., 1998). Finally, B_{max} was expressed per mg protein; therefore, the significant difference in RVM was not a factor. However, it is interesting to note that the larger RVM in Chilko sockeye salmon amplifies the difference in the total number of receptors on the ventricle.

7.3.2 Temperature Effects on B_{max}

The increase in B_{max} with warming in Chilko sockeye salmon was a novel response for fish and was not seen in Nechako sockeye salmon. Two previous studies with rainbow trout showed the opposite pattern, namely, an 11% decrease in B_{max} per °C increase in temperature (Gamperl et al., 1998; Keen et al., 1993).

Notably, the acclimation duration in the present study (4 days) was much shorter than previous studies (typically >3 weeks, Gamperl et al., 1998; Hanson et al., 2005; Keen et al., 1993). The assay used in the present study cannot determine whether the additional β-adrenoceptors were synthesized *de novo* or whether they were simply released from vesicles contained within the cell. Moreover, the time interval required to alter β_2-AR expression is unknown for fish and is an area of research should be pursued in future studies.

A case has been made for the importance of β_2-AR to *stimulate* maximum cardiac performance during exercise and at high temperature in salmon (Butler et al., 1986; Hanson et al., 2006; Hanson and Farrell, 2007; Randall and Perry, 1992) and to *protect* maximum cardiac function against a harmful acidotic and hypoxic venous environment, especially at high temperatures (Hanson and Farrell, 2007; Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977). *In vitro* and *in situ* perfused heart studies have consistently shown that acidic and hypoxic conditions lead to impaired cardiac contraction (Dridezic and Gesser 1994; Farrell et al., 1986; (Farrell et al., 1988b; Farrell and Milligan, 1986; Gesser et al., 1982; Gesser and Jorgensen, 1982; Kalinin and Gesser, 2002) and adrenergic stimulation plays a key role in maintaining or enhancing cardiac function under these conditions (Driedzic and Gesser, 1994; Hanson et al., 2006; Hanson and Farrell, 2007; Nielsen and Gesser, 2001).

When the ventricular cell-surface β_2-AR is activated, the signalling pathway ultimately increases intracellular calcium delivery to the cardiomyocytes, which enhances both the force and rate of cardiac contraction. Thus, calcium handling in the cardiomyocytes may play a critical role in temperature tolerance in sockeye salmon. Calcium cycling and sarcoplasmic reticulum function has been demonstrated to be critical for the broad temperature tolerance in bluefin tuna (Castilho et al., 2007; Landeira-Fernandez et al., 2004; Shiels et al., 2011). Specifically, bluefin tuna have more sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2) in their cardiomyocytes, which regulates Ca^{2+} uptake into the sarcoplasmic reticulum, compared to warmer species that do not have to cope with a wide range of temperatures (Castilho et al., 2007; Landeira-Fernandez et al., 2004). Future studies should examine whether Chilko sockeye salmon similarly have more SERCA2 compared to populations with a more narrow optimal thermal range.

Given that the reduction in aerobic scope at high temperatures can likely be attributed to a limitation in maximum cardiovascular performance (Chapter 5), increased B_{max} could lead to superior thermal tolerance. Consequently, the exceptionally high and broad thermal tolerance of Chilko sockeye salmon may be due, at least in part, to elevated B_{max}. This hypothesis should be further investigated using perfused heart studies with Chilko and Nechako sockeye salmon.

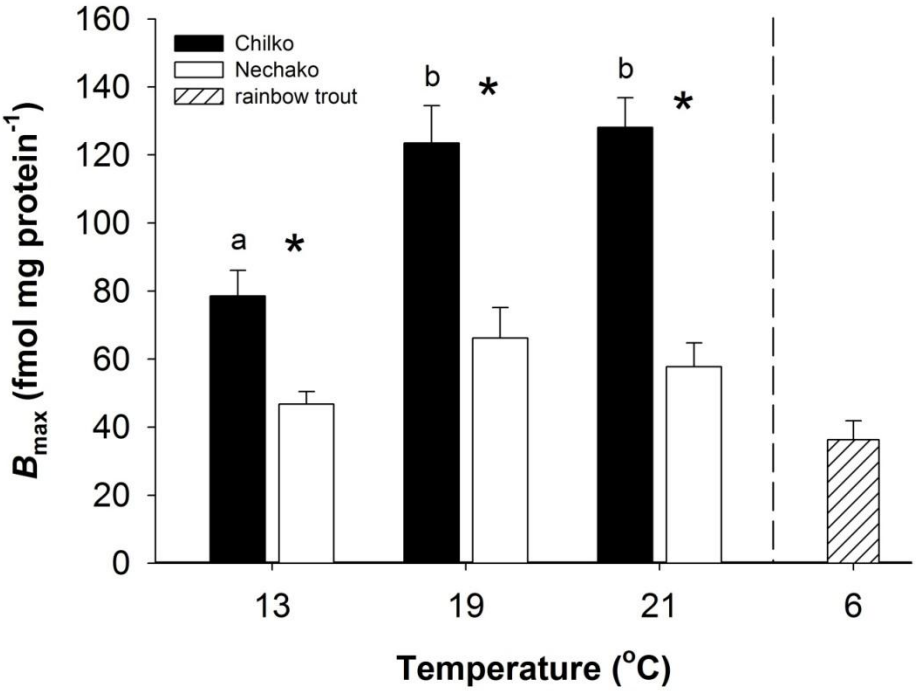

Figure 7.1. Ventricular β-adrenoceptor density (B_{max}). Significant differences between populations are indicated by * ($p < 0.001$). Significant differences between temperature treatments existed only for Chilko sockeye salmon and are indicated by differing letters. Rainbow trout were included as a reference group to confirm the assay technique.

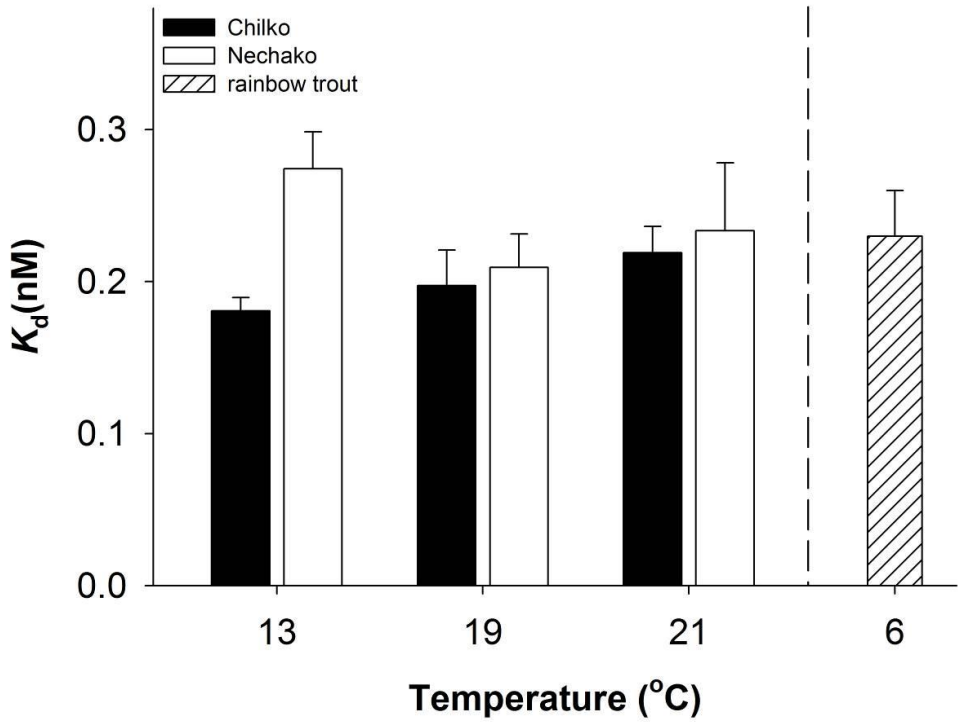

Figure 7.2. Ventricular β-adrenoceptor [3H]CGP-12177 dissociation constant (K_d). No significant differences were detected among sockeye salmon groups. Rainbow trout acclimated to 6°C in freshwater were included as a reference group to confirm the assay technique.

Table 7.1. Gross morphology for fish used in β-AR experiment. Relative ventricular mass (RVM) and gonadosomatic index (GSI) are indicated. Mean ± SEM are presented. Significant differences within and between populations are indicated by differing letters and an asterisk, respectively. N-values for GSI are indicated in parentheses.

	Chilko	Nechako	Rainbow trout
n	16	18	7
body mass (kg)	2.14 ± 0.10	2.31 ± 0.10	1.88 ± 0.08
fork length (cm)	58.0 ± 0.7	58.5 ± 0.6	47.1 ± 1.2
condition factor	1.09 ± 0.02	1.14 ± 0.03	1.81 ± 0.08
RVM %	0.156 ± 0.003	0.145 ± 0.004*	0.118 ± 0.009
GSI (males) %	2.3 ± 0.2 (9)^{a}	1.4 ± 0.1 (10)^{y}*	1.8 ± 0.5
GSI (females) %	5.1 ± 0.3 (7)^{b}	3.8 ± 0.2 (8)^{z}*	

CHAPTER 8: CONCLUSIONS

The general objective of this thesis was to examine the physiological basis for thermal tolerance among sockeye salmon populations. I hypothesized that thermal limits are set at a local level by physiological limitations in aerobic performance due to cardiac collapse.

In support of this hypothesis, my research suggests that sockeye salmon populations in the Fraser River watershed have physiologically adapted to meet the specific challenges of their local upriver migration conditions. Thermal optima for each population coincided with the river temperatures typically encountered during upstream migration. Temperatures exceeding the population-specific thermal optimum resulted in severely impaired aerobic scope and swimming performance. My research further suggests that fish are unable to swim at warm temperatures due to insufficient oxygen supply to meet the swimming muscles' demand, triggered via a cardiac limitation. Finally, I identified that thermal tolerance differs across sockeye salmon populations and suggest a potential mechanism for enhanced thermal tolerance in Chilko sockeye salmon. All told, important management and conservation implications may emerge from my research. I identified a possible cause for in-river mortality associated with warm temperatures in sockeye salmon and I identified certain populations most vulnerable to climate change.

8.1 Local Adaptation in Fraser River Sockeye Salmon Populations

The lifetime fitness of millions of sockeye salmon that annually return to the Fraser River depends on a physically demanding upriver migration. During this once-in-a-lifetime event, fish swim continuously against a fast flowing river for several weeks at ground speeds of 20 to 40 km

day^{-1} (English et al., 2005). Feeding ceases in the ocean and upriver swimming is fuelled entirely by endogenous energy stores. Because sockeye salmon return to natal spawning grounds with remarkable fidelity, the Fraser River is home to more than 100 genetically and geographically distinct populations (Beacham et al., 2005), each of which experiences variable upriver migration conditions, depending on when they enter the river and where they spawn. Thus, populations vary in migration distance (100 to 1100 km), elevation gain (10 to 1200 m), river temperature (9° to 22°C), and river flow (2000 to 10,000 m^3 s^{-1}). Reproductively isolated populations can potentially adapt to the environmental conditions that induce maximal aerobic challenges, which for sockeye salmon likely occur during the upriver spawning migration. Indeed, local migratory conditions apparently exert strong selection pressure for adaptation because morphological and behavioural characteristics (gross somatic energy, body morphology, egg number and swimming behaviour) correlate with river migration distance, elevation gain and/or work (distance x elevation gain) in sockeye salmon (Crossin et al., 2004; Hinch and Rand, 2000). Therefore, I hypothesized that physiological adaptation in sockeye salmon occurs locally at the population level, reflecting the specific river migration conditions.

I applied an established conceptual and mechanistic framework for understanding temperature effects on aquatic ectotherms, the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis (Pörtner 2002, Pörtner and Knust, 2007, Pörtner and Farrell, 2008). OCLTT attributes the decline in aerobic scope (the difference between resting and maximal oxygen consumption rates) above an animal's optimal temperature (T_{opt}) to capacity limitations of the organs systems that deliver oxygen to the tissues. The expectation is that local adaptations should extend to multiple levels of the cardiorespiratory system, explaining intraspecific variation in thermal tolerance and aerobic scope.

Eight sockeye salmon populations spanning a range of river migration difficulties were used to varying degrees in my study. Migration difficulty was quantified using various environmental river characteristics: distance, elevation gain, temperature, migration rate, duration, work, river slope and migration effort. I predicted that migration distance, elevation gain and work would exert the strongest selection pressure on aerobic scope given their importance in selecting for morphological traits (Crossin et al., 2004). I measured individual cardiorespiratory performance (N = 97) as a function of temperature in four populations. Aerobic scope curves for each population were significantly related to the historic range in river temperature they experienced, a finding consistent with two additional Fraser River sockeye salmon populations (Farrell et al., 2008; Lee et al., 2003c). The coastal Weaver sockeye salmon experience the coldest temperatures and had the lowest T_{opt} (14.5°C) whereas the upriver populations experience similar river temperatures and accordingly had a similar T_{opt} (range 16.4-17.2°C). The upriver Chilko population displayed an unusually broad optimal thermal range that corresponded with the lower temperatures encountered during their difficult migration in the Chilcotin watershed and the high temperatures encountered while migrating through Hells Gate. In addition, significant differences in maximum aerobic scope among the populations were positively correlated with the distance to the spawning ground. These results suggest population level adaptation of maximum aerobic scope to selection imposed by river conditions encountered during migration.

Given that cardiac capacity and aerobic scope are tightly related (Farrell, 2009), I expected populations with the greatest migratory demands to display similar adaptations in cardiac morphology and performance. Relative ventricular mass (RVM), percentage compact myocardium (% compact; the proportion of the ventricle supplied with coronary blood flow) and

relative dry compact mass (RDCM) significantly differed among populations. All three morphological variables were significantly greater for upriver compared with coastal populations, suggesting that the hydraulically challenging sections of the river may impose selection on heart morphology. In addition, correlations between cardiac morphology and migration difficulty, and maximum aerobic scope with RDCM, provide promising evidence for local adaptation to river conditions on an even finer scale (Endler, 1986; Schluter, 2000; Taylor, 1991). Furthermore, aerobic scope, cardiac scope and scope for heart rate were all positively correlated, and varied in parallel with river temperature, suggesting that the temperature dependence of cardiac performance is linked to that of aerobic capacity at the population level. In contrast, maximum cardiovascular performance did not significantly differ among upriver populations, though notably, coastal populations were not included in the analysis. I predict that cardiovascular performance in coastal populations would be reduced compared to upriver populations, mimicking the trend observed with aerobic scope.

Altogether, this is the first ever large-scale study to demonstrate how wild fish within a single watershed are physiologically fine-tuned to their migration environment. I found a strong relationship between the difficulty of river migration and the cardiorespiratory physiology and cardiac morphology of the populations examined. Furthermore, optimal water temperature for aerobic swimming matched the typical water temperatures historically encountered by each population.

The failed attempt to transplant coastal sockeye salmon to upriver spawning grounds in order to help re-establish populations decimated by the 1913 Hells Gate rockslide (Ricker, 1972) provides a cautionary tale to managers. It is becoming clear that coastal populations are not adapted for the more arduous upriver migration and are ill-equipped to complete the more

difficult migration (Taylor, 1991). Combining my results with those found in the literature (Crossin et al., 2004; Gilhousen, 1980; Lee et al., 2003c), the upriver, more athletic populations (Early Stuart, Nechako, Chilko and Quesnel) can be characterized as having more somatic energy at the start of their migration, fewer eggs, a smaller, more fusiform body shape, higher aerobic scope, more energetically efficient swimming behaviour and larger hearts with more compact myocardium compared to coastal populations (Weaver and Harrison). Lower Adams sockeye salmon fall somewhere in-between these extremes.

8.2 Mechanism of Cardiorespiratory Collapse at High Temperature

Sockeye salmon exposed to temperatures above their population-specific T_{opt} had severely impaired aerobic swimming performance. However, the mechanism of this decline in aerobic scope is poorly understood. Using the OCLTT hypothesis as a framework, I predicted that an oxygen limitation could occur at the level of the gills, the heart or the muscle. By simultaneously measuring oxygen consumption, cardiac output and arterial and venous oxygen status in fish swimming to U_{crit}, I comprehensively examined these possibilities for the first time.

Corroborating earlier work for sockeye salmon swimming at ~75% of U_{crit} (Steinhausen et al., 2008), my data showed that scope for f_H collapsed at a lower temperature than either aerobic scope, cardiac scope or scope for V_s. Thus, my data give weight to the idea that reduced scope for f_H above T_{opt} is the triggering factor that limits maximum $\dot{Q}$ and the capacity of the cardiorespiratory system to transport oxygen. There was no evidence of a gill limitation since P_{aO2} and C_{aO2} remained constant at temperatures above T_{opt}. Furthermore, there did not appear to be an immediate diffusion limitation at the muscle since P_{vO2} and C_{vO2} did decline with further

warming above T_{opt}, though a diffusion limitation may have developed at the tissues at the warmer temperatures (Wagner, 1996). All told, the initiating step leading to a mismatch between oxygen supply and demand at the swimming muscle appears to be a cardiac limitation due to reduced scope for heart rate.

8.3 Potential Mechanism for Enhanced Thermal Tolerance

I sought a mechanistic explanation for the observed intraspecific variation in thermal tolerance. Cardiac adrenergic stimulation protects salmonid cardiac function at low temperatures (Keen et al., 1993; Shiels et al., 2002) and against the negative effects of acidosis and hypoxia during exercise (Hanson and Farrell, 2007), but protection diminishes at high temperatures associated with declining aerobic scope (Hanson and Farrell, 2007; Keen et al., 1993). Therefore, I hypothesized that the unusually broad and high thermal tolerance of the Chilko population would reflect a greater density of adrenaline-binding ventricular β-adrenoceptors compared with the co-migrating Nechako population that has a narrower and lower thermal tolerance. I determined ventricular β-adrenoceptor density (B_{max}) and binding affinity (K_d) in fish that had been held for 4 d at 13, 19 or 21°C. At all three temperatures, Chilko had a significantly higher B_{max} compared with Nechako sockeye salmon (K_d did not differ) and over twice that previously measured for salmonids. In contrast to rainbow trout (Gamperl et al., 1998; Keen et al., 1993), B_{max} increased significantly when Chilko sockeye salmon were warmed to 19 and 21°C from 13°C. Thus, not only did Chilko sockeye salmon have a greater B_{max} compared to Nechako, they actually increased B_{max} in response to warming. Consequently, elevated ventricular β-adrenoceptor expression for Chilko sockeye salmon may provide greater cardiac capacity and

protection at temperature extremes, expanding their thermal tolerance compared with the Nechako population.

8.4 Conservation and Management Implications

Warm river temperatures have been repeatedly associated with high in-river mortality in ecologically, economically and culturally important Fraser River sockeye salmon. Mortality clearly differs across populations and among years in sockeye salmon (Hinch and Martins, 2011). My results support the hypothesis that continued increases in summer river temperatures will result in population-specific responses of sockeye salmon (Farrell et al., 2008).

The sockeye salmon populations included in my study clearly differ in T_{crit} (when aerobic scope is zero and fish survival is passive, time-limited and supported by anaerobic metabolism). While upstream migration is obviously impossible at T_{crit}, exactly how much aerobic scope is required for successful river migration is unknown. A biotelemetry study with Weaver sockeye salmon suggests that at least 50% of aerobic scope is needed [<10% of fish reached their spawning area at 18-21°C when aerobic scope is 0-68% of maximal (Farrell et al., 2008; Mathes et al., 2010)]. However, given that all the upriver populations studied here have 89-97% of maximum aerobic scope at the upper 90th percentile of historic temperatures encountered, perhaps ~90% of aerobic scope is necessary over a broader time scale for upriver populations experiencing greater migration difficulty. Accordingly, temperatures exceeding the population-specific upper T_p (temperature corresponding to 90% of maximum aerobic scope, which includes current temperature maxima of 21.5°C) could limit successful migrations due to a functional collapse in aerobic scope. Empirically, no sockeye salmon population has initiated river

migration at a temperature exceeding 21°C (Hyatt et al., 2003), nor has a historic mean migration temperature been above 19°C (Hodgson and Quinn., 2002). However, Chilko sockeye salmon may emerge as "superfish" with greater resilience to climate change by being able to maintain cardiorespiratory performance at higher temperatures. Conversely, Weaver and Nechako populations appear especially susceptible to high temperature. If Weaver sockeye salmon continue to enter the Fraser River up to six weeks earlier than normal (Cooke et al., 2004), exposing themselves to such high temperatures, high en-route mortality will continue (Cooke et al., 2004; Farrell et al., 2008; Mathes et al., 2010,).

In summary, while warming water temperatures are undoubtedly a global issue for fishes at the species level, I propose a concern at the population level for Fraser River sockeye salmon. Since current warming trends in the Fraser River (1.9°C during the last 60 years) are expected to continue (Ferrari et al., 2007; Morrison et al., 2002), survival of sockeye salmon populations will require some combination of behavioural adaptations (to avoid high temperatures by entering the river when it is cooler) and physiological adaptations (a higher T_p to increase high temperature tolerance). Substantial shifts in entry timing are unlikely due to energy and time constraints to achieve highly conserved spawning dates. On the other hand, warming river temperatures could exert strong selective pressure for physiological adaptation. Physiological adaptation requires trait heritability, trait variability and differential fitness. Evidence of all three have been presented here: local adaptation of cardiorespiratory traits, individual variability in these traits and zero lifetime fitness for fish failing to complete their upriver migration. The salmonid genome clearly has the capacity for higher thermal tolerance [current thermal extremes are documented for redband trout (*Oncorhynchus mykiss*) which experienced 15-27°C diurnally, acutely tolerated 29°C and demonstrated a plateau in aerobic scope at 26°C (Rodnick et al.,

2004)], suggesting that there is potential for future physiological adaptation in Fraser River sockeye salmon. I suggest that adaptations at the level of the heart that sustain cardiac performance at high temperatures, such as the increased ventricular β-adrenoceptor density displayed in Chilko sockeye salmon, could be beneficial in this regard. The current challenge is determining whether the rates and extents of physiological adaptation for Fraser River sockeye salmon will allow them to adapt quickly enough to cope with the current warming trend.

8.5 Future Directions

This thesis has identified numerous future directions for further research into questions surrounding the physiological basis of thermal tolerance and local adaptation in fishes.

First, the correlational evidence relating physiological variability to migration difficulty presented here only provides promising, but not definitive, evidence of local adaptation. Therefore, breeding studies should be conducted to look for more conclusive evidence of adaptation. In addition, this thesis only examined a single, brief stage of the life cycle of a sockeye salmon. Indeed, the upriver migration represents only ~2% of a sockeye salmon's entire life. It would be interesting to examine thermal tolerance and physiological variability across populations in other life stages. In addition, cardiovascular physiology should be examined in adult sockeye salmon from coastal populations to determine if the trends for aerobic scope and cardiac morphology extend to cardiac performance.

This thesis also opens up a myriad of questions regarding cardiorespiratory collapse at high temperature. Comparisons of ultrastructure in heart and skeletal muscle (e.g. capillary and mitochondria density) across populations varying in thermal tolerance and athletic ability would

provide insight into the role of muscle morphology in limiting oxygen diffusion at high temperature and with exercise. Furthermore, the cause of the bradycardia and cardiac disrhythmia during high temperature swimming is unknown. Studies using atropine to block vagal tone should be conducted to determine if the bradycardia and disrhythmia are under cholinergic control. Notably, I was unable to investigate the possibility that thermal tolerance differs between male and female sockeye salmon, which is an area of research that should be pursued. Studies measuring blood flow distribution would also be beneficial, though there are problems with microsphere technique in fish (Farrell et al., 2001b). Direct measurement of gonadal blood flow during swimming and high temperature exposure would provide insight into important trade-offs between swimming and gonad development since all tissues cannot be simultaneously perfused. Finally, the capacity for migrating adult sockeye salmon to acclimate and the role of phenotypic plasticity in temperature tolerance is still poorly understood. Temperature studies should be used to examine the possibility and timecourse for acclimation from the gene to whole animal level.

A major concern emerging from my thesis is that populations are already experiencing temperatures at their upper thermal limit. Since Fraser River temperatures are expected to continue to warm along the same trajectory (~2°C over 60 years, Ferrari et al., 2007; Morrison et al., 2002), populations will have to adapt in order to cope. However, we don't know which or if any populations will be able to adapt quickly enough to keep pace with the warming temperatures. Therefore, studies examining the rate and extent of physiological adaptation are necessary.

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