# DISTRICTS' RESPONSE TO NMFS COMMENTS ON THE DRAFT REPORT FOR THE THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

## DON PEDRO PROJECT
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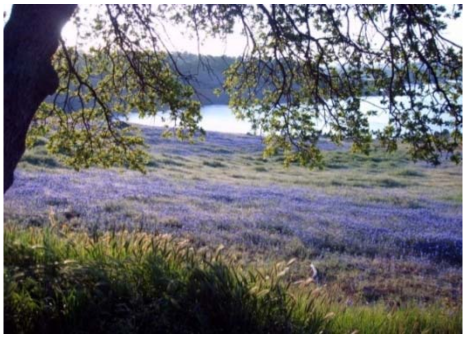

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# 1.0 BACKGROUND

In January 2015, Turlock Irrigation District and Modesto Irrigation Districts (collectively, the Districts) released a draft report for the Thermal Performance of Wild Juvenile *Oncorhynchus mykiss* in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature (Farrell et al. 2015) to relicensing participants for review and comment. Comments on Farrell et al. 2015 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. The report was subsequently finalized in February 2017 (Farrell et al. 2017). Farrell et al. 2017 includes copies of the abovementioned comment letters (as Appendix 5) and the Districts' responses to these comments (as Appendix 6).

In February 2017, the National Marine Fisheries Services (NMFS) provided additional comments on Farrell et al. 2015. The Districts herein provide a response to NMFS' comments.

## 2.0 RESPONSE TO COMMENTS

Thank you for the detailed, thorough and critical comments on the report (Farrell et al. 2015). It is unfortunate that, because of the timing of the review, the NMFS did not have the benefit of reviewing our revised report (Farrell et al., 2017), which includes our detailed responses to CDFW's comments. We would encourage NMFS to review this document before moving on to our specific response below. Please also read the peer-reviewed published manuscript of this study (Verhille et al. 2016). NMFS' February 2017 comment letter, Farrell et al. 2017, and Verhille et al. 2016 are attached to this document as Attachments A, B, and C, respectively, for your convenience.

As we outline below, many of the issues seem to stem from a mismatch in expertise between the experimenters and the reviewers. We apologize for any lack of clarity on our part and for taking for granted that all reviewers would possess a similar baseline and core understanding of fundamental tenants of thermal biology and physiology of fishes because this communication barrier appears to have caused several misinterpretations.

To be clear, our experiments are fundamental physiology. The theoretical and technical approach is based on well-accepted physiological and ecological principles established by Fred Fry and others over a 60-year period. What is different today is that technology and methodology are more reliable than 60 years ago, which makes our data all the more robust. Therefore, we feel that neither our data quality, nor our experimental approach should be challenged in the way it has been in the NMFS review. These direct challenges have necessitated us to defend our science, aggressively in places. Indeed, after a normal and thorough review by anonymous experts in the field, our work now appears in the peer-reviewed scientific literature (Verhille et al. 2016). Please understand that we view this exchange as positive and essential because we stand behind the numbers we have carefully generated.

What troubled us most is the overreliance by the NMFS reviewers on the publication and opinions of Clark et al. (2013a). Rarely do scientific papers draw letters to the editor, but Clark et al. (2013a) drew two independent letters of concern that were subsequently published in the same journal, along with a response by Clark et al. (2013b). Clark et al. (2013a) felt very strongly that the OCLTT hypothesis cannot explain all of fish biology, and that other performance measures should be used in addition to aerobic scope. They did not suggest alternative metrics for aerobic scope, and replacements still have not emerged 4 years on. What Clark et al. (2013a) have never challenged is that aerobic scope (and related measurements) is a useful metric that tells us much about a fish. In fact, Dr. Clark continues to measure aerobic scope in many of his subsequent works, as do a wide spectrum of the fish community. Therefore, we are not alone in using this approach and perhaps a search of aerobic scope in the scientific literature will support this assertion. Perhaps the new review by Portner et al. (2017) will provide further assurances of the value and reliability of measuring aerobic scope (see Attachment D).

Consequently, the issue at hand is not the utility of measuring aerobic scope or how accurately it was measured, but rather what can be inferred from such measurements. We have gone to great lengths in our responses to both CDFW, in the revised Farrell et al. 2017 report, in our published manuscript, and now in our responses to NMFS' comments below to further articulate what our data should and should not be used for in the context of managing lower Tuolumne fish and the 7DADM for *O. mykiss*. More generally, Farrell (2016) has dealt with some of these issues, noting that while we have ample resources to measure a fish's capacity to obtain oxygen, we have an imperfect understanding of how and when a fish apportions this capacity to the various life supporting activities that require oxygen. Thus, an important knowledge gap exists between what we can measure and what we would like to know for management purposes.

We do recognize the importance of our data for management purposes, and because of this, fundamental misunderstandings, misconceptions and misinterpretations must be addressed. It is paramount to us that we are as clear as possible on each of these points, necessitating the following dialog consistent with a rigorous scientific exchange. What we feel is the most important scientific fact to emerge, and of direct relevance to management, is that the measurements made by us on Tuolumne *O. mykiss* indicate that we must set this stock/strain of *O. mykiss* apart from other strains for which we have similar measurements. This suggests to us that local adaptation may have occurred and that the Tuolumne *O. mykiss* show a thermal physiology that is more like that of the subspecies of *O. mykiss* that has adapted to desert regions in the USA and that of *O. mykiss* selectively bred from an unknown Californian *O. mykiss* stock for a harsh, hot and arid new life in lakes of Western Australia.

The idea of local adaptation within a species is not a new concept, but it is one that EPA 2003 left unaddressed when setting temperature guidance criteria some 15 years ago. Science has marched on and important advances have been made in our understanding of thermal tolerance of fishes. Indeed, we now have similar thermal performance data in hand for a California strain of Chinook salmon (Poletto et al. 2016).

## Specific Responses:

In the hope of aiding NMFS to better understand our findings and our interpretations, rather than addressing the 28 pages of comments, point by point, and line by line, we group the comments into 4 general topics, which we address collectively: 1. Data quality; 2. Excessive critique beyond expertise; 3. Overemphasis on a single review (Clark et al 2013a); and 4. General statistics. We close by addressing the reviewer's 10 specific questions (taken from pp. 9-10 of the review).

### 1. The quality of the data is frequently questioned by the reviewers

Our data are of the highest quality. Our research team is composed of internationally recognized experts in the thermal physiology of fishes drawn from inside and outside California. We stand by the core data on absolute aerobic scope (AAS) of TR *O. mykiss* in our Report. Indeed, the work has already been internationally peer-reviewed to be published (Verhille et al. 2016). Therefore, the methodology and results have been taken through the rigors of the time-tested scientific evaluation process for acceptance by the wider scientific community. Moreover, the data appear in open-access literature for all to read. Consequently, any questions as to the quality of the data or the reliability of the experimental approach raised by the reviewers are now mute points. Indeed, until contradictory data are introduced to refute these data, they must stand.

Therefore, we have refrained from another detailed defense of the quality of the results despite the misleading comments and innuendo found in the NMFS review.

If additional detail is needed for the purposes of clarity so that our Report can reach a broader readership and better serve scientists with a limited understanding of thermal performance in fishes, this can be done easily through the addition of supplementary information and explanations in footnotes. The data on AAS presented in the Report are now *de facto* scientifically sound.

**2. Excessive, unreasonable, and overtly misleading commentary revealing a disconnect between the expertise of the NMFS reviewers with that needed to evaluate our data.**

The reviewers openly acknowledge that they do not understand either the study design and measurement principle, or the permitting restrictions imposed on the study that is reported.
With respect to the first point, the reviewers state:

> *From NMFS: "We have not conducted aerobic scope experiments ourselves, and so the list below is to highlight areas where we did not fully understand the study design, methodological approach or data analysis."*

If the reviewers do not understand the basic methodology, how are they able to perform a reliable scientific review? Perhaps this explains why we found the review excessively long, containing much digression and excessive quotations.

Of greater concern, however, are spurious claims that border on falsehoods when compared to what was said in our Report. Since spurious claims are obstructive to the goal of moving towards better management of TR *O. mykiss*, they must be noted. Unfortunately, in providing specific examples below, the reviewers force us to criticize the work of other scientists without them being able to defend themselves, which is not how science should proceed.

a) The reviewers' present unreasonable experimental demands given the permitting limitations for the experimental work, which are followed by comments that reveal insufficient understanding for proper data review and evaluation.

For example, the NMFS review contains many suggestions of additional experimental work that should have been done on TR *O. mykiss.* To be clear once more, the study permit allowed us to use up to 50 fish, and no more. In addition, only 2 fish could be out of the river for a period of 24 h, and there could be no more than 3 fish mortalities, which greatly restricts the experimental design. Nevertheless, even with this limited number of fish, we generated scientifically sound and novel information of the thermal physiology of TR *O. mykiss*. These data led us to the conclusion that the TR population of *O. mykiss* shows features that differ from typical Northwest Pacific *O. mykiss* populations. Instead, the TR population of *O. mykiss* possesses features that closely resemble those *O. mykiss* populations known to have a superior thermal tolerance.

Yet, the reviewer clearly misunderstood our constraints and misrepresents our experimental design with comments such as:

> *From the NMFS: "Two O. mykiss individuals were tested at each exposure temperature, rather than testing the same individual over the range of exposure temperatures"*

What we actually did was test 2 fish each day using the same test temperature and varied the temperature on a daily basis over a wide range, but using new fish as the study permit required. Why cannot the reviewer simply accept that a solid experimental design was used to derive a complex curve by treating the dependent variable (i.e., temperature) as a continuous variable within the constraints of what the permitting allowed. Instead, the review goes on to suggest that we should conduct thermal acclimation and growth studies when such studies were impossible within our study permit.

Another example is the section of the NMFS review that was introduced by:

> *From the NMFS: "We reviewed the Report in the light of the recommendations of Clark et al. (2013a), intended to ensure that swim-tunnel aerobic metabolism measurements are sound and comparable between studies."*

It is then followed by:

> *"Clark et al. (2013a) recommend conducting aerobic scope tests beyond what the fish experience in nature. We understand ESA collection permit restrictions prevented tests at temperatures higher than 25°C (p. 15), but these warmer conditions occur often in the lower Tuolumne River. How did this experimental constraint affect the test results? For example, the experiments yielded an average aerobic scope that remained within 5% of optimum from 17.8°C to 24.6°C. Since the upper range value of this estimate is near 25°C, are additional data points (from exposures at temperatures >25°C) necessary to produce a curve that more precisely estimates an optimum temperature or range for aerobic scope?"*

Additional data points at sustained temperatures above 25°*C* would not be helpful in our efforts to define the optimal temperature range because it is possible that more fish would have died when test at temperatures above 25°*C*. We clearly show that *O. mykiss* began to die when exhaustively exercised at 25°C. As conservation-minded scientists, we did not want to kill any more fish, so why would we want to use higher temperatures when the writing was already on the wall: 25°C is too warm for TR *O. mykiss* to exhaustively exercise. Besides, only 3 fish deaths were possible for the entire set of experiments, as dictated by our study permit.

b) We do not see the point of the reviewers recommending that other stocks beside TR *O. mykiss* be considered. We already have 40-yr old data on growth of Minnesotan *O. mykiss* (Hokanson et al. 1977), but current data for TR *O. mykiss* suggest they are inapplicable to warmer Californian rivers. This, quite frankly, is the point that is most important in all of this. The scientific literature tells us that the performance of Minnesotan fish is very unlikely to reflect TR *O. mykiss*. Thus, from a management perspective, what you need are data for TR *O. mykiss*, and this is what we have provided.

Critically, you must understand that what we have shown here is strong evidence that LTR *O. mykiss* can be fundamentally different, with respect to their thermal performance, compared with other *O. mykiss* stocks found around the world. The extension of this is important. It is simply not appropriate, and the literature no longer supports, managing specific populations as if they are all equal. This is inconsistent with our understanding of thermal performance in fishes, and further, is inconsistent with evolutionary principles and local thermal adaptation. In the absence of data, we should be conservative. In the presence of data, we should be continually working towards using the data to improve our practices, working towards population-specific thermal management.

c) We agree with the NMFS that more than one performance metric should be incorporated for the determination of protective water temperature criteria.

> From the NMFS, p. 7: "*As explained below, the NMFS does not support the use of any single performance metric (aerobic scope or another measure) to determine protective water temperature criteria. We support using multiple lines of evidence gathered from studies of several physiological and ecological measures (EPA 2003; McCullough et al. 2009).*"

However, the critical issue quickly becomes: which suite of performance criteria do you need, tested over what ecologically-relevant timescale, for which lifestage and/or population of fish etc.? We maintain that AS is a very useful metric, adding important information to the management tool box, and we present our expert interpretations for what these data tell us about TR *O. mykiss* relative to other *O. mykiss* populations. The lines of evidence used to set the EPA (2003) criteria that are applied to TR *O. mykiss* were not derived from studies using TR *O. mykiss*. Our physiological study along with other ecological observations for TR *O. mykiss* are building the multiple lines for evidence that support the conclusion that TR *O. mykiss* are locally adapted to higher water temperatures than the *O. mykiss* populations used to develop the EPA (2003) criteria.

d) The NMFS comment on p. 4 is misleading.

> *From the NMFS: "We found the Report's experimental approach and framework relied heavily on acceptance of the OCLTT hypothesis as an established fact or law."*

This is nonsense. A hypothesis is tested, not proven. This is the basis of the scientific method. What is a fact for fishes is that all activities require oxygen, either immediately to directly support that activity, or shortly after during the recovery phase. This is the only fact that we rely on in this regard.

Furthermore, our data do not have to be framed by the OCLTT hypothesis. If we remove from our Report all reference to OCLTT, it would not affect any of our data. Therefore, trying to discredit the OCLTT framework is not only unproductive, it is flawed. The OCLTT hypothesis is a contemporary extension of the Fry paradigm, which has long been accepted by fish biologists (see Claireaux and Chabot 2016) and encompasses the Fry polygon.

Moreover, the reviewers openly admit a poor understanding of what Fred Fry measured in fish and then applied to their ecology, just like both Brett and Elliott did many years afterwards with the following comments:

> *From the NMFS: "In reviewing the Report, we found the need for much more explanation of how aerobic scope (the difference between resting and maximum metabolic rates) by itself can be assumed to define a fish's capacity to carry out its essential life functions like swimming, defending territory, catching prey and feeding, digesting a meal, growing, and avoiding predators (p. 4), or of how this capacity or potential translates to vital rates (e.g., size-based fecundity, mortality rate, etc.) that affect population-level status."*

The reviewer then uses a sleight of hand to shift ownership of a well-established fact by stating:

> *From the NMFS: "The Report asserts that aerobic scope* <u>*defines*</u> *a fish's capacity to perform its essential life functions*".

Our statement holds true because, as we explain below, the operable word is "capacity", unlike the reviewers' emphasis of the wrong word ("defines"). Indeed, to challenge this definition reveals a lack of understanding of simple bioenergetic principles. $O_2$ is the currency of life for animals.

$$6O_2 + C_6H_{12}O_6 = 6CO_2 + 6H_2O.$$

Simply put, an animal generates $CO_2$ and water using food and $O_2$, which in the process of mitochondrial respiration generates ATP to fuel all essential life functions.

Importantly, this defining principle says nothing about which of the life functions the $O_2$ is used for at any given moment in time. What it does imply, however, is that if an animal cannot supply sufficient $O_2$ for an activity, it cannot perform the activity as well or at all. This simple concept has been endlessly demonstrated by exposing animals to environmental hypoxia, which reduces $O_2$ supply and at some level reduces what an animal can do. Thus, if the maximum CAPACITY to supply $O_2$ (i.e., AAS) is a known entity, you then know what an animal can POTENTIALLY do. This very principle was adopted in our work and is one that was established almost a century ago for human studies and over 60 years ago for fish studies.

What we can also do with the growing literature base in this area is make stronger conclusions and inferences about what the ecological significance of AS capacity means between species or among populations of the same species. Comparisons of relative AS values among species and populations is incredibly powerful.

While AAS does not tell us directly how a fish apportions its capacity to use $O_2$ on a given day, or among competing activities (Farrell, 2016), of all activities in fish, digestion and exercise are perhaps the best understood, $O_2$-demanding functions which we try to interpret in our report.

## 3. Overemphasis on a single review article, Clark et al. 2013a

The Review leans heavily on the words and recommendations of the Clark et al. (2013a & 2013b). Indeed, the critique of the experimental approach mostly relies on these contributions to the literature, which are cited to excess. It is important to clarify what these two works represent. First, Clark et al. (2013a) is a review (i.e., no original data are contained therein) that takes particular exception to the universality of the OCLTT hypothesis. Furthermore, these authors recommend the use of "multiple performance measures" without stating what these new "performance metrics" might be. Indeed, their review closes with:

> *"… it does provide a timely reminder that the OCLTT hypothesis is not universally applicable and there are other performance metrics that may work independently or synergistically with attributes such as aerobic scope to govern the thermal preferences and fitness of water-breathing organisms. A fruitful direction for future research is to investigate which performance metric(s) becomes progressively limited as ectothermic animals are shifted away from their preferred temperature (i.e. the temperature they select when given the opportunity)."*

By making this statement (and from reading the entire review), the three authors of Clark et al. (2013a) could not provide a substitute "performance metric" for ASS at that time. Furthermore, an alternative has not emerged in the scientific literature to date. More importantly, some of the opinions contained in Clark et al. (2013a) were challenged by **two** independent letters to the journal's Editor from two distinct research teams (Farrell 2013; Portner and Giomi, 2013). These two letters were published alongside another letter to the Editor (Clark et al. 2013b) in response to the two challenges. Thus, all three letters are opinions and are NOT a peer-reviewed source. All three letters address the validity of the opinions expressed in Clark et al. (2013a), which represents the perspective of just three scientists. Thus, Clark et al. (2013a) was not accepted carte blanche by the scientific community, in part because it offered no alternative to AAS.

## 4. Comments of the reviewers suggest a fundamental lack of understanding of statistics

Our concern is clearly revealed with the following quote:

> *From the NMFS: "We noted the imprecise fit (r2 = 0.099) of the curve through the aerobic scope data points (Figure 4, p. 37). In contrast, the fit of the curve for the resting (routine) metabolic rate was good (r2 = 0.8), and better than for maximum metabolic rate (r2 = 0.49). Are these findings common in aerobic scope studies? How does the imprecise fit of the aerobic scope curve affect the reliability of the estimates of optimal temperature for aerobic scope, the comparability of the results with other studies, or its use in setting protective temperature criteria?"*

Scientists understand that a regression shows the robustness of the relationship between two variables, X and Y. A horizontal line through data means that no such relationship exists between X and Y, and this would be reflected in a very low r2. Our point of presenting the statistic (r2 = 0.099) is that AAS **does not** statistically change appreciably over much of the temperature range that we tested, i.e., TR *O. mykiss* maintain a near maximal capacity to supply oxygen over a surprisingly wide temperature range.

Thus, by raising this as an issue, the reviewers, possibly inadvertently, are admitting that TR *O. mykiss* at 22°C and 18°C must have the same capacity to deliver oxygen to tissues, which is the primary conclusion of our Report. What then follows in our logic is that if there is no difference in aerobic capacity between these two temperatures, why are regulations using 18°C as a cut off temperature? Instead, this thermal limit was derived primarily on a growth study performed 40 years ago and with *O. mykiss* that had been transplanted and hatchery-reared in Minnesota rather than a population from the TR that had been fully acclimatized to summer river temperatures, which was the case for our data. Here, we do not want to be overly disrespectful and critical of the historical data (Hokanson et al. 1977), but we must respectfully acknowledge that the scientific understanding of fish thermal physiology has moved on technically and intellectually since 1977.

## 5. Answers to specific questions from NMFS, p. 9 onwards.

(1) Can you explain this study-design decision, and how it might have affected the resulting estimates of the optimal temperature for aerobic scope?

We believe oxygen is needed for all activities of a fish. Therefore, we measured the fish's capacity to supply oxygen as a function of temperature and treating temperature as a continuous variable so that a complex curve could be fitted to the data for no more than 50 fish.

(2) Could the short-term (several hours of) temperature acclimation described above have affected the test results, or are longer acclimation intervals (e.g. weeks) required to affect aerobic scope responses?

Aerobic scope curves can respond to thermal acclimation (e.g. over weeks at a new temperature), but the magnitude of the response is often species and/or population dependent. We do not feel that acclimation was a factor in the present experiments. Thus, 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 because it is well known that thermal acclimation is used by fishes to "improve performance" at the new acclimation temperature. It is a valid comment to query the 'acclimatization' state of wild caught fish. The typical pattern is that as fish are acclimated/acclimatized to higher temperatures, the performance curve (if it shifts at all) will shift in the direction that promotes better performance at warmer temperatures (i.e. the thermal optima will move to a higher temperature as acclimation/acclimatization temperatures increase). 75 percent of the fish tested in this study were sourced from locations with capture temperatures between 12.7 and 17.1°C. Therefore, if the TR *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.

(3) How could the long-term temperature acclimation experienced by the individuals tested, and the inability to test the less tolerant individuals in the Tuolumne River population, have affected the test results?

There are several ideas conflated in this question and the balance of the paragraph that follows. The reviewers' terminology is imprecise. The idea that thermal acclimation/acclimatization could influence aerobic scope is addressed above. The suggestion by the reviewers that acclimation of individuals (which, importantly, is a process that happens to an individual within its lifetime) is somehow affecting our results in a way that would lead us to conclude that our data are not representative of other important individuals called the 'less tolerant, non-surviving individuals" in the population doesn't make sense. These ideas do not go together. The reviewer also mentions 'long term exposures' but the time course they are referring to is vague.

Of course, we are only testing survivors, as would be the case for any experiment performed on a living organism. Our data do not directly address the 'tolerance' or 'performance' of the non-surviving members of a population, which in and of itself, doesn't make sense. Assuming that some fish from a population don't survive because they are 'less tolerant' is quite the speculation. There are many reasons why fish die. Anytime organisms are sampled from a population for study, a fundamental assumption is always that the fish sampled and the variation measured, is representative of the population.

(4) Since the upper range value of this estimate is near 25°C, are additional data points (from exposures at temperatures >25°C) necessary to produce a curve that more precisely estimates an optimum temperature or range for aerobic scope?

Fish began dying at 25°C. We know from the voluminous literature on the thermal limits of fishes under test conditions and time courses of exposure similar to those used here that more fish would die beyond 25°C. It is possible that some individuals may have survived at warmer temperatures, but the purpose of the study design was not to kill fish. If you are interested in revealing the fundamental mechanisms underlying fish failure at high temperatures (which is largely the focus of the OCLTT debate and of Clark et al. 2013a), you must explore the limits of these curves. If you are interested in evaluating protective thermal management targets and you are constrained by permitting, testing fish approaching lethal limits is unethical. The range of temperatures over which fish demonstrate performance capacity is what is important because a fish in nature does not spend its entire life at an optimal temperature for aerobic scope.

(5) Are these findings common in aerobic scope studies?

Our study findings are consistent with the results from other aerobic scope studies (e.g. Parsons 2011).

(6) How does the imprecise fit of the aerobic scope curve affect the reliability of the estimates of optimal temperature for aerobic scope, the comparability of the results with other studies, or its use in setting protective temperature criteria?

Dealt with above in the "Comments of the reviewers suggest a fundamental lack of understanding of statistics" section.

(7) How did the steps taken (repeated stimulation with high-velocity bursts to encourage swimming) affect the aerobic scope results, and the estimates of the optimal temperature for aerobic scope?

This is a critique that we could have made mention of more clearly in the report. More than one method to elicit and measure MMR is regularly used in aerobic scope studies, appears as a suitable method in the peer-reviewed literature, and the validity of our application of it here is further evidenced by the peer-reviewed publication of our research (Verhille et al. 2016). Please see the published figures which include different symbols for the different methods to acquire MR. Visual inspection shows that there was no pattern between method and result.

(8) We noted the maximum metabolic rate was found to be lowest for fish exhibiting this behavior (p. 19); did this affect the precision of the maximum metabolic rate and aerobic scope results?

Please see Verhille et al. 2016, Figure 1. Visual inspection shows that there was no pattern between method and result. Note, an underestimate of MMR would lead to a reduced AAS which would be an overly conservative result and could not lead to an overestimate of metabolic capacity.

(9) How does using an exponent from a fish other than *O. mykiss* affect the aerobic scope results, the estimates of optimal temperature for aerobic scope, or the comparability of the results with other studies?

Scaling metabolic data has no internal effect on our study because the range of body mass of our fish was too small to use a scaling exponent or an ANCOVA with body mass. However, what scaling does allow for is a better comparison with existing data where body mass varies AMONG studies, as is the case when we compare our results with other data for our quality control purposes and interpretation. In fact, few data exist for active and resting mass exponents for a rainbow trout population at the size range investigated in this study. Wieser (1985; JEB 118:133-142) determined mass exponents of 0.08 to 7 g rainbow trout at much cooler temperatures (4°C to 12°C), and found similar mass exponents to what we applied. We applied a mass exponent of 0.95, which is the midpoint between the resting and active mass exponents reported in Lucas et al 2014, and, according to Wieser (1985), the midpoint between the resting and active mass exponents for fish at 12°C was 1.03. The decision to base the mass exponent on the more recent work by Lucas et al (2014), despite it being a different species, was based on methodologies. Active metabolic rate was achieved by electrical shock in the Wieser (1985) work, whereas, Lucas et al. (2014) provoked activity through manual chases, which we considered to be a more similar approach to our critical swimming velocity tests.

(10) Did these fish undergo aerobic testing prior to death, and if so what were the exposure temperatures?

We did not report any metabolic data for these fish because none were taken, see Appendix 4 of the report. Chlorine is part of our tunnel cleaning procedure and we made a terrible mistake in not neutralizing this fully before introducing fish W37 and W 38 into the tunnels for testing. They were dead before any measurements were made. The third fish died after the test, and again the data were not used in mathematically deriving the curve for aerobic scope. It has been known since the 1950's that fish can exhaust themselves at high temperature and experience delayed mortality as a consequence.

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# DISTRICTS' RESPONSE TO NMFS COMMENTS ON THE DRAFT REPORT FOR THE THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

## ATTACHMENT A

## NMFS' FEBRUARY 2017 COMMENT LETTER

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**UNITED STATES DEPARTMENT OF COMMERCE**
**National Oceanic and Atmospheric Administration**
NOAA FISHERIES SERVICE
WEST COAST REGION
650 Capitol Mall, Suite 5-100
Sacramento, California 95814-4706

February 6, 2017

In response refer to:
WF:P-2299/P-14581.WCR:FERC

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

Re: National Marine Fisheries Service's Comments on the Study, "*Thermal Performance of Wild Juvenile Oncorhynchus mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature* (Farrell *et al*. 2015), is filed to the Administrative Record of the Federal Energy Regulatory Commission's Projects, New Don Pedro (P-2299) and LaGrange (P-14581), located on the Tuolumne River, California.

Dear Secretary Bose:

NOAA Fisheries Service (NMFS) provides our Review of the Study, *Thermal Performance of Wild Juvenile Oncorhynchus mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature* (Farrell *et al*. 2015). NMFS' Review is hereby filed to the Administrative Records of the Federal Energy Regulatory Commission's Projects, New Don Pedro (P-2299) and LaGrange (P-14581), located on the Tuolumne River, California.

If you have any questions regarding the attached document, please contact William Foster (916-930-3617).

Sincerely,

Steve Edmondson
FERC Branch Supervisor
NMFS, West Coast Region

Document Attached
CC: FERC Service Lists for P-2299 an P-14581

# A REVIEW BY
# THE NATIONAL MARINE FISHERIES SERVICE
# CALIFORNIA CENTRAL VALLEY OFFICE

## INTRODUCTION

The following is a review by the National Marine Fisheries Service (NMFS) of *Thermal Performance of Wild Juvenile Oncorhynchus mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature* (Report) (Farrell et al. 2015), prepared for the Turlock Irrigation District (TID) and the Modesto Irrigation District (MID). The investigations were conducted in 2014, for a water and aquatic resources (W&AR) study during the Federal Energy Regulatory Commission (FERC) re-licensing proceeding for the Don Pedro Hydroelectric Project (FERC No. 2299). In study W&AR-14 (Enclosure A), TID and MID plan to collect information about the site-specific temperature responses of steelhead or rainbow trout (*Oncorhynchus mykiss*), for use in a reassessment of the thermal guidelines (EPA 2003) applied in the lower Tuolumne River and elsewhere in California's Central Valley. It is expected that TID and MID will file either the Report or a modified version in the administrative record for the Don Pedro FERC re-licensing proceeding.

Both resident *O. mykiss* (i.e. "rainbow trout") and the anadromous life-history form (i.e. "steelhead") exist in the Tuolumne River downstream of the La Grange Dam (Zimmerman et al. 2009). The Tuolumne River steelhead belong to the California Central Valley (CCV) steelhead distinct population segment (DPS), listed as "threatened" under the federal Endangered Species Act (ESA) (Federal Register Notice, January 5, 2006). The juvenile offspring of steelhead also belong to the DPS, and are protected under the ESA even prior to their seaward migration. Due to the impassable La Grange Dam (height 131 feet), CCV steelhead have access to only the fifty-two miles of the "lower Tuolumne River" from the San Joaquin River upstream to the Dam; this segment is ESA-designated "critical habitat" for the CCV steelhead DPS (Federal Register Notice, September 2, 2005).

For the CCV steelhead, the model of Lindley et al. (2006) predicts the historical habitat suitable for the species was limited by summertime conditions; due to the relatively lower elevations and warmer conditions in the lower Tuolumne River, the model predicts all 324 km (201 miles) of historically-suitable habitat was in the higher and colder upper Tuolumne River watershed (Lindley et al. 2006, pp. 9-10). Present-day dams that impound large reservoirs can maintain higher base flows and lower stream temperatures for some distance below the dam during summer than would be found in those reaches in the absence of the dam. In the Tuolumne River, flows from the drainage area above the La Grange Dam are impounded in four reservoirs: Hetch Hetchy, Lake Eleanor, Cherry Lake, and Don Pedro. The Don Pedro Reservoir is a short distance (2.6 miles) upstream of the La Grange Dam, and holds a large capacity of nearly 2 million acre-feet. Cold releases from Don Pedro quickly transit the small, riverine reservoir impounded by the

La Grange Dam, where in summer large volumes are diverted from the lower Tuolumne River into canals, appreciably lowering the flows downstream.

The NMFS is concerned about flow management that could affect the adult and juvenile steelhead in the lower Tuolumne River, including the application or modification of water temperature criteria, standards, or basin-plan beneficial uses by anadromous fishes. The goal of the NMFS is to recover the CCV steelhead DPS, including the Tuolumne River population. Because water temperature affects the health of individual fish, it also affects entire populations and species assemblages (Richter and Kolmes 2005). In reviewing the Report, we sought to understand how the temperature-dependent aerobic scope responses of individual juvenile *O. mykiss* might inform actions to improve the Tuolumne River steelhead population, and thereby promote recovery.

## STUDY BACKGROUND

Swimming tunnel respirometer experiments were conducted with juvenile *O. mykiss* in a streamside facility on the lower Tuolumne River, downstream of the La Grange Dam (river mile (RM) 52). The test fish (100-200 millimeter fork length, N=48) were captured in the following lower Tuolumne River locations (number): RM 50.7 (36); RM 51.6 (8); RM 50.4 (2); and RM 49.1 (2). Water pumped from the River was passed through the test facility, while its temperatures were variably controlled between 13 degrees Centigrade (°C) to 25°C. An individual fish was placed in the swim tunnel, and water velocities were adjusted to create the equivalent of an “aquatic treadmill” that required it to swim at various speeds. To measure the oxygen consumed by a fish during a test, the exchange of fresh (aerated) water with the tunnel was intermittently turned off; the rate of oxygen-level decline within the closed chamber provided an estimate of the fish’s aerobic metabolism (at the given activity level and water temperature).

The experimental approach adopts the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis, which proposes that the extremes of thermal tolerance are set by an animal’s inability to supply oxygen to its tissues above and beyond a basic routine need. “Aerobic scope” is the difference between the minimum and maximum oxygen consumption rates. Accordingly, the Tuolumne River tests measured how much oxygen was routinely consumed by a resting fish, its routine metabolic rate (RMR)[1], followed by tests of how much oxygen could be maximally extracted from the water by a fast-swimming fish, termed its maximum metabolic rate (MMR). By subtracting RMR from MMR, the difference is the “absolute aerobic scope” (AAS) for a test

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[1] NMFS notes that the oxygen consumption rate for fish as measured in lab experiments is not equal to the metabolic rate (Nelson 2016). It may be time to start considering *M*O2 as its own measurement and not a surrogate for metabolic rate. As the price and availability of better technology for direct calorimetry of aquatic organisms improves, more laboratories will want to use it and the term metabolic rate should be reserved for their results. As these direct calorimetric results on fish accumulate, more informed comparisons between the two techniques will be made and the understanding of energy flow through fishes improved. Energy, after all, is the currency of fish life and its accurate measurement has implications across the entirety of fish biology (Nelson 2016).

fish - a measure of the capacity of a fish to supply oxygen to its tissues above and beyond a basic routine need.

The Report explains the biological significance of aerobic scope is that AAS defines a fish's capacity to perform the activities essential to carry out its life functions, such as swimming, catching prey and feeding, digesting a meal, growing, avoiding predators, and defending territory (p. *i*; p. 4, pp. 21-22). Consistent with the OCLTT hypothesis, the Report provides Figure 1 (p. 33), a schematic plot depicting how theoretical aerobic scope values (y-axis) vary with water temperatures (x-axis); the point where the aerobic scope versus temperature curve reaches its peak is defined as the optimal temperature ($T_{opt}$). The authors also compute factorial aerobic scope (FAS = MMR/RMR), a ratio described as another way of expressing a fish's aerobic capacity across a range of water temperatures; the authors explain that FAS values of 2 or greater are biologically significant because they indicate an aerobic capacity sufficient for a fish to properly digest a full stomach (p. *ii*; pp. 4-5, p. 20, pp. 21-22), potentially grow faster at a higher temperature (p. 22), or maintain station in a flowing river (p. 21).

The aerobic scope test results were used to determine an optimal temperature range for the Tuolumne River *O. mykiss* population, via plots of the swim tunnel metabolic measurements versus test temperatures, using best-fit mathematical models (lines or curves) along with their confidence intervals (Figure 4, p. 37). The experiments yielded a $T_{opt}$ for AAS of 21.2°C, an average AAS that remained within 5% of this peak from 17.8°C to 24.6°C, and FAS values > 2 for all fish up to 23°C (p. *ii*, pp. 23-24). The study results were compared with the 18°C criterion[2] recommended by the U.S. Environmental Protection Agency (EPA 2003), and the Report concluded the test data "…provide sound evidence to establish alternative numeric criteria that would apply to the Tuolumne River *O. mykiss* population below La Grange Diversion Dam." (p. 24). The Report recommends "…that a conservative upper performance limit of 22°C, instead of 18°C, be used to determine a 7DADM value for this population." (p. 24).

## COMMENTS AND CONCERNS

### The Tuolumne River experiments do not test the OCLTT hypothesis; rather, this hypothesis is adopted as if it were widely accepted that aerobic scope alone defines a fish's capacity to perform its essential life functions.

The theoretical rationale for the OCLTT hypothesis holds that the biochemical and physiological capacities of fishes have evolved such that aerobic scope is maximized within a given temperature range ($T_{opt}$ for AAS) in order to optimize fitness-related performance, such as growth, reproduction and locomotion, while performance diminishes as aerobic scope decreases at higher and lower temperatures (Clark et al. 2013a). We found the Report's experimental

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[2] The EPA recommends 18°C for the protection of Salmon/Trout Migration plus Non-Core Juvenile Rearing, where "Trout" refers to Steelhead and coastal cutthroat (EPA 2003, p. 25). The numeric criterion value (18°C) is the recommended maximum of the 7-day average of the daily maximum temperature (7DADM).

approach and framework relied heavily on acceptance of the OCLTT hypothesis as an established fact or law:

> "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. (p. *i*).
>
> "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." (p. 4).
>
> "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)." (p. 5).

The Report asserts that aerobic scope <u>defines</u> a fish's capacity to perform its essential life functions:

> "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.). (p. 4).
>
> "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, <u>AAS defines a fish's capacity to perform the activities essential to carry out its life functions</u>." (p. 4). [Underline emphasis added].

While the Report adopts the OCLTT hypothesis, we observe that all experts do not agree with a wide application of the theory, and a healthy debate is ongoing about the ecological relevance of aerobic scope to overall fitness (Clark et al. 2013a; Farrell 2013; Pörtner and Giomi 2013; Clark et al. 2013b). Clark et al. (2013a) warn that the OCLTT hypothesis should not be assumed to be a law, but should undergo more testing (like all hypotheses). Clark et al. (2013b) observe that the temperature where aerobic scope is maximal ($T_{opt}$ for AAS) cannot be assumed to be the same as the optimal temperature for a fish ($T_{opt}$); they propose that the "onset of loss of performance" of an animal can occur where aerobic scope is maximal because other metrics of performance can be markedly reduced at the $T_{opt}$ for AAS. What seems a reasonable alternative to the OCLTT hypothesis is that the optimal temperature of the animal will be context dependent, governed by interacting optimal temperatures of different physiological and ecological performance metrics (Clark et al. 2013b).

The studies of Hokanson et al. (1977) illustrate how different thermal optima can exist for different endpoints. In this research, 50-day laboratory tests were conducted with *O. mykiss* fed excess rations and exposed to seven constant temperatures (between 8 and 22°C) and six diel temperature fluctuations (±3.8°C about mean temperatures from 12 to 22°C). For constant temperature treatments, the maximum specific growth rate occurred at 17.2°C, with an average specific mortality rate of 0.35% per day at this optimum temperature and lower. Specific growth rate at a fluctuating temperature of 22.2±3.8°C was zero, and mortality rate was 42.8% per day during the first 7 days. In experiments under a fluctuating regime of 15.5 to 17.3°C, the average specific mortality was 0.36% per day. Combining data on specific growth and mortality rates, the authors were able to predict the yield for a hypothetical population under the temperature regimes; they determined a population would exhibit zero increase over 40 days at a constant 23°C and a fluctuating temperature of 21±3.8°C because under these temperature conditions, specific growth rate balances specific mortality rate. Considering these laboratory results and corroborating field information, they recommended a mean weekly temperature of 17±2 °C for *O. mykiss*, so that maximum yield is not appreciably reduced for populations living in the fluctuating temperature regimes normally found in the natural environment. In their results interpretation, Hokanson et al. (1977) considered both growth and survival endpoint responses (and their interaction), recognizing that population production involves a balance between individual growth and mortality rate of the population.

The temperatures affecting *O. mykiss* smoltification must also be considered to assess steelhead production. Smoltification involves the physiological, morphological, biochemical, and behavioral changes before and during seaward migration and ocean entry. Myrick and Cech (2005) studied food consumption and growth endpoints versus temperature in *O. mykiss* (from the Nimbus State Fish Hatchery, American River, California); while they found temperatures approaching 19°C would increase growth rates, they referred to multiple studies suggesting that if juvenile steelhead are exposed to temperatures much above 11°C during the presmolt and smolt periods, their ability to osmoregulate and survive in salt water would be significantly impaired. They recommended study of how long juvenile *O. mykiss* can be exposed to higher temperatures before returning to temperatures that are better suited for smolting (p. 328). The EPA (2003) recommends a maximum 7DADM of 14°C for the protection of waters where and when the early stages of steelhead smoltification occurs or may occur (p. 31). The Report found aerobic scope maximal at 21.2°C, with an average that remained within 5% of this peak from 17.8°C to 24.6°C; however, when *O. mykiss* smoltification is included as an endpoint these temperatures are much higher than those deemed protective.

The CCV steelhead simulation model of Satterthwaite et al. (2010) incorporates survival-based as well as growth-based variables. Their model suggests the greatest management concern with respect to preserving anadromy is reduced survival of emigrating smolts during their downstream migration (freshwater, estuarine, and ocean). They emphasize the potential importance to *O. mykiss* anadromy of large changes in both freshwater survival or growth rates; both factors are emphasized, and both are affected by temperature. Several other variables are also at play in the model, including a temperature-dependent measure of basal metabolic rate (Table 1, p. 4).

In reviewing the Report, we found the need for much more explanation of how aerobic scope (the difference between resting and maximum metabolic rates) by itself can be assumed to <u>define</u> a fish's capacity to carry out its essential life functions like swimming, defending territory, catching prey and feeding, digesting a meal, growing, and avoiding predators (p. 4), or of how this capacity or potential translates to vital rates (e.g., size-based fecundity, mortality rate, etc.) that affect population-level status. As stated earlier, the goal of the NMFS is to increase the Tuolumne River steelhead population. If aerobic scope data can promote this goal, we need to better understand how it is linked to the field-based, population-level data used to determine steelhead viability (Lindley et al. 2007). In Enclosure B, we provide a reproduced table from McCullough et al. 2009 (p. 92) that arranges research topics concerning the thermal biology of fishes within their various levels of biological organization. We used the table to place the Tuolumne River aerobic scope experiments in the context of other research that investigates physiological or ecological endpoints. Assessed in this broader context, the Tuolumne River experiments appear to evaluate *O. mykiss* response at the physiological level (aerobic scope performance) and extrapolate the biological significance of the test results across levels #2, #3, and #4 (consistent with the acceptance of OCLTT theory); the Report concludes its test results warrant a new water quality criterion for the lower Tuolumne River (#5 policy-level implications). The Report is not clear about the linkages; how is the aerobic scope performance of individual Tuolumne River *O. mykiss* a sufficient measure of population-level fitness under the thermal regime? How is the singular aerobic scope measure sufficient to establish a protective water temperature criterion?

Clark et al. (2013a) observe that aerobic scope is likely to be an important mechanism contributing to the ecology of fishes because it should set the limit for the magnitude of oxygen-demanding processes that can be performed simultaneously. However, they caution that aerobic scope may continue to increase until temperature approaches lethal levels and then decline rapidly as death ensues, thereby providing little insight into the preferred temperature or performance of aquatic ectotherms (p. 2771). MMR has often been reported to first increase with temperature, then plateau or even decrease due to detrimental effects of high temperature on convective oxygen transport (Fry & Hart, 1948; Farrell et al., 2009; Portner, 2010, as cited in Norin and Clark 2016), causing a bell-shaped relationship between aerobic scope and temperature. It is becoming clearer, however, that MMR, AAS, or both does not always plateau or decrease at high temperatures in fishes (Norin and Clark 2016). Many studies show that in general, fishes appear to exhibit a continuous increase in MMR, AAS, or both until the point where temperature is at the upper end of the natural range and close to or at lethal levels (Norin and Clark 2016), including the study on the Central Valley Chinook salmon juveniles (Poletto et al. 2016). Recent evidence suggests that there is considerable individual variation in the thermal sensitivity of MMR, AAS, or both (Poletto et al. 2016), with high-MMR individuals responding less to heating compared with low-MMR individuals (Norin and Clark 2016). In fact, the aerobic scope for the Central Valley juvenile Chinook salmon continues to increase at water temperatures up to 25 or 26°C, where substantial mortality occurred (Poletto et al. 2016). We are concerned that negative effects also could occur at temperatures much lower than near-lethal levels, where aerobic scope is continuing to increase with temperature.

As explained below, the NMFS does not support the use of any single performance metric (aerobic scope or another measure) to determine protective water temperature criteria. We support using multiple lines of evidence gathered from studies of several physiological and ecological measures (EPA 2003; McCullough et al. 2009).

**The Report makes numerous comparisons between its Tuolumne River aerobic scope results and the 18°C summer criterion recommended by the EPA (2003), but the EPA's criterion was derived from several types of studies and not aerobic scope experiments.**

The Report establishes how metabolic rate measurements and related computations (e.g., AAS, FAS, etc.) were used to derive an optimal (peak) temperature and optimal temperature range for aerobic scope. Then, in numerous instances these numerical results are compared and contrasted with the EPA's 18°C criterion. These comparisons appear to assume the EPA's criterion was based on aerobic scope studies, but our understanding is the EPA (2003) did not use aerobic scope information to establish its recommended summer 18°C criterion. The Report does not establish how EPA used aerobic scope information to develop their criterion, or if they used aerobic scope information at all. It also does not adequately acknowledge or discuss the other types of thermal suitability information the EPA (2003) used to derive its 18°C criterion. For these reasons, we found several of the Report's comparisons to be invalid, and list examples below:

> "Interestingly, by setting the 7DADM criterion for salmon and trout migration as 20°C, rather than 18°C, EPA (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." (p. 2).
>
> "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." (p. 5).

> "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… Maximum metabolic rate (MMR) will increase with test temperature and reach a peak around 18°C according to the EPA criterion…Absolute aerobic scope (AAS) has a $T_{opt}$ around 18°C according to the EPA criteria…AAS will decline at a temperature just above 18°C…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." (p. 6).
>
> "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." (p. 15).
>
> "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." (p. 20).
>
> "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." (p. 20).
>
> "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)." (p. 24).

In these examples, the Report suggests the EPA (2003) conducted or reviewed aerobic scope experiments using *O. mykiss* from the Pacific Northwest, and these test data were used in the derivation of its 18°C criterion. If this is so, the Report should describe or cite the experiments the EPA conducted or considered, what the results were, and how the EPA used the aerobic scope data to derive its 18°C criterion. The EPA also considered the results of several other types of studies to establish its thermal criteria (EPA 2003, Table 1, p. 16), and so the Report should at least discuss them when comparing recommendations.

**We reviewed the Report in the light of the recommendations of Clark et al. (2013a), intended to ensure that swim-tunnel aerobic metabolism measurements are sound and comparable between studies.**

We have not conducted aerobic scope experiments ourselves, and so the list below is to highlight areas where we did not fully understand the study design, methodological approach or data analysis.

- Two *O. mykiss* individuals were tested at each exposure temperature, rather than testing the same individual over the range of exposure temperatures. Clark et al. (2013a) note the latter design allows repeated-measures analyses. Can you explain this study-design decision, and how it might have affected the resulting estimates of the optimal temperature for aerobic scope?

- The Tuolumne River temperatures where test fish were captured ranged between 12.7 and 17.1°C, and the fish were held in tanks (prior to testing) in Tuolumne River water between 12.5 and 13.6°C (p. 9). The time from fish capture in the river to placement into holding tanks ranged up to 2 hours, and fish remained in holding tanks for up to another 3 hours before transfer to a swim tunnel respirometer. In the swim tunnel, fish were held for another 1 hour, underwent a 1-hour training swim, and then recovered for 1 hour, all at 13±0.3°C. Then, water temperatures were increased to the test temperature for each pair of fish (ranging from 13 to 25°C), at least 8 hours before swimming tests began. (p. 10). Experts have noted the physiological mechanisms of thermal acclimation in modulating aerobic scope are not well understood (Clark et al. 2013b, p. 2778). Could the short-term (several hours of) temperature acclimation described above have affected the test results, or are longer acclimation intervals (e.g. weeks) required to affect aerobic scope responses?

- Experts also note there appear to be few studies of the potential thermal acclimation of metabolism during long-term exposures (Clark et al. 2013b, p. 2778). The test fish were sampled from the small population of age-1 and older survivors of exposures to the warm thermal conditions in the lower Tuolumne River, over one or more summers. It seems the aerobic scope responses of the less-tolerant individuals in a population are relevant if this response information is to be used to establish thermal criteria protective at the population level. Obviously the individuals that do not survive (over one or more summers) could not be tested with the field-based study design used. How could the long-term temperature acclimation experienced by the individuals tested, and the inability to test the less tolerant individuals in the Tuolumne River population, have affected the test results?

- Clark et al. (2013a) recommend conducting aerobic scope tests beyond what the fish experience in nature. We understand ESA collection permit restrictions prevented tests at temperatures higher than 25°C (p. 15), but these warmer conditions occur often in the lower Tuolumne River. How did this experimental constraint affect the test results? For example, the experiments yielded an average aerobic scope that remained within 5% of optimum from 17.8°C to 24.6°C. Since the upper range value of this estimate is near 25°C, are additional data points (from exposures at temperatures >25°C) necessary to produce a curve that more precisely estimates an optimum temperature or range for aerobic scope?

- We noted the imprecise fit ($r^2 = 0.099$) of the curve through the aerobic scope data points (Figure 4, p. 37). In contrast, the fit of the curve for the resting (routine) metabolic rate was good ($r^2 = 0.8$), and better than for maximum metabolic rate ($r^2 = 0.49$). Are these findings common in aerobic scope studies? How does the imprecise fit of the aerobic scope curve affect the reliability of the estimates of optimal temperature for aerobic scope, the comparability of the results with other studies, or its use in setting protective temperature criteria?

- During tests at faster swimming speeds (to determine maximum metabolic rate), roughly 50% of the test fish used their caudal fin to prop themselves on the downstream screen to avoid swimming (p. 12). How did the steps taken (repeated stimulation with high-velocity bursts to encourage swimming) affect the aerobic scope results, and the estimates of the optimal temperature for aerobic scope? We noted the maximum metabolic rate was found to be lowest for fish exhibiting this behavior (p. 19); did this affect the precision of the maximum metabolic rate and aerobic scope results?

- To account for individual variation in body mass, metabolic rate was corrected for fish mass using the exponent 0.95, a value halfway between the life-stage-independent exponent determined for resting and active zebrafish (p. 11). We noted Cech et al. (1990) use the exponent 0.67 in their tests with multiple species, including *O. mykiss* (p. 97). How does using an exponent from a fish other than *O. mykiss* affect the aerobic scope results, the estimates of optimal temperature for aerobic scope, or the comparability of the results with other studies?

- Appendix 2 (p. 47) lists two fish mortalities (W37 and W38) that were attributed to chloride residue in the swim tunnels, but it is not clear how this cause of death was determined. Did these fish undergo aerobic testing prior to death, and if so what were the exposure temperatures?

**The Report's conclusions are unclear about how the aerobic scope results are to be used.**

As mentioned in the "Introduction," the Tuolumne River investigations were conducted as part of a study (W&AR-14) for the FERC re-licensing proceeding, one that intends to provide information to reassess the temperature guidelines (EPA 2003) for protection of the *O. mykiss* in the lower Tuolumne River. In this context, we found the wording of the Report's "Conclusion" (p. 24) could be interpreted to recommend either:

1) A maximum 7DADM value of 22°C should be applied as the new summer criterion to protect the lower Tuolumne River *O. mykiss* population, replacing the EPA's recommended maximum 7DADM of 18°C.

2) The aerobic scope study results (including the upper performance limit of 22°C) should be used in a new criterion-setting process, to determine an alternative to the 18°C criterion recommended by the EPA.

Below, we consider both possible interpretations of the Report's conclusions, and provide comments and suggestions for each.

**If the Report's conclusion meant to recommend replacement of the EPA's current summer criterion (a maximum 7DADM of 18°C) with one based on the Tuolumne River aerobic scope studies (a maximum 7DADM of 22°C), we disagree.**

The Report's "primary purpose" statement establishes that the swim tunnel test data (metabolic rate measurements and related computations, e.g., AAS, FAS, etc.) were the single line of evidence for determining <u>the</u> optimal temperature range for juvenile *O. mykiss* in the Tuolumne River:

> "The primary purpose of this study is to determine the thermal performance of the subadult (100-200 mm fork length; FL) *O. mykiss* population inhabiting 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.). <u>Thus, MR measurements were used to determine **the** optimal temperature range for Tuolumne River *O. mykiss*</u>." (p. 4). [Underline and bold emphasis added].
>
> "In the present study, we use 95% of the peak AAS value to set <u>**the**</u> optimal thermal range (Figure 1; the two temperatures that bracket $T_{opt}$ are termed a Pejus temperature, $T_p$)." (p. 5). [Underline and bold emphasis added].

The Report's sole reliance on aerobic scope data is justified by an assertion that AAS <u>defines</u> a fish's capacity to perform essential life function activities (p. 4). We do not find adequate justification in the Report (or referenced therein) to agree that aerobic scope information alone defines a fish's capacity to carry out its life functions. As discussed above, more explanation is warranted about how a measure of the aerobic <u>capacity</u> to perform a life-history function (in short-term tests, under a given temperature regime) is adequate to assess how well a temperature regime promotes population-level fitness in the natural environment.

The EPA's (2003) criterion-setting approach for juvenile salmon and *O. mykiss* considered multiple lines of evidence gathered from studies of several physiological and ecological metrics. These included:

- The water temperatures (over a one-week exposure) causing death;

- The optimal water temperatures for growth, under limited and unlimited food supplies;
- The thermal preferences of rearing juveniles, in both laboratory and field studies;
- The water temperatures causing impairments to juvenile smoltification; and
- The water temperatures leading to minimal, elevated, and high disease risks.
  (EPA 2003, Table 1, p. 16).

The EPA noted the importance of ecological as well as physiological considerations when establishing numeric water temperature criteria (EPA 2003, p. 18). Accordingly, they considered not only "physiological optimum temperatures" (where functions like growth, swimming, heart performance, etc. are optimized), but also "ecological optimum temperatures" which they described as those where fish do best in the natural environment - considering food availability, competition, predation, and fluctuating temperatures. The EPA summarized their considerations for salmon and trout juvenile rearing:

> "EPA looked at both laboratory and field data and considered both physiological and ecological aspects. Optimal growth under limited food rations in laboratory experiments, preference temperatures in laboratory experiments where fish select between a gradient of temperatures, and field studies on where rearing predominately occurs are three independent lines of evidence indicating the optimal temperature range for rearing in the natural environment. As highlighted in Tables 1 and 2 (and shown in detail in the technical issue papers[3]) these three lines of evidence show very consistent results, with the optimal range between 8 - 12°C for bull trout juvenile rearing and between 10 - 16°C for salmon and trout juvenile rearing." (EPA 2003, p. 19). [Footnote added].

The EPA recommended a maximum 16°C 7DADM for salmon and trout "core" juvenile rearing, in a river basin's mid-to-upper reaches with moderate to high juvenile densities. The 16°C criterion is to: (1) safely protect juvenile salmon and trout from lethal temperatures; (2) provide upper optimal conditions for juvenile growth under limited food during the period of summer maximum temperatures and optimal temperatures for other times of the growth season; (3) avoid temperatures where juvenile salmon and trout are at a competitive disadvantage with other fish; (4) protect against temperature-induced elevated disease rates; and (5) provide temperatures that studies show juvenile salmon and trout prefer and are found in high densities (EPA 2003, p. 27). As mentioned earlier, the historical habitat suitable for summer-rearing *O. mykiss* was in the higher and colder upper Tuolumne River watershed (Lindley et al. 2006). In the Tuolumne River, the La Grange Dam (at RM 52) currently blocks the *O. mykiss* in its lower elevations from reaching the upper watershed.

The EPA recommended a maximum 18°C 7DADM criterion for salmon and trout migration plus "non-core" juvenile rearing, recognizing that juvenile salmonids will experience waters that have a higher temperature than their optimal thermal range. This criterion is to: (1) safely protect

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[3] The 3-year interagency cooperation that produced *EPA Region 10 Guidance for Pacific Northwest State and Tribal Temperature Water Quality Standards* (EPA 2003) also produced 6 scientific issue papers, 2 scientific peer review panel reports, and the integration of public comments.

against lethal conditions for both juveniles and adults; (2) prevent migration blockage conditions for migrating adults; (3) provide optimal or near optimal juvenile growth conditions (under limited food conditions) for much of the summer, except during the summer maximum conditions, which would be warmer than optimal; and (4) prevent adults and juveniles from high disease risk and minimize the exposure time to temperatures that can lead to elevated disease rates (EPA 2003, p. 28). This criterion is applicable in a river basin's mid-to-lower reaches, with moderate to low juvenile densities (downstream of the core juvenile rearing reaches).

In the Tuolumne River, all of the habitat currently accessible to rearing juvenile *O. mykiss* is "non-core" (due to the impassable La Grange Dam). This point is highly relevant to the management of the thermal regime in the lower Tuolumne River because its steelhead population is now confined to the lower reaches, where flow and temperature management must accommodate both its fall-run Chinook salmon and its steelhead. While individual *O. mykiss* that express the steelhead life history smolt and migrate to saltwater, they do so only after remaining in freshwater (and over-summering) for 1 to 3 years. In contrast, fall-run Chinook juveniles are predominantly "ocean-rearing" fish that reside a shorter time in freshwater and do not over-summer in-river. Myrick and Cech (2004) recognize how the extended, over-summer residence time before smolting for *O. mykiss* (compared to Chinook salmon) make them more vulnerable to alterations of the natural thermal regime, and to the management of thermal regimes designed to protect juvenile fall-run Chinook salmon (p. 115).

As mentioned above, the EPA also recommend a maximum 7DADM of 14°C for *O. mykiss* smoltification. The interval for *O. mykiss* smoltification in the lower Tuolumne River could extend from spring to early summer. Dams and diversions in the watershed cause large-scale alterations in the magnitude, frequency, timing, duration, and rate-of-change of streamflows in the lower Tuolumne River (McBain and Trush 2000; Anderson 2009), and these flow alterations affect the temperature regime during the *O. mykiss* smoltification and out-migration intervals (Gordus 2009). Anderson (2009, p. 14) explains how snowmelt floods in the Tuolumne River once provided large-magnitude, sustained flows peaking from late April to early June, then receding in July and August. With the onset of impoundment and diversion, snowmelt floods have been largely eliminated from the annual hydrograph and replaced with FERC license-required spring "pulse flows" intended to stimulate smolt emigration. The snowmelt recession limb of the annual Tuolumne River hydrograph has also been altered; the duration and rate of the snowmelt recession plays an important role in the juvenile rearing and smolt outmigration life stages. Before the large dams and diversions were in place, these recession flows connected the snowmelt peak flows to the summer baseflows, and generally extended into July during dry years and into August in wetter years. The receding limb of the spring pulse flows that now occur in the lower Tuolumne River (under the FERC license) is often early, short, and steep (Anderson 2009, p. 14), resulting in diminished flood plain inundation duration (Gard 2009, Table 1, p. 9) as well as warmer water temperatures in the late spring and early summer months (Gordus 2009).

In their temperature criteria-setting considerations, the EPA (2003) recognized that dams can significantly reduce the river flow rate, thereby causing juvenile migrants to be exposed to high temperatures for a much longer time than they would under a natural flow regime (p. 7).

**If the Report's conclusions are meant to recommend the future development of a site-specific alternative to the EPA's recommended criterion (18°C maximum 7DADM), we do not find adequate explanation of how the Tuolumne River aerobic scope results should be used in that process.**

The California State Water Quality Control Board (State Board) has applied the EPA criteria (2003) to evaluate long-term, field-based assessments of temperature suitability for juvenile *O. mykiss* (and other salmonids) in the lower Tuolumne River[4]; the temperature data and analysis were submitted to the EPA, who found the information supports a listing of the lower Tuolumne River on the Federal Clean Water Act § 303(d) list of impaired water bodies (due to unsuitably high water temperatures for the life stages of anadromous fishes). Consequently, the EPA is requiring the State Board to develop a total maximum daily load (TMDL) for temperature in the lower Tuolumne River, by the year 2021.

The EPA (2003) recognized that situations exist where their general guidance can be supplanted with site-specific criteria (p. 34). The NMFS supports options within EPA's guidance for the development of alternative criteria, where the general numeric criteria are unattainable or inappropriate (Enclosure C). If the Report is recommending the use of its Tuolumne River aerobic scope results in the development of a new temperature criterion, it is not clear how. Neither the Report, nor the Turlock and Modesto Irrigation Districts' Study W&AR-14 (which plans to assess the existing lower Tuolumne River temperature criteria) describe plans or a process to coordinate with the State Board or the EPA to develop alternative criteria.

We do not hold that aerobic metabolism studies alone can define the singular optimum temperature for a fish, or establish protective temperature criteria for *O. mykiss*. Below, we list and discuss topics for consideration in any process to set new temperature criteria or develop a temperature-related TMDL for the lower Tuolumne River.

1. **Review the long-term dataset of water temperatures measured in the lower Tuolumne River during the summer.**

The California Department of Fish and Wildlife collected water temperature data from 1998 through 2006, from several locations in the lower Tuolumne River (Gordus 2009). The data clearly indicate summer temperatures in the lower Tuolumne River exceed the EPA's summer recommendations (2003) for juvenile *O. mykiss* rearing, over several miles of the lower

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[4] The California Department of Fish and Wildlife collected data from 1998 through 2006, using in situ devices (probes) deployed at various locations in the lower Tuolumne River, and that continuously measure and record water temperatures. The analysis of the data applied the EPA criteria (2003) as the benchmarks to determine the impairments of the anadromous fish beneficial uses established by the State Board.

Tuolumne River, and do so across multiple water-year types and summer weather conditions. It is likely additional data are available from years earlier than 1996, and new data have been collected since 2006. This long-term dataset could be used to extend the thermal suitability analysis, for use in any process for setting new temperature criteria. For example, it could be used with flow gage and air temperature data to assess how lower Tuolumne River water temperatures change under various water-year types, discharge regimes (from the La Grange Dam), and atmospheric conditions, including the conditions during the recent California drought.

2. **Review information about the general trends observed in *O. mykiss* distribution and abundance in thermal "zones" of rivers that grade from colder to warmer, including in California.**

The Report discusses the redband rainbow trout (*O. mykiss gairdneri*) found in eastern Oregon and Idaho (p. *ii*., pp. 2-3) where summer temperatures can reach 26ºC or greater (Zoellick 1999). While the <u>presence</u> of this cold-water fish is documented at these high temperatures, studies of its distribution and abundance suggest strong negative correlations between trout densities and stream temperatures. In the John Day River basin (Oregon), Li et al. (1994) found negative correlations between redband trout densities of all age-classes and maximum stream temperatures (Table 3, p. 634), and a negative relationship ($r = –0.71$, $P < 0.05$) between trout biomass:invertebrate biomass ratios and stream temperatures (Figure 7, p. 637). Ebersole et al. (2001) found redband trout in twelve studied northeast Oregon stream reaches, with some individuals present where mean daily maximum temperatures were 25°C; however, the authors reported a negative correlation ($r = –0.70$, $P = 0.01$) between mean trout density and mean daily maximum water temperatures (Figure 3, p. 6). Zoellick (2004) studied the redband trout in two southwestern Idaho creeks, and found trout density negatively correlated ($r = –0.76$, $P = 0.03$) with increases in water temperature in both creeks (Figure 4, p. 24); density and biomass were greater in the stream with lower daily maximum water temperatures (range 18° to 22°C) than in the stream with higher temperatures (range 20.2° to 26°C) (Figure 3, p. 23).

The Report also notes the presence of *O. mykiss* in southern California streams where ambient summer water temperatures routinely exceed 25°C. However, the *O. mykiss* population sizes and densities in these warmer streams and stream reaches are low, and negatively correlated with temperature. Douglas (1995) studied the *O. mykiss* in the Santa Ynez River watershed (Santa Barbara County, California), where extensive runoff occurs during winter rains but the higher flows of winter/spring rapidly diminish by summer. As a result, much of the mainstem Santa Ynez and most of the smaller streams dry up by late summer or fall each year, including areas where winter and spring *O. mykiss* spawning occurs. Surface water at those times is limited to large, isolated pools or groundwater seepage flows in the upper reaches of some streams (p. 9). The author found the juvenile *O. mykiss* density negatively correlated ($r = –0.61$, $P < 0.05$) with the temperature difference between shallow (warmer) and deep (cooler) habitats within the same stratified pools (Table 2, p. 27). Various aspects of temperature (range and maxima for all habitats, and the difference in mean temperatures of shallow and deep pool sub-habitats) showed strong negative correlations (range -0.50 to -0.68) with the density of adult trout (Table 3, p. 28).

This study of *O. mykiss* densities was limited to the upper reaches of the Santa Ynez watershed because mean water temperatures were 21°C a short distance (~2 kilometers) below Bradbury Dam, and then rose to over 25°C farther downstream and reached maxima near 29°C around Lompoc, California (p. 56-58).

Matthews and Berg (1997) examined summer (August) *O. mykiss* distribution in two adjacent pools in Sespe Creek (Ventura County, California) where water temperature reached 28.9°C. In "Pool 1" water temperatures ranged from 21.5°C at the bottom to 28.9°C at the surface. After 5 August, trout were no longer found in this pool, suggesting they had moved out of the high temperature water, or died. In the adjacent "Pool 2" surface water temperatures were as high as 27.9°C, but temperatures on the bottom remained cooler (17.5 to 21.0°C) than in Pool 1, and *O. mykiss* aggregations were consistently observed in this lower stratum throughout the study period despite lower dissolved oxygen values. Regression analysis showed mean *O. mykiss* numbers per pool region were inversely related to water temperature and dissolved oxygen: the mean number of trout per region decreased as dissolved oxygen and temperature increased (significant regression coefficients, $P < 0.05$) (Table II, p. 67; Figure 8, p. 68).

Eaton et al. (1995) assembled a large field-based database, the "Fish and Temperature Database Matching System" (FTDMS), to match spatial and temporal stream temperature records with fish sampling events; this information was used to estimate the yearly temperature regimes used by 30 freshwater fishes common across the U.S. (including *O. mykiss*). An impressive total of 207,846 weekly mean fish/temperature datasets from 29 states were generated and analyzed. The results showed good agreement between assignments of species to coldwater, coolwater, or warm water "guilds" based on earlier laboratory mortality test data. The coldwater "trout-salmon-whitefish" species (including *O. mykiss*) were found at distinctly lower temperatures than the others, comprising the 9 lowest FTDMS data points on a plot including all 30 fishes (Figure 2, p. 13), with an obvious separation (near 23°C) between the coldwater guild and the others.

In a study of 130 sites in California's San Joaquin River watershed (including the Tuolumne River), Moyle and Nichols (1973) used correlative analyses (between environmental variables and species abundances) to identify four distinct fish species "associations." They found the *O. mykiss* (rainbow trout) association overlapped the least with the others; strong correlative relationships were observed between the distribution and abundance of *O. mykiss* and the variables temperature and elevation (which were highly negatively correlated with one another). In general, the "Rainbow Trout Association" was found in the colder, higher-elevation permanent streams; the "California Roach Association" was found in the small, warm intermittent tributaries to the larger streams; the "Native Cyprinid-Catostomid Association" was found in the larger low elevation streams; and the "Introduced Fishes Association" was found in the low-elevation, intermittent streams that had been highly modified.

From 1993 to 1995, Brown (2000) sampled twenty sites in the lower San Joaquin River drainage (including four sites in the lower Tuolumne River) to characterize fish communities and their associations with measures of water quality and habitat quality. His results indicate the native fish community can persist in the human-modified stream reaches below the major foothill dams, but the downstream range of the community appeared to be limited, particularly in the Merced and Tuolumne rivers. Also, although the native community is still present, introduced species may be present at the same sites in low to moderate percentage abundances (p. 263).

We acknowledge that correlative relationships do not establish or confirm causation, and so we sought out studies that examined the linkages between aerobic metabolism and the distribution or abundance of *O. mykiss*. Cech et al. (1990) studied the metabolic responses and tolerance limits of several California fishes to temperature and dissolved oxygen. Their experiments were conducted in flow-through respirometers, with the following test fishes: *O. mykiss*, Sacramento sucker, Sacramento squawfish (common name changed to Sacramento pikeminnow), hardhead, California roach, riffle sculpin, and tule perch; all are native Californian species that comprise recognized species associations. Resting metabolic responses were measured because they were thought to be a basic property of each species, and could be used to compare species under similar conditions of temperature and hypoxia; the authors explain that active metabolic rates would not be comparable because the rate of energy usage during activity depends on the type of activity (thermodynamic path) more than some basic property of individual species (p. 97). When the test results were compared with relevant field observations of occurrence and distributional limits (e.g. Moyle and Nichols 1973), the authors found good correspondence, suggesting a major role for temperature and dissolved oxygen as ecological determinants of California stream fish distributions. Based on their data and other physiological studies, they concluded the effect of temperature on *O. mykiss* metabolism prevents them from inhabiting the squawfish-sucker-hardhead and California roach zones during the warmer seasons (except that *O. mykiss* could be found in these lower-elevation zones if cold outflows existed downstream of dams) (p. 101).

The general trends observed in *O. mykiss* distribution and abundance in the warmer thermal “zones” of rivers and streams, including in California, suggest the species may exist there in reduced numbers, but will not thrive in the warmer conditions. During any process for setting new temperature criteria for the lower Tuolumne River, consideration should be given to the already-limited size of the colder-water zone directly downstream of the La Grange Dam (Gordus 2009), and the potential adverse consequences to *O. mykiss* of further reducing the downstream extent of this zone if warmer criteria are adopted.

### 3. Review the long-term snorkeling data for *O. mykiss* to understand the site-specific distribution of the population in the lower Tuolumne River.

A preferred temperature range is that which the fish most frequently inhabits when allowed to freely select temperatures in a thermal gradient (McCullough 1999, p. 10). Among the independent lines of evidence considered by the EPA during its criteria-setting process (2003) were preference temperatures indicated by laboratory experiments (where fish select between a gradient of temperatures), and field studies indicating the stream locations where juvenile salmonid rearing predominately occurs. The temperatures selected by fish in the field may be important companion studies to conduct alongside aerobic scope experiments because one cannot assume that a fish will occupy or seek out temperatures where aerobic scope is maximal. For example, Norin et al. (2014) found that while the $T_{opt}$ for aerobic scope was 38°C for a tropical fish, when given the opportunity the fish selected a median temperature of 31.7±0.5°C, and spent only 10±3% of the time at temperatures >36°C.

In the Tuolumne River, the respirometer experiments yielded a $T_{opt}$ for aerobic scope of 21.2°C and an average aerobic scope that remained within 5% of this peak from 17.8°C to 24.6°C (p. *ii*, pp. 23-24). However, the *O. mykiss* in the lower Tuolumne River do not appear to seek out temperatures that maximize their aerobic scope. Snorkeling surveys have been conducted annually (sometimes multiple times per year) in the lower Tuolumne River over the past 31 years (1982-2014) (TID and MID 2015). These long-term snorkel survey data (TID and MID 2015, Figure 3; reproduced in Enclosure D) clearly show that *O. mykiss* over-summering in the lower Tuolumne River are found in the colder waters (Gordus 2009) near their upstream migration limit (the La Grange Dam).

This is consistent with the behavior of the redband trout of Eastern Oregon. Anderson et al. (2011) studied their seasonal migrations in the Donner and Blitzen River (Oregon), using radio telemetry and passive integrated transponder (PIT) tags. During both summers of the study, temperatures in the lower river exceeded the 24.3°C ultimate upper incipient lethal temperature of the redband trout (Bear et al. 2007), and approached its 29.4°C critical thermal maximum (Rodnick et al. 2004). While the authors expected to observe adult (mature) trout moving upstream in spring (to find suitable spawning habitats), they did not expect to also observe the long-distance upstream migrations (at the same time of year) by sub-adult (immature) trout (p. 10); they interpreted these movements as actions to find favorable thermal conditions and good foraging opportunities. Gamperl et al. (2002) found that redband trout from the Little Blitzen River and nearby Bridge Creek both exhibited a preferred temperature of approximately 13°C.

A visual overview of Figure 3 (Enclosure D) suggests the lower Tuolumne River *O. mykiss* in summer are consistently clustered in the uppermost ~5 miles (from RM 46.9 upstream to RM 51.6). By performing computations with the survey data from 1997-2014, we found 79% of the *O. mykiss* snorkel observations were in this uppermost 4.7 miles of the lower Tuolumne River. When 1997-2014 snorkel data from only the warmest months (July and August) are considered, 86% of the *O. mykiss* observations were in the upper 4.7 miles. The last two years of available snorkeling data (2013-2014) were recorded in very warm and dry (drought) conditions; in these two years, 96% of the *O. mykiss* observations were in the upper 4.7 miles.

The Report refers to fishery survey data gathered since 1997 to define the extent of "primary rearing habitat" for *O. mykiss*, which the authors consider to be the 12.4 miles from RM 39.6 extending upstream to near the La Grange Dam at RM 52 (p. 1). We adopted the Report's temporal (1997) and spatial (RM 39.6) divisions to further review the snorkel data, and found that surveys from 1997-2014 reported 99% of the *O. mykiss* were observed in the uppermost 12.4 miles of the lower Tuolumne River. This result calls in to question the Report's use of RM 39.6

to delineate the downstream limit of the primary *O. mykiss* habitat; this location appears to define the downstream extent of the total habitat because very few (only 1%) of individuals were observed in the sites downstream of this location. We suggest a more realistic downstream demarcation for the primary *O. mykiss* summer habitat is well above RM 12.4, and closer to RM 5. Also, the Report noted maximum water temperatures at RM 12.4 have exceeded 27°C during the summer months (p. 1), a near-lethal temperature for *O. mykiss*; such high temperatures are a plausible explanation for the upstream, clustered distribution of *O. mykiss* consistently observed in the snorkeling data.

The model produced by Lindley et al. (2006) predicts that all 201 miles of stream habitat in the Tuolumne River watershed suitable for summer occupancy by *O. mykiss* was historically found upstream of the La Grange Dam (which now blocks *O. mykiss* in the lower Tuolumne River from reaching the upper watershed). If RM 12.4 is considered the present downstream extent of suitable summer habitat for *O. mykiss* in the Tuolumne River, then the existing habitat accessible to CCV steelhead is a low 6% of the historical amount. If RM 5 is considered the present downstream extent of suitable summer habitat, then the existing accessible steelhead habitat is an even lower 2.5% of the historical amount. Also notable is that access for juvenile salmonids to rearing habitats in permanently-flowing tributaries has been entirely blocked by the La Grange Dam.

Any process of setting new temperature criteria for the lower Tuolumne River should consider the potential negative effects of reducing even further the size of the already-limited colder water habitat below the La Grange Dam (Gordus 2009).

4. **Review the lower Tuolumne River snorkeling data to understand the abundance of *O. mykiss*, to assess the site-specific population status and trends.**

Our review of the summarized Tuolumne River snorkel surveys suggests the numbers of *O. mykiss* have been, and continue to be, very low under the flow and thermal regimes (TID and MID 2015, Table 2; reproduced in Enclosure E). Over the 31 years monitored, the numbers of *O. mykiss* observed (survey data in all sites combined) averaged 182 per year. We noted the remarkably low numbers from 1987-95, when in 7 of the 9 years surveyed zero individual *O. mykiss* were observed in any of the snorkeled locations, which spanned ~25 miles of the River. During the best two years of this interval, a single *O. mykiss* was observed in 1992, and 3 individuals were observed in 1995 (Table 2).

The more recent data (from 1997 to the present) suggest some (modest) improvement, with the observed number averaging 306 per year (all sites combined). However, the numbers are still very low in most years, and the data are uneven; as recently as 2014, only 53 *O. mykiss* were observed in 12 survey sites that spanned ~20 miles of the lower Tuolumne River.

We understand observations from snorkel surveys will underestimate the actual numbers of over-summering *O. mykiss* present. However, the numbers observed are so low they undeniably suggest a lower Tuolumne River generally depauperate in *O. mykiss*, especially when one considers the long-term duration (30+ years) of the surveys and how they have been conducted over several miles of the lower Tuolumne River.

During any process of setting new temperature criteria for the lower Tuolumne River, the snorkel, water temperature, and flow-gage datasets could be used to further explore relationships between summer flows/temperatures and the abundances and distributions of *O. mykiss*. Fish densities could be calculated (by dividing observed or estimated fish numbers by the river miles surveyed), allowing comparisons with other California and western U.S. streams (Platts and McHenry 1988); the existing snorkel numbers suggest very low salmonid densities in the lower Tuolumne River. Continued monitoring of the responses could occur to evaluate responses as flow release adjustments occur.

5. **Consider that unless the very low size of the total *O. mykiss* population surviving over the summer in the lower Tuolumne River is substantially increased, a viable CCV steelhead population cannot be achieved.**

A common misconception is that declines in anadromous fish populations are caused either by factors in their freshwater habitat or by changes in their marine environment – but the effects are not mutually exclusive (Lindley 2009). Regarding the freshwater effects (i.e., in the lower Tuolumne River), CCV steelhead juveniles must remain in their natal rivers (and over-summer) for 1 to 3 years before they smolt and migrate seaward (Myrick and Cech 2004). Therefore, the in-river conditions during summer (including water temperature) are key factors limiting survival to the age of smoltification.

The lower Tuolumne River snorkeling data indicate the over-summer survival of the total *O. mykiss* population is very low, much too low to achieve a viable steelhead population (measured by adult returns, see Lindley et al. 2007). This is because only a fraction of the (already very low) total juvenile *O. mykiss* population can be expected to smolt; to that fraction, the low natural rates of smolt-to-returning-adult survival must be considered. Mortality during the smolt migration and early ocean occupancy may exceed 95% (Kendall et al 2015). Unless several thousand more sub-adult *O. mykiss* are produced, and survive in the lower Tuolumne River at least one summer until they smolt, only a small number of adult steelhead can be expected to return.

The available data about adult steelhead returning to the Tuolumne River reflect this situation. Since 1940, very low numbers of adults have been observed passing upstream in the lower Tuolumne River. Older accounts report 66 steelhead passed upstream of the former Dennett Dam (RM 16.2) between October 1 and November 30, 1940, and 5 steelhead passed in late October of 1942 (CDFG 1993, in Stillwater Sciences 2013, p. 5-35 to 5-36). More recent counts at the lower Tuolumne River weir (installed in 2009, at RM 24.5) indicate very few steelhead enter the Tuolumne River:

2009: One *O. mykiss* was documented passing upstream on November 7, 2009 (Stillwater Sciences 2013, p. 4-1).

2010: During monitoring from September 9, 2010 to December 1, 2010, no *O. mykiss* were recorded passing upstream through the weir (FISHBIO 2011, p. 11).

2011: Four *O. mykiss* were recorded passing through the weir between September 16, 2011 and December 31, 2011 (FISHBIO 2012, Table 3, p. 11). One individual was recorded as an ad-clipped fish. We note these fish may not have been adult steelhead because of their small sizes, ranging from length 14.2 to 16.5 inches.

2012: Three *O. mykiss* (no adipose fin clips) were recorded passing through the weir between September 24, 2012 and December 31, 2012 (FISHBIO 2013, Table 3, p. 13); however, we note these fish may not have been adult steelhead because of their small sizes, ranging from length 14.5 to 16 inches.

2013: No *O. mykiss* were recorded passing the weir between September 24, 2013 and December 31, 2013 (FISHBIO 2014, p. 11).

2014: No *O. mykiss* were recorded passing the weir between September 29, 2014 and December 31, 2014, (FISHBIO 2015, p. 12); during a second monitoring interval from January 1, 2014 to May 7, 2014, no *O. mykiss* were recorded passing the weir (p. 16).

Collectively, this information suggests that very low numbers of adult steelhead return to the lower Tuolumne River. Although the weir monitoring has mostly focused on the study of fall-run Chinook, the monitoring period of late September through December since 2011 covers the peak immigration of adult steelhead to Central Valley rivers, including the lower Tuolumne River. Within a weir monitoring season, there may be smaller temporal gaps for many reasons, including high flow conditions that may affect weir operations or prevent them altogether. Also, the weir location may be upstream of some existing steelhead spawning habitat (FISHBIO 2015, p. 2). This information suggests that the actual numbers of immigrating adult steelhead may be higher than reported. In addition, rotary screw trap monitoring data indicate that a few to no steelhead smolts leave the lower Tuolumne River (Stillwater Sciences 2013, p. 3-1; p. 4-1), while we acknowledge that screw traps may not be efficient at capturing steelhead smolts.

It is not completely understood why some *O. mykiss* migrate to the ocean while others do not. Life history expression is thought to be partly under genetic control (Pearse et al. 2014), as well as influenced by environmental factors. Sloat and Reeves (2014) investigated (in laboratory stream mesocosms) the influences of individual condition, standard metabolic rate, and rearing temperature on anadromous versus resident life history expression. Given the high relevancy of this study, we provide below an overview of the experimental methods. Juvenile *O. mykiss* spawned from anadromous parents (Clackamas River Hatchery, Oregon) were separated into “dominant” and “subordinate” behavioral groups, based on competitive outcomes. Fish considered “dominant” were the individuals that established and defended feeding territories, while those considered “subordinate” dispersed downstream within the artificial stream mesocosm. Within each separated group, standard metabolic rate was measured in a randomly selected subset of individuals, using intermittent-flow respirometry (in tests similar to the Report’s “swim tunnel” metabolic experiments). Fish belonging to the dominant group were found to exhibit higher standard metabolic rates than fish from the subordinate group. The high and low standard metabolic rate groups were then reared under alternative “warm” and “cold” thermal regimes to complete the experiments. Rearing occurred until fish exhibited phenotypic traits associated with either anadromous (smolting) or resident (sexual maturation) life histories. The warm regime consisted of seasonally adjusted temperatures between 6 and 18°C, and the cold regime ranged between 6 and 13°C. Individual body size was tracked throughout the experiment, to determine how standard metabolic rate, rearing temperature, and individual growth trajectories influenced life history expression. Whole-body lipid content was determined in a random selection of 30 males and 30 females per life history type, per temperature treatment. This lipid data was collected because temperature-driven changes in energy storage may have important consequences for salmonid life histories; juveniles exposed to lower stream temperatures tend to allocate more energy towards storage, at a cost to somatic growth, and energy reserves (in the form of lipids) are needed to initiate the sexual maturation process.

The study results suggest that warmer temperature regimes that maximize growth at the expense of energy storage will reduce the probability of freshwater maturation (resident life history expression). However, the authors cautioned against the interpretation that warmer thermal regimes will result in a higher abundance of anadromous individuals (steelhead) because the numerical abundance of salmonids commonly decreases with increasing temperature. They warn that an increase in the proportion of smolts within *O. mykiss* populations at higher temperatures may be offset by decreases in total population size (p. 499). It is also noteworthy that the maximum exposure temperature in the “warm” regime did not exceed 18°C.

The low total *O. mykiss* population size in the lower Tuolumne River, along with its low steelhead population, appear consistent with the scenario observed by Sloat and Reeves (2014). So, while improvements in smolt survival (in-river, through the Delta and Bay, and in the ocean) will be necessary to improve the Tuolumne River steelhead population (and for DPS recovery), these efforts will be insufficient unless the in-river total *O. mykiss* population is increased substantially.

Accordingly, any process for setting new temperature criteria should consider the adverse effects upon the Tuolumne River steelhead population of the very low production and over-summer survival of *O. mykiss*, which has been consistently observed in the lower Tuolumne River (TID and MID 2015, Table 2).

## 6. Review information about thermal stress in *O. mykiss*, and consider the linkages between biomarker studies and population-level effects.

Research into the underlying molecular and physiological processes that result in different degrees of thermal tolerance might lead to improved models of thermal tolerance (McCullough et al. 2009, p. 93). We sought out clues to understanding why the total population size of *O. mykiss* in the lower Tuolumne River is so low, and why its *O. mykiss* seek out summer water temperatures below the reported optimum temperature for aerobic scope. As explained above, *O. mykiss* are also observed at low densities in the warmer zones of other streams, and have been observed moving (seasonal migrations and diel movements) to colder habitats when conditions warm to near the upper range of their optimal temperature for aerobic scope. If these observations are not explained by aerobic scope performance, perhaps other metrics or endpoints could be considered in a new criterion-setting process.

The Report includes "important indications" in the Tuolumne River experiments that some of the tested *O. mykiss* were stressed at temperatures of 23-25°C; for example, 4 out of 13 (31%) individuals tested at 23-25°C had a FAS < 2, two fish regurgitated their stomach contents at 25°C, and the only fish mortality occurred during recovery after testing at 25°C (p. 20). Field and lab research using physiological indicators of thermal stress in individual fish appear promising, and could complement studies such as water temperature and population-size monitoring.

Feldhaus (2006) studied the link between summer temperatures and physiological performance indices for juvenile redband rainbow trout in the John Day River watershed (Eastern Oregon); the goal was to define summer water temperatures as physiologically "suitable," "marginal," or "unsuitable." The studies suggest that while these fish can tolerate summer daily maximum temperatures in excess of 22ºC, they are physiologically compromised as indicated by the induction of heat-shock proteins, a biomarker for thermal stress (p. 50-51; Iwama et al. 1998).

Kammerer and Heppell (2012) conducted studies of the wild redband trout in the John Day River watershed, in two systems with contrasting thermal regimes (colder and warmer) to test the hypothesis that trout are physiologically healthy below a certain temperature threshold, and above this threshold performance declines. They discuss (p. 2) how fish exposed to high temperatures undergo a cellular stress response, in which individual cells mount a host of responses to cope with the stress; heat-shock proteins are described as "cellular guardians" produced in the liver that reduce damage to other cellular components when an animal is exposed to high temperatures. The authors found relative heat-shock protein levels in liver and fin tissue increased with temperatures (Figure 4, p. 8), and were correlated with reduced fish size and changes in body fat, particularly above a 23 °C thermal threshold (p. 11).

In a follow-up laboratory study, Kammerer and Heppell (2013) assessed the long-term effects of elevated summer stream temperatures on growth, the cellular stress response, and whole-body lipids in juvenile summer steelhead (Skamania–Columbia basin hatchery stock). Replicated groups of fish were exposed to three different temperatures, 15, 23, and 25ºC, for 25 consecutive days followed by a 55-day recovery period at 15ºC. At temperatures ≥ 25ºC, steelhead consumed significantly less food per day and had elevated feed conversion rates, but experienced slower growth, reduced body size, lower body fat, and elevated heat-shock protein levels relative to fish exposed to 23ºC and 15ºC. Also, growth decreased 24.4% and 27.1% for length and mass, respectively, between 15ºC and 23ºC, and an additional 60% and 56.5%, respectively, between 23ºC and 25ºC. Mortalities occurred only in the 25ºC exposures, beginning 10 days after the start of the experiments. While growth increments and lipid levels recovered to control levels after water temperature was reduced, body size of the fish exposed to 25ºC lagged throughout the experiment. Heat-shock protein levels were detectable up to 25 days after the thermal stress. The authors concluded the results show that thermal stress affects performance, and they suggested a thermal threshold of 23ºC, after which exposed steelhead incur a “physiological debt.”

Werner et al. (2005) measured expression of heat-shock protein 72 in white muscle tissue of juvenile steelhead parr (*O. mykiss*) in the Navarro River watershed (California), and concluded the fish were experiencing cellular stress when maximum daily water temperatures reached 20-22.5ºC. In a related laboratory study, Viant et al. (2003) performed long-term (10-week) exposures of juvenile *O. mykiss* to 15 and 20ºC, and measured metabolic condition, stress response and growth rate endpoints. In addition to heat-shock proteins, they analyzed multiple “traditional” biomarkers of metabolic condition (using nuclear magnetic resonance-based metabolite profiling) in muscle and liver. The authors found their laboratory results support the conclusion from earlier Navarro River field study, that heat-shock protein 72 is an indicator of thermal stress in juvenile steelhead parr; in fish that experienced a temperature increase from 15 to 20ºC, heat-shock protein levels remained elevated in 15% of these fish for 10 weeks. The authors also found good correlations between increased heat-shock protein synthesis and reduced metabolic condition. While they did not observe reduced steelhead growth rates, they noted the highest exposure temperature was 20ºC and the sufficient food provided likely supported the increased metabolic demand at that temperature or lower.

In their studies, Kammerer and Heppell (2012) found good correlations between liver- and fin-tissue heat shock protein 70 in *O. mykiss*, which suggests a non-lethal sampling method (fin clips) could be used to measure thermal stress. This is relevant because concerns exist over even small “take” of the rare, ESA-listed *O. mykiss* in the lower Tuolumne River, which could occur during scientific collection. Before setting new temperature criteria to protect the lower Tuolumne River *O. mykiss*, heat shock proteins or other biomarkers could be examined in fish living in the existing (baseline) summer temperature conditions, to understand if they are experiencing thermal stresses.

### 7. Consider that at higher temperatures in the lower Tuolumne River, food supply may limit the energy available for growth and survival of *O. mykiss*, and exert population-level effects.

Bioenergetics models rely on the balanced energy equation of Winberg (1956), as cited in Hartman and Brandt (1995): $G = C – (R + SDA) – F – U$, where all units are in energy and: G is growth; C is consumption; R is metabolism; SDA is specific dynamic action (or the heat increment associated with biochemical transformation of ingested food into forms that can be metabolized); F is egestion (the removal of indigestible food); and U is excretion (the elimination of waste products resulting from metabolic processes). First, it is evident from this energy equation that metabolism is not the only temperature-dependent variable influencing growth. Second, it would be incorrect to assume growth can be maintained as temperature increases, even if R (or aerobic scope, MMR – RMR) is unchanged or higher at the warmer temperature, unless energy (food) consumption is increased.

Weber et al. (2014) applied a bioenergetics mass balance equation similar to the one above, in their study of *O. mykiss* habitat growth potential in the John Day River basin (Oregon); their focus was on measurements of (invertebrate) food abundance and stream temperature. They observed nearly five-fold differences in the abundances of benthic and drifting invertebrate assemblages among the reaches studied. Their modeling demonstrated that relationships between temperature and salmonid physiological rates are insufficient when attempting to estimate the growth potential of a stream habitat – they found consideration of local invertebrate food availability can greatly increase the accuracy of bioenergetics-based predictions of salmonid growth (p. 1165).

Kendall et al. (2014) review the processes and patterns of anadromy and residency in steelhead and rainbow trout, including the interactions among genetics, individual condition, and environmental influences; what is striking is the broad role of stream temperature acting within and between these influences. The genetic basis for smolt transformation is discussed; as explained earlier, the processes of smolt transformation are also influenced by temperature. The expression of genes associated with physiological traits such as metabolism and food conversion efficiency is discussed; both metabolism and food conversion efficiency are temperature-dependent. In reviewing the role of individual fish condition on *O. mykiss* anadromy and residency, growth, size, and lipid content are discussed; temperature influences all of these factors. Stream temperature itself is discussed as an environmental variable, along with others that are themselves not independent of temperature, such as stream flow, habitat, food supply, competitor density, and freshwater migration challenges (Figure 4, p. 329). The reviewers recommend future investigation of the effects of temperature on *O. mykiss* anadromy and residency; they especially single out the need to better understand the role of energy allocation (p. 337).

The Report relies solely on data from its lower Tuolumne River aerobic scope experiments to assess the scope for growth for *O. mykiss* under a thermal regime. The experiments yielded FAS (factorial aerobic scope = MMR/RMR) values > 2 for all fish up to 23°C (p. ii, pp. 23-24). The Report explains that FAS values of 2 or greater are biologically significant because they indicate an aerobic capacity sufficient for a fish to properly digest a full stomach (p. *ii*; pp. 4-5, p. 20, pp. 21-22), and to potentially grow faster at a higher temperature (p. 22). However, demonstration of aerobic scope sufficient for digestion up to 23°C does not guarantee *O. mykiss* growth or survival in the lower Tuolumne River at this temperature. As discussed above, juvenile *O. mykiss* experiencing longer-term thermal stress (indicated by heat-shock protein synthesis) at higher temperatures (23-25ºC) may consume less food, experience slower growth and reduced body size, and have lower lipid content (Kammerer and Heppell 2013). At lower, but sub-optimal exposure temperatures (~20ºC) heat-shock protein synthesis and metabolic indicators also signal stress in *O. mykiss*, but reduced growth may not occur <u>if sufficient food is available</u> (Werner et al. 2005).

Any process to develop new Tuolumne River temperature criteria should include bioenergetics considerations, which suggest *O. mykiss* growth would likely decline if food availability and consumption were kept constant while lower Tuolumne River temperatures increased. Also, the bioenergetics equation discussed above does not consider additional temperature-influenced factors such as competition for food and space between cold-water *O. mykiss* and fishes that are more warm-adapted. For example, temperature-dependent competition between *O. mykiss* and Sacramento pikeminnow (*Ptychocheilus grandis*) has been shown to reduce the condition and abundance of juvenile *O. mykiss* at higher temperatures (Reese and Harvey 2002). This raises the possibility of negative competitive effects with other fishes (native and introduced) sympatric with the *O. mykiss* in the lower Tuolumne River.

### 8. Review information about the abundances and distributions of native and non-native fishes in the lower Tuolumne River, consider the potential negative effects to *O. mykiss* of competition and predation, and how these effects might worsen under a warmer thermal regime.

As discussed above, the development of existing water temperature criteria for juvenile *O. mykiss* considered not only "physiological optimum temperatures" (where functions like growth, swimming, heart performance, etc. are optimized), but also "ecological optimum temperatures" where fish do best in the natural environment -- considering food availability, competition, predation, and fluctuating temperatures (EPA 2003, p. 18). While the distribution and abundance of fish species is partially dependent upon physiological responses, this pattern is also controlled by the interactions among competing species and predator-prey relationships (McCullough et al. 2009, p. 102).

We previously discussed research documenting negative correlations between *O. mykiss* abundances and maximum temperatures in small creeks in the high deserts of Oregon and Idaho, and in southern California. In these systems, the negative effects of interspecific competition or predation are likely small because few fishes are present, and *O. mykiss* are themselves the only or top predator. In contrast, the Tuolumne River is a large river with a drainage area upstream of the La Grange Dam of ~1,550 square miles. Brown and Ford (2002) describe how these California rivers (including the Tuolumne River) with large reservoirs can maintain native species for varying distances below the dams, with nonnative warm-water species dominating their lower reaches (p. 332).

Surveys in the lower Tuolumne River by seining, snorkeling, and electrofishing found 33 fish taxa, 12 native to California and 21 introduced species (Ford and Brown 2001, Table 9, p. 279). The native *O. mykiss*, hitch, prickly sculpin, Sacramento blackfish, Sacramento splittail, and tule perch were relatively rare, never exceeding 1% of the total catch with any of the sampling methods (Table 10, p. 282); introduced species were present throughout the lower Tuolumne River, and were dominant downstream of Waterford (RM 32). Predatory fishes such as bass and catfish were present. The authors discuss their lower Tuolumne River fish survey results in the context of the potential negative thermal effects on juvenile salmonids that occur below lethal levels; they include the physiological changes that affect growth, disease resistance, predator avoidance, and smolting, as well as ecological effects such as increased predator activity or increased food requirement (without an increase in food supply). Since temperatures in the lower Tuolumne River often vary within the range at which these effects seem to occur, these effects (and their combinations) may be important influences on the survival of young salmonids (p. 311).

Yearly snorkel surveys since 1982 have also consistently documented the presence of native cool water and introduced warm water fishes in the lower Tuolumne River (TID and MID 2015). After 1996, these species have been observed in lower numbers in upstream sites, which has been attributed to the higher minimum summer flow releases (p. 4). However, as recently as 2014, the numbers of Sacramento pikeminnow observed were far greater than the numbers of *O. mykiss* (Table 1, p. 16), and warm water fishes observed were bluegill sunfish (*Lepomis macrochirus*), redear sunfish (*L. microlophus*), green sunfish (*L. cyanellus*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*M. dolomieu*), and spotted bass (*M. punctulatus*).

In the Report, we found inadequate discussion of how the aerobic metabolism responses of lower Tuolumne River *O. mykiss* might inform their competitive or predator/prey interactions with the high number of cool- and warm-water fishes also present. Cech et al. (1990) illustrate how the routine metabolic responses of multiple fishes (measured in swim tunnel respirometers at various temperatures) might be used to evaluate their spatial overlap in rivers and streams. Any process to develop new temperature criteria should consider that colder summer conditions in the lower Tuolumne River could reduce the spatial overlap of juvenile *O. mykiss* with their potential competitors or predators, while a warmer thermal regime is likely to have the opposite result.

9. **Consider that recent genetic studies of *O. mykiss* in California indicate the test fish used in the Tuolumne River aerobic performance experiments are introgressed by fish with coastal steelhead ancestry.**

Pearse and Garza (2015) collected genotype data from >1,900 *O. mykiss* sampled at one or more locations in 15 tributary sub-basins of the Sacramento and San Joaquin rivers, including locations above and below barriers to anadromy. In the Tuolumne River, samples were collected in both the upper watershed (above the impassable La Grange and Don Pedro dams) and the lower Tuolumne River. They analyzed the genetic diversity and population structure differentiation between populations above and below dams, and the relationship of Central

Valley *O. mykiss* populations to coastal California steelhead. The introgression by both hatchery rainbow trout strains (which have primarily native Central Valley ancestry) and imported coastal steelhead stocks was also evaluated.

Central Valley *O. mykiss* above and below dams within the same tributary were not found to be each other's closest relatives, and the study found no relationship between genetic and geographic distance among below-barrier populations. The results suggest introgression by hatchery rainbow trout strains does not appear to be widespread among above-barrier populations. However, the clustering of the lower Tuolumne River (below-barrier) population with Nimbus Fish Hatchery and American River samples indicates the lower Tuolumne River *O. mykiss* have been introgressed by fish with coastal steelhead ancestry (p. 13). The authors advise that future conservation, restoration, and mitigation efforts should take these findings into account when working to meet recovery planning goals.

Any process to develop new temperature criteria should be done in the light of this new genetic information, and consider that the aerobic scope experiments were performed with *O. mykiss* captured in the lower Tuolumne River. In the future, *O. mykiss* different from the existing lower Tuolumne River stock may be used in steelhead recovery efforts in the watershed.

### 10. Consider that water temperature is not the only factor negatively affecting the lower Tuolumne River *O. mykiss* and steelhead populations, but it is a very important one.

In closing, although water temperature is the factor predominantly discussed in this review, we recognize it is not the only factor capable of negatively affecting the lower Tuolumne River *O. mykiss* and steelhead populations. Degradation or loss of habitat unrelated to temperature (e.g., due to impassable dams, channel modifications, gravel supply interruptions, large wood supply interruptions, etc.), as well as hatchery operations and other factors have played a role.

However, a protective temperature regime will be necessary to achieve the ESA recovery goals for the lower Tuolumne River steelhead population. We also recognize that policy decisions will be population-focused, and based on a weight-of-the-evidence rather than on a case for local adjustment to high river temperature that rests on a single study or finding.

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# DISTRICTS' RESPONSE TO NMFS COMMENTS ON THE DRAFT REPORT FOR THE THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

## ATTACHMENT B

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

# 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]

February 2017

Prepared for:

Turlock Irrigation District – Turlock, California
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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 |
| % $O_2$Sat | 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 ($P=5.83\times10^{-13}$) relationship (Figure 4A), where:

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

   x = temperature (°C).

   Thus, RMR at 13°C averaged 2.18 ± 0.45 (95% CI) mg $O_2$ $\text{kg}^{-0.95}$ $\text{min}^{-1}$ and reached 5.37 ± 0.41 (95% CI) mg $O_2$ $\text{kg}^{-0.95}$ $\text{min}^{-1}$ 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 ($P=8.94\times10^{-7}$) relationship (Figure 4B), where:

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

   x = temperature (°C)

   Thus, MMR at 13°C averaged 6.62 ± 1.03 (95% CI) mg $O_2$ $\text{kg}^{-0.95}$ $\text{min}^{-1}$ and increased up to 11.22 ± 0.86 (95% CI) mg $O_2$ $\text{kg}^{-0.95}$ $\text{min}^{-1}$ 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 ($P=2.92 \times 10^{-4}$) 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:
   $\text{MR (mg O}_2\text{ kg}^{-0.95}\text{ min}^{-1}) = 0.75 \text{ (TBF)} + 1.05$

   For fish tested at 20°C, this relationship was:
   $\text{MR (mg O}_2\text{ kg}^{-0.95}\text{ min}^{-1}) = 1.04 \text{ (TBF)} + 1.89$

   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 $20^{o}$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 18°C and 20°C, but decreased when measured at 25°C, the only other test temperature examined above 20°C (Chen et al. 2015). These authors report that 90% of peak AAS was maintained between 13°C and 20°C. 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

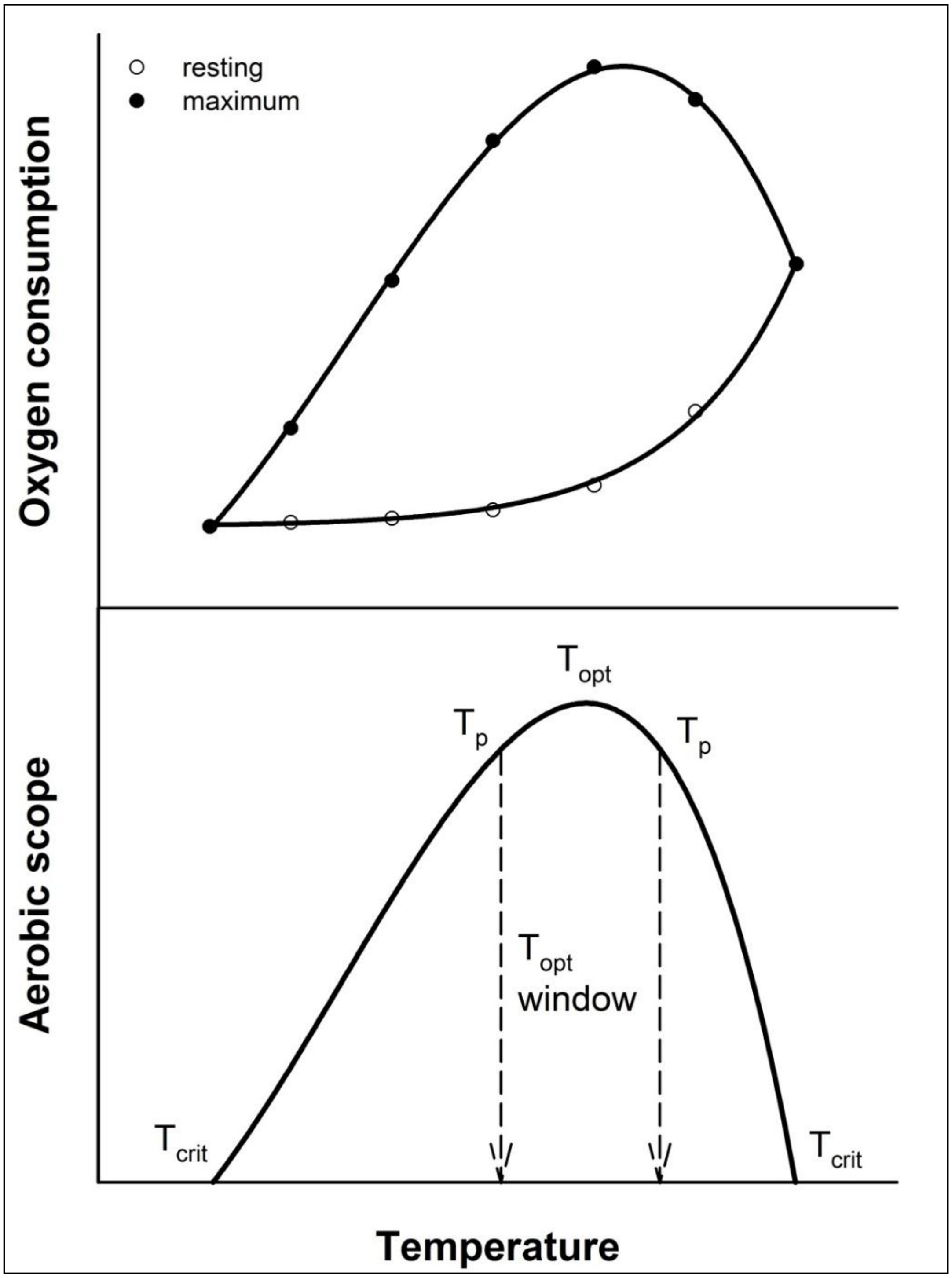
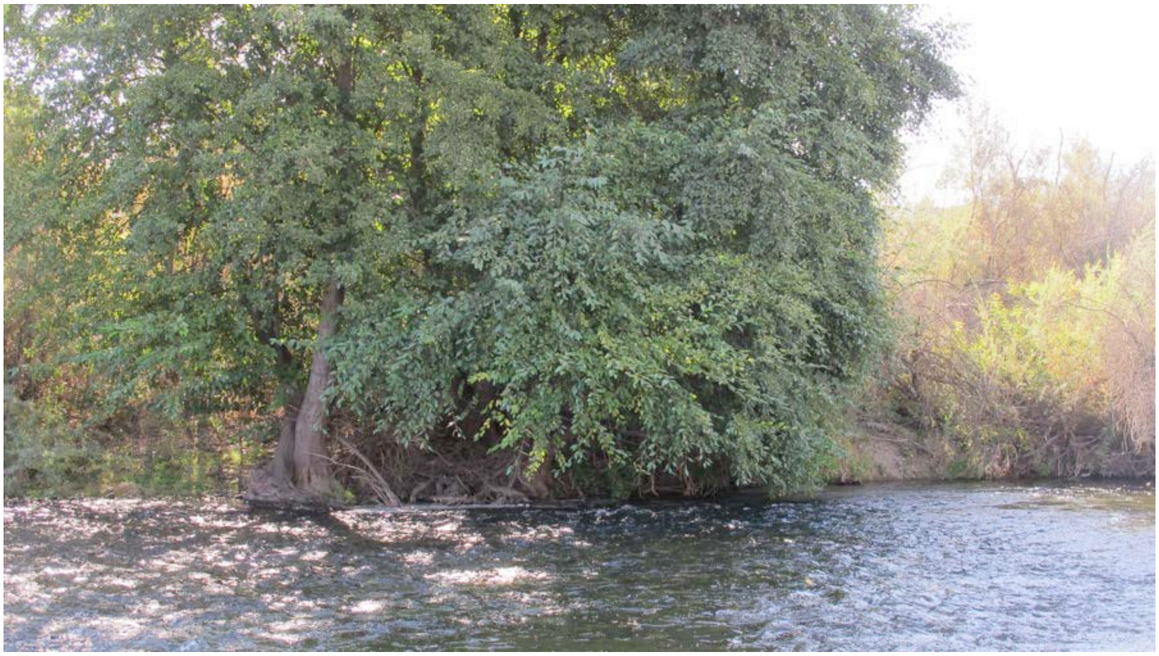


**Figure 1.** Schematic representation of the resting metabolic rate (= routine; RMR) and maximum metabolic rate (MMR) and aerobic scope (AS = MMR-RMR) for a temperature specialist. See text for details. $T_{opt}$ = optimum temperature, $T_p$ = pejus temperatures which set the thermal window or range 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.**

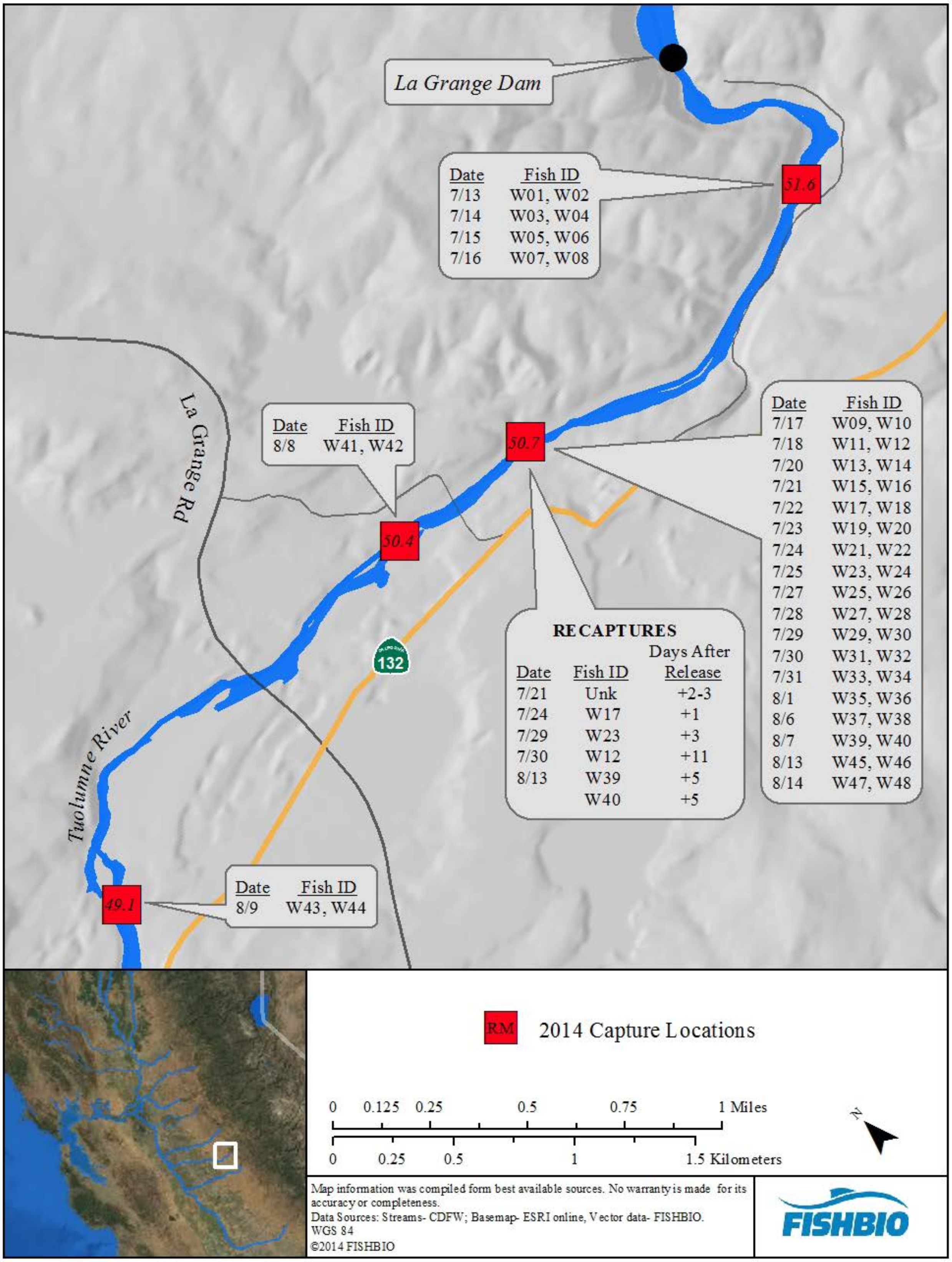


**Figure 3.** **Map of capture and recapture locations of all Tuolumne River *O. mykiss* test fish.**

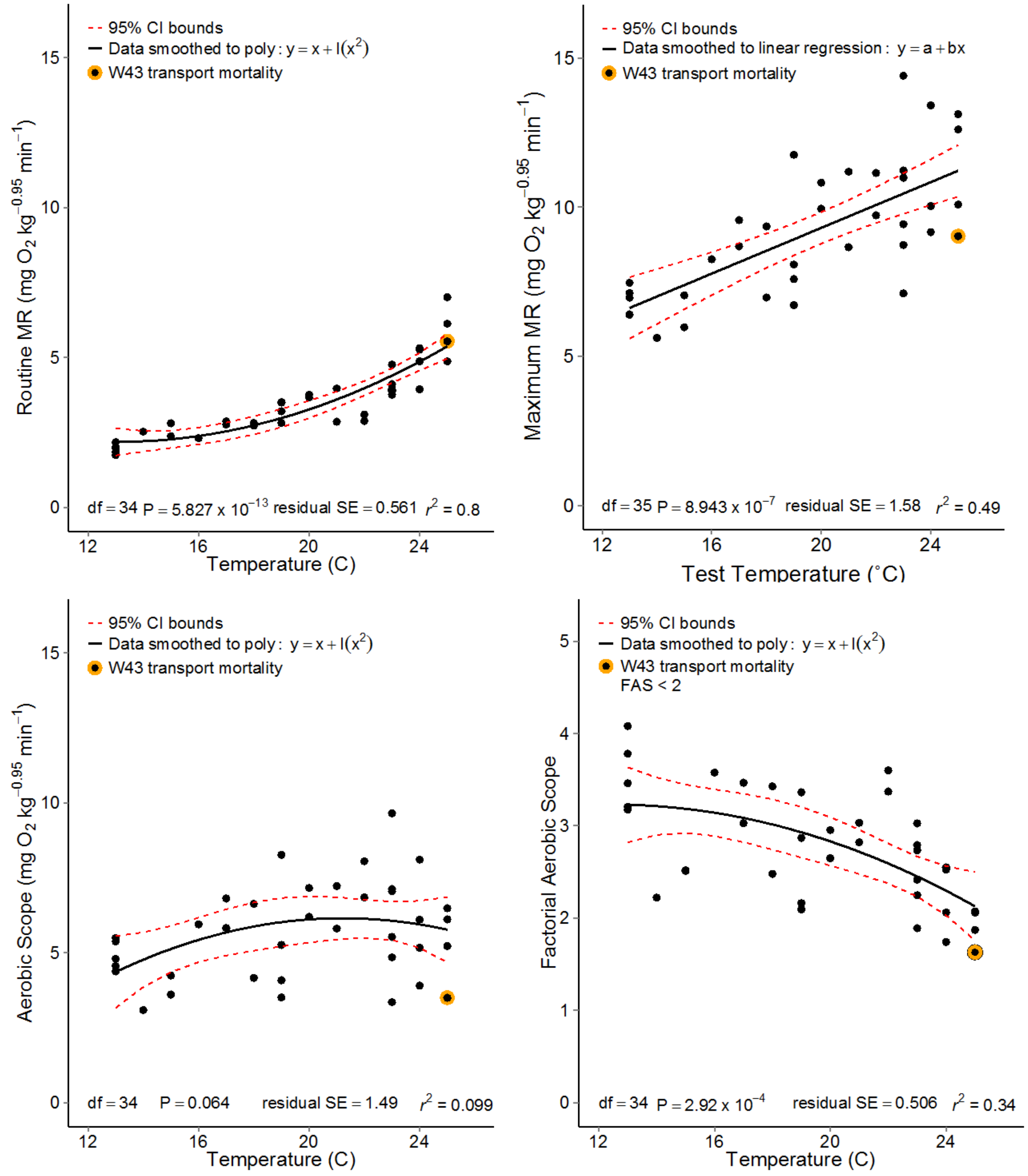


**Figure 4.** **The relationships between test temperature and the routine (RMR) and maximum metabolic rate (MMR) of Tuolumne River *O. mykiss*. Absolute aerobic scope (AAS) and factorial aerobic scope (FAS) were derived from the MR measurements. Each data point represents an individual fish tested at 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.**

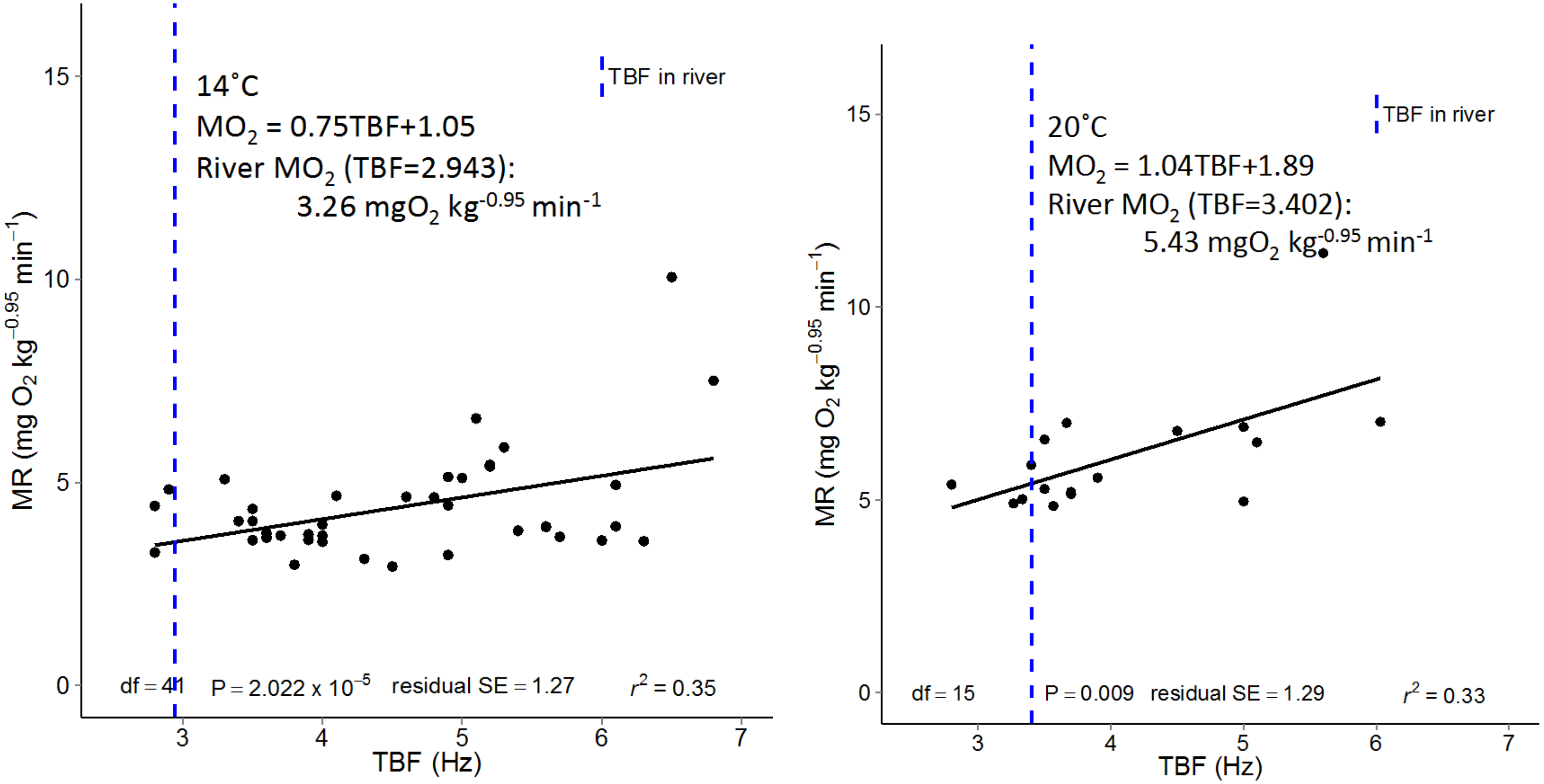


**Figure 5.** **The relationship between tail beat frequency (TBF; Hz) and metabolic rate (MR; mg $O_2$ kg$^{-0.95}$ min$^{-1}$) measured when Tuolumne River *O. mykiss* were swimming continuously in a swim tunnel at 14 °C or 20°C. The solid black line represents the linear regression based on the data 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 | CTmax | Heating rate | Mass | Length | Strain | Reference |
|---|---|---|---|---|---|---|
| **Temperature (°C)** | **(°C)** | **(°C min-1)** | **(g)** | **(cm)** | **Source** | |
| 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.

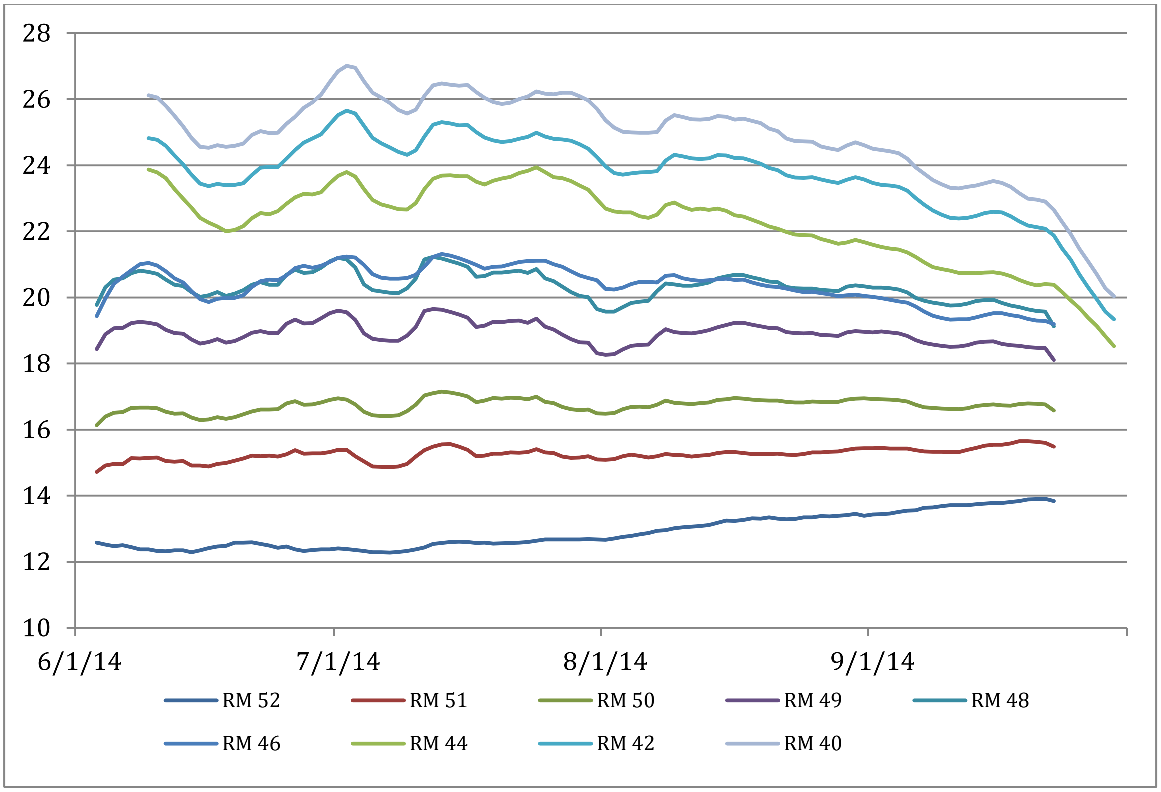
# APPENDICES

# 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<br>W - 120.44421 | 7/13 | 9:45 | 12.9 | N - 37.66574<br>W - 120.44421 | 7/14 | 15:35 | 14.4 | 4 FW Riffle (side channel #3) | 51.5 | |
| W02 | N - 37.66574<br>W - 120.44421 | 7/13 | 11:24 | 13.2 | N - 37.66574<br>W - 120.44421 | 7/14 | 15:36 | 14.4 | 4 FW Riffle (side channel #3) | 51.5 | |
| W03 | N - 37.66532<br>W - 120.44482 | 7/14 | 11:04 | 13.5 | N - 37.66518<br>W - 120.44509 | 7/15 | 17:25 | 14.1 | 4 FW Riffle (side channel #3) | 51.5 | |
| W04 | N - 37.66538<br>W - 120.44470 | 7/14 | 11:08 | 13.5 | N - 37.66518<br>W - 120.44509 | 7/15 | 17:25 | 14.1 | 4 FW Riffle (side channel #3) | 51.5 | |
| W05 | N - 37.66524<br>W - 120.44424 | 7/15 | 9:50 | 12.8 | N - 37.66544<br>W - 120.44449 | 7/16 | 13:07 | 14.6 | 4 FW Riffle (side channel #3) | 51.5 | |
| W06 | N - 37.66536<br>W - 120.44474 | 7/15 | 10:53 | 12.9 | N - 37.66544<br>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<br>W - 120.44449 | 7/16 | 9:52 | 12.9 | N - 37.66510<br>W - 120.44515 | 7/17 | 13:16 | 14 | 4 FW Riffle (side channel #3) | 51.5 | |
| W08 | N - 37.66544<br>W - 120.44449 | 7/16 | 10:10 | 12.7 | N - 37.66510<br>W - 120.44515 | 7/17 | 13:16 | 14 | 4 FW Riffle (side channel #3) | 51.5 | |
| W09 | N - 37.66586<br>W - 120.45826 | 7/17 | 9:10 | 13.5 | N - 37.66581<br>W - 120.45829 | 7/18 | 14:36 | 16 | 11 FW Riffle | 50.7 | |
| W10 | N - 37.66586<br>W - 120.45826 | 7/17 | 9:24 | 13.5 | N - 37.66581<br>W - 120.45829 | 7/18 | 14:36 | 16 | 11 FW Riffle | 50.7 | |
| W11 | N - 37.66581<br>W - 120.45829 | 7/18 | 8:40 | 13.7 | N - 37.66581<br>W - 120.45829 | 7/19 | 14:49 | 15.5 | 11 FW Riffle | 50.7 | |
| W12 | N - 37.66581<br>W - 120.45829 | 7/18 | 8:40 | 13.7 | N - 37.66581<br>W - 120.45829 | 7/19 | 14:49 | 15.5 | 11 FW Riffle | 50.7 | |
| W13 | N - 37.66579<br>W - 120.45832 | 7/20 | 8:48 | 13.4 | N - 37.66585<br>W - 120.45823 | 7/21 | 13:59 | 15.3 | 11 FW Riffle | 50.7 | |
| W14 | N - 37.66579<br>W - 120.45832 | 7/20 | 8:48 | 13.4 | N - 37.66585<br>W - 120.45823 | 7/21 | 13:59 | 15.3 | 11 FW Riffle | 50.7 | |
| W15 | N - 37.66585<br>W - 120.45823 | 7/21 | 8:35 | 13.3 | N - 37.66579<br>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<br>W - 120.45823 | 7/21 | 8:35 | 13.3 | N - 37.66579<br>W - 120.45834 | 7/22 | 13:47 | 15.0 | 11 FW Riffle | 50.7 | |
| W17 | N - 37.66579<br>W - 120.45834 | 7/22 | 10:23 | 13.6 | N - 37.66579<br>W - 120.45839 | 7/23 | 14:13 | 15.4 | 11 FW Riffle | 50.7 | |
| W18 | N - 37.66579<br>W - 120.45834 | 7/22 | 10:28 | 13.6 | N - 37.66579<br>W - 120.45839 | 7/23 | 14:13 | 15.4 | 11 FW Riffle | 50.7 | |
| W19 | N - 37.66579<br>W - 120.45834 | 7/23 | 10:10 | 13.5 | N - 37.66574<br>W - 120.45786 | 7/24 | 14:29 | 15.3 | 11 FW Riffle | 50.7 | |
| W20 | N - 37.66579<br>W - 120.45834 | 7/23 | 10:27 | 13.5 | N - 37.66574<br>W - 120.45786 | 7/24 | 14:29 | 15.3 | 11 FW Riffle | 50.7 | |
| W21 | N - 37.66579<br>W - 120.45828 | 7/24 | 9:00 | 13.5 | N - 37.66571<br>W - 120.45794 | 7/25 | 14:00 | 15.4 | 11 FW Riffle | 50.7 | 7/24- recaptured PIT tag #114752 |
| W22 | N - 37.66579<br>W - 120.45828 | 7/24 | 9:00 | 13.5 | N - 37.66571<br>W - 120.45794 | 7/25 | 14:00 | 15.4 | 11 FW Riffle | 50.7 | |
| W23 | N - 37.66582<br>W - 120.45830 | 7/25 | 9:05 | 13.5 | N - 37.66582<br>W - 120.45830 | 7/26 | 13:33 | 15.1 | 11 FW Riffle | 50.7 | |
| W24 | N - 37.66582<br>W - 120.45830 | 7/25 | 9:05 | 13.6 | N - 37.66582<br>W - 120.45830 | 7/26 | 13:33 | 15.1 | 11 FW Riffle | 50.7 | |
| W25 | N - 37.66565<br>W - 120.45826 | 7/27 | 8:15 | 13.6 | N - 37.66565<br>W - 120.45826 | 7/28 | 14:15 | 14.5 | 11 FW Riffle | 50.7 | |
| W26 | N - 37.66565<br>W - 120.45826 | 7/27 | 8:15 | 13.6 | N - 37.66565<br>W - 120.45826 | 7/28 | 14:15 | 14.5 | 11 FW Riffle | 50.7 | |
| W27 | N - 37.66565<br>W - 120.45826 | 7/28 | 9:15 | 13 | N - 37.66565<br>W - 120.45826 | 7/29 | 14:15 | 14.9 | 11 FW Riffle | 50.7 | |
| W28 | N - 37.66565<br>W - 120.45826 | 7/28 | 9:15 | 13 | N - 37.66565<br>W - 120.45826 | 7/29 | 14:15 | 14.9 | 11 FW Riffle | 50.7 | |
| W29 | N - 37.66565<br>W - 120.45826 | 7/29 | 9:30 | 13.3 | N - 37.66574<br>W - 120.45788 | 7/30 | 16:30 | 14.7 | 11 FW Riffle | 50.7 | 7/29- recaptured PIT tag #114809 |
| W30 | N - 37.66565<br>W - 120.45826 | 7/29 | 9:18 | 13.3 | N - 37.66574<br>W - 120.45788 | 7/30 | 16:30 | 14.7 | 11 FW Riffle | 50.7 | |
| W31 | N - 37.66565<br>W - 120.45826 | 7/30 | 9:00 | 13.3 | N - 37.66565<br>W - 120.45826 | 7/31 | 13:38 | 15.1 | 11 FW Riffle | 50.7 | 7/30- recaptured PIT tag #114734 |
| W32 | N - 37.66565<br>W - 120.45826 | 7/30 | 9:07 | 13.3 | N - 37.66565<br>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<br>W - 120.45826 | 7/31 | 9:05 | 13.1 | N - 37.66565<br>W - 120.45826 | 8/1 | 13:42 | 15.0 | 11 FW Riffle | 50.7 | |
| W34 | N - 37.66565<br>W - 120.45826 | 7/31 | 9:05 | 13.1 | N - 37.66565<br>W - 120.45826 | 8/1 | 13:42 | 15.0 | 11 FW Riffle | 50.7 | |
| W35 | N - 37.66565<br>W - 120.45826 | 8/1 | 9:02 | 13.2 | N - 37.66565<br>W - 120.45826 | 8/2 | 15:40 | 15.8 | 11 FW Riffle | 50.7 | |
| W36 | N - 37.66565<br>W - 120.45826 | 8/1 | 9:30 | 13.2 | N - 37.66565<br>W - 120.45826 | 8/2 | 15:40 | 15.8 | 11 FW Riffle | 50.7 | |
| W37 | N - 37.66565<br>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<br>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<br>W - 120.45826 | 8/7 | 9:08 | 13.5 | N - 37.66668<br>W - 120.46420 | 8/8 | 17:31 | 15.8 | 11 FW Riffle | 50.7 | |
| W40 | N - 37.66565<br>W - 120.45826 | 8/7 | 9:30 | 13.5 | N - 37.66668<br>W - 120.46420 | 8/8 | 17:31 | 15.8 | 11 FW Riffle | 50.7 | |
| W41 | N - 37.66643<br>W - 120.46432 | 8/8 | 11:18 | 15.5 | N - 37.66643<br>W - 120.46432 | 8/9 | 16:00 | 16.7 | 14 BC Riffle | 50.4 | |
| W42 | N - 37.66643<br>W - 120.46432 | 8/8 | 11:35 | 14.6 | N - 37.66643<br>W - 120.46432 | 8/9 | 16:00 | 16.7 | 14 BC Riffle | 50.4 | |
| W43 | N - 37.66426<br>W - 120.48132 | 8/9 | 11:40 | 17.1 | N - 37.66308<br>W - 120.48160 | 8/10 | 15:13 | 18.0 | 25 BC Riffle | 49.1 | Mortality- post- swim test transport |
| W44 | N - 37.66426<br>W - 120.48132 | 8/9 | 11:40 | 17.1 | N - 37.66308<br>W - 120.48160 | 8/10 | 15:13 | 18.0 | 25 BC Riffle | 49.1 | |
| W45 | N - 37.66565<br>W - 120.45826 | 8/13 | 10:25 | 14.4 | N - 37.66565<br>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<br>W - 120.45826 | 8/13 | 10:59 | 13.9 | N - 37.66565<br>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<br>W - 120.45826 | 8/14 | 9:08 | 13.6 | N - 37.66565<br>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<br>W - 120.45826 | 8/14 | 9:15 | 13.6 | N - 37.66565<br>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 | | |
| 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 | | |
| 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

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.

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[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*).

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[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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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.

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[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
Attn: Peter Barnes
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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.

cc: Ms. Kimberly D. Bose, Secretary
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Received 9-6-2016
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August 31, 2016

Rose Staples, Executive Assistant
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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 <u>does not</u> 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 <u>are always less than</u> 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.

## <u>Discussion</u>

### 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?

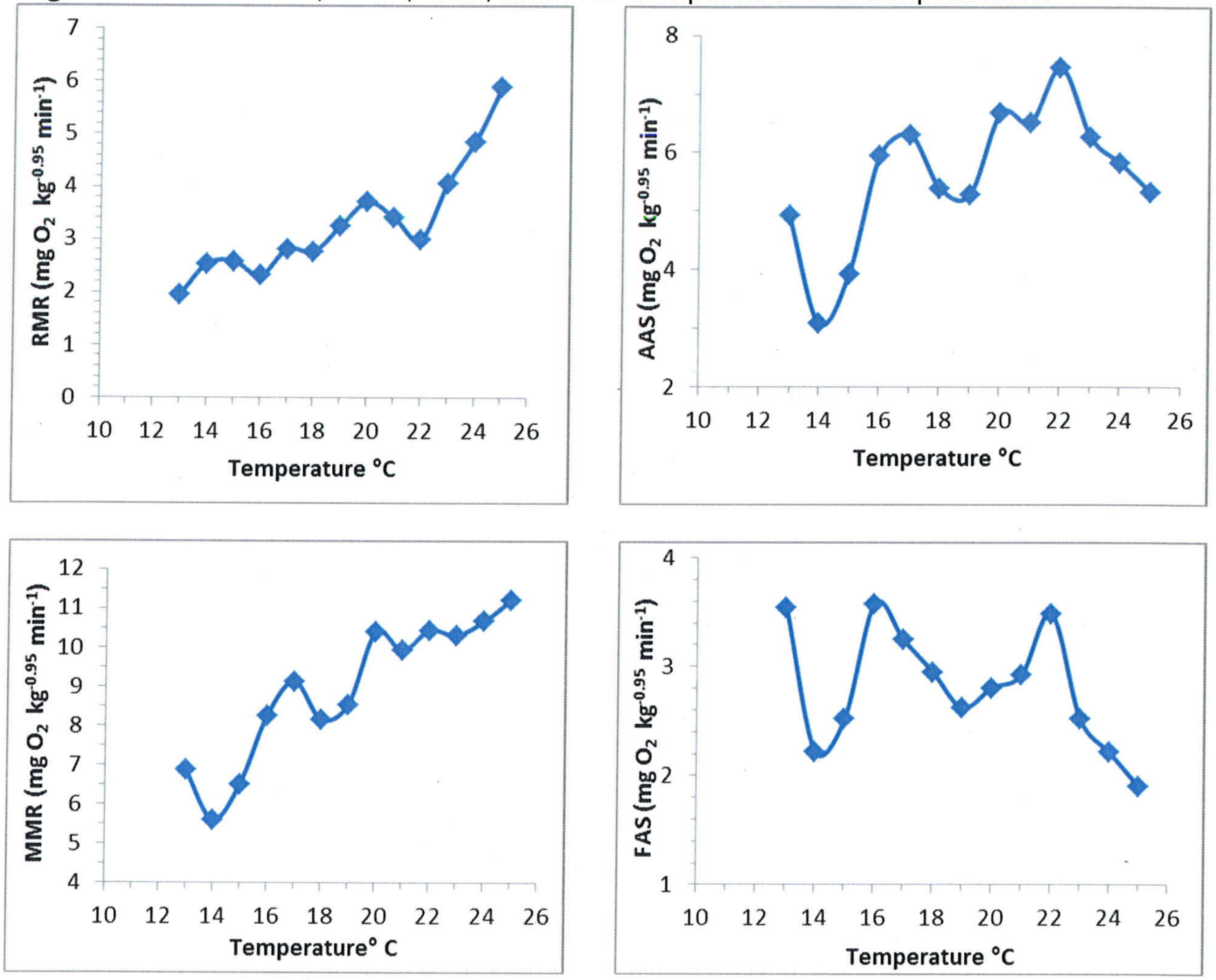
As stated in USEPA (2001) Issue Paper 4, Page 8, *a*s metabolic demands and oxygen consumption increase, gill ventilation must also rise proportionately (Heath and Hughes 1973). Further USEPA (2001) Issue Paper 4, Page 5 states:

> *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 cannot 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
Regional Manager

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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$^{o}$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.

| Comment # (page #) | Comment | Districts' Response |
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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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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<br>(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.<br>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.<br>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).<br>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<br>(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.<br><br>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<br>(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<br>(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<br>(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<br>(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<br>(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<br>(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:<br>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?*<br>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.<br>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.<br>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.<br>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<br>(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<br>(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.<br><br>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.<br><br>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.<br><br>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.<br>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.<br>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.<br>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<br>(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.<br>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<br>(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.<br>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<br>(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.<br>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).<br>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.<br>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.<br>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.<br>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!<br>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.<br>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.<br>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.<br>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.<br><br>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<br>(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.<br><br>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.<br>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.<br>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).<br>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.<br>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.<br>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.<br>If there is no peak for ASS we must then talk about a thermal range for peak AAS, which is what we do.<br>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.<br>Please see previous response to acute/chronic criteria.<br>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.<br>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!!!<br>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.<br>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).<br>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.<br>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.<br>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.<br>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.<br>We clearly do not measure chronic temperatures so the distinction is meaningless for this Report.<br>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.<br>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.<br>Tcrit would be when ASS fell to 0, but we never saw this with the present experiments.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>Our experiment was measuring the optimal temperature range for Tuolumne River *O. mykiss*, not acute CTmax temperatures for these fish.<br>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.<br>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).<br>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.<br>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.<br><br>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<br>(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.<br><br>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.<br><br>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<br>(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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br><br>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.<br><br>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.<br>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.<br>*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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>The idea that these fish are stressed has been repeatedly dealt with above. |

| Comment # (page #) | Comment | Districts' Response |
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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.<br>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.<br>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. |

| Comment # (page #) | Comment | Districts' Response |
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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.<br>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 |

| Comment # (page #) | Comment | Districts’ Response |
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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.<br>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.<br>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.<br>Some of the fish swam for multiple hours at a high rate at 21.2°C and higher temperatures.<br>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.<br>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.<br>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 |

| Comment # (page #) | Comment | Districts' Response |
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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!)<br>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.<br>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 |

| Comment # (page #) | Comment | Districts' Response |
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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.<br>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.<br>See response to CDFW-1 specific to the repetitive comment regarding chronic/acute criteria.<br>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. |

| Comment # (page #) | Comment | Districts' Response |
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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 killograms); 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

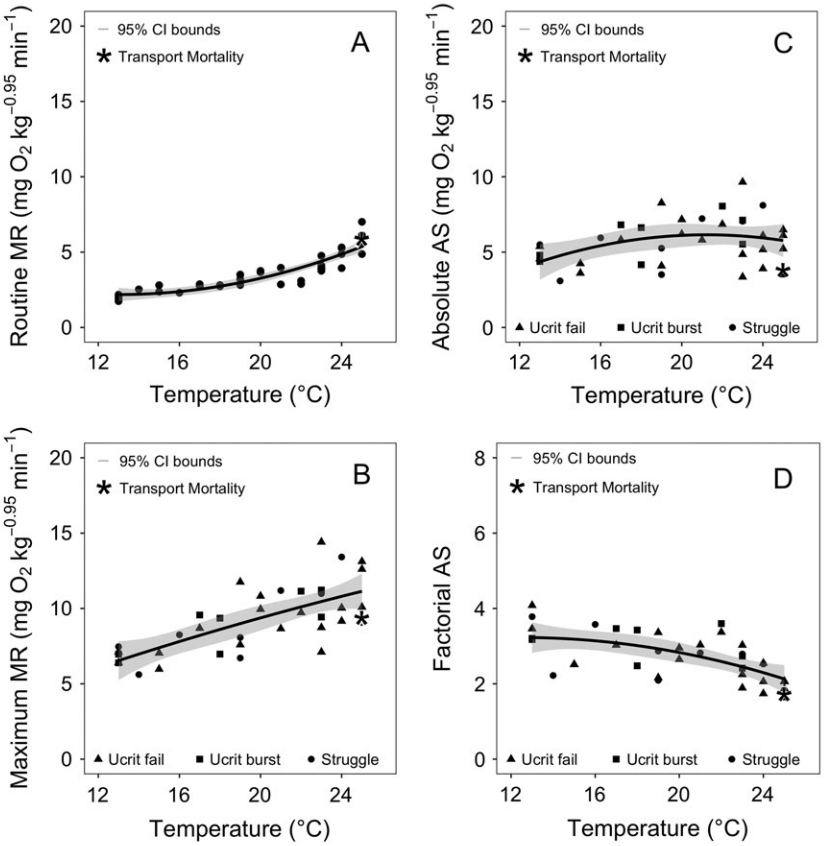

**Figure 1:** The relationships between test temperature and the routine (RMR; **A**) and maximal metabolic rate (MMR; **B**) of Tuolumne River *Oncorhynchus mykiss*. The three methods used to measure MMR (see Materials and methods section) are distinguished by different symbols. Absolute aerobic scope (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

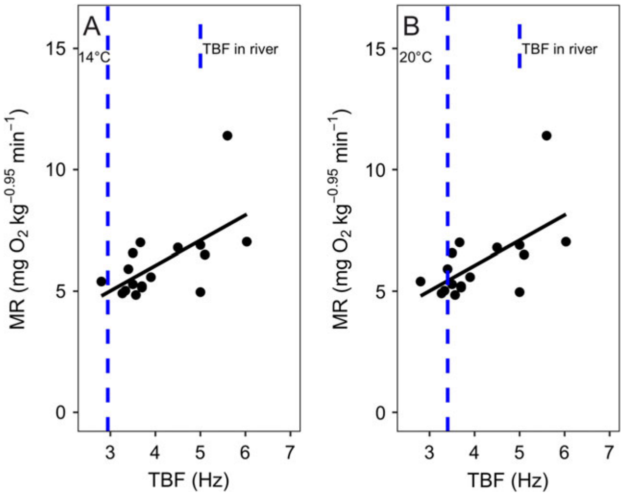

**Figure 2:** The relationship between tail beat frequency (TBF; in hertz) and metabolic rate (MR; in mg $O_2$ $kg^{-0.95}$ $min^{-1}$) measured when Tuolumne River *Oncorhynchus mykiss* were swimming continuously in a swim tunnel at 14 (**A**) or 20°C (**B**). The continuous black line represents the 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 $O_2$ $kg^{-0.95}$ $min^{-1}$.

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.

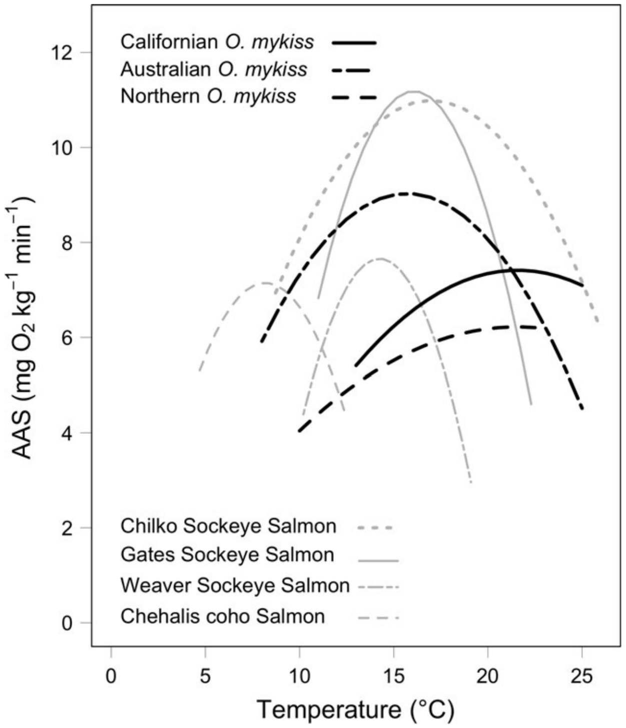

**Figure 3:** Absolute aerobic scope (AAS) for three strains of *Oncorhynchus mykiss*, i.e. a northern strain (Fry, 1948), an Australian strain (Chen *et al.*, 2015) and the California strain reported in this manuscript, compared with AAS measurements of Chehalis Coho salmon (*Oncorhynchus kisutch* Walbaum 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.

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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# DISTRICTS' RESPONSE TO NMFS COMMENTS ON THE DRAFT REPORT FOR THE THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

## ATTACHMENT C

## 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 killograms); 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

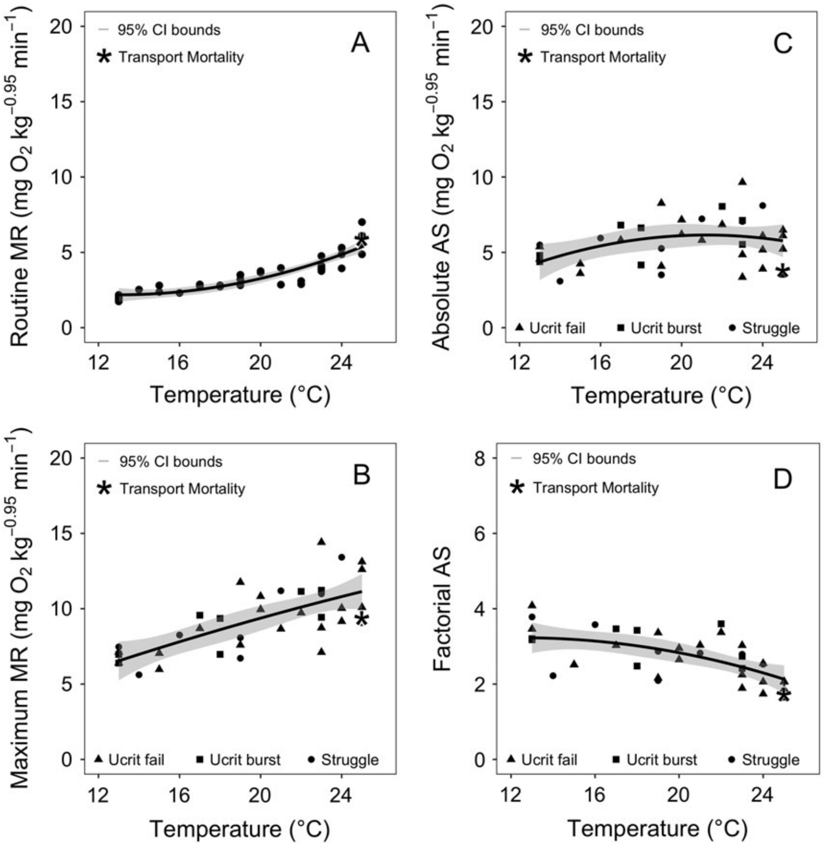

**Figure 1:** The relationships between test temperature and the routine (RMR; **A**) and maximal metabolic rate (MMR; **B**) of Tuolumne River *Oncorhynchus mykiss*. The three methods used to measure MMR (see Materials and methods section) are distinguished by different symbols. Absolute aerobic scope (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

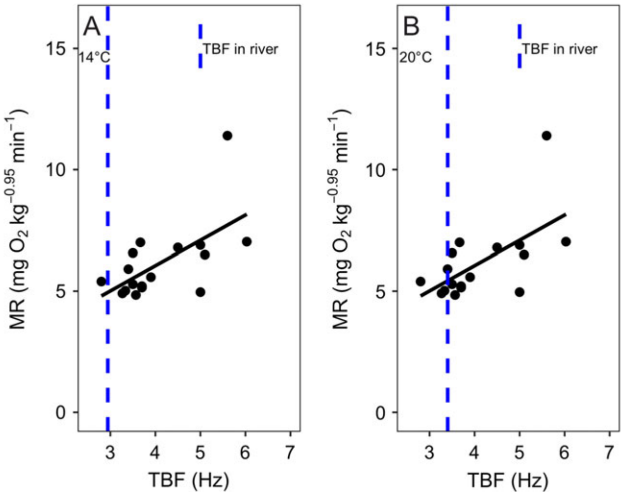

**Figure 2:** The relationship between tail beat frequency (TBF; in hertz) and metabolic rate (MR; in mg $O_2$ $kg^{-0.95}$ $min^{-1}$) measured when Tuolumne River *Oncorhynchus mykiss* were swimming continuously in a swim tunnel at 14 (**A**) or 20°C (**B**). The continuous black line represents the 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 $O_2$ $kg^{-0.95}$ $min^{-1}$.

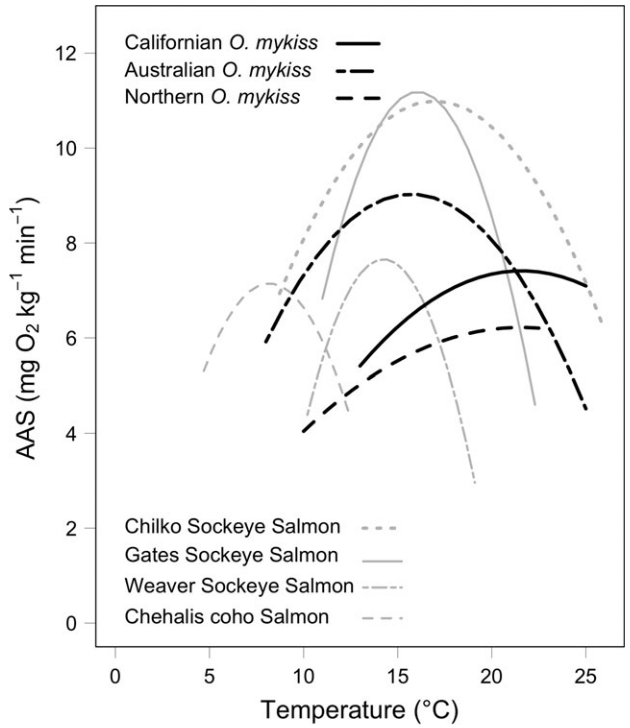

**Figure 3:** Absolute aerobic scope (AAS) for three strains of *Oncorhynchus mykiss*, i.e. a northern strain (Fry, 1948), an Australian strain (Chen *et al.*, 2015) and the California strain reported in this manuscript, compared with AAS measurements of Chehalis Coho salmon (*Oncorhynchus kisutch* Walbaum 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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# DISTRICTS' RESPONSE TO NMFS COMMENTS ON THE DRAFT REPORT FOR THE THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

## ATTACHMENT D

## OXYGEN- AND CAPACITY-LIMITED THERMAL TOLERANCE: BRIDGING ECOLOGY AND PHYSIOLOGY

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COMMENTARY

# Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology

**Hans-O. Pörtner*, Christian Bock and Felix C. Mark**

## ABSTRACT

Observations of climate impacts on ecosystems highlight the need for an understanding of organismal thermal ranges and their implications at the ecosystem level. Where changes in aquatic animal populations have been observed, the integrative concept of oxygen- and capacity-limited thermal tolerance (OCLTT) has successfully characterised the onset of thermal limits to performance and field abundance. The OCLTT concept addresses the molecular to whole-animal mechanisms that define thermal constraints on the capacity for oxygen supply to the organism in relation to oxygen demand. The resulting 'total excess aerobic power budget' supports an animal's performance (e.g. comprising motor activity, reproduction and growth) within an individual's thermal range. The aerobic power budget is often approximated through measurements of aerobic scope for activity (i.e. the maximum difference between resting and the highest exercise-induced rate of oxygen consumption), whereas most animals in the field rely on lower (i.e. routine) modes of activity. At thermal limits, OCLTT also integrates protective mechanisms that extend time-limited tolerance to temperature extremes – mechanisms such as chaperones, anaerobic metabolism and antioxidative defence. Here, we briefly summarise the OCLTT concept and update it by addressing the role of routine metabolism. We highlight potential pitfalls in applying the concept and discuss the variables measured that led to the development of OCLTT. We propose that OCLTT explains why thermal vulnerability is highest at the whole-animal level and lowest at the molecular level. We also discuss how OCLTT captures the thermal constraints on the evolution of aquatic animal life and supports an understanding of the benefits of transitioning from water to land.

**KEY WORDS: Organisational complexity, Sublethal thermal limits, Aerobic power budget, Aerobic performance, Oxygen supply, Oxygen demand, Temperature adaptation, Water breather, Air breather**

## Introduction

Given the impacts of climate warming on ecosystems, it is critical that we increase our understanding of organismal thermal ranges, responses and tolerances. Our understanding has long been insufficient, as studies have often focused on estimates of critical thermal maxima ($CT_{max}$) or lethal limits ($LT_{50}$; see Glossary). In fishes, these upper and lower limits and the range between them correlate to varying degrees with latitude, and probably also with latitude-associated temperature regimes (re-assessed by Pörtner and Peck, 2010). However, lethal limits are often more extreme than the temperatures that an animal will experience in its environment. Thus, there is a variable 'safety margin' between ambient temperature extremes and lethal temperatures (Sunday et al., 2012, 2014). Negative effects of changing temperature may occur within this margin, impacting ecology and therefore requiring identification. The physiological mechanisms causing heat or chill coma and death have been investigated for more than a century, thanks to the desire to identify the primary mechanism of temperature-associated death, yet a comprehensive mechanism-based understanding of this process has not been established.

Because temperature has a pervasive influence on all levels of biological organisation (Hochachka and Somero, 2002), research should address how mechanisms across these levels combine to shape the thermal limitations of an organism in the context of the ecosystem. The oxygen- and capacity-limited thermal tolerance (OCLTT) concept (Box 1), developed over the last two decades, has been proposed to meet these challenges and to provide a framework explaining how physiological mechanisms co-define an animal's fundamental and realised thermal niches (see Glossary), with a focus on critical life stages (for early summaries of OCLTT, see Pörtner, 2001, 2002; for thermal niches, see Pörtner et al., 2010; Deutsch et al., 2015; Payne et al., 2016). The basic idea underlying the OCLTT is that once temperatures approach limiting values, constraints on the capacity of an animal to supply oxygen to tissues to meet demand cause a progressive decline in performance (e.g. Pörtner and Giomi, 2013; Giomi et al., 2014), with consequences at the ecosystem level (e.g. Del Raye and Weng, 2015; Payne et al., 2016). OCLTT considers that most routine performances are fuelled sustainably by aerobic metabolism in excess of standard metabolic rate (SMR) and largely exclude anaerobic metabolism.

The aim of this Commentary is to summarise (and update) the key elements of the OCLTT concept. We first discuss the use of the OCLTT to understand species' responses to climate change. We then highlight pitfalls that can result from (over-)simplification, from different uses of terms and from combining OCLTT with traditional concepts (e.g. $CT_{max}$), especially when bypassing the transition from sublethal to lethal thermal limits (see Glossary). We summarise the physiological variables that were measured when developing the OCLTT concept and that should be tested further in order to assess OCLTT, and discuss different understandings of the term 'capacity'. Finally, we consider the evolutionary modulation of the OCLTT.

## Using OCLTT to understand species' responses to climate change

The limits of a species' realised niche are thought to determine its large-scale temperature-dependent biogeography, as well as the animals' responses to warming. Combined with consideration of OCLTT, such principles may allow us to explain the currently observed biogeographical shifts of marine animals (Poloczanska et al., 2013). Individuals that undergo biogeographical shifts have experienced non-lethal thermal constraints; however, organisms that stay behind may eventually be lethally affected. Both processes

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### List of symbols and abbreviations

| | |
|---|---|
| $Ca_{O_2}$ | arterial oxygen content |
| $Cv_{O_2}$ | venous oxygen content |
| CO | cardiac output |
| $CT_{max}$ | critical thermal maximum |
| $LT_{50}$ | lethal temperature causing 50% mortality |
| $\dot{M}_{O_2}$ | oxygen consumption rate |
| OCLTT | oxygen- and capacity-limited thermal tolerance |
| $P_{O_2}$ | partial pressure of $O_2$ |
| $Pa_{O_2}$ | arterial partial pressure of $O_2$ |
| $Pv_{O_2}$ | venous partial pressure of $O_2$ |
| SMR | standard metabolic rate |
| $T_c$ | critical temperature |
| $T_d$ | denaturation temperature |
| $T_{opt}$ | optimum temperature |
| $T_p$ | pejus temperature |

contribute to local extinction (e.g. Jones et al., 2009). While individuals survive non-lethal thermal constraints, the resulting reductions in available energy will jeopardise reproduction and thus population survival. Furthermore, the borders of the realised niche also depend on species interactions (e.g. changing availability of prey organisms). Current knowledge suggests that the borders of thermal niches can shift as a result of acclimation of individuals between seasons or adaptation over generations; this might allow organisms to tolerate rising temperatures without changes in distribution. However, data reveal ongoing distribution shifts for different species, indicating that the capacities for acclimation and adaptation are limited or that these processes are too slow to prevent biogeographical shifts (evidence reviewed and assessed by IPCC AR5: Poloczanska et al., 2014; Pörtner et al., 2014).

OCLTT has been used to explain the physiology underpinning the climate responses of individual species, as observed in the field, at biogeographical borders of critical life stages or under extreme seasonal conditions. Information on species-specific thermal niches can be derived from data on temperature-dependent steady-state animal performance (see Glossary) under the routine conditions of a species – relevant performances can range from steady-state swimming of salmon during spawning migrations (Eliason et al., 2011), to growth (including feeding) rates of benthic and demersal fish (Pörtner and Knust, 2007), to ventilatory and motor activities as in amphipods (Jakob et al., 2016) – and, more generally, they relate to the scope of routine oxygen demand and associated shifts in species abundance (see Pörtner and Knust, 2007) or biogeographical boundaries (Deutsch et al., 2015). With the possible exception of salmon migrating upstream (Fig. 1), routine performance in the centre of the thermal range usually does not fully exploit the available aerobic power budget (*sensu* Guderley and Pörtner, 2010; see Glossary).

As a result of acclimation or local adaptation and the associated trade-offs, the resulting total thermal performance curves (relating to aerobic power budget and encompassing various performances) differ between species and even between populations of the same species (Box 2, Fig. 1A), leading to different metabolic and performance characteristics across latitudes (Pörtner, 2006; Pörtner et al., 2008; Schröer et al., 2009). As a result of phenotypic diversity and plasticity, a species' biogeographical range is made up of overlapping niches of populations or individuals (Fig. 1A). In addition, differences in thermal curves and optima for individual performances may result from individuals being in different physiological modes when performing (e.g. due to different feeding or hormonal status), such that trade-offs in energy

### Glossary

**Active thermal tolerance**
This occurs in the range of temperatures permanently tolerated. It involves aerobic performance and associated aerobic metabolism fuelling the energy demands of maintenance and additional functions (e.g. growth, reproduction) and behaviours (e.g. roaming, foraging, mating).

**Aerobic power budget**
The full amount of excess aerobic energy available above maintenance that is recruited from mitochondrial metabolism. It encompasses and is traditionally estimated from aerobic scope for exercise (see below). However, muscles may not be able to fully exploit that aerobic power budget, or may push energy demand beyond routine power budget, through anaerobic contributions and transient mobilisation of functional reserves. Furthermore, trade-offs in energy allocation may occur, affecting the balance between behaviours and exercise, growth of reproductive and somatic tissue and repair processes.

**Aerobic scope**
The difference between resting and the highest exercise-induced rate of oxygen consumption. In brief, aerobic scope for exercise is a measure of aerobic power budget with the need to consider the complexities and functional constraints discussed under 'aerobic power budget'.

**Critical thermal maximum ($CT_{max}$)**
The high temperature extreme leading to the onset of spasms (unorganised locomotion), close to the lethal temperature.

**Functional capacity**
The ability to routinely and permanently maintain a certain rate of functioning, supporting a specific level and kind of performance as needed under routine conditions at the ecosystem level.

**Functional reserve**
Additional performance capacity activated by hormonal action, e.g. catecholamines (fight and flight response).

**Functional scope**
The ability to increase the rate of a specific function or set of functions above those at rest, supporting a specific level and kind of performance as needed under routine conditions at the ecosystem level.

**Fundamental thermal niche (*sensu* Hutchinson)**
The temperature range within which physiological functioning of a species allows tolerance under resting conditions (covering active and passive ranges). Temperature effects may be influenced by specific effects of other environmental factors.

**Lethal temperature limit ($LT_{50}$)**
The temperature extreme (cold or warm) causing 50% mortality. It should be noted that the value of $LT_{50}$ found is influenced by the experimental protocol, especially the duration of exposure to step-wise increased or decreased temperature.

**Passive thermal tolerance**
The range of temperatures sustained passively by an organism through exploitation of residual aerobic and anaerobic metabolism, antioxidative defence, metabolic depression and the heat shock response. As these resources are depleted over time and feeding is constrained, passive tolerance is time limited.

**Realised thermal niche (*sensu* Hutchinson)**
The range of temperatures within which physiological functioning sustains Darwinian fitness and persistence of a species under routine conditions, including species interactions. Temperature effects may be influenced by specific effects of other environmental factors.

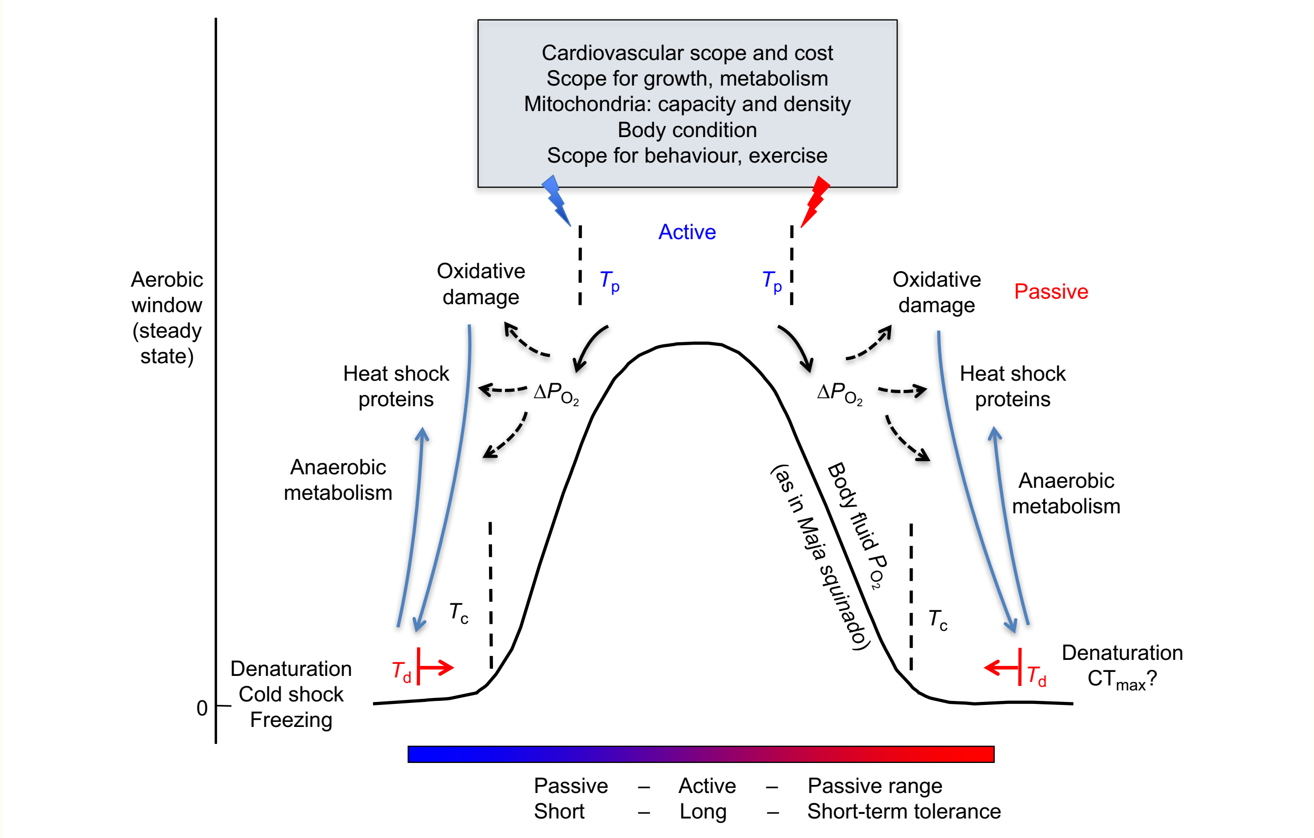
**Steady-state routine performance**
The rate of performance (feeding, behaviours, reproduction) that an organism displays routinely and permanently to maintain fitness in its natural environment.

**Sublethal limits**
Constraints in maintaining a functional rate under changing environmental conditions, e.g. warming, with negative implications at the ecosystem level.

**Symmorphosis**
According to this theory, the components of an organism match its overall functional scope, building on a quantitative match of design and functional parameters. The functional capacity of a complex system such as an animal's body must cope with the highest functional demand (after Weibel et al., 1991).

### Box 1. The OCLTT concept

Selected indicators of oxygen- and capacity-limited thermal tolerance (OCLTT) provide a systems view of multiple interlinked parameters characterising the thermal range of an aquatic animal species and its aerobic window (at steady state) (e.g. Pörtner, 2002, 2012; Pörtner et al., 2010, updated according to findings by Deutsch et al., 2015). The example given in the figure is for a warm temperate aquatic animal. The solid line shows the principal pattern of (mixed) body fluid $P_{O_2}$ against temperature in *Maja squinado* (Frederich and Pörtner, 2000). Note that temperature-dependent patterns of body fluid $P_{O_2}$ are not uniform between species and do not closely follow temperature-dependent changes in metabolic rate or performance. The range of active thermal tolerance (see Glossary) is limited on both sides by pejus temperatures ($T_p$; the box lists processes supporting active tolerance, which become constrained beyond $T_p$). Towards warm and cold extremes, the transition to passive thermal tolerance (see Glossary) is indicated by a decline in (venous) $P_{O_2}$ (solid black arrows), causing oxidative stress (Heise et al., 2006), heat shock response and, finally, transition to anaerobic metabolism (e.g. Kyprianou et al., 2010; Pörtner and Knust, 2007; dashed black arrows). The model proposes that, in a systemic to molecular hierarchy of thermal tolerance thresholds, these progressive transitions from sublethal to acutely lethal conditions [characterised by critical thermal maximum ($CT_{max}$), cold shock, denaturation (at denaturation temperature, $T_d$)] involve feedback between whole-organism and molecular levels. Blue arrows indicate the link between oxidative stress, heat-induced molecular damage and heat shock protein expression (for further details on these interactions, see Kassahn et al., 2009). This whole-organism feedback may narrow molecular thermal windows, such that $T_d$ is reached at lesser extremes of temperature (red arrows shifting upper and lower $T_d$). The passive tolerance range is a component of the niche used routinely by organisms experiencing extreme temperatures (e.g. in the intertidal zone). Rather than widening the active thermal range at a cost, they minimise metabolic costs and tolerate extremes anaerobically. Extended thermal tolerance is then achieved by protective mechanisms such as metabolic depression, anaerobic metabolism beyond the critical temperature ($T_c$), antioxidative defence and the use of chaperones such as heat shock proteins (e.g. Tomanek and Somero, 2002).



budgets may occur (see Holt and Jorgensen, 2015; for an early example of how starvation modifies thermal performance and optima in salmonids, see Brett, 1971).

OCLTT principles are presently integrated into models predicting the effects of climate on species' distributions and the consequences for ecosystems (e.g. Jones and Cheung, 2014; Deutsch et al., 2015). The concept has recently been extended to incorporate the combined effects of various climate change-associated drivers, such as ocean warming combined with acidification and hypoxia (Pörtner, 2010, 2012). It should also be noted that the pattern of acclimatisation to one climate-related driver such as temperature can be modified by the combined effects of multiple drivers (Anttila et al., 2015), indicating that highly complex mechanisms shape sublethal and lethal thermal constraints.

## Complexity shaping thermal limits: pitfalls when addressing OCLTT

Early in the development of the OCLTT concept it was suggested that thermal constraints are first noticeable at the highest level of organisational complexity (i.e. the whole animal), before affecting lower hierarchical levels (e.g. cellular and molecular levels; Pörtner, 2002). Recently, Storch et al. (2014) developed a 'complexity index' to compare the largely different thermal limits found across marine organism domains. They found a relationship between sublethal (and lethal) thermal limits and the number of body and cell

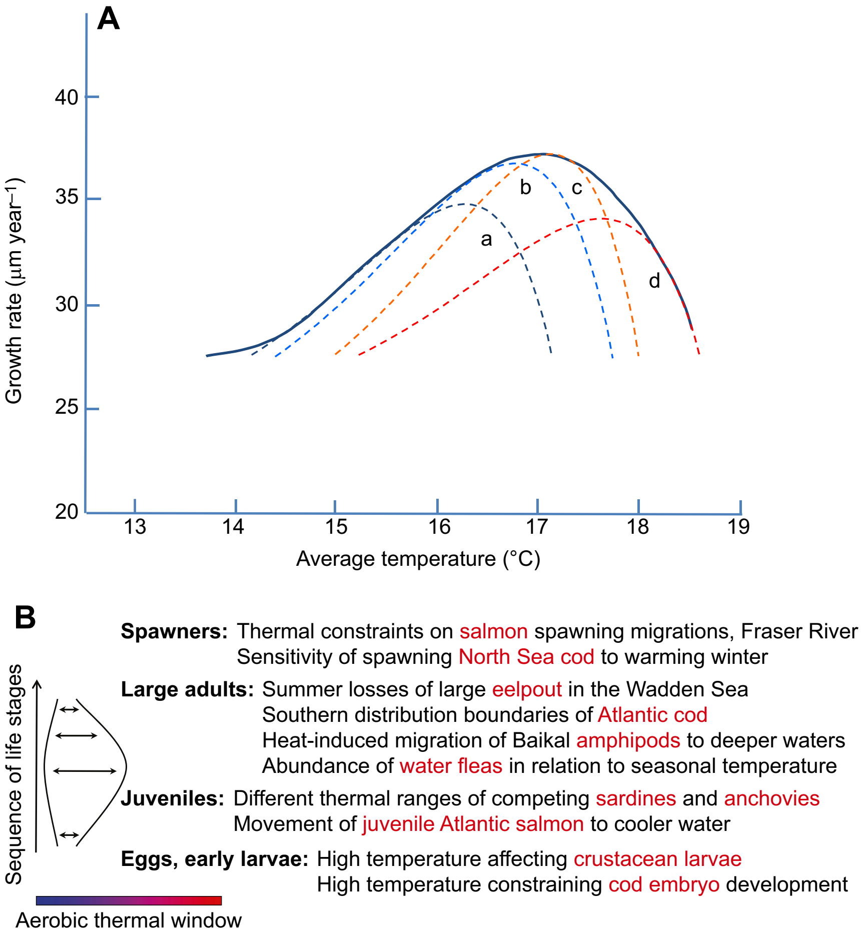


**Fig. 1. Dynamics of thermal windows between populations and during ontogeny.** (A) Temperature-dependent growth rates (the increment per year) read from otoliths in field populations of banded morwong, *Cheilodactylus spectabilis*, around Australia and New Zealand (after Neuheimer et al., 2011). The overall growth curve may well reflect the combination of different growth curves of individual (hypothetical) populations (a–d), indicating phenotypic diversity of thermal optima and ranges resulting from local acclimatisation or adaptation, as seen in cod, *Gadus morhua.* Trade-offs in energy budget result, and these lead to shifts in growth rate and performance capacities between populations (e.g. Pörtner et al., 2008; see Box 2). (B) Ontogenetic dynamics of thermal windows [curves indicate changes in upper and lower oxygen- and capacity-limited thermal tolerance (OCLTT) limits over time and between life stages; thermal window widths are indicated by arrows; Pörtner and Farrell, 2008] in relation to observed ecosystem/life-history constraints or to field phenomena explained by laboratory evidence for the respective life stage (after Perry et al., 2005; Breau et al., 2011; Eliason et al., 2011, 2013; Deutsch et al., 2015; Dahlke et al., 2016; Jakob et al., 2016; Pörtner and Knust, 2007; Pörtner et al., 2008; Storch et al., 2011; Schwerin et al., 2010; Takasuka et al., 2007).

compartments (separated by structure or function) as a proxy of complexity. The comparatively low thermal limit to animal life, i.e. above about 45°C (or somewhat less in water), would result from functional integration of a large number of compartments at the whole-organism level (Storch et al., 2014). This immediately rules out the possibility that one individual key protein or mechanism has an exclusive role in whole-organism limitation. Instead, constraints felt at lower levels of biological organisation should be embedded into the whole-organism context (see Pörtner, 2012). In heat-tolerant microbes, proteins do function up to 120°C, and there is no reason to assume that animal proteins could not evolve heat limits above the thermal limit of animal life.

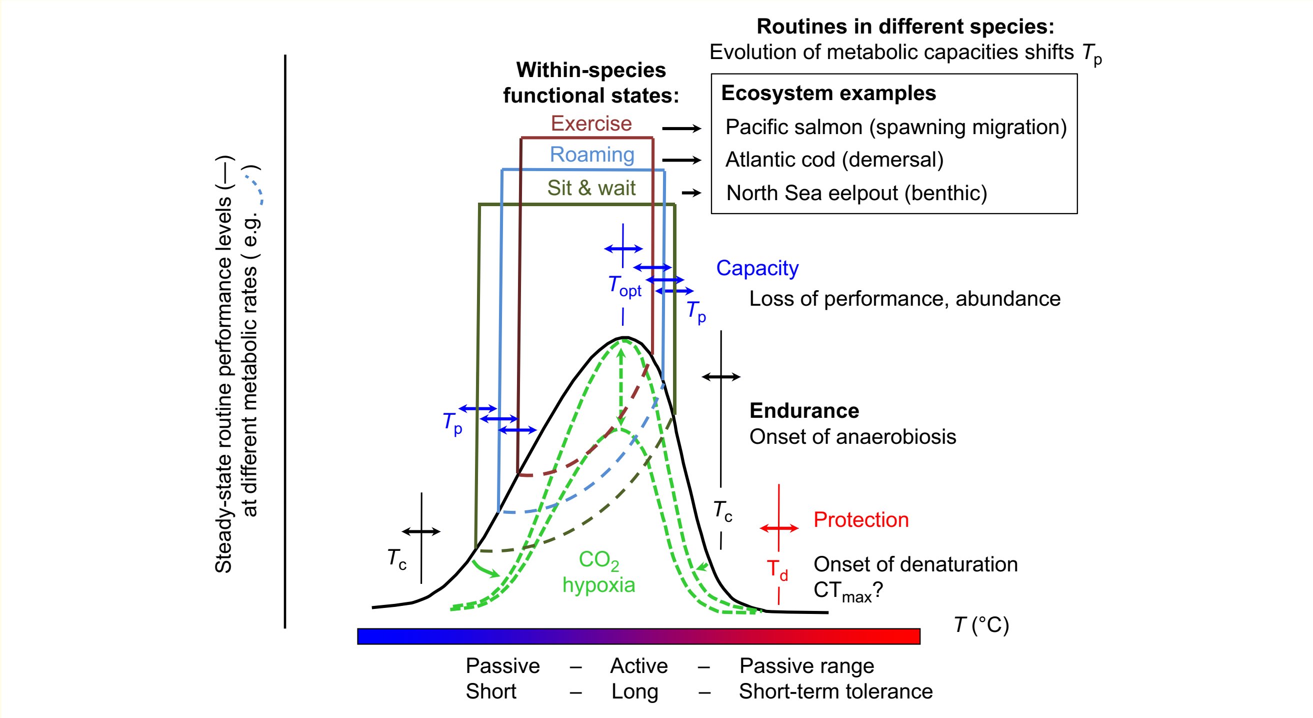
For the benefit of optimum functioning, whole-organism and molecular thermal ranges would be interdependent on evolutionary time scales, with molecular thermal ranges being somewhat wider than whole-animal thermal ranges (Pörtner et al., 2012). Lower whole-animal thermal limits would promote the functional optimisation of proteins within the low range of animal body temperatures, resulting in molecular limits beyond but close to whole-organism thermal limits (e.g. Somero, 2010). At the same time, whole-animal constraints can feed back to the protein level, e.g. through oxidative stress (see Kassahn et al., 2009). Consequently, whole-organism limits ‘trickle down’ to limits at lower organisational levels, such that individual molecular or organellar functions may become limited at less extreme temperatures *in situ* than when extracted from the whole-organism or tissue context (Pörtner et al., 2012; cf. Iftikar and Hickey, 2013; Leo et al., 2017). Characterising the role of aerobic metabolism and underlying mechanisms in thermal limitation (Schulte, 2015) thus requires considering how these mechanisms interact with others (e.g. antioxidative defence) and whether whole-organism phenomena feed back to these mechanisms (Box 1). This level of complexity may explain why thermal biology has not had a coherent framework and also why experimental work building on reductionist hypotheses (e.g. the idea that thermal damage to one kind of protein causes whole-organism heat death) comes with potential pitfalls. Although experiments must necessarily be reductionist, researchers should strive to embed experimental findings into concepts that capture the full complexity of the mechanisms involved, in an ecological context.

Matches or mismatches in oxygen supply and demand affect all tissues and cells, and thus the largest conceivable number of body compartments in an animal (Storch et al., 2014). Despite the underlying role of functional complexity in OCLTT-induced sublethal thermal constraints, however, some recent studies have exclusively focused on $LT_{50}$ in an attempt to investigate the OCLTT concept; for example, by asking whether oxygen availability can shift $LT_{50}$ or whether a maximally stimulated and exploited cardiovascular system has the capacity to supply oxygen until this point. $LT_{50}$ and $CT_{max}$ are conventional measures of ultimate tolerance limits (e.g. Lutterschmidt and Hutchison, 1997). These limits lie at the edge of or outside the range of aerobic power budget (Zakhartsev et al., 2003; Pörtner and Knust, 2007; Chen et al., 2015), beyond critical limits ($T_c$, where there is a transition to anaerobic metabolism; Box 1) and close to the co-evolving denaturation temperature ($T_d$) (see Farrell, 2009, and below). Such testing raises concerns, as it bypasses the sublethal thresholds at the core of OCLTT, such as pejus temperature ($T_p$, the onset of capacity limitation and hypoxaemia) and $T_c$, and their ecological relevance (primarily of $T_p$), which has been demonstrated in field studies

### Box 2. Performance and OCLTT

Within the thermal range constrained by the $P_{O_2}$ profile (Box 1), the kinetic stimulation of metabolic processes and energy-dependent functions by warming (and their inhibition upon cooling) leads to an asymmetric whole-organism total performance curve supported by aerobic power budget. The curve has a functional optimum close to the upper $T_p$. The power budget is shared between growth, immune response, reproduction and exercise during different behaviours (e.g. migration, competition, foraging) at different routine steady-state levels (e.g. sit and wait, roaming). Thermal limitation begins with reduced performance, and tolerance becomes progressively more time limited once functional scope falls below a limiting threshold beyond $T_p$. At optimum temperature ($T_{opt}$), maximised steady-state functional scope results from optimum oxygen supply at baseline oxygen demand, which rises exponentially above $T_{opt}$ (dashed exponential curves, see Fig. 2). Capacity constraints lead to a mismatch in oxygen supply and demand beyond $T_p$. Depending on the level of routine performance and associated energy demand, $T_p$ and $T_c$ are dynamic within species. They are influenced by oxygen demand versus supply and by the temperature dependence of underlying metabolic costs (dashed exponential lines linking upper and lower $T_p$). The figure illustrates shifts in upper and lower $T_p$ depending on functional rates and associated metabolic demands. Different species use different routine functional rates, and their $T_p$ values are adjusted during their evolution to match ambient conditions. Both ambient hypoxia and elevated carbon dioxide levels may modify thermal windows (Walther et al., 2009; Zittier et al., 2012) and performance optima (green dashed lines). The graph depicts acute performance levels and limitations in response to short-term temperature fluctuations; during acclimatisation or adaptation (double-headed arrows), these may lead to shifts and changing widths of thermal windows (Fig. 1). The exact position of $CT_{max}$ in relation to $T_c$ and $T_d$ is unknown.

Thermal acclimatisation and adaptation shift thermal limits through changing membrane composition or capacities of enzymes and mitochondria, or through mechanisms protecting molecular integrity (influencing $T_d$; see Pörtner, 2012). For example, in the warmth, reduced oxygen demand and increased $T_p$ and $T_c$ are expected following a decrease in tissue mitochondrial density, capacity and proton leakage costs. The resulting unidirectional shifts of both upper and lower tolerance thresholds, and the changing width of the thermal window involve molecular and cellular adjustments shaping the metabolic capacity of tissues and the functional capacity of relevant energy-consuming processes and their maintenance costs.



(e.g. Pörtner and Knust, 2007; Eliason et al., 2011; Jakob et al., 2016). By addressing the effect of oxygen on lethality, such studies also overlook the contribution and capacity of mechanisms contributing to passive thermal tolerance (see Glossary; e.g. heat shock response, anaerobic metabolism, metabolic depression and antioxidative defence) as well as its time limitation beyond $T_p$ and $T_c$, before lethal effects set in at around $T_d$ (Box 1; Pörtner, 2010, 2012; cf. Peck et al., 2009). More generally, passive thermal tolerance starts once hypoxaemia and/or $Q_{10}$-dependent rate limitations (in the cold) constrain routine performance beyond $T_p$ (Box 1).

Considering the step-by-step development of OCLTT from upper $T_p$ to $T_c$ – and the increasing involvement of further mechanisms limiting thermal tolerance – leads to the prediction that any effects of experimentally increasing oxygen availability are progressively reduced and may become small at $CT_{max}$ or $LT_{50}$; such systematic investigation of oxygen effects across thermal thresholds would be useful. The existence of a whole-organism to molecular hierarchy of thermal sensitivity also suggests that increasing oxygen availability may not cause large shifts in $CT_{max}$ or $LT_{50}$, as oxygen may not (fully) alleviate the extreme (e.g. denaturation) limits that co-evolved at lower levels of organisation. For example, under ambient hyperoxia, excess oxygen may alleviate thermal stress by reducing the costs of oxygen supply, thereby causing shifts in sublethal but not necessarily in lethal limits (e.g. Mark et al., 2002; Pörtner et al., 2006; Ekström et al., 2016). By contrast, ambient hypoxia or reduced oxygen supply capacity would exacerbate thermal stress and, because of stronger feedback from whole-organism to molecular levels, e.g. through enhanced oxidative stress, may reduce upper $CT_{max}$ or $LT_{50}$ to some extent.

In line with the prediction that reduced oxygen supply capacity may reduce $CT_{max}$, recent studies in fish have identified a role for haematocrit in thermal tolerance. In support of OCLTT, Beers and

Sidell (2011) found a positive correlation between haematocrit and $\mathrm{CT_{max}}$ across Antarctic fish species. A recent study in sea bass manipulated the haematocrit and found a small but significant decline in $\mathrm{CT_{max}}$ at low haematocrit (Wang et al., 2014). However, $\mathrm{CT_{max}}$ and haematocrit were not correlated, possibly as a result of the effects of reduced sensitivity to oxygen close to $\mathrm{CT_{max}}$, as discussed above, combined with high data variability. The specific mechanisms causing the shift in $\mathrm{CT_{max}}$ or the reasons for the high variability in the relationship between $\mathrm{CT_{max}}$ and haematocrit remain insufficiently explored (see below). Overall, sublethal thermal constraints are more likely than lethal limits to be responsive to changing oxygen availability, and are likely to be more closely related to tissue functional capacity (see Glossary) and energy budget.

### Variables indicative of OCLTT

In light of the above discussion, it seems prudent to avoid focusing exclusively on $\mathrm{CT_{max}}$ or $\mathrm{LT_{50}}$ in tests of the OCLTT concept. Instead, we should specifically identify sublethal thermal constraints from respiratory and metabolic variables under resting or routine conditions (Table 1). Breakpoints in the temperature dependence of these variables by their nature are ‘softer’ indicators of thermal limitation than a ‘hard’ endpoint such as lethal collapse. For example, warming causes SMR to rise exponentially until a breakpoint temperature beyond which SMR no longer increases (indicating the $T_c$ to be surpassed) (e.g. Melzner et al., 2006; Giomi and Pörtner, 2013; see Fig. 2). Increasing ambient oxygen levels can lower the slope of the exponential rise in SMR and cause significantly lowered oxygen consumption rates at high temperatures – as seen in resting fish (Mark et al., 2002) or in amphibious crabs exposed to air (Giomi et al., 2014) – thereby increasing $T_c$.

Excess oxygen leads to reduced blood flow and thus lowers the cost of cardiovascular activity. This implies that, conversely, lower ambient oxygen levels cause metabolism to rise more strongly with increasing temperature, as a result of increased cardiovascular circulation. Similarly, anaemia can cause an increase in cardiac output (as in anaemic sea bass; see Wang et al., 2014); however, the cost increment in cardiovascular activity may remain small. By reducing viscosity (Farrell, 1991), a lower haematocrit may compensate for the cost increment, balancing the oxygen shortage caused by the reduced haematocrit. Generally, the patterns of heart rate and cardiac output indicate sublethal thermal limitation as they do not increase sufficiently to match the warming-induced rise in $O_2$ demand and to keep the aerobic power budget large, a lag setting in well below $\mathrm{CT_{max}}$ (e.g. Wang et al., 2014). Haematocrit may thus be better correlated with sublethal constraints than with $\mathrm{CT_{max}}$ (e.g. Buckley et al., 2014).

So how might variables underlying the OCLTT best be investigated? Analyses of the OCLTT should mimic natural conditions and consider routine activities displayed by the animal in the field, as well as minimising stress phenomena that would transiently mobilise functional reserves (see Glossary), e.g. through release of catecholamines, which stimulate cardiovascular circulation, glycogenolysis or anaerobic metabolism. Such stimulation supports time-limited thermal tolerance but has negative consequences for other components of the energy budget, e.g. growth. It should be noted that not all species display continuous motor activity; thus, measurements of steady-state aerobic scope (see Glossary; Farrell, 2013) for exercise may not always be possible when testing OCLTT. Tissue oxygenation and oxygen supply to sensitive aerobic organs such as the heart (Ekström et al., 2017) or liver may closely trace OCLTT under routine conditions, but such estimates are usually not available. In fish, measurements of venous rather than arterial $P_{O_2}$ appear appropriate to indicate thermal constraints, because of venous perfusion of the heart in most species (Farrell and Clutterham, 2003; Lannig et al., 2004; Ekström et al., 2016; Farrell, 2009) and the proximity of venous blood to tissues; these analyses would ideally be complemented by those of venous oxygen content ($C\mathrm{v}_{O_2}$). In crustaceans, measurements of oxygen partial pressure in (mixed

**Table 1. Variables analysed and interpreted as indicators of OCLTT in animals**

| Parameter | $T_{opt}$ | $T_p$ | $T_c$ | $T_d$ |
|---|---|---|---|---|
| Maximum aerobic scope: maximum growth, maximum exercise | ✓ | | | |
| $\mathrm{CO_{max}}$ (exercise) | ✓ | | | |
| Reduced performance* (exercise, growth, CO) | | ✓ | | |
| $P\mathrm{v}_{O_2}$ /$C\mathrm{v}_{O_2}$ | | ✓ | ✓ | |
| BP $P\mathrm{v}_{O_2}$ /$C\mathrm{v}_{O_2}$ | | ✓ | ✓ | |
| BP ventilation | | ✓ | ✓ | |
| BP heart rate | | ✓ | ✓ | |
| BP stroke volume | | ✓ | ✓ | |
| BP $\dot{M}_{O_2}$ | | ✓ | ✓ | |
| $\mathrm{CO_{max}}$ (rest, routine) | | | ✓ | |
| Mitochondrial functioning (permeabilised fibres) | | ✓? | ✓? | |
| Anaerobic end products (especially succinate) | | | ✓ | |
| Cardiac arrhythmia/bradycardia | | | ✓ | |
| $\mathrm{CT_{max}}$ | | | | ✓? |

See Box 1 and 2, and Storch et al. (2014). Indicators are mostly respiratory parameters that have been used to assess different oxygen- and capacity-limited thermal tolerance (OCLTT)-related terms and thresholds (Box 1, Box 2; $T_{opt}$, $T_p$, $T_c$, $T_d$), as indicated with a tick. A general conclusion from available studies is that the physiological condition of the experimental animals needs to be well defined and to match (long- and short-term) ecosystem conditions. Otherwise, trade-offs in aerobic energy (power) budget may have consequences for individual performances and their thermal constraints. Note that the assessment benefits from an integrative analysis of various processes and taxon- or even species-specific patterns, as not all indicators may display obvious thresholds in all taxa or species. $\dot{M}_{O_2}$, oxygen consumption rate; CO, cardiac output; BP, breakpoint; $P\mathrm{v}_{O_2}$, venous partial pressure of $O_2$; $C\mathrm{v}_{O_2}$, venous oxygen content; ?, proof of concept needed. *Ideally, total aerobic performance (not directly measurable because of trade-offs in energy budget), but often referred to as growth (Fig. 1) or exercise.

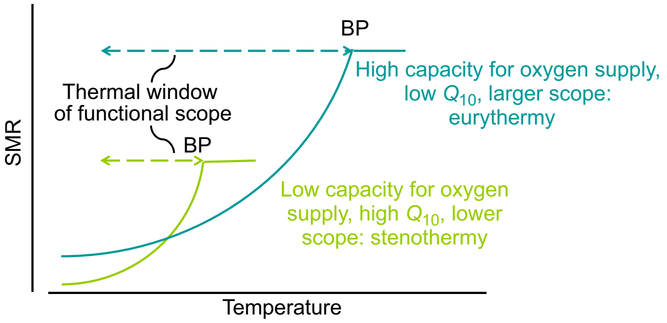


**Fig. 2. Conceptual graph illustrating the thermal operating ranges of two hypothetical systems with different temperature-dependent capacities.** The schematic diagram shows how low- and high-capacity systems (green and blue curves, respectively) have different baseline costs (standard metabolic rate, SMR) and ranges of operation if exposed to workload (e.g. due to warming) until reaching different capacity limits (under routine conditions, indicated by breakpoints, BP). Through similar principles and differences in thermal responses ($Q_{10}$), OCLTT distinguishes stenotherms from eurytherms (here exemplified through narrow versus wide temperature ranges and differences in the temperature dependence of the workloads; see Pörtner, 2006). In each case, the thermal window width of functional scope is represented by the length of the dashed arrows.

arterial and venous) haemolymph and of succinate concentrations in tissues can reveal the development of extreme hypoxaemia at thermal extremes (Frederich and Pörtner, 2000). The accumulation of anaerobic metabolites (such as succinate) beyond $T_c$ indicates oxygen-deficient mitochondria. The ecologically important transition phases from earliest sublethal limitation (at $T_p$) to $T_c$ precede $CT_{max}$ (see Zakhartsev et al., 2003) – but their mechanistic link to $CT_{max}$ clearly deserves further study. In the future, continuous recordings of tissue oxygenation may also support such analyses.

Regulatory responses are likely to involve various molecular factors (Kassahn et al., 2009). For example, hypoxia-inducible factor 1 (HIF-1) contributes to improving anaerobic capacity as well as oxygen supply through erythropoesis and may, thereby, enhance the capacity for heat and cold endurance. Cold adaptation may be supported by thyroid hormones (Little et al., 2013). Depending on the animal phylum, these changes contribute to adjustments of oxygen-transport capacity by both ventilation and circulation (invertebrates) or mainly circulation (fishes), as well as to adjustments in mitochondrial density, capacity, oxygen demand and energy budget (all tissues).

### Addressing capacity, performance and energy budget

It should be noted that the term ‘capacity’ is used in different ways. The original OCLTT literature emphasises the interdependence and trade-offs between the rates and capacities of functions supplying and consuming oxygen and associated energy (e.g. cardiovascular systems, as reflected in cardiac output; or mitochondrial ATP synthesis or transmembrane ion transport in all tissues), their baseline costs (e.g. resting contractile cardiac activity, mitochondrial proton leakage or transmembrane ion leakage) and the resulting performance at various levels of biological organisation, in unstressed animals at rest or during routine activities in variable or stable climates. However, recent studies intending to test OCLTT have focused on the cardiovascular system and pushed it to its limits to determine whether it has the maximum capacity to provide sufficient oxygen to the organism until $LT_{50}$ (Gräns et al., 2014; Wang et al., 2014). This approach probably activates functional reserves (see above), which is possible only transiently. At first sight, both views may appear valid, yet OCLTT emphasises sublethal thermal limitation and whole-organism consequences and trade-offs under resting and routine conditions. The concept addresses the subtle links and constraints in baseline costs, net functional scope (see Glossary) and resulting whole-organism performance capacity (see Fig. 2), as well as any shifts in net energy allocation to routine activities, without transient activation of functional reserves.

Conceptually, functional properties of the fish heart illustrate core aspects of the term ‘capacity’ and the associated functional and thermal limits, as considered in the OCLTT concept for species with different modes of life and under different temperature regimes. Oxygen consumption of the body ($\dot{M}_{O_2}$, excluding gas exchange via the skin) equals cardiac output (CO) multiplied by the difference in arterial and venous oxygen concentration (Fick’s principle):

$$\dot{M}_{O_2} = \mathrm{CO} \times (Ca_{O_2} - Cv_{O_2}). \quad (1)$$

Cardiac output is the product of stroke volume and heart rate, and reflects the ‘functional capacity’ of the heart. Stroke volume is the blood volume pumped by the fish ventricle during one contraction. Any increase in oxygen demand (e.g. during routine exercise) is reflected in an increase in cardiac output. For most fishes, the contribution of stroke volume to oxygen supply is usually higher than that of heart rate, e.g. during sustained (routine) swimming (Farrell, 1991).

Functional capacity has three interdependent aspects: (1) how much performance can be achieved, depending on (2) how the respective system is set up and (3) at what cost. A high-capacity cardiovascular system comprises a larger heart with higher resting cardiac output than a low-capacity system (Farrell, 1991). Active fishes usually have a larger relative heart mass (Santer, 1985; Farrell, 1991). Accordingly, exercise training can produce isometric cardiac growth as seen in rainbow trout (Farrell et al., 1990). Cardiac output depends on the volume of the heart and the pressure generated by wall tension. When higher cardiac output is needed (e.g. during warming), heart rate and, possibly, stroke volume increase, at an energetic cost (i.e. increased oxygen consumption). A larger stroke volume entails an increase in ventricle radius and greater filling during diastole.

Morphologically, the fish heart can be simplified as a sphere with defined volume ($V$) and diameter ($D$, wall thickness) of the myocardial muscle. The workload ($W$) of a contracting sphere equals:

$$W = p \times V, \quad (2)$$

where $p$ is pressure.

Energy requirements (oxygen demand) of the heart are mainly determined by wall tension ($T$) as defined by Laplace’s law:

$$T = p \times r/2D, \quad (3)$$

where $r$ is the ventricle radius. The equation illustrates that a thicker myocardial muscle produces higher pressure and then output, at the expense of higher baseline costs due to larger, mitochondria-rich tissue mass. The energy turnover during contraction is related to the contractile shortening of heart muscle fibres. In the assumed spherical heart, the length of a circular heart fibre may equal the heart circumference, $L=2\pi r$, with a shrinking radius representing the contractile shortening of the muscle fibre. Because $V=4/3\pi r^3$, the same absolute value of contractile shortening in a large, high-capacity heart (with a large radius) will produce a stroke volume larger than that of a small, low-capacity heart, at rest and during exercise. Similarly, for the same stroke volume, larger hearts need a smaller contraction of the muscle fibre than smaller hearts, resulting in a lower increase in relative cellular oxygen/energy demand for the same increase in performance, and a larger functional and energy reserve in larger hearts to maximise performance. For the same increase in workload, a small-volume heart will thus be stimulated to a greater extent and limited sooner than a large-volume heart (Fig. 2), a conclusion supported by the larger maximum stroke volumes of isolated perfused hearts of active fish (larger hearts) versus sluggish fish (smaller hearts; Farrell, 1991).

According to OCLTT, the capacity of the heart plays a role in determining thermal tolerance and associated energy turnover. As outlined above, excess oxygen can cause reduced blood flow and visibly lower whole-organism oxygen demand in the warmth, possibly by reducing the rising cost of cardiovascular activity. Increasing temperature induces increased cardiac output (e.g. Lannig et al., 2004; Farrell, 2009; Franklin et al., 2013), building on different baseline costs of maintenance and with different exponential slopes in hearts of different sizes according to a species’ active or passive lifestyle (Fig. 2).

Even within-species variability as seen in European sea bass indicates that larger hearts in fish with lower SMR support higher temperature tolerance and faster recovery from exercise than smaller hearts in fish with higher SMR (Ozolina et al., 2016). This emphasises that there is poorly understood variability in the patterns depicted in Fig. 2. The following hypothetical picture emerges: the comparison of sluggish versus active fish appears analogous to that of cold stenothermal versus cold eurythermal fish (or ectotherms in general). To meet the same absolute or relative increase in oxygen demand, a low-capacity, low-cost system as in a sluggish fish (or a cold-adapted stenotherm) would experience a stronger stimulus than a higher-capacity system as in an active fish (or in a temperate eurytherm), causing a greater percentage cost increment in the sluggish fish/stenotherm and thus contributing to a higher $Q_{10}$ and earlier thermal limitation under routine conditions. This pattern is also mirrored in the low-capacity, low-cost mitochondria seen in cold-adapted stenotherms versus high-capacity, high-cost mitochondria as in cold-adapted eurytherms (Pörtner, 2006; Fig. 2). In line with these findings, more active, mobile Antarctic stenotherms are indeed more heat tolerant than sessile sluggish ones (Peck et al., 2009; for the role of cold adaptation and eurythermy in the evolution of high-energy turnover endotherms, see Pörtner, 2004; Clarke and Pörtner, 2010). Further observations are also in line with these emerging principles. Population-specific adaptation to various temperature regimes in salmon involves different heart sizes and adrenoceptor densities (Eliason et al., 2011, 2013). Acclimation of individual fish to temperature also involves changes in cardiac performance of fish. Acclimation to warming in salmon causes an increase in maximum heart rate, meeting the rising baseline cost (Anttila et al., 2014).

The mechanical picture drawn from this simplified approach will thus be modified by potential cellular or morphological differences, such as in the oxidative capacity of mitochondria (see Pörtner, 2006), pacemaker activity, and size and capillarisation of the heart, or blood viscosity. For example, a low contribution of blood oxygen transport to aerobic scope is compensated for to some extent by the evolution of relatively large hearts, as in Antarctic icefishes (Farrell, 1991). The interplay of all of these factors will shape the contribution of the cardiovascular system to the species-specific oxygen and capacity limitation of the whole organism.

In general, maintenance costs (measured as SMR) are relatively low within the optimal thermal range but rise exponentially towards the upper limit of thermal tolerance, constraining functional (aerobic) scope. Although for some performances (like growth or reproduction) or routine activities (roaming and feeding) aerobic scope is not fully exploited, rising maintenance costs will still introduce constraints on aerobic power budget. Thus, functional scope, e.g. of the heart, is highest at the thermal optimum ($T_{\mathrm{opt}}$) when maintenance costs are still relatively low. At temperatures below $T_{\mathrm{opt}}$, functional scope is depressed by cooling more than maintenance costs are, finally leading to the failure of oxygen supply to meet demand as seen in warm temperate animals at critically low temperatures (Frederich and Pörtner, 2000). High-capacity systems (e.g. tuna), while having a higher baseline cost (Fig. 2), come with the benefit of easily buffering demand under routine conditions, e.g. during warming or exercise or both, with a smaller percentage increment in cost and limitation setting in at higher temperatures than for the same condition in a low-capacity system (as in hagfish or in Antarctic icefish, considering the loss of haemoglobin in the latter). Here, baseline costs are lower but percentage increments are higher, and the system runs into capacity limitations at lesser extremes. Because of the interdependence of capacity and cost, the percentage increment of cost per degree of warming is thus highest in energy-saving, low-capacity systems, such as polar or winter stenotherms (e.g. Pörtner, 2006; Wittmann et al., 2008; Pörtner et al., 2013), emphasising a link between energy turnover, mode of life and the level of eurythermy (see Pörtner, 2004; Peck et al., 2009; Clarke and Pörtner, 2010). It should be noted that temperate-zone animals may be able to exploit the energetic benefits of being either winter stenotherms or spring and summer eurytherms through seasonal acclimatisation (e.g. Wittmann et al., 2008).

Looking at capacity just in terms of its maximum exploitability thus misses the role of underlying design and its plasticity under routine conditions, as well as the subtleties in the functional transitions and limitations. In a living animal, an early subtle indication of capacity limitation can be the presence of a breakpoint temperature (Fig. 2). This more complex approach to capacity captures the progressive development of thermal limitation from the earliest constraints to lethal temperatures (Box 1), as well as the difference between stenotherms and eurytherms (Pörtner, 2006).

In this context, measurements of aerobic scope for exercise as an estimate of aerobic power budget have to be interpreted very carefully, as analyses of aerobic scope using critical swimming speed ($U_{\mathrm{crit}}$) protocols in fish can include exploitation of non-sustainable short-term functional reserves that rely on hormonal (adrenergic) stimulation or anaerobic processes, beyond the onset of kick-and-glide swimming (Lurman et al., 2007). As the degree of mobilisation of anaerobic reserves can have a strong behavioural component (Peake and Farrell, 2006), the use of fatigue-based exercise protocols may overestimate aerobic capacity, thereby again missing earliest functional constraints at pejus limits (Table 1).

It is also important to note that $T_{\mathrm{p}}$ and $T_{\mathrm{c}}$ may shift depending on the routine performances used and their steady-state energy demand at the ecosystem level (Box 1). For some species in a specific life phase (e.g. spawning migrations in salmon), $T_{\mathrm{p}}$ and $T_{\mathrm{c}}$ are best determined during full exploitation of aerobic scope for exercise. For other species and life phases (and more widely), this should be during their lower levels of routine activities (e.g. Atlantic cod; Deutsch et al., 2015). In species regularly experiencing temperature extremes (such as at low tide in the intertidal zone), their capacity to exploit the passive tolerance range may become important in shaping fitness. Ideally, for assessing ecologically relevant $T_{\mathrm{p}}$ and $T_{\mathrm{c}}$ values, mode of life and associated energy demand, life phase and habitat challenges require consideration.

## Evolutionary modulation of OCLTT

The OCLTT framework has identified phenomena of thermal limitation in various animal phyla against ecological and evolutionary backgrounds (e.g. Pörtner et al., 2005; Knoll et al., 2007). It has been suggested to be an early evolutionary principle in animals that has been modified according to life stage (see Pörtner and Farrell, 2008) or climate zone (Pörtner, 2006; Beers and Sidell, 2011; Pörtner et al., 2013), or during the evolution of air breathing (Giomi et al., 2014).

OCLTT varies with ontogeny, the associated development of organ functioning, metabolic plasticity and the organ's resulting capacity and body size. Thermal windows are typically narrow during early life and adult spawning stages and wider during juvenile and young adult stages (Pörtner and Farrell, 2008; Poletto et al., 2017; Fig. 1B). Such bottlenecks constrain where early and spawning life stages can live, and expose them to strong evolutionary pressures, leading to adaptive changes with functional consequences for the next life stage. These are virtually unexplored. Knowing the life history of a species in the context of habitat fluctuations is thus relevant to fully identify evolutionary bottlenecks and their consequences for physiology and biogeography.

In permanently oxygen-rich polar waters, adaptation may have alleviated thermal constraints on the cold side of the thermal window (Wittmann et al., 2012; Pörtner et al., 2013), leaving residual cold limitation to kinetic constraints on functional capacity. Improved cold tolerance was facilitated by excess oxygen dissolved in cold water, which supported low metabolic rates by allowing a lowered oxygen supply capacity and cost. However, this would come at the price of enhanced heat intolerance (Pörtner et al., 2013). In addition, excess oxygen supply at stable low temperatures may have enabled the loss of myoglobin and haemoglobin functions in Antarctic icefish, which lowered oxygen supply capacity and increased heat intolerance even further (Beers and Sidell, 2011).

The situation is less clear for the evolutionary adaptation to breathing air, which has 30-fold higher oxygen levels than water. The transition to terrestrial life often required the evolution of completely new gas-exchange systems (lungs, trachea), while convective ‘blood’-bound oxygen supply to tissues persisted in most taxa (except for some adult insects). The symmorphosis principle (see Glossary; Weibel et al., 1991) suggests that these new convective oxygen-supply systems also evolved with their capacity limits set to cope with temperature extremes. In lower vertebrates, some evidence in fact indicates a progressively limited scope for oxygen uptake by the lungs towards higher temperatures (see fig. 6 in Pörtner, 2002; Jackson, 2007). In contrast, crustaceans still use their gills when in air and are therefore suitable models to investigate the potential benefits of air breathing for thermal tolerance. In fact, findings in amphibious crabs corroborate that oxygen supply costs are reduced in air and that this causes enhanced heat tolerance (Giomi et al., 2014). Insects may also have exploited this route; while aquatic larvae are subject to OCLTT principles (Verberk and Calosi, 2012), the tracheal oxygen supply to tissues in terrestrial adults supports elevated heat tolerance at minimised oxygen-supply costs.

Recent studies of thermal tolerance in air breathers may not have considered this diverse evolutionary background, or the pitfalls mentioned above. A study of the tropical toad *Rhinella marina* reported evidence for thermal acclimation at two temperatures (Seebacher and Franklin, 2011) but did not explore the related changes in steady-state cardiovascular functions or in tissue oxygenation (or venous oxygen tensions as determined by Pörtner et al., 1991). Such evidence is required when investigating a role for oxygen in thermal limitation. A later study of the same species by Overgaard et al. (2012) found a typical exponential increase in resting oxygen consumption, which entered the steep phase beyond 30°C, in line with a transition to pejus range (see Fig. 2). However, activity was enforced and non-steady state; and data variability was high and may not have provided sufficient resolution to determine limiting thresholds. A later study on python (Fobian et al., 2014) drew conclusions from measurements of temperature-dependent arterial oxygen tensions, which remained high even at high temperatures. It needs to be considered that arterial oxygen values are often not suitable to investigate OCLTT, as also seen in fish, for example, where arterial $P_{O_2}$ ($Pa_{O_2}$) remained high when venous $P_{O_2}$ ($Pv_{O_2}$) fell with rising temperatures (see Sartoris et al., 2003). In line with OCLTT, however, the python developed a warming-induced reduction in aerobic scope, which was determined as the difference between metabolic rates of fasting and digesting snakes. The drop in scope was paralleled by a levelling off in temperature-dependent arterial $P_{O_2}$ and oxygen consumption in digesting snakes (see fig.1 of Fobian et al., 2014). As a corollary, future studies addressing sublethal constraints in oxygen supply towards extreme temperatures in air require careful consideration of best practices for studying OCLTT (Pörtner, 2014).

Further study of the role of OCLTT in animal evolution and ontogeny is needed. For example, the mechanisms of thermal adaptation and limitation have been poorly investigated across all life stages of a species or in both aquatic and terrestrial animals from the sub-tropics and the tropics. These organisms are probably adapted to ambient temperatures closer to metazoan heat limits of about 45°C, suggesting a steep transition from optimum temperatures via sublethal to lethal limits. This makes it more difficult to accurately identify sublethal thresholds, analogous to the study of cold limitation in (sub-)polar organisms. Thermal adaptation to the warmth would cause a down-regulation of metabolic rate at high oxygen diffusivity, which might alleviate oxygen-dependent constraints. Nonetheless, some data in tropical fish indicate constraints on aerobic scope for exercise at high temperatures (Munday et al., 2009). That said, the available data are presently too limited to clearly identify typical patterns of complexity limits in both tropical and subtropical aquatic and terrestrial ectotherms.

## Conclusions

The ad hoc mixing of OCLTT with classical concepts in thermal biology such as $CT_{max}$ requires care, as such studies tend to overlook the subtle limits to aerobic metabolism and performance, and the systemic to molecular hierarchy of thermal tolerance.

For understanding ecological patterns, the functional rates of organisms need to be explained. Darwinian fitness does not depend on one performance trait only, but various traits come together in their additive energy cost. Fitness is thus related to routine metabolism, reflecting how aerobic power budget is used by different performances simultaneously and at the required minimum level and above. Therefore, OCLTT should not be simplified to comprise aerobic scope for exercise only. OCLTT is about a cause and effect understanding for different performances and their role in fitness and share in energy budget of a specific life stage. Such cause and effect understanding is at the core of physiological studies in an ecological context. This principal understanding is also an asset when making predictions on the fate of populations in a distant future.

The OCLTT concept explains the first line of thermal limitation at the whole-organism level in animals, and may represent an evolutionary constraint that was modified depending on life stage and climate, and during transition to life in air. Neglecting to consider the links between levels of biological organisation will lead to insufficient explanations of thermal limits that fall behind what the OCLTT concept has already achieved. It is possible that the combination of OCLTT and molecular limits shape lethal limits and their response to oxygen availability. As experiments can rarely resolve all facets of complex phenomena, addressing such complexity requires the parallel development of theoretical background and experimental approaches at multiple levels; current theories of evolutionary biology illustrate these requirements (e.g. Angilletta, 2009). Thermal physiology should strive to interpret experimental results in light of an organism’s ontogeny and ecology, using comprehensive, ecologically relevant concepts (Bozinovic and Pörtner, 2015). Conversely, reductionist lines of interpretation should remain coherent with the widest possible conceptual framework.

Sublethal thermal limitation according to OCLTT can also vary depending on activity level (e.g. resting, roaming or high rates of steady-state energy use); thus, it is important to consider which situation is typical for a species and its critical life stage(s) in the wild. Whether limitations to routine metabolic scope set biogeographical borders and, thus, limits to the realised niche needs to be investigated more widely. In the context of thermal biology and climate change, we require integrative, ecosystem-oriented and evolutionary modes of interpretation, ideally combining field and experimental data. With this aim, the OCLTT concept has connected ecological and physiological findings in animals, and we hope that these connections will be developed further in the future.

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**Competing interests**
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