THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

FINAL REPORT
DON PEDRO PROJECT

Prepared for:
Turlock Irrigation District – Turlock, California
Modesto Irrigation District – Modesto, California

February 2017

Thermal Performance of Wild Juvenile *Oncorhynchus mykiss* in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature[1]

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FOREWORD

In July 2011, as part of the Don Pedro Hydroelectric Project (No. 2299) Federal Energy Regulatory Commission (FERC) relicensing proceeding, Turlock Irrigation Districts (TID) and Modesto Irrigation District (MID) (collectively, the Districts) proposed to study the influence of temperature on juvenile Tuolumne River *Oncorhynchus mykiss*, as part of a suite of investigations described in the Temperature Criteria Assessment (Chinook and *Oncorhynchus mykiss*) (W&AR-14) Study Plan, as provided in the Districts' Proposed Study Plan. In its December 2011 Study Plan Determination, FERC determined that the Districts were not required to complete the Temperature Criteria Assessment (Chinook and *Oncorhynchus mykiss*), but indicated that empirical data collected on the thermal capability of Tuolumne River fish would be considered in the Don Pedro Project relicensing proceeding.

The Districts elected to complete an investigation of the thermal performance of juvenile *O. mykiss* in the lower Tuolumne River, given the importance that empirical evidence on this subject would have in the relicensing proceeding. In June 2014, the Districts finalized the Local Adaptation of Temperature Tolerance of *O. mykiss* Juveniles in the Lower Tuolumne River Study Plan and posted the document to the Don Pedro Project relicensing website. On June 30, 2014, the Districts invited relicensing participants to attend, prior to the start of fieldwork, a site visit to observe the onsite laboratory set-up and a demonstration of the study approach and the equipment to be used. The demonstration, held on July 10, 2014, was attended by a representative from the California Department of Fish and Wildlife and members of the public. Fieldwork for the study began later that month and was completed in August. In January 2015, the Districts sent a draft study report to relicensing participants for 30-day review and comment. Comments on the draft study report were received from the Tuolumne River Trust, the California Sportfishing Protection Alliance, the State Water Resources Control Board, and the California Department of Fish and Wildlife (Appendix 5). The Districts provide responses to these comments in Appendix 6 of this report.

In November 2016, this study was published in the peer reviewed journal *Conservation Physiology*. The journal article is appended to this report as Appendix 7.

EXECUTIVE SUMMARY

The purpose of this study was to investigate the thermal performance of juvenile *Oncorhynchus mykiss* that populate the lower Tuolumne River in the Central Valley region of California with respect to the seasonal maximal water temperatures they experience during the summer months.

The study tested the hypothesis that the Tuolumne River *O. mykiss* population below La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that exist in the river during the summer. The basis for this hypothesis is peer-reviewed scientific literature that indicates that salmonid species, including *O. mykiss*, can adjust to local thermal conditions. In the current study, *O. mykiss* were locally caught and tested, and then returned safely within ~ 1 day of capture to the Tuolumne River.

The experimental approach acknowledged the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis, which proposes that the extremes of thermal tolerance are set by a fish's inability to supply oxygen to its tissues above and beyond a basic routine need. The experimental approach also acknowledged that every activity of a fish in a river (swimming, catching prey and feeding, digesting a meal, avoiding predators, defending territory, etc.) requires oxygen above and beyond a basic routine need and that salmonids have evolved to maximize their oxygen supply when they fuel muscles during exhaustive swimming. Consequently, the tests performed here directly measured how much oxygen can be maximally extracted from the water by a fish (its maximum metabolic rate; MMR) and how much oxygen is routinely needed by that fish to exist (its routine metabolic rate; RMR). These measurements were performed in a swim tunnel respirometer (the equivalent of an aquatic treadmill) at different test temperatures ranging from 13°C to 25°C. Then, by subtracting RMR from MMR, we determined over this temperature range the capacity of *O. mykiss* to supply oxygen to tissues above and beyond a basic routine need, which is termed the absolute aerobic scope (AAS = MMR - RMR) and defines the fish's capacity to perform the activities essential to complete its life history. Factorial aerobic scope (FAS = MMR/RMR) was also calculated as another way of expressing a fish's aerobic capacity. These measurements were performed over a wide range of test temperatures (13°C to 25°C), which allowed us to determine the dependence of aerobic capacity on water temperature. These short-term direct measurements of temperature effects on fish metabolism did not assess the potentially beneficial physiological and biochemical changes that would be associated with thermal acclimation during longer-term growth studies (i.e., weeks).

As expected, the routine need for oxygen of these fish (RMR) increased exponentially with test temperature from 13°C to 25°C (36 different fish each tested at a single temperature). For these same fish, MMR also increased over the same range of test temperatures, but to a lesser degree. As a result, the absolute capacity to supply oxygen to tissues above routine needs (AAS) reached a peak at 21.2°C (as modeled for all fish by a mathematical equation). Moreover, there was a wide temperature range around this optimum where AAS changed very little. For example, the statistical 95% confidence limit for peak AAS extended between 16.4°C and 25°C. Likewise, 95% of the numerical peak for AAS (i.e., 5.84 mg O_2 $kg^{-0.95}$ min^{-1}) could be maintained between 17.8°C and 24.6°C. By being able to maintain peak AAS across a range of test temperatures that clearly spans the 7-Day Average of the Daily Maximum (7DADM) criterion of 18°C set out by

EPA (2003) for Pacific Northwest *O. mykiss*, Tuolumne River *O. mykiss* population has a broader range of thermal performance than previously thought.

Thus, the physiological measurements presented in this report supports the hypothesis that the *O. mykiss* population found in the Tuolumne River downstream of La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that typify the summer months. Indeed, all fish that were tested from 13°C to 24°C recovered quickly from an exhaustive swim test and then were successfully returned to the river. Some of these test fish were inadvertently recaptured up to 11 days later in their original river habitat and appeared to be in excellent condition when visually inspected. Also, three of the four fish tested at 25°C were successfully returned to the river after their arduous experimental tests. The upper thermal performance limit (i.e., the temperature where AAS is zero) for Tuolumne River *O. mykiss* could not be determined with the present experiments due to conditions set forth by the National Marine Fisheries Service (NMFS), but the present data suggest that it must lie above 25°C.

The conclusion of the study is that the thermal range over which the Tuolumne River *O. mykiss* population can maintain 95% of their peak aerobic capacity is 17.8°C to 24.6°C. Moreover, up to a temperature of 23°C, all test fish could at least double their routine oxygen uptake (a FAS value >2.0), which we suggest is sufficient aerobic capacity for the fish to properly digest a meal. Finally, based on a video analysis of the swimming activity of *O. mykiss* in the Tuolumne River, fish at ambient water temperatures were predicted to have an excess aerobic capacity well beyond that needed to swim and maintain station against the river current in their usual habitat.

These results support the hypothesis that the thermal performance of wild *O. mykiss* from the Tuolumne River represents an exception to that expected based on the 7DADM criterion set out by EPA (2003) for Pacific Northwest *O. mykiss*. Moreover, given that the average AAS remained within 5% of peak performance up to a temperature of 24.6°C and that all Tuolumne River *O. mykiss* maintained a FAS value >2.0 up to 23°C, we recommend that a conservative upper aerobic performance limit of 22°C, instead of 18°C, be considered in re-determining a 7DADM for this population.

This wide range of thermal performance for *O. mykiss* from the Tuolumne River is consistent with that found for *O. mykiss* populations already known to be high-temperature tolerant, such as the redband strain of rainbow trout (*O. mykiss gairdneri*) in the high deserts of Eastern Oregon and Idaho, steelhead trout from the south coast of California, and selected and hatchery-maintained strains of *O. mykiss* in Western Australia and Japan. Whether the high thermal performance that was demonstrated for the *O. mykiss* of the Tuolumne River downstream of La Grange Diversion Dam arose through genetic selection or physiological acclimatization was beyond the purpose and scope of the present study.

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7DADM	7-Day Average of the Daily Maximum
95% CI	95% Confidence Limits
AAS	Absolute Aerobic Scope (MMR-RMR)
AS	Aerobic Scope
BP	Barometric pressure
CTmax	Critical Thermal maximum
EPA	U.S. Environmental Protection Agency
ESA	Endangered Species Act
FAS	Factorial Aerobic Scope (MMR/RMR)
FL	Fork Length of the fish
ILT	Incipient Lethal Temperature
M	Mass of fish
MMR	Maximum Metabolic Rate
MR	Metabolic Rate
O_2	Oxygen
O_2(A)	Tunnel water oxygen concentration at beginning of seal
O_2(B)	Tunnel water oxygen concentration at end of seal
OCLTT	Oxygen- and Capacity-Limited Thermal Tolerance
PIT	Passive Integrated Transponder
RM	River Mile
RMR	Routine Metabolic Rate
T	Time
TBF	Tail Beat Frequency
T_{crit}	Critical Temperature when performance (e.g., aerobic scope) reaches zero
T_{opt}	Optimal Temperature when performance (e.g., aerobic scope) reaches a peak
T_p	Pejus Temperature when performance (e.g., aerobic scope) decreases from its peak. In the present study, T_p is set when absolute aerobic scope decreases to 95% of the peak capacity at T_{opt}
V	Tunnel volume
$\alpha(O_2)$	Solubility of oxygen in water
% O_2Sat	Percent saturation of oxygen in water

INTRODUCTION

The Tuolumne River has been significantly affected by human activity since the mid-1800s, including in-channel and overbank mining of gold and gravel, urban and agricultural encroachment, and water resource development. Summertime water diversions from the Tuolumne River near La Grange, CA have been occurring for over 120 years. These changes have contributed to a unique river habitat for the *O. mykiss* population that lives in the Tuolumne River downstream of the La Grange Diversion Dam located at River Mile 52 (RM 52). Year round, the Don Pedro Dam located near RM 54 releases cool water to the river (10-13°C) even during the hottest periods in summer. As this water flows downstream it can gain or lose thermal energy depending on its surrounding environment. In summer months, the average river temperature increases appreciably with distance downstream of the dam (see Appendix 1). At RM 49, for example, river temperature peaked at 20.2°C in July 2014. However, cooler river temperatures are associated with cloud cover and over night, and deeper ponds in the river do show some thermal stratification. In 2013, a detailed study of summertime temperatures in the Tuolumne River was performed between ca. RMs 3-37 (HDR 2014).

Based on observations from monitoring surveys conducted since 1997 (Ford and Kirihara 2010; Stillwater Sciences 2012), *O. mykiss* rearing habitat extends from RM 52 to ca. RM 30, with spawning habitat in 2013 documented from RM 50 to about RM 39 (FISHBIO 2013). Review of this information suggests that primary rearing habitat for *O. mykiss* since 1997 has been concentrated upstream of RM 39.6, where peak water temperatures have occasionally exceeded 27°C during the summer months. Therefore, the realized habitat of *O. mykiss* during summer presently covers a distance of ca. 12.4 river miles, where water temperature varies within the range of 11°C to 28°C. Any difference between where a fish actually lives (the realized habitat) and its fundamental habitat is determined by behavior (Matthews and Berg 1997).

Thermal Tolerance and Thermal Performance

Fundamental habitat of any animal is determined in part by its thermal tolerance limits to warm and cold. Even humans, who normally regulate body temperature at 37°C (98.4°F), quickly succumb if body temperature cannot be maintained below 45°C in extreme heat. However, the body temperature of a fish such as *O. mykiss* in the Tuolumne River is not regulated in the same way as that of humans. Instead, it is always the same as the surrounding river temperature, except for brief (seconds to minutes), non-steady states whenever a fish moves rapidly between regions of thermal stratification. Nevertheless, a fish warmed or cooled beyond its thermal limits will rapidly succumb, just like a human.

Scientists commonly measure the thermal tolerance limit of a fish using either incipient lethal temperature (ILT) or critical thermal maximum (CTmax) tests. An upper ILT test acutely exposes fish to a suite of elevated temperatures and reports the temperature at which 50% of the test fish succumb. In contrast, an upper CTmax test warms (ca. 0.3°C per min) a fish until it can no longer maintain its upright orientation and reports the temperature when 50% of the fish roll over. Fish can rarely live for more than a few minutes at its CTmax.

While CTmax values have been widely used to distinguish thermal tolerance differences among fish species, CTmax does not always discriminate more subtle physiological adjustments in thermal tolerance expected within a fish species in response to season and/or genetic differences. For example, a CTmax value of 29°C is reported for trout acclimated to temperatures ranging from 12 to 20°C (Table 1). While CTmax values for *O. mykiss* can certainly be similar over a wide range of thermal acclimation temperatures and populations, there are exceptions because CTmax can increase in some studies of thermal acclimation of *O. mykiss* (Table 1), as it does when killifish are thermally acclimated (Fangue et al. 2006). The sub-species redband trout has the highest CTmax for the genus *O. mykiss* and red-band trout live in dessert environment. Any insensitivity of the CTmax measurement likely stems from relatively short test exposure times (min) and the rapid but sometimes variable warming rates that are employed when measuring CTmax. Regardless, CTmax is always higher than the temperature that a fish can tolerate for hours to days and certainly higher than the temperature at which a fish can no longer swim aerobically.

Consequently, despite its relative ease of measurement, CTmax, which is a measure of thermal tolerance, is increasingly being replacing by fish biologists with metrics that measure thermal performance. Metrics such as growth are preferred because they have some ecological relevance but have the disadvantage of requiring 30 or more days for a fish to achieve sufficient growth to determine its optimal temperature (or range of temperatures) for growth. Also, growth studies indirectly assess the effects of temperature on fish energetics and usually require rearing fish in controlled conditions that do not account for the full range of bioenergetic functions necessary for survival in nature (e.g. foraging, migration, competition, predation avoidance).

An alternative metric for performance acknowledges that all activities of a fish ultimately require oxygen (O_2). Therefore, it is possible to directly assess a fish's need for and capacity to deliver oxygen and use these measures as an ecologically relevant metric of fish performance. Furthermore, by making such measurements over a range of temperature, as first done some 60 years ago (e.g., Fry 1947), it is possible to accurately characterize the thermal effects on a fish's ability to deliver oxygen to its tissues, which is a direct measurement of energetic capacity to support the bioenergetic functions necessary for survival in nature. Unlike growth studies that require wild fish to be removed from their natural environment into a controlled artificial environment for months, studies of oxygen uptake can be performed in days. While methods to characterize fish thermal performance using oxygen uptake have an extremely long history, watershed managers have only started to embrace these thermal performance metrics over the past decade. As a result, existing regulatory criteria tend not to have considered these metrics, which can be measured at a local scale.

7-day Average of the Daily Maxima (7DADM)

One of the thermal criteria used by EPA to protect fish is the 7-day average of the daily water temperature maximum (7DADM). The explicit recommendation in EPA (2003) for juvenile *O. mykiss* in summer rearing habitats is a 7DADM <18°C. A key study that influenced the current 7DADM criterion for *O. mykiss* from the Pacific Northwest is the growth study of Hokanson et al. (1977), which was reviewed in Issue Paper 5 (EPA 2001). Growth is considered as a very powerful integrator of environmental, behavioral and physiological influences of a fish's fitness.

Hokanson et al. (1977) measured growth of juvenile *O. mykiss* from the Great Lakes in Minnesota using constant and fluctuating (a daily temperature oscillation of ± 3.8°C) thermal regimes. *O. mykiss* grew maximally at 16-18°C, termed the optimum temperature (T_{opt}) for growth. However, there are a number of concerns with applying these results to *O. mykiss* from the Tuolumne River. Foremost, *O. mykiss* are not native to Minnesota; they are an introduced species. Second, the thermal and other environmental conditions in Minnesota are far from similar to those encountered by *O. mykiss* in the Tuolumne River (below we show clear scientific support for local thermal adaptation of fishes, including *O. mykiss*). Moreover, the work of Hokanson et al. (1977) pre-dated the routine statistical packages that can place a statistical 95% confidence interval (CI) around data such as growth and oxygen uptake. This is an important data gap because EPA (2003) states that: "*Each salmonid life stage has an optimal **temperature range** (our emphasis). Physiological optimum temperatures are those where physiological functions (e.g., growth, swimming, heart performance) are optimized. These temperatures are generally determined in laboratory experiments.*" Therefore, this key study established a temperature optimum for growth rather than a thermal range for peak growth performance. EPA (2003) recommends 20°C as the 7DADM criterion for salmon and trout migration. Curiously, this criterion acknowledges that Pacific Northwest *O. mykiss* have sufficient aerobic scope for the energetic demands of river migration at a temperature that is 2°C higher than the 7DADM for growth in juveniles (18°C). River migration can be the most energetically challenging activity a salmonid can undertake and certainly requires more energy allocation than is used for feeding and growth. A juvenile salmonid in a river or stream will hold station and use darting behavior to opportunistically capture food drifting downstream. Thus they need energy for periodic sprint and burst activities, plus the cost of digesting and assimilating the captured food (specific dynamic action or heat increment of digestion). Furthermore, Hokanson et al. (1977) discovered that "*At temperatures in excess of the growth optimum, mortality rates were significantly higher during the first 20 days of this experiment than the last 30 days.*" The implication of this observation is that a proportion of the test fish were either initially better suited for high temperature or became better suited after living for 20 days at a supra-optimal temperature when compared to the fish that died during the initial 20-day period.

In view of this uncertainty surrounding the applicability of the 7DADM for *O. mykiss* to *O. mykiss* in the Tuolumne River, we now review some of the literature that supports the possibility for local physiological acclimation or genetic adaptation to warm temperature within the *O. mykiss* genus.

Current Evidence for Local Physiological Acclimatization and Genetic Selection

Thermal acclimation is a physiological process whereby an ectothermic animal, such as a fish, can potentially perform better after being placed in a new environment. Thermal acclimation involves a suite of physiological and biochemical changes that occur over a period of several weeks. Thus, if a fish living in say 14ºC water is transferred to 20ºC, its performance would progressively improve as it acclimates to the new temperature. This processes is referred to as thermal plasticity within a species. The extensive knowledge on thermal acclimation among fish species dates back well into the 1940s. Thermal plasticity, however, has limits that vary from species to species, which is a result of thermal adaptation within a species.

As early as the late 1960s, Bidgood and Berst (1969) used upper ILT data to conclusively demonstrate that juvenile *O. mykiss* from four anadromous Great Lakes populations could thermally acclimate, i.e., warm acclimation increased their upper ILT. Likewise in California (CA) there is wide variation in the thermal performance curves for hatching success among different strains of *O. mykiss* (Myrick and Cech 2001). While this variability includes the Eagle Lake and Mt. Shasta strains, these two strains had been shown earlier to have a similar CTmax (Myrick and Cech 2000). Thus, in the early 2000s, evidence for thermal acclimation was extensive within the species *O. mykiss*.

Evidence for thermal adaptation within the species *O. mykiss* was limited at the time of Issue Paper 5 (EPA 2001). Nevertheless, the work did acknowledge the possibility of genetic adaptation by asking is there enough evidence for genetic variation within a species to warrant geographically-specific or stock-specific water temperature standards. The conclusion was *"The literature on genetic variation in thermal effects indicates occasionally significant but very small differences among stocks and increasing differences among subspecies, species, and families of fishes. Many differences that had been attributed in the literature to stock differences are now considered to be statistical problems in analysis, fish behavioral responses under test conditions, or allowing insufficient time for fish to shift from field conditions to test conditions"*. In fact, Issue Paper 5 (EPA 2001) cited (see its Table 1) Sonski (1983), who identified the T_{opt} for growth of redband trout (*O. mykiss gairdineri*) as 20°C, which is the highest value for the genus *O. mykiss*. Therefore, evidence did exist in the literature prior to 2001 that the genus *O. mykiss* can perhaps be genetically adapted to local environmental conditions.

Since 2001, the peer-reviewed scientific literature has provided ample and convincing support for thermal adaptation at the population level and among a wide variety of fish species (e.g., killifish populations on the Atlantic coast, Fangue et al., 2006; stickleback populations in the Pacific Northwest, Barrett et al., 2011). Importantly, included are salmon and trout species belonging to the *Oncorhynchus* genus. For example, Eliason et al. (2011) showed that populations of adult sockeye salmon (*O. nerka*) in British Columbia's Fraser River watershed are adjusted to perform best at the local temperature conditions that they experience during their spawning river migration. Indeed, their maximum aerobic swimming capacity is also well matched with the range of hydraulic challenges that the different populations face migrating upstream to their spawning area (Eliason et al. 2013).

In addition, wild populations of redband trout, a sub-species of *O. mykiss*, inhabit natural desert environments in Oregon and Idaho where summer stream water temperatures can exceed 30°C. New thermal performance studies provide evidence for local thermal adaptation of redband trout (Rodnick et al. 2004) and the redband trout's ability to genetically adapt when acclimated to a common set of experimental conditions has found support (Narum et al. 2010, 2013, Narum and Campbell 2015).

O. mykiss is an introduced fish species on every continent except Antarctica. Moreover, selective breeding of *O. mykiss* has been effective in selecting for high temperature tolerance. For example, Hartman and Porto (2014) found evidence for temperature-dependent growth and differences in feeding performance among three *O. mykiss* strains. Also, severe thermal exposures in a hatchery program in Western Australia have produced in just over 20 generations a line of *O. mykiss* that is thermally tolerant (Morrissy 1973; Molony 2001; Molony et al. 2004; Chen et al. 2015). During summer extremes, the juvenile *O. mykiss* continue to swim and feed even when water temperature reaches 26°C (Michael Snow, Department of Fisheries, Government of Western Australia, pers. comm.). The founder *O. mykiss* population for this thermally tolerant line was transplanted during the last century from CA with the intention of setting up a recreational fishery for *O. mykiss* in Western Australia. Japanese researchers have similarly selected a strain of rainbow trout that show high thermal tolerance (Ineno et al., 2005).

Therefore, clear and compelling scientific knowledge exists for local adjustments and genetic selection of high thermal performance of *O. mykiss*. This new knowledge has been largely added to the scientific literature subsequent to the 18°C 7DADM being identified for *O. mykiss* in the Pacific Northwest by the EPA (2003). EPA (2003) did acknowledge that local adjustment was possible and that well-designed studies could be used to identify site-specific thermal adjustments. The present study aims to provide such evidence for the *O. mykiss* population inhabiting the lower Tuolumne River.

Justification and Purpose of the Study

The primary purpose of this study is to determine the thermal performance of the sub-adult (100-200 mm fork length; FL) *O. mykiss* population that inhabits the lower Tuolumne River (LTR) to assess any local adjustment in thermal performance. Thermal performance was assessed as the range of temperatures over which juvenile *O. mykiss* can increase aerobic metabolic rate (MR) beyond basic needs. This aerobic capacity could be used for any of the normal daily activities of *O. mykiss* in the Tuolumne River during its normal life history (swimming, catching prey and feeding, digesting a meal, growing, avoiding predators, defending territory, etc.). Thus, MR measurements were used to determine the optimal temperature range for Tuolumne River *O. mykiss.*

This experimental approach is consistent with the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis that has emerged as a conceptual model to assess thermal performance of aquatic animals and determine the fundamental thermal range for their distributions (Pörtner and Knust 2007; Pörtner and Farrell 2008). The OCLTT hypothesis proposes that the extremes of thermal tolerance will be set by a fish's inability to supply oxygen to its tissues above a basic routine need. The ecological relevance of the OCLTT hypothesis is exemplified through performance measurements in eelpout (*Zoarcidae viviparous*) and spawning Pacific salmon. The temperature at which oxygen supply to the tissues of eelpout becomes limiting closely corresponds with the temperatures where growth performance and abundance of eelpout decrease in the German Wadden Sea (Pörtner and Knust 2007). In spawning Pacific salmon, temperature ranges for upstream migration success correspond with the temperature range across which absolute aerobic scope is maximal (Eliason et al. 2011). More recently, Chen et al. (2015) demonstrated a broad thermal range for absolute aerobic scope in the thermally tolerant *O. mykiss* strain from Western Australia.

Salmonids are examples of fish that have evolved to maximize oxygen supply to exhaustive swimming muscles. Therefore, our experimental approach directly measured MR under two states: routine metabolic rate (RMR), representing how much oxygen is needed by an individual *O. mykiss* to exist in the Tuolumne River and maximum metabolic rate (MMR), representing how much oxygen can be maximally extracted from the water for its tissues, typically when swimming. The capacity of the fish to supply oxygen to tissues above and beyond a basic routine need is then calculated by subtracting RMR from MMR, which is termed the absolute aerobic scope (AAS = MMR - RMR). Therefore, AAS defines a fish's capacity to perform the activities essential to carry out its life functions.

Factorial aerobic scope (FAS = MMR/RMR) is another way of expressing aerobic capacity by characterizing how much a fish can increase oxygen uptake beyond routine needs (RMR). A key activity for survival in nature, namely feeding and digestion, is expected to require up to a doubling of a fish's RMR for a large meal (Jobling 1981; Alsop and Wood 1997; Fu et al. 2005; Luo and Xie 2008), i.e., an FAS value of 2 allows for proper digestion of a large meal.

Measurements of fish MR were obtained using the equivalent of an aquatic treadmill (a swimming tunnel respirometer) and at different test temperatures (from 13°C to 25°C). By mathematically modeling these data, the optimal temperature (T_{opt}) for the peak AAS could be established for juvenile *O. mykiss*. The T_{opt} window (or thermal range) is defined by Parsons (2011) as *"the range in temperatures where maximum aerobic scope is maintained*". In the present study, we use 95% of the peak AAS value to set the optimal thermal range (Figure 1; the two temperatures that bracket T_{opt} are termed a Pejus temperature, T_p). If, as predicted by the OCTTL hypothesis, a cardiorespiratory limitation exists for exercising salmonids during warming, AAS will decrease below 95% of peak AAS beyond the upper T_p, and often rapidly over just a few degrees before lethal temperatures are reached (Farrell 2009). The critical temperature (T_{crit}) is the temperature when there is no aerobic scope and therefore aerobic activities beyond basic needs, including swimming, are impossible. Thus, whenever a fish is warmed beyond its T_p, maximum oxygen delivery progressively fails to quantitatively keep up with the need for increased oxygen delivery just to maintain the resting state (Farrell 2009). As a result, the factorial aerobic scope (AMR/RMR) decreases with temperature. Thus, an important index when considering FAS is the temperature when FAS decreases below a value of 2 because it would not be possible to double RMR for the digestion of a large meal (Jobling 1981; Alsop and Wood 1997; Fu et al. 2005; Luo and Xie 2008).

Thus, the primary study goal is to determine if there is evidence for local temperature 'adjustment' in Tuolumne River *O. mykiss* by establishing the temperatures that set the thermal range for T_{opt} (at 95% of peak) and determining how rapidly AAS declines between the upper T_p and T_{crit} for Tuolumne River *O. mykiss*. This information should help define more accurate criteria for thermal performance of juvenile *O. mykiss* rearing in the lower Tuolumne River. Specifically, the temperature indices and the shape of the aerobic scope curve derived in the present study can also be compared with those of other *O. mykiss* populations and with the EPA (2003) recommendations.

While the curve relating AAS with temperature has been coined a Fry aerobic scope curve (Fry 1947), curves that describe the effect of temperature on a measure of organismal performance (e.g., RMR, MMR, AAS, growth) are more generally called thermal reaction norms (Huey and Kingsolver 1979; Schulte et al. 2011). Reaction norms typically have a shape in which the performance index increases with increasing temperature, reaches a peak at some intermediate temperature, and declines with a further temperature increase. Importantly, the specific shape and position of these performance curves can vary among species and in response to thermal variation in a fish's environment. The magnitude and timescale of environmental temperature exposure are both critical and persistent differences in local thermal conditions over evolutionary time scales may result in compensatory adaptive changes in local populations (Hochachka and Somero 2002). On a shorter time scale, and if temperature varies on a daily or seasonal basis at a given locality, fish may compensate for the temperature difference over weeks to months - termed thermal acclimatization for natural settings or simply thermal acclimation when only temperature is manipulated under controlled laboratory conditions. Fish can also respond immediately (seconds to hours) to acute thermal challenges using either behavioral (e.g., attraction and avoidance), or physiological and biochemical responses (e.g., changes in heart rate and heat shock proteins).

Although the theoretical basis for how patterns of thermal performance can be shaped by local thermal regimes is now well understood and this theory provides the framework for the present study, our study was not designed to distinguish between the mechanisms of local thermal adaptation (which implies a proven genetic change) and acclimatization. Consequently, rather than using the term 'adaptive', we say that the fish are acclimatized to the local conditions and will use the general term that fish are 'well adjusted' to local environmental conditions, if we find that to be the case. However, fish were sampled from the coldest section of their habitat and their response to acute warming was examined. Therefore, our short-term, direct measurements of temperature effects on fish oxygen uptake could not assess the likely beneficial effects of thermal acclimation due to conditions for fish removal set forth by the National Marine Fisheries Service (NMFS).

EPA (2003) also states that: "*Ecological optimum temperatures are those where fish do best in the natural environment considering food availability, competition, predation, and fluctuating temperatures. Both (sic lab-based and field based measurements) are important considerations when establishing numeric criteria.*" Importantly, Issue Paper 5 (EPA 2001) comments that "*Field testing of fish survival under high temperatures is not usually done. If such methods were feasible, the improved realism would be helpful.*" Therefore, the present experiments established a field laboratory beside the Tuolumne River so that the thermal performance of wild *O. mykiss* acclimatized to field conditions could be tested without prolonged transport and holding of fish.

Predictions and Alternate Predictions

Given the EPA (2003) 7DADM and the current scientific literature, it is possible to make two types of contrasting predictions for the upper thermal performance of wild *O. mykiss* captured from the Tuolumne River: a) predictions based on the EPA (2003) 7DADM criterion, and b) alternative predictions based on contemporary literature for local thermal adjustment.

Predictions Derived From EPA (2003)

Based on the EPA (2003) 7DADM criteria alone, one would predict that wild *O. mykiss* captured from the Tuolumne River for the present tests would show the following:

1. Routine metabolic rate (RMR) will increase exponentially until the test temperature approaches the upper thermal limit for *O. mykiss* (i.e., CTmax), which depending on the *O. mykiss* strain and acclimation temperature, is 26°C to 32°C (see Table 1).
2. Maximum metabolic rate (MMR) will increase with test temperature and reach a peak around 18°C according to the EPA criterion.
3. Absolute aerobic scope (AAS) has a T_{opt} around 18°C according to the EPA criteria.
4. AAS will rapidly decline at a temperature just above 18°C.
5. Factorial aerobic scope (FAS) will decline with increasing temperature, reaching a value < 2 (i.e., MMR is less than twice RMR) at a temperature just above 18°C.

Alternative Predictions for a Thermally Adjusted Population

Based on recent peer-reviewed studies, the present study tested the hypothesis that the Tuolumne River *O. mykiss* population below La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that exist in the river during the summer. One would then predict that the results of the present study would show the following:

1. RMR will increase exponentially until the test temperature approaches the upper thermal limit for *O. mykiss* (i.e., CTmax), which is ca. 26°C to 32°C depending on the study.
2. MMR will increase with test temperature and reach a peak that is above 18°C.
3. AAS will have a T_{opt} that is above 18°C.
4. AAS will decline at a temperature above 18°C.
5. FAS will decline with increasing temperature, but maintain a value > 2 at temperatures above 18°C.

METHODS

Permitting Restrictions that Influenced the Experimental Design

Wild Tuolumne River *O. mykiss* were collected under National Marine Fisheries Service Section 10 permit # 17913 and California Fish and Wildlife Scientific Collecting Permit Amendments. No distinction was made between resident (rainbow trout) and anadromous (steelhead) life history forms, and both are referred to as *O. mykiss* throughout this document. For permitting purposes, these fish are considered as "ESA-listed California Central Valley steelhead, *O. mykiss*".

Fish collection (to a maximum of 50 fish) was allowed between RM 52.2 and RM 39.5, and between June 1 and September 30, 2014. Fish collections were not allowed at river water temperatures that exceeded 70°F (21.1°C). Incidental fish recaptures were authorized in addition to the initial take limit (n=50), with these reported as 'additional take' under the NMFS permit reporting conditions. Because indirect fish mortality was limited to 3 fish, no more than 2 fish were captured per day as a precautionary measure to limit indirect mortalities. Also, temperatures were not tested randomly and most of the highest temperatures were tested last to preclude premature termination of the work should there be high-temperature related mortality.

Preliminary experiments were performed with hatchery reared *O. mykiss* to ensure that all the equipment was fully functional and properly calibrated prior to testing wild fish. All experimental procedures were approved by the University of California Davis' Institutional Animal Care and Use Committee (Protocol # 18196). All fish capture and handling activities were conducted by experienced FISHBIO personnel.

Fish Collection, Transport, and Holding

Fish capture was conducted via seine net (0.32 cm nylon mesh, 1.8 m high, 9 m long). Several precautions were used during capture activities in order to minimize handling of non-target fish. These included 1-2 snorkelers in the water identifying *O. mykiss* of the target size range (100-200 mm) prior to seine sweeps, as well as the use of a mesh size allowing fish smaller than the target fork length to avoid capture. Captured fish within the target range were transferred to a partially submerged transport tank via a large scoop net to minimize handling and avoid air exposure during transfer. Each captured fish was scanned for presence of a PIT tag to ensure that the fish had not been tested previously. Upon capture, a water temperature logger (Onset Computer Corporation) was placed in the transport tank with the fish recording temperature at 15-min intervals through the duration of the fish holding/testing period. These loggers remained in the water with the fish throughout all transport, experimental protocols and handling until fish were returned to the river.

In total, 48 *O. mykiss* were captured between July 11 and August 13, 2014 (Appendix 2). Each fish was given a unique identification ('W' for wild, followed by a number between 01 and 48). Two fish were captured and tested daily using four capture locations (Figure 2). The fish ID, capture location (River Mile, RM), and any recaptures are shown in Figure 3 and summarized in Appendix 3. Most of the test fish (36) were captured from a single site (RM 50.7), 8 fish were captured at RM 51.6, 2 at RM 50.4 and 2 at RM 49.1 (Figure 3). Instantaneous water temperature and dissolved oxygen (DO) levels were recorded at the time of capture, and varied between 12.7 and 17.1°C. Temperature loggers were placed at RM 40, 42, 44, 46, and 48-50 from early June to late September, 2014. From the logged temperature data, 7DADM at each RM location was calculated and plotted in Appendix 1. Additional information about release locations, water temperatures, time of day, and general comments are summarized in (Appendix 2).

Fish were placed individually into 13-l plastic transport tanks, modified with numerous 0.8 cm diameter holes drilled at least 2.0 cm from the bottom to ensure sufficient water movement through the transport container. The fish, inside its transport tank, was placed into an individual insulated Yeti cooler filled with 25 l fresh river water and driven to the experimental field site (< 20-min journey). Water temperature and DO were re-measured in the transport tanks on arrival and fish were transferred from the coolers to outdoor holding tanks (300 l) filled with flow-through Tuolumne River water between 12.5 and 13.6°C. This water-to-water fish transfer minimized handling stress and eliminated air exposure.

The holding tanks received river water passed through a coarse foam filter then a 18-l gas equilibration column for aeration. This water was split between the holding tanks and the sump tank supplying the swim tunnels. Oxygen content in all vessels remained above 80% air saturation at all times. Time from fish capture in the river to placement into holding tanks ranged from 60 to 120 min. Fish remained in holding tanks for 60 to 180 min before being transfer to a swim tunnel respirometer.

Swim Tunnel Respirometry

Individual fish were tested in one of two, 5-l automated swim tunnel respirometers (Loligo, Denmark). As with the holding tanks, swim tunnels were supplied with Tuolumne River water but via a fine pressurized 20-µm pleated filter; then a 180-l temperature-controlled sump, which operated as a partial recirculating system; and an 18-l gas equilibration column. The sump was continuously refreshed with air-equilibrated river water, turning over the entire system every 80-90 min. Additionally, an aquarium grade air pump supplied air stones in each tunnel bath for aeration. For temperature control, water from the sump was circulated through a 9500 BTU Heat Pump (Aqua Logic Delta Star. Model DSHP-7), and returned to the sump through a high volume pump (model SHE1.7, Sweetwater®, USA), where two proportional temperature controllers (model 72, YSI, Ohio) were mated to one 800 W titanium heater each (model TH-0800, Finnex, USA), resulting in temperature control precision of ± 0.5°C across a temperature range of 12 to 26°C. To prevent buildup of ammonia waste in the water, ammonia-absorbing zeolite was kept in the system's sump and replaced weekly. Swim tunnel water baths were refreshed with the aerated sump water approximately every 20 min.

Water oxygen saturation was monitored using dipping probe mini oxygen sensors, one per tunnel, connected to AutoResp software through a 4-channel Witrox oxygen meter (Loligo). Water temperature in the swim tunnel was monitored with a temperature probe connected through the Witrox system and temperature loggers (see Fish collection, transport, and holding).

To limit disturbance of fish, swim tunnels were enclosed with black shade cloth. Above each tunnel, video cameras with infrared lighting (Q-See, QSC1352W, China) were mounted to continuously monitor and record (Panasonic HDMI DVD-R, DMR-EA18K, Japan) fish during swims and overnight routine metabolism measurements.

Measuring Metabolic Rates

All routine and swimming metabolic rates were measured using intermittent respirometry, a well-established technique that is the gold standard in fish biology (reviewed in Steffensen 1989; Cech 1990). A flush pump connected each tunnel chamber with an aerated external bath to allow control of tunnel sealing (during oxygen measurements) and flushing with fresh, aerated water. The pump was controlled automatically through AutoResp software and a DAQ-PAC-WF4 automated respirometry system (Loligo).

When the flush pump for the swim tunnel was off, no gas or water exchange occurred within the tunnel and so the oxygen level in the tunnel water declined due to fish respiration. Therefore, the rate at which oxygen declined in the tunnel was an estimate of aerobic metabolism. Oxygen drop (in mg O_2) was calculated for a minimum 2-min period when the tunnel was sealed. To restore oxygen levels in the swim tunnel, a flush pump connected to the external water bath refreshed tunnel water for periods of 2 to 5 min. Oxygen levels were never allowed to fall below 80% saturation. Swim tunnels were bleached and rinsed weekly to prevent accumulation of bacteria. At the beginning and end of the 2-month experiment, background oxygen consumption measures of both tunnels without fish were performed. No oxygen consumption for these controls was detected, even at the highest test temperature (25°C).

Two-point temperature-paired calibrations at 100% and 0% oxygen saturation were performed weekly on the oxygen probes. The 100% calibration was performed in aerated distilled water. The 0% calibration was performed in 150 ml distilled water with 3 g of sodium sulfite (Na_2SO) dissolved. Percent oxygen saturation was converted to oxygen concentration ($[O_2]$, mg O_2 l^{-1}) using the formula:

$$[O_2] = \%\ O_2Sat/100 \times \alpha(O_2) \times BP.$$

Where $\%O_2Sat$ is the percent oxygen saturation of the water read by the oxygen probes; $\alpha(O_2)$ is the solubility coefficient of oxygen in water at the water temperature (mg O_2 l^{-1} $mmHg^{-1}$); BP is barometric pressure in mmHg.

Metabolic rate (MR in mg O_2 $kg^{-0.95}$ min^{-1}) for resting and swimming fish was calculated according to the formula:

$$MR = \{[(O_2(A) - O_2(B)) \times V] \times M^{-0.95}\} \times T^{-1}$$

Where $O_2(A)$ is the oxygen concentration in the tunnel at the beginning of the seal (mg O_2 l^{-1}); $O_2(B)$ is the oxygen concentration in the tunnel at the end of the seal (mg O_2 l^{-1}); V is the volume of the tunnel (l); M is the mass of the fish (kg); T is the duration of the seal (min).

To account for individual variation in body mass, MR was allometrically corrected for fish mass using the exponent 0.95. This value is halfway between the life-stage-independent exponent determined for resting (0.97) and active (0.93) zebrafish (Lucas et al. 2014).

Experimental Protocol

Fish were placed individually into the swim tunnels between 1300 h and 1600 h on the day of capture. Water temperature in the swim tunnels was set to 13 ± 0.3°C (i.e., close to the habitat water temperature) and fish were given a 60-min adjustment period to this temperature prior to a 60-min training swim. Each tunnel was equipped with a variable frequency drive motor designed to generate a laminar water flow through the swimming section of the tunnel (calibrated to water velocity using a digital anemometer with a 30-mm vane wheel flow probe; Hönzsch, Germany). During the training swim, water flow velocity was gradually increased until the fish moved off of the tunnel floor and began to swim (usually at ca. 30 cm s^{-1}). Once the fish began swimming, water velocity was further increased to 5-10 cm s^{-1} above the initial swimming speed and held for 50 min. To complete the training swim, water velocity was increased to a maximum of 50 cm s^{-1} for the last 10 min, which was the expected maximum swimming velocity of 150 mm fish at 13°C (Alsop and Wood 1997). Previous studies have shown that training swim protocols result in better swimming performance in critical swimming velocity tests performed the next day (Jain et al. 1997).

Fish then recovered for 60 min at 13±0.3°C before water temperature was increased to the test temperature for each pair of fish (ranging from 13 to 25°C). Water temperature was increased in increments of 1°C 30 min^{-1} and the time that the test temperature was reached was noted, which for the highest test temperature (25°C) took ca. 24 h. Thus, all fish in the study reached their test temperature at least 8 h before swimming tests began the following morning. Measurements of MR began 30 min after the fish reached the test temperature and continued until 0700 h. The lowest four MR measurements collected during this overnight period were averaged to estimate RMR.

Critical swimming velocity tests at the test temperature began between 0800 h and 0900 h for each fish. MMR was measured in two phases: a critical swimming velocity test followed by a burst swimming test. For the critical swimming velocity test, water velocity was again gradually increased until the fish moved off of the chamber floor and began to swim. Once a fish was swimming consistently, water velocity was gradually increased to 30 cm s^{-1} over a 10-min period and then held at 30 cm s^{-1} for 20 min. If a higher initial swimming velocity was required to elicit continual swimming, the fish was held at this initial velocity for 20 min as its first test velocity. Water velocity was then increased in increments of 3 to 6 cm s^{-1} every 20 min until the fish failed to swim continuously. The velocity increment was set to ~10% of the previous test velocity, i.e., if the previous test velocity was between 20 to 39 cm s^{-1}, the velocity increment was 3 cm s^{-1}; when the previous test velocity was between 40 to 49 cm s^{-1}, the velocity increment was 4 cm s^{-1}. Active metabolic rate was monitored at each test velocity by closing the tunnel for either two 7-min or one 17-min measurement periods after the first 3 min of being flushed with fresh water. Water in the tunnel never dropped below 80% air saturation, which is an oxygen level expected to be considered normoxic. At the end of a measurement period, the next test velocity began with a 3-min flush period. Whenever a fish fell back in the swimming chamber and made full body contact with the downstream screen in the tunnel, water velocity was lowered to 13 to 17 cm s^{-1} for 1 min, and the 20-min timer stopped. After a 1-min recovery, the test velocity was gradually restored over a 2-min period and then the 20-min timer was restarted. Failure velocity was defined when the fish fell back to the downstream screen a second time during the same test velocity. The time of this failure velocity was noted.

For each test velocity, video recordings were observed for quantification of tail beat frequency (TBF measured in Hz). Three 10-s sections of video, where the fish was continuously holding station without contact with the downstream screen, bottom or side of the tunnel were identified. If three replicates were not possible throughout the entire 20-min interval, two replicates were used. If only one replicate was possible, that interval was not quantified. For each of the three (or two) sections, video was slowed to 1/4 to 1/8 of real time speed, and the number of tail beats were counted over 10 s of real time. The 2 or 3 replicates were then averaged. The same methodology was applied to video recordings taken of fish swimming in the river at temperatures of 14°C and 20°C during the study period.

Approximately 50% of the wild fish did not respond as expected to the critical swimming velocity protocol, but instead used their caudal fin to prop themselves on the downstream screen to avoid swimming. This behavior was regularly observed at test velocities well above the measured maximum swimming velocity for other fish. Consequently, to estimate MMR for these fish, swimming activity was evoked by rapidly increasing water velocity to a transient velocity stimulus of 70 to 100 cm s^{-1} (increase over 10 s and hold for 30 s or less), then decreasing the velocity back to the test velocity. Fish tended to briefly burst swim off of the downstream screen when velocities exceeded 70 cm s^{-1}. After the transient velocity increase, the fish was allowed to swim without interference (at the test velocity) as long as it continued to swim. For some fish, it was necessary to apply the transient velocity stimulus several times to keep the fish swimming. These fish were otherwise swum identically to fish that swam continuously; i.e., with 20-min test velocity periods and with metabolic rate measurements taken during each test velocity period. Failure for these fish was considered to occur when the fish did not swim upstream to prevent contact with the downstream screen, despite the water velocity being increased to 100 cm s^{-1} and returning to test velocity three times. After a critical swimming velocity trial was terminated, all fish were allowed to recover at velocities of 13-17 cm s^{-1} for 20 min.

The subsequent burst swimming test entailed a series of metabolic rate measurements taken at higher, short-duration (30-s) water velocities. To begin the burst swimming test, the water velocity was reset to the initial critical swimming velocity test increment specific to the individual fish—i.e., the first velocity increment at which the fish swam continuously for 20 min. The burst swimming protocol involved swimming a fish at its initial critical swimming velocity test increment for up to 10 min before the water velocity was rapidly increased over ca. 10 s to the maximum speed the fish could swim without contacting the downstream screen and held for ca. 30 s (or less if the fish fell back on to the downstream screen). After the 30-s burst, the velocity was decreased back to the initial critical test velocity for ca. 30 s. This protocol was repeated multiple times for at least 5 min and up to 10 min. Metabolic rate was measured for these fish by flushing the tunnel for the first 3 min of the 10-min continuous swim, then sealing the tunnel for the remaining time. Similarly, the tunnel was flushed for no more than the first

3 min of the 10-min burst swim, and sealed for the remaining time. After completion of the burst swim protocol, fish were allowed at least 60 min of recovery at the test temperature.

Following the 1-h recovery period after the swim tests, water temperature in the tunnels was lowered to ca. 13-15°C over a 30-min period. Fish were then transferred into the individual transport tanks and placed in the flow through holding tanks before measurement and tagging procedures. Fish were anaesthetized for < 5 min with CO_2 (produced by dissolving 2 Alka-Seltzer tablets in 3 l river water) and without losing gill ventilatory movements. The fork length (FL, mm) and mass (g) for each fish was measured, and half duplex PIT (Oregon RFID) tags were placed into the abdominal cavity of the fish through a 1-mm incision through the body wall, just off center of the linea alba. All equipment was sterilized with NOLVASAN S prior to tagging, and wounds were sealed with 3M VetBond. Fish were returned to the transport coolers filled with 13-15°C river water to revive (observed to swim and maintain equilibrium) before being transported to the river capture site for release. At the release site, river water was gradually added to the transport cooler to equilibrate the fish to river water temperature at a rate of 1-2°C h^{-1} before release. Once the acclimated to the river temperature, fish were allowed to swim away volitionally.

To summarize, prior to release back to the river, all fish were subjected to:

- a 1-h adjustment period in the swim tunnel at 13°C;
- a 1-h training swim at 13°C that began at ca. 1600 h;
- a 1-h recovery period at 13°C before the water temperature was warmed to the test temperatures;
- holding at the test temperature for at least 8 h before testing for MMR;
- swimming at various activity levels for minimally 2 h and maximally 6 h until they reached exhaustion;
- a 1-h recovery period at test temperature;
- decrease from test temperature to 13-15°C over 30 min; and
- morphometric measurement and tagging.

Data Quality Control, Model Selection and Analyses

Routine metabolic rate quality control (QC) was performed by visually inspecting over night video recordings for fish activity. Data from any fish showing consistent activity over night was discarded. Data from three fish (W7, W8, and W17) were discarded based on this criterion. RMR was calculated by averaging the lowest 4 metabolic rate measurements from 30 min after the fish reached the test temperature to 0700 the next morning.

There were two methods of establishing MMR: 1) Swimming (critical swimming velocity and burst performance), and 2) Agitated behavior (i.e., random movements and struggling) in the tunnel. QC criteria for MMR involved assessment of fish behavior in the tunnel via the video, and MR response to incremental increases in tunnel speed. MMR was reported as the single highest MR measurement. The highest MRs observed in this study were concurrent with fish exhibiting intense agitation. For fish not exhibiting intense agitation, the swimming MMR was used as overall MMR. Four of these ‘non-agitated’ fish (W2, W13, W14, and W15) were discarded due to failure of MR to increase incrementally despite continuous station-holding swimming with tunnel velocity increases of more than 15 cm s^{-1}.

Four different relationships were examined: 1) RMR versus test temperature, 2) MMR versus test temperature, 3) AAS versus test temperature, and 4) FAS versus test temperature. Model fitting was performed in R (http://cran.r-project.org) using the 'lm' function. Four different models were tested: linear, quadratic, antilog base 2, and log base 2 model. To select the model that best described each data set, the r^2 and residuals of each model type were compared. The model with the highest r^2 was chosen, except, when the r^2 of different models were identical, the model with the lowest residual SE was chosen. Confidence intervals and predicted values based on the best-fit model were calculated using the 'predict' function, also in R.

RESULTS

The experimental data table, including raw RMR, MMR, AAS, and FAS data for individual fish are presented in Appendix 4.

1. Routine metabolic rate (RMR) increased exponentially over the range of test temperatures from 13°C to 25°C. This thermal response was fitted with a statistically significant ($P=5.83x10^{-13}$) relationship (Figure 4A), where:

   RMR (mg O_2 $kg^{-0.95}$ min^{-1}) = $5.9513 - 0.5787x + 0.02x^2$

   x = temperature (°C).

   Thus, RMR at 13°C averaged 2.18 ± 0.45 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1} and reached 5.37 ± 0.41 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1} at 25°C. Consequently, the fish's oxygen demand (cost of basic living) increased by 2.5-fold over the 12°C range for test temperature.

   These results for RMR are consistent with our prediction #1 derived from EPA (2003) criteria and the identical alternative prediction #1. They state that RMR should increase exponentially until the test temperature approaches the upper thermal tolerance limit for *O. mykiss*, which according to published CTmax values is 26°C to 32°C (see Table 1). This prediction could not be fully tested because permitting restrictions prevented test temperatures higher than 25°C, a temperature that is clearly lower than the CTmax because fish survived and even swam for several hours at 25°C.

2. Maximum metabolic rate (MMR) increased linearly with test temperature up to the maximum test temperature of 25°C. This thermal response was fitted with a statistically significant ($P=8.94x10^{-7}$) relationship (Figure 4B), where:

   MMR (mg O_2 $kg^{-0.95}$ min^{-1}) = $1.6359 + 0.3835x$

   x = temperature (°C)

   Thus, MMR at 13°C averaged 6.62 ± 1.03 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1} and increased up to 11.22 ± 0.86 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1} at the highest test temperature (25°C). Consequently, the maximum oxygen delivery at 25°C was 1.7-times greater than that at 13°C.

   These results for MMR are inconsistent with our prediction #2 derived from EPA (2003) criteria where MMR was expected to peak near to 18°C. Instead, these MMR results are consistent with our alternative prediction #2 that the Tuolumne River population of *O. mykiss* is locally adjusted to warmer temperature, as demonstrated by peak MMR occurring at least 7°C higher than 18°C.

3. Absolute aerobic scope (AAS) was largely independent of test temperature over the range 13-25°C. Indeed, it was only at the two extremes of test temperature that any change in

AAS was statistically discernable. Because of the weak dependence of AAS on test temperature, the best statistical model for these AAS data only approached statistical significance (P=0.06; Figure 4C) where:

AAS (mg O_2 $kg^{-0.95}$ min^{-1}) = $-5.7993+1.1263x -0.0265x^2$

x = temperature (°C).

This mathematical relationship generated a T_{opt} at 21.2°C with a peak AAS of 6.15 ± 0.71(95% CI) mg O_2 $kg^{-0.95}$ min^{-1}.

These results for AAS are inconsistent with our prediction #3 based on EPA (2003) criteria, but are consistent with our alternative prediction #3 that the Tuolumne River population of *O. mykiss* is locally adjusted by having T_{opt} for AAS that is greater than 18°C, i.e., 21.2°C.

4. Contrary to our prediction #4 and our alternative prediction #4, AAS did not significantly decline above the optimal temperature. In fact, the numerical change in average AAS was surprisingly small over the entire test temperature range. Thus, rather than having a well-defined peak to the AAS curve, as expected for fish with a narrow thermal range and as schematically depicted in Figure 1, the results revealed a rather flat curve more similar to one typical of a temperature generalist. Simply, *O. mykiss* in the lower Tuolumne River were able to maintain peak AAS over a wide range of test temperatures well above 18°C. This fact can be best illustrated by two metrics, the thermal range for the statistical 95% CI of AAS and the T_{opt} window for 95% of the peak AAS (i.e., 5.84 mg O_2 $kg^{-0.95}$ min^{-1}).

   The statistical 95% confidence limits for peak AAS extend from 16.4°C to 25°C. Consequently, the numerical decrease in average AAS from 6.15 ± 0.71(95% CI) mg O_2 $kg^{-0.95}$ min^{-1} at T_{opt} to 5.78 ± 1.09(95% CI) mg O_2 $kg^{-0.95}$ min^{-1} at 25°C was only 6% and did not reach statistical significance. Indeed, the AAS measured at 24.5°C (5.89 ± 1.05 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1}) was numerically identical to that measured at 18°C (5.89 ± 0.80 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1}). But when measured at 13°C, AAS was 4.36 ± 1.21(95% CI), which was below the 95% CI for the peak AAS value. The numerical 95% peak AAS could be maintained from 17.8°C to 24.6°C, which is a more conservative thermal range for T_{opt}.

5. Although individual variability in FAS was considerable, on average the Tuolumne River population of *O. mykiss* could at least double their RMR across the entire test temperature range from 13 to 25°C. On an individual fish basis, a FAS value exceeding 3.5 was achieved in individual fish tested at 13, 16, and 22°C. Factorial aerobic scope (FAS) declined with temperature. This thermal response was fitted with a statistically significant ($P=2.92x10^{-4}$) relationship (Figure 4D) where

   FAS = $2.1438 + 0.1744x -0.0070x^2$

x = temperature (°C).

Consequently, the average FAS at 13°C was 3.32 ± 0.41 (95% CI) and decreased to 2.13 ± 0.33 (95% CI) at 25°C. This result is inconsistent with our prediction #5 derived from EPA (2003) criteria, but consistent with our alternative prediction #5 that FAS will remain above a value of 2 at temperatures well above 18°C. Indeed, all individual fish tested up to 23°C had a FAS value >2, with only 4 out of 14 fish tested at 23°C, 24°C and 25°C having a FAS value <2.

6. During swim tests at test temperatures of 14°C and 20°C, a statistically significant linear relationship ($P=2.05 \times 10^{-5}$ for 14°C and 0.009 for 20°C) was determined between MR and Tail Beat Frequency (TBF) (Figure 5).

   For fish tested at 14°C, this relationship was:

   $$MR\ (mg\ O_2\ kg^{-0.95}\ min^{-1}) = 0.75\ (TBF) + 1.05$$

   For fish tested at 20°C, this relationship was:

   $$MR\ (mg\ O_2\ kg^{-0.95}\ min^{-1}) = 1.04\ (TBF) + 1.89$$

   Video analysis of fish in the lower Tuolumne River at 14°C and 20°C revealed that a fish holding station against a river current required a TBF of 2.94 and 3.40 Hz, respectively. From these TBF values, it was possible using Figure 5 to interpolate a MR associated with *O. mykiss* holding station in normal habitat against the Tuolumne River current. These values were 3.26 mg O_2 $kg^{-0.95}$ min^{-1} at 14°C and 5.43 mg O_2 $kg^{-0.95}$ min^{-1} and 20°C. These estimates indicate that the cost of holding station increased MR by 1.50- and 2.04-fold, respectively, and used up about half of the available FAS (67% and 49%, respectively) at these two temperatures. This meant that the remaining FAS was 2.0 at 14°C and 1.7 at 20°C.

7. After exhaustive exercise, fish quickly recovered their RMR without any visible consequences when they were inspected before being returned to the river. After a 60-min recovery period, MR either had returned to RMR, or was no more than 20% higher than RMR. There were only two exceptions to this generality. Two fish tested at 25°C regurgitated rather large meals of aquatic invertebrates during the recovery from the swim test, and one of these fish died abruptly during the recovery period. No other fish mortality occurred as a result of testing the fish.

   Further evidence of post-release recovery was provided by the six fish that were inadvertently recaptured 1 to 11 days after they had been tested and returned to the river (Figure 3, Appendix 3). All these fish were recaptured in their same habitat unit and within 20 m of the original capture location. All recaptured fish were visually in good condition. Three of these recaptured fish had been tested at one of the highest test temperatures, 23°C.

DISCUSSION

Data Quality

This report contains the first metabolic rate data for the Tuolumne River *O. mykiss* population, which were used to characterize their capacity for aerobic performance over a wide test temperature range, one that extended above 18°C. The absolute values for RMR and MMR can be compared with the scientific literature even though caution is needed whenever differences exist in body mass, acclimation temperature, populations and species among the studies.

As a generality, a doubling or tripling of RMR is considered a normal biological response for an acute 10°C temperature change (Schmidt-Nielsen 1994). For the Tuolumne River *O. mykiss* population, RMR increased by 2.5-times for a 12°C change, from 2.18 mg O_2 $kg^{-0.95}$ min^{-1} at 13°C to 5.37 mg O_2 $kg^{-0.95}$ min^{-1} at 25°C. By comparison, a study of thermally acclimated and smaller sized (5-7 g) Mount Shasta and Eagle Lake *O. mykiss* found that RMR was similar (2.3-2.8 mg O_2 $kg^{-0.95}$ min^{-1}) at 14°C, but lower (2.9-3.1 mg O_2 $kg^{-0.95}$ min^{-1}) at 25°C (Myrick and Cech 2000, Table 2). Similar RMR values are reported in a wide range of studies for juvenile salmonids (Table 2). Also, when compared with other field-based measurements, but on wild adult salmon (coho, pink and sockeye) at temperatures of 10-16°C (2.9 – 4.3 mg O_2 kg min^{-1}; Farrell et al. 2003), the RMR measured in this study for *O. mykiss* was again lower at these temperatures.

The main methodological challenge with accurately measuring RMR in fish is eliminating spontaneous locomotory activity, which can potentially elevate MR in salmonids more so than any other activity. (Note: An overestimate of RMR reduces the AAS estimate). Therefore, considerable effort was used to select the minimum MR rate measurements to estimate RMR and to use video analysis to confirm that the fish were inactive during the MR measurement, an additional quality control measure that was introduced by Cech (1990). As a result, the variance for RMR of Tuolumne River *O. mykiss* was small despite the fact that the measurements were field-based. The variance was much less than that reported for a field study with adult sockeye salmon (individual RMR values varied by about 2-times) where the experimental protocol was limited to only one RMR measurement (Lee et al. 2003). As a result of this low variance, the statistical model explained 80% of the variance in RMR. Therefore, we are confident in the RMR measurements generated for this report.

Normally, RMR is measured in a post-absorptive state (i.e., following a period of starvation for usually 24 h) because the digestive process is an activity that requires an increase in RMR (Jobling 1981). In the present study, however, the digestive state of the wild fish could not be controlled because the fish would take a day or longer to fully digest a meal and return to a post-absorptive state (Jobling 1981). In fact, feces were regularly found in the swim tunnels after the overnight acclimation period, which indicated that fish in the river were feeding and that the digestive process had continued for at least part of the overnight period. Therefore, although the present measurement of RMR could have been elevated by a variable contribution for digestion, our RMR values still agree with, or fall below, comparable literature values, suggesting that digestion was not a major contributor to the RMR values measured here. Nevertheless, we cannot be certain that we measured standard metabolic rate (SMR), which is more typically used in traditional laboratory experiments to assess AAS. SMR would be lower than RMR, which would result in an underestimate of AAS and FAS when compared with literature that used SMR for these estimates.

The methodological challenge with accurately measuring MMR in wild fish is that fish vary in their willingness to participate in forced activity because they are naive to the holding conditions and to the actual swim challenge. Thus, while it is impossible to overestimate MMR and AAS, MMR and AAS can be underestimated if a fish chooses not to swim maximally. While it is possible that MMR, and therefore AAS, were underestimated in this field study, we gave the wild fish a training swim and then used four different testing protocols to generate a MMR measurement to minimize this complication. Indeed, because some of the wild Tuolumne River *O. mykiss* were reluctant to perform a U_{crit} protocol, a burst swimming protocol was used to generate MMR. The four protocols were:

1. continuous swimming with incremental increases in velocity;
2. a combination of continuous swimming and short velocity bursts to push fish off of the downstream screen;
3. a 10-min burst protocol of alternating 30 s of a very high velocity burst with 30 s of low velocity burst (aimed at maintaining moderate swimming); and
4. spontaneous intense activity during RMR measurements (rarely used, but sometimes MR was greater than the for other 3 protocols).

For Tuolumne River *O. mykiss*, the linear regression of MMR versus temperature estimated that MMR at 13°C was 6.62 mg O_2 $kg^{-0.95}$ min^{-1} and increased to 11.22 mg O_2 $kg^{-0.95}$ min^{-1} at 25°C. The statistical model for MMR explained 50% of the individual variance for the *O. mykiss* tested. We are unaware of any data in the literature assessing the response of MMR to warming in juvenile *O. mykiss*, other than the recent study on thermally tolerant *O. mykiss* (~30 g) tested in Western Australia. These fish had a peak AAS of ~10 mg O_2 kg^{-1} min^{-1} that was similar when tested at both 18ºC and 20ºC, but decreased when measured at 25ºC, the only other test temperature examined above 20ºC (Chen et al. 2015). These authors report that 90% of peak AAS was maintained between 13ºC and 20ºC. Also, the average MMR value 7.4 mg O_2 $kg^{-0.95}$ min^{-1} here at 15°C is at the high end of the range (2.9 to 8.3 mg O_2 $kg^{-0.95}$ min^{-1}) reported in the literature for smaller (2-13 g) *O. mykiss* (Table 2). Also at 15°C, we found an average AAS of 5.1 mg O_2 $kg^{-0.95}$ min^{-1} and FAS of 3.2, both of which were on the high end of the range of reported values in the literature (1.8-5.8 mg O_2 $kg^{-0.95}$ min^{-1} and 2.2-5.8, respectively, Table 2). When compared with similar field measurements on wild adult salmon (coho, pink and sockeye) at temperatures of 10-16°C (8.6-12.6 mg O_2 kg min^{-1}; Farrell et al. 2003), the MMRs measured here overlap with the lower end of this range. The individual variation for MMR was greater than that for RMR in Tuolumne River *O. mykiss*, but less than the individual variation reported for MMR values in a field study of adult sockeye salmon (Lee et al. 2003). It is interesting that the variation in MMR correlated with behavior, such that the fish that displayed frequent spontaneous activity during RMR and U_{crit} tests had the highest MMR within a temperature group. Fish that swam continuously throughout a U_{crit} test without many extra stimuli to encourage swimming invariably had the next highest MMR. The lowest MMR was for fish that propped themselves with their caudal fin to avoid swimming despite repeated stimuli with short velocity bursts and this behavior may have resulted in an underestimate of MMR.

Reaction norms, defined by the shape of the response curves in Figure 4, allow for proper mathematical and statistical consideration of the thermal range of performance, a concept that is fully endorsed by EPA (i.e., the 7DADM designation "*recognizes the fact that salmon and trout juveniles will use waters that have a higher temperature than their optimal thermal range.*"). Indeed, given the rather flat reaction norm centered around a T_{opt} of 21.2°C shown here for the Tuolumne River *O. mykiss*, it is certainly more appropriate to talk about a thermal range of performance. Thus, given the good agreement with existing literature for MR measurements combined with the fact that the shape of the response curves will be independent of the methodological concerns noted above, we are confident in using these response curves to test the predictions based on EPA (2003) and our alternative predictions.

Evidence for Local Thermal Adjustment

Our predictions based on EPA (2003), as listed above, assumed that the Tuolumne River *O. mykiss* population would perform similarly to Pacific Northwest *O. mykiss* populations used to set the 7DADM by EPA (2003). Our alternative predictions, however, allow for the possibility of local thermal adjustment to a warmer river habitat. Collectively, the results show clear deviations from our predictions based on EPA (2003), and consistency with the alternative predictions, which suggests the likelihood that the Tuolumne River *O. mykiss* population is locally adjusted to warm thermal conditions. In particular, the T_{opt} for AAS was 21.2°C, markedly higher than 18°C. Furthermore, AAS at 18°C was numerically the same as that at 24.5°C. Therefore, we discovered that the Tuolumne River *O. mykiss* population has a wide thermal range for optimal performance. Indeed, one fish was inadvertently recaptured in good visual condition from its original habitat location in the Tuolumne River 11 days after being tested at 23°C for 14 h and performing demanding swim tests. All the same, given that the CTmax could not be determined in the present work and that MMR increased up to the highest test temperature (25°C), it was impossible to determine the upper thermal limit when MMR collapses, which means that alternate metrics must be used to set the upper thermal limit for the Tuolumne River *O. mykiss* population.

The present work provides three useful metrics of the optimal temperature range for the Tuolumne River *O. mykiss* population. Using the T_{opt} of 21.2°C for the mathematical peak of AAS, the least conservative metric is the thermal range that is encompassed by the 95% CI for peak AAS, which set the thermal optimum range between 16.4°C and 25°C. The next metric, which was nearly as conservative as the first, is the thermal range where AAS remained numerically within 5% of the peak AAS at 21.2°C, which set the thermal optimum range between 17.8°C and 24.6°C. The small difference between these two temperature ranges is more a result of the individual variation in the data. The third and most conservative metric defines the temperature range where the FAS value for every fish tested was >2, which would set the thermal optimum range between 13 and 22°C, although the average FAS value was 2.13 at 25°C. Thus, the performance of the Tuolumne River *O. mykiss* population remained sufficiently elevated well beyond 18°C, which is compelling evidence of local adjustment to warm conditions.

Yet, there were important indications that the thermal testing and intensive swim imposed on them outside of their normal habitat over a 24-h period taxed a small percentage of individuals at temperatures of 23-25°C. In the present study, the telltale signs were that 4 out of 13 individuals tested at 23-25°C had a FAS < 2. Similar to the present study, Chen et al. (2015) report that FAS was 1.4-1.8 at 25°C for the thermally tolerant *O. mykiss* in Western Australia. In the next section, we suggest that fish need a FAS value of about 2 for proper digestion of a meal. Interestingly, two fish regurgitated their stomach contents at 25°C, a symptom common during extreme athletic exertion in humans when metabolic rate over-taxes oxygen supply. Such individual variability in upper thermal performance is not unexpected. Indeed, Hokanson et al. (1977) reported heightened mortality only during the initial 20 days of a growth trial for *O. mykiss* at supra-optimal temperatures. Lastly, the only fish mortality occurred in the recovery period (a phenomenon known as 'delayed mortality') after one fish was tested at 25°C.

Ecological Relevance of the Present Findings

Establishing ecological relevance of physiological data, such as those collected for the present report, has always been a challenge because of the multiple factors that influence fish distributions, behaviors and performance in the wild. Here, we measured the aerobic capacity of the Tuolumne River *O. mykiss* population in a field setting to improve the ecological relevance by minimizing fish transport and handling. After a rapid recovery from our exhaustive swim and thermal tests (as seen the 60-min recovery of MR after the swimming test), test fish appeared to reestablish their original habitat in the Tuolumne River because a portion of them were inadvertently recaptured in the river within 20 m of their original capture site. This excellent recovery behavior from intense testing seemed to be independent of the test temperature because fish were recaptured after a wide range of test temperatures (16-23°C; see Appendix 3)

To provide ecological relevance to physiological findings some 60 years ago, Fry (1947) introduced the concepts of a fish being metabolically loaded and metabolically limited to explain environmental effects on fishes. Simply put, a metabolic load from an environmental factor increases the oxygen cost of living (e.g., it costs energy to detoxify a poison, or, as in the present study case, a thermal increase in RMR). Conversely, a metabolic limit from an environmental factor decreases the MMR, leaving less oxygen available for activities. More broadly, the allocation of energy and tradeoffs is now a fundamental tenant of ecological physiology, especially in fishes (see review by Sokolova et al. 2012). Like all other temperature studies with fish, we found that RMR increased between 13 and 25°C, but there was nothing untoward in the magnitude of this thermal response (a 2.5-times increase in RMR over this temperature range).

MMR increased with temperature from 13 to 25°C, which would mean that as fish encounter higher temperatures, they have the capacity to perform an activity at a higher absolute rate, i.e., swim faster to capture food or avoid predators, digest meals faster, detoxify chemicals faster, etc. They certainly swam harder with temperature in the present study. Thus, the Tuolumne River *O. mykiss* population can perform to a higher capacity level at 25°C compared with either 13°C or 18°C. The temperature that the Tuolumne River *O. mykiss* population is predicted to have its highest absolute capacity for aerobic activity, the T_{opt} for AAS, was 21.2°C.

FAS, which measures the capacity for a proportional increase in RMR, typically decreases with temperature in fishes (Clark et al. 2011), as was the case here. Thus, the present finding for FAS was not unexpected. Moreover, being able to maintain FAS above 2 (i.e., being able to at least double its RMR; FAS = 2) may relevance for two important ecological activities for fish: digesting a full stomach and maintaining station in a flowing river.

Many laboratory studies with fish have examined the metabolic cost of digesting a full stomach (i.e., ad libitum feeding in a laboratory). The peak oxygen cost of digesting a meal increases with temperature and meal size, but peak MR does not increase by more than 2-fold at the temperatures used here and with a typical meal size (2% of body mass per feeding) for a salmonid in culture (e.g., Jobling 1981; Alsop and Wood 1997; Fu et al. 2005; Luo and Xie 2008). Therefore, a FAS value of 2 can be used as an index that a fish has the aerobic capacity to digest a full meal, and all individual fish achieved this performance up to 23°C. As a result of high temperature, a fish would digest the same meal with a similar overall oxygen cost but at a faster rate. This means that the fish could eat more frequently and potentially grow faster at a higher temperature with a FAS >2. Thus, the important ecological consideration is whether or not there is sufficient food in the Tuolumne River to support the highest MR associated with high temperature. All available studies suggest that the Tuolumne River population is not food limited, including direct studies of Tuolumne River Chinook salmon diet (TID/MID 1992, Appendix 16), long-term benthic macro-invertebrate sampling data collected from 1988–2008 (e.g., TID/MID 1997, Report 1996-4; TID/MID 2009, Report 2008-7), as well as the relatively high length-at-age for *O. mykiss* sampled in 2012 (Stillwater Sciences 2013). Indeed, the *O. mykiss* sampled for the current study were apparently feeding well in the river during summer months given the high condition factors (see Appendix 2), feces being regularly found in the swim tunnel and two test fish regurgitating rather large meals post-exhaustion. We do not know, however, whether a wild fish would eat meals as large as 2% of body mass, as in laboratory studies.

Here, we took advantage of the video analysis of the swimming behaviors of individual *O. mykiss* in the Tuolumne River habitat to provide a second evaluation of the ecological relevance of MR data. This analysis revealed a common set of swimming behaviors that *O. mykiss* used to maintain station in the water current, as well as darting behaviors used either to protect their territory or to grab food floating down the river. Because maintaining station against a water current requires a sustained swimming activity that is functionally analogous to steady swimming at one of the velocity increments in the swim tunnel, it was possible to estimate the tail beat frequency (TBF) while performing this normal river activity. Then, using Figure 5, the TBF for station holding was compared with the TBF used while swimming in the swim tunnel to determine a MR. Thus, the estimated oxygen cost of maintaining station in the Tuolumne River by *O. mykiss* at 14°C was found to increase metabolism to 1.5-times RMR, leaving fish with a FAS of 2, and therefore plenty of aerobic scope for additional activities besides maintaining station. Similarly at 20°C, maintaining station increased metabolic rate to twice RMR, and the remaining FAS was 1.7. Therefore, by combining laboratory and field observations, we can conclude that the Tuolumne River *O. mykiss* population at 20°C have an aerobic capacity to easily maintain station in their normal river habitat and additionally nearly double their RMR for other activities, or relocating to a lower water flow area to perform other activities.

According to Issue Paper 5 (EPA 2001) "*Acclimation is different from adaptation. Adaptation is the evolutionary process leading to genetic changes that produce modifications in morphology,* *physiology, and so on. Acclimation is a short-term change in physiological readiness to confront daily shifts in environmental conditions. The extent of the ability to tolerate environmental conditions (e.g., water temperature extremes) is limited by evolutionary adaptations, and within these constraints is further modified by acclimation*." Here we could not evaluate the possibility that the Tuolumne River *O. mykiss* population can thermally acclimate to warmer river temperatures as the summer progresses, due to the restrictions on the number of fish removed from the river (a maximum of 50 individuals) and their habitat temperature. Since the instantaneous temperature in the habitat where the test fish were captured was between 12.7 and 17.1°C (see Appendix 1), the upper thermal performance determined here may have underestimated thermal performance if the Tuolumne River *O. mykiss* can acclimate to temperatures higher than the river temperature they were captured in. In this regard, the thermal acclimation study of Mount Shasta and Eagle Lake *O. mykiss* (Myrick and Cech 2000) is particularly informative. Growth rate of the Mount Shasta strain was fastest at acclimation temperatures of 19 and 22°C, temperatures that bracket the T_{opt} for AAS determined here for Tuolumne River *O. mykiss.* However, growth of the Mount Shasta strain stopped at 25°C, which is consistent with our result that FAS approached a value of 2 at 25°C. In contrast, growth rate for the Eagle Lake strain was fastest at 19°C and decreased at 22°C. The Eagle Lake strain actually lost weight at 25°C, which indicated that food intake was not keeping pace with the energy requirements to sustain the RMR at this temperature, perhaps because of a limitation on AAS. Thus, the Mount Shasta strain of *O. mykiss* was better able to thermally acclimate to temperatures above 20°C than the Eagle Lake strain.

With clear evidence that California strains of *O. mykiss* grow optimally at acclimation temperatures >18°C and that local differences among strains amount to as much as a 3°C shift in the optimum temperature for growth, there already existed a precedent that the thermal range for optimal performance can reach 22°C for local populations of *O. mykiss*. Indeed, the new data presented here adds to this evidence of local adjustments of *O. mykiss* to warm river habitats, because while T_{opt} for AAS was 21.2°C, AAS remained within 5% of the peak AAS up to 24.6°C and all fish maintained a FAS value >2 up to 23°C.

CONCLUSION

High quality field data were generated on the physiological performance of Tuolumne River *O. mykiss* acutely exposed to a temperature range of 13 to 25°C. These data on the RMR, MMR, AAS, and FAS were consistent with higher thermal performance in Tuolumne River *O. mykiss* compared to those data used to generate the 7DADM value of 18°C using Pacific Northwest *O. mykiss* (EPA 2003). These new data are consistent with recent peer-reviewed literature that points to local thermal adjustments among salmonid populations. Therefore, these new data provide sound evidence to establish alternative numeric criteria that would apply to the Tuolumne River *O. mykiss* population below La Grange Diversion Dam. Given a measured T_{opt} for AAS of 21.2°C, and that the average AAS remained within 5% of this peak performance up to 24.6°C, and all fish maintained a FAS value >2 up to 23°C, we recommend that a conservative upper performance limit of 22°C, instead of 18°C, be used to re-determine a 7DADM value for this population.

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FIGURES

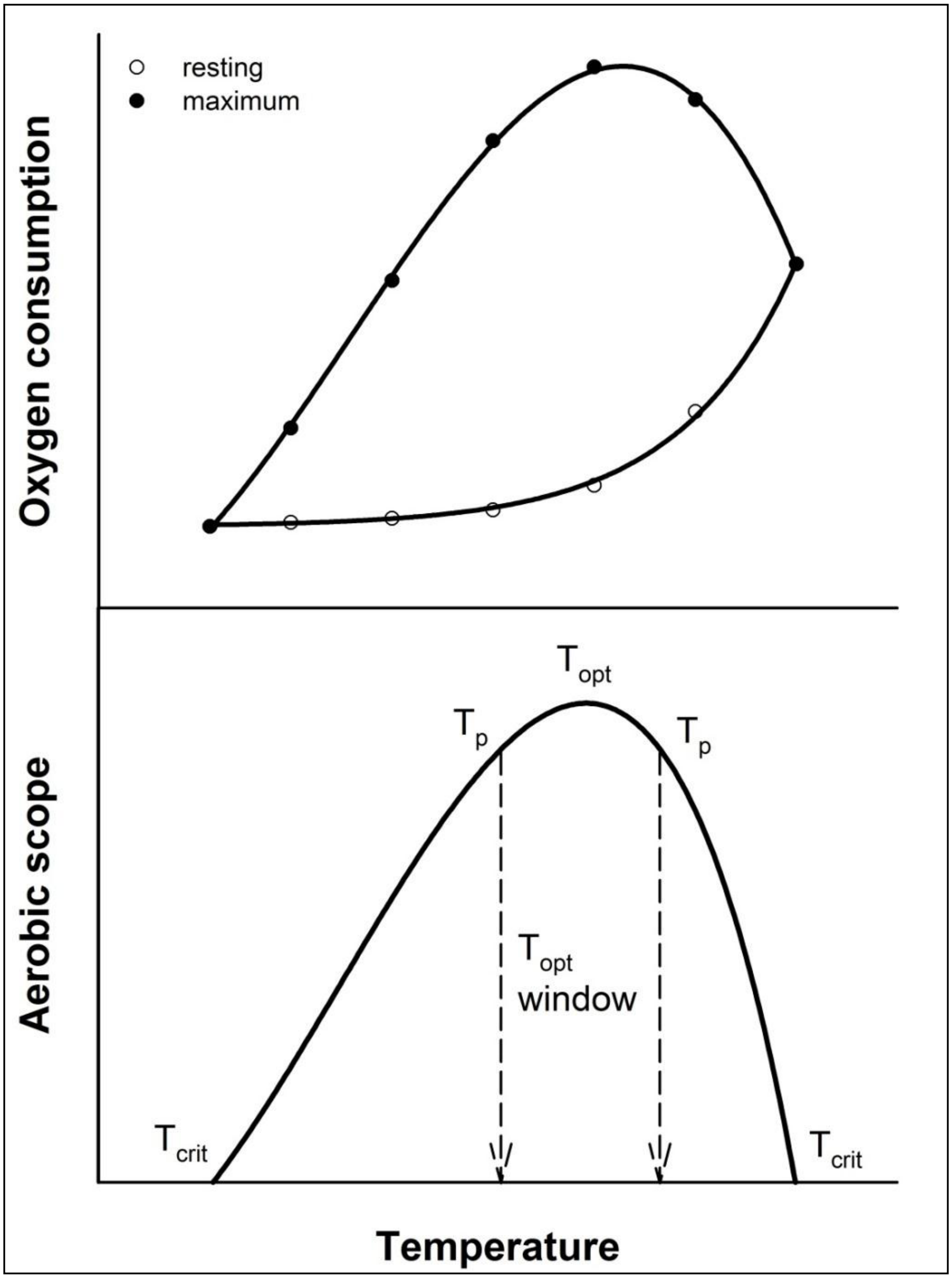


Figure 1. Schematic representation of the resting metabolic rate (= routine; RMR) and maximum metabolic rate (MMR) and aerobic scope (AS = MMR-RMR) for a temperature specialist. See text for details. T_{opt} = optimum temperature, T_p = pejus temperatures which set the thermal window or range in which 95% of the peak value for AS can be maintained; T_{crit} = critical temperatures where there is no aerobic scope. (Parsons 2011).

(a) RM 51.6

(b) RM 50.7

(c) RM 50.4

(d) RM 49.1

Figure 2. Representative photographic images (a-d) of the four capture locations for Tuolumne River *O. mykiss*, by river mile.

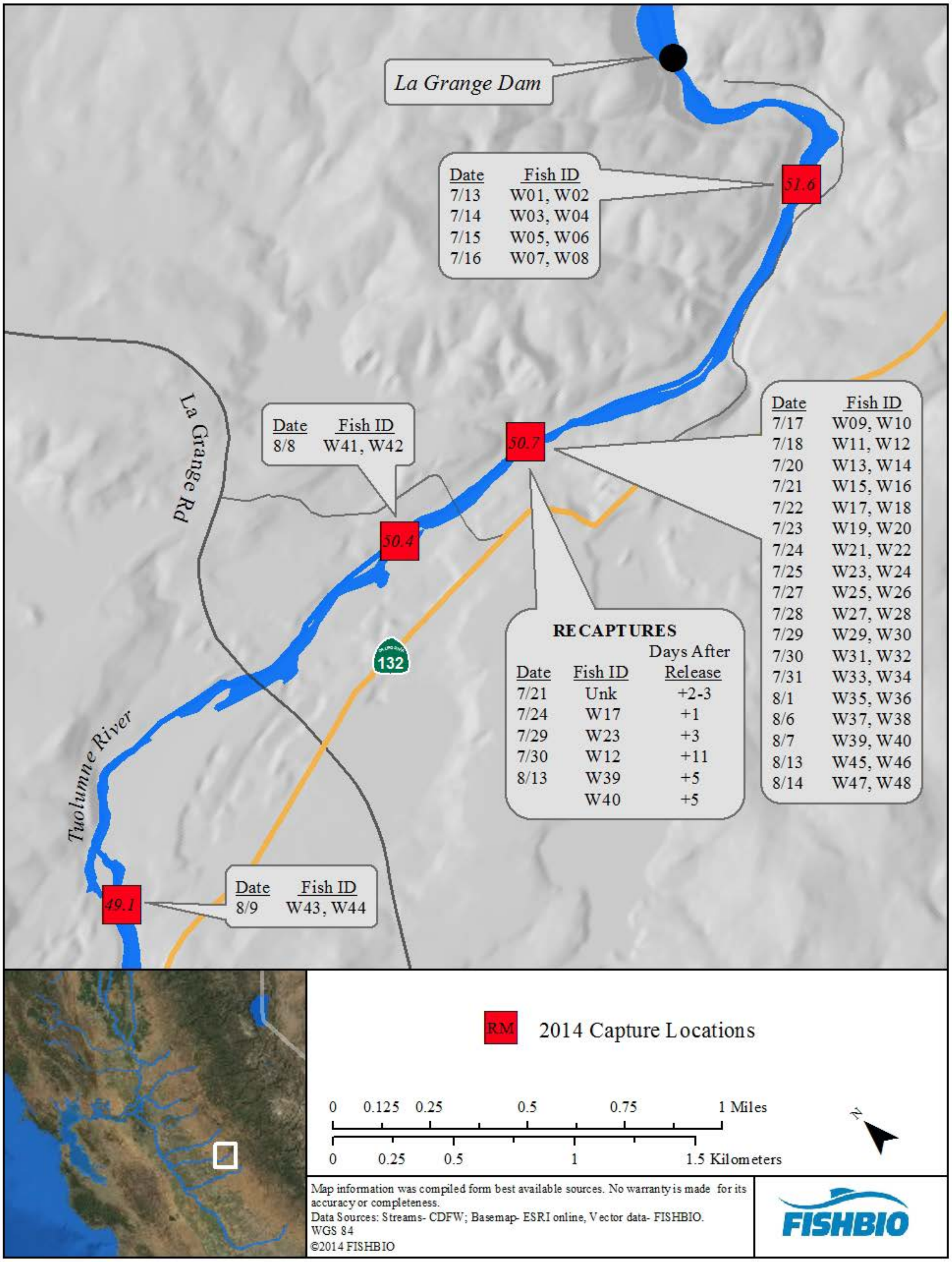


Figure 3. Map of capture and recapture locations of all Tuolumne River *O. mykiss* test fish.

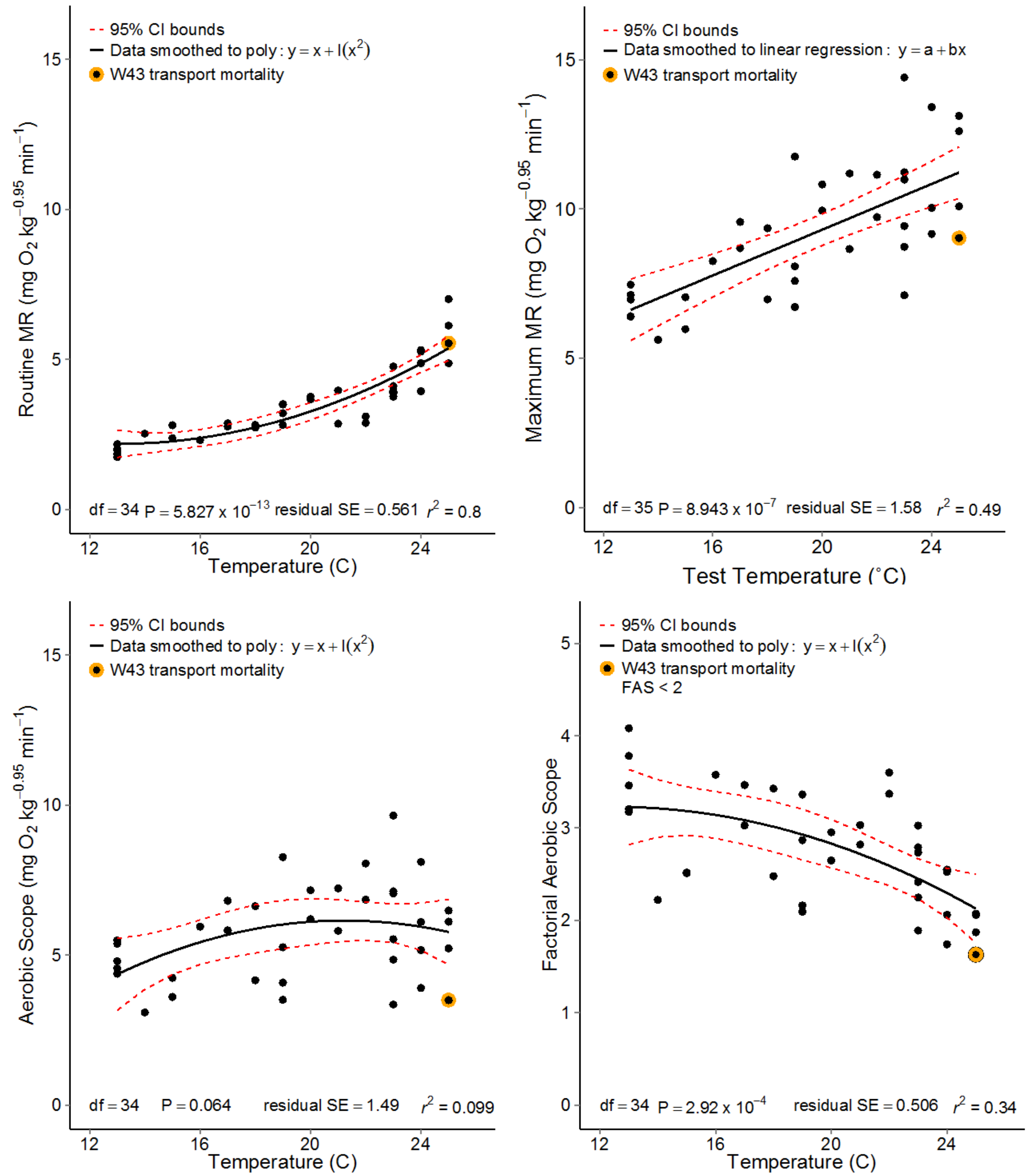


Figure 4. The relationships between test temperature and the routine (RMR) and maximum metabolic rate (MMR) of Tuolumne River *O. mykiss*. Absolute aerobic scope (AAS) and factorial aerobic scope (FAS) were derived from the MR measurements. Each data point represents an individual fish tested at one temperature. These data were given a best-fit mathematical model (solid line or curve) and the 95% confidence intervals for this line are indicated by the broken lines.

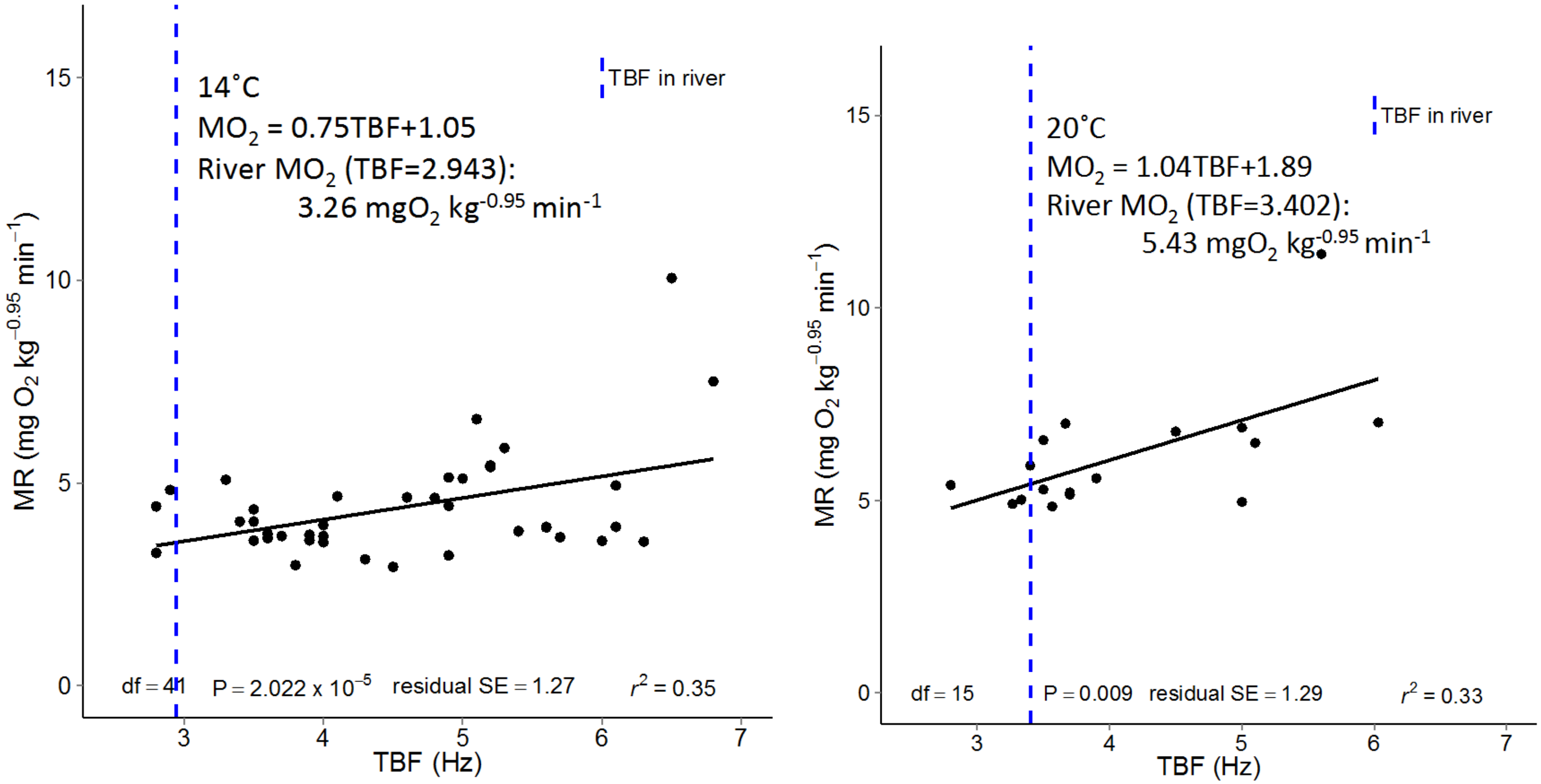


Figure 5. **The relationship between tail beat frequency (TBF; Hz) and metabolic rate (MR; mg O_2 kg$^{-0.95}$ min^{-1}) measured when Tuolumne River *O. mykiss* were swimming continuously in a swim tunnel at 14 °C or 20°C. The solid black line represents the linear regression based on the data for N=7 fish at 14°C and N=5 fish at 20oC). The blue dashed lines represent the estimated TBF (2.94 Hz at 14°C and 3.40 Hz at 20°C (bottom graph) taken from videos of *O. mykiss* maintaining station in a water current in their normal Tuolumne River habitat.**

TABLES

Table 1. Literature values of critical thermal maximum (CTmax) for *O. mykiss* populations.

Acclimation Temperature (°C)	CTmax (°C)	Heating rate (°C min-1)	Mass (g)	Length (cm)	Strain Source	Reference
8	26.9 ± 0.12	0.1		11 – 18	Washington	Becker and Wolford 1980
9.8	27.9 ± 0.05	0.3		15,3 ± 0.25	Pennsylvania	Carline and Machung 2001
10	28.5 ± 0.28	0.02				Lee and Rinne 1980
10	28.0 ± 0.12	0.3	~15	~10	Missouri	Currie et al. 1998
10	27.7 ±0.08	0.3	12.9 ± 0.6		California	Myrick and Cech 2000
11	27.5	0.3	8.0 ± 1.6		California	Myrick and Cech 2005
11 *	29.0 ± 0.05	0.3	2.4 ± 0.5		British Columbia	Scott 2012
13	27.9 ±0.14	0.33		21.8±0.4	Ontario	Leblanc et al. 2011
14	28.5±0.11	0.3	13.8 ± 0.8		California	Myrick and Cech 2000
14	29.4 ±0.1	0.03%	41 - 140		Oregon	Rodnick et al. 2004
15	29.4 ± 0.08	0.3				Strange et al. 1993
15	29.1 ± 0.09	0.3	~15	~10	Missouri	Currie et al. 1998
15	27.7 ±0.03	0.0014 #	89.9 ± 5.4	11.9 – 0.3	North Carolina	Galbreath et al. 2006
15	28.4	0.3	9.3 ± 2.0		California	Myrick and Cech 2005
15	~29.65	0.083 &			Miyazaki, Japan	Ineno et al. 2005
15	29.0 ± 0.02	0.3/0.1	30.2 ± 0.3	13.0 ± 0.4	Western Australia	Present study
18	31.2	0.3		4.1 – 20	Arizona	Recsetar et al. 2012
19	29.6	0.3	14.3 ± 2.9		California	Myrick and Cech 2005
19	29.9 ± 0.17	0.3	11.8 ± 0.7		California	Myrick and Cech 2000
20	29.35 ± 0.19	0.02				Lee and Rinne 1980
20	29.8 ±0.12	0.3	~2	~4	Missouri	Currie et al. 1998
20	~30.4	0.083 &			Miyazaki, Japan	Ineno et al. 2005
20	31.14±0.03	0.3		10.8±0.1	Hatchery (British Columbia)	Hartman and Porto 2014
20	31.20±0.03	0.3		11.9±0.1	Hatchery (Virginia)	Hartman and Porto 2014
20	31.29±0.02	0.3		9.5±0.1	Hatchery (Maryland)	Hartman and Porto 2014
22	30.9 ± 0.13	0.3	9.29 - 0.99		California	Myrick and Cech 2000
25	31.75 ± 0.1	0.3	6.1 - 0.63		California	Myrick and Cech 2000

*fish held at 10 ~12°C.

& temperature was increased at 5°C h^{-1}.

% temperature was increased at 2°C h^{-1}.

temperature was increased at 2°C day^{-1}.

Table 2. Literature values for routine metabolic rate (RMcR), maximum metabolic rate (MMR), absolute aerobic scope (AAS) and factorial aerobic scope (FAS) of juvenile salmonid fishes.

Species	Source[1] (test location)	Mass (g)	Temperature (°C)		Metabolic rates (mg O_2 $kg^{-0.95}$ min^{-1})			FAS	Reference
			Acclimate	Test	RMR	MMR	AAS		
Rainbow trout	Hatchery (L)	13	15	15	0.5	2.9	2.4	5.8	Alsop and Wood 1997
	Hatchery (L)	6	15	15	1	2.8	1.8	2.8	Alsop and Wood 1997
	Hatchery (L)	2-3	15	15	3.9	8.7	4.8	2.2	Scarabello et al. 1991
	Hatchery (L)	6	15	15	2.5	8.3	5.8	3.3	Scarabello et al. 1992
	Hatchery (L)	18	17	17	3.9	7	3.1	1.8	McGeer et al. 2000
	Eagle Lake Wild[2] (L)	6.9	10	10	2.6				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	7.2	14	14	2.8				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	14.1	19	19	2.6				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	13.4	22	22	2.9				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	5	25	25	3.1				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	10	10	10	2				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	7.5	14	14	2.3				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	24.5	19	19	2.2				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	15	22	22	2.4				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	5.4	25	25	2.9				Myrick and Cech 2000
Steelhead trout	Wild (H/F)	1.7	8.3	8.3	1.8-3.4	5.7-9.1			Van Leeuwen et al. 2011
	Hatchery (H/F)	3.3	12.3	12.3	1.9-3.6	5.5-9.7			Van Leeuwen et al. 2011
Rainbow trout	Wild[2] (L) (territorial)	60-80		13	0.6-1.9				Sloat and Reeves 2014
	Wild[2] (L) (dispersing)	60-80		13	0.6-1.5				Sloat and Reeves 2014
Rainbow cutthroat hybrid	Hatchery (F)	20-70	9.5-11	9.5-11	2.3				Rasmussen et al. 2012
Cutthroat trout	West slope Wild (F)	20-100	9.5-11	9.5-11	2.6				Rasmussen et al. 2012
Redband trout	Wild Bridge Creek (F)	92 (150-200 mm)	12-24*	13	1.8	8.5	6.7	4.7	Gamperl et al. 2002
	Wild Bridge Creek (F)	108 (150-200 mm)	12-24*	24	4.5	14	9.5	3.1	Gamperl et al. 2002
	Wild Little Blitzen River (F)	58	12-18*	13	2.4	12	9.6	5.0	Gamperl et al. 2002
	Wild Little Blitzen River (F)	71	12-18*	24	5.6	14	8.4	2.5	Gamperl et al. 2002

Species	Source[1] (test location)	Mass (g)	Temperature (°C)		Metabolic rates (mg O_2 $kg^{-0.95}$ min^{-1})			FAS	Reference
			Acclimate	Test	RMR	MMR	AAS		
	Wild 12 Mile Creek (F)	56	19-30 (23.4)*	24	4.7	18.3	13.6	3.9	Rodnick et al. 2004
	Wild Rock Creek (F)	50	12-27 (18.7)*	24	4.7	18	13.3	3.8	Rodnick et al. 2004
	Wild Bridge Creek (F)	63	13-21 (17)*	24	4.6	15.6	11	3.4	Rodnick et al. 2004
Sockeye salmon	Wild (L)	37 (170 mm)	5	5	0.9	7.6	6.7	8.4	Brett 1964
	Wild (L)	33(160 mm)	10	10	1.4	8.7	7.3	6.2	Brett 1964
	Wild (L)	55 (190 mm)	15	15	1.7	14.2	12.5	8.4	Brett 1964
	Wild (L)	63 (190 mm)	20	20	2.1	13.1	11	6.2	Brett 1964
	Wild (L)	52 (180 mm)	24	24	0.8	12.7	11.9	15.9	Brett 1964
	Wild (L)	20-60	5.3	5.3	0.5	6.9	6.4	13.8	Brett and Glass 1973
	Wild (L)	19-60	15	15	0.9	9.9	9	11.0	Brett and Glass 1973
	Wild (L)	20-50	20	20	1.7	12.5	10.8	7.4	Brett and Glass 1973
Coho salmon	Wild (H/F)	3.9	8.3	8.3	1.5-3.1	3.6-6.2			Van Leeuwen et al. 2011
	Hatchery (H/F)	5.4	12.3	12.3	1.1-2.3	3.8-6.5			Van Leeuwen et al. 2011
	Wild[2] (F)	40-100	9.5-11	9.5-11	3.2				Rasmussen et al. 2012
	Wild (L)	4.3	14	14	1.6				Van Leeuwen et al. 2012
Redband trout	Wild 12 Mile Creek (F)	94	19-30 (23)*	14	1.6				Rodnick et al. 2004
	Wild 12 Mile Creek (F)	94	19-30 (23)*	24	2.3				Rodnick et al. 2004
	Wild 12 Mile Creek (F)	94	19-30 (23)*	26	4.8				Rodnick et al. 2004
	Wild 12 Mile Creek (F)	94	19-30 (23)*	28	5.6				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	14	1.8				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	24	3.7				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	26	5.7				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	28	6.1				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	14	2.3				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	24	4.2				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	26	5.6				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	28	6.7				Rodnick et al. 2004

[1] L = laboratory; H = hatchery; F=Field.
[2] Spawned in a hatchery.
*Acclimations to cycled temperature regime of range indicated, and average in brackets if reported.

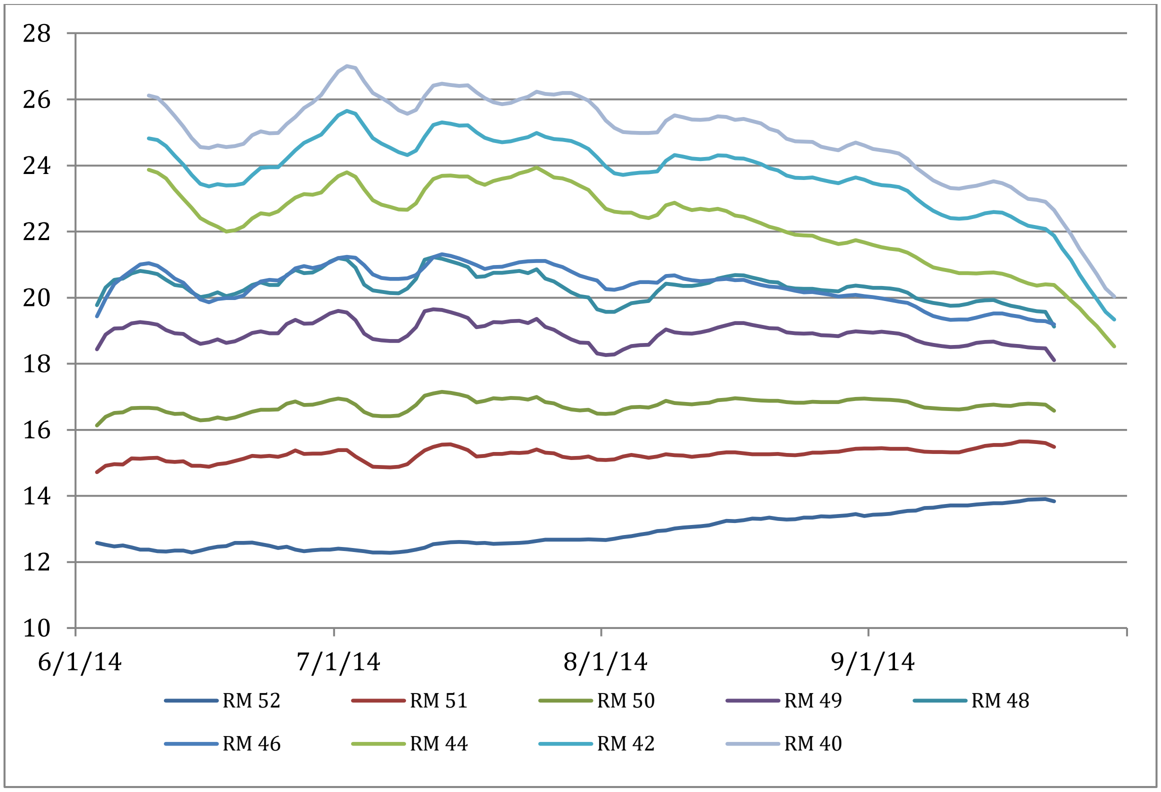
APPENDICES

THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

APPENDIX 1

TUOLUMNE RIVER 7-DAY AVERAGE OF MAXIMUM DAILY TEMPERATURES

Appendix 1. Tuolumne River 7-day average of maximum daily temperatures (7DADM) from June 1 to September 30, 2014. Thermograph data provided by TID (Patrick Maloney).



THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

APPENDIX 2

CAPTURE RELEASE TABLE

Appendix 2. Capture release table. Fish capture and release locations and physical conditions.

Fish ID	Capture				Release				Habitat Unit ID (Stillwater 2010)	Est. RM	Comments
	Coordinates	Date	Time	Temp (°C)	Coordinates	Date	Time	Temp (°C)			
W01	N - 37.66574 W - 120.44421	7/13	9:45	12.9	N - 37.66574 W - 120.44421	7/14	15:35	14.4	4 FW Riffle (side channel #3)	51.5	
W02	N - 37.66574 W - 120.44421	7/13	11:24	13.2	N - 37.66574 W - 120.44421	7/14	15:36	14.4	4 FW Riffle (side channel #3)	51.5	
W03	N - 37.66532 W - 120.44482	7/14	11:04	13.5	N - 37.66518 W - 120.44509	7/15	17:25	14.1	4 FW Riffle (side channel #3)	51.5	
W04	N - 37.66538 W - 120.44470	7/14	11:08	13.5	N - 37.66518 W - 120.44509	7/15	17:25	14.1	4 FW Riffle (side channel #3)	51.5	
W05	N - 37.66524 W - 120.44424	7/15	9:50	12.8	N - 37.66544 W - 120.44449	7/16	13:07	14.6	4 FW Riffle (side channel #3)	51.5	
W06	N - 37.66536 W - 120.44474	7/15	10:53	12.9	N - 37.66544 W - 120.44449	7/16	12:00	13.4	4 FW Riffle (side channel #3)	51.5	Fish not measured or PIT tagged to limit handling
W07	N - 37.66544 W - 120.44449	7/16	9:52	12.9	N - 37.66510 W - 120.44515	7/17	13:16	14	4 FW Riffle (side channel #3)	51.5	
W08	N - 37.66544 W - 120.44449	7/16	10:10	12.7	N - 37.66510 W - 120.44515	7/17	13:16	14	4 FW Riffle (side channel #3)	51.5	
W09	N - 37.66586 W - 120.45826	7/17	9:10	13.5	N - 37.66581 W - 120.45829	7/18	14:36	16	11 FW Riffle	50.7	
W10	N - 37.66586 W - 120.45826	7/17	9:24	13.5	N - 37.66581 W - 120.45829	7/18	14:36	16	11 FW Riffle	50.7	
W11	N - 37.66581 W - 120.45829	7/18	8:40	13.7	N - 37.66581 W - 120.45829	7/19	14:49	15.5	11 FW Riffle	50.7	
W12	N - 37.66581 W - 120.45829	7/18	8:40	13.7	N - 37.66581 W - 120.45829	7/19	14:49	15.5	11 FW Riffle	50.7	
W13	N - 37.66579 W - 120.45832	7/20	8:48	13.4	N - 37.66585 W - 120.45823	7/21	13:59	15.3	11 FW Riffle	50.7	
W14	N - 37.66579 W - 120.45832	7/20	8:48	13.4	N - 37.66585 W - 120.45823	7/21	13:59	15.3	11 FW Riffle	50.7	
W15	N - 37.66585 W - 120.45823	7/21	8:35	13.3	N - 37.66579 W - 120.45834	7/22	13:47	15.0	11 FW Riffle	50.7	7/21- recaptured a PIT tagged fish #114779, 114769, or 114734

Fish ID	Capture				Release				Habitat Unit ID (Stillwater 2010)	Est. RM	Comments
	Coordinates	Date	Time	Temp (°C)	Coordinates	Date	Time	Temp (°C)			
W16	N - 37.66585 W - 120.45823	7/21	8:35	13.3	N - 37.66579 W - 120.45834	7/22	13:47	15.0	11 FW Riffle	50.7	
W17	N - 37.66579 W - 120.45834	7/22	10:23	13.6	N - 37.66579 W - 120.45839	7/23	14:13	15.4	11 FW Riffle	50.7	
W18	N - 37.66579 W - 120.45834	7/22	10:28	13.6	N - 37.66579 W - 120.45839	7/23	14:13	15.4	11 FW Riffle	50.7	
W19	N - 37.66579 W - 120.45834	7/23	10:10	13.5	N - 37.66574 W - 120.45786	7/24	14:29	15.3	11 FW Riffle	50.7	
W20	N - 37.66579 W - 120.45834	7/23	10:27	13.5	N - 37.66574 W - 120.45786	7/24	14:29	15.3	11 FW Riffle	50.7	
W21	N - 37.66579 W - 120.45828	7/24	9:00	13.5	N - 37.66571 W - 120.45794	7/25	14:00	15.4	11 FW Riffle	50.7	7/24- recaptured PIT tag #114752
W22	N - 37.66579 W - 120.45828	7/24	9:00	13.5	N - 37.66571 W - 120.45794	7/25	14:00	15.4	11 FW Riffle	50.7	
W23	N - 37.66582 W - 120.45830	7/25	9:05	13.5	N - 37.66582 W - 120.45830	7/26	13:33	15.1	11 FW Riffle	50.7	
W24	N - 37.66582 W - 120.45830	7/25	9:05	13.6	N - 37.66582 W - 120.45830	7/26	13:33	15.1	11 FW Riffle	50.7	
W25	N - 37.66565 W - 120.45826	7/27	8:15	13.6	N - 37.66565 W - 120.45826	7/28	14:15	14.5	11 FW Riffle	50.7	
W26	N - 37.66565 W - 120.45826	7/27	8:15	13.6	N - 37.66565 W - 120.45826	7/28	14:15	14.5	11 FW Riffle	50.7	
W27	N - 37.66565 W - 120.45826	7/28	9:15	13	N - 37.66565 W - 120.45826	7/29	14:15	14.9	11 FW Riffle	50.7	
W28	N - 37.66565 W - 120.45826	7/28	9:15	13	N - 37.66565 W - 120.45826	7/29	14:15	14.9	11 FW Riffle	50.7	
W29	N - 37.66565 W - 120.45826	7/29	9:30	13.3	N - 37.66574 W - 120.45788	7/30	16:30	14.7	11 FW Riffle	50.7	7/29- recaptured PIT tag #114809
W30	N - 37.66565 W - 120.45826	7/29	9:18	13.3	N - 37.66574 W - 120.45788	7/30	16:30	14.7	11 FW Riffle	50.7	
W31	N - 37.66565 W - 120.45826	7/30	9:00	13.3	N - 37.66565 W - 120.45826	7/31	13:38	15.1	11 FW Riffle	50.7	7/30- recaptured PIT tag #114734
W32	N - 37.66565 W - 120.45826	7/30	9:07	13.3	N - 37.66565 W - 120.45826	7/31	13:38	15.1	11 FW Riffle	50.7	

Fish ID	Capture				Release				Habitat Unit ID (Stillwater 2010)	Est. RM	Comments
	Coordinates	Date	Time	Temp (°C)	Coordinates	Date	Time	Temp (°C)			
W33	N - 37.66565 W - 120.45826	7/31	9:05	13.1	N - 37.66565 W - 120.45826	8/1	13:42	15.0	11 FW Riffle	50.7	
W34	N - 37.66565 W - 120.45826	7/31	9:05	13.1	N - 37.66565 W - 120.45826	8/1	13:42	15.0	11 FW Riffle	50.7	
W35	N - 37.66565 W - 120.45826	8/1	9:02	13.2	N - 37.66565 W - 120.45826	8/2	15:40	15.8	11 FW Riffle	50.7	
W36	N - 37.66565 W - 120.45826	8/1	9:30	13.2	N - 37.66565 W - 120.45826	8/2	15:40	15.8	11 FW Riffle	50.7	
W37	N - 37.66565 W - 120.45826	8/6	9:18	13.4	--	--	--	--	11 FW Riffle	50.7	Mortality- due to chloride residue in tunnel
W38	N - 37.66565 W - 120.45826	8/6	9:28	13.4	--	--	--	--	11 FW Riffle	50.7	Mortality- due to chloride residue in tunnel
W39	N - 37.66565 W - 120.45826	8/7	9:08	13.5	N - 37.66668 W - 120.46420	8/8	17:31	15.8	11 FW Riffle	50.7	
W40	N - 37.66565 W - 120.45826	8/7	9:30	13.5	N - 37.66668 W - 120.46420	8/8	17:31	15.8	11 FW Riffle	50.7	
W41	N - 37.66643 W - 120.46432	8/8	11:18	15.5	N - 37.66643 W - 120.46432	8/9	16:00	16.7	14 BC Riffle	50.4	
W42	N - 37.66643 W - 120.46432	8/8	11:35	14.6	N - 37.66643 W - 120.46432	8/9	16:00	16.7	14 BC Riffle	50.4	
W43	N - 37.66426 W - 120.48132	8/9	11:40	17.1	N - 37.66308 W - 120.48160	8/10	15:13	18.0	25 BC Riffle	49.1	Mortality- post- swim test transport
W44	N - 37.66426 W - 120.48132	8/9	11:40	17.1	N - 37.66308 W - 120.48160	8/10	15:13	18.0	25 BC Riffle	49.1	
W45	N - 37.66565 W - 120.45826	8/13	10:25	14.4	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish not PIT tagged to limit handling after study termination per NMFS Section 10 permit conditions
W46	N - 37.66565 W - 120.45826	8/13	10:59	13.9	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish not PIT tagged to limit handling after study termination per NMFS Section 10 permit conditions
W47	N - 37.66565 W - 120.45826	8/14	9:08	13.6	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish released w/o testing per NMFS Section 10 permit conditions
W48	N - 37.66565 W - 120.45826	8/14	9:15	13.6	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish released w/o testing per NMFS Section 10 permit conditions

THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

APPENDIX 3

PIT CODE AND RECAPTURE TABLE

Appendix 3. PIT code and recapture table. Only five out of seven recapture fish are included in this table because PIT IDs were not recorded for two of the recaptured fish. See Figure 3 for details on the two unidentified recaptured fish, and recapture location for all recaptured fish. Days post-release is the number of days after release the PIT was recaptured.

Fish ID	PIT	Test Temp (˚C)	PIT recap freq	Days post release
W01	114756	13		
W02	114745	13		
W03	114743	13		
W04	114720	13		
W05	114764	15		
W06	--	15		
W07	114755	19		
W08	114807	19		
W09	114779	21		
W10	114773	21		
W11	114769	23		
W12	114734	23	1	11
W13	114750	17		
W14	114759	17		
W15	114741	14		
W16	114766	14		

Fish ID	PIT	Test Temp (˚C)	PIT recap freq	Days post release
W17	114752	16	1	1
W18	114808	16		
W19	114803	20		
W20	114723	20		
W21	114786	22		
W22	114730	22		
W23	114809	18	1	3
W24	114714	18		
W25	114787	23		
W26	114725	23		
W27	526260	17		
W28	526292	17		
W29	526299	24		
W30	526275	24		
W31	526297	19		
W32	526212	19		

Fish ID	PIT	Test Temp (˚C)	PIT recap freq	Days post release
W33	526226	13		
W34	526211	13		
W35	526285	25		
W36	526263	25		
W37	--	--		
W38	--	--		
W39	526255	23	1	5
W40	526298	23	1	5
W41	526227	24		
W42	526235	24		
W43	526284	25		
W44	526252	25		
W45	--	19		
W46	--	19		
W47	--	--		
W48	--	--		

THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

APPENDIX 4

EXPERIMENTAL DATA TABLE

Appendix 4. Experimental data table. RMR: routine metabolic rate; MMR: maximum metabolic rate; AAS: absolute aerobic scope; FAS: factorial aerobic scope; K: condition factor (mass x 10^5 / FL^3).

Fish ID	Test Temp (°C)	RMR (mg O_2 $kg^{-0.95}min^{-1}$)	MMR (mg O_2 $kg^{-0.95}min^{-1}$)	AAS (mg O_2 $kg^{-0.95}min^{-1}$)	FAS	FL (mm)	Mass (g)	K	Body Depth (mm)	Body Width (mm)	Quality Control
W01	13	1.97	7.46	5.49	3.78	112	15.7	1.12	21	9	
W02	13	2.25				110	13.3	1.00	19	9	DISCARD; tunnel leak confirmed
W03	13	1.85	6.40	4.55	3.46	102	10.9	1.03	19.5	11	
W04	13	1.75	7.12	5.37	4.08	102	10.6	1.00	19.5	14	
W05	15	2.80	7.05	4.24	2.51	113	13.4	0.93	22.0	11	
W06	15	2.37	5.98	3.61	2.52	~160	~29.2	0.87			
W07	19		9.79			126	21.4	1.05	22.5	12	DISCARD; activity during RMR
W08	19		6.41			100	10.5	1.07	18.0	9	DISCARD; activity during RMR
W09	21	3.96	11.19	7.23	2.82	125	20.2	1.03	24.0	12	
W10	21	2.86	8.66	5.80	3.03	197	79.6	1.04	36.0	20	
W11	23	3.94	10.99	7.05	2.79	132	24.3	1.06	21.0	12	
W12	23	3.88	8.73	4.85	2.25	131	25.1	1.12	24.0	13	
W13	17	1.89				141	29.4	1.05	26.0	14	DISCARD; no MR increase with velocity 33 to 53 cms^{-1}
W14	17	2.47				142	29.9	1.04	23.0	10	DISCARD; no MR increase with velocity 30 to 46 cms^{-1}
W15	14	2.14				129	22.2	1.03	26.0	11	DISCARD; no MR increase with velocity 32 to 46 cms^{-1}
W16	14	2.53	5.61	3.08	2.22	137	28.4	1.10	24.0	12	
W17	16		8.13			135	27.6	1.12	26.0	13	DISCARD; activity during RMR
W18	16	2.31	8.26	5.95	3.58	133	25.9	1.10	25.0	10	
W19	20	3.75	9.95	6.19	2.65	147	38.4	1.21	28.0	11	
W20	20	3.66	10.83	7.16	2.96	134	28.1	1.17	25.0	11	
W21	22	3.09	11.15	8.06	3.61	124	21.7	1.14	21.0	10	

Fish ID	Test Temp (°C)	RMR (mg O_2 kg$^{-0.95}$min^{-1})	MMR (mg O_2 kg$^{-0.95}$min^{-1})	AAS (mg O_2 kg$^{-0.95}$min^{-1})	FAS	FL (mm)	Mass (g)	K	Body Depth (mm)	Body Width (mm)	Quality Control
W22	22	2.89	9.73	6.84	3.37	115	15.8	1.04	19.0	8	
W23	18	2.73	9.35	6.62	3.42	164	47.1	1.07	30.0	18	
W24	18	2.81	6.97	4.16	2.48	133	22.6	0.96	21.0	13	
W25	23	4.11	11.23	7.12	2.73	121	18.7	1.06	20.0	11	
W26	23	3.90	9.43	5.53	2.42	129	23.4	1.09	23.0	12	
W27	17	2.76	9.57	6.81	3.47	134	24.9	1.03	21.0	13	
W28	17	2.87	8.69	5.81	3.02	122	19.9	1.10	24.0	12	
W29	24	5.31	13.41	8.10	2.52	104	13.0	1.16	18.0	10	
W30	24	5.26	9.17	3.91	1.74	115	16.5	1.08	19.0	12	
W31	19	2.81	8.07	5.26	2.87	138	29.0	1.10	24.0	10	
W32	19	3.21	6.71	3.51	2.09	140	27.2	0.99	28.0	11	
W33	13	2.17	6.97	4.80	3.21	117	16.4	1.02	19.0	8	
W34	13	2.02	6.40	4.38	3.17	105	12.2	1.05	19.0	7	
W35	25	4.87	10.09	5.21	2.07	130	27.4	1.25	26.0	10	
W36	25	7.01	13.12	6.11	1.87	111	12.4	0.91	17.0	7	
W37	Mortality- due to chloride residue in tunnel										
W38	Mortality- due to chloride residue in tunnel										
W39	23	3.76	7.11	3.36	1.89	101	12	1.02	17.0	6	
W40	23	4.76	14.41	9.65	3.03	122	18.5	1.16	20.0	10	
W41	24	4.87	10.04	5.17	2.06	131	23.1	1.03	22.0	12	
W42	24	3.94	10.04	6.10	2.55	138	25.5	0.97	22.0	12	
W43	25	5.54	9.03	3.49	1.63	107	14.5	1.18	19.0	8	
W44	25	6.13	12.61	6.48	2.06	113	14.9	1.03	19.0	8	
W45	19	3.49	11.76	8.27	3.37	~101	~11.5	1.12	~16	~10	
W46	19	3.51	7.59	4.08	2.16	~108	~13.1	1.04	~17	~10	

Fish ID	Test Temp (°C)	RMR (mg O_2 $kg^{-0.95}min^{-1}$)	MMR (mg O_2 $kg^{-0.95}min^{-1}$)	AAS (mg O_2 $kg^{-0.95}min^{-1}$)	FAS	FL (mm)	Mass (g)	K	Body Depth (mm)	Body Width (mm)	Quality Control
W47	Fish released w/o testing per NMFS Section 10 permit conditions										
W48	Fish released w/o testing per NMFS Section 10 permit conditions										

THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

APPENDIX 5

COMMENTS RECEIVED ON THE DRAFT STUDY REPORT

Tuolumne River Trust

CALIFORNIA SPORTFISHING PROTECTION ALLIANCE CSPA * Since 1983 *

March 2, 2015

Ms. Rose Staples
HDR, Inc.
rose.staples@hdrinc.com

Re: Comments on January 31, 2015 draft of *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature.*

Dear Ms. Staples,

The California Sportfishing Protection Alliance (CSPA) and the Tuolumne River Trust (TRT) submit the following comments on the January 31, 2015 draft of *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature* ("Study").

Overview

Based on our review of the Study and some of the background material cited in the Study, including the EPA (2003) *Region 10 Guidance For Pacific Northwest State and Tribal Temperature Water Quality Standard* that the Study in significant part seeks to address, it appears to us that the Study proposes to recommend to regulators a change in the established EPA (2003) temperature benchmark for a 7DADM value for the population of *O. mykiss* in the lower Tuolumne River based on site-specific evidence.

The EPA (2003) guidelines recognize that site-specific thermal criteria for salmonids may be developed that are more appropriate for specific locations and populations than are the general criteria promulgated in the guidelines. Evaluation of physiological response in a target population is an appropriate approach to development of site-specific conditions. We accept the premise of the Study that site-specific physiological study of the response of fish to water temperature may demonstrate that such response in a specific population is different than broader, more general and geographically unspecific studies of the response of fish to water temperature have shown.

Neither CSPA nor the Tuolumne River Trust has fisheries physiologists on staff, and neither has the resources to hire a consulting fisheries physiologist at this time. We therefore have no comment at this time on the experimental approach adopted within the Study, the value of the metrics adopted, or the execution of the Study. We may bring in an outside consultant at a later point in the ILP process to evaluate these and other technical aspects of the Study.

Instead, we confine our comments to the implicit and explicit argument that Study results can "be used to determine a 7DADM value for this population." (Study Conclusion, p. 24).

The Study does not evaluate the physiological response of the population of *O. mykiss* in the lower Tuolumne River over time.

There are limitations to the Study that the Study does not acknowledge. Chief among these limitations is that the Study does not evaluate physiological response of the population of *O. mykiss* in the lower Tuolumne River over time. On the contrary, 75% of the test fish were sourced from a location one mile downstream of La Grange Powerhouse, where temperatures at capture ranged from 12.7° C to 17.1° C. While the Study is critical of Hokanson (1977) for an issue concerning confidence intervals, the Study does not address Hokanson's use of a 40-day period to evaluate physiological response. Other studies (e.g. Brett 1956; Bidgood 1969) similarly address long-term exposure to less-than-optimal thermal conditions. The Study does not acknowledge this limitation. It is akin to trying to determine the best overall athletic performance in a decathlon based on performance in the sprint alone.

Thermal conditions in the summer in most of the lower Tuolumne River are much more comparable to a marathon than a sprint. In the absence of adequate flow, grinding ambient temperatures with daily highs greater than 90° F for four months, and greater than 100° F on multiple days, create long-term water temperatures that are stressful to juvenile and adult *O. mykiss*. A City of San Francisco biologist has acknowledged on the record in this proceeding that *O. mykiss* populations in the lower Tuolumne River are substantially smaller than populations downstream of rim dams in the Sacramento River drainage, where water temperatures are generally much lower than temperatures in the lower Tuolumne River.[1] A change in the 7DADM value for the population of *O. mykiss* in the lower Tuolumne River is not warranted based on the evidence presented. The document should therefore be re-cast as a study, rather than walking what appears to us to be a gray line between a study and a position paper that advocates a departure from established guidance.

Before any adjustment to the established (EPA 2003) temperature benchmark for a 7DADM value for the population of *O. mykiss* in the lower Tuolumne River is considered based on site-specific conditions and response, further investigation and evaluation would be required. The Study should explicitly state this, and should describe additional evidence needed before any change in the 7DADM value for the population *O. mykiss* in the lower Tuolumne River might appropriately be evaluated.

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[1] *See* Dr. Ronald Yoshiyama, "*Commentary on Evaluating the Temperature-Related Flow Requirements of Steelhead-Rainbow Trout (Oncorhynchus Mykiss) in the Lower Tuolumne River: A Literature Review and Synthesis*," eLibrary no. 20120807-5082 (July 5, 2012), p. 2: "The actual numbers of adult and juvenile trout in the lower Tuolumne River were not accurately known until recently. Routine fish monitoring by the Districts indicates relatively low numbers of trout have been present over the past 1-2 decades--i.e., far below the numbers occurring in the Sacramento River mainstem and tributaries."

We discuss additional limitations of the Study and additional evidentiary needs below.

The Study results alone do not warrant site-specific summer water temperature criteria for *O. mykiss* in the lower Tuolumne River.

The Study is careful in its language not to state outright that its results *alone* can be used to develop alternative summer temperature criteria for the lower Tuolumne River. The Executive Summary states:

> Moreover, given that the average AAS remained within 5% of peak performance up to a temperature of 24.6°C and that all Tuolumne River *O. mykiss* maintained a FAS value >2.0 up to 23°C, we recommend that a conservative upper performance limit of 22°C, instead of 18°C, *be used to determine* a 7-Day Average of the Daily Maximum (7DADM) value. (Study, p. ii, emphasis added).

The Conclusion states in greater context:

> High quality field data were generated on the physiological performance of Tuolumne River *O. mykiss* acutely exposed to a temperature range of 13 to 25°C. These data on the RMR, MMR, AAS, and FAS were consistent with higher thermal performance in Tuolumne River *O. mykiss* compared to that used to generate the 7DADM value of 18°C using Pacific northwest *O. mykiss* (EPA 2003). These new data are consistent with recent peer-reviewed literature that points to local thermal adjustments among salmonid populations. Therefore, these data provide sound evidence to establish alternative numeric criteria that would apply to the Tuolumne River *O. mykiss* population below La Grange Diversion Dam. Given a measured Topt for AAS of 21.2°C, and that the average AAS remained within 5% of this peak performance up to 24.6°C, and all fish maintained a FAS value >2 up to 23°C, we recommend that a conservative upper performance limit of 22°C, instead of 18°C, *be used to determine* a 7DADM value for this population. (Study, p. 24, emphasis added)

The use of the passive voice ("be used to determine") is at once imprecise as to the nature and context of such use and imprecise as to who will or should use it. In our view, the appropriate use of the Study results would be to 1) evaluate their limitations; 2) develop additional investigations that might be necessary to scientifically justify consideration of adjusting thermal criteria for the population of *O. mykiss* in the lower Tuolumne River, 3) enumerate and evaluate regulatory and policy issues that might be involved in adjusting these criteria; and 4) assemble these necessary components and, based on this ensemble, develop a process for considering and evaluating site-specific water temperature criteria.

However, the Study provides no such context and proposes no such process. While the Study does not explicitly say that its results alone can be used to develop alternative summer temperature criteria for the lower Tuolumne River, the Districts have already used the results of the Study to advocate that temperatures greater than those of the EPA (2003) criteria be considered appropriate to determine amount of usable habitat in the lower Tuolumne. The draft

Lower Tuolumne River Instream Flow Study—Evaluation of effective usable habitat area for over-summering O. mykiss distributed by the Districts' consultants to relicensing participants on February 27, 2015 adopts a higher range of suitable temperatures for over-summering O. mykiss *based on the present Thermal Performance Study*:

> Although the majority of historical (1996–2009) snorkel survey observations of *O. mykiss* in the lower Tuolumne River have occurred at temperatures of 20°C (68°F) or below (Ford and Kirihara 2010), *O. mykiss* have been routinely observed occupying Tuolumne River habitats at temperatures ranging from 11–25°C (52–77°C). Using wild juvenile *O. mykiss* collected from the Tuolumne River in the summer of 2014, a recently completed thermal performance study (Farrell et al. 2014) found a peak in the absolute aerobic scope (AAS) vs. temperature curve at 21.2°C (70°F), higher than the 19°C (66°F) growth rate optimum identified by Myrick and Cech (2001). Because Farrell et al. (2014) also found that the AAS of the wild *O. mykiss* test fish remained within 5% of the peak AAS between 17.8°C (64°F) to 24.6°C (76°F), these site-specific empirical data with broader temperature thresholds were selected for evaluation of thermal suitability for *O. mykiss*. In the current study, the temperatures of 18°C (66.4°F), 20°C (68°F), 22°C (71.6°F), and 24°C (75.2°F) were evaluated over each of the summer months (June through September) when these temperatures can be exceeded in the lower Tuolumne River.[2]

In skipping from study to study, any caveats and limitations that might be present or implied disappear. In order to avoid such misuse, the authors of the current Study should be more explicit in its caveats and should describe the limitations of its conclusions.

The Study may be limited because it analyzes a single lifestage.

The Study examines only the juvenile lifestage of *O. mykiss* in the lower Tuolumne River. The Clean Water Act requires that the most sensitive resources be protected. It is not clear whether the adult lifestage, which is also present during the summer time period, is more, equally or less sensitive to high water temperatures. Before adjustments of summer temperature criteria for *O. mykiss* in the lower Tuolumne River could be considered, an evaluation of the physiological response of adult *O. mykiss* in the lower Tuolumne River would need to conducted, in addition to completing the evaluation of the physiological response of juveniles.

The Study makes comparisons between *O. mykiss* in the lower Tuolumne River and populations that are more permanent and defined and that have more common characteristics.

The Study draws comparisons with other populations of rainbow trout that have demonstrated higher temperature tolerances than the figures given for juvenile rearing in the EPA (2003) Criteria. Several of these are cited in the EPA document, including redband trout in Eastern Oregon, southern California coastal steelhead, and trout introduced in Australia.

[2] Stillwater Sciences, 2015, *Lower Tuolumne River Instream Flow Study—Evaluation of effective usable habitat area for over-summering O. mykiss*. Draft Report. Prepared by Stillwater Sciences, Davis, California for Turlock Irrigation District, Turlock California and Modesto Irrigation District, Modesto, California. Distributed to relicensing participants via e-mail by Ms. Rose Staples on February 27, 2015, pp. 2-3.

Certainly at least the redband and southern California steelhead are more likely to share common ancestry and even genetics than the fish in the lower Tuolumne River, where the population was extremely small due to low project flows until 1995. The current Tuolumne population is likely a combination of residual lower river fish, wild or hatchery fish washed down from La Grange (themselves possibly the result of production in La Grange Reservoir or originating in Don Pedro Reservoir), and some number of anadromous individuals of unknown origin and their progeny. It is further likely that the population is being replenished from these sources on an ongoing basis, and that some portion of the fish that are there in several years will have little directly in common with the current population. This is particularly likely under dry or drought conditions, when a greater proportion of the existing population may be expected to perish. Managing a changing population based on ascribed thermal tolerances of an existing population is questionable both scientifically and as policy.

It is likely that the present population in the lower Tuolumne is temperature tolerant because it has had to be in order to survive, and that improved thermal conditions would create a larger population. Improved thermal conditions would certainly increase the volume of suitable habitat by pushing thermal limitations further downstream. It is a policy as well as a scientific question whether to manage to the highest suitable temperature (whatever that may be) or to manage to what is likely to produce a stronger population. On a policy and recreational basis, it is hard to justify a small population managed for small fish. If the population were more robust, the argument for managing to a higher temperature would be more credible.

There is no bioenergetics study of O. mykiss in the lower Tuolumne River that would support management for water temperatures higher than those recommended in EPA (2003) guidance.

The Districts declined in 2011 to conduct a bioenergetics study of O. mykiss in the lower Tuolumne River as recommended by the Department of Fish and Wildlife.[3] The Commission did not order this study. The current Study recognizes: "the important ecological consideration is whether or not there is sufficient food in the Tuolumne River to support the highest MR associated with high temperature." (Study, p. 22). The Study supports the hypothesis that sufficient food is present only with anecdotal data:

> All available studies suggest that the Tuolumne River population is not food limited, including direct studies of Tuolumne River Chinook salmon diet (TID/MID 1992, Appendix 16), long-term benthic macro-invertebrate sampling data collected from 1988–2008 (e.g., TID/MID 1997, Report 1996-4; TID/MID 2009, Report 2008-7), as well as the relatively high length-at-age for *O. mykiss* sampled in 2012 (Stillwater Sciences 2013). Indeed, the *O. mykiss* sampled for the current study were apparently feeding well in the river during summer months given the high condition factors (see Appendix 2), feces being regularly found in the swim tunnel and two test fish regurgitating rather large meals post-exhaustion. (*ibid*).

[3] *See* California Department of Fish and Wildlife, *Comments on Proposed Study Plan*, eLibrary 20111024-5118, p. 55 ff., proposed Bioenergetics Study.

It is one thing to say that there is apparently sufficient food in the lower Tuolumne for the small population of *O. mykiss* located in a relatively small section of the river. It is quite another to argue in the absence of a targeted study that food production is great enough to support a larger population at the highest metabolic rate associated with high water temperatures. There is no evidence to support such a finding. If food is indeed unusually abundant, why is the *O. mykiss* population in the lower Tuolumne River neither greatly abundant nor characterized by large numbers of large fish?

Conclusion and recommendations

The summer water temperature criteria that are apparently recommended in the Study, and that are more definitively recommended based on the present Study in the just-released draft study entitled *Lower Tuolumne River Instream Flow Study—Evaluation of effective usable habitat area for over-summering O. mykiss*, are not warranted by the evidence the Study has collected. If the Districts wish to persist in seeking to define site-specific summer water temperature criteria for the lower Tuolumne River, they should affirmatively address the scientific and policy issues we have described above. In brief, these are

1. Follow-up site specific physiological studies must address elevated water temperatures over an extended period of time, ideally over an entire summer.
2. Follow-up site specific physiological studies must be conducted on adult as well as juvenile *O. mykiss*.
3. Follow-up site specific physiological studies must address the likely multiple sources and ongoing replenishment of the *O. mykiss* population of the lower Tuolumne River.
4. The Districts should perform a bioenergetics study for juvenile and adult *O. mykiss* in the lower Tuolumne River.

In addition, the Study should be edited so that the Executive Summary and the Conclusion place the value of the findings in the appropriate context of how they might inform a comprehensive review of site-specific summer thermal conditions in the lower Tuolumne River.

Please contact Chris Shutes if you have any questions. Thank you for the opportunity to comment on the draft of the Study entitled *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature*.

Respectfully submitted,

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State Water Resources Control Board

MAR 1 8 2015

Ms. Rose Staples
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Portland, ME 04103
Via email: Rose.Staples@hdrinc.com

Dear Ms. Staples:

COMMENTS ON THE THERMAL PERFORMANCE OF WILD JUVENILE ONCORHYNCHUS MYKISS IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE REPORT; NEW DON PEDRO HYDROELECTRIC PROJECT; FEDERAL ENERGY REGULATORY COMMISSION PROJECT NO. 2299

On January 30, 2015 the State Water Resources Control Board (State Water Board) received the *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature Report* (Report). This Report was developed by Turlock Irrigation District and Modesto Irrigation District (collectively referred to as Districts[1]) as part of the Federal Energy Regulatory Commission (FERC) relicensing of the Don Pedro Hydroelectric Project (Project). The Project is also referred to as FERC Project No. 2299. The Report is a result of Water and Aquatic Resource (W&AR) Study Plan 14: Temperature Criteria Assessment (Study Plan 14) developed by the Districts. Study Plan 14 was not required by FERC in its Final Study Plan Determination and is not supported by the State Water Board, California Department of Fish and Wildlife (CDFW), United States Fish and Wildlife Service (USFWS), or the National Marine Fisheries Services (NMFS).

Throughout the relicensing process, State Water Board staff maintained that the relicensing studies and environmental impact analyses should use the temperature criteria for salmonids outlined in the 2003 United States Environmental Protection Agency (USEPA) *Region 10 Guidance for Pacific Northwest State and Tribal Temperature Water Quality Standards* (USEPA Guidance) (USEPA 2003). The Tuolumne River from Don Pedro Reservoir to the San Joaquin River was listed as impaired for temperature under Section 303(d) of the Clean Water Act (CWA) in 2008. The 2003 USEPA Guidance for salmonids was used as the evaluation guideline for five of the six lines of evidence used to support the Section 303(d) listing of the Tuolumne River for temperature. As such, State Water Board staff has consistently provided comments requesting that any salmonid related protection, mitigation and enhancement measures developed through the relicensing process follow the 2003 USEPA Guidance.

State Water Board staff reviewed the Report and provides the following comments. State Water Board staff relies upon the specialized expertise of CDFW, USFWS, and NMFS when dealing with aquatic and terrestrial species. Therefore, it is essential that these agencies continue to be actively involved in the development of any *Oncrohynchus mykiss* (*O. mykiss*) temperature criteria specific to the Tuolumne River.

[1] Districts also refers to the consultants that represent them.

The Report often compares its results to the 2003 USEPA Guidance. The Report does not clearly and accurately introduce the 2003 USEPA Guidance, its goals, and development. The 2003 USEPA Guidance was developed as part of a collaborative process between states, tribes, and federal agencies. One of the stated goals of the 2003 USEPA Guidance is to provide temperature guidance that

> "meets the biological requirements of native salmonid species for survival and recovery pursuant to the Endangered Species Act (ESA), provides for the restoration and maintenance of surface water temperature to support and protect native salmonids pursuant to the CWA, and meets the salmon rebuilding needs of federal trust responsibilities with treaty tribes."

It is important to understand that the 2003 UESPA Guidance was developed using numerous peer-reviewed studies and published papers. Properly understanding the 2003 USEPA Guidance and its goals is essential when comparing information contained in the USEPA Guidance and the Report. Knowledge of the 2003 USEPA Guidance goals assists in the understanding of the Report's limitations, and provides an example regarding how one may approach collaborative development of similar studies in the future.

The Report does not explicitly state that its results alone demand a change in the 7-Day Average of the Daily Maximum (7DADM) temperature outlined in the 2003 USEPA Guidance. Rather, the Report states that this information should be used to determine a 7DADM value specific to Tuolumne River *O. mykiss*. However, the Report does not outline a process to be used to determine a scientifically acceptable and defensible 7DADM specific to the Tuolumne River *O. mykiss*.

As previously stated in this comment letter, the 7DADM developed in the 2003 USEPA Guidance was developed as part of a collaborative effort and relied upon numerous peer-reviewed studies and published reports. State Water Board staff recommends that any process to develop temperature criteria specific to the Tuolumne River follow a similar process as the EPA Guidance. Two additional examples of the recommended process include: *The Final Staff Report for the Klamath River Total Maximum Daily Loads (TMDLs) Addressing Temperature, Dissolved Oxygen, Nutrient, and Microcystin Impairments in California* (NCRWQCB 2010), and *The Effects of Temperature on Steelhead Trout, Coho Salmon, and Chinook Salmon Biology and Function by Life Stage; Implications for Klamath Basin TMDLs* (NCRWQCB 2005).

It is important to point out that the Report focuses on increased water temperature effects on only one parameter (oxygen consumption) and one life stage (juvenile) for *O. mykiss*. Study Plan 14 and the Report do not evaluate long term effects of increased water temperature as well as the other lifestages of *O. mykiss*. Questions that might be evaluated as part of a more comprehensive study include, but are not limited to:

- What is/are the effect(s) of increased temperature conditions on other life stages of *O. mykiss* or the long-term effects of this short-term exposure on *O. mykiss*?
- How does temperature influence other factors which may affect salmonids, such as food availability and disease?

These are a couple of questions that need to be answered prior to considering changes to temperature criteria. Additionally, Study Plan 14 and the Report only consider increased temperature effects on fish persisting in the Tuolumne River under current conditions. Study Plan 14 and the Report fail to examine the effects of increased river temperatures on the recovery of *O. mykiss* populations in the Tuolumne River. In 2006, NMFS listed California Central Valley Steelhead as threatened under the federal ESA. With a listed species, it is important than any subsequent studies also address the ability of the species, *O. mykiss* in this instance, to increase in population size under the proposed temperature(s).

As public agencies with responsibility over the Project, the Districts will act as the lead agency in the development of the California Environmental Quality Act (CEQA) document for relicensing of the Project. As a responsible agency, the State Water Board will rely upon the Districts CEQA document when issuing its CWA Section 401 water quality certification for the Project. State Water Board staff understands the desire to review temperature related information for Tuolumne River salmonids, but is concerned that the Districts will use this limited information in the development of the CEQA document. Further research and consultation is necessary before this Report can be used to advocate for higher water temperature criteria in the Tuolumne River. Study Plan 14 and its associated Report do not justify abandonment of the 2003 USEPA Guidance and shall not be substituted for the 2003 USEPA Guidance.

If you have questions regarding this letter, please contact me at (916) 445-9989 or by email at Peter.Barnes@waterboards.ca.gov. Written correspondence should be directed to:

State Water Resources Control Board
Division of Water Rights
Water Quality Certification Program
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Sincerely,

Peter Barnes
Engineering Geologist
Water Quality Certification Program

References

USEPA. 2003. EPA Region 10 Guidance for Pacific Northwest State and Tribal Temperature Water Quality Standards. April 2003

North Coast Regional Water Quality Control Board (NCRWQCB). 2005. The Effects of Temperature on Steelhead Trout, Coho Salmon, and Chinook Salmon Biology and Function by Life Stage; Implications for Klamath Basin TMDLs. August 2005.

North Coast Regional Water Quality Control Board (NCRWQCB). 2010. Final Staff Report for the Klamath River Total Maximum Daily Loads (TMDLs) Addressing Temperature, Dissolved Oxygen, Nutrient, and Microcystin Impairments in California. March 2010.

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Received 9-6-2016
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August 31, 2016

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Subject: **Comments to *Thermal performance of wild juvenile Oncorhynchus mykiss in the lower Tuolumne River: A case for local adjustment to high river temperature report Don Pedro Project January 2015* (Study)**

Dear Ms. Staples:

The California Department of Fish and Wildlife (Department) has reviewed the above study report. It is noteworthy that the Department has been informed by Dr. Nann Fangue that a revised study report was completed in May 2015; however, the Department has not received this new version for review. Therefore, we recognize that the new version may have already addressed one or more of our comments as presented below.

The authors conducted an aerobic scope laboratory swim tunnel test for juvenile wild rainbow trout (*O. mykiss*) at temperatures ranging from 13°C to 25°C. Juvenile trout were captured from the Tuolumne River, acclimated to the test facilities and then swim tunnel tested at various water temperatures overnight pending study design. Metabolic oxygen consumption was measured at rest and during swimming by increasing water flows in a swim tunnel. The Department provides the following comments regarding the above mentioned study report.

General Comments:

Survival stress tests (i.e. thermal tolerance tests) are tests that are conducted using a few individuals from a population and exposing them to water temperature regimes that, can vary in degree, time of exposure, and pre-test acclimation water temperature test starting point so that the survival rate(s) for these individual fish can be identified. If a sufficient number of individuals within a distinct population segment have been tested, survival rates obtained from individually tested fish can be inferred to represent the survival rates for the entire population. Survival stress tests for individuals are common, while population thermal tests are rare due to the need to test many fish. The aerobic study that the authors conducted is basically an acute water temperature stress test that attempts to produce individual fish water temperature survival rates using oxygen consumption as the survival metric.

An aerobic study is basically an acute water temperature survival stress test that requires pushing fish to complete exhaustion, then using oxygen consumption as the measurement metric to document when exhaustion occurs. The study design identifies acute exposure to stressful warmer water temperatures at the individual level; therefore, the study cannot inform development and/or revision of population level chronic water temperature criteria. In their report, the authors compare their acute water temperature results to the United States Environmental Protection Agency's chronic population criteria (USEPA 2003)[1] which is inappropriate.

Anadromous salmonids populations throughout the Pacific Northwest (including California) are declining primarily because of poor reproductive success and recruitment back into the population (Yoshiyama et al 2001)[2] . The intent of the USEPA (2003) analysis was to reverse that trend by presenting chronic population water temperature criteria. Chronic criteria and population criteria are always lower than acute and individual criteria. The authors presented higher acute/individual water temperature criteria based on a single study, but failed to extrapolate the results to a lower chronic population criteria that would be protective for reproductive success and recruitment to maintain a sustainable (i.e. viable) population. Survival rates are based on amount of time exposed, as well as temperature exposure, and are extremely well described in the scientific literature.

The USEPA, in their document entitled "Summary of Technical Literature Examining the Physiological Effects of Temperature on Salmon" (EPA 2001)[3,] further emphasizes the importance of short and long term exposure to temperatures:

> *What are lethal temperature effects?*
> *National Academy of Sciences (NAS) (1972) recommendations for water temperature exposure for protection of aquatic life specify maximum acceptable temperatures for prolonged exposures (≥ 1 week), winter maximum temperatures, short-term exposure to extreme temperature, and*

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[1] U.S. Environmental Protection Agency 2003. *USEPA Region 10 Guidance for Pacific Northwest State and Tribal Temperature Water Quality Standards.* EPA 910-B-03-002. Region 10 Office of Water, Seattle, WA.

[2] Yoshiyama, R.M., E.R. Gerstung, F.W. Fisher, and P.B. Moyle. 2001. *Historical and Present Distribution of Chinook Salmon in the Central Valley Drainage of California.* California Department of Fish and Game. Fish Bulletin 179 (1): 71-176

[3] U.S. Environmental Protection Agency. 2001. Issue Paper 5, Summary of Technical Literature Examining the Physiological Effects of Temperature on Salmonids (page 12).

suitable reproduction and development temperatures. Lethal effects are thermal effects that cause direct mortality within an exposure period of less than 1 wk. Prolonged exposure temperatures and temperatures that interfere with normal reproduction and development can result in mortality or reduction in population fitness or production, but the effects may be delayed or indirect, or result from impairment of function or reduction in suitable habitat or food quantity and quality available.

Specific Comments:

Executive Summary

Page i, second paragraph. The authors stated, "The study tested the hypothesis that the Tuolumne River *O. mykiss* population below La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that exist in the river during the summer". What is the authors' definition of "locally adjusted"?

Page i, third paragraph, last sentence. The authors state, "Therefore, the experimental approach also acknowledges that every activity of a fish in a river (swimming, catching prey and feeding, digesting a meal, avoiding predators, defending territory, etc.) requires oxygen consumption above a basic routine need and that salmonids have evolved to maximize their oxygen supply when they fuel muscles during exhaustive swimming". This statement leads to three questions; 1) This test appears to study basic survival, but does the study address reproductive success and recruitment? 2) Does this experimental design measure activities related to spawning, immune function and general overall stress? and 3) Isn't this the case for all vertebrates, that an animal's physiological function evolved to fuel their muscle under non-resting (exercise) or stress conditions?

Page i, fourth paragraph. The authors state "As expected for a fish, RMR [Routine Metabolic Rate] increased exponentially with increasing test temperature from 13°C to 25°C (36 different fish, each at a single test temperature)". Basically the RMR is a fish in a resting state, thus if their RMR increased with temperature in a resting state, this indicates the fish are becoming stressed in the warmer temperatures without exertion. They analyzed their results using a mathematical model. What would the results look like if the results were analyzed using standard statistical analysis for each temperature group? Further they presented temperature ranges from 16.4°C to 25°C and 17.8°C to 24.6°C, suggesting the higher temperatures are protective for basic survival. This leads to the question, do the authors agree that the 16.4°C and 17.8°C temperature levels (i.e. lower end of range) to be a more protective temperature at a chronic population exposure level to provide optimal reproductive success and recruitment rather than the higher temperature's the author are advocating? It's vitally important to remember that just because a fish or a fish population **survives** at a certain temperature; it does not automatically mean that the fish or the fish population **thrives** at the same temperature range. The ability to "thrive", carries with it the ability to successfully grow and reproduce at sufficient levels that keep the both the individual fish, and the fish population, in good condition (i.e. adequate reproductive viability).

The authors further state, "Thus, the maintenance of AAS [Absolute Aerobic Scope] across nearly the entire test temperature range clearly shows that the Tuolumne River *O. mykiss* population has a broad range of thermal performance". Isn't this case for all vertebrates? The authors further state, "Indeed, the AAS of the Tuolumne River *O. mykiss* population was atypical when compared with cold-adjusted, *O mykiss* from the Pacific Northwest, whose thermal performance optimum is reported as 18°C" (USEPA 2003). What exactly is meant by "atypical"? What is meant by "cold-adjusted" fish from the Pacific Northwest when all salmonids are cold water fish that evolved in cold waters that originated from snow melt and ground water seepage into the river systems? The reference to the USEPA (2003) 18°C as a thermal performance optimum is incorrect. The USEPA (2003) report did not discuss thermal performance, but rather concentrated developing sub-lethal chronic population criteria to improve reproductive success and recruitment to reverse a declining population trend. It is inappropriate, and therefore not scientifically valid, to compare acute individual results to chronic population criteria. The last sentence suggesting the upper thermal performance is above 25°C is pure speculation on part of the authors and should be deleted.

Page ii, first paragraph. What do the authors mean when indicating that the fish are locally adjusted? The fish are blocked by a series of dams, preventing them to migrate upstream to cooling temperatures, so they have no choice but to live in a warmer environmental regime. The authors also stated they lost 1 of 4 fish acutely exposed to 25°C. Do the authors agree that 25% fish exposed to 25°C would die, especially if they are chronically exposed to this and higher temperatures?

Page ii, second paragraph. The authors state, "The conclusion of the study is that the thermal range over which the Tuolumne River *O. mykiss population* can maintain a 95% of peak aerobic activity from 17.8°C to 26.6°C". How long can these fish withstand this activity? In the last sentence they state that "Finally, based on a video analysis of the swimming activity of *O. mykiss* in the Tuolumne River, fish at ambient water temperatures were predicted to have excess aerobic capacity well beyond that needed to swim and maintain station against the river current in their usual habitat". However, don't all vertebrates have excess aerobic capacity to survive and meet the basic needs of survival; how are these trout any different from any other living creature? Just because a fish can survive a short duration elevated temperature exposure event (i.e. minutes) does not mean that it can withstand the same elevated temperature for a long exposure event (i.e. days, weeks, and/or months).

A human analogy helps us understand key physiological concepts and keep them separate. For example, an Olympic marathon runner can run 26.2 miles in approximately two hours; however, this same runner cannot maintain the same pace for days, weeks, and months. The point here is that the Olympic runner is training for an acute event but in so doing he/she is not enabling him/herself to maintain an acute pace over a chronic period of time (days, weeks, and months). The ability of fish to survive an acute event is not indicative of a fish's ability to survive a chronic event. As was stated above, acute tolerance is always higher than chronic tolerance. USEPA set chronic criteria while the authors of this report conducted an acute study. At best, this study's results may be used to inform development of acute level criteria (i.e. temperature tolerance over short duration) but is does not translate to predicting a chronic level criterion (i.e. temperature tolerance over long durations).

Page ii, last paragraph. The USEPA (2003) criterion is not an upper performance level for fish. The authors are comparing acute results to a chronic value, an individual result to a population criteria, and survival to reproductive success and recruitment, which are all inappropriate comparisons. The authors need to conduct the same test in other rainbow trout stocks throughout the Pacific Northwest to make a similar comparison to this study before rendering a conclusion that the Tuolumne River rainbow trout have evolved higher population acute water temperature tolerance. The authors recommend "...we recommend that a conservative upper performance limit of 22°C, instead of 18°C, be used to determine a 7-Day Average of the Daily Maximum (7DADM) value". However, for cold water fish, such as trout, it would be more appropriate, conservatively speaking, to use the lower water temperatures values (17.8°C) the authors presented in their study. Their comparison to the redband trout is also inappropriate because the redband trout evolved under a totally different set of environmental conditions compared to coastal rainbow trout/steelhead. Coastal rainbow trout evolved across thousands of years in river systems that originate in high mountain elevations and connect to the Pacific Ocean. Today's rainbow trout have been exposed to river systems, blocked by dams for less than 100 years, which is insufficient on the evolutionary scale to adapt to today's river water conditions.

Introduction

Page 1, first paragraph. The authors' state, "However, cooler river temperatures are associated with cloud cover and over night [sic], and deeper ponds in the river do show some thermal stratification". Did the authors document the daily temperature difference during the hot summers, and identify and document any cool refugia or deep pools locations and measure water temperatures?

Page 1, second paragraph. The location in river miles was discussed as to where rainbow trout are commonly found with temperatures ranging from 11°C to 28°C. This is true; however, these fish have no other choice but to live under these environmental conditions because their natural migratory route to cooler high elevation waters is blocked by dams. If a fish can survive under a set of environmental (i.e. acute and chronic) conditions, including "thriving" (i.e. reproductive success over many generations etc.), then this fish has demonstrated that it has the capacity to withstand higher temperatures. However, not knowing the environmental conditions which other fish populations are actually exposed to and not knowing their population viability, the justification for changing temperature criteria based upon other fish stocks is scientifically invalid.

Thermal Tolerance and Thermal Performance

The entire section discusses acute thermal tolerance in relation to survival, but does not present any information about chronic exposures in relation to reproductive success and recruitment to maintain a sustainable population. On page 2, paragraph 1, last sentence, the authors state "Regardless, CT_{max} is always higher than the temperature that a fish can tolerate for hours to days and certainly higher that the temperature at which a fish can no longer swim aerobically". The CT_{max} is a lethal temperature, at which point a fish can no longer swim aerobically. The tunnel test conducted by the authors accomplished the same end point where the fish were pushed to exhaustion and could no longer swim aerobically. So how does the tunnel test as presented by the authors differ from CT_{max} as stated in this paragraph?

7-day Average of the Daily Maxima (7DADM)

The USEPA (2003) criterion is discussed in this section.

Page 2, second paragraph, last sentence. The authors state, "Interestingly, by setting the 7DADM criterion for salmon and trout migration as 20°C, rather than 18°C, USEPA (2003) acknowledged that juvenile Pacific Northwest *O. mykiss* have sufficient aerobic scope for the energetic demands of river migration even at a temperature 2°C above the 7DADM for juvenile growth". However, the authors failed to mention the 20°C migration criteria is conditioned with a provision to restore or provide the natural thermal regime; or to provide or restore cold water refugia. Examples of cold water refugia or natural cool regime would include the confluence of cold tributaries at the main stem river or where groundwater exchanges with the river flow (hyporheic flow) that would provide cold water refugia for fish to escape maximum temperatures. Waters in tributaries for large rivers in the Central Valley have been diverted, eliminating cold water refugia at the confluence of these tributaries and groundwater pumping in the valley has lowered groundwater levels, thus removing natural cool ground water seeps into the valley's rivers. [4]

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[4] Corbett, F., T. Harter, and M. Sneed. 2011. *Subsidence due to excessive groundwater withdrawal in the San Joaquin Valley, California.* American Geophysical Union. Fall Meeting Abstract #H23H-1397.

Justification and Purpose of the Study

Page 4, first paragraph, last sentence. The authors state, "Thus, MR [Metabolic Rate] measurements were used to determine the optimal temperature range for Tuolumne River *O. mykiss*". Can the authors provide a definition for what they consider "optimal temperature range" and differentiate an acute and chronic optima range? Do the authors consider the hottest temperature as optimal or would a cold water fish be in excellent condition at a lower temperature from a chronic exposure perspective?

Page 5, the first paragraph describes the "aquatic treadmill" similar to Parsons (2011) and Figure 1 that is presented on page 33 in this Study report. The peak T_{opt} in Figure 1 appears to be the maximum acute temperature (T_{max}) at the peak of maximum oxygen consumption and not necessarily an optimal temperature. From the peak temperature to higher temperatures, oxygen consumption decreases, suggesting the fish is exhausted and no longer capable of absorbing oxygen similar to what occurs in hyperventilation with humans. It is vitally important to remember that water at higher temperatures have lower oxygen concentrations, which is noteworthy because oxygen crosses the cellular membrane via a concentration gradient. Thus, lower oxygen concentrations in the water decrease the concentration gradient forcing the fish to use more energy to pull oxygen across their gill membrane, similar to hyperventilation of a human at the 8,000-foot elevation where the oxygen concentration is lower than that which occurs at lower elevations. Clark et al. (2013)[5] Figure 1 B (page 2772) demonstrates that T_{opt} is midway up the aerobic scope and not at the peak of the slope. They further state "*T_{optAS} provides little insight into the preferred temperature or performance of aquatic ectotherms, but rather aerobic scope continues to increase until temperatures approaches lethal levels, beyond which aerobic scope declines rapidly as death ensues.*" We agree with Clark et al. (2013)[5] that the curves peak should be considered a T_{max}, not a T_{opt}.

Page 5, second paragraph, last sentence. The authors state, "Specifically, the temperature indices and the shape of the aerobic scope curve derived in the present study can also be compared with those of other *O. mykiss* populations and with the EPA (2003) recommendations". It is inappropriate to compare results from an acute stress test conducted for basic survival needs and then make inferences to a population needing protection at the chronic criterion level. Again, acute level does not equate to chronic level when it comes to conducting tests and/or developing protective criteria. The USEPA criteria are chronic not acute; therefore, any reference to USEPA criteria in

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[5] Clark et al. 2013. *Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations.* The Journal of Experimental Biology 216:2771-2782.

this report for purposes of changing chronic criteria is unfounded and is therefore not scientifically valid.

Page 5, last paragraph. This paragraph summarizes Fry (1947)[6] as presented in Parsons 2011.

> Parsons (2011) states:
> *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).*

The "tunnel" experiment is an acute test that measures acclimation rather than adaptation. Central Valley salmonids evolved across thousands of generations to adapt to their living environment before the construction of dams. Fish that exist today have **not** evolved under today's environmental conditions because the time period has been too short for adaptation. Yes, *O. mykiss* can acclimate on an acute basis, but cannot adapt on a chronic basis in the less than 140 years since the construction of dams which blocked their historic spawning grounds.

Similar to Parson (2011) description, resistance or adaptation is a result of the evolutionary process that takes generations to develop and cause a genetic change across those generations in a population[7]. Tolerance or acclimation is a result of an individual, or a group of individuals, repeated exposure across the life of the individual that causes a physiological change. Individual based temperature exposure tolerance does not expand to all individuals in the population, but population based exposure adaptation transfers to all individuals within the population. Population thresholds are designed to protect a population; whereas, an individual threshold is designed to protect an individual or small group of individuals. A population threshold will have minimal health effects for all the individuals, including the most weak, in that population.[8,9]

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[6] Fry, F. E. J. 1947. *Effects of the environment on animal activity.* Publ. Ontario Fish. Res. Lab. 55, 1-62.

[7] Guthrie, F. E. 1980. *Resistance and tolerance to toxicants*. Pages 357-375 in E. Hodgson and F. E. Guthrie, editors. Introduction to Biochemical Toxicology. Elsevier, NY. 437pp.

[8] U. S. Environmental Protection Agency (USEPA). 1989. *Glossary of terms related to health, exposure, and risk assessment.*

[9] Air Risk Information Support Center. Research Triangle Park, N.C: Air RISC. Air Risk Information Support Center. Research Triangle Park, N.C: Air RISC.

Therefore, the population exposure threshold tends to be lower in value (i.e. more restrictive) than the individual exposure threshold. In summary, population thresholds are always less than an individual threshold and chronic thresholds are always less than acute thresholds. Thus, the reported fish water temperature experiment address individual level, but have limited usefulness as a basis for a full understanding of resistance or adaptation at the population level. As such, the tunnel stress test provides great information about tolerance and acclimation at the individual level, but is inappropriate to extrapolate the results to adaptation for chronic population exposure criteria.

Predictions Derived From EPA (2003)

Page 6. The authors proposed predictions based the USEPA (2003) criteria are irrelevant because the USEPA (2003) criteria were not based on an acute stress test. Is data presented in Table 1 based on acute or chronic tests? The USEPA (2003) 18°C criterion is not based on maximum metabolic rate (MMR) acute test as presented in Figure 1, but is a chronic criterion which is lower than acute criterion. The USEPA (2003) never stated an AAS T_{opt} metric, nor discussed this study design, to develop a chronic population criterion.

Alternative Predictions of Thermal Adjustment

Page 6. On what are the predictions based? Again, this study design is an acute stress test. It is well known that *O. mykiss* can survive in temperature above 18°C, but the study design does not answer the questions as to what is the chronic population threshold for reproductive success and recruitment to maintain sustainable populations across many future generations. The study design also does not address how well the *O. mykiss* immune system functions to ward off disease or how well a cold water fish can escape a warm water predator, especially when the water temperature are in the optimal range for the warm water predator. This study design can measure individual cold water fish short sprint energy to avoid a predator, but does not indicate how long a cold water fish can escape in a predatory warm water fish optimal temperature zone.

Fish Collection, Transport, and Handling

Pages 8 to 9. Most of the study fish were caught in the upper coolest reaches of the river. However, if these fish are adjusted to warm temperatures, why were they present in the coolest waters of the river? The fact that most of the fish were found and captured in the coolest waters of the river is indicative that, at the population level, *O. mykiss* in the lower Tuolumne River are seeking cooler water to reside in even though warmer water is available to them.

Experimental Protocols

Page 11, last paragraph. The authors state, "Water velocity was then increased in increments of 3 to 6 cm s^{-1} every 20 min until the fish failed to swim continuously". Is this an acceptable fisheries technique to allow an animal to work to the point of complete exhaustion? Would it be better to do a timed test by stopping the test before the fish is completely exhausted?

Page 12, third paragraph. The authors state, "Approximately 50% of the wild fish did not respond to the critical swimming velocity protocol but instead used their caudal fin to prop themselves on the downstream screen to avoid swimming". Is this a sign the fish were already stressed before the experimentation started and possibly a result of too warm temperatures to begin with?

Data Quality Control, Model Selection and Analyses

Page 13, last paragraph. The authors state, "Routine metabolic rate quality control (QC) was performed by visually inspecting over night [sic] video recordings for fish activity" and that "data from any fish showing consistent activity over night [sic] was disguarded". Why were the data discarded? Was the fish activity a sign of stress before the experiments started? In addition the authors state, "For fish exhibiting intense agitation, the swimming MMR was used as overall MMR." Four of these 'non-agitated' fish (W2, W13, W14, and W15) were disgarded due to failure of MR to increase incrementally; despite continuous station-holding swimming with tunnel velocity increases of more than 15 cm s^{-1}". Were these fish already stressed? How does inclusion of these data influence study results? It is important that data not be "selected" in order to bias study results. Scientific integrity requires that data not be thrown out for invalid reasons, including if the results cannot be explained or if they are different than expected.

Results

Page 15, Number 1, third paragraph, second sentence. The authors state, "They state that Routine Metabolic Rate (RMR) should increase exponentially until the test temperature approaches the upper thermal tolerance limit for *O. mykiss,* which according to published CT_{mas} values is 26°C to 32°C (see Table 1)". Who is "they"? If "they" is the USEPA, this is an incorrect statement because the USEPA did not include RMR studies in their review.

Myrick and Cech's Table 1[10] had significant less food consumption and decreased growth rates and increased mortality in their 25°C test fish compared to their 10°C, 14°C, and 19°C exposed fish. In their Table 2 results, fish consumed significantly less oxygen at 25°C compared to fish exposed to 10°C, 14°C, and 19°C. In their discussion they conclude:

> *Because thermal resistance in fish is closely correlated with exposure time (Elliott and Elliott 1995), fish with higher critical thermal maxima can tolerate longer exposures to sub-lethal temperatures, giving them a better chance of escaping to thermal refuges (Matthews et al. 1994) or surviving diel extremes.*
>
> *We observed no differences between Eagle Lake and Mt. Shasta trouts' thermal tolerances. Critical thermal maxima for both strains appeared to be lower than those reported for other rainbow trout acclimated to low (10–11 ◦C), or medium (14–15 ◦C) temperatures, but were similar to those reported for other salmonids acclimated to higher (19–20 ◦C) temperatures (Table 5). With the possible exception of lake trout (Salvelinus namaycush) (Grande and Andersen 1991), Arctic charr (S. alpinus) (Lyytikäinen et al. 1997) and other salmonids restricted to high latitudes, salmonids appear to have very similar thermal tolerances, irrespective of origin (Table 5).*

O. mykiss can survive in acute warm temperatures as demonstrated by the authors, but cold water fish still need cold water refugia sometime during the day. According to Myrick and Cech (2000) there is very little thermal difference between fish stocks per their comparison of other research studies (see Table 5) under similar experimental conditions.

Page 15, Number 2. The authors state, "These results for MMR are inconsistent with our prediction #2 derived from EPA (2003) criteria where MMR was expected to peak near to 18°C". This statement is irrelevant because the authors are comparing chronic population criteria to acute individual results. Again, chronic and population thresholds are always less than acute and individual thresholds.

Page 16, Number 3, third paragraph. The authors state, "These results for AAS are inconsistent with our prediction #3 based on EPA (2003) criteria, but are consistent with our alternative prediction #3 that the Tuolumne River population of *O. mykiss* is locally adjusted by having T_{opt} for AAS that is greater than 18°C i.e., 21.2°C." This statement is

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10 Myrick, C. A., and J. J. Cech Jr. 2000. Temperature influences on California rainbow trout physiological performance. Fish Physiol. Biochem 22:245-254

irrelevant because the authors are comparing a chronic population criterion to acute individual results. Again, chronic and population thresholds are always less than acute and individual thresholds.

Page 16, Number 4, last sentence. The authors state, "The numerical 95% peak AAS could be maintained from 17.8°C to 24.6°C, which is a more conservative thermal range for T_{opt}". However, based on the authors results, and because rainbow trout are a cold water fish, a true conservative thermal range would be from 16.4°C to 17.8°C.

Pages 16 to 17, Number 5. Same comment as for comparing an acute stress test results to a chronic population criterion. The author state, "Indeed, all individual fish tested up to 23°C has a FAS [Factorial Aerobic Scope] value >2, with only 4 out of 14 fish tested at 23°C, 24°C, and 25°C having a FAS value <2." A chronic population threshold is formulated to protect the weakest individuals in a population, so by using a lower criterion these 4 weaker fish should have better physiological function and survival.

Page 17, Number 7. The authors state, "Two fish tested at 25°C regurgitated rather large meals of aquatic invertebrates during the recovery from the swim test, and one of these fish died abruptly during the recovery period". Since, fish were exposed to an exhaustive state, this causes us to question whether or not this an appropriate testing technique where the test has to force an animal to complete exhaustion, especially for a group of fish that may be already stressed due to having to live in environmental conditions of altered flows and habitats that they did not evolve with.

Discussion

Data Quality

Page 18, first paragraph. This provides a brief summary of the results and comparison to other aerobic studies. The Department completed an analysis of variance as presented in the following Table 1 using the Study's data presented in Appendix 4.

Table 1. Mean RMR, MMR, AAS and FAS for rainbow trout from the Tuolumne River, summer 2014. Means with the same letters within each column are not significantly difference ($P<0.05$) using analysis of variance.

Temperature (°C)	N	RMR ($mgO_2\ kg^{-0.95}\ min^{-1}$)	MMR $mgO_2\ kg^{-0.95}\ min^{-1}$)	AAS $mgO_2\ kg^{-0.95}\ min^{-1}$)	FAS
≤18	13	2.38	7.37	4.99[a]	3.15[a]
19-20	8	3.41	9.35[a]	5.94[a]	2.74[a,b]
22-25	16	4.58	10.64[a]	6.06[a]	2.41[b]

RMR = Routine Metabolic Rate; MMR = Maximum Metabolic Rate; AAS = Absolute Aerobic Scope; FAS = Factorial Aerobic Scope.

Temperatures at and below 18°C were significantly different for RMR, MMR, and FAS compared to the highest temperatures at and above 22°C. For RMR, which is a fish at rest, is this an indication the fish at the warmer temperatures were already stressed before the experiment started?

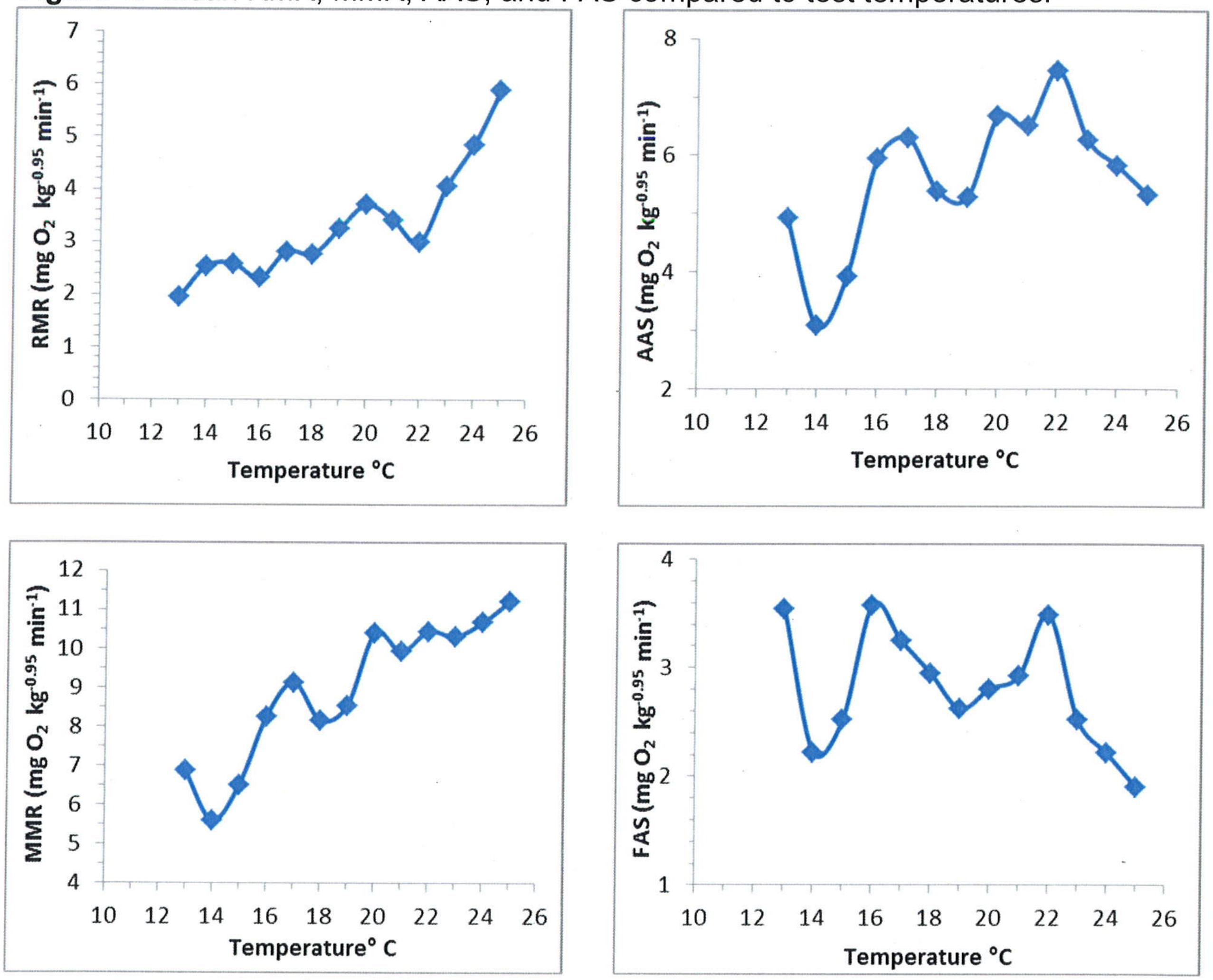
As stated in USEPA (2001) Issue Paper 4, Page 8, *a*s metabolic demands and oxygen consumption increase, gill ventilation must also rise proportionately (Heath and Hughes 1973). Further USEPA (2001) Issue Paper 4, Page 5 states:

> *There is an important relationship between temperature and the dissolved oxygen (DO) needs of fish. As temperature increases, metabolic rates increase, increasing the demand for oxygen by an organism. At the same time, the DO available to the organism decreases. Therefore, at times of the year when fish may experience temperature stress they also may experience stress from low DO levels.*

There is an inverse relationship between water temperature and oxygen concentration. As temperature increases, oxygen decreases. As such, at the warmer temperature with less oxygen, are the fish stressed to the point they are hyperventilating, thus increasing their metabolism trying to pull in as much oxygen as possible from a low oxygen environment.

The Department also graphed the mean results for each temperature as presented in Appendix 4.

Figure 1. Mean RMR, MMR, AAS, and FAS compared to test temperatures.



Note at 22°C for RMR, AAS, and FAS and at 21°C for MMR there is a sudden change in the slope of the graph. Does this change in slope indicate there is a sudden change in the physiological function of the fish and a clinical sign that the fish are highly stressed? A highly stressed animal is considered to be in poor condition.

Page 19, Protocol Number 2. The authors state, “2 a combination of continuous swimming and short velocity bursts to push fish off of the downstream screen”. Was this an indication the fish was already tired and stressed at the beginning of the experiment?

Evidence for Local Thermal Adjustment

Page 20, first paragraph. The authors state, "Our predictions based on EPA (2003), as listed above, assumed that the Tuolumne River *O. mykiss* population would perform similarly to Pacific Northwest *O. mykiss* populations used to set the 7DADM by USEPA (2003)". The predictions based on USEPA are irrelevant because the USEPA did not perform tunnel stress techniques or use such data to develop their chronic population criteria recommendations. The authors recommend using 21.2°C rather than 18°C, but they are comparing an acute/individual result to a chronic/population recommendation. Have the authors considered other techniques to determine what cold water fish, such as *O. mykiss* can chronically sustain normal/optimal physiological function, including immune function, reproductive success and recruitment, at their recommended temperature of 21.2°C? It is well understood that cold water fish can simply survive at warmer temperatures to a point, but what about their entire life cycle needs at the individual and population levels? The authors mention these test fish have a wide optimal thermal performance range, but this is true for all living organisms; what do the authors consider "optimal"?

In the same paragraph, last sentence, the authors also state, "However, given that the CT_{max} could not be determined in the present work and that MMR increased up to the highest test temperature (25°C), it was impossible to determine the upper thermal limit when MMR collapses, which means that alternate metrics must be used to set the upper thermal limit for the Tuolumne River *O. mykiss* population". Since the "upper thermal limit" is survival based, can the author's present reproductive success and recruitment base criteria with this type of testing?

Page 20, second paragraph. The authors state, "The present work provides three useful metrics of the optimal temperature range". What is meant by "optimal temperature range"? T_{opt} appears to be more of a temperature maximum (T_{max}) than a T_{opt}. A temperature maximum does not necessarily mean it is an optimal temperature. Fry (1947) page 56, Figure 27, does not state the peak of activity as optimal, but refers to the "potential range of activity" and the "scope for activity". Fry further reduces the area of the activity curve by discussing "controlling factors". The USEPA (2001) as presented below provides a number of "controlling factors". Did the authors for this study consider controlling factors as described by Fry to adjust their activity curve?

In USEPA (2001) Issue Paper 4, Page 2 states:

> *A wide range of biological, chemical, and physical factors can challenge the physiological systems of fish. Various stressors such as handling, fright, forced swimming, anesthesia, rapid temperature changes, and scale loss all elicit a stress response characterized by physiological changes, which tend to be similar for all stressors (Wedemeyer and McLeay 1981). The stress response proceeds as follows: the central nervous system triggers the release of stress hormones (i.e., corticosteroids), changes*

> *occur in blood chemistry and hematology (i.e., reduced blood clotting time), and metabolism may be altered, which in turn can result in tissue changes (nitrogen balance and oxygen debt) followed by loss of electrolytes (Wedemeyer and McLeay 1981). These responses are expressed through changes in predator avoidance, growth, parr-smolt transformation, spawning success, migratory behavior, and incidence of disease. There also is a reduction in tolerance to subsequent stressors (Wedemeyer and McLeay 1981). At the population level, stress response may reduce recruitment and species abundance and diversity.*

In USEPA (2001) Issue paper 5, Page 57 states:

> *Thermal stress is any temperature change that significantly alters biological functions of an organism and lowers probability of survival (Elliott 1981). Stress was categorized by Fry (1947 as cited by Elliott 1981) and Brett (1958) as lethal (leading to death within the resistance time), limiting (restricting essential metabolites or interfering with energy metabolism or respiration), inhibiting (interfering with normal functions such as reproduction, endocrine and ionic balance, and feeding functions), and loading (increased burden on metabolism that controls growth and activity). The latter three stresses can be lethal when continued over a long period (Elliott 1981).*

Page 20, last paragraph, first sentence. The authors state, "Yet, there were important indications that a small percentage of individuals were taxed at 23-25°C by the thermal testing and intensive swim imposed on them outside of their normal habitat over a 24-h period." In the fourth sentence they further state, "In the present study, the telltale signs were that 4 of 13 individuals [31%] tested at 23-25°C had a FAS <2." This supports the concept that a chronic population base threshold is to protect the weakest individuals in a population and <u>cannot</u> be formulated by using just one simple acute stress test.

Pages 21 to 22, top line. In the same paragraph, the authors state, "Lastly the only fish mortality occurred in the recovery period (a phenomenon known as 'delayed mortality') after one fish was tested at 25°C". What is the point of mentioning 'delayed mortality'? The end result is one of four fish (25%) died at the highest temperature when forced to swim until completely exhausted.

Ecological relevance of the Present Findings

Page 21, third paragraph. The authors state, "MMR increased with temperature from 13 to 25°C, which would mean that as fish encounter higher temperatures, they have the capacity to perform an activity at a higher absolute rate, i.e., swim faster to capture food or avoid predators, digest meals faster, detoxify chemicals faster, etc.". Are the authors saying rainbow trout are better off at 25°C instead of <19°C? Their interpretation does not make any sense. We agree a fish will have burst of energy no matter what the temperature, however, the question remains how long can they maintain this energy consumption under chronic warm temperatures at 21.2°C? It takes energy to reproduce, how does exposure to chronic warm temperature impact reproduction success and recruitment into the population? Clark et al. (2013), page 2779, stated that there is a range of optimal temperatures for different processes and life histories and these optimal temperatures are different from T_{optAS}. They used an example for adult pink salmon where a T_{optAS} is at 21°C, but if reproduction occurred at 21°C would fail because the optimal temperature for spawning is <14°C. They further stated on page 2780, that fish have different physiological functions at different optimal temperatures as presented in their Figure 7B.

Page 22, first paragraph, third sentence. The authors state "As a result of high temperature, a fish would digest the same meal with a similar overall oxygen cost but at a faster rate". This study did not measure how fast fish can digest their food at increasing water temperatures, therefore this statement stating that a fish would digest their food at a faster rate at higher temperatures is an assumption based on speculation. As the authors discussed, this study design measured oxygen demand to demonstrate fish have extra burst energy from a resting state to seek and catch food, but does not include measuring the rate of digestion. All animals digest their food during the resting state, otherwise their digestive tract would cramp-up during high activity.

Page 22, last paragraph. The authors state "Here we did not evaluate the possibility that the Tuolumne River *O. mykiss* population can thermally acclimate to warmer river temperatures as the summer progresses, due to the available sample of a maximum of 50 individuals and their habitat temperature." Actually the authors did evaluate if Tuolumne River trout can acclimate, because this was an acute stress test designed for that purpose. Up to a limit, animals can acclimate to an acute environmental change, but how do these animals reproduce successfully under chronic environmental changes such as migratory routes being blocked and under different water flow regimes that they did not evolve with?

Conclusions

Page 24. As previously stated, the USEPA did not use acute tunnel stress test to evaluate a chronic population criterion. They included a number of factors as part of their evaluation. It is inappropriate to compare results from an acute individual test to a chronic population threshold. Since *O. mykiss* are a cold water fish, it would be more appropriate and conservative to use their lower range results (16.4 and 17.8°C) to protect this fish, particularly where reproduction success appears to be low because the population has been declining for decades since the dams were constructed[11].

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[11] Yoshiyama et al, 2001

Figures

Page 33, Figure 1. The T_{opt} appears to be an acute maximum temperature at the peak of maximum oxygen consumption and not necessarily an optimal temperature. From the peak temperature and higher, oxygen consumption decreases, suggesting the fish is exhausted and no longer capable of absorbing oxygen similarly to hyperventilation. See comment above for Page 20, second paragraph.

Page 37, Figure 4. See comment above for Page 20 second paragraph. Per Fry (1947) page 56, Figure 27, did the authors for this study consider controlling factors to adjust their activity curve? For the Factorial Aerobic Scope curve, the peak is approximately 13°C and decreases as temperatures increases. Clark et al. (2013) Figure 6, page 2778, presents a similar Factorial Aerobic Scope curve where the T_{optAS} is at the peak of the curve representing the lowest temperature at 11°C. Using Clark et al. (2013) concept, the authors Figure 4 peak at 13°C should be considered the T_{opt} for Tuolumne River rainbow trout, not the maximum temperatures.

Page 44, Appendix 1. Were *O. mykiss* observed, or attempts made to capture fish, between River Mile 39.5 (permit limit location) and River Mile 49? River water temperatures were above 18°C, so it would be worthwhile information to know if a healthy number of rainbow trout occupied this area. River Mile 48 appears to be below the 21.1°C permit requirement.

Page 47, Appendix 2. Fish W43 died. Did this fish die from delayed Capture Myopathy as a result of handling and exposure to high temperatures? Capture Myopathy results in the death of a captured wild animal during or after the animal has been captured and released.

Page 49, Appendix 4. All the data should be included for peer review, particularly for the fish that were discarded. What would the analysis look like if the discarded fish data was included? According to the Quality Control column the discarded fish were removed because of activity during RMR or no MR increase. Does this indicate the fish were already stressed? Which fish died?

Page 50. Four of 14 fish tested at 23°C, 24°C and 25°C had a FAS < 2. These results of less than 2 at the highest test temperatures indicate these fish were highly stressed at these temperatures.

We appreciate the opportunity to review and comment on the report. If you have any questions regarding these comments please contact Dr. Andrew Gordus, Staff Toxicologist, at the address or telephone number provided on this letterhead.

Sincerely,

for Julie A. Vance
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THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

APPENDIX 6

RESPONSE TO COMMENTS ON THE DRAFT STUDY REPORT

Overarching Reply Comments
To CDFW's Review of the Current Study

On August 31, 2016, California Fish & Wildlife (CDFW) provided comments on the draft report entitled "Thermal Performance of Wild Juvenile *O. mykiss* of the Lower Tuolumne River" issued in January 2015. It is evident from the comments received from the reviewers that the study team has not been clear enough in describing:

a) the quality of the experimental work and the scientific rigor that was applied;

b) the applicability of the data generated relative to the larger question regarding the conservation of *O. mykiss* in the Tuolumne River; and

c) what types of data could provide further insight into the thermal ecology of Tuolumne River *O. mykiss*

Therefore, in addition to our detailed reply comments provided in this Appendix 6, we offer the following discussion to deal with certain issues that were raised in the CDFW review comments, issues that lie both within and outside of the primary purpose of our report. Hopefully, along with our detailed response document to the comments, this will better explain why we took the particular study approach that we did, namely using a temperature-dependent metabolic performance measure (i.e. aerobic scope), the ecological value of which is supported by a large volume of scientific literature. Further, the researchers conducting the study applied state-of-the-art methods and measurement techniques. Therefore, the information generated is applicable to the management of Tuolumne River *O. mykiss*.

1) What does absolute aerobic scope (AAS) tell us? AAS is a capacity measure or index that has comparative value. We measure this 'capacity' or 'potential', if you like. It is clear that the present experiments were not intended to directly address how such capacity would or could be used by the fish. Indeed, very few fish studies have even attempted to study capacity allocation given the inherent difficulties of such an effort. Nevertheless, the most important guiding principle is that if a fish has no aerobic capacity, no activities can be performed other than those dealing with basic survival (basal metabolism in human terms). Conversely, if aerobic capacity is evident, as we discovered across a wide range of test temperatures for Tuolumne River *O. mykiss*, then that capacity is available for use for activity across the temperature range.

   AAS is a well-grounded scientific measurement that has only improved with time since its first inception by Fred Fry 60 years ago. We now have better measurement equipment available, as was used in this study, that gives us more reliable, more accurate and more frequent recordings, plus we have video to monitor the fish. Furthermore, we have a much greater appreciation of where errors can be introduced, how large they might be and how they can be avoided. Indeed, an entire special issue of an International journal (Journal of Fish Biology) was devoted to this topic in 2016, which attests to the rigor of the experimental approach we adopted. In the conduct of this study, we followed published principles and guidelines, i.e., our study was state-of-the-art. Few studies, including those used by EPA 2003, have tested wild fish. We tested wild Tuolumne River *O. mykiss* to ensure direct relevance of the data. AAS simply characterizes what capacity is available; further experiments would be informative to characterize how Tuolumne River *O. mykiss* allocate this capacity, including the potentially interactive effects of thermal acclimation, growth or reproduction. Thus, while comments and criticisms along these lines may potentially be relevant to the broader management of Tuolumne River *O. mykiss*, they indicate a misunderstanding of the purpose and use of our study. The present study demonstrates the fact that Tuolumne River *O. mykiss* have the capacity for the performance of ecologically relevant traits across the wide range of relatively higher temperatures experienced in the lower Tuolumne River.

2) One thing that is clear from our work and of critical importance is that the study populations included in the EPA criteria documents are 'northerly' populations. This should not be in dispute. The only work on southern populations comes from Dr. Joseph Cech's lab and post-dates the EPA document which was used to set the 7DADM. Also clear is that the *O. mykiss* benchmark temperatures were established over a dozen years ago and considerable new science has amassed on thermal effects on fishes. Indeed, it may be the most intensely studied topic within fish biology over the past 10 years.

3) Since the early 2000's or so, population-specific thermal sensitivity research, especially for fishes, has expanded greatly, including further methodological and interpretive advancements. It is now widely accepted that local populations of fish of the same species can differ in thermal sensitivity, and it has been consistently demonstrated that their sensitivity is usually matched to their native or local thermal regimes whenever this has been properly tested. These observations are consistent with what is termed local thermal adaptation. Therefore, a logical link should be that thermal regulatory criteria should acknowledge the local population's thermal sensitivity. The main point here is that any regulatory guideline should properly reflect the fish species and location to which they are intended to apply. Indeed, the EPA 2003 supporting document directly acknowledges this, but also notes there was insufficient data *at that time* to provide an informed opinion. The database on local adaptation within a species has now changed enormously. Thus, whenever evidence for local adaptation of a particular population of fish emerges, it is entirely reasonable to challenge the applicability of a more general guideline. This study tested wild Tuolumne River *O. mykiss* to ensure direct relevance of the data.

   Population-specific performance is seen in many traits: growth, lethal limits, swimming performance, metabolic performance and aerobic scope, and each of these traits can be shaped by temperature at a variety of interacting timescales [i.e. acute (seconds to minutes), acclimatory (days to weeks; perhaps 'chronic' using the CDFW reviewer's terminology), and adaptive]. Indeed, these are complex traits, and ecologists agree that these traits have implications at the level of the population. We acknowledge that there is debate about specific 'implications', but we try to be clear and precise, as well as conservative, as to what our data on Tuolumne River *O. mykiss* have revealed for the first time.

As a general rule and an example, positive growth rates occur under conditions that are conducive to survival. Exactly how growth and survival translates to population dynamics requires considerably more detailed study beyond measuring growth rate, and perhaps modeling, which is never perfect without reliable input variables. Natural selection directly operates at the level of the individual, and effects become manifest at the population level. Therefore, understanding effects on individuals and knowing the physiological mechanisms that operate within individuals are key pieces of knowledge to obtain before attempts to extrapolate to the population level can be made with confidence.

Consequently, we performed experiments that targeted individual, wild juvenile fish and probed mechanisms of the thermal tolerance that are well established in the mainstream fish literature. To reiterate, we performed our experiments on wild Tuolumne River fish (not hatchery fish as used for EPA 2003), captured from their native habitat and tested streamside. This experimental design is particularly powerful in estimating innate, real-time AAS capacity for this specific population.

4) AAS allows us to make comparisons. For example, we can safely conclude that the lower Tuolumne *O. mykiss* population does comparatively better at warmer temperatures than northern *O. mykiss* populations because we have shown aerobic performance across a temperature range that includes temperatures higher than those tolerated in northern populations. Consequently, our data only addresses the ‘blanket’ 7DADM guideline for all *O. mykiss* populations across the US, in one specific manner: we no longer have confidence in the growth studies used by EPA in 2003 to set guidelines for lower Tuolumne River *O. mykiss* because our AAS data for lower Tuolumne rainbow trout clearly show that this population is unlike and definitively different from more northern populations. Consequently, it is a confidence issue. Of course, any new guidelines should only be considered in close consultation with the EPA, and using the best available science and its modern interpretation. We did not suggest otherwise in the report and continue to hold this viewpoint. This is what the data are telling us, nothing more and certainly nothing less.

5) What our data should NOT be used for is to pick a new thermal criterion based solely on our aerobic scope curve. In fact, we do not suggest revising the 7DADM based solely on our AAS curve. We simply state that we believe our data are suggestive of local thermal adaptation in Central Valley fish and inconsistent with a blanket criterion for the population under consideration. Because the Tuolumne River *O. mykiss* fish outperform northerly populations at warm temperatures, the inference is that the current guidelines are overly conservative.

6) We also assume, perhaps incorrectly, that all of the scientists working on thermal requirements of fishes would appreciate, without repeated statement, the fact that this study addresses physiological mechanisms related to temperature and temperature alone, and to juvenile fish alone. Nowhere did we extrapolate our findings to other life stages because we are aware of, and therefore sensitive to, some species of fish showing stage-specific thermal sensitivity. We also know that multiple stressors can interact (e.g. temperature sensitive metabolism x food) in additive or synergistic ways, so nowhere do we suggest

that our data are the sole requirement to determine a 7DADM. However, the value of population-specific, site-specific data should not be underestimated.

7) Additional data that may be helpful to managing Tuolumne River *O. mykiss* might include: comparative thermal sensitivity literature from other studies and other populations; knowledge of food resources available to the fish in question; and life-stage sensitivities that could reveal a 'weak link' in life history. Of course, this is not exhaustive, but it does acknowledge possible additional information that would be useful. We understand that at least some of this data is already available.

8) While we never suggest that a new 7DADM value be extracted solely from our data, we do suggest that the current value is conservative for Tuolumne River juvenile *O. mykiss*. Also, we know as a fact that a higher thermal tolerance than that reflected by the EPA 2003 7DADM exists within the *O. mykiss* genome because publications on local thermal selection (e.g. Australian and Japanese rainbow trout) conclusively illustrate that these populations feed and grow at temperatures well in excess of 20°C.

   It is true that we do not know the growth capabilities of Tuolumne River *O. mykiss* but given our new understanding of FAS values, the Tuolumne River *O. mykiss* have sufficient aerobic capacity to eat a large meal, they had food in their stomachs when captured, have an abundance of food in the Tuolumne River, and have been videoed swimming to capture food passing by at temperature well above the EPA recommended 7DADM. This all provides additional evidence that juvenile *O. mykiss* captured from the lower Tuolumne River are feeding and growing in the current thermal regime. Growth studies would be useful to confirm rates of growth, but the present study supports the Tuolumne River *O. mykiss'* significant capacity for growth. If there is any doubt that a higher thermal tolerance than that reflected by the 7DADM exists within the *O. mykiss* genome, we simply have to turn to the established physiological literature on a variety of *O. mykiss* that through natural selection live in the deserts of Idaho and Oregon, namely the redband trout, *Oncorhynchus mykiss gairdneri*. This variety deals with, as well as swims and feeds in, summer temperatures that can reach 26°C.

It is our hope that these remarks clarify some of the apparent misunderstanding of the design and purpose of the study. Below we respond to individual comments received on the draft report.

A Note about 'Acute' and 'Chronic' Temperature Response

There is often much discussion and debate about 'acute' and 'chronic' temperature response in fish. For the purposes of this discussion, we view 'acute' as relevant over the timescale of seconds to hours to days. Over longer timescales, weeks to months, temperature is considered 'chronic'. However, some scientists reserve the term chronic to a certain portion of the lifecycle of a test animal, e.g., mammalian toxicology.

Binning the effects of temperature on fishes into categories like 'acute' and 'chronic' is not a straightforward task and to attempt to do so is a dramatic oversimplification of both experimental methodologies and organismal biological, physiological and behavioral responses. This is in part because fish can acclimate to a new temperature and this acclimation can follow different time courses depending on the process being studied, and because a fish is rarely exposed to a single, static temperature for many weeks. Thus, while there are very good experimental reasons to control the acclimation temperature for groups of fishes before testing (as you would do in laboratory acclimation studies of thermal tolerance or growth), these tests are artificial and eliminate naturally-occurring thermal oscillations as well as fish behavioral selection of particular thermal habitat.

We argue that it is much more insightful to understand the biologically-relevant oscillations in environmental temperatures of a particular system, which likely include daily fluctuations, fluctuations occurring over seasons, and/or variation in spatial temperature distributions. It is also critical to understand how these temperature profiles interact with the response variable that you are measuring (e.g. molecular responses as compared to organismal growth – each of which will have a distinct response pattern and response time). For example: heat shock proteins show an acclimation response in a matter of hours, whereas whole animal physiology can take weeks to acclimate. Lastly, one of the more challenging tasks for scientists is to understand how fish behaviorally utilize their thermal habitat as a reflection of their physiological capacities and limits.

Consequently, how fish respond, physiologically and behaviorally, to environmental temperature change is a function of previous thermal history (e.g. seasonal acclimation), the magnitude and timescale of the thermal change (e.g. how high did the temperature rise, how quickly), and the duration of the exposure (e.g. how long was the new thermal exposure). Regulations should incorporate data that speak to each of these aspects. We point out that the 7DADM is neither an "acute" or "chronic" regulation, but it is in fact designed to incorporate temperature oscillations. Incorporating thermal heterogeneity into fish habitat, when done properly, is certainly more appropriate than managing to a static (chronic) thermal target (which we all should be able to agree is completely artificial to fishes that evolved in habitats with thermal variability).

Importantly, no single study exists, or can be designed, that completely incorporates the complexities of thermal exposures and measured endpoints to 'spit out' the perfect thermal regulatory criteria for a particular species. Thus, regulations are based on a collection of data/experiments spanning so called 'chronic' and 'acute', biologically relevant thermal exposures and incorporating a variety of well-studied and understood endpoints. Or, in some cases, when data are not available for strong support, regulations should be reasonably protective.

With specific respect to the study conducted on the Lower Tuolumne River, the fish were seasonally acclimated to the prevailing summer river conditions. We knew the temperature at which they were captured, but not the temperatures that they had experienced or for how long they had experienced them. We minimized the potential effects of thermal acclimation of processes that take many hours or weeks (fish were tested immediately, i.e. within hours, following capture from the river). Lastly, metabolic performance capacity was measured as a function of an incremental warming protocol that lasted no longer than 6 hours of exposure to a test temperature between 13 and 25°C, depending on the individual.

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TRT/CSPA-1 (p. 2)	The Study does not evaluate the physiological response of the population of *O. mykiss* in the lower Tuolumne River over time. There are limitations to the Study that the Study does not acknowledge. Chief among these limitations is that the Study does not evaluate physiological response of the population of *O. mykiss* in the lower Tuolumne River over time. On the contrary, 75% of the test fish were sourced from a location one mile downstream of La Grange Powerhouse, where temperatures at capture ranged from 12.7°C to 17.1°C. While the Study is critical of Hokanson (1977) for an issue concerning confidence intervals, the Study does not address Hokanson's use of a 40-day period to evaluate physiological response. Other studies (e.g. Brett 1956; Bidgood 1969) similarly address long-term exposure to less-than-optimal thermal conditions. The Study does not acknowledge this limitation.	We could have been clearer about stating the design and intent of the study. The Report has been amended accordingly. However, the study plan prepared for the study and reviewed previously by the commenter spelled out the specific design and purpose of the study. This never changed. We do not understand the commenters' concern regarding lack of evaluating responses over time. This was not the objective of the Report as clearly explained in the original study plan. Indeed, the permits issued by the resource agencies for fish removal would NOT permit more than 2 fish to be studied at a time and over time – this was the maximum number of fish that could be removed from the river. To reliably measure growth rate, at least 40 days would be needed to detect responses and rates because the fish have to change their mass by a reliably detectable amount. This was never intended, as explained in the study plan. Nor could we have done this within the limitations of the permits issued. Instead, we measured oxygen uptake, which uses a different time scale, and it can be reliably measured over periods of minutes. Also, we went to great lengths to follow and analyze oxygen uptake over a nearly 24-hour period to examine its variability and ensure our estimates of RMR were as accurate as possible for a field study. Also, we carefully measured maximum oxygen uptake in the manner used by both Fry and Brett (who was Fry's student), but using modern technology with greater accuracy and precision. Therefore, we can state with confidence what the fish's

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		capacity was in terms of delivering oxygen to tissues over a broad temperature range. If, however, the comment concerning "over time" is that it might be beneficial to study fish that were acclimated to different temperatures (14°C was the coolest temperatures found in the lower Tuolumne River during 2014 study period), this is a valid comment. Nevertheless, it is well known that thermal acclimation is used by fishes to "improve performance" at the new acclimation temperature. Therefore, if the lower Tuolumne River *O. mykiss* used in the present study can be shown to acclimate to water temperatures warmer than they were experiencing at the time of the experiments, we have then provided a conservative estimate of temperature effects on the fish performance by looking only at the effect of a rapid rise in water temperature from river temperature to which they were acclimated. The concern raised does not change the outcome of our results, but does introduce the possibility that this fish population could do even better at warmer temperature if they were allowed to first acclimate. The comment of the reviewer goes on to be critical of our critique of the general application of Hokanson's data on rainbow trout that were studied in the American midwest to a population of rainbow trout in the Central Valley, CA. The fact is, as proven by the study, Tuolumne *O. mykiss* juveniles displayed a physiology and thermal tolerance quite different from more northern populations of rainbow trout. In fact, we point out that they are more similar to *O. mykiss* populations that have adapted to desert streams!

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		We agree that the experimental approach used in this study differs fundamentally from the approach used by EPA to formulate its temperature recommendations for the Pacific Northwest. Three points of clarification are below. 1. Our criticism of Hokanson (1977) is two-fold. Foremost, today's knowledge of local adaptation of a wide range of species from sticklebacks, through killifish to salmonids tells us that it may be inappropriate to apply studies that are geographically separated by large distances and differing climates. For example, use of data from studies of trout from central USA to the same species locally adapted to California river systems may be inappropriate. Indeed, this was the primary driver for the present study. Our commentary on the work of Hokanson (1977) is valid, as it does not criticize the quality of the data per se, rather the application of the results. Our concern about confidence limits in Hokanson (1977) is a minor one, driven in part because against our a priori predictions of fish performance, we found that the Tuolumne River fish were unexpectedly tolerant of acute changes in temperature and performed similarly over a wide range of temperatures. This introduces the statistical issue of when does warm temperature create an unfavorable fish performance. We think confidence limits are needed. Without confidence limits for a study such as Hokanson (1977), which is nearly 40 years old and did not have access to the statistical tools now

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		commonly available, we cannot retrospectively interrogate these older data. 2. Lastly, how these fish exploit the local thermal gradients in the lower Tuolumne River was not part of the objectives of the current study. Nevertheless, it is possible to speculate. Perhaps they behave similar to the sockeye salmon that Brett studied in the 1970's, by diurnally moving to warm reaches to feed and returning to cooler reaches to digest their food. This type of behavior would take advantage of warm habitats. In any event, whether such behavior occurs or does not occur has no effect on the conclusions of the present study.
TRT/CSPA-2 (p. 2)	A City of San Francisco biologist has acknowledged on the record in this proceeding that *O. mykiss* populations in the lower Tuolumne River are substantially smaller than populations downstream of rim dams in the Sacramento river drainage, where water temperatures are generally much lower than temperatures in the lower Tuolumne River.	Comparisons of differing *O. mykiss* population sizes from spatially distinct river systems citing a statement by a "City of San Francisco biologist" lacks scientific rigor and should be disregarded. Just as one example, the two rivers under comparison would have substantially different geomorphological histories and structures which may be a more important factor affecting population sizes. Other factors may also play key roles in abundance such as total spawning area, differing food sources, predation pressures, fishing activities, etc. These issues have nothing to do with temperature in this system.
TRT/CSPA-3 (p. 2)	Before any adjustment to the established (EPA 2003) temperature benchmark for a 7DADM value for the population of *O. mykiss* in the lower Tuolumne River is considered based on site-specific conditions and response, further investigation and evaluation would be required. The Study should explicitly state this, and	It should be noted that EPA (2003) does not provide specific temperature recommendations to the lower Tuolumne River or any California river system. It is a general recommendation that applies to all populations of rainbow trout. The report's discussion is not intended as the basis for changing the EPA (2003) 7DADM recommendations.

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	should describe additional evidence needed before any change in the 7DADM value for the population *O. mykiss* in the lower Tuolumne River might appropriately be evaluated.	Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. Minimally, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study. This report is not intended to preempt consultation with EPA. We strongly believe that the new data collected here are a firm basis for opening such a dialogue about site-specific temperature criteria in general as well as for the Tuolumne River *O. mykiss*. We suspect that EPA would welcome this dialogue being opened as their 2003 report acknowledged the possibility of local adaptation. EPA 2003 simply cited that the scientific evidence at that time was weak. The scientific evidence is now much stronger.
TRT/CSPA-4 (p. 3)	The Study results alone do not warrant site-specific summer water temperature criteria for *O. mykiss* in the lower Tuolumne River.	The Districts assert that the site-specific empirical evidence that exists for Tuolumne River *O. mykiss* warrants considerable weight when compared to data from completely different regions of the country. Also, see response to TRT/CSPA-3
TRT/CSPA-5 (p. 3)	In our view, the appropriate use of the Study results would be to 1) evaluate their limitations; 2) develop additional investigations that might be necessary to scientifically justify consideration of adjusting thermal criteria for the population of *O. mykiss* in the lower Tuolumne River, 3) enumerate and evaluate regulatory and policy issues that might be involved in adjusting these criteria; and 4) assemble these necessary components and, based on this ensemble, develop a process for considering and evaluating site-specific water temperature criteria.	While evaluating natural background provisions and use attainability exceptions to the EPA (2003) 18°C 7DADM recommendations are beyond the scope of the current study, the Districts (TID/MID 2014, Attachment A) previously demonstrated that potential re-operation of the Don Pedro Project to meet EPA (2003) temperature recommendations was infeasible under a range of potential scenarios evaluated, including "without dams" scenarios. Given the infeasibility of meeting the EPA 18°C 7DADM benchmark and that the results of the current study

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		demonstrated near-optimum physiological performance and active feeding at temperatures well above 18°C, consideration of site-specific exceptions to this recommendations are warranted. We are pleased to read that the reviewer appears to agree with us that further steps are warranted. Therefore, the real issue is not what is contained in the report, but rather what should follow from it.
TRT/CSPA-6 (p. 4)	The authors of the current Study should be more explicit in its caveats and should describe the limitations of its conclusions.	As noted above in the response to TRT/CSPA-1 the data generated here would in our opinion represent the most conservative estimate of temperature effects by only looking at the effect of a rapid change from acclimation temperatures. To speculate on how well *O. mykiss* from the lower Tuolumne River might perform if they were allowed to acclimate to even higher water temperatures is beyond the scope of the present study. We agree that we could have been clearer about stating the limitations of the study, but it is clear that these limitations are more pertinent to the future actions and not the conclusions that are based on our data.
TRT/CSPA-7 (p. 4)	The Study examines only the juvenile lifestage of *O. mykiss* in the lower Tuolumne River. The Clean Water Act requires that the most sensitive resources be protected. It is not clear whether the adult lifestage, which is also present during the summer time period, is more, equally or less sensitive to high water temperatures. Before adjustments of summer temperature criteria for *O. mykiss* in the lower Tuolumne River could be considered, an evaluation of the physiological response of adult *O. mykiss* in the lower Tuolumne River would need to conducted, in addition to completing the evaluation of the physiological response of juveniles.	At no time in the report do we state that our results for juvenile fish are directly applicable to other life stages of this species. Although some studies have examined the relative thermal tolerance of juvenile and adult salmonid life stages, evaluation of the thermal performance of adult *O. mykiss* was outside the scope of the study plan. The decision to use juvenile vs adult-sized fish was made on the basis of higher relative abundance and the ability to capture them with beach seines vs angling that may result in reduced swimming performance and necessitate longer recovery times for adult fish that were captured by that method.

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		Again, future steps might be to study other life stages, but this possibility does not challenge the present results. We would also like to note that in order to advance to the adult life stage, fish must survive the juvenile life stage. And juveniles are evidence of a successful life stage in this river system. It would be an odd, and unsustainable, biological adjustment to have juvenile fish be well acclimated to local conditions only to prove fatal when it reaches the adult life stage.
TRT/CSPA-8 (p. 4)	The Study makes comparisons between *O. mykiss* in the lower Tuolumne River and populations that are more permanent and defined and that have more common characteristics. The current Tuolumne population is likely a combination of residual lower river fish, wild or hatchery fish washed down from La Grange (themselves possibly the result of production in La Grange Reservoir or originating in Don Pedro Reservoir), and some number of anadromous individuals of unknown origin and their progeny. It is further likely that the population is being replenished from these sources on an ongoing basis, and that some portion of the fish that are there in several years will have little directly in common with the current population. This is particularly likely under dry or drought conditions, when a greater proportion of the existing population may be expected to perish. Managing a changing population based on ascribed thermal tolerances of an existing population is questionable both scientifically and as policy.	This comment is highly speculative. Moreover, we do not fully understand what the reviewer means by "populations that are more permanent and defined and that have more common characteristics". The population that we have studied and that is protected is a resident of the river system, one that has a barrier upstream in the form of a dam and a potential thermal barrier downstream. How they arrived there and how they adapted is not a concern of this Report. This Report focuses on the thermal capacity of the fish that currently reside in the river and are protected by current regulations.

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		Thus, while the supposition that future lower Tuolumne River *O. mykiss* populations may have little directly in common with the current population is interesting, it does not affect the conclusions of our study since we are limited to testing the current population. Furthermore, if the intent is to do future stocking of this river system with fish from either a hatchery or another wild source, then similar experiments could be performed on those populations. The use of 'wild' or 'local' fish for the Tuolumne River would then have to be redefined.
TRT/CSPA-9 (p. 5)	There is no bioenergetics study of *O. mykiss* in the lower Tuolumne river that would support management for water temperatures higher than those recommended in EPA guidance. The Districts declined in 2011 to conduct a bioenergetics study of *O. mykiss* in the lower Tuolumne River as recommended by the Department of Fish and Wildlife. The Commission did not order this study.	The EPA guidance was not based on any bioenergetics studies. While the Districts were not required to undertake a direct bioenergetics study in FERC's May 21, 2013 study determination, it should be understood that all bioenergetics (activities) require oxygen. **The current study characterized the maximal capacity to deliver oxygen for any and all activities**. Indeed, for any energetic model the currency can be oxygen or Joules. Regardless, the sum of all the bioenergetics inputs cannot in the long term exceed feeding input (the fish would be starving) or the maximum aerobic capacity (which is exactly what we measured). In addition, the multitude of factors that go into a bioenergetics study would require a large number of individuals to be removed from the river, well in excess of the authorized fish take, and many of these fish would have to be sacrificed for such a study. The current study design allowed direct examination of physiological performance without the need for either high levels of fish take or sacrificing fish. Indeed, we successfully returned all but three of the study fish to the river. Further, the *O. mykiss*

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		population studies and resulting in-river population model does include a bioenergetics component. To be clear, the study was implicitly designed to minimize the impact of fish removal from the river for experimentation.
TRT/CSPA-10 (p. 6)	Follow-up site specific physiological studies must address elevated water temperatures over an extended period of time, ideally over an entire summer.	We do not understand the commenters' concern regarding lack of evaluating responses over time. This was not the objective of the Report, as clearly pointed out in the study plan. Indeed, the permits issued for fish removal would NOT permit more than 2 fish to be studied at a time and over time – this was the maximum number of fish that could be removed from the river. Perhaps the following clarifies matters. To reliably measure growth rate, at least 40 days would be needed to detect a response because the fish have to change their mass by a reliably detectable amount. We clearly could not do this with the permits issued, nor did the study plan (previously reviewed by CDFW) ever suggest this was the intent of the study. Instead, as detailed in the study plan, we measured oxygen uptake, which uses a different time scale, and it can be reliably measured over periods of minutes. Also, we went to great lengths to follow and analyze oxygen uptake over a nearly 24-hour period to examine its variability and ensure our estimates of RMR were as accurate as possible for a field study. Also, we carefully measured maximum oxygen uptake in the manner used by both Fry and Brett, but using modern technology with greater accuracy and precision. Therefore, we can state with confidence what the fish's capacity was in terms of delivering oxygen to tissues over a broad temperature range.

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		If, however, the comment concerning "over time" is that we need to study fish that were acclimated to different temperatures (14°C was the coolest temperatures found in the lower Tuolumne River during the 2014 study), this is a valid comment. Nevertheless, it is well known that thermal acclimation is used by fishes to "improve performance" at the new acclimation temperature. Therefore, if the lower Tuolumne River *O. mykiss* used in the present study can be shown to acclimate to water temperatures warmer than they were experiencing at the time of the experiments, we have then provided a conservative estimate of temperature effects on the fish performance by looking only at the effect of a rapid change in water temperature from river temperature to which they were acclimated. This concern does not change the outcome of our results, but does introduce the possibility that this fish population could do even better at warmer temperature if they were allowed to first acclimate.
TRT/CSPA-11 (p. 6)	Follow-up site specific physiological studies must be conducted on adult as well as juvenile *O. mykiss*.	The decision to use juvenile vs adult-sized fish was made on the basis of higher relative abundance and the ability to capture them with beach seines vs angling that may result in reduced swimming performance and necessitate longer recovery times for adult fish that were captured by that method. However, we would note that in order to advance to the adult life stage, fish must survive the juvenile life stage. And juveniles are evidence of a successful life stage in this river system. It would be an odd, and unsustainable, biological adjustment to have juvenile fish be well acclimated to local conditions only to prove fatal when it reaches the adult life stage.

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		The suggestion to study Tuolumne River adult *O. mykiss* would seem to indicate agreement with the Districts' assertion that the EPA 2003 recommended temperatures are invalid because the EPA research involved no site-specific studies nor even results of research on CA *O. mykiss*, neither juvenile nor adult. Note also that Hokanson (1977) on which EPA 2003 recommendation is based, did not consider multiple life stages.
TRT/CSPA-12 (p. 6)	Follow-up site specific physiological studies must address the likely multiple sources and ongoing replenishment of the *O. mykiss* population of the lower Tuolumne River.	See response to TRT/CSPA-8 above. However, the commenter is suggesting that *O. mykiss* from different locations in the lower Tuolumne (even within a mile of each other) would have differing thermal capacities, while the EPA 2003 paper proposes that all *O. mykiss* populations in the entire Pacific NW and CA should be considered to have the same thermal capability. We agree with the commenter that site-specific empirical information is a much better measure of performance. Nonetheless, our Report concerns one specific population.
TRT/CSPA-13 (p. 6)	The Districts should perform a bioenergetics study for juvenile and adult *O. mykiss* in the lower Tuolumne River.	See response to TRT/CSPA-9 and -12.
TRT/CSPA-14 (p. 6)	The Study should be edited so that the Executive Summary and the Conclusion place the value of the findings in the appropriate context of how they might inform a comprehensive review of site-specific summer thermal conditions in the lower Tuolumne River.	The Executive Summary has been amended to address this comment. Also, see response to TRT/CSPA-3. Again, the present study suggests that applying the EPA 2003 recommendation to the Tuolumne River *O. mykiss* population is overly conservative.
SWRCB-1 (p. 1)	Study Plan 14 was not required by FERC in its Final Study Plan Determination and is not supported by the State Water Board, California Department of Fish and	In its December 2011 SPD, FERC stated that it would consider additional empirical evidence from the Tuolumne River. The development, evaluation and application of

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	Wildlife (CDFW), United States Fish and Wildlife Service (USFWS), or the National Marine Fisheries Service (NMFS)."	empirical evidence that would reduce uncertainties regarding temperature-related effects on Tuolumne River salmonids is the primary purpose of this study. FERC's emphasis on empirical evidence has further encouraged the Districts to identify and consider new evaluations that could contribute to more focused understanding of potential influences of temperature on LTR salmonids, which led to the development of this study approach and report.
SWRCB-2 (p. 2)	The report does not explicitly state that its results alone demand a change in the 7DADM temperature outlined in the 2003 USEPA Guidance. Rather the report states that this information should be used to determine a 7DADM value specific to Tuolumne River *O. mykiss*. However, the report does not outline a process to be used to determine a scientifically acceptable and defensible 7DADM specific to the Tuolumne River *O. mykiss*.	The recommendation lies well beyond the objective of the present report. See response to TRT/CSPA- 3 and -4.
SWRCB-3 (p. 2)	"State Water Board staff recommends that any process to develop temperature criteria specific to the Tuolumne River follow a similar process as the EPA Guidance. Two additional examples of the recommended process include: The Final Staff Report for the Klamath River Total Maximum Daily Loads (TMDLs) Addressing Temperature, Dissolved Oxygen, Nutrient, and Microcystin Impairments in California (NCRWQCB 2010), and The Effects of Temperature on Steelhead Trout, Coho Salmon, and Chinook Salmon Biology and Function by Life Stage; Implications for Klamath Basin TMDLs (NCRWQCB 2005)."	The references provided by the SWRCB deal with TMDL development, a different process than that required for amending the present temperature guidance. In any event, the Districts look forward to working with the SWRCB on temperature issues on the lower Tuolumne River.

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SWRCB-4 (p. 2)	"It is important to point out that the Report focuses on increased water temperature effects on only one parameter (oxygen consumption) and one life stage (juvenile) for *O. mykiss*. Study Plan 14 and the Report do not evaluate long term effects of increased water temperature as well as the other life stages of *O. mykiss*. Questions that might be evaluated as part of a more comprehensive study include, but are not limited to: 1. What is/are the effect(s) of increased temperature conditions on other life stages of *O. mykiss* or the long-term effects of this short-term exposure on *O. mykiss?* 2. How does temperature influence other factors which may affect salmonids, such as food availability and disease?	We thank the commenter for explicitly stating what our Report achieved. It would seem from this that the reviewer has no difficulty with accepting our data. Again it seems that the reviewer is making suggestions for future steps, which we have commented on above: See response to TRT/CSPA-1 & TRT/CSPA-9. Regardless, it is important to remember that the present study significantly expands the knowledge base regarding *O. mykiss* on the lower Tuolumne River. There were no studies for this population prior to the present study, which was a rigorous and comprehensive examination of thermal performance on juvenile wild fish. Indeed, the reviewer does not challenge the quality of the data in hand. We would argue that empirical data are a better indicator of thermal performance than largely unrelated information the applicability of which is difficult to measure. Related to temperature's influence on other factors, prior studies of food sources on the Tuolumne under the existing temperature and flow regime have indicated healthy BMI populations and that prior studies have not found any significant disease issues with Tuolumne River salmonids.
SWRCB-5 (p. 2)	Study Plan 14 and the Report only consider increased temperature effects on fish persisting in the Tuolumne River under current conditions. Study Plan 14 and the report fail to examine the effects of increased river temperatures on the recovery of *O. mykiss* populations in the Tuolumne River.	The current study was able to examine the effect of increased temperature on juvenile *O. mykiss* persisting in the Tuolumne River under current conditions. While examination of questions related to conditions affecting future *O. mykiss* populations in the lower Tuolumne River are beyond the scope of the current study, the present study indicates an ability of the local population to adjust to local conditions.

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		The Districts are uncertain as to what is meant by "recovery of *O. mykiss"* under "increased river temperatures".
CDFW-1 (p. 2)	The study design identifies acute exposure to stressful warmer water temperatures at the individual level; therefore, the study cannot inform development and/or revision of population level chronic water temperature criteria. In their report, the authors compare their acute water temperature results to the United States Environmental Protection Agency's chronic population criteria (USEPA 2003) which is inappropriate.	The reviewer has a fundamental misunderstanding of the study design and purpose. The reviewer thinks we were conducting an acute survival study not testing metabolic capacity (through eliciting maximum metabolic rates using swim tests) at chronic water temperatures. We ask that the reviewer please read the overarching statement where we clearly explain what AAS measures tell us, and how these data relate to the 7DADM. Also, see our response to TRT/CSPA-3: The report discussion is not intended as the basis for changing the EPA (2003) 7DADM recommendations. Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. Minimally, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study. Please note that the casual use of 'stress' and 'stressful' should be avoided. Defining 'stress' in fish requires rigorous experiments at a population-specific level, careful endpoint selection and interpretation, and well-described exposure conditions. Assuming that our test conditions were 'stressful' and assuming that warm temperatures are necessarily 'stressful' to Tuolumne River *O. mykiss* is an unsupported statement. Our data, in fact, suggest that at temperatures much higher than 18C, the tested fish maintain maximum AS. We do not see how a fish that is "stressed"

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		would be able to maintain its AAS given that "stress" is a metabolic load (see Fry, Beamish and Brett reviews of this in the last century) that necessarily limits aerobic performance.
CDFW-2 (p. 2)	Anadromous salmonids populations throughout the Pacific Northwest (including California) are declining primarily because of poor reproductive success and recruitment back into the population (Yoshiyama et al 2001).	It must be noted that this is not what the referenced paper said or concluded.
CDFW-3 (p. 2)	The intent of the USEPA (2003) analysis was to reverse that trend by presenting chronic population water temperature criteria.	This is the reviewer's interpretation of the intent of EPA (2003) but this was not the stated rationale for the document. In fact, the EPA (2003) report did acknowledge that "local adjustment was possible and that well-designed studies could be used to identify site-specific thermal adjustments". This was one of the reasons for conducting the present study, which we believe was well-designed and well-executed. It produced definitive and reliable data. Importantly, the 7DADM criteria incorporates information to estimate thermal optima and performance breadth using a diversity of thermal performance metrics (e.g. lethal limits, longer term thermal experiments related to growth, and many others) that operate several timescales of thermal exposure. The focus by the reviewer in casting the EPA 7DADM criteria as exclusively chronic is misleading and incorrect. Please see our response to TRT/CSPA-3, as well as the overarching response document at the front of this response to comments.

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CDFW-4 (p. 2)	Chronic criteria and population criteria are always lower than acute and individual criteria. The authors presented higher acute/individual water temperature criteria based on a single study, but failed to extrapolate the results to a lower chronic population criteria that would be protective for reproductive success and recruitment to maintain a sustainable (i.e. viable) population. Survival rates are based on amount of time exposed, as well as temperature exposure, and are extremely well described in the scientific literature.	As stated above, the reviewer's fundamental misunderstanding appears to be that we were conducting a survival study not a test of metabolic capacity. There is no need to extrapolate our study results to a lower chronic temperature criteria since we were making direct measurements of the optimal temperature range for Tuolumne River *O. mykiss* based on their metabolic capacity. Indeed, we would argue that there is no reliable methodology to extrapolate from acute to chronic studies. However, if a fish cannot perform after an acute temperature change, it is unlikely to perform well with a chronic change unless it can thermally acclimate. We show that the fish do well with an acute thermal change, and these data do not appear to be in dispute. Please see the overarching response document at the front of these responses to comments where we explain what should and should not be gleaned from our data as well as specific remarks on how our data 'scale' up to population level functions. Also see our response to CDFW-3 regarding chronic criteria.
CDFW-5 (p. 3)	Executive Summary, page i, second paragraph. The authors stated, "The study tested the hypothesis that the Tuolumne River *O. mykiss* population below La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that exist in the river during the summer". What is the authors' definition of "locally adjusted"?	"Locally adjusted" was defined on page 6 as a hypothesis that can be tested by confirming or refuting by evaluating the predictions on page 7 of our report. In short, the hypothesis is: Tuolumne River *O. mykiss* are "locally adjusted" if they have higher metabolic capacity (absolute aerobic scope) at temperatures above 18^{o} C. A finding like this would be in contrast to the earlier data based solely on northern fish data. Locally adjusted is a term that includes, and does not distinguish between, local adaptation and local

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		acclimation/acclimatization. The assignment of 'adaptive' happens at the level of the population, not for individuals. This is in contrast to acclimation/acclimatization, which are traits/responses that change at the individual level, over the course of a particular individual's lifetime. A simple example would be how season influences fur thickness in bears. This is a trait that varies by individual, by season. Please see the last sentence of the executive summary where we explicitly state that our study does not distinguish between these two explanations as this was not our objective. Also, please see the entire section in the Introduction (Current Evidence for Local Physiological Acclimatization and Genetic Selection) articulating how acclimation/acclimatization/adaptation is defined. Thus, we used a term that encompassed both mechanistic explanations.
CDFW-6 (p. 3)	Executive summary, page i, third paragraph, last sentence. The authors state, "Therefore, the experimental approach also acknowledges that every activity of a fish in a river (swimming, catching prey and feeding, digesting a meal, avoiding predators, defending territory, etc.) requires oxygen consumption above a basic routine need and that salmonids have evolved to maximize their oxygen supply when they fuel muscles during exhaustive swimming". This statement leads to three questions; 1) This test appears to study basic survival, but does the study address reproductive success and recruitment? 2) Does this experimental design measure activities related to spawning, immune function and general overall stress? and 3) Isn't this the case for all vertebrates, that an animal's physiological function evolved to fuel their	As described in the study plan prepared for this study and submitted for review prior to conducting the study, we did not aim to study basic survival. Therefore the reviewer misunderstands the study objectives. We measure aerobic capacity. Survival would require a minimum of SMR, but this study shows that these fish had the capacity to more than double their metabolic rate at specific temperatures. This information can be used to assess whether there is the capacity for activities well beyond survival, such as growth, immune function, reproduction, predator avoidance, etc. We cannot comment on how the fish use this capacity. These are behavioral decisions made by this fish not by us. However, it we know that the energetic cost of an activity is maximally a doubling of metabolic rate, and this fish has this capacity, it is reasonable to then conclude that the fish has the capacity to undertake this activity.

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	muscle under non-resting (exercise) or stress conditions?	Regarding question (3), the issue is not whether an animal's physiological function has evolved to fuel their muscles during exercise, the issue is their capacity to fuel their muscles at different temperatures.
CDFW-7 (pp. 3 and 4)	Executive Summary, page i, fourth paragraph. The authors state "As expected for a fish, RMR [Routine Metabolic Rate] increased exponentially with increasing test temperature from 13°C to 25°C (36 different fish, each at a single test temperature)". Basically the RMR is a fish in a resting state, thus if their RMR increased with temperature in a resting state, this indicates the fish are becoming stressed in the warmer temperatures without exertion. They analyzed their results using a mathematical model. What would the results look like if the results were analyzed using standard statistical analysis for each temperature group? Further they presented temperature ranges from 16.4 °C to 25°C and 17.8°C to 24.6°C, suggesting the higher temperatures are protective for basic survival. This leads to the question, do the authors agree that the 16.4°C and 17.8°C temperature levels (i.e. lower end of range) to be a more protective temperature at a chronic population exposure level to provide optimal reproductive success and recruitment rather than the higher temperature's the author are advocating? It's vitally important to remember that just because a fish or a fish population survives at a certain temperature; it does not automatically mean that the fish or the fish population thrives at the same temperature range. The ability to "thrive", carries with it the ability to	The commenter's statement "*thus if their RMR increased with temperature in a resting state, this indicates the fish are becoming stressed in the warmer temperatures without exertion*" is simply and fundamentally incorrect. We know of no theoretical reasoning or literature to support such a claim. Arrhenius in the 1920's showed that all rate functions, including many biological ones, increase with an exponent of 2-3. To suggest otherwise reveals a fundamental lack of understanding about how temperature affects ectotherms. Why does RMR go up with increasing temperature? It has to do with simple laws of thermodynamics – fish are ectotherms, their body reflects water temperature. As fish/molecules warm up, they collide more frequently. Biochemical rates, such as ATP turnover, increase. Thus RMR increases. The 'amount' or how temperature sensitive this process is varies across species and reflects variation in biochemistry (I could go on a long tangent here about protein evolution etc.) and we express this temperature sensitivity with calculated temperature quotients, or Q10s. This is an expression for how much a rate (like a metabolic rate) changes with every 10C change in temperature. Ecologically relevant Q10s are usually between 1.5 and 3 in fishes. The lower the Q10, the less temperature sensitive a species is and the less MR changes as temperature changes.

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	successfully grow and reproduce at sufficient levels that keep the both the individual fish, and the fish population, in good condition (i.e. adequate reproductive viability). The authors further state, "Thus, the maintenance of AAS [Absolute Aerobic Scope] across nearly the entire test temperature range clearly shows that the Tuolumne River *O. mykiss* population has a broad range of thermal performance". Isn't this case for all vertebrates? The authors further state, "Indeed, the AAS of the Tuolumne River *O. mykiss* population was atypical when compared with cold-adjusted, *O. mykiss* from the Pacific Northwest, whose thermal performance optimum is reported as 18°C" (USEPA 2003). What exactly is meant by "atypical"? What is meant by "cold-adjusted" fish from the Pacific Northwest when all salmonids are cold water fish that evolved in cold waters that originated from snow melt and ground water seepage into the river systems? The reference to the USEPA (2003) 18°C as a thermal performance optimum is incorrect. The USEPA (2003) report did not discuss thermal performance, but rather concentrated developing sub-lethal chronic population criteria to improve reproductive success and recruitment to reverse a declining population trend. It is inappropriate, and therefore not scientifically valid, to compare acute individual results to chronic population criteria. The last sentence suggesting the upper thermal performance is above 25°C is pure speculation on part of the authors and should be deleted.	Another incorrect interpretation of increasing RMR with increasing temperature is that when you see an increase in MR, it indicates stress. If a fish were stressed with acute warming the increase in oxygen uptake would have an even higher exponent. However, because MMR does not similarly increase with stress, AAS must then decrease with acute warming due to the following equation; AAS = [MMR – (RMR +stress)]. Therefore the fact that AAS was maintained across temperature despite an increase in RMR with temperature argues AGAINST the very claim the reviewer is making. Interestingly, fish in a variety of situations can behaviorally and deliberately seek out warm temperatures in order to avoid the 'dampening' effects of cool temperatures on activities such as growth. Thus seeking warm water is not 'stressful'. For example, if you want to get big, and if you have access to lots of food, you might select warm temperatures to process food at a faster rate, smoltify sooner etc. The perspective that cold is always 'less stressful' than warm is pervasive throughout the CDFW comments, and is not ecologically relevant and has no biological basis. Fish have evolved with a physiology suited to historic thermal conditions - not the ones imposed by CDFW, EPA, and other regulators. This is called Natural Selection. While Darwin and others advanced this idea centuries ago, biologist are only now beginning to appreciate natural selection at a mechanistic level. To suggest otherwise does not

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		acknowledge the seasonal/temporal fluctuations in temperature that are part of a fish's life history! Issues regarding the casual use of 'stress', the 7DADM as a chronic criteria, and misunderstandings about what is meant by local adjustment have been dealt with in the clarifier statement at the beginning of this attachment and in responses above. The question put forward after "18°C" (USEPA 2003)" is a rhetorical question since the reviewer provided the results of his "standard statistical analysis" later in his review. The statistical questions are dealt with below in the methods/results. Related to this comment, it is incorrect and purposely misleading to suggest we are advocating for higher temperatures, we are reporting on the results of our study which show that these fish have the capacity to conduct various energetically demanding tasks at temperatures above 18°C. The report discussion is not intended as the basis for changing the EPA (2003) 7DADM recommendations. Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. As a minimum, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study. Related to the question about thermal response of "all vertebrates" the short answer is "no". Some vertebrates can have a much narrower range of temperatures where thermal performance, as indexed by AAS, is much narrower (e.g.

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		Fraser River sockeye salmon). This is another point where fundamental misunderstanding of the thermal performance literature for fishes has apparently led this reviewer down a confused path. What the study and the data demonstrate is a relatively flat curve, which is consistent with capacity being temperature INSENSITIVE and acclimation/adaptation to a rather wide thermal range. Our use of "atypical" means different from the *O. mykiss* which are the basis for EPA's 18°C criteria. The reviewer clearly believes that all salmonids are genetically programmed for cold water and that there has not been sufficient time for any to become locally adjusted (warm or cold). Scientific evidence indicates that salmonids from different locations within the Pacific Northwest and Canada have different optimal temperatures for day to day existence, migration and feeding in freshwater environments (Parsons 2011; plus other references).
CDFW-8 (p. 4)	Executive Summary, page ii, first paragraph. What do the authors mean when indicating that the fish are locally adjusted? The fish are blocked by a series of dams, preventing them to migrate upstream to cooling temperatures, so they have no choice but to live in a warmer environmental regime. The authors also stated they lost 1 of 4 fish acutely exposed to 25°C. Do the authors agree that 25% fish exposed to 25°C would die, especially if they are chronically exposed to this and higher temperatures?	The explanation of what was meant by "locally adjusted" is provided above. No, the authors do not agree that "that 25% of fish exposed to 25°C would die, especially if they are chronically exposed to this and higher temperatures". Our research did not attempt to answer this question, and it would be inappropriate to try to do so. A different type of study with more fish tested at higher temperatures and more sensitive mortality endpoints would be required to answer this question. What we did show instead was that if fish were at 25°C and swum to exhaustion, 25% died. This observation is very different from the assertion regarding survival, which was not

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		measured. Of course, "survival" is time-dependent. A CTmax measurement is used by some to measure "survival, but this is a matter of a few minutes. Even the fish that died in our experiments at 25°C lasted longer than a few minutes! Thus, the debate around "survival" and temperature has a long and unresolved history, and thermal resistance and tolerance are better terms at a mechanistic level. This is part of the reason why modern day physiologists measure AAS to assess thermal performance. The study was never intended to define specific chronic thermal exposure limits. We remind the reviewer that our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. At a minimum, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study. It is unclear to us what is unclear about this very specific articulation of the study goal and main finding.
CDFW-9 (pp. 4 and 5)	Executive Summary, page ii, second paragraph. The authors state, "The conclusion of the study is that the thermal range over which the Tuolumne River *O. mykiss* population can maintain a 95% of peak aerobic activity from 17.8°C to 26.6°C". How long can these fish withstand this activity? In the last sentence they state that "Finally, based on a video analysis of the swimming activity of *O. mykiss* in the Tuolumne River, fish at ambient water temperatures were predicted to have excess aerobic capacity well beyond that needed to swim and maintain station against the river current in their usual habitat". However, don't all vertebrates have	Some of the fish could maintain this level of activity for hours in the swim tunnel but in the wild most of their lives occur at much lower activity levels and peak activity only occurs for a few seconds to feed or avoid predators. Also, please see clarifying document at the beginning of this attachment where the issue of energy allocation is explained and the value of understanding AAS capacity as a comparative metric is restated. However, no animal ever lives for prolonged periods near its maximum AAS. Therefore, we agree with the contention. Jared Diamond for example suggested that maximum

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	excess aerobic capacity to survive and meet the basic needs of survival; how are these trout any different from any other living creature? Just because a fish can survive a short duration elevated temperature exposure event (i.e. minutes) does not mean that it can withstand the same elevated temperature for a long exposure event (i.e. days, weeks, and/or months). A human analogy helps us understand key physiological concepts and keep them separate. For example, an Olympic marathon runner can run 26.2 miles in approximately two hours; however, this same runner cannot maintain the same pace for days, weeks, and months. The point here is that the Olympic runner is training for an acute event but in so doing he/she is not enabling him/herself to maintain an acute pace over a chronic period of time (days, weeks, and months). The ability of fish to survive an acute event is not indicative of a fish's ability to survive a chronic event. As was stated above, acute tolerance is always higher than chronic tolerance. USEPA set chronic criteria while the authors of this report conducted an acute study. At best, this study's results may be used to inform development of acute level criteria (i.e. temperature tolerance over short duration) but is does not translate to predicting a chronic level criterion (i.e. temperature tolerance over long durations).	sustained performance in lactating mammals was limited by food movement across the gut; high endurance athletes and lumberjacks appear to have similar problems. Biologists who more broadly measure daily energy expenditures in wild animals rarely find that metabolic rate is on average 2X basal rates. Thus, the finding that Tuolumne River *O. mykiss* have a FAS of >2 for much of the thermal range we studied must have impressed this reviewer. Yes, each vertebrate species will have excess aerobic capacity to perform and survive at some range of temperatures. The issue we specifically address in this Report is "what is this range of temperatures". There are obviously lots of differences between Tuolumne River trout and other living creatures, but the only difference relevant in this study is the optimum temperature range for Tuolumne River *O. mykiss* compared to that for other populations of *O. mykiss*. Tuolumne River *O. mykiss* have been observed living and feeding in a river which has higher water temperatures than most other *O. mykiss* populations. See our discussion of comparative context for help in understanding this point. With regard to the human analogy paragraph: This comment confuses several important concepts that we have explained above and in the clarifying document at the beginning of the attachment. The first part is about how metabolic energy is allocated and we've responded to this already. The next bit introduces acute and chronic exposures, which we've addressed above as well.

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CDFW-10 (p. 5)	Executive Summary, page ii, last paragraph. The USEPA (2003) criterion is not an upper performance level for fish. The authors are comparing acute results to a chronic value, an individual result to a population criteria, and survival to reproductive success and recruitment, which are all inappropriate comparisons. The authors need to conduct the same test in other rainbow trout stocks throughout the Pacific Northwest to make a similar comparison to this study before rendering a conclusion that the Tuolumne River rainbow trout have evolved higher population acute water temperature tolerance. The authors recommend " ... we recommend that a conservative upper performance limit of 22°C, instead of 18°C, be used to determine a 7-Day Average of the Daily Maximum (7DADM) value". However, for cold water fish, such as trout, it would be more appropriate, conservatively speaking, to use the lower water temperatures values (17.8°C) the authors presented in their study. Their comparison to the redband trout is also inappropriate because the redband trout evolved under a totally different set of environmental conditions compared to coastal rainbow trout/steelhead. Coastal rainbow trout evolved across thousands of years in river systems that originate in high mountain elevations and connect to the Pacific Ocean. Today's rainbow trout have been exposed to river systems, blocked by dams for less than 100 years, which is insufficient on the evolutionary scale to adapt to today's river water conditions.	We agree that USEPA 2003 criterion was not the upper performance level, but it was the optimal temperature for peak growth. See explanation above, regarding that fact that our experiment was measuring the optimal temperature range for Tuolumne River *O. mykiss*, not acute CTmax temperatures for these fish. If there is no peak for ASS we must then talk about a thermal range for peak AAS, which is what we do. The reviewer repeatedly returns to the idea that trout are a cold-water species that cannot adapt to warm conditions. We agree that most trout populations are post-glacial invaders, but there is a groundswell of evidence that indicates exceptions to the rule. Red band trout are a documented exception. So are the hatchery-selected rainbow trout in Western Australia and Japan. We believe Tuolumne River *O. mykiss* are another exception based on the data presented in our Report. Please see previous response to acute/chronic criteria. The reviewer appears to be very certain that 100 years of exposure to higher water temperatures in not sufficient for rainbow trout to become locally adjusted to higher temperatures than other rainbow trout populations. In the report, we thoroughly review the published literature that addresses thermal adaptation among rainbow trout populations and that demonstrates supports for local thermal adaptation. The reviewer cannot be correct with their assertion, as it has taken far shorter for this to occur. Moreover, geneticists are increasingly of the belief that Gene

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		X Environment effects can take over in about 7 generations and be evident in as little as 2 generations. It is also not clear why the reviewer is focused on potential adaptation happening only over the last 100 years in California in response to dams. There are many natural systems in California (pre-dam) where fish would have encountered warm temperatures that would be comparatively warmer than northern latitudes. In drought years, trout can be trapped in shrinking ponds that get quite warm. Some survive and the survivors add resilience to the population. Please look into some of the portfolio effect literature. The opinion presented here by the reviewer is only one perspective. To think that we have been imposing artificial high temperature selection on California fish over the last 100 years is incomplete and quite likely incorrect. An argument could be made that constant, year-round cold-water access for fish immediately below dam is 'unnatural' selection and could contribute to the loss of high temperature resilience by dampening selective high temperature signals that would have, historically, occurred naturally.
CDFW-11 (p. 5)	Introduction, page 1, first paragraph. The authors' state, "However, cooler river temperatures are associated with cloud cover and over night [sic], and deeper ponds in the river do show some thermal stratification". Did the authors document the daily temperature difference during the hot summers, and identify and document any cool refugia or deep pools locations and measure water temperatures?	It is well known from the literature, human behavior and animal behavior that air temperature cools with cloud cover or at night. Groundwater seeps into rivers also provide cool refugia. To argue otherwise is folly. Indeed, the whole idea behind a 7DADM is that temperature fluctuates overnight and from day to day!!! Extensive studies were performed of water temperatures of the lower Tuolumne River and the reviewer is referred to

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		these studies that have been previously provided to CDFW as part of the relicensing process.
CDFW-12 (pp. 5 and 6)	Introduction, Page 1, second paragraph. The location in river miles was discussed as to where rainbow trout are commonly found with temperatures ranging from 11°C to 28°C. This is true; however, these fish have no other choice but to live under these environmental conditions because their natural migratory route to cooler high elevation waters is blocked by dams. If a fish can survive under a set of environmental (i.e. acute and chronic) conditions, including "thriving" (i.e. reproductive success over many generations etc.), then this fish has demonstrated that it has the capacity to withstand higher temperatures. However, not knowing the environmental conditions which other fish populations are actually exposed to and not knowing their population viability, the justification for changing temperature criteria based upon other fish stocks is scientifically invalid.	From these comments it seems that the reviewer agrees with our contention that this fish population has a limited and constrained habitat. Also they must be surviving, growing and reproducing in this environment. Therefore, they are likely adapted to the local conditions through natural selection. We are simply showing that this population has an excess aerobic capacity to perform over much of this thermal range. This is an important advance of knowledge, especially since it is not widely shared among other more cold-adapted rainbow trout populations, including those introduced to the midwest of the USA and were used by Hokanson (1977). Please also note: The report discussion is not intended as the basis for changing the EPA (2003) 7DADM recommendations. Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. Minimally, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study.
CDFW-13 (p. 6)	The entire [Thermal Tolerance and Thermal Performance] section discusses acute thermal tolerance in relation to survival, but does not present any information about chronic exposures in relation to reproductive success and recruitment to maintain a sustainable population. On page 2, paragraph 1, last sentence, the authors state "Regardless, CT max is always higher than the temperature that a fish can	See explanation above, regarding that fact that our experiment was measuring the optimal temperature range for Tuolumne River *O. mykiss*, not acute CTmax temperatures or survival for these fish. CTmax measures thermal tolerance; AAS measures capacity. The fact that when a fish is about to die at CTmax can be

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	tolerate for hours to days and certainly higher that the temperature at which a fish can no longer swim aerobically". The CT_{max} is a lethal temperature, at which point a fish can no longer swim aerobically. The tunnel test conducted by the authors accomplished the same end point where the fish were pushed to exhaustion and could no longer swim aerobically. So how does the tunnel test as presented by the authors differ from CT_{max} as stated in this paragraph?	higher than when FAS is 2 seems to be a reasonable statement. Please also see previous comments regarding acute and chronic metrics, and how our study relates to population metrics like reproduction and survival.
CDFW-14 (p. 6)	7-day Average of the Daily Maxima (7DADM), page 2, second paragraph, last sentence. The authors state, "Interestingly, by setting the 7DADM criterion for salmon and trout migration as 20°C, rather than 18°C, USEPA (2003) acknowledged that juvenile Pacific Northwest *O. mykiss* have sufficient aerobic scope for the energetic demands of river migration even at a temperature 2°C above the 7DADM for juvenile growth". However, the authors failed to mention the 20°C migration criteria is conditioned with a provision to restore or provide the natural thermal regime; or to provide or restore cold water refugia. Examples of cold water refugia or natural cool regime would include the confluence of cold tributaries at the main stem river or where groundwater exchanges with the river flow (hyporheic flow) that would provide cold water refugia for fish to escape maximum temperatures. Waters in tributaries for large rivers in the Central Valley have been diverted, eliminating cold water refugia at the confluence of these tributaries and groundwater pumping in the valley has lowered groundwater levels, thus removing natural cool ground water seeps into the	We have removed this statement from the Report to avoid potentially misleading any reader. This is not an important issue to us, but was meant to illustrate that even the blanket 7DADM had exceptions. We note that the reference to the Corbett report is misleading as it did not draw this conclusion.

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	valley's rivers. (Corbett, F., T. Harter, and M. Sneed. 2011. Subsidence due to excessive groundwater withdrawal in the San Joaquin Valley, California. American Geophysical Union. Fall Meeting Abstract #H23H-1397.)	
CDFW-15 (p. 7)	Justification and Purpose of the Study, page 4, first paragraph, last sentence. The authors state, "Thus, MR [Metabolic Rate] measurements were used to determine the optimal temperature range for Tuolumne River *O. mykiss*". Can the authors provide a definition for what they consider "optimal temperature range" and differentiate an acute and chronic optima range? Do the authors consider the hottest temperature as optimal or would a cold water fish be in excellent condition at a lower temperature from a chronic exposure perspective?	Please carefully review Figure 1, including the legend. The requested information is stated clearly there. We clearly do not measure chronic temperatures so the distinction is meaningless for this Report. We clearly do not consider the hottest test temperature as optimal and that is also clear from Figure 1.
CDFW-16 (p. 7)	Justification and Purpose of the Study, page 5, the first paragraph describes the "aquatic treadmill" similar to Parsons (2011) and Figure 1 that is presented on page 33 in this Study report. The peak T_{opt} in Figure 1 appears to be the maximum acute temperature (T_{max}) at the peak of maximum oxygen consumption and not necessarily an optimal temperature. From the peak temperature to higher temperatures, oxygen consumption decreases, suggesting the fish is exhausted and no longer capable of absorbing oxygen similar to what occurs in hyperventilation with humans. It is vitally important to remember that water at higher temperatures have lower oxygen concentrations, which is noteworthy because oxygen crosses the cellular membrane via a concentration gradient. Thus, lower oxygen	This appears to be one of the key sources of the reviewer's confusion on the purpose of our study. The maximum acute temperature (Tcrit in Figure 1) is not at the peak of maximum oxygen consumption. To be clear on the definitions that we have used, the temperature at which peak ASS occurs is DEFINED as Topt. This is the accepted definition. Tcrit would be when ASS fell to 0, but we never saw this with the present experiments. Furthermore, the statistics argue that there is no specific peak AAS as such, only a large thermal range over which there is no statistically significant change in AAS. Thus, there is not

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	concentrations in the water decrease the concentration gradient forcing the fish to use more energy to pull oxygen across their gill membrane, similar to hyperventilation of a human at the 8,000-foot elevation where the oxygen concentration is lower than that which occurs at lower elevations. Clark et al. (2013) Figure 1 B (page 2772) demonstrates that T_{opt} is midway up the aerobic scope and not at the peak of the slope. They further state "T_{optAS} provides little insight into the preferred temperature or performance of aquatic ectotherms, but rather aerobic scope continues to increase until temperatures approaches lethal levels, beyond which aerobic scope declines rapidly as death ensues." We agree with Clark et al. (2013) that the curves peak should be considered a T_{max}, not a T_{opt}.	a Topt as such, only a range over which a peak AAS is maintained. We are not therefore dealing with a mountain peak, but instead a prairie plateau! In this regard, the broad thermal performance of AAS more closely resembles the eurythermal killifish and goldfish. As a former postdoctoral supervisor of Tim Clark and co-author, Dr. Farrell is very familiar with his research and publications. We agree that AAS only tells us what capacity exists. Thermal preference, as pointed out by Clark et al. 2013, is a completely separate issue and should not be confused with Topt. However, if a fish wants to maximize the capacity to perform activities then it should choose Topt. The fish may or may not choose or prefer this temperature, but then there can be situations when they cannot – e.g. overwinter in the Tuolumne River, when the fish are likely to acclimate to cooler seasonal temperatures. Clearly a steelhead trout in the Pacific Ocean could not prefer a Topt of say 18°C for growth (as dictated by the 7DADM simply because such conditions do not exist within their known habitat range at sea. They are found at much lower temperature.
CDFW-17 (pp. 7 and 8)	Justification and Purpose of the Study, page 5, second paragraph, last sentence. The authors state, "Specifically, the temperature indices and the shape of the aerobic scope curve derived in the present study can also be compared with those of other *O. mykiss* populations and with the EPA (2003) recommendations". It is inappropriate to compare results from an acute stress test conducted for basic	See explanation above, regarding that fact that our experiment was measuring the optimal temperature range for Tuolumne River *O. mykiss*, not acute CTmax temperatures for these fish. We fundamentally disagree with the reviewer on this point and again refer to how we are interpreting our work and how it relates to the EPA criteria. The report is not intended as

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	survival needs and then make inferences to a population needing protection at the chronic criterion level. Again, acute level does not equate to chronic level when it comes to conducting tests and/or developing protective criteria. The USEPA criteria are chronic not acute; therefore, any reference to USEPA criteria in this report for purposes of changing chronic criteria is unfounded and is therefore not scientifically valid.	the basis for changing the EPA (2003) 7DADM recommendations. Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. Minimally, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study. Given the wealth of published literature on the ecological relevance of AAS measures, we think it rather inappropriate for a comment like 'not scientifically valid' to appear in this forum.
CDFW-18 (pp. 8 and 9)	Justification and Purpose of the Study, page 5, last paragraph. This paragraph summarizes Fry (1947) as presented in Parsons 2011. The "tunnel" experiment is an acute test that measures acclimation rather than adaptation. Central Valley salmonids evolved across thousands of generations to adapt to their living environment before the construction of dams. Fish that exist today have not evolved under today's environmental conditions because the time period has been too short for adaptation. Yes, *O. mykiss* can acclimate on an acute basis, but cannot adapt on a chronic basis in the less than 140 years since the construction of dams which blocked their historic spawning grounds. Similar to Parson (2011) description, resistance or adaptation is a result of the evolutionary process that takes generations to develop and cause a genetic change across those generations in a population (Guthrie 1980). Tolerance or acclimation is a result of an individual, or	We never make any claim that this fish population is adapted. We simply say that the evidence is in support of local adaption. We do not want to sound like a broken record, as almost all of these issues are variously and repeatedly dealt with in the responses above and in the clarifying document. There are a couple of points we must emphasize. Our experiment was measuring the optimal temperature range for Tuolumne River *O. mykiss*, not acute CTmax temperatures for these fish. If *O. mykiss* can't acclimate on a chronic basis to warmer water, we should not find them living and feeding in these warm water locations where they are observed in the Tuolumne River. Lastly, populations are made up of individuals. If the individual does not have the AAS to perform, the population

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	a group of individuals, repeated exposure across the life of the individual that causes a physiological change. Individual based temperature exposure tolerance does not expand to all individuals in the population, but population based exposure adaptation transfers to all individuals within the population. Population thresholds are designed to protect a population; whereas, an individual threshold is designed to protect an individual or small group of individuals. A population threshold will have minimal health effects for all the individuals, including the most weak, in that population (USEPA 2989; Air RISK). Therefore, the population exposure threshold tends to be lower in value (i.e. more restrictive) than the individual exposure threshold. In summary, population thresholds are always less than an individual threshold and chronic thresholds are always less than acute thresholds. Thus, the reported fish water temperature experiment address individual level, but have limited usefulness as a basis for a full understanding of resistance or adaptation at the population level. As such, the tunnel stress test provides great information about tolerance and acclimation at the individual level, but is inappropriate to extrapolate the results to adaptation for chronic population exposure criteria.	will cease to exist. Furthermore, natural selection acts on individuals and the results are reflected in populations. Therefore, if we do not understand the effects of temperature at the level of individuals, we have no hope of properly understanding the population effects.
CDFW-19 (p. 9)	Predictions Derived from EPA (2003), page 6. The authors proposed predictions based the USEPA (2003) criteria are irrelevant because the USEPA (2003) criteria were not based on an acute stress test. Is data presented in Table 1 based on acute or chronic tests?	We agree with the reviewer on what the USEPA 2003 report does and does not contain. This does not mean that we cannot make predictions, which is all we do. The report discussion is not intended as the basis for

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	The USEPA (2003) 18°C criterion is not based on maximum metabolic rate (MMR) acute test as presented in Figure 1, but is a chronic criterion which is lower than acute criterion. The USEPA (2003) never stated an AAS T_{opt} metric, nor discussed this study design, to develop a chronic population criterion.	changing the EPA (2003) 7DADM recommendations. Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. Minimally, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study. This report is not intended to preempt consultation with EPA. We strongly believe that the new data collected here are a firm basis for opening such a dialogue about site-specific temperature criteria in general as well as for the Tuolumne River *O. mykiss*.
CDFW-20 (p. 9)	Alternative Predictions of Thermal Adjustment, page 6. On what are the predictions based? Again, this study design is an acute stress test. It is well known that *O. mykiss* can survive in temperature above 18°C, but the study design does not answer the questions as to what is the chronic population threshold for reproductive success and recruitment to maintain sustainable populations across many future generations. The study design also does not address how well the *O. mykiss* immune system functions to ward off disease or how well a cold water fish can escape a warm water predator, especially when the water temperature are in the optimal range for the warm water predator. This study design can measure individual cold water fish short sprint energy to avoid a predator, but does not indicate how long a cold water fish can escape in a predatory warm water fish optimal temperature zone.	We agree with the reviewer on what the USEPA 2003 report does and does not contain. This does not mean that we cannot make alternative predictions, which is all we do. The reviewer continues to blindly refer to this population of rainbow trout as a cold-water species, when they clearly live in river temperatures reaching 24°C. The report discussion is not intended as the basis for changing the EPA (2003) 7DADM recommendations. Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. Minimally, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study.
CDFW-21 (p. 9)	Fish Collection, Transport, and Handling, pages 8 to 9. Most of the study fish were caught in the upper coolest	The choice to collect fish from cool reaches had nothing to do with the distribution of fish in the river with respect to

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	reaches of the river. However, if these fish are adjusted to warm temperatures, why were they present in the coolest waters of the river? The fact that most of the fish were found and captured in the coolest waters of the river is indicative that, at the population level, *O. mykiss* in the lower Tuolumne River are seeking cooler water to reside in even though warmer water is available to them.	temperature. Jumping to this conclusion is incorrect, unsupported, and misleading. The distribution of *O. mykiss* within the Tuolumne River is affected by many factors, only one of which is temperature, e.g there may be more predators downstream. Prior studies on the Tuolumne River have documented *O. mykiss* in warmer water. All of these studies have been provided to CDFW and we refer the reviewer to the many submittals on this subject. The decision to collect fish from relatively cold reaches was twofold. First, by collecting fish from cooler reaches, they were more likely to have a relatively cool thermal history (acclimatization) as compared to fish from warmer reaches. Because thermal history has a positive relationship with performance (i.e. if fish are acclimated to warmer temperatures, performance at warmer temperatures improves), testing cold-acclimatized fish should lead us to the most conservative AAS curve, with respect to temperature, that we could obtain. Certainly, when data are to be used for thermal criterion discussion, conservatism is desired for fish protection. Also it makes for easier comparison with existing data on rainbow trout. Secondly, collecting fish from cooler areas minimized our chances of capture-related mortalities (due to rapid temperature change during capture, release, or transport) during the peak of summer. This was an unnecessary risk that we thought best to avoid so that our study would not be shut down early. Our experiment showed that there is a wide range of temperatures where Tuolumne River *O. mykiss* have the

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		aerobic scope to live and thrive. *O. mykiss* are found in the Tuolumne River at the range of water temperatures tested in our experiment. The distribution of *O. mykiss* within the Tuolumne River is affected by numerous factors only one of which is temperature.
CDFW-22 (p. 10)	Experimental Protocols, page 11, last paragraph. The authors state, "Water velocity was then increased in increments of 3 to 6 cm s^{-1} every 20 min until the fish failed to swim continuously". Is this an acceptable fisheries technique to allow an animal to work to the point of complete exhaustion? Would it be better to do a timed test by stopping the test before the fish is completely exhausted?	This experimental design has been used in numerous studies and approved by university research protocols. Please refer to the clarifying document and the cited special issue on methodology. Importantly, this comment reveals a fundamental misunderstanding related to why we are using swimming tests and exhaustion. To properly measure AAS, we need a method to estimate MMR and swimming to exhaustion happens to be one way to do this. Swimming fish for an extended period of time and a submaximal swimming velocity would not elicit maximum metabolic rates. A human example might help. Would a jogger out for a 20 min casual, timed run reach MMR? No. Would a runner chased to exhaustion exhibit MMR near the endpoint, yes. Even the cited and supported work by Clark et al. 2013 uses this methodology and Clark and Norin, 2016 used the approach used by us. It seems that the reviewer is adopting double standards.
CDFW-23 (p. 10)	Experimental Protocols, page 12, third paragraph. The authors state, "Approximately 50% of the wild fish did not respond to the critical swimming velocity protocol but instead used their caudal fin to prop themselves on the downstream screen to avoid swimming". Is this a sign the fish were already stressed before the	It is unclear what the reviewer considers stress. See previous comments regarding the pitfalls of using this term casually. This was not a sign of 'stress' and there was no discernable pattern between tail propping with test temperature. Making the speculation that this behavior had something to do with

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	experimentation started and possibly a result of too warm temperatures to begin with?	elevated test temperatures is incorrect. It is common for some fish to be 'non participants' in tests like these. We see this in all species that I'm familiar with and the literature provides many examples. These fish are wild and a swim tunnel setting is novel. Some individuals require different motivations (stepwise velocity increases, bursting velocities etc.) to help to orient them to the current and elicit sustained swimming. In salmonids, tail propping is often seen at low velocities to save energy rather than swimming, but as velocities continue to increase, salmon will then begin to swim. Once they start swimming, they almost always perform to exhaustion. This fish are built for continuous swimming.
CDFW-24 (p. 10)	Data Quality Control, Model Selection and Analyses, page 13, last paragraph. The authors state, "Routine metabolic rate quality control (QC) was performed by visually inspecting over night [sic] video recordings for fish activity" and that "data from any fish showing consistent activity over night [sic] was discarded". Why were the data discarded? Was the fish activity a sign of stress before the experiments started? In addition the authors state, "For fish exhibiting intense agitation, the swimming MMR was used as overall MMR." Four of these 'non-agitated' fish (W2, W13, W14, and W15) were disgarded due to failure of MR to increase incrementally; despite continuous station-holding swimming with tunnel velocity increases of more than 15 cm s^{-1}". Were these fish already stressed? How does inclusion of these data influence study results? It is important that data not be "selected" in order to bias study results. Scientific integrity requires that data not	It is unclear what the reviewer considers stress. See previous comments regarding the pitfalls of using this term casually. Please review our definition of RMR and reread comments on the effect of stress on RMR and AAS in the previous comments. It is clear that metabolic measure of active fish is NOT the physiological state representative of RMR. Active fish have metabolic rates somewhere between RMR and MMR and are not we were measuring in order to calculate AAS. We are keenly aware of the fact that rigorous science includes all valid data points and rigorous scientists do not 'throw out' valid data. All of our methods are consistent with published methods, see the previously referenced special issue of Journal of Fish Biology. Our rationale is clear and justified and supported by the scientific (published) literature, which we've cited. The insinuation that there is even a hint of a

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	be thrown out for invalid reasons, including if the results cannot be explained or if they are different than expected.	scientific integrity issue of the co-authors of this study is completely out of line, inappropriate, and disappointing. Certainly, there is a less combative way to query the inclusion or exclusion of specific data points.
CDFW-25 (pp. 10 and 11)	Results, Page 15, Number 1, third paragraph, second sentence. The authors state, "They state that Routine Metabolic Rate (RMR) should increase exponentially until the test temperature approaches the upper thermal tolerance limit for *O. mykiss*, which according to published CT_{mas} values is 26°C to 32°C (see Table 1)". Who is "they"? If "they" is the USEPA, this is an incorrect statement because the USEPA did not include RMR studies in their review. Myrick and Cech's Table 1 had significant less food consumption and decreased growth rates and increased mortality in their 25°C test fish compared to their 10°C, 14°C, and 19°C exposed fish. In their Table 2 results, fish consumed significantly less oxygen at 25°C compared to fish exposed to 10°C, 14 °C, and 19°C. *O. mykiss* can survive in acute warm temperatures as demonstrated by the authors, but cold water fish still need cold water refugia sometime during the day. According to Myrick and Cech (2000) there is very little thermal difference between fish stocks per their comparison of other research studies (see Table 5) under similar experimental conditions.	"They" is referring to the predictions not to any group or report. The sentence should have started with "These predictions" not "They".
CDFW-26 (p. 11)	Results, page 15, Number 2. The authors state, "These results for MMR are inconsistent with our prediction #2 derived from EPA (2003) criteria where MMR was	Chronic/acute criteria are dealt with in CDFW-1 and in the clarifying document.

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	expected to peak near to 18°C". This statement is irrelevant because the authors are comparing chronic population criteria to acute individual results. Again, chronic and population thresholds are always less than acute and individual thresholds.	
CDFW-27 (pp. 11 and 12)	Results, page 16, Number 3, third paragraph. The authors state, "These results for AAS are inconsistent with our prediction #3 based on EPA (2003) criteria, but are consistent with our alternative prediction #3 that the Tuolumne River population of *O. mykiss* is locally adjusted by having T_{opt} for AAS that is greater than 18°C i.e., 21.2°C." This statement is irrelevant because the authors are comparing a chronic population criterion to acute individual results. Again, chronic and population thresholds are always less than acute and individual thresholds.	Chronic/acute criteria are dealt with in CDFW-1 and in the clarifying document.
CDFW-28 (p. 12)	Results, page 16, Number 4, last sentence. The authors state, "The numerical 95% peak AAS could be maintained from 17.8°C to 24.6°C, which is a more conservative thermal range for T_{opt}". However, based on the authors results, and because rainbow trout are a cold water fish, a true conservative thermal range would be from 16.4°C to 17.8°C.	How does this reviewer know what is "true" for Tuolumne River *O. mykiss*? The 16.4-17.8 °C range is more conservative but not necessarily the "true conservative thermal range". Why not pick, 17.8 to 17.9 to be more conservative still. Such selection is arbitrary and has no statistical basis. Statistically AAS does not change over the range of temperatures stated. Therefore we adopt a scientific rigor that the reviewer does not appear to appreciate. Furthermore, the reviewer has repeatedly ignored the results of our tests of Tuolumne River *O. mykiss* and based his whole review on the assumption that our study fish are no different from other *O. mykiss* populations.

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		Again the reviewer ignores the data by calling this population a cold-water species when data show that they perform equally well at temperatures in excess of 20°C. It's also important to mention that 'coldwater fish' is not at all a precise term. What is cold, what is warm? Modern thermal ecology literature suggests that some Om populations are simply not as 'cold water' as previously thought.
CDFW-29 (p. 12)	Results, pages 16 to 17, Number 5. Same comment as for comparing an acute stress test results to a chronic population criterion. The author state, "Indeed, all individual fish tested up to 23°C has a FAS [Factorial Aerobic Scope] value >2, with only 4 out of 14 fish tested at 23°C, 24°C, and 25°C having a FAS value <2." A chronic population threshold is formulated to protect the weakest individuals in a population, so by using a lower criterion these 4 weaker fish should have better physiological function and survival.	Chronic/acute criteria are dealt with in CDFW-1 and in the clarifying document.
CDFW-30 (p. 12)	Results, page 17, Number 7. The authors state, "Two fish tested at 25°C regurgitated rather large meals of aquatic invertebrates during the recovery from the swim test, and one of these fish died abruptly during the recovery period". Since, fish were exposed to an exhaustive state, this causes us to question whether or not this an appropriate testing technique where the test has to force an animal to complete exhaustion, especially for a group of fish that may be already stressed due to having to live in environmental conditions of altered flows and habitats that they did not evolve with.	Same comment on casual use of 'stress' and 'stressed out'. What, scientifically, do these qualitative judgment statements mean? They have no value. We have already explained above why we use exhaustion to elicit MMR. We either measure it or we do not. We are dealing with wild fish and cannot hold them outside of the river to ensure a full post-prandial state. We simply cannot believe that the reviewer holds the following premise "a group of fish that may be already stressed due to having to live in environmental conditions of altered flows and habitats that they did not evolve with." How on earth would the reviewer reach such a speculative

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		conclusion without data to back it up? The amount of supposition and lack of scientific data to back such assertions is a total shock.
CDFW-31 (p. 12)	Discussion, Data Quality, page 18, first paragraph. This section provides a brief summary of the results and comparison to other aerobic studies. The Department completed an analysis of variance as presented in the following Table 1 using the Study's data presented in Appendix 4. Temperatures at and below 18°C were significantly different for RMR, MMR, and FAS compared to the highest temperatures at and above 22°C. For RMR, which is a fish at rest, is this an indication the fish at the warmer temperatures were already stressed before the experiment started?	It is simply not possible to respond to a comment about a re-analysis of the data when no statistical details are given. The statistical test, how the treatment groups were defined, n values, degrees of freedom, alpha level, and p-values are all missing. Note: imprecise use of stress and lack of understanding of the simple effects of temperature on biological rates are repeated once more.
CDFW-32 (p. 13)	There is an inverse relationship between water temperature and oxygen concentration. As temperature increases, oxygen decreases. As such, at the warmer temperature with less oxygen, are the fish stressed to the point they are hyperventilating, thus increasing their metabolism trying to pull in as much oxygen as possible from a low oxygen environment.	The movement of oxygen from water into the blood of a fish is governed by the partial pressure of oxygen in the water and not by oxygen concentration. Therefore the reviewer does not understand the basic principles of oxygen movement into fish by suggesting that the decrease in oxygen concentration with temperature triggers hyperventilation. Fish ventilation responds to oxygen partial pressure not concentration in the water. If the reviewer thinks otherwise they have been misled. Furthermore, oxygen concentration decreased by only 10% per 10°C. Therefore the decrease is around 15% for the entire range of test temperatures. Despite this minor change, the fish increase MMR and maintain AAS, so this is a non-issue. The idea that these fish are stressed has been repeatedly dealt with above.

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CDFW-33 (pp. 13 and 14)	The Department also graphed the mean results for each temperature as presented in Appendix 4. Note at 22°C for RMR, AAS, and FAS and at 21°C for MMR there is a sudden change in the slope of the graph. Does this change in slope indicate there is a sudden change in the physiological function of the fish and a clinical sign that the fish are highly stressed? A highly stressed animal is considered to be in poor condition.	Our response to CDFW-31 applies here too. It is simply not possible to respond to a graph that we do not have. The equation, the R2 and p value are needed. We are not sure of what clinical sign that the reviewer refers to.
CDFW-34 (p. 14)	Discussion, Data Quality, page 19, Protocol Number 2. The authors state, "2 a combination of continuous swimming and short velocity bursts to push fish off of the downstream screen". Was this an indication the fish was already tired and stressed at the beginning of the experiment?	Of course a fish is tired when it is exhausted. That is why it has reach MMR and we stop the experiment. The fish nevertheless were observed to recover quickly.
CDFW-35 (p. 15)	Evidence for Local Thermal Adjustment, page 20, first paragraph. The authors state, "Our predictions based on EPA (2003), as listed above, assumed that the Tuolumne River *O. mykiss* population would perform similarly to Pacific Northwest *O. mykiss* populations used to set the 7DADM by USEPA (2003)". The predictions based on USEPA are irrelevant because the USEPA did not perform tunnel stress techniques or use such data to develop their chronic population criteria recommendations. The authors recommend using 21.2°C rather than 18°C, but they are comparing an acute/individual result to a chronic/population recommendation. Have the authors considered other techniques to determine what cold water fish, such as *O. mykiss* can chronically sustain normal/optimal physiological function, including immune function, reproductive success and recruitment, at their	Please see the clarifying document at the beginning of this attachment where we reiterate what our study results reveal and should be used for. That the reviewer suggests "The authors mention these test fish have a wide optimal thermal performance range, but this is true for all living organisms;" suggest a complete lack of understanding of thermal biology. Some Antarctic ice fish die at about 4°C.

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	recommended temperature of 21.2°C? It is well understood that cold water fish can simply survive at warmer temperatures to a point, but what about their entire life cycle needs at the individual and population levels? The authors mention these test fish have a wide optimal thermal performance range, but this is true for all living organisms; what do the authors consider "optimal"?	
CDFW-36 (p. 15)	Evidence for Local Thermal Adjustment, page 20, first paragraph, last sentence. The authors also state, "However, given that the CT_{max} could not be determined in the present work and that MMR increased up to the highest test temperature (25°C), it was impossible to determine the upper thermal limit when MMR collapses, which means that alternate metrics must be used to set the upper thermal limit for the Tuolumne River *O. mykiss* population". Since the "upper thermal limit" is survival based, can the author's present reproductive success and recruitment base criteria with this type of testing?	Reproductive success and recruitment were not part of this study's purpose.
CDFW-37 (p. 15)	Evidence for Local Thermal Adjustment, page 20, second paragraph. The authors state, "The present work provides three useful metrics of the optimal temperature range". What is meant by "optimal temperature range"? T_{opt} appears to be more of a temperature maximum (T_{max}) than a T_{opt}. A temperature maximum does not necessarily mean it is an optimal temperature. Fry (1947) page 56, Figure 27, does not state the peak of activity as optimal, but refers to the "potential range of activity" and the "scope for activity". Fry further reduces the area of the activity curve by discussing	Please see response to CDFW-15. The thermal performance literature has evolved from the fundamental work of Fry to appreciate that there are many forms of thermal performance curves (not a static performance curve, incorporating all metrics for a particular species) like CDFW reviewer is presenting. These curves are shaped by many factors, including the 5 (or 6 depending on the citation) classifications of factors that Fry delineated in his work. They are also shaped by timescale (acute, acclimatory, adaptive), the fish's life history, the fish's

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	"controlling factors". The USEPA (2001) as presented below provides a number of "controlling factors". Did the authors for this study consider controlling factors as described by Fry to adjust their activity curve?	evolutionary thermal history, etc. We do not disagree with the reviewer on the importance of these aspects and nowhere do we discount any of these details. It is interesting that Fry 1947, while an absolutely critical piece of early work, is also not augmented by the reviewer with more recent literature on thermal performance curves, their utility, and their interpretations. We cite several. Please revisit the clarifying document where we clearly articulate how our results articulate with the 7DADM.
CDFW-38 (p. 16)	Evidence for Local Thermal Adjustment, page 20, last paragraph, first sentence. The authors state, "Yet, there were important indications that a small percentage of individuals were taxed at 23-25°C by the thermal testing and intensive swim imposed on them outside of their normal habitat over a 24-h period." In the fourth sentence they further state, "In the present study, the telltale signs were that 4 of 13 individuals [31 %] tested at 23-25°C had a FAS <2." This supports the concept that a chronic population base threshold is to protect the weakest individuals in a population and cannot be formulated by using just one simple acute stress test.	See explanation above, regarding that fact that our experiment was measuring the optimal temperature range for Tuolumne River *O. mykiss*, not acute CTmax temperatures for these fish. It is incorrect for the reviewer to repeatedly call our test an 'acute stress test' for many reasons. As stated above, the optimal temperature range for Tuolumne River *O. mykiss* suggested by our swim tunnel tests is lower than the temperatures at which 4 fish had FAS values less than 2.
CDFW-39 (p. 16)	Evidence for Local Thermal Adjustment, pages 21 to 22, top line. In the same paragraph, the authors state, "Lastly the only fish mortality occurred in the recovery period (a phenomenon known as 'delayed mortality') after one fish was tested at 25°C". What is the point of mentioning 'delayed mortality'? The end result is one of four fish (25%) died at the highest temperature when forced to swim until completely exhausted.	"Delayed mortality" is just a term used to describe this category of mortality. Certainly, given the repeated interest in the duration of thermal exposure by the reviewer, the importance of expressing whether the death was immediate in response to an acute 25°C exposure or if the response was the result of a more prolonged high temperature exposure can be appreciated.

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CDFW-40 (pp. 16 and 17)	Ecological Relevance of the Present Findings, page 21, third paragraph. The authors state, "MMR increased with temperature from 13 to 25°C, which would mean that as fish encounter higher temperatures, they have the capacity to perform an activity at a higher absolute rate, i.e., swim faster to capture food or avoid predators, digest meals faster, detoxify chemicals faster, etc.". Are the authors saying rainbow trout are better off at 25°C instead of <19°C? Their interpretation does not make any sense. We agree a fish will have burst of energy no matter what the temperature, however, the question remains how long can they maintain this energy consumption under chronic warm temperatures at 21.2°C? It takes energy to reproduce, how does exposure to chronic warm temperature impact reproduction success and recruitment into the population? Clark et al. (2013), page 2779, stated that there is a range of optimal temperatures for different processes and life histories and these optimal temperatures are different from T_{optAS}. They used an example for adult pink salmon where a T_{optAS} is at 21°C, but if reproduction occurred at 21°C would fail because the optimal temperature for spawning is <14°C. They further stated on page 2780, that fish have different physiological functions at different optimal temperatures as presented in their Figure 7B.	No, we are saying that *O. mykiss* have greater aerobic scope at 21.2°C than at the other temperatures tested. Therefore, they would be better off from a physiological energy perspective at temperatures near 21.2°C than the other temperatures tested. Some of the fish swam for multiple hours at a high rate at 21.2°C and higher temperatures. Please review the clarifying document for our perspectives about how our data do and do not address the 7DADM EPA criteria and how our data relate to energy allocation. Please also note: The report discussion is not intended as the basis for changing the EPA (2003) 7DADM recommendations. Instead, our work simply suggests that the current value of 18°C lacks merit for the current *O. mykiss* population found in the lower Tuolumne River. Minimally, 18°C as the 7DADM value is a very conservative upper thermal limit based on the results of the current study. The reviewer indicates that he does not think our interpretation makes sense. However, it is equally possible that our interpretations do make sense and we have either not communicated them simply and clearly enough or the reviewer is not understanding the study goals and interpretations. We have tried very hard to restate how are data are or are not relevant to the comments from CDFW.
CDFW-41 (p. 17)	Ecological Relevance of the Present Findings, page 22, first paragraph, third sentence. The authors state "As a result of high temperature, a fish would digest the same	The commenter's statement regarding the relationship between digestion rate and temperature is based on basic physiology of fishes and reveals a fundamental lack of

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	meal with a similar overall oxygen cost but at a faster rate". This study did not measure how fast fish can digest their food at increasing water temperatures, therefore this statement stating that a fish would digest their food at a faster rate at higher temperatures is an assumption based on speculation. As the authors discussed, this study design measured oxygen demand to demonstrate fish have extra burst energy from a resting state to seek and catch food, but does not include measuring the rate of digestion. All animals digest their food during the resting state, otherwise their digestive tract would cramp-up during high activity.	understanding of the effects of temperature on another physiological rate process in fishes – digestion. (Please read the works of Jobling in the 1980-90's and Fu in 2000's. Indeed ecologists have postulated that some fishes actively feed in very warm areas so they can digest more!) We did not study nor use the terminology 'extra burst energy' as suggested by the reviewer. These are the reviewer's words and should not be mistaken for ours. We also note that the last sentence is a bit misapplied in using anthropomorphic words like 'cramp up' in an attempt to describe some sort of physiological relationship or process. Regarding the comment that "[a]ll animals digest their food during the resting state", we assume the reviewer is familiar with the behaviors of pelagic fish. Many swim continuously and eat and swim at the same time. Indeed, filter feeders must swim to feed. We note that the reviewer did not have any comment on the middle paragraph on page 22 of our report, where we provided clear evidence of *O. mykiss* maintaining their station in the Tuolumne River at 20°C where their metabolic rate (derived from tail beat frequency) was twice their RMR but substantially below their MMR at that temperature. The commenter is disregarding data that we provide while preferring to offer spurious speculation.
CDFW-42 (p. 17)	Ecological Relevance of the Present Findings, page 22, last paragraph. The authors state "Here we did not evaluate the possibility that the Tuolumne River *O. mykiss* population can thermally acclimate to warmer river temperatures as the summer progresses, due to the	This comment reveals fundamentally incorrect ideas and misunderstandings about what acute versus acclimatory processes are and what time scales are relevant. We kindly ask the reviewer to review the various report documents noting the definitions of these terms. Please also review the

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	available sample of a maximum of 50 individuals and their habitat temperature." Actually the authors did evaluate if Tuolumne River trout can acclimate, because this was an acute stress test designed for that purpose. Up to a limit, animals can acclimate to an acute environmental change, but how do these animals reproduce successfully under chronic environmental changes such as migratory routes being blocked and under different water flow regimes that they did not evolve with?	study design. To evaluate thermal acclimation experimentally, you must control and/or measure the fish's thermal acclimation history so that it is known. With those data in hand you can then attribute results to the effect of acclimation. Our fish were wild-caught and acclimatized to the river where thermal history was not measured. Thus, we did not test acclimation. The reviewer comments here regarding 'acute' are not relevant and the later part of the last sentence regarding migration routes and flows are also not relevant. If permitting had allowed us to keep each fish out of the river for 4 weeks, we would have measured acclimation. So the experiments could be performed with our mobile physiology lab, but was not tested because of the available permit.
CDFW-43 (p. 17)	Conclusions, page 24. As previously stated, the USEPA did not use acute tunnel stress test to evaluate a chronic population criterion. They included a number of factors as part of their evaluation. It is inappropriate to compare results from an acute individual test to a chronic population threshold. Since *O. mykiss* are a cold water fish, it would be more appropriate and conservative to use their lower range results (16.4 and 17.8°C) to protect this fish, particularly where reproduction success appears to be low because the population has been declining for decades since the dams were constructed (Yoshiyama et al., 2001).	See explanation above, regarding that fact that our experiment was measuring the optimal temperature range for Tuolumne River *O. mykiss*, not acute CTmax temperatures for these fish. See response to CDFW-1 specific to the repetitive comment regarding chronic/acute criteria. It is nice to see the reviewer finally acknowledge here that the 7DADM incorporates several types of thermal performance data in setting the criteria. One of the strengths of our study is that we have used a state-of-the-art approach (i.e. AAS) to contribute to this understanding. We have clarified how our data relate to the 7DADM in the clarifying document.

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		Note that we do not think it to be appropriate to casually call *O. mykiss* cold water fish without using more precise and descriptive terminology. The literature has expanded over the last 10-20 years to show that at least some populations of *O. mykiss*, including those in this study, may not all be equally 'cold-water' as previously grouped.
CDFW-44 (p. 18)	Figures, page 33, Figure 1. The T_{opt} appears to be an acute maximum temperature at the peak of maximum oxygen consumption and not necessarily an optimal temperature. From the peak temperature and higher, oxygen consumption decreases, suggesting the fish is exhausted and no longer capable of absorbing oxygen similarly to hyperventilation. See comment above for Page 20, second paragraph.	Addressed above.
CDFW-45 (p. 18)	Figures, page 37, Figure 4. See comment above for Page 20 second paragraph. Per Fry (1947) page 56, Figure 27, did the authors for this study consider controlling factors to adjust their activity curve? For the Factorial Aerobic Scope curve, the peak is approximately 13°C and decreases as temperatures increases. Clark et al. (2013) Figure 6, page 2778, presents a similar Factorial Aerobic Scope curve where the T_{optAS} is at the peak of the curve representing the lowest temperature at 11°C. Using Clark et al. (2013) concept, the authors Figure 4 peak at 13°C should be considered the T_{opt} for Tuolumne River rainbow trout, not the maximum temperatures.	Addressed above.
CDFW-46 (p. 18)	Figures, page 44, Appendix 1. Were *O. mykiss* observed, or attempts made to capture fish, between River Mile 39.5 (permit limit location) and River Mile 49? River water temperatures were above 18°C, so it	Not during the 2014 study but *O. mykiss* were observed in the Tuolumne River below river mile 49 in 2015 and in other years.

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	would be worthwhile information to know if a healthy number of rainbow trout occupied this area. River Mile 48 appears to be below the 21.1°C permit requirement.	
CDFW-47 (p. 18)	Figures, page 47, Appendix 2. Fish W43 died. Did this fish die from delayed Capture Myopathy as a result of handling and exposure to high temperatures? Capture Myopathy results in the death of a captured wild animal during or after the animal has been captured and released.	The fish that died after the swim tunnel test was one of two fish that regurgitated their stomach contents after being tested at 25°C. We don't know the reason for this mortality but it was likely associated with the excess metabolic demand resulting from digesting a full stomach of food combined with the test used to determine MMR using the swim tunnel. The other fish that regurgitated their stomach contents after the swim tunnel test did not die.
CDFW-48 (p. 18)	Figures, page 49, Appendix 4. All the data should be included for peer review, particularly for the fish that were discarded. What would the analysis look like if the discarded fish data was included? According to the Quality Control column the discarded fish were removed because of activity during RMR or no MR increase. Does this indicate the fish were already stressed? Which fish died?	Addressed above. We followed state-of-the-art peer reviewed methods, and will not comment further on the reviewer's arbitrary assignment of the descriptor 'stressed' to any of our study animals without any supporting data.
CDFW-49 (p. 18)	Figures, page 50. Four of 14 fish tested at 23°C, 24°C and 25°C had a FAS< 2. These results of less than 2 at the highest test temperatures indicate these fish were highly stressed at these temperatures.	Addressed above.

THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

APPENDIX 7

HIGH THERMAL TOLERANCE OF A RAINBOW TROUT POPULATION NEAR ITS SOUTHERN RANGE LIMIT SUGGESTS LOCAL THERMAL ADJUSTMENT

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Research article

High thermal tolerance of a rainbow trout population near its southern range limit suggests local thermal adjustment

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Transformation of earth's ecosystems by anthropogenic climate change is predicted for the 21st century. In many regions, the associated increase in environmental temperatures and reduced precipitation will have direct effects on the physiological performance of terrestrial and aquatic ectotherms and have already threatened fish biodiversity and important fisheries. The threat of elevated environmental temperatures is particularly salient for members of the *Oncorhynchus* genus living in California, which is the southern limit of their range. Here, we report the first assessments of the aerobic capacity of a Californian population of wild *Oncorhynchus mykiss* Walbaum in relationship to water temperature. Our field measurements revealed that wild *O. mykiss* from the lower Tuolumne River, California maintained 95% of their peak aerobic scope across an impressive temperature range (17.8–24.6°C). The thermal range for peak performance corresponds to local high river temperatures, but represents an unusually high temperature tolerance compared with conspecifics and congeneric species from northern latitudes. This high thermal tolerance suggests that *O. mykiss* at the southern limit of their indigenous distribution may be locally adjusted relative to more northern populations. From fisheries management and conservation perspectives, these findings challenge the use of a single thermal criterion to regulate the habitat of the *O. mykiss* species along the entirety of its distribution range.

Key words: aerobic scope, fish, metabolic rate, *Oncorhynchus mykiss*, swimming, temperature

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Introduction

Rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) is regarded as a cold-water fish species with an indigenous range stretching across an immense temperature gradient, from the subarctic climate region of the Bering Sea to the Mediterranean climate region of Northern Baja California (Reyes, 2008). Despite this large temperature gradient and distribution range, the optimal temperature range for wild *O. mykiss* aerobic performance capacity has been determined only for indigenous populations inhabiting temperate climates. Local adaptation of thermal performance exists within the teleosts (Angilletta, 2009), but has never been shown for wild *O. mykiss* populations across their native range. Without knowledge of the variation in thermal performance among populations of *O. mykiss*, fish conservation managers apply regulatory water temperature criteria derived for higher latitude populations of O. *mykiss* for protection of lower latitude populations.

The present study considered the thermal performance of a population of O. *mykiss* located in a river near the southern limits of its native range and was prompted by a number of recent events. Foremost, global indicators show that 2014 and 2015 were the warmest years on record for the earth's climate (Blunden and Arndt, 2015; NOAA National Centers for Environmental Information, 2016). Animal populations, such as Californian O. *mykiss*, which exist at the latitudinal extremes of their biogeographical range, are expected to experience the most profound negative effects of such climate changes (Lassalle and Rochard, 2009). Second, for a fish that tends to favour pristine, cold water in most of its native habitat, native O. *mykiss* populations inhabiting the extremely warm summer temperatures of Californian rivers are evidence of considerable phenotypic plasticity (or genetic variability) within the species, allowing acclimation (or adaptation) to much warmer environmental temperature regimes. Indeed, severe thermal exposures in southern Western Australia have produced a line of introduced, hatchery-reared O. *mykiss* (Morrissy, 1973; Molony, 2001; Molony *et al.*, 2004) that swim and feed at 26°C (Michael Snow, Department of Fisheries, Government of Western Australia, personal communication) and retain 50% of their peak aerobic capacity at 25°C (Chen *et al.*, 2015). Interestingly, the founder population for this thermally tolerant hatchery strain was transplanted from California during the last century for recreational fisheries. Thus, with climate change continuing to shift baseline river water quality and availability (Sousa *et al.*, 2011; Swain *et al.*, 2014), especially in central California, where the intensification of weather extremes is triggering water crises and extreme droughts (Dettinger and Cayan, 2014), knowledge of the local thermal requirements of vulnerable key fish species becomes ever more pressing (Moyle *et al.*, 2011).

Fish can adjust to warmer habitat temperatures by relocating to a cooler refuge (if available), thermally acclimating or thermally adapting (Farrell and Franklin, 2016); responses that all operate at different time scales. Indeed, the suggestion that fish might tailor their metabolic rate to habitat temperature has a long and strong history across a wide range of aquatic habitats and species (Fry, 1947, 1971; Brett and Groves, 1979; Elliott, 1982; Jobling, 1994; Hochachka and Somero, 2002; Donelson *et al.*, 2012). In fact, local thermal adaptation has been thoroughly characterized for other fish species, such as stickleback populations (Barrett *et al.*, 2011), temperate killifish (Fangue *et al.*, 2006) and tropical killifish (McKenzie *et al.*, 2013). Even within the genus *Oncorhynchus*, Fraser River watershed populations of sockeye salmon (O. *nerka* Walbaum 1792) have apparently tuned their thermal performance to meet the energetic needs of their once-in-a-lifetime upstream migration (Farrell *et al.*, 2008; Eliason *et al.*, 2011, 2013). The ability of O. *mykiss* to acclimate thermally is well documented (Myrick and Cech, 2000), and there appears to be the genetic potential for thermal adaptation given the successful selective breeding of O. *mykiss* lines that perform well at high temperatures (Australian lines, Molony *et al.*, 2004; Japanese lines, Ineno *et al.*, 2005). Nevertheless, assessments of the aerobic capacity in relation to water temperature of wild O. *mykiss* at the southern extent of their range in California are lacking. What is known for two Californian strains of O. *mykiss* (Eagle Lake and Mount Shasta; Myrick and Cech, 2000) is that the thermal performance curves for hatching success differ (Myrick and Cech, 2001) despite similar upper thermal tolerance values (CTmax). In addition, the Eagle Lake and Mount Shasta strains of O. *mykiss* grew fastest at different acclimation temperatures (19 and 22°C, respectively), but growth ceased at 25°C in both strains (Myrick and Cech, 2000).

The accumulating evidence for variation in thermal performance within and among Pacific salmon and rainbow trout populations seems incongruous with the criteria used by the US Environmental Protection Agency (EPA) to regulate water temperatures. The EPA uses a regulatory 7 day average of the daily water temperature maximum (7DADM) of 18°C for all juvenile O. *mykiss* over their entire native US range from southern California into Alaska (US Environmental Protection Agency, 2003). One way to bring greater insight into population-specific thermal tolerance and to take local adaptation and acclimation into consideration for regulatory purposes is to use a well-established non-lethal approach to study the thermal physiology of O. *mykiss* populations inhabiting unusually warm habitats. Therefore, we examined O. *mykiss* that inhabit the Tuolumne River below La Grange Diversion Dam, which is the most downstream habitat for O. *mykiss* in a watershed that drains ~2500 km^2 of the Western Sierra Nevada mountain range. This river reach is characterized by a longitudinal thermal gradient, which increases from 12°C to occasionally as high as 26°C during summer warming over a ~25 km stretch of river. By measuring metabolic scope for activity (Fry, 1947), we tested the hypothesis that O. *mykiss* residing below the La Grange Diversion Dam on the Tuolumne River may be locally adapted to the summer habitat temperatures that can reach 26°C. Mechanistically, our experimental approach builds on a fish's ultimate requirement to have the capacity to supply oxygen for all activities (e.g. for foraging, digestion, growth, migration, predator avoidance and reproduction). The capacity to provide oxygen beyond basic needs is termed absolute aerobic scope (AAS), which, in field situations (e.g. Pörtner and Knust, 2007; Nilsson *et al.*, 2009; Gardiner *et al.*, 2010; Eliason *et al.*, 2011; Rummer *et al.*, 2014), can be estimated from the difference between routine metabolic rate (RMR) and maximal metabolic rate (MMR). Thus, by measuring RMR and MMR over a wide range of water temperatures, the portion of the temperature range where AAS (i.e. the capacity for aerobic activity) is maximized can be defined. Such information is lacking for wild O. *mykiss* in central California. For the present study, a temporary respirometry laboratory was built beside the Tuolumne River. This laboratory allowed wild juvenile

O. mykiss to be tested at temperatures between 13 and 25°C before they were returned to their original habitat within 24 h, as required by the experimental permits.

This study has implications beyond the thermal needs for resident aquatic species because this segment of the Tuolumne River is part of a watershed that provides municipal water to >2.4 million residents of the San Francisco Bay Area and agricultural irrigation water to the Central Valley (Turlock Irrigation District and Modesto Irrigation District, 2011). The recent drought in central California has left reservoirs at historic lows (California Department of Water Resources, 2015) and has challenged the capacity to balance the environmental water flow needs of aquatic biota with the human requirements from this watershed for domestic, agricultural and recreational use. Juvenile *O. mykiss* living below the La Grange Diversion Dam have been observed exploiting summer Tuolumne River temperatures from 12 to 26°C over 25 river km (HDR Engineering, Inc., 2014). There are no additional cool-water inputs (except for rare summer rains), resulting in progressive warming of the water released from the Dam as it flows downstream. Establishing the optimal temperature range for aerobic performance of wild Californian *O. mykiss* will provide fish conservation managers with scientific support for temperature criteria that allow for optimization of this balance between human and fish requirements.

Materials and methods

Permitting restrictions that influenced the experimental design

Wild Tuolumne River *O. mykiss* were collected under National Marine Fisheries Service Section 10 permit no. 17913 and California Fish and Wildlife Scientific Collecting Permit Amendments. No distinction was made between resident (rainbow trout) and anadromous (steelhead) life-history forms. For permitting purposes, these fish are considered as ESA-listed California Central Valley steelhead, *O. mykiss*. Fish collection (up to a maximum of 50 individuals) was allowed only between river kilometer (RK) 84.0 and RK 63.6, and capture temperatures could not exceed 21.1°C. This permit allowed only two fish to be captured and tested each day, and all fish had to be returned to their original river habitat. Given that indirect fish mortality was limited to three fish, a precautionary measure included testing fish at the highest temperatures last (i.e. not randomly assigning test temperature). Additionally, the permit restricted test temperatures to ≤25°C. All experimental procedures were approved by the Institutional Animal Care and Use Committee (protocol no. 18196; the University of California Davis).

Fish collection, transport and holding

Two wild *O. mykiss* were collected daily [a total of 44 fish; 22.4 g (SEM = 1.78, range 10.5–79.6 g) and 125.7 mm (SEM = 2.88)] from four primary locations on the Tuolumne River (Supplementary material, Fig. S1). The two fish were immediately scanned for a passive integrated transponder (PIT) tag to preclude re-testing a fish. The fish were transferred directly to a 13 litre container partly submerged in the river before being driven to the streamside field laboratory (<20 min) in insulated coolers filled with 25 litres of fresh river water. A water temperature logger (recording every 15 min; Onset Computer Corporation, USA) remained with the fish until testing was completed and the fish was returned to the river. At the field laboratory, located immediately downstream from the La Grange Diversion Dam, fish were transferred to holding tanks (300 litres) filled with flow-through Tuolumne River water (directly from the dam) that had passed through a coarse foam filter and then an 18 litre gas-equilibration column for aeration (12.5–13.6°C, >80% air saturation). Thus, field-acclimatized fish were placed into the holding tanks within 60–120 min of capture and remained there for 60–180 min before being transferred to one of two 5 litre automated swim tunnel respirometers (Loligo, Denmark). Routine and maximal metabolic rates were then measured at temperatures between 13 and 25°C (1°C increments).

Swim tunnel respirometers

The swim tunnel respirometers received aerated Tuolumne River water from an 80 litre temperature-controlled sump that was refreshed every 80–90 min. Water temperature was regulated within ±0.5°C of the test temperature by passing sump water through a 9500 BTU Heat Pump (Model DSHP-7, Aqua Logic Delta Star, USA) with a high-volume pump (model SHE1.7, Sweetwater®, USA). Additionally, two proportional temperature controllers (model 72, YSI, USA) each ran an 800 W titanium heater (model TH-0800, Finnex, USA) located in the sump. The water temperature in the swim tunnels was monitored with a temperature probe connected through a four-channel Witrox oxygen meter (Loligo). All temperature-measuring devices were calibrated bi-weekly to ±0.1°C of a National Institute of Standards and Technology certified glass thermometer. Ammonia build-up was prevented by zeolite in the sump, which was replaced weekly. Water oxygen saturation in each swim tunnel was monitored continuously using a dipping probe mini oxygen sensor connected to AutoResp software (Loligo) through the Witrox system (Loligo). Video cameras with infrared lighting (Q-See, QSC1352W, China) continuously recorded (Panasonic HDMI DVD-R, DMR-EA18K, Japan) fish behaviour in the swim tunnels, which were shaded by black cloth to limit fish disturbance. A variable frequency drive motor generated laminar water flow through the swimming section (calibrated using a digital anemometer with a 30 mm vane wheel flow probe; Hönzsch, Germany) in each swim tunnel.

Metabolic rate measurement

Routine and active metabolic rates of fish in the swim tunnel respirometers were measured using intermittent respirometry

(Steffensen, 1989; Cech, 1990; Chabot *et al.*, 2016; Svendsen *et al.*, 2016). The swim tunnel was automatically sealed during measurements and flushed with fresh, aerated sump water between measurements (AutoResp software and a DAQ-PAC-WF4 automated respirometry system, Loligo). Oxygen removal from the water by the fish (in milligrams of oxygen) was measured for a minimal period of 2 min when the swim tunnel was sealed, without oxygen levels falling below 80% air saturation. No background oxygen consumption was detected without fish (performed at the end of each day with both swim tunnels; Rodgers *et al.*, 2016) even at the highest test temperature (25°C). Each oxygen probe was calibrated weekly at the test temperatures using 100% (aerated distilled water) and 0% (150 ml distilled water with 3 g dissolved Na_2SO_3) air-saturated water.

Oxygen uptake was calculated according to the following formula:

$$\text{Oxygen uptake}\left(\text{in mg } O_2 \text{kg}^{-0.95}\text{ min}^{-1}\right) = \left\{\left[\left(O_2(t_1) - O_2(t_2)\right) \times V\right] \times M^{-0.95}\right\} \times T^{-1},$$

where $O_2(t_1)$ is the oxygen concentration in the swim tunnel at the beginning of the seal (in milligrams of oxygen per litre); $O_2(t_2)$ is the oxygen concentration in the tunnel at the end of the seal (in milligrams of oxygen per litre); V is the volume of the swim tunnel (in litres); M is the mass of the fish (in kilograms); and T is the duration of the measurement (in minutes). Allometric correction for variable body mass used the exponent 0.95, which is halfway between the life-stage-independent exponent determined for resting (0.97) and active (0.93) zebrafish (Lucas *et al.*, 2014).

Experimental protocol

Fish were introduced between 13.00 and 16.00 h each day into a swim tunnel at 13 ± 0.3°C, which was close to the river temperature at which most fish were caught, and left for 60 min before a 60 min training swim (Jain *et al.*, 1997), during which water flow velocity was gradually increased to 5–10 cm s^{-1} higher than when swimming started (typically at 30 cm s^{-1}) and held for 50 min before a 10 min swim at 50 cm s^{-1} (the anticipated maximal prolonged swimming velocity for a 150 mm fish at 13°C; Alsop and Wood, 1997). Recovery for 60 min preceded the incremental increases in water temperature (1°C per 30 min) up to the test temperature. Oxygen uptake (10–30 min, depending on the test temperature, and followed by a 5–10 min flush period) was continuously measured throughout the night until 07.00 h. Estimates of RMR for each of the 44 tested fish were calculated by averaging the lowest four oxygen uptake measurements at the test temperature for the minimum 8 h overnight period (Chabot *et al.*, 2016). Visual inspection of the video recordings confirmed that fish were quiescent during these measurements with the exception of three fish that were discarded owing to consistent activity throughout the night (Crocker and Cech, 1997), which reduced the RMR measurements to 41 fish.

Critical swimming velocity and burst swimming protocols (Reidy *et al.*, 1995; Killen *et al.*, 2007; Clark *et al.*, 2013; Norin and Clark, 2016) were used to determine MMR. They began between 08.00 and 09.00 h and lasted 2–6 h. For the critical swimming velocity test, water velocity was gradually increased until the fish continuously swam at 30 cm s^{-1} for 20 min. Water velocity was incrementally increased every 20 min by 10% of the previous test velocity (3–6 cm s^{-1}) until the fish was no longer able to swim continuously and fell back to make full body contact with the downstream screen of the swimming chamber. The fish recovered for 1 min at 13–17 cm s^{-1}, the lowest velocity setting of the swim tunnel, before restoring the final water velocity over a 2 min period and restarting the 20 min timer. Fatigue was defined as when the fish made full body contact with the downstream screen of the swim tunnel a second time at the same test velocity or failed to resume swimming. Active metabolic rate was measured at each test velocity using a 3 min flush period and a 7–17 min measurement period. All fish swam for 20 min at one water velocity, but almost 50% of the wild fish used their caudal fin to prop themselves on the downstream screen of the swim tunnel to avoid swimming faster, which required a secondary measurement of maximal metabolic rate using a burst swimming protocol. For the burst swimming protocol, tunnel velocity was set to and held for 10 min at the highest critical swimming velocity test increment where that fish had continuously swum. Afterwards, water velocity was rapidly (over 10 s) increased to 70–100 cm s^{-1}, which invariably elicited burst swimming activity for 30 s or less, when water velocity exceeded 70 cm s^{-1}. This protocol was repeated multiple times for 5–10 min, while oxygen uptake was measured continuously. The MMR was assigned to the highest active metabolic rate measured with the active respirometry methods. Occasionally, fish exhibited intense struggling behaviours with an even higher oxygen uptake, which was assigned MMR. The MMR was not estimated for four fish, which failed to swim and raise their metabolic rate appreciably with any of the methods, resulting in a total of 37 fish with RMR and MMR measurements. Absolute aerobic scope (AAS = MMR − RMR) and factorial aerobic scope (FAS = MMR/RMR) were calculated.

All fish recovered in the swim tunnel at a water velocity of 13–17 cm s^{-1} and at the test temperature for 1 h while measuring oxygen uptake. Water temperature was then decreased to ~13°C over a 30 min period before the fish was removed, measured, PIT tagged and put into a holding tank before release at the capture site. Fish were individually anaesthetized for <5 min with CO_2 (2 Alka-Seltzer tablets dissolved in 3 litres of river water) for morphometric measurements [fork length (FL), in millimetres; and body mass, in grams], condition factor calculation (CF = body mass × $10^5/FL^3$), and PIT tagging. Half duplex PIT (Oregon RFID) tags were placed into the abdominal cavity via a

1 mm incision through the body wall, just off-centre of the linea alba. All equipment was sterilized with NOLVASAN S prior to tagging, and incisions were sealed with 3M VetBond. Revived fish were immediately transported to the coolers filled with 13–15°C river water. At the release site, river water was gradually added to the cooler to equilibrate the fish to river water temperature at a rate of 1–2°C h^{-1} before fish were allowed to swim away voluntarily.

Measurements of tail beat frequency

The tail beat frequency (TBF; number of tail beats per 10 s, reported in Hz) of fish swimming continuously and holding station without contacting the downstream screen of the respirometer was measured using the average of two or three 10 s sections of video recordings played back at either one-quarter or one-eighth of real time. The TBF was then related to swimming speed and temperature. Tail beat frequencies of undisturbed fish holding station in the Tuolumne River were measured from footage from underwater video cameras anchored within 1 m of *O. mykiss* schools and left to record for up to 4 h (GoPro Hero 4). The TBFs were determined using the same methodology applied to respirometer video recordings ($n = 15$ at 14°C and $n = 1$ at 20°C).

Data analysis

A statistical model was fitted to individual data [performed in R (R Core Development Team, 2013) using the 'lm' function] to determine the best relationships between the test temperature and RMR, MMR, AAS and FAS. The statistical model (linear, quadratic, antilogarithic base 2 and logarithmic base 2 were tested) with the highest r^2 and lowest residual SE being reported. Confidence intervals and predicted values based on the best-fit model were calculated in R using the 'predict' function. Variances around metabolic rate measurements are reported as 95% confidence intervals (CIs).

Results

As anticipated, basic oxygen needs (RMR) increased exponentially by 2.5-fold from 13 to 25°C (from 2.18 ± 0.45 (95% CI) to 5.37 ± 0.41 mg O_2 $kg^{-0.95}$ min^{-1}). This thermal response was modelled by: RMR (in mg O_2 $kg^{-0.95}$ min^{-1}) = 5.9513 − 0.5787 (temperature, in °C) + 0.0200 (temperature, in °C)^2 ($P < 0.001$, $r^2 = 0.798$; Fig. 1A). The MMR increased linearly by 1.7 times (from 6.62 ± 1.03 to 11.22 ± 0.86 mg O_2 $kg^{-0.95}$ min^{-1}) from 13 to 25°C. This thermal response was modelled by: MMR (in mg O_2 $kg^{-0.95}$ min^{-1}) = 1.6359 + 0.3835 (temperature, in °C) ($P < 0.001$, $r^2 = 0.489$; Fig. 1B). Given that MMR almost kept pace with the thermal effect on RMR, AAS had a rather flat reaction norm that was largely independent of the test temperature range. This thermal response was modelled by: AAS (in mg O_2 $kg^{-0.95}$ min^{-1}) = −5.7993 + 1.1263 (temperature, in °C) − 0.0265 (temperature, in °C)^2 ($P = 0.060$, $r^2 = 0.098$; Fig. 1C). Using this model, peak AAS (6.15 ± 0.71 mg O_2 $kg^{-0.95}$ min^{-1}) was centred at 21.2°C. Nevertheless, the unexpected flat reaction norm meant that 95% of peak AAS was maintained from 17.8 to 24.6°C, which is a broad thermal window for peak AAS that extends well beyond the 7DADM value of 18°C for *O. mykiss*.

Factorial aerobic scope is a useful metric of whether or not a fish might have the required aerobic capacity to perform a specific activity, e.g. a doubling of RMR (i.e. FAS = 2) might be needed to digest a full meal properly (Jobling, 1981; Alsop and Wood, 1997; Fu *et al.*, 2005; Luo and Xie, 2008). As expected, FAS decreased with temperature (Clark *et al.*, 2013), a thermal response modelled by: FAS = 2.1438 + 0.1744 (temperature, in °C) − 0.0070 (temperature, in °C)^2 ($P < 0.001$, $r^2 = 0.344$; Fig. 1D).

In addition, given the need to integrate AAS or FAS within an ecological framework (see Overgaard *et al.*, 2012; Clark *et al.*, 2013; Farrell, 2013, 2016; Pörtner and Giomi, 2013; Ern *et al.*, 2014; Norin *et al.*, 2014), we used measurements of TBF to estimate the oxygen cost required by a wild *O. mykiss* to maintain station in the river currents of typical habitats in the Tuolumne River. A steady TBF used for this activity at ambient temperatures of 14 and 20°C was 2.94 and 3.40 Hz, respectively (see supplemental video, available online). Using respirometer swimming data to relate TBF to oxygen uptake at 14 and 20°C ($P < 0.001$, $r^2 = 0.35$; and $P = 0.009$, $r^2 = 0.33$, respectively; Fig. 2), in-river TBF values of 2.94 and 3.40 Hz corresponded to 3.26 and 5.43 mg O_2 $kg^{-0.95}$ min^{-1}, respectively. Therefore, we suggest that wild fish observed holding station in the Tuolumne River increased RMR by 1.5 times at 14°C and by 2.0 times at 20°C, an activity that would use 49 and 67%, respectively, of the available FAS measured at these two temperatures.

Fish recovery after exhaustive swimming tests was quick and without any visible consequences. The RMR at the end of the 60 min recovery period was either elevated by no more than 20% or fully restored; an observation consistent with previous laboratory studies of *O. mykiss* recovery (Jain *et al.*, 1997; Jain and Farrell, 2003). Two fish tested at 25°C were the only exceptions. These two fish regurgitated their gut contents during recovery and one then died abruptly. Inadvertent fish recapture provided some information on fish survival after being returned to the river. Six PIT-tagged fish were recaptured at 1–11 days post-testing within 20 m of their original capture location; all were visually in good condition, and three of these fish had been tested at 23°C.

Discussion

The present study is the first to consider the thermal response for an *O. mykiss* population so close to the southerly boundary of the natural distribution range for indigenous *O. mykiss*. We clearly show that 95% of peak AAS was maintained over an unexpectedly broad thermal window (17.8–24.6°C) and that all fish tested could maintain an

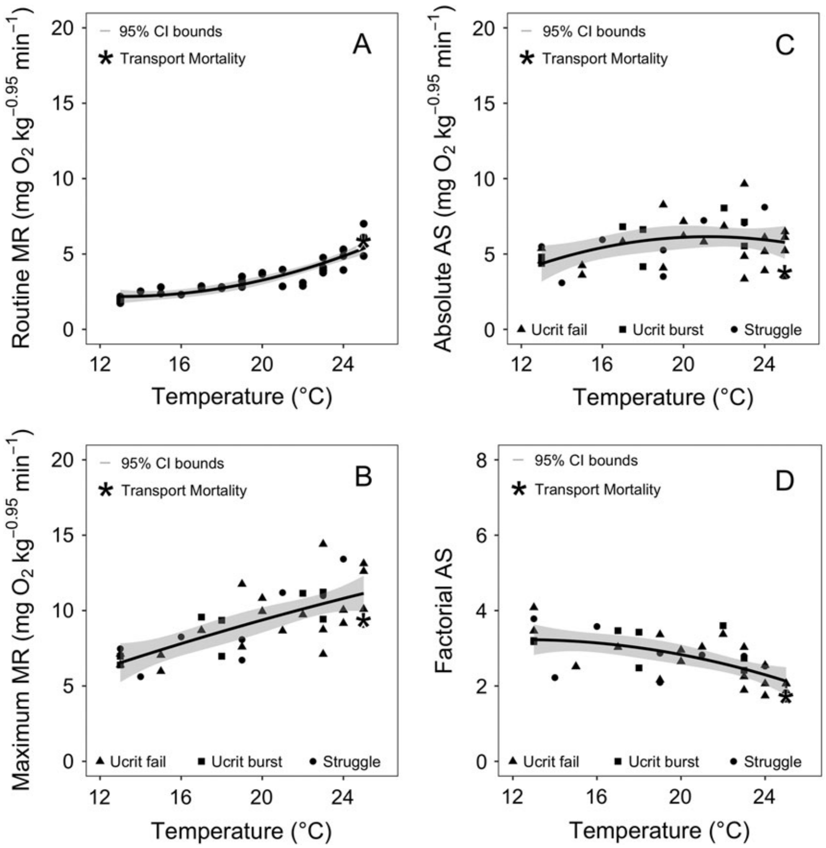

Figure 1: The relationships between test temperature and the routine (RMR; **A**) and maximal metabolic rate (MMR; **B**) of Tuolumne River *Oncorhynchus mykiss*. The three methods used to measure MMR (see Materials and methods section) are distinguished by different symbols. Absolute aerobic scope (AAS; **C**) and factorial aerobic scope (FAS; **D**) were derived from the metabolic rate measurements. Each data point represents an individual fish tested at one temperature. These data were given a best-fit mathematical model (continuous line or curve), and the 95% confidence intervals for each line are indicated by the shaded area. The RMR and FAS were smoothed to a polynomial fit of the form $y = x + I(x^2)$, where *y* is RMR or FAS, *x* is temperature, and *I* is a constant. The MMR and AAS were smoothed to a linear fit of the form $y = x + c$, where *c* is a constant. For RMR, degrees of freedom (d.f.) = 34, $P < 0.001$, residual standard error (RSE) = 0.561 and $r^2 = 0.798$. For MMR, d.f. = 35, $P < 0.001$, RSE = 1.580 and $r^2 = 0.489$. For AAS, d.f. = 35, $P = 0.060$, RSE = 1.490 and $r^2 = 0.098$. For FAS, d.f. = 34, $P < 0.001$, RSE = 0.506 and $r^2 = 0.344$. The asterisk indicates the one fish that died abruptly after the swimming test.

FAS >2.0 up to 23°C. Moreover, we place these findings into an ecological context by suggesting that the level of FAS at temperatures at least as high as 20°C may be more than adequate to maintain station in the local water current of the Tuolumne River and probably to digest a meal properly and optimize growth, which is a very powerful integrator of environmental, behavioural and physiological influences on a fish's fitness. Moreover, fish were tested on site and returned afterwards to the river, making the work locally relevant for the *O. mykiss* population, sensitive to conservation needs and globally relevant by addressing the following broad question: are fish at the extreme edges of their biogeographical range more physiologically tolerant because of the thermal extremes they experienced there?

The present results show good quantitative agreement with various previous studies with *O. mykiss* that have measured some of the variables measured in the present study. For example, the 2.5-fold exponential increase in RMR from 13 to 25°C (from 2.18 ± 0.45 (95% CI) to 5.37 ± 0.41 mg O_2 $kg^{-0.95}$ min^{-1}) compares well with laboratory studies of RMR reported at 14°C (2.3–2.8 mg O_2 $kg^{-0.95}$ min^{-1}; Myrick and Cech, 2000) for 7 g Mount Shasta and Eagle Lake *O. mykiss*, and at 25°C (~6.5 mg O_2 per $kg^{-0.95}$ min^{-1}; Chen *et al.*, 2015) for 30 g Western Australian *O. mykiss*. Therefore, concerns about handling stress and specific dynamic action were minimal. Likewise, MMR increased linearly by 1.7 times (from 6.62 ± 1.03 to 11.22 ± 0.86 mg O_2 $kg^{-0.95}$ min^{-1}) from 13 to 25°C, comparing well with previous laboratory measurements of MMR reported at 15°C (2.8–8.7 mg O_2 $kg^{-0.95}$ min^{-1}) for 2–13 g *O. mykiss* (Scarabello *et al.*, 1992, Alsop and Wood, 1997) and with the peak MMR at 20°C (~11.13 mg O_2 per $kg^{-0.95}$ min^{-1}) for Australian *O. mykiss* (Chen *et al.*, 2015). As a consequence of MMR nearly keeping pace with the thermal

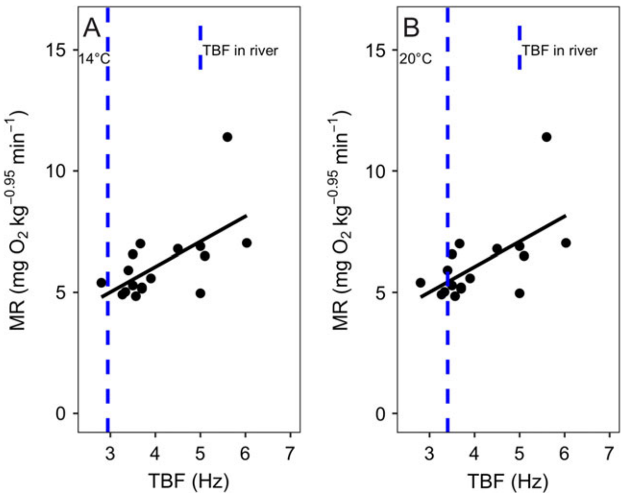

Figure 2: The relationship between tail beat frequency (TBF; in hertz) and metabolic rate (MR; in mg O_2 $kg^{-0.95}$ min^{-1}) measured when Tuolumne River *Oncorhynchus mykiss* were swimming continuously in a swim tunnel at 14 (**A**) or 20°C (**B**). The continuous black line represents the linear regression based on the data for $n = 7$ fish at 14°C and $n = 5$ fish at 20°C. The vertical dashed lines represent the estimated TBF (2.94 Hz at 14°C and 3.40 Hz at 20°C) taken from videos of *O. mykiss* maintaining station in a water current in their normal Tuolumne River habitat. At 14°C, the relationship between TBF and MR followed the equation MR = 0.75TBF + 1.05, with degrees of freedom (d.f.) = 41, $P < 0.001$, residual standard error (RSE) = 1.27 and $r^2 = 0.35$. According to this formula, the MR for the TBF measured in the river (2.943 Hz) at 14°C was estimated to be 3.26 mg O_2 $kg^{-0.95}$ min^{-1}. At 20°C, the relationship between TBF and MR followed the equation MR = 1.04TBF + 1.89, with d.f. = 15, $P = 0.009$, RSE = 1.29 and $r^2 = 0.33$. According to this formula, the MR for the TBF measured in the river at 20°C (3.402 Hz) was estimated to be 5.43 mg O_2 $kg^{-0.95}$ min^{-1}.

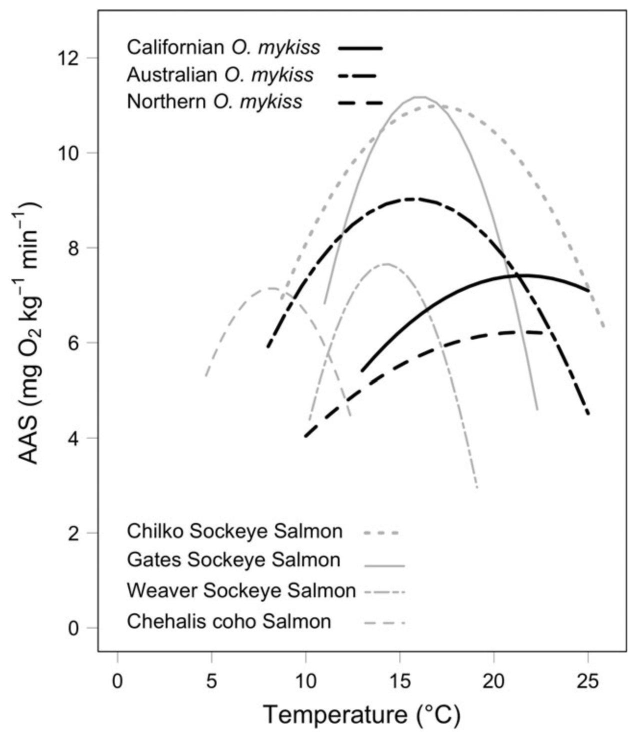

Figure 3: Absolute aerobic scope (AAS) for three strains of *Oncorhynchus mykiss*, i.e. a northern strain (Fry, 1948), an Australian strain (Chen *et al.*, 2015) and the California strain reported in this manuscript, compared with AAS measurements of Chehalis Coho salmon (*Oncorhynchus kisutch* Walbaum 1792) and Gates Creek, Weaver Creek (*Oncorhynchus nerka* Walbaum 1792; Lee *et al.*, 2003a) and Chilko Creek sockeye salmon (Eliason *et al.*, 2011). The best-fit line of the relationship between AAS and temperature of the species and populations from other publications was predicted using a second-order polynomial linear regression performed on the raw data (Lee *et al.*, 2003a; Chen *et al.*, 2015) or data extracted from plots (Fry, 1948; Eliason *et al.*, 2011) from the original publications. Coefficient estimates from the linear regression analysis were then used to determine the peak aerobic scope and the temperatures corresponding to the peak and 95% of peak AAS.

effect on RMR, AAS was largely independent of test temperature. Directly comparing our AAS values with other studies revealed that our result for AAS at 15°C (5.10 mg O_2 $kg^{-0.95}$ min^{-1}) was at the high end of previous laboratory measurements of AAS (1.8–5.8 mg O_2 $kg^{-0.95}$ min^{-1}) for *O. mykiss* at 15°C (Scarabello *et al.*, 1992, Alsop and Wood, 1997; McGeer *et al.*, 2000), but lower than peak AAS (~7.3 mg O_2 per $kg^{-0.95}$ min^{-1}) at 20°C in Australian *O. mykiss* (Chen *et al.*, 2015). Likewise, our FAS values were bracketed by values obtained in previous laboratory studies. At 24°C, FAS (2.13 ± 0.33) was greater than that reported at 25°C (1.8) for Western Australian *O. mykiss* (Chen *et al.*, 2015), but compared with FAS values for juvenile rainbow trout (1.8–5.8) at 13°C (Scarabello *et al.*, 1992, Alsop and Wood, 1997, McGeer *et al.*, 2000), our FAS at 13°C (3.32 ± 0.41) was in the middle of the range.

To place the present data for Californian *O. mykiss* into perspective, we have compared (Fig. 3) their reaction norm with those published for juveniles of northern *O. mykiss* (data from Fry, 1948) and Australian hatchery-selected *O. mykiss* (data from Chen *et al.*, 2015), as well as adult northern populations of selected Pacific salmon populations (data from Lee *et al.*, 2003a and Eliason *et al.*, 2011). Among the native *O. mykiss* populations, the Lower Tuolumne River juvenile Californian *O. mykiss* are likely to experience the highest temperatures during summer (up to 26°C), although the introduced Australian *O. mykiss* population had experienced selection temperatures ≥25°C (Chen *et al.*, 2015). Notably, AAS at 24°C for Tuolumne River *O. mykiss* is greater than other *O. mykiss* populations and only bettered by the Chilko sockeye salmon population, one of several sockeye salmon populations that are known to have a peak AAS at the modal temperature for their upstream spawning migration (Eliason *et al.*, 2011, 2013). Thus, the present data are in line with evidence of intraspecific matching of metabolic rate to local water temperatures within the *Oncorhynchus* genus. Although the peak AAS of the Australian *O. mykiss* population was 50% greater than for the other two *O. mykiss* populations, Tuolumne River *O. mykiss* had the broadest and highest thermal window (17.8–24.6°C) among the *O. mykiss* populations (20.5–22.4°C from Fry, 1948; and 12.8– 18.6°C from Chen *et al.* 2015).

Whether the matching of Tuolumne River *O. mykiss* metabolic performance to local habitat temperatures is a result of thermal acclimation or local adaption, as in the Western Australian *O. mykiss*, will need study well beyond the present work. Thermal acclimation usually results in fish performing better at the new temperature. For example, thermal acclimation offsets the effect of acute warming on RMR in 5–7 g Mount Shasta and Eagle Lake *O. mykiss* (2.3–2.8 mg O_2 $kg^{-0.95}$ min^{-1} at 14°C and 2.9–3.1 mg O_2 $kg^{-0.95}$ min^{-1} at 25°C; Myrick and Cech, 2000), which would normally double RMR over this temperature range, as observed here. Warm acclimation can also increase upper thermal tolerance limits, as it did for four anadromous Great Lakes populations of juvenile *O. mykiss* (Bidgood and Berst, 1969). Given that our fish were captured at and, presumably, thermally acclimatized to between 14 and 17°C, it would be of interest to test wild fish with a warmer thermal acclimation history. But even without thermal acclimation, the present data suggest that Tuolumne River *O. mykiss* and those for northern *O. mykiss* (Fry, 1948; see Fig. 3) have the aerobic capacity temporarily, if not regularly, to exploit temperatures well above 18°C, which is the upper thermal limit suggested by EPA guidance documents (US Environmental Protection Agency, 2003).

Nevertheless, we caution that such local tailoring may not be evident in all salmonid species. For example, the thermal physiology of Atlantic salmon (*Salmo salar* Linnaeus 1758) from northern and southern extremes of their European range did not show any major difference (Anttila *et al.*, 2014). All the same, a sub-species of redband trout (*O. mykiss gairdneri*), which are apparently adapted to high summer temperatures of North American desert streams (Narum *et al.*, 2010; Narum and Campbell, 2015), are likewise capable of high levels of swimming performance up to 24°C (Rodnick *et al.*, 2004) and higher swimming performance for a warm vs. a cool creek population (Gamperl *et al.*, 2002). Redband trout have been observed actively feeding at 27–28°C (Sonski, 1984; Behnke, 2010), but thermal selection of wild redband trout is centred between 13 (Gamperl *et al.*, 2002) and 17°C (Dauwalter *et al.*, 2015). How *O. mykiss* behaviourally exploit the steep summer thermal gradient in the Tuolumne River below the La Grange Diversion Dam (from 12 to 26°C over 25 km; HDR Engineering, Inc., 2014) is another unknown. Even without these important details, Tuolumne River *O. mykiss* appear physiologically to be tolerant of the thermal extremes they experience.

The capacity of a fish to deliver oxygen to support activities in water of varying quality is a concept originally introduced for fishes >60 years ago (Fry, 1947). The oxygen- and capacity-limited thermal tolerance hypothesis broadens this concept and provides a mechanistic explanation (Pörtner, 2001; Pörtner and Farrell, 2008; Deutsch *et al.*, 2015), but is currently under debate (Overgaard *et al.*, 2012; Clark *et al.*, 2013; Farrell, 2013; Pörtner and Giomi, 2013; Ern *et al.*, 2014; Norin *et al.*, 2014). An accepted fact is that a metabolic load from an environmental factor (e.g. temperature) can increase the oxygen cost for living (i.e. RMR). Consequently, like all other temperature studies with fish, the magnitude of the 2.5-fold increase in RMR observed here over a 12°C temperature range (between 13 and 25°C) was expected. However, temperature did not limit MMR, which increased linearly with acute warming, and the peak MMR was not resolved. The statistical models, which were based on individual responses and 1°C temperature increments from 13 to 25°C, predicted a peak AAS at 21.2°C for Tuolumne River *O. mykiss* and a FAS >2.0 up to 23°C. As the allocation of energy and trade-offs are recognized and fundamental tenants of ecological physiology, especially in fishes (Sokolova *et al.*, 2012), we suggest that being able to at least double RMR has ecological relevance for two behaviours that are likely to influence survival of *O. mykiss*, maintaining station in a flowing river and processing a large meal.

Snorkeling in the Tuolumne River provided visual observations of *O. mykiss* maintaining station in the river current for prolonged periods that were punctuated by hiding under the river bank and by darting behaviours to capture prey and to protect their position. Maintaining station required a steady TBF similar to the situation in the swim tunnel respirometer, which allowed us to estimate a metabolic cost of maintaining station in typical Tuolumne River habitats at 14 and 20°C (a 1.5- to 2-fold increase in RMR) and the aerobic scope available for additional activities (1.7–2 times RMR). Although darting behaviours are likely to be fuelled anaerobically, *O. mykiss* must (and were clearly able to) repay the post-exercise excess oxygen debt (Lee *et al.*, 2003b) while maintaining station in the river current. The rapid recovery of RMR after exhaustive exercise in the swim tunnel suggests that *O. mykiss* had the capacity to repay post-exercise excess oxygen debt rapidly at temperatures as high as 24°C. Although digestion of a meal at high temperatures proceeds more rapidly and with a higher peak metabolic rate, the total oxygen cost of the meal remains similar. Thus, fish can theoretically eat more frequently and potentially grow faster at a higher temperature provided there is a sufficient FAS for digestion within the overall AAS. Given that peak metabolic rate during digestion of a typical meal for a salmonid does not necessarily double RMR at the temperatures used here (e.g. Jobling, 1981; Alsop and Wood, 1997; Fu *et al.*, 2005; Luo and Xie, 2008), an FAS value of 2 should be a reasonable index, and all *O. mykiss* tested had this capacity up to 23°C. Indeed, the fish were apparently feeding well in the river, given a high condition factor (1.1 SEM = 0.01), the faecal deposits found in the swim tunnel and two fish regurgitating meals when tested at 25°C. Meal regurgitation would be consistent with an oxygen limitation, given that aquatic hypoxia impairs digestion in *O. mykiss* (Eliason and Farrell, 2014). Indeed, feeding and growth are suppressed at supra-optimal temperatures (Hokanson *et al.*, 1977; Brett and Groves, 1979; Elliott, 1982; Myrick and Cech, 2000, 2001). Taken together, these data suggest that Tuolumne River *O. mykiss* were doing well in their habitat and had the aerobic capacity to do so.

Our metabolic measurements, which show good quantitative agreement with controlled laboratory *O. mykiss* studies, represent a major challenge to the use of a single thermal criterion to regulate *O. mykiss* habitat when determining conservation criteria along the entire Pacific coast and perhaps elsewhere. The 7DADM of 18°C for *O. mykiss* draws heavily on a growth study performed in Minnesota (Hokanson *et al.*, 1977). Therefore, it will be important to examine whether the peak AAS at 21.2°C for Tuolumne River *O. mykiss* is associated with a peak growth rate. In this regard, the peak growth rate of another Californian *O. mykiss* population (the Mount Shasta strain) occurred at acclimation temperatures (19–22°C; Myrick and Cech, 2000) above the 7DADM and within the thermal window for 95% peak AAS for Tuolumne River *O. mykiss*. The Mount Shasta *O. mykiss* strain also stopped growing at 25°C, the same temperature at which FAS for Tuolumne River *O. mykiss* approached 2. In contrast, the Californian Eagle Lake *O. mykiss* strain grew fastest at 19°C and lost weight at 25°C (Myrick and Cech, 2000). Thus, the Mount Shasta and Tuolumne River *O. mykiss* populations are better able to acclimate thermally to temperatures >20°C than the Eagle Lake strain. With clear evidence that a California strain of *O. mykiss* can grow faster at acclimation temperatures >18°C and that strains may differ in their the optimal temperature for growth by as much as 3°C, there is a precedent that local populations of *O. mykiss* can perform well above 18°C. Our findings also highlight the need for future experiments that consider replicate populations from throughout the species range to assess how widespread intraspecific variation in aerobic scope in *O. mykiss* might be. Continual development and refinement of the metrics used to best inform regulatory criteria should be an ongoing pursuit, particularly if regional standards are to be implemented and if the criteria move away from what may now be considered conservative. Probabilistic modelling approaches associated with a diversity of water temperature standards should be developed in order for managers to understand the balance between standards that are conservative compared with those that are more risky.

The capacity to balance the essential environmental requirements of aquatic biota with human requirements is becoming increasingly challenging across the globe because of recent increases in severe drought and record high temperature occurrences, a trend that climate change models project will continue. We suggest that broadly applying regulatory criteria, such as the 18°C 7DADM criterion for Pacific Northwest *O. mykiss* populations, to all North American *O. mykiss* is no longer realistic and, in the present case, overly conservative. The high degree of thermal plasticity discovered here for the Tuolumne River *O. mykiss* population, which corresponds to local thermal conditions, adds to the accumulating evidence of the capacity for local adaptation among populations within the *Oncorhynchus* genus, including *O. mykiss*. Importantly, this work clearly illustrates that, owing to thermal plasticity, broad application of a single temperature criterion for fish protection and conservation is not scientifically supported, especially for fish populations at the extreme limits of the species' indigenous range.

Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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