

Thermal performance in juvenile hatchery *Oncorhynchus tshawytscha*:  
aerobic scope tests over a range of environmental temperatures

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Prepared by:

Jamillynn B. Poletto, Ph.D.  
Postdoctoral Research Fellow  
University of California, Davis  
Department of Wildlife, Fish, and Conservation Biology  
Davis, CA

Dennis E. Cocherell  
Senior Research Associate  
University of California, Davis  
Department of Wildlife, Fish, and Conservation Biology  
Davis, CA

Nann A. Fangué, Ph.D.  
Associate Professor of Fish Physiological Ecology  
University of California, Davis  
Department of Wildlife, Fish, and Conservation Biology  
Davis, CA

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## Introduction

Temperature is well known to both indirectly and directly affect the behavior and physiology of fishes, influencing the geographical distribution of a species, as well specific physiological processes such as metabolic rate and growth (Moyle and Cech, 2002; Schmidt-Nielson, 1999). As such, understanding how changes in environmental temperature may influence early life history stages in fish is fundamental in predicting subsequent size- and condition-dependent processes, such as dispersal, survival, and migration. Consequently, fish biologists are increasingly evaluating metrics that measure thermal physiological performance, especially when the metric is of important ecological relevance such as growth or other bioenergetics functions that ultimately require the delivery of oxygen to tissues. Methods to characterize fish thermal performance date back some 60 years (e.g., Fry 1947), though the use of these measures for watershed management is a relatively recent practice. However, the United States Environmental Protection Agency (U.S. EPA) has identified physiological performance as an important metric to consider, stating (2003): “Each salmonid life stage has an optimal temperature range. Physiological optimum temperatures are those where physiological functions (e.g., growth, swimming, heart performance) are optimized. These temperatures are generally determined in laboratory experiments.” These data are critical for managers seeking to link the survival of fishes with environmental temperature regimes, pinpoint temperature ranges for optimal survival, and help target future restoration sites that will be important for the recovery of native fish populations.

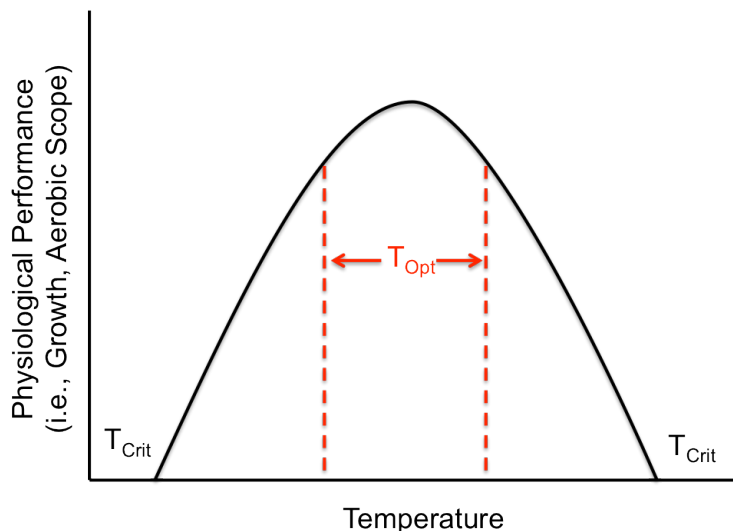
All activities of a fish ultimately require oxygen ( $O_2$ ). Therefore, it is possible to directly assess a fish's need for and capacity to deliver oxygen to its tissues, and use these measures as an ecologically relevant metric of fish performance. These measures can be made over a range of temperatures to characterize the influence of temperature on a fish's ability to increase its aerobic metabolic rate (MR) beyond basic needs (termed aerobic capacity or aerobic scope, AS). The aerobic capacity of a fish provides energy that can be used for any daily activities performed during their normal life history that extend beyond maintenance of life to include ecologically relevant and important functions (i.e., swimming, foraging, digestion, growth, predator avoidance, territorial defense, immune function, reproduction, etc.). Metabolic rate measurements therefore are an important component of the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis. The OCLTT hypothesis states that the extremes of the thermal tolerance of an animal will be determined by aerobic metabolism of active tissues above a basic routine need. Once an animal is no longer able to supply oxygen to active tissues above a maintenance level, the animal will no longer be able to tolerate temperatures above or below this limit. This hypothesis has emerged as a conceptual model to assess thermal performance of aquatic animals, and to determine the fundamental thermal range for a particular species (Pörtner and Knust 2007, Pörtner and Farrell 2008).

To assess thermal aerobic capacity, two evaluations of performance are generally measured: routine (or resting) metabolic rate (RMR), and maximum metabolic rate (MMR). RMR is a measurement of how much oxygen is required for basic persistence of the animal, while MMR measures the upper limit of how much oxygen can be extracted from the water for aerobic metabolism, typically when swimming at or near maximum capabilities. Aerobic capacity is defined as the ability of the animal to deliver oxygen to its tissues above a basic need,

and is calculated by subtracting RMR from MMR (termed absolute aerobic scope [AAS = MMR – RMR]). Therefore, AAS defines the maximum capacity available to an animal to perform the activities essential for survival. Factorial aerobic scope (FAS) is another way of expressing aerobic capacity, and is calculated by dividing MMR by RMR (FAS = MMR/RMR). FAS characterizes how many times an animal can increase its MR above resting, and therefore can be used for important comparisons among species, populations, and individuals. At minimal, a fish needs to double its RMR to be able to perform activities crucial for survival, such as foraging and digestion (Alsop and Wood 1997, Fu et al. 2005, Jobling 1981, Luo and Xie 2008). Therefore, a critical FAS value below which fishes are not expected to persist is 2.

As with most physiological responses, metabolic rate and aerobic capacity in fishes changes as a function of environmental temperatures, such that as temperatures increase, aerobic capacity increases until reaching a peak, which can be sustained across some range of temperatures (Figure 1, the thermal optima; Brett et al., 1969). As temperatures continue to increase above this optimum, aerobic capacity begins to decrease, eventually leading to increases in mortality (Houde 1989, Hofmann and Fischer 2003). Similarly, at temperatures below optimal, metabolic rates are slowed (Hofmann and Fischer, 2003, Houde, 1989) or even inhibited. This change in physiological performance in response to an environmental variable is generally known as a reaction norm (Huey and Kingsolver 1979, Schulte et al. 2011). Thermal reaction norms created for aerobic scope can be compared and integrated with those obtained for other measures of physiological performance to create a more thorough understanding of thermal tolerance, and the temperatures at which sub-lethal effects can be observed. Thus, it is crucial to examine metabolism and aerobic capacity over a wide range of temperatures in order to accurately understand and estimate the relationship between temperature and metabolism, especially for fish of different sizes or life history stages (Jobling 2003).

As with many measures of physiological performance, metabolic rates and aerobic scope are not static and can be modified by environmental variables such as ontogeny, time of year, or disease. One notable variable that can affect aerobic scope is thermal history, or the temperature conditions experienced by an individual over weeks to months (termed “acclimatization” in the wild, or “acclimation” in the laboratory). In response to exposure to different temperature conditions, the optimal temperature range for peak aerobic scope can shift, such that



**Figure 1. Schematic representation of thermal tolerance.  $T_{opt}$  = optimum temperature range over which 95% of the peak performance can be maintained;  $T_{crit}$  = critical temperatures where performance is minimal.**

acclimatization to elevated temperatures can result in an optimal temperature range that is shifted to warmer temperatures than those observed for fish acclimated to lower temperatures. Conversely, acclimation to colder temperatures may shift the optimal temperature range for aerobic scope to lower temperatures. This plasticity in thermal physiological performance is important for the persistence of individuals, as well as the population. Physiological plasticity allows for continued physiological performance in the face of changing environmental conditions. These changes may be predictable, such as temperature changes that accompany seasonal shifts, or unpredictable changes, such as those observed due to climate change. Similarly, populations may have the ability to adapt to new environmental conditions over thousands of generations, and can exhibit local adaptation in thermal physiology. Variation in aerobic scope among individuals of a population can mediate population persistence, as some individuals outcompete others in the face of changing environmental conditions. Thus, it is important to characterize aerobic scope for individuals under different acclimatization or acclimation histories and among populations.

In heavily-altered ecosystems, anthropogenic factors coupled with global climate change have drastically changed available habitats for many native fishes (Cloern and Jassby 2012, Moyle et al. 2013). Water projects such as large hydropower dams have altered flow and temperature regimes (Poff et al. 1997, Bunn and Arthington 2002, Marchetti and Moyle 2001), and reduced or degraded native spawning and rearing habitats (Sheer and Steel 2006, Pelicice and Agostinho 2008), while global climate change has resulted in warmer overall river temperatures (Cloern and Jassby, 2012). As ecologically relevant environmental variables like temperature have shifted, fish populations have subsequently declined (Cloern and Jassby, 2012; Hanak et al., 2015). Native California fishes have been disproportionately affected by these changes, and precipitous population declines have been observed for several species, some of which are listed under the Endangered Species Act (ESA) (Moyle et al. 2013).

Despite documented correlations between fish declines and changes in environmental variables such as temperature (Moyle and Leidy, 1992; Moyle et al. 2010; Quiñones and Moyle 2014), there has been a profound lack of data available on the actual physiological and/or behavioral mechanisms driving fish population declines. Physiological performance data, such as information on aerobic scope, may help provide mechanistic explanations for both how and why fish population declines are occurring. Furthermore, as populations decline and management becomes more crucial for population persistence, thermal limits, optimal thermal ranges, and data on how key physiological processes, such as metabolic rate, change in response to environmental variables becomes more important. Therefore, the objectives of our study were to quantify the aerobic scope of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) acclimated to two different rearing temperatures (14 or 20°C) and tested over a temperature range of 12-26°C.

Chinook salmon are native to the Pacific coast of North America, and the species is comprised of several evolutionary distinct units (ESUs) with unique behavioral and reproductive characteristics (NMFS 2009). Many ESUs show considerable variation in the timing of maturation and/or entry into fresh water for spawning, the characteristics of spawning locations (including temperature), and the variables associated with emigration of juvenile smolts. In the Central Valley of California, Chinook salmon populations are the focus of many important conservation and management programs, and state water management programs are geared

towards optimizing conditions for salmonid spawning and migration. Therefore, it is important to understand how Chinook salmon respond to environmental variables such as temperature, and how physiological performance can be affected by thermal history. Understanding the variation associated with thermal performance, such as aerobic scope, both within and among populations can help further our understanding of how this species is affected by critically important environmental variables, such as temperature.

Our results can be used by agency scientists working to manage the early life history stages of Chinook salmon, and will provide more accurate and detailed data where critical knowledge gaps exist. This work provides a range of temperatures over which Chinook salmon exhibit maximal aerobic capacity and thermal performance in laboratory conditions. These data can then be used in combination with field studies and laboratory studies of other ecologically relevant variables, such as temperature impacts to growth, smoltification, and predation, to develop target temperature ranges that can be used by managers to improve Chinook salmon survival. These ranges provide key insights into how temperature can affect specific physiological processes, such as metabolism, in addition to how temperature may affect overall survival of individuals. Coupled with spatial and temporal data of environmental variables throughout the watershed, our data could be critical in developing more effective management plans that may increase Chinook salmon populations. Our data therefore contributes to the growing body of data from both field and laboratory studies that allows for the development of a framework with which to understand temperature effects on salmon populations. Our approach has the potential to provide invaluable management advice on areas to target for habitat restoration, and provide temperature ranges over which Chinook salmon populations will flourish or suffer.

## **Methods**

### *Fish Transport and Acclimation*

Juvenile fall-run Chinook salmon ( $n = 300$ ) were transported to the University of California Davis (UC Davis) Center for Aquatic Biology and Aquaculture from the California Department of Fish and Wildlife Mokelumne River Hatchery (Clements, CA) in early May 2015. Fish were transported in fresh well water ( $14^{\circ}\text{C}$ ) via an aerated transport tank that maintained oxygen levels above 80% saturation. Prior to rearing in acclimation temperatures, fish were held at UC Davis in outdoor flow-through (3 l/min) 1.5 m diameter tanks supplied with water from a fresh, non-chlorinated well, and fish were fed daily to satiation with pelleted trout diet (mix of 2mm Skretting commercial trout feed and 3/64" Rangen sturgeon feed).

Fish were then transferred to flow-through 1.5m diameter tanks, and transitioned to target acclimation temperatures (10, 14, or  $20^{\circ}\text{C}$ ) at a rate of  $1^{\circ}\text{C}$  per day. Water temperatures were controlled by mixing ambient ( $18^{\circ}\text{C}$ ) and chilled ( $9^{\circ}\text{C}$ ) water for the 10 and  $14^{\circ}\text{C}$  tanks, and by using 800-watt titanium heaters (Model TH-0800, Finnex, USA) and temperature controllers (Model 72, YSI, Ohio) for the  $20^{\circ}\text{C}$  tanks. All dissolved oxygen levels were maintained above 80% saturation in the rearing tanks for the duration of the experimental period. Mean water temperatures for each acclimation group are listed in Table 1. Two replicate tanks held fish at  $10^{\circ}\text{C}$  ( $n = 30$  fish per tank), 4 replicate tanks held fish at  $14^{\circ}\text{C}$  ( $n = 30$  fish per tank), and 5 replicate tanks held fish at  $20^{\circ}\text{C}$  ( $n = 30$  fish per tank). All fish were held at acclimation

temperatures for a minimum of three weeks prior to initiation of metabolic rate measurements. Once target acclimation temperatures were reached, each tank of fish was fed at a rate of 2.0% body mass/per fish/per day, and absolute feed amounts were adjusted every 1.5 – 2 weeks to account for fish growth.

| Target Acclimation Temp (°C) | n Tanks | Water Temp (°C) |
|------------------------------|---------|-----------------|
| 10                           | 2       | 10.9 ± 0.12     |
| 14                           | 4       | 14.8 ± 0.06     |
| 20                           | 5       | 19.3 ± 0.09     |

**Table 1. Water temperatures for the 3 different temperature acclimation groups. Fish were reared in acclimation conditions for at least 3 weeks prior to the measurement of metabolic rates.**

### *Aerobic Scope Measurements*

#### Experimental Design

For the 14 and 20°C acclimation groups, measurements of aerobic scope were taken over a range of swimming (testing) temperatures from 12-26°C, at 1°C increments. Metabolic rates (resting and maximum metabolic rates) were measured for an individual fish at one swimming temperature, and four (n = 4) fish from each acclimation group were tested at each swimming temperature. For the 10°C acclimation group, a total of four fish (n=4) were tested at each of the following swimming temperatures: 12, 18, and 24°C.

#### Swim Tunnel Respirometry

Fish were tested in one of three 5-liter automated swim tunnel respirometers (Loligo, Denmark) two of which were controlled using a single computer system (two-tunnel system), and one of which was controlled using a separate system (single-tunnel system). Acclimation and testing temperatures were randomized between the two systems. For all three tunnels, water was pumped into an aerated water bath surrounding the swim tunnel from a designated sump unique to each system using a water pump (Danner, Model 18B), and was returned to the sump after circulation through the system. Sump water was continuously refreshed with fresh well water from a designated non-chlorinated well, and was supplied with air stones for additional aeration. Temperature in the respirometers was controlled by circulating water through a chiller (Aqua Logic Delta Star Model DSHP-7) and pumping it back to the sump using a high-volume water pump (Model SHE1.7, Sweetwater©, USA). In addition, each sump contained two 800-watt titanium heaters (Model TH-0800, Finnex, USA) connected to variable temperature controllers (Model 72, YSI, Ohio). These two methods were used simultaneously to achieve water temperature control within the swim tunnels with a precision of ± 0.5°C. Swim tunnels and associated pumps were bleached and cleaned each week to reduce potential bacterial growth in the system.

Oxygen saturation values within the swim tunnels were measured using mini fiber optic oxygen probes (1 per tunnel) and were continuously monitored and recorded by AutoResp software (ver. 2.2.2). The oxygen probes were connected to the AutoResp software via a Witrox-4 oxygen meter (Loligo, Denmark) for the two-tunnel system, and a Witrox-1 oxygen

meter for the single-tunnel. Oxygen probes were calibrated each week using a two-point, temperature-paired technique calibrating at 0 and 100% oxygen saturation. Therefore, dissolved oxygen was measured in percent saturation, since absolute values at each water temperature were different. Water velocity in the swim tunnels was generated using a DAQ-M data acquisition device and a VFD controller (SEW Eurodrive, Model 4x and 12x). Velocity (precision of < 1 cm/s) for each tunnel was controlled remotely through the use of the AutoResp program.

To reduce disturbance and/or experimenter influence on the fish during metabolic rate measurements, all swim tunnels were surrounded by black shade cloth and black plastic sheeting. Infrared cameras (Q-See, QSC1352W, China) were mounted directly overhead each tunnel, and were connected to both a television monitor and a DVR recorder. The cameras recorded behavior of each individual fish during metabolic rate measurements, and television monitors allowed for the observation of each fish without additional disturbance.

Metabolic measurements for both resting and maximum metabolic rates were made using intermittent respirometry. For each swim tunnel, a flush pump (Danner, Model 2) circulated water from the aerated water bath into the swim chamber, and was controlled automatically through AutoResp software and a DAQ automated respirometry system. When the flush pump for a swim tunnel was off, the tunnel was sealed and no gas or water exchange occurred within the tunnel. When the flush pump was on, fresh, aerated water was circulated through the tunnel from the surrounding water bath. Therefore, metabolic rates could be measured during periods in which the tunnel was sealed. During tunnel sealing, 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 drops (in mg O<sub>2</sub>) were calculated for a minimum 2 min period when the tunnel was sealed. When the flush pump was circulating water from the water bath, oxygen levels were restored within the swim chamber as gas and water flowed through the tunnel (approximately 2-5 mins). Oxygen levels were never allowed to fall below 80% saturation.

Percent saturation was converted to oxygen concentration ([O<sub>2</sub>], mg O<sub>2</sub> l<sup>-1</sup>) using the formula:

$$[O_2] = \% O_2\text{Sat}/100 \times \alpha(O_2) \times BP,$$

where %O<sub>2</sub>Sat 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<sub>2</sub> l<sup>-1</sup> mmHg<sup>-1</sup>); BP is barometric pressure in mmHg.

Metabolic rate (MR in mg O<sub>2</sub> kg<sup>-0.95</sup> min<sup>-1</sup>) for resting and swimming fish was calculated using the formula:

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

where O<sub>2</sub>(A) is the oxygen concentration in the tunnel at the beginning of the seal (mg O<sub>2</sub> l<sup>-1</sup>); O<sub>2</sub>(B) is the oxygen concentration in the tunnel at the end of the seal (mg O<sub>2</sub> l<sup>-1</sup>); V is the volume of water in the tunnel (l); M is the mass of the fish (kg); T is the duration of the seal (min).

All metabolic rates were corrected for fish mass to account for individual variation in size 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).

#### Resting Metabolic Rate (RMR)

Prior to any metabolic rate measurement, fish were transferred into individual 0.5 x 1.0 m rectangular, flow-through holding tanks, and fasted for 24 hours prior to the initiation of metabolic measurements. Holding tanks were supplied with aerated acclimation water from the same source as the acclimation tanks, and water temperatures were maintained at acclimation temperatures. After the 24-hour fasting period, individual fish were transferred into a swim tunnel between the hours of approximately 1500 and 1700. Following transfer, fish were given a 1-hour acclimation period during which the temperature of the tunnel was the same as their acclimation water. Following the acclimation period, the tunnel temperature was adjusted to a predetermined testing temperature (between 12 and 26°C) at a rate of 1°C per 30 minutes (2°C per hour). The two-tunnel system shared one chiller, and therefore both tunnels were set to identical testing temperatures, while the single-tunnel system could be operated at an independent temperature. After each testing temperature was reached, fish were allowed a 30-min acclimation period prior to the initiation of resting metabolic rate (RMR) measurements. RMR measurements were taken automatically overnight using AutoResp software. Measurement periods (during which the tunnels were sealed) were 2400 seconds (40 mins) in duration. Flush period durations were adjusted according to temperature, with warmer temperatures flushing for longer durations to ensure adequate oxygen saturation in the chambers. A small pump recirculated water within the swim chamber at a velocity that allowed fish to keep oxygenated water around their gills, but that did not elicit movement (< 5 cm/s). Video equipment continuously monitored fish behavior to ensure that little or no activity occurred.

#### Maximum Metabolic Rate (MMR)

Maximum metabolic rate (MMR) measurements were initiated the morning following RMR measurements between the hours of ca. 800 and 900. A modified critical swimming velocity protocol was used to swim the fish until exhaustion. In cases where exhaustion was not achieved, or fish were resistant to swimming, a burst swimming protocol was used to elicit MMR. Modified critical swimming tests were initiated after gradually increasing the water velocity in the swimming chamber from 0 to 30 cm/s over a period of approximately 2 minutes. The fish then remained at this initial velocity step (30 cm/s) and each subsequent step for 20 minutes. After 20 mins, the water velocity was increased by approximately 10% of the previous test velocity (i.e., 3 cm/s if the previous step ranged between 30-39 cm/s, 4 cm/s if between 40-49 cm/s, etc.) until the fish reached exhaustion and was unable to continue to swim. Active metabolism was measured during each step by sealing off the swim tunnel and recording the drop in oxygen saturation. This seal period was maintained for as long as possible without allowing the water air saturation to drop below 80%. For fish with high metabolic rates, multiple seal periods were measured during the respective velocity step. When the oxygen saturation approached 80% or the velocity increment approached 20 min, fresh water was flushed into the tunnel until saturation was above 95%.



If a fish impinged upon the back screen (stopped swimming and became trapped), a rest period was given in which water velocity was decreased to approximately 15 cm/s for 1 min, and then gradually increased to the testing velocity over a period of 2 mins. A fish was considered exhausted if it was unable to resume swimming behavior after an impingement, or if a fish impinged 2 times during the same velocity step. At this point, the time and velocity of failure was noted, the tunnel was flushed, and the velocity was decreased to RMR conditions. If a fish required a burst swimming protocol to achieve MMR, fish were first given a 20 min resting period in which no water velocity was present in the swim chamber. After this period, fish were swum at 30 cm/s for 10 mins, and subsequently subjected to high water velocities. The velocity was rapidly increased above normal swimming velocities (ca. 60 cm/s) over a period of 10 secs, requiring the fish to burst swim in the chamber. Bursting velocity was held for a maximum duration of 30 seconds, after which the velocity was decreased to 30 cm/s. This process was repeated for at least 5 minutes, and up to 10 minutes.

Following exhaustion, the fish was allowed to recover at RMR conditions for at least 1 hour. Following the recovery period, water temperature was returned to acclimation temperature at 2°C an hour. After the acclimation was reached, fish were removed from the swim tunnel, and placed into a short-term (24-hour) recovery tank. Following the 24-hour recovery, mass (g), fork length (cm) and total length (cm) were measured and recorded. Fish were then transferred to a designated long-term recovery tank.

### *Growth*

Growth was assessed for fish acclimated to 14 and 20°C by weighing and measuring each tank of fish once acclimation temperatures were reached, and by weighing and measuring individuals after the completion of the metabolic rate test. Therefore, mass and length were recorded throughout the experimental period, and the date of these measurements as well as the number of days the fish were at the acclimation temperature were recorded. Growth for fish acclimated to 10°C was also assessed in this manner, but was supplemented by additional measurements in which fish (n = 17) were randomly selected from 10°C acclimation tanks, weighed and measured, and returned to the tank.

## **Results**

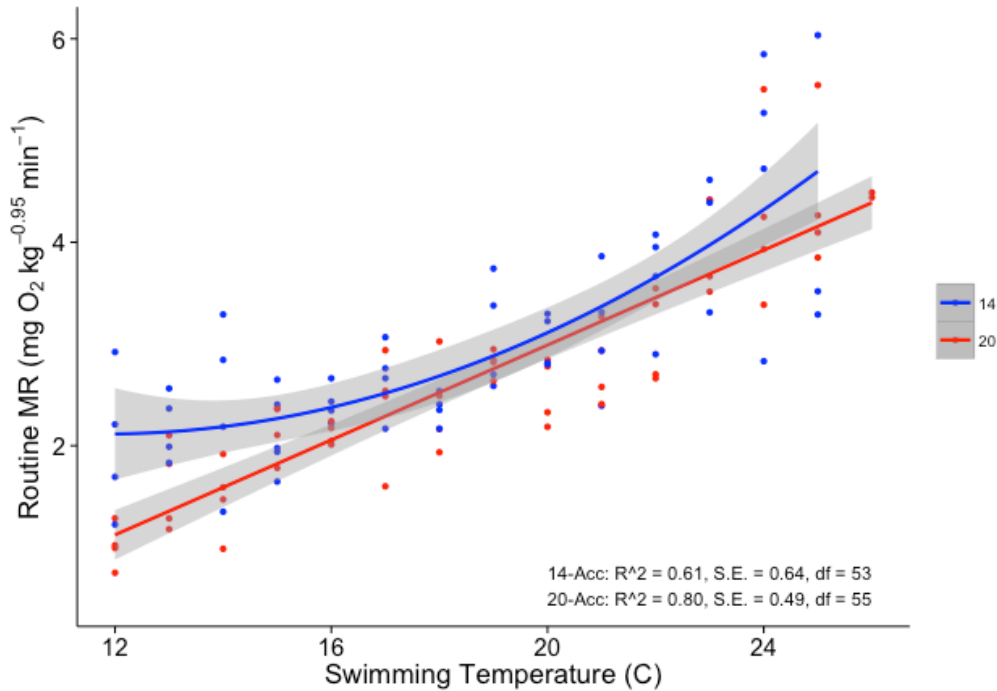
### *Mortality*

Very little mortality was observed during the course of experimental trials, except at elevated temperatures (Supplementary Table 5). When tested at 25°C, 1 out of the 4 fish acclimated to 14°C exhibited mortality during the course of metabolic measurements, though no fish acclimated to 20°C died at this testing temperature. However, when tested at 26°C, 3 out the 4 fish acclimated to 14°C exhibited mortality, and 1 of the 4 lost equilibrium during metabolic measurements. Similarly, 2 of the 4 fish acclimated to 20°C exhibited mortality at 26°C. Only one other mortality occurred during metabolic measurements throughout the duration of the experiment (a 20°C-acclimated fish tested at 18°C). No measurements obtained from fish prior to mortality or loss of equilibrium were used to calculate metabolic rates or aerobic scope values.

## Metabolic Measurements

### Resting Metabolic Rate (RMR)

The resting metabolic rate (RMR) of juvenile Chinook salmon increased as swimming temperatures increased from 12 to 26°C for both acclimation groups. RMR was significantly affected by both acclimation temperature ( $df = 1$ ,  $F = 7.30$ ,  $p = 0.008$ ) and by swimming temperature ( $df = 14$ ,  $F = 19.9$ ,  $p = 2.2 \times 10^{-16}$ ), but there was no significant interaction between the two ( $df = 13$ ,  $F = 0.65$ ,  $p = 0.81$ ). The significant effects of acclimation temperature and swimming temperature indicate that both past thermal history of the fish and the temperature at which the RMR was obtained (swimming temperature) influenced RMR, but the non-significant interaction suggests that the swimming temperature affected the RMR of both acclimation groups similarly.



**Figure 2. Resting (or routine) metabolic rates (RMR) for hatchery juvenile Chinook salmon acclimated to 14 and 20°C. Each point represents the RMR for one individual fish, and the solid line represents the best fitting line for the data; equations for best-fit curve are given in the text. The shaded area surrounded each line represents the standard error of the curve. '14-Acc' and '20-Acc' represent values for 14 and 20°C acclimation groups, respectively. There was a significant effect of acclimation temperature on RMR values ( $p = 0.008$ ).**

### 14°C Acclimation Group

The RMR of fish acclimated to 14°C increased exponentially over the range of swimming temperatures tested (12 to 25°C, due to mortality at 26°C). This response was fitted with a statistically significant ( $p = 5.46 \times 10^{-12}$ ) relationship (Figure 2):

$$\text{RMR (mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}) = 4.17439 - 0.34926x + 0.01481x^2,$$

where x = temperature (°C).

The mean RMR for 14°C-acclimated fish tested at 12°C was  $2.01 \pm 0.37 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , while the mean RMR at 25°C was  $4.28 \pm 0.88 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ . This equates to roughly a 2.1-fold increase in RMR over a 13°C temperature increase, which is consistent with typical  $Q_{10}$  values that range between 1.5 and 3.0 in fishes. These results support predictions that physiological rates (such as metabolic rates) will increase as temperatures increase, though our data did not show an upper range of temperatures whereby metabolism declined below optimal values as predicted in Figure 1. Instead, RMR values continued to increase at temperatures just below the lethal temperatures of 25 or 26°C.

### 20°C Acclimation Group

The RMR of fish acclimated to 20°C increased linearly over the range of swimming temperatures tested (12 to 26°C). This response was fitted with a statistically significant ( $p = 2.2 \times 10^{-16}$ ) relationship (Figure 2):

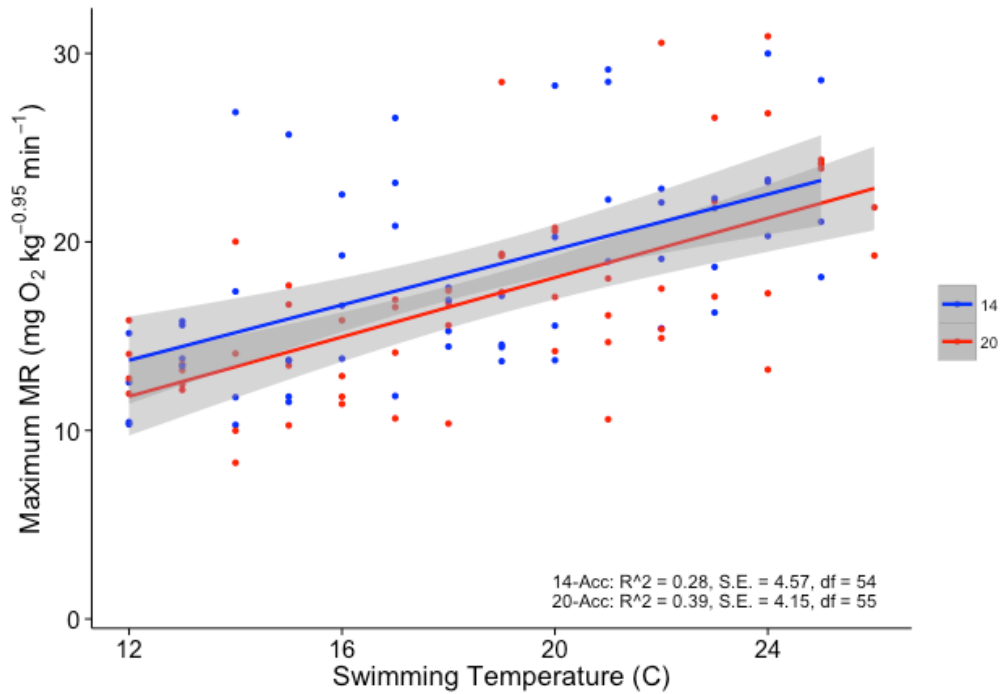
$$\text{RMR (mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}) = -1.6767 + 0.2333x,$$

where x = temperature (°C).

The mean of RMR for 20°C-acclimated fish tested at 12°C was  $1.01 \pm 0.11 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , while the mean RMR at 25°C was  $4.44 \pm 0.38 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ . While data was obtained from these fish at 26°C, 50% mortality at this swimming temperature was observed, and we are reporting metabolic rates obtained at 25°C for direct comparison with measures obtained from fish acclimated to 14°C. Over a 13°C test temperature range, the RMR of fish acclimated to 20°C increased nearly 4.5-fold, a higher rate of increase than that observed for fish acclimated to 14°C. This difference is driven by the differences observed at low swimming temperatures, as the RMR values observed for temperatures exceeding 23°C were similar (Supplementary Table 1).

### *Maximum Metabolic Rate (MMR)*

The maximum metabolic rate (MMR) of juvenile Chinook salmon increased linearly as swimming temperatures increased from 12 to 26°C for both acclimation groups. MMR was not significantly affected by acclimation temperature ( $df = 1$ ,  $F = 2.44$ ,  $p = 0.12$ ), but was significantly affected by swimming temperature ( $df = 14$ ,  $F = 4.33$ ,  $p = 1.0 \times 10^{-5}$ ). There was no significant interaction between acclimation group and swimming temperature ( $df = 13$ ,  $F = 1.58$ ,  $p = 0.11$ ), indicating that the effect of swimming temperature on MMR was similar for both acclimation groups.



**Figure 3. Maximum metabolic rates (MMR) for hatchery juvenile Chinook salmon acclimated to 14 and 20°C. Each point represents the MMR for one individual fish, and the solid line represents the best fitting line for the data; equations for best-fit curve are given in the text. The shaded area surrounded each line represents the standard error of the curve. '14-Acc' and '20-Acc' represent values for 14 and 20°C acclimation groups, respectively. There was no significant effect of acclimation temperature on MMR values ( $p = 0.12$ ).**

#### 14°C Acclimation Group

The MMR of fish acclimated to 14°C increased linearly over the range of swimming temperatures tested (12 to 25°C, due to mortality at 26°C). This response was fitted with a statistically significant ( $p = 1.51 \times 10^{-5}$ ) relationship (Figure 3):

$$\text{MMR (mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}) = 4.9155 + 0.7337x,$$

where  $x$  = temperature (°C).

The mean of MMR for 14°C-acclimated fish tested at 12°C was  $12.12 \pm 1.14 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , while the mean MMR at 25°C was  $22.59 \pm 3.11 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , a 1.9-fold increase over the swimming temperature range. MMR continued to increase as swimming temperature increased until mortalities were observed at 25 and 26°C.

#### 20°C Acclimation Group

The MMR of fish acclimated to 20°C increased linearly over the range of swimming temperatures tested (12 to 26°C). This response was fitted with a statistically significant ( $p = 1.55 \times 10^{-7}$ ) relationship (Figure 3):

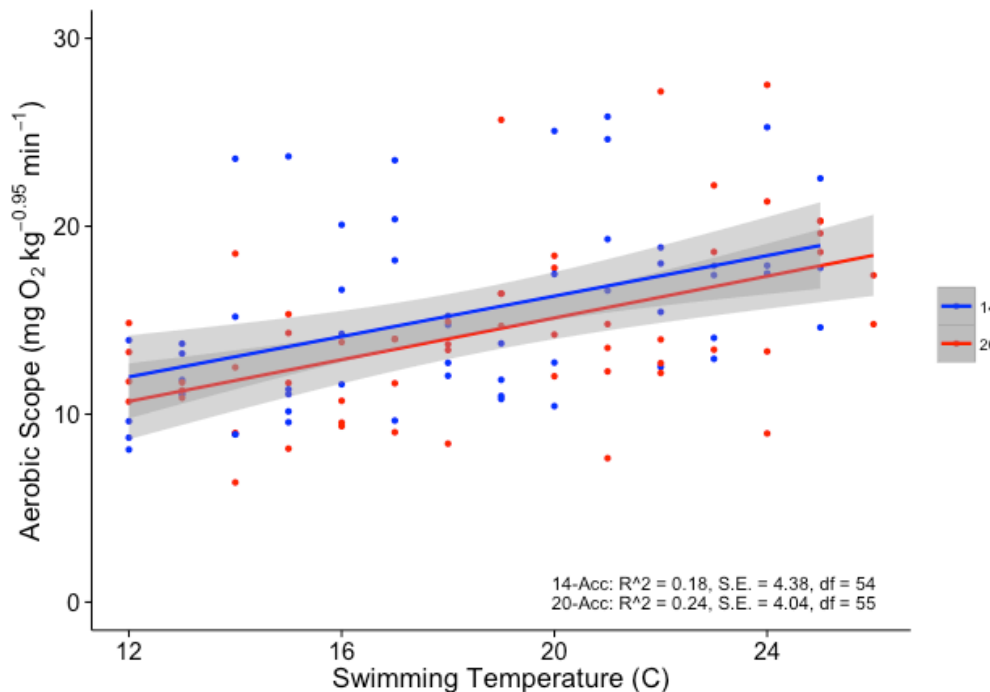
$$\text{MMR (mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}) = 2.3395 + 0.7885x,$$

where  $x$  = temperature ( $^{\circ}\text{C}$ ).

The mean of MMR for  $20^{\circ}\text{C}$ -acclimated fish tested at  $12^{\circ}\text{C}$  was  $13.65 \pm 0.85 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , while the mean MMR at  $25^{\circ}\text{C}$  was  $24.14 \pm 0.10 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , 1.8-fold increase over the range of temperatures tested. This rate of increase was very similar to that obtained for fish acclimated to  $14^{\circ}\text{C}$ , and reflects the lack of difference in MMR observed between the two acclimation groups. This is further supported by the similar values obtained for MMR as temperature increased, as shown in Supplementary Table 2.

### *Absolute Aerobic Scope (AAS)*

The absolute aerobic scope (AAS) of juvenile Chinook salmon acclimated to 14 and  $20^{\circ}\text{C}$  both increased linearly as swimming temperatures increased from 12 to  $26^{\circ}\text{C}$ . The AAS was not significantly affected by acclimation temperature ( $df = 1$ ,  $F = 1.58$ ,  $p = 0.21$ ), but swimming temperature significantly affected the AAS of both acclimation groups ( $df = 14$ ,  $F = 2.51$ ,  $p = 0.005$ ). The interaction between acclimation and swimming temperature was not statistically significant ( $df = 13$ ,  $F = 1.73$ ,  $p = 0.07$ ), suggesting a similar increase in AAS as swimming temperature increased for both groups.



**Figure 4. Absolute aerobic scope (AAS) values for hatchery juvenile Chinook salmon acclimated to 14 and  $20^{\circ}\text{C}$ . Each point represents the AAS for one individual fish, and the solid line represents the best fitting line for the data; equations for best-fit curve are given in the text. The shaded area surrounded each line represents the standard error of the curve. '14-Acc' and '20-Acc' represent values for 14 and  $20^{\circ}\text{C}$  acclimation groups, respectively. There was no significant effect of acclimation temperature on AAS values ( $p = 0.21$ ).**

### 14°C Acclimation Group

The AAS of fish acclimated to 14°C increased linearly over the range of swimming temperatures tested (12 to 25°C, due to mortality at 26°C). This response was fitted with a statistically significant ( $p = 0.0006$ ) relationship (Figure 4):

$$\text{AAS (mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}) = 5.5259 + 0.5379x,$$

where  $x$  = temperature (°C).

The mean AAS for 14°C-acclimated fish tested at 12°C was  $10.11 \pm 1.31 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , while the mean AAS at 25°C was  $18.31 \pm 2.30 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ . AAS therefore increased 1.8-fold over the temperature range tested, and no clear peak in AAS was observed.

### 20°C Acclimation Group

The AAS of fish acclimated to 20°C increased linearly over the range of swimming temperatures tested (12 to 26°C). This response was fitted with a statistically significant ( $p = 5.97 \times 10^{-5}$ ) relationship (Figure 4):

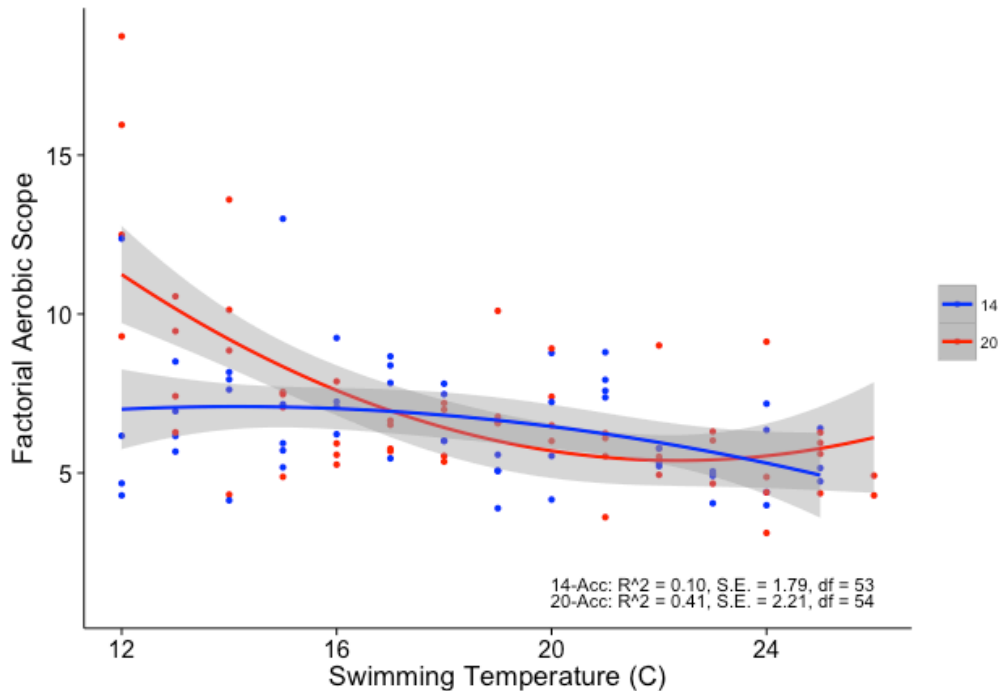
$$\text{AAS (mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}) = 4.0162 + 0.5552x,$$

where  $x$  = temperature (°C).

The mean AAS for 20°C-acclimated fish tested at 12°C was  $12.64 \pm 0.92 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ , while the mean AAS at 25°C was  $19.70 \pm 0.39 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$ . This 1.6-fold increase in AAS over the testing temperatures was slightly lower but still similar to the 1.8-fold increase observed for fish acclimated to 14°C, and reflects the lack of differentiation in AAS observed for the two acclimation groups (Supplementary Table 3).

### *Factorial Aerobic Scope (FAS)*

The factorial aerobic scope (FAS) of both the 14 and 20°C acclimation groups decreased as swimming temperatures increased. There were no significant differences in FAS between acclimation groups ( $df = 1$ ,  $F = 2.80$ ,  $p = 0.1$ ), though both swimming temperature and the interaction between swimming temperature and acclimation group were significant ( $df = 14$ ,  $F = 3.87$ ,  $p = 4.74 \times 10^{-5}$ ;  $df = 13$ ,  $F = 3.08$ ,  $p = 0.0009$ ). The significant interaction suggest that while FAS decreased as swimming temperatures increased in both groups, the rate of decrease was different for the two acclimation groups (Figure 5).



**Figure 5. Factorial aerobic scope (FAS) values for hatchery juvenile Chinook salmon acclimated to 14 and 20°C. Each point represents the FAS for one individual fish, and the solid line represents the best fitting line for the data; equations for best-fit curve are given in the text. The shaded area surrounded each line represents the standard error of the curve. '14-Acc' and '20-Acc' represent values for 14 and 20°C acclimation groups, respectively. There was no significant effect of acclimation temperature on FAS values ( $p = 0.10$ ). No fish tested exhibited a FAS value of less than 2. The lowest FAS value was 3.11, obtained from a 20°C acclimated fish tested at 24°C.**

#### 14°C Acclimation Group

The FAS of fish acclimated to 14°C decreased logistically over the range of swimming temperatures tested (12 to 25°C, due to mortality at 26°C). This response was fitted with a statistically significant ( $p = 0.03$ ) relationship (Figure 5):

$$\text{FAS} = 3.4295 + 0.5175x - 0.0183x^2$$

where  $x$  = temperature (°C).

The mean FAS for 14°C-acclimated fish tested at 12°C was  $6.88 \pm 1.88$ , while the mean FAS at 25°C was  $5.43 \pm 0.50$ . The decrease in FAS is relatively shallow, and reflects a loss of FAS over a 13°C temperature range of roughly 25%.

#### 20°C Acclimation Group

The FAS of fish acclimated to 20°C decreased logistically over the range of swimming temperatures tested (12 to 26°C). This response was fitted with a statistically significant ( $p = 3.27 \times 10^{-7}$ ) relationship (Figure 5):

$$\text{FAS} = 32.6594 - 2.4390x + 0.05454x^2$$

where  $x$  = temperature ( $^{\circ}\text{C}$ ).

The mean FAS for  $20^{\circ}\text{C}$ -acclimated fish tested at  $12^{\circ}\text{C}$  was  $14.12 \pm 2.05$ , while the mean FAS at  $25^{\circ}\text{C}$  was  $5.54 \pm 0.42$ . While the decrease in FAS for fish acclimated to  $20^{\circ}\text{C}$  over the range of temperatures tested was greater than that for fish acclimated to  $14^{\circ}\text{C}$  (a 60% decrease vs.  $\sim 25\%$ , respectively), FAS values were similar between the two acclimation groups except at low temperatures. The relatively high FAS values observed for fish acclimated to  $20^{\circ}\text{C}$  were driven by the very low RMR values obtained for  $20^{\circ}\text{C}$ -acclimated fish at temperatures below roughly  $15^{\circ}\text{C}$ . However, the differences in the loss of FAS between acclimation groups as temperatures increased is supported by the significant interaction between acclimation and swimming temperatures, and suggests that the two acclimation groups are differentially affected by changes in temperature (Supplementary Table 4).

#### *Metabolic Measurements for Fish Acclimated to $10^{\circ}\text{C}$*

Due to the small sample size for fish acclimated to  $10^{\circ}\text{C}$  (tested at only three swimming temperatures), the data were not statistically analyzed for response to swimming temperatures or for comparison to data from  $14^{\circ}\text{C}$ - and  $20^{\circ}\text{C}$ -acclimated fish. The data are summarized in Supplementary Tables 1-4.

The RMR for fish acclimated to  $10^{\circ}\text{C}$  increased from  $1.90 \pm 0.12 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$  at  $12^{\circ}\text{C}$  to  $4.40 \pm 0.25 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$  at  $24^{\circ}\text{C}$ . This 2.3-fold increase in RMR as temperature increases is similar to the 2.1-fold increase observed in  $14^{\circ}\text{C}$ -acclimated fish, and the mean values observed at 12 and  $24^{\circ}\text{C}$  for fish acclimated to  $10^{\circ}\text{C}$  approximate those observed for fish acclimated to  $14^{\circ}\text{C}$  (Supplementary Table 1).

The MMR for fish acclimated to  $10^{\circ}\text{C}$  increased from  $12.80 \pm 0.98 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$  at  $12^{\circ}\text{C}$  to  $24.35 \pm 1.88 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$  at  $24^{\circ}\text{C}$ . These values are remarkably similar to MMR values obtained at these temperatures for both  $14^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  acclimation groups, and is consistent with the finding that acclimation temperature did not significantly affect MMR.

Similarly, the AAS for fish acclimated to  $10^{\circ}\text{C}$  increased from  $10.90 \pm 1.05 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$  at  $12^{\circ}\text{C}$  to  $19.95 \pm 1.81 \text{ mg O}_2 \text{ kg}^{-0.95} \text{ min}^{-1}$  at  $24^{\circ}\text{C}$ ; these values which closely matched those obtained for the  $14^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  acclimation groups as well. The similarity in these values suggests that very low acclimation temperatures did not have an effect on AAS, and supports the finding that there were no significant differences in AAS between acclimation groups.

Finally, the FAS for fish acclimated to  $10^{\circ}\text{C}$  decreased from  $6.89 \pm 0.91$  at  $12^{\circ}\text{C}$  to  $5.56 \pm 0.45$  at  $24^{\circ}\text{C}$ . These values are very similar to those obtained for fish acclimated to  $14^{\circ}\text{C}$  ( $6.88 \pm 1.88$  and  $5.43 \pm 0.50$  at 12 and  $24^{\circ}\text{C}$ , respectively), and resulted in a similar decrease in FAS of roughly 25% over the temperatures tested.

The data obtained from fish acclimated to  $10^{\circ}\text{C}$  supports the overall findings that acclimation temperature had a minimal impact on the metabolism of juvenile Chinook salmon,



and suggests that recent thermal history, especially in colder temperatures (i.e., 10-14°C), may not significantly affect metabolic performance for short-term (hours) thermal exposures for this species.

### ***Growth***

The mass- and fork length-specific growth rates (g/day and cm/day, respectively) are listed in Table 2. There were no significant differences in the initial mass or fork length of fish acclimated to different temperatures ( $p > 0.05$  for both comparisons). Similarly, there were no significant differences in the mass specific growth rate ( $df = 2, F = 1.71, p = 0.19$ ) or the fork length specific growth rate ( $df = 2, F = 1.37, p = 0.26$ ) for fish acclimated to different temperatures.

| <b>Acclimation Temperature</b> | <b>Initial Mass (g)</b> | <b>Initial Fork Length (cm)</b> | <b>Mass SGR</b> | <b>Fork Length SGR</b> |
|--------------------------------|-------------------------|---------------------------------|-----------------|------------------------|
| 10                             | 8.8 ± 0.22              | 8.7 ± 0.08                      | 0.151 ± 0.01    | 0.037 ± 0.001          |
| 14                             | 8.6 ± 0.17              | 8.8 ± 0.06                      | 0.195 ± 0.01    | 0.042 ± 0.002          |
| 20                             | 8.5 ± 0.14              | 8.7 ± 0.06                      | 0.173 ± 0.01    | 0.037 ± 0.002          |

**Table 2. Initial sizes and specific growth rates (SGR) observed throughout the course of the aerobic scope experiment. There were no significant differences in the initial mass or fork length of fish acclimated to different temperatures, and there were no significant differences in the SGR of mass or the SGR of fork length for fish acclimated to different temperatures ( $p > 0.05$  for all comparisons).**

### **Discussion**

The results from this experiment represent an assessment of the effect of thermal acclimation on the aerobic scope of juvenile fall-run Chinook salmon tested over a wide range of environmental temperatures. The temperature range over which Chinook salmon were tested (12-26°C) encompasses cool temperatures experienced in upper tributary riverine environments where juvenile Chinook salmon rearing grounds are located, and temperatures potentially experienced in the Sacramento-San Joaquin Delta, through which Chinook salmon smolts must migrate. Therefore, our results provide information about the physiological performance capabilities of Chinook salmon under a range of environmentally relevant temperatures. The thermal performance of fish in this study, however, should be interpreted with caution, as hatchery fish tested in the laboratory likely exhibit different physiological capabilities than both hatchery fish in the wild (due to optimal laboratory conditions, such as the lack of predators, abundant food availability, and high dissolved oxygen content) and their wild counterparts. However, these results underscore the importance of the effects of temperature on physiological performance, as well as the remarkable capacity of juvenile Chinook salmon to maintain physiological performance over a wide range of environmental temperatures. Furthermore, these results contribute to a body of knowledge on the physiological capabilities of Chinook salmon that can be used to inform management actions.

To test the effect of thermal history on aerobic capacity, we compared the metabolic rates and aerobic scope of juvenile Chinook salmon acclimated to 14 or 20°C. Overall, acclimation temperature had little effect on the thermal performance of Chinook salmon. Acclimation temperature significantly affected RMR, though the MMR, AAS, and FAS of juvenile Chinook salmon were statistically indistinguishable between acclimation groups. The difference in RMR between acclimation groups was driven largely by the RMR of fish acclimated to 20°C at low testing temperatures – temperatures lower than 15°C. At these low temperatures, fish acclimated to 20°C exhibited metabolic rates between roughly 1-2 mg O<sub>2</sub>/kg<sup>0.95</sup>/min, which were lower than those exhibited by fish acclimated to 14°C tested at the same temperatures. This difference could be explained, in part, by physiological changes that accompanied the 3-week acclimation in waters with different temperatures. For fish acclimated to 14°C, testing temperatures between 12-15°C were similar to their recent thermal history. Therefore, metabolic enzymes and associated metabolic pathways were likely functioning at or near a high capacity. Conversely, the relatively large temperature discrepancy between the thermal conditions experienced by fish acclimated to 20°C and testing temperatures between 12-15°C resulted in depressed physiological function and reduced metabolic rates.

The main effect of thermal history had no significant effect on MMR, AAS, or FAS, and these values were similar for fish acclimated to 14 and 20°C. The similarity in thermal performance of juvenile Chinook salmon acclimated to different temperatures extended to fish acclimated to 10°C. While metabolic rates obtained from 10°C-acclimated fish were not statistically compared to those obtained from 14- and 20°C-acclimated fish, the values observed were remarkably similar (Supplementary Tables 1-4). Previous studies using incipient lethal temperature methods (i.e. measures of thermal resistance related to survival times at different temperatures, ILT) have shown effects of acclimation temperature in Atlantic (*Salmo salar*; Elliott 1991), Chinook (Brett 1952), and sockeye (*O. nerka*; Brett 1952, 1971) salmon, especially as the magnitude of the difference between acclimation temperature groups increased (i.e., 5 versus 25°C). Others have shown no difference in lethal temperatures in Atlantic salmon when acclimation temperatures were more similar (i.e., 15 versus 20°C; Elliott and Elliott 1995). In fact, all studies on fishes to date show some degree of thermal acclimation in thermal resistance (i.e. ILT) and thermal tolerance (i.e. CTM) measures (reviewed in Beitinger and Bennett 2000; Schulte et al. 2011), but the magnitude of the acclimation response varies among species and with life history, thermal niche (i.e. eurytherms vs. stenotherms), and ontogeny (Komoroske et al. 2014). It is also important to note, however, that these studies quantify thermal tolerance and resistance (measures that relate to short term survival of individuals) and it is not expected that all traits (e.g. swimming performance and metabolic rate) would respond to thermal acclimation in a similar way. In fact, eurythermal fishes such as killifishes have a very limited thermal acclimation response with respect to swimming performance (Fangue et al. 2008), but show substantial thermal acclimation with respect to thermal tolerance values (Fangue et al. 2006). It may actually be advantageous to compensate for temperature variation across an ecologically relevant temperature range, since fishes are ectotherms that are subject to environmental temperatures for body temperature and rate functions. Therefore, the ability to compensate for temperature changes and maintain consistency in critically important functions such as metabolism and swimming may be an important physiological capability.

The lack of a significant main effect of acclimation temperature on the majority of metabolic rate measurements could be due to several different, though not mutually exclusive, mechanisms. It is possible that the duration of acclimation (3 weeks) was not long enough to induce the physiological changes associated with differential thermal performance capabilities. Despite evidence that suggests that a 3-week acclimation period is sufficient to induce physiological change (Bennett et al. 1998; Doudoroff 1942; Fanguie et al. 2014), the actual amount of time required to observe such alterations may be species-specific. Another potential explanation could be due to the environments experienced during the juvenile life history stage of Chinook salmon. The life history strategy of Chinook salmon exposes juveniles to both cooler riverine temperatures associated with rearing grounds located in the upper reaches of the watershed, and warmer water temperatures in the bays and estuaries through which they must swim as they outmigrate to the ocean. Due to the wide range of natural temperatures experienced by juvenile Chinook salmon, both 14 and 20°C could be important temperatures for this species to maintain physiological performance. Thus, the metabolic rates and aerobic capacity exhibited by fish acclimated to different temperatures may have been within their physiological capacity, and lead to the observed lack of difference in performance.

Despite acclimation temperature having no significant overall effect on aerobic scope of juvenile Chinook salmon, there was a significant interaction between acclimation temperature and testing temperature for FAS (Figure 5). As testing temperatures increased, the FAS of fish acclimated to 14 and 20°C both decreased, though the rate of decrease differed between the two groups. FAS values for fish acclimated to 20°C decreased more rapidly as swimming temperatures increased compared to fish acclimated to 14°C, for which the decrease in FAS was much more modest. This difference again was driven primarily by differences in FAS values at swimming temperatures  $\leq 15^\circ\text{C}$ . At these low temperatures, the FAS values of 20°C-acclimated fish were much greater than those observed for 14°C-acclimated fish, though these differences disappeared as testing temperatures increased. The significant interaction, however, between acclimation and testing temperatures indicates that the acclimation conditions may have influenced some aspect of thermal performance in response to temperature. This is supported by the differences observed in RMR values between the acclimation groups. Further investigation into these potential effects is required to more thoroughly understand the influence of thermal history on metabolic rates and aerobic scope in juvenile salmon. Measuring thermal performance at swimming temperatures less than 12°C is one aspect future studies should focus on, since 12°C was the lowest temperature at which fish were tested in the present study.

For both acclimation groups, there was a lack of a clear temperature range over which aerobic scope was optimal/maximal. AAS values continued to increase over the full range of temperatures tested in both groups, and a peak AAS value was not observed. Indeed, for both acclimation groups, AAS increased with increasing test temperatures until mortality began to occur. For 14°C-acclimated fish, mortality rates abruptly increased at 25°C, and no metabolic rate measurements (100 % mortality or imminent mortality [loss of equilibrium]) could be obtained for fish at this testing temperature. Similarly, 20°C-acclimated fish exhibited 50% mortality when tested at 25°C. However, prior to these increases in mortality rates, the AAS for both acclimation groups did not plateau or decrease, as would be predicted by the OCLTT hypothesis. Instead, it appears that Chinook salmon were able to maintain maximal oxygen extraction from the water at temperatures immediately near those that resulted in mortality. This

relationship was consistent with that obtained for RMR and MMR, for which no peak performance value or optimal temperature range was observed. These results suggest that the aerobic capacity of juvenile hatchery fall-run Chinook salmon is relatively temperature-insensitive for acute temperature exposures over the range of tested temperatures. This is consistent with the results obtained for FAS values; no individual exhibited a FAS of less than 2, which is the minimum value thought required for digestion and growth (Verhille et al. 2015a). Juvenile Chinook from both acclimation groups maintained FAS values of greater than 3 over the range of swimming temperatures tested, indicating that these fish would be able to maintain aerobic metabolism and sustain growth during acute temperature exposures of up to 24-25°C (when mortality rates abruptly increased). There is some speculation as to whether a minimum FAS value is an accurate number for all species, or if this should be determined on a species- or population-specific level. More research on several species, particularly in the *Oncorhynchus* genus, is required to accurately assess this value.

Relative thermal insensitivity has been documented in previous studies on aerobic scope in salmonids. The thermal range over which AAS was statistically consistent for hatchery *O. mykiss* was 16-25°C (Verhille et al. 2015b), which was also similar to data obtained for wild *O. mykiss* tested on the Lower Tuolumne River (Verhille et al. 2015a). The lack of a clear peak of thermal performance in aerobic scope documented in juvenile Chinook in the current study is therefore consistent with data that has been previously obtained, particularly for populations of the *Oncorhynchus* genus located in the Central Valley of California. Whereas thermal optima and peak performance in aerobic scope has been documented for some salmonid species, notably sockeye salmon (*O. nerka*; Eliason et al. 2011), the physiological performance of fall-run hatchery salmon may be less constrained over a wide range of environmental temperatures. The variation documented between salmonid species in thermal sensitivity may reflect evolutionary histories, local environmental conditions, or differences in life history strategies. Further comparisons between species and populations are needed to elucidate the mechanisms resulting in differences in thermal sensitivity of aerobic scope.

The inter-individual variation observed in metabolic rate measurements and aerobic scope values in this study and in previous work (Millidine et al. 2009, Metcalfe et al. 2016, Norin and Malte 2012) highlights the importance of quantifying physiological performance in response to environmental variables. The high degree of variation and the range of variation observed for thermal performance between individuals are important to investigate in future studies. The coefficient of variation for all thermal performance metrics ranged from as low as 0.54 % for RMR to as high as 27.3% for FAS. This variation could be due to relaxed selective pressure associated with hatchery populations, which would allow for the survival of individuals with potentially low fitness and low physiological performance capabilities. A comparison of thermal performance with wild populations would be necessary to determine if the observed variation is due to hatchery practices, and warrants further investigation. Conversely, the observed variation could be an adaptive trait of this population of Chinook salmon, and could indicate adaptive potential. Variation in physiological performance allows for the increased probability of population persistence in the face of environmental variability. If environmental conditions shift or rapidly change, variation in individual physiological capacity indicates that some proportion of the population could survive and reproduce in the new environment. Thus, the variation observed in the present study may indicate that fall-run Chinook salmon have the

ability to persist as climate change occurs, and that management actions may be effective even as temperatures continue to rise. Similarly, inter-individual variation within a population allows for specific management targets to be set – a specific percentage of the population could be targeted for protection by management actions if deemed appropriate. Therefore, quantifying not only physiological performance but also the *variation* in that performance is critical for future effective management of salmonids.

Quantifying physiological performance in response to environmental variables is crucial for implementing effective conservation and management actions, and elucidates the mechanisms that link the environment to larger scale processes, such as changes in population levels (Horodysky et al. 2015). Our results can be used to better manage the early life history stages of Chinook salmon, and to further our knowledge of how changing environmental conditions will affect native fish populations. Future work should continue to assess thermal physiological performance in different populations of Chinook salmon, including that of wild fish both in the field and the laboratory, to help build a more comprehensive understanding of the physiological mechanisms driving salmonid populations in California.

### **Summary and Future Studies**

Overall, the aerobic scope of juvenile hatchery Chinook salmon was relatively temperature insensitive, and fish in this laboratory setting were able to maintain physiological performance from 12 to 25°C, when mortality rates then abruptly increased. It is possible that fish tested in our laboratory study may perform differently than those in the wild, due to the increased metabolic costs of predator avoidance, foraging, and finding optimal habitat. Importantly, however, aerobic scope data can be paired with results from other studies investigating critically important temperature-mediated functions, such as heart rate, growth or swimming performance data, to maximize our ability to understand how temperature affects juvenile salmonids. Taken together, these results will contribute to the expanding body of knowledge assessing the effects of temperature on physiological performance in salmonids. Future studies should include assessments of aerobic scope from different populations of Chinook salmon to determine if performance is affected by local temporal or spatial adaptation, and to determine if the inter-individual variation observed in this study is conserved in wild populations of fish, or due to the hatchery origin of the fish tested here. Future studies could also increase the number of individuals tested at elevated temperatures to further explore the upper limits of performance, and should explore the ecological relevance of a FAS value of 2 specifically for Chinook salmon.

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## Supplementary Tables

| Acclimation<br>Temp | Swimming<br>Temp | n | Mean RMR (mg O <sub>2</sub> /kg <sup>0.95</sup> /min) | SE   |
|---------------------|------------------|---|---|------|
| 10                  | 12               | 4 | 1.90  | 0.12 |
| 10                  | 18               | 4 | 3.02  | 0.72 |
| 10                  | 24               | 4 | 4.40  | 0.25 |
| 14                  | 12               | 4 | 2.012   | 0.36 |
| 14                  | 13               | 4 | 2.19  | 0.17 |
| 14                  | 14               | 4 | 2.42  | 0.42 |
| 14                  | 15               | 5 | 2.12  | 0.18 |
| 14                  | 16               | 4 | 2.42  | 0.09 |
| 14                  | 17               | 4 | 2.66  | 0.19 |
| 14                  | 18               | 4 | 2.37  | 0.08 |
| 14                  | 19               | 4 | 3.10  | 0.28 |
| 14                  | 20               | 4 | 3.03  | 0.13 |
| 14                  | 21               | 4 | 3.13  | 0.31 |
| 14                  | 22               | 4 | 3.65  | 0.26 |
| 14                  | 23               | 4 | 4.18  | 0.30 |
| 14                  | 24               | 4 | 4.67  | 0.65 |
| 14                  | 25               | 3 | 4.28  | 0.88 |
| 20                  | 12               | 4 | 1.01  | 0.11 |
| 20                  | 13               | 4 | 1.60  | 0.22 |
| 20                  | 14               | 4 | 1.49  | 0.19 |
| 20                  | 15               | 4 | 2.15  | 0.14 |
| 20                  | 16               | 4 | 2.12  | 0.05 |
| 20                  | 17               | 4 | 2.39  | 0.28 |
| 20                  | 18               | 4 | 2.40  | 0.24 |
| 20                  | 19               | 4 | 2.81  | 0.07 |
| 20                  | 20               | 4 | 2.53  | 0.16 |
| 20                  | 21               | 4 | 2.80  | 0.19 |
| 20                  | 22               | 4 | 3.08  | 0.23 |
| 20                  | 23               | 3 | 3.87  | 0.28 |
| 20                  | 24               | 4 | 4.27  | 0.45 |
| 20                  | 25               | 4 | 4.44  | 0.38 |
| 20                  | 26               | 2 | 4.47  | 0.02 |

**Supplementary Table 1. Resting metabolic rates (RMR) for hatchery juvenile Chinook salmon. Fish acclimated to 10°C were tested at 12, 18 and 24°C, while fish acclimated to 14 and 20°C were tested at swimming temperatures between 12 and 26°C.**

| Acclimation Temp | Swimming Temp | n | Mean MMR (mg O <sub>2</sub> /kg <sup>0.95</sup> /min) | SE    |
|------------------|---------------|---|---|-------|
| 10               | 12            | 4 | 12.80   | 0.98  |
| 10               | 18            | 4 | 18.54   | 4.50  |
| 10               | 24            | 4 | 24.35   | 1.88  |
| 14               | 12            | 4 | 12.12   | 1.14  |
| 14               | 13            | 4 | 14.65   | 0.60  |
| 14               | 14            | 4 | 16.58   | 3.76  |
| 14               | 15            | 5 | 15.29   | 2.64  |
| 14               | 16            | 4 | 18.06   | 1.861 |
| 14               | 17            | 4 | 20.60   | 3.15  |
| 14               | 18            | 4 | 16.05   | 0.72  |
| 14               | 19            | 4 | 14.94   | 0.76  |
| 14               | 20            | 4 | 19.46   | 3.25  |
| 14               | 21            | 4 | 24.71   | 2.47  |
| 14               | 22            | 4 | 19.86   | 1.68  |
| 14               | 23            | 4 | 19.76   | 1.42  |
| 14               | 24            | 4 | 24.20   | 2.05  |
| 14               | 25            | 3 | 22.60   | 3.11  |
| 20               | 12            | 4 | 13.65   | 0.85  |
| 20               | 13            | 4 | 12.83   | 0.32  |
| 20               | 14            | 4 | 13.09   | 2.61  |
| 20               | 15            | 4 | 14.52   | 1.69  |
| 20               | 16            | 4 | 12.80   | 1.00  |
| 20               | 17            | 4 | 14.56   | 1.453 |
| 20               | 18            | 4 | 15.024  | 1.60  |
| 20               | 19            | 4 | 21.11   | 2.50  |
| 20               | 20            | 4 | 18.15   | 1.56  |
| 20               | 21            | 4 | 14.86   | 1.58  |
| 20               | 22            | 4 | 19.59   | 3.703 |
| 20               | 23            | 3 | 21.94   | 2.74  |
| 20               | 24            | 4 | 22.06   | 4.10  |
| 20               | 25            | 4 | 24.14   | 0.10  |
| 20               | 26            | 2 | 20.56   | 1.28  |

**Supplementary Table 2. Maximum metabolic rates (MMR) for hatchery juvenile Chinook salmon. Fish acclimated to 10°C were tested at 12, 18 and 24°C, while fish acclimated to 14 and 20°C were tested at swimming temperatures between 12 and 26°C.**

| Acclimation Temp | Swimming Temp | n | Mean AS ( mg O <sub>2</sub> /kg <sup>0.95</sup> /min ) | SE   |
|------------------|---------------|---|--|------|
| 10               | 12            | 4 | 10.90  | 1.05 |
| 10               | 18            | 4 | 15.53  | 4.04 |
| 10               | 24            | 4 | 19.95  | 1.81 |
| 14               | 12            | 4 | 10.11  | 1.31 |
| 14               | 13            | 4 | 12.46  | 0.62 |
| 14               | 14            | 4 | 14.16  | 3.47 |
| 14               | 15            | 5 | 13.16  | 2.66 |
| 14               | 16            | 4 | 15.64  | 1.80 |
| 14               | 17            | 4 | 17.93  | 2.97 |
| 14               | 18            | 4 | 13.69  | 0.77 |
| 14               | 19            | 4 | 11.84  | 0.68 |
| 14               | 20            | 4 | 16.42  | 3.23 |
| 14               | 21            | 4 | 21.59  | 2.19 |
| 14               | 22            | 4 | 16.21  | 1.43 |
| 14               | 23            | 4 | 15.57  | 1.22 |
| 14               | 24            | 4 | 19.53  | 1.92 |
| 14               | 25            | 3 | 18.31  | 2.30 |
| 20               | 12            | 4 | 12.64  | 0.92 |
| 20               | 13            | 4 | 11.23  | 0.17 |
| 20               | 14            | 4 | 11.60  | 2.63 |
| 20               | 15            | 4 | 12.37  | 1.60 |
| 20               | 16            | 4 | 10.86  | 1.03 |
| 20               | 17            | 4 | 12.17  | 1.18 |
| 20               | 18            | 4 | 12.62  | 1.43 |
| 20               | 19            | 4 | 18.30  | 2.49 |
| 20               | 20            | 4 | 15.62  | 1.51 |
| 20               | 21            | 4 | 12.06  | 1.56 |
| 20               | 22            | 4 | 16.51  | 3.57 |
| 20               | 23            | 3 | 18.08  | 2.54 |
| 20               | 24            | 4 | 17.79  | 4.13 |
| 20               | 25            | 4 | 19.70  | 0.39 |
| 20               | 26            | 2 | 16.09  | 1.30 |

**Supplementary Table 3. Aerobic scopes (AS) for hatchery juvenile Chinook salmon. Fish acclimated to 10°C were tested at 12, 18 and 24°C, while fish acclimated to 14 and 20°C were tested at swimming temperatures between 12 and 26°C.**

| Acclimation Temp | Swimming Temp | n | Mean FAS | SE   |
|------------------|---------------|---|----------|------|
| 10               | 12            | 4 | 6.89     | 0.91 |
| 10               | 18            | 4 | 6.34     | 1.46 |
| 10               | 24            | 4 | 5.56     | 0.45 |
| 14               | 12            | 4 | 6.88     | 1.88 |
| 14               | 13            | 4 | 6.82     | 0.62 |
| 14               | 14            | 4 | 6.70     | 0.95 |
| 14               | 15            | 5 | 7.40     | 1.44 |
| 14               | 16            | 4 | 7.45     | 0.64 |
| 14               | 17            | 4 | 7.58     | 0.73 |
| 14               | 18            | 4 | 6.83     | 0.48 |
| 14               | 19            | 4 | 4.90     | 0.36 |
| 14               | 20            | 4 | 6.43     | 1.00 |
| 14               | 21            | 4 | 7.92     | 0.32 |
| 14               | 22            | 4 | 5.43     | 0.12 |
| 14               | 23            | 4 | 4.74     | 0.23 |
| 14               | 24            | 4 | 5.48     | 0.77 |
| 14               | 25            | 3 | 5.43     | 0.50 |
| 20               | 12            | 4 | 14.12    | 2.05 |
| 20               | 13            | 4 | 8.43     | 0.97 |
| 20               | 14            | 4 | 9.23     | 1.92 |
| 20               | 15            | 4 | 6.74     | 0.63 |
| 20               | 16            | 4 | 6.16     | 0.59 |
| 20               | 17            | 4 | 6.15     | 0.25 |
| 20               | 18            | 4 | 6.27     | 0.48 |
| 20               | 19            | 4 | 7.50     | 0.87 |
| 20               | 20            | 4 | 7.21     | 0.64 |
| 20               | 21            | 4 | 5.37     | 0.61 |
| 20               | 22            | 4 | 6.31     | 0.92 |
| 20               | 23            | 3 | 5.67     | 0.51 |
| 20               | 24            | 4 | 5.38     | 1.31 |
| 20               | 25            | 4 | 5.54     | 0.42 |
| 20               | 26            | 2 | 4.61     | 0.31 |

**Supplementary Table 4. Factorial aerobic scopes (FAS) for hatchery juvenile Chinook salmon. Fish acclimated to 10°C were tested at 12, 18 and 24°C, while fish acclimated to 14 and 20°C were tested at swimming temperatures between 12 and 26°C.**

| Acclimation<br>Temp | Swimming<br>Temp | n<br>Mortality | n Tested | n Bursted |
|---------------------|------------------|----------------|----------|-----------|
| 10                  | 12               | 0              | 4        | 0         |
| 10                  | 18               | 0              | 4        | 0         |
| 10                  | 24               | 0              | 4        | 0         |
| 14                  | 12               | 0              | 4        | 0         |
| 14                  | 13               | 0              | 4        | 2         |
| 14                  | 14               | 0              | 4        | 0         |
| 14                  | 15               | 0              | 5        | 0         |
| 14                  | 16               | 0              | 4        | 0         |
| 14                  | 17               | 0              | 4        | 1         |
| 14                  | 18               | 0              | 4        | 1         |
| 14                  | 19               | 0              | 4        | 0         |
| 14                  | 20               | 0              | 4        | 0         |
| 14                  | 21               | 0              | 4        | 0         |
| 14                  | 22               | 0              | 4        | 0         |
| 14                  | 23               | 0              | 4        | 0         |
| 14                  | 24               | 0              | 4        | 0         |
| 14                  | 25               | 1              | 4        | 1         |
| 14                  | 26               | 3 (1 LOE)      | 4        | 0         |
| 20                  | 12               | 0              | 4        | 0         |
| 20                  | 13               | 0              | 4        | 0         |
| 20                  | 14               | 0              | 4        | 0         |
| 20                  | 15               | 0              | 4        | 0         |
| 20                  | 16               | 0              | 4        | 1         |
| 20                  | 17               | 0              | 4        | 1         |
| 20                  | 18               | 1              | 5        | 1         |
| 20                  | 19               | 0              | 4        | 2         |
| 20                  | 20               | 0              | 4        | 0         |
| 20                  | 21               | 0              | 4        | 0         |
| 20                  | 22               | 0              | 4        | 0         |
| 20                  | 23               | 0              | 3        | 1         |
| 20                  | 24               | 0              | 4        | 1         |
| 20                  | 25               | 0              | 4        | 0         |
| 20                  | 26               | 2              | 4        | 0         |

**Supplementary Table 5. Summary of all experiments performed. Mortality refers to number of fish (n) that died during any point in the experiment, and no values from experiments that resulted in mortality or loss of equilibrium (LOE) were used to calculate metabolic rates. The number of fish that required a bursting protocol to obtain maximum metabolic rates are also shown.**