Bioenergetics of early life history anadromous fishes in the Sacramento-San Joaquin River Basin

By

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ABSTRACT

Bioenergetics is the study of how energy flows through and transforms within organisms. Variables such as temperature and food availability have variable effects on growth and metabolism. There is an increasing interest in determining fish bioenergetics in conjunction with behavior and life-history traits to predict species-specific responses to climate change.

The Sacramento-San Joaquin River Basin, California, USA, supports multiple species of anadromous fishes – Chinook salmon, green sturgeon, and white sturgeon – which return to these freshwater natal habitats to spawn. Many of the physical and ecological characteristics of this watershed have been impacted by physical modifications and climate change. Specifically, dams and channelization have led to changes in water temperature, flow, and altered food web dynamics. Early life stages of fish are particularly vulnerable, and declining recruitment is thought to contribute to precipitous population decline.

Each chapter addresses an aspect of fish bioenergetics, using metabolism as a foundation due to its representation of the sum of biochemical processes used for energy intake and support for life functions. Chapter 2 examined the metabolic cost of digestion in juvenile Chinook salmon across a broad range of temperatures, finding that the digestive response largely retains its energy efficiency at all temperatures. Chapter 3 determined the scaling of metabolic rate and differences in developmental trajectories across early ontogeny of green sturgeon, finding that rearing in suboptimal cold temperature is metabolically costly and leads to large size-at-age delays. Chapter 4 investigated the relationship between metabolic rate and locomotory activity in juvenile white sturgeon under nutritional stress, finding that the relationship is context dependent, requiring sufficient exposure to nutritional stress.

Collectively, my research investigated stage- and species-specific aspects of larval and juvenile fish bioenergetics that can help us understand how various environmental stressors may impact fish populations. The intersection of bioenergetics, behavior, and organismal performance is critical for continued insight on species- and ecosystem-wide responses to perturbations, because it illuminates the diversity of physiological and behavioral responses available to fish. By investigating these responses with respect to current and expected climate-induced conditions, we can better craft management strategies to conserve these commercially and culturally important native California fishes.

CHAPTER 1

Background

Diadromous fish – which migrate between freshwater and marine habitats – are in decline across North America (Waldman and Quinn, 2022). In California, anadromous fish such as salmon and sturgeon, which spawn in freshwater, are impacted by decades of extensive engineering projects, which have altered flow and temperature regimes, degraded habitats, and eliminated access to historical spawning areas (Yoshiyama *et al.*, 1998; Moyle *et al.*, 2013). These changes to coastal and inland freshwater habitats impact early life history stages, affecting recruitment – the process by which young fish eventually join and contribute to the adult population (Goto *et al.*, 2015). Early life stages are often more vulnerable to environmental variables such as temperature (Portner and Farrell, 2008), and more susceptible to starvation (Post and Evans, 1989) or predation (French *et al.*, 2010; Baird *et al.*, 2020). Understanding the mechanisms underlying the impacts of anthropogenic change in aquatic ecosystems on early life history stages is critical to improving population numbers and understanding which environmental factors might be leading to population declines.

The Sacramento San Joaquin River Basin (SSJRB) in California, USA, simultaneously supports habitat for multiple Endangered Species Act (ESA) listed anadromous fish species (Lindley *et al.*, 2006; Heublein, 2017) and water supply for 27 million people and 750,000 acres of farmland (Pathak *et al.*, 2018). As a result, water is meticulously stored, managed, and released from dams for multi-use benefit (Escriva-Bou *et al.*, 2016). The ESA protects imperiled plants and animals, prohibiting the take of listed species and any destruction of habitat that affects the species (National Research Council, 1995). The National Marine Fisheries Service (NMFS) and the US Fish and Wildlife Service initiate federal reviews of listed species to

determine critical habitat, population impacts, and whether actions need to be undertaken to prevent their extinction. As climate change will cause California's water supply to be less predictable, mismatches between water supply and water demand are likely to cause increased conflict in the future. Historically, insufficient water allocated for habitat maintenance of ESA-listed fish species has resulted in the curtailing or reapportioning of water rights from other users, because federal courts have affirmed that habitat modification may constitute a taking under the ESA (Estes, 1992). When such an event occurs, NMFS strives to settle on a compromise such as limiting the curtailment to stage-specific timing of the protected species' life cycle. In order to efficiently allocate a limited water supply, detailed knowledge of how current and future environmental conditions affect protected species is important (Tanaka *et al.*, 2006). More specifically, stage- and species-specific needs of early ontogeny anadromous fishes, which spawn and rear in these impacted freshwater river basins like the SSJRB, are critical to understand and least well-studied.

Temperature is a key abiotic factor affecting many physiological, ecological, and behavioral aspects of ectothermic organisms (Angilletta *et al.*, 2002). Furthermore, environmental temperature has cascading effects on aquatic ecosystems and their organisms through changes in growth/developmental rates and altered food web dynamics through temperature's connection with flow and hydrodynamics in riverine systems. Individual organisms can respond to these changes via physiological acclimatization (Crozier and Hutchings, 2014), behavioral thermoregulation (Brewitt *et al.*, 2017), or divergence in behavioral tendencies (Killen *et al.*, 2013). Physiological acclimatization is the ability of fish to undergo physiological changes at multiple levels of organization, such as upregulating or downregulating the expression of different genes or synthesizing different protein isoforms, which may operate

more efficiently under the new set of conditions (Moyes and Schulte, 2008). Another way to cope with environmental temperature can simply be to move to a different habitat. Behavioral thermoregulation may be used when an individual chooses to move, weighing the costs and benefits (e.g. prey availability, thermal refugia, predation risk) of different habitats in a heterogeneous ecosystem such as a river basin with many tributaries, side channels, and seasonal wetlands (Brewitt *et al.*, 2017; Cordoleani *et al.*, 2022). Such behavioral shifts are thought to have evolved in synchrony with physiological traits to increase fitness, although behavioral shifts are not uniform in response within a species. Variability in behavioral shifts may be the result of differential sensitivity to stressors among phenotypes. Altogether, the diversity of physiological and behavioral responses available to fishes in response to a stressor are thus important to study in conjunction.

My dissertation investigates relationships between physiology, bioenergetics, and behavior in three species of anadromous fishes in the SSJRB: Chinook salmon, green sturgeon, and white sturgeon. Chinook salmon are a commercially and culturally important fish species in California, with multiple distinct runs that exhibit different life history timing. Several of these runs are listed as threatened or endangered. Chinook salmon typically live 2-4 years, with freshwater spawning and egg incubation occurring over 3-4 months, followed by an additional 3-5 months of juvenile rearing before outmigrating to the ocean where the brunt of their adult growth and development occurs. Because of the variation in run life histories, Chinook salmon inhabit the SSJRB nearly year-round. In contrast, Green and white sturgeon are both much longer-lived, requiring 10-16 years to reach sexual maturity and attaining maximum ages of 70-100 years, although fish older than 20 have been exceedingly rare to catch in the SSJRB. Sturgeons are among the most ancient of ray finned fishes, retaining many ancestral features not

found in modern bony fishes (teleosts). Green sturgeon are separated into Distinct Population Segments (DPS), with the federally listed southern DPS spawning solely in the SSJRB, and the northern DPS spawning in two watersheds in northern California and Oregon. Green sturgeon typically spend up to a year in freshwater before migrating downstream estuarine areas until >30 cm total length when they can tolerate seawater. White sturgeon are a California state Species of Special Concern due to concerns that their populations are declining more quickly than expected, although there is currently still an open recreational fishery. They have a similar freshwater rearing period to green sturgeon, migrating down to estuarine areas within the first year. However, white sturgeon are less marine-oriented than green sturgeon, preferring to reside as adults in the freshwater estuary with occasional forays into coastal waters.

By virtue of their run/population life history diversity and anadromous lifestyle, Chinook salmon, green sturgeon, and white sturgeon all lead complex lives for which there are still many knowledge gaps. In particular, the early life stages of both sturgeon species in the SSJRB are not well understood with regards to habitat use, population size, and recruitment. Within the green sturgeon federal Species Recovery Plan, stage-specific and habitat-specific threats are ranked along with data sufficiency (National Marine Fisheries Service, 2018). Almost all threats for eggs and larvae/juveniles in the Sacramento River Basin have low data sufficiency. My research fills some of these gaps using whole body metrics such as metabolic rate, length-weight relationships, and behavior to investigate how current and future water temperatures and food availability may impact these species.

Chapter 2 explores the energetics of digestion in juvenile Chinook salmon across a range of ecologically relevant temperatures (13-24°C). I assessed the proportion of a fish's energy budget utilized for digestion by measuring the increased uptake of oxygen, putting it in context

to their total aerobic capacity. I tested the hypothesis that temperature affects the rate and energy use of digestion, with potential ramifications for energy budgeting decisions. I found that digestion in juvenile Chinook salmon is resilient to changes in temperature, suggesting that maintaining homeostasis in digestive capability is an adaptation to the highly heterogeneous environment in which they spend their early life.

Chapter 3 explores the carryover effects of low incubation and rearing temperatures on early ontogeny of green sturgeon. Using allometric scaling relationships in metabolic rate and differences in length-weight relationships, I assessed how energy use changes through early ontogeny. I hypothesized that suboptimal low temperature (11°C) for embryo incubation and larval rearing is detrimental to early life history green sturgeon, reducing growth rates and increasing metabolic rate. I found support for this prediction, with cold rearing temperature leading to large size-at-age differences and increased oxygen demand. However, cold embryo incubation temperature had no lasting effects, and length-weight relationships were similar, indicating similar developmental trajectories, despite size-at-age differences.

Chapter 4 explores the effect of nutritional stress on the relationship between metabolic rate and locomotor activity in juvenile white sturgeon. Using metabolic rate and behavioral assays, I assessed how increased nutritional stress over six weeks revealed context-dependent effects between metabolic rate and locomotion. I hypothesized that individual differences in metabolic rate correlates to individual differences in behavior, and that nutritional stress can alter these relationships. I found partial support for this hypothesis, with metabolic rate and locomotion correlating after application of the greatest duration of nutritional stress.

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CHAPTER 2

The effect of temperature on specific dynamic action of juvenile Fall-run Chinook salmon, *Oncorhynchus tshawytscha*

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Abstract

Juvenile Fall-run Chinook salmon (*Oncorhynchus tshawytscha*) in the Sacramento San Joaquin River Basin (SSJRB) experience temporally and spatially heterogenous temperature regimes, between cool upper tributaries and the warm channelized Delta, during freshwater rearing and outmigration. Limited water resources necessitate human management of dam releases, allowing temperature modifications. The objective of this study was to examine the effect of temperature on specific dynamic action (SDA) – or the metabolic cost associated with feeding and digestion – which is thought to represent a substantial portion of fish energy budgets. Measuring SDA with respect to absolute aerobic scope (AAS), estimated by the difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR), provides a snapshot of its respective energy allocation. Fish were acclimated to 16℃, raised or lowered to each acute temperature (13, 16, 19, 22, or 24℃), then fed a meal of commercial pellets weighing 2% of their wet mass. We detected a significant positive effect of temperature on SMR and MMR, but not on AAS. As expected, there was no significant effect of temperature on the total O_2 cost of digestion, but unlike other studies, we did not see a significant difference in duration, peak metabolic rate standardized to SMR, time to peak, percent of meal energy utilized, nor the ratio of peak O_2 consumption to SMR. Peak O_2 consumption represented 10.4 – 14.5% of AAS leaving a large amount of aerobic capacity available for other activities, and meal energy utilized for digestion ranged from 5.7 – 7.2%, leaving substantial remaining energy to potentially assimilate for growth. Our juvenile Fall-run Chinook salmon exhibited thermal stability in their SDA response, which may play a role in maintaining homeostasis of digestive capability in a highly heterogeneous thermal environment where rapid growth is important for successful competition with conspecifics and for avoiding predation.

Introduction

Chinook salmon (*Oncorhynchus tshawytscha*) are an anadromous fish species native to the North Pacific Ocean that have faced major population reductions under increasing anthropogenic stress (Noakes *et al.*, 2000; Yoshiyama *et al.,* 1998). The Sacramento-San Joaquin River Basin (SSJRB) of California supports some of the most heavily impacted populations of Chinook salmon, where there are four seasonal runs: fall, late-fall, winter, and spring – the latter two of which are endangered and threatened, respectively (Moyle *et al.*, 2017). Within the SSJRB, which encompasses the southernmost range for Chinook salmon, extensive engineering projects have altered flow and temperature regimes, degraded habitats, and eliminated access to historical spawning areas (Yoshiyama *et al.*, 1998). Chinook salmon are predicted to be further impacted by climate change as decreases in reservoir storage will reduce river flows and increase water temperatures (Moyle *et al.* 2017). For ectotherms such as fish, temperature is a critical variable that affects virtually all aspects of an organism's physiology and biochemistry (Hochachka & Somero, 2002; Huey & Kingsolver, 1993). Paradoxically, the fate of Chinook salmon in the SSJRB depends on human management because water temperatures are now artificially regulated by dam releases (Yates *et al.*, 2008).

Temperature-dependent bioenergetic processes such as metabolism ($\dot{M}O_2$) are commonly measured in fishes as a proxy for physiological performance (Fry, 1971). One such metabolic performance metric is absolute aerobic scope (AAS) – the difference between the minimum and maximum metabolic rate (MMR) as measured by oxygen consumption rate (Farrell, 2016). The minimum metabolic rate, termed standard metabolic rate (SMR), represents a fish's basic need for oxygen, while MMR is a fish's capacity to deliver additional oxygen to support activities beyond this basic need (Chabot *et al.*, 2016*b*). Thus, AAS provides an estimate of the surplus

energy available to an organism that can be invested into fitness-related functions (e.g. growth, digestion, locomotion, avoiding predation, reproduction, etc.), providing a snapshot of a fish's energy budget under specific measurement conditions (Clark *et al.*, 2013). Because this energy surplus is finite, fish must make tradeoffs among various functions such as growth, development, and digestion (Sokolova *et al.*, 2012). Understanding how energy balance changes with respect to temperature may be helpful in predicting tolerance limits, population success, and response to future climate impacts for Chinook salmon in California and other fishes (Jutfelt *et al.* 2021; Poletto *et al.*, 2017; Pörtner 2010; Steell, *et al.* 2019; Zillig *et al.*, 2021).

Feeding and digestion are thought to represent a substantial portion of fish energy budgets, as the resulting increase in $\dot{M}O_2$ can last for hours or days (Soofiani & Hawkins, 1982). The metabolic cost of feeding is referred to most commonly in the literature as specific dynamic action (SDA) and is defined as the increase in metabolism associated with "ingestion, digestion, absorption and assimilation of a meal" (Kleiber, 1975; Secor, 2009). A fish's SDA is measured by continuously recording metabolic rate after feeding, providing a complete profile of the postprandial metabolic response (Fig. 1, Table 1). SDA is known to be affected by many factors such as meal size, meal composition, feeding frequency, hypoxia, and body temperature (Eliason *et al.*, 2007; Eliason & Farrell, 2014; Fu *et al.*, 2005; Jobling, 1981, 1983; Steell *et al.*, 2019; Tirsgaard *et al.*, 2015). Body temperature is a primary determinant of the shape and dyamics of the SDA response. Warmer temperatures increase peak $\dot{M}O_2$ during digestion (SDA_{peak}), increase peak $\dot{M}O_2$ standardized to baseline ($Peak_{net}$), shorten the duration of the postprandial response (SDA_{dur}), and reduce the time to peak (t_{peak}), effectively temporally compressing the SDA response (Eliason *et al.* 2011; Jobling, 1981; McCue, 2006; Sandblom *et al.* 2014). In contrast, warming temperatures have negligible effects on the total cost (SDA_{cost}) and ratio of SDA_{cost} to

meal energy content (SDA_{coef}) of the postprandial response (McCue, 2006). Importantly, the temporal compression of SDA with increasing temperatures is expected to reduce the proportion of AAS remaining during SDA_{peak} – termed postprandial residual aerobic scope (PRAS) – and may have major implications for fitness as tradeoffs must be made between using AAS for SDA or locomotion, growth, and other processes (Jutfelt *et al.*, 2021; LeGrow and Beamish, 1986; Sandblom *et al.* 2014).

While studies investigating the effect of temperature on SDA in fishes are not uncommon, there are only a handful investigating the effect in the *Oncorhynchus* genus (Eliason *et al.*, 2007, 2008; Eliason & Farrell, 2014; Thorarensen & Farrell, 2006), and none in juvenile Chinook salmon. Additionally, assessing the effect of temperature on SDA, AAS, and PRAS together has been described for very few species (Sandblom *et al.*, 2014; Pang *et al.* 2010, 2011). In particular, the effect of temperature on SDA is important to understand for juvenile Chinook in the SSJRB due to the diversity of runs and life histories in this watershed. Despite increasingly severe and frequent drought conditions that will raise water temperatures and strain reservoir capacities, a rigid temperature criterion of 13.3℃ (seven-day average of daily maximums, 7-DADM) for endangered winter-run Chinook embryo rearing during summer forces a wider temperature range than is natural for the dam-truncated watershed (USFWS, 1999; Zillig *et al.*, 2021). The lower Sacramento River mainstem regularly experiences temperatures exceeding 20℃ by late spring, and although juvenile Chinook can tolerate short-term exposures to sublethal temperatures (25℃+), the duration of exposure is expected to increase in the future (Myrick and Cech, 2004). As increasing water temperatures are expected to reduce PRAS, understanding how temperature affects SDA, AAS, and PRAS is necessary to contextualize the portion of AAS dedicated to feeding and digestion, and to define the role of feeding in juvenile

Chinook energy budgets (Norin & Clark, 2017). The aim of this study was to measure the effects of temperature on SDA variables and AAS in juvenile Fall-run Chinook salmon across a range of ecologically relevant temperatures experienced in the SSJRB.

Materials and methods

Experimental animals

Juvenile Fall-run Chinook salmon were transported from Coleman National Fish Hatchery (Anderson, CA, USA) via an aerated transport tank that maintained oxygen levels of >90% of air saturation. All fish were from the same cohort, experienced the same rearing conditions, and the number of families was unknown. Fish (n = 200) were transferred to the Center for Aquatic Biology and Aquaculture (University of California, Davis, CA, USA) on June 25, 2017, and were reared in two (590-L) tanks with air-equilibrated well-water flow-through (3 L min^{-1}). Well-water salinity was <0.5 practical salinity unit and temperature was kept at 16°C under natural photoperiod conditions for Davis, CA (38.5 N, 121.7 W) for at least three weeks prior to experimentation. Fish were fed 3-mm commercial pellet feed (50% protein, 12% oil, 9% moisture, 3% fiber, 12% ash, 14.6 kJ/g digestible energy, Skretting, Toole, Utah, USA) ad libitum ration over a 12 h period daily. Experimental fish were size selected from the source tank because SDA responses can only be directly compared between conspecifics of a certain age/size that are consuming identical meals (McCue 2006). Mean body mass (p = 0.070) and total length (p = 0.098) did not differ significantly among the five temperature treatments (Table 2). All experimental protocols and fish care methods were approved by the UC Davis Institutional Animal Care and Use Committee, protocol #18196.

Respirometry

$\dot{M}O_2$ (mg O_2 min^{-1} kg^{-1}) of individual fish was measured using intermittent flow respirometry using a 7-chamber system fabricated at UC Davis. Each 1.5-L acrylic respirometry chamber was mounted in a 284-L aerated and UV-sterilized water bath surrounded by black curtains to minimize disturbance. The intermittent flow cycle was set such that each flush period was 5 min, the wait period was 1 min, and the recirculating closed period was 7-10 min depending on temperature, during which the oxygen content of the water was recorded every second using a fiber-optic oxygen dipping probe (Loligo Systems, Viborg, Denmark) inserted into the respirometer through a water-tight rubber stopper. Oxygen levels within the respirometry chamber were not allowed to decline to <80% saturation at the end of each measurement period to ensure the fish did not become hypoxic and stressed (Svendsen *et al.* 2016). Each respirometer had a DC recirculation pump to maintain water mixing during the measurement period and to minimize flow disturbances to the fish. Flush and recirculation periods were controlled using Autoresp™ software (Loligo Systems, Viborg, Denmark). $\dot{M}O_2$ values were calculated from the linearly declining O_2 content of the water inside the respirometer during each closed period and limited to slopes with a $R^2 > 0.96$ (Svendsen *et al.* 2016). Prior to each experiment, oxygen probes were calibrated with oxygen-free distilled water and fully aerated distilled water. Oxygen-free distilled water was created by adding 1g sodium sulfite (Na_2SO_3; Spectrum Chemical Manufacturing Corp., CA, USA) to 100 ml of distilled water, while fully aerated distilled water was created by bubbling ambient air into 100 ml of water for 20 min. Both calibration measurements were conducted inside the experimental water bath to reduce temperature fluctuations.

Following a three-week acclimation to 16°C, individual fish were tested at one of five acute temperatures: 13, 16, 19, 22, or 24°C. Due to natural diel fluctuations in the facility's well

water source, water temperatures had a fluctuation of up to ± 1.0°C. The experimental protocol was identical for each acute temperature. In total, 9-12 fish per acute temperature were included (Table 2). Fish were fasted for 24 h in individual holding tanks before being placed randomly in a respirometry chamber at the acclimation temperature of 16°C. Figure 1 presents a representative trace of $\dot{M}O_2$ data over the course of an SDA experiment for an individual fish.

After a 1-h adjustment period to the respirometer, temperature in the water bath was either held at 16°C or changed at 2°C/h to 13, 19, 22 or 24°C. Upon reaching the acute temperature, $\dot{M}O_2$ measurements began, to provide data for SMR estimates. Because attempts to coerce the fish to feed voluntarily in the respirometer were unsuccessful, a force-feeding protocol was used to administer the meal (personal communication from Dr. Erika J. Eliason, University of California, Santa Barbara). The next morning (-24 h in Fig. 1), each fish underwent a sham-feeding procedure (completely identical to force-feeding but without food ingestion) to habituate the fish to the process of force-feeding and to assess the handling effect on $\dot{M}O_2$ (Eliason *et al.* 2007, 2008). After an additional 24 h (0 h in Fig. 1), the fish was again removed, and force fed a meal using 3 mm pellets consistent in caloric content, composition, and digestible energy content (McCue, 2006). Target meal sizes were 2% of wet body mass because pilot experiments showed that larger rations often resulted in partial or total regurgitation. Additionally, mean meal sizes of 2.18% and 1.16% were measured for wild and hatchery juvenile Chinook salmon, respectively, from the Nisqually River delta, Puget Sound, Washington, justifying our target meal size (Davies *et al.*, 2018). Ultimately, mean meal sizes were 1.81% ± 0.03 for all fish and did not differ significantly among temperature treatments ($p = 0.15$). The force-feeding protocol consisted of lightly anesthetizing fish with a buffered solution of tricaine methanesulfonate (0.03 g/L; MS-222; Syndel, Ferndale, Washington, USA) until loss of equilibrium, followed by measurement of

wet mass and manual administration of a meal with rubber-tipped forceps (Eliason *et al.* 2008). Fish were then returned into their respirometers and postprandial $\dot{M}O_2$ was measured for 72 h (0 – 72 h in Fig. 1). Any pellets regurgitated were siphoned out, counted, and multiplied by the known mean mass of a dry pellet, to eliminate bias introduced by hydrated pellets (Eliason *et al.* 2007). One quarter of fish regurgitated pellets within the respirometer, and was typically limited to one or two pellets, equivalent to 0.02 – 0.04 g, or 4 – 8% of the intended meal size. Regurgitation did not trend with temperature. At the end of the 72-h period, fish were removed and manually chased to exhaustion with a hand net until they no longer responded to contact of the net with their caudal fin (usually between 3-6 min), then returned to respirometry chambers immediately for a MMR measurement (Cutts *et al.* 2002, Svendsen *et al.* 2012). At the end of the experiment, fish were euthanized in a lethal buffered tricaine methanesulfonate (0.5 g/L) solution, then measured to the nearest 0.01g and 1.0 mm.

Background microbial $\dot{M}O_2$ in each respirometer chamber was measured at three time points in each experiment: during the sham feeding procedure, during the feeding procedure, and post-experiment. $\dot{M}O_2$ values for individual fish were corrected by grouping background $\dot{M}O_2$ values by acute temperature, fitting an exponential model to each dataset, then subtracting the predicted values from each fish's $\dot{M}O_2$ trace (Svendsen *et al.* 2016).

Data and statistical analysis

$\dot{M}O_2$ was recorded using Autoresp^{TM} software (Loligo Systems, Viborg, Denmark) and data analyses performed using R studio (version 3.6.1; http://R-project.org/). $\dot{M}O_2$ values included in analysis were required to have an $R^2 > 0.96$, resulting in an average loss of 7.5% of total $\dot{M}O_2$ values collected. For SMR estimates, $\dot{M}O_2$ values were filtered to remove the hours representing handling stress as indicated by the sham feeding protocol, and the 48 h after feeding

(time zero, Fig. 1) to remove the elevated values of the SDA response period. SMR and all variables of SDA were calculated using the *fishMO2* package and R script provided by Chabot *et al.* (2016*a*). This script also included the R package *quantreg* (Koenker, 2011) which fit nonparametric quantile regressions to the data to estimate SMR and SDA, where values of tau (τ), the penalty parameter (λ), and the tolerance value were set at 0.2, 12, and 5%, respectively, based on recommendations given by Chabot *et al.* (2016a). For estimating the SDA curve, a non-parametric quantile approach was used, which allows some percentage of the observations, set by τ, to fall below the estimated line (Chabot *et al.*, 2016a, 2016b). Chabot *et al.* (2016b) recommend choosing the value of τ based on the optimal method used to estimate SMR. In this study, the recommended method for calculating SMR was non-parametric quantile regression in 43 fish and mean of the lowest normal distribution in 11 fish. This justified using the same value of 0.2 for both non-parametric quantile regression calculations of SMR and τ for SDA calculations. Setting $\tau = 0.2$ allowed 20% of the $\dot{M}O_2$ values to fall below the estimated SMR and SDA lines. λ was set to 12, as it is recommended to be larger than the duration of an activity cycle, which for most fish is one per day lasting half a day or less (Chabot *et al.*, 2016a). The tolerance value of 5% terminated the SDA curve when the quantile fit reached SMR + 5% (Fig. 1; Chabot *et al.*, 2016*a*). The effect of sham feeding on $\dot{M}O_2$ was assessed by inspecting data for each individual fish. Because sham feeding typically elevated $\dot{M}O_2$ and subsided after 4 h, a 4 h period was removed before fitting the model, and SDA was assumed to follow a straight line joining the origin of the SDA response (time zero, Fig. 1) to the first value predicted by the fitted line, as recommended by Chabot *et al.* (2016*a*). SDA variables were calculated from these fitted curves to describe the post-feeding $\dot{M}O_2$ metrics (Table 1) following Secor (2009). SDA_{cost} was converted to kJ from the area bounded by SDA and SMR (polygon in Fig. 1) by assuming that 1

g of oxygen is associated with the release of 13.6 kJ of energy (Cho *et al.*, 1982). In contrast, MMR was limited to one value per fish due to having one opportunity to elicit MMR.

All metabolic and SDA variables were grouped according to temperature treatment and examined for differences using a one-way ANOVA, with differences between groups tested using Tukey's honest significant difference when relevant. Results were considered significant at $p < 0.05$. All values are reported as mean ± SE unless otherwise noted. Data analyses were completed using R (R Core Team, 2019). Correlations between water temperature and SMR, MMR, and AAS were fitted with polynomial regression lines using lowest AIC model selection (Fig. 2a). An exception was MMR, where a linear regression model fit best. However, we have chosen to also report a polynomial regression line for MMR because it is more ecologically logical, and the difference in AIC between the linear and polynomial regression models was less than .5 AIC units.

Results

Typically, mean $\dot{M}O_2$ showed an elevated and somewhat variable pattern following the sham and actual feedings (Fig. 1). The mean postprandial increase was of much greater mean duration than that following the sham feeding and peaked (t_{peak}) at 19.3, 13.2, 15.4, 15.0, and 14.0 h at 13, 16, 19, 22, and 24℃, respectively, though it was not significantly different among temperature treatments ($p = 0.123$, Table 2). The duration of the SDA response (SDA_{dur}) trended negatively with temperature from 43.4 to 36.6 h, but was not significant among temperature treatments ($p = 0.602$). As expected, SDA_{peak} increased with temperature ($p < 0.001$) except between 16 and 19℃ ($p = 0.908$). However, when SMR was subtracted from peak values ($Peak_{net}$), there were no significant differences among treatments ($p = 0.183$). The ratio of peak to SMR (SDA_{scope}) decreased with temperature from 1.36 to 1.24 and was significantly different

(p = 0.0411) between 16 and 24℃ (p = 0.046). The mean energetic cost of the SDA response (SDA_{cost}) as well as the mean percentage of the ingested meal energy consumed by SDA_{cost} (SDA_{coef}) were not significantly different (p = 0.666 and p = 0.743, respectively) (Table 2).

Mean SMR increased significantly ($p < 0.001$) with increasing temperature at each of the five tested temperatures except between 16 and 19℃ (p = 0.504), and was fitted to the equation SMR (mg O_2 min^{-1} kg^{-1}) = $4.791 – 0.443x + 0.018x^2$ where x is temperature in ℃ (Fig. 2a, Table 2). Mean MMR was significant (p = 0.015) between 13 and 22℃ (p = 0.035), and 13 and 24℃ (p = 0.016), and was fitted to the equation MMR (mg O_2 min^{-1} kg^{-1}) = $4.550 + 0.479x – 0.008x^2$ where x is temperature in ℃ (Fig. 2a, Table 2). Mean AAS ranged from 7.2 to 8.3 mg O_2 min^{-1} kg^{-1} but was not significantly different (p = 0.54) and was fitted to the equation AAS (mg O_2 min^{-1} kg^{-1}) = $– 0.241 + 0.921x – 0.026x^2$ where x is temperature in ℃ (Fig. 2a, Table 2). In the context of AAS, the peak SDA response comprised 10.4 to 14.4% of AAS, leaving a mean PRAS of 6.2 to 7.3 mg O_2 min^{-1} kg^{-1}, or 85.5 to 89.6% of AAS, although neither was significantly different among treatments (p = 0.244 and p = 0.57, respectively) (Fig. 2a, 2b, Table 2).

Discussion

SDA variables

The SDA responses from our salmon were remarkably similar across all temperature treatments, with SDA_{cost}, $Peak_{net}$, t_{peak}, SDA_{dur}, SDA_{coef}, and SDA_{scope} all not significantly different. SDA_{peak} was significantly different across temperatures, but this significance disappeared once standardized to individual fish's SMR ($Peak_{net}$) and corresponded to postprandial increases of 124 – 136% of SMR. Therefore, we conclude that the SDA response in this population of juvenile Fall-run Chinook salmon shows a large degree of thermal

independence. This level of thermal insensitivity was unexpected, given the influence ambient temperature exerts on the biologic rates of ectotherms. Our temperatures represented an ecologically relevant range experienced by juvenile Fall-run Chinook salmon in upper tributary rearing grounds (10-14℃) and in the Sacramento-San Joaquin Delta (~25℃) as they outmigrate, suggesting that juvenile Fall-run Chinook have substantial plasticity in their digestive response (Columbia Basin Research, University of Washington, 2022).

While not significant, our fish did exhibit a slight trend towards increased SDA_{peak} and decreased SDA_{dur} of the metabolic response with increasing temperature. Effects of temperature on SDA_{peak} and SDA_{dur} are documented in many ectotherm species, but inconsistent in magnitude across species or even within the same family, as evidenced by the differing thermal susceptibility of these metrics in three cyprinid species (Pang *et al.* 2010). Variability in SDA response of fishes is also explained by lifestyle (i.e., active swimmers vs. sit-and-wait ambush predators) due to differences in the capacity of the central cardio-respiratory, digestive, and locomotor systems (Jutfelt *et al.* 2021, Pang *et al.* 2011). Steell *et al.* (2019) observed higher metabolic rates during meal digestion rather than from exhaustive exercise in tropical lionfish, with SDA_{peak} exceeding active metabolic rate by as much as 1.7 times. Even small meals occupied 64% of AAS in lionfish at 26℃, whereas our salmons' peak occupied only 10.4 to 14.4% of AAS at all temperatures.

One can also express SDA_{peak} as a percentage of MMR, or the oxygen-transporting capacity of the cardiovascular system. For our fish, SDA_{peak} corresponded to 30.5 – 46.6% of MMR, leaving a substantial amount of aerobic capacity available. Studies in juvenile rainbow trout where SDA was compared to MMR rather than AAS, have suggested higher percentages of MMR consumption at SDA_{peak}. LeGrow and Beamish (1986) estimated SDA_{peak} for 10 – 15 g

rainbow trout fed 2% of their body mass with diets of varying protein and lipid contents at 15℃, to be between 60 and 80% of MMR. However, MMR was calculated using an equation given by Rao (1969) and MMR can vary considerably between individuals. Similarly, for 10 – 20 g juvenile rainbow trout (*O. mykiss*) at 15℃, Alsop and Wood (1997) measured satiation-fed (~3% of body mass on average) metabolic rate as approximately 70% of MMR, although fish were fed as a group within a tank prior to individual transfer to respirometers so exact meal size was unknown. Eliason *et al.* (2007) estimated average SDA_{peak} in adult rainbow trout (503.4 ± 10.7 g, mean ± SEM) to consume 53% (minimum) and 69% (average) of MMR, using a MMR given by Kiceniuk and Jones (1977).

Generally, it appears both juvenile and adult salmonids can utilize a large proportion of their aerobic scope for digestion if needed, though our study suggests juvenile Chinook in the SSJRB require less aerobic capacity to digest a meal. Definitive conclusions on trends between juvenile and adult fish require further study due to differences in methods, diet composition, and species. Additionally, it is possible that the dynamics of SDA changes with age due to dietary protein needs and the role of protein synthesis in the postprandial increase in metabolic rate (Seth *et al.,* 2009). In well-studied rainbow trout aquaculture, the optimal dietary protein level for optimal growth decreases from 50% to 35% from very young trout to adult maintenance diet (Hilton and Slinger, 1981). For adult rainbow trout, Eliason *et al*. (2007) found that isoenergetic diets with varying protein and lipid levels, which significantly alter protein utilization and deposition, had no effect on SMR, SDA_{peak}, t_{peak}, or SDA_{cost}. The substantial aerobic capacity remaining in our juvenile Chinook salmon at SDA_{peak} at all temperatures suggests that digestion is an important function and may be attributed to their need to grow rapidly at this life stage. Measurements of $\dot{M}O_2$ in fed and fasted Chinook salmon and rainbow trout (*Oncorhynchus*

mykiss) forced to swim at critical swimming speeds found that MMR remained the same, but that critical swimming speed was lower in the fed fish (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). For these *Oncorhynchus spp.*, the metabolic processes associated with digestion and assimilation are prioritized, potentially at the expense of maximum sustained swimming performance.

Our salmons' relatively small and constant SDA_{coef} of 5.7 to 7.2% indicates that a small proportion of ingested meal energy went toward the SDA response, leaving substantial absorbed energy remaining to be allocated to growth (LeGrow and Beamish, 1986). However, the pellet diet we provided is an energetically high-density food (14.6 kJ/g digestible energy), which likely led to lower SDA_{coef} values than would be found with fish consuming natural prey items. Davis *et al.* (2018) assessed gut contents of juvenile Chinook salmon from the Nisqually River delta in Puget Sound, Washington and estimated energy density of stomach contents to be 5.32 ± 2.94 kJ/g and 4.47 ± 2.62 kJ/g for wild and hatchery fish, respectively, and stomach fullness as a percent of fish wet weight to be 2.18 ± 3.58% and 1.16 ± 2.80%. Given our salmons' meal size of 2% corresponding to a mean of 0.51g and with a mean SDA_{cost} of 0.47 kJ, values of SDA_{coef} with more realistic meal energy densities would be 17.4% and 21.0% for wild and hatchery fish. Although Davis' *et al.* (2018) data are from a different watershed, similar types of prey are consumed by juvenile Fall-run Chinook salmon in the lower Mokelumne River and yolo bypass, both of which are located within the SRB (Goertler *et al.* 2018; Merz, 2002). Additionally, juvenile salmon augment their foraging behavior by preferentially consuming calorically valuable prey and consuming a greater quantity of prey when calorically valuable prey are not available (Goertler *et al.* 2018). These estimated SDA_{coef} values are also within the range of 11.9 – 32.3% for Biwa trout (*O. rhodurus*) fed 1.0 – 3.3% of body weight with rainbow trout (*Salmo*

gairdneri) or ayu (*Plecoglossus altivelis*) fillets (Miura *et al.* 1976). Due to the variability of SDA_{coef} depending on meal energy density and size, we caution comparing SDA_{coef} values between studies without taking into account these details. Investigating the SDA response in juvenile Chinook using natural prey items is an avenue for further study.

Our salmons' SDA_{scope} of 1.24 to 1.39 was lower than the 1.5 to 2.5 times SMR range reported for many different fish (Jobling 1981; McCue 2006; Secor 2009), and SDA_{dur} was similar to that reported for the congeneric rainbow trout (*O. mykiss*) fed a meal of 2% body mass (Eliason *et al.* 2007; LeGrow and Beamish, 1986; Medland and Beamish, 1985). Fish likely face a tradeoff between ingesting large, infrequent meals vs. smaller, more frequent meals due to the inverse relationship between SDA_{dur} and SDA_{peak}. SDA_{dur} typically increases with increasing meal size and is variable depending on fish size and meal composition (Jobling, 1981). However, it is thought that SDA is dominated by relatively fixed metabolic costs created by the upregulation of digestive processes, so it is possible regular feeding reduces the costs of constantly up- and down-regulating the digestive system (Boyce and Clarke, 1997). Fish fed multiple meals have mixed results, with juvenile cod (*Gadus morhua*) exhibiting a cumulative effect of increased $\dot{M}O_2$ with each meal and a maximum observed after the third or fourth meal (Soofiani and Hawkins, 1982). In contrast, lionfish had reduced costs when feeding frequently vs. feeding singularly (Steell *et al.*, 2019). For juvenile Chinook salmon, we suspect the dynamics of multiple feedings to more resemble that of juvenile cod due to greater similarities in size, prey choice, and lifestyle. Because we measured a single instance of feeding using easily digestible pellets, our SDA values likely represent the lower end of postprandial energy consumption for juvenile Chinook in the wild.

SMR, MMR, AAS, and PRAS

Our salmons' SMR, MMR, and AAS (Fig. 2a, Table 2) were consistent with those from previous reports for the same species of a similar mass tested from 12 to 26°C, although MMR was elicited using an incremental swimming protocol in contrast to the chase protocol in the present study (Poletto *et al.*, 2017). Juvenile Chinook had relatively constant aerobic capacities over the range of acute temperatures, which was maintained by matching the increase in SMR with an equivalent increase in MMR (Fig. 2a). Thermal insensitivity of AAS has been documented in another Californian *Oncorhynchus* species, with hatchery and wild *O. mykiss* tested on the Lower Tuolumne River showing an ability to maintain 95% of maximum AAS across a wide temperature range of 17.8 – 24.6°C (Verhille *et al.*, 2016). Previous studies on salmonids from more northern latitudes showed an AAS peak or plateau at high temperatures, which then plummets when critical temperatures are reached (Farrell, 2016). However, in the present study and for other *Oncorhynchus* species located in the Central Valley of California, clear peaks or plummets are lacking (Verhille *et al.*, 2016). We attempted to measure SDA at 25°C, but found that exposure to this temperature for greater than 24 h proved fatal. Mortality from chronic exposure to temperatures above 24°C is well documented in juvenile salmonids, although the underlying mechanism is not well understood (Myrick & Cech, 2002). Interestingly, maintaining a high level of swimming performance and aerobic capacity up to nearly lethal temperatures has been shown in multiple juvenile Chinook populations from a range of latitudes along the West coast of the United States, as well as in adult sockeye salmon (*O. nerka*) from the Fraser River, British Columbia, Canada (Eliason *et al.* 2013; personal communication from Dr. Zillig, University of California, Davis). Our attempts to test fish at 25°C may have been additionally hampered by a lack of ram ventilation due to static respirometers rather than swim tunnels.

By measuring both SDA variables and AAS in individual fish, we could assess PRAS, an ecologically relevant metric of available excess energy after consumption of a meal. Our salmon's PRAS of 6.2 – 7.3 mg O_2 min^{-1} kg^{-1} was equivalent to a remaining scope for activity of 85.5 – 89.6% of AAS – quite a large proportion. The remaining energy must fuel all other activities for a given fish and it is possible that additional stressors, strenuous activity, or warmer, sublethal temperatures could reduce PRAS (Jutfelt *et al.* 2021). However, it is suggested that juvenile salmonids modify their behavior to maximize AAS via foraging in prey-dense mainstem habitats, followed by retreating to cooler thermal refugia such as tributaries (Brewitt *et al.*, 2017).

Limitations and assumptions

One limitation of our study is that we did not investigate assimilation efficiency (AE) – the fraction of ingested food that is incorporated into biological tissue. AE is measured by calculating absorption minus defecation and excretion during an organism's gut transit time, and can be affected by food type, frequency of ingestion, and temperature (Pouil *et al.*, 2018). In addition, the absorption of specific nutrients and elements can also vary with temperature. For example, Van Campenhout et al. (2007) showed that decreasing the temperature from 25°C to 15°C in common carp (*Cyprinus carpio*) caused no change in cadmium AE, but a significant decrease in zinc AE. Although an increase in temperature typically increases enzymatic activity and decreases gut transit time, resulting in no change in AE, some lizard species exhibit reduced assimilation efficiency at extreme temperatures (Plasman *et al.*, 2019). Thus, the PRAS maintained across our test temperatures may not be indicative of the use-value of the meal provided, potentially affecting growth. Additionally, in the wild, digestion is affected by

behavioral mediation such as movement to different temperatures, reduction of meal size, increased meal frequency, and intentional regurgitation (Jutfelt *et al.,* 2021).

Our study was conducted with hatchery juvenile Chinook under temperature-controlled, well-oxygenated conditions, with optimal feed and without additional environmental stressors. In contrast, wild fish must obtain prey, escape predators, choose suitable habitat, and cope with variable environmental conditions, creating much more complex dynamics when it comes to prioritizing energetic demands. Recently, there has been an improved understanding of how the introgression of hatchery- and wild-origin fish has reduced fitness (Araki *et al.,* 2008), eroded life history diversity (Carlson and Satterthwaite, 2011), and resulted in drastically increased hatchery contributions to spawning populations (Willmes *et al.,* 2018). However, little is known about the direct consequences on digestion, energetics, or physiological response to temperature. Unfortunately for the fate of wild Chinook in the Central Valley, over 90% of fish captured in the ocean fishery in 1992 and 2002 for Fall-run Chinook salmon were of hatchery origin (Barnett-Johnson *et al.,* 2007) – a consequence of over a half-century of large-scale hatchery propagation (Sturrock *et al.,* 2019). For our study, this makes our use of hatchery-origin fish more relevant than in other watersheds where wild fish retain a larger genetic difference from their hatchery counterparts, although it does not discount the importance of social and behavioral cues in energy use that may differ for the two settings.

Lastly, our study was conducted with one acclimation temperature of 16℃, with acute exposure to test temperatures. Acclimation temperature is known to affect thermal performance curves, with physiological responses occurring at time scales ranging from minutes to weeks (Schulte *et al.,* 2011). For juvenile Fall-run Chinook sourced from Coleman hatchery, AAS in fish acclimated to 11, 16, and 20℃ and measured at acute temperatures from 8 to 25℃ had

similarly shaped responses among acclimation temperatures, with more dispersion between acclimation temperatures occurring at the lower range of acute temperatures (Zillig *et al.*, in press, CJFAS). This suggests that across these acclimation and acute temperatures, the patterns observed in our SDA metrics may not vary dramatically with changes in acclimation temperatures, although this would need to be confirmed in future studies. For much longer-term acclimations lasting days to weeks, metabolic thermal compensation may occur, where a sudden change to a new thermal condition alters metabolic metrics (such as SMR, MMR, and AAS) but the fish is able to compensate to some degree over time (Sandblom *et al.*, 2014). Ultimately, neither acclimation nor acute temperature changes take into account the behavior of wild fish and their decisions in thermal regulation.

Conclusions

The results of our study suggest that moderate temperatures (13 – 24℃) seen throughout the SSJRB are not a critical factor when it comes to the cost of digestion in Fall-run juvenile Chinook salmon. However, extended periods of sublethal temperatures (24℃+), are likely to increase in frequency and duration, lowering survival among juvenile Chinook. We believe prey availability is likely to be a more important factor, as evidence suggests that abundant prey resources may mitigate the negative effects of elevated temperature on fish growth (Brewitt *et al.*, 2017; Lusardi *et al.*, 2020). Additionally, physiological plasticity in the form of thermal acclimation is well documented for Chinook salmon. Palmisano *et al.* (2000) found that Chinook salmon increased heat-shock protein 90 expression in heart, muscle, brain, and gill tissues after a 5 h exposure to 21.6℃, indicating an acute compensatory mechanism. Such rapid compensatory mechanisms and the importance of growth in juvenile Chinook salmon may explain the minimal effects of temperature on our SDA variables. In conclusion, juvenile Chinook salmon are

exposed to both cool riverine temperatures in upper-watershed rearing grounds and to warmer temperatures within the estuaries and bay as they migrate to the ocean. The diversity of life history strategies among runs of Chinook salmon in the SSJRB result in juveniles rearing within the watershed nearly year-round (Brandes and McLain, 2000). The thermal stability of their SDA responses may play a role in maintaining homeostasis in digestive capability in a highly heterogeneous environment, where rapid growth is important for successful competition with conspecifics and for avoiding predation (Beamish and Mahnken, 2001; Sogard, 1997).

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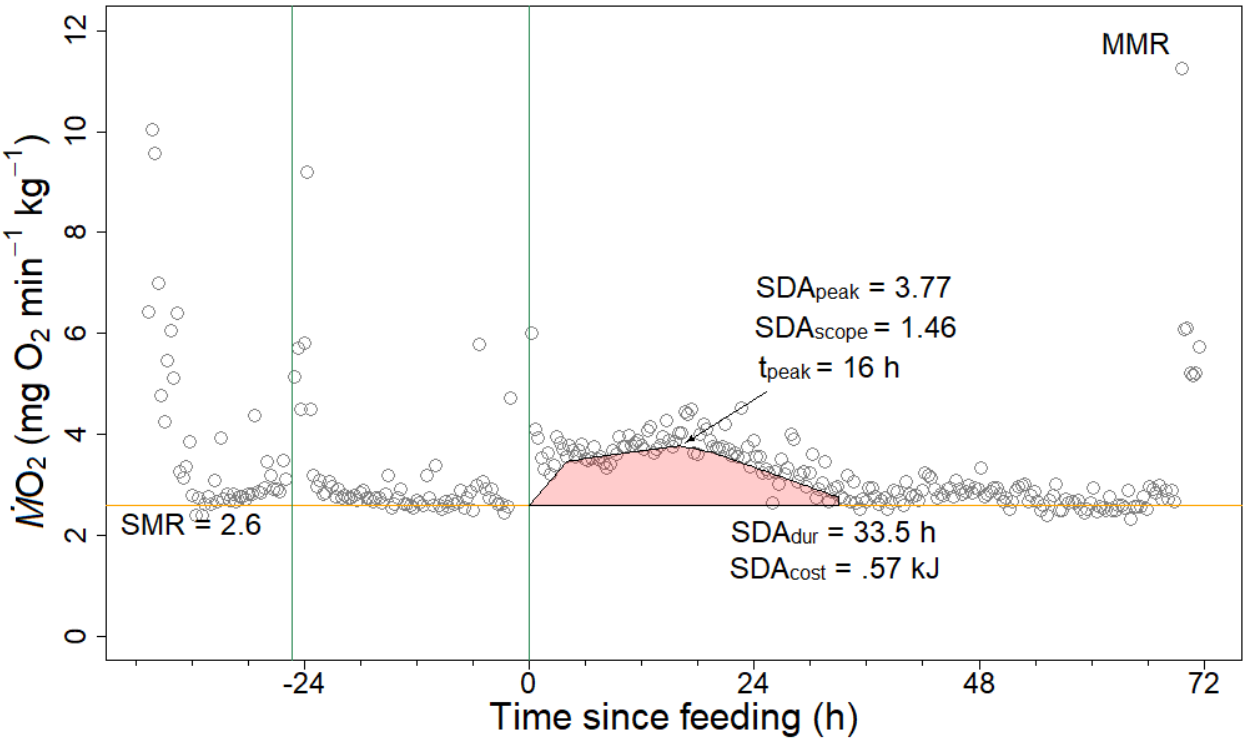

Figure 1.1: A representative continuous recording of the $\dot{M}O_2$ (mg O_2 min^{-1} kg^{-1}) from a juvenile Chinook salmon (25.9 g, 12.9 cm fork length) acclimated to 16°C and tested at 16°C using a respirometry system at UC Davis. At the 1st vertical bar (Time: -24), the fish was removed, sham-fed and returned to the vessel. At the 2nd vertical bar (Time: 0), the fish was removed and force-fed 2% of its wet body weight with formulated pellets and returned to the vessel for up to 72 h. A quantile regression is used to estimate the SMR from pre-feeding $\dot{M}O_2$, indicated by the horizontal line, whereas SDA is estimated from post-feeding $\dot{M}O_2$. SDA is considered terminated when the regression converged with the SMR + 5%. The duration of the SDA response is 33.5 h and is noted as SDA_{dur}. SDA_{cost} is estimated by integrating the area between the curve and SMR (marked polygon area) and is reported in kJ by assuming 1 g of O_2 is associated with the release of 13.6 kJ of energy (Cho *et al.*, 1982). SDA_{scope} (mg O_2 min^{-1} kg^{-1}) and t_{peak} (h) are indicated by the arrow. MMR was collected at the end of the experiment by chasing the fish in a bucket to exhaustion and then returning it immediately to the respirometry chamber.

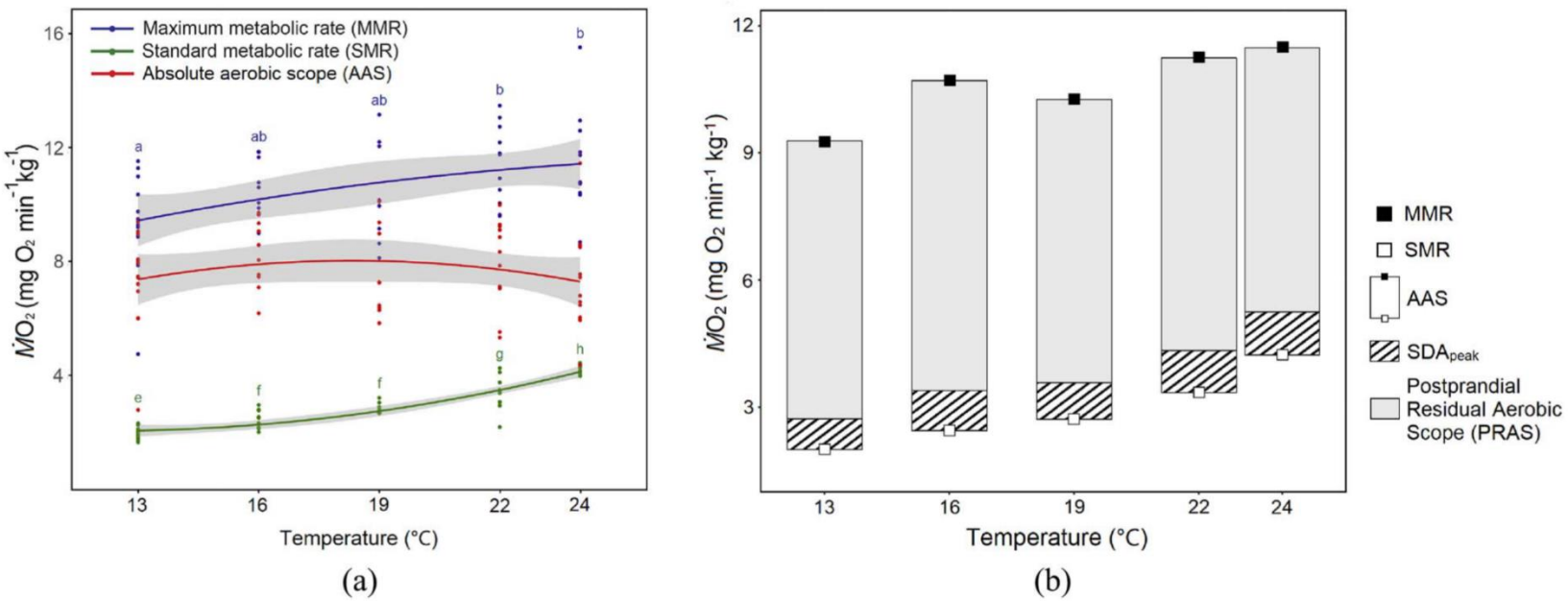

Figure 2.1: (a) The effect of temperature on standard metabolic rate (SMR), maximum metabolic rate (MMR), and absolute aerobic scope (AAS) in juvenile Chinook salmon reared at 16℃ and tested at 13, 16, 19, 22, and 24℃. Solid blue dots and line represent MMR fit to a second order polynomial described by MMR (mg O_2 min^{-1} kg^{-1}) = 4.550 + 0.479x – 0.008x^2 where x is temperature in ℃. Solid green dots and line represent SMR fit to a second order polynomial described by SMR (mg O_2 min^{-1} kg^{-1}) = 4.791 – 0.443x + 0.018x^2 where x is temperature in ℃. Solid red dots and line represent AAS fit to a second order polynomial described by AAS (mg O_2 min^{-1} kg^{-1}) = – 0.241 + 0.921x – 0.026x^2 where x is temperature in ℃. **(b)** Relationships between SDA_{peak}, SMR, MMR, and AAS at 13, 16, 19, 22, and 24℃. SMR and MMR are represented by the open and closed squares, respectively, and AAS is the difference between the two, represented by the gray rectangle. SDA_{peak} is represented by the hatched area.

Table 1.1: Definition of variables used to quantify the postprandial metabolic response to feeding.

Variable	Definition
SMR (mg O_2 min^{-1} kg^{-1})	Baseline metabolic rate of postabsorptive individuals
SDA_{cost} (kJ)	Total O_2 cost of the postprandial response, calculated as the area under the $\dot{M}O_2$ curve bounded by SMR and converted to kJ using an oxycalorific coefficient (Cho *et al.* 1982)
SDA_{peak} (mg O_2 min^{-1} kg^{-1})	Postprandial peak in $\dot{M}O_2$ (mg O_2 min^{-1} kg^{-1})
$Peak_{net}$ (mg O_2 min^{-1} kg^{-1})	SDA_{peak} minus SMR
t_{peak} (h)	Time from feeding to SDA_{peak} in hours
SDA_{dur} (h)	Duration of time from feeding to when $\dot{M}O_2$ is no longer significantly greater than baseline (SMR + 5%) (Chabot *et al.* 2016*a*)
SDA_{scope}	Ratio of SDA_{peak} to SMR
SDA_{coef} (%)	SDA_{cost} divided by the digestible energy content of the meal

Table 2.1: Temperature effects on postprandial metabolism in juvenile Chinook salmon. Values are reported as means ± s.e. Different superscript letters represent significantly different values between acute temperature treatments ($p < 0.05$; Tukey's test).

Variable	Temperature				
	13°C	16°C	19°C	22°C	24°C
n	12	10	9	12	11
SMR ($mg\ O_2\ min^{-1}\ kg^{-1}$)	2.0 ± 0.06^{a}	2.4 ± 0.10^{b}	2.7 ± 0.14^{b}	3.3 ± 0.16^{c}	4.2 ± 0.05^{d}
MMR ($mg\ O_2\ min^{-1}\ kg^{-1}$)	9.3 ± 0.53^{a}	10.7 ± 0.33^{ab}	10.3 ± 0.59^{ab}	11.2 ± 0.42^{b}	11.5 ± 0.54^{b}
AS ($mg\ O_2\ min^{-1}\ kg^{-1}$)	7.3 ± 0.52	8.3 ± 0.38	7.5 ± 0.52	7.9 ± 0.43	7.2 ± 0.55
Wet Mass (g)	28.1 ± 0.68	26.3 ± 1.26	29.8 ± 1.43	26.4 ± 0.60	28.9 ± 0.99
Fork Length (cm)	13.4 ± 0.11	13.0 ± 0.18	13.6 ± 0.19	13.2 ± 0.12	13.5 ± 0.13
Total Length (cm)	14.4 ± 0.12	14.0 ±0.16	14.5 ± 0.21	14.2 ± 0.12	14.5 ± 0.14
SDA_{cost} (kJ)	0.40 ± 0.04	0.51 ± 0.09	0.49 ± 0.07	0.47 ± 0.04	0.50 ± 0.05
SDA_{peak} ($mg\ O_2\ min^{-1}\ kg^{-1}$)	2.7 ± 0.11^{a}	3.4 ± 0.14^{b}	3.6 ± 0.19^{b}	4.3 ± 0.18^{c}	5.3 ± 0.11^{d}
$Peak_{net}$	0.73 ± 0.06	0.94 ± 0.09	0.88 ± 0.14	1.00 ± 0.08	1.02 ± 0.11
t_{peak} (h)	19.3 ± 1.8	13.2 ± 2.2	15.4 ± 1.7	15.0 ± 1.6	14.0 ± 1.3
SDA_{dur} (h)	43.4 ± 2.7	39.0 ± 4.1	39.5 ± 3.0	38.2 ± 2.8	36.6 ± 3.4
SDA_{scope}	1.36 ± 0.03^{ab}	1.39 ± 0.04^{a}	1.33 ± 0.06^{ab}	1.31 ± 0.03^{ab}	1.24 ± 0.03^{b}
SDA_{coeff} (%)	5.7 ± 0.5	7.2 ± 1.1	6.4 ± 0.9	6.5 ± 0.5	6.4 ± 0.7
PRAS ($mg\ O_2\ min^{-1}\ kg^{-1}$)	6.5 ± 0.5	7.3 ± 0.3	6.7 ± 0.5	6.9 ± 0.5	6.2 ± 0.5
Remaining scope for activity (%)	89.6 ± 0.9	88.6 ± 1.0	88.2 ± 1.9	86.6 ± 1.6	85.5 ± 1.5

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CHAPTER 3

Persistent effects of low incubation temperature on growth and metabolism of larval Green Sturgeon (*Acipenser medirostris*)

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Abstract

Southern Distinct Population Segment (sDPS) Green Sturgeon spawn solely in one stretch of the Sacramento River in California. Management of this spawning habitat is complicated by cold water temperature requirements for the conservation of winter-run Chinook Salmon. This study assessed carryover effects of low incubation temperature on the scaling relationships in growth and metabolism in northern DPS Green Sturgeon used as a proxy for sDPS green sturgeon across embryo to early juvenile life stages. Fish were incubated and reared at 11℃ and 15℃, with a subset experiencing a reciprocal temperature transfer post-hatch, to assess recovery from cold incubation or to simulate a cold-water dam release which would chill incubating eggs. Metabolic rate and growth of embryos and larvae were measured to 118 days post hatch. Incubation and rearing at 15℃ relative to incubation and rearing at 11℃ resulted in fish of the same age (118 dph) with a ca. 37-fold difference in weight. Log-transformed length-weight relationships indicated few differences in developmental growth trajectories or transitions from prelarval to larval and from larval to early juvenile morphologies. However, considerable size-at-age differences persisted between rearing temperatures, with 11℃ fish requiring 120 days post-hatch to achieve 1 g in mass relative to 60 days required for 15℃ fish. The scaling relationship between log-transformed whole-body metabolism and mass exhibited a steeper slope and thus an increased oxygen requirement with size in 11℃ reared fish. Understanding how cold temperatures affect Green Sturgeon ontogeny is necessary to refine our larval recruitment estimations for this threatened species.

Introduction

Green Sturgeon (*Acipenser medirostris*) are anadromous, long-lived, and late maturing fish found in the northern Pacific Ocean along the coast of North America (Moyle 2002; Moser et al. 2016). Despite a latitudinally-broad range from Ensenada, Mexico to the Bering Sea off the Alaskan coast, they are considered a species of concern due to the widespread damming of rivers, which has eliminated access to historical spawning areas and altered flow and temperature regimes (Yoshiyama et al. 1998; Moser et al. 2016). Currently, the only known rivers where Green Sturgeon spawn are the Rogue River in Oregon (USA), and the Klamath and Sacramento Rivers in California (National Marine Fisheries Service 2018). Genetic analyses have shown a strong differentiation between populations spawning in the Rogue and Klamath Rivers, and the Sacramento River (SR), separating the species into a northern (nDPS) and a southern distinct population segment (sDPS), respectively (Israel et al. 2004; Adams et al. 2007). In 2006, sDPS Green Sturgeon were listed as a threatened species under the Endangered Species Act (ESA) due to population decline, habitat loss and degradation, and the presence of a single spawning reach remaining in the SR which exposed the sDPS to increased risk of extinction from stochastic events (National Marine Fisheries Service 2018). Therefore, understanding the role of environmental characteristics on Green Sturgeon early life history and recruitment is vital to predicting species persistence and management.

Direct evidence of spawning by sDPS Green Sturgeon, verified using egg mat sampling, has been documented in sequential years within a 94 km stretch of the mainstem SR from the Glenn-Colusa Irrigation District pumping station at river kilometer (rkm) 331 – calculated as the distance upstream from Suisun Bay in the Sacramento-San Joaquin River Delta – to Inks Creek at rkm 426 (Brown 2007; Poytress et al. 2015; National Marine Fisheries Service 2018).

Additionally, in 2011 sDPS Green Sturgeon were observed to have spawned in the Feather River, a large tributary of the SR (Seesholtz et al. 2015). Models suggest that in the absence of impassable dams, historical Green Sturgeon spawning habitat would have included the mainstem Sacramento and San Joaquin Rivers, and several major tributaries including the lower Feather, American, and Yuba Rivers (Mora et al. 2009). Currently, the mainstem SR spawning area for Green Sturgeon is downstream of an adjacent stretch of spawning habitat for endangered winter-run Chinook Salmon (*Oncorhynchus tshawytscha*), which historically spawned in now-inaccessible high elevation, cold, spring-fed streams (Lindley et al. 2004). This habitat elimination led to an ESA listing for winter-run Chinook Salmon in 1994 – earlier than the 2006 listing for sDPS Green Sturgeon – resulting in management actions that prioritize critical temperature thresholds for winter-run Chinook Salmon. Managers seek to maintain SR water temperatures below 13.3℃ (56°F) from approximately May to July via cold-water releases from Shasta dam (Fig. 1; California Department of Water Resources 1988; U.S. Environmental Protection Agency 2003). These temperatures are lower than historic mainstem SR temperatures and the optimal incubation temperatures of 14-17℃ for Green Sturgeon embryos reared in laboratory conditions (California Department of Water Resources 1988; Van Eenennaam et al. 2005). Van Eenennaam et al. (2005) showed that incubation temperatures of 11℃ resulted in decreased hatch rates and shorter hatchlings in nDPS Green Sturgeon – used as a proxy for sDPS Green Sturgeon because of their listing status. Similarly, Poletto et al. (2018) found that juvenile nDPS Green Sturgeon reared at temperatures 11 or 13℃ elicited reduced growth rates compared to fish reared at 16 or 19°C. Neither of these studies investigated the effects of cold temperature across early life history stages (i.e. embryo incubation to pre-larval, larval, and early juvenile stages), a data gap we intend to fill also using nDPS Green Sturgeon.

Temperature affects virtually all aspects of physiology in ectotherms including growth and metabolism (Hochachka and Somero 2002). Understanding the effect of temperature on metabolism provides a baseline understanding of energy budgets and resource allocation that have implications for growth, maximum performance (Schulte 2015), behavior (Killen et al. 2013), and life history strategy (Metcalfe et al. 1995; Killen et al. 2010). Colder temperatures typically reduce metabolism and subsequently growth, increasing a fish's vulnerability to predation and reducing recruitment (Anderson 1988). While mechanisms affecting juvenile survival such as fish passage devices (Steel et al. 2022) and predation risk (Baird et al. 2020) have been investigated for larger size classes, little is known about mechanisms affecting larval and early juvenile life stages. sDPS-specific monitoring is limited, and using low numbers of fish caught as bycatch in other species' monitoring efforts complicate analysis (Poytress et al. 2014). Wild sDPS Green Sturgeon embryos and early larval stages are especially understudied because known spawning habitats are characterized by high flows, turbid waters, and cobble substrates that make locating larvae difficult. For these reasons and due to their listing status, nDPS Green Sturgeon will be used in this study. Much of the temperature thresholds, habitat requirements, ontogenetic developmental trajectories, and early life behavior of sDPS Green Sturgeon are assumed to be similar to those of nDPS Green Sturgeon studied in the laboratory, despite their genetic separation. Exposing nDPS Green Sturgeon to temperatures below laboratory-based optimums for growth may more accurately represent the conditions experienced by wild sDPS Green Sturgeon in the current dam-manipulated management regime and provide an assessment of how carryover effects from cold water temperatures may affect them down the line. Environmental conditions encountered during early life have the potential to affect phenotypic traits later in life – termed carryover effects – and can have implications for performance and

fitness (Burggren and Mueller 2015; Saboret and Ingram 2019). Chronic decreases in metabolism and growth due to cold temperatures can impact survival (Wilson et al. 2021), outmigration timing (Gosselin et al. 2021), foraging and swimming ability (Baker et al. 2014), and salinity tolerance (Allen and Cech 2007; Allen et al. 2011). Additionally, these carryover effects can result from relatively short exposures if they occur during critical developmental windows (Mueller et al. 2015). The purpose of this study was to assess carryover effects of incubation temperature on allometric scaling relationships in both metabolism and growth with age, by using reciprocal temperature transfers after hatch between an optimal and low incubation temperature. Embryos were incubated at an optimal (15°C) and low (11°C) temperature until 1 day post-hatch (dph), at which time half of each treatment underwent a reciprocal temperature switch. We predicted that low incubation temperatures would dramatically slow growth, reducing size-at-age and leading to differences in length-weight relationships between treatments. Upon the reciprocal temperature switch, we expected to see compensatory growth among fish moved to optimal temperature, continued stunting among fish remaining at the colder temperature, and depression of growth among fish moved from optimal to the colder temperature condition.

Materials and methods

Experimental animals

nDPS Green Sturgeon embryos were obtained from a broodstock program maintained at the UC Davis Center for Aquatic Biology and Aquaculture's (CABA) Putah Creek Facility. Green Sturgeon adults were originally sourced throughout 1999-2005 from wild Klamath River basin nDPS Green Sturgeon in collaboration with the Yurok Tribe's gill-net subsistence fishery and spawned annually (Van Eenennaam et al. 2008). In March 2019, fertile F2 adults (one

female and 3 males) were tank spawned following methods described by Van Eenennaam et al. (2012). Injections of gonadotropin-releasing hormone analog and White Sturgeon (*A. transmontanus)* pituitary extracts were used to induce spawning. The next day approximately 500 eggs were ramped down to 11℃ ± 0.5 at a rate of 2℃ per day while the other 500 eggs were maintained at 15℃ ± 0.5. Embryos were incubated at 15℃ and 11℃ until 1 dph, at which time half of each treatment underwent a reciprocal temperature switch, resulting in four total treatments: incubation and rearing at 15℃, incubation and rearing at 11℃, incubation at 11℃ and rearing at 15℃, and incubation at 15℃ and rearing at 11℃, hereafter listed as 15℃, 11℃, 11 – 15℃, and 15 – 11℃ treatments, respectively. The incubation period prior to reciprocal temperature transfers lasted seven days for both 11℃ and 15℃ groups.

Embryos were incubated at 11℃ or 15℃ inside floating mesh baskets within two 350-L temperature-controlled flow-through rearing tanks supplied with aerated water from a dedicated well. Embryos were arranged in a single layer to maximize oxygenation and prevent clumping and the spread of fungus on dead eggs. Dead and fungus covered eggs were removed at least three times per day. Well-water salinity was 0.4 parts per thousand and fish were exposed to natural photoperiod conditions for Davis, CA (38.5 °N). Once sturgeon began to hatch and swim freely, they were moved out of the floating mesh baskets and into rearing tanks. Each treatment was evenly separated into two 470 L replicate tanks used for the remainder of the experiment. Since early developmental stages rely on endogenous yolk reserves (Kamler 2008), fish were not fed until approximately 14 dph, although food was provided at ca.12 dph to orient larvae to chemical cues (Van Eenennaam et al. 2012). Once feeding was detected, larvae were fed *ad libitum* with semi-moist commercial Starter Crumble feed (Skretting, USA) and excess uneaten feed and feces were removed daily. Minimum feed rates were calculated according to optimal

feed rate models for White Sturgeon (Deng et al. 2003; Lee et al. 2014) using mean wet mass and water temperature. Feed rates were updated biweekly to account for fish growth and routinely exceeded to ensure *ad libitum* feed availability. All experimental protocols and fish care were approved by the UC Davis Institutional Animal Care and Use Committee, protocol #21834.

Growth

Length and weight were measured to the nearest 0.01mm using digital calipers (Neiko tools), and 0.0001g using an analytical balance (Model A-200DS, Denver Instrument Company) for individual Green Sturgeon from 1 dph to 118 dph in increasing increments to reflect exponential growth. Length and weight were collected following metabolic rate ($\dot{M}O_2$) trials and additional data was gathered using random samples of eight fish from each of two replicate treatment tanks. In total, 132, 188, 107, and 165 fish were measured for 15℃, 11℃, 11 – 15℃, and 15 – 11℃ treatments, respectively (Table 1).

Metabolic rate

Oxygen consumption – an indirect measure of aerobic metabolic rate ($\dot{M}O_2$) – of individual embryos was measured using closed-system respirometry in two 24-well microplate systems (Loligo Systems, Denmark) with flow-through water baths supplied separately by 11℃ and 15℃ 350-L temperature-controlled reservoirs. Each 940µl well contained an optical oxygen sensor spot (PreSens, Germany), which was read using a 24-channel optical fluorescence oxygen reading device and MicroResp^{TM} automated microplate respirometry software (Loligo Systems, Denmark). The microplate system and water bath were kept on an electric rocking platform which gently agitated the contents in the microplate wells to ensure oxygen mixing. Each well's oxygen sensor spot was calibrated individually with oxygen-free water and fully aerated distilled

water at both 11℃ or 15℃, specific to the following trial. Oxygen-free distilled water was created by adding 1g sodium sulfite (Na_2SO_3) to 100 ml of distilled water. Fully aerated distilled water was created by bubbling ambient air into 100 ml of water for 20 min. Both calibration measurements were conducted while the microplates were inside the water bath to reduce temperature fluctuations. $\dot{M}O_2$ was calculated from the slope of the linear regression fit to each well's declining O_2 content during the closed period (10 – 60 minutes). Due to the nature of closed respirometry, each embryo produced one $\dot{M}O_2$ measurement, which was only included in analysis if the $R^2 > 0.95$. Experiments were concluded before the oxygen concentration declined below 6.0 mg O_2 L^{-1} – an oxygen concentration that ensures survival and routine metabolism in European sturgeon (*A. sturio*) embryos exposed for 48 h (Delage et al. 2020). Background bacterial respiration was assessed by leaving 6 of the 24 wells empty per plate run. Slopes of the 6 blank wells were averaged and subtracted from the remaining 18 wells containing embryos (Svendsen et al. 2016).

$\dot{M}O_2$ of individual larvae was measured by intermittent flow respirometry using an 8-chamber system constructed at University of California, Davis. Each chamber consisted of a glass cylinder with a rubber stopper on each end. Rubber stoppers were pierced with stainless steel tubing fitted with high grade gas impermeable silicone tubing which provided recirculating and flush water flow via two 8-channel low-flow peristaltic pumps (Model BT100-1L, Langer Instruments, USA). To balance rate of oxygen consumption, temperature and recirculating flow rate we used two sizes of respirometry chambers (7.77 ± 0.07 mL and 20.78 ± 0.20 mL, mean ± Std Dev) to accommodate the larvae and early juveniles as they grew (Svendsen et al. 2016). Each chamber had an optical oxygen sensor spot (PreSens, Germany) affixed to the inside of the glass chamber wall with silicone glue, which was read through the glass using fiber optic cables

and two 4-channel oxygen meters (Witrox 4, Loligo systems, Denmark). The intermittent flow cycle was set such that chambers never fell below 80% O_2 saturation (8.82 and 8.07 mg O_2 L^{-1} at 11℃ and 15℃, respectively) regardless of temperature to ensure the chambers did not become hypoxic (Svendsen et al. 2016). Flush and recirculation periods were controlled using Autoresp^{TM} software (Loligo systems, Denmark). The respirometry chambers and water bath were cleaned and dried daily, and peristaltic pump tubing was bleached, neutralized, and rinsed weekly to prevent bacterial buildup on surfaces. Each respirometry chamber's sensor spot was calibrated individually with oxygen-free distilled water and fully aerated distilled water every two weeks.

After a 24 h fasting period to eliminate increased postprandial oxygen consumption (Dabrowski et al. 1987), fish were transferred to respirometers and held for ten measurement cycles on average (600-1200 seconds per measure period), corresponding to roughly 4 h per trial. Preliminary trials showed that two measurement periods were sufficient for fish to recover from handling stress, so the first two measurement periods were removed from analysis. Additionally, data from each trial was visually inspected and measurement periods removed if there were exceedingly variable measurements indicative of equipment abnormalities, such as negative oxygen consumption rate values. Removed measurement periods totaled approximately 7% of all measurement periods. RMR was calculated as the average of the three lowest slopes after any removal of measurement periods (Verhille et al. 2016; Poletto et al. 2017; Zillig et al. 2022). After the trial, sturgeon were euthanized in a lethal solution of tricaine methanesulfonate (0.5 g L^{-1}) buffered with sodium bicarbonate (0.42 g L^{-1}) and salt (6.0 g L^{-1}), then measured to the nearest 0.0001g and 0.01 mm. Background bacterial respiration was assessed by taking a minimum of three measurement periods after removal of fish, which were averaged and

subtracted from the respective chamber's fish respiration rate. This protocol was completed twice per day, resulting in 16 individual sturgeon $\dot{M}O_2$ assessments per day. $\dot{M}O_2$ is affected by exogenous factors such as temperature as well as endogenous factors such as circadian rhythm, so morning and afternoon $\dot{M}O_2$ measurements were compared within treatments. As morning and afternoon $\dot{M}O_2$ measurements were not significantly different in three of four treatments, they were combined for analysis. Further, our measurements were taken during daylight hours to minimize natural circadian rhythm peaks in $\dot{M}O_2$, as Green Sturgeon larvae are primarily nocturnal (Kynard et al. 2005). Similarly, Svendsen et al. (2014) found that Lake Sturgeon (*A. fulvescens*) exhibited peak $\dot{M}O_2$ at dawn with a continued decrease to a metabolic low 3 h after exposure to daylight.

Data and statistical analysis

Growth was compared by log transforming both wet body mass and total length, then fit with piecewise linear regression using the *segmented* package (Muggeo, 2003, 2008). The Davies' test from the *segmented* package was used to analyze each treatment for a non-zero difference-in-slope parameter, providing an estimate of two statistically significant inflection points. Linear models were iteratively fit to identify the two inflection point locations and confidence intervals, as well as regression parameters for the three linear segments separated by the inflection points, hereafter referred to as growth stanzas (Table 1, Fig. 2). Inflection points were compared in a pairwise fashion and considered significant by assessing whether the confidence interval for the difference in means did not include zero. Each of the three individual stanza slopes were compared between treatments by pairwise comparison using the Tukey method in the R package *emmeans*. Results were considered significant at $p < 0.05$.

Condition factor was calculated two ways for each length and weight measurement for additional comparison between treatments. Fulton's condition factor (K_f) was calculated using the equation $K_f = 100\frac{W}{L^3}$ where W = wet mass in grams and L = length in cm, assuming isometric growth represented by a coefficient of 3 derived from the slope of length-weight relationships (LWRs) in many fish species (Froese 2006). Allometric condition factor (K_a) was calculated using the equation $K_a = 100\frac{W}{L^b}$, where W = wet mass in grams, L = length in cm, and b is the slope of the LWR for each treatment and growth stanza (Cone 1989). K_a allows comparison of condition factor while preserving differences between growth stanzas. Both condition factor and growth were also expressed with respect to accumulated thermal units (ATUs) to account for differences in temperature exposure. One thermal unit is defined as one degree Celsius experienced in a 24-hour period, thus an embryo incubated at 15℃ would accumulate 15 thermal units per day (Boyd et al. 2010). Accumulation of ATUs commenced once embryo final acclimation temperatures were reached.

$\dot{M}O_2$ was recorded using MicroResp™ and AutoResp™ software for embryos and larvae, respectively (Loligo Systems, Denmark), and data analyses performed using R (version 4.2.1). Differences between mass-specific $\dot{M}O_2$ of embryos incubated at 11℃ and 15℃ were assessed using T-tests. For larvae, whole-body $\dot{M}O_2$ and wet body mass were log transformed and tested in *segmented* for any potential inflection points. As none were found, data for each treatment were fit to the equation $log\ \dot{M}O_2 = log\ a + b\ log\ x$, where $\dot{M}O_2$ is in mg O_2 individual^{-1} h^{-1}, and x is wet mass in grams. Slopes compared by pairwise comparison using the Tukey method in the R package *emmeans*. Slopes from the first growth stanza were compared to the expected value of 1 for larval fishes using T-tests (Bochdansky and Leggett 2001).

Results

Growth of log-log transformed wet body mass and total length spanning prelarval, larval, and early juvenile stages (to 118 dph) exhibited three distinct growth stanzas and two inflection points (Fig. 2, Table 1). At the first inflection point, fish length was not significantly different between treatments that shared incubation temperatures, while fish length at the second inflection point was not significantly different among any of the treatments. Each of the three stanzas were fit to the equation $log\ length = log\ a + b\ log\ mass$, where *length* is in mm and *mass* is in g. Among treatments pairwise comparisons of first stanza slopes were not significantly different, neither were any pairwise comparisons of third stanza slopes. However, the second stanza of the 15°C group was significantly different from the 11°C group (p = 0.007) and the 11 – 15°C group (p = 0.046) (Table 1). Altogether, there was very little difference in developmental trajectories with respect to size at developmental transitions, but an increasingly massive size-at-age difference between the 15°C and 11°C rearing temperatures persisted. The age at which fish achieved 1 g wet mass was ~60 dph for 15°C reared fish and ~120 dph for 11°C fish (Fig. 2).

Condition factor varied widely depending on both the method of calculation (K_f vs. K_a) and growth stanza, although patterns were similar (Fig. 3). Through ontogeny, fish exhibited the highest K_f and K_a values during prelarval endogenous stages (first growth stanza), lowest values during larval stage (second growth stanza), and moderate values after transition to early juvenile morphology (third growth stanza). Due to the fixed growth coefficient of 3, K_f was continuous through growth, while K_a was grouped by stanza. K_f ranged from 0.42 to 1.40, while K_a ranged from 0.11 to 3.25. It must be noted that the condition factor values (K_f or K_a) can only be compared within method of calculation and not between.

Mass specific $\dot{M}O_2$ of embryos incubated at 11℃ and 15℃ were not significantly different ($p = 0.1417$, Fig. 4). Larvae incubated and reared at 15℃ were described by $log\ \dot{M}O_2 = log\ 1.84 + 0.53 \times log\ x$; larvae incubated at 11℃ and reared at 15℃ were described by $log\ \dot{M}O_2 = log\ 1.84 + 0.56 \times log\ x$; larvae incubated at 15℃ and reared at 11℃, described by $log\ \dot{M}O_2 = log\ 2.32 + 1.27 \times log\ x$; and larvae incubated and reared at 11℃ were described by $log\ \dot{M}O_2 = log\ 2.27 + 1.16 \times log\ x$ (Fig. 5). Pairwise comparisons of treatment slopes showed significant differences ($p < 0.0001$) between all treatments except those that shared rearing temperatures: 15℃ and 11 – 15℃ ($p = 0.98$) and 11℃ and 15 – 11℃ ($p = 0.90$). When the first stanzas' slopes were compared to the value of $b = 1$, all treatments were significantly different (15℃, $p < 0.0001$; 11 – 15℃, $p < 0.0001$; 15 – 11℃, $p = 0.0149$) except the 11℃ treatment ($p = 0.1890$).

Discussion

Growth

Incubation temperatures did not affect the final length and weight achieved by 118 dph for treatments sharing rearing temperatures (Fig. 6a-d). However, 11℃ rearing severely delayed Green Sturgeon larvae growth, especially during the larval and early juvenile stages, (second growth stanza and second inflection point, Fig. 2), at which the 15℃ reared fish rapidly increased in size (Fig. 6a-d). Stunted growth has cascading effects on foraging ability, swimming ability, and osmoregulation, all of which could contribute to poor recruitment. For example, salinity tolerance in nDPS Green Sturgeon increases linearly with body length and weight (Allen et al. 2011), in conjunction with a rapid increase in mitochondria-rich cell size in the gills between 15 and 45 cm total length (Allen et al. 2009). Similarly, nDPS Green Sturgeon critical swimming velocity increases with size in larval fish ages 20 to 60 dph (Verhille et al. 2014).

Reduced size-at-age also increases the period of vulnerability to predation (Baird et al. 2020). While the predicted effects of climate change are expected to increase water temperatures on average, it is still important to consider the effects of managed artificially cool water on sDPS Green Sturgeon, especially in overlapping spawning habitats and timing with winter-run Chinook Salmon (Fig. 1).

Despite considerably different size-at-age relationships among treatments, LWRs revealed three similar developmental stages (Fig. 2). The three growth stanzas correspond to prelarval, larval, and early juvenile stages and the two inflection points represent the transition between developmental periods (Table 1). These developmental changes likely have survival and recruitment implications as changes in form reflect changes in function and habitat (Fuiman and Werger 2002). Changes in slope across stanzas are known to be influenced by both endogenous and exogenous factors such as developmental stages, temperature, or starvation (Froese 2006). Anecdotal accounts on Green Sturgeon morphology from past work rearing fish at cold temperatures has suggested that cold temperatures lead to fish with large heads and skinny bodies, but our LWRs exhibited remarkably similar trajectories across treatments. In total, our treatments exhibited minimal differences in slope (indicative of growth trajectory) and inflection points (indicative of size at transition between developmental stages), despite severely slowed growth in both 11℃ reared treatments. Total lengths corresponding to the first inflection point were significantly different between the two treatments with 11℃ incubation temperatures (11℃, 11 – 15℃) and the two treatments with 15℃ incubation temperatures (15℃, 15 – 11℃), indicating that cold incubation temperatures can supress growth rate of prelarval fish. However, by the second inflection point, none of the total lengths were significantly different between treatments. Additionally, slopes were not significantly different between treatments within the

first and third stanzas, respectively, and only two pairwise treatments in the second stanza had significantly different slopes (Table 1).

While growth stanzas in larval Green Sturgeon are not a new discovery, they have not been assessed at low temperatures. Gisbert and Doroshov (2006) reared fish up to 50 dph at 16°C and found three stanzas in their LWRs with slopes of 0.76, 2.35, and 3.38 and inflections at 20 and 25 mm. Interestingly, the slopes became steeper with size and both inflection points occurred close to the size at which our first inflection points occurred (23 – 25 mm). The limited size range (10 – 45 mm) observed in Gisbert and Doroshov (2006) could explain this discrepancy. In an effort to replicate their analysis, we limited our data to 50 dph and repeated the analysis. We found only one inflection point for all treatments except 11 – 15°C, which exhibited two inflection points and slope steepness patterns very similar to the original data of our study. As our full dataset substantially expands the range of sizes studied (14 – 200 mm) it is possible that differences in the scale of data would affect the fitting of piecewise linear regression. Additionally, our fish originated from one mother with three fathers and it has been shown that maternal effects can exert a strong influence on early life developmental trajectories (Lindholm et al. 2006). Differences in developmental trajectories across different mothers is a future avenue for study.

As expected, due to the similarities in LWR across treatments, patterns for K_f and K_a were similar across treatments when comparing fish of the same length (Fig. 3, right panels). When comparing by ATU, the pattern was delayed in cold-reared fish, suggesting a lag in development associated with rearing at 11°C (Fig. 3, left panels). Kappenman et al. (2009) measured K_f values of ~0.28 to 0.33 in juvenile Shovelnose Sturgeon (*Scaphirhynchus platorynchus*) reared to 87 dph at temperatures from 8 to 30°C, with optimal K_f values between

16 and 20°C. Ultimately, standalone condition factor metrics from wild Green Sturgeon of these life stages are not likely to be informative due to the size-at-age differences and lack of thermal history information.

Overall, reduced size-at-age observed in cold-reared fish and similar LWR and condition factors suggest that length measurements – a widely-measured, non-lethal metric of field-based sDPS Green Sturgeon growth – cannot be correlated accurately to age without prior knowledge of a fish's temperature history. Importantly, length measurements are the only individual-based data collected for sDPS Green Sturgeon in the SR, because they are captured incidentally by rotary screw traps optimized for and targeting outmigrating salmonid smolts. Length measurements are then compared to optimal growth charts to back calculate age and estimated spawning dates. Furthermore, these incidental captures are the primary source of information on wild larval sDPS Green Sturgeon habitat utilization, habitat type, and timing of downstream migration. For instance, summaries of sDPS Green Sturgeon incidental captures increasingly used to assess relative abundance and species trends exhibited median lengths of 29mm (between 1994 – 2000) and 27.3 mm (between 2002-2012) (Gaines and Martin 2001; Poytress et al. 2014). Both sizes fall within the second stanza of larval sturgeon growth (Fig. 2), which was the most variable across treatments, and would lead to age estimates of 23 to 56 dph in our treatments, highlighting the inherent uncertainty of length-to-age predictions. We suggest measuring weight along with length for all incidental captures of Green Sturgeon juveniles in the SR to better understand how fish are developing in this watershed, and to build a dataset of LWRs across years and environmental conditions for comparison.

Metabolic rates

To our knowledge, this study is the first to report metabolic rates for Green Sturgeon embryos. Given the influence of temperature on ectotherms, embryos followed expected trends with lower metabolic rates at 11℃ compared to 15℃, though they were not significantly different (Fig. 4; Hochachka & Somero 2002; Fry, 1971). All fish hatched within a 24 h period despite the estimated post-fertilization to peak hatch times of ~150 h for incubation at 15℃ and ~220 h for incubation at 11℃ (Van Eenennaam et al. 2005). Incubation differences were likely obscured by the 48 h initial temperature ramp at a rate of 2℃ per day from the spawning temperature of 15℃ to incubation temperature of 11℃. Van Eenennaam et al. (2005) found that 11℃ incubation reduced survival and larvae hatched at shorter lengths than embryos reared at more optimal temperatures (14 – 16℃), despite not differing in dry weight. This was consistent in our hatch lengths, which averaged 14.2 and 17.5 mm ($p < 0.001$) for 11℃ and 15℃ fish, respectively. In Shortnose Sturgeon (*A. brevirostrum*) and Atlantic Sturgeon (*A. oxyrhunchus*), larvae reared at lower than optimal temperatures also hatched at shorter lengths and exhibited slower yolk-sac utilization rates, but temperature did not affect size at completion of yolk absorption (Hardy and Litvak 2004). Interestingly, yolk utilization efficiency was independent of temperature in both species, suggesting that sturgeon are capable of balancing the increasing metabolic requirement of warmer temperatures with a reduction in developmental time and vice versa when in colder temperatures, supporting the similar developmental trajectories seen in our growth data (Fig. 2).

$\dot{M}O_2$ values reported from the log-log relationship between larvae and wet mass are assumed to represent routine metabolic rate rather than standard metabolic rate, and are presented as whole-individual (Fig. 5a) and mass-specific values (Fig. 5b) (Chabot et al. 2016; Peck and Moyano 2016). Although larvae were separated from food prior to experiments,

somewhat confined within the respirometry chambers, and measured during low activity daylight hours, our measurements inevitably included growth and some activity, as larvae were never completely quiescent regardless of acclimation duration during preliminary trials (Kynard et al. 2005; Svendsen et al. 2014). The slope of the relationship between whole-individual $\dot{M}O_2$ and wet mass are used to explain the metabolic scaling of fish. Attempts to describe a universal value of *b* for the metabolic scaling of fish suggest that juveniles and adults scale with exponents close to 0.8 or 0.9, while larval fish scale isometrically with an exponent of 1 due to organogenesis, rapid development, and locomotion costs (Giguère et al. 1988; Clarke and Johnston 1999; Glazier 2005; Jerde et al. 2019). In marine fish larvae, the metabolic scaling exponents range from 0.60 to 1.20, with a mean of 0.87. However, within individual species much more variation is typically seen, showing bi- or tri-phasic relationships in metabolic scaling (Glazier 2005). Consequently, the broader scaling relationships are often only evident where the data spans 6 or 7 orders of magnitude in mass and such datasets are uncommon (Post and Lee 1996; Bochdansky and Leggett 2001).

Temperature influenced how whole-individual $\dot{M}O_2$ scaled with wet mass across prelarval to early juvenile stages of Green Sturgeon (Fig. 5a). Treatments sharing 11°C rearing temperatures (11 – 11°C and 15 – 11°C) exhibited steeper mass scaling exponents of 1.16 and 1.27, respectively, while those sharing 15°C rearing temperatures (11 – 15°C and 15 – 15°C) had exponents of 0.56 and 0.53, respectively (*b* values in Fig. 5a). Our *b* values are between or lower than the exponents given for Green Sturgeon attributed to exogenous and endogenous feeding stages (1.04 and 1.64, respectively) reared at 16°C (Gisbert et al. 2001), and within the range of 0.42 – 1.54 observed in 6 to 24 dph White Sturgeon reared at 14°C in either gravel substrate or no substrate (Boucher et al. 2018). Larger *b* values indicate that total metabolism increases more

quickly per unit mass, while a *b* of 1 indicates isometric metabolic scaling with mass. High mass exponents (i.e. *b*) in early life teleosts are widespread but not universal, with exponents greater than 1.0 more common in pre-feeding larvae (Rombough 1988). The larger *b* values in our 11°C reared fish relative to 15°C suggest that these baseline functions and growth at suboptimal low temperature is more energetically costly (Fig. 5a).

The location of the inflection points in metabolic scaling appear to vary dramatically among species and are hypothesized to correlate to ontogenetic changes in the mass scaling of respiratory surfaces (e.g., cutaneous surfaces and gill lamellar surface area) and not with size at metamorphosis (Post and Lee 1996). We did not detect any statistically significant inflection points in our $\dot{M}O_2$ relationships, which did span metamorphosis from prelarval to larval (first inflection point, Fig. 2) and larval to early juvenile stages (second inflection point, Fig. 2). When compared to the expected larval exponent of 1, all treatments except 11°C were significantly different (Fig. 5a), possibly due to the large size of larval Green Sturgeon relative to that of many marine larval fishes to which this trend seems to apply (Giguère et al. 1988).

Our mass-specific $\dot{M}O_2$ values suggest that cold rearing temperatures are more energetically costly and may contribute to dramatically slowed growth, as evidenced by the positive relationship of mass-specific $\dot{M}O_2$ with wet mass in cold-reared fish (Fig. 5b). Typically, a decrease in mass-specific metabolic rate is expected with an increase in mass and is believed to be related to the increase in the ratio of low metabolic activity tissues to high metabolic activity tissues (e.g. organs) (Oikawa et al. 1991). The increased metabolic requirements in the 11°C reared fish could increase the likelihood of starvation and alter fitness-relevant behaviors (e.g. exposure to predation during increased foraging) during the early life stages (Killen et al. 2007).

Ecological Consequences

Survival is a direct function of growth, mediated through size-dependent predation (Anderson 1988). For wild sDPS Green Sturgeon, survival rates from eggs to juveniles is unknown, and monitoring efforts are a byproduct of salmonid monitoring, and fail to capture the needed information on population sizes and dynamics. The significant delay in growth from rearing at cold temperature increases the duration of predation vulnerability for Green Sturgeon (Goto et al. 2015). Baird *et al.* (2020) found that predation rates on juvenile Green Sturgeon decreased with increasing size, though size classes tested were greater than 50 mm. The Green Sturgeon studied herein metamorphosed into juveniles and developed sharp scutes at lengths between 33 – 36 mm (second inflection point, Fig. 2) and were at the cusp of 50 mm around 118 dph when reared at 11°C, approximately twice the age of fish reared at 15°C. These smaller size classes are likely more vulnerable to predation due to lack of defenses, and prolonged exposure to gape-limited predators. In White Sturgeon, the presence of rocks for cover decreased predation on 14 – 17 mm larvae, consistent with our understanding of preferred spawning habitat being composed of cobble substrate with fast water velocities that prevent silting of interstitial spaces (Gadomski & Parsley, 2005; National Marine Fisheries Service, 2018). On top of delayed growth and associated increased predation-risk, wild larval sDPS Green Sturgeon were observed to have more empty stomachs at colder temperatures, potentially indicating reduced foraging activity or food availability (Zarri and Palkovacs 2019). Similarly, Poletto et al. (2018) found that combining multiple stressors of cold rearing and restricted feed resulted in lowest relative condition in juvenile nDPS Green Sturgeon reared to ~65 dph.

Future Management

In most years, when cold water is available, water temperatures are kept artificially cold (<13.3°C, U.S. Environmental Protection Agency 2003) in the SR from April to June to protect

incubating and rearing juvenile winter-run Chinook Salmon. The management prescribed cold-water releases from Shasta reservoir influence approximately 80 kilometers of the downstream SR, 60 km of which contain adult distribution and confirmed spawning habitat of the sDPS Green Sturgeon (Fig. 1, Heublein et al. 2009; Poytress et al. 2015). This overlap likely exposes wild sDPS Green sturgeon larvae to suboptimal low temperatures during sensitive developmental stages, highlighting a conservation conflict between optimal sDPS Green Sturgeon and winter-run Chinook Salmon management goals.

Given the long-lasting carryover effects of reduced size-at-age due to cold temperature incubation and rearing on sDPS Green Sturgeon shown here, we suggest that more suitable spawning and rearing habitat should be created further downstream to improve growth, survival, and recruitment. This would expand spawning habitat to warmer waters and reduce the conflict between managing the same stretch of river for both winter-run Chinook Salmon and sDPS Green Sturgeon. Currently, substrate augmentation via gravel additions is a viable management strategy for salmonid spawning habitat, but such methods have not been investigated for sturgeon (Stillwater Sciences 2007). To date, there is a lack of information on egg-to-larva survival, juvenile recruitment, and mortality estimates for all life stages. Our study suggests that artificially suppressed water temperatures may be more detrimental to sDPS Green Sturgeon than previously thought and confound age estimation of wild-caught fish in Sacramento River spawning habitat.

Acknowledgements

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undergraduate students at UC Davis for assisting with spawning and providing eggs from the broodstock.

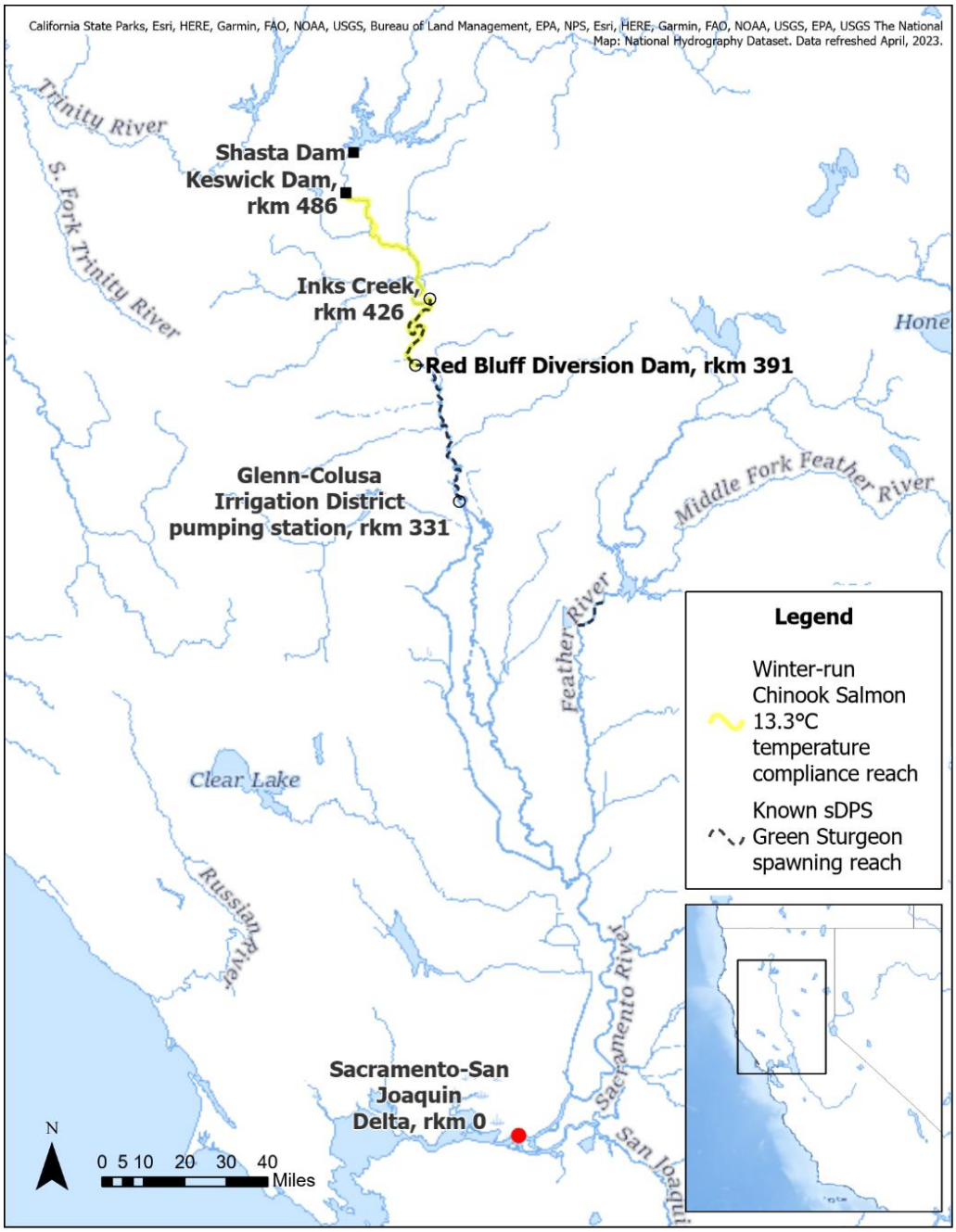

Figure 2.1: Map of the Sacramento River basin in California, showing the confirmed distribution of sDPS Green Sturgeon spawning reaches and the winter-run Chinook Salmon temperature compliance area. River kilometers (rkm) are calculated as the distance in km upstream from the head of Suisun Bay in the Sacramento-San Joaquin River Delta. Map created using ArcGIS® software by Esri. Basemap sources: California State Parks, Esri, HERE, Garmin, FAO, NOAA, USGS, Bureau of Land Management, EPA, NPS, National Hydrography Dataset, refreshed April, 2023.

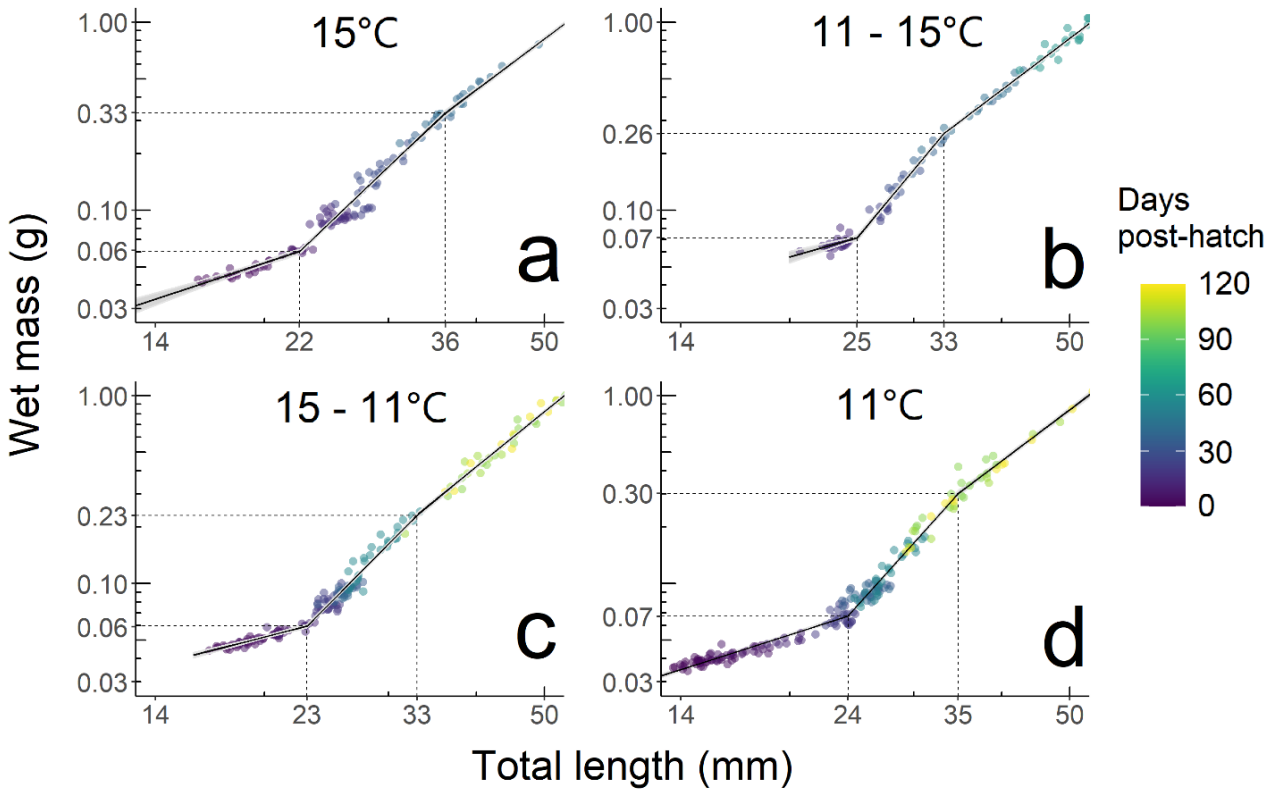

Figure 2.2: Length-weight relationships of Green Sturgeon larvae **(a)** incubated and reared at 15°C, **(b)** incubated at 11°C and reared at 15°C, **(c)** incubated at 15°C and reared at 11°C, and **(d)** incubated and reared at 11°C plotted on log-log transformed axes. Piecewise linear regression estimated using the *segmented* package in R revealed three growth stanzas separated by two inflection points in all treatments. Each stanza is fit to the equation $log\ length = log\ a + b\ log\ mass$, where length is in millimeters and mass is in grams. Colored points represent raw data with age shown by color gradient, lines represent the predicted linear regression, and gray ribbons represent standard error of the fit. Inflection points are indicated by dotted vertical and horizontal lines indicating associated lengths and weights at the axes.

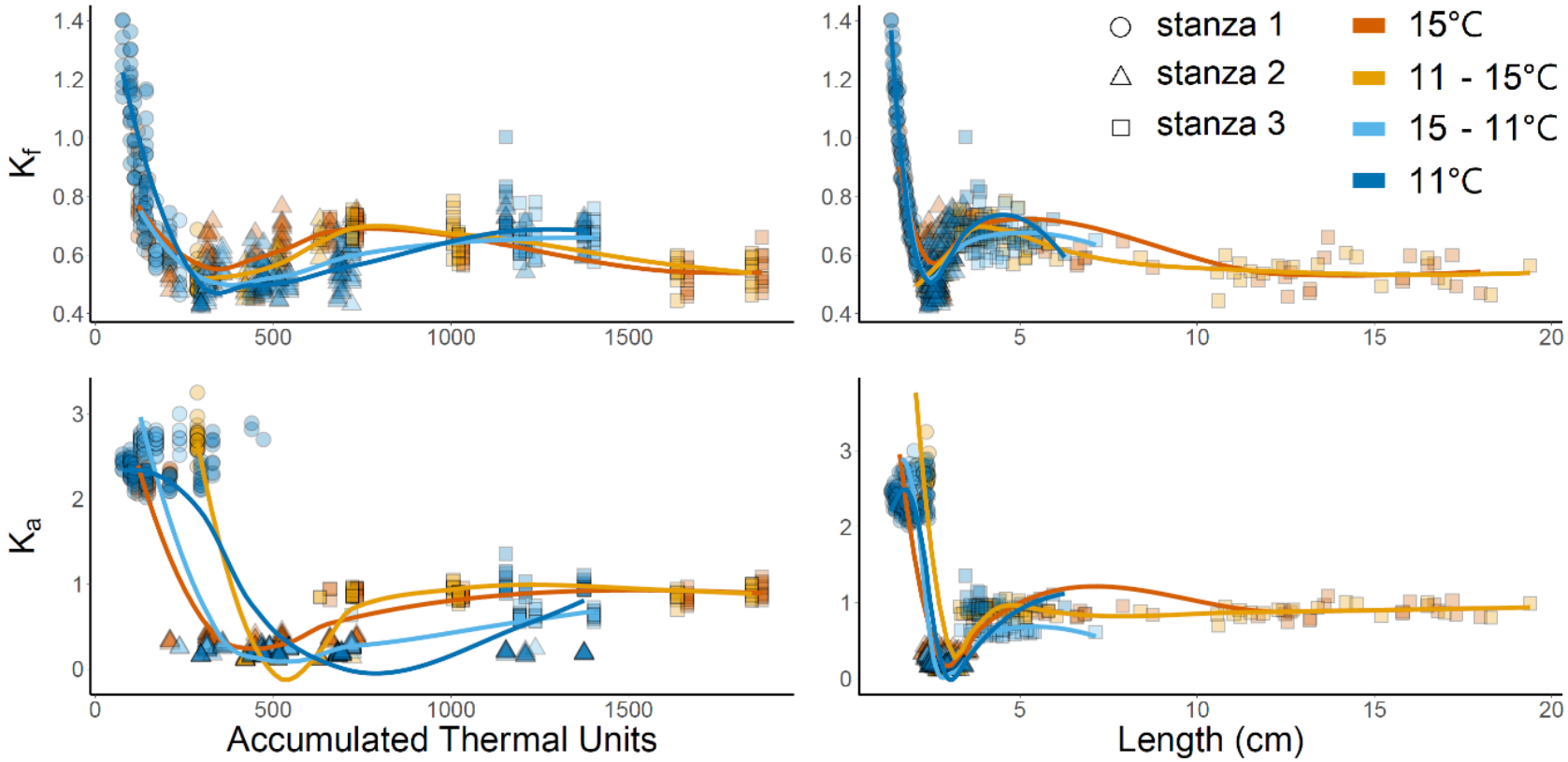

Figure 2.3: Fulton's (K_f) and Allometric (K_a) condition factors by accumulated thermal units (left panels) and length in cm (right panels) of prelarval, larval, and early juvenile Green Sturgeon exposed to different rearing conditions: incubation and rearing at 15℃ (dark orange), incubation at 11℃ and rearing at 15℃ (light orange), incubation at 15℃ and rearing at 11℃ (light blue), and incubation and rearing at 11℃ (dark blue). Lines are fit using Loess smoothing. Shapes indicate which growth stanza a growth measurement falls within each treatment's LWR, with circles representing stanza 1, triangles representing stanza 2, and squares representing stanza 3.

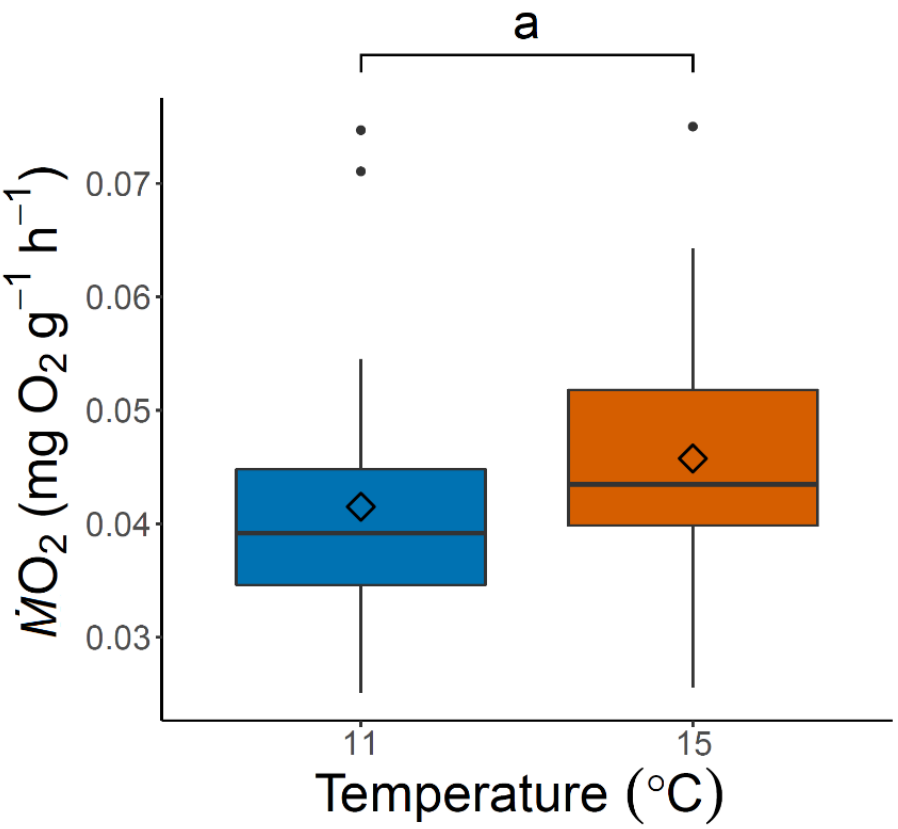

Figure 2.4: Mass-specific metabolic rate (mg O_2 g^{-1} h^{-1}) of embryos incubated at 11°C and 15°C, shown in blue and orange, respectively. The center line of the boxplots represents the median, the box represents the inter-quartile range (IQR), the whiskers extend 1.5 times IQR, and diamonds represent the mean. Mass-specific metabolic rate was not significantly different (p = 0.1417) between the two incubation temperatures.

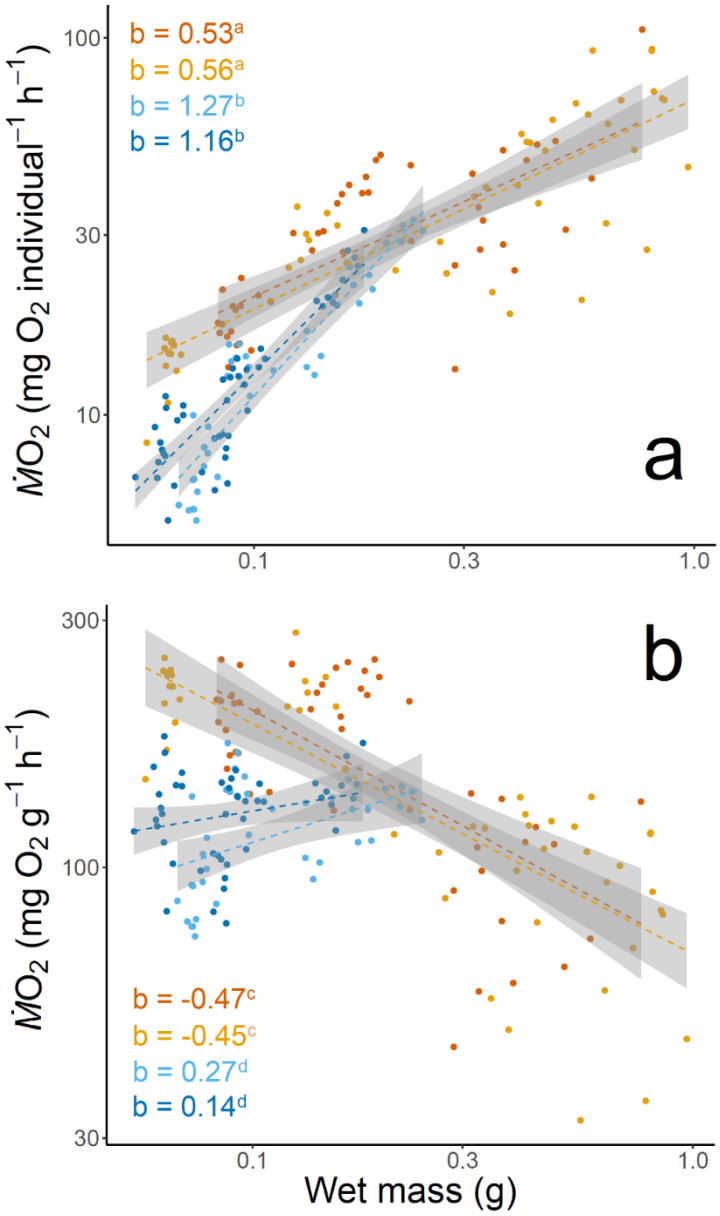

Figure 2.5: Whole-individual metabolic rate (**a:** mg O_2 individual^{-1} h^{-1}) and mass specific metabolic rate (**b:** mg O_2 g^{-1} h^{-1}) against fish wet mass (g) of Green Sturgeon through ontogeny to 63 dph, reared at 11°C or 15°C, plotted on log-log transformed axes. Relationships were fit using the equation $log\ \dot{M}O_2 = log\ a + b\ log\ x$, where x is wet mass in g. For whole-individual metabolic rates (a): dark orange points and line represent larvae incubated and reared at 15°C, described by $log\ \dot{M}O_2 = log\ 1.84 + 0.53\ log\ x$. Light orange points and line represent larvae incubated at 11°C and reared at 15°C, described by $log\ MO_2 = log\ 1.84 + 0.56\ log\ x$. Light blue points and line represent larvae incubated at 15°C and reared at 11°C, described by $log\ \dot{M}O_2 = log\ 2.32 + 1.27\ log\ x$. Dark blue points and line represent larvae incubated and reared at 11°C, described by $log\ \dot{M}O_2 = log\ 2.27 + 1.16\ log\ x$. For mass-specific metabolic rates (b): dark orange points and line represent larvae incubated and reared at 15°C, described by $log\ \dot{M}O_2 = log\ 1.84 - 0.20\ log\ x$. Light orange points and line represent larvae incubated at 11°C and reared at 15°C, described by $log\ \dot{M}O_2 = log\ 1.83 + 0.19\ log\ x$. Light blue points and line represent larvae incubated at 15°C and reared at 11°C, described by $log\ \dot{M}O_2 = log\ 2.32 + 0.12\ log\ x$. Dark blue points and line represent larvae incubated and reared at 11°C, described by $log\ \dot{M}O_2 = log\ 2.25 + 0.06\ log\ x$. Slope values, b, for each treatment are indicated in each treatment's respective color with letters indicating significance between values via pairwise comparison.

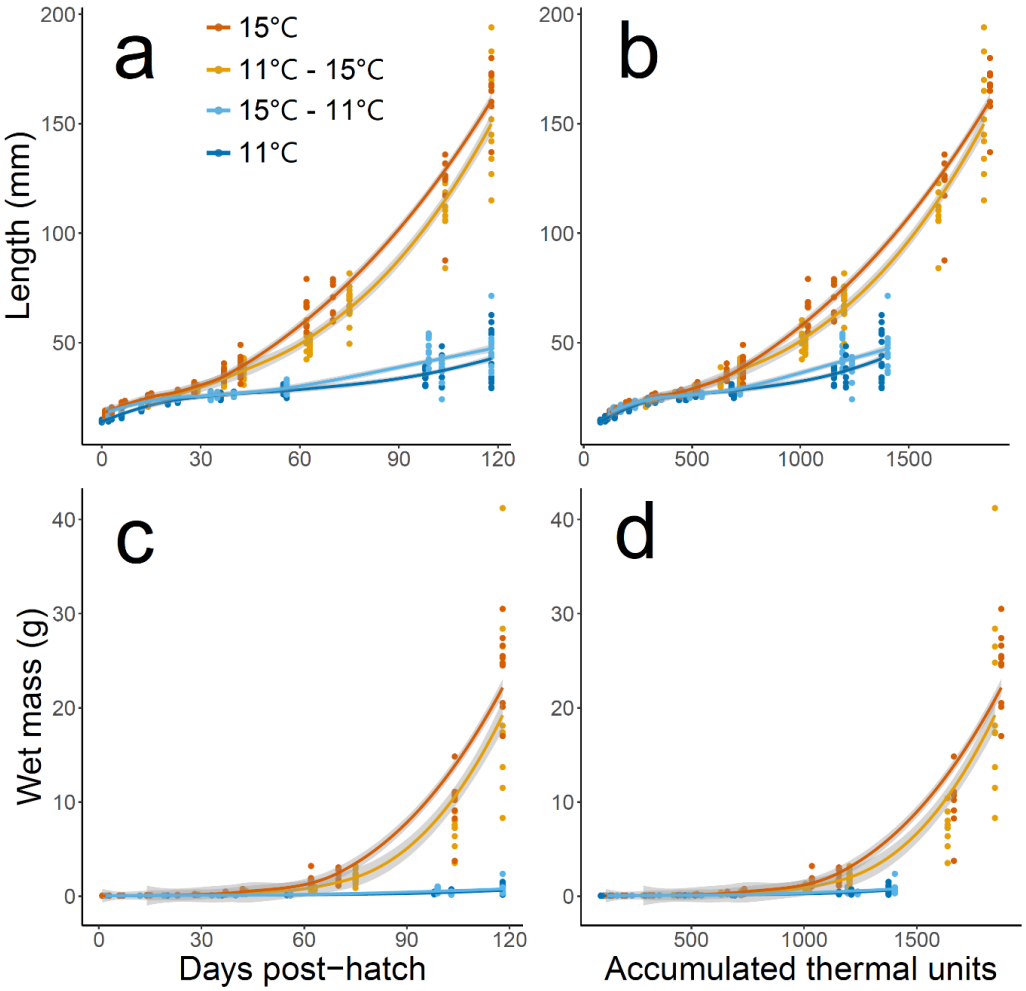

Figure 2.6: Increase in length and mass of Green Sturgeon from hatch to 118 days post-hatch by **(a, c)** age and **(b, d)** accumulated thermal units (ATUs) for each treatment. Dark orange points and lines represent larvae incubated and reared at 15℃ (n = 132). Light orange points and lines represent larvae incubated at 11℃ and reared at 15℃ (n = 107). Light blue points and lines represent larvae incubated at 15℃ and reared at 11℃ (n = 165)). Dark blue points and lines represent larvae incubated and reared at 11℃ (n = 188). Data is smoothed using loess and standard error is indicated in gray.

Table 2.1: Inflection points and slopes presented in length (mm) and mass (g) estimated from the log-log transformed relationship between length and mass in Green Sturgeon from hatch to 1 g wet mass and 50 mm total length for each temperature treatment. Inflection points are presented with 95% confidence intervals, while slopes are presented with standard errors. Inflection point and slope superscripts indicate statistical significance in pairwise comparisons between treatments. N-values represent the number of fish included in the regression for each stanza.

	15°C		**11 - 15°C**		**15 - 11°C**		**11°C**	
First inflection point *Total length (mm) and wet mass (g)*								
	mm	*g*	*mm*	*g*	*mm*	*g*	*mm*	*g*
Lower CI (95%)	21.53	0.0575	24.23	0.0692	22.49	0.0583	23.80	0.0661
Estimate	22.45[a]	0.0605	24.92[b]	0.0713	22.97[a]	0.0595	24.23[b]	0.0675
Upper CI (95%)	23.41	0.0637	25.64	0.0735	23.47	0.0607	24.67	0.0690
Second inflection point *Total length (mm) and wet mass (g)*								
	mm	*g*	*mm*	*g*	*mm*	*g*	*mm*	*g*
Lower CI (95%)	32.44	0.2237	31.75	0.2119	29.80	0.1581	32.67	0.2347
Estimate	36.16	0.3288	33.10	0.2557	33.16	0.2362	34.70	0.3019
Upper CI (95%)	40.31	0.4835	34.51	0.3085	36.90	0.3528	36.86	0.3883
First stanza								
First slope	1.241		1.064		0.961		1.200	
Std. error	0.225		0.442		0.135		0.056	
n	25		24		52		85	
Second stanza								
Second slope	3.551[c]		4.500 [d]		3.757 [cd]		4.168 [d]	
Std. error	0.101		0.211		0.110		0.110	
n	66		25		78		79	
Third stanza								
Third slope	2.809		2.816		3.035		2.811	
Std. error	0.028		0.019		0.089		0.114	
n	41		58		35		24	

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CHAPTER 4

Nutritional stress as a revealing factor in the relationship between standard metabolic rate and locomotor activity in juvenile white sturgeon (*Acipenser transmontanus*)

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Abstract

White sturgeon (*Acipenser transmontanus*) populations in the Sacramento-San Joaquin River Basin are in decline due to habitat impacts, variable recruitment, and altered food availability, all of which are exacerbated by climate change. The minimal metabolic expenditure required to maintain homeostasis, termed standard metabolic rate (SMR), is thought to have broad ecological relevance because it correlates with other important measures of metabolic demand and a range of fitness-related behavioral traits. SMR is repeatable within individuals, yet variable among individuals, and it is thought that this variation may underlie variation in behavior. Additionally, SMR has been shown to exhibit phenotypic flexibility in the presence of changing food availability. The objective of this study was to assess how nutritional stress may affect the relationship between SMR and locomotor activity in juvenile white sturgeon. We reared white sturgeon at 15°C under an optimal feed rate (OFR, 5.3% bodyweight/day) and low feed rate (LFR, 2.6% bodyweight/day) for six weeks, measuring SMR and locomotor activity at the three- and six-week timepoints. OFR fish were significantly larger than LFR fish at both timepoints, but mass-specific SMR was not significantly different across treatment or time. We found that only fish under the greatest nutritional stress (six weeks at LFR) showed a significant relationship between SMR and locomotor activity, supporting the idea of context-dependent responses to stressors. As changing climate is projected to impact food web dynamics and food availability, understanding how nutritional stress affects physiological and behavioral traits in white sturgeon may help to predict their response to future environmental change.

Introduction

White sturgeon (*Acipenser transmontanus*) are anadromous, long-lived, and late maturing fish native to the West coast of North America. They primarily reside in the waterways and estuaries of the Fraser River (British Columbia), Columbia River (Washington and Oregon), and the Sacramento-San Joaquin River Basin (SSJRB) in California. Like most major river systems, the SSJRB suffers from altered flow and temperature regimes due to extensive engineering projects and impassable dams (Yoshiyama *et al.*, 1998; Moyle *et al.*, 2013). White sturgeon spawning and larval rearing in the SSJRB is threatened by this infrastructure as well as climate change, with the frequency of high spring and winter outflows and the intensity of drought events being correlated to variable recruitment levels (Fish, 2010). White sturgeon are not currently listed, but are categorized as a state Species of Special Concern due to habitat impacts and negative predicted population growth rates (Blackburn *et al.*, 2019). A basic understanding of how environmental factors relate to the physiology and behavior of white sturgeon is critical for effective management.

Metabolic rate is a commonly measured physiological trait, as it represents the collective biochemical processes by which organisms transform energy to support life functions and is thought to have evolved in close synchrony with traits relating species' niches and their fitness (Brandl *et al.*, 2023). Standard metabolic rate (SMR), the baseline rate of energy usage required to maintain physiological homeostasis, affects fitness-related traits and thus are expected to be under multiple and often antagonistic selective pressures (Burton *et al.*, 2011; Killen *et al.*, 2016). SMR is variable within species, yet also repeatable across age and mass in many taxa (Nespolo and Franco, 2007). This variation in SMR was largely considered noise or measurement error but, thanks in part to advances in dissolved oxygen measuring technology,

has more recently been acknowledged as consistent between-individual differences in physiology (Metcalfe *et al.*, 2016). Additionally, SMR has been shown to exhibit phenotypic flexibility across days to weeks, with SMR increasing when food availability is increased, and decreasing when food levels decline (juvenile brown trout, Auer *et al.*, 2015, 2016a). Phenotypic diversity and flexibility can be indicative of genetic diversity and the ability of a population to cope and adapt to environmental change. Understanding the ecological and behavioral consequences of variability in SMR may help fisheries managers predict future outcomes to climate impacts.

Physiology and behavior are intertwined, as internal state can affect decision-making in organisms. SMR, for example, has been correlated with growth (Álvarez and Nicieza, 2005), food availability (Auer *et al.*, 2016b), and behaviors such as aggression and boldness (Metcalfe *et al.*, 1995). However, the link between SMR and behavior is not clear, and the effect size, directionality, and any resulting fitness consequences appear to be context-dependent (Armstrong et al. 2011). One hypothesis explaining some of this variation is that an observable link between SMR and behavior may depend on the level of stress the animal is experiencing, with the physiological stressor strengthening or revealing a relationship between the two (Killen *et al.*, 2011, 2012). A common environmental stressor experienced by animals is insufficient nutrition. In European sea bass (*Dicentrarchus labrax*), metabolic rate correlated with risk-takin behavior, but only in fish that had experienced food deprivation (Killen *et al.*, 2011). Thus, nutritional stress may affect the physical state of an organism, leading to altered metabolic budgeting decisions and divergence in behavioral tendencies (Killen *et al.*, 2013).

Changes to flow and temperature in the SSJRB alter food web dynamics, and the impact on white sturgeon is not fully understood. For sympatric threatened green sturgeon, evidence

suggests that river temperature and discharge related to dam releases in the upper Sacramento River reduce the prevalence of preferred zooplankton prey in larval and early juvenile stomach contents (Zarri and Palkovacs, 2019). Nutritional stress and its relationship with behavior is understudied in juvenile white sturgeon and may contribute to poor recruitment. Thus, our goal was to determine whether nutritional stress causes shifts in SMR or alters the relationship between SMR and locomotor activity. We hypothesized that ration size would affect SMR over time, predicting that the amount of food positively correlates with SMR, as metabolic flexibility may be especially important for somatic growth during the juvenile stage – a key determinant of fitness because of its effects on body size (Sogard, 1997). Additionally, we hypothesized that locomotor activity was linked to individual differences in SMR, predicting that individuals with high SMRs will be more active than those with low SMRs because greater activity is needed to support the added energetic demands of a high SMR (Mueller and Diamond, 2001; Speakman *et al.*, 2004). Lastly, we hypothesized that the relationship between SMR and locomotor activity would be affected by nutritional stress, predicting that any correlation between the two would become stronger with greater nutritional stress under the assumption that the relationship may be context dependent (Burton *et al.*, 2011; Killen *et al.*, 2011, 2012).

Materials and methods

Experimental animals

White sturgeon embryos were transported from Sterling Caviar Farm (Sacramento, California) to the Center for Aquatic Biology and Aquaculture at UC Davis in June 2018. All fish were from the same cohort and the number of families was unknown. At hatch, 300 larvae were split evenly among six 300-L replicate flow-through well-water tanks and reared at 15°C. Well-water salinity was 0.4 parts per thousand (ppt) and fish were exposed to natural

photoperiod conditions for Davis, CA (38.5 °N). Since early developmental stages rely on endogenous yolk reserves (Kamler, 2008), fish were not fed until approximately 14 days post-hatch (dph), although food was provided at ca.12 dph to orient larvae to chemical cues (Van Eenennaam *et al.*, 2012). Once feeding was detected, larvae were fed *ad libitum* with semi-moist commercial Starter Crumble feed (Skretting, USA) and excess uneaten feed and feces were removed daily. Feed rates were calculated according to optimal feed rate models for white sturgeon (Deng *et al.*, 2003; Lee *et al.*, 2014) using mean wet mass and water temperature and were updated biweekly to account for fish growth and routinely exceeded to ensure *ad libitum* feed availability. All handling, care, and experimental procedures used were reviewed and approved by the UC Davis Institutional Animal Care and Use committee (IACUC, protocol #21834).

At the onset of the experiment at 50 dph, three tanks received an optimal feed rate (OFR) of 5.3% bodyweight/day and three tanks were reduced to a low feed rate (LFR) of 2.6% bodyweight/day. Feed rates continued to be updated biweekly to account for fish growth. Tank water temperatures were kept at 14.9 ± 0.4°C, monitored using iButton® temperature loggers (model DS1922L, Maxim Integrated Products, Inc., USA) with a sampling rate of 600 s. Respirometry and locomotor activity trials were conducted after 3 and 6 weeks of ration exposure.

Respirometry

Oxygen consumption – an indirect measure of metabolic rate ($\dot{M}O_2$, mg O_2 h^{-1}) – of individual juveniles was measured using intermittent flow respirometry in an 8-chamber system at CABA. Each chamber (146.05 ± 1.25 mL) consisted of a glass jar with a rubber stopper on the end. The rubber stopper was pierced with two stainless steel tubes fit with high grade gas

impermeable silicone tubing which provided recirculating and flush water flow via one 8-channel low-flow peristaltic pump (recirculating flow, Model BT100-1L, Langer Instruments, USA) and one submersible aquarium pump with an 8-channel manifold and check valves to prevent backflow (flush flow). Chambers were submerged in a 78-L water bath with 15°C flow-through well water. Each chamber had an optical oxygen sensor spot (PreSens, Germany) affixed to the inside of the glass chamber wall with silicone glue, which was read through the glass using fiber optic cables and two 4-channel oxygen meters (Witrox 4, Loligo systems, Denmark). The intermittent flow cycle was set such that chambers never fell below 80% O_2 saturation at the end of each measurement to ensure the fish did not become hypoxic and stressed (Svendsen *et al.*, 2016). Flush and recirculation periods were controlled using Autoresp^{TM} software (Loligo systems, Denmark). For each trial, seven fish were randomly chosen from rearing tanks and placed in isolation for a fasting period of 24 h to ensure digestion and absorption of nutrients from daily feeding would not elevate $\dot{M}O_2$ (Chabot *et al.*, 2016). Fish were then transferred to respirometers, with one respirometer left empty to measure background bacterial respiration (Svendsen *et al.*, 2016). After a 1 h adjustment period to the respirometer, $\dot{M}O_2$ measurements began and were conducted for the next ~22 h. The respirometry chambers and water bath were cleaned and dried after every trial, and peristaltic pump tubing was bleached, neutralized, and rinsed weekly to prevent bacterial buildup on surfaces. Each respirometry chamber's sensor spot was calibrated individually with oxygen-free distilled water and fully aerated distilled water every two weeks.

Locomotor activity

Locomotor activity was measured in a circular behavioral arena made from flexible 2 mm white pvc sheeting in a 1 m x 1.5 m water table, allowing the size of the arena to be scaled to the

total length of each fish (8:1, diameter:fish). The entire water table was encircled by curtains to reduce any disturbance to the fish during behavioral trials. Video was recorded using a GoPro situated ~0.5 m above the arena with a linear field of view and captured in 1080p/30 frames s^{-1}. Video analyses were conducted using with EthoVision® XT 14. After fish total length was measured, individual fish were placed in a mesh holding area at the center of the arena and allowed 5 mins to acclimate, which was deemed sufficient via pilot studies and previous white sturgeon behavioral trials (Hansen 2017, unpublished). The mesh holding area was lifted out of the arena remotely by string to prevent disturbances, and the fish was allowed to explore the arena for 10 mins. Locomotor activity was measured as the total distance moved during the 10 min trial divided by the total length of the fish. The water table was drained and cleaned with alcohol after each trial to remove any remaining cues from other fish.

Data and statistical analysis

Metabolic rate was recorded using Autoresp™ software (Loligo Systems, Viborg, Denmark) and data analyses performed using R (version 4.2.1). On average, 50 $\dot{M}O_2$ measurements were collected per fish and values included in analysis were required to have an $R^2 > 0.96$, resulting in an average removal of 12.3% of $\dot{M}O_2$ measurements. For each trial, background bacterial respiration was subtracted from individual fish $\dot{M}O_2$ values. SMR was calculated from mass specific $\dot{M}O_2$ values ($mgO_2\ h^{-1}\ g^{-1}$) following recommendations and R script provided by Chabot *et al.* (2016), which provided a best estimate of SMR using multiple calculation methods. For our data, SMR estimations were best using non-parametric quantile regression in 39 fish and mean of the lowest normal distribution in 13 fish.

Differences in wet mass, total length, SMR and whole body $\dot{M}O_2$ under each feed rate were compared using Welch T-tests at three and six weeks(Fig. 1, Fig. 3). Total length and wet

mass were log-log transformed to assess the scaling of growth over time between feed rates, using the R package *emmeans* to compare slopes by pairwise comparison with the Tukey method. Relationships between SMR, activity level and nutritional stress were compared using linear models with activity and feed rate as interacting fixed predictors. Models were compared separately within week, as models incorporating the entire both time periods resulted in many extraneous pairwise comparisons that were irrelevant. Results were considered significant at $p < 0.05$.

Results

At three weeks and six weeks, mean wet mass (mean ± SD in g; week 3: OFR = 1.98 ± 0.30, LFR = 1.15 ± 0.21; week 6: OFR = 3.76 ± 1.14, LFR = 1.89 ± 0.40) was significantly larger in OFR fish (week 3: difference = 0.83 g, 95% CI [0.60, 1.05], t(20) = 7.68, $p < 0.001$; 95% CI [1.96, 4.58]; week 6: difference = 1.87 g, 95% CI [1.18, 2.56], t(24) = 5.60, $p < 0.001$; 95% CI [1.19, 3.17]) (Fig. 1a). Similarly, at both three and six weeks, mean total length (mean ± SD in cm; week 3: OFR = 7.93 ± 0.50, LFR = 6.76 ± 0.32; week 6: OFR = 9.89 ± 1.02, LFR = 8.03 ± 0.71) was significantly larger in OFR fish (week 3: difference = 1.17 cm, 95% CI [0.80, 1.54], t(20) = 6.65, $p < 0.001$; 95% CI [1.62, 4.04]; week6: (difference = 1.86 cm, 95% CI [1.15, 2.57], t(24) = 5.39, $p < 0.001$; 95% CI [1.13, 3.07]) (Fig. 1b). Log-log transformed length and wet mass indicated significant positive effects of feed rate (beta = 2.71, 95% CI [2.46, 2.96], t(44) = 22.12, $p < 0.001$, Std. beta = 0.90). However, comparisons of the slopes indicated that feed rate did not significantly affect growth trajectories (t(44) = -0.452, p 0.653) during the 6-week duration (Fig. 2).

SMR, which is mass specific, was not significantly affected by feed rate at three (mean ± SD in mg O_2 g^{-1} h^{-1}; OFR = 0.20 ± 0.05, LFR = 0.20 ± 0.06; t(20) = -0.11, $p = 0.913$) or six

weeks (mean ± SD in mg O_2 g^{-1} h^{-1}; OFR = 0.22 ± 0.06, LFR = 0.20 ± 0.04; t(24) = 1.18, p = 0.248) (Fig. 3a). In contrast, mean whole-body $\dot{M}O_2$ at three and six weeks (mean ± SD in mg O_2 individual^{-1} h^{-1}; week 3: OFR = 0.38 ± 0.07, LFR = 0.23 ± 0.07; week 6: OFR = 0.77 ± 0.13, LFR = 0.37 ± 0.11) was significantly larger in OFR fish as expected (week 3: difference = 0.15 mg O_2 individual^{-1} h^{-1}, 95% CI [0.09, 0.22], t(20) = 4.84, p < 0.001; 95% CI [1.00, 3.11]; week 6: difference = 0.40 mg O_2 individual^{-1} h^{-1}, 95% CI [0.31, 0.50], t(24) = 8.66, p < 0.001; 95% CI [2.16, 4.61]) (Fig. 3b). At three weeks, there was no significant interaction of locomotor activity and feed rate (β = 7.83e-03, t(18) = 0.51, p = 0.671), nor was there a significant main effect of LFR (β = -0.02, t(18) = -0.43, p = 0.671) or locomotor activity (β = -1.45e-03, t(18) = -0.13, p = 0.895) on SMR (Fig. 4). At 6 weeks, the interaction of locomotor activity and feed rate (β = 0.02, t(22) = 2.32, p = 0.030) and the main effect of LFR (β = -0.08, t(22) = -2.59, p = 0.017) were significant, while locomotor activity did not have a significant effect on SMR (β = -0.01, t(22) = -1.47, p = 0.157).

Discussion

Feed rate, SMR and locomotor activity

Only fish under the greatest nutritional stress (six weeks at LFR) showed a significant relationship between SMR and locomotor activity (Fig. 4). These results suggest that while metabolic demand can play a role in determining behavior, the extent of these effects may be context dependent and vary, for example, with factors such as the level of nutritional stress (see Killen *et al.*, 2011). LFR fish with relatively higher SMRs may have engaged in increased activity in attempts to acquire food for maintaining their more metabolically demanding machinery. This supports findings by Killen et al. (2011) in which juvenile European sea bass (*Dicentrarchus labrax*) deprived of food for seven days revealed a significant positive

correlation between metabolic rate and activity – measured as the number of transitions between one covered and three open areas of a raceway – when it did not exist prior to food deprivation. Additionally, the authors found a significant positive correlation between instantaneous mass loss and metabolic rate, further supporting that relatively higher SMR values require more energy to maintain. Furthermore, there were among-individual differences in behavioral changes pre- and post-food deprivation. Our study used different individuals between the three- and six-week measurements, revealing only overall trends and not consistent individual differences in the effect of nutritional stress on the relationship between SMR and locomotor activity. This is a particularly interesting avenue for future study, as it is thought that consistent individual differences in physiological state are an important factor that promote the formation of individual differences in personality (Biro and Stamps, 2008; Houston, 2010; Mittelbach *et al.*, 2014).

While the effect of nutritional stress on SMR and locomotor activity is relatively subtle in this study, optimal feed rates are laboratory-based rations for maximum growth and not realistic to what fish experience in the wild. We knew our feed rates were sufficient to create stunted growth between the OFR and LFR fish (Poletto *et al.*, 2018), but lack of wild juvenile white sturgeon diet studies limits comparison to actual feed rates. It is likely that wild white sturgeon experience fluctuations in food availability that have the capacity to influence behavior on a more consistent and extreme basis than seen here, such that our LFR ration represents a greater food availability than typical wild diets. Additionally, body size may affect these behavioral responses, as smaller fish are at greater risk of predation and their higher mass-specific metabolic rates make them more prone to starvation (Post and Evans, 1989).

SMR and feed rate

Contrary to our prediction, LFR fish did not exhibit a reduced SMR relative to OFR fish at either three or six weeks (Fig. 3a), while whole-body $\dot{M}O_2$ behaved as expected, with OFR fish growing larger and thus having a greater quantity of respiring tissue at both timepoints (Fig. 3b). A decrease in SMR after accounting for differences in mass following food deprivation or increase in SMR after increased ration has been shown in coho salmon (*Oncorhynchus kisutch*) after six weeks (Van Leeuwen *et al.*, 2012), Atlantic salmon (*Salmo salar*) after four weeks (O'Connor *et al.*, 2000), and juvenile brown trout (*Salmo trutta*) after four weeks (Auer *et al.*, 2015). In brown trout, these shifts were additionally linked with differences in somatic growth, with individuals who had greater increases in SMR exhibiting faster growth (Auer *et al.*, 2015). These individual differences in response to food deprivation or gain support the idea that consistent individual differences in $\dot{M}O_2$ can be correlated with other physiological changes. Van Leeuwen et al. (2012) suggest that the reduction in SMR under food deprivation is likely due to the involuntary reduction in anabolic and catabolic processes associated with reduced food consumption and growth, rather than a voluntary suppression of metabolism due to low food. Interestingly, starvation physiology of rainbow trout (*O. mykiss*) showed that digestive somatic index declined during starvation, indicating a quicker mobilization of energy reserves from the digestive tract with respect to the overall body mass, while Adriatic sturgeon (*A. naccarii*) mobilized energy from muscle and liver tissues (Furne and Sanz, 2018). Different tissues and organs show considerable variation in mass specific metabolic rates, so nutritional stress with regards to SMR may present differently in sturgeon than other teleosts (Metcalfe *et al.*, 2023). Flexibility in energy metabolism is thought to be important for maximizing growth rates under challenges imposed by variability in food availability, which is a common occurrence in nature for most fishes.

The lack of a response in SMR to low feed in our sturgeon, despite large differences in body size, was unexpected. Extending the time at ration may have resulted in significant differences, but evidence also suggests that sturgeon do not display physiological responses to stress typical to other teleost species. For example, juvenile pallid sturgeon (*Scaphirhynchus albus*) and hybrid pallid × shovelnose (*S. albus* × *platorynchus*) exposed to a 30 s handling exposure failed to evoke increases in plasma cortisol, lactate or glucose, though a significant increase in plasma cortisol was elicited after a 6 h severe confinement stressor with handling (Barton *et al.*, 2000). In Atlantic sturgeon (*A. oxyrhynchus*) and shortnose sturgeon (*A. brevirostrum*), $\dot{M}O_2$ values were 10-20% lower than teleosts of comparable size and post-exercise $\dot{M}O_2$ only increased two-fold. Additionally, the physiological response to exercise subsided relatively rapidly compared to teleosts (Kieffer *et al.*, 2001). Feed restriction in green sturgeon fingerlings, however, did perturb metabolites related to energy metabolism, osmolality regulation, and antioxidation capacity within kidney, liver, and muscle tissues (Lin *et al.*, 2019). Altogether, this suggests that physiological changes resulting from nutritional stress in sturgeon are better studied using molecular techniques that can individually assess organ function rather than whole-body metrics such as SMR, and that primitive fishes should not be assumed to behave physiologically as teleosts do.

Growth

Feed rate significantly affected length and wet mass after both three and six weeks at ration, which was expected as juvenile sturgeon exhibit rapid rates of growth (Fig. 1a, 1b). Body size has important implications for fish performance and survival in nature. In sympatric green sturgeon, predation decreases with increases in size (Baird *et al.*, 2020), while critical swimming velocity (Verhille *et al.*, 2014) and salinity tolerance (Allen *et al.*, 2011) increase with increasing

size. Nutritional levels can also alter heat shock protein levels, affecting critical thermal maximum (Verhille *et al.*, 2016). In contrast, log-log transformed length and wet mass exhibited no difference in the rate of growth under each treatment, indicated by the slope of the length-weight relationship (Fig. 2). This tendency for similar growth trajectories in sturgeon is not fully understood but was also observed in larval and early juvenile green sturgeon (Lo et al. 2023, in review). As all fish for this study were from the same cohort, it is possible that parentage exerted some uniform influence on early-life growth and is an interesting avenue for further study.

Conclusion

The relationship between physiological traits and behavior is an important aspect of individual response that should be investigated, as behavioral shifts are often the first response to a perturbation that an organism can undertake. Additionally, these shifts in behavior may be adaptive responses to increase the probability of survival and could assist in species-specific conservation strategies. Nutritional stress is a ubiquitous environmental stressor, and white sturgeon are just one of several declining anadromous fish species in the SSJRB that are likely to be increasingly impacted by continued food web changes. This study provides evidence that SMR and locomotory activity in white sturgeon juveniles exhibits a context-dependent response to sufficient nutritional stress. Assessing how other types of stressors may affect this relationship is an interesting avenue of future study.

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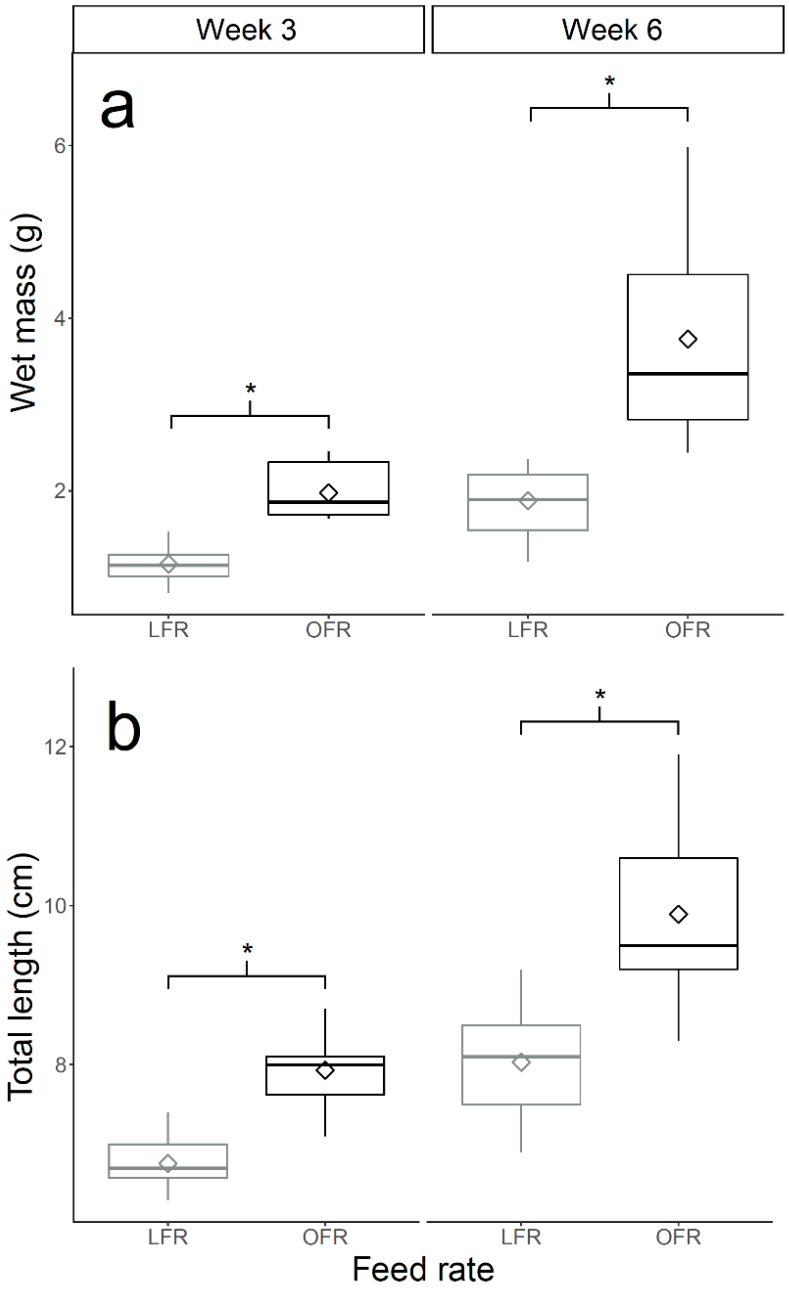

Figure 3.1: Juvenile white sturgeon (*Acipenser trasmontanus*) growth (**a:** wet mass in g and **b:** total length in cm) at 3 and 6 weeks reared at 15°C fed optimal feed ration (OFR) and low feed ration (LFR, 50% of OFR). The center line of the boxplots represents the median, the box represents the inter-quartile range, the whiskers extend 1.5 times IQR, and diamonds represent the mean. Asterisks indicate $p < 0.05$.

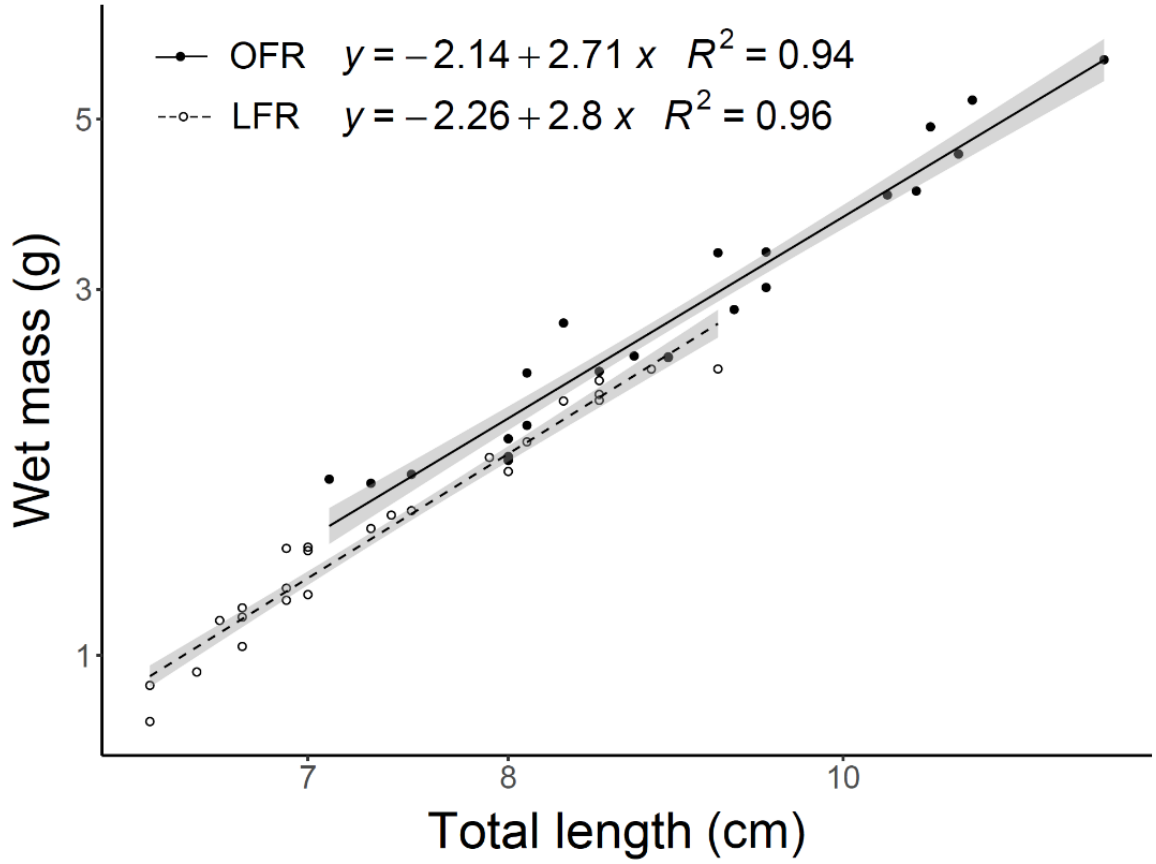

Figure 3.2: Length-weight relationships of juvenile white sturgeon (*Acipenser transmontanus*) reared at 15°C fed optimal feed ration (OFR, closed circles and solid line) and low feed ration (LFR, 50% of OFR, open circles and dashed line), plotted on log-log transformed axes. Lines represent least squares regressions and gray ribbons represent standard error of the fit.

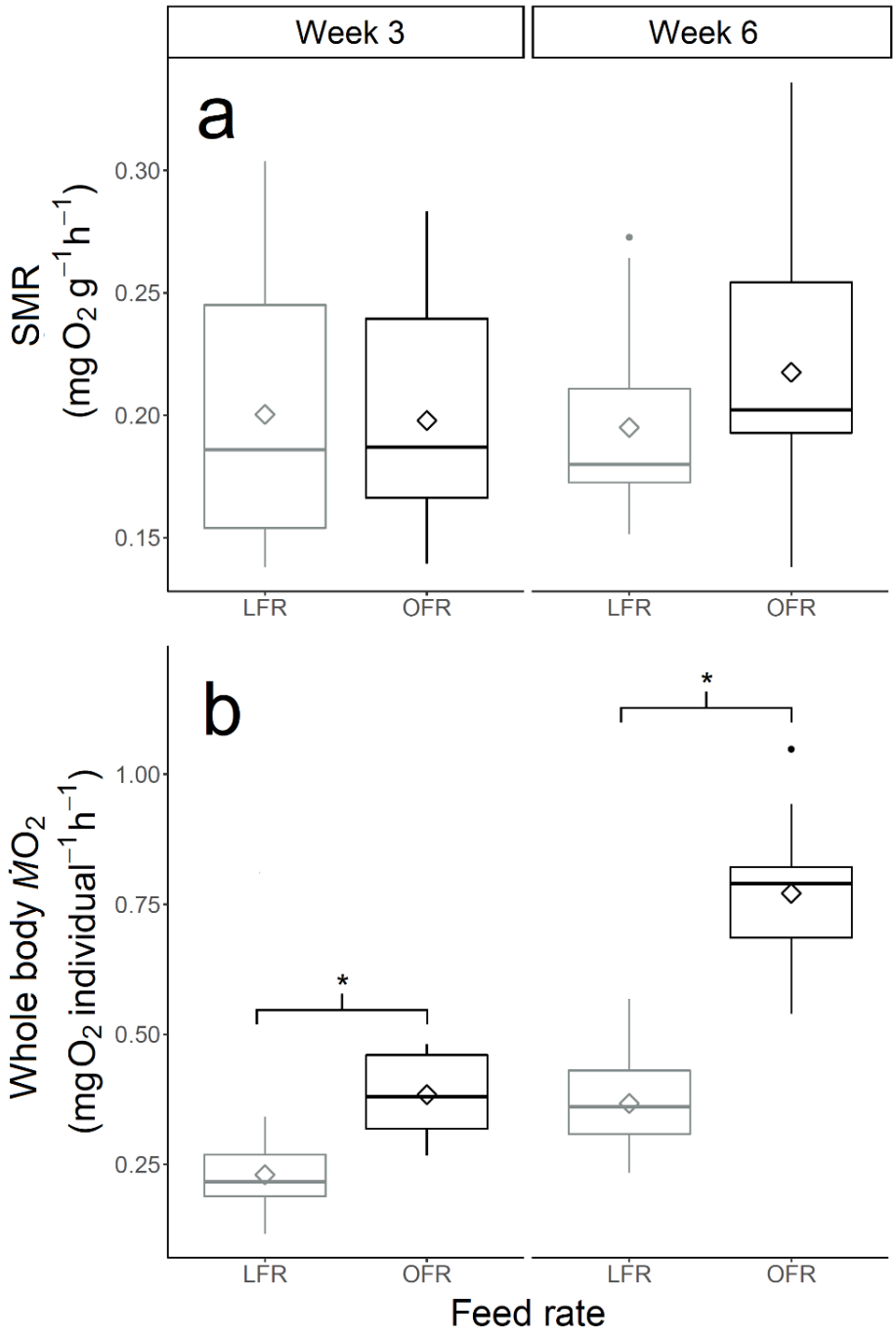

Figure 3.3: Metabolic rates (**a:** Standard metabolic rate, SMR, mg O_2 g^{-1} h^{-1}; **b:** Whole-body metabolic rate, mg O_2 $individual^{-1}$ h^{-1}) of juvenile white sturgeon (*Acipenser transmontanus*) reared at 15°C and fed optimal feed ration (OFR, in black) and low feed ration (LFR, 50% of OFR, in gray). The center line of the boxplots represents the median, the box represents the inter-quartile range (IQR), the whiskers extend 1.5 times IQR, and diamonds represent the mean. Asterisks indicate $p < 0.05$.

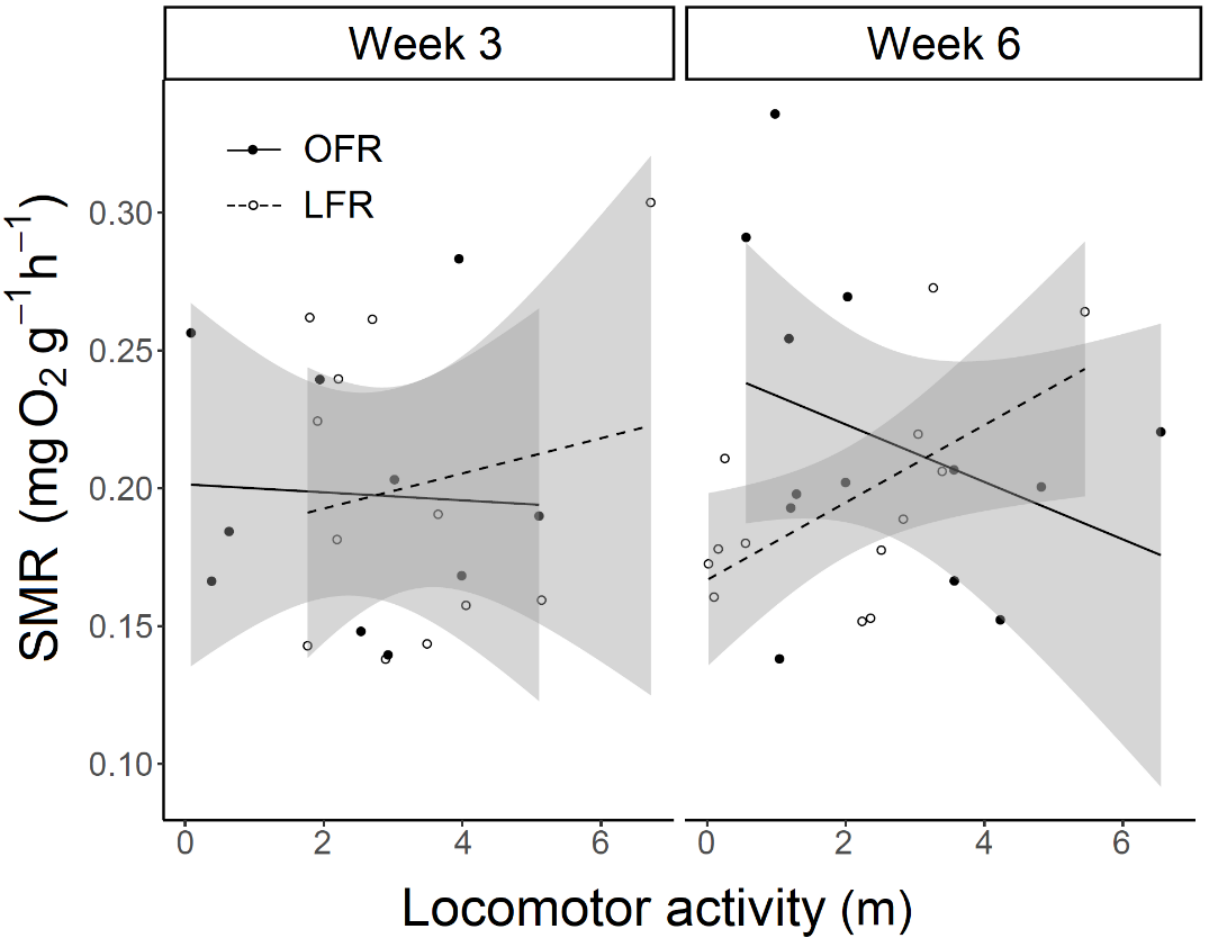

Figure 4: The relationship between SMR and locomotor activity of juvenile white sturgeon (*Acipenser transmontanus*) reared at 15°C and fed optimal feed ration (OFR, closed circles and solid line) and low feed ration (LFR, 50% of OFR, open circles and dashed line). Neither feed rate exhibited significant relationships between SMR and locomotor activity at 3 weeks, nor did OFR fish at 6 weeks. The significant linear least squares regression for LFR fish at 6 weeks is represented by: locomotor activity = 0.167 + 0.014 (SMR), $p = 0.028$.

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CHAPTER 5

General Discussion

My dissertation research has furthered our understanding of the effects of temperature on bioenergetic processes in anadromous fishes. Specifically, there was a lack of information on the energetic requirements of early life history stages, especially in sturgeon species. Metabolism, the sum of the biochemical processes that organisms use for both energy intake and to support all its life functions formed the foundation for this research. My dissertation research investigated effects of temperature and nutrition on aspects of metabolism such as digestion, growth, and energy allocation to behavior. As much of the anadromous fish habitat in California is declining in quality due to anthropogenic impacts, it is important to understand the capacity of their physiological response, especially under conditions that reflect the degrading habitats. My research adds to the growing literature acknowledging broader and cascading physiological, behavioral, and ecological impacts that elevated temperature exerts on the lives of ectotherms.

The responses of anadromous fishes to climate-change related stressors such as temperature is a major focus of management agencies working in the Sacramento-San Joaquin River Basin (SSJRB). For Chinook salmon, this often refers to warming temperatures approaching their upper thermal limit due to blocked access to historical habitats with cooler waters than the mainstem Sacramento River. It is becoming more apparent that population-specific criteria may need to be determined to replace the current rigid temperature criteria derived from northerly stocks of Chinook salmon (U.S. Environmental Protection Agency, 2003; Zillig *et al.*, 2021). In contrast, sturgeon thermal criteria lack supporting data, and the effects of current cold water dam releases is not known. My research aimed to fill some of these gaps by investigating stage- and species-specific responses to thermal and nutritional stress.

Chapter 2 demonstrated that juvenile Chinook salmon have significant digestive capability across a broad range of temperatures, providing novel data quantifying the energy required for digestion and presenting it in the context of fish energy budgets. These results suggested that juvenile Chinook are well adapted to the heterogeneous conditions found in freshwater habitats and that the cost of digestion with respect to energy supply is likely not a concern in water temperatures up to 24°C. This supports findings that juvenile Chinook salmonids from British Columbia exhibit peak growth at warm temperatures (18.9-20.5°C) fed maximal rations (Brett *et al.*, 1982) and similarly, that juvenile Chinook from the Sacramento River exhibited the same growth rates until daily temperatures exceeded 20°C (Marine and Cech, 2004). However, these warm water growth capabilities necessitate adequate food availability, which is a growing concern in dammed, leveed, and otherwise modified waterways (Jeffres *et al.*, 2020; Lusardi *et al.*, 2020). Approximately 95% of historic floodplain wetlands in the Central Valley of California have been drained or are no longer accessible to aquatic species (Frayer *et al.*, 1989), eliminating seasonal floodplain habitat that is significantly more productive and increases daily growth rates of juvenile Chinook salmon relative to main-river channels (Cordoleani *et al.*, 2022). Future avenues of study include assessing the cost of digestion under fluctuating temperature regimes or in thermal preference apparatuses, to refine costs of digestion under more natural conditions and to understand the behavioral use of thermal refugia, which occurs in the wild (Brewitt *et al.*, 2017). The costs of digestion quantified in Chapter 2 can be incorporated into bioenergetic models to better understand processes such as fish growth, nutrient cycling, food web dynamics, and habitat selection specified for juvenile Chinook salmon in the SSJRB (Trudel *et al.*, 2005; MacFarlane, 2010).

Chapter 3 expanded limited knowledge on basic physiology of green sturgeon early life history stages, by examining how human-managed suboptimal low water temperature (11°C) causes dramatically slowed growth and increased metabolic expenditure. The effect of low temperature on sDPS green sturgeon ontogeny has long been an afterthought due to their later listing under the Endangered Species Act relative to winter-run Chinook salmon, which inhabit and spawn in adjacent stretches of the upper Sacramento River. In 2009, an independent scientific review of the National Marine Fisheries Service's (NMFS) Biological Opinion on the long-term operations of the Central Valley Project and State Water Project specifically cited a lack of evaluation of the effects of artificially cool summer temperatures on sturgeon development (CALFED science review panel, 2009). At the 2021 5-year review for the species, flow and temperature guideline criteria were still not designated, remaining as a medium threat and high priority for research and development (Vick, 2021). My research helped to fill this gap by providing baseline information on how cold incubation and rearing temperatures affect the earliest life stages of green sturgeon. The results caution against age calculations without thermal history information, and suggest that weight measurements would be a valuable additional metric to collect in conjunction with length to better understand developmental trajectories through length-weight relationships in wild-caught sDPS green sturgeon juveniles. With regards to federally listed species for which species recovery plans exist, systematically addressing identified threats and data insufficient areas should be a priority for researchers looking to improve species-specific conservation outcomes.

Chapter 4 addressed behavioral and physiological changes in juvenile white sturgeon in response to nutritional stress. When a fish is exposed to a stressor or perturbation, shifts in behavior are often the first line of defense, and these behaviors may be adaptive for increasing

the probability of survival. Additionally, behavioral shifts may be more easily interpreted within an ecological context relative to physiological traits (Lockridge, 1981). I found that nutritional stress caused shifts in the relationship between metabolic rate and locomotory activity, though further research is needed to assess how food availability in the wild may compare to the level of nutritional stress investigated here. Additionally, understanding that certain behavioral responses can be context-dependent is important to grasp the breadth of response to a singular stressor. This study utilized video tracking technology, which has greatly improved for fish in recent years. Additionally, the ability to quantify fish behavior objectively is allowing more complex analysis (Kane *et al.*, 2004; Li *et al.*, 2022). Conservation behavior is increasingly acknowledged as a method to inform common management interventions such as habitat restoration, spatial planning, and protected areas (Cooke *et al.*, 2023).

Together, my thesis investigates novel species-specific aspects of bioenergetics on physiology and behavior. As Chinook salmon, green sturgeon, and white sturgeon are all in decline, it is critical to improve our understanding of how they cope with changing temperature and nutrition going forward to improve management strategies. Further, other sturgeon and salmonid species are in decline all over the world. Many avenues of future research remain, especially with regards to migratory fish species in other impacted watersheds.

Over 60% of the world's longest free-flowing rivers are impeded by dams or man-made infrastructure (Zhang and Gu, 2023). These dams impact the flow of nutrients (Maavara *et al.*, 2015), reduce habitat quality and connectivity (Barbarossa *et al.*, 2020), and extirpate aquatic insects via unnatural artificial flow regimes (Kennedy *et al.*, 2016). A boom in hydropower is expected globally as the demand for electricity rises with the modernization of developing countries, mainly taking place in South America, South and East Asia, and the Balkan region

(Zarfl *et al.*, 2019). On the opposite end, dam removals have allowed fish to recolonize previously inaccessible reaches. This was observed when the Elwha dam in Washington, USA was removed, prompting 8 anadromous fish species to return within 2.5 years (Duda *et al.*, 2021). As such, opportunities abound for comparing the characteristics of pre-/post-dam and pre-/post-removal in conjunction with the physiology and behavior of the anadromous fish species that will be impacted. Understanding how intraspecific variation can cause fish to vary in the acquisition and utilization of energy, the breadth of this variation, and how the environment modulates links between metabolic traits and behaviors may allow us to predict how fish may respond to such large-scale changes. Both future dams and dam removal provide opportunities to inform one another and to help managers improve dam building practices, water management, and habitat restoration. As climate change continues to impact aquatic ecosystems globally, continuing to link physiology and behavior will be critical as we strive to best mitigate the impacts on fishes.

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