UNIVERSITY OF CALIFORNIA

Santa Barbara

Conservation Physiology of Fishes in the Anthropocene

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

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Jacey Cassandra Van Wert

DEDICATION

To my late sister, Delany Alexandra Van Wert, who died from a brain aneurysm on May 7, 2010, at the age of 12. I would not be the person I am today without the 12 years I had with you. I hold on tight to our memories in our Philomena home in Niskayuna, New York with our brothers, dogs, and friends. But even more, I know what Bethany Beach, Delaware, meant to you. Though we only made this trip once a year, we loved it because it brought family together, with sunlight, warmth, and safety. We dug for clams, made mud pies, and floated in the soft waves together. While you rest in the ocean waters, I find myself drawn to the sea. I am here now, earning my PhD having studied aquatic ecosystems and spending much of my time in the water. We both felt drawn to the natural world- through animals and nature- and I know you wanted the best for our world. So this is for you.

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VITA OF JACEY CASSANDRA VAN WERT

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ABSTRACT

Conservation Physiology of Fishes in the Anthropocene

by

Jacey Cassandra Van Wert

Human activities threaten aquatic ecosystems and their biodiversity to the extent that we are now living in an era known as the Anthropocene. Fish respond to environmental threats (in part) by changing their physiology, which is reflected across many levels of biological organization. My research focused on the physiology of fishes in vulnerable systems to gain insights into the mechanisms underlying fish responses to human-induced stressors. Specifically, I worked in two threatened systems – coral reef fishes and Pacific salmon – which, although seemingly different, are highly productive and closely tied to the daily lives of humans. These systems are also similarly threatened by warming and fishing activities. In this thesis, I assessed: 1) the nutrient contribution of fish feces to coral reefs, 2) the acclimation capacity and thermal tolerance of a coral reef fish during simulated marine heatwaves, 3) population-specific thermal tolerance of pre-spawning Chinook salmon, and 4) the importance of the heart for aerobic performance in coho salmon. From community-level nutrient inputs down to the cellular function of the heart, my thesis demonstrates that we must consider performance across biological scales when thinking about the conservation and resilience of aquatic ecosystems.

PREFACE

The data presented in the chapters within this thesis are published or in press for peer-review publication. Further details, including supplementary information, can be found at the DOI for each publication. In all four chapters, JC Van Wert was the primary contributor to the experimental design, data collection, data analysis, and manuscript preparation.

Chapter 1: Van Wert JC, Ezzat L, Munsterman K, Landfield K, Schiettekatte N, Parravicini V, Casey J, Brandl S, Burkepile DE, Eliason EJ. (2023). Fish feces reveal diverse nutrient sources for coral reefs. *Ecol*. https://doi.org/10.1002/ecy.4119.

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1.1 Abstract

Consumers mediate nutrient cycling through excretion and egestion across most ecosystems. In nutrient-poor tropical waters such as coral reefs, nutrient cycling is critical for maintaining productivity. While the cycling of fish-derived inorganic nutrients via excretion has been extensively investigated, the role of egestion for nutrient cycling has remained poorly explored. We sampled the fecal contents of 570 individual fishes across 40 species, representing six dominant trophic guilds of coral reef fishes in Mo'orea, French Polynesia. We measured fecal macro- (proteins, carbohydrates, lipids) and micro- (calcium, copper, iron, magnesium, manganese, zinc) nutrients and compared the fecal nutrient quantity and quality across trophic guilds, taxa, and body size. Macro- and micronutrient concentrations in fish feces varied markedly across species. Genera and trophic guild best predicted fecal nutrient concentrations. In addition, nutrient composition in feces was unique among species within both trophic guilds (herbivores and corallivores) and genera (*Acanthurus* and *Chaetodon*). Particularly, certain coral reef fishes (e.g., *Thalassoma hardwicke*, *Chromis xanthura*, *Chaetodon pelewensis* and *Acanthurus pyroferus*) harbored relatively high concentrations of micronutrients (e.g., Mn, Mg, Zn and Fe, respectively) that are known to contribute to ocean productivity and positively impact coral physiological performances. Given the nutrient-rich profiles across reef fish feces, conserving holistic reef fish communities ensures the availability of nutritional pools on coral reefs. We therefore suggest that better integration of consumer egestion dynamics into food web models and

ecosystem-scale processes will facilitate an improved understanding of coral reef functioning.

1.2 Introduction

Nutrient cycling is important in driving ecosystem function and sustaining biological diversity (Cherel et al., 2011; DeAngelis et al., 1989; Ratnarajah et al., 2014; Stears et al., 2018; Williams et al., 2018). Animals cycle nutrients by sequestering, transporting, transforming, and releasing nutrients as waste products by excretion (elimination of assimilated food) and egestion (elimination of unassimilated food) (Atkinson et al., 2017; Vanni, 2002). For example, wildebeest transport nitrogen across the Serengeti, while baleen whales concentrate and deposit trace metals in pelagic waters (McNaughton et al., 1988; Ratnarajah et al., 2014). However, biodiversity loss is increasing at an alarming rate due to local and global disturbances with the potential to alter system-wide nutrient dynamics (Barnosky et al., 2011; Pereira et al., 2012). Despite the importance of animals in cycling nutrients across many ecosystems, we know remarkably little about how individual species within communities and their associated traits (e.g., taxonomy, diet) may influence their role for system-wide nutrient cycling (but see Allgeier et al., 2017; Peters et al., 2019; Wing et al., 2021). This is particularly true in highly diverse ecosystems such as coral reefs, which host a quarter of the global marine biodiversity (Carpenter et al., 2008; Plaisance et al., 2011).

Coral reefs are among the most productive ecosystems on Earth (Hatcher, 1988). In these oligotrophic systems, primary producers take up dissolved inorganic nutrients as rapidly as they are released because near-reef concentrations are typically low (Souter & Lindén, 2000). Hence, nutrient cycling by consumers is vital in maintaining high productivity (Allgeier et al., 2017; De Goeij et al., 2013). Coral reef fishes comprise some of the highest biomass of

consumers on coral reefs (Jackson et al., 2001; Sorokin, 1993), and their high biodiversity (e.g., taxonomic and functional diversity) can sustain critical ecosystem processes (Brandl et al., 2019; Lefcheck et al., 2019). Fish are involved in important top-down ecosystem functions, including predation and the removal of algae and detritus (Bellwood et al., 2004; Brandl et al., 2019; Green & Bellwood, 2009; Schiettekatte et al., 2022; Tebbett et al., 2022). However, reef fishes also support coral reefs via bottom-up processes by supplying inorganic nutrients (nitrogen [N] and phosphorus [P]) to the reef ecosystem via excretion and egestion (Allgeier et al., 2017; Burkepile et al., 2013; Holbrook et al., 2008; Meyer et al., 1983). In fact, schooling fish that shelter within corals may stimulate coral growth by up to 21% through the provisioning of N and P (Meyer & Schultz., 1985), and these nutrients may enhance resistance to thermal stress in corals (Chase et al., 2018; Shantz et al., 2023). Although much focus has been on the inorganic nutrients derived from fish excretion (Allgeier et al., 2017; Burkepile et al., 2013; Munsterman et al., 2021; Schiettekatte et al., 2022), fish feces (via egestion) also harbor key nutrients (Bray et al., 1981; Pinnegar & Polunin, 2006) that may play significant roles in coral reef ecosystem functioning (Meyer & Schultz, 1985; Rempel et al., 2022; Schiettekatte et al., 2023; Williams et al., 2018). The scarce literature available on the subject suggests that coral reef fish feces may contain macronutrients (i.e., proteins, carbohydrates, lipids) and micronutrients (e.g., zinc, iron, magnesium) that vary in concentration across species and trophic guilds, which may greatly alter the value of fecal material for other organisms (Bailey & Robertson, 1982; Crossman et al., 2005). However, this has only been investigated across a small number of reef fish species. It stands to reason that the identity and concentration of both macro- and micronutrients in feces (hereinafter referred to as the “nutrient profile”) may largely depend

on taxonomy, trophic guild (broadly defined here by fish diet), and life phase of coral reef fishes. As such, nutrients derived from the feces of different fishes could represent a diverse pool of resources for corals and other reef macro and microorganisms. In particular, certain minerals (Mg, Mn, Fe) appear to mitigate coral bleaching during thermal stress and may be especially important in resilience to climate change (Biscéré et al., 2018; Ferrier-Pagès et al., 2018; Houlbrèque & Ferrier-Pagès, 2009). Gaining additional insights into the diversity and abundance of nutrients supplied by reef fishes via egestion may reveal a more nuanced picture of the functional roles of fishes as nutrient recyclers.

Here, we compare fecal nutrient profiles across a diverse range of coral reef fish species. Specifically, we quantify macro- (proteins, carbohydrates, lipids) and micro- (calcium, copper, iron, magnesium, manganese, and zinc) nutrients, in addition to water content and ash, for 570 individuals across 40 species, 10 families, and six trophic guilds in Mo'orea, French Polynesia. We selected these nutrients because they are involved in fundamental biochemical and physiological processes (e.g., photosynthesis and cellular respiration) across many organisms and are identified as critical nutrients for many coral reef taxa (Ferrier-Pagès et al., 2018). The objectives of our study are to (1) characterize the macro- and micronutrients in the feces of a diverse reef fish community and (2) determine how fecal nutrient profiles vary across fish traits, including body size, taxonomy (family, genus, species), and trophic guild.

1.3 Methods

1.3.1 Sample collection

We collected fishes around Mo'orea, French Polynesia (17.5388° S, 149.8295° W) between July and August 2018 and August 2019 in two separate datasets. Concerning the spelling of Mo'orea, we followed the *Te Fare Vanā'a* transcription system that is adhered to by a large segment of the Tahitian community, but also recognize other community members follow the *Raapoto* transcription system where the island name is spelled without the 'eta (i.e., Moorea) (see mcr.lternet.edu/spelling of Tahitian_place_names). Dataset 1 includes fish individuals (N = 317) that were collected at the North Shore forereef, fringing reef, and backreef habitats across 14 sites. A subset of fishes from Dataset 1 (N = 34) was collected from roadside stands during this sampling time, but the precise collection sites around Mo'orea are unknown and these collection sites were classified according to stand location (e.g., East or West). Dataset 2 includes fish individuals (N = 253) collected on the northern, eastern, and western shores of Mo'orea across the forereef, reef crest, backreef, and fringing reef habitats across 48 sites. In total, we collected 570 individuals across 40 species (minimum four individuals per species) of fish and 61 different sites. These species represent 70% of non-elasmobranch fish biomass on coral reefs in Mo'orea (Brooks, 2022). These fishes represent 10 families (Acanthuridae, Balistidae, Chaetodontidae, Cirrhitidae, Holocentridae, Labridae, Lutjanidae, Monacanthidae, Pomacentridae, Serranidae), spanning six trophic guilds (corallivores, detritivores, herbivores, invertivores, piscivores, and planktivores) as guided by the MCR LTER (Brooks, 2022), FishBase (Froese & Pauly, 2022) and Parravicini et al. (2020). Fishes were collected via spearfishing between 0945 and 1500 and were transported on ice back to either the University of California Gump Research Station (Dataset 1) or the Centre de

Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) in Mo'orea (Dataset 2). In the lab, fish were weighed (g) and measured for fork length (Dataset 1) or total length (Dataset 2) (cm). Feces were removed from the last 4 cm of the large intestine and were either kept in 1.5 mL Eppendorf vials at −20°C and transported back to University of California Santa Barbara in the United States (Dataset 1, N = 317) and freeze-dried for >36 h each to measure water content, and ground using a conical glass homogenizing pestle, or both frozen and freeze-dried for >24 h each at CRIOBE Mo'orea and transported to the CRIOBE in Perpignan, France, where samples were ground to a fine powder using a homogenizer (Dataset 2, N = 253).

1.3.2 Macronutrient quantification

Macronutrients (protein, lipid, carbohydrate) and ash were assessed only for Dataset 1. For protein and carbohydrate analysis, we measured 10 mg of homogenized sample into 2 mL screw cap vials, diluted each sample with MilliQ water with a dilution factor of 100, and homogenized samples at 6 m/s for four 30 s cycles (Fisher Brand Bead Mill 24) with ~10 mg 0.5 mm zirconium oxide beads. These homogenates were stored in −20°C until further use. To measure total protein, we used a modified bicinchoninic (BCA) assay (Barbarino & Lourenço, 2005; Mann & Gallager, 1985). Using a thawed aliquot (50 µL) of the homogenate, we precipitated the protein from the sample or bovine albumin serum (BSA) standard with 72% trichloroacetic acid (TCA) and removed the supernatant to eliminate potential interferences, including lipids and free amino acids. We then followed a modified microplate BCA assay protocol (Thermoscientific Pierce BCA Kit) to measure absorbance at 562 nm in triplicate in a spectrophotometer multi-mode plate reader (SpectraMax id3, Molecular Devices). We measured carbohydrate using a modified version of the phenol-

sulfuric acid method to determine total sugar in glucose equivalents. We extracted the carbohydrate from the samples (250 µL aliquot of the homogenate thawed, re-homogenized for 30 s at 6 m/s) and standard using cold 15% TCA, incubated samples at 4°C for 30 min, spun in a micro centrifuge (1000 rpm, 10 min), and collected the supernatant containing carbohydrates. We then estimated the carbohydrate concentration using the phenol-sulfuric acid method (DuBois et al., 1956) with a modified microplate method (Masuko et al., 2005). We measured absorbance in triplicates at 490 nm with glucose as the standard (SpectraMax id3, Molecular Devices).

To measure lipid content, we followed the methods of Mann and Gallager (1985) and Johnson et al. (2017). We measured samples in duplicates (40–50 mg; 5–10 mg when minimal sample available) into solvent-washed test tubes, added and vortexed with 100 µL water and 1.5 mL chloroform: methanol (1:2). Samples were then incubated at 4°C for 10 min and centrifuged (4000 rpm, 5 min), with the supernatant removed to a separate test tube. The remaining sample was re-extracted in 1.5 mL chloroform: methanol (2:1) and the supernatants were pooled, mixed with 950 µL NaCl (0.7%), and incubated at 4°C for 30 min. To separate the phases, samples were then centrifuged (4000 rpm, 5 min). The lower phase was measured for volume, and 1 mL was then deposited onto a pre-weighed aluminum weighing boat and dried overnight. The lipid was re-weighed, then weight was extrapolated to the entire bottom layer volume to determine lipid concentration. For ash, we pre-combusted aluminum weighing boats at 450°C for 6 h and pre-heated the samples in an oven at 100°C overnight to ensure full water loss. We combusted pre-weighed samples in a muffle furnace for 6 h at 450°C and then reweighed samples to obtain ash content.

1.3.3 Micronutrient quantification

Micronutrients (calcium [Ca], copper [Cu], iron [Fe], magnesium [Mg], manganese [Mn], and/or zinc [Zn]) were assessed in duplicate from Dataset 1 (N = 157) and a subset of Dataset 2 (N = 65) following modified methods of Ratnarajah et al. (2018). We chose these micronutrients because they have been identified as critical nutrients for many coral reef taxa, especially reef-building corals (Ferrier-Pagès et al., 2018). Briefly, we manually homogenized each sample and weighed 8–40 mg subsample into metal-free acid-cleaned 50 mL polypropylene vials (Ultimate Clean Cup, Environmental Express). Samples were acid-digested (2–5 mL concentrated nitric acid; Plasma Pure Grade, Fisher Scientific and 0.125 mL of 30% hydrogen peroxide) overnight. They were then heated to 90°C for 2 h, cooled, and diluted to 5% nitric acid. Identical procedures were followed for blanks. Samples were then analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) at the Bren School of Environmental Science and Management at University of California, Santa Barbara facilities. A separate subset of samples from Dataset 2 (N = 185) were analyzed at University of Michigan for micronutrients (Ca, Cu, Fe, Mg, Mn, and/or Zn) and prepped and measured by ICP-MS according to Rempel et al. (2022). Both procedures followed similar protocols and resulted in similar measurements; therefore, micronutrients were pooled. When measurements were returned as negatives, they contained nutrient concentrations too low to detect and were reported as 0.

1.3.4 Data analyses

All statistical analyses were performed in R (version 2022.02.0) using the packages vegan (Oksanen et al., 2022), pairwiseAdonis (Martinez Arbizu, 2020) and lme4 (Bates et al., 2014). We assessed relationships between macro-/micronutrients and reef fish trophic guilds

or species by using non-metric multidimensional scaling (NMDS) ordinations on log-transformed nutrients, based on a Bray–Curtis dissimilarity index (Bray & Curtis, 1957), using the "envfit" function to visualize patterns and identify correlations between nutrients and trophic guilds or species. We then conducted separate NMDS analyses within specific trophic guilds (corallivores and herbivores) and genera (*Chaetodon* and *Acanthurus*) because these groups had large sample sizes, are abundant around Mo'orea, and play key roles in coral reef ecosystem functioning (Brooks, 2022; Tebbett et al., 2022). To test the effects of trophic guilds and species on nutrient contents, we computed permutational analyses of variance (PERMANOVAs) based on Bray–Curtis dissimilarity index and 999 permutations with "site" included as a random effect. Pairwise differences were tested using the function "pairwiseAdonis," and p-values were adjusted according to a Bonferroni–Holm correction to account for multiple comparisons.

Next, to determine which variables best predicted nutrient content, we ran a series of mixed effects models with fixed effects (family, genus, species, body mass, and trophic guild) tested independently, additively, and interactively for each macro- and micronutrient. There were 61 unique collection sites, classified by distinct GPS coordinates or distinct roadside stand spots, so we included "site" as a random effect. Collinear variables (family, genus, and species) were not tested additively or interactively with each other. Complementary models were compared using the Bayesian Information Criterion (BIC), where the lowest BIC score was accepted as the best fit; however, only models with $\Delta BIC < 7$ were considered in the analyses (Jerde et al., 2019).

Data residuals were tested for normality, homogeneity, and moderate correlation, and data were log transformed when they did not fit normality assumptions. Outliers (N = 15) were

removed if values were outside 1.5× interquartile range across all individuals and within a species. If values for one individual were determined to be outliers for more than two macro- or micronutrients, this suggests that samples were contaminated or not processed correctly, so all nutrients of that type (either macro- or micro-) were removed for that individual (Grubbs, 1950; Wing et al., 2021). We standardized the length metrics across the two datasets, converting all lengths to total lengths using published species-specific scaling parameters (Brooks & Adam, 2019). We sampled 570 individuals across 40 species to capture the coral reef fish community; however, some of our sampled species were underrepresented for certain fecal nutrients. We therefore present sample sizes with mean ± SD values and graphically present results from species with ≥ four individuals for major macronutrients (carbohydrate, lipid, protein) or micronutrients (Ca, Cu, Fe, Mg, Mn, Zn), resulting in 26 species for macro- and 39 species for micronutrients. For NMDS ordinations, we include species with ≥ four individuals each measured for all major macro- or micronutrients.

1.4 Results

1.4.1 Feces macronutrient composition

The concentration of fecal macronutrients varied substantially across 26 coral reef fish species (Figure 1.1). Ash and water were the primary components of fish feces across all species (Figure 1.1A). However, the mean protein concentration of feces varied 33-fold, from 1.4% dry weight (DW) in *Acanthurus olivaceus* to 45.7% DW in *Naso lituratus*. The mean carbohydrate concentration varied 13-fold, from 0.5% DW in *Scarus psittacus* to 6.3% DW in *Acanthurus nigrofuscus.* For lipids, *Amanses scopas* contained the lowest concentration at

1% mean DW, compared to *Odonus niger* at 9% mean DW, a 9-fold difference. Meanwhile, *N. lituratus* had the lowest mean ash content at 31.5% DW compared to *A. scopas* at 89.5% DW.

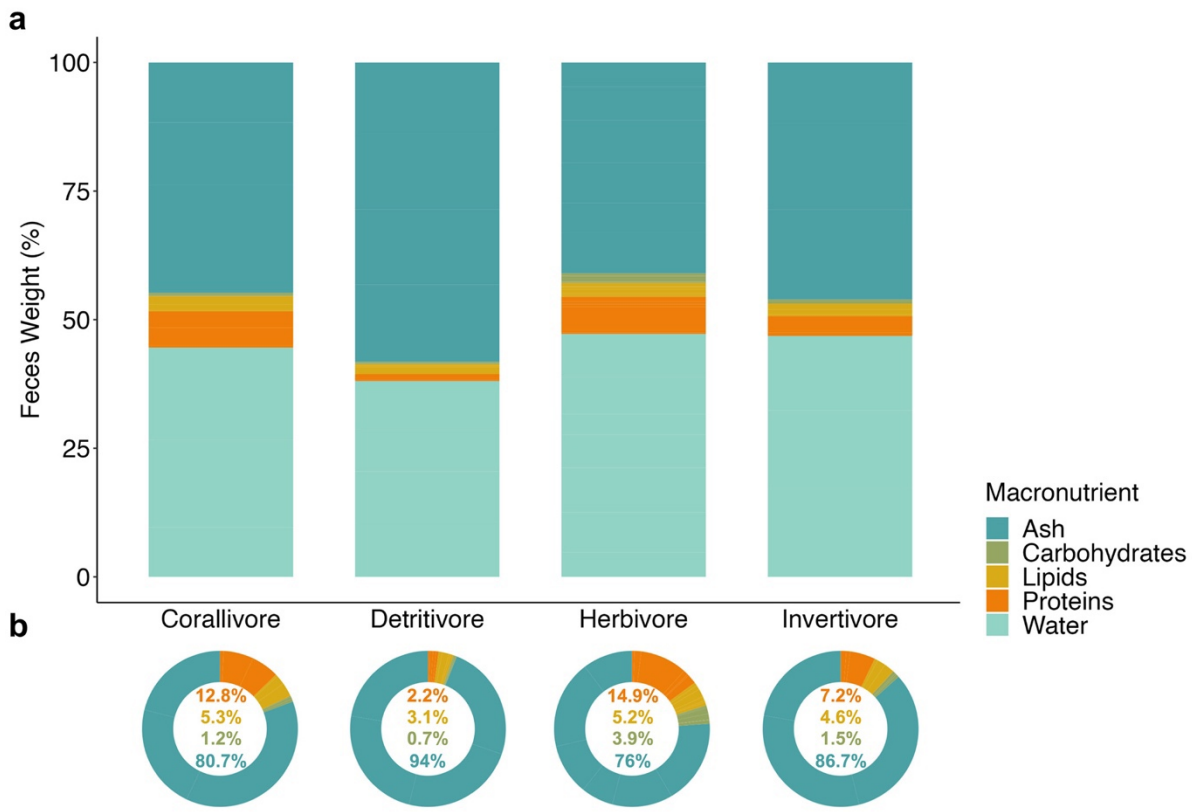

Figure 1.1. Mean macronutrient content across trophic guilds normalized to (A) 100% feces weight and (B) 100% feces dry weight (excludes water). Data represent species with $N \geq 4$ individuals per species and depicts normalized means for three corallivore species, four detritivore species, six herbivore species, and three invertivore species.

Macronutrient concentrations (protein, carbohydrate, lipid) varied by trophic guild (Figure 1.1A-B). For instance, we found that invertivores and detritivores egested the highest relative proportion of ash. Corallivore and herbivore feces contained relatively high

proportions of protein. We did not have an adequate sample size to measure macronutrients in planktivores or piscivores.

Our NMDS ordination analyses according to macronutrients revealed clustering by the five trophic guilds (Figure 1.2A) and the trophic guild had a significant effect on macronutrient concentration (PERMANOVA, $F_4 = 9.59$, $R^2 = 0.20$, $p < 0.001$). Detritivores were distinct from all trophic groups in their fecal macronutrients (pairwiseAdonis, $p < 0.05$ for all comparisons), as well as herbivores, corallivores, and invertivores from one another (pairwiseAdonis, $p < 0.05$ for all comparisons), while other trophic groups did not statistically differ from one another.

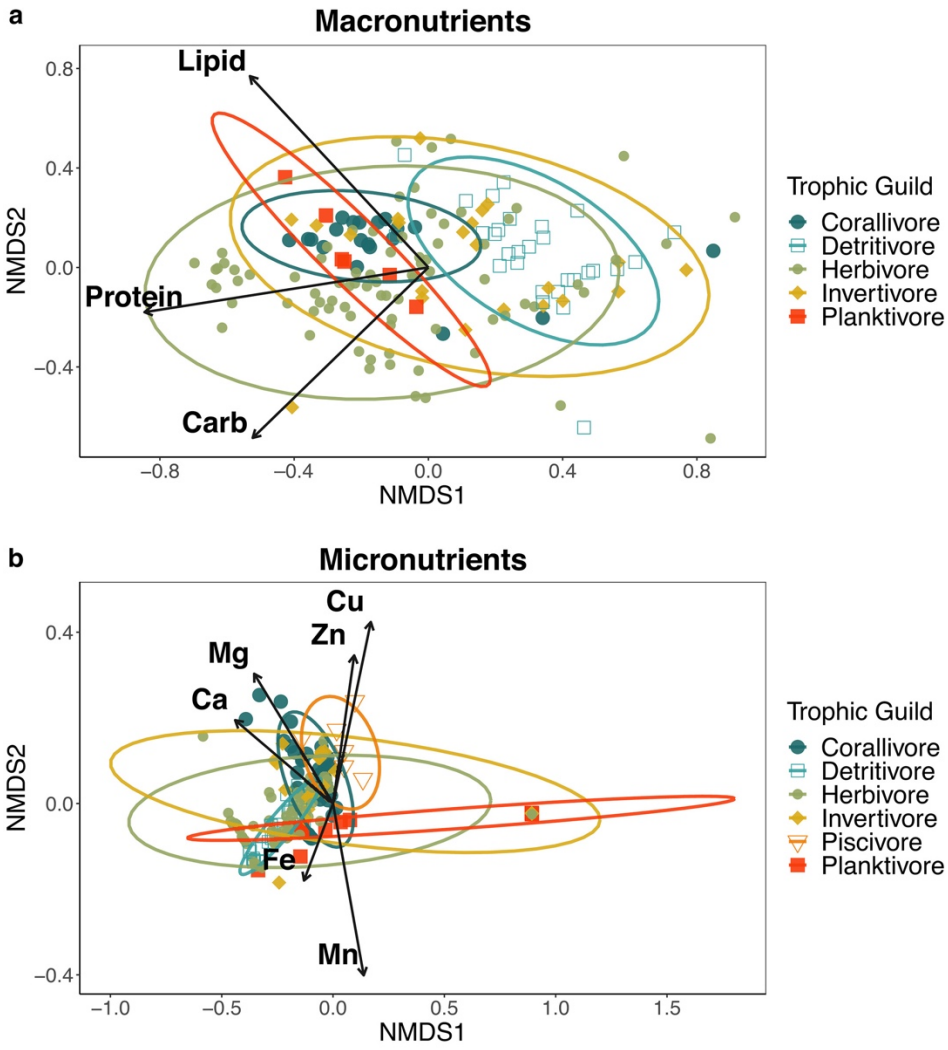

Figure 1.2. Non-metric multidimensional scaling (NMDS) ordinations of fecal (A) macronutrients (carb [carbohydrates], protein, lipid) (k = 2, stress = 0.119, N = 159) and (B) micronutrients (calcium [Ca], copper [Cu], iron [Fe], magnesium [Mg], manganese [Mn], and zinc [Zn]) across individuals (k = 2, stress = 0.041, N = 249) by trophic guild. Plots are based on the Bray–Curtis dissimilarity index and nutrients are shown as vectors, scaled down by 50% in (b). Ellipses depict 95% confidence interval. Each data point represents an individual. The macronutrient plot excludes piscivores due to a small sample size.

The concentration of macronutrients egested by fishes was best predicted by genus or trophic guild, and/or in conjunction with mass. Trophic guild was the best predictor for lipid concentration ($\chi^2 = 39.57$, df = 5, $p < 0.001$) as well as for carbohydrate concentration in conjunction with body mass (log[mass]: $\chi^2 = 13.86$, df = 1, $p < 0.001$; trophic guild: $\chi^2 = 46.03$, df = 5, $p < 0.0001$). In contrast, genus was the best predictor of ash content ($\chi^2 = 164.94$, df = 17, $p < 0.0001$) as well as for protein concentration either alone (genus: $\chi^2 = 666.89$, df = 18, $p < 0.0001$), or in conjunction with body mass (log[mass]: $\chi^2 = 14.68$, df = 1, $p < 0.0001$; genus: $\chi^2 = 677.85$, df = 18, $p < 0.0001$).

1.4.2 Feces micronutrient composition

Fecal micronutrients varied extensively across 39 coral reef fish species (Figures 1.2 and 1.3). Mean copper concentrations in feces ranged from 0 ppm in *Chlorurus spilurus*, *Ctenochaetus flavicauda* and *Scarus psittacus* to 163 ppm in *Thalassoma hardwicke* feces (Figure 1.3). A ~1800-fold difference was measured in mean Fe concentrations, from 24 ppm in *Amanses scopas* to 43,015 ppm in *Acanthurus pyroferus* feces. For Mg, *A. scopas* had the lowest fecal concentration with a mean of 3116 ppm while *Chromis xanthura* had a mean of 65,863 ppm Mg, a 21-fold difference (Figure 1.3). Species such as *T. hardwicke* had no measurable fecal Ca, whereas *A. scopas* contained a mean of 398,207 ppm of fecal Ca (Figure 1.3). On the other hand, *A. scopas* showed the lowest mean concentration of Mn at 0.6 ppm while *T. hardwicke* had the highest mean concentration of fecal Mn at 167 ppm (Figure 1.3). The mean Zn concentration varied ~1800-fold, from 0.4 ppm in *Scarus psittacus* feces to 784 ppm in *Chaetodon pelewensis* feces (Figure 1.3).

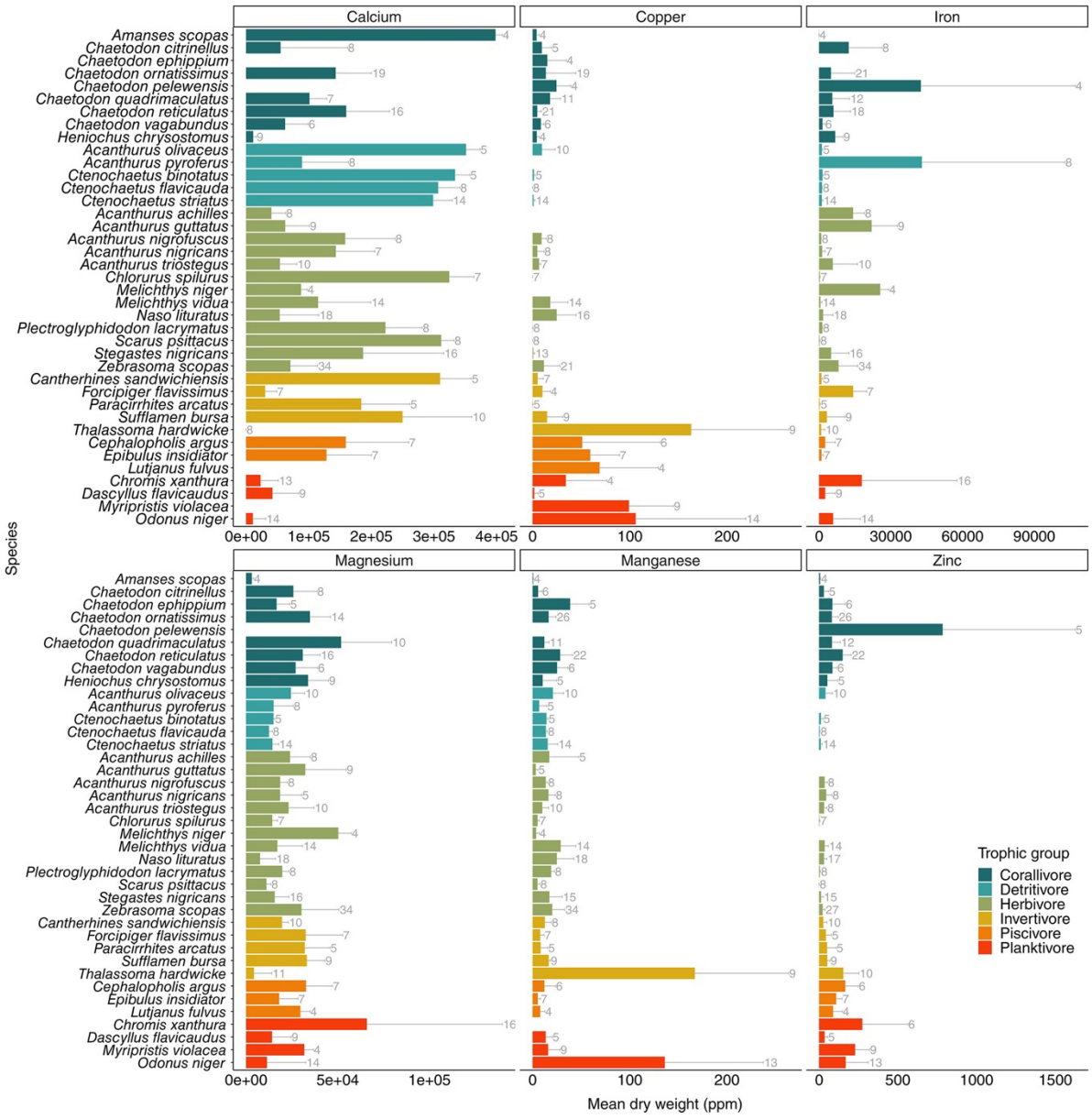

Figure 1.3. Mean dry weight (ppm) of calcium, copper, iron, magnesium, manganese, and zinc measured in feces of 39 reef fish species across six trophic guilds in descending order (corallivore, detritivore, herbivore, invertivore, piscivore, planktivore). Error bars represent SD. Data represents species with $N \geq 4$ individuals per species per nutrient and numbers in gray represent sample size and for cases where the nutrient was not measured for the species, no number or bar is shown.

1.4.3 Variation in micronutrients across trophic guilds

Examining fecal micronutrients across trophic guilds (Figure 1.3) revealed trends that also emerged on the NMDS ordinations (Figure 1.2B). We observed limited clustering among trophic guilds (Figure 1.2). The ordination showed a tight clustering among piscivores, detritivores, and corallivores compared to the other trophic guilds (Figure 1.2B). Trophic guilds significantly differed in micronutrient concentrations (PERMANOVA, $F_5 = 8.52$ $R^2 = 0.17$, $p < 0.001$), including differences in detritivore fecal nutrients compared to all trophic guilds (pairwiseAdonis, $p < 0.05$ for all comparisons) and differences in corallivore and planktivore, corallivore and invertivore, and herbivore and planktivore fecal nutrients (pairwiseAdonis, $p < 0.05$ for above comparisons).

There was no single best variable (species, genus, family, mass, or trophic guild) that consistently predicted the concentration of micronutrients egested by fishes. Trophic guild was the best predictor for Ca ($\chi^2 = 75.92$, df = 5, $p < 0.0001$) and Cu ($\chi^2 = 96.54$, df = 5, $p < 0.0001$) concentrations. In contrast, genus was the best predictor for Fe ($\chi^2 = 173.06$, df = 22, $p < 0.0001$), Mn ($\chi^2 = 249.04$, df = 22, $p < 0.0001$), and Zn ($\chi^2 = 277.96$, df = 23, $p < 0.0001$) concentrations, whereas family was the best predictor for Mg concentration ($\chi^2 = 66.89$, df = 10, $p < 0.0001$). Body mass was a common (though non-significant) additive predictor for nutrients; Cu concentrations increased with body mass whereas Fe, Ca, Mn, and Zn concentrations decreased with body mass.

1.4.4 Variation in micronutrient quantities within trophic guilds and within genera

In addition to the differences in nutrient profiles across trophic guilds, differences in fecal micronutrient profiles also are apparent within trophic guilds (i.e., herbivore Figure 1.4A, corallivore Figure 1.4B). The NMDS ordination for fecal micronutrient concentrations of herbivore species revealed clustering (Figure 1.4A), and micronutrient concentrations significantly differed across herbivore species (PERMANOVA $F_9 = 8.99$, $R^2 = 0.50$, $p < 0.001$). Distinct clusters separated *N. lituratus* from *C. spilurus*, *P. lacrymatus*, *S. psittacus*, and *S. nigricans*, as well as *S. psittacus* from *A. nigrofuscus*, *A. triostegus*, *N. lituratus*, *P. lacrymatus* and *Z. scopas* for example (pairwiseAdonis, $p < 0.05$ for above comparisons) (Figure 1.4A). *A. nigrofuscus* also differed from *A. triostegus* and *C. spilurus*, as well as *S. nigricans* from *Z. scopas* (pairwiseAdonis, $p < 0.05$ for above comparisons) (Figure 1.4A). When investigating patterns of micronutrient concentrations within the herbivorous genus *Acanthurus*, we observed that the four species formed distinct clusters (Figure 1.4C; PERMANOVA $F_3 = 11.17$, $R^2 = 0.61$, $p < 0.001$) and all four *Acanthurus* species significantly differed in micronutrient concentrations (pairwiseAdonis, $p < 0.05$ for all comparisons).

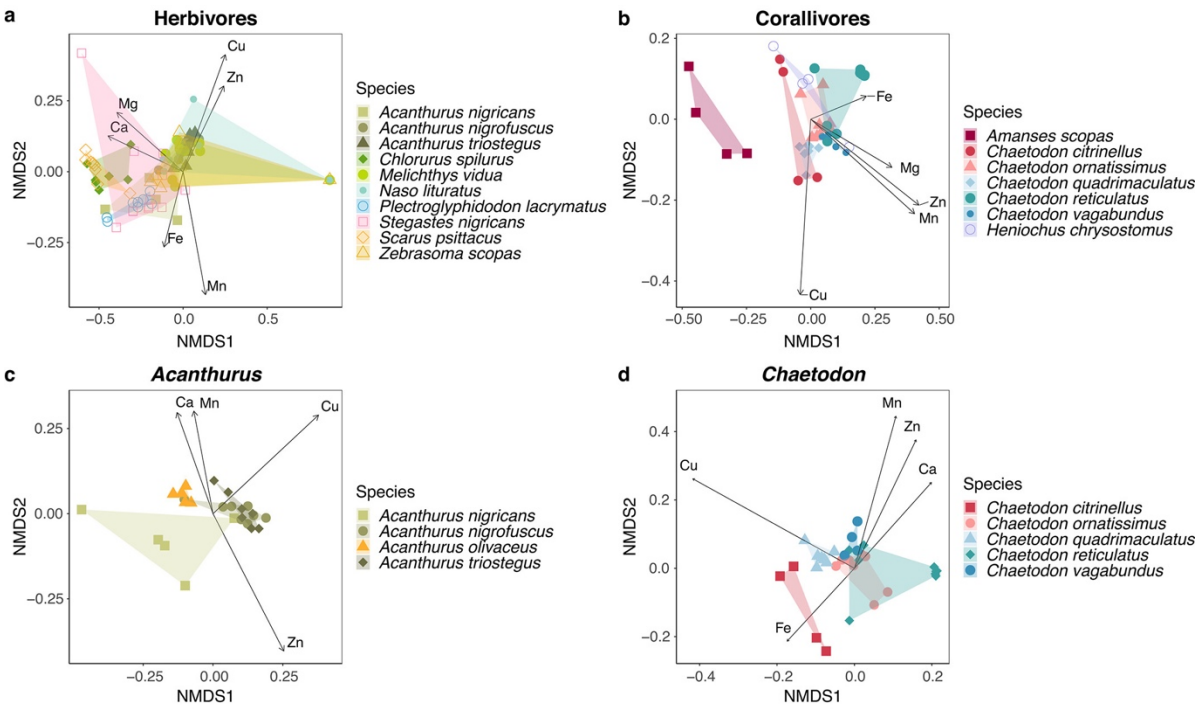

Figure 1.4. NMDS ordinations based on significant ($p < 0.05$, based on a permutation test) fecal micronutrients (calcium [Ca], copper [Cu], iron [Fe], magnesium [Mg], manganese [Mn], and zinc [Zn]) in species within the (A) herbivore trophic guild ($k = 2$, stress = 0.025), (B) corallivore trophic guild ($k = 2$, stress = 0.118), (C) genus *Acanthurus* ($k = 2$, stress = 0.041), and (D) genus *Chaetodon* ($k = 2$, stress = 0.078). Each polygon represents the nutrient profile of a single fish species, with each point corresponding to an individual. Colors represent each species. Plots are based on the Bray–Curtis dissimilarity index and nutrients are shown as vectors, scaled down by 50%.

For corallivores, the NMDS ordination for fecal micronutrient concentrations across seven species also revealed clustering (Figure 1.4B), and these species differed significantly in their micronutrient concentrations (PERMANOVA, $F_6 = 22.45$, $R^2 = 0.80$, $p < 0.001$). *Chaetodon quadrimaculatus* differed significantly in micronutrient concentrations from *C. reticulatus*,

and *C. vagabundus* (pariwiseAdonis, $p < 0.05$ for above comparisons) (Figure 1.4B). Additionally, *A. scopas* significantly differed from *C. ornatissimus* and *C. reticulatus*, and *H. chrysostomus* differed from *C. ornatissimus* (pairwiseAdonis, $p < 0.05$ for above comparisons) for example. When we investigated ordination patterns of micronutrient concentrations within the genus *Chaetodon*, there was tight clustering, and these micronutrient profiles differed significantly across species (PERMANOVA, $F_4 = 8.19$, $R^2 = 0.55$, $p < 0.001$) (Figure 1.4D). The micronutrient profile of *C. quadrimaculatus* differed from *C. citrinellus*, *C. reticulatus*, and *C. vagabundus*, in addition to *C. citrinellus* differing from *C. ornatissimus* and *C. reticulatus* (pariwiseAdonis, $p < 0.05$ for above comparisons) (Figure 1.4D).

1.5 Discussion

High productivity on coral reefs is facilitated by the efficient internal cycling of energy and nutrients. Reef fishes are a dominant consumer group on reefs, but how they recycle different types of nutrients in their feces has not been studied in detail. We measured the fecal nutrient composition from 570 coral reef fish individuals spanning 40 species and representing 70% of non-elasmobranch fish biomass around Mo'orea, French Polynesia (Brooks, 2022). We found that feces are diverse in nutrient quantity and quality across fish species. The best predictor variable (body size, taxonomy, and trophic guild) for macro- and micronutrient concentrations varied for each nutrient, highlighting the complexity of interactions in nutrient recycling in coral reef ecosystems. We measured biologically important minerals for corals (Cu, Fe, and/or Zn) (Ferrier-Pagès et al., 2018) in the feces of corallivores, planktivores, invertivores, and piscivores, and we demonstrate significant

variability in concentrations in herbivores and corallivores. The composition of fecal nutrients varied substantially across trophic guilds as well as across species within the same trophic guilds, especially for herbivores and corallivores, and across species within the genera *Acanthurus* and *Chaetodon*. Thus, when considering the nutrients that fishes recycle in their feces, some trophic guilds and even species within these trophic guilds contribute unique nutrient profiles (Figures 1.4 and 1.5). The variation in fecal nutrient concentrations across fish species underscores the diversity of reef fish functional roles and reinforces their importance for nutrient supply and ecosystem functioning on coral reefs.

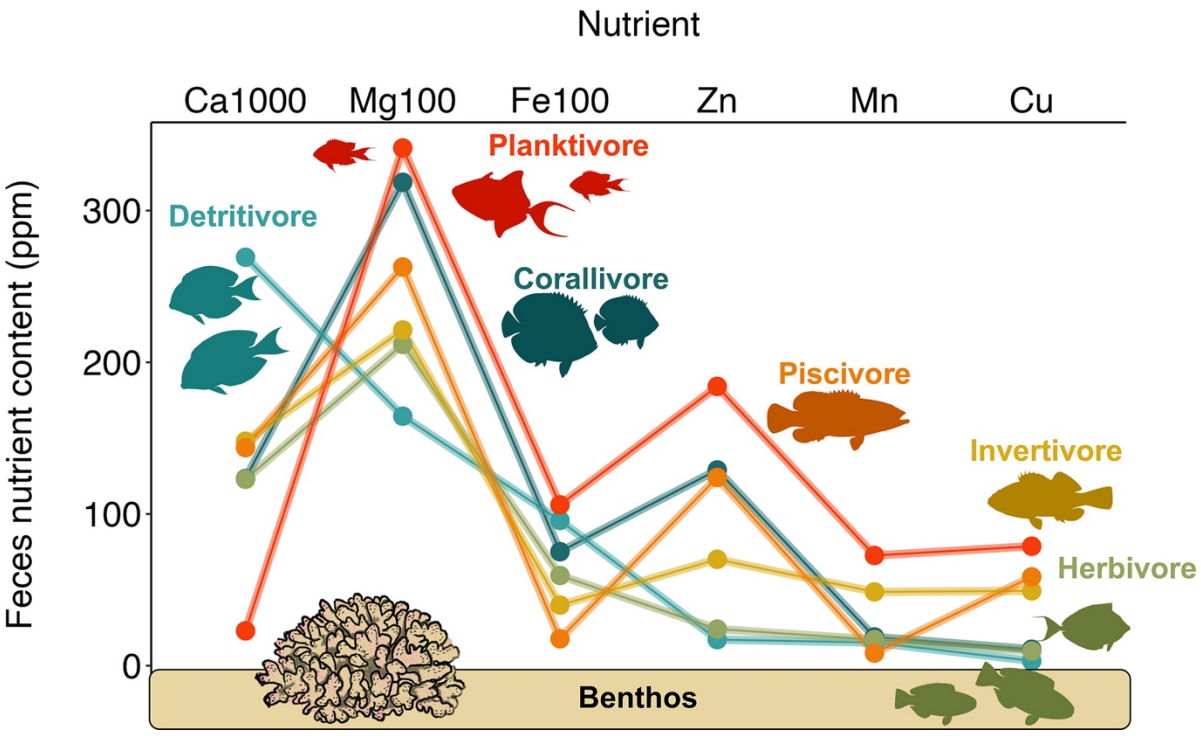

Figure 1.5. Summary schematic demonstrating mean micronutrient (Ca, Mg, Fe, Zn, Mn, Cu) contribution by primary trophic guilds of coral reef fishes in ppm. To maintain the *y*-axis scale Ca, Mg, and Fe are represented as fractions of original values by 1000 (Ca) and 100 (Mg and Fe). The benthos is included below to contextualize the approximate quantity of

nutrients that may disperse into the water column and land on benthos. Trophic guilds are represented by species within each guild (fishualize: Schiettekatte et al., 2019).

Compared to the breadth of knowledge in terrestrial systems (Beard et al., 2002; Leslie et al., 2008; Pastor et al., 1993; Veldhuis et al., 2018), our understanding of how aquatic consumers recycle nutrients via their feces is surprisingly small. Although the egestion of nitrogen, phosphorous, and carbon is increasingly incorporated into bioenergetic models for aquatic consumers (Atkinson et al., 2017; Schiettekatte et al., 2020, 2023; Williams et al., 2018), fecal micronutrient composition has remained largely unexplored, especially for reef fishes where only a limited set of species and trophic groups have been investigated (Bailey & Robertson, 1982; Crossman et al., 2005). Our findings align with previous results, as we found similar concentrations of ash, protein, carbohydrate, and lipid concentrations in the feces of the few overlapping species, including *Acanthurus olivaceus* and *Ctenochaetus striatus* (Bailey & Robertson, 1982). However, we found higher protein concentrations in species such as *Naso lituratus*. Herbivores show highly variable amino acid absorption efficiencies (Crossman et al., 2005), which may explain the high and variable protein values reported here. Similar to Schiettekatte et al. (2023), which compares CNP across reef fish feces, we found that the quality of fish feces varied strongly across and within trophic guilds. The consistency of fecal nutrient concentrations in the same species across different studies, despite relatively different locations (Mo'orea, Palau, and the Great Barrier Reef), reflects the shared nutritional requirements and broad feeding behaviors across these species (Bailey & Robertson, 1982; Crossman et al., 2005).

Fecal nutrient content represents components from unassimilated food. Thus, we can directly link species' diets to their fecal nutrient content. Given that fine-scale partitioning has been demonstrated in coral reef fish diets (Casey et al., 2019), we would expect this to translate to differences in fecal nutrient profiles. Corallivores, for example, feed on coral polyps, which are rich in lipids (8%–40% DW) (Stimson, 1987) and proteins (15%–23% DW) but are characterized by lower concentrations in carbohydrates (0.62% DW) (Ben-David-Zaslow & Benayahu, 1999). Moreover, coral tissue can contain relatively high levels of trace metals such as Zn (467 ppm DW) and Mg (533 ppm DW) (Hanna & Muir, 1990), and dead coral skeleton consist of 3.20% Mg (32,000 ppm) (Goldberg et al., 2019). Consistent with their trophic guild, we found that obligate corallivores, such as *Chaetodon pelewensis* and *C. reticulatus*, harbored higher fecal concentrations of Zn and Mg than the facultative corallivore *C. vagabundus* that has a more generalized diet including algae (Froese & Pauly, 2022), which is generally lower in these elemental concentrations (Rempel et al., 2022). Dietary content also mirrored fecal nutrients of *Chromis xanthura*, which consume planktonic diets that are generally rich in Fe and Zn and exhibited high fecal concentrations of these nutrients (Twining et al., 2004).

Although we show significant effects of taxonomy, body size, and trophic guild on fecal nutrient recycling, we found high intra-specific variability in nutrient concentrations for some species (e.g., *Acanthurus pyroferus*, *Thalassoma hardwicke*, *Odonus niger*) and could not account for all extrinsic and intrinsic factors that influence diet and metabolism (Ben-David-Zaslow & Benayahu, 1999; Lowman et al., 2021; Stimson, 1987). For example, some fishes may switch their diet across seasons and physio-chemical water conditions (Clements & Choat, 1993; Johnson et al., 2017). In addition, local stressors such as marine pollution

(e.g., nearshore reefs exposed to pollution in Mo'orea, see Adam et al., 2021) can lead to significant changes in benthic composition on coral reefs (Adam et al., 2021; Bonanno & Orlando-Bonaca, 2018; Hanna & Muir, 1990), ultimately affecting diet and muscle tissue nutrient content (Robinson et al., 2022). These environmental factors likely influence some taxa, life stages, or trophic guilds to shape patterns of nutrient recycling. Our finding that feces nutrient composition was generally not well predicted by broader taxonomy (family) and instead often showed high inter- and intra-specific variability in composition, contrasts with the finding that family is the best predictor of excretion rates in fish (Allgeier et al., 2021; Vanni et al., 2002). Thus, studying egestion as a form of fish-mediated nutrient supply demonstrates that each fish may contribute to nutrient recycling in distinct ways.

We identify diverse nutrient profiles, demonstrating that a suite of critical nutrients is recycled and potentially provided to benthic organisms such as corals, algae, and microorganisms via fish feces. Corals in particular, require an array of micro- and macronutrients (e.g., N, P, Mg, Mn, Fe, Cu, and Zn) to sustain their metabolism (Ferrier-Pagès et al., 2018). For instance, nutrients produced by fishes can have positive effects on coral growth and were shown to increase coral thermal tolerance (Meyer et al., 1983; Shantz et al., 2023). In addition, Mg, Mn, and Fe are known to ensure proper photo-physiological performance of coral symbiotic dinoflagellates, especially during periods of thermal stress (Ferrier-Pagès et al., 2018). Heterotrophic feeding supplies coral dinoflagellate symbionts with Mg, Mn, and Fe, which comprise antioxidant enzyme structures that scavenge reactive oxygen species (ROS) produced in excess during thermal stress (Ferrier-Pagès et al., 2018; Weis, 2008). Mn and Fe are also critical for photosynthesis because of their implication in chlorophyll production and amino acid synthesis, and elements such as Cu and Zn are

cofactors to critical enzymes involved in metabolism (Morel et al., 1994; Twining & Baines, 2013). The organic nutrients released from egestion as particulate matter may serve as food for heterotrophs including corals (Hatcher, 1988; Robertson, 1982). These nutrients may also provide a substrate for bacterial decomposition (Turner, 2002) that may make some of these nutrients more bioavailable to other organisms. However, the bioavailability of nutrients will largely determine their contribution to nutrient recycling on coral reefs. Future research needs to assess the bioavailability of fish-derived nutrients through feces to corals, algae, and other macro- and micro-organisms.

Compared to other marine species, coral reef fish feces are rich in certain nutrients. We found that some coral reef fish species contained one to two orders of magnitude greater fecal Fe concentrations (e.g., *Acanthurus pyroferus* 43,015 mg kg^{-1}) than found in sperm whales (757 mg kg^{-1}) and predatory seabirds, like the south polar skua (3928 mg kg^{-1}). We further found that a small-bodied wrasse, *Thalassoma hardwicke*, had a four-fold greater fecal Mn concentration than multiple species of whales, seabirds, and seals (167 mg kg^{-1} vs. 22–43 mg kg^{-1}) (Ratnarajah et al., 2014; Wing et al., 2021). Despite their small size compared to the above marine animals, coral reef fishes represent a high proportion of biomass within their ecosystem; thus, their total fecal output represents a large nutrient contribution. Thus, the loss of species or trophic guilds has the potential to shift food webs and further alter nutrient recycling (Allgeier et al., 2017; Halvorson & Atkinson, 2019; Peters et al., 2019). Overfishing often removes larger and/or higher trophic level fish (piscivores, herbivores, detritivores) on coral reefs, and the loss of these individuals could cascade down to benthic communities and impact nutrient recycling (Allgeier et al., 2016; Micheli et al., 2014; Mumby et al., 2006; Zaneveld et al., 2016). Species that are targeted by small-scale

fisheries around Mo'orea (Nassiri et al., 2021) tend to have nutrient-rich fecal profiles. For instance, fisheries-targeted piscivores (e.g., *Epibulus insidiator* and *Cephalopholis argus*) showed relatively high fecal Zn concentrations in our study while targeted detritivores (e.g., *Acanthurus olivaceus*) typically contained relatively high fecal Ca concentrations. Species like *Chlorurus spilurus* and *Naso lituratus* are also commonly targeted by recreational fishing (Nassiri et al., 2021), and while they are both herbivores, these species supply distinct fecal micronutrient profiles. The loss of any of these species could directly translate to the loss of certain key nutrient recycling pathways, with negative consequences for coral reef community structure and functioning.

1.6 Conclusion

Here, we highlight a missing functional link of nutrient recycling in a key group of consumers, coral reef fishes, and reveal the diversity of nutrients cycled by reef fishes through egestion. Many closely related coral reef fish species have minimal overlap in their trophic niches (Brandl et al., 2020; Casey et al., 2019; Eurich et al., 2019; Pozas-Schacre et al., 2021). Similarly, our results show that reef fish feces differ in nutrient profiles at the level of trophic guilds as well as, in some cases, at the species-level. By enabling the recycling of a diversity of macro- and micronutrients, fish communities may confer benefits to many other species that inhabit coral reefs. However, global and local stressors (e.g., pollution, overfishing) are changing reef landscapes and fish assemblages, threatening the nutrient recycling pathway provided by reef fishes. Thus, it is important for both terrestrial and marine environments to continue to reveal the underappreciated functional roles that many groups of animals play in nutrient cycling.

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Chapter 2: Despite plasticity, heatwaves are costly for a coral reef fish

2.1 Abstract

Climate change is intensifying extreme weather events, including marine heatwaves, which are prolonged periods of anomalously high sea surface temperature that pose a novel threat to aquatic animals. Tropical animals may be especially vulnerable to marine heatwaves because they are adapted to a narrow temperature range. If these animals cannot acclimate to marine heatwaves, the extreme heat could impair their behavior and fitness. Here, we investigated how marine heatwave conditions affected the performance and thermal tolerance of a tropical predatory fish, arceye hawkfish (*Paracirrhites arcatu*s), across two seasons in Mo'orea, French Polynesia. We found that the fish's daily activities, including recovery from burst swimming and digestion, were more energetically costly in fish exposed to marine heatwave conditions across both seasons, while their aerobic capacity remained the same. Given their constrained energy budget, these rising costs associated with warming may impact how hawkfish prioritize activities. Additionally, hawkfish that were exposed to hotter temperatures exhibited cardiac plasticity by increasing their maximum heart rate but were still operating within a few degrees of their thermal limits. With more frequent and intense heatwaves, hawkfish, and other tropical fishes must rapidly acclimate, or they may suffer physiological consequences that alter their role in the ecosystem.

2.2 Introduction

As global biodiversity faces more frequent and intense stressors associated with climate change, species must acclimate, adapt, move, or die (Aitken et al., 2008). Given that adaptation occurs over generations and relocation requires both dispersal capacity and available habitat conditions, acclimation may be the only coping mechanism available to many species (Seebacher et al., 2015). In contrast to long-term ocean warming, marine heatwaves ensue rapidly, with anomalous sea surface temperatures surpassing average temperatures by approximately 2 - 4°C for ≥ 5 days to multiple weeks (Frölicher et al., 2018). Thus, an animal's capacity to acclimate to these conditions is fundamental to its survival, and these challenges are likely to be particularly acute for species in tropical ecosystems such as coral reefs that exist near their thermal maxima (Magel et al., 2020).

Coral reef fish evolved in the stable thermal environment of the tropics and perform optimally within narrow temperature ranges, living close to their thermal limits (Tewksbury et al., 2008). Fish physiology may be sensitive to temperature changes at the seasonal scale (Johansen et al., 2015; Tran and Johansen, 2023). This begets the question of how these fish cope with marine heatwaves during seasonal extremes. Performance across levels of biological organization can be compromised at extreme temperatures (Tewksbury et al., 2008; Nyboer and Chapman, 2018). Aerobic scope (i.e., the difference between standard metabolism and maximal metabolism) reflects the capacity of the fish to perform essential behaviors, including swimming, digestion, defense, and reproduction (Farrell, 2016). Reef fishes are critical for maintaining coral reefs by providing ecological services such as recycling nutrients via egestion and excretion, guarding territories, and clearing space (Brandl et al., 2019), and all these activities require sufficient aerobic capacity. Previous work shows

that small increases in temperature can have sublethal effects on fish, including reducing their aerobic scope (Gardiner et al., 2010; Johansen and Jones, 2011; Munday et al., 2009; Nilsson et al., 2009; Rummer et al., 2014)

At the same time, other vital processes may become more energetically costly at high temperatures, such as digestion (specific dynamic action, SDA) (Sandblom et al., 2014) and recovery from exertion (Kraskura et al., 2021). Thus, during marine heatwaves, fish may be less able to perform certain activities (i.e., swimming, eating, reproduction, or territoriality) within their constrained energy budget (Holt and Jørgensen, 2015; Norin and Clark, 2017). Furthermore, if digestive costs increase during marine heatwaves, fish may need to reduce their consumption rate to maintain scope (Jutfelt et al., 2021). Fish presence increases coral performance during heat stress by excreting nutrients that enhance coral growth and coral nutritional reserves (Shantz et al., 2023; Ezzat et al., 2015; Holbrook et al., 2008), making fish's ability to survive and perform during heatwaves especially critical. If fish cannot fulfill their ecological roles because of constrained physiological performance during a marine heatwave, reef ecosystems may be threatened (Pörtner and Peck, 2010).

The function of the heart may be particularly vulnerable to marine heatwaves, compromising its key role in transporting oxygen, nutrients, wastes, hormones, and immune cells. As whole animal oxygen demand increases with warming, the heart ensures sufficient oxygen delivery to the tissues primarily via an increase in heart rate (Eliason and Anttila, 2017; Farrell et al., 2009; Fry, 1947). At some critical temperature however, maximum heart rate (f_{Hmax}) can no longer increase and eventually becomes arrhythmic (Casselman et al., 2012). Heart failure, therefore, is considered a primary mechanism that regulates the upper thermal limits of fish (Eliason and Anttila, 2017). In warm-temperate systems, marine

heatwaves have already exceeded the cardiac thermal limits in a sparid fish and may compromise its survival and distribution (van der Walt et al., 2021). Cardiac thermal limits have yet to be elucidated in coral reef fish. Accordingly, measuring the thermal limits of the heart in coral reef fish may help us assess the vulnerability of reef fish to climate change.

In this study, our overarching goal was to determine how marine heatwaves impact the performance of a common coral reef fish. The arceye hawkfish (*Paracirrhites arcatus*) is a "perching" ambush predator that depends on its ability to briefly perform at high exertion levels to capture prey, making this species an excellent model organism for assessing digestive, recovery, and cardiac performance under heatwave conditions (DeMartini, 1996). The work took place in Mo'orea, French Polynesia, where daily maximum ocean temperatures typically ranged from 26 to 29°C annually but reached temperatures beyond 29°C during marine heatwave events (Figure 2.1). Our first objective was to (1) determine if various whole-organism physiological performance traits are impaired after a week-long heatwave event during seasonal extremes. We assessed the metabolic rates, aerobic scope, exercise recovery, and SDA of fish acclimated to Austral winter (27, 31°C) and Austral summer (28, 29, 33°C) marine heatwave conditions under current and Intergovernmental Panel on Climate Change (IPCC) projections (i.e., +1, +4, or + 5°C). Our second objective was to (2) determine if relative meal size (2 vs. 4% body mass) increases costs for fish acclimated to higher temperatures. Our final goal was to (3) assess whether the cardiac performance and cardiac upper thermal limits are impaired in hawkfish under heatwave scenarios. Collectively, these metrics provide insight into how hawkfish may be able to perform essential activities, including hunting and maintaining their dominance over coral heads during a marine heatwave.

2.3 Methods

2.3.1 Site

This work took place at the University of California Gump Research Station on the volcanic high island of Mo'orea, French Polynesia. Ocean temperature data (Figure 2.1) were collected continuously (every 20 min) from 2005 to 2021 on a thermistor (SBE 39) mounted to the backreef of Mo'orea LTER2 (-17.476993, -149.802713) at 2 m depth as part of the Mo'orea Coral Reef Long-Term Ecological Research (LTER) time series (Leichter et al., 2022). One marine heatwave event associated with bleaching (Speare et al., 2021) occurred Jan-Jul 2019 (Figure 2.1A) (Leinbach et al., 2023), and returned to average conditions approximately one week prior to the animal collections for Austral winter 2019 experiments (collection details below).

2.3.2 Animal collection and husbandry

Arceye hawkfish (N = 75, 1 - 20 g) were collected on SCUBA or snorkel with clove oil (1:9, clove oil to 95% ethanol), hand nets, and slurp guns from the backreef (1 - 3 m depth) on the north shore of Mo'orea, French Polynesia (-17.476477, -149.818766) in two different seasons: the Austral winter (Jul – Aug 2019) and Austral summer (Nov – Dec 2022). Fish were transferred to a large cooler supplied with air stones (> 90% water air saturation) and transported back to the outdoor wet lab within 2 h. Following transport, fish were immediately transferred to shaded outdoor aquaria (500 l) supplied with ambient seawater pumped directly from the ocean and maintained at ambient water conditions for a minimum of 24 h and a maximum of two weeks before subjecting fish to their thermal acclimation treatments. Due to logistical constraints, holding time could not be tracked for individual fish

and therefore could not be accounted for statistically, however, fish were randomly selected for acclimation to account for this. Fish in the wild consume a generalist diet of invertebrates and small fish (Leray et al., 2015) but for consistency, were fed chopped scallops (*Argopecten purpuratus*) daily *ad libitum*. A subset of fish (N = 8) were captured one morning during Austral summer and underwent the cardiac thermal tolerance tests within 7 h of capture to represent Austral summer "wild" fish acclimatized to 28°C (see below for details).

Fish were transferred into 65 l aquaria beneath an outdoor awning (4 - 6 fish per tank; 3 tanks per acclimation temperature), with three to four dead *Pocillopora* sp. coral heads included per tank to serve as shelter for these coral-associated fishes. Water temperature was either maintained at ambient temperature (winter: 27°C, summer: 28°C) or raised to the marine heatwave temperature (winter: 31°C, summer: 29 or 33°C) by 2°C per hour. These temperatures represent the current maximum and projected climate change (summer: +1, +5°C; winter: + 4°C) temperatures for this population during the different seasons (IPCC, 2023). Tanks were maintained above 80% of oxygen saturation and subject to the natural photoperiod (13 h light:11 h dark). Given that a marine heatwave may last from five days to several weeks (Frölicher et al., 2018), fish were acclimated to their temperature treatment for one week before respirometry trials (Nilsson et al., 2009). Fish acclimated to 33°C were fed twice daily to ensure they had the same access to food relative to their metabolism as fishes at lower temperatures. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara (protocol #955-955.1) and are in accordance with relevant guidelines (including ARRIVE guidelines) and regulations.

2.3.3 Intermittent flow respirometry

Oxygen consumption rates (MO_2) were measured in a custom-made intermittent flow respirometry system to measure maximum metabolic rate (MMR), recovery, standard metabolic rate (SMR), and specific dynamic action (SDA) in individual fish. A header tank maintaining ambient (27-28°C) or heated water (29, 31, or 33°C) in an open circuit supplied 102 L tubs or a water table containing submerged respirometers custom-made from polyvinyl tupperware (Lock & Lock; Seoul, South Korea). Respirometers (13 x 8.7 x 5.5 cm, 566 total l; or 13.4 x 9 x 5.8 cm, 690 total l) were plumbed with PVC tubing to recirculation pumps (Eheim Universal 300 pump, flow rates averaged 2 l min^{-1}) and flush pumps (Eheim Universal 600 pump, flow rate to each respirometer averaged 1.1 l min^{-1}). Because of the relatively small size of *P. arcatus,* flush pumps were divided between two to four respirometers. FireSting robust Oxygen probes (PyroScience, Aachen, Germany) were fitted into the recirculation loop and measured oxygen levels continuously. Recirculation pumps continuously pumped water throughout a closed loop, and flush pumps were set on a timer to automatically turn on intermittently to flush fresh seawater into the respirometers and ensure dissolved oxygen did not reach below 70%. Shade cloth covered the respirometers to prevent disturbance and excess light exposure.

To account for bacterial respiration, background was measured in all respirometers for a minimum of three cycles before and after each full set of respirometry trials (before MMR and after SDA). Additionally, the entire setup was drained, rinsed, and cleaned with freshwater and bleach between each trial of 8 - 12 fish to minimize bacterial growth.

Fish were fasted 24 h before respirometry to assume a post-absorptive state (Roche et al., 2013). This was an assumption made prior to having estimated SDA duration in hawkfish

and was maintained for standardization. Trials began between 11:00 – 16:00. Maximum metabolic rate (MMR) was measured at the beginning of the respirometry trial, except for two trials (N = 4 fish (27°C), N = 4 fish (31°C)) where MMR measurements followed SMR measurements due to logistical constraints, but this timing did not affect MMR measurements at either temperature (T-tests, P = 0.631 (27°C), P=0.621 (31°C)). MMR was induced by hand-chasing an individual fish in a bucket for 3 min followed by 1 min of air exposure in a hand net and immediately placing the individual in a respirometer (Norin and Clark, 2016). MO_2 was measured for 5-7 min to measure MMR and then chambers were flushed with fresh seawater. Following MMR measurements for each fish, the flush pumps were automated to reoxygenate the respirometers with fresh seawater in 15 min intervals of flush:measure cycles (e.g., 5 min flush: 10 min measurement; 6 min flush:9 min measurement). Fish were kept in respirometers overnight for 18 – 20 h for recovery and SMR measurements, with flush cycles modified as needed to maintain oxygen saturation above 70%.

Digestion is a metabolically expensive process that involves the breakdown, transport, and synthesis of food molecules (McCue, 2006). The oxygen cost of digestion and assimilation is termed the specific dynamic action (SDA). Fish would not freely feed in their respirometers or in isolation in a tank, so they were gavage-fed for SDA measurements, which is a common approach for delivering food to fishes for SDA experiments (Chabot et al., 2016). After the overnight recovery measurements, each fish was removed from the respirometer, anesthetized in clove oil (2019: 20 mg l^{-1}, 2022: 10 mg l^{-1}, 1:9 clove oil to 95% ethanol), weighed, and gavage fed 2 or 4% of their body weight in scallops with forceps. These different relative meal sizes were chosen to mimic a relatively small and large meal

and are typical in digestion studies (Secor, 2009). Food loss was monitored and estimated in an aerated recovery bucket for 10 min before returning the fish to the respirometer. Any regurgitated food was weighed and re-fed to the individual, which was typically necessary a second time for about 50% of the fish and a third time for 5% of the fish. Fish were again monitored for 10 min before being returned to their respective respirometer. MO_2 measurement cycles began after all fish per trial were fed, which occurred within a two-hour time frame. MO_2 was measured for 40 - 44 h to estimate SDA (flush:measure cycles as described above for recovery and SMR).

Following SDA, fish were either euthanized (immersed in MS-222, 500 mg l^{-1}) and dissected (N = 32), sham-fed (N = 8), or underwent the cardiac thermal tolerance test (N = 30). Fish were sham fed to determine the duration of the stress and handling response induced by the anesthesia (2019: 20 mg l^{-1}, 2022: 10 mg l^{-1}, 1:9 clove oil to 95% ethanol) and gavage procedure during which forceps were used to open their mouths to mimic gavage feeding. Fish were recovered in an aerated bath, a subset (N = 2) underwent the gavage procedure a second time and then fish were returned to respirometers to measure MO_2 for 5 – 18 h. To verify that fish recovered from clove oil within 5 h during the first year of trials, the longer time frame was selected for the following year.

2.3.4 MO_2 analysis

MO_2 data were analyzed and visualized in R (version 4.2.1) using the package *AnalyzeResp*[80]. Mass-specific MO_2 (units: mg O_2 kg^{-1} min^{-1}) was calculated from the change in concentration of O_2 over time (ΔO_2) in the respirometer using $MO_2 = (\Delta O_2 * (v_R - v_F))/m$, where v_R is the respirometer volume, v_F is the volume of the fish (L, assuming 1 kg = 1 L), and m is the fish mass (kg). All measurement period dissolved oxygen regressions were

visually assessed to ensure each O_2 slope was linear and negative. All MO_2 values were corrected for microbial background respiration. Background respiration was calculated based on a first-order exponential curve calculated between the average initial and end background measurements for each set of respirometers and then subtracted from the slope of each associated MO_2 measurement. At the onset of trials, background respiration levels typically accounted for 10% of the observed respiration rates for the fish and grew to as high as 60% by the trial's conclusion. MO_2 values were assessed for body mass effects on oxygen consumption rates (SMR and MMR). Using the Bayesian Information Criterion (BIC), we determined that SMR values were isometric, whereas MMR values scaled allometrically and required a scaling correction. The two best-fit models (ΔBIC <7) used the hawkfish data (scaling coefficient = 0.69) or the universal scaling coefficient for fish (scaling coefficient = 0.89) (Jerde et al., 2019). Due to the small range in body mass, we opted for the universal metabolic scaling coefficient of 0.89 for MMR and used 5 g as the common body mass. The corrected MMR was used in all subsequent calculations and statistics.

MMR was selected as the first MO_2 measurement post-exhaustive exercise. This was the highest MO_2 value for all fish except for 11% of fish (N = 6) which experienced the highest MO_2 post-feeding during the 3 h recovery from anesthesia and handling. For these individuals, the first MO_2 post-exercise was still selected as 'MMR'. MMR was calculated using a sliding window analysis ($\geq$ 120 s), where each sliding window began at the start of the measurement period and moved in 1 s increments across the measurement cycle, selecting the steepest ΔO_2 with an $R^2 > 0.9$ as MMR (Little et al., 2020; Prinzing et al., 2021). Whether this exhaustive exercise elicited true MMR is unclear, but manual chasing to exhaustion provides the most reliable measure of MMR in species that do not swim for

prolonged periods (Norin and Clark., 2016), such as hawkfish. SMR was calculated as the lowest 10% quantile of all validated MO_2 measurements post-exhaustive exercise and post-feeding with $R^2 > 0.95$. Absolute aerobic scope (AAS) values were calculated as MMR – SMR for each individual, and factorial aerobic scope (FAS) as MMR/SMR. AAS represents the aerobic capacity of the fish to perform activities beyond standard (e.g., growth, swimming, digestion) (Fry, 1947; Clark et al., 2013). FAS represents the aerobic capacity of the fish relative to its standard rate of oxygen uptake (Little et al., 2020).

Recovery from exercise is a metabolically expensive process that restores homeostasis by clearing lactate and restoring oxygen stores, glycogen, high-energy phosphates, and osmoregulatory balance (Wood, 1991). During recovery, fish are vulnerable and may forgo important activities (e.g., forage, compete for territory, find mates) (Birnie-Gauvin et al., 2023). Hawkfish are ambush predators and therefore, immediate recovery may be a more relevant metric and was estimated rather than the total excess post oxygen recovery (EPOC) (Clark et al., 2013; Marras et al., 2010). Short-term recovery was calculated in three ways: (1) MO_2 over time, by fitting individual biexponential curves to each acclimation temperature as determined by the BIC. (2) Time to FAS = 2, as MMR/MO_2 for each individual during the recovery following exercise and pooled in 10 - 15 min time blocks for each treatment. Time to reach FAS = 2 was selected as a recovery threshold because this is the point at which fish can fully digest a meal and likely resume foraging (Eliason et al., 2022; Adams et al., 2022; Eliason et al., 2008). (3) %AAS, calculated as $(MMR-MO_2)/AAS$ at each MO_2 measurement for each fish. Logarithmic growth curves were fitted to the recovery data using the nls function from the *stats* package (R Core Team, 2022). The threshold of 75% AAS was selected as a standardized metric under the assumption that

hawkfish would resume normal activity (e.g., hunt, compete for territories, swim rapidly) between 50-90% AAS (Jain et al., 1998). How hawkfish or species with similar life histories prioritize metabolic demands at supra-optimal temperatures is unknown, but based on work with more active species, this threshold is a moderate starting point to compare recovery across temperatures (Farrell, 2016).

Based on the sham feeding trials, clove oil and handling had a 2-3 h effect on MO_2. Therefore, MO_2 measurements included in the SDA analyses for each fish began 3 h after feeding. SDA_{peak} was calculated as the maximum postprandial MO_2, and the associated time from feeding to reach peak SDA was termed 'time-to-SDA_{peak}'. The duration of SDA (SDA_{dur}) was calculated as the number of hours between time fed and the first point of MO_2 to reach the lowest 10% quantile of recorded postprandial MO_2 values (SMR_{SDA}). SMR_{SDA} was statistically the same as SMR calculated as described above (t-test, $P = 0.554$). SDA was calculated by integrating the area beneath postprandial MO_2 minus SMR_{SDA} and began at the time of feeding with a line extrapolated from SMR to the first analyzed MO_2 measurement (i.e., 3 h) (Figure 2.2). The remaining scope for activity during SDA_{peak} indicates the percentage of energy fish have available to them during the most metabolically expensive part of digestion. This was calculated as (mean SDA_{peak} – mean SMR)/(mean AAS)*100 for each treatment (temperature x relative meal size). SDA_{peak} /SMR indicates the proportion of energy allocated during the most metabolically expensive part of digestion. An SDA_{peak}/SMR = 2 suggests fish need to double their MO_2 to digest a meal. The cost of SDA (SDA_{coeff}) represents the percentage of energy consumed and was calculated as $SDA_{coeff} = (E_{SDA}/E_{meal}) \times 100$, where E_{SDA} is the energy spent on SDA, assuming 1 g of O_2 is associated with the release of 13.6 kJ of energy (Cho et al., 1982), and E_{meal} is the energy of the scallop

meal, calculated as the mass of scallop fed multiplied by its average gross energy density (3.87 kJ g^{-1}) and a 0.8 correction factor to account for indigestible energy (Jobling, 1983).

2.3.5 Cardiac thermal tolerance test

Cardiac function governs whole-organism performance and its response to a controlled acute temperature increase (i.e., the cardiac thermal tolerance test) reveals the acclimation potential of populations to respond to climate change scenarios (Eliason et al., 2022). In summer 2022, a subset of fish from respirometry (N = 30) and from the wild (N = 8) underwent the cardiac thermal tolerance test (Casselman et al., 2012; Hardison et al., 2021; Anttila et al., 2014). Since fish were transferred from the respirometer to the cardiac thermal tolerance test, we could ensure fish were starved for 40-44 h. However, for the 'wild' treatment, we could only verify fish hadn't eaten since the morning of collection (2-7 h). Fish were individually removed from a respirometer or the "wild" holding tank, anesthetized in seawater containing 80 mg l^{-1} MS-222 buffered with $NaHCO_3$, and then placed ventral side up in a sling in a water bath (10 l seawater containing buffered 65 mg l^{-1} MS-222). Water was circulated past the gills for constant irrigation, and an airstone maintained oxygenation. Stainless steel needle tip electrodes (ADInstruments Inc., Colorado Springs, CO, USA) were inserted beneath the skin to detect an ECG signal that was amplified with a Dual Bio Amp amplifier (ADInstruments Inc.) and filtered (filters: 60 Hz Notch filter; mains filter; low pass: 2 kHz; high pass: 10 Hz; range: 2 mV) (Hardison et al., 2021; Schwieterman et al., 2022).

After a 15 min equilibration period at the acclimation temperature, atropine sulfate was injected intraperitoneally (1.2 mg kg^{-1} in 0.9% NaCl) to block vagal tone, and 15 min later, isoproterenol was injected intraperitoneally (4 μg kg^{-1} in 0.9% NaCl) to maximally stimulate β-adrenoreceptors. These drug concentrations were tested prior to the experiment to

ensure a double dose did not further increase heart rate (f_H; beats min^{-1}). After another 15 min, the test began, and water was heated by 1°C every 6 min by running recirculating heated water through a stainless-steel coil in each water bath. At each 1°C interval, f_{Hmax} and temperature were stabilized and recorded. The test ended after the onset of cardiac arrhythmia (T_{ARR}), as indicated by a transition from rhythmic to arrhythmic beating or a missed QRS peak and precipitous decline in heart rate for all tested fish[28]. Individuals were then euthanized (immersed in MS-222, 500 mg l^{-1}), measured for total length, and dissected to verify their digestion status.

2.3.6 Cardiac thermal tolerance test analysis

ECG analyses were performed in LabChart Software (AD Instruments, Dunedin, New Zealand). f_{Hmax} was calculated for each temperature increment from a continuous 15 s measurement. Due to the relatively high acclimation temperatures and limited data points, the Arrhenius breakpoint temperature was not determined. Peak maximum heart rate (f_{Hmax}) was determined from the highest f_{Hmax} recorded over a 15 s measurement, and peak temperature (T_{PEAK}) was defined as the temperature corresponding to peak f_{Hmax}. T_{ARR} was determined as the temperature when the heartbeat transitioned from rhythmic to arrhythmic beating, when the trace missed a QRS peak, or when there was a precipitous decline in f_{Hmax} (Casselman et al., 2012). Of the 38 tests, 3 were deemed unusable due to poor ECG conductivity. Thermal safety margin (TSM) was calculated as T_{ARR} – max environmental temperature. Polynomial curves were fitted to f_{Hmax} data and compared using BIC, where the fit with the lowest BIC score was assigned the best-fit model.

2.3.7 Statistical analyses

Data were analyzed using R version 4.2.1. Values are presented as mean ± standard error of mean (SEM), and statistical significance was set at $P < 0.05$. Metrics were investigated for normality using Shapiro–Wilk tests and quantile–quantile plots, and for heteroscedasticity using Levene's test.

To examine how whole-animal physiological performance varied across acclimation temperatures, we used a one-way ANOVA to compare SMR, MMR, AAS, and FAS separately across seasons. Fish fed 2% BM were also assessed for differences in SDA, SDA_{peak}, SDA_{dur}, time-to-SDA_{peak}, and SDA_{coeff} between acclimation temperatures each season using independent t-tests. To determine if relative meal size (2 vs 4% BM) increased costs disproportionately, SDA, SDA_{peak}, SDA_{dur}, time-to-SDA_{peak}, and SDA_{coeff} values were compared across acclimation temperatures and relative meal sizes using a two-way ANOVA. Finally, to assess the difference in upper thermal tolerance cardiac limits across acclimation temperatures, a one-way ANOVA was used to compare f_{Hmax}, T_{PEAK}, and T_{ARR} across acclimation temperatures. All ANOVAs were followed by post-hoc Tukey HSD. For nonsignificant interactions, the interaction was removed from the model. When data are collected from two separate field seasons (Austral winter and summer), data are presented separately by season.

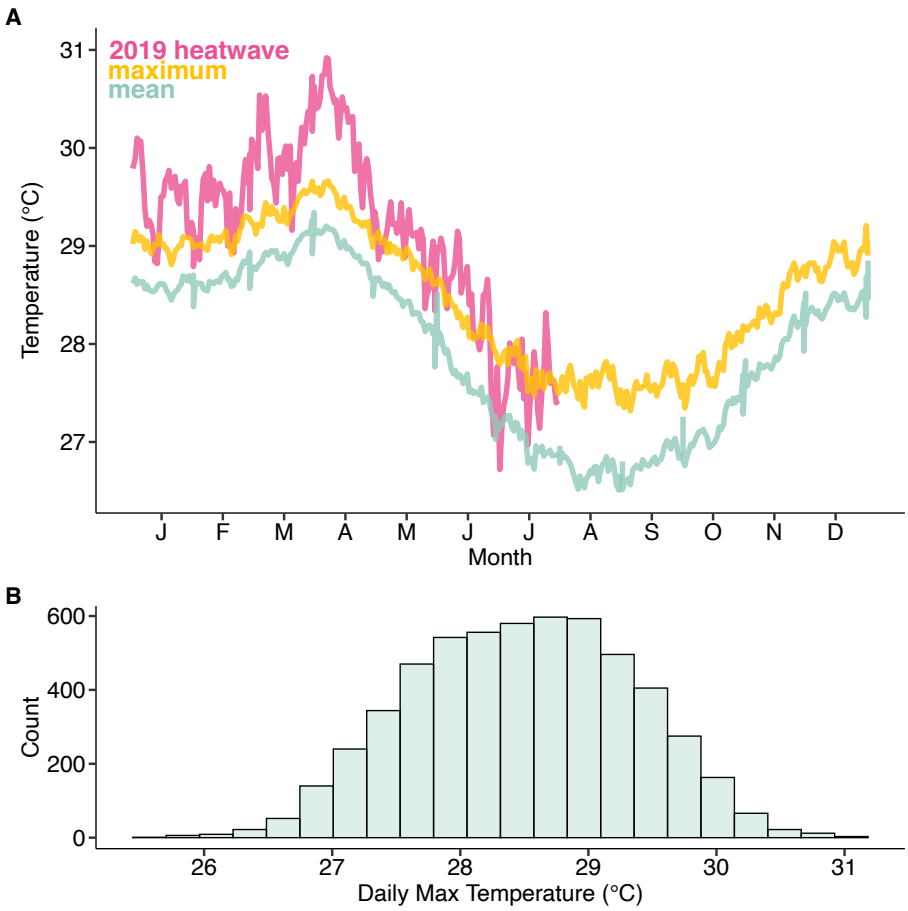

Figure 2.1. Temperatures (°C) on Mo'orea backreef between 2005-2021. (A) The daily mean (turquoise) and maximum (yellow) temperatures averaged across 2005 – 2021, and daily maximum (pink) temperatures during a marine heatwave in 2019; (B) overall count in daily maximum temperatures over the 16-year timeframe. Temperature data is from LTER2 site (Leichter et al., 2022), 500 m from the hawkfish collection site.

2.4 Results

2.4.1 Oxygen consumption rates

We assessed the whole-organism physiological performance of hawkfish under heatwave conditions via various metabolic performance traits (Figure 2.2). We found that SMR generally increased with acclimation temperature, though the only significant increase occurred in fish acclimated to 33°C, which was 60% greater than fish acclimated to 28°C (Figure 2.3A). In contrast, MMR was consistent across acclimation temperatures in the winter but increased in the summer by 27% from ambient to 33°C ($P = 0.026$, ANOVA). AAS did not vary across acclimation temperatures in the summer ($P = 0.461$, ANOVA) or winter ($P = 0.809$, ANOVA) (Figure 2.3B-C).

Another whole-organism physiological metric we examined was post-exercise recovery, which differed across acclimation temperatures. Fish acclimated to 27°C had a rapid recovery (sharp decline in MO_2), whereas fish acclimated to 33°C had a slower recovery (slow decline in MO_2) (Figure 2.4A). FAS available to fish surpassed 2 in 10 min for 27°C fish, 100 min for 28°C fish, 55 min for 29°C fish, 10 min for 31°C fish and 145 mins for 33°C fish. Fish recovered to 75% of AAS at 33 min for 27°C fish, 180 min for 28°C fish, 60 min for 29°C fish, 69 min for 31°C fish, and 188 min for 33°C fish (Figure 2.4B-F). Meanwhile, only 27°C acclimated fish recovered to 100% AAS within 180 min after the exhaustive exercise (Figure 2.4B-F). The variability in %AAS recovered generally increased with temperature (27°C: 16.7% (coefficient of variation); 28°C: 19.1%, 29°C: 19.4%, 31°C: 18.1%, 33°C: 21.6%) (Figure 2.4B-F).

The final metric we examined to assess whole-organism physiological performance was SDA when fed 2% BM (Table 2.1). When comparing SDA across acclimation

temperatures, we found an effect of temperature on SDA_{peak} in the winter. SDA_{peak} increased by nearly 50% with increasing acclimation temperature, from 4.95 mg O_2 kg^{-1} min^{-1} at 27°C to 7.26 mg O_2 kg^{-1} min^{-1} at 31°C ($P = 0.003$; Table 2.1). There was no effect of temperature on SDA or SDA_{dur}. Overall, the average remaining scope for activity available to fish during SDA_{peak} during the digestion of a 2% BM meal decreased by 47%, from 8.95 to 4.75 mg O_2 kg^{-1} min^{-1} (Table 2.1; Figure 2.5A). FAS was greatest in the winter at 27 and 31°C and decreased in the summer at 29, and 33°C (Figure 2.5C). Thus, while MO_2 could be increased beyond SMR by nearly 6-fold at 27 and 31°C, the fish in the summer acclimated to 29°C could only increase MO_2 above SMR by 4-fold and the 33°C fish by 3-fold ($P < 0.01$, t-test) (Figure 2.5C). SDA_{peak} as a ratio of SMR remained close to 2 across all acclimation temperatures and was highest at 29°C in the summer.

When we tested if relative meal size (2 vs 4% body mass) would increase costs for fish acclimated to 33°C, we did not find a strong response. SDA was not greater in fish fed 4% BM (52.56 mg O_2 kg^{-1}) compared to their counterpart fed 2% BM (36.6 mg O_2 kg^{-1}) at 33°C ($P = 0.292$; Figure 2.5A-B). Also surprisingly, relative meal size did not impact SDA_{peak} ($P = 0.660$). Overall, there was an effect of acclimation temperature, with the highest SDA_{peak} at 33°C reaching 9.98 mg O_2 kg^{-1} min^{-1} in fish fed 4% BM (Table 2.1). Meanwhile, fish fed 4% BM acclimated to ambient temperature had a marginally greater FAS than those acclimated to 33°C ($P = 0.053$) and SDApeak/SMR also remained close to 2 (Figure 2.5D).

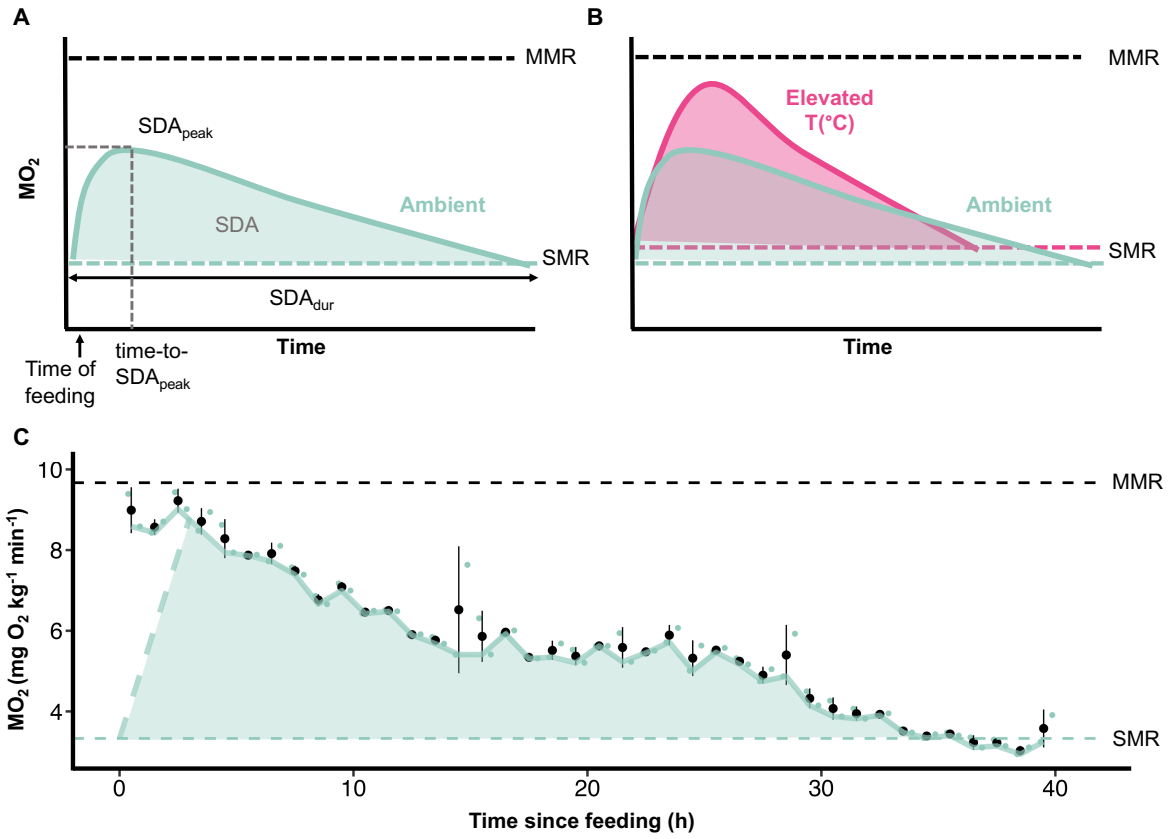

Figure 2.2. Conceptual and representative diagrams of SDA after a single feeding event at hour 0. (A) The line represents the MO_2 values post-feeding. SDA is the integral under the curve between postprandial MO_2 and standard metabolic rate (SMR, turquoise dashed line) over SDA_{dur} (the duration between feeding and the first value to fall below SMR). Peak SDA (SDA_{peak}) is the maximal postprandial MO_2 value (not pooled) following feeding and time-to-SDA_{peak} is the associated time (h) until peak SDA. (B) expected MO_2 trace for fish under elevated temperatures in comparison to ambient temperatures. (C) a representative trace of MO_2 for an ambient fish with each black point as mean MO_2 ± SEM pooled for every hour and each turquoise point as an individual measurement. The turquoise dashed line indicates where the SDA calculations begin to control for the effect of handling (anesthetic and gavage), and the horizontal lines indicate the MMR and SMR values for this individual fish.

The turquoise solid line connects the lowest MO_2 of each hour. The SDA is calculated as the shaded area between the dashed turquoise line, the solid turquoise line, and SMR until SDA ends.

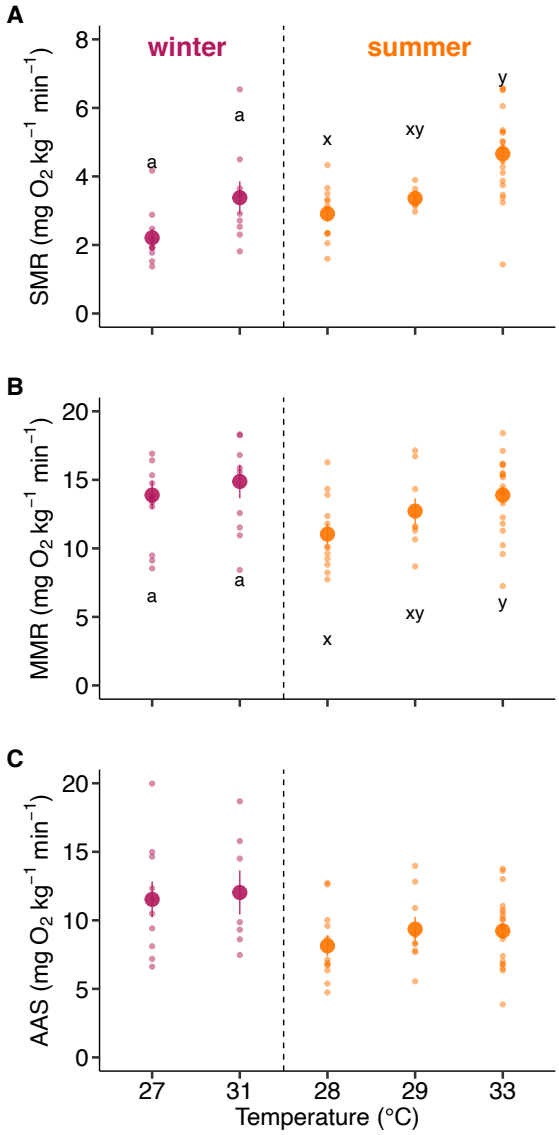

Figure 2.3. Oxygen uptake rate (mg O_2 kg^{-1} min^{-1}) in hawkfish acclimated during the winter (pink; 27 and 31°C) and summer (orange; 28, 29 and 33°C). (A) Standard Metabolic Rate (SMR, mg O_2 kg^{-1} min^{-1}). (B) Maximum Metabolic Rate (MMR, mg O_2 kg^{-1} min^{-1}). (C)

Absolute Aerobic Scope (AAS, mg O_2 kg^{-1} min^{-1}). Large data points and error bars represent mean ± SEM, and data from individuals are plotted as small data points. Statistics are assessed for summer (orange) and winter (pink) separately. Lowercase letters indicate significant differences ($P < 0.05$) between acclimation temperatures within a season (ANOVA). Note that 27 and 28°C represent ambient temperatures in the wild for the winter and summer, respectively, thus both acting as controls for the respective seasons.

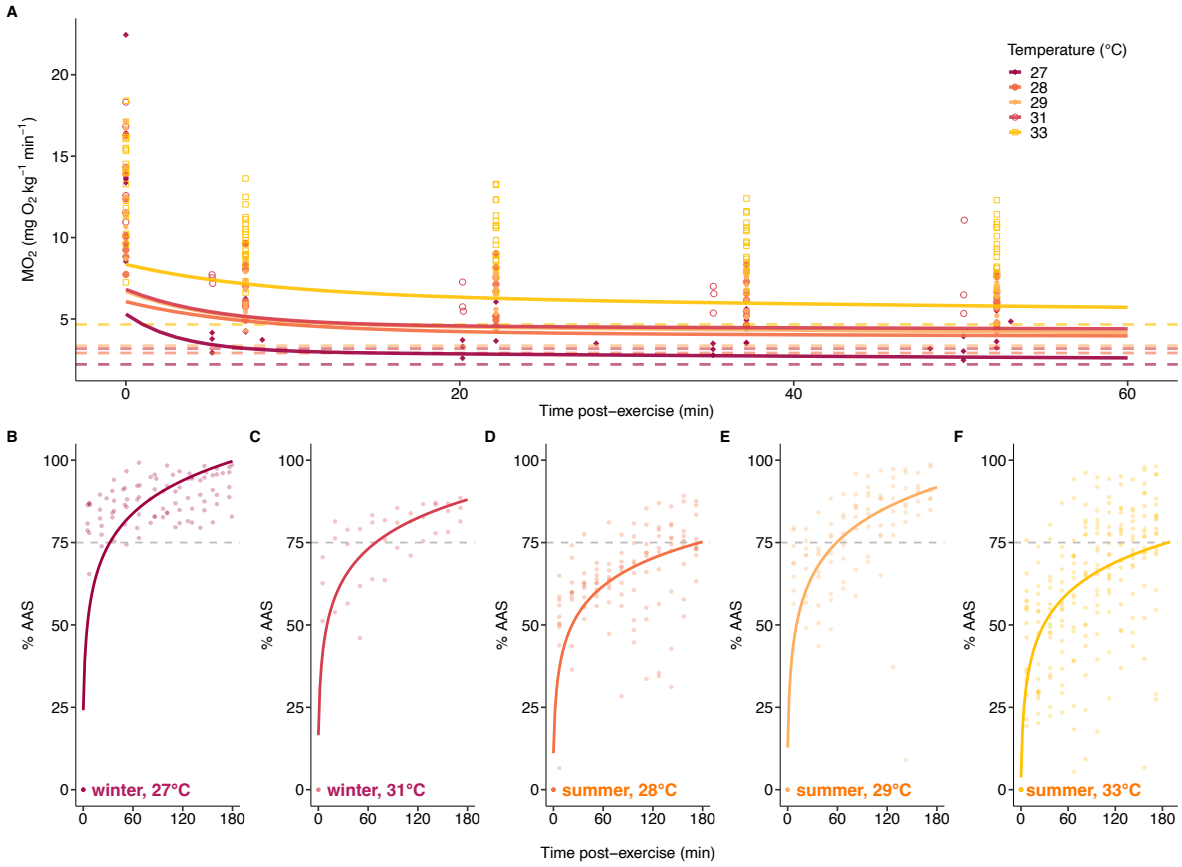

Figure 2.4. Recovery after exhaustive exercise for fish acclimated during the winter (27 and 31°C) and summer (28, 29 and 33°C). (A) Metabolic rate (MO_2) post chase during the first 60 min of recovery. Biexponential curves are fit for each acclimation temperature and each data point represents a measurement at a timepoint for an individual fish. Dashed lines

represent average SMR for that acclimation temperature. (B-F) Percent absolute aerobic scope (%AAS) recovered during the first 180 min following exhaustive exercise for fish acclimated in the winter (pinks): 27 [N = 8] and 31°C [N = 4] and summer (oranges): 28 [N = 10], 29 [N = 9], and 33°C [N = 19]). Values at 0 indicate MMR. Logarithmic growth curves are fit to each acclimation temperature as described in the methods. The grey horizontal line marks the point at which each treatment reaches 75% AAS. Individual data points are presented for each fish at each timepoint.

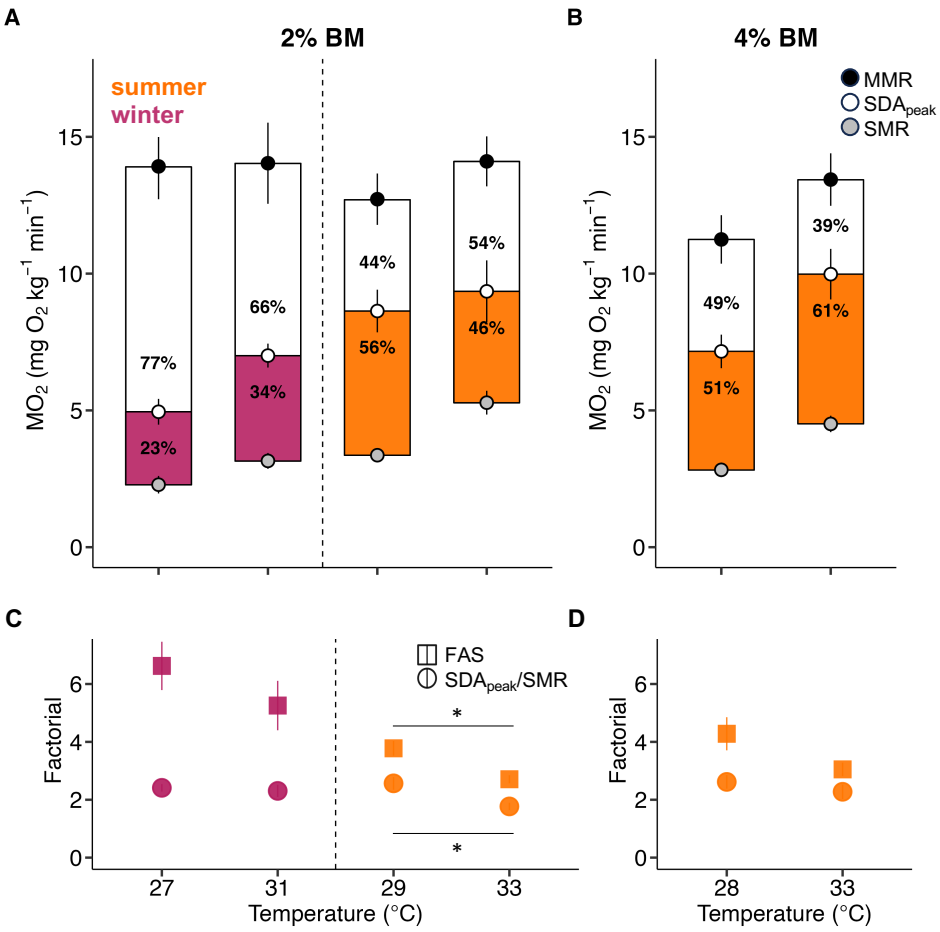

Figure 2.5. Scope for activity during SDA. Data shown for acclimation temperatures fed 2% body mass (BM) (27 [N = 10], 29 [N = 9], 31 [N = 7], and 33°C [N = 7]) (first panel) or 28°C and 33°C 4% BM (28 [N = 10], and 33°C [N = 11]) (second panel). (A-B) Mean metabolic rates are shown for hawkfish at rest (SMR; gray), at maximal activity (MMR; black), and at maximal digestion (SDA_{peak}; white) with SEM as the error. The colored bars (pink and orange) and associated percentages indicate the mean percent of scope used for SDA, and the remaining area in white and its associated percentage is the mean scope for

activity. These values are not statistically compared and are reported for illustrative purposes. (C-D) Factorial of MMR/SMR (FAS; square) and SDA_{peak}/SMR (circle). The difference between FAS and SDA_{peak}/SMR indicates the extent to which fish experience a metabolic constraint. Statistics are assessed for summer (orange) and winter (pink) separately. Asterisks indicate significant differences ($P < 0.05$) between acclimation temperatures within a season (t-test).

2.4.2 Cardiac performance

We assessed the cardiac performance and upper thermal limits under heatwave scenarios using the cardiac thermal tolerance test. For each acclimation temperature, f_{Hmax} followed the expected shape of an acute thermal performance curve (TPC), where it increased until T_{PEAK}, at which point f_{Hmax} declined with rising temperatures until the onset of cardiac arrhythmia (T_{ARR}) (Figure 2.6A). The TPC was more broadly shaped for wild fish, with a less apparent T_{PEAK} and more rapid onset of T_{ARR}. There was evidence to support an effect of temperature on model selection for f_{Hmax}, with a fourth-order polynomial curve determined to be the best-fit model by BIC. These models demonstrated that warm acclimation increased cardiac performance (i.e., f_{Hmax}) and increased the upper thermal limits of the heart (i.e., T_{PEAK} and T_{ARR}) (Figure 2.6A-C). Peak f_{Hmax} ranged from 347.03 ± 23.05 bpm in fish taken directly from the wild (28°C acclimatization) up to 415.50 ± 8.18 bpm in 33°C acclimated fish, representing a 20% increase (Figure 2.6, Table 2.2). Although upper thermal limits (T_{PEAK}, T_{ARR}) generally increased with warm acclimation, they only significantly differed between wild/28°C acclimated and 33°C acclimated for T_{PEAK} ($\Delta T = 2$ - 2.7°C) and between wild and

33°C for T_{ARR} (ΔT = 1.7°C) (Figure 2.6B-C, Table 2.2). The thermal safety margin increased with acclimation temperature and was 28% greater in fish acclimated to 33°C compared to wild-caught fish (P = 0.0113; Figure 2.6D).

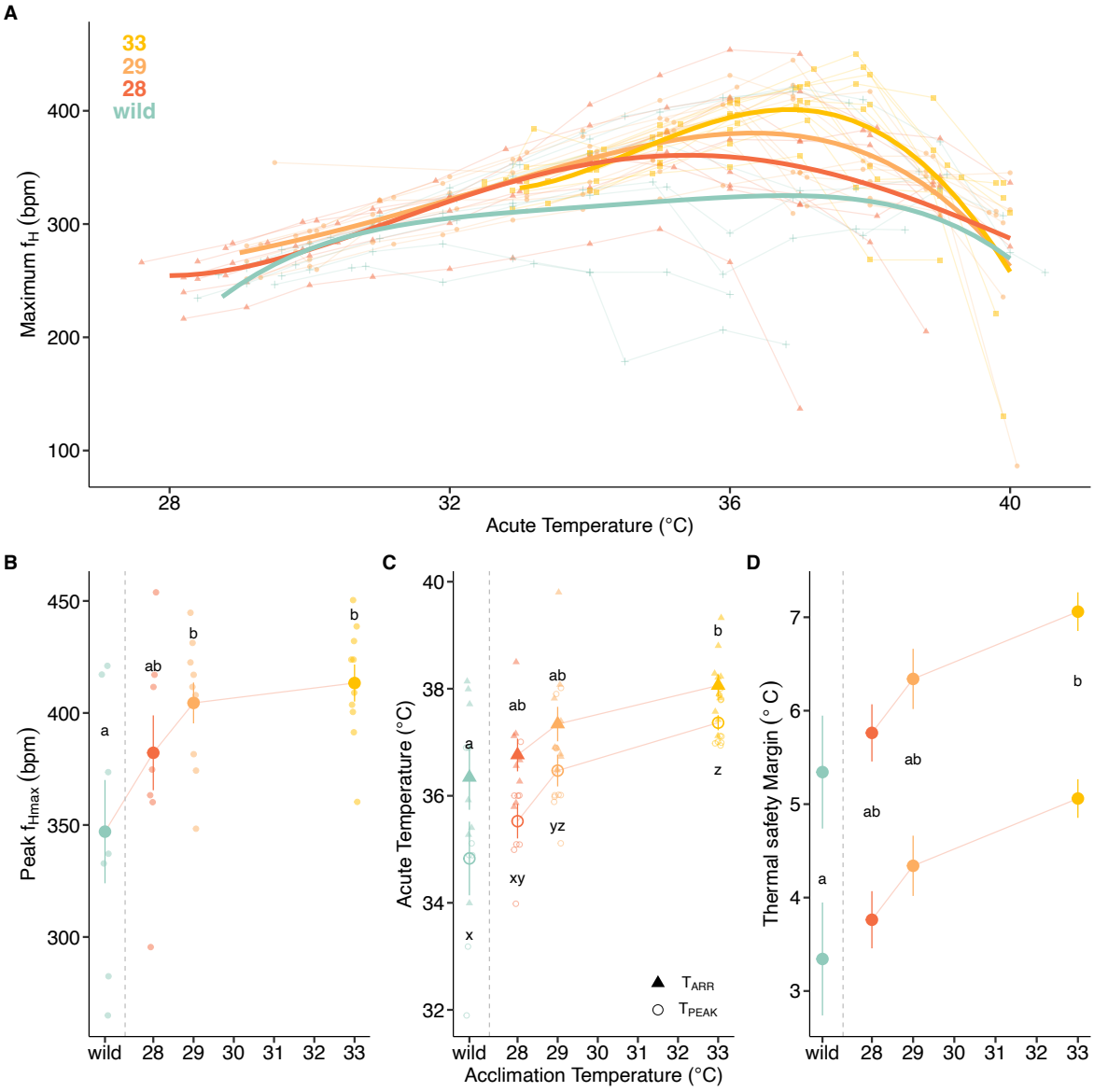

Figure 2.6. Acute cardiac thermal performance curve and associated metrics of wild-caught (28°C, turquoise), and lab-acclimated hawkfish: 28°C (red-orange), 29°C (orange), and 33°C (yellow). (A) Individual performance curves and data points overlaid with thermal

performance curves modeled as a fourth-order polynomial across each acclimation temperature as determined by the best-fit model by BIC. (B) Peak maximum heart rate (f_{Hmax}) in bpm. (C) temperature at f_{Hmax} (T_{PEAK}; circles, significance letters below) and temperature at which arrhythmias began ($T_{ARR;}$ triangles, significance letters above), and (D) thermal safety margin presented as the difference between T_{ARR} and maximum environmental temperature during a marine heatwave of 31°C (top) or 33°C (bottom). (B-D) Values are presented as means ± SEM and individual points represent values for individuals. Different lowercase letters indicate significant differences among acclimation temperatures (ANOVA, Tukey HSD). The gray dashed vertical line separates wild-caught fish from lab-acclimated fish.

2.5 Discussion

This study provides evidence that hawkfish have a plastic response to simulated marine heatwave conditions. While temperature acclimation had a moderate impact on certain metabolic metrics (SMR, MMR, AAS, and FAS), it had a more pronounced effect on other important physiological processes. Recovery and digestion were found to be more costly even 1°C above ambient conditions, and this appears to be exacerbated during the summer season. When testing the effect of relative meal size on digestion, we found that doubling the meal size did not increase digestion costs or SDA_{peak} at 33°C. This study is also, to our knowledge, the first study to report data on coral reef fish cardiac performance during an acute temperature challenge, revealing that hawkfish have a plastic cardiac response to marine heatwave conditions, but this plasticity plateaus with increasing heatwave conditions. Overall, our study provides evidence that coral reef fish have impaired physiological

performance under marine heatwave conditions, and experience important limitations with respect to digestion and recovery. These changes in physiology may impact their behavior, and could severely compromise their fitness and survival, altering their ecological roles within coral reef ecosystems during marine heatwaves.

Elevated temperatures are commonly known to impair the aerobic scope of coral reef fish (Gardiner et al., 2010; Johansen and Jones, 2011; Nilsson et al., 2009; Rummer et al., 2014). However, this trend does not always hold true (Cook, 2021; Norin et al., 2014; Scheuffele et al., 2021) as observed here for arceye hawkfish. We found that the aerobic scope of the hawkfish was not significantly impaired at higher acclimation temperatures or across seasons, supporting the notion that aerobic scope may not always be the most informative performance metric across certain temperature ranges, or for certain species (Clark et al., 2013). While examining a larger temperature range could show impaired aerobic scope in hawkfish at temperatures beyond those tested here, it may be more valuable to examine important aerobic activities within the context of aerobic scope at current and predicted future temperatures. In our study, hawkfish acclimated to warmer temperatures had less available scope for other activities during digestion and recovery, particularly during an extreme heatwave simulation (33°C). This indicates that both recovery and digestion processes were more thermally sensitive to temperature changes than aerobic scope. Thus, the available scope for hawkfish to perform their ecological function as perching ambush predators on coral reefs is likely to be significantly reduced when digesting or recovering from exhaustive exercise at warming temperatures. Similar temperature-dependent effects on different performance metrics have been observed in other species with different ecological

roles, including the swimming energetics of triggerfish and parrotfish, as well as the activity patterns of coral trout (Korsmeyer et al., 2002; Johansen et al., 2014).

As predators that perch on coral to hunt prey in exposed reef environments and engage in aggressive territorial interactions (Kane et al., 2009), hawkfish rely on the capacity to rapidly swim and recover in a timely manner. Hawkfish acclimated to 29°C took twice as long to recover to 75% AAS (the hypothesized threshold when hawkfish would be able to fully resume normal activities), and nearly six times as long at 33°C compared to ambient winter conditions (27°C). This indicates that fish exposed to more extreme marine heatwaves may have constrained energy to perform aerobic activities such as hunting and guarding territories (i.e., they have a reduced capacity to perform their ecological functions) and they remain vulnerable for an extended duration (Birnie-Gauvin et al., 2023). Somewhat surprisingly, fish in the summer acclimated to ambient conditions (28°C) had impaired recovery compared to fish acclimated to the ambient winter conditions (27°C). This could indicate seasonal effects on performance, with small differences in temperature having somewhat pronounced effects on recovery. Alternatively, this pattern could be related to differences in their thermal histories. The winter-tested fish (27°C) had experienced a true heatwave, reaching 31°C in the wild only a few months prior to collection, and may have been pre-acclimatized to warmer conditions (Figure 2.1A).

Processing food increases metabolic rate because it requires the ingestion, digestion, absorption, and assimilation of nutrients (McCue, 2006; Secor, 2009; Jobling, 1983). Depending on the meal, conditions, and species, these costs can demand a vast scope, and this is especially true for predators (Steell et al., 2019). Hawkfish that digested a meal of the same size (2% BM) at 33°C compared to 27°C had twice the metabolic cost during the peak

of digestion (SDA_{peak}), leaving 50% less aerobic scope available to them. The finding that higher temperatures induce greater metabolic rates during digestion aligns with the work done on other warm-adapted fishes, including lionfish (*Pterois* spp.) (Steell et al., 2019), southern catfish (*Silurus meridionalis*) (Luo and Xie, 2008), yellowfin tuna (*Thunnus albacares*) (Klinger et al., 2016), and the Caribbean neon goby (*Elacatinus lobeli*) (Di Santo and Lobel, 2016). Meal size (2 vs 4% BM) did not increase SDA_{peak} or SDA costs at 33°C but ambient fish fed 4% BM had greater SDA_{peak} than those fed 2% BM. However, we did not test the same treatment (27 or 28°C) across both 2 and 4% BM SDA trials, limiting our ability to assess if bigger meals disproportionately increase costs for fish acclimated to warmer temperatures. Additionally, the protein content was 35% higher in the scallop in 2022 compared to 2019, which would also increase SDA (Cho et al., 1989). Notably, the fish in our study were gavage-fed, which means they did not freely choose their relative meal size. However, the SDA_{peak}/SMR remained ~2 across acclimation temperatures and relative meal sizes, indicating fish need to double their MO_2 to digest a meal. Given the small factorial scope available to the fish during digestion (MMR/SMR - SDA_{peak}/SMR) with the 4% BM ration size and at the warmer temperatures, hawkfish may choose smaller meals to retain scope for activity in the wild. This aligns with the finding that ectotherms eat less at supra-optimal temperatures (Jobling, 1981) potentially reducing their SDA_{peak} response to preserve aerobic scope for other activities (Jutfelt et al., 2021). If fish opt for smaller meals in higher temperatures to preserve energy for activity, their overall consumption decreases. This could have significant implications for their ecological role as predators and nutrient recyclers in the coral reef ecosystem (Brandl et al., 2019; Van Wert et al., 2023), which is particularly critical to corals during marine heatwaves (Shantz et al., 2023).

The ectotherm heart plays a crucial role in circulating blood containing oxygen, nutrients, wastes, hormones, and immune cells throughout the body. It is sensitive to temperature change and is considered the first organ system that fails under high temperatures, making it an ideal system for studying thermal tolerance[25]. Under acute warming, fish increase their heart rate to improve oxygen delivery to tissues[26,27]. In response to a prolonged thermal event, fish may undergo cardiac remodeling, which generally happens over the course of days to weeks (Keen et al., 2017). In the case of a heatwave, which is prompt and temporary, the cardiac response is ideally rapid and reversible. By measuring the cardiac thermal limits of fish acclimated to different temperatures for one week, we captured the acclimation abilities of hawkfish to a relevant marine-heatwave timescale, where full thermal acclimation may not have yet occurred (Hardison et al., 2023).

The thermal tolerance limits of coral reef fish have been typically assessed in previous literature using measures such as CT_{max} or LT_{50}, which have revealed limits from 34 to 44°C depending on the species, life stage, or acclimation conditions (Comte and Olden, 2017; Eme and Bennett, 2009; Giomi et al., 2019; Habary et al., 2017; Illing et al., 2020). While these metrics are informative in certain contexts (Desforges et al., 2023), they lack a direct link to the actual mechanisms driving thermal tolerance limits (Lefevre et al., 2021), and occur at temperatures beyond which the heart has gone arrhythmic (Hardison et al., 2021), such that the fish is no longer functional. Instead, the cardiac thermal tolerance test may be more informative of functional thermal tolerance limits. Here, we showed that fish had a plastic response to warmer conditions by increasing their f_{Hmax}, T_{ARR}, and T_{PEAK}, but they hit an upper thermal ceiling between 29 and 33°C at which point cardiac performance was fixed. While the TSM for a 31°C heatwave scenario ranged from 6-7°C at the upper

acclimation temperatures, hawkfish were increasingly unable to fully compensate. Though the CT_{max} of arceye hawkfish in Mo'orea has not been measured to our knowledge, the CT_{max} of the closely related whitespot hawkfish (*Paracirrhites hemistictus*), in Indonesia acclimated to 27.8°C was 40.2°C (Eme and Bennett, 2009), representing a 4°C difference from the T_{ARR} of fish acclimated to 28°C in our experiment. Even so, cardiac impairments (i.e., reduced scope for heart rate, diminished oxygen delivery) begin at temperatures below T_{ARR}, making the functional thermal tolerance narrower than observed here (van der Walt et al., 2021).

Of note is the finding that arceye hawkfish had some of the highest peak f_{Hmax} measured in fish thus far, reaching 415 bpm at 37.6°C. In fact, their heart rates are greater than what would be expected given the peak maximum heart rates of other similar-sized fishes, including opaleye (*Girella nigricans*) acclimated to 20°C: 205 bpm at 30°C (Hardison et al., 2021), goldfish (*Carassius auratus*) acclimated to 28°C: 210 bpm at 33.1°C (Ferreira et al., 2014), cyprinids (*Danio* spp.) acclimated to 28°C: 256-323 bpm at 33-34°C (Sidhu et al., 2014), and killifish (*Fundulus heteroclitus*) acclimated to 33°C: 244 bpm at 36.8°C (Safi et al., 2019). In South Africa, a sparid fish is already experiencing temperatures beyond its cardiac limits during a marine heatwave, threatening its survival and distribution (van der Walt et al., 2021). Hawkfish, and likely other coral reef fish, are operating at a high and narrow temperature range where the heart nears its functional limits. The plasticity of the heart will determine whether these fish survive marine heatwaves and can function at such extremes, or if fish will need to move to cooler or deeper waters. While the plasticity of tropical fish hearts is understudied, research on salmonid (*Oncorhynchus* spp.) and zebrafish (*Danio* spp.) hearts indicates potential acclimation mechanisms ranging from changes at the molecular to the morphological level (Eliason and Anttila, 2017). Warm acclimation changes

cardiac mitochondrial metabolism and adrenergic sensitivity (Eliason and Anttila, 2017). Over days to weeks, changes include a reduction in the relative ventricular mass, an increase in compact myocardium and capillary density within the compact myocardium, and changes in collagen fiber densities (Keen et al., 2017). Although we do not examine the specific mechanism here, we show that arceye hawkfish have some capacity for cardiac thermal acclimation under heatwave conditions, but that plasticity reaches a ceiling beyond ~29°C. Whether similar patterns exist in other coral reef species remains to be determined.

Conservation physiology informs us about how fish may respond to environmental and anthropogenic changes (Cooke et al., 2013; Illing and Rummer, 2017). In addition to examining the acclimation response to marine heatwave conditions, we demonstrated the importance of assessing the cardiac thermal tolerance of non-acclimated fish captured directly from the wild. Despite having been acclimatized to a similar mean temperature of 28°C, wild-caught fish had a lower peak f_{Hmax}, T_{ARR}, and T_{PEAK} than laboratory fish acclimated to 28°C. This may be related to differences in thermal variation (Schwieterman et al., 2022), diet (Hardison et al., 2023), or other holding effects. Alternatively, fish may have had residual effects from the clove oil used to capture fish 3-8 h earlier in the day, though this is known to reduce T_{PEAK} and f_{Hmax} and not T_{ARR} (Casselman et al., 2012).

A fish's prior thermal experience may also impact its ability to perform during a marine heatwave. If fish are thermally acclimated for an extended period, fish may be able to fully compensate via physiological and morphological changes. The shorthorn sculpin, for example, fully thermally compensated certain performance metrics after eight weeks of acclimation to a warmer temperature (Sandblom et al., 2014). In contrast, prolonged exposure to suboptimal temperatures could have deleterious impacts (e.g., impaired growth

rates, gonad development, immune response, and swim performance) (Johansen and Jones, 2011; Donelson et al., 2010; Genin et al., 2020; Grans et al., 2014). Unfortunately, we are unable to discern the 2019 marine heatwave effects from summer and winter conditions, and we are limited in our ability to directly compare performances between seasons due to differing temperature treatments. Even still, we found evidence for differences in metabolic performance between the two experimental timeframes. Seasonal variation in metabolism may be one possibility, where fish have reduced resting metabolic rates in the winter (Chipps et al., 2000). There is also the possibility that fish have elevated metabolism related to their spawning season (Beamish, 1964). Although the spawning season for arceye hawkfish remains unknown to us, the spawning season for long-nosed hawkfish (*Oxycirrhites typus,* Papua New Guinea) is in the summer (Donaldson and Colin, 1989). On the other hand, the 2019 marine heatwave event could have had a legacy on fish performance, or fish that did not adapt relocated to deeper, cooler waters or died, and the most warm-adapted fish remained. Thus, despite the small difference of approximately 1°C in winter versus summer conditions, hawkfish may be more vulnerable to marine heatwaves in the summer when their metabolic demands are greatest, as they become more territorial and develop their gonads.

2.6 Conclusion

The plasticity of coral reef fish will largely determine how they fare in acute rapid environmental challenges posed by climate change. Although hawkfish showed some acclimation response, the hawkfish acclimated to 33°C were compromised in activities that are fundamental to their role as predators including recovery from burst swimming and peak digestion. Finally, we found that cardiac plasticity plateaued at 29°C. If hawkfish physiological performance is constrained during a marine heatwave in the wild, they may

choose to adjust their diet type, diet quantity, or sacrifice energy toward other important activities. This would inherently alter how hawkfish interact with the reef and their important roles as invertivores and nutrient recyclers. The impact of such heatwaves on behavior and ecosystem function in the wild may have unanticipated consequences on the reef community, and these impacts may occur across fish species.

Table 2.1. Summary statistics for SDA metabolism.

Acclimation temperature (°C)	BM fed (%)	n	SDA (mg O_2 kg^{-1})	SDA_{dur} (h)	SDA_{peak} (mg O_2 kg^{-1} min^{-1})	time-to-SDA_{peak} (h)	SDA_{coeff} (%)
27	2	10	31.23 ± 4.24	28.1 ± 1.42	4.95 ± 0.47	13.6 ± 2.94	0.69 ± 0.09
28	4	10	57.44 ± 8.43	33.28 ± 1.01	7.16 ± 0.61	6.15 ± 1.6	1.26 ± 0.19
29	2	9	48.58 ± 6.96	31.94 ± 1.44	8.63 ± 0.78	6.61 ± 2.9	1.07 ± 0.15
31	2	8	30.3 ± 4.78	24.12 ± 2.38	7.26 ± 0.46	7.94 ± 1.73	0.67 ± 0.11
33	2	7	36.62 ± 12.77	25.83 ± 6.99	9.35 ± 1.13	3.5 ± 0.94	0.8 ± 0.28
	4	11	52.56 ± 6.28	25.94 ± 1.46	9.98 ± 0.92	5.23 ± 1.52	1.15 ± 0.14

SDA metrics (sample size (n), SDA, SDA_{dur}, SDA_{peak}, time-to-$SDA_{peak,\ and}$ SDA_{coeff}) across 5 acclimation temperatures and 2 feeding treatments (2% and 4% body mass (BM) of scallop).

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Chapter 3: Population variability in thermal performance of pre-spawning adult Chinook salmon

3.1 Abstract

Climate change is causing large declines in many Pacific salmon populations. In particular, warm rivers are associated with high levels of premature mortality in migrating adults. The Fraser River watershed in British Columbia, Canada, supports some of the largest Chinook salmon (*Oncorhynchus tshawytscha*) runs in the world. However, the Fraser River is warming at a rate that threatens these populations at critical freshwater life stages. A growing body of literature suggests salmonids are locally adapted to their thermal migratory experience, and thus, population-specific thermal performance information can aid in management decisions. We compared the thermal performance of pre-spawning adult Chinook salmon from two populations, a coastal fall-run from the Chilliwack River (125 km cooler migration) and an interior summer-run from the Shuswap River (565 km warmer migration). We acutely exposed fish to temperatures reflecting current (12°C, 18°C) and future projected temperatures (21°C, 24°C) in the Fraser River and assessed survival, aerobic capacity (resting and maximum metabolic rates, absolute aerobic scope (AAS), muscle and ventricle citrate synthase), anaerobic capacity (muscle and ventricle lactate dehydrogenase) and recovery capacity (post-exercise metabolism, blood physiology, tissue lactate). Chilliwack Chinook salmon performed worse at high temperatures, indicated by elevated mortality, reduced breadth in AAS, enhanced plasma lactate and potassium levels and elevated tissue lactate concentrations compared with Shuswap Chinook salmon. At water temperatures exceeding the upper pejus temperatures (T_{pejus}, defined here as 80% of

maximum AAS) of Chilliwack (18.7°C) and Shuswap (20.2°C) Chinook salmon populations, physiological performance will decline and affect migration and survival to spawn. Our results reveal population differences in pre-spawning Chinook salmon performance across scales of biological organization at ecologically relevant temperatures. Given the rapid warming of rivers, we show that it is critical to consider the intra-specific variation in thermal physiology to assist in the conservation and management of Pacific salmon.

3.2 Introduction

Pacific salmon (*Oncorhynchus* spp.) are fundamental to the ecosystems, economy and culture of the Northeast Pacific (Naiman et al., 2002; Jacob et al., 2010; Gislasin et al., 2017). Their lifetime fitness depends on their ability to migrate from the Pacific Ocean to natal freshwater spawning grounds on finite energy reserves to then spawn and die (i.e. they are semelparous; Mesa and Magie, 2006; Farrell et al., 2008). Salmon return to their natal spawning grounds with high fidelity, which in turn maintains many genetically and geographically distinct populations. These populations experience different environmental conditions during their upriver migration depending on when they enter the river and where they spawn, resulting in populations that are locally adapted to their migration conditions (Lee et al., 2003b; Eliason et al., 2011). However, warming rivers are causing mass mortalities of adult spawning salmonids across species and populations throughout their ranges (Gilhousen, 1990; Schreck et al., 1994; Keefer et al., 2008; Scholz et al., 2011; Martins et al., 2012; Bowerman et al., 2018; von Biela et al., 2022). Therefore, there is an

immediate need to better understand the thermal physiology of Pacific salmon across species and among populations.

Chinook salmon (*O. tshawytscha*) have wide life history diversity and many genetically distinct spawning populations that provide the opportunity for differences in average thermal experiences and adaptation (Bourret et al., 2016). They are broadly distributed across a range of thermal environments from the warm Central Valley, California, across cool sub-arctic Alaska and back around the Pacific Rim to Japan, with populations declining and federally listed or assessed as endangered or threatened throughout their range (e.g. United States—ESA, 2022; Canada—COSEWIC, 2020). Central within their range, more than 50 distinct Chinook salmon populations return annually to migrate up the Fraser River, in British Columbia, Canada (Beacham et al., 2002). Fraser River summer water temperature has increased by over 2°C since the 1950s, reaching over 22°C, (Foreman et al., 2001; Patterson et al., 2007) and is projected to continue increasing (Morrison et al., 2002; Ferrari et al., 2007; Grant et al., 2019). Current knowledge suggests that fish are most vulnerable to warming temperatures as embryos/eggs and spawning adults (Pörtner and Farrell, 2008; Dahlke et al., 2020), yet it remains unclear how elevated temperatures affect migrating adult Chinook salmon. In salmonids, physiology and morphology are strongly tied to thermal history, and adult sockeye (*O. nerka*) and embryo and juvenile Chinook salmon are locally adapted to their natal streams (Beacham and Murray, 1989; Beacham and Withler, 1991; Eliason et al., 2011). Yet, we know remarkably little about the migrating adult life stage, nor how populations vary in thermal performance at this life stage and the mechanisms that underlie vulnerability, making conservation and management of Chinook salmon a challenge.

To determine the thermal sensitivity of migrating adult Chinook salmon, we examine the survival, metabolic capacities and recovery differences between two populations with distinct thermal histories. Survival is a clear indicator of success, especially for a semelparous species that has a single opportunity to spawn. Metabolism is also an important metric, because as water temperatures increase, so does aerobic cellular metabolism and therefore whole animal oxygen consumption rate (MO_2, a proxy for metabolic rate) (Fry, 1971; Pörtner, 2001, 2010; Dillon et al., 2010). But fish have a maximal capacity for aerobic metabolism, termed maximum metabolic rate (MMR) (Norin and Clark, 2016). As baseline maintenance metabolism or standard metabolic rate (SMR) increases with temperature, fish have a lower capacity to deliver oxygen to tissues to support aerobic activities such as swimming and migration (Farrell, 2016). This "capacity" is known as their "absolute aerobic scope" (AAS) and is the difference between their MMR and SMR (Fry, 1947; Farrell et al., 2009; Pörtner, 2010; Eliason et al., 2011; Schulte, 2015). Accordingly, AAS increases as a function of temperature until it is maximized, and fish are at their optimal thermal temperature ($Topt_{AAS}$) and have a maximum capacity to perform aerobic activities, before declining at high temperatures (Farrell, 2016). As salmonids' AAS is reduced due to increasing river temperatures, they may not be able to maintain the work needed to migrate upstream and complete spawning. In addition to aerobic swimming, migrating adult salmon must also use anaerobic burst swimming to negotiate hydraulic challenges, avoid predation, dig redds (nests), spawn and defend territories (Rand and Hinch, 1998; Healey et al., 2003; Jain and Farrell, 2003; Berejikian et al., 2007). Salmon can sustain aerobic swimming for extended periods, supporting migrations of hundreds of kilometers; however, they can only maintain anaerobic exercise for shorter durations. They must then restore homeostasis and

metabolically recover by clearing lactate and restoring glycogen, high-energy phosphates, oxygen stores and osmoregulatory balance (Wood, 1991; Milligan, 1996; Kieffer, 2000; Jain and Farrell, 2003; Lee et al., 2003a; Suski et al., 2007; Raby et al., 2015), a measurement termed "excess post exercise oxygen consumption" (EPOC) (Gaesser and Brooks, 1984). To complete upstream migration, salmon need to minimize both the duration and energetic costs of recovery (Claireaux et al., 2000; Suski et al., 2007; Eliason and Farrell, 2016). However, warming river temperatures may prolong recovery time (Prystay et al., 2017; Kraskura et al., 2021), which has clear fitness costs and could result in migration failure in Pacific salmon (Jain and Farrell, 2003; Lee et al., 2003a; Eliason et al., 2011; Burnett et al., 2014b; Raby et al., 2015).

Our objective was to compare the thermal performance of maturing and migrating adult Chinook salmon from the Fraser River. We compared two populations that experience different migration distances and challenges, including different thermal regimes: fall-run Coastal Chinook salmon from the Chilliwack population (125 km cooler migration), and summer-run Interior Chinook salmon from the Shuswap population (565 km warmer migration). We acclimated salmon to ambient conditions (12°C) and exposed salmon to acute warming temperatures either mimicking current, or future projected temperatures expected with climate change. We used intermittent respirometry to measure resting metabolic rate (RMR, a proxy to SMR), MMR following a chase and air exposure protocol, AAS and post-exercise recovery. We also assessed cardiac, red and white muscle enzyme activities (lactate dehydrogenase, citrate synthase), circulating blood plasma ion levels (K^+, Cl^-, Na^+) and metabolite levels (lactate) to evaluate how populations differed in anaerobic and aerobic metabolic capacities and post-exercise recovery. Our hypothesis was that

differences in physiological capacities would explain thermal sensitivities, matching historic riverine thermal conditions. We predicted that summer-run, interior Shuswap Chinook salmon would perform better at high temperatures, as indicated by higher survival rates, greater AAS breadth, a greater recovery capacity (e.g. lower lactate concentrations and plasma ion levels) and greater capacity for anaerobic and aerobic activity [e.g. greater AAS capacity, higher LDH and CS activities], compared with the fall-run, coastal Chilliwack Chinook salmon population. This work will help to elucidate mechanisms underlying intraspecific variability in thermal performance in ectotherms. In addition, by quantifying the thermal performance of adult Chinook salmon populations with different migration histories we can help inform conservation efforts of Chinook salmon across their geographic range.

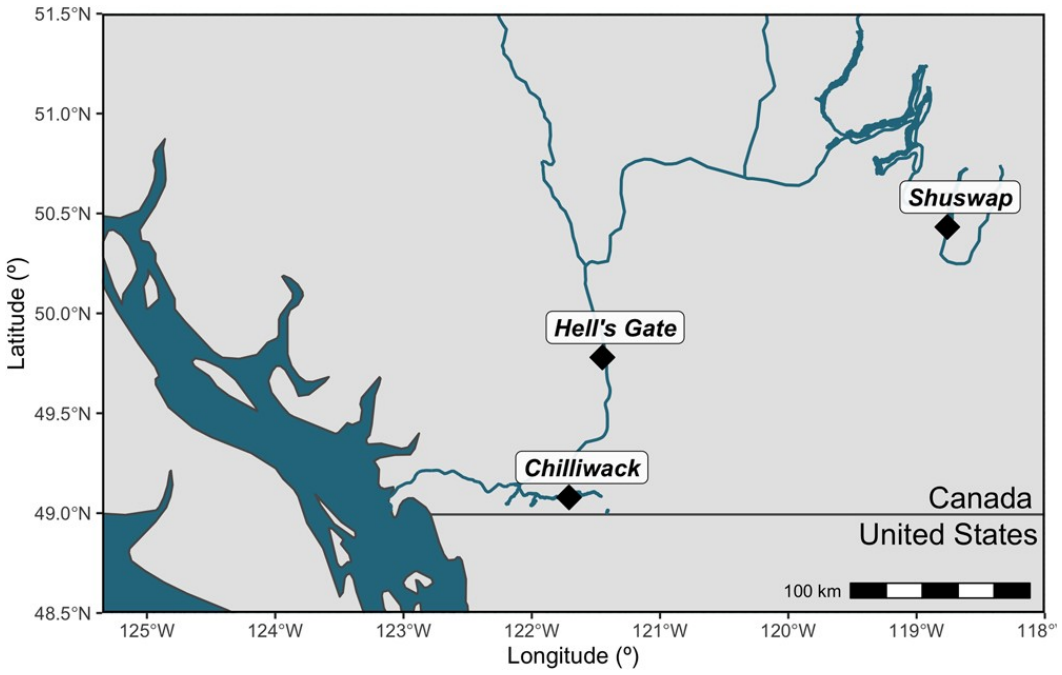

Figure 2.1. Map of Fraser River, British Columbia, Canada watershed. Spawning grounds for Chilliwack (Coastal) and Shuswap (Interior) Chinook salmon (*O. tshawytscha)* and the

primary hydraulic challenge separating the Coastal and Interior populations (Hell's Gate) are marked (black diamond).

3.3 Methods

3.3.1 Fish collection and holding

Chilliwack (N = 47, fork length (FL, snout to fork in tail in mm) = 632 ± 143 mm, mean ± SEM) and Shuswap (N = 38, FL = 673 ± 126 mm) Chinook salmon were collected *en route,* close to their respective spawning grounds: dip-netted at Chilliwack Hatchery (49.078550, −121.709216) October 7th to 24th 2019 and caught using seine-nets downstream of Mabel Lake (50.605414, −118.822505) September 17th to 27th 2019, respectively (Figure 2.1). These populations experience different conditions during their spawning migrations in the Fraser River watershed. The "Coastal" Chilliwack Chinook salmon have a relatively easy migration (125 km). They are a fall-run and enter the Fraser River in September and arrive at Chilliwack Hatchery (220 m elevation) in October for spawning, encountering cooler temperatures (Daily mean = 15.5°C in 2019, Historic mean = 15°C from 1950–2018, Current maximum daily mean = 21.5°C) (Patterson et al., 2007; Fraser River Ewatch, 2021). The "Interior" Shuswap Chinook salmon have a longer migration (565 km) and must pass through Hell's Gate and the Fraser Canyon, a 200 km stretch of challenging swimming through fast waterflow, both posing considerable physical challenges, especially in warmer conditions (Martins et al., 2012). Shuswap Chinook salmon are a summer-run and enter the Fraser River in July, soon enter the South Thompson River and migrate to their spawning grounds downstream of Mabel Lake (450 m elevation) in mid to late September and

complete spawning by mid-October (Shearing, 2013). Because of their early entry, Shuswap Chinook salmon experience warmer conditions (Daily mean = 18.5°C in 2019, Historic mean = 18°C from 1950–2018, Current maximum daily mean = 22.8°C) in the lower Fraser River and South Thompson Rivers (Patterson et al., 2007; Fraser River Ewatch, 2021).

After capture, the fish were transported by truck in a holding tank (2700 L, stocking density ≤ 15 fish per tank, > 90% air saturation) to the Cultus Lake Laboratory in Chilliwack, British Columbia, Canada (Fisheries and Oceans Canada). Fish were transferred to large outdoor holding tanks (5.3 m diam, 8000 L; stocking density ≤ 11 fish per tank) supplied with flow-through, sand-filtered and UV-sterilized freshwater from Cultus Lake. In each tank, air stones maintained oxygen > 90% air saturation and a water pump generated a circular current. The water temperature was maintained at 11–12°C by mixing warmer shallow water with colder deep lake water. Each tank had a transparent window to allow fish to maintain a natural diurnal cycle during the holding period. Fish were held for a minimum of 1 day and a maximum of 17 days prior to experimentation and were not fed. All experimental protocols were approved by the Animal Care Committee at the University of British Columbia (protocol #A17–0160).

3.3.2 Intermittent flow respirometry

Five respirometers (54.5 or 98 L), custom-built from semi-transparent polyvinyl chloride tubes with a removable screw-on lid, held individual fish during the intermittent flow respirometry protocol for recordings of MO_2. Fish were assigned to a respirometer according to size in order to maintain a 15:1 to 25:1 respirometer volume: animal mass. Water was continuously recirculated through the respirometer with a water pump (Eheim 1200 or Lifeguard Quiet One Pro 3000) and a flush circuit comprising a time-controlled

flush pump (Lifeguard Quiet One Pro 5000, 45 L min^{-1} or Current USA E-Flux 3170, 48 L min^{-1}) replenished the water and returned the oxygen levels to normoxia after each MO_2 measurement. Oxygen (mg L^{-1}) and temperature (°C) within the respirometers were recorded using a robust fiber optic oxygen sensor and temperature sensor (Pyroscience, Germany) placed in a PVC recirculating loop, which was connected to a FireSting optic O_2 (and temperature) meter (Pyroscience, Germany). During a reading period, the flush pump was turned off and the decline in O_2 levels due to the respiration of the fish was recorded. Chamber mixing was achieved by recirculation pumps as well as the ventilation and tail movements of the fish. The three experimental tanks holding the respirometers were sheltered with a tarp to minimize disturbance and the individual fish were oriented with their heads in the opaque caps, minimizing visual disturbances.

Each experimental session started in the morning by transferring fish to an experimental thermal exposure tank (1.95 m diam, 1970 L, stocking density = 2 to 3 fish) maintained at 12°C. The temperature was then increased by 2°C h^{-1} until it reached the randomly assigned test temperature (12, 18, 21, or 24°C) and then held at the test temperature for 1 h. Fish were transferred using dip nets into an exercise tank (1.8 m diam, 2000 L) receiving a high flow-through of water maintained at the test temperature. Each fish underwent two exercise measurements: MMR_{1h}, after 1 h of acute thermal exposure; and MMR_{18h}, after 18 h of acute thermal exposure. MMR_{1h} occurred 13:00–14:30 and the MMR_{18h} occurred 8:00–9:00 the following day. To determine MMR, the fish were exercised to exhaustion by manually "chasing" the fish to elicit swimming or burst swimming for 3 min, by lightly following or touching the individuals' tail, followed by 1 min air exposure in a dip net (Gale et al., 2014; Little et al., 2020a). Following air exposure, fish were

immediately (within 120 s) transferred into respirometers submersed in flow-through experimental tanks (181 cm diam, 42 cm depth) maintained at the test temperature (via mixture of heated water and cold lake water) to measure MO_2 following the chase protocol and during the subsequent recovery over the following 18–20 h during which RMR was determined (more details below). After 18–20 h at the exposure temperature, the fish were removed from the respirometer and underwent another identical chase exercise protocol as described above, and then immediately returned to the respirometer to measure immediate MO_2 (MMR_{18h}). The first MO_2 measurement following MMR_{1h} and MMR_{18h} consisted of a 4–6 min closed DO measurement during which the flush pump was turned off allowing assessments of MO_2, and then by flushing the respirometer to fully reoxygenate the respirometer. This was followed by automated 10- or 15-min MO_2 cycles, comprising 6–9 min flushing periods to fully reoxygenate the respirometer followed by 4–6 min flush-off periods until the removal of the fish. The timing of the MO_2 cycles was adjusted to ensure that the O_2 remained above 75% air saturation. Test temperatures were selected to reflect current Fraser River temperatures on the lower (12°C) and upper-range (18°C) and future projected temperatures (21, 24°C) (Fraser River Ewatch, 2021). The overall brief temperature duration (i.e. 1 h of temperature exposure and 18 h of temperature exposure) was specifically chosen to mimic an acute, short-term ecologically relevant heat stress event (Rodnick et al., 2004; Hague et al., 2011).

Recovery occurs over multiple timescales, including partial, but rapid, initial recovery (within minutes to hours, important for repeat bouts of maximum swim performance) (Farrell et al., 1998; Jain et al., 1998; Jain and Farrell, 2003; Lee et al., 2003a) and full recovery (up to 16 hours, important for repairing cellular damage and restoring

metabolites, SMR, resting heart rate) (Milligan, 1996; Zhang et al., 2018). We sampled fish after 1 h of recovery when the initial rapid recovery phase was expected to be complete and under optimal conditions fish would be able to resume swimming (e.g. Eliason et al., 2013b), however full recovery would not be expected. Following the MMR_{18h} measurement and 1 h recovery period, the fish were removed from the respirometer and euthanized by a blunt cranial blow followed by severing the spinal cord with a scalpel. Fish exhibiting signs of morbidity throughout the experiment (e.g. loss of equilibrium, gasping at surface of exposure tank) were immediately euthanized. Trials occurred over 26 days and systems were cleaned between each population using a diluted Virkon disinfectant. Probe calibration occurred weekly using two-point calibrations (aerated water for 100%, sodium sulfite for 0%).

3.3.3 Terminal sampling and body morphometrics

Each fish was measured for mass (to the nearest gram) and fork length (to the nearest mm). A caudal blood sample (~3 ml) was collected (21 G needle, lithium heparinized BD Vacutainer, BD, Franklin Lake, NJ, USA) and immediately placed on ice for a maximum of one hour. Hematocrit was measured in duplicate and the remaining blood was centrifuged at 1200 g for 5 min to separate the blood plasma, which was immediately flash frozen in liquid nitrogen and stored at −80°C for later analyses. Heart (bisected across the valve to the apex) and muscle tissues (two samples ~ 0.5 cm thick containing both red and white muscle posterior to the dorsal fin) were freeze clamped in liquid nitrogen and stored at −80°C for later analyses. Organ masses were recorded for ventricle, gonads, liver and spleen and the adipose fin was marked as present (i.e. wild fish) or absent (i.e. hatchery origin fish). The gonadal somatic index (GSI), relative ventricular mass (RVM), splenosomatic index (SSI),

hepatosomatic index (HSI) were calculated by dividing organ mass (gonads, ventricle, spleen, or liver) by the fish total body mass * 100.

3.3.4 Blood and tissue analyses

Plasma and tissue metrics reveal possible mechanisms underlying the performance of individuals. Potassium and sodium were analyzed using an XP Five-channel Flame Photometer (BWB Technologies, UK), chloride using a Chlorocheck Digital Chloridomter (EliTech Group, France) and osmolality using a 3200 Osmometer (Advanced Instruments, USA). Elevated or depressed ions indicate an ionic imbalance and, particularly following exhaustive exercise, can disrupt muscle contraction and inhibit swimming (Wood, 1991; Holk and Lykkeboe, 1998). Plasma lactate and glucose were measured using a 2300 Stat Plus Glucose and L-Lactate analyzer (YSI, USA) according to established methods (Farrell et al., 2001). Glucose is a finite energy reserve that fuels metabolism and is mobilized during stressful events (Kubokawa et al., 1999). Hormones were run in a FLUOstar Omega multi-mode microplate reader (BMG Labtech, USA). Cortisol was analyzed via Cortisol ELISA kits (Neogen, USA) and read for absorbance at 650 nm, followed by the addition of 50 µl 1 N HCl and measured at 450 nm. Cortisol has many functions and elevated levels have been linked to impaired performance in Pacific salmon (Milligan, 1996). Testosterone and 17B-estradiol were extracted from plasma using diethyl ether and quantified with ELISA kits (Neogen, USA) according to manufacturer instructions. Reproductive hormones promote sexual maturation and indicate the reproductive status of individuals (Idler et al., 1961). All plasma samples were run in duplicate.

Lactate results from anaerobic glycolysis and indicates physiological recovery status from anaerobic exercise (Milligan, 1996). To measure tissue lactate, frozen ventricle and white muscle samples were ground under liquid nitrogen using a mortar and pestle and weighed (~20 mg), treated with ice-cold 8% $HClO_4$ and sonicated on ice with three 5 s bursts. The homogenate was centrifuged at 10000 g for 10 min at 4°C and the supernatant was neutralized using 3 M K_2CO_3, centrifuged again at 10000 g for 10 min at 4°C and extracts were aliquoted (~400 µl) and stored at −80°C until analyses. Samples were assayed in triplicate on a FLUOstar Omega Microplate reader with a lactate standard curve to measure the concentration of lactate using LDH to catalyze the oxidation of lactate with the reduction of NAD^+ at 340 nm (Richards et al., 2002).

If oxygen supply is limited during exercise or post-exercise recovery, aerobic and anaerobic metabolic proxies indicate both the ability and capacity to sustain performance (Brett, 1964). The capacity for aerobic (e.g. CS) and anaerobic metabolism (e.g. LDH) in tissues with high ATP demand (e.g. heart, red muscle, white muscle) might be locally adapted to thermal conditions and enzyme activity levels measured at different assay temperatures across groups (sex, populations, species) could reveal differences in thermal adaptation (Little et al., 2020b). Enzyme activities (CS and LDH) were measured from the ventricle, white and red muscle homogenates to determine the thermal performance of these tissues across 8, 12, 18, 24 or 25 and 28°C using established methods (Moyes et al., 1997; Martínez et al., 2006; Little et al., 2020b). Frozen tissues were sliced, weighed (~25 mg) and homogenized in buffer (0.1% Triton, 50 mmol l^{-1} HEPES, 1 mmol L^{-1} EDTA, pH 7.4) with 0.5 mm zirconium oxide beads in a bead beater (Fisherbrand Bead Mill 24 Homogenizer) kept at 4°C for two 6 m s^{-1} 30 s cycles with 1 min on ice in-between. Aliquots were

separated (~300 µl each) and stored at −80°C until analyses for LDH and CS. All samples were read in triplicate on a FLUOstar Omega multi-mode microplate reader (BMG Labtech, Germany) at 340 nm to measure the disappearance of NADH for LDH activity, or 412nm to measure the production of 5-thio-2-nitrobenzoic acid, a proxy for CS activity. Activity levels were calculated with an extinction coefficient of 6.22 and 13.6 $mmol^{-1}$ cm^{-1} for LDH and CS, respectively. Absorbance readings were normalized using the Pathlength sensor.

3.3.5 Data and statistical analyses

Fish with low hematocrit (<20%) were excluded from the study (4 fish total). The MO_2 data were analyzed and visualized in RStudio (RStudio Team, 2020) using custom code (Kraskura, 2022). The mass-specific MO_2 (expressed in mg O_2 kg^{-1} min^{-1}) was calculated from the change in the concentration of O_2 over time (ΔO_2, i.e. the slope of a fitted regression line over the course of each measurement cycle) in the sealed respirometer using $MO_2 = (\Delta O_2 * (v_R - v_F))/m$, where v_R is respirometer volume, v_F is mass of the fish (L, assuming 1 kg = 1 L) and m is the fish mass (kg).

MMR was calculated in three ways: MMR_{1h}, MMR_{18h} and $MMR_{OVERALL}$ (see below). MMR_{1h} and MMR_{18h} were calculated from the first measurement cycle following the exhaustive exercise protocol using a sliding window analysis (90 s minimum). Specifically, each ≥90 s sliding window began at the start of the measurement period and moved in 1 s increments across the measurement cycle, and the steepest ΔO_2 with an $R^2 > 0.9$ was used as MMR (Little et al., 2020a). Depending on life stage and behavior, exhaustive exercise does not always evoke the highest MO_2, which is what defines MMR (Raby et al., 2020) so we also estimated $MMR_{OVERALL}$ by choosing the maximum MO_2 value measured during the experiment for each fish with more than 60 overnight

recovery MO_2 values. Because there was a minimal effect of time exposed to temperature, we used the $MMR_{OVERALL}$ in our primary analyses to best estimate maximum MO_2.

RMR typically occurred during nighttime and was calculated as the lowest 10% quantile of all validated MO_2 measurements with $R^2 > 0.85$. RMR was calculated for individuals that had at least 60 validated MO_2 measurements. All regressions were visually assessed to ensure slopes were linear, negative and with no artificial irregularities. Fish that died during RMR measurements were not included in the RMR estimate. AAS values were calculated as the $MMR_{OVERALL}$ – RMR for each individual, and factorial aerobic scope (FAS) values were calculated as $MMR_{OVERALL}$/RMR. In cases where fish did not survive the experiment (from the beginning of the acute 1 h thermal exposure to the end of the MMR_{18h} recovery period), an individual was considered a mortality and AAS and FAS were treated as values of 0 because a dead fish is assumed to have zero aerobic scope. Further, it is clear from moribund fish that aerobic capacity is severely diminished. While it is rare to include mortalities in estimates of aerobic scope, these fish died during the experimental procedures (i.e. during the respirometry measurements or 1 h thermal exposure period before the respirometry trial began) and thus are part of the full, complete dataset. We also present results from survivors only but point out that this method could be misleading and may greatly overestimate the aerobic capacities of these populations.

Allometric scaling of metabolism was considered because of the large range in body mass, 1.4 to 7.2 kg. Mixed models were used to test the significance of the following main predictor variables: population and test temperature (with and without the interaction) and $\log_{10}$(body mass). Model selection criteria (Bayesian Information Criterion, BIC) were used to determine the best-fit model for each performance metric and confirmed allometric

scaling of metabolism. The significance of each main effect was tested using ANOVA. For visualization purposes, the values are presented as a mass-specific (mg O_2 kg^{-1} min^{-1}) adjusted to represent a 3.5 kg fish using metabolic scaling coefficients estimated by mixed models (scaling exponent of 0.67 for RMR and 0.58 for MMR_{1h}, MMR_{18h} and $MMR_{OVERALL}$).

Background microbial respiration rates were measured for 30 min before and after each experiment for each respirometer. However, the microbial respiration was determined to be negligible and therefore not incorporated into analyses.

We measured short-term recovery with three estimates: (1) Percent of AAS (%AAS), calculated as the MO_2 recovered as a function of the AAS following each exercise calculated at time points 0, 10, 20, 30, 40 and 50 min; (2) percent of MMR (%MMR), calculated as the MO_2 value as a function of the MMR_{1h} or MMR_{18h} following the respective exhaustive exercise, calculated at time points 0, 10, 20, 30, 40 and 50 min; and (3) the time to recover to 50% of respective MMR ($recMMR_{50}$) (Kraskura et al., 2021). Individuals without a distinct measurement between 48 and 52% of MMR were excluded from $recMMR_{50}$ analyses. EPOC was analyzed by smoothing MO_2 measurements using a cubic smoothing spline function (smooth.spline, R package 'stats') for overnight recovery measurements starting at MMR_{1h}. EPOC was calculated as the area integrated beneath the curve minus the area of the integrated RMR, with values pooled and calculated into the first five hourly time blocks. Fish that did not have > 60 measurements or did not complete EPOC (return to RMR; one individual) were excluded from this analysis.
Temperature coefficients (Q_{10}) for RMR values were calculated based on temperature treatment group means for each population using the equation,

$$Q10 = \frac{RX}{R12}^{\left(\frac{10}{TX-T12}\right)}$$

where R12 and RX are the RMR values measured at corresponding temperature T12 (12°C) and TX (18°C, 21°C or 24°C).

The optimal temperature for AAS ($Topt_{AAS}$) was measured as the maximum AAS values based on the polynomial model calculated for AAS and the upper T_{pejus} (°C) was defined as the maximum temperature at which AAS remained above 80% of the maximum AAS.

All data were analyzed for statistics using RStudio version 1.2.1335(RStudio Team, 2020). Statistical significance was accepted at $P < 0.05$. Values are presented as mean ± standard error of mean (SEM) unless otherwise stated. Values were assessed for normality using residual plots and quantile-quantile plots and log_{10}-transformed if necessary to fit normality assumptions. All data were measured for homoscedasticity using Levene's Test. For survival rates, a binomial two-parameter log-logistic function was fit and a likelihood ratio test compared the model with the fixed effect (population) to a null model. Body mass differences across test temperatures within and across populations were assessed using a two-way ANOVA. The effects of sex and population on body metrics (GSI, RVM, SSI, HSI) were assessed using a two-way ANOVA with sex and population as factors. MMR_{1h}, MMR_{18h}, $MMR_{OVERALL}$, RMR, AAS, FAS, $recMMR_{50}$, plasma variables and tissue lactate were modeled for interactive effects of temperature and population and analyzed using two-way ANOVA's and significant main effects or interactions were further explored using a Tukey's HSD post hoc test (R package 'emmeans'). When an interaction was not significant, the interaction was dropped and the model was re-run to test for main effects. To fit parametric test assumptions, $recMMR_{50}$ values were log10-transformed. The AAS and

FAS were also modeled to a second order polynomial regression for the entire dataset (mortalities included as 0's). To determine the effect of time exposed to test temperature on post-chase MMR (MMR_{1h} vs MMR_{18h}), a linear mixed-effects model fit by Maximum Likelihood was used. The main effects were exposure time (1 *vs*. 18 h), population and temperature treatment as categorical predictors, and the random effect was fish ID because individual fish were measured for MMR at both time points. The significance of each main effect was tested using a type II two-way ANOVA (R package 'nlme'; Pinheiro et al., 2022).

Recovery data (%AAS, %MMR, hourly EPOC and cumulative EPOC) were non-independent across time and were analyzed using repeated measures ANOVA. We used linear mixed models to account for individual-specific trends, with individual fish as a random effect to account for repeated measures across each timepoint (R package 'lme4') (Bates et al., 2015). For the short-term recovery (1 h) following exhaustive exercises MMR_{1h} and MMR_{18h}, both %AAS and %MMR values were pooled in 10 min blocks for averages within each temperature treatment for MMR_{1h} and MMR_{18h}. The timepoints (10 min intervals), temperature treatment and population and their interactions were all included as fixed effects. The best fit model as determined using BIC did not include the interaction between population and any covariates. The significance of each fixed effect was then measured using a two-way ANOVA (type III). Hourly and cumulative EPOC were log10-transformed to comply with parametric test assumptions.

Kinetic enzyme activities were analyzed for differences using mixed effect models with effect terms: population, assay temperature and their interaction. Fish ID was used as a cluster variable to account for repeated measures across different assay temperatures using a two-way ANOVA (type III).

3.4 Results

3.4.1 Body morphometrics

Body mass was approximately the same across all treatments in Chilliwack (3.31 ± 0.25 kg, N = 47) and Shuswap (3.56 ± 0.21 kg, N = 38) Chinook salmon (pop*temp: $F_3 = 0.191$, P = 0.902). Though we attempted to control for reproductive status by collecting all fish near spawning grounds, there was an interactive effect of population and sex on GSI ($F_1 = 19.410$, P < 0.0001, N = 85). Sexual maturity, as determined by greater GSI, varied between populations. Female Chilliwack Chinook salmon had a lower GSI compared with female Shuswap salmon (19.74 ± 1.37% [N = 5] *vs*. 23.47 ± 1.13% [N = 12] respectively; P = 0.0013), whereas male Chilliwack salmon had a greater GSI than male Shuswap salmon (7.07 ± 0.29%; [N = 42] *vs.* 5.25 ± 0.20% [N = 26], respectively; P = 0.0017).

Males had a greater RVM than females in both Chilliwack and Shuswap Chinook salmon (males 0.20 ± 0.00% and 0.20 ± 0.01% vs. females 0.17 ± 0.01 and 0.17 ± 0.00%, respectively, across populations; $F_1 = 10.856$, P = 0.001, N = 85). Both population and sex independently influenced SSI, where male Chilliwack salmon had a 32% greater mean SSI compared with male Shuswap salmon (0.21 ± 0.01 (N = 42) *vs*. 0.16 ± 0.01% (N = 26)), respectively, but there were no major difference in SSI between females (Chilliwack: 0.11 + 0.01% (N = 5) *vs*. Shuswap: 0.12 + 0.01% (N = 12)) (sex: $F_1 = 15.393$, P < 0.001; pop: $F_1 = 8.218$, P = 0.005). In contrast, there were no differences in HSI between populations or sex (Chilliwack Chinook salmon: 1.79 ± 0.08% *vs*. Shuswap Chinook salmon: 1.50 ± 0.15%; pop: $F_1 = 2.152$, P = 0.146; sex: $F_1 = 0.522$, P = 0.472).

3.4.2 Effects of warming on survival

Both populations suffered mortalities throughout the experiment (i.e. starting at the acute 1 h temperature exposure before respirometry through to 1 h post MMR_{18h}) at 18, 21 and 24°C. The mortality rates at 18°C were similar but low, and differences in survivorship became evident at 21°C, where 47% of Chilliwack Chinook salmon and 10% of Shuswap salmon died (Figure 3.2). Differences in survivorship were stark at 24°C, with 100% mortality in Chilliwack Chinook salmon and 63% mortality in Shuswap Chinook salmon. There were not enough female Chinook salmon in either population to evaluate statistical differences in sex survival rates.

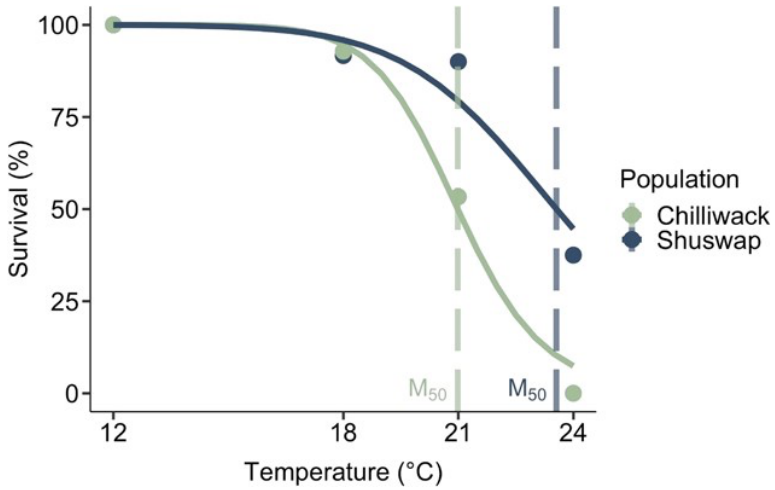

Figure 3.2. Chinook salmon population survival across test temperatures. The percent of surviving individuals from the Chilliwack (green symbols; $N = 47$) and Shuswap (blue symbols; $N = 38$) Chinook salmon (*O. tshawytscha*) populations are plotted as data points fitted with a two-parameter log-logistic function. Vertical dashed line indicates M_{50}, the temperature at which 50% mortality was predicted to occur in each population ($M_{50} = 21$°C, 95% CI [20.0, 22.0°C] Chilliwack, 23.6°C, 95% CI [21.2, 25.9°C] Shuswap).

Survival rate decreased in both populations with increasing temperature (Figure 3.2). Population as a fixed effect on the survival rate model had a marginally better fit than the null model ($\chi 2_4 = 4.9452$, $P = 0.0844$; Figure 3.2). Fifty percent of mortalities (M_{50}) occurred at lower temperatures for Chilliwack Chinook salmon, with an M_{50} of 21.0°C, 95% CI [20.0, 22.0°C] compared with an M_{50} of 23.6°C, 95% CI [21.2, 25.9°C] for Shuswap Chinook salmon (Figure 3.2). Mortality occurred following the first exhaustive exercise event (MMR_{1h}) resulting in fewer MMR_{18h} measurements at several temperatures (Chilliwack: N = 5 mortalities during recovery at 21°C, N = 4 at 24°C; Shuswap: N = 1 at 18°C and N = 3 at 24°C). Differences in sample sizes were also due to the inability to use some MMR values based on requirements described above.

3.4.3 Metabolic performances

There were no effects of test temperature or population, when comparing MMR_{1h} and MMR_{18h} values, though there was an effect of time exposed to test temperature (temp: $X^2_3 = 2.159$, $P = 0.540$; Pop: $X^2_1 = 0.230$, $P = 0.631$; time: $X^2_1 = 3.815$, $P = 0.051$; Figure 3.3). Some fish (39%; N = 14 for Chilliwack and N = 10 for Shuswap of 61 statistically eligible fish) experienced their highest MO_2 values during their overnight recovery, and for those fish, values were 26% and 40% higher than MMR_{1h} for Chilliwack and Shuswap Chinook salmon, respectively (Figure 3.3). Therefore, the remainder of the results presented use $MMR_{OVERALL}$ values.

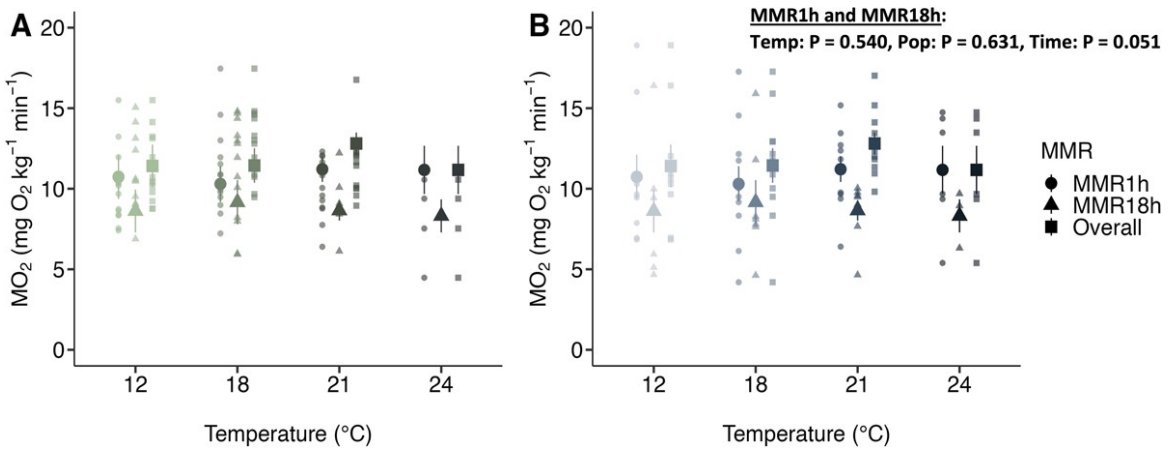

Figure 3.3. MMR from three separate measurements. The MMR during 1 h exposure (MMR_{1h}), 18 h exposure (MMR_{18h}) and calculated from maximum values including during overnight recovery ($MMR_{OVERALL}$) in (A) Chilliwack (green symbols) and (B) Shuswap (blue symbols) Chinook salmon (*O. tshawytscha*) acutely exposed to 12, 18, 21, or 24°C. Large data points are mean MMR values ± SEM and small data points are individual MMR values. Values are corrected using the metabolic scaling coefficient of 0.58. Note that there is no data for MMR_{18h} for Chilliwack fish at 24°C because of mortalities. Statistical results from repeated measures two-way ANOVA accounting for MMR_{1h} and MMR_{18h} values are presented with variables: test temperature (Temp), population (Pop), MMR time (Time), fish id (ID)) in panel B (MMR ~ Temp + Pop + Time, random = ~ 1|ID).

At the acclimation temperature (12°C), there are minimal differences in metabolic rates (RMR, $MMR_{OVERALL}$, AAS, FAS) between populations (Figure 3.4). Chilliwack Chinook salmon and Shuswap Chinook salmon had similar RMR values ($P = 0.053$, 2.70 ± 0.24 *vs*. 2.06 ± 0.21 mg kg^{-1} h^{-1}, respectively) and there were no differences in $MMR_{OVERALL}$ ($P = 0.545$, 11.26 ± 0.55 *vs*. 11.41 ± 1.33 mg kg^{-1} h^{-1}, respectively Figure 3.4). The resulting AAS also showed no difference between Chilliwack and Shuswap Chinook

salmon populations at 12°C (P = 0.565, 8.56 ± 0.51 vs. 9.35 ± 1.14 mg kg^{-1} h^{-1}, respectively), though Shuswap Chinook salmon had a greater FAS than Chilliwack Chinook salmon at 12°C (*P* = 0.002, 4.45 ± 0.36 vs. 5.54 ± 0.33, respectively; Figure 3.4).

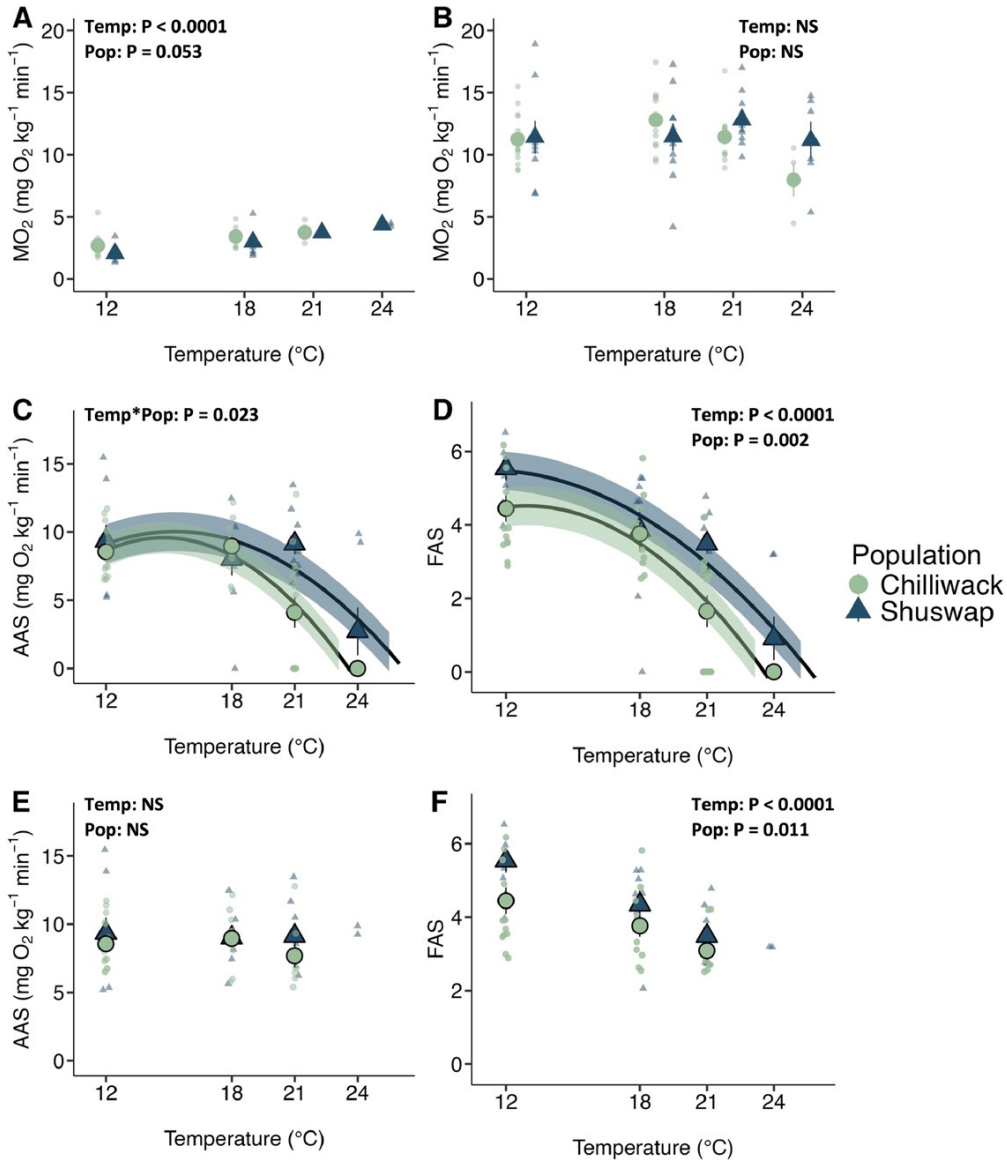

Figure 3.4. Effects of warming on metabolic rates in Chilliwack Chinook salmon (*O. tshawytscha*) (green circles) and Shuswap Chinook salmon (blue triangles) acclimated to 12°C and tested at different temperatures (18, 21, 24°C). (A) Resting metabolic rate, (B) MMR ($MMR_{OVERALL}$), (C) AAS of all fish (mortalities included as 0), (D) FAS of all fish

(mortalities included as 0), (E) AAS of surviving fish, (F) FAS of surviving fish. Significant effects (test temperature (Temp), population (Pop)) denote statistical results for RMR, $MMR_{OVERALL}$, AAS and FAS. A second order polynomial regression is modeled as (AAS ~ temp + I($Temp^2$) and (FAS ~ Temp + I($Temp^2$) with SEM shaded for (C) and (D)). Values are corrected to a common body mass of 3.5 kg using the metabolic scaling coefficient of 0.58 for $MMR_{OVERALL}$ (mg O_2 kg^{-1} min^{-1}), 0.67 for RMR (mg O_2 kg^{-1} min^{-1}), AAS as $MMR_{OVERALL}$ -RMR (mg O_2 kg^{-1} min^{-1}), FAS as $MMR_{OVERALL}$/RMR (unit-less) and expressed as mean ± SEM. Individual data points represent values for individual fish. Mortalities in (C) and (D) occurred during the experiment and are indicative of zero scope (Chilliwack: 12°C [N = 0], 18°C [0], 21°C [7], 24°C [4]; Shuswap: 12°C [N = 0], 18°C [1], 21°C [0], 24°C [5]). Note that there are no RMR data at 24°C for Chilliwack Chinook salmon due to mortality, and SEM is too small to be seen for some values. Also note there are no mean or SEM for 24°C in (E) and (F) due to N = 2.

Temperature effects were apparent across several metrics. RMR increased with increasing test temperatures (18, 21 and 24°C) in both Chilliwack and Shuswap Chinook salmon ($F_3 = 12.886$, $P < 0.0001$; Figure 3.4). RMR values were not reported for Chilliwack Chinook salmon at 24°C because of high mortality. Shuswap Chinook salmon displayed a higher thermal sensitivity relative to Chilliwack Chinook salmon (RMR $Q10_{12–18} = 1.86$ vs. 1.49, $Q10_{12–21} = 1.93$ vs. 1.44, respectively, and $Q10_{12–24} = 1.87$ (Shuswap only)). However, neither test temperature nor population origin affected $MMR_{OVERALL}$ in either population

(temp: $F_3 = 1.938$, $P = 0.131$; pop: $F_1 = 0.371$, $P = 0.545$), with mean values ranging from 7.99 to 12.80 mg O_2 kg^{-1} min^{-1} in Chilliwack Chinook salmon and 11.17 to 12.81 mg O_2 kg^{-1} min^{-1} in Shuswap Chinook salmon (Figure 3.4).

Aerobic scope was assessed in two ways: (1) incorporating fish that died during the experimental period as zero aerobic scope values (Figure 3.4C, D) only from survivors (Figure 3.4E, F). When incorporating fish that died, AAS varied among temperature treatments in Chilliwack and Shuswap Chinook salmon ($F_3 = 16.096$, $P < 0.0001$; Figure 3.4C). For Chilliwack Chinook salmon, AAS was both greatest and unchanged between 12 and 18°C (8.56 ± 0.51 and 8.96 ± 0.58 mg O_2 kg^{-1} min^{-1}, respectively). However, due to increasing RMR and relatively unchanged $MMR_{OVERALL}$ with increasing temperature, AAS declined at 21 and 24°C in both populations, with no scope remaining in Chilliwack Chinook salmon at 24°C because of mortalities, and very limited scope in Shuswap Chinook salmon at 24°C (2.73 ± 1.76 mg O_2 kg^{-1} min^{-1}). The thermal range for $Topt_{AAS}$ and upper T_{pejus} was 14.75–18.70 and 15.30–20.15°C reaching 9.6 and 10.0 mg O_2 kg^{-1} min^{-1} in Chilliwack and Shuswap Chinook salmon respectively (Figure 3.4C, Figure 3.5). Test temperature also affected FAS ($F_3 = 32.343$, $P < 0.001$; Figure 3.4D). In Chilliwack Chinook, FAS decreased from 12°C to 18°C (4.45 ± 0.36 and 3.76 ± 0.30, respectively) and fish had more than two times the amount of FAS available than at 21°C (1.65 ± 0.43). Shuswap Chinook salmon had the highest FAS overall at 12°C (5.54 ± 0.33) but more rapidly declined with increasing temperatures, dropping by 30% at 18°C (3.86 ± 0.59) and nearly 40% by 21°C (3.49 ± 0.24). When aerobic scopes were assessed for surviving fish only (Figure 3.4E, F), there was no effect of temperature ($F_2 = 0.356$, $P = 0.702$) or population ($F_1 = 1.500$, $P = 0.226$) on AAS. However, there were significant effects of

temperature ($F_2 = 12.871$, $P < 0.0001$)) and population ($F_1 = 6.968$, $P = 0.011$) on FAS (Figure 3.4F).

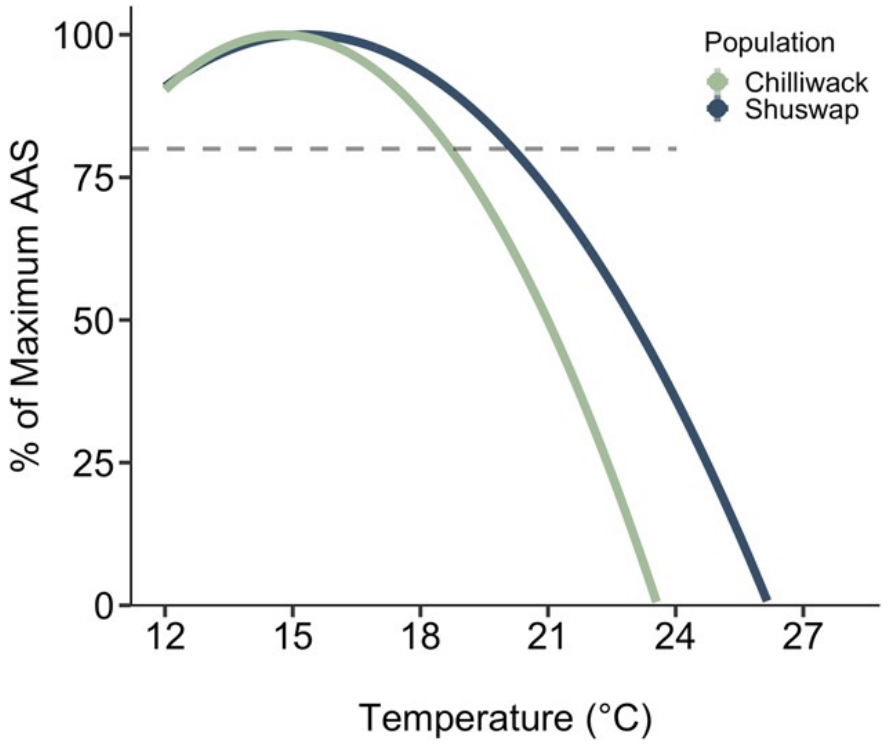

Figure 3.5. Predicted river temperature thresholds for Fraser River Chinook salmon (*O. tshawytscha*) populations. AAS is modeled as percent of maximum AAS for Chilliwack (green) and Shuswap (blue) Chinook salmon across temperatures based on a second order polynomial regression (AAS ~ Temp + I(Temp^2)). Pacific salmon are hypothesized to need 80% of maximum AAS (horizontal dashed line) to successfully spawn, corresponding to the upper T_{pejus} at 18.70 ± 2.96°C (SD) (Chilliwack Chinook salmon) and 20.15 ± 3.70°C (Shuswap Chinook salmon)

3.4.4 Post-exercise recovery

Short-term (1 h) recovery after exercise was never complete, even at 12°C (Figure 3.6). Both the rapid (1 h) and acute (18 h) temperature exposure times resulted in drastic

performance differences in both populations, most noticeably at the extreme upper temperatures (Figure 3.6). Test temperature impacted %AAS after MMR_{1h} ($P < 0.0001$) but not MMR_{18h} ($P = 0.263$; Figure 3.6). Timepoint affected %AAS after MMR_{1h} and MMR_{18h} ($P < 0.0001$) whereas there was no effect of population on %AAS after either respective chase ($P = 0.132$, 0.129; Figure 3.6). There was also a significant two-way interaction between the timepoint and temperature treatment following MMR_{1h} (time*temp: $\chi 2_1 = 11.807$, $P < 0.001$). In both populations and at both exposure times, fish held at the lowest test temperatures (12, 18°C) recovered more rapidly, with more than 50% AAS within the first 10 min. In Chilliwack Chinook salmon, fish held at the higher test temperatures (21°C, 24°C) recovered more slowly and did not return to 50% AAS within the first 50 min of recovery, whereas the Shuswap Chinook salmon tested at the higher test temperatures recovered to 50% AAS after both chases within the 50 min recovery (Figure 3.6). The recovery time required to reach 50% of the MMR following the first exhaustive chase MMR_{1h} displayed similar patterns as described here for %AAS.

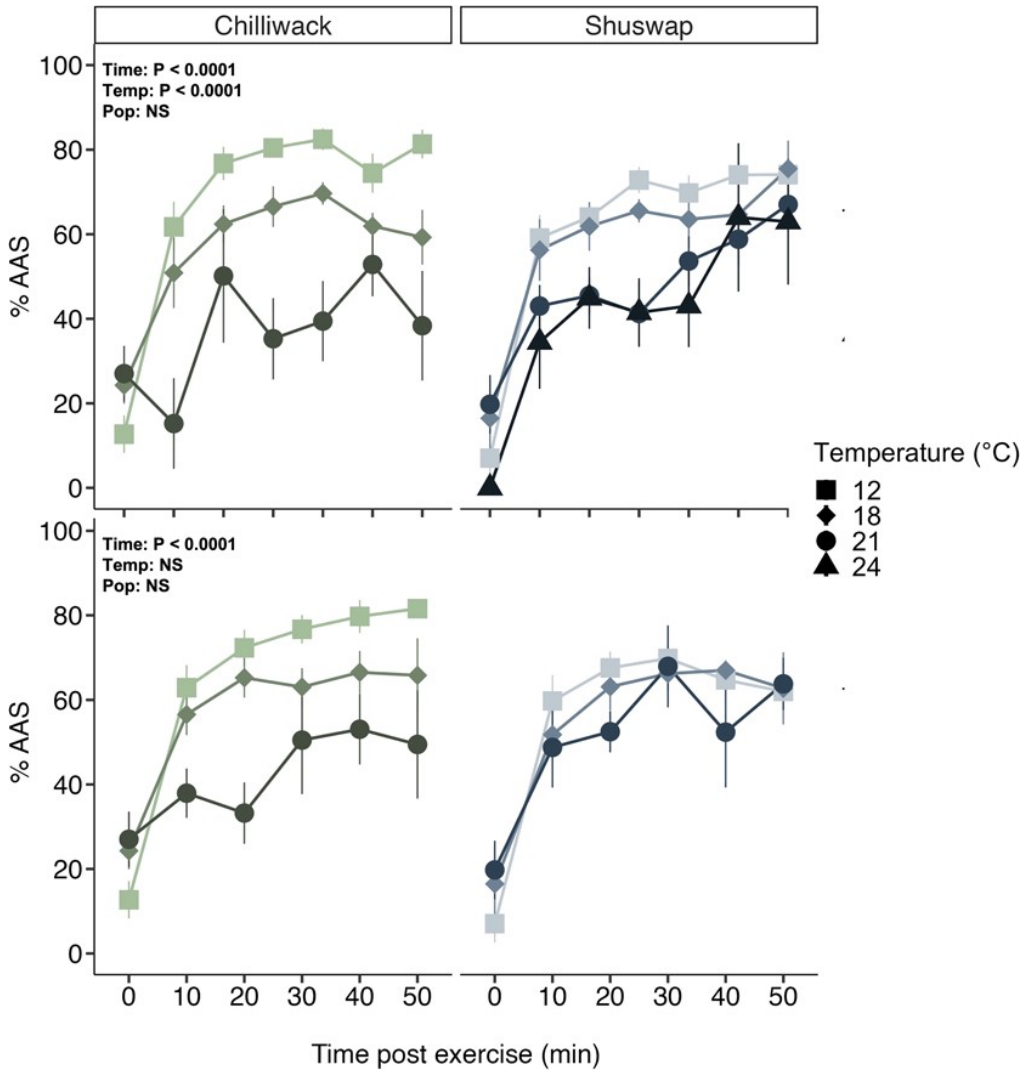

Figure 3.6. Short-term recovery following exhaustive exercise in Chinook salmon (*O. tshawytscha*). Short-term recovery measured as the MO_2 recovered as a function of AAS during the 50 min of recovery following (A) MMR_{1h} and (B) MMR_{18h} for Chilliwack (green symbols; first column) and Shuswap (blue symbols; second column) Chinook salmon acclimated to 12°C and tested at 12 (square), 18 (diamond), 21 (circle) and 24°C (triangle). Values are pooled every 10 min as mean percent of AAS ± SEM. Significant effect of each effect term (timepoint (Time), test temperature (Temp), population (Pop)) (ANOVA) denote statistical results for recovery following each exhaustive exercise (MMR_{1h} or MMR_{18h}). Note that there are no recovery values for 24°C Chilliwack Chinook salmon at either chase

timepoint (MMR_{1h}, MMR_{18h}) because of mortality following the first exhaustive exercise recovery period and no data for 24°C Shuswap Chinook salmon at the 18 h exhaustive exercise because of low sample size ($N = 2$). Note that percent AAS at 0 min does not begin at 0% because AAS is calculated based on $MMR_{OVERALL}$.

Intermediate (5 h) recovery from MMR_{1h} as measured by EPOC acquired each h over five h varied due to a two-way interaction between hourly timepoints and test temperature (time*temp: $\chi^2_{12} = 41.401$, $P < 0.01$), though there was no effect of population ($\chi^2_1 = 0.353$, $P = 0.552$).

3.4.5 Blood chemistry & hormones

In general, blood values sampled after 1 h of recovery following exhaustive exercise (MMR_{18h}) varied across populations and temperature treatments. There were significant effects of both population and temperature on plasma lactate at the 1 h recovery timepoint post MMR_{18h}, with increases in lactate with increasing temperature from 14.40 to 20.52 mmol L^{-1} in Chilliwack Chinook salmon and 12.29 to 17.77 mmol L^{-1} in Shuswap Chinook salmon (pop: $F_1 = 3.189$, $P = 0.079$; temp: $F_3 = 12.761$, $P < 0.001$; Table 3.1, Figure 3.7C). Male salmon testosterone levels significantly decreased with elevated temperature across populations, declining by 49% and 76% from 12 to 21°C in Chilliwack and Shuswap Chinook salmon, respectively (pop*temp: $F_2 = 4.278$, $P = 0.020$; Table 3.1). There were also significant effects of population on estradiol in males, with estradiol concentrations 30% higher in Chilliwack Chinook salmon than Shuswap Chinook salmon at 12°C (pop: $F_1 = 26.022$, $P < 0.001$; Table 3.1). Male Shuswap Chinook salmon had lower cortisol levels

compared with male Chilliwack Chinook salmon across all temperatures (pop: $F_1 = 14.597$, $P < 0.001$; temp: $F_3 = 1.118$, $P = 0.351$; Table 3.1). Hematocrit significantly differed across temperature treatments and between populations (pop: $F_1 = 15.564$, $P < 0.001$; temp: $F_3 = 4.530$, $P = 0.006$; Table 3.1). Potassium levels significantly increased across temperatures, from 2.57 to 3.72 mmol L^{-1} and 2.46 to 4.30 mmol L^{-1} in Chilliwack and Shuswap Chinook salmon, respectively (temp: $F_3 = 4.086$, $P = 0.010$; Table 3.1). Sodium levels were significantly higher in Chilliwack Chinook salmon than Shuswap Chinook salmon, but there was no effect of temperature treatment (pop: $F_1 = 6.713$, $P = 0.012$; temp: $F_3 = 0.113$, $P = 0.952$). Plasma chloride, osmolality and glucose sampled at the 1 h recovery post MMR_{18h} were not significantly different between populations or across temperatures ($P = 0.238$–0.649; Table 3.1).

3.4.6 Population differences and effects of warming on cellular processes

Lactate concentrations sampled 1 h after the exhaustive chase generally increased with test temperature in both the cardiac and white muscle. In the heart, there was a significant effect of test temperature on lactate concentrations ($F_3 = 7.40$, $P < 0.001$; Figure 3.7A). For both populations, the 21 and 24°C exposed fish had higher cardiac lactate concentrations than individuals exposed to 12°C (Figure 3.7A). There was a significant interactive effect of population and test temperature on lactate concentrations in the white muscle ($F_2 = 4.36$, $P = 0.0175$), where lactate concentrations were significantly higher in Shuswap Chinook salmon compared with Chilliwack salmon tested at 18°C ($P = 0.0187$; Figure 3.7B). White muscle lactate concentrations were also significantly higher at 24°C *vs*. 12°C tested Shuswap Chinook salmon ($P = 0.0238$; Figure 3.7B).

In the cardiac, white and red muscle, LDH activity increased with assay temperature in both populations (cardiac: $\chi^2_4 = 494.58$, $P < 0.0001$; white: $\chi^2_4 = 729.30$, $P < 0.0001$; red: $\chi^2_4 = 1310.65$; $P < 0.0001$; Figure 3.8A-C). However, population origin had no effect on LDH activity in cardiac, white, or red tissues (Figure 3.8A-C). In red muscle, CS activity increased with assay temperature in both populations ($\chi^2_4 = 558.71$, $P < 0.001$), and enzyme activity appears to increase more quickly with assay temperature in Chilliwack than Shuswap Chinook salmon (Pop*AssayTemp, $\chi^2_4 = 10.35$, $P = 0.035$; Figure 3.8E,). In cardiac tissue, CS activity increased with increasing assay temperature ($\chi^2_4 = 976.25$, $P < 0.0001$), but in contrast, there was no effect or interactive effect of population (Pop: $\chi^2_1 = 2.85$, $P = 0.10$; Pop*Temp $\chi^2_2 = 0.05$, $P = 0.95$; Figure 3.8D).

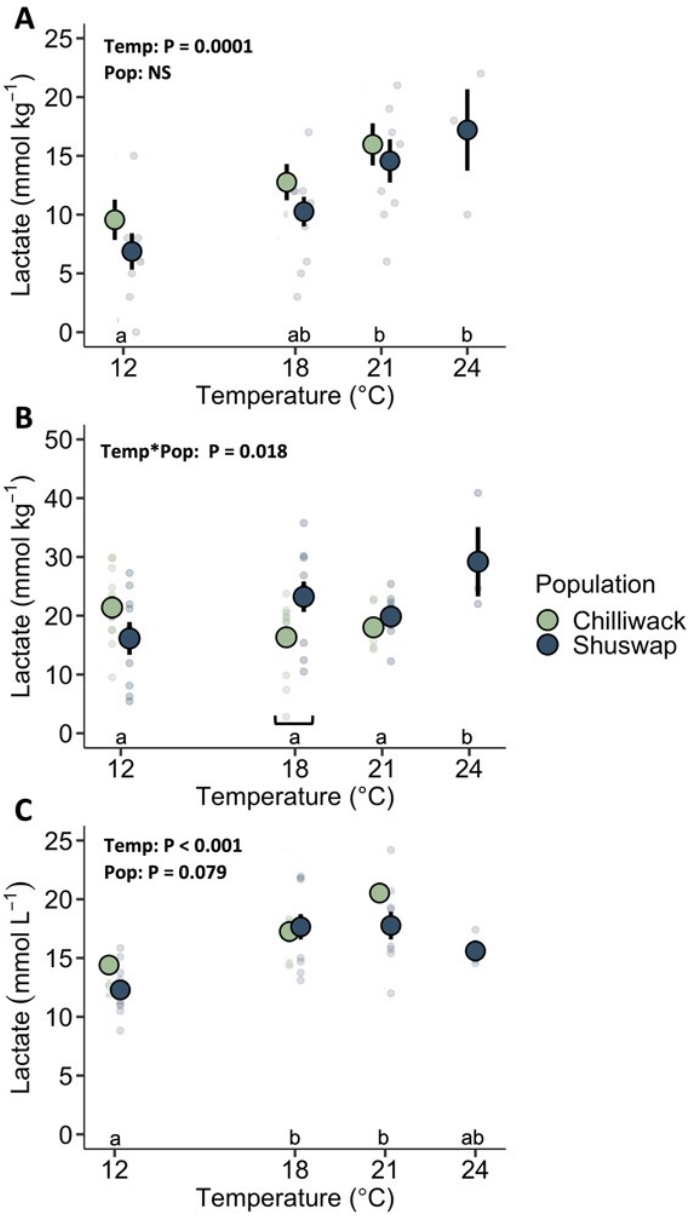

Figure 3.7. Lactate concentration in Chinook salmon (*O. tshawytscha*) (A) cardiac tissue, (B) white muscle and (C) plasma after 1 h recovery. Concentration (cardiac and white muscle: mmol kg^{-1}; plasma: mmol L^{-1}) measured from individuals acclimated to 12°C and tested at 12, 18, 21 and 24°C is presented as mean ± SEM. Faded individual data points represent values from individual fish, with associated population color. Significant two-way interaction (test temperature (Temp) * population (Pop)) (ANOVA; Table 1) or effect terms independently (ANOVA) denote statistical results for each tissue, where letters represent a

significant difference between test temperatures and bar represents a significant difference between populations at a test temperature.

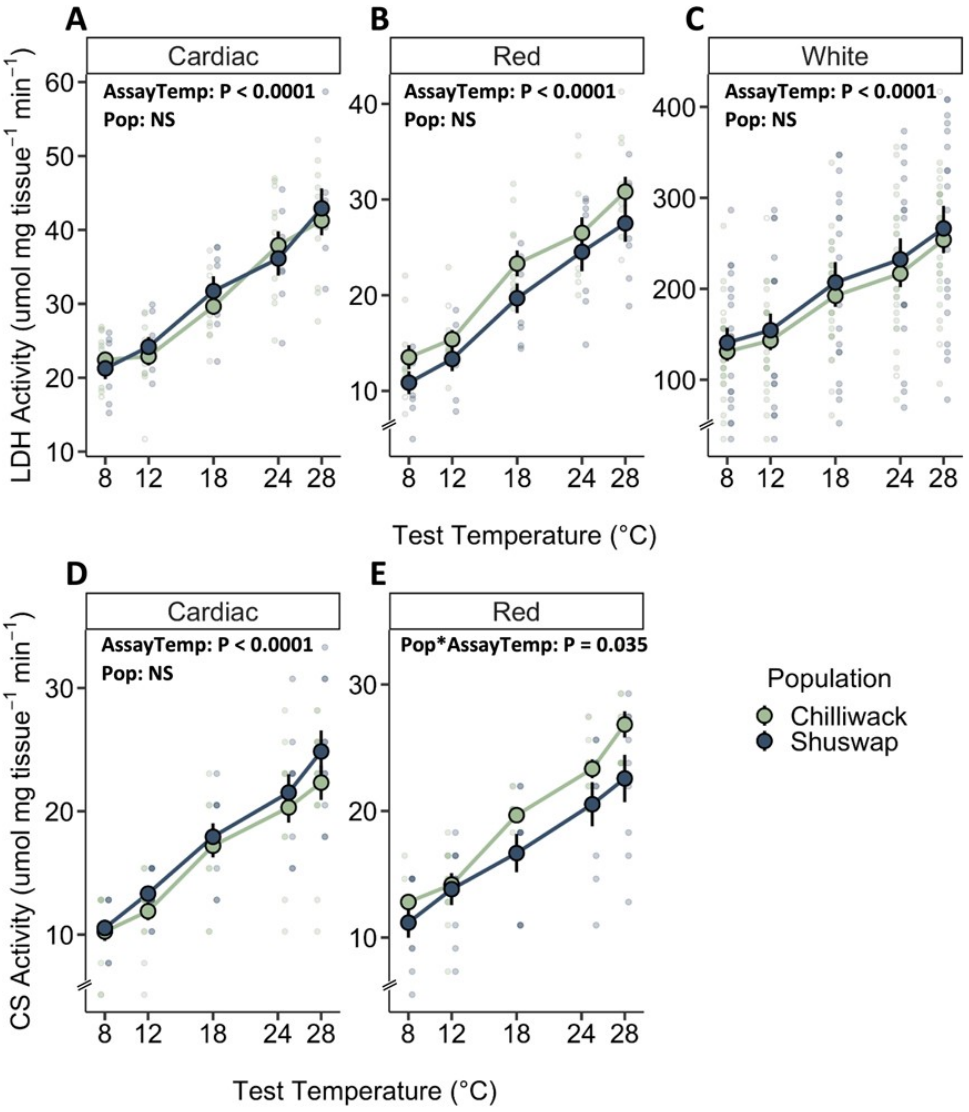

Figure 3.8. LDH and CS activity in cardiac and skeletal muscle after 1 h recovery in Chilliwack (green symbols) and Shuswap (blue symbols) Chinook salmon. LDH activity (μmol mg tissue^{-1} min^{-1}) across assay temperatures (8, 12, 18, 24, 28°C) is measured from individuals acclimated to 12°C from (A) heart, (B) red muscle and (C) white muscle and CS activity (μmol mg tissue^{-1} min^{-1}) across assay temperature (8, 12, 18, 25, 28°C) is

measured from individuals acclimated to 12°C from (D) heart and (E) red muscle. Values are presented as mean activity levels ± SEM and faded individual data points represent values from individual fish, with associated population color. Significant effect terms (assay temperature (AssayTemp), population (Pop)) (ANOVA) denote statistical results for each tissue. Note the difference in y-axis scale.

3.5 Discussion

In this study, we compared intraspecific differences in physiological performances and thermal performance in two populations of Chinook salmon from the Fraser River in British Columbia, Canada. Shuswap Chinook salmon are an interior summer-run population that enters the Fraser River earlier and historically encounters a warmer, longer, more challenging migration whereas Chilliwack Chinook salmon are a coastal fall-run population and enter the river later and encounter a shorter, and historically cooler, migration. We found that Shuswap Chinook salmon were generally more tolerant to high temperatures than Chilliwack Chinook salmon as indicated by better survival rates, wider AAS breadth, and quicker recovery at higher temperatures. However, both populations currently encounter temperatures that approach their upper thermal limits at this pre-spawning life stage, which suggests they may not have adapted at a pace that has kept up with warming.

3.5.1 Coastal and interior Chinook salmon differ in thermal performance

Our findings indicate that migration history (temperature and physical challenges) plays an important role in population thermal performance and physiological capacities. Intraspecific variability is not uncommon in other salmonids (Lee et al., 2003b; Eliason et

al., 2011; Chen et al., 2013, 2015, 2018; Stitt et al., 2014; Verhille et al., 2016; Whitney et al., 2016; Poletto et al., 2017; Abe et al., 2019; Anttila et al., 2019; Zillig et al., 2021; Anlauf-Dunn et al., 2022; Zillig et al., 2022) and in other fish species such as killifish and Atlantic cod (Fangue et al., 2006; Lucassen et al., 2006). Our findings complement previous work in adult sockeye salmon and egg/embryo Chinook salmon from coastal *vs*. interior populations (Beacham and Murray, 1989; Beacham and Withler, 1991; Eliason et al., 2011). Populations with longer and more physically challenging spawning migrations have adapted to their up-river migration conditions with larger somatic energy reserves at the onset of migration, smaller gonadal investment, higher AAS and $Topt_{AAS}$ and greater heart performance as indicated by larger RVM, improved coronary supply, elevated heart rate and greater SERCA activity compared with short-migrating conspecifics (Crossin et al., 2004; Eliason et al., 2011; Cooke et al., 2012; Anttila et al., 2019). Indeed, in this study male Shuswap Chinook salmon had smaller gonadal investment than Chilliwack Chinook salmon (5.3 vs 7.1 GSI%), and while the opposite appeared to be the case for females, sample sizes were likely too small to detect differences and populations may have not been equally at the same level of sexual maturity, with loose eggs observed in the Shuswap but not Chilliwack female fish (pers. obs). However, we did not see a difference in RVM between populations. Nevertheless, our study does not distinguish whether differences in performance metrics are necessarily due to adaptation or acclimation to thermal regimes and/or physical challenges, particularly since the fish were collected near their spawning grounds.

We found similar aerobic capacities but different thermal breadths between these Chinook salmon populations. The AAS values at $Topt_{AAS}$ (9.6 and 10.0 mg O_2 kg^{-1} min^{-1} in Chilliwack and Shuswap, respectively) correspond with the AAS of other adult salmon

(chum 10.3–10.7 mg O_2 min^{-1} kg^{-1} (Abe et al., 2019); pink 7.7–18.3 mg O_2 min^{-1} kg^{-1} (Clark et al., 2011); rainbow trout 9.2 mg O_2 min^{-1} kg^{-1} (Chen et al., 2015); sockeye 7.7–11.8 mg O_2 min^{-1} kg^{-1} (Eliason et al., 2011)) and these AAS values are also comparable when only accounting for survivors (Figure 3.4E). Notably, MMR and AAS were consistent whether assessed 1 h or 18 h after acute thermal exposure, demonstrating rapid thermal compensation. Interestingly, the MMR values measured post-chase underestimated AAS in pre-spawning Chinook salmon since 39% of fish demonstrated their highest MO_2 value during overnight recovery. This demonstrates the importance of examining all MO_2 slopes and using careful interpretation when estimating MMR (Killen et al., 2017). To our knowledge, this study is the first to measure AAS in maturing salmon obtained near their spawning grounds. The optimal upper thermal windows (defined by $Topt_{AAS}$ and upper T_{pejus}) of 14.75 to 18.70 ± 2.96°C (SD) and 15.30 to 20.15 ± 3.70°C in the Chilliwack and Shuswap populations demonstrate a wider thermal breadth in Shuswap salmon. Generally, these temperature ranges are consistent with previous findings for migrating adult Chinook salmon thermal limits, which wait at river mouths and slow their migration when river temperatures are too warm (20–23.9°C Columbia River; 19–21°C Sacramento River) (Fish and Hanavan, 1948; Hallock et al., 1970; Richter and Kolmes, 2005; Goniea et al., 2006; Keefer et al., 2018; for review see McCullough, 2001). We did not find major differences in $Topt_{AAS}$ between the populations and this might be because we did not test fish within the thermal range that is likely to be "optimal" (14–17°C) as measured in sockeye and coho (*O. kisutch*) salmon (Eliason et al., 2011; Kraskura et al., 2021) and instead focused on testing fish at the supraoptimal temperatures to define temperatures where performance would collapse.

We took an integrative approach across biological levels of organization to identify mechanisms for differences in physiological and thermal performance by sampling tissue from fish after 1 h of recovery following MMR_{18h}. While we expected to find greater capacity for aerobic and anaerobic metabolism in Shuswap Chinook salmon (e.g. higher activity levels of CS and LDH), we saw no population differences in CS or LDH activity across assay temperatures that are in line with the AAS findings. However, high inter-individual variability in enzyme activities indicated individual differences in physiological performance. Indeed, individuals may differ in their performance and thermal capacity via cellular plasticity and local adaptation (Taylor, 1991). Previous work has found population-specific and sex-specific differences in the activity or density of enzymes associated with the metabolic or cardiac function of salmon (Rodnick et al., 2008; Eliason et al., 2011; Anttila et al., 2019; Little et al., 2020b), which points to the utility of cellular level investigation and variety of mechanisms that may underlie differences in organismal performance.

3.5.2 Recovery is impaired in both populations at projected river temperatures

Recovery is critical for salmon during their once-in-a-lifetime spawning migration and inability to recover may lead to premature death and failure to complete migration (Makiguchi et al., 2011; Burnett et al., 2014a). It is suggested that salmon need 80–90% of AAS to complete their spawning migration (Farrell et al., 2008; Eliason et al., 2011) and salmon need to recover to 50% of their AAS to repeat their swim performance (Farrell et al., 1998, 2003; Jain et al., 1998; Jain and Farrell, 2003; MacNutt et al., 2006). In accordance with our hypothesis, Shuswap salmon had improved recovery capacities at higher temperatures compared with Chilliwack salmon. Chilliwack salmon tested at 18°C required an extra 1 h to reach 50% MMR compared with Shuswap salmon. Further, Chinook salmon

tested at 21°C were only at 15% (Chilliwack) and 43% (Shuswap) AAS after 1 h of recovery. Given that salmon on the spawning grounds are often in their last hours to days of life, this time lost to recovery is significant.

Temperatures above 21°C hindered recovery for both populations, as exhibited by lactate and potassium levels. Previous studies identified "threshold values" for plasma lactate, where levels must decrease below a threshold of 10–13 mmol L^{-1} before an individual can repeat swim performance in the lab (sockeye salmon: Farrell et al., 1998; rainbow trout: Jain and Farrell, 2003). In the wild, ocean telemetry tracking studies have found that sockeye salmon will perish if plasma lactate exceeds 18–20 mmol L^{-1} during capture (Crossin et al., 2009). After 1 h of recovery following exhaustive exercise, Chilliwack Chinook salmon had higher plasma lactate levels than Shuswap Chinook salmon at each temperature, indicating Chilliwack salmon utilized anaerobic metabolism to a greater extent in response to the chase protocol and/or had impaired ability to clear lactate. However, these populations had similar tissue LDH activities, indicating there was no difference in lactate clearance. Plasma lactate exceeded the above thresholds and suggests that Chinook salmon are either more tolerant to high lactate levels and/or are past their threshold and would be unable to repeat swim at the time of sampling (Farrell et al., 1998; Jain and Farrell, 2003). Additionally, hyperkalemia (5 mmol L^{-1} K^{+} perfusate) reduces cardiac output by 30% (Hanson et al. (2006). As indicated by cardiac lactate and plasma potassium levels here, temperatures beyond 21°C could have compromised cardiac function in both populations.

3.5.3 Management implications

The Fraser River is the greatest producer of Pacific salmon in Canada and supports a major share of Canadian Chinook salmon populations (Northcote and Atagi, 1997; Beacham et al., 2002). However, the Fraser River and its tributaries are warming at an alarming rate and current maximum temperatures (daily means) during the upriver migration to the spawning grounds are 21.5°C for Chilliwack and 22.8°C for Shuswap Chinook salmon (Fraser River EWatch, 2021). These temperatures exceed the upper T_{pejus} of these two populations of pre-spawning adult Chinook salmon (Chilliwack, 18.7°C; and Shuswap, 20.2°C; Figure 3.5), which mark the maximum temperature below which fish are predicted to perform near optimally. Accordingly, Chilliwack and Shuswap Chinook salmon may have a functional warming tolerance (max environmental temperature-upper T_{pejus}) (Anlauf-Dunn et al., 2022) of 2.8 and 2.6°C, respectively, *en route* to the spawning grounds. It is evident that current and in the near future, Fraser River temperatures are approaching the functional limits of Chinook salmon.

Indeed, here we included both the aerobic scopes of all fish (Figure 3.4C, D) and survivors only (Figure 3.4E, F) to prevent misinterpretation of the results(Patterson et al., 2016). While it is uncommon to calculate aerobic scope using mortalities because the recorded data from moribund fish does not represent SMR or MMR, a mortality indicates that the individual has zero aerobic capacity and would not sufficiently swim upstream or successfully spawn (Eliason and Farrell, 2016). Mortalities occurred across high temperatures (18, 21, 24°C) in our experiment and discounting these mortalities would greatly overestimate the aerobic capacity of a population. As the purpose of this study is in part, to provide the science to managers and stakeholders about the ability of these Chinook

salmon to perform during their final life stage, it is essential that we include all individuals in the experiment to accurately estimate the aerobic capacity of each population.

Acclimation to warming by chronic thermal exposure can increase AAS by depressing SMR (Seebacher et al., 2015; Sandblom et al., 2016) and therefore might increase the energy available to successfully migrate and spawn. We measured the physiological performance of salmon in response to short-term, acute temperature exposures. In the wild, pre-spawning salmon experience big temperature changes in this acute timeframe because they are generally restricted to their spawning grounds with limited space, thermal heterogeneity and time because they are senescing (e.g. Donaldson et al., 2009). This contrasts with the 2–4-week chronic acclimation studies that are more typical in a laboratory. The inter-individual variability that we observed in physiological performance across biological levels within populations suggests differences in acclimation capacity and requires further investigation. For example, female salmon are more vulnerable to secondary stressors than male salmon and are returning to the spawning grounds in lower numbers than historically (Hinch et al., 2021). While our study did not have adequate numbers to comprehensively compare sex-specific performance, the premature mortalities in the few female individuals we measured leave room for further inquiry into sex-specific vulnerabilities.

3.6 Conclusion

Our work demonstrates that pre-spawning adult Chinook salmon are vulnerable to warming river temperatures. These populations, being separated by several months in migration onset and hundred kilometers of migratory distance, displayed intraspecific and inter-individual variability in physiological performance at acute but temporally relevant

thermal exposures scaling from the cell to population level. Thermal performance paralleled migratory history: Coastal Chilliwack Chinook salmon that migrate during cooler seasons are less tolerant to high temperatures than Interior Shuswap Chinook salmon that migrate during warmer seasons. Even still, based on physiological thermal performance and current temperature exposure risks, these populations have roughly equivalent vulnerability to river warming. Both populations currently experience river temperatures that are almost 3°C warmer than their functional warming tolerance. Maintaining a diverse portfolio of physiological traits within and between populations can increase resilience and support the variation needed for adaptive change (Zillig et al., 2021). Therefore, protecting physiological diversity is essential to Pacific salmon conservation (Cordoleani et al., 2021).

Table 3.1. Population-specific blood chemistry parameters in Chinook salmon (*O. tshawytscha*) 1 h post-MMR_{18h} (chase and air exposure) acclimated to 12°C and tested at 12, 18, 21 and 24°C for Chilliwack HIS and Shuswap (S) Chinook salmon populations (Pop).

Physiological Variable	Pop	12°C	18°C	21°C	24°C	Pop		Temp		Pop * Temp	
						$F_{(df)}$	P-value	$F_{(df)}$	P-value	$F_{(df)}$	P-value
Lactate (mmol L^{-1})	C	14.40 ± 2.92[a] (14)	17.25 ± 3.25[b] (13)	20.52 ± 2.06[b] (8)	NA	3.189_1	0.079	12.761_3	**<0.001**	1.640_2	0.203
	S	12.29 ± 2.31[a] (9)	17.66 ± 3.41[b] (10)	17.77 ± 3.54[b] (9)	15.60 ± 1.57[ab] (3)						
Glucose (mmol L^{-1})	C	6.11 ± 1.81 (14)	6.74 ± 2.42 (13)	6.41 ± 1.35 (8)	NA	0.346_1	0.559	0.818_3	0.489	0.352_2	0.705
	S	7.01 ± 2.43 (9)	7.16 ± 4.04 (10)	6.00 ± 1.73 (9)	5.01 ± 0.17 (3)						
Testosterone (ng ml^{-1}) – male	C	10.13 ± 5.70* (13)	10.10 ± 8.47 (11)	5.18 ± 1.51 (8)	NA	0.113_1	0.739	4.595_3	**0.007**	4.278_2	**0.020**
	S	18.48 ± 13.72[a]* (5)	5.41 ± 1.12[b] (7)	4.42 ± 0.51[b] (7)	3.28 ± 0.93[b] (3)						
Estradiol (ng ml^{-1}) – male	C	0.65 ± 0.11 (13)	0.63 ± 0.10 (11)	0.58 ± 0.09 (8)	NA	26.022_1	**<0.001**	1.000_3	0.401	0.441_2	0.646
	S	0.50 ± 0.04 (5)	0.45 ± 0.02 (7)	0.47 ± 0.10 (7)	0.42 ± 0.23 (3)						

Cortisol (ng ml^{-1}) – male	C	576.87 ± 291.01 (13)	593.25 ± 214.31 (11)	460.22 ± 109.24 (8)	NA	14.597_1	**<0.001**	1.118_3	0.351	1.573_2	0.218
	S	192.26 ± 74.11 (5)	407.75 ± 192.13 (7)	327.30 ± 138.62 (7)	194.31 ± 110.73 (3)						
Hematocrit (%)	C	58.00 ± 6.73[a] (14)	62.23 ± 6.76[ab] (13)	68.75 ± 6.56[b] (8)	NA	15.564_1	**<0.001**	4.530_3	**0.006**	0.882_2	0.419
Sodium, Na^+ (mmol L^{-1})	S	52.00 ± 8.43[a] (9)	56.20 ± 6.29[ab] (10)	57.00 ± 10.51[b] (9)	62.67 ± 8.02[ab] (3)						
	C	151.76 ± 17.98 (14)	150.63 ± 9.73 (13)	153.39 ± 14.42 (8)	NA	6.713_1	**0.012**	0.113_3	0.952	0.663_2	0.519
	S	139.58 ± 19.21 (9)	146.83 ± 9.70 (10)	140.93 ± 10.50 (9)	142.37 ± 3.21 (3)						
Chloride, Cl^- (mmol L^{-1})	C	121.36 ± 14.21 (14)	116.14 ± 5.43 (13)	112.75 ± 8.96 (8)	NA	0.248_1	0.621	0.551_3	0.649	1.370_2	0.262
	S	116.20 ± 17.38 (9)	121.75 ± 9.30 (10)	117.39 ± 13.13 (9)	122.17 ± 5.97 (3)						
Potassium, K^+ (mmol L^{-1})	C	2.57 ± 0.95[a] (14)	3.10 ± 1.31[ab] (13)	3.72 ± 1.64[b] (8)	NA	0.0004_1	0.985	4.086_3	**0.010**	0.066_2	0.936
	S	2.46 ± 1.36[a] (9)	3.09 ± 0.88[ab] (10)	3.91 ± 1.75[b] (9)	4.30 ± 0.75[ab] (3)						
Osmolality (mOsm kg^{-1})	C	311.21 ± 26.46 (14)	313.54 ± 9.30 (13)	313.75 ± 10.86 (8)	NA	1.418_1	0.238	0.597_3	0.620	0.521_2	0.597
	S	300.78 ± 28.40 (9)	314.10 ± 9.45 (10)	306.22 ± 15.96 (9)	312.00 ± 4.58 (3)						

Inside parentheses are sample size. Represented are means and standard deviation values for each treatment group, and ANOVA results. Physiological variables that were significantly affected by population, temperature or the interaction of population and temperature are bolded, letters indicate significant post hoc HD results across temperatures and asterisks indicate significant post hoc HD results across populations ($P < 0.05$). Temp, temperature; Pop, population; df, degrees of freedom.

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Chapter 4: Coronary circulation enhances the aerobic performance of wild Pacific salmon

4.1 Abstract

Female Pacific salmon often experience higher mortality than males during their once-in-a-lifetime up-river spawning migration, particularly when exposed to secondary stressors (e.g. high temperatures). However, the underlying mechanisms remain unknown. One hypothesis is that female Pacific salmon hearts are more oxygen-limited than males and are less able to supply oxygen to the body's tissues during this demanding migration. Notably, female hearts have higher coronary blood flow, which could indicate a greater reliance on this oxygen source. Oxygen limitations can develop from naturally occurring coronary blockages (i.e., coronary arteriosclerosis) found in mature salmon hearts. If female hearts rely more heavily on coronary blood flow but experience similar arteriosclerosis levels as males, they will have disproportionately impaired aerobic performance. To test this hypothesis, we measured resting (RMR) and maximum metabolic rate (MMR), aerobic scope (AS) and acute upper thermal tolerance in coho salmon (*Oncorhynchus kisutch*) with an intact or artificially blocked coronary oxygen supply. We also assessed venous blood oxygen and chemistry (cortisol, ions, and metabolite concentrations) at different time intervals during recovery from exhaustive exercise. We found that coronary blockage impaired MMR, AS, and the partial pressure of oxygen in venous blood (PvO_2) during exercise recovery but did not differ between sexes. Coronary ligation lowered acute upper thermal tolerance by 1.1°C. Though we did not find evidence of enhanced female reliance on coronary supply, our findings highlight the importance of coronary blood supply for

mature wild salmon, where migration success may be linked to cardiac performance, particularly during warm water conditions.

4.2 Introduction

Biological sex influences how organisms, populations, and communities perform in response to environmental change (Gissi et al., 2023; Pörtner and Farrell, 2008). Performance disparities between sexes can result from differences in physiology, morphology, and behavior and are frequently more pronounced at the sexually mature life stage when species often exhibit sexual dimorphism (Hanson et al., 2008). Changes in sex-specific performance raise a potential conservation concern because maintaining functional sex ratios is crucial for sustaining a population (Kappeler et al., 2023). This is especially true for semelparous species, such as Pacific salmon (*Oncorhynchus* spp). Over the last few decades, sockeye salmon (*O. nerka*) have experienced female-biased mortality during their physically challenging, once-in-a-lifetime upriver spawning migration, particularly in association with additional stressors including high or low flows, fishing interactions, handling, and high temperatures (Hinch et al., 2021). There is also evidence that coho (*O. kisutch*) and Chinook (*O. tshawytscha*) salmon exhibit female biased mortality during their maturing river migration life stage (Hinch et al., 2021). Despite river migration mortality levels for female sockeye salmon being 2 to 8 times higher than those of males under stressful environmental conditions (Hinch et al., 2021), the mechanisms underpinning sex-specific mortality have not been resolved.

During their aerobically challenging spawning migration, salmon are simultaneously undergoing reproductive maturation and swimming upriver, supported exclusively by endogenous energy stores (Eliason and Farrell, 2016). Their aerobic scope (AS) represents the available aerobic capacity to support challenges like migration and is determined as the

difference between resting (RMR) and maximum metabolic rates (MMR) (Farrell, 2009; Fry, 1947). During warming, blood oxygen transport increases to meet heightened metabolic demands of systemic tissues, but cardiovascular and thus metabolic performance (i.e., cardiorespiratory performance) becomes constrained at critically high temperatures in athletic fish including salmonids (Ekström et al., 2023; Eliason et al., 2013a; Farrell et al., 2009; Holt and Jorgensen, 2015). One hypothesis suggests that the constraint of cardiorespiratory performance relates to the fact that cardiac tissues become oxygen-limited at such extremes (Ekström et al., 2016; Eliason et al., 2013a; Farrell, 2009; Steinhausen et al., 2008).

The heart supplies oxygen to working tissues to support the enhanced aerobic demand during migration. The heart itself is highly dependent on aerobic metabolism to sustain cardiac function, and the oxygen supply to the heart is facilitated through two sources: the coronary artery delivers oxygen-rich arterial blood directly from the gills to the coronary vasculature of the ventricular compact myocardium, and the venous circulation delivers leftover oxygen to the ventricular spongy myocardium after the systemic tissues have first been supplied (Farrell, 2002a). The compact myocardium can range from 20-50% of the total ventricular mass (Brijs et al., 2017; Eliason et al., 2011), suggesting the importance of coronary circulation can vary across salmon species, populations, life-stages, and sexes. The coronary artery provides distinct advantages to enhance performance (Farrell and Smith, 2017). As salmon increase their swimming speed, encounter higher temperatures, or experience environmental hypoxia, there is a corresponding increase in coronary blood flow to the compact myocardium of the ventricle (Axelsson and Farrell, 1993; Ekström et al., 2017; Gamperl et al., 1995). This indicates the coronary perfusion of the heart is important during these environmental and physiological challenges. However, as adult

salmon age they develop lesions from the thickening of the myointimal layer in the coronary artery (coronary arteriosclerosis) that likely decrease coronary blood flow (Brijs et al., 2020; Farrell, 2002b; Farrell et al., 1990). This is proposed to occur from vascular damage caused by the repeated overstretching of the coronary artery during each heartbeat (Saunders et al., 1992). While the impact of partial coronary occlusion is unknown, several studies conducting a surgical intervention to block the flow of oxygenated blood to the compact myocardium (by coronary ligation), have revealed that coronary blockage impairs cardiac conductivity during resting conditions (Brijs et al., 2020; Zena et al., 2021; Zena et al., 2024), and cardiac performances (e.g. stroke volume, cardiac output, and ventral aortic blood pressure generation) during swimming, acute warming, or environmental hypoxia in rainbow trout, *Oncorhynchus mykiss* (Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021; Steffensen and Farrell, 1998). In addition, coronary ligation reduced the critical thermal maximum (CT_{max}) (Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021), functional thermal maximum (FT_{max}) (Ekström et al., 2023), cardiac and aerobic scope, (Ekström et al., 2018), and maximum sustained swimming speed (U_{crit}) (Farrell and Steffensen, 1987) in salmonids.

We speculate that coronary ligation may have a bigger impact on the cardiorespiratory performance of females than males. Females have smaller ventricles (Clark et al., 2008; Little et al., 2020b) and a higher resting heart rate compared to males (Sandblom et al., 2009), suggesting females may have diminished cardiorespiratory capacity compared to males. Females also accumulate more cardiac lactate than males following handling stress (sockeye salmon; Eliason et al., 2020), but this is likely not due to an impaired PvO_2 supply to the spongy myocardium (coho salmon; Little et al., 2023). The lower activity levels of cardiac

lactate dehydrogenase in females may limit their ability to cope with hypoxia and metabolize lactate (coho salmon; Little et al., 2020b). Females have higher coronary blood flow, yet at high temperatures, they are unable to increase coronary blood flow to the same extent as males (rainbow trout; Ekström et al., 2017). During a swim test where the temperature was ramped up, Ekström et al. (2023) found that female coho salmon generally had lower cardiac output, stroke volume, and MO_2 compared to males. Females with coronary ligations had lower PvO_2 compared to coronary-ligated males when they quit swimming (FT_{max}; Ekström et al., 2023). Thus, females may be more constrained in terms of their cardiorespiratory performance due to coronary limitations. Specifically, there may be an oxygen limitation to the compact myocardium that is potentially causing increased female mortality (Eliason et al., 2023). It remains unknown whether wild female salmon rely more heavily on coronary circulation for basic cardiorespiratory performance compared to males. The rainbow trout in Ekström et al. (2017) were of hatchery origin and may have fundamental morphological and physiological differences (Gamperl and Farrell, 2004). The wild coho in Ekström et al. (2023) underwent an experiment with the combined stress of forced swimming and warming water, and various cardiorespiratory metrics were sampled. However, the importance of coronary supply to support basic metabolism and exercise recovery across sexes remains undiscerned.

Building upon prior research using the same population as Ekstrom et al. (2023), we examined the role of coronary circulation on sex-specific performance, to determine whether male and female coho salmon differ in their reliance on coronary oxygen supply to support cardiorespiratory aerobic performance during exercise and recovery, or rapid environmental warming. We measured aerobic performance (RMR, MMR, AS), recovery post-exercise (excess post oxygen consumption; EPOC), the partial pressure of oxygen in venous blood

(PvO_2), as well as cortisol, ions, and metabolite concentrations during short-term recovery, and the acute upper thermal tolerance in salmon that were either coronary-ligated (no coronary blood flow to the heart) or sham-operated (blood flow intact). We hypothesized that ligation of the coronary artery would reduce aerobic performance, impair recovery, and lower thermal tolerance across both sexes, but that this would be exacerbated for female salmon.

4.3 Methods

4.3.1 Fish collection and holding

Adult coho salmon (N = 41 [N = 22 female, 19 male], fork length = 55.70 ± 0.83 cm, mean ± SEM; Table 1) were dip-netted at the Chilliwack River Hatchery on Sep 30, Oct 7, and Oct 24, 2019. These fish had recently completed their upstream migration (daily average water temperature 15.5°C) from the ocean preparing to spawn but had not fully completed sexual maturation as evidenced by their silver colouration. The fish were transported by truck 20 km within a holding tank (2700 L, stocking density ≤15 fish, >90% water air saturation) to the Fisheries and Oceans Canada Cultus Lake Salmon Laboratory in Chilliwack, British Columbia, Canada, where they were transferred to outdoor circular holding tanks (5.3 m diameter, 8000 L; stocking density ≤ 11 fish). These tanks were supplied with flow-through freshwater from Cultus Lake, which was filtered through sand and UV-treated, maintained at 11-12°C, and >90% air saturation. A directional current was maintained in each holding tank via a submersed pump to mimic river conditions. The holding tanks had lids with transparent windows which allowed for a natural diurnal cycle. Fish were held for 1-13 days and were not fed because they naturally stop feeding during their upstream migration. All experimental

protocols were approved by the University of British Columbia Animal Care Committee (#A17-0160).

4.3.2 Surgical procedure

The surgical interventions were performed the day before the respirometry experiment. Fish were anesthetized in 12°C freshwater containing MS-222 (Tricaine methanesulfonate, 150 mg kg^{-1}, buffered with $NaHCO_3$, 300 mg kg^{-1}) before measuring body mass. The fish was placed on its side on wet foam and a lower dose of anesthesia (MS-222, 75 mg kg^{-1}, buffered with $NaHCO_3$, 150 mg kg^{-1}) at 12°C continuously irrigated the gills. Fish were randomly assigned to one of two treatments: "coronary-ligated" or "sham-operated" (control). In both treatments, a small incision was made in the *isthmus* (Farrell and Steffensen, 1987). In the coronary-ligated group, the coronary artery was dissected free and ligated by tying a 6-0 silk suture around the artery, permanently restricting coronary blood flow to the ventricle. In the sham-operated group, the procedure was identical except that no suture was tied around the coronary artery. In all fish, a PE-50 cannula filled with heparinized saline (0.9% NaCl, 150 IU ml^{-1}) was inserted into the *sinus venosus* to allow for the sampling of venous blood throughout the experiment (Ekström et al., 2023). The cannula was secured along the side and dorsal ridge posterior to the dorsal fin using 2-0 silk sutures. The fish was then placed in a recovery tank (1.95 m diam, 1970 L, stocking density ≤ 2 fish) and recovered overnight (12°C, >90% air saturation).

4.3.3 Experimental protocol

The experimental protocol began the following day at 13:00, at which time individual fish (N = 4) were transferred to a circular exercise tank (1.8 m diam, 2000 L) receiving a high flow-

through of water maintained at 12°C. Fish were manually "chased" to elicit burst swimming for three min until exhaustion (Gale et al., 2014) and then immediately placed in custom-built respirometers (54.5 L) (see Van Wert et al., 2023 for respirometer details) in a flow-through experimental tank (1.8 m diam, 2000 L). The chase protocol for this population of coho salmon has been shown to elicit a similar MMR response as compared to that acquired when swimming fish to exhaustion in a swim tunnel (Little et al., 2020a).

Upon transfer to the respirometer, the cannula suture along the dorsal fin was carefully cut and the cannula was wired through a 'snorkel' on the top of the respirometer. The respirometer lid was sealed shut and the oxygen consumption rate (MO_2) was measured for 4-6 min to estimate MMR. Additionally, a ~0.7 ml blood sample was taken using a heparinized syringe and the collected volume of blood was replaced with a similar volume of saline (0.9% NaCl). Following the first MO_2 measurement, a pump was automated to turn on in 10- or 15- min MO_2 cycles, comprising of 6-9 min flushing periods to reoxygenate the respirometer, and a 4-6 min flush-off period to measure oxygen consumption. MO_2 cycles continued overnight and were adjusted to ensure O_2 remained above 80% air saturation. A blood sample was also taken at 15 min and 60 min following the exercise, and the following morning at 08:00, ~18 h since chase ('rest'). Due to the increased activity levels of fish during the chase and recovery, cannulas that were dislodged from some fish, and fish that were bleeding (N = 8) were euthanized and dissected as described below. The experimental tank was sheltered with a tarp to minimize external disturbances.

After approximately 20 h of MO_2 measurements, an acute thermal ramping protocol began. Water temperature was increased at 0.1°C min^{-1} until a temperature was reached at which the fish exhibited signs of loss of equilibrium (termed 'CT_{max}'; note that the ramping

rate for CT_{max} varies across studies and can influence final values (Desforges et al., 2023) but for consistency in terminology with previous related studies (Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021), we opted to use 'CT_{max}' here). Again, due to the increased activity levels of fish during the acute thermal ramping test, cannulas were dislodged in some fish, and fish that exhibited premature bleeding from the cannula (N = 11) were immediately euthanized and dissected as described below. A total of 22 fish (N = 13 female, 9 male) underwent the entire acute thermal ramping test, and at this point, blood was sampled from the cannula and fish were removed from the respirometer and euthanized by cranial blow and severing of the spinal cord. Fork length, and masses of body, ventricle, liver, spleen, and gonads were measured. Ventricles were then bisected from the valves to the apex and stored in 70% ethanol for later determinations of the percent compact myocardium using methods described by Farrell et al (2007).

4.3.4 Blood metrics

PvO_2 was determined immediately after each blood sample by injecting a 300 µl aliquot into a chamber with an integrated robust fiber optic O_2 sensor connected to a FireSting O_2 meter (PyroScience, Germany). The chamber was sealed and placed into the experimental tank. A PvO_2 value was estimated when O_2 plateaued at ~3 min. The blood was then re-pooled with the original sample. All blood samples were then stored on ice for a maximum of 1 h. Hematocrit was measured in duplicate and the remaining blood was centrifuged at 1200 g for 5 min to separate the plasma, which was flash-frozen in liquid nitrogen and stored at −80°C for future analyses.

Plasma samples were assessed for cortisol, lactate, glucose, K^+, and Na^+ in duplicate. Cortisol was measured in a FLUOstar Omega multimode microplate reader (BMG Labtech,

USA) using Cortisol ELISA kits (Neogen, USA) and read for absorbance at 650 nm, followed by the addition of 50 µl 1N HCl and measured at 450 nm. Lactate and glucose were measured using a 2300 Stat Plus glucose and L-Lactate analyzer (YSI, USA) (Farrell et al., 2001). Potassium and sodium were measured using an XP Five-channel Flame Photometer (BWB Technologies, UK).

4.3.5 Data and statistical analysis

Body morphometrics including relative ventricular mass (RVM), gonadal size index (GSI), hepatosomatic index (HIS), and splenosomatic index (SSI) were calculated by dividing the specific organ mass by the total body mass *100. Percent compact myocardium was determined as the total dried compact myocardium over the total dried ventricle tissue (spongy + compact myocardium) *100.

MO_2 data were analyzed in RStudio (RStudio Team, 2020) using *AnalyzeResp* (Kraskura, 2022). The mass-specific MO_2 (mg O_2 kg^{-1} h^{-1}) was calculated from the linear decline in O_2 concentration over the course of each measurement cycle (ΔO_2) in the respirometer according to $MO_2 = (\Delta O_2 * (v_R - v_F))/m$, where v_R is respirometer volume, v_F is mass of the fish (L, assuming 1 kg = 1 L) and m is the fish mass (kg). Body mass of coho was limited to a narrow range and did not significantly influence metabolic rate. Therefore, isometric metabolic scaling was used to express mass-specific metabolism. MMR was calculated from the MO_2 measurement following the exhaustive using a sliding window analysis (180 s minimum). The ≥ 180 s sliding window began at the start of the measurement period and moved across the measurement in 1 s increments. The steepest ΔO_2 with an $R^2 > 0.95$ was extracted as MMR (Little et al., 2020). RMR occurred overnight and was calculated as the lowest 10% quantile of all validated MO_2 measurements with $R^2 > 0.90$. Individuals

were only assessed for RMR if they had at least 60 validated MO_2 measurements. All regressions were visually assessed for negative linearity. For each fish, AAS was calculated as MMR subtracted by RMR and the factorial aerobic scope (FAS) as the ratio of MMR divided by RMR. EPOC was calculated for each fish as the difference between the area under the MO_2 curve (AUC) from MMR until MO_2 returned to RMR and the area under RMR using the spline method (*DescTools,* Signorell et al., 2017). EPOC duration was calculated as the time until EPOC ended.

All data were statistically analyzed using R version 2022.12.0+353 (2022) with a significance level of $\alpha = 0.05$. All metrics were tested for normality using Shapiro-Wilk test and quantile-quantile plots and for heteroscedasticity using Levene's test. Data that did not pass normality were $\log_{10}$ transformed (RMR, RVM, GSI, HIS, SSI, percent compact myocardium, RMR, EPOC, cortisol, glucose, and PvO_2 at CT_{max}) and reassessed for normality. To compare morphometrics across treatments and sexes, we tested for differences in body mass, FL, RVM, GSI, HIS, SSI, and percent compact myocardium using ANOVA's (type II). To test the effect of coronary ligation on performance across sexes, we tested for differences in MMR, RMR, AAS and FAS using ANOVA's (type III) with the interaction between sex and ligation treatment. The interaction was not significant for these four metrics, so sex and treatment were tested for significance without the interaction (type II). Short-term recovery data (recovery MO_2, PvO_2, and blood metrics) were non-independent across time and were analyzed using repeated measures ANOVA's. Based on BIC model selection, sex, treatment, and timepoint were tested as fixed effects in a linear mixed model with fish ID as a random effect. The significance of these interactions was tested using ANOVA (type III) and Tukey's HSD *post hoc* tests (*emmeans*). Long-term recovery data (EPOC and EPOC

duration) were assessed using one-way ANOVAs with treatment as the independent variable and sex was not included as a factor due to the low sample size. To test the effect of coronary ligation on acute thermal tolerance and PvO_2, we used an ANOVA but excluded sex as a factor due to the low sample size. All ANOVA residuals were sufficiently normal.

4.4 Results

4.4.1 Morphology

Body mass and fork length did not differ between treatment groups or sexes (Table 4.1). RVM was greater in males than females in both treatment groups ($T = 2.74$, $P = 0.009$), and was greater in coronary-ligated fish compared to sham-operated fish ($T = -2.07$, $P = 0.045$; Table 4.1). In addition, females had a greater HIS than males ($T = -6.57$, $P < 0.001$) and coronary-ligated fish overall had a greater HIS than sham-operated fish ($T = -2.32$, $P = 0.026$; Table 4.1). Females had a higher GSI than males ($T = -19.45$, $P < 0.001$), whereas females had a lower SSI than males ($T = 2.68$, $P = 0.011$; Table 4.1). The proportions of compact myocardium were similar across sexes ($T = -0.22$, $P = 0.828$), but the percent compact myocardium was marginally greater in coronary-ligated fish compared to sham-treated fish ($31.2 \pm 0.9\%$ *vs*. $29.0 \pm 0.8\%$, respectively; $T = -1.77$, $P = 0.085$; Table 4.1).

4.4.2 Metabolic rates

Coronary-ligated fish had a reduced MMR by 16% ($F_1 = 5.631$, $P = 0.025$), AAS by 21% ($F_1 = 5.151$, $P = 0.034$), and FAS by 20% ($F_1 = 5.331$, $P = 0.032$) compared to sham-operated fish (Figure 4.1, Table 4.2). Though RMR did not differ across treatment groups ($F_1 = 0.032$, $P = 0.860$), RMR was statistically lower in females compared to males across treatments ($F_1 = 4.661$, $P = 0.040$; Figure 4.1, Table 4.2). Additionally, MMR was significantly lower in

females compared to males across treatments (F_1 = 5.718, P = 0.025; Figure 4.1, Table 4.2). However, there was no significant interaction between coronary ligation treatment and sex for all metabolic metrics.

4.4.3 Recovery

Throughout the first hour of recovery following exhaustive exercise, MO_2 steadily declined (χ^2 = 342.123, P < 0.001) (Figure 4.2A). Treatment (χ^2 = 7.176, P = 0.007) and sex (χ^2 = 6.605, P = 0.010) affected short-term MO_2, but there was no interaction between the two (χ^2 = 0.248, P = 0.618; Figure 4.2). Females particularly had lower MO_2 than males (T = -2.419, P = 0.020). Both timepoint (χ^2 = 26.949, P < 0.001) and treatment (χ^2 = 7.699, P = 0.006) had a significant effect on PvO_2 during recovery but there was no significant effect of sex (χ^2 = 0.770, P = 0.380) nor was there an interaction between these factors (χ^2 = 0.525, P = 0.469). Immediately after the exhaustive exercise, sham-operated fish had a PvO_2 of 25.2 ± 1.9 torr, whereas coronary-ligated fish had a PvO_2 of 21.3 ± 1.2 torr (Figure 4.2B). At 15 min into recovery, PvO_2 remained low at 23.0 ± 2.1 torr in coronary-ligated fish compared to sham-operated fish (35.7 ± 2.9 torr), which in sham-operated fish had increased to values similar to resting PvO_2 (33.9 ± 2.8 torr) following full recovery (Figure 4.2B). By 60 min, the PvO_2 in coronary-ligated fish had increased to nearly resting values (33.1 ± 4.0 torr at 60 min *vs*. 31 ± 1.4 torr when fully recovered) (Figure 4.2B). The long-term recovery costs (EPOC) (F_1 = 1.197, P = 0.286) and the full EPOC recovery durations (F_1 = 0.036, P = 0.852) were similar across treatments (Table 4.2).

Plasma lactate, glucose, sodium, and cortisol concentrations, as well as hematocrit significantly varied across recovery timepoints (0, 15, 60, rest) (P < 0.001; Figure 4.3). Plasma lactate was highest at 60 min post-exercise compared to all other recovery timepoints (χ^2 =

204.393, df = 4, P <0.001; Figure 4.3A), with levels at 16.1 mmol l^{-1} (sham-operated) and 19.5 mmol l^{-1} (coronary-ligated). For plasma glucose, there was a significant interaction between timepoint and sex (χ^2 = 41.952, df = 4, P < 0.001), where males had higher plasma glucose at 60 min (P = 0.038; Tukey's HSD). Plasma sodium concentrations varied across sexes (χ^2 = 3.875, df = 1, P = 0.049), which were generally lower in female fish compared to male fish and reached as low as 142 mmol l^{-1} in coronary-ligated female salmon at 60 min post-exercise (Figure 4.3D). As expected, sex also affected plasma cortisol levels during recovery with significantly higher levels measured in females compared to males (χ^2 = 41.528, df = 1, P < 0.001; Figure 4.3E). In contrast, sex did not influence lactate, potassium, or hematocrit concentrations (P > 0.001; Figure 4.3) and the ligation treatment did not impact blood metrics (P > 0.001; Figure 4.3).

4.4.4 Acute thermal limits

Coronary ligation significantly reduced the acute thermal tolerance of coho by 1.1°C from 26.9°C (sham-operated) to 25.8°C (coronary-ligated (F_1 = 6.752, P = 0.018; Figure 4.4A). Due to limited sample size (sham-operated female: 7, male: 2; coronary-ligated female: 6, male: 7), female and male thermal tolerance could not be statistically compared (Figure 4.4A). The PvO_2 at CT_{max} were 12.0 ± 4.1 torr for sham-treated fish (N = 8) and 14.6 ± 6.2 (N = 6) for coronary-ligated fish and did not differ between treatments (F_1 = 0.659, P = 0.433; Figure 4.4B). Again, due to limited sample size for females and males, the PvO_2 at CT_{max} could not be statistically compared (Figure 4.4B).

After the acute thermal ramping protocol, fish had elevated lactate concentrations in both sham-operated and coronary-ligated fish compared to resting levels (14.0 ± 1.1 mmol l^{-1} and 12.9 ± 1.0 mmol l^{-1}, compared to 0.9 ± 0.1 mmol l^{-1} and 3.3 ± 2.6 mmol l^{-1}, respectively;

Fig 3A). Additionally, after CT_{max}, potassium concentration was greater than all other time points ($P < 0.001$) at ~5 mmol l^{-1} in both treatments (Figure 4.3C). Hematocrit levels after CT_{max} were also equivalent to levels reached immediately after the exhaustive exercise ($P = 0.999$), nearing 55% and greater than at rest ($P < 0.001$; Figure 4.3F). After CT_{max}, cortisol was also elevated above resting levels ($P < 0.001$; Fig 4.3E). Meanwhile, glucose and sodium concentrations were similar to resting values ($P > 0.05$; Figure 4.3B, D).

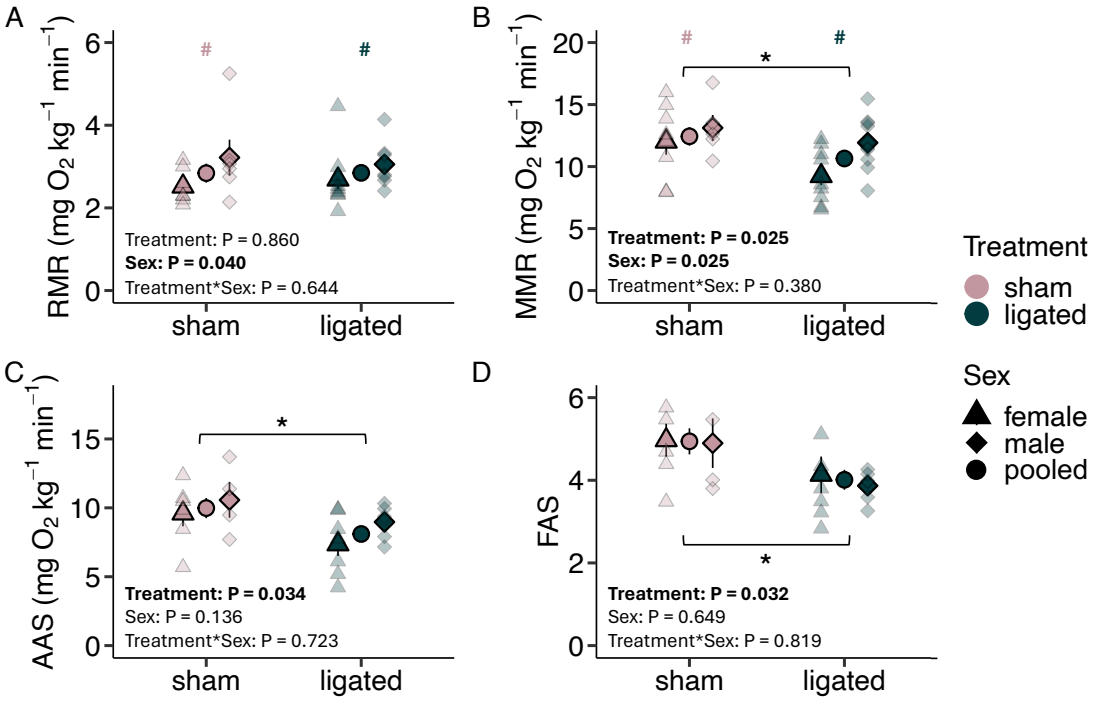

Figure 4.1. Effects of coronary ligation on the oxygen uptake rates in female and male coho salmon (*Oncorhynchus kisutch)*. (A) Resting metabolic rate (RMR), (B) maximum metabolic rate (MMR), (C) absolute aerobic scope (AAS), and (D) factorial aerobic scope (FAS). Female individuals are represented by triangles and male individuals by diamonds. The solid, larger data point denotes the mean and SEM for each sex (triangles and diamonds) or pooled (circles), at each treatment (coronary-ligated *vs.* sham-operated). P-values are reported for

results from two-way ANOVAs with independent variables (treatment, sex) bolded if significant, with asterisks (*) for significant differences between treatments and hashtags (#) for significant differences between sexes within a treatment. See Table 4.2 for sample sizes.

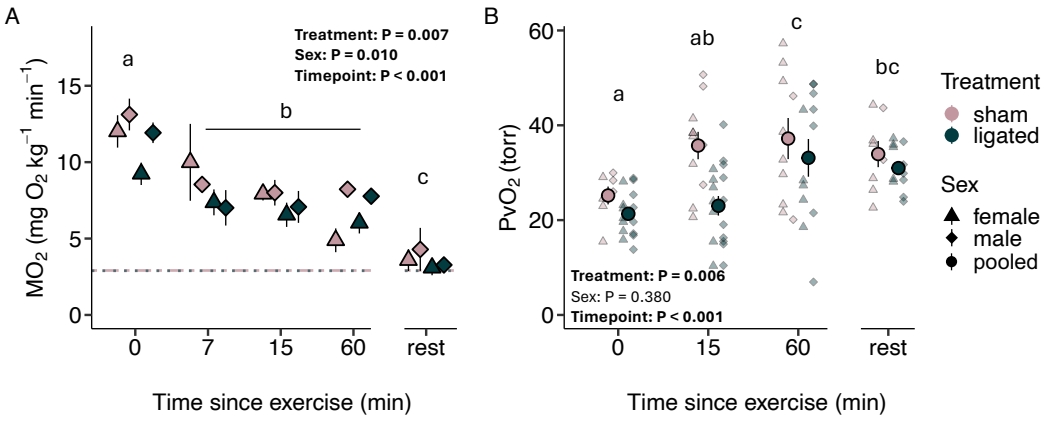

Figure 4.2. Effects of coronary ligation on the aerobic recovery and venous oxygen supply in coho salmon (*Oncorhynchus kisutch)*. (A) Mean MO_2 for female (triangle) and male (diamond) sham-operated (pink) and coronary-ligated (blue) fish with the horizontal dotted lines representing the RMR for sham-operated (2.96 mg O_2 kg^{-1} min^{-1}) and coronary-ligated fish (2.92 mg O_2 kg^{-1} min^{-1}). Sample sizes at time 0: sham N = 13 (8 female, 5 male), ligated N = 19 (9 female, 10 male); time 7: sham N = 6 (3 female, 3 male), ligated N = 7 (3 female, 4 male); time 15: sham N = 6 (3 female, 3 male), ligated N = 8 (3 female, 5 male); time 60: sham N = 5 (2 female, 3 male), ligated N = 10 (4 female, 6 male); rest: sham N = 9 (5 female, 4 male), ligated N = 14 (7 female, 7 male). (B) The partial pressure of venous O_2 at 0, 15, 60 min, and 18 h (rest) after exhaustive exercise, with the smaller transparent points as the female (triangle) and male (diamond) individual values, and the solid, larger datapoint as the mean and SEM for each treatment (sham-operated *vs*. coronary-ligated) at every

timepoint since exhaustive exercise. P-values are reported for results from linear mixed models, with individual fish as a random effect to account for repeated measures across each timepoint with independent variables (treatment, sex, timepoint) and tested for significance using repeated measures ANOVA. Values are bolded if significant. For B, values for female and male salmon are pooled within each treatment group (circle) because they are statistically the same. Different letters indicate significant differences across timepoints ($p < 0.05$) by Tukey HSD.

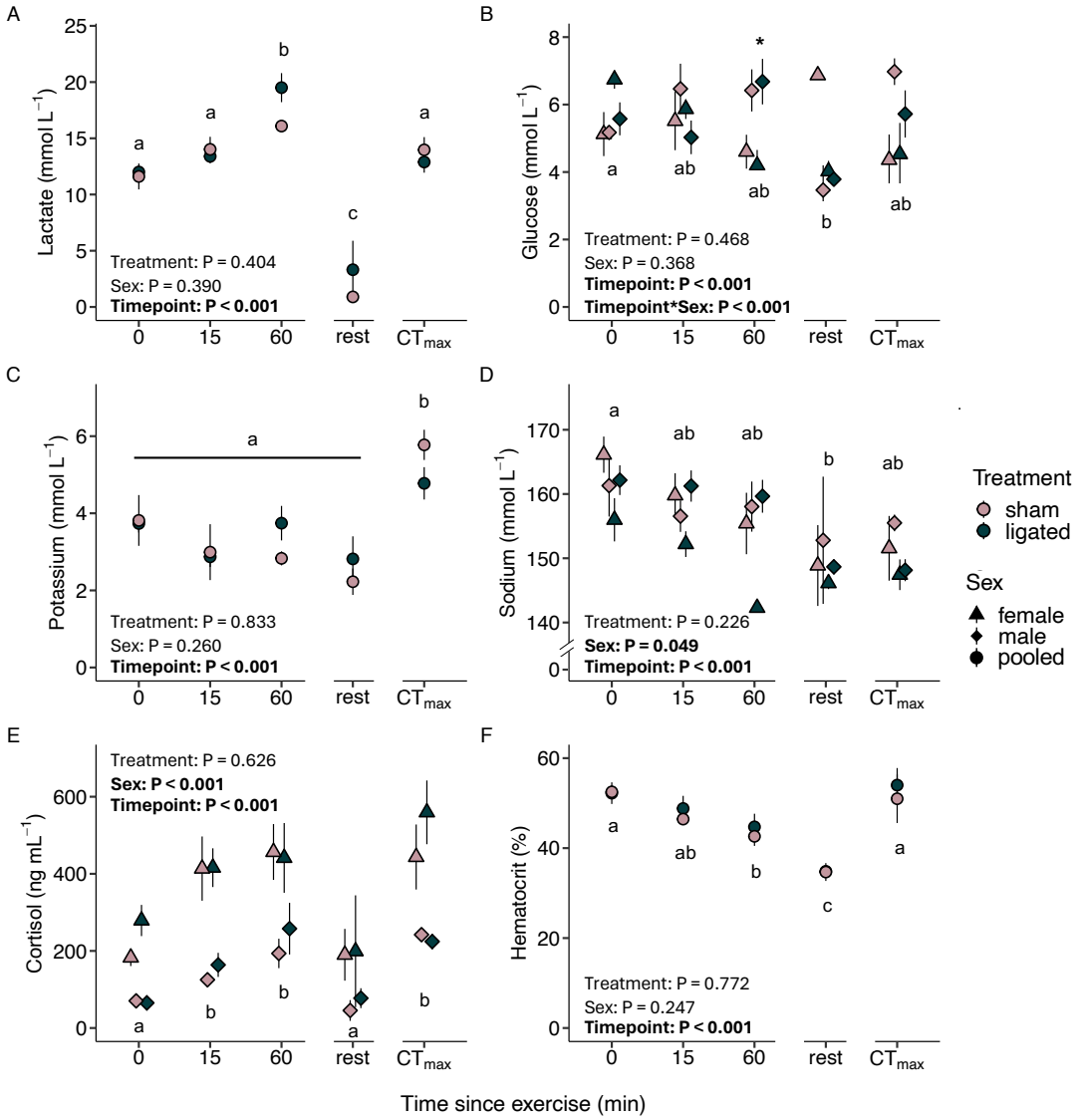

Figure 4.3. Effects of coronary ligation on blood chemistry in coho salmon (*Oncorhynchus kisutch*) during recovery from an exhaustive exercise and an acute thermal ramping test. (A) Lactate, (B) glucose, (C) potassium, (D) sodium, (E) cortisol concentrations as well as (F) hematocrit are represented at five experimental timepoints: following exercise (0, 15, and 60 min post-exercise), recovery after 18 h (rest), and after the acute thermal ramping protocol (CT_{max}). Points denote the mean and SEM for each treatment (sham-operated *vs*. coronary-ligated) at every timepoint since the exercise with sample sizes at time 0: sham N = 7 (4 female, 3 male), ligated N = 14 (7 female, 7 male); time 15: sham N = 12 (8 female, 4 male), ligated N = 17 (7 female, 10 male); time 60: sham N = 13 (8 female, 5 male), ligated N = 16 (6 female, 10 male); rest: sham N = 8 (6 female, 2 male), ligated N = 12 (6 female, 6 male); CT_{max}: sham N = 8 (6 female, 2 male), ligated N= 13 (6 female, 7 male). Sample sizes for hematocrit values were time 0: sham N = 11 (6 female, 5 male), ligated N = 12 (5 female, 7 male); time 15: sham N = 12 (7 female, 5 male), ligated N = 13 (5 female, 8 male); time 60: sham N = 11 (6 female, 5 male), ligated N = 11 (4 female, 7 male); rest: sham N = 7 (5 female, 2 male), ligated N = 7 (4 female, 3 male); CT_{max}: sham N = 7 (5 female, 2 male), ligated N= 10 (4 female, 6 male). P-values are reported for results from linear mixed models, with individual fish as a random effect to account for repeated measures across each timepoint with independent variables (treatment, sex, timepoint) and tested for significance using repeated measures ANOVA. Female and male salmon are pooled within each treatment group except for glucose, sodium, and cortisol concentrations where sex or its interaction is a significant factor (females: triangle, males: diamond). Different letters indicate significant differences across timepoints ($p < 0.05$) and asterisks (*) indicate significant differences between sexes at a timepoint determined by Tukey's HSD.

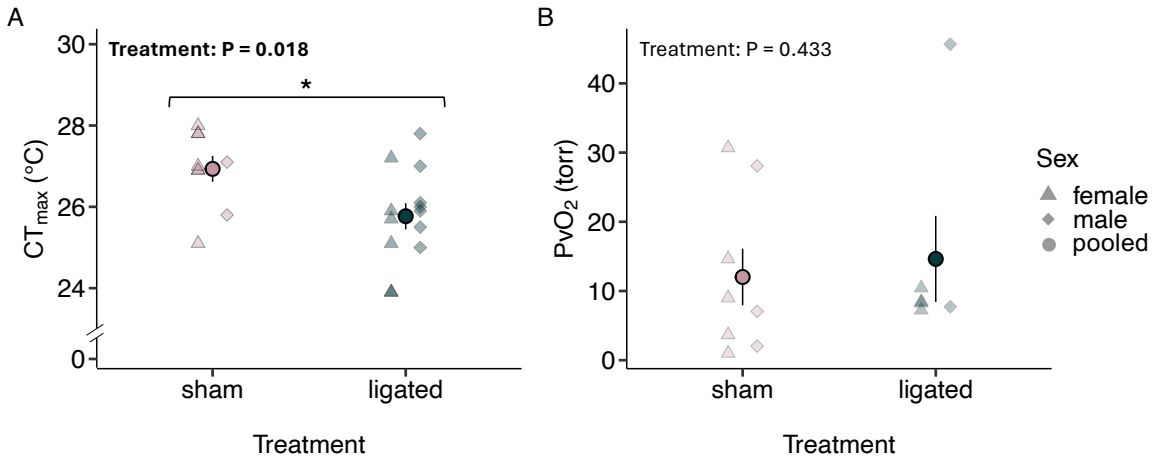

Figure 4.4. Effect of coronary ligation on the acute thermal tolerance of coho salmon (*Oncorhynchus kisutch*). Female individuals are represented by triangles, male individuals are represented by diamonds, and pooled means as circles. **(**A) Acute thermal limit (CT_{max}) (sham: N = 9 total, 7 female, 2 male; ligated: N = 13 total, 6 female, 7 male) and (B) PvO_2 at CT_{max} (sham: N = 8 total, 5 female, 3 male; ligated: N = 6 total, 4 female, 2 male) of sham-operated and coronary-ligated salmon. Due to low sample sizes, sex could not be included in statistical analyses and sex was pooled. The solid, circular point denotes the pooled mean and SEM for each treatment (coronary-ligated *vs*. sham-operated), and individual data points are represented for females (triangle) and males (diamond). P-values are reported for results from a one-way ANOVA.

4.5 Discussion

Coronary blockage by surgical ligation impaired the aerobic performance of female salmon, but contrary to our hypothesis, female salmon do not seem to rely on the coronary supply more than their male counterparts. Rather, coronary blockage impaired aerobic performance across sexes, exhibited by a reduced MMR, AAS, FAS, and blood venous oxygen content during recovery from exercise. Salmon with coronary blockages demonstrated a lower acute thermal tolerance by 1.1°C compared to sham-operated fish, but due to the limited sample size, sex-specific differences could not be discerned.

4.5.1 Coronary oxygen supply is not more important in supporting female metabolism

We found a similar relative importance of coronary perfusion across sexes. This does not help to explain the high mortality of female salmon relative to males during their up-river spawning migration (Hinch et al., 2021). We still cannot discount an oxygen limitation to the female hearts as a mechanism underlying these increased mortalities, in part, because we do not have up-to-date information on the severity of coronary arteriosclerosis in wild migrating sockeye salmon. Although the severity of coronary arteriosclerosis was recorded to be similar between sexes in 1960s (Robertson et al., 1961), the female-biased mortalities emerged decades later (Hinch et al., 2021). Although females and males similarly rely on their coronary arteries, this does not discount that females could have more severe coronary arteriosclerosis and therefore less oxygen supply to the compact myocardium. Other hypothesized mechanisms also remain, such as the higher oxygen demand needed for gonad development and support in females, where males may have higher aerobic scope compared to females (Clark et al., 2011), or their

differences in physiological recovery from anaerobic activity (Burnett et al., 2014; Eliason et al., 2020).

4.5.2 Coronary blockage compromises metabolic performance in wild pre-spawning coho salmon

Our findings indicate that coronary ligation severely reduced MMR in both sexes, resulting in a 26% decrease for females and a 10% for males. While fish were not necessarily swum to their MMR as in Ekström et al. (2023) and differences could not be discerned across sexes, sham-treated fish reached ~12 mg O_2 kg^{-1} $min^{-1,}$ and coronary ligated fish reached ~7 mg O_2 kg^{-1} min^{-1}, a ~50% difference. And at FT_{max}, coronary ligation diminished MO_2 by 38%. This impairment would likely constrain their capacity to swim and endure upriver migration conditions. This finding therefore suggests that coronary arteriosclerosis may potentially result in severe consequences on the migratory capacity of salmon. Spawning Pacific salmon are recognized to develop coronary arteriosclerosis during their migration, with nearly universal incidence and up to a 48% occlusion of the coronary artery (Farrell, 2002b; Farrell et al., 1990). Theoretically, such severe blockage of the coronary artery could lead to a substantial (~70%) decline in coronary blood flow to the heart (Brijs et al., 2020). Our findings suggest that fish with an obstructed coronary blood flow would have a lower maximal aerobic performance which would likely compromise their swim ability during migration. This is especially important in the context of spawning migration, where some areas in the river may require maximum swimming speed, endurance, or jumping capabilities (Thorstad et al., 2008) that are achieved through aerobic and anaerobic processes (Beamish, 1978). The coronary blockage in our study blocked 100% of blood flow to the coronary artery, which is more severe than arteriosclerotic lesions observed in Farrell et al. (1990), where the

artery was 48% occluded. However, those estimates came from salmon that survived the migration to the spawning grounds. It is possible that fish that did not survive the spawning migration had more severe arteriosclerosis (Farrell, 2002b).

Aerobic scope is considered a fundamental fitness trait and is of particular importance to migrating salmon, which presumably need 80-90% of their scope to complete their migration (Eliason et al., 2011; Farrell et al., 2008). In contrast to our prediction, aerobic scope was impacted equally across sexes. The aerobic scope in sham-treated fish matched that of a previous study on the same coho salmon population (no surgery performed), at ~10 mg O_2 kg^{-1} min^{-1} (Kraskura et al., 2021). However, coronary ligations reduced the aerobic scope by 19%, which corroborates previous findings in rainbow trout (*O. mykiss*) in which coronary ligation led to a 29% reduction in aerobic scope (Ekström et al., 2018). Thus, our findings suggest that cases of severe coronary arteriosclerosis will compromise coronary blood flow and impair aerobic scope, which would most likely reduce the migratory success of salmon during their once-in-a-lifetime migration (Eliason and Farrell, 2016). Further, the proportion of compact myocardium (relative ventricular mass) of the coho population in this study (30%) is relatively low compared to populations with a more strenuous migration, e.g. sockeye salmon (*O. nerka*; 45% compact myocardium, see Eliason et al., (2011)). Thus, populations with greater proportions of compact myocardium might rely on more coronary O_2 supply and even with similar levels of pathology, might be even more compromised.

Direct impairment of the compact myocardium by coronary occlusion would reduce cardiac output and tissues would have to extract a greater portion of available blood oxygen to meet aerobic demands, resulting in lower PvO_2 supplied to the heart. The low PvO_2 supply to the spongy myocardium would further compromise cardiac function, and thus aerobic

capacity. Indeed, during and immediately after exhaustive exercise, the PvO_2 and thus O_2 supply to the inner spongy myocardium declined to 20 - 30 torr across both treatments, which is consistent with values previously measured in fatigued salmon (Ekström et al., 2023; Eliason et al., 2013b; Farrell and Clutterham, 2003; Little et al., 2023; Steinhausen et al., 2008). In some coronary-ligated individuals, the PvO_2 reached as low as 7-10 torr, which surpasses the threshold (19 torr) for bradycardia and arrhythmia following exercise (Ekström et al., 2023; Wallbom et al., 2023) and the threshold (10 torr) that induces cardiac collapse in salmonids (Clark et al., 2008; Davie and Farrell, 1991; Hanson and Farrell, 2007). In combination with the reduced PvO_2 and the need for increased cardiac output during exercise (Ekström et al., 2023), the PO_2 gradient between the luminal blood and cardiac mitochondria was likely insufficient to adequately support cardiac pumping (Davie and Farrell, 1991). According to Fick's principle, MO_2 equals the cardiac output multiplied by the difference in arterial and venous oxygen content. MO_2 levels in coronary-ligated fish did not increase above sham-treated fish with the corresponding low PvO_2 at 0 and 15 min, but this could be the result of changes in several cardiorespiratory variables Tissues may have extracted more oxygen from the blood, leading to a greater difference between arterial and venous oxygen content, or less oxygen could have been delivered to tissues due to reduced cardiac output, leading to increased oxygen extraction and lower PvO_2. In fact, during recovery from swimming, cardiac output would be expected to decline (Eliason et al., 2013b). Prior work showed evidence that coronary ligation causes cardiac arrhythmia and bradycardia that severely constrains cardiac function and cardiovascular O_2 transport to systemic tissues following and during exhaustive exercise, which ultimately constrains metabolic capacity (Ekström et al., 2023; Wallbom et

al., 2023; Zena et al., 2024). Our current findings reinforce that during exercise, salmon are highly dependent on the delivery of oxygen to the heart via coronary circulation.

Migrating salmon use anaerobic (glycolytic) burst swimming to navigate high flows and rapids (Rand and Hinch, 1998). Given their limited energy stores and narrow time window to spawn, salmon must be able to recover in a timely and effective manner (Birnie-Gauvin et al., 2023). Fish had increased plasma lactate concentration during the 1 h post-exercise recovery period. This elevated lactate coincided with a lower PvO_2 at 1 h in the coronary-ligated fish, suggesting that ligated fish had a greater reliance on glycolysis to meet tissue energy demands, or that the normal recycling of lactate as a fuel for cardiac metabolism was compromised reducing lactate clearance rates. The accumulation of lactate was concurrent with declining sodium levels and elevated potassium levels, which could also be associated with extracellular metabolic acidosis, leading to impaired cardiac contractility in fish (Hanson and Farrell, 2007). Similarly, plasma lactate accumulated in swimming coho at FT_{max} and coincided with elevated potassium levels (Ekström et al., 2023). Interestingly, plasma lactate accumulated to a lesser extent in the FT_{max} coho study, reaching 5 mmol l^{-1} compared to nearly 20 mmol l^{-1} here (Ekström et al., 2023). This may be due to differences in the exercise protocols used (exhaustive chase here *vs*. swim tunnel in Ekström et al., 2023) (Milligan and Wood, 1986). Nevertheless, the coronary blockage might have impeded the capacity to sustain cardiac performance through different mechanisms that are still not entirely elucidated but are evidenced here and in previous work.

The coronary-ligated fish also had larger RVM and greater percent compact myocardium, which conflicts with findings that compact myocardium decreases in coronary-ligated rainbow trout after three days (Zena et al., 2021). However, these coho salmon may have had

a varied response to coronary ligation, and the heart may have remained in the inflammatory response stage three days since coronary ligation, which is characterized by an infiltration of inflammatory cells to the damaged heart area for repair (Grivas et al., 2014; Zena et al., 2021). Fish also appeared to have responses to coronary ligation beyond the cardiorespiratory system. The liver is involved in metabolism and energy storage (Harper and Wolf, 2009), and the greater HSI in coronary-ligated fish compared to sham-treated fish indicates that the liver underwent hyperplasia or hypertrophy, a common stress response.

4.5.3 Coronary circulation improves acute thermal tolerance

The performance metrics described above could be especially critical during environmental challenges, including warming water temperatures, increased or decreased flow rates, or hypoxic conditions. In our study, building upon prior research conducted with coho salmon of the same population (Ekström et al., 2023) we observed that coronary ligation lowered acute temperature tolerance by 1.1°C. Similar effects were found in rainbow trout with coronary-ligated coronary arteries (Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021). Ekström et al. (2023), swam coho, from the same population as this study, in a swim tunnel to measure thermal limits (CT_{swim}) and found that coronary ligation substantially lowered thermal limits by 4.4°C. This underscores the significance of coronary circulation in coping with the combination of energetic demands of intense swimming and elevated temperatures. One underlying mechanism contributing to the lower thermal tolerance in coronary-ligated rainbow trout was a decline in stroke volume and cardiac output, culminating in the failure of cardiac function at lower temperatures (Ekström et al., 2019; Morgenroth et al., 2021). At the acute thermal limits, the blood PvO_2 of both sham-operated and coronary-ligated fish neared 12-14 torr and reached below 10 torr for nine fish. These values nearing

the 10 torr threshold for cardiac collapse in salmonids suggest thermal tolerance was in part, limited by the heart (Clark et al., 2008; Davie and Farrell, 1991; Hanson and Farrell, 2007). We didn't necessarily expect differences in PvO_2 between treatments because the blood was sampled at CT_{max} for each fish.

The CT_{max} values measured here (25-27°C) are far higher than the thermal tolerances of salmonids measured using more ecologically relevant techniques (Mayer et al., 2023). CT_{max} tests ramp temperatures at an acute rate until loss of equilibrium, thus indicating an upper thermal limit indicative of death, not functional thermal tolerance (Blasco et al., 2020; Desforges et al., 2023). Indeed, a more functional test swam fish during acute warming (CT_{swim}) in the same coho population with the same sham and coronary ligation treatments and found lower functional limits, ranging from 15-25°C across treatments (Ekström et al., 2023). Sex-specific differences in survival and performance are revealed at temperatures below 20-21°C (Hinch et al., 2021). Current Fraser River watershed temperatures rarely exceed 22°C (Fraser River EWatch, 2023), and elevated *en route* mortality is observed when temperatures >18°C for many salmon populations (Martins et al., 2012). Though the CT_{max} values do not provide ecologically relevant thermal thresholds, they can be used to compare across treatments and studies to reveal relative thermal tolerance. The mechanisms responsible for the differences in CT_{max} between the treatments may similarly impact physiological performance at lower temperatures and have long-term impacts. Therefore, care should be taken when interpreting these findings, but the implications remain.

4.6 Conclusion

The productivity (e.g. recruits per spawner) of Pacific salmon has been in decline in their southern range for the past 30 years (Peterman and Dorner, 2012) with adult returns to spawning grounds at record low abundances in many populations. Those individuals who do succeed to spawn must demonstrate exceptional cardiac performance. If salmon have impaired cardiorespiratory fitness, such as reduced MMR, aerobic scope, and recovery rates when coronary blood flow is restricted, they may be less likely to complete their spawning migration. This raises the question of how these effects extend to the wild and across salmonid populations and species (Farrell, 2023). Although coronary circulation is a secondary oxygen supply to the heart in certain fish, unlike its primary role in supporting hearts in birds and mammals (Farrell et al., 2012), we highlight its potential significance in wild migrating salmon. We propose that the ramifications of coronary arteriosclerosis for salmon could become more severe as they confront growing and novel challenges during their upstream migrations in the face of climate change. This underscores the value of studying heart function at the foundational level and within the framework of climate change.

Table 4.1. Morphological traits for the coho salmon (*Oncorhynchus kisutch)* in the two treatment groups (sham-operated *vs*. coronary-ligated) for females, males, and pooled. Values are represented as mean ± SEM.

Metric	Treatment					
	Sham			Ligated		
	female	**male**	**pooled**	**female**	**male**	**pooled**
Body mass (kg)	2.04 ± 0.19	2.12 ± 0.19	2.08 ± 0.13	1.85 ± 0.11	1.69 ± 0.19	1.77 ± 0.11
FL (cm)	56.6 ± 1.6	57.9 ± 2.4	57.1 ± 1.3	55.2 ± 1.22	54.2 ± 1.8	54.7 ± 1.1
RVM (%)	0.18 ± 0.01$^{\#}$	0.21 ± 0.01$^{\#}$	0.2 ± 0.01*	0.21 ± 0.02$^{\#}$	0.24 ± 0.01$^{\#}$	0.22 ± 0.01*
GSI (%)	16.38 ± 1.07$^{\#}$	4.48 ± 0.35$^{\#}$	11.48 ± 1.59	15.02 ± 0.99$^{\#}$	4.62 ± 0.24$^{\#}$	9.82 ± 1.19
HSI (%)	1.75 ± 0.16$^{\#}$	1.15 ± 0.05$^{\#}$	1.5 ± 0.12*	2.12 ± 0.15$^{\#}$	1.27 ± 0.03$^{\#}$	1.69 ± 0.12*
SSI (%)	0.13 ± 0.01$^{\#}$	0.17 ± 0.02$^{\#}$	0.15 ± 0.01	0.15 ± 0.01$^{\#}$	0.19 ± 0.02$^{\#}$	0.17 ± 0.01
Compact myocardium (%)	28.86 ± 1.25	29.26 ± 0.84	29.01 ± 0.82*	31.67 ± 1.35	30.8 ± 1.23	31.24 ± 0.9*
N	10	7	17	12	12	24

Abbreviations: FL = fork length, RVM = relative ventricle mass, GSI = gonadal size index, HSI = hepatosomatic index, SSI = spleen somatic index, N = sample size. Asterisks (*) denote statistically significant ($P < 0.05$) differences between treatment groups, and hashtags (#) denote significant differences between sexes within a treatment group.

Table 4.2. Metabolic performances for the coho salmon (*Oncorhynchus kisutch)* in the sham-operated and coronary-ligated groups for females, males, and pooled sexes. Values are represented as mean ± SEM with sample size in parentheses.

Metric	Treatment					
	Sham			Ligated		
	female	**male**	**pooled**	**female**	**male**	**pooled**
RMR (mg O_2 kg^{-1} min^{-1})	2.52 ± 0.16 (7)[#]	3.22 ± 0.43 (6)[#]	2.84 ± 0.23 (13)	2.69 ± 0.24 (9)[#]	3.06 ± 0.22 (7)[#]	2.85 ± 0.17 (16)
MMR (mg O_2 kg^{-1} min^{-1})	12.01 ± 1.06 (8)[#]	13.12 ± 1.04 (5)[#]	12.44 ± 0.75 (13)[*]	9.24 ± 0.73 (9)[#]	11.92 ± 0.67 (10)[#]	10.65 ± 0.58 (19)[*]
AAS (mg O_2 kg^{-1} min^{-1})	9.59 ± 0.94 (6)	10.57 ± 1.29 (4)	9.98 ± 0.73 (10)[*]	7.36 ± 0.85 (7)	8.97 ± 0.49 (6)	8.1 ± 0.54 (13)[*]
FAS	4.97 ± 0.39 (6)	4.90 ± 0.6 (4)	4.94 ± 0.32 (10)[*]	4.13 ± 0.44 (7)	3.87 ± 0.15 (6)	4.01 ± 0.24 (13)[*]
EPOC (mg O_2 kg^{-1})	1345.79 ± 390.66 (5)	2426.65 ± 750.12 (4)	1826.17 ± 415.01 (9)	1109.37 ± 190.95 (7)	1364.24 ± 230.42 (7)	1236.8 ± 148.04 (14)
EPOC duration (min)	462.5 ± 97.7 (5)	646.58 ± 125.02 (4)	544.31 ± 79.39 (9)	542.21 ± 82.31 (7)	510.79 ± 80.91 (7)	526.5 ± 55.62 (14)

Abbreviations: RMR = resting metabolic rate, MMR = maximum metabolic rate, AAS = absolute aerobic scope, FAS = factorial aerobic scope, EPOC = excess post oxygen consumption. Hashtags (#) indicate differences across sexes and asterisks (*) indicate differences between treatments.

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