

**IN-RIVER ASSESSMENT OF SWIMMING AND  
FEEDING BEHAVIORS AND INFERENCES OF  
METABOLIC STATE OF *ONCORHYNCHUS MYKISS* IN  
THE LOWER TUOLUMNE RIVER**

**STUDY REPORT  
DON PEDRO PROJECT  
FERC NO. 2299**



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**September 2017**

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## 1.0 INTRODUCTION

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The temperature range for optimal performance is an important variable influencing the fundamental habitat of fish populations (Fry 1947; Huey and Kingsolver 1979; Schulte et al. 2011), and is easily measured in a tightly controlled laboratory setting. A 2014 streamside laboratory study quantified the upper thermal performance limit and temperature range for peak metabolic performance of wild Lower Tuolumne River (LTR) juvenile (100 mm to 200 mm) *O. mykiss* (Farrell et al. 2017; Verhille et al. 2016). Aerobic metabolic rate was assessed by directly measuring 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). To determine the capacity of *O. mykiss* to supply oxygen to tissues above and beyond a basic routine need, RMR was subtracted from MMR. This capacity is termed the absolute aerobic scope (AAS = MMR - RMR) and defines the fish's capacity to perform the activities essential to life (swimming, catching prey and feeding, digesting a meal, avoiding predators, defending territory, etc.). As RMR increases with temperature, tasks like holding station take up an increasing proportion of available AAS, resulting in reduced ability to perform other essential tasks simultaneously. The relationship between aerobic capacity and water temperature described by performing these measurements over a wide range of temperatures (13°C to 25°C) showed peak AAS to occur at 21.2°C and average AAS to remain within 5 percent of this peak up to 24.6°C.

When assessing temperature influences on fish survival and distribution in nature, it is essential to integrate the temperature responses of aerobic scope described in the swim tunnel study into an ecological framework that accounts for the biotic and abiotic factors that interact with temperature effects on fish metabolic capacity (Overgaard et al. 2012; Clark et al. 2013a; Norin et al. 2014; Ern et al. 2014; Pörtner and Giomi 2013; Farrell 2013, 2016) used by juvenile Pacific salmon to carry out daytime activities (Puckett and Dill 1985). As station holding and feeding are essential activities for the long-term survival of *O. mykiss* in nature, quantification of temperature effects on the metabolic demands of and capability to perform these two activities provides improved understanding of the influence of water temperature. This 2015 study was designed to build upon the findings of the 2014 streamside laboratory study by investigating whether high summer water temperatures prevent juvenile *O. mykiss* in the LTR from performing essential activities.

## 2.0 STUDY GOALS AND OBJECTIVES

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The goal of this study was to build upon findings of the 2014 swim tunnel study by integrating the temperature responses of aerobic scope into an ecological framework accounting for factors that interact with temperature effects on LTR *O. mykiss* metabolic capacity. The cumulative energetic demands of holding station (i.e., the swimming behavior required to hold position in the river current) and feeding have been suggested to be more than 80 percent of the energy budget (Puckett and Dill 1985), but this assertion has never been rigorously tested. Furthermore, swimming effort is very much context specific and salmonids are known to increase their routine metabolic rate up to 10-fold with maximum swimming activity. For such individuals it would seem unlikely that they spend most of their life expending energy at 80 percent of maximum. Therefore, the issue at hand is how often and at what speed do fish swim in nature, especially when foraging?

One approach to investigating these questions is to build from the strong correlation between metabolic rate of steady swimming, non-schooling fish, and tail beat frequency (Feldmeth and Jenkins 1973; Herskin and Steffensen 1998; Lowe 2001; Steinhausen et al. 2005; Webber et al. 2001). This robust relationship has allowed scientists to reliably use tail beat frequency (TBF) to estimate metabolic rates (Puckett and Dill 1985; Wardle et al. 1989). This approach was previously used to estimate metabolic rates of *O. mykiss* in another California stream (Feldmeth and Jenkins 1973). An objective of this study was to quantify in-river TBF of juvenile *O. mykiss* to estimate field metabolic rates.

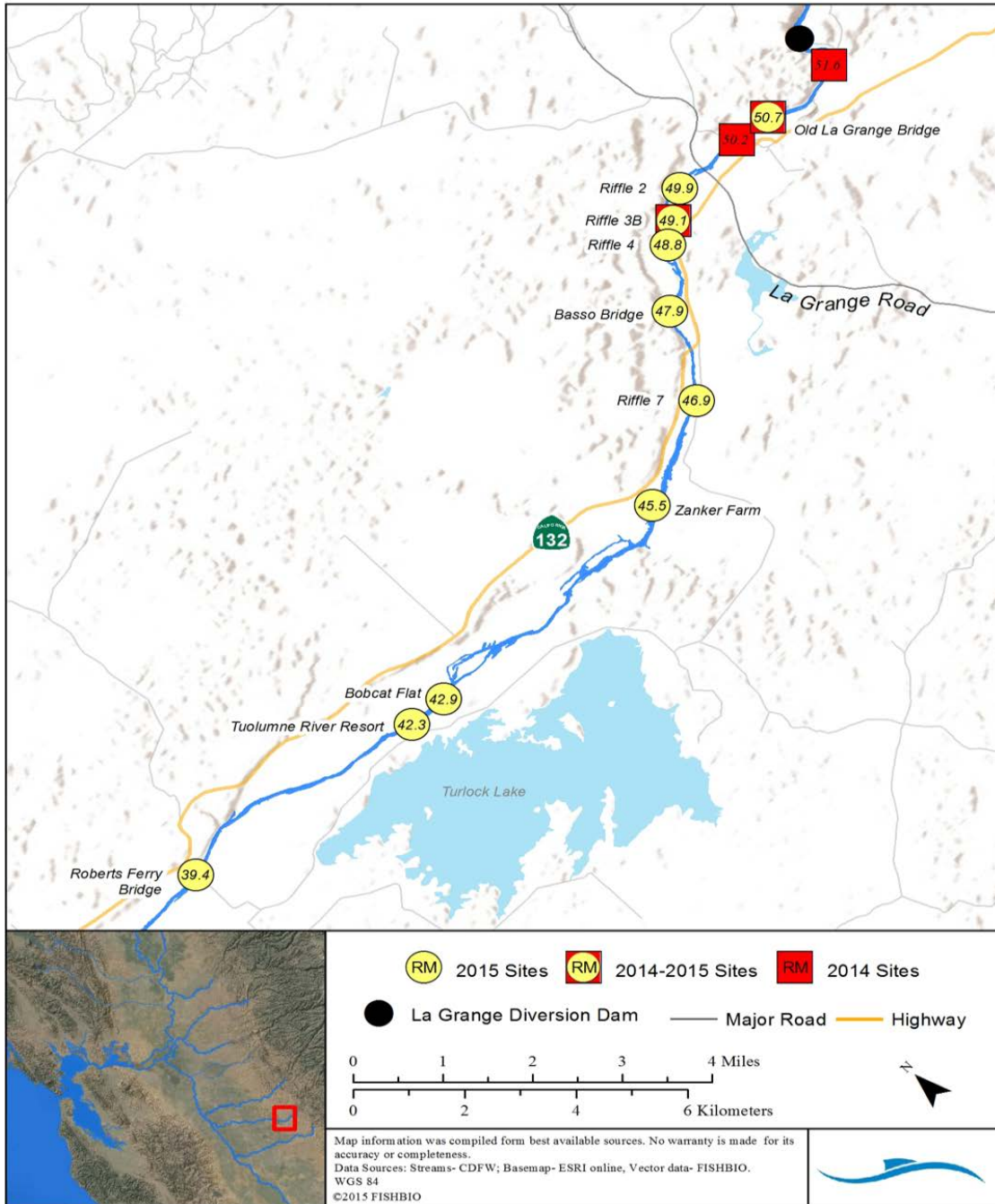
The metabolic demands of holding station and feeding are necessarily additive, unless the digestive process is entirely suppressed when a fish swims even moderately. As supra-optimal temperatures reduce metabolic capacity, activities other than feeding may be prioritized leading to reduced growth (Brett 1979; Elliott 1982; Hokanson et al. 1977). Estimates of food consumption (i.e., quantity, quality, or assimilation of the captured food) cannot be performed on free-swimming fish. However, prey strike frequencies provide valuable information on the capacity for feeding activity, and have been previously measured for juvenile salmonids in streams (Feldmeth and Jenkins 1973; Nislow et al. 1998; Puckett and Dill 1985; Quigley et al. 2006). One objective of this study was to quantify in-river prey strike frequencies of juvenile LTR *O. mykiss*.

The following questions were examined to improve understanding of LTR juvenile *O. mykiss* metabolic rates and feeding behavior at summer water temperatures:

- Is the observed TBF of LTR *O. mykiss* holding station in water currents at typical LTR habitats less than the maximum TBF measured for LTR *O. mykiss* holding station in the 2014 swim tunnel study?
- Using TBF as the predictor, how does the in-river metabolic rate of LTR *O. mykiss* holding station compare with the maximum metabolic rate measured at the same temperature for LTR *O. mykiss* in the 2014 swim tunnel study?
- Do observed prey strike frequencies in typical habitats of LTR *O. mykiss* decrease with increasing LTR water temperature?

### 3.0 STUDY AREA

The study area (Figure 3.0-1) was downstream of the La Grange Diversion Dam on the Tuolumne River, and extended from the Old La Grange Bridge (RM 50.7) to the Roberts Ferry Bridge (RM 39.4).



**Figure 3.0-1. Map of the lower Tuolumne River with sample sites from the 2015 tail beat frequency study and the 2014 swim tunnel study (Farrell et al. 2017) indicated.**

## 4.0 METHODS

Metabolic state and feeding behavior were quantified from in-river video recordings over a range of river temperatures and river locations. Tail beat frequency (TBF) was measured from these videos and used to predict the in-river metabolic rate while fish were holding station in the river. Metabolic rates were predicted from a mathematical model (based on metabolic and TBF measurements in Farrell et al. 2017) that related metabolic rate to TBF measured at different temperatures. Similar approaches of using TBF measurements to estimate active metabolic rate have been applied to sockeye salmon (*Oncorhynchus nerka*; Hinch and Rand 1998) and *O. mykiss* (Feldmeth and Jenkins 1973). Feeding frequency measurements have previously been used to assess temperature effects on in-river Chinook salmon (*Oncorhynchus tshawytscha*) feeding (Quigley and Hinch 2006). In-river feeding frequency of LTR *O. mykiss* was measured from the same video recordings as TBF measurements.

### 4.1 In-River Video Recording

Ten sites were selected from locations between RM 39.4 and RM 50.7 of the LTR where *O. mykiss* had been previously observed during summer index surveys (Ford and Kirihaara 2010; Table 4.1-1 and Figure 3.0-1). Site selection considered historical fish counts at each site, accessibility, and a desire to observe fish in water temperatures ranging from 13°C to 25°C. Sites 1-9 were each surveyed by snorkeling during June 8-9, June 29-30, July 20-21, and August 18-19. No juvenile *O. mykiss* were observed at site 10 (RM 39.4) during surveys in June, so this site was not re-visited in July and August. The timing of site surveys was systematically ordered to limit potential effects of time of day. Odd numbered sites were surveyed on the first day of each event, beginning at the most upstream site and proceeding to the most downstream site. Then, on the second day of the sampling time point, the even numbered sites were surveyed beginning at the most downstream site. Each survey day began between 09:00 and 10:00, while the last site survey for the day began between 12:00 and 15:00.

**Table 4.1-1. River mile locations of snorkel survey and video recording sites used for counts of 100 mm to 200 mm juvenile *O. mykiss* and measurements of tail beat frequency and prey strike frequency.**

Site	Location	River Mile
1	Old La Grange Bridge	50.7
2	Riffle 2	49.9
3	Riffle 3B	49.1
4	Riffle 4A	48.8
5	Basso Bridge	47.9
6	Riffle 7	46.9
7	Zanker Farm	45.5
8	Bobcat Flat	42.9
9	Tuolumne River Resort	42.3
10	Roberts Ferry Bridge	39.4

The purpose of snorkel surveys at each site was to locate *O. mykiss* and guide positioning of underwater GoPro® video cameras. If 100 mm to 200 mm *O. mykiss* (the size range tested in the



2014 study) were observed, one or two underwater GoPro® video cameras were secured within 2 m to record undisturbed behavior in each habitat. The GoPro® battery life limited uninterrupted video footage to 2-4 h for each unit. Video footage was used to measure TBF and fish feeding behavior in the river.

## 4.2 River Conditions

A handheld Pro ODO, YSI meter (Yellow Springs, Ohio, USA) measured instantaneous water temperature and dissolved oxygen during each snorkel survey at each site. Water temperatures were also continuously monitored during the study period with thermographs recording hourly water temperatures at five of the survey sites (Figure 5.3-1, provided later in this study report). All thermographs and the YSI meter were confirmed to be within  $\pm 0.5^{\circ}\text{C}$  before the first survey and after the last survey. The YSI meter was calibrated for dissolved oxygen measurements, according to manufacturer's recommendations, at the beginning of each survey day.

Thermographs (HOBO Water Temp Pro v2; Onset, Computer, Bourne, Massachusetts, USA) were attached to each camera to record water temperatures at 10-min intervals. The time settings of the cameras and thermographs were synchronized, which allowed each TBF measurement to be matched with water temperature.

Flow records from the La Grange gauge (11289650) were also downloaded from the United States Geological Survey (USGS) water data website.

## 4.3 Measuring Tail Beat Frequency

Fish did not remain in the field of view for the entire recording period, and TBF of in-river juvenile *O. mykiss* (100 – 200 mm TL) was measured in Hz by counting the number of beats in 10-s sections of video recordings. The number of sections (replicates as shown in Attachment A, Table A-1) varied depending on the number of 10-s sections that individual fish remained in the field of view. TBF measurements were only made for *O. mykiss* in the same size range as those tested in the 2014 study to ensure valid application of the mathematical model relating TBF to metabolic rate (see section 4.4 Estimating In-River Metabolic Rates). Clear distinctions among individual fish were not always possible, especially at sites with large numbers of fish. Whenever possible, size and distinguishing features were used to identify individuals. When a fish exited the video frame of view, it tended to rapidly return to its previous position. Therefore, a fish entering the frame of view was assigned the same ID as a recently exited fish if it moved to the same location within  $\leq 60$  s of the original fish exiting. If a fish entered the frame of view  $> 60$  s after the initial fish exited or moved to a different location, the individual was assigned a new fish ID.

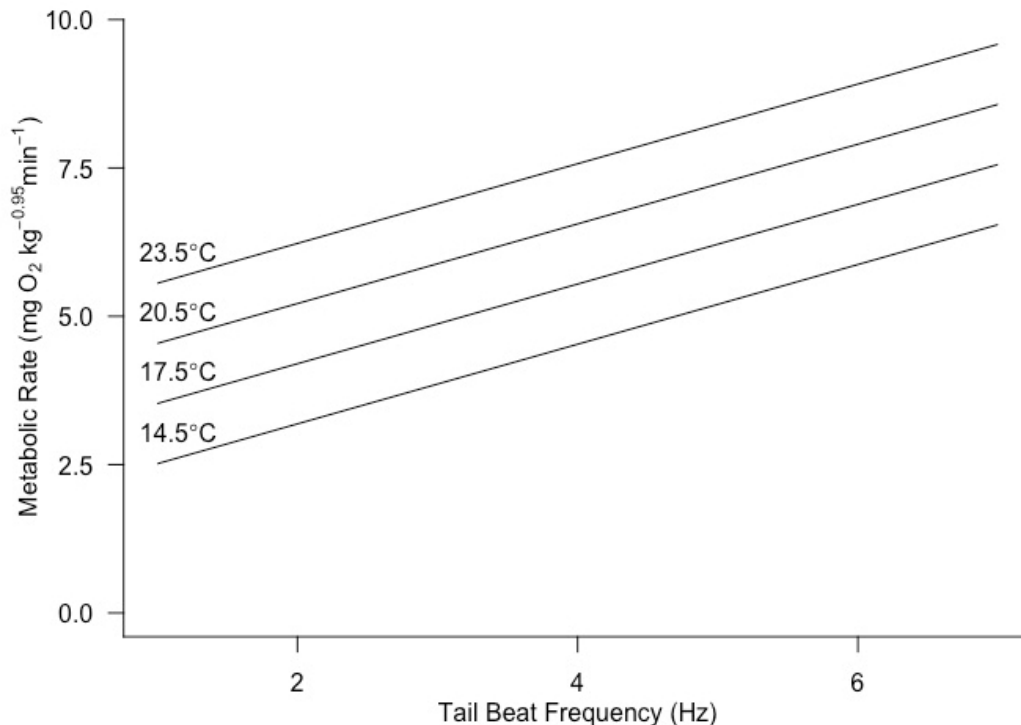
## 4.4 Estimating In-River Metabolic Rates

The 2014 swim tunnel study provided data to estimate TBF by developing a mathematical model of the relationship between TBF and metabolic rate for 100 mm to 200 mm *O. mykiss* swimming at different velocities and temperatures (see Farrell et al. 2017 for detailed methods). Briefly, video recordings were used to quantify TBF (measured in Hz) at each swimming velocity by

counting the number of beats in 10-s sections of video, while the fish was continuously holding station in the water current imposed by the swim tunnel. A mixed effects model predicting metabolic rate of juvenile *O. mykiss* from TBF and temperature was estimated using TBF and metabolic rate measurements made in the swim tunnel at temperatures ranging from 13°C to 23°C (Figure 4.5-1). Modeling was performed in R (R Core Team 2014), using the ‘lmer’ function of the lme4 package. The model included the temperature term as both a random effect, to allow random variation of the temperature effect on the intercept, and a fixed effect, to include temperature as a predictor of metabolic rate. Metabolic rate of LTR *O. mykiss* swimming in a swim tunnel respirometer was related to TBF and to temperatures up to 24.9°C (Figure 4.5-1). In-river metabolic rates were generated from the model using the ‘predict’ function of the base R package and based on in-river TBF measurements and the river water temperature during the video recording.

#### 4.5 Estimating Prey Strike Frequencies

Prey strikes, defined as a rapid, vertical or horizontal movement and an immediate return to the original river position, were also quantified from video recordings. Fish did not remain in the field of view for the entire recording period so prey strikes were counted in 30-60 s of continuous video. Observations from segments shorter than 60 s were converted to a count of prey strikes  $\text{min}^{-1}$ . In many, but not all cases, fish were observed targeting a suspended particle, then striking and ingesting it. This assessment, therefore, is a quantification of the ability of the fish to mount and sustain a feeding behavior (a strike). It does not measure the quantity, quality, or digestibility of the food obtained.



**Figure 4.5-1. General linear mixed effects model predictions of metabolic rates from tail beat frequencies ranging from 1 to 8 Hz at four temperatures.**

## 5.0 RESULTS

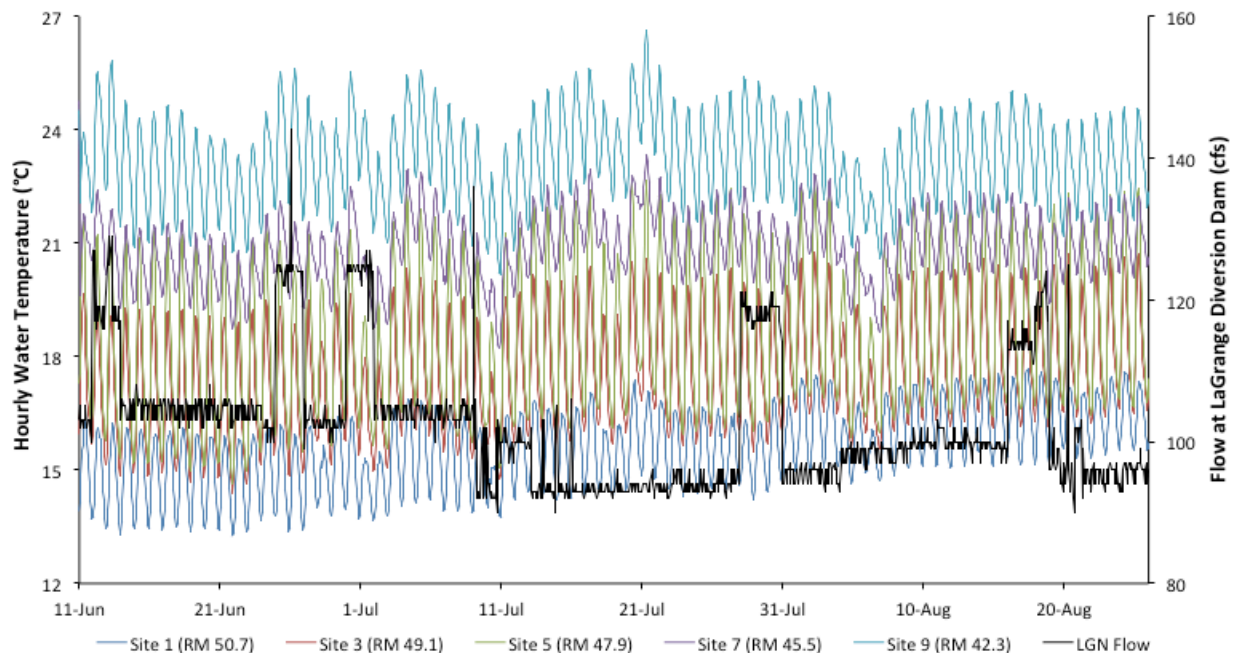
### 5.1 In-River Video Capture

Video recordings for TBF measurements were performed at every site at every visit when 100 mm and 200 mm TL *O. mykiss* were observed during the snorkel survey. No juvenile *O. mykiss* were observed at site 8 (RM 42.9) or site 10 (RM 39.4) so no video footage was recorded at these sites (Figure 3.0-1). *O. mykiss* were also not observed in site 2 (RM 49.9) during the first survey. Video recordings were obtained during each visit to sites 6 (RM 46.9), 7 (RM 45.5), and 9 (RM 42.3).

### 5.2 River Conditions

Flows at La Grange were approximately 100 cfs from early June through early July and approximately 95 cfs through the remainder of the summer study period (Figure 5.2-1). Flows were increased to approximately 115-120 cfs during five brief periods during this time period (Figure 5.2-1).

Water temperatures increased with distance downstream from La Grange Diversion Dam, but the range of temperatures occurring at a given site were relatively consistent throughout the study period (Figure 5.2-1). Water temperatures in the study area ranged from 13.3°C to 26.6°C, consistent with the target range for this study, which was based on the range of water temperatures evaluated during the 2014 swim tunnel study.



**Figure 5.2-1. Lower Tuolumne River water temperatures and flow during June through August 2015.**

### 5.3 Tail Beat Frequency Measurements

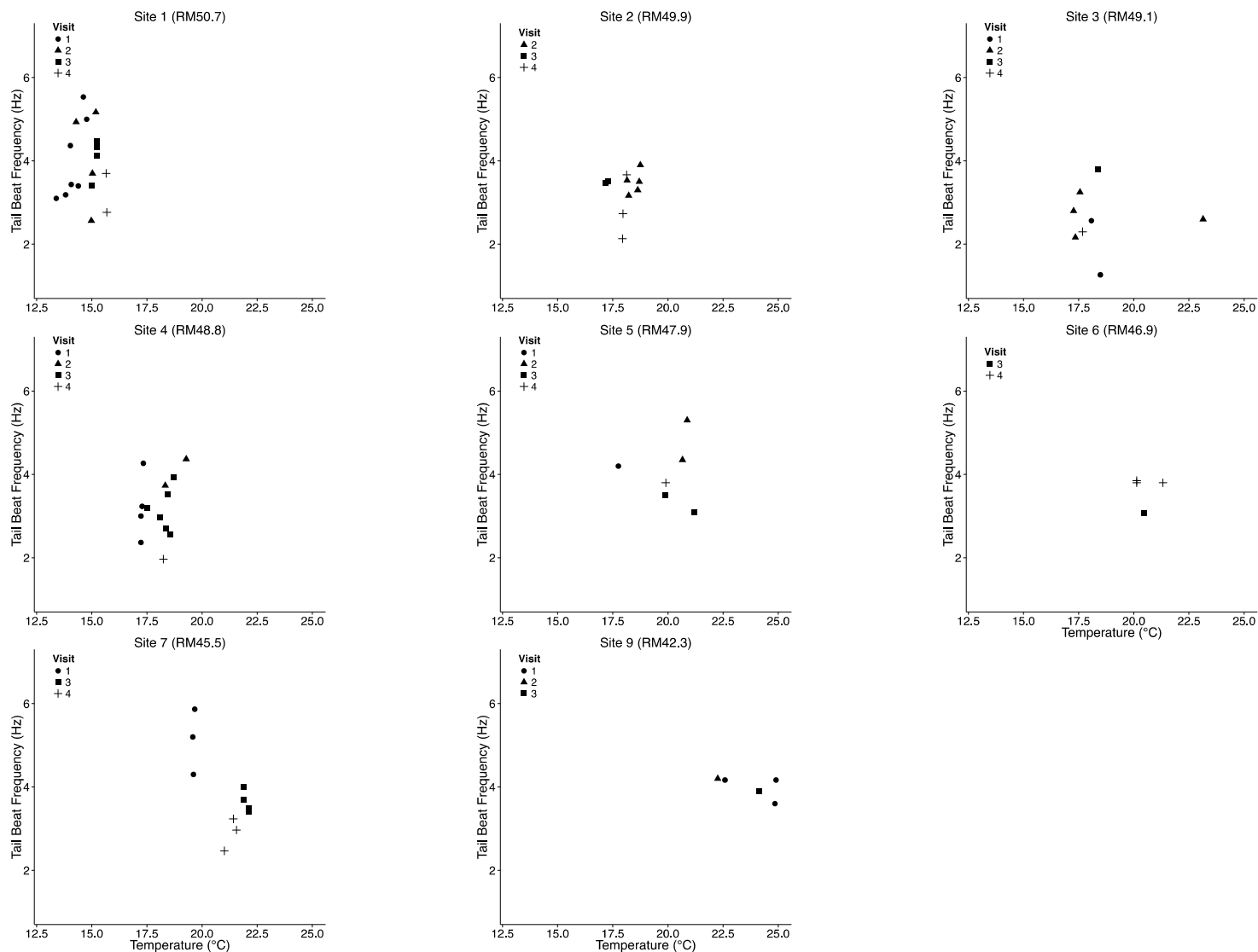
Measurements of TBF for individual fish are provided in Attachment A, Table A-1 and Figure 5.3-1. The number of fish successfully measured for TBF ranged from one to seven per visit per site, and the number of replicate TBF measurements per fish ranged from 1 to 21. As a result, the number of replicate measurements of in-river TBF per site and per visit ranged from 1 to 35.

TBF was measured at river temperatures ranging from 13.4°C to 24.9°C over the course of the summer study period. The in-river TBF measurements were placed into one of four temperature ranges that each bracketed a 3°C range (14.5±1.5°C, 17.5±1.5°C, 20.5±1.5°C, and 23.5±1.5°C). The number of fish used for these four temperature bins was 17, 31, 17, and 7, respectively. TBF measured from video records of *O. mykiss* in the LTR averaged 3.6 Hz (range: 1.3 to 5.9 Hz) and was largely independent of river water temperatures over the range of 14.5±1.5°C to 23.5±1.5°C (Figure 5.3-2).

The effects of temperature on TBF were assessed by performing an ANOVA between TBF and temperature classification followed by the Tukey method to examine if there were statistically significant differences among the different temperatures. An ANOVA found a significant effect of temperature on TBF only at 17.5±1.5°C. This value was significantly lower than the measurements at 14.5±1.5°C (P=0.002) and 20.5±1.5°C (P=0.002), but was not significantly different from the measurements at 23.5±1.5°C (P=0.087).

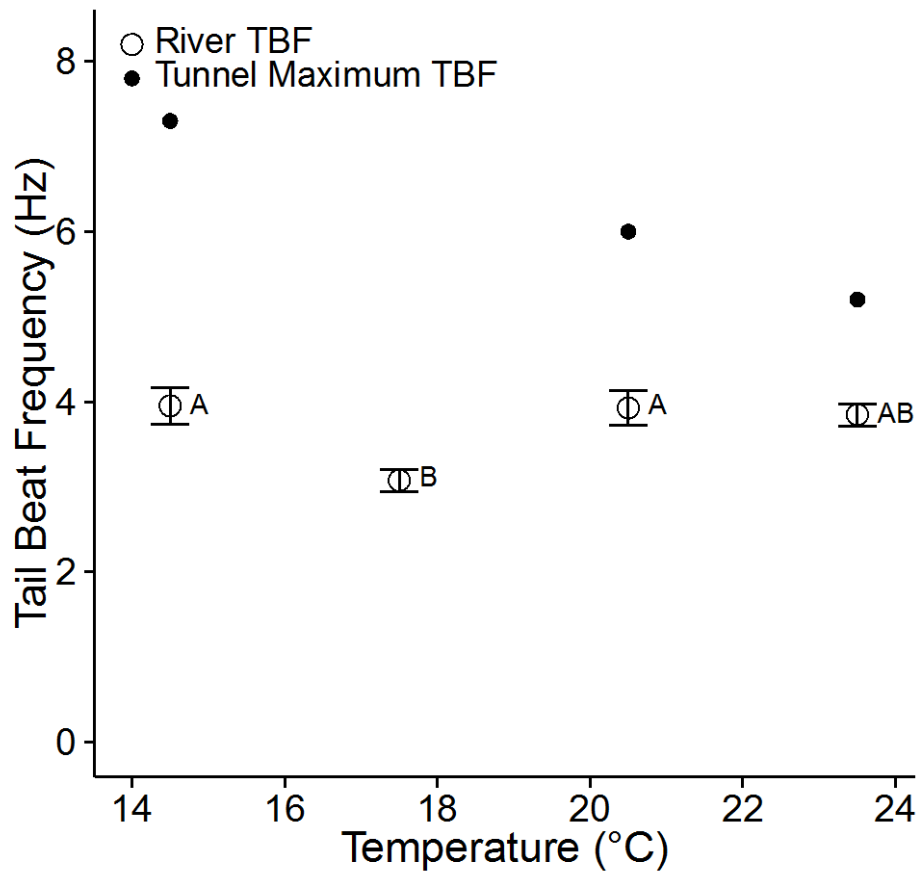
#### Objective #1

TBF measured in-river was always less than the maximum TBF in the swim tunnel for any given temperature (Figure 5.3-2). Therefore, across the entire temperature range, a fish still had the capacity to increase TBF by 1.3-fold to 1.5-fold when compared with the maximum TBF in the swim tunnel. In fact, video analysis showed that LTR *O. mykiss* also performed darting behaviors for feeding, defense of territory, or escape from threats regardless of the river temperature. These TBF measurements and visual observations suggest that LTR *O. mykiss* had a capacity for activity above and beyond station holding at all the temperatures for the in-river observations (Figure 5.3-2).



Note: Visit 1 was on June 8-9, visit 2 was on June 29-30, visit 3 was on July 20-21, and visit 4 was on August 18-19.

**Figure 5.3-1. Mean tail beat frequency versus water temperature for every individual juvenile *O. mykiss* at each site and each site visit.**



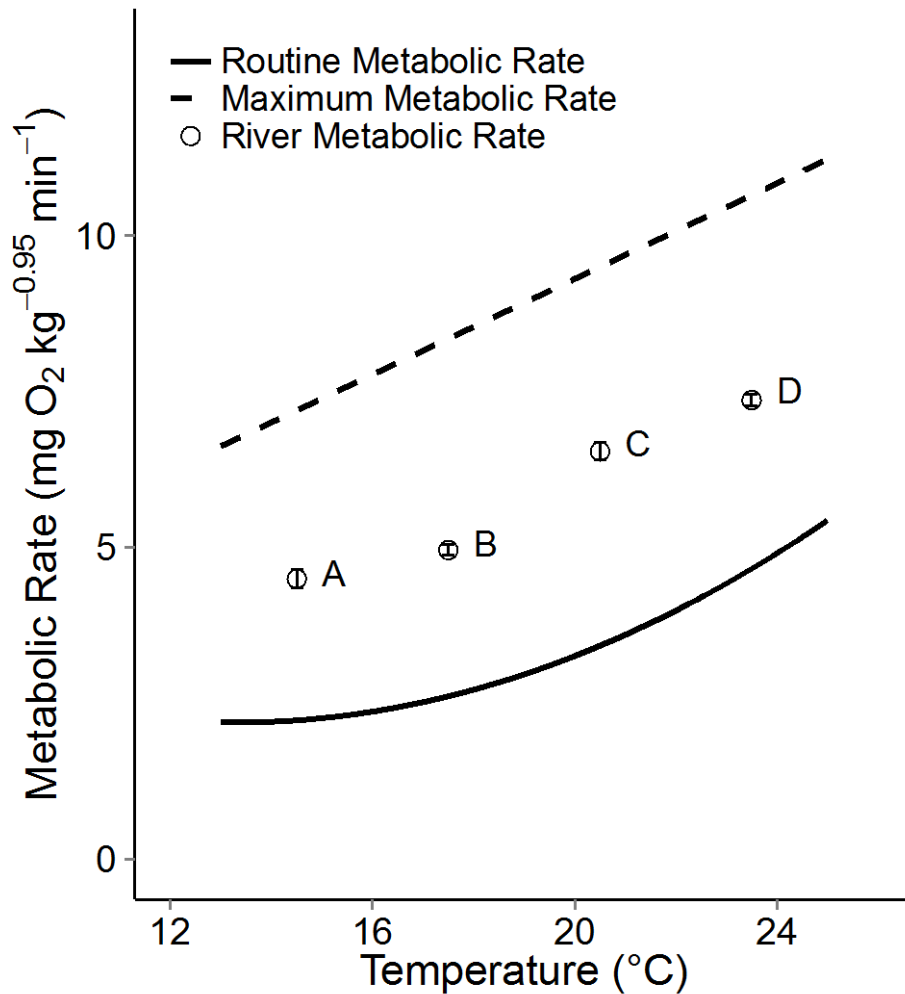
Note: Four temperature bins are: (1) 14.5±1.5°C; n=17 in-river fish, (2) 17.5±1.5°C; n=31 in-river fish, (3) 20.5±1.5°C; n=17 in-river fish, and (4) 23.5±1.5°C; n=5 in-river fish. Different letters demarcate significantly different in-river TBF among temperature classifications.

**Figure 5.3-2. Mean in-river tail beat frequency (TBF; ±standard error of the mean) and maximum swim tunnel TBF of *O. mykiss* pooled into four temperature bins.**

#### 5.4 Estimates of In-River Metabolic Rates

The estimated metabolic rates derived from in-river TBF increased with water temperature and ranged from 4.5±1.4 mg O<sub>2</sub> kg<sup>-0.95</sup> min<sup>-1</sup> at 14.5°C to 7.4±1.3 mg O<sub>2</sub> kg<sup>-0.95</sup> min<sup>-1</sup> at 23.5°C (Figure 5.4-1). By subtracting the RMR obtained in the swim tunnel study from these in-river metabolic rates, it is possible to estimate the cost of holding station in-river. The cost of holding station ranged from 2.3 mg O<sub>2</sub> kg<sup>-0.95</sup> min<sup>-1</sup> to 2.8 mg O<sub>2</sub> kg<sup>-0.95</sup> min<sup>-1</sup> over RMR, representing a 1.6- to 2.0-fold increase over RMR at river temperatures between 14.5°C and 23.5°C. The metabolic cost of holding station differed significantly among all river temperatures (Table 5.4-1).

The capacity to further increase metabolic rate above that needed to maintain station in-river was calculated by subtracting in-river metabolic rates from MMR. This capacity (2.7 mg O<sub>2</sub> kg<sup>-0.95</sup> min<sup>-1</sup> to 3.2 mg O<sub>2</sub> kg<sup>-0.95</sup> min<sup>-1</sup>) was found to be independent of the observed river temperatures between 14.5°C and 23.5°C.



Note: Four temperature bins are: (1)  $14.5 \pm 1.5^\circ\text{C}$ ;  $n=17$  fish, (2)  $17.5 \pm 1.5^\circ\text{C}$ ;  $n=31$  fish, (3)  $20.5 \pm 1.5^\circ\text{C}$ ;  $n=17$  fish, and (4)  $23.5 \pm 1.5^\circ\text{C}$ ;  $n=5$  fish. The modeled relationships for average routine (solid line) and maximum (dashed line) metabolic rates with temperature are plotted from data in Farrell et al. (2017). Different letters demarcate significantly different in-river metabolic rates among temperature classifications.

**Figure 5.4-1. Predicted in-river metabolic rate ( $\pm$  standard error of the mean, open circles) of *O. mykiss* at the four temperatures bins.**

**Table 5.4-1. P values for Tukey comparisons of metabolic cost of holding station at river water temperatures.**

	17.5°C	20.5°C	23.5°C
14.5°C	0.040	<0.001	<0.001
17.5°C		<0.001	<0.001
20.5°C	<0.001		<0.001
23.5°C	<0.001	<0.001	

## 5.5 Prey Strike Frequencies

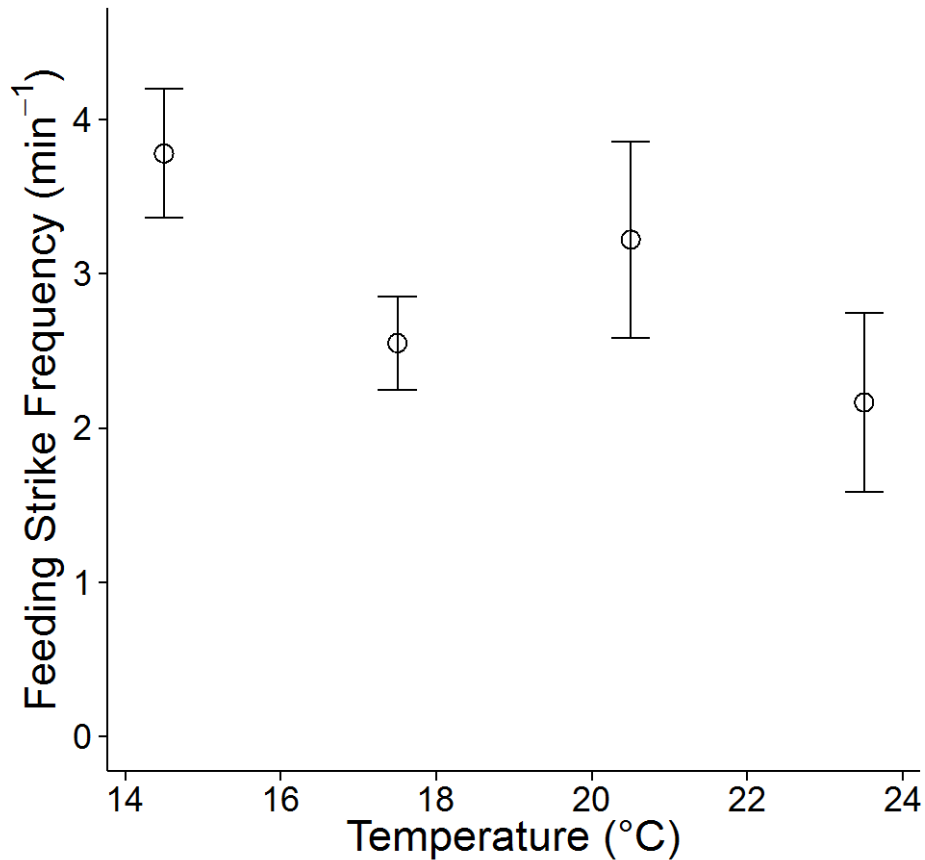
Prey strike frequencies were measured for 57 individuals and replicate measurements were recorded for some individuals (Attachment B, Table B-1). As a result, a total of 165 measurements of in-river prey strike frequency were obtained. As with TBF, prey strike frequency was not quantified during some visits because video did not contain sections meeting the minimum criteria for analysis (see Methods).

Prey strike frequencies of individual fish ranged from 0.5 strikes  $\text{min}^{-1}$  to 8.0 strikes  $\text{min}^{-1}$ , and strike frequencies varied within and between sites and visits. Due to the low number of replicates within each site and during each visit, prey strike frequencies were pooled across sites and visits. Linear regression analysis found no statistically significant relationship between prey strike frequency and site ( $P=0.860$ ), but a statistically significant relationship was identified between prey strike frequency and visit ( $P=0.048$ ) ( $r^2=0.07$ , residuals = 1.63). However, given that the slope of the relationship is so low and the low amount of variation explained by visit, the relationship is of little value and not likely biologically significant.

Prey strikes were observed at river water temperatures ranging from 13.8°C to 24.9°C, and three fish were observed feeding at temperatures greater than 24°C. In-river prey strike frequency was measured on 15, 25, 12, and 5 fish, respectively, for 14.5±1.5°C, 17.5±1.5°C, 20.5±1.5°C, and 23.5±1.5°C. The effects of temperature on prey strike frequency were assessed using ANOVA between prey strike frequency and temperature classification.

Over the 9°C range in river water temperature from 14.5°C to 23.5°C, mean prey strike frequency decreased from 3.8±0.4 strikes  $\text{min}^{-1}$  to 2.2±0.6 strikes  $\text{min}^{-1}$  (Figure 5.5-1). Although the magnitude of mean prey strike frequency was lower at 23.5°C than 14.5°C, and the range of prey strike frequencies at 14.5°C did not overlap with the range of prey strike frequencies observed at 23.5°C, the one-way ANOVA showed no statistically significant effect of temperature on prey strike frequency ( $p=0.158$ ).





Note: Four temperature bins are: (1)  $14.5 \pm 1.5^\circ\text{C}$ ;  $n=15$  fish, (2)  $17.5 \pm 1.5^\circ\text{C}$ ;  $n=25$  fish, (3)  $20.5 \pm 1.5^\circ\text{C}$ ;  $n=12$  fish, and (4)  $23.5 \pm 1.5^\circ\text{C}$ ;  $n=5$  fish.

**Figure 5.5-1.** Mean feeding behavior ( $\pm$  standard error of the mean) of *O. mykiss* at four temperature bins calculated across all river sites and dates. ANOVA showed no statistically significant effect of temperature on prey strike frequency ( $p=0.158$ ).

## 6.0 DISCUSSION

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This study expands on a 2014 study, which recommended an upper thermal tolerance limit of 22°C for juvenile LTR *O. mykiss* based on the degree to which fish could increase aerobic metabolic rate, beyond basic needs. The strong correlation between TBF and metabolic rate of steady swimming, non-schooling fish (Feldmeth and Jenkins 1973; Herskin and Steffensen 1998; Lowe 2001; Steinhausen et al. 2005; Webber et al. 2001) has allowed scientists to reliably use TBF to predict metabolic rates of free-swimming fish (Puckett and Dill 1985; Wardle et al. 1989), including *O. mykiss* in a California stream (Feldmeth and Jenkins 1973). This 2015 study was designed to build upon the findings of the 2014 streamside laboratory study by investigating whether high summer water temperatures prevent juvenile *O. mykiss* in the LTR from performing essential activities, specifically swimming and feeding.

While in-river metabolic rate measurements could not take into account variability in river flow speeds that likely existed among sites and visits, the non-significant effect of site on TBF (Figure 5.3-1, and linear regression model  $P=0.072$ ) suggests differences in flow experienced by the fish were unlikely to have influenced the relationship between in-river metabolic rate and temperature. However, fish are proficient at selecting river flow speeds to efficiently maintain station in their habitat with minimal metabolic expenditure (Hockley et al. 2014; Smith et al. 2014). This behavior was observed at sites where flows were likely relatively high due to channel narrowing (e.g., site 9 at RM 42.3), where fish sheltered themselves from the high flows by using the spaces between rocks. Thus, the lack of variability in TBF among sites may simply reflect *O. mykiss* selecting microhabitats with rather similar water velocity profiles.

This study found support for the predictions that *O. mykiss* TBFs in LTR currents did not exceed the maximum TBFs at the same temperatures for LTR *O. mykiss* in a swim tunnel study. Consequently, in-river metabolic rates of station holding *O. mykiss*, as predicted from in-river TBF observations, were always lower than MMR measured in the swim tunnel study. Comparisons of estimated in-river metabolic rates with maximum metabolic rates measured in swim tunnels during the 2014 study showed the majority of fish holding station in the river flow (i.e., those located at  $14.5\pm 1.5^{\circ}\text{C}$ ) would have the capacity for a further 1.6-fold increase in their metabolic rate, whereas the few fish found at  $23.5\pm 1.5^{\circ}\text{C}$  could increase metabolic rate by a further 1.4-fold. These results suggest that at temperatures ranging from 13.4 to 24.9°C juvenile *O. mykiss* possessed metabolic capacity above and beyond that required to hold position in typical LTR summer currents, allowing them to carry out other activities, such as feeding, which are necessary for survival.

Beyond the ability to maintain station over a wide range of river temperatures, video data clearly revealed that LTR *O. mykiss* regularly (at least once every 2 min and as frequently as every 9 s) performed darting behaviors for feeding, defense of territory, or escape from threats over the full range of river temperatures. While prey strike frequencies decreased from 3.8 strikes  $\text{min}^{-1}$  at 14.5°C to 2.2 strikes  $\text{min}^{-1}$  at 23.5°C, no statistically significant effect of temperature on prey strike frequency was identified. Since temperature effects on feeding strike frequency were primarily assessed across different sites, habitat and food abundance were variable, which may have reduced the power to detect a feeding strike frequency response to temperature.

Locomotion associated with darting behaviors is likely fuelled anaerobically, meaning there is no immediate oxygen cost, but a metabolic oxygen debt must accrue that must eventually be repaid through elevated aerobic metabolism (Lee et al. 2003; Scarabello et al. 1992). In the swim tunnel study (Farrell et al. 2017), the oxygen debt accrued during the arduous 2-6 h long swim tests was rapidly repaid by all fish at 13°C to 25°C. At the end of a 60-min recovery period RMR was either fully restored or increased by no more than 20 percent. The regular darting behaviors and high feeding rates observed in the in-river video recordings suggest that even though *O. mykiss* were expending energy to continuously maintaining station in the water current, they had sufficient aerobic capacity remaining to repay the oxygen debt associated with darting behaviors and potentially digesting any food that they captured.

In summary, *O. mykiss* were observed performing normal and undisturbed swimming and feeding at river temperatures ranging from 13.4 to 24.9°C. Across this range of water temperatures, this study did not find evidence that metabolic performance of juvenile LTR *O. mykiss* was thermally limited. This finding is congruent with the recommended upper thermal tolerance limit of 22°C for juvenile LTR *O. mykiss* based on the previous swim tunnel study.

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**IN-RIVER ASSESSMENT OF SWIMMING AND FEEDING BEHAVIORS  
AND INFERENCES OF METABOLIC STATE OF *ONCORHYNCHUS*  
*MYKISS* IN THE LOWER TUOLUMNE RIVER**

**ATTACHMENT A**

**TAIL BEAT FREQUENCY (TBF) MEASUREMENTS FOR INDIVIDUAL  
*O. MYKISS* OBSERVED**

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**Table A-1. Tail beat frequency (TBF) measurements for individual *O. mykiss* observed.**

Fish ID	Date	Visit	Site	Mean Thermograph Temperature (°C)	Temperature Classification (°C)	Mean TBF (Hz±SD)	Replicate Measurements
1	8-Jun	1	1	13.8	14.5	3.2(±0.4)	21
2	8-Jun	1	1	14.4	14.5	3.4	1
3	8-Jun	1	1	13.4	14.5	3.1	1
4	8-Jun	1	1	14.0	14.5	4.4(±0.3)	3
5	8-Jun	1	1	14.1	14.5	3.4(±0.4)	3
6	8-Jun	1	1	14.6	14.5	5.5(±0.1)	3
7	8-Jun	1	1	14.8	14.5	5.0(±0.3)	3
12	8-Jun	1	3	18.1	17.5	2.6(±0.5)	3
14	8-Jun	1	3	18.5	17.5	1.3(±0.2)	6
16	8-Jun	1	9	24.9	23.5	4.2(±0.5)	3
17	8-Jun	1	9	24.9	23.5	3.6(±0.4)	3
18	9-Jun	1	9	22.6	23.5	4.2(±0.3)	3
20	9-Jun	1	7	19.6	20.5	4.3(±0.3)	3
21	9-Jun	1	7	19.6	20.5	5.2	1
22	9-Jun	1	7	19.7	20.5	5.9(±0.6)	3
24	9-Jun	1	5	17.8	17.5	4.2(±0.2)	3
27	9-Jun	1	4	17.3	17.5	3.2(±0.3)	3
28	9-Jun	1	4	17.2	17.5	2.4(±0.1)	3
29	9-Jun	1	4	17.3	17.5	4.3(±0.4)	3
30	9-Jun	1	4	17.2	17.5	3.0(±0.2)	3
33	29-Jun	2	9	22.3	23.5	4.2(±0.7)	3
36	29-Jun	2	5	20.7	20.5	4.4(±1.3)	2
37	29-Jun	2	5	20.9	20.5	5.3	1
43	29-Jun	2	4	19.3	20.5	4.4(±0.6)	3
45	29-Jun	2	2	18.1	17.5	3.5(±0.4)	3
46	29-Jun	2	2	18.2	17.5	3.2(±0.3)	3
48	29-Jun	2	2	18.6	17.5	3.3	1
49	29-Jun	2	2	18.7	17.5	3.5	1
50	29-Jun	2	2	18.8	17.5	3.9	1
52	30-Jun	2	1	15.2	14.5	5.2(±0.5)	3
53	30-Jun	2	1	14.3	14.5	4.9(±0.3)	3
54	30-Jun	2	1	15.0	14.5	3.7(±0.4)	3
57	30-Jun	2	1	15.0	14.5	2.6(±0.2)	3
60	30-Jun	2	3	17.3	17.5	2.8	1
61	30-Jun	2	3	17.4	17.5	2.2(±0.2)	3
62	30-Jun	2	3	17.6	17.5	3.3(±0.1)	2
63	30-Jun	2	3	17.6	17.5	2.6	1
64	30-Jun	2	4	18.3	17.5	3.7(±1.0)	3
66	20-Jul	3	1	15.2	14.5	4.3(±0.7)	3
67	20-Jul	3	1	15.2	14.5	4.5(±0.3)	3
68	20-Jul	3	1	15.2	14.5	4.1(±0.5)	3
71	20-Jul	3	1	15.0	14.5	3.4(±0.6)	3
75	20-Jul	3	3	18.4	17.5	3.8(±0.1)	3

<b>Fish ID</b>	<b>Date</b>	<b>Visit</b>	<b>Site</b>	<b>Mean Thermograph Temperature (°C)</b>	<b>Temperature Classification (°C)</b>	<b>Mean TBF (Hz±SD)</b>	<b>Replicate Measurements</b>
77	20-Jul	3	5	19.9	20.5	3.5	1
77	20-Jul	3	5	21.2	20.5	3.1	1
78	20-Jul	3	7	21.9	20.5	3.7	1
78	20-Jul	3	7	22.1	23.5	3.5(±0.1)	2
79	20-Jul	3	7	21.9	20.5	4	1
79	20-Jul	3	7	22.1	23.5	3.4	1
82	21-Jul	3	9	24.2	23.5	3.9	1
83	21-Jul	3	2	17.3	17.5	3.5(±0.7)	3
84	21-Jul	3	2	17.2	17.5	3.5(±0.2)	3
85	21-Jul	3	4	17.5	17.5	3.2(±0.3)	3
88	21-Jul	3	4	18.1	17.5	3.0(±0.6)	3
89	21-Jul	3	4	18.4	17.5	2.7(±0.3)	3
90	21-Jul	3	4	18.4	17.5	3.5(±0.3)	3
91	21-Jul	3	4	18.6	17.5	2.6(±0.3)	3
92	21-Jul	3	4	18.7	17.5	3.9(±0.4)	3
93	21-Jul	3	6	20.5	20.5	3.1(±0.3)	3
94	18-Aug	4	1	15.7	14.5	3.7(±0.7)	3
95	18-Aug	4	1	15.7	14.5	2.8(±0.5)	3
101	18-Aug	4	3	17.7	17.5	2.3(±0.0)	3
102	18-Aug	4	5	19.9	20.5	3.8(±0.2)	3
103	18-Aug	4	7	21.4	20.5	3.2(±0.9)	3
104	18-Aug	4	7	21.6	20.5	3.0(±0.3)	3
105	18-Aug	4	7	21.0	20.5	2.5(±0.3)	3
108	19-Aug	4	2	18.0	17.5	2.7(±0.3)	3
109	19-Aug	4	2	18.1	17.5	3.7(±0.6)	3
110	19-Aug	4	2	17.9	17.5	2.1(±0.2)	3
111	19-Aug	4	4	18.2	17.5	2.0(±0.1)	3
112	19-Aug	4	6	21.3	20.5	3.8(±0.1)	2
113	19-Aug	4	6	20.1	20.5	3.8(±0.7)	2
114	19-Aug	4	6	20.1	20.5	3.9(±0.2)	2

**IN-RIVER ASSESSMENT OF SWIMMING AND FEEDING BEHAVIORS  
AND INFERENCES OF METABOLIC STATE OF *ONCORHYNCHUS*  
*MYKISS* IN THE LOWER TUOLUMNE RIVER**

**ATTACHMENT B**

**PREY STRIKE FREQUENCY MEASUREMENTS FOR INDIVIDUAL  
*O. MYKISS* OBSERVED**

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**Table B-1. Prey strike frequency measurements for individual *O. mykiss* observed.**

Fish ID	Date	Visit	Site	Mean Thermograph Temperature (°C)	Temperature Classification (°C)	Mean Feeding Frequency (strikes min-1±SD)	Replicate Measurements
1	8-Jun	1	1	13.8	14.5	3.4(±2.2)	11
4	8-Jun	1	1	14.0	14.5	3.0(±1.0)	3
5	8-Jun	1	1	14.1	14.5	5.3(±0.6)	3
6	8-Jun	1	1	14.6	14.5	4.3(±1.2)	3
7	8-Jun	1	1	14.8	14.5	2.7(±0.6)	3
12	8-Jun	1	3	18.1	17.5	3.0(±1.7)	3
14	8-Jun	1	3	18.5	17.5	1.3(±1.2)	3
16	8-Jun	1	9	24.9	23.5	2.7(±0.6)	3
17	8-Jun	1	9	24.9	23.5	2.0(±2.6)	3
18	9-Jun	1	9	22.6	23.5	1.7(±1.5)	3
20	9-Jun	1	7	19.6	20.5	4.7(±2.1)	3
21	9-Jun	1	7	19.6	20.5	1.0	1
22	9-Jun	1	7	19.7	20.5	1.3(±1.5)	3
27	9-Jun	1	4	17.3	17.5	1.3(±0.6)	3
28	9-Jun	1	4	17.2	17.5	1.7(±1.2)	3
29	9-Jun	1	4	17.3	17.5	1.0(±1.0)	3
30	9-Jun	1	4	17.2	17.5	1.3(±1.5)	3
33	29-Jun	2	9	22.3	23.5	0.5(±0.7)	2
36	29-Jun	2	5	20.7	20.5	1.3(±1.2)	3
43	29-Jun	2	4	19.3	20.5	2.3(±1.5)	3
45	29-Jun	2	2	18.1	17.5	2.7	1
46	29-Jun	2	2	18.2	17.5	1.3(±1.2)	3
49	29-Jun	2	2	18.7	17.5	4.0	1
52	30-Jun	2	1	15.2	14.5	6.3(±1.2)	3
53	30-Jun	2	1	14.3	14.5	5.3(±0.6)	3
54	30-Jun	2	1	15.0	14.5	2.3(±0.6)	3
57	30-Jun	2	1	15.0	14.5	0.7(±1.2)	3
62	30-Jun	2	3	17.6	17.5	3.0	1
64	30-Jun	2	4	18.3	17.5	2.8(±1.3)	3
66	20-Jul	3	1	15.2	14.5	2.7(±0.6)	3
67	20-Jul	3	1	15.2	14.5	2.3(±0.6)	3
68	20-Jul	3	1	15.2	14.5	5.2(±4.1)	3
71	20-Jul	3	1	15.0	14.5	3.7(±2.1)	3
75	20-Jul	3	3	18.4	17.5	1.8(±0.4)	3
78	20-Jul	3	7	21.9	20.5	8.0	1
78	20-Jul	3	7	22.1	23.5	4.0(±1.4)	2
79	20-Jul	3	7	22.0	20.5	2.0	1
83	21-Jul	3	2	17.3	17.5	5.3(±2.3)	3
84	21-Jul	3	2	17.2	17.5	5.7(±2.3)	3
85	21-Jul	3	4	17.5	17.5	0.7(±0.6)	3
88	21-Jul	3	4	18.1	17.5	2.0(±0.0)	3
89	21-Jul	3	4	18.4	17.5	0.7(±1.2)	3

<b>Fish ID</b>	<b>Date</b>	<b>Visit</b>	<b>Site</b>	<b>Mean Thermograph Temperature (°C)</b>	<b>Temperature Classification (°C)</b>	<b>Mean Feeding Frequency (strikes min-1±SD)</b>	<b>Replicate Measurements</b>
90	21-Jul	3	4	18.4	17.5	1.3(±0.6)	3
91	21-Jul	3	4	18.6	17.5	4.3(±3.1)	3
92	21-Jul	3	4	18.7	17.5	5.0(±1.7)	3
93	21-Jul	3	6	20.5	20.5	4.0(±2.6)	3
94	18-Aug	4	1	15.7	14.5	6.1(±1.6)	3
95	18-Aug	4	1	15.7	14.5	3.3(±1.2)	3
101	18-Aug	4	3	17.7	17.5	2.3(±0.6)	3
102	18-Aug	4	5	19.9	20.5	2.8(±2.0)	3
103	18-Aug	4	7	21.4	20.5	5.3(±1.5)	3
104	18-Aug	4	7	21.6	20.5	2.7(±0.6)	3
105	18-Aug	4	7	21.0	20.5	4.0(±1.0)	3
108	19-Aug	4	2	18.0	17.5	1.7(±0.6)	3
109	19-Aug	4	2	18.1	17.5	3.7(±0.6)	3
110	19-Aug	4	2	17.9	17.5	3.3(±1.5)	3
111	19-Aug	4	4	18.2	17.5	4.7(±2.1)	3