Laboratory-based measures of temperature preference and metabolic thermal sensitivity provide insight into the habitat utilisation of juvenile California Horn Shark *(Heterodontus francisci)* and Leopard Shark *(Triakis semifasciata)*

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ABSTRACT

Laboratory-based studies examining fish physiological and behavioural responses to temperature can provide important insight into species-specific habitat preferences and utilisation, and are especially useful in examining vulnerable life stages that are difficult to study in the wild. Here, we couple shuttle box behavioural experiments with respirometry trials to determine the temperature preferences and metabolic thermal sensitivity of juvenile California Horn Shark (*Heterodontus francisci*) and Leopard Shark (*Triakis semifasciata*). As juveniles, these two species often occupy similar estuarine habitats, but display contrasting behaviours and activity levels – *H. francisci* are relatively sedentary, while *T. semifasciata* are more active and mobile. Our results show that juvenile *H. francisci* and *T. semifasciata* have comparable thermal preferences and occupy similar temperature ranges, but *H. francisci* metabolism is more sensitive to acute changes in temperature as expressed through a higher Q_{10} (*H. francisci* = 2.58; *T. semifasciata* = 1.97; temperature range: 12-24°C). Underlying chronic temperature acclimation to both warm (21°C) and cool (15°C) representative seasonal temperatures did not appear to significantly affect these parameters. We discuss our results in the context of field studies examining known distributions, habitat, and movement patterns of *H. francisci* and *T. semifasciata* to better understand the role of temperature in species-specific behaviour. Our results suggest that juvenile *H. francisci* likely target thermally stable environments such as estuaries that are close to their preferred temperature, while juvenile *T. semifasciata* metabolism and behaviour appear less dependent on temperature.

Keywords: shark, juvenile, metabolism, temperature preference, temperature acclimation, thermal sensitivity

INTRODUCTION

Temperature is an important driver of fish habitat use, and although many species make exploratory movements across broad temperature ranges, they often use physiological or behavioural controls to target a preferred internal body temperature (Fry, 1947; Jobling, 1981). For example, elasmobranchs (sharks and rays) are known to behaviourally thermoregulate by physically moving across temperature gradients to target bio-energetically favourable thermal conditions (Carey et al., 1990; Grubbs et al., 2007; Heupel & Simpendorfer, 2008; Kessel et al., 2014; Klimley, 1993; Stokesbury et al., 2005). This thermoregulatory behaviour is often associated with processes such as foraging, digestion, and parturition (Hight & Lowe, 2007; Sims et al., 2006; Wallman & Bennett, 2006; Watanabe et al., 2021). Selected thermal habitat is largely dependent on the thermal sensitivity of the fish's underlying metabolism. Interspecific variability in the thermal sensitivity of metabolism is often expressed through the Q_{10} temperature coefficient (a measure of how a physiological rate changes across a 10°C temperature change) and suggests certain species are likely to show greater physiological or behavioural responses to fluctuations in environmental temperature than others (Schmidt-Nielsen, 1997). Understanding species-specific behavioural and physiological responses to temperature is important for understanding fish habitat utilisation in the face of changing temperatures, whether it be in response to broader-scale changes such as anthropogenic-induced climate change or more local changes such as habitat alteration associated with human encroachment (e.g., changing bay or estuary circulation patterns through development and dredging or thermal effluents).

Insights into elasmobranch behavioural thermoregulation are often derived from *in situ* movement data from tagging and tracking studies used to examine habitat utilisation (Andrzejaczek et al., 2018; da Silva et al., 2021; Campana et al., 2011; Matern & Hopkins, 2000; Speed et al., 2012; Thums et al., 2013; Vaudo & Heithaus, 2013). However, isolating the direct effect of temperature on fish movements and behaviour can be difficult as various environmental and biological drivers of habitat utilisation can act simultaneously and may co-vary with temperature. For instance, adult female Leopard

Shark *Triakis semifasciata* Girard 1855 are known to often aggregate in warm shallow waters, but the increased temperatures also correlate with other potentially advantageous conditions including reduced wave height, proximity to foraging grounds, and refuge from males (Hight & Lowe, 2007; Nosal et al., 2013). Laboratory-based studies allow for the isolation of single variables such as temperature and can thereby provide a more direct understanding of the drivers of wild behavior and movement (Casterlin & Reynolds, 1979; Dabruzzi et al., 2013; Gervais et al., 2018; Lear et al., 2017; Nay et al., 2021; Wallman & Bennett, 2006). In addition, laboratory work allows for examination of juvenile animals that are often poor candidates for field tagging studies (due to increased vulnerability or sensitivity associated with tagging procedures and electronic tag size) and may have different temperature preferences than adults (Lafrance et al. 2005). In this study, we isolate temperature as a single variable in a controlled setting to investigate how temperature preference and the thermal sensitivity of metabolism compare between juveniles of two co-occurring shark species with different activity levels.

The California Horn Shark *Heterodontus francisci* (Girard 1855) and *T. semifasciata* are two common mesopredators of southern California that occupy relatively warm, shallow embayments during their early life stages (Talent, 1985; Ebert, 2003). For many sharks, such embayments and estuaries are thought to serve as nursery grounds (Castro, 1993; Merson & Pratt, 2001; Espinoza et al., 2011), in which the shallow and protected waters generally provide relatively warm, bioenergetically-favourable conditions for growing juveniles, abundant prey, and refuge from larger predators (Bass, 1978; Branstetter, 1990; Heupel et al., 2007; Springer, 1967). Although their shallow coastal habitat preferences may be similar, juvenile *H. francisci* and *T. semifasciata* appear to utilize nursery habitats in different ways. *H. francisci* is a benthic species that is generally inactive during the day, often resting within crevices of shallow rocky reefs (Nelson & Johnson, 1970; Talent, 1985; Ebert, 2003; Meese & Lowe, 2020a; Meese & Lowe, 2020b). At night, *H. francisci* will emerge from their shelter sites and forage over mud, sand flats, or eelgrass beds. During these foraging movements, *H. francisci* experience a wider range of temperatures than during the day and will take frequent breaks to rest (tagged sharks spent ~50% of the night at rest; Meese & Lower 2020b). In contrast, *T. semifasciata* is a more mobile demersal

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species, which spends most of its time actively swimming both during the day and night (Manley, 1995; Ackerman et al., 2000; Ebert, 2003; Hight & Lowe, 2007). Like *H. francisci*, *T. semifasciata* is more active at night (mean movement rate greatly increases; Manley, 1995; Ackerman et al., 2000) and will forage over mud, sand flats, or eelgrass beds of the surf and subtidal zone (Ebert, 2003; Talent, 1985). While adult female *T. semifasciata* are often thought to aggregate in bioenergetically-favourable thermal habitats which may increase gestation rates, tagging studies also show that adults regularly travel relatively large distances over short periods of time, while often experiencing a wide range of temperatures (Carlisle & Starr, 2009; Hight & Lowe, 2007; Hopkins & Cech, 2003; Nosal et al., 2013; Nosal et al., 2014). Thus, as co-occurring species with contrasting behaviours and activity levels, *H. francisci* and *T. semifasciata* serve as good model organisms to understand how temperature may drive habitat utilisation.

Here we examine the adaptive thermal biology of juvenile *H. francisci* and *T. semifasciata* through laboratory-based study of behavioural thermoregulation and metabolism. First, we used a shuttle box experiment to determine the temperature preferences of juvenile *H. francisci* and *T. semifasciata*. Second, to assess the effects of temperature on metabolism, we measured resting oxygen consumption (a proxy for resting metabolic rate) concurrently with acute (< 24 h) changes in temperature chosen to represent short-term movement across thermal regimes experienced by both study species within estuaries and embayments of southern California. We hypothesised that juvenile *H. francisci* and *T. semifasciata* would have similar preferred temperatures consistent with their observed co-occurrence in shallow estuarine habitats, but that juvenile *T. semifasciata* metabolism would be less sensitive to changes in temperature than that of juvenile *H. francisci* due to their more active daily lifestyle and likelihood of regularly encountering larger temperature fluctuations. We describe our laboratory-based results in reference to *in situ* field studies and surveys to better understand how temperature influences the habitat preference and movements of *H. francisci* and *T. semifasciata*.

METHODS

Ethical Statement

The care and use of experimental animals complied with United States animal welfare laws, guidelines and policies as approved by the Institutional Animal Care and Use Committees of the University of California, San Diego (Protocol #S00080) and National Oceanic and Atmospheric Administration (NOAA) Southwest Fisheries Science Center (SWFSC) (Protocol # SW1801). One shark died in captivity, five were euthanised for a separate study, and the rest were released back into the wild in accordance with California Fish and Wildlife Scientific Collecting Permit SC-13908.

Collection and Holding

We collected *H. francisci* (37-49 cm total length (TL), n=5 males and n=5 females) and *T. semifasciata* (70-90 cm TL, n=5 males and n=3 females) from Mission Bay (32°46'30.8"N 117°13'47.5"W) and San Diego Bay (32°39'40.2"N 117°08'13.4"W) CA, respectively, as bycatch in Hubbs SeaWorld Research Institute's White Seabass Gillnet Survey in June, 2018 (California Fish and Wildlife Scientific Collecting Permits SC-2481 and SC-13908). We size-selected captured individuals to only retain immature individuals, as determined by length of maturity estimates (Ebert, 2003; Kusher et al., 1992), and confirmed via the absence of clasper calcification in males. Immediately following capture, we temporarily held sharks indoors at Scripps Institution of Oceanography, La Jolla, CA, in tanks containing flow-through ambient seawater until they began eating, at which point we moved them across the street to the indoor SWFSC Experimental Aquarium for shuttle box and respirometry trials. At SWFSC, we held *H. francisci* in a $300 \times 150 \times 90$ cm ($l \times w \times h$) oval tank (approximately 3200 L), while we held *T. semifasciata* in a 300×90 cm ($d \times h$) round tank (approximately 6350 L), both continuously fed with flow-through filtered and UV and ozone-sterilized seawater. Fish were held under a 12:12 h photoperiod for the duration of the study to eliminate any light-induced seasonal cues in order to help isolate temperature as a single variable. We fed sharks a diet of California Market Squid *Doryteuthes*

opalescens (Berry 1911) and Pacific Chub Mackerel *Scomber japonicus* Houttuyn 1782 until satiation every 72 h. All sharks were fasted for 72 h prior to both shuttle box and respirometry trials, consistent with previous shark research to avoid any metabolic effects associated with specific dynamic action (Miklos et al., 2003; Lear et al., 2017; Whitney et al., 2016).

Because both metabolic rate and temperature preference are known to change with temperature and thermal acclimation length for some elasmobranch species (Carlson & Parsons, 1999; Mortensen et al., 2007), we conducted two successive series of experimental trials in which we acclimated the sharks for a minimum of six weeks, first to a representative summer temperature ("warm" treatment targeted at 21°C), and, second, to a representative winter temperature ("cold" treatment targeted at 15°C). We chose these chronic temperature acclimations to align with mean summer and winter sea surface temperature (SST) data measured from NOAA Station SDBC1 (32°42'51" N 117°10'26" W) in San Diego Bay (NOAA National Data Buoy Center) from 2012-2017. Because we collected the sharks in early summer, we first conducted the warm acclimation experiments, which due to water flow rates and tank dynamics were actually 20.2 ± 0.1°C for *T. semifasciata* and 20.7 ± 0.5°C for *H. francisci*. All ten *H. francisci* and all eight *T. semifasciata* were used in these warm acclimation experiments. Following the warm acclimation experiments, we slowlt lowered the water temperature to the targeted 15°C (~0.5°C/day) and then acclimated individuals for a minimum of six weeks to the cold treatment (actual temperature: 14.9 ± 0.5°C for *T. semifasciata* and 15.3 ± 0.4°C for *H. francisci*) before beginning the cold acclimation experiments. Only five of the *H. francisci* and seven *T. semifasciata* were used (five *H. francisci* were used in a non-related study and one *T. semifasciata* died while in captivity between chronic acclimation treatments). From the start of the "warm acclimation" to the end of the "cold acclimation" (32 weeks), *H. francisci* and *T. semifasciata* increased in mass by 22.9 ± 3.74% (mean percent mass increase $\pm$ SE) and 29.2 ± 5.37%, respectively (Table 1).

Shuttle box System

Following acclimation to each mean temperature ("warm" or "cold" acclimation), we assessed shark temperature preference using an automated shuttle box system (Loligo Systems Inc., Tjele, Denmark). The shuttle box consisted of two 130 x 60 cm (*d x h*) circular chambers connected by a 35 x 30 x 60 cm (*l x w x h*) shuttling channel (Figure 1), where one of the circular chambers contained water that was always kept 1.5°C warmer (warm side) than the other (cool side). Each chamber was connected to an external buffer tank for temperature control. Water temperature in each buffer tank was maintained by pumping water through recirculating closed loops composed of stainless steel heating / cooling coils immersed in water baths of either 30 or 6°C water. Pumps to each heating / cooling bath were modulated by a temperature sensor placed within the return tubing from the shuttle box to the buffer tank (Figure 1). The in-flow and out-flow into each chamber of the shuttle box created a slight circular current (flow rate was ~6.8 L min^{-1}) within each chamber to promote mixing and temperature uniformity, while mixing between the warm and cold chambers was minimised by the shuttle channel. The total volume of water in the system, including chambers, hoses, and buffer tanks, was approximately 600 l. During an experiment, a shark would behaviourally thermoregulate by "shuttling" back and forth between the warm and cool sides. When the shark swam to the warm side, it triggered a system-wide temperature increase of 2.0°C per hour, while maintaining the 1.5°C difference between the warm and cool sides. When the fish switched to the cool side, system-wide cooling was triggered at the same rate. The position of the fish was monitored by an overhead video camera (uEye USB camera, Imaging Development Systems, Dimbacher, Germany) connected to a computer with accompanying animal tracking software (Shuttlesoft, Loligo Systems Inc., Tjele, Denmark). This software automatically controlled the pumps leading to the heating / cooling coils, and consequently the system water temperatures, in response to fish movement via a Daq-M data acquisition and control box (Loligo Systems Inc., Tjele, Denmark). Dissolved oxygen concentration was maintained throughout the system at saturation by air stones located in the buffer tanks.

In order to control for the possibility that a fish might have a tank side preference that could affect experimental results, we switched the warm and cool sides of the shuttle box system for half of all the trials and randomly assigned sharks to a starting side when we first introduced them into the system. We

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placed each shark in the shuttle box system with the system maintaining a static difference of 1.5°C around the acclimation temperature (~20.75°C cool side and ~22.25°C warm side during 21°C warm acclimation experiments, ~14.75°C cool side and ~16.25°C for the 15°C cold acclimation experiments). Once placed in the shuttle box, the shark was continuously tracked for 68-96 h.

Temperature Preference

Due to the thermal rate coefficient of sharks obtained in previous studies (Stevens & Fry 1974; Hight & Lowe 2007) and the small mass of the sharks used in this study, we assumed internal body temperature and the occupied water temperature were the same. Thus, as in other studies, we report the final temperature preferendum as the median occupied water temperature within an individual trial (Figure 2; Macnaughton et al., 2018; Stol et al., 2013). To account for individual variability in learning to navigate the shuttle box, we ran a broken stick model using *segmented* package (Muggeo, 2017) in R to determine the time when the shark began targeting its preferred temperature (Figure 2). We determined the preferred temperature [i.e., median occupied temperature (Macnaughton et al., 2018; Stol et al., 2013)] from this break to the end of the trial (typically resulting in 12-48 h of purposeful behavioural thermoregulatory shuttling behaviour). In addition to the median preferred temperature, we determined the preferred temperature range as the lower and upper bounds between which sharks spent 95% of their time once the shark began targeting its preferred temperature. We determined the temperature preference, lower preferred temperature bounds, and upper preferred temperature bounds separately for each individual following both cold and warm acclimations.

Respirometry Trials

In order to examine the effect of short-term (acute) temperature changes on resting metabolism at different chronic temperature acclimation treatments, we measured resting oxygen consumption rate for both species via closed respirometry. We ran respirometry trials for warm-acclimated (21°C) individuals at 16°C, 20°C and 24°C, and cold-acclimated (15°C) individuals at 12°C in addition to 16°C, 20°C, and

24°C. Our respirometry system was composed of a cylindrical acrylic holding chamber proportional to the size of the individual (5.7-16.4 L for *H. francisci* and 40.0-52.5 L for *T. semifasciata*; Loligo Systems, Inc.) with a recirculating loop containing a fibre optic oxygen sensor and temperature sensor connected to a Fibox 4 fibre optic oxygen transmitter (PreSens Precision Sensing GmbH, Regensburg, Germany). The respirometer was placed within a buffer tank that we used to maintain the desired temperature and provide aerated seawater for flushing the system between trials (measurement periods).

Following a 72 h fasting period, we placed a shark into the respirometer at its chronic warm or cold temperature acclimation (15°C or 21°C). We then slowly adjusted the temperature (0.5°C h^{-1}) down to the starting experimental temperature (either 12°C for cold acclimation or 16°C for warm acclimation) controlled by an automated temperature mixing station. We held each individual at the starting temperature for 12 hours prior to measuring oxygen consumption to account for initial handling and acute temperature stress (Miklos et al., 2003; Luongo & Lowe, 2018). For each measurement trial, we manually closed the inflow and outflow valves of the respirometer to seal it off from the surrounding buffer tank, and we recorded the dissolved oxygen level (% air saturation) within the recirculating loop every five seconds until it was brought down to between 70-80% air saturation. Following each trial, we flushed the respirometer with oxygen saturated seawater from the buffer tank and repeated the process. We conducted a minimum of two trials (range: 2-7 trials) at each temperature (trial length range 7-90 mins depending on species and temperature), after which we slowly raised the system temperature (~0.5°C h^{-1}) and repeated the trials at the next temperature following another 12-hour acclimation period at the designated temperature. We ran all respirometry trials at each temperature during daylight hours as the sharks were typically less active during the day, and this also allowed us to continuously monitor the sharks during data acquisition. From the start of the respirometry experiment at the low temperature to the end of the experiment at the warm temperature the sharks were in the respirometer for ~72 h and ~96 h for the warm acclimation and cold acclimation experiments, respectively. Once we collected respirometry measurements at all temperatures, we removed the shark and resealed the respirometer for an additional 1.5 hours at the warmest experimental temperature (24°C) to measure background microbial respiration.

We estimated the rate of oxygen depletion within the respirometer for each shark individual at each temperature using a rolling regression (Chabot et al. 2021) of the five-second resolution data over lengths of 10% air saturation drawdown within each trial using code modified from Prinzing et al (2021). Shark M_{O_2} estimates were calculated for each oxygen depletion regression slope by multiplying by the volume of the respirometer while accounting for the volume of the shark (the displacement volume of the shark was determined using estimates from Luongo and Lowe (2018) where 1 kg displaces 0.957 L of water). We filtered out M_{O_2} estimates outside two standard deviations of the mean and calculated resting M_{O_2} as the 25% quantile ($q_{0.25}$) using "calcSMR" (Chabot et al., 2016). This method allowed us to include trials with some sporadic movements and fluctuations in oxygen consumption (however, for one warm-acclimated *H. francisci* and two cold-acclimated *T. semifasciata* individuals some trials were omitted at warmer temperatures due to non-stop activity and signs of stress resulting in a continuously elevated oxygen consumption rate (Ferry-Graham & Gibb, 2001)). We corrected all resting M_{O_2} estimates for the background respiration assuming a linear increase in bacterial respiration over time within the chamber and adjusting the measured background respiration for changes in temperature using a Q_{10} of 2.0 (in all cases background respiration was calculated as less than 5% of the total oxygen consumption rate for the shark). The mean measured change in mass of the shark from before and after the 3-4 d respirometry experiments was ~2% (n=22), and therefore any changes to metabolism associated with change in body mass during the trial were considered negligible (Sims & Davies, 1994; Ferry-Graham & Gibb, 2001; Miklos et al., 2003).

In order to more directly compare resting M_{O_2} between individuals, we mass adjusted the oxygen consumption rate of each shark to the mean mass of each species for each temperature acclimation treatment using a scaling exponent of 0.80 (Brett & Groves 1979; Wegner et al 2018; Luongo & Lowe, 2018) as:

$$M_{O_2} = a_n \underline{M}^{0.80} \qquad\qquad (1)$$

where the mass-adjusted oxygen consumption (M_{O_2} in mg O_2 h^{-1} kg^{-1}) for each individual was a function of scaling intercept (*a*) for each individual (*n*), a_n (= $M_{O_{2(n)}}$ / $M_{(n)}^{0.80}$), and $\underline{M}$ is the mean mass of all individuals during that acclimation period (either warm or cool acclimation).

We determined the metabolic thermal sensitivity quotient (Q_{10}) by the Schmidt-Nielsen (1997) equation:

$$Q_{10} = \left(\frac{Mo_{2(2)}}{Mo_{2(1)}}\right)^{\frac{10}{T_2 - T_1}} \tag{2}$$

where $M_{O_{2(1)}}$ is the oxygen consumption rate at temperature T_1 and $M_{O_{2(2)}}$ is the oxygen consumption rate at temperature T_2.

Statistical Analyses

We ran linear mixed effects models (LMEs) to examine the potential significant differences in temperature preference, lower preferred temperature bound, and upper preferred temperature bound (separate response variables) between species, temperature acclimation, and sex (fixed factors) using the *lme4* (Bates et al., 2015) and *lmerTest* packages (Kuznetsova et al., 2017) in R. We also ran an LME to examine potential significant differences in M_{O_2} (response variable) between temperature acclimation (cold vs. warm), experimental temperature (12°C, 16°C, 20°C, 24°C), and sex (all as fixed factors). The difference in M_{O_2} between the two species was evident across all temperatures, and therefore, we excluded species as a fixed factor for this model. To account for the repeated measures of individuals at the two acclimation temperatures, we treated individual as a random effect for all LMEs. Visual Q-Q plots and Shapiro-Wilk normality tests showed there was a normal distribution among the residuals for which we applied the LMEs. We calculated variance inflation factor (VIF) to assess multi-collinearity and found the variables were not strongly correlated (VIF <5) for all LMEs. Analysis of Variance (ANOVA)

was also performed to obtain the F-value of each LME. We used the *multcomp* package (Hothorn et al., 2008) for multiple pairwise comparisons with Bonferroni adjustments to p-values to examine specific differences found by the ANOVAs. All analyses were conducted in R version 3.6.1.

RESULTS

The preferred temperature and occupied temperature range of *H. francisci* and *T. semifasciata* determined through shuttle box experimentation did not differ significantly between species, acclimation treatment, or sex (Table 2; ANOVA $p > 0.05$ for all variables and interactions). *H. francisci* preferred $20.6 \pm 0.8°C$ and $18.0 \pm 1.9°C$ (mean $\pm$ SE) after warm and cold acclimations, respectively, while *T. semifasciata* preferred $18.4 \pm 1.4°C$ and $17.5 \pm 1.1°C$ after warm and cold acclimations, respectively. Likewise, both species generally showed similar lower and upper bound temperatures around their preferred mean temperature (Table 2), although the effect of acclimation on the upper bound temperature was near the significance threshold (p= 0.05524), likely due to warm-acclimated horn sharks which exhibited a trend of exploring warmer upper bound temperatures. Although not statistically different, there was a general trend in which females from both species preferred warmer temperatures and had a higher shifted thermal range than males in both acclimation treatments (Table 3).

T. semifasciata had higher resting oxygen consumption rates (M_{O_2}) relative to *H. francisci* at all respirometry trial temperatures (12°C, 16°C, 20°C, and 24°C), regardless of the acclimation treatment (Table 4). Mean M_{O_2} significantly increased across respirometry trial temperatures from 12-24°C under both acclimation treatments for both *T. semifasciata* (ANOVA, $p<0.001$) and *H. francisci* (ANOVA, $p<0.001$; Figure 3). Resting oxygen consumption at a given temperature did not differ significantly between warm and cold acclimation treatments nor between sexes for either *H. francisci* or *T. semifasciata* (ANOVA, $p > 0.05$ for all variables and interactions). Resting oxygen consumption rates of *H. francisci*

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and *T. semifasciata* from this study are shown in comparison to those determined in previous studies (Scharold et al., 1989; Miklos et al., 2003; Luongo & Lowe, 2018) in Table 4. Across all experimental temperatures from 12-24°C, the Q_{10} for *H. francisci* (2.58) was higher than that of *T. semifasciata* (1.97; Table 5).

DISCUSSION

Experimental approaches to understand behavioural thermoregulation and physiological responses to temperature allow for insight into fish habitat selection. We provide a comprehensive perspective of behavioural thermoregulation in juvenile *H. francisci* and *T. semifasciata* by reporting oxygen consumption measurements in conjunction with laboratory-based temperature preference measures using the same experimental animals. In combination with previous field studies, the laboratory-based measures of thermal preference and metabolic sensitivity to temperature (Q_{10}) collected here help discern how environmental temperature influences the habitat selection of juvenile *H. francisci* and *T. semifasciata*. Specifically, we found that juvenile *H. francisci* and *T. semifasciata* prefer similar temperatures and occupied similar temperature ranges regardless of chronic temperature acclimation suggesting that both species have a largely seasonal-independent thermal optimum in which they prefer to reside. Likewise, resting metabolic rates and the metabolic thermal sensitivity (Q_{10}) did not vary between chronic temperature acclimation treatments within either species. However, *H. francisci* showed greater metabolic sensitivity (higher Q_{10}) with acute changes in water temperature relative to *T. semifasciata*, which likely reflects their more limited daily movements and site fidelity within rocky refuge. Therefore, our findings suggest it may be beneficial for juvenile *H. francisci* to select for more thermally-stable habitat when compared with juvenile *T. semifasciata*.

Juvenile California Horn Sharks

Juvenile *H. francisci* preferred temperatures of 18.0 ± 1.9°C during cold acclimation and 20.6 ± 0.8°C during warm acclimation and spent the majority of time in waters greater than ~16°C regardless of the acclimation treatment. Despite the 2.6°C difference in mean temperature preference during these chronic temperature acclimations, the large intraspecific variability between individuals resulted in preferred temperatures and ranges that were not significantly different. However, warm acclimated *H. francisci* explored warmer waters (mean upper thermal preferred range ± SE: 24.2 ± 0.8°C) than cold acclimated *H. francisci* (20.6 ± 2.3°C) suggesting that some seasonal changes to temperature preference and physiological optima are possible, although a larger sample size is needed to enhance statistical power. The preferred temperatures and temperature ranges measured for juvenile *H. francisci* in this study are consistent with water temperatures experienced by juvenile *H. francisci* across their geographic distribution, which extends from central California, USA, to southern Mexico, including the Gulf of California (Ebert, 2003).

On smaller scales, our data support the previous supposition that *H. francisci* daily habitat selection is largely temperature driven (Ebert, 2003; Meese & Lowe, 2020a; Meese & Lowe, 2020b). Field surveys and tracking studies show that *H. francisci* spend most daylight hours (~90%) resting, often exhibiting site fidelity and homing behaviour to specific rocky outcrops that serve as rest or shelter sites (Meese & Lowe, 2020a; Meese & Lowe, 2020b; Strong, 1990). The temperature preference of warm-acclimated juvenile *H. francisci* estimated in our study (20.6 ± 0.8°C, range: 16.6-24.2°C) almost exactly reflects the thermal range (mean ± SD: 20.3 ± 1.6°C, range: 16.3–23.2°C) and shallow waters selected by immature *H. francisci* during the summer at Catalina Island in southern California as determined through day-time field population surveys (Meese & Lowe, 2020b). The consistency between our laboratory-derived results in which temperature was isolated as a single variable with those determined for wild *H. francisci* through field surveys directly supports the hypothesis that temperature is likely a strong driving force of juvenile *H. francisci* habitat selection. While additional lab-based studies are needed for adult *H. francisci*, field studies suggest that juveniles select slightly warmer and shallower resting sites relative to mature individuals (Meese & Lowe 2020b), which likely helps promote somatic growth.

At night, *H. francisci* are known to leave their daytime resting sites to forage. While limited data exist for juvenile nighttime movements, active telemetry studies show adult *H. francisci* continue to stay close to their preferred temperature (Meese & Lowe, 2020a). Specifically, active tracking by Meese & Lowe (2020a) showed that adult *H. francisci* resting during the day in shallow waters (0.2-35.0 m) with a mean temperature of 18.1 ± 0.3°C appeared to continue to track the same mean temperature (18.0 ± 0.5°C) while exhibiting directed movements to specific foraging areas away from their areas of shelter at night. This was despite occasional movements into deeper or shallower depths where they experienced larger overall temperature fluctuations (range: 10.0-23.8°C) than during the day. Thus, while some elasmobranchs alternate between environments with different mean temperatures that are specific to resting and foraging (DiGirolamo et al., 2012; Hopkins & Cech, 1994; Matern et al., 2000; Sims et al., 2006; Wallman & Bennett, 2006), *H. francisci* do not appear to exhibit this pattern. Instead, our laboratory-based results confirm that *H. francisci* actively track their preferred temperature.

The active tracking of a mean preferred temperature by *H. francisci* even during foraging likely reflects their relatively high metabolic sensitivity to short-term changes in temperature as represented by a Q_{10} of 2.58 from 12-24°C found in this study after a relatively short 12-hour temperature acclimation period. In contrast, Luongo & Lowe (2018) found a much lower Q_{10} of 2.01 over the same temperature range for juvenile *H. francisci* following a longer acclimation period of several weeks. A lower Q_{10} in response to prolonged temperature change would be advantageous for dealing with seasonal temperature changes, as water temperatures within their range drop well below their preferred thermal optima in the winter. In addition to their relatively high metabolic thermal sensitivity, juvenile *H. francisci* likely have low thermal inertia—the ability of the animal to resist changes in temperature—due to their small body size, meaning their body temperature will conform relatively quickly to the temperature of the surrounding environment. While little is known about the fine-scale movements of juvenile *H. francisci*, these factors suggest juveniles are also likely to limit movements across broad thermal regimes during periods of activity (i.e., foraging) and will likely remain in areas with more stable temperatures close to

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their thermal preference, such as in estuaries. Such limited movements, particularly in shallow environments, are also likely advantageous in avoiding predation (Wetherbee et al., 2007).

Juvenile Leopard Sharks

Our study shows that juvenile *T. semifasciata* generally have similar thermal preferences to juvenile *H. francisci*, preferring a mean temperature of $17.5 \pm 1.1°C$ following cold acclimation and $18.4 \pm 1.4°C$ following warm acclimation, while spending 95% of their time between ~16 and 21°C regardless of acclimation treatment. These temperatures are generally similar to those experienced by adult *T. semifasciata* (mean range: $17.4 – 22.7$ °C) tracked in a study by Nosal et al. (2013) along the coast of southern California during summer months. Despite similarity in thermal preference to *H. francisci*, juvenile *T. semifasciata* exhibited lower metabolic thermal sensitivity ($Q_{10} = 1.97$ from 12-24°C) than *H. francisci*, suggesting juvenile *T. semifasciata* are likely less reliant on staying within a narrow range of preferred water temperature. Specifically, as more active and mobile mesopredators, *T. semifasciata* are more likely to move across thermal gradients while foraging and a lower Q_{10} would thus minimise the metabolic impacts of sudden temperature changes. In addition to lower thermal sensitivity, the larger body size and higher thermal inertia of larger *T. semifasciata* may further facilitate movements through heterogeneous thermal environments over short periods of time by limiting changes to internal body temperature (Hight & Lowe, 2007). The reduced influence of temperature on *T. semifasciata* behaviour is evidenced by field studies showing correlation between their fine-scale *in situ* movements and a variety of other factors, including tide, photoperiod, salinity, wave height, and dissolved oxygen (Ackerman et al., 2000; Carlisle & Starr 2009; Manley, 1995; Nosal et al., 2013). Although temperature may not be the main driver of fine-scale movements, the seasonal abundance of *T. semifasciata* in shallow embayments and calm coastal regions is largely thought to be temperature driven (Carlisle & Starr 2009; Hight & Lowe, 2007; Hopkins & Cech 2003; Nosal et al., 2013).

Temperature preference and thermal sensitivity of *T. semifasciata* may also vary across their geographic range. In aquatic ectotherms, metabolic rate and optimal temperature generally decrease with

latitude due to the decrease in environmental temperature (Brown et al., 2004; Clarke, 2003; DeLong et al., 2018; Munch & Salinas, 2009). Further, intraspecific thermal sensitivity (Sylvestre et al., 2007) and thermal tolerance (Pörtner & Peck, 2010) can differ across latitudinal gradients in which there are distinct differences in thermal habitat. Evidence suggests there may be different populations of *T. semifasciata* along the coast of California (Barker et al., 2015; Lewallen et al., 2007), which could possibly be driven by local adaptations to differences in selective pressures, including temperature. *T. semifasciata* are found from the Gulf of California, Mexico, where sea surface temperatures can reach above 30°C, to Oregon, USA, where sea surface temperatures can drop below 8°C (NOAA National Data Buoy Center). In southern California, sea surface temperature typically ranges from 14-22°C and can reach extremes below 12°C and above 26°C, while in northern California, sea surface temperatures typically range from 10-16°C and can drop below 8°C (NOAA National Data Buoy Center). The extent of the *T. semifasciata* range suggests that individuals found farther north may prefer and/or tolerate colder temperatures than those examined in the present study and that there may be population-level differences in thermal optima. Our study reports a lower thermal sensitivity (Q_{10} = 1.97 from 12-24°C) than what has been reported in juvenile *T. semifasciata* captured from Elkhorn Slough, CA, a tidal estuary in the northern geographic extent of the *T. semifasciata* range (Miklos et al., 2003: Q_{10} = 2.51 from 12-24°C). Additionally, we show that juvenile *T. semifasciata* from southern California avoid temperatures below 16°C, while *T. semifasciata* in northern California regularly reside at temperatures below 16°C and only appear to leave preferred habitat when water temperatures drop below 10°C (Hopkins & Cech, 2003; Carlisle & Starr, 2009). Such disparities indicate clear regional differences in temperature preference that likely affect both fine-scale movements and seasonal abundance within nearshore environments, and can ultimately lead to region-specific habitat utilisation and conservation considerations.

Sex Comparisons

While not statistically significant, juvenile females of *H. francisci* and *T. semifasciata* appeared to prefer warmer temperatures relative to juvenile males in both the warm and cold chronic temperature

acclimation treatments. Our study was constrained by a low number of individuals from each sex, and a greater sample size is needed to statistically confirm this pattern, particularly across a larger size class to encompass all ontogenetic life stages. However, sexual segregation has been observed in many shark species and is believed to be associated with sex-specific reproductive demands (e.g., gamete production, gestation of embryos), differences in somatic growth requirements, and avoidance of overly aggressive male mating behaviour (Jacoby et al., 2011; Klimley, 1987; Mucientes et al., 2009; Sims, 2005; Wearmouth & Sims, 2008). Klimley (1987) suggested that in shark species where females grow larger than males, sexes will segregate by thermal habitat both prior to and post maturation to increase food consumption and maximise growth rate, which would ultimately increase fecundity. Females of both *H. francisci* and *T. semifasciata* grow larger than males and reach sexual maturity at a larger size (Ebert, 2003), and therefore immature females may target warmer waters to increase body temperature and growth rate in order to reach maturity faster (Economakis & Lobel, 1998). However, movement studies for juveniles of both species are needed to determine if there are functional differences in thermal habitat utilisation between the sexes at this early life stage.

The preferred temperatures of the warm-acclimated immature female *T. semifasciata* we report in this study (mean ± SE; 19.9 ± 1.5°C) are similar to occupied temperatures of adult females aggregating in the wild (mean ± SD; 20.6°C ± 1.0°C from Nosal et al., 2013 and 21.8 ± 1.2°C from Hight and Lowe, 2007), and thus it is possible that this sex specific thermal preference may extend across ontogeny. Although we showed a similar trend of female preference for warmer temperature in *H. francisci*, our findings were non-significant and sex-specific differences in temperature or depth distribution have not been observed in the field (Meese & Lowe, 2020b). However, the closely related Port Jackson Shark *Heterodontus portjacksonii* (Meyer 1793) is known to exhibit sexual and ontogenetic habitat segregation (McLaughlin & O'Gower, 1971), suggesting additional field and laboratory investigations of sex-specific differences may reveal temperature preference and movement across ontogeny within *H. francisci*.

Implications

Various shark species, including both *H. francisci* and *T. semifasciata*, utilise warm, shallow coastal waters such as estuaries and bays during early life stages in order to optimise growth and fitness (Castro, 1993; Espinoza et al., 2011; Merson & Pratt, 2001). Almost 60% of the global human population lives along the coast, which leads to increased pressure on estuarine habitats (Gleason et al., 2011). This is certainly the case in southern California (Gillanders et al., 2003), where unique geomorphology and dynamic tectonics have led to small and shallow estuaries (14 of 16 estuaries in San Diego County are <10 km^2) with low freshwater input (Emmett et al., 2000; California Department of Fish and Game Marine Resources Region, 2014). Changes in estuarine ecosystems (e.g., through human encroachment and climate change), coupled with the small size of the estuaries in southern California and the less suitable riverine-based estuaries of northern California (Hughes et al., 2014), may have significant impacts on fishes such as *H. francisci* and *T. semifasciata*. Thus, it is imperative to better understand how juvenile sharks utilise these coastal environments and assess the relative importance of local estuaries and bays as viable nursery grounds.

On smaller scales, increases to temperature can affect the activity and distribution of fishes within their nearshore coastal and estuarine habitats (Grady et al., 2019; Sunday et al., 2012). Warmer water temperature increases energetic demands and can result in increased time spent foraging and potential exposure to predators (Dell et al., 2011; Rall et al., 2010). Species with lower metabolic thermal sensitivities, such as *T. semifasciata,* are likely more resilient to changes in thermal habitat than co-occurring species with higher metabolic thermal sensitivity such as *H. francisci.* A predictive energetic model by Luongo & Lowe (2018) suggests that *H. francisci* in southern California could have experienced a 23% increase in mean standard metabolic rate from 2012-2017 due to the rise in mean sea surface temperature across this time period. Anthropogenic-induced climate change is expected to exacerbate extreme increases in temperature within small and shallow estuaries, potentially resulting in the loss of this essential habitat. For example, *T. semifasciata* are known to actively avoid water temperatures warmer than 26 ºC (Carlisle & Starr, 2009; Hight & Lowe, 2007), with temperatures of 27ºC beginning to have deleterious effects on heart function (Cox & Wegner, unpublished). Even at 24ºC,

several *T. semifasciata* and *H. francisci* used in the current study were visibly restless within the respirometer, precluding the ability to acquire resting oxygen consumption measurements for some individuals. Further, a previous behavioural experiment on two juvenile *H. francisci*, reported that both sharks actively avoided water temperatures of 31ºC regardless of whether internal body temperature (i.e., brain stem) was warm (26ºC) or cool (12ºC) (Crawshaw & Hammel, 1973). Temperatures can exceed 30°C in the Gulf of California and 26°C within waters off southern California (NOAA National Data Buoy Center; Walker et al., 2020), and such warm pockets are expected to expand in the future. Conversely, sharks near the northern extent of their range are expected to experience a decrease in the occurrence of lower temperatures, which may open up new habitat and lead to northward range expansion as is already observed for several marine species (Cheung et al., 2009; Hobbs, 2011; Nicolas et al., 2011; Walker et al., 2020). Changes in species composition in the California Current is strongly correlated with climate change (Doney et al., 2011), and the rise in temperature over the last few decades has been linked to the increase in relative abundance of *H. francisci* and the decrease in relative abundance of swell sharks *Cephaloscyllium ventriosum* (Garman 1880) along Catalina Island (Grover, 1972; Ebert, 2003). Warming ocean temperatures can also lead to changes in prey availability, which can alter fitness. For example, northward shifts in the distribution of California Market Squid (*D. opalescens*), a prey species for both *H. francisci* and *T. semifasciata*, during the 2015-2016 El Niño Southern Oscillation [ENSO] (Van Noord & Dorval, 2017), may have contributed to lower body condition (girth to length ratio) observed in *H. francisci* that year (Meese & Lowe, 2020a). During the 2015-2016 ENSO, *H. francisci* also exhibited a decrease in site fidelity, which may have been a consequence of increased foraging effort required by the decrease in available prey and increased temperatures (Meese & Lowe 2020a).

Future Directions

Baseline assessments linking physiology and behaviour are critical for understanding species-specific and life-stage-specific drivers of habitat selection, particularly in the face of changing environmental conditions. Our results for juvenile *H. francisci* and *T. semifasciata* suggest some ability

for both species to acclimatise to temporal changes in temperature on the scale of weeks to months, but the observation of a consistent preferred temperature, despite chronic temperature acclimation to typical seasonal temperatures, suggests there are limits to short-term physiological plasticity and preferences. As observed in other species (Di Santo & Bennett, 2011; Lowe, 2001; Luongo & Lowe, 2018; Neer et al., 2006; Whitney et al., 2016), both *H. francisci* and *T. semifasciata* displayed large intraspecific variability in oxygen consumption rate, which may be attributed to individual differences in cellular physiology (Norin & Malte, 2012; Salin et al., 2016). This intraspecific variability suggests there may be a proportion of the population of each species that is better suited to shifting environmental conditions, but the degree to which these species may be resilient needs further investigation.

Our work indicates that thermal dependence is largely contingent on the activity levels and ecology of the shark species in question. Co-occurring species, like *H. francisci* and *T. semifasciata*, may overlap in time and space, yet their habitat utilisation can differ due to their metabolic sensitivity to temperature change. It is important to note that the preferred temperature and thermal range of both *H. francisci,* and *T. semifasciata* were not at the temperatures that incurred the lowest metabolic costs, but instead at temperatures with elevated resting metabolic rates. Thus, the selection for preferred habitat is likely based on the culmination of various physiological drivers that result in the most benefit to the organism. Likewise, resting metabolic rate is only a portion of metabolic performance and the effects of temperature on other aspects of energy dynamics such as maximum metabolic rate and aerobic scope are likely important components of thermal tolerance for many species (Pörtner, 2010; Schulte, 2015), particularly for more active species that do not truly rest. Thermal habitat selection in the wild also likely includes balancing the advantages (e.g., food availability, predator avoidance) and disadvantages (e.g., changes in physical variables) of the residing habitat while minimising the energetic cost associated with the environmental temperature. Temperature preference and associated physiological parameters may also change with ontogeny and geographic distribution and it is crucial that future studies focus on collecting baseline physiological, bioenergetic, and behavioural data to help refine approaches for conservation and fisheries management. It is also imperative that future studies use a synergistic approach in pairing

laboratory-based experimentation with *in situ* tracking, particularly during early life stages, to more fully

resolve spatiotemporal models and elucidate true drivers of habitat use.

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AUTHOR CONTRIBUTIONS

Z.R.S., P.A.H., and N.C.W. conceptualised the study and the methodological approach. Z.R.S conducted the investigation under supervision from P.A.H and N.C.W. Both Z.R.S and T.S.P. analysed the data. Data validation was done by N.C.W. The original draft was written by Z.R.S. with editorial contributions by T.S.P., P.A.H, and N.C.W.

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FIGURES AND TABLES

Table 1. *H. francisci* and *T. semifasciata* size data immediately preceding shuttle box and respirometry trials following both the warm (21°C) and cold (15°C) chronic temperature acclimation periods.

Species	Sex	Individual	TL (cm) Warm Acclimation	Mass (kg) Warm Acclimation	TL (cm) Cold Acclimation	Mass (kg) Cold Acclimation	% Mass Gained
	F	1	45.6	0.738			
	F	2	48.2	0.852			
	F	3	41.1	0.496	41.9	0.669	34.8
California Horn	F	4	48.7	0.755			
Shark	F	5	37.6	0.393	38.7	0.439	11.7
(*Heterodontus*	M	6	41.1	0.515	42.6	0.642	24.9
francisci)	M	7	43.7	0.746	45.2	0.860	15.3
	M	8	38.8	0.443	39.3	0.565	27.7
	M	9	38.2	0.408			
	M	10	37.1	0.433			
	Mean ± SE		**42.0 ± 1.3**	**0.578 ± 0.052**	**41.5 ± 1.1**	**0.635 ± 0.062**	**22.9 ± 3.7**
	F	1	88	2.72	96.0	3.62	33.1
	M	2	75.6	1.68			
	F	3	89.9	2.70	95.0	3.30	22.2
Leopard Shark	F	4	70.2	1.42	79.3	2.07	45.8
(*Triakis*	F	5	84.5	2.32	91.5	2.81	21.1
semifasciata)	M	6	77.8	2.10	84.2	2.20	4.8
	M	7	72.1	1.44	77.5	1.84	27.8
	F	8	76.2	1.86	82.0	2.78	49.5
	Mean ± SE		**79.3 ± 2.4**	**2.03 ± 0.16**	**86.5 ± 2.7**	**2.66 ± 0.23**	**29.2 ± 5.4**

Total length (TL) and mass are reported for the same individuals under each temperature acclimation.

Table 2. Preferred temperatures and temperature ranges (means ± SE) of *H. francisci* and *T. semifasciata* compared between two chronic temperature acclimation treatments.

				Occupied Temperature Range (°C)	
Species	**Acclimation**	**n**	**Preferred Temperature (°C)**	**Lower Bound**	**Upper Bound**
Horn Shark	Warm (21°C)	10	20.6 ± 0.8	16.6 ± 0.9	24.2 ± 0.8
(*Heterodontus francisci*)	Cold (15°C)	5	18.0 ± 1.9	16.1 ± 0.9	20.6 ± 2.3
Leopard Shark	Warm (21°C)	8	18.4 ± 1.4	16.3 ± 1.0	20.7 ± 1.4
(*Triakis semifasciata*)	Cold (15°C)	7	17.5 ± 1.1	15.9 ± 1.1	19.8 ± 1.4

Preferred temperatures were calculated as means of the median preferred temperature of each individual. Temperature ranges were determined as the bounds between which sharks spent 95% of their time. There were no significant differences within and across each species.

Table 3. Preferred temperatures and temperature ranges (means ± SE) of *H. francisci* and *T. semifasciata* compared between two chronic temperature acclimation treatments and sex.

Species	Acclimation	Sex	Preferred Temperature (°C)	Occupied Temperature Ranges (°C)	
				Lower Bound	Upper Bound
California Horn Shark (*Heterodontus francisci*)	Warm (21°C)	Male (n=5)	19.6 ± 1.3	15.0 ± 0.9	23.4 ± 1.3
	Warm (21°C)	Female (n=5)	21.6 ± 0.8	18.1 ± 1.4	24.9 ± 1.1
	Cold (15°C)	Male (n=2)	17.7 ± 2.7	16.7 ± 2.5	18.6 ± 3.1
	Cold (15°C)	Female (n=3)	18.5 ± 3.1	15.2 ± 2.3	23.5 ± 3.0
Leopard Shark (*Triakis semifasciata*)	Warm (21°C)	Male (n=3)	15.7 ± 2.0	13.8 ± 1.4	18.9 ± 3.0
	Warm (21°C)	Female (n=5)	20.0 ± 1.7	17.8 ± 0.9	21.9 ± 1.4
	Cold (15°C)	Male (n=2)	17.0 ± 1.2	15.3 ± 1.1	19.7 ± 1.3
	Cold (15°C)	Female (n=5)	17.7 ± 1.5	16.1 ± 1.4	19.9 ± 1.9

Preferred temperatures were calculated as means of the median preferred temperature of each individual. Temperature ranges were determined as the upper and lower temperatures between which sharks spent 95% of their time. There were no significant differences across sexes within each species.

Table 4. Mean resting oxygen consumption rate (mg O_2 h^{-1} kg^{-1}) for *H. francisci* and *T. semifasciata* at each experimental temperature and chronic temperature acclimation treatment in comparison to oxygen consumption rates reported from previous studies.

Species	Study	Mass (kg)	Mean M_{O_2} (mg O_2 h^{-1} kg^{-1}) ± SE			
			12°C	16°C	20°C	24°C
California Horn Shark (*Heterodontus francisci*)	Present study (acc. 15°C)	0.58	16.1 ± 2.1	22.3 ± 1.9	39.1 ± 4.4	50.1 ± 3.0
	Present study (acc. 21°C)	0.63		18.9 ± 1.4	28.2 ± 3.2	43.3 ± 4.7
	Mean	**0.60**		**20.0 ± 1.2**	**31.8 ± 2.9**	**55.4 ± 3.2**
	Luongo & Lowe (2018)	0.60		22.5 ± 1.5	29.8 ± 1.6	
Leopard Shark (*Triakis semifasciata*)	Present study (acc.15°C)	2.03	150 ±15.7	220 ± 22.3	284 ± 20.7	338 ± 32.1
	Present study (acc. 21°C)	2.66		156 ± 11.1	207 ± 18.9	269 ± 25.3
	Mean	**2.32**		**186 ± 14.3**	**243 ± 16.9**	**273 ± 21.1**
	Miklos et al. (2003)	2.32[†]	98.2	165	232	299
	Scharold et al. (1989)	2.32[†]		195 ± 52.2		

[†]For more direct comparison, oxygen consumption rates for *T. semifasciata* were scaled to the mean mass of individuals in this study (2.32 kg) using equation 1 and a scaling exponent of 0.8. *H. francisci* from Luongo & Lowe (2018) were acclimated to the trial temperatures (14°C, 16°C, 20°C, and 22°C for 2-11 weeks prior to oxygen consumption measurements (mean mass of 0.60 kg – same as the present study). *T. semifasciata* from Miklos et al. (2003) were collected between 12-14°C and held for 5-7 days in flow through seawater at these temperatures prior to respirometry trials (mean mass of 0.97 kg). Measurements of oxygen consumption rate for *T. semifasciata* from Scharold et al. (1989) were conducted at 14-18°C (mean mass not reported; range 2.2-5.8 kg).

Table 5. Thermal sensitivity of oxygen consumption (Q_{10}) in *H. francisci* and *T. semifasciata* across temperature and chronic temperature acclimation treatments (estimated using equation 2).

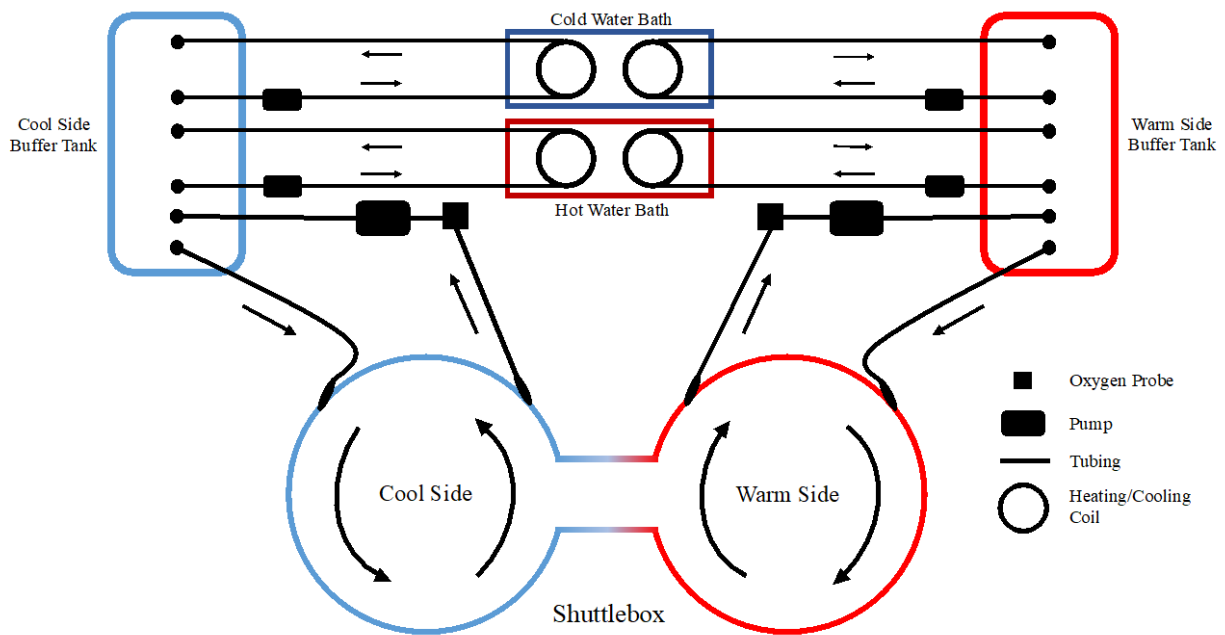| Species	Acclimation	Metabolic Thermal Sensitivity (Q_{10})	
		16-24°C	12-24°C
California Horn Shark (*Heterodontus francisci*)	Warm (21°C)	2.75	-
	Cold (15°C)	2.82	2.58
Leopard Shark (*Triakis semifasciata*)	Warm (21°C)	1.71	-
	Cold (15°C)	1.99	1.97

Figure 1. Schematic (top-down view) of the shuttle box system (bottom) and associated buffer tanks, heating/cooling coils, and water baths (top) used to control temperature. Water from the warm (red) side of the shuttle box was held 1.5°C warmer than water in the cold (blue) side; both sides of the shuttle box synchronously increased or decreased in temperature depending on the shark's position within the system, which was monitored from above by a camera and Shuttlesoft software (Loligo Inc.).
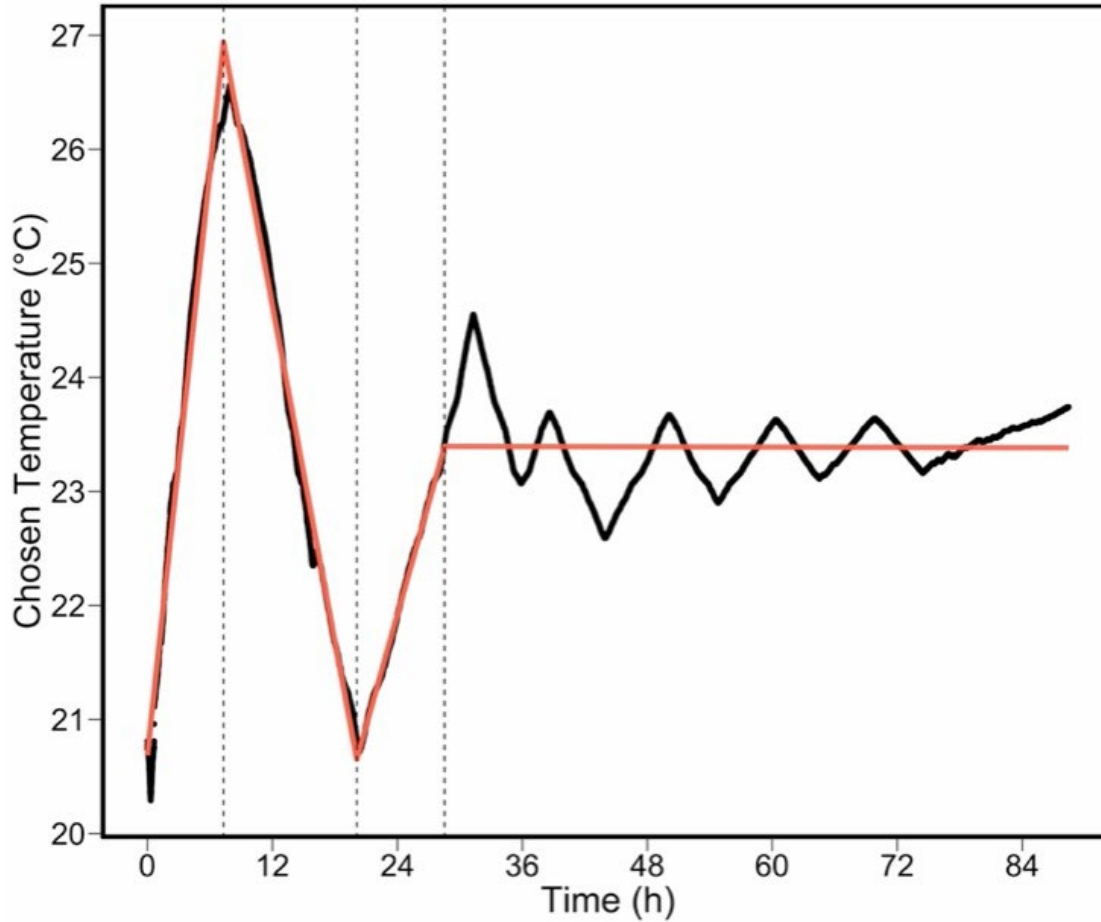
Figure 2. Example of the water temperature experienced by a 0.408 kg *H. francisci* individual over an 88-hour period in the shuttle box. The broken stick model (solid red line) used to find the breakpoints (dashed lines) in slopes is overlaid on the graph. The final breakpoint (dashed line farthest to right) indicates the point at which the individual started to target its preferred temperature. All points after this dashed line were then used to determine the final preferred median temperature and temperature ranges (95% time spent).
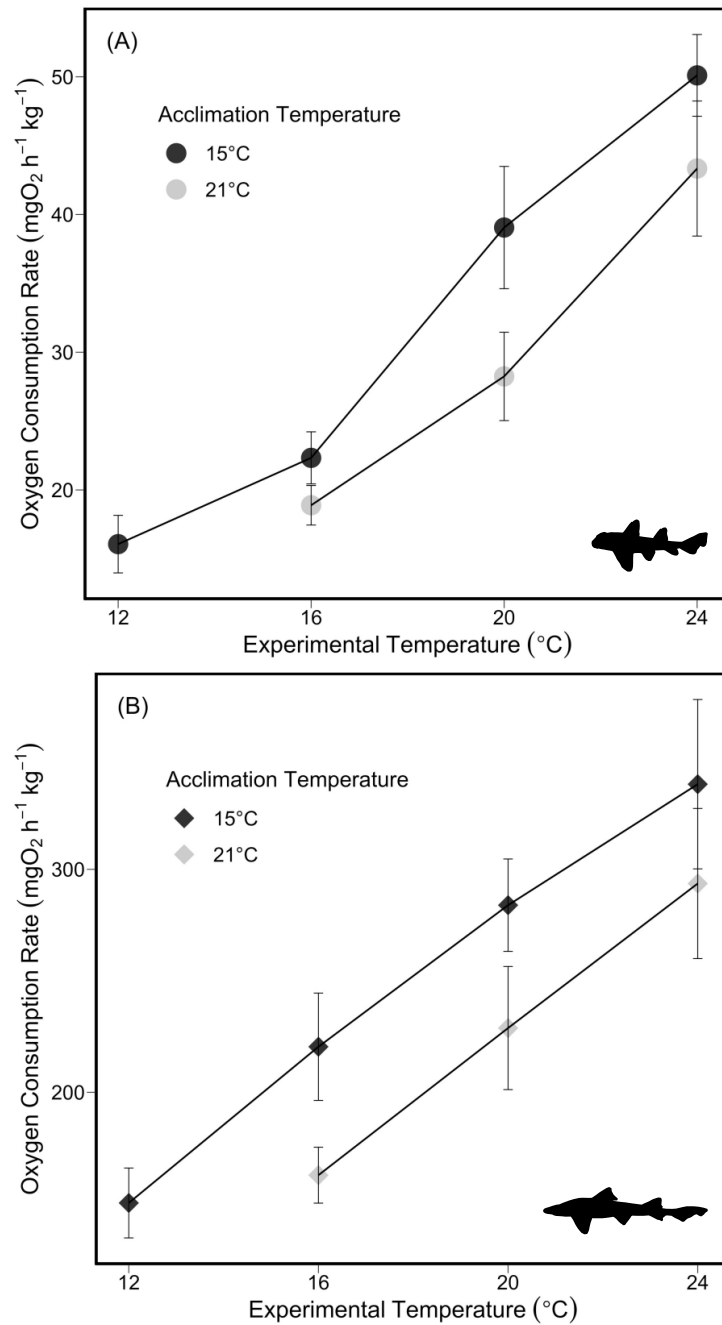
Figure 3. Mean (± SE) resting oxygen consumption rates (M_{O_2}) at each experimental temperature during each acclimation treatment for (A) *H. francisci* and (B) *T. semifasciata*. A) For warm-acclimated *H. francisci*, n=10 at 12°C, 16°C, and 20°C, n=9 at 24°C; for cold-acclimated *H. francisci*, n=5 at all experimental temperatures. B) For warm-acclimated *T. semifasciata*, n=8 at all experimental temperatures; for cold-acclimated *T. semifasciata*, n=7 at 12°C, 16°C, and 20°C, n=5 at 24°C.