BEHAVIORAL TEMPERATURE PREFERENCE AND PHYSIOLOGICAL CRITICAL THERMAL LIMITS OF THE NON-NATIVE INDO-PACIFIC SERGEANT *ABUDEFDUF VAIGIENSIS*, ENDEMIC HAWAIIAN SERGEANT *ABUDEFDUF ABDOMINALIS*, AND HYBRIDS FROM Oʻ‌AHU, HAWAIʻI

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ABSTRACT

Sea surface temperatures have been increasing globally as has the frequency of marine heat waves due to climate change. In the Hawaiian Archipelago, sea surface temperatures have been increasing since the 1940s. Tropical marine ectotherms often have a core temperature close to their environmental temperatures and settle in environments near their upper thermal range. Increased sea surface temperature has been shown to threaten coral reef fishes when surpassing their thermal tolerances. I chose to investigate the sergeant major species group (*Abudefduf abdominalis*, *A. vaigiensis*, and hybrids) which are an abundant coral reef fish in the Hawaiian Islands. The endemic Hawaiian sergeant, *A. abdominalis*, and the invasive Indo-Pacific sergeant, *A. vaigiensis*, first reported in the islands in the 1990s, were formally documented hybridizing in 2007, although hybridization likely occurred before. I assessed differences in thermal biology in relation to genetic similarity to the pure, parental species (*A. abdominalis* and *A. vaigiensis*) by using Structure Q_{Ava} as an estimate of the proportion of the genome in each individual that came from *A. vaigiensis* versus *A. abdominalis*. I performed temperature preference (T_{pref}) experiments, which assessed an individual's behavioral choice for certain temperatures, in an annular chamber on forty sergeant majors. Additionally, I performed critical thermal maximum (CT_{max}) experiments, which assess an individual's physiological thermal limit, on thirty-six sergeant majors. There was no effect of genetic similarity to *A. vaigiensis* on CT_{max}, but mass had an effect of a 1.4°C decrease on CT_{max} as mass increased from 8.7 to 124.6 g. Additionally, there was an effect of 1.7°C increase in the size of the thermal preference range, interquartile range (IQR), over the mass range in this study (8.7 to 124.6 g = 116 g). I found genetic similarity to *A. vaigiensis* to have significant effect of 1.2°C increase in median T_{pref}, a 2.2 °C increase in lower avoidance temperature (the first quartile), and a 1.5°C decrease in the size of the IQR; moreover,

both *A. vaigiensis* and hybrids showed a preference for temperatures from 26 - 28°C. By using Structure Q_{Ava} as an estimate of the proportion of the genome in each individual that came from *A. vaigiensis* versus *A. abdominalis*, I found genetic similarity to *A. vaigiensis* lead to higher median T_{prefs}, higher lower avoidance temperatures, and decreased size of the thermal preference ranges, though there was no effect on CT_{max}. Although I could not directly compare across species groups, testing for differences in thermal biology in relation to genetic similarity to the pure, parental species suggest that the invasive *A. vaigiensis* have a preference for warmer temperatures than the native *A. abdominalis*, which may have aided *A. vaigiensis*' expansion into Hawaiian habitats with increasing SST.

Behavioral temperature preference and physiological critical thermal limits of the non-native Indo-Pacific sergeant *Abudefduf vaigiensis*, endemic Hawaiian sergeant *Abudefduf abdominalis*, and hybrids from Oʻahu, Hawaiʻi

by

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This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Marine Science at Hawai'i Pacific University. We the undersigned have examined this document and have found that it is complete and satisfactory in all respects, and all revisions required by the final examining committee have been made.

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Chapter 1: Effects of increased sea surface temperature on tropical reef fish

Introduction

One of the greatest challenges facing marine organisms is increased sea surface temperatures (SST) as a result of human-induced climate change. Currently, we are in a geological era referred to as the Anthropocene, which is defined as the period where human activities are the dominant force influencing the environment and climate (Lewis & Maslin, 2015). Evidence suggests that human activities are impacting climate on scales similar to ancient geological events like volcanic activity and meteorite strikes (Hoegh-Guldberg & Bruno, 2010). This era is designated by the alteration of global nitrogen cycles through the use of fertilizers, increased production of CO_2 by industrial and commercial processes, and the sixth mass extinction event due to environmental changes and habitat destruction (Lewis & Maslin, 2015). The ocean acts as a sink, absorbing CO_2, and also heat, leading to higher SST (Heinze et al., 2015; Reid et al., 2009). Persistent increases in average SST also leads to more frequent and severe marine heatwaves (MHW).

MHW are climate events with extended periods of regional ocean warming and are defined by sea surface temperature (SST) exceeding the seasonal 90th percentile which persist for at least five consecutive days based on a long-term (typically 30-year) baseline; defined thresholds and time can be specific to the location being measured (Hobday et al., 2016; Oliver et al., 2021; Pearce et al., 2011). The causes for MHW are variable with the most notable cause being atmospheric state (determined by the interaction of radiation, fluid dynamics and chemistry), anomalous ocean heat transport, retreat in Arctic sea ice (Oliver et al., 2021), and tropical-extratropical teleconnection, a process in which climate variability in one region causes a response in a distant region, through physical mechanisms like jet streams, Rossby waves, or ocean currents (Stan et al., 2017). MHW cause bottom-up effects on keystone species like corals (Couch et al., 2017). MHW are known to cause corals to bleach or cause mass mortalities which affects the species that rely on corals to feed, forage, and shelter (Couch et al., 2017). MHW impact every level of an ecosystem, and fishes have multiple reactions to these lengthened periods of increased SST.

Fish are ectotherms and their body temperature reflects environmental temperatures. They are, therefore, susceptible to increased SST due to climate change. In general, tropical fish are adapted to a narrow range of temperatures because over evolutionary time the tropical seas have had narrow and stable temperature ranges (Pinsky et al., 2020). A fish's temperature tolerance correlates with their geographical range limits and thermal niche within their local environment (Stuart-Smith et al., 2017; Gvoždík, 2018). Temperature changes can impact key aspects of fish life histories: development, growth rate, swimming ability, reproduction, and behavior (Munday et al., 2008; Ern et al., 2023). For example, coral reef fishes grow to smaller maximum sizes and live shorter lives in warmer environments, which also cause size or age at maturation to decrease (Munday et al., 2008). Fish larvae have shown increased growth rates in higher temperatures (Fennie et al., 2023); however, adults are limited by food intake, and so this relationship becomes more complicated as several variables need to be considered: metabolic rate, food availability, nutritional quality, and population density (Munday et al., 2008).

In order to avoid increased SST, several responses to increased SST by fishes have been observed: deep reef refuge (Pereira et al., 2018) or range extensions or shifts to higher latitudes (Feary et al., 2014). Mortality events have been recorded during MHW due to acute thermal stress (Tietbohl et al., 2025); however, mortality can also be linked to reduced hypoxia tolerance due to increased SST (Nilsson et al., 2010). Genetics also play a major role in temperature tolerances as different species of fish have different heat shock proteins (HSP), which help to stabilize protein structure and prevent denaturing due to heat stress events (Basu et al., 2002). Investigating how fish adapt to water temperature changes will help predict hierarchical ecological responses: ecosystem complexity, ecosystem function, community structure, and species longevity as climate change intensifies.

Goal statement: This review aims to explain how increased ocean temperatures affect tropical reef fishes by (1.) discussing climate change and marine heatwaves and their impacts on coral reef ecosystems, by (2.) discussing physiological responses of marine ectotherms to temperature changes and by (3.) categorizing how tropical reef fishes may compensate for the effects of increased temperatures: deep reef refuge, migration, and physiological adaptations (Figure 1).

Theme A. Climate change and reef warming

General climate change ~ Atmospheric CO_2 is increasing at a rate of ~1.9 ppm/year almost entirely due to human-generated emissions, with 45% of CO_2 emissions remaining in the atmosphere, 25% absorbed by the oceans, and 30% absorbed by land (Raupach & Canadell, 2010). When CO_2 enters the ocean, it reacts with water to form bicarbonate (HCO_3^-) and protons (H^+) which decreases pH (Doney et al., 2009). In seawater, lower pH decreases calcium carbonate ($CaCO_3$) saturation, making the formation of shells and coral skeletons more challenging for marine organisms (Hönisch et al., 2012). Increases of CO_2 in the atmosphere has also caused global temperatures to increase by ~0.2°C per decade, due to the greenhouse effect (Hoegh-Guldberg & Bruno, 2010). Most of the heat has been absorbed by the oceans, which has increased average sea surface temperatures (SST) by 0.6°C from 1910 to 2010 (Hoegh-Guldberg

& Bruno, 2010). Even if global warming is limited to 1.5°C, the Intergovernmental Panel on Climate Change (IPCC) predicts that the tropics will still face periods of higher temperatures, or extreme heatwaves (Intergovernmental Panel on Climate Change, 2018). MHWs have caused SST temperature anomalies of over 5°C (Oliver et al., 2021).

Impacts on coral reefs~ Coral reefs, which are home to thousands of fish species, are mostly found in tropical latitudes, and these reefs are under threat due to ocean acidification and increased SST. Coral reefs are both structurally and biologically complex , providing the foundation for high diversity and abundance of organisms (Couch et al., 2017). Coral bleaching is the loss of the endosymbionts (*Symbiodinium* spp.) due to environmental stresses like elevated temperatures (Douglas, 2003; Couch et al., 2017). Endosymbionts have a mutualistic relationship with coral by performing photosynthetic processes that provide the coral with nutrients while the symbiont receives shelter from the coral (Davy et al., 2012; Roth, 2014). Sometimes, if the stress is limited and temporary, corals may recover by reassimilating their endosymbionts after bleaching or by meeting their energy requirements by some other mechanism like feeding (Grottoli et al., 2006), but often the bleached corals cannot recover and face mass mortalities (Suggett & Smith, 2020). Decreased live coral cover, changes in the species composition of remaining corals, and a loss of habitat complexity by bleaching, combine to decrease the abundance and diversity of reef fish assemblages (Pratchett et al., 2008).

Locations of warming events~ Coral bleaching events due to heat stress have become more frequent and more severe since the 1980s (Couch et al., 2017; Suggett & Smith, 2020). The severity of these events often correlates to the type of coral species present, abundance (Darling et al., 2019) geographical location, environmental factors (Suggett & Smith, 2020), and previous thermal stress, making some reefs more susceptible than others (Guest et al., 2012). These bleaching events can be localized, such as the 2014 event in Papahānaumokuākea Marine National Monument in the central Pacific (Couch et al., 2017), or occur on global scales, such as the 2010 global bleaching event that impacted corals from the Indian Ocean to the Caribbean (Guest et al., 2012). In 2019, there was a MHW that caused 80% of coral reefs in Kāneʻohe Bay, Hawaiʻi to bleach and algae cover to increase (Yadav et al., 2023). Heat susceptible corals like *Pocillopora* spp. saw 77% mortality, while a more heat tolerant coral like *Porite compressa* saw 19% mortality (Yadav et al., 2023). In 2023, the reefs north of Kauaʻi and the Northwest Hawaiian Islands (NWHI) were under the coral bleaching watch advisory (NOAA Coral Reef Watch, 2023).

Theme B. Physiological effects of increased SST on tropical ectotherms

Reef fish thermal physiology~ Fish are ectotherms and can therefore only survive in a defined range of water temperatures. Thermal tolerance is the range of temperatures a physiological system can sustain a predefined level of capacity, and can tell us the animal's temperature limits (Ern et al., 2023). These limits can be observed *in situ* when mass mortalities or range extensions/shifts of fish populations occur due to SST changes (Genin et al., 2020; U.S. Environmental Protection Agency, 2024). *Ex situ*, these limits can be defined by performing upper and lower critical temperature (CT_{min} and CT_{max}) experiments (Ern et al., 2023; Raby et al., 2025). CT_{max} is the temperature where animals exhibit heat failure when exposed to a gradual increase in temperature and CT_{min} represents the corresponding lower-temperature threshold, the temperature at which animals exhibit cold-induced loss of motor control or equilibrium during gradual cooling (Ern et al., 2023).

The currently accepted “homeostatic maintenance” hypothesis states that the animal accumulates heat stress and eventually biological mechanisms to maintain homeostasis are lost until thermal injury and heart failure is experienced (Desforges et al., 2022; Ern et al., 2023). Different species have different but finite tolerances to heat stress (survivable) and heat failure (unsurvivable long-term) (Ern et al., 2023). T_{crit} is defined as the critical temperature for aerobic scope; above or below these temperatures, the oxygen supply capacity of the cardiorespiratory system cannot maintain standard metabolic rate making long term survival is barely possible (Ern et al., 2023). Heat directly affects molecular reaction rates (bottom-up), and negative impacts from heat come from insufficient oxygenation of the tissues leading to ATP deficiency (top-down) (Schulte, 2015).

Hypoxia tolerance of fishes has also been shown to be affected by increased SST. A study in Australia looked at two coral reef fish species, the Doederlein's cardinalfish (*Ostorhinchus doederleini*) and the lemon damselfish (*Pomacentrus moluccensis*) (Nilsson et al., 2010; Figure 2.). They found that neither species was able to acclimate their metabolic rates and hypoxia tolerance to increase SST; an increase in 3°C caused the lower critical oxygen level ($[O_2]_{crit}$; the lowest oxygen concentration at which resting metabolic rate can be maintained) to increase by 71% for the cardinal fish species (Nilsson et al., 2010; Figure 2.). The study indicated that increased oxygen demands were required at increased SST.

Temperature preference~ Several methods exist to assess a marine ectotherm’s temperature preference (T_{pref}). Most depend on an ectotherm's natural behavior to move between available temperatures (Reynolds & Casterlin, 1979). For example, a shuttle box design has two distinct temperature zones created by two tanks with a narrow connection that allows the fish two temperature choices (Nay et al., 2020; Harman et al., 2021; Christensen et al., 2021). If the species selects the warmer tank, the temperature will increase systematically over time, and if the species selects the cooler tank the inverse will happen (Nay et al., 2020). This process allows the species to actively control the temperature of its environment (Nay et al., 2020; Christensen et al., 2021). Other thermal preference experimental setups include: rectangular chambers with horizontal temperature gradients, cylindrical chambers with vertical gradients, and an annular chamber with gradients around a circle (Myrick et al., 2004).

The annular chamber is a ring shaped tank with a channel for the animal to swim 360° in a circle. The water in the circular channel is warmed and cooled to create a gradient of temperatures within the channel, and the animal's position is then recorded in the tank over a predefined amount of time and correlated to the temperature in that location (Myrick et al., 2004; McMahon et al., 2008). The experiment assumes that the animal will spend most of its time in areas/temperatures that it prefers (Reynolds & Casterlin, 1979). T_{pref} experiments in have been done on a variety of species including *Crangon crangon, Chromis atripectoralis*, and *Oncorhynchus mykiss* (Myrick et al., 2004; McMahon et al., 2008; Reiser et al., 2013, Nay et al., 2020). The results from these experiments may indicate the temperature at which the fish is best adapted, or where fish might move if temperatures change.

Critical thermal maximum~ Critical thermal maximum (CT_{max}) experiments are used to measure the upper thermal tolerance limit, or the temperature at which heat failure occurs (Morgan et al., 2018; Figure 3.). These experiments are helpful in estimating the temperature at which a species can no longer survive; with global warming and increased frequency of MHW, these experiments may help predict threats to and expirations of species. To perform these experiments, generally, fish (multiple or individual depending on size) are exposed to gradual increases in temperature over time until there is loss of equilibrium (LOE), the point where coordinated movement stops due to a failure to control and contract the muscles responsible for maintaining balance (Ern et al., 2023).

The rate at which the temperature is raised, the starting acclimation temperature, and the size of the fish can influence CT_{max} and intraspecific variability (Nati et al., 2021). A study conducting CT_{max} experiments on zebrafish, *Danio rerio*, used small thermocouple (thermometers) inserted into the dorsal muscle of the fish to test if there was a difference in body and water temperature at a thermal ramping rate of 0.3°C min^{-1}; they found a lag of less than 0.2°C between the body temperature and water temperature (Morgan et al., 2018). A different study on shortnose sturgeons found no impact of heating rate in CT_{max} experiments (Bard et al., 2019). Different studies on shortnose sturgeons, *Acipenser brevirostrum*, have used a range of heating rates from 6 – 15°C h^{-1} (0.1 - 0.25°C min^{-1}) (Zhang & Kieffer, 2014; Spear & Kieffer, 2016; Zhang et al., 2017). Tolerance to fast ramping rates during CT_{max} experiments were shown to correlate with tolerance to slow warming over a day (Åsheim et al., 2020), indicating that rapid CT_{max} measurements can be used in predicting fitness at realistic timescales for heat waves (Ern et al., 2023). The rate at which water is warmed during CT_{max} experiments is highly variable across different studies (Jobling, 1981); one study found higher heating rates resulted in higher CT_{max} values and suggest these experiments are more indicative of fish experiencing abrupt changes in temperature (Becker & Genoway, 1979). Faster ramping rates may also indicate the thermal tolerance of critical organs like the brain (Jutfelt et al., 2019) and heart (Ekström et al., 2014; Ekström et al., 2016); while slow ramping rates (days to months) may allow for acclimation (Desforges et al., 2022).

Overall, my research for this review indicates that the warming rates used in CT_{max} experiments are highly variable, but recently a preprint manuscript was released by collaborators between Canada, Denmark, Finland, Norway, Sweden, and Australia; the manuscript is a guide to standardize CT_{max} experiments on aquatic ectotherms (Raby et al., 2025). According to collaborators, determining the ramping rate for CT_{max} experiments should be based on the variability in thermal inertia across tissues in the animal during heating or cooling (Raby et al., 2025). Pilot experiments use a thermocouple inserted into deep muscle tissue of the animal to track the thermal change internally while also measuring the thermal change in the external environment (Raby et al., 2025). However, other tissues, such as the brain which are highly vascularized and close to the external surfaces of the fish, may have a greater effect on thermal tolerance than thermal inertia felt across deep muscle tissues (Jutfelt et al. 2019; Raby et al., 2025). Raby et al. (2025) discourage the use of a post-hoc correction on CT_{max} for heat-transference time as the mechanisms and relevant tissues behind these effects are not fully understood. Additionally, Raby et al. (2025) suggest a ramping rate of 0.3°C min-1 or 18°C h^{-1} for CT_{max} experiments of most aquatic ectotherms, but they suggest a slightly slower rate for larger animals, on the order of ~100 g or more to minimize the thermal lag between internal tissues and the environment.

Mass and critical thermal maximum

Although body size/mass has been shown to influence thermal tolerance, it is not clear how (Gomez Isaza & Rodgers, 2024). There are several hypotheses that aim to explain how size/mass influences thermal tolerance (Gillooly et al., 2001; Pörtner, 2010; Ern et al., 2023; (Chung & Schulte, 2020; Leiva et al., 2024). The first is that thermal tolerance may be restricted by the effect of size and temperature on metabolic rates in ectotherms (Gillooly et al., 2001).

Total metabolic rate increases with mass allometrically while mass-specific metabolic rate decreases with mass meaning smaller animals burn more energy per gram of body weight than larger animals. Temperature increases metabolic rate by ~2–3× for every 10°C rise for ectotherms until temperature gets too high and starts to denature enzymes (Gunderson, 2024). Together mass and temperature affect thermal tolerance; small individuals heat and cool quickly (low thermal inertia), due to their high mass-specific metabolic rates, which allows for rapid acclimation, but a short extreme temperature spike can easily push their body temperature to a lethal level (Gunderson, 2024). Larger individuals heat and cool more slowly (high thermal inertia) and are more buffered to rapid environmental changes; however, if the individual starts to overheat, it cannot dissipate heat quickly (Gunderson, 2024). The second is that oxygen transport, linked to metabolism (and therefore size), fails once a certain body temperature is reached and therefore can be used to define thermal tolerance (Pörtner, 2010). The third is diffusion distance (which increases with body size), specifically, the longest distance between the supply endpoint and the cells defines thermal tolerance by influencing how fast molecules move (Gillooly et al., 2001). The fourth is that acute heat stress is intimately tied to enzyme function (larger teleosts rely more on efficient enzyme function due to lower mass-specific metabolic rates, so the rate at which enzymes are working, per gram of tissue, is typically lower in large fish and they respond more slowly to acute stress), and therefore, can be used to define thermal tolerance (Ern et al., 2023). Finally, the fifth is that mitochondrial dysfunction sets the upper thermal limits of ectotherms because mitochondria lose their ability to efficiently convert oxygen and energetic substrates into ATP at a certain temperature (large teleosts have lower surface-area-to-volume ratios, which makes oxygen diffusion and delivery less efficient at the tissue level which can impair mitochondrial oxygen supply during acute heat stress) (Chung &

Schulte, 2020; Leiva et al., 2024). Additionally, there is no one specific effect of body mass on thermal tolerance (Gomez Isaza & Rodgers, 2024). Clark et al. (2017) and Di Santo & Lobel, (2017) found that large masses resulted in lower thermal tolerance, while Illing et al. (2020) and Moyano et al. (2017) found that large masses resulted in higher thermal tolerances. Mass' effect on thermal tolerance varied across studies, but mass was always considered as variable affecting thermal tolerance due to its known physiological effects.

Acclimation and thermal tolerance **-** Acclimation is often included as a factor when accessing a species thermal tolerance. A study on thermal tolerance across fish species and life stages found acclimation dynamics strongly affect which species are impacted by a changing climate (De Bonville et al., 2025). De Bonville et al. (2025) describe three acclimation components. The first being acclimation capacity which refers to a fish's ability to change their thermal limit after experiencing increased temperature in their environment (Einum et al., 2019; De Bonville et al., 2025). The second was response induction time which measures the length of exposure to a different environmental temperature before a change in the fish's thermal limit is seen (De Bonville et al., 2025). The third acclimation dynamic was time to full acclimation which refers to the length of time needed to completely acclimate by adjusting the fish's physiology to reach a new steady state (De Bonville et al., 2025). These three dynamics could explain fish geographic distributions and their risk to heatwaves. It's important to note that these dynamics change for an individual throughout its different life stages (Dufty et al., 2002; Fischer et al., 2014); specifically, declining thermal plasticity with age and size (Rohr et al., 2018). Some species are able to increase their thermal tolerance after short exposures to warm temperatures before they reach full acclimation; for example a significant increase in CT_{max} was found for stickleback, *Gasterosteus aculeatus*, after 3 h of warm water exposure and for wrasse, *Ctenolabrus rupestris*, after 6 h of warm water exposure (De Bonville et al., 2025). Still some species do not see significant increases in CT_{max} until full acclimation is reached like flounders, *Platichthys flesus*, after 4 days of exposure (De Bonville et al., 2025). It was suggested that stickleback and wrasse which reside in shallower waters with less thermal refuges have increased selection for rapid thermal physiological plasticity (De Bonville et al., 2025) compared to flounders who may seek refuge in the benthic environment by burying themselves in sand (Ziegler & Frisk, 2019). In addition to looking across species for differences in acclimation dynamics, they also examined differences in dynamics between adults and juveniles within a species (De Bonville et al., 2025). They found negligible differences in acclimatation dynamics and thermal tolerances between adult and juvenile zebrafish, *Danio rerio*, possibly because they coexist in similar shallow tropical environments (De Bonville et al., 2025).

Theme C. Observed responses to increased SST on tropical ectotherms *in situ*

Retreat/migration~ Many factors impact a species ability to migrate to new environments. Feary et al. (2014) completed a review explaining why some coral reef species can successfully shift latitudinally and why others cannot. This review investigated variables such as: large-scale ocean currents, ocean eddies, environmental constraints to distributional shifts, population density, latitudinal distribution, life-history traits associated with vagrancy, resource constraints to vagrant success, habitat association, settlement preferences, dietary preferences, and functional groups. A review on all of these topics would be redundant, so I am choosing to only focus on latitudinal distribution because migration of tropical fish species is often limited by latitude.

Temperate fish species have been observed moving to new habitats higher in latitudes (Feary et al., 2014); however, tropical species are often limited to current latitudinal distributions and temperature tolerances (Munday et al., 2008). Many species already occupy the whole latitudinal range of their coral reefs (Feary et al., 2014), or they only occur on the high latitude edges of their reef (Paxton et al., 2019). Retreating to higher latitudes may not be an option for some tropical species. However, a study by Feary et al. (2014), looked at the proportional abundance of seven butterflyfish species in high latitude, low latitude, and temperate regions found that tropical vagrant species (species that breed within tropical coral reef habitats and their larvae settle in temperate reef habitats) had the highest natal populations in relatively high latitude regions of the extent of coral reefs, meaning vagrants originated from higher latitudes in the tropical-temperate transition region; this indicates that high latitude reef populations may be a source of tropical vagrants. Another factor influencing the ability of tropical reef fish to settle in temperate environments is pelagic larval duration (PLD); a study on the damselfish, *Abudefduf vaigiensis,* in southeastern Australia, found that the population of *A. vaigiensis* in the study had an extended PLD which aided their expansion into temperate environments (Rigg et al., 2025). However, if a species is physically able to migrate to a high latitude reef or a temperate region, they will still be limited by their tolerance to lower temperatures in winter; fishes with wider thermal tolerances for lower temperatures are associated with successful vagrant species (Downie et al., 2025).

Deep reef refuge~ Deep reefs (> 25 m) are generally less affected by temperature fluctuations and anthropogenic disturbances like overfishing and pollutants and therefore, may act as a refuge for thermally stressed fish on shallow reefs (Pereira et al., 2018). Usually, deep reefs are closely linked to shallow reef areas, creating a continuous or semi-continous belt along a depth gradient (Hinderstein et al., 2010). However, depth affects light attenuation can therefore affect coral composition and cover (Pereira et al., 2018). As previously mentioned, fish abundance and diversity are affected by coral cover and composition (Pratchett et al., 2008). In order for deep reefs to act as refuges, physical and environmental conditions must be similar to the shallow reef that fishes emigrate from (Bongaerts et al., 2010). A study done on Atlantic reefs off the coast of Brazil, suggests deep reef refuge has occurred (Pereira et al., 2018). Benthic composition, habitat features, and quality were the same on shallow reefs (~4 m) and deep reefs (> 25 m), but there were strong differences in reef fish communities. The abundance, species richness, and size classes of reef fish communities increased on deep reefs (Pereira et al., 2018). Differences in reef fish communities, but not in benthic communities suggest to the authors that the deep reefs in this study can be used for refuge. A different study in the south Atlantic found limited support for the deep reef refuge hypothesis (Medeiros et al., 2021). Medeiros et al. (2021) found that there were 15 species exclusive to the shallow reefs, deep reefs had more rare species, and deep reefs had less common or dominant species, indicating that exclusive species local extinction was unlikely to be reversed by immigrants from deep reefs (Medeiros et al., 2021). However, the authors stressed that particular species, functions, and lineages do find refuge in deep reefs, but not whole fish communities (Medeiros et al., 2021).

Adaptations~ The prime candidates for local adaptation to climate change are small, short-lived species (Munday et al., 2008). Existing phenotypic plasticity within different species may promote acclimatization to increased SST, and connectivity between established populations and migratory populations may allow for genetic adaptation by gene flow (Basu et al., 2002; Munday et al., 2008; Smith et al., 2020). Species that are most at risk typically are those near the latitudinal margins of developed reef or live on isolated island groups (Munday et al., 2008).

These populations have less opportunity for gene flow and cannot physically move to new locations with better environmental conditions. A study on rainbowfish, *Melanotaenia duboulayi*, across three ecotypes, subtropical, temperate, and desert, found that populations from historically warmer regions, subtropical, had phenotypic plasticity of key stress-related genes, including heat shock proteins (HSPs) that supports acclimatization to increasing SST (Smith et al., 2020).

In hope that species can adapt to increased SST, several studies have looked at a fish's ability to respond to increased water temperature (Ekström et al., 2016; Donelson et al., 2016; Chen et al., 2021; Wang et al., 2022; Watson et al., 2024). One study measured transgenerational plasticity (TGP), or how environmental conditions of the parent affect the development of the offspring, and developmental plasticity, the ability of an organism to alter its development in response to the environment, at three different temperatures, present-day = 28.5°C, +1.5°C = 30.0°C, and +3.0°C = 31.5°C, over two generations, F1, F2, on the damselfish, *Acanthochromis polycanthus* (Donelson et al., 2016). They found that warming at +1.5°C across two generations enhanced reproduction; there was also evidence for TGP in egg and offspring attributes that would not be possible for developmental plasticity alone in these generations (Donelson et al., 2016). However, fish developing in warmer environments at +3.0°C across two generations were unable to reproduce at all (Donelson et al., 2016). Fish populations that experience a slight increase in temperature across multiple generations may have the ability to maintain or enhance reproduction while fish populations that experience a larger increase in temperature across multiple generations may have reduced reproduction.

To investigate how temperature affects gene expression across generations, one study looked at five reproductive genes in the brain of *A. polycanthus* (*Fshb, Lhb, Gnrh1, Gnrhr and*

Ddc) before and after three different acclimation treatments, +0.0°C, +1.5°C, and +3.0°C, across two generations, F1 and F2 (Veilleux & Donelson, 2018). In females the *Fshb* gene, which affects the process of yolk formation in the ovaries, was significantly lower for the two treatment groups than the control, but all other genes (*Lhb, Gnrh1, Gnrhr and Ddc*) did not exhibit a change in expression across treatments. In males the levels of *Fshr* and *Lhcgr*, which regulate reproductive function by initiating developmental processes, decreased in the testes of male subjects in both experimental treatments (Veilleux & Donelson, 2018). They conclude that the ability to acclimate reproductive capacity relies on the *Fshr* and *Lhcgr* expression in males and the *Fshb* expression in females (Veilleux & Donelson, 2018). Over generational time, fish with the ability to maintain the *Fshb, Fshr,* and *Lhcgr* expression as temperature increases may have an increased reproductive capacity compared to fish that have decreased *Fshb, Fshr,* and *Lhcgr* expression.

Increased temperature does not only impact the reproductive capacity of an animal, but it also affects their metabolic processes. Heat shock proteins (HSP) are essential in various aspects of protein metabolism. There are three main families of proteins: Hsp90 (85–90 kDa), Hsp70 (68–73 kDa), and low molecular weight heat shock proteins (16–47 kDa) (Basu et al., 2002). HSP can be up-regulated in cells that are exposed to a wide variety of stressors, particularly those that denature proteins like temperature (Welch, 1993) which suggests that cellular stress responses play a role in survival and health of fish (Basu et al., 2002). HSP have been found to boost immune responses and increase disease resistance in crustaceans (Kumar et al., 2022), and HSP can help promote growth in marine invertebrates at temperatures above their average (Choresh et al., 2004). Data indicate that HSP expression is subject to acclimatization, and that HSP concentrations and types present may fluctuate in response to variations in seasonal temperature in the aquatic environment (Basu et al., 2002). HSP may therefore play an important role in long-term adaptation to increased temperature (Parsell & Lindquist, 1993).

Conclusions

Climate change is increasing SST (Hoegh-Guldberg & Bruno, 2010), causing more frequent and severe MHWs, which negatively impact corals the habitats that many reef fish occupy (Couch et al., 2017), and negatively affects the physiology of reef fishes (Ern et al., 2023). Ocean acidification by increased concentrations of CO_2 and coral bleaching and mortality due to increased SST act synergistically and decrease ecosystem health and function (Raupach & Canadell, 2010; Hönisch et al., 2012; & Couch et al., 2017). Reef fish who rely on coral reef ecosystems for feeding, foraging, refuge, and other life history processes are negatively impacted by coral loss due to climate change (Pratchett et al., 2008).

In addition to being impacted by habitat loss, ocean acidification, and increased SST due to climate change, fish are also directly affected by increased SST. All ectothermic fish that have been examined for thermal limits, have shown thermal tolerances and limits that when surpassed, result in death (T_{crit} and CT_{max}) (Clark et al., 2017; Ospina & Mora, 2004; Ern et al., 2023). Increased SST has also been shown to increase oxygen consumption rates and increase lower critical oxygen inducing hypoxic events in fishes (Nilsson et al., 2010). Generally, we know heat affects molecular reaction rates (bottom-up), and that negative effects from temperature come from insufficient oxygenation of the tissues causing ATP deficiency (top-down) (Schulte, 2015).

Increased SST in tropical reef fish environments has been shown to influence adaptation strategies including migration to higher latitude environments (Munday et al., 2008; Feary et al., 2014), deep reef refuge (Pereira et al., 2018; Medeiros et al., 2021), and adaptations such as transgenerational plasticity (Donelson et al., 2016), gene expression (Veilleux & Donelson, 2018), and changed HSP expression (Welch, 1993; Basu et al., 2002). These responses are variable among species and can be specific to certain reef populations, and therefore, these adaptational responses can not be considered as ubiquitous for all species. Other responses to increased SST must be investigated.

Unresolved

With predicted increases in SST due to climate change, understanding tropical reef fish thermal ranges is becoming increasingly important. With experimental approaches such as CT_{max} and T_{pref} experiments, we can start to define these ranges on a species and population level. CT_{max} experiments have been performed on *Plectropomus leopardus* (Messmer et al., 2017), *Acanthochromis polyacanthus*, *Dascyllus aruanus, Dischistodus perspicillatus* (Clark et al., 2017), *Amphiprion melanopus, Lates calcarifer* (Illing et al., 2020), *Apogon pacifici, Apogon dovii, Thalassoma lucassanum, Halichoeres dispilus, Lutjanus guttatus, Cirrhitichtys oxycephalus,* and *Coricopterus urospilus* (Ospina & Mora, 2004), and several other species. Still these experiments have only been performed on a small percentage of the 6,000-8,000 species residing in coral reef ecosystems (Basic information about coral reefs, 2025). In order to gain a more comprehensive understanding of fish temperature ranges, additional CT_{max} and T_{pref} experiments should be performed. Experimenting on all tropical fish species is not feasible; I recommend focusing future experiments on threatened and endangered species as well as species in families that have no or few recorded CT_{max} and/or T_{pref} experiments.

In addition to *ex situ* experimentation, *in situ* surveys before, during, and after MHWs and increased SST events should be conducted. Surveys would reveal changes in coral reef populations relating to abundance and diversity that could indicate mass mortality or migration. These surveys may also help inform CT_{max} and T_{pref} experiments. As previously discussed, small, short-lived species are expected to adapt to increased SST, so a focus on large long-lived species as well as coral obligate species should especially be noted during surveys. Corals are also extremely threatened by increased SST which jeopardizes the habitats where many tropical fish species reside. Therefore, surveys should also note changes in benthic coral communities.

References

Åsheim E. R., Andreassen, A. H., Morgan, R., & Jutfelt, F. (2020). Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish. *J Exp Biol,* 223, doi:10.1242/jeb.229195.

Bard, B., & Kieffer, J. D. (2019). The effects of repeat acute thermal stress on the critical thermal maximum (CT_{max}) and physiology of juvenile shortnose sturgeon (*Acipenser brevirostrum*). *Canadian Journal of Zoology*, *97*(6), 567–572. https://doi.org/10.1139/cjz-2018-0157.

Basic information about coral reefs. (2025). *Environmental Protection Agency (EPA)*. https://www.epa.gov/coral-reefs/basic-information-about-coral-reefs#:~:text=Coral%20reefs%20are%20among%20the,point%20in%20their%20life%20cycle.

Basu, N., Todgham, A. E., Ackerman, P. A., Bibeau, M. R., Nakano, K., Schulte, P. M., & Iwama, G. K. (2002). Heat shock protein genes and their functional significance in fish. *Gene*, 295(2), 173-83, doi: 10.1016/s0378-1119(02)00687-x.

Becker, C. D., & Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology Fish*, 4, 245-256.

Bongaerts, P., Ridgway, T., Sampayo, E. M., & Hoegh-Guldberg, O. (2010). Assessing the 'deep reef refugia' hypothesis: Focus on Caribbean reefs. *Coral Reefs*, 29(2), 309–327. https://doi.org/10.1007/s00338-009-0581-x.

Chen, Q., Feng, C., Liu, W., Zhang, J., & Li, D. (2021). Effects of thermal acclimation regimes on growth performance, antioxidant capacity and heat shock protein 70 expression in Schizothorax prenanti. *Journal of Thermal Biology*, 100, 103027. https://doi.org/10.1016/j.jtherbio.2021.103027.

Choresh, O., Loya, Y., Müller, W. E., Wiedenmann, J., & Azem, A. (2004). The mitochondrial 60-kDa heat shock protein in marine invertebrates: Biochemical purification and molecular characterization. Cell Stress Chaperones. 2004;9:38–48. doi: 10.1379/1466-1268.

Christensen, E. A. F., Andersen, L. E. J., Bergsson, H., Steffensen, J. F., & Killen, S. S. (2021). Shuttle-box systems for studying preferred environmental ranges by aquatic animals, *Conservation Physiology*, 9(1). https://doi.org/10.1093/conphys/coab028.

Chung, D. J., & Schulte, P. M. (2020). Mitochondria and the thermal limits of ectotherms. *Journal of Experimental Biology*, 223.

Couch, C. S., Burns, J. H. R., Liu, G., Steward, K., Gutlay, T. N., Kenyon, J., Eakin, C. M., & Kosaki, R. K. (2017). Mass coral bleaching due to unprecedented marine heatwave in Papahānaumokuākea Marine National Monument (Northwestern Hawaiian Islands). *PLOS One*, *12*(9), e0185121. https://doi.org/10.1371/journal.pone.0185121.

Clark, T. D., Roche, D. G., Binning, S. A., Speers-Roesch, B., & Sundin, J. (2017). Maximum thermal limits of coral reef damselfishes are size dependent and resilient to near-future ocean acidification. *J Exp Biol,* 220(19), 3519–3526. doi: https://doi.org/10.1242/jeb.162529.

Darling, E.S., McClanahan, T.R., Maina, J. *et al.* (2019). Social–environmental drivers inform strategic management of coral reefs in the Anthropocene. *Nature Ecology & Evolution* 3, 1341–1350 (2019). https://doi.org/10.1038/s41559-019-0953-8.

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews, 76*(2), 229–261. https://doi.org/10.1128/MMBR.05014-11.

De Bonville, J., Andreassen, A. H., Cowan, Z., Silva-Garay, L., Leeuwis, R. H. J., Åsheim, E. R., Speers-Roesch, B., Raby, G. D., Binning, S. A., & Jutfelt, F. (2025). Dynamics of thermal tolerance plasticity across fish species and life stages. *Journal of Thermal Biology*, 127, 0306-4565, https://doi.org/10.1016/j.jtherbio.2024.104024.

Desforges, J. E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K. M., Eliason, E. J., Dressler, T. L., McKenzie, D. J., Bates, A. E., Lawrence, M. J., Fangue, N., & Cooke, S. J. (2022). The ecological relevance of critical thermal maxima methodology for fishes. *Journal of Fish Biology*, 1-17, 10.1111/jfb.15368.

Di Santo, V., & Lobel, P. S. (2017). Body size and thermal tolerance in tropical gobies. *Journal of Experimental Marine Biology and Ecology*, 487, 11–17.

Donelson, J. M., Wong, M., Booth, D. J., & Munday, P. L. (2016). Transgenerational plasticity of reproduction depends on rate of warming across generations. *Evol Appl* 9: 1072–1081.

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean acidification: The other CO_2 problem. *Annual Review of Marine Science*, 1, 169–192. https://doi.org/10.1146/annurev.marine.010908.163834.

Douglas, A. E. (2003). Coral bleaching – how and why? *Marine Pollution Bulletin, 46*(4), 385–392. https://doi.org/10.1016/S0025-326X(03)00037-7.

Downie, A. T., Champion, C., & Booth, D. J. (2025). Physiological traits for predicting poleward extensions in tropical fishes: from lab to management. *Global Change Biology*, 31, 1-27. https://doi.org/10.1111/gcb.70213.

Dufty, A.M., Clobert, J., & Møller, A.P. (2002). Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evol*ution*,* 17, 190–196.

Einum, S., Ratikainen, I., Wright, J., Pelabon, C., Bech, C., Jutfelt, F., Stawski, C., & Burton, T. (2019). How to quantify thermal acclimation capacity? *Global Change Biology*. 25, 1893–1894.

Ekström, A., Jutfelt, F., & Sandblom, E. (2014). Effects of autonomic blockade on acute thermal tolerance and cardioventilatory performance in rainbow trout, Oncorhynchus mykiss. *Journal of Thermal Biology*, 44, 47–54.

Ekström, A., Axelsson, M., Sandblom, E., & Gräns, A. (2016). Cardiac thermal plasticity improves heat tolerance in European perch, Perca fluviatilis. *Journal of Comparative Physiology B*, 186(4), 497–507. https://doi.org/10.1007/s00360-016-0975-4.

Ern, R., Andreassen, A. H., & Jutfelt, F. (2023). Physiological Mechanisms of Acute Upper Thermal Tolerance in Fish. *Physiology*, *38*(3), 141–158. https://doi.org/10.1152/physiol.00027.2022.

Feary, D. A., Pratchett, M. S., J Emslie, M., Fowler, A. M., Figueira, W. F., Luiz, O. J., Nakamura, Y., & Booth, D. J. (2014). Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish and Fisheries*, *15*(4), 593–615. https://doi.org/10.1111/faf.12036.

Fennie, H. W., Grorud-Colvert, K., & Sponaugle, S. (2023). Larval rockfish growth and survival in response to anomalous ocean conditions. *Sci Rep* 13, 4089, https://doi.org/10.1038/s41598-023-30726-5.

Fischer, B., van Doorn, G.S., Dieckmann, U., & Taborsky, B. (2014). The evolution of age-dependent plasticity. *American Naturalist*, 183, 108–125.

Genin, A., Levy, L., Sharon, G., Raitsos, D. E., & Diamant, A. (2020). Rapid onsets of warming events trigger mass mortality of coral reef fish. *Proceedings of the National Academy of*

Sciences of the United States of America, 117(41), 25378–25385. https://doi.org/10.1073/pnas.2009748117.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293, 2248–2251.

Gomez Isaza, D. F., & Rodgers, E. M. (2024). Upper thermal limits are 'hard-wired' across body mass but not populations of estuarine fish. *Journal of Thermal Biology*, 125. https://doi.org/10.1016/j.jtherbio.2024.103970.

Grottoli, A., Rodrigues, L., & Palardy, J. (2006). Heterotrophic plasticity and resilience in bleached corals. *Nature* 440, 1186–1189. https://doi.org/10.1038/nature04565.

Guest, J. R., Baird, A. H., Maynard J. A., Muttaqin, E., Edwards, A. J., Campbell, S. J., Yewdall, K., Affendi, Y. A., & Chou, L. M. (2012). Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PLoS ONE 7(3): e33353. https://doi.org/10.1371/journal.pone.0033353.

Gunderson, A.R. (2024). Disentangling physiological and physical explanations for body size-dependent thermal tolerance. *Journal of Thermal Biology*. 227(5). https://doi.org/10.1242/jeb.245645.

Gvoždík, L. (2018). Just what is the thermal niche? *Oikos*, 127, 1701–1710. https://doi.org/10.1111/oik.05563.

Harman, A. A., Fuzzen, M., Stoa, L., Boreham, D., Manzon, R., Somers, C. M., & Wilson, J. Y. (2021). Evaluating tank acclimation and trial length for dynamic shuttle box temperature preference assays in aquatic animals. *Journal of Experimental Biology*, 224 (12): jeb233205. doi: https://doi.org/10.1242/jeb.233205.

Heinze, C., Meyer, S., Goris, N., Anderson, L., Steinfeldt, R., Chang, N., Le Quéré, C., & Bakker, D. C. E. (2015). The ocean carbon sink – impacts, vulnerabilities and challenges. *Earth System Dynamics*, 6, 327–358, https://doi.org/10.5194/esd-6-327-2015, 2015.

Hinderstein, L. M., Marr, J. C., Martinez, F. A., Dowgiallo, M. J., & Puglise, K. A. (2010). Mesophotic coral ecosystems: Characterization, ecology, and management. *Coral Reefs*, 29(2), 247–251. https://doi.org/10.1007/s00338-010-0614-5.

Hobday A.J., Alexander L.V., Perkins-Kirkpatrick S.E., Smale D.A., Straub S.C., Oliver E.C.J., Benthuysen J.A., Burrows M.T., Donat M.G., Feng M., Holbrook N.J., Moore P.J., Scannell H.A., Sen Gupta A., Wernberg T. A hierarchical approach to defining marine heatwaves. (2016). *Progress in Oceanography*, 141, 227–238. doi: 10.1016/j.pocean.2015.12.014.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, 328(5985), 1523–1528. https://doi.org/10.1126/science.1189930.

Hönisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R. C., Greene, S. E., Kiessling, W., Ries, J., Zachos, J. C., Royer, D. L., Barker, S., Marchitto, T. M., Moyer, R., Pelejero, C., Ziveri, P., Bijma, J., & Williams, B. (2012). The geological record of ocean acidification. *Science,* 335(6072), 1058–1063. https://doi.org/10.1126/science.1208277.

Illing, B., Downie, A. T., Beghin, M., & Rummer, J. L. (2020). Critical thermal maxima of early life stages of three tropical fishes: Effects of rearing temperature and experimental heating rate. *Journal of Thermal Biology,* 90*,* 0306-4565, https://doi.org/10.1016/j.jtherbio.2020.102582.

Intergovernmental Panel on Climate Change. (2018). Global Warming of 1.5 °C. An IPCC Special Report on the impacts of global warming of 1.5 °C above pre-industrial levels and related global greenhouse gas emission pathways *[V. Masson-Delmotte et al. (eds.)]. World Meteorological Organization*. https://doi.org/10.1017/9781009157940.

Jobling, M. (1981). Temperature tolerance and the final preferendum - rapid methods for the assessment of optimum growth temperatures. *Journal of Fish Biology*, 19, 439-455.

Jutfelt, F., Roche, D. G., Clark, T. D., Norin, T., Binning, S. A., Speers Roesch, B., Amcoff, M., Morgan, R., Andreassen, A. H., & Sundin, J. (2019). Brain cooling marginally increases acute upper thermal tolerance in Atlantic cod. *Journal of Experimental Biology*, 222(19), jeb208249.

Kumar, V., Roy, S., Behera, B. K., & Das, B. K. (2022). Heat Shock Proteins (Hsps) in Cellular Homeostasis: A Promising Tool for Health Management in Crustacean Aquaculture. *Life (Basel)*, 12(11), 1777. doi: 10.3390/life12111777.

Leiva, F. P., Santos, M., Rezende, E. L., & Verberk, W. C. E. P. (2024). Intraspecific variation of heat tolerance in a model ectotherm: the role of oxygen supply capacity. *Functional Ecology*. https://doi.org/10.1111/1365-2435.14485.

Lewis, S. L., & Maslin, M. A. (2015). Defining the Anthropocene. *Nature*, *519*(7542), 171–180. https://doi.org/10.1038/nature14258.

McMahon, T. E., Bear, E. A., & Zale, A. V. (2008). Use of an annular chamber for testing thermal preference of westslope cutthroat trout and rainbow trout. *Journal of Freshwater Ecology*, *23*(1), 55–63. https://doi.org/10.1080/02705060.2008.9664557.

Medeiros, A. P. M., Ferreira, B. P., Alvarado, F., Betancur-R, R., Soares, M. O., & Santos, B. A. (2021). Deep reefs are not refugium for shallow-water fish communities in the southwestern Atlantic. *Ecol Evol*, 11, 4413–4427. https://doi.org/10.1002/ece3.7336.

Messmer, V., Pratchett, M. S., Hoey, A. S., Tobin, A. J., Coker, D. J., Cooke, S. J. & Clark, T. D. (2017). Global warming may disproportionately affect larger adults in a predatory coral reef fish. *Glob Change Biol,* 23, 2230-2240. https://doi.org/10.1111/gcb.13552.

Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CT_{max} is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports*, *8*(1), 7099. https://doi.org/10.1038/s41598-018-25593-4.

Moyano, M., Candebat, C., Ruhbaum, Y., Alvarez-Fernandez, S., Claireaux, G., Zambonino-Infante, J.-L., & Peck, M.A. (2017). Effects of warming rate, acclimation temperature and ontogeny on the critical thermal maximum of temperate marine fish larvae. *PLoS One*, 12, e0179928.

Munday, P. L., Jones, G. P., Pratchett, M. S., & Williams, A. J. (2008). Climate change and the future for coral reef fishes. *Fish and Fisheries*, *9*(3), 261–285. https://doi.org/10.1111/j.1467-2979.2008.00281.x.

Myrick, C. A., Folgner, D. K., & Cech, J. J. (2004). An annular chamber for aquatic animal preference studies. American Fisheries Society 133: 427-433.

Nati, J. J. H., Svendsen, M. B. S., Marras, S., Killen, S. S., Steffensen, J. F., McKenzie, D. J., & Domenici, P. (2021). Intraspecific variation in thermal tolerance differs between tropical and temperate fishes. *Scientific Reports, 11*, Article 21272. https://doi.org/10.1038/s41598-021-00695-8.

Nay, T. J., Johansen, J. L., Rummer, J. L., Steffensen, J. F., Pratchett, M. S., & Hoey, A. S. (2020). Habitat complexity influences selection of thermal environment in a common coral reef fish. Conservation Physiology DOI: 10.1093/conphys/coaa070. eCollection 2020.

Nilsson, G. E., Östlund-Nilsson, S., & Munday, P. L. (2010). Effects of elevated temperature on coral reef fishes: Loss of hypoxia tolerance and inability to acclimate. *Comparative*

Biochemistry and Physiology Part A: Molecular & Integrative Physiology, *156*(4), 389–393. https://doi.org/10.1016/j.cbpa.2010.03.009.

NOAA Coral Reef Watch. (2023). Pacific climate update – Coral bleaching heat stress analysis and seasonal guidance through June 2023. *National Oceanic and Atmospheric Administration.* https://coralreefwatch.noaa.gov/satellite/analyses_guidance/pacific_cbts_ag_20230308.php.

Oliver, E. C. J., Benthuysen, J. A., Darmaraki, S., Donat, M. G., Hobday, A. J., Holbrook, N. J., Schlegel, R. W., & Sen Gupta, A. (2021). Marine heatwaves. *Annual Review of Marine Science*, *13*(1), 313–342. https://doi.org/10.1146/annurev-marine-032720-095144.

Ospina, A. F., & Mora, C. (2004). Effect of body size on reef fish tolerance to extreme low and high temperatures. *Environmental Biology of Fishes,* 70, 339–343 https://doi.org/10.1023/B:EBFI.0000035429.39129.34.

Parsell, D. A., & Lindquist, S. (1993). The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu Rev Genet*, 27, 437-96. doi: 10.1146/annurev.ge.27.120193.002253.

Paxton, A. B., Peterson, C. H., Taylor, J. C., Adler, A. M., Pickering, E. A., & Silliman, B. R. (2019). Artificial reefs facilitate tropical fish at their range edge. *Commun Biol,* 2, 168, https://doi.org/10.1038/s42003-019-0398-2.

Pearce, A. F., Lenanton, R. C., Jackson, G., Moore, J., Feng, M., & Gaughan, D. (2011). The "marine heat wave" off Western Australia during the summer of 2010/11 (Fisheries Research Report No. 222). *Department of Fisheries, Western Australia*.

Pereira, P. H. C., Macedo, C. H., Nunes, J. D. A. C. C., Marangoni, L. F. D. B., & Bianchini, A. (2018). Effects of depth on reef fish communities: Insights of a "deep refuge hypothesis" from Southwestern Atlantic reefs. *PLOS ONE*, *13*(9), e0203072. https://doi.org/10.1371/journal.pone.0203072.

Pinsky, M. L., Selden, R. L., & Kitchel, Z. J. (2020). Climate-Driven Shifts in Marine Species Ranges: Scaling from Organisms to Communities. *Annual Review of Marine Science,*12(1), 153-179, https://doi.org/10.1146/annurev-marine-010419-010916.

Pörtner, H. -O. (2010). Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, 213, 881–893.

Pratchett, M. S., Munday, P. L., & Wilson, S. K. (2008). Effects of climate-induced coral bleaching on coral-reef fishes: ecological and economic consequences. *Oceanography and Marine Biology: An Annual Review* 46, 251–296.

Raby, G. D., Morgan, R., Andreassen, A. H., Stewart, E. M. C., de Bonville, J., Hoots, E. C., Kuchenmüller, L., Metz, M., Rowsey, L. E., Green, L., Griffin, R. A., Martin, S., Bauer Reid, H., Ern, R., Åsheim, E. R., Cowan, Z., Leeuwis, R. H. J., Blewett, T. A., Speers-Roesch, B., Clark, T. D., Binning, S. A., Sundin, J., & Jutfelt, F. (2025). Measuring critical thermal maximum in aquatic ectotherms: a practical guide. *EcoEvoRxiv*. https://doi.org/10.6084/m9.figshare.28319774.v1.

Raupach, M. R., & Canadell, J. G. (2010). Carbon and the anthropocene. *Current Opinion in Environmental Sustainability*, *2*(4), 210–218. https://doi.org/10.1016/j.cosust.2010.04.003.

Reid, P. C., Fischer, A. C., Lewis-Brown, E., Meredith, M. P., Sparrow, M., Andersson, A. J., Antia, A., Bates, N. R., Bathmann, U., Beaugrand, G., Brix, H., Dye, S., Edwards, M., Furevik, T., Gangstø, R., Hátún, H., Hopcroft, R. R., Kendall, M., Kasten, S., Keeling, R., Le Quéré, C., Mackenzie, F. T., Malin, G., Mauritzen, C., Ólafsson, J., Paull, C., Rignot, E., Shimada, K., Vogt, M., Wallace, C., Wang, Z., & Washington, R. (2009). Chapter 1 Impacts of the Oceans on Climate Change, Advances in Marine Biology. *Academic Press,* 56, 1-150, https://doi.org/10.1016/S0065-2881(09)56001-4.

Reiser, S., Temming, A., Eckhardt, A., & Herrmann, J. P. (2013). Automation and critical evaluation of an annular chamber for aquatic ectotherm temperature preference experiments. Methods in Ecology and Evolution 4: 531-541.

Reynolds, W. W., & Casterlin, M. E. (1979). Behavioral thermoregulation and the “Final Preferendum” paradigm. *American Zoologist*, 19(1), 211-224.

Rigg, A.L., Fowler, A.M., Nagelkerken, I., & Booth, D. J. (2025). Faster larval growth and shorter pelagic duration enhance the post-settlement persistence of a common range-extending coral-reef fish in a temperate ecosystem. *Coral Reefs* https://doi.org/10.1007/s00338-025-02627-8.

Rohr, J.R., Civitello, D.J., Cohen, J.M., Roznik, E.A., Sinervo, B., & Dell, A.I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letter*, 21, 1425–1439.

Roth, M. S. (2014). The engine of the reef: Photobiology of the coral–algal symbiosis. *Frontiers in Microbiology,* 5, 422. https://doi.org/10.3389/fmicb.2014.00422.

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J Exp Biol,* 218, 1856-1866, doi:10. 1242/jeb.118851.

Smith, S., McCairns, R. J. S., Sasaki, M., Bernatchez, L., & Beheregaray, L. B. (2020). Adaptation of plasticity to projected maximum temperatures and across climatically defined bioregions in rainbowfish ecotypes. *Proceedings of the National Academy of Sciences, 117*(9), 5359–5368. https://doi.org/10.1073/pnas.1921124117.

Spear, M. C., & Kieffer, J. D. (2016). Critical thermal maxima and hematology for juvenile Atlantic (*Acipenser oxyrinchus* Mitchell 1815) and shortnose (*Acipenser brevirostrum* Lesueur, 1818) sturgeons. *J. Appl. Ichthyol.* 32: 251–257. doi:10. 1111/jai.13002.

Stan, C., Straus, D. M., Frederiksen J.S., Lin, H., Maloney E. D., & Schumacher, C. (2017). Review of tropical-extratropical teleconnections on intraseasonal time scales. *AGU Publications*, 902-937. DOI 10.1002/2016RG000538.

Stuart-Smith, R. D., Edgar, G. J., & Bates, A. E. (2017). Thermal limits to the geographic distributions of shallow-water marine species. *Nature Ecology & Evolution*, 1, 1846. https://doi.org/10.1038/s41559-017-0353-x.

Suggett, D. J., & Smith, D. J. (2020). Coral bleaching patterns are the outcome of complex biological and environmental networking. *Global Change Biol*ogy 26: 68–79. https://doi.org/10.1111/gcb.14871.

Tietbohl, M. D., Genevier, L. G. C., Krieger, E. C., Kattan, A., Wang, Y., Gokul, E. A., Rodriguez Bravo, L. M., Palm, L., Mele, G., Hoteit, I., & Johnson, M. D. (2025). Extreme marine heatwave linked to mass fish kill in the Red Sea. *Science of the Total Environment, 975*, 179073. https://doi.org/10.1016/j.scitotenv.2025.179073.

U.S. Environmental Protection Agency. (2024). Climate Change Indicators: Marine species distribution. *Retrieved August 13, 2025, from EPA website*:

https://www.epa.gov/climate-indicators/climate-change-indicators-marine-species-distribution.

Veilleux, H. D., & Donelson, J. M. (2018). Reproductive gene expression in a coral reef fish exposed to increasing temperature across generations. *Conservation Physiology*, 6(1). https://doi.org/10.1093/conphys/cox077.

Wang, M., Qiu, C., Zhao, L., Zhang, J., & Wang, Y. (2022). *Effects of thermal acclimation on intestinal microbiota, histopathology, and HSP70 expression in juvenile Oxygymnocypris stewarti under heat stress*. Frontiers in Microbiology, 13, 898145. https://doi.org/10.3389/fmicb.2022.898145.

Watson, S.‑A., Scott, J. J. M., Munday, P. L., Clark, T. D., & Rummer, J. L. (2024). Upper thermal tolerance plasticity in fishes is highly variable among species, life stages and acclimation durations. *Journal of Experimental Biology*, 227(4), jeb246528. https://doi.org/10.1242/jeb.246528.

Welch, W. J. (1993). How cells respond to stress. *Scientific American*, 269, 56-64.

Yadav, S., Roach, T. N. F., McWilliam, M. J., Caruso, C., De Souza, M. R., Foley, C., Allen, C., Dilworth, J., Huckeba, J., Santoro, E. P., Wold, R., Simpson, J., Miller, S., Hancock, J. R., Drury, C., & Madin, J. S. (2023). Fine-scale variability in coral bleaching and mortality during a marine heatwave. *Frontiers in Marine Science*, *10*, 1108365. https://doi.org/10.3389/fmars.2023.1108365.

Zhang, Y., & Kieffer, J.D. (2014). Critical thermal maximum (CT_{max}) and hematology of shortnose sturgeons (Acipenser brevirostrum) acclimated to three temperatures. *Can. J. Zool.* 92(3): 215–221. doi:10.1139/cjz-2013-0223.

Zhang, Y., Loughery, J.R., Martyniuk, C.J., & Kieffer, J.D. (2017). Physiological and molecular responses of juvenile shortnose sturgeon (Acipenser brevirostrum) to thermal stress. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 203: 314–321. doi:10.1016/j.cbpa.2016.10.009.

Ziegler, C.M., & Frisk, M.G. (2019). Flatfish utilize sediment blanket to facilitate thermoregulation. *Marine Ecology Progress Series*, 609, 179–186.

Figures

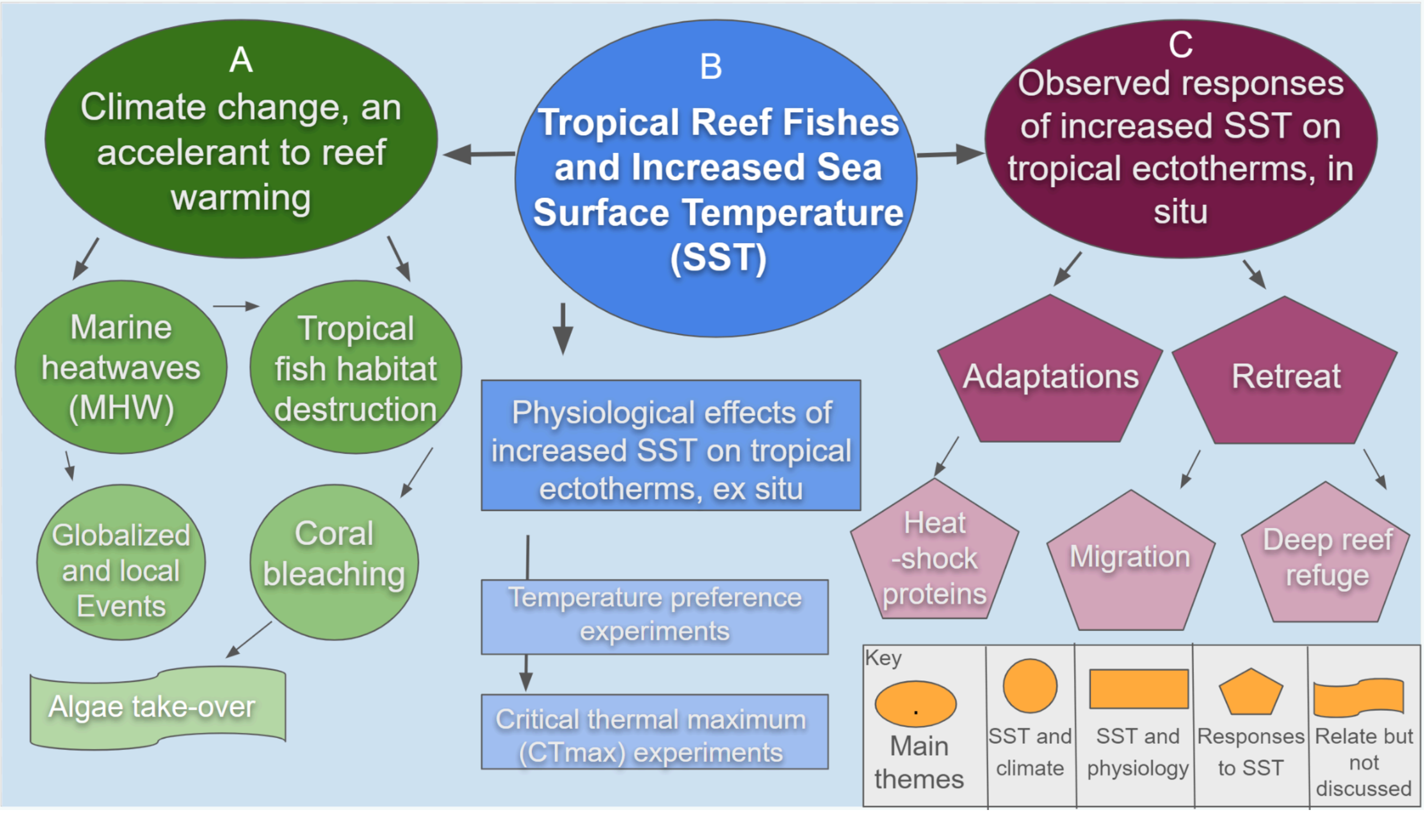

Figure 1. Review Approach: There are three main themes indicated by the oval shape A. B. and C. The details of each theme are correlated by color and by shape as indicated by the key.

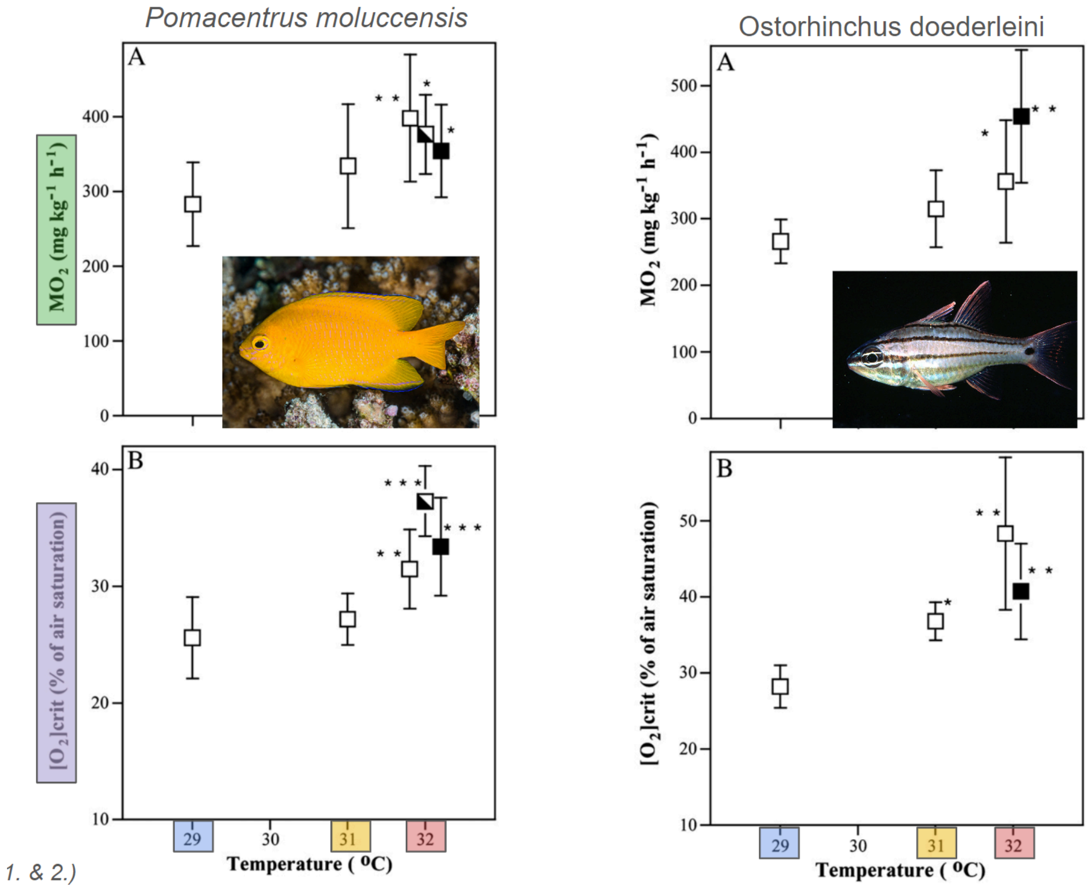

Figure 2. Resting oxygen consumption rate (MO2; green), the lowest concentration of oxygen at which resting MO2 can be maintained ([O2]crit; purple), for three acclimation treatments (29°C [open square], 32°C (10 days) [half-filled square], 32°C (22 days)) filled square at three experimental temperatures (29°C, 31°C, 32°C), for two species of reef fish *Pomacentrus moluccensis* & *Ostorhinchus doederleini*. Adapted from *Nilsson et al., 2010*.

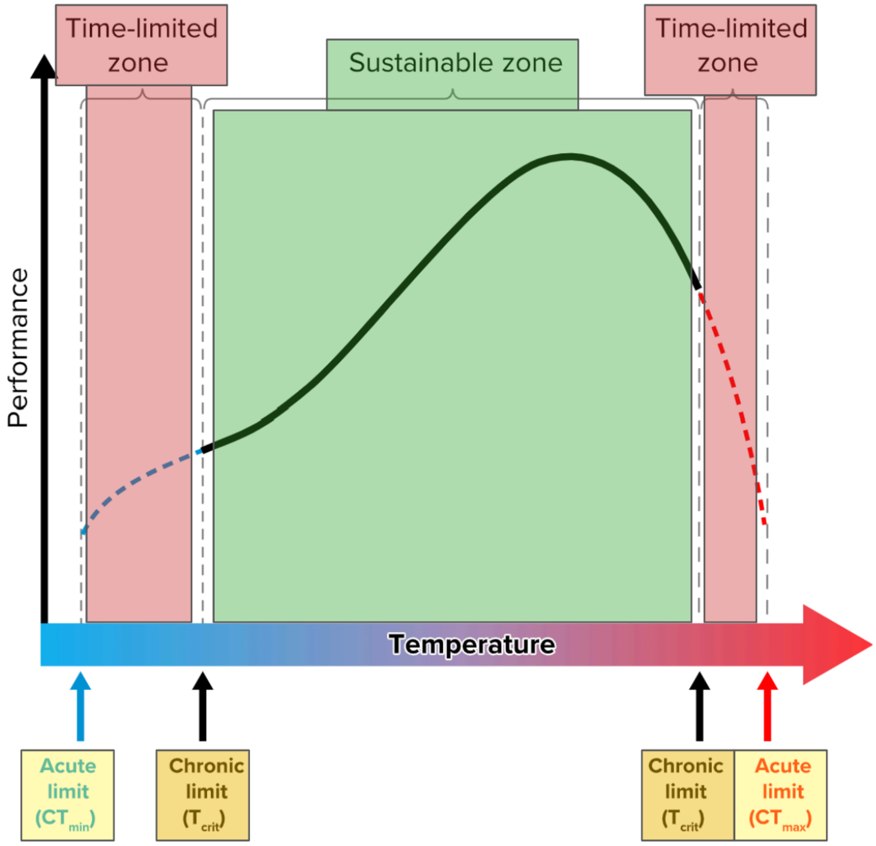

Figure 3. General performance of tropical fish in response to temperature. Green: Sustainable Zone: fish can sustain function in these temperatures long-term, Red: Time-limited Zone: short-term survival possible, but puts stress on the fish, Orange: T_{crit}: long-term survival barely possible, Yellow:CT_{max}/CT_{min} only short-term survival is possible. Adapted from *Ern et al., 2023*.

Chapter 2: Behavioral temperature preference and physiological critical thermal limits of the non-native Indo-Pacific sergeant *Abudefduf vaigiensis*, endemic Hawaiian sergeant *A. abdominalis*, and hybrids from O‘ahu, Hawai‘i

Abstract

Climate change has caused increases in sea surface temperature (SST) globally and has increased the frequency of marine heatwaves (MHW), which threatens tropical reef ecosystems by impacting biodiversity and population distributions. The main objective of my study was to assess differences in thermal biology between the endemic Hawaiian sergeant major, *Abudefduf abdominalis* and the invasive Indo-Pacific sergeant major, *Abudefduf vaigiensis*, and their hybrids by evaluating behavioral and physiological responses to SST. I hypothesized that if differences in thermal biology, specifically temperature preference (T_{pref}) and upper thermal limit (critical temperature maximum, CT_{max}), along with increasing SSTs have aided the expansion of the tropical Indo-Pacific sergeant into the habitat of the sub-tropical Hawaiian sergeant, then T_{pref} and CT_{max} of *A. vaigiensis* will be higher than *A. abdominalis*. I also examined the responses of the hybrids, for comparison to the pure, parental species and hypothesized that hybrid T_{pref} and CT_{max} will fall in between T_{pref} and CT_{max} measurements of the pure, parental species. Structure Q_{Ava} was used as an estimate of the proportion of the genome in each individual that came from *A. vaigiensis* versus *A. abdominalis* by assigning individuals to populations based on differences in multilocus genotype data. There was no effect of genetic similarity to *A. vaigiensis* on CT_{max} (mean ± S.E., from *A. abdominalis* 37.7 ± 0.2°C to *A. vaigiensis* 38.1 ± 0.1°C), but mass had an effect of a 1.4°C decrease on CT_{max} as mass increased from 8.7 to 124.6 g . Additionally, the size of the thermal preference range, or interquartile range (IQR), increased by 1.7°C with mass. I

found genetic similarity to *A*. *vaigiensis* to have a significant effect of a 1.2°C increase in median T_{pref} from 26.3 ± 0.4 °C for *A. abdominalis* to 27.6 ± 0.3 °C for *A. vaigiensis*. In addition there was a 2.2 °C increase in lower avoidance temperature (Q1) from 24.6 ± 0.5°C to 26.7 ± 0.3°C and a 1.5°C decrease in interquartile range (IQR) from 3.2 ± 0.3°C to 1.9 ± 0.2°C for *A. abdominalis* to *A. vaigiensis*, respectively. These results partially support my hypothesis and suggest that the invasive *A*. *vaigiensis* have a preference for warmer temperatures than the native *A. abdominalis*, which may have aided *A. vaigiensis*' expansion into Hawaiian habitats with increasing SST.

Introduction

Global climate change, specifically increased sea surface temperatures (SST) and increased frequency of marine heatwaves (MHW), can have negative impacts on tropical reef fishes' physiology (mitochondrial activity, electron transport, fatty acid metabolism, and cellular stress responses) and physical performance (aerobic scope) (Alfonso et al., 2020; Bernal et al., 2020). Increased SST and increased frequency of MHW have also been shown to affect reef fish distributions (Magel et al., 2020). Reef fishes, like most smaller marine ectotherms (those less than 1 kg and without a *rete mirabilia*) have a core temperature close to their environment (within a few tenths of a Celsius degree; Reynolds & Casterlin, 1979). Tropical fish species tend to occupy environments near their upper thermal range, which causes these organisms to be threatened by slight increases in sea surface temperature (Rowe et al., 2018; Alfonso et al., 2020). Some fish species are extending their distribution poleward into temperate environments (Downie et al., 2025), and ocean gyres have been found to carry larvae hundreds of kilometers away from their natal reef (Cowen et al., 2006; Christie et al., 2010). For example, genetic evidence found that yellow tang, *Zebrasoma flavescens*, larvae have been able to drift 180 km from marine protected areas (MPAs) to less populated reefs that are open to fishing (Christie et al., 2010). However, successful larval settlement depends on numerous factors, including natal population abundance, parental care, distance from the spawning site, transport processes, swimming ability, predation, habitat suitability, and the capacity to reproduce in new environments (Feary et al., 2014). Despite these limitations to range expansion, many tropical fish species have been making poleward shifts in their distributions (Feary et al., 2014).

A species of reef fish, the Indo-Pacific sergeant, *Abudefduf vaigiensis*, has expanded into the waters of the Hawaiian Archipelago and is hybridizing with the endemic Hawaiian sergeant, *Abudefduf abdominalis* (Coleman et al., 2014; Maruska & Peyton, 2007). The *A. vaigiensis* species can be found all across the Indo-Pacific ranging from the Red Sea and the east coast of Africa to Japan and northern New Zealand (Randall, 2007), and was first reported in 1991 in the Hawaiian Archipelago (Mundy, 2005; Randall, 2007). Conversely, *A. abdominalis*, is endemic to the Hawaiian Archipelago (Randall, 2007). The reason for *A. vaigiensis* expansion into the Hawaiian Archipelago is unknown, but *A. vaigiensis'* native range experiences warmer median sea surface temperatures than in the Hawaiian Archipelago (Froese & Pauly, 2024). The median SST experienced by *A. vaigiensis* across its global range is 27.2°C, while for *A. abdominalis* along the Hawaiian Island chain the median SST experienced is 25.3°C (Froese & Pauly, 2024). According to the Hawaiian Ocean Time-series, the maximum and minimum SST was 26°C and 23°C, respectively, in the year 1990 one year before *A. vaigiensis* was first documented in Hawaii (Winn et al., 1990). Hawaiian surface air temperature and the frequency of heat waves in the archipelago, which contribute to increase SST and MHW, have been increasing since the 1980s (Zhu & Li, 2017). As *A. vaigiensis* expanded into the Hawaiian Archipelago in the 1990s, it is possible that rising SST may have aided in that expansion.

In addition, the distinct temperature differences between the warmer Main Hawaiian Islands, MHI, and the cooler Northwest Hawaiian Islands, NWHI, may be influencing the relative species ratios of the non-native species and native species across the archipelago. *Abudefduf vaigiensis* and *A. abdominalis* do not exist in equal ratios across the Hawaiian Archipelago; in the MHI population densities of *A. vaigensis* are equal to or higher than *A. abdominalis*, but *A. abdominalis* outnumbers *A. vaigiensis* by 2:1 in the NWHI (Coleman et al., 2014). Eight months of the year, the NWHI are cooler than the MHI by more than 1°C (Rooney et al., 2008). The summer season SST in the NWHI is equal to or slightly warmer than the MHI due to decreased trade winds and mixing (Rooney et al., 2008). In the winter season the NWHI experiences a SST 6°C lower than the MHI (Rooney et al., 2008). On average, SST in the NWHI is ~24.3°C and is ~25.5°C in the MHI SST (Gove et al., 2022; PacIOOS, n.d.). From 1900 to 2018, average SST in the MHI has increased by 1°C as well as experienced five marine heat waves in the last 20 years (NOAA Integrated Ecosystem Assessment Program, n.d.). Any biological differences between species, as defined by thermal preference and thermal limits, may explain why the non-native and endemic species exist in different ratios across the archipelago and why *A. vaigiensis* was able to establish populations in the Hawaiian Archipelago.

To determine if temperature is a factor in *A. vaigiensis* colonization and distribution across the Hawaiian Archipelago, temperature preference and thermal tolerance experiments were performed on *A. vaigiensis*, the endemic *A. abdominalis,* and their hybrids. In their natural environments, fish experience temperatures that they prefer, tolerate, or resist, and the temperatures that they prefer can be correlated to optimal growth (Jobling, 1981). 'Final preferendum' or temperature preference (T_{pref}) is defined as the temperature that a fish will behaviorally choose when exposed to a thermal gradient over time, and T_{pref} experiments can indicate the optimal temperatures for fish, or where fish might move if temperature changes (Fry, 1947; Reynolds & Casterlin 1979). A fish's T_{pref} can be a narrow or wide range of temperatures depending on a population's natural evolution regarding habitat and food availability, inter/intra-competition, and predation intensity within their environment (Beitinger & Fitzpatrick, 1979). The upper thermal tolerance limit, or critical thermal maximum (CT_{max}) is the temperature at which movement failure occurs (Morgan et al., 2018). CT_{max} is defined as the "thermal point" where locomotion is disrupted, there is an inability to coordinate movement, and death is inevitable if kept in that thermal condition (Cowels & Bogert, 1944; Ern et al., 2023).

CT_{max} can be used to assess performance during exposure to higher temperatures (Desforges et al., 2022; Ørsted & Overgaard, 2022). CT_{max} and critical thermal minimum (CT_{min}, lower "thermal point" where locomotion is disrupted) are known to be "survival bottlenecks" for ectotherms as SST above and below a species CT_{max} and CT_{min} are lethal (Vinagre et al., 2019; Downie et al., 2025). To determine CT_{max}, fish are exposed to gradual increases in temperature over time until there is loss of equilibrium (LOE) which is the point where coordinated movement stops due to a failure to control and contract the muscles responsible for maintaining balance (Ern et al., 2023).

I hypothesized that if differences in thermal biology, specifically temperature preference and upper thermal limit, along with increasing SSTs have aided the expansion of the tropical Indo-Pacific sergeant into the habitat of the sub-tropical Hawaiian sergeant, then T_{pref} and CT_{max} of *A. vaigiensis* will be higher than *A. abdominalis*. I also examined the responses of the hybrids, for comparison to the pure, parental species and hypothesized that hybrid T_{pref} and CT_{max} will fall in between T_{pref} and CT_{max} measurements of the pure, parental species.

Materials and Methods

To address my hypothesis, that thermal biological differences, temperature preference and upper thermal limit, has aided the expansion and establishment of the tropical Indo-Pacific sergeant into the habitat of the sub-tropical Hawaiian sergeant, I examined the T_{pref} of both *Abudefduf vaigiensis*, *Abudefduf abdominalis* and their hybrids, by using an annular preference chamber, and determined CT_{max} through gradual increases in temperature until LOE. Species and hybrids were identified using fin clips to perform DNA analysis.

Collection and Treatment

A total of 40 *Abudefduf* spp. were collected on Oʻahu from Heʻeia Kea Boat Harbor, Kaupō Beach Park, and Aloha Tower Pier 7. Thirty of these were collected from Heʻeia Kea Boat Harbor due to the abundance and catchability in that location. Fish were collected using a hook and line with bread balls as bait. Collected fish were placed into 19 L buckets, with no more than four per bucket, fitted with lids and air stones to aerate the water during transportation to Hawaiʻi Pacific University's Makapuʻu Campus in Waimānalo, HI. To protect against the introduction of pathogens to the Makapuʻu Campus and water system, each fish was treated with a 5 min freshwater dip followed by a 30 min treatment with 100 ppm hydrogen peroxide and 0.5 ml/L of 1% methylene blue solution in seawater. After this treatment, the fish were held in individual 14 L flow-through indoor tanks supplied with a continuous flow (~1 L/min) of seawater at the Hawaiʻi Pacific University Makapuʻu Campus. The fish were kept at ambient temperatures (~26.5°C), each with an air stone, a PVC tube (housing structure for the fish), a 12 h:12 h light-dark photoperiod, and were allowed to acclimate for at least one week before experimentation. The fish were fed ~1 gram of 3 mm sinking pellets (New Life Spectrum Large Fish Formula) once per day. The fish were fasted for 24 h before experimentation.

Species Identification

DNA extraction - After T_{pref} and CT_{max} experiments, I euthanized the fish with an overdose of tricaine methansulfonate (250 mg/L in buffered seawater). The fork length was measured and the number of both dorsal and anal soft fin rays were counted. A fin clip from the caudal fin was placed in a sample tube filled with 70% ethanol (EtOH). To isolate total genomic DNA from finclip samples, I performed the HotSHOT DNA extraction method (Meeker et al.,

2007) or I used the OMEGA Bio-tek E.Z.N.A. Tissue DNA Kit (OMEGA Bio-tek, Norcross, GA, USA).

PCR **-** Protocols and procedures for PCR were based on Coleman et al. (2014), Hassan et al. (2002), Song et al. (1998), and Taberlet et al. (1992). 120 bp of the nuclear gene for gonadotropin-releasing hormone 3 (GnRH3-3) was amplified using the primers GnRH3F (5'-GCCCAAACCCAAGAGAGACTTAGACC-3') and GnRH3R (5'-TTCGGTCAAAATGACTGGAATCATC-3'), and 129bp of the nuclear gene for glyceraldehyde-3-phosphate dehydrogenase were amplified using the primers Gpd2-F5 (5'-AGCCTGGAGGCACGACGACA-3') and Gpd2-R5 (5'AGGCAGAACGGATGATGCAGGA-3'). These genetic regions were identified as having fixed DNA base pair differences between the two species, and had previously been used to distinguish the two species and identify their hybrids (Coleman et al., 2014). The GnRH3 and Gpd2 reaction mixtures for PCR were as follows: 1 ul of the forward and reverse primer, 0.5 ul of BSA (4 mg/ml), 10 ul Go-Taq green, 5.5 ul of milliQ water, and 2 ul of DNA extract. The thermocycler steps for GnRH3 and Gpd2 were as follows: 94°C for 5 min, then 35 cycles of denaturing at 94°C for 30 sec, then 58°C for 30 sec, an extension at 72°C for 30 sec, a final extension at 72°C for 10 min, and finally the samples were held at 15°C (T100 Thermal cycler, *BIO-RAD*). To identify maternal lineage, the ~574 bp cytochrome b gene (cyt b) was amplified using the primers Cytb-F (5'-GTGACTTGAAAAACCACCGTTG-3') and Cytb-R (5'-CACTGAACTTTTTGGTGGCAAC-3'). The Cytb reaction mixture for PCR was as follows: 1 ul of the forward and reverse primer, 0.5 ul of BSA (4 mg/ml), 10 ul Go-Taq green, 5.5 ul of milliQ water, and 2 ul of DNA extract. The steps for the thermocycler were as follows: 94°C for 4 min, then 35 cycles of denaturing at 94°C for 1 min, annealing at 50°C for 45 sec, an extension at 72°C for 45 sec, a final extension at 72°C for 10 min, and lastly the samples are held at 15°C (T100 Thermal Cycler, *BIO-RAD*). The Cytb gene for each individual was amplified to identify the maternal lineage.

Sequencing **-** To confirm the successful PCR amplification of sufficient quality DNA for each individual fish for each genetic assay, I ran each PCR product through a 2% agarose gel electrophoresis at 100 V, 1 Amp, 1 Watt for 30 min. I included a negative control PCR product to test for contaminants within my products, and I used a 1000 bp DNA ladder to visually confirm the size of the PCR product on the gel documentation system, (ChemiDoc XRS+, *BIO-RAD*). Samples were purified by incubating with 2 ul Exonuclease I and Shrimp Alkaline Phosphatase (ExoSAP) to 5 ul PCR product at 37°C for 30 min 85°C for 15 min, and held at 14°C. I sent the purified PCR product to the University of Hawai‘i Advanced Studies of Genomics, Proteomics and Bioinformatics sequencing facility, for sequencing. PCR products were Sanger sequenced on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Hybrid identification **-** Sequences for Cytb, Gpd2, and GnRH3 primers were edited and aligned in Geneious Prime (Geneious Prime 2023.1, Biomatters Ltd.). Sequences from forty pure *A. abdominalis* and forty pure *A. vaigiensis* were obtained from Richard Coleman at the Rosenstiel School of Marine, Atmospheric, and Earth Science of the University of Miami (Coleman et al., 2014). I aligned the eighty pure species samples and my forty samples to a single pure *A. abdominalis* sequence for each primer: GnRH3, Gpd2, and Cytb. The alignments were sent to the University of Miami for analysis using the programs PHASE v.2.1 (Stephens & Donnelly, 2003), DNASP v.5.10 (Librado & Rozas, 2009), STRUCTURE v.2.3.2 (Pritchard et al., 2000; Hubisz et al., 2009), and NEWHYBRIDS v.1.1 (Anderson & Thompson, 2002). These programs were employed using the same protocol as described in Coleman et al. (2014).

The allelic states of heterozygous sites in GnRH3 and Gpd2 were estimated with the Bayesian program PHASE v.2.1 (Stephens & Donnelly, 2003) as implemented in DNASP v.5.10 (Librado & Rozas, 2009) using the procedure described in Coleman et al. (2014). Pure parental species and hybrids were discerned using an assignment test with two methods: STRUCTURE and NEWHYBRIDS (Coleman et al., 2014).

STRUCTURE is a Bayesian method that categorizes individuals into distinct populations to estimate ancestry. The number of genetic clusters (K) was based on the results of STRUCTURE HARVESTER v.0.6.93 (Earl & von Holdt, 2012). For my data, $K = 2$, where cluster one was pure *A. vaigiensis* and cluster two was pure *A. abdominalis*. STRUCTURE analysis determined the estimated membership coefficients (Q) for each individual. Q-values were used to distinguish purebreds from hybrids; $Q \geq$ threshold ≤value (Tq) are purebred and all others are hybrids. Tq was determined by running a simulation of parental and hybrid classes using HYBRIDLAB v.1.0 (Nielsen et al., 2006) following Hasselman et al. (2014). For my data, $Tq = 0.94$ for both clusters. Each individual was given two Q-values, one for each cluster ($Q_{Ava}=$ *A. vaigiensis* and $Q_{Aab}=$ *A. abdominalis*). Q_{Ava} was used as an estimate of the proportion of the genome in each individual that came from *A. vaigiensis* versus *A. abdominalis*. The values of Structure Q_{Ava} were used for statistical analysis as a measure of genetic admixture between the two parent species.

NEWHYBRIDS uses Markov chain Monte Carlo simulations in a Bayesian setting to calculate posterior probabilities, P, of an individual belonging to one of six clusters Parental 1 (P1), Parental 2 (P2), F1 (P1 9 P2), F2 (F1 9 F1), P1 9 F1 backcross (Bx1), or P2 9 F1 backcross (Bx2). The analysis was performed following the protocol from Coleman et al. (2014). A threshold probability value, Tp, was determined for NEWHYBRIDS using HYBRIDLAB as described above. For my data, Tp = 0.85 for the six clusters. $P \geq 0.85$ for the Parental 1 and Parental 2 clusters were defined as purebred. $P < 0.85$ for the Parental 1 and Parental 2 clusters were defined as hybrids.

When grouping individuals into species and hybrid groups, results for both STRUCTURE and NEWHYBRIDS were considered. Purebreds were defined as having a $P \geq 0.85$ for either Parental 1 or Parental 2 cluster (NEWHYBRIDS) AND $Q_{Ava} \geq 0.94$ or $Q_{Aab} \geq 0.94$ (STRUCTURE). If $Q < Tq$ for STRUCTURE, $P < Tq$ NEWHYBRIDS, or both, the individual was classified as a hybrid. Hybrid relatedness to the parent species was assessed using Structure Q_{Ava}.

Temperature Preference

Annular temperature chamber **-** In order to determine thermal behavior, I tested both species of *Abudefduf* and the hybrids using an annular preference chamber with a temperature gradient of 22 to 32°C over a 24 h period. The chamber design was based on Myrick et al. (2004) and Schram et al. (2013) and is fully described in van Hall (2021) and van Hall & Korsmeyer (in press) (Fig. 1; Fig. 2).

The annular preference chamber was a circular tank with four concentric walls, making three separate channels and an opening for temperature sensors at the center (Figs. 1 & 2). The circular shape is useful for fish with delicate fins and creates a uniform interior among all subsections. The first, outermost channel had a diameter of 90 cm and received water from five temperature-controlled reservoirs. The second channel was the swimming channel, where the fish were placed and remained during the experiment. The walls of the swimming channel were perforated with holes to allow water flow from the mixing channel and to the third, innermost effluent channel, which returned it to the temperature-controlled reservoirs. The swimming channel was 10 cm wide with a water depth of 10 cm and a central circumference of 220 cm. The first channel, which receives water, and the third channel that returns water have eight partitions to separate the water from the temperature-controlled reservoirs. The swimming channel lacks these partitions, but had 0.8 mm wide clear polycarbonate dividers that extended into the top 1 cm of water in the swim channel to reduce shearing forces and water mixing.

Each of the eight primary sections of the annular chamber, labeled A-H, were divided into three subsections, creating a total of 24 temperature zones/subsections: A1, A2, A3, B1, B2, etc. During an experiment, the temperatures in the eight primary sections were monitored continuously by *DS18b20* digital temperature sensors and recorded to the computer. Additionally, after the 24 h preference experiment, the temperature of the 24 subsections were recorded by hand using a digital thermometer (Component Design Northwest, model DTW450L), mid-channel at mid-depth, three times and then averaged for each of the subsections.

Reservoirs **-** There were five 45 L temperature-controlled reservoirs connected to the annular chamber (Fig. 2). The water temperature in these reservoirs was controlled by a total of 1,600 Watts (W) of submersible heaters and two ¼ horsepower (hp) water chillers. Submersible heaters and water circulated through titanium coils submerged in a chilled water bath were controlled by *Bayite* temperature controllers and allowed for a specific temperature to be maintained in each reservoir. Two chillers, *Tank Chiller Line TK 1000* and *Arctica Titanium Chiller*, were used to cool the water in a ~115 L water bath. Each reservoir contained an airstone to ensure fully aerated water within all sections of the annular chamber. Water was pumped from the reservoirs to the annular chamber at ~5.0 L/min to create a temperature gradient from 22 to 32°C in the swim channel.

Camera setup **-** To record the fish during the experiment, a USB camera (1 megapixel, 3.6 mm lens, ELP USB100W05MT-DL36) was centered 191 cm above the chamber. There were eight 2 W LED lights hung in a ring 162 cm above the swim channel to ensure uniform light during the day (0600-1800 h). At night (1800-0600 h), the camera's built-in infrared lights and one 1.5 W blue LED were used to illuminate the chamber. The bottom of the swim channel was covered with white vinyl prismatic reflective sheeting (3M) to enhance the contrast of the fish against the background. The annular temperature chamber was surrounded by a black curtain to exclude outside disturbances and reduce bias of subsection selection by visual surroundings. The software program *H264 Web 4.0* was used to capture a 9000-frame video every 15 min for the duration of the experiment.

Experimental design **-** To perform T_{pref} experiments, the annular preference chamber was filled with seawater at ambient temperature (~26.5°C) and a fish was placed into one of the primary sections using a random number generator (between 1 and 8) representing one of the primary sections A to H. The fish was allowed to acclimate for one hour with no temperature gradient and no video recording. After the hour acclimation, a control 'no-gradient' usage period (no temperature gradient) was video recorded. After the one hour 'no-gradient' usage period, the temperature controllers for the reservoirs were turned on, allowing the reservoirs and the chamber to warm or cool to their set temperatures. Once a stabilized temperature gradient from 22 to 32°C was achieved (~1 hour), the time was noted and the recording of the 24 h preference experiment began.

After 24 h, the fish was removed from the chamber and was air exposed (< 1 min) to measure fork length (cm), depth (cm), and mass (g). The fish was returned to its original flow-through tank. The temperature gradient in the annular chamber and the temperature of the reservoirs was recorded using the hand-held digital thermometer. After four experiments were performed in the annular chamber, the polarity of the temperature gradient was flipped to minimize bias of any tank feature influencing the fish's location among replicates. After each experiment, the annular chamber and reservoirs were sanitized with bleach and rinsed with freshwater.

Data processing **-** The videos of the 'no-gradient' usage and the '24 h' periods were analyzed using *idTracker.es* (Pérez-Escudero et al., 2014) or *FastTrack.exe* (Gallois & Candelier, 2021). Both software programs lay a coordinate grid over top of the video recording from the experiments. The software tracked each fish's position (x and y coordinates) in the annular chamber and used each frame as a time reference. The software provided the coordinates, time series, and a visual display of the fish's motion throughout time. Based on the tracked coordinates, the location of the fish every 20 seconds was assigned to a location of one of the 24 subsections, and the temperature in that subsection was assigned to that position at that time. We looked at the fish's position every 20 seconds to minimize autocorrelation between samples and to ensure each measurement was semi-independent. Fish were tracked using the same method as the experimental period for the 'no-gradient' usage period, but there was no temperature gradient present. Although the temperature was uniform throughout the chamber during the 'no gradient' usage period, fish positions were assigned to the eventual temperatures of the zones after the gradient was established. These data were used to determine if there was a bias in zone selection that was not related to the temperature gradient and for comparison of zone usage during the 24 h preference test.

Temperature Preference Statistical Analysis

Individual preference **-** The median temperature selected by the fish represented the T_{pref} with the first and third quartile temperatures (Q1 and Q3, respectively) representing the lower and upper thermal preference zone (Magnuson et al., 1979). The median has commonly been used to report thermal preference and the interquartile range IQR (Q1 - Q3) has been used to report preference as thermal range, as preference cannot be defined as one temperature (Magnuson et al., 1979; Behrens et al., 2012; Reiser et al., 2013). Quartiles in these experiments are often used to define the temperatures that the individual will avoid (Magnuson et al., 1979; Schurmann et al., 1991). The first quartile, Q1, in my experiments was defined as lower avoidance temperature (T_{avoid}) and the third quartile, Q3, was defined as the upper T_{avoid}. The interquartile range (IQR) represented the size of their thermal preference range. The median, Q1, Q3, and IQR were determined for each hour of the experiment, as well as for two specific time periods: the no temperature gradient/control ('no-gradient' usage) and the full '24 h' preference selection after the gradient was established.

Model comparisons - For comparison, a median, Q1, Q3, and IQR was also determined for each individual based on the available temperatures in all 24 subsections during testing. These 'available temperatures' data modelled the fish spending an equal amount of time at each subsection based on 180 equally distributed values (to match the number of observations in one hour). The 'available temperatures' model represented what the results would look like if the fish was moving at random and not showing a T_{pref} ('non-choosing'), and this was included in the statistical analyses as one of the 'time periods' for each fish. The 'available temperatures' and 'no-gradient' usage periods acted as controls for comparison to the experimental data.

Fish temperature selections, as defined by the median, Q1, and Q3, during each hour of the preference experiment were analyzed with a quantile regression to test for significant difference between both the ‘available temperatures’ and ‘no-gradient’ usage periods. For each hour of the ‘24 h’ preference period, the distribution of temperatures was tested to ensure that they were significantly different from both the ‘available temperatures’ and ‘no-gradient’ usage periods, indicating that the fish was selecting for temperatures. If the hour median, Q1, and Q3, together, were not significantly different from either the ‘available temperatures’ or ‘no-gradient’ usage periods, then the hour was removed and declared a ‘non-choosing’ hour (Behrens et al., 2012; Christensen et al., 2021, van Hall, 2021; van Hall & Korsmeyer, in press). Including ‘non-choosing’ hours would skew the median towards the median tank temperature (~27°C) and would not represent thermal preference selection. If fish were actively selecting for the median tank temperature, those hours would still be included because the Q1 or Q3 would be significantly different from the ‘available temperatures’ distribution, so they would not be considered ‘non-choosing.’

Mass was included as a covariate in all statistical analyses because mass was found to have a significant effect on both IQR and CT_{max} and, therefore, could have influenced the T_{pref} Q1, median, and Q3. In summary, for each fish, or replicate, a median, Q1, Q3, and IQR was determined for each time ‘period’: ‘available temperatures,’ ‘no-gradient’ usage, and ‘24 h preference.’ Any ‘non-choosing’ hours were removed before analysis.

Marginal linear model - Marginal linear models (MLM; West et al., 2014) were used to test T_{pref} median, Q1, Q3, and IQR for the effect of time period, mass and Structure Q_{Ava}, as an estimate of the proportion of the genome in each individual that came from *A. vaigiensis* versus *A. abdominalis*. The following interactions were also included in the MLM: period × Q_{Ava} and period × mass. The three time periods included the two controls (‘available temperatures’ and ‘no-gradient’ usage), and the combined ‘24 h’ preference test. Period was included as a repeated measure with individuals as subjects. For the MLM, the mass term was first centered by subtracting the average mass (32.8 g) from all mass values. The covariance structure chosen was Compound Symmetry: Heterogeneous based on the lowest value of Hurvich and Tsai’s Criterion (AIC_C). Follow up pairwise comparisons among periods were conducted at the average Structure Q_{Ava} (=0.62) and were corrected for multiple comparisons using the Holm-Bonferroni sequential correction. For plotting, the mass-corrected IQR was calculated to standardize all individual masses to a common mass, 32.8 g, using the mass coefficient from the MLM [mass-corrected IQR = IQR - 0.015 * (32.8 - mass)], removing mass as a factor to examine the relationship between IQR and Q_{Ava} alone.

Compositional analysis - The second method, compositional analysis of habitat use, corrected for potential biases in the availability of temperature ranges within the annular chamber (Schram et al., 2013; van Hall, 2021; van Hall & Korsmeyer, in press). I performed a compositional analysis to compare temperature use to available temperatures and for differences among the species/hybrids using *R-studio* with the "*adehabitatHS*" package installed; I modified code written by E. van Hall & Korsmeyer (in press), to fit my data (RStudio Team, 2023; Aebischer et al., 1993; Calenge, 2006; Schram et al., 2013; van Hall, 2021; van Hall & Korsmeyer, in press). Available temperatures in the annular chamber were divided into five 2°C bins from 22 - 32°C (i.e., < 24, 24-26, 26-28, 28-30, > 30°C) and preference ratios (proportion used / proportion available) were calculated to determine relative usage of the available temperature zones during all control and experimental periods on account that the temperature zones were not equally distributed throughout the annular preference chamber. A preference ratio greater than 1.0 indicated that that zone's proportional usage was higher than the proportion available, or a preference for that temperature zone. A preference ratio less than 1.0 indicated selection against that temperature zone. Preference ratios in the ‘no-gradient’ usage and ‘24 h’ preference period were tested to determine if these periods were non-random using the ‘compana’ command in the adehabitatHS package in RStudio (RStudio Team, 2023; Calenge, 2006). If there was non-random usage during either period, temperature bins were ranked from least to most preferred, and the program determined which ranks were significantly different (Aebischer et al., 1993; van Hall & Korsmeyer, in press). Lambda was used as a measure of habitat selection, where lower values suggest stronger selection, and significant *p*-values indicated significant selection where fish would actively prefer or avoid certain temperature zones during a certain period. Geometric means were used to present the overall preference ratios in the figures because of the log-normal distributions of the probability ratios (van Hall, 2021; van Hall & Korsmeyer, in press).

Critical Thermal Maximum (CT_{max})

Tank setup - In order to determine the upper critical temperature of both species and the hybrids, I conducted CT_{max} experiments with a heating rate of 0.1°C min^{-1} (6°C h^{-1}) The design for the CT_{max} chamber was adapted from those used by Dr. Jacob Johansen’s Fish Resilience Program at the Hawai‘i Institute of Marine Biology, University of Hawai‘i (Vaughan et al., 2025) (Figs. 3 & 4).

Modified, insulated water coolers were used for the experimental tank and the warm water reservoir (Figs. 3 & 4). The experimental tank contained two submersible water pumps positioned in diagonally opposite corners to ensure good circulation and mixing during temperature ramping. An additional pump in the experimental tank directed water through an aeration tower and bubble trap to maintain air-equilibrated oxygen saturation. During experimentation, the fish were held in perforated plastic baskets (24.0 cm x 11.5 cm x 12.5 cm; L x W x H) with floats (cut up pool noodles) attached to the top of each corner, so they did not interfere with the recirculating pumps at the base of the tank. The size of the baskets prevented the fish from continuous forward swimming and using inertia to prevent the onset of LOE. Each basket was covered with clear plastic mesh secured by a rubber band. The temperature in the experimental tank was increased by pumping in heated water from the reservoir tank. The overflow of the experimental tank led to the reservoir, where water temperature was maintained at 45°C using *Baylite* temperature controllers. The reservoir contained an air stone, two heaters totaling 1000 W, and a pump, *EHEIM: 1048*, to bring heated water to the experimental tank. The tubing supplying the experimental tank with heated water extended to the bottom of the experimental tank to ensure that the heated water did not directly contact any fish until it was mixed and circulated by the recirculating pumps. Temperature inside the experimental tank was monitored by a digital temperature probe and recorded by a custom designed computer program (*LabVIEW* 2020, National Instruments, 2020). The software controlled a computer-actuated solenoid to activate the pump from the reservoir to regulate the rate of heating in the experimental tank to 6°C h^{-1} (0.1°C min^{-1}). Overflow from the experimental tank was returned to the reservoir. A USB camera affixed over the experimental tank was used to monitor and record the fish during the experiment.

Experimental design - The size of the experimental tank allowed me to test three individuals at a time using three separate floating baskets. A single fish was placed in each basket in the experimental tank 24 h before the experiment began allowing the fish to acclimate to the experimental conditions. During this time the experimental tank received a flow-through of water from the same source as the holding tanks (~26.5°C). The experiment began by stopping the flow of incoming seawater, and activating the software to begin pumping heated seawater from the reservoir into the experimental tank to raise temperature by 0.1°C min^{-1} (6°C h^{-1}). As the fish were warmed, they eventually experienced LOE. The temperature and time during LOE was noted for each fish. To ensure that each fish was being pulled out of the experiment at the correct time/temperature, I defined LOE as the point at which coordinated swimming failed and the fish rolled to its side for at least 5 seconds. After the fish experienced LOE, the basket they were in was quickly removed from the experimental tank and were placed into their original flow-through holding tank for recovery. The temperature at LOE (CT_{max}) was verified by reviewing the video recording and simultaneously recorded tank temperatures.

Data processing and statistical analysis - A multiple linear regression was performed to test the effect of estimated membership proportion of the genome to *A. vaigiensis*, Structure Q_{Ava}, on CT_{max}, with mass as a covariate (*SPSS* v.28, IBM Corp., 2021). For plotting, a mass-corrected CT_{max} was calculated to standardize all individual masses to the average mass, 32.8 g, removing mass as a factor to examine the relationship between CT_{max} and Q_{Ava} alone. This correction was done by using the mass coefficient (-0.0118) from the regression analysis [mass-corrected CT_{max} = CT_{max} - 0.0118 * (32.8 - mass)].

RESULTS

DNA Analysis

Twenty-four individuals were classified as hybrids with Q-values < Tq (0.94) for STRUCTURE and $P < Tp$ (0.85) for NEWHYBRIDS. Thirteen individuals were classified as *A. vaigiensis* with $Q \geq 0.94$ (STRUCTURE) and $P \geq 0.85$ (NEWHYBRIDS). One individual was classified as *A. abdominalis* with $Q \geq 0.94$ (STRUCTURE) and $P \geq 0.85$ (NEWHYBRIDS). Two individuals were classified as *A*. *abdominalis* with $Q \geq 0.94$ (STRUCTURE), but were classified as hybrids by $P \leq 0.85$ (NEWHYBRIDS). Since STRUCTURE and NEWHYBRIDS analyses did not result in the same classification, these two individuals were conservatively classified as hybrids. The species totals were 26 hybrids, 13 *A*. *vaigiensis*, and 1 *A*. *abdominalis* (Table 1).

Temperature Preference

Fish behavior - Fish behaviors during the first hour of experimentation, the 'no-gradient' usage period, varied greatly among the forty individuals. Many fish explored all areas of the swimming channel; however, some fish remained in relatively the same spot that they were randomly placed for the 'no-gradient' usage period. Selection of a certain region in the swimming channel during the 'no-gradient' usage period was not anticipated. This time interval acted as an experimental control, and showed preference and avoidance for a certain region in the swimming channel when no temperature gradient was established. The results for the 'no-gradient' usage period accounted for selection of regions of the annular chamber based on variables other than temperature. If fish showed the same location distribution during hours of the '24 h' preference period as in 'no-gradient'usage period, those hours were removed from further statistical analyses ('non-choosing' hours).

Swimming behavior during the experimental period ('24 h' preference) also varied. Out of the forty individuals tested, twenty-five individuals expressed temperature selection across all 24 hours of the experiment, and fifteen individuals had at least one 'non-choosing' hour that was not significantly different from either the 'available temperatures' or 'no-gradient' usage period. Three fish displayed 'non-choosing' behavior for more than half of the 24 h experiment. The single *A*. *abdominalis* only showed preference during seven hours (17 'non-choosing' hours) (Fig. 5). *Abudefduf vaigiensis* only had two individuals with 'non-choosing' hours; one individual had two ‘non-choosing’ hours (13:00 and 20:00) and the other individual only had a single ‘non-choosing’ hour (12:00) (Fig. 6). There were a total of ten hybrid individuals who expressed at least one ‘non-choosing’ hour (Fig. 7). There were two hybrids with several ‘non-choosing’ hours. The first hybrid only showed preference for eight hours (16 ‘non-choosing’ hours), and the second only showed preference for ten hours (14 ‘non-choosing’ hours) of the ‘24 h’ period (Fig. 7).

Median and quartiles analysis

Medians - The average (±s.d.) 24 h median T_{pref} for *A. vaigiensis* was 27.4 ± 0.9°C and was 27.0 ± 1.4°C for hybrids (Table 2). The single *A. abdominalis* ‘24 h’ median T_{pref} was 24.9°C (Table 2). Because of the high variability among the hybrids, the Structure Q_{Ava} value was used in the statistical models as an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero). In addition, because of the wide range of masses that were not equally distributed among the species groups, mass was included as a covariate.

Overall, there were no significant effects of mass, periods (‘available temperatures’, ‘no-gradient’ usage, & ‘24 h’ preference), or Q_{Ava}, or the interactions of period × Q_{Ava} and period × mass on median T_{pref} (MLM, p>0.05; Table 3). Although the overall interaction of period × Q_{Ava} was not significant [F(2, 51.2)=2.7, p = 0.08], there was a significant simple interaction of ‘24 h’ preference period × Q_{Ava}, compared with the ‘available temperatures’ period × Q_{Ava} (p = 0.04, Table 3). This result suggests an effect of Q_{Ava} on median T_{pref} only during the 24 h preference test with an effect size of a 1.2°C increase in median T_{pref} as Q_{Ava} ranges from 0 (*A. abdominalis*) to 1.0 (*A. vaigiensis*) (based on Q_{Ava} and ‘24 h’ period × Q_{Ava}

parameter estimates (Table 3; Fig. 8)). Estimated marginal means (±SE) for median T_{pref} at the average mass (= 32.8 g) was 26.3 ± 0.4°C for *A. abdominalis* (Structure Q_{Ava} = 0) and 27.6 ± 0.3°C for *A. vaigiensis* (Structure Q_{Ava} = 1) (Table 3; Fig. 8).

Pairwise comparisons of medians across time periods at the average Q_{Ava} (0.62) and average mass (32.8 g) revealed no significant difference between the 'no-gradient' usage and the 'available temperatures' control ($p = 0.116$, mean difference (MD) = 0.4°C), but a significant difference between the '24 h' preference period and 'available temperatures' ($p = 0.036$, MD = 0.5°C). The medians during the 'no gradient' usage and '24 h' preference were very similar and not significantly different ($p = 0.74$, MD = 0.1°C) (Table 2). These results suggest that the fish were selecting a median T_{pref} that was 0.5°C higher on average than what was modeled in 'available temperatures.'

First quartile, Q1 - For the first quartile (Q1), or lower T_{avoid}, during the '24 h' preference test, the average (±s.d.) for *A. vaigiensis* was 26.6 ± 0.8°C and 25.6 ± 1.8°C for hybrids (Table 2). The single *A. abdominalis* '24 h' lower T_{avoid} was 23.7°C (Table 2). Overall, there was no significant effect of mass, or the interaction period × mass, but the interaction period × Q_{Ava} was significant [$F(2, 38.6) = 5.05$, $p = 0.011$], indicating that the effect of Q_{Ava} on Q1 varied with the time period. The only significant simple interaction was for the '24 h' period × Q_{Ava}, compared with the 'available temperatures' period × Q_{Ava} ($p = 0.003$, Table 4). This result indicates a significant effect of Q_{Ava} on Q1 during the '24 h' preference test, with an effect of a 2.2 °C increase in lower T_{avoid} (Q1) as Q_{Ava} ranges from 0 (*A. abdominalis*) to 1.0 (*A. vaigiensis*) (Table 4; Fig. 8). Estimated marginal means (±SE) for the lower T_{avoid} (Q1) at the average mass (= 32.8

g) was 24.6 ± 0.5°C for *A. abdominalis* (Structure Q_{Ava}= 0) and was 26.7 ± 0.3°C for *A. vaigiensis* (Structure Q_{Ava}= 1) (Table 4; Fig. 8).

Pairwise comparisons of periods at the average Q_{Ava} and mass found significant differences in lower T_{avoid} between the '24 h' preference and 'available temperatures' periods ($p < 0.001$, MD = 2.1°C) and between '24 h' and 'no-gradient' usage periods ($p = 0.002$, MD = 1.1°C,). There was also a significant difference between 'available temperatures' and the 'no-gradient' usage periods (p = 0.001). These results suggest that the fish were selecting a lower T_{avoid} that was 2.1°C higher than the 'available temperatures' Q1.

Third quartile, Q3 - The upper edge of the T_{pref} zone (Q3), or upper T_{avoid}, during the '24 h' period average (±s.d.) was 28.4 ± 1.0°C for *A. vaigiensis* and was 28.3 ± 1.2°C for hybrids (Table 2). The single *A. abdominalis* '24 h' upper T_{avoid} (Q3) was 26.6°C (Table 2). Overall, there was no significant effect of mass, Q_{Ava}, the interactions period × mass, and period × Q_{Ava}, but time period was significant [F(2, 58.4) = 7.65, $p = 0.001$] (Table 5; Fig. 8). Estimated marginal means (±SE) of the upper T_{avoid} (Q3) at the average mass was 27.8 ± 0.4°C for *A. abdominalis* (Structure Q_{Ava}= 0) and was 28.6 ± 0.3°C for *A. vaigiensis* (Structure Q_{Ava}= 1), though they were not significantly different (Table 5; Fig. 8).

Pairwise comparisons of periods at the average Q_{Ava} and mass showed significant differences in upper T_{avoid} between '24 h' and 'available temperatures' periods ($p < 0.001$, MD = -1.1°C) and between '24 h' and 'no-gradient' usage periods ($p = 0.006$, MD = -0.7°C). There was also a significant difference between 'available temperatures' and 'no-gradient' usage periods ($p = 0.041$, MD = -0.4°C). These results suggest that the fish were selecting an upper T_{avoid} that was 1.0°C lower than the 'available temperatures' Q3.

Interquartile range, IQR - The average (±s.d.) size of the interquartile range (IQR), or T_{pref} zone, during the '24 h' period was 1.7 ± 0.6°C for *A. vaigiensis* and was 2.7 ± 1.4°C for hybrids (Table 2). The single *A. abdominalis* '24 h' IQR was 2.9°C (Table 2). There were significant interactions between both period × mass [F(2, 37.5) = 5.214, $p = 0.010$] and the period × Q_{Ava} [F(2, 37.5) = 5.659, $p = 0.007$]. There were significant simple interactions of '24 h' period × mass ($p = 0.045$, Table 6) and of the 'no-gradient' usage period × mass ($p = 0.029$, Table 6), compared with the 'available temperatures' period × mass. These results suggest an effect of mass on IQR, or T_{pref} zone, during the '24 h' period with an effect size of a 1.7°C increase over the mass range in this study (8.7 to 124.6 g = 116 g) (Table 6; Fig. 9). Conversely, the effect of mass on IQR during the 'no-gradient' usage period was of a 2.1°C decrease (Table 6). There was also a significant simple interaction between the '24 h' period × Q_{Ava}, compared with the 'available temperatures' period × Q_{Ava} ($p = 0.004$, Table 6) with an effect size of a 1.5°C decrease in IQR as Q_{Ava} ranges from 0 (*A. abdominalis*) to 1.0 (*A. vaigiensis*) (Fig. 10). This result indicates that the relatedness to each species impacts the temperature zones selected by the fish. Estimated marginal means (±SE) of the interquartile range (IQR) T_{pref} at average mass was 3.2 ± 0.3°C for *A. abdominalis* (Structure $Q_{Ava} = 0$) and was 1.9 ± 0.2°C for *A. vaigiensis* (Structure $Q_{Ava} = 1$) (Table 6; Fig. 10).

Pairwise comparisons of periods revealed there was a significant difference in IQR for both the 'no-gradient' usage ($p < 0.001$, MD = -1.4°C) and the '24 h' preference period ($p < 0.001$, MD = -3.2°C) compared with 'available temperatures.' The IQR was significantly smaller during the '24 h' period compared to 'no-gradient' usage ($p < 0.001$, MD = -1.8°C). .

This result suggests that the fish were selecting a temperature zone that was narrower from what was available in the annular chamber.

Compositional analysis - A non-parametric rank-based comparison of habitat selection was used to compare zone usage during the ‘no-gradient’ usage and ‘24 h’ periods for *A. vaigiensis* and hybrids as groups (Aebischer et al., 1993). *Abudefduf abdominalis* preference ratios were calculated but were not analyzed with this method as there was only one individual. Zones were defined by the available temperatures within the annular chamber and grouped as five 2°C bins from 22 - 32°C (i.e., < 24, 24-26, 26-28, 28-30, > 30°C). Preference ratios (proportion used / proportion available) were calculated to determine relative usage of the available temperature zones during the control and experimental periods on account that the temperature zones were not available equally.

For *A. vaigiensis*, the ‘no-gradient’ usage was non-random (lambda = 0.236; $p = 0.014$; Fig. 11). Despite the uniform temperatures in the ‘no-gradient’ period and regular reversal of the temperature polarity in the subsequent preference test, the eventual temperature zone < 24°C was significantly less used than the other zones, with an average preference ratio of 0.74 ($p < 0.05$; Fig. 11). These results suggest that *A. vaigiensis* individuals were not equally using all available space within the annular chamber when there was no temperature gradient established.

The zone usage during the ‘24 h’ preference period for *A. vaigiensis* was also non-random (lambda = 0.0419; $p = 0.002$; Fig. 11), but showed greater preferences that were different than during the ‘no-gradient’ period. The 26 - 28°C zone was significantly more preferred than all other zones (preference ratio 1.96, $p < 0.05$; Fig. 11). The least preferred temperature zone was < 24°C which was significantly different from all other zones (preference ratio 0.098, $p < 0.05$; Fig. 11). These results suggest that *A. vaigiensis* individuals were selecting for the mid-range temperature zones when a temperature gradient was established during the '24 h' period.

Similarly, for hybrids, the 'no-gradient' usage period was non-random (lambda = 0.591; $p = 0.016$), but with only small preference deviations from 1.0 (Fig. 12). The eventual '26 - 28°C,' '28 - 30°C,' and '> 30°C' were not significantly different from each other ($p > 0.05$; Fig. 12). The eventual '24 - 26°C' zone was avoided, and was significantly different from all other zones (preference ratio 0.69, $p < 0.05$; Fig. 12). The least 'preferred' eventual temperature zone was '< 24°C' (preference ratio 0.64, $p < 0.05$; Fig. 12). These results suggest that the hybrid individuals were not truly moving at random in the entire available space within the annular chamber when there was no temperature gradient established.

Zone usage during the '24 h' preference period for hybrids was also non-random (lambda = 0.213; $p = 0.002$), but with different usage than during the 'no-gradient' usage period. The 26 - 28°C was the most preferred temperature zone compared with all other zones (preference ratio 1.63, $p < 0.05$; Fig. 12). Other zones, 28 - 30°C, 24 - 26°C, > 30°C, showed avoidance and were not significantly different from each other ($p > 0.05$; Fig. 12). The least preferred temperature zone was < 24°C, which was significantly different from all other zones (preference ratio 0.28, $p < 0.05$; Fig. 12). These results suggest that the hybrid individuals were selecting for similar mid-range temperature zones as *A. vaigiensis*, on average, when a temperature gradient was established. The single pure *A. abdominalis* tested most preferred the 24 - 26°C thermal range (preference ratio 1.85) and showed the most avoidance for the 28 - 30°C thermal range (preference ratio 0.28).

Critical Thermal Maximum (CT_{max})

I performed a total of 36 CT_{max} experiments on 23 hybrids, one *A. abdominalis*, and 12 *A. vaigiensis* (Table 1). The average (± s.d.) CT_{max}, or upper thermal limit, for *A. vaigiensis* was 38.4 ± 0.1°C and was 37.7 ± 0.1°C for hybrids. The single *A. abdominalis* tested had a CT_{max} of 37.8°C. The multiple linear regression of CT_{max} with both Q_{Ava} and mass as factors was significant [F(2, 33) = 12.936, $R^2 = 0.439$, $p < 0.001$] with mass having a significant negative relationship (b = -0.0118, $p = 0.001$; Table 7). These results show that mass had an effect of a 1.4°C decrease on CT_{max} as mass increased from 8.7 to 124.6 g (Fig. 13). There was, however, no significant relationship between CT_{max} and Q_{Ava} ($p > 0.05$) (Table 7; Fig. 14). Estimated marginal mean for CT_{max} was 37.7 ± 0.2°C for *A. abdominalis* and was 38.1 ± 0.1°C for *A. vaigiensis*, although these results were not significantly different (Table 7; Fig. 14).

Discussion

To test if differences in thermal biology between species and increasing SST in the Hawaiian Archipelago has aided in the expansion of Indo-Pacific sergeant major, *A. vaigiensis*, into the habitat of native Hawaiian sergeant major, *A. abdominalis*, I determined T_{pref} and CT_{max} for a range of pure and hybrid individuals. T_{pref} experiments demonstrate a species behavioral choice for certain temperatures (Beitinger & Fitzpatrick, 1979; Reynolds & Casterlin, 1979), and CT_{max} experiments identify a species physiological upper thermal limit (Morgan et al., 2018; Ern et al., 2023). I had originally planned to compare results across species groups; however, since I caught only a single pure *A. abdominalis* this was not possible. Instead, I used Structure Q_{Ava} as an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero). This method still allowed me to examine differences in thermal biology but due to genetic relatedness rather than between species. I hypothesized that if differences in thermal biology, specifically temperature preference and upper thermal limit, along with increasing SSTs have aided the expansion of the tropical Indo-Pacific sergeant into the habitat of the sub-tropical Hawaiian sergeant, then T_{pref} and CT_{max} of *A. vaigiensis* will be higher than *A. abdominalis*. I also examined the responses of the hybrids, for comparison to the pure, parental species and hypothesized that hybrid T_{pref} and CT_{max} will fall in between T_{pref} and CT_{max} measurements of the pure, parental species. I found a significant positive effect of body mass on IQR and a negative effect on CT_{max} (Figs. 9 & 13), but differences in CT_{max} were not related to Q_{Ava} (Fig. 14). However, I found that the T_{pref} median and lower quartile (Q1), or lower T_{avoid}, was higher, and the preference zone (IQR) was narrower, the closer the genetic relatedness was to *A. vaigiensis* (Q_{Ava} = 1) (Figs. 8 & 10).

Although I could not compare average T_{pref} and CT_{max} results across species groups, these results partially support my hypothesis and suggest that the invasive *A. vaigiensis*, have a preference for warmer temperatures than the native *A. abdominalis*, which may have aided *A. vaigiensis*' expansion into Hawaiian habitats with increasing SST.

Temperature Preference

The usage of space in the preference chamber during 'no-gradient' usage periods were non-random for both *A. vaigiensis* and hybrids (Figs. 11 & 12), and mapping the eventual temperatures resulted in medians that were different from available temperatures, indicating unequal usage (Table 3). This outcome was not expected and suggests that some areas of the chamber may have been preferred even without a temperature gradient present, even though steps were taken to avoid this, such as introducing fish in random locations and reversing the polarity of the gradient. However, preference and avoidance that was shown during this period were smaller and different than what was shown during the ‘24 h’ preference test, therefore it appears once the temperature gradient was established, the temperatures were the primary factor affecting the fish’s location.

The lower T_{avoid} (Q1), median, upper T_{avoid} (Q3), and IQR were similar between both species and hybrids, and compositional analysis found the 26 - 28°C temperature zone was most preferred by both *A. vaigiensis* and hybrids, while preference ratios for the single *A. abdominalis* revealed a preference for a slightly lower range 24 - 26°C (Table 2; Fig. 11; Fig. 12). Similar preferences were expected as several studies have found that fish preferred temperatures similar to the temperatures they experience in their natural habitats (Nay et al., 2015; Norin et al., 2014; Habary et al., 2017), and both species and hybrids coexist within the same habitats and experience the same fluctuations in temperature. The temperature ranges preferred by both species and hybrids fall within average SST in Hawai‘i, 26 – 27 °C (Kappel et al., 2017; Wedding et al., 2018). The least preferred zone by both *A. vaigiensis* and hybrids was < 24°C, showing avoidance of low temperatures (< 24°C) , similar to SST on O‘ahu in winter seasons, 23 - 24°C (University of Hawai‘i PacIOOS, n.d.; Kappel et al., 2017). The second most avoided zone was > 30°C, similar to increased SST due to marine heat waves (MHW), specifically, the MHW known as “The Blob” that saw SSTs around West Hawai‘i rise to as high as 30.3 °C in 2015 (Hawai‘i Integrated Ecosystem Assessment, n.d.). Although *A. vaigiensis* and hybrids are able to tolerate the temperatures at either end of the 22 - 32°C temperature range, these temperatures are not preferred and would be selected against when given the choice in the natural habitats. T_{pref} has been used as an approximation of the optimal temperature for physiological and biochemical processes (Habary et al., 2017; Jobling, 1981; Magnuson et al., 1979), so one can infer that the avoidance of temperatures < 24°C and > 30°C by *A. vaigiensis* and hybrids may be due to decreased physiological functions. For example, a study on mahi-mahi, *Coryphaena hippurus*, found reduced aerobic scope and swimming performance when acclimated to sub- or supra-optimal temperature (20 or 32°C) when compared to aerobic scope and swimming performance at their optimal temperature of 28°C (Heuer et al., 2021).

The results from *A. vaigiensis*, hybrids, and the single *A. abdominalis* could not be compared statistically, so Structure Q_{Ava}, which described genetic relatedness to pure *Abudefduf vaigiensis* species, was used to test differences in thermal biology, specifically differences in T_{pref} and CT_{max}. There was a strong effect of genetic relatedness to pure *Abudefduf* species on the lower T_{avoid} (Q1) but no effect on the upper T_{avoid} (Q3). These results indicate that both species have a similar upper T_{avoid}, and there are no significant differences in thermal biology in the upper preference range, which may be why I did not find significant effect of Q_{Ava} on CT_{max}. Additionally, the size of T_{pref} range, IQR, did become more narrow as genetic relatedness to *A. vaigiensis* increased. Narrowing of the IQR was due to increases of the lower T_{avoid} (Q1) for individuals more genetically similar to *A. vaigiensis*. These results indicate that *A. vaigiensis* species preferred a higher and more select range of temperatures compared to *A. abdominalis* species.

Previous studies have conducted T_{pref} experiments on Hawaiian reef fish. Medvick & Miller (1979) used a horizontal thermal gradient tank to study behavioral thermal preference on Hawaiian sergeant, *A. abdominalis*, and found average T_{pref} for adults to be 25.9°C. That T_{pref} is higher than the median T_{pref} found for the single pure *A. abdominalis* measured in my research (median T_{pref} = 24.9°C) and lower than the median T_{pref} found from the marginal linear model for *A. abdominalis* (median T_{pref} at structure Q_{Ava} of 0 = 26.6°C). In a study of T_{pref} of another Hawaiian reef fish, the yellow tang, *Zebrasoma flavescens*, using the same annular chamber as the present study, found daytime preferences to be 27.4°C (van Hall, 2021; van Hall & Korsmeyer, in press). These previous studies on T_{pref} on Hawaiian reef fish discussed here are similar to average SST in Hawaiʻi of 26 – 27 °C (Kappel et al., 2017; Wedding et al., 2018).

Critical Thermal Maximum (CT_{max})

CT_{max} has been defined as the upper "thermal point" where locomotion is disrupted (Cowels & Bogert, 1944). When measuring CT_{max} of fish, the "thermal point" is exhibited as the loss of equilibrium (LOE) or failure to control and contract the muscles responsible for maintaining balance (Lutterschmidt & Hutchinson, 1997; Ern et al., 2023). A review on standardizing CT_{max} experiments, suggested a ramping rate of 0.2–0.5 °C min^{-1} or 12–30 °C h^{-1} for fish up to 100 g and a slightly slower ramping rate of 0.1–0.3 °C min^{-1} or 6–18 °C h^{-1} for larger animals (Raby et al., 2025). The ramping rate used during my experimentation was 0.1°C min^{-1} or 6°C h^{-1} which should have accounted for the large range in masses of fish tested (Table 1).

Although on average, there was an increase in CT_{max} with higher Structure Q_{Ava}, or relatedness to *A. vaigiensis*, this relationship was not significant once the effect of body mass was taken into account (Table 7; Fig. 14). These results indicate there may be a relationship between genetic similarity to *A. vaigiensis* and CT_{max}, but further testing must be conducted in order to confirm this relationship. CT_{max} and CT_{min} (critical thermal minimum: lower "thermal point" where locomotion is disrupted) results are closely related to distributions of fish species globally (Payne et al., 2021; Sunday et al., 2012; Downie et al., 2025). The differences in preference of the lower thermal range due to genetic relatedness suggests that there may be differences in the critical thermal minimum (CT_{min}), and this should be investigated in future studies. I expect that *A. abdominalis* would have a lower CT_{min} as this species has existed in

Hawai‘i’s cooler waters longer than *A. vaigiensis* and, therefore, may be more tolerant to colder temperatures. Thermal tolerance corresponds to latitudinal ranges (Sunday et al., 2012), and ectotherms from warmer, tropical latitudes have higher CT_{max} and a wider range of heating tolerance than temperate ectotherms (Payne et al., 2021). Since the invasive sergeant major, *A. vaigiensis*, and the native sergeant major, *A. abdominalis*, in this research, were collected from the same sites, there may not be a difference in thermal tolerances between species.

Ontogenetic Stage and Mass Effects on Thermal Tolerance

Sergeant majors, regardless of species groups or hybridization, had a significant decrease in thermal tolerance (CT_{max}) with increasing mass (Fig. 13). Individuals with large body sizes (> 100 g) will have higher thermal inertia than smaller fish and therefore, there may be a lag in body temperature as environmental temperature is ramped in CT_{max} experiments (Stevens & Sutterlin, 1976; Schurmann et al., 1991). As a result, the larger individuals tested may have a slightly lower CT_{max} than what was reported (Fig. 13). Previous studies have estimated the lag in body temperature with body size to correct the CTmax, but this is not recommended as these corrections require many assumptions regarding mechanisms behind thermal inertia and relevant tissues (Raby et al., 2025).

Body mass and ontogenetic stage have been established as key factors contributing to intraspecific variation of temperature tolerance (Gunderson, 2024). A study on 694 marine and freshwater fish species from all climate zones found ontogenetic stage to be a “thermal bottleneck” in that embryos and spawning adults had a narrower thermal tolerance range than larvae and nonreproductive adults (Dahlke et al., 2020). The study found that as fish develop, changes in cardiorespiratory functions influence their thermal tolerance, with early life stages being more oxygen-limited and thus more vulnerable to temperature changes (Dahlke et al., 2020). Individuals used in my research were preserved after fin clips were collected; therefore ontogenetic stages (non-reproductive vs reproductive adults) of individuals tested could be identified through dissections, though many of the fish tested were too small to be reproductive. In a future study, with more individuals across a larger size range, the effect of the ontogenetic stage on T_{pref} and CT_{max} could be assessed.

Although body size/mass has been shown to influence thermal tolerance, it is not clear how (Gomez Isaza & Rodgers, 2024). There are several hypotheses that aim to explain how size/mass influences thermal tolerance (Gillooly et al., 2001; Pörtner, 2010; Ern et al., 2023; Chung & Schulte, 2020; Leiva et al., 2024). In addition to not knowing how mass influences thermal tolerance, there is no one specific effect of body mass on thermal tolerance (Gomez Isaza & Rodgers, 2024). Larger body sizes has been correlated with lower thermal tolerance (Clark et al., 2017; Di Santo & Lobel, 2017), while other studies have found that a larger body size results in a higher thermal tolerance (Illing et al., 2020; Moyano et al., 2017), and still some studies have found no relationship between body mass and thermal tolerance (Ospina & Mora, 2004; Recsetar et al., 2012). Most studies, however, show either no effect or a negative effect of mass on CT_{max} (McKenzie et al., 2021). There was a significant negative effect of mass on CT_{max} in the *Abudefduf* spp. in the present study (Table 7; Fig. 13).

Impacts of Ocean Warming

Tropical coral reef ecosystems are being impacted by increasing SST and increased frequency of MHW due to climate change (Feary et al., 2014). Many tropical reef fish have narrow thermal ranges and reside in habitats where SST has reached or is close to reaching their upper thermal limit (Rowe et al., 2018; Alfonso et al., 2020) forcing them to extend or shift distributions for refuge in new habitats (Downie et al., 2025). The process where tropical species extend their distributions poleward to temperate environments due to increasing SST and boundary current is defined as tropicalization (Downie et al., 2025). Still, some species and populations are not able to adapt to increased SST and experience mass mortality events during periods of extreme increased SST or MHW (Genin et al., 2020).

Abudefduf vaigiensis has been observed extending their distributions into higher latitudes on Australia's eastern coast during the summer season and is able to persist in this new environment as seasonal winter temperatures fell below 18°C (Downie et al., 2025). Winter SSTs are considered a “survival bottleneck” for tropical species with only 13% of tropical species surviving overwinter in Sydney (34° S) and only 8% surviving overwinter in Merimbula (37° S) (Booth et al., 2018). *Abudefduf vaigiensis* persistence in these new temperate environments suggests that their physiology was plastic enough across ontogenetic stages, larvae to adult, so that they could tolerate cooler SSTs (Downie et al., 2025). A study on *A. vaigiensis* early life history traits after settlement in temperate waters found that shorter pelagic larval duration (PLD) was correlated to faster-growing larvae and longer post-settlement persistence (Rigg et al., 2025). Rapid larval growth and shorter planktonic durations reduces exposure to pelagic mortality risks and likely enhanced compatibility with temperate conditions in order to access new and possibly more suitable cooler or temperate environments (Rigg et al., 2025). Juvenile *A. vaigiensis* have been observed selecting for high-protein diets under lower temperature conditions in order to decrease feeding activity while maintaining their metabolic rates (Rowe et al., 2018). Other factors that were found to aid tropical species tropicalization are larvae capacity to shift metabolic pathways or having metabolic pathways developed enough to respond to cold stress; however, the ability of young reef fish to switch metabolic pathways in response to cold stress is uncertain amongst tropical species and should be studied further (Downie et al., 2025). Other biological factors that may influence a tropical species' ability to persist in temperate environments include neuroendocrine responses, blood chemistry, osmoregulation, immune and cardiac system responses, whole organism health, and changes in the population (Barton, 2002). *Abudefduf vaigiensis'* persistence in temperate environments along Australia's eastern coast suggests that this species has some of the aforementioned tropicalization characteristics. Generally, Hawai'i reefs experience cooler SST than other reef environments across the Indo-Pacific, *A. vaigiensis'* native environment (Guidry & Mackenzie, 2013; Yao & Wang, 2022).

Sergeant Major Distributions and Temperature Across the Hawaiian Islands

The results from my research on the Indo-Pacific, native Hawaiian, and hybrid sergeant majors indicate there are slight biological differences in T_{pref} along the genetic relatedness spectrum from *A. abdominalis* to *A. vaigiensis*. There was a significant increase of 1.2°C on median T_{pref} as genetic similarity to *A. vaigiensis* increased. Eight months of the year, there is a ≥ 1°C difference in SST in the MHI compared to the NWHI (Rooney et al., 2008). The marginal linear model indicates the genetic composition of an individual fish affected the fish's lower avoidance temperature (Q1) more than other aspects of the fish's preferred temperature (Table 3). *Abudefduf vaigiensis* lower T_{avoid} was 2.7°C higher than present day average winter SST in the MHI (24.0°C), and was 3.7 - 4.7°C higher than present day average winter SST in the NWHI (22 – 23°C) (Table 3; PacIOOS, n.d.). In comparison, *A. abdominalis* lower T_{avoid} was 0.6°C and 1.6 - 2.6°C higher than winter temperatures in the MHI and NWHI, respectively (Table 3; PacIOOS, n.d.).

When sampled between 2009 and 2012, population densities of *A. vaigiensis* were equal to or higher than *A. abdominalis* in the MHI where SST are warmer, but *A. vaigiensis* were outnumbered 2:1 in the NWHI where SST are cooler (Coleman et al., 2014). By synthesizing other research with their own surveys, Rooney et al. (2008) found the Pearl Hermes, Midway, and Kure Atolls, the northernmost atolls, to be oceanographically distinct from the rest of the archipelago. Population densities (individuals/m_2) of *A. abdominalis* outnumber *A. vaigiensis* by 667:149 on Pearl & Hermes Atoll, 1,390:15 on Midway Atoll, and 285:51 on Kure Atoll (Coleman et al., 2014). Comparatively, population densities of *A. vaigiensis* outnumbered *A. abdominalis* on island of O‘ahu, 268:107 and Maui 41:39 (Coleman, 2014). Hybrid frequency in the MHI was greater at 14.8% compared with 0 - 6.7% across the NWHI (Coleman et al., 2014). This difference in lower T_{avoid} and the difference in SST along the island chain may be more important in explaining the distribution of *A. vaigiensis* and *A. abdominalis* across the Hawaiian Archipelago.

While temperature may be influencing population densities of these species and hybrids across the archipelago, other variables such as physical oceanographic factors and biological traits of the species must be considered. For example, Wren et al. (2016) used a Lagrangian bio-physical particle tracking model to assess particle distribution across the archipelago to simulate larvae dispersal. They found 96.3% of particles transported from the MHI ultimately settled in the MHI, while 3.7% settled in the NWHI (Wren et al., 2016). Comparatively, 95.1% of particles transported from the NWHI settled in the NWHI, while 4.8% settled in the MHI (Wren et al., 2016). These results suggest larval dispersal for the two regions, MHI and NWHI, are more closely linked to themselves than to each other. However, it’s important to note that these results are based on a standard PLD, eddy diffusivity, and ocean state, so percent of successful settlement will vary by species and by location. Physical oceanographic factors like mesoscale eddies, currents, and retentive hydrodynamics can influence larval settlement

(Lindo-Atichati et al., 2020). Physical oceanographic factors and biological traits of the species, in addition to temperature, should be considered when considering the cause of population density differences for sergeant majors across the Hawaiian Archipelago.

While the present study was not designed to examine current ratios of sergeant major populations, my limited sample from catch locations across Oʻahu during 2023 - 2025 found *A. vaigiensis* outnumbered *A. abdominalis* by 13:1, with hybrids accounting for 65% of samples (Table 1). Only attaining a single native sergeant major, *A. abdominalis*, was not anticipated, and suggests that the population density of non-native Indo-Pacific sergeant major, *A. vaigiensis* has further increased relative to *A. abdominalis*, and that the proportion of hybrids has increased as well. Future studies should re-examine population densities of *A. vaigiensis*, *A. abdominalis*, and hybrids across the MHI and NWHI in order to assess changes in the state of hybridization over time.

Unresolved and Future Research

There were two results from my experiments that found no effect of species or hybrid relatedness. The first was the effect of Structure Q_{Ava}, the estimate of genetic similarity or dissimilarity to *A. vaigiensis* / *A. abdominalis*, on upper T_{avoid}, Q3 (Table 5; Fig. 8). The second was the effect of Structure Q_{Ava} on CT_{max} (Table 7; Fig. 14). Both relationships were positive, but they were not statistically significant (Fig. 8; Fig. 14). It is possible that the relationship between Q_{Ava} and upper T_{avoid} and between Q_{Ava} and CT_{max} may become significant after testing more individuals, specifically testing more *A. abdominalis* individuals. Mass was found to have a significant negative relationship with CT_{max}, so even with more *A. abdominalis* tested a relationship between Q_{Ava} and CT_{max} may not be revealed as the effect of mass may over power the effect of Q_{Ava} on thermal tolerance. Regardless, future studies should investigate *A. abdominalis* T_{pref} and CT_{max} in order to make comparisons across species groups in addition to testing the effect of Structure Q_{Ava}.

Species identification, across a smaller sample size, revealed different population densities of *A. vaigiensis* and *A. abdominalis* on O‘ahu than Coleman et al. (2014). Research similar to Coleman et al. (2014) should be revisited in order to assess changes in population density between species and changes in the state of hybridization across the islands over time. Additionally, sergeant majors from other islands in the MHI and NWHI should be tested for T_{pref} and CT_{max} in order to make comparisons across populations and to test if thermal biological differences are contributing to differences in population densities across the Hawaiian Archipelago.

Other studies should focus on acclimation effects on thermal preference and tolerance for sergeant majors. Acclimation was not a factor in my research; however, some species are able to increase their thermal tolerance after short exposures to warm temperatures before they reach full acclimation, and that acclimation dynamics strongly affect which species are impacted by a changing climate (De Bonville et al., 2025). The capacity to acclimate to warmer temperatures may be indicative of sergeant major species survival to extreme climate change and should be studied.

Conclusions

By using Structure Q_{Ava} as an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero), I found significant trends that align with my hypothesis, and found differences in thermal biology due to genetic relatedness to either *A. vaigiensis* or *A. abdominalis*.

There was a significant positive effect of genetic similarity to *A. vaigiensis* on both median T_{pref} and the lower T_{avoid}, Q1. There was also a significant negative effect of genetic similarity on IQR, resulting in a narrower thermal preference zone for individuals more genetically related to *A. vaigiensis* than individuals more genetically related to *A. abdominalis*.

My results indicate that there are slight differences in thermal biology between *A. vaigiensis* and *A. abdominalis* and further testing should be conducted. Before the 1990s, the non-native sergeant, *A. vaigiensis*, lived in tropical environments across the Indo-Pacific. Climate change has caused higher average SST, more persistent warming, and increased frequency MHW across the Indo-Pacific (Yao & Wang, 2022). The species distribution of *A. vaigiensis* has been expanding into higher latitude, cooler temperate environments along Australia's eastern coast, suggesting that higher latitudes there have become more suitable for this species (Downie et al., 2025). Generally, Hawaiian reefs experience a broader range of SST across seasons and experience less-frequent extreme peaks in SST in comparison to other reefs in the Indo-Pacific (Guidry & Mackenzie, 2013; Yao & Wang, 2022). When *A. vaigiensis* was first established in Hawaiian waters in the 1990s, it may have been accidental or chance introduction by marine debris (Benadon et al., 2023), but now, with increasing average SST in Hawaiʻi's reefs due to climate change, these environments are becoming more suitable for *A. vaigiensis*. This invasive species has been shown, in this research, to have a higher and narrower T_{pref} range than those less genetically similar to *A. vaigiensis* (hybrids and *A. abdominalis*) indicating that differences in thermal biology may have aided in their expansion into and persistence in Hawaiʻi's reefs.

References

Aebischer, N. J., Robertson, P. A., & Kenward, R. E. (1993). Compositional analysis of habitat use from animal radio-tracking data. *Ecology*, 74(5), 1313-1325. doi:10.2307/1940062.

Alfonso, S., Gesto, M., & Sadoul, B. (2020). Temperature increase and its effects on fish stress physiology in the context of global warming. *Fish Biology*, 98 1496-1508. https://doi.org/10.1111/jfb.14599.

Anderson, E. C., & Thompson, E. A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics, 160*(3), 1217–1229.

Barton, B. A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology,* 42, 517–525. https://doi.org/ 10.1093/icb/42.3.517.

Behrens, J. W., Gräns, A., Therkildsen, N. O., Neuenfeldt, S., & Axelsson, M. (2012). Correlations between hemoglobin type and temperature preference of juvenile Atlantic cod *Gadus morhua*. *Journal of 624 Experimental Marine Biology and Ecology*, 413, 71-77.

Beitinger, T. L. & Fitzpatrick, L. C. (1979). Physiological and ecological correlates of preferred temperature in fish. *American Zoology*, 19, 319-329.

Benadon, C., Zabin, C. J., Haram, L. E., Carlton, J. T., Maximenko, N., Nelson, P., Crowley, M., & Ruiz, G. M. (2023). Marine debris facilitates long-distance dispersal of fish species. *Marine Biology,* 171(2), Article 43. https://doi.org/10.1007/s00227-023-04365-3.

Bernal, M. A., Schunter, C., Lehmann, R., Lightfoot, D. J., Allan, B. J. M., Veilleux, H. D., Rummer, J. L., Munday, P. L., & Ravasi, T. (2020). Species-specific molecular responses of wild coral reef fishes during a marine heatwave. *Science Advances, 6*(12), eaay3423. https://doi.org/10.1126/sciadv.aay3423.

Booth, D. J., Beretta, G. A., Brown, L., & Figueira, W. F. (2018). Predicting success of range-expanding coral reef fish in temperate habitats using temperature - abundance relationships. *Frontiers in Marine Science,* 5. https://doi.org/10.3389/fmars.2018.00031.

Calenge, C. (2006). The package “adehabitat” for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling*, 197(3-4), 516-519. doi:10.1016/j.ecolmodel.2006.03.017.

Christensen, E. A. F., Andersen, L. E. J., Bergsson, H., Steffensen, J. F., & Killen, S. S. (2021). Shuttle-box systems for studying preferred environmental ranges by aquatic animals. *Conservation Physiology,* 9, coab028.

Christie, M. R., Tissot, B. N., Albins, M. A., Beets, B. N., Apostolaki, Y., Stamoulis, K. A., Toonen, R. J., & Bowen, B. W. (2010). Larval connectivity in an effective network of marine protected areas. *PLOS ONE*, 5(12), e15715. https://doi.org/10.1371/journal.pone.0015715.

Chung, D. J., & Schulte, P. M. (2020). Mitochondria and the thermal limits of ectotherms. *Journal of Experimental Biology*, 223.

Clark, T. D., Roche, D. G., Binning, S. A., Speers-Roesch, B., & Sundin, J. (2017). Maximum thermal limits of coral reef damselfishes are size dependent and resilient to near- future ocean acidification. *Journal of Experimental Biology*, 220, 3519–3526.

Coleman, R. R., Gaither, M. R., Kimokeo, B., Stanton, F. G., Bowen, B. W., & Toonen, R. J. (2014). Large-scale introduction of the Indo-Pacific damselfish *Abudefduf vaigiensis* into Hawaiʻi promotes genetic swamping of the endemic congener *A. abdominalis*. *Molecular Ecology,* 23, 5552-5565.

Cowen, R. K., Paris, C. B., & Srinivasan, A. (2006). Scaling of connectivity in marine populations. *Science, 311*(5760), 522–527.

Cowles, R. B., & Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Bulletin of the American Museum of Natural History, 83*, 261–296.

Dahlke, F. T., Wohlrab, S., Butzin, M., & Portner, H. -O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science,* 369, 65–70.

De Bonville, J., Andreassen, A. H., Cowan, Z., Silva-Garay, L., Leeuwis, R. H. J., Åsheim, E. R., Speers-Roesch, B., Raby, G. D., Binning, S. A., & Jutfelt, F. (2025). Dynamics of thermal tolerance plasticity across fish species and life stages. *Journal of Thermal Biology*, 127, 0306-4565, https://doi.org/10.1016/j.jtherbio.2024.104024.

Desforges, J. E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K. M., Eliason, E. J., Dressler, T. L., McKenzie, D. J., Bates, A. E., Lawrence, M. J., Fangue, N., & Cooke, S. J. (2022). The ecological relevance of critical thermal maxima methodology for fishes. *Journal of Fish Biology*, 1-17, 10.1111/jfb.15368.

Di Santo, V., & Lobel, P. S. (2017). Body size and thermal tolerance in tropical gobies. *Journal of Experimental Marine Biology and Ecology*, 487, 11–17.

Downie, A. T., Champion, C., & Booth, D. J. (2025). Physiological traits for predicting poleward extensions in tropical fishes: from lab to management. *Global Change Biology*, 31, 1-27. https://doi.org/10.1111/gcb.70213.

Earl, D. A., & von Holdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources, 4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7.

Ern, R., Andreassen, A. H., & Jutfelt, F. (2023). Physiological mechanisms of acute upper thermal tolerance in fish. *Physiology*, 38(3), 141–158. https://doi.org/10.1152/physiol.00027.2022.

Feary, D. A., Pratchett, M. S., Emslie, M. J., Fowler, A. M., Figueira, W. F., Luiz, O. J., Nakamura, Y., & Booth, D. J. (2014). Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish and Fisheries*, *15*(4), 593–615. https://doi.org/10.1111/faf.12036.

Froese, R., & Pauly, D. (2024). FishBase. World Wide Web electronic publication. www.fishbase.org.

Fry, F. E. J. (1947). Effects of the environment on animal activity. *University of Toronto Studies, Biological Series*, 55, 1–62.

Gallois, B., & Candelier, R. (2021). FastTrack: An open-source software for tracking varying numbers of deformable objects. *PLOS Computational Biology,* 17.2, e1008697.

Geneious Prime 2023.1. (2023). Biomatters Ltd., Auckland, New Zealand. Available from https://www.geneious.com.

Genin, A., Levy, L., Sharon, G., Raitsos, D. E., & Diamant, A. (2020). Rapid onsets of warming events trigger mass mortality of coral reef fish, *Proceedings of the National Academy of Sciences*, 117 (41), https://doi.org/10.1073/pnas.2009748117.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293, 2248–2251.

Gomez Isaza, D. F., & Rodgers, E. M. (2024). Upper thermal limits are 'hard-wired' across body mass but not populations of estuarine fish. *Journal of Thermal Biology*, 125. https://doi.org/10.1016/j.jtherbio.2024.103970.

Gove, J., Maynard, J., Lecky, J., Tracey, D., Allen, M., Asner, G., Conklin, E., Couch, C., Hum, K., Ingram, R., Kindinger, T., Leong, K., Oleson, K., Towle, E., van Hooidonk, R., Williams, G., & Hospital, J. (2022). 2022 Ecosystem Status Report for Hawaiʻi. *Pacific Islands Fisheries Science Center*, PIFSC Special Publication, SP-23-01, 91p. doi:10.25923/r53p-fn97.

Guidry, M. W., & Mackenzie, F. T. (2013). Future climate change, sea level rise, and ocean acidification: Implications for Hawaiʻi and Western Pacific fisheries management. *University of Hawaiʻi Sea Grant College Program*. https://seagrant.soest.hawaii.edu.

Gunderson, A.R. (2024). Disentangling physiological and physical explanations for body size-dependent thermal tolerance. *Journal of Thermal Biology*. 227(5). https://doi.org/10.1242/jeb.245645.

Habary, A., Johansen, J. L., Nay, T. J., Steffensen, J. F., & Rummer, J. L. (2017). Adapt, move or die–how will tropical coral reef fishes cope with ocean warming? *Global Change Biology*, 23(2), 566-577.

Hassan, M., Lemaire, C., Fauvelot, C., & Bonhomme, F. (2002). Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish. *Molecular Ecology Notes* 2, 334–340.

Hasselman, D. J., Argo, E. E., McBride, M. C., Bentzen, P., Schultz, T. F., Perez-Umphrey, A. A., & Palkovacs, E. P. (2014). Human disturbance causes the formation of a hybrid swarm between two naturally sympatric fish species. *Molecular Ecology, 23*(5), 1137–1152. https://doi.org/10.1111/mec.12674.

Hawaiʻi Integrated Ecosystem Assessment. (n.d.). *Why was 2015 so hot in Hawaiʻi?* NOAA. Retrieved July 20, 2025, from NOAA's Integrated Ecosystem Assessment website.

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources, 9*(5), 1322–1332.

Heuer, R. M., Stieglitz, J. D., Pasparakis, C., Enochs, I. C., Benetti, D. D., & Grosell, M. (2021). The effects of temperature acclimation on swimming performance in the pelagic mahi‑mahi (*Coryphaena hippurus*). *Frontiers in Marine Science,* 8, 654276. https://doi.org/10.3389/fmars.2021.654276.

IBM Corp. (2021). *IBM SPSS Statistics for Windows* (version 28) [Computer software]. IBM Corp. https://www.ibm.com/products/spss-statistics.

Illing, B., Downie, A., Beghin, M., & Rummer, J. (2020). Critical thermal maxima of early life stages of three tropical fishes: effects of rearing temperature and experimental heating rate. *Journal of Thermal Biology*, 90, 102582.

Jobling, M. (1981). Temperature tolerance and the final preferendum - rapid methods for the assessment of optimum growth temperatures. *Journal of Fish Biology*, 19, 439-455.

Kappel, C.V., K.A. Selkoe, and Ocean Tipping Points (OTP). (2017). Sea surface temperature (SST) long-term mean, 2000-2013 - Hawaiʻi. *Distributed by the Pacific Islands Ocean Observing System (PacIOOS)*. http://pacioos.org/metadata/hi_otp_all_sst_avg.html.

Leiva, F. P., Santos, M., Rezende, E. L., & Verberk, W. C. E. P. (2024). Intraspecific variation of heat tolerance in a model ectotherm: the role of oxygen supply capacity. *Functional Ecology*. https://doi.org/10.1111/1365-2435.14485.

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451–1452. https://doi.org/10.1093/bioinformatics/btp187.

Lindo‑Atichati, D., Jia, Y., Wren, J. L. K., Antoniades, A., & Kobayashi, D. R. (2020). Eddies in the Hawaiian Archipelago Region: Formation, characterization, and potential implications on larval retention of reef fish. *Journal of Geophysical Research: Oceans, 125*(5), e2019JC015348. https://doi.org/10.1029/2019JC015348.

Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: History and critique. *Canadian Journal of Zoology*, 75(10), 1561–1574.

Magel, J. M. T., Dimoff, S. A., & Baum, J. K. (2020). Direct and indirect effects of climate change‑amplified pulse heat stress events on coral reef fish communities. *Ecological Applications, 30*(6), e02124. https://doi.org/10.1002/eap.2124

Magnuson, J. J., Crowder, L. B., & Medvick, P. A. (1979). Temperature and ecological resource. *American Zoologist*, 19(1): 331-343.

Maruska, K. P., & Peyton, K. A. (2007). Interspecific spawning between a recent immigrant and an endemic damselfish (Pisces: Pomacentridae) in the Hawaiian Islands. *Pacific Science*, *61*(2), 211–221. https://doi.org/10.2984/1534-6188(2007)61[211:ISBARI]2.0.CO;2.

McKenzie, D. J., Zhang, Y., Eliason, E. J., Schulte, P. M., Claireaux, G., Blasco, F. R., Binning, S. A., Clark, T. D., Farrell, A. P., Gilmour, K. M., Healy, T. M., Lefevre, S., Lefevre, F., Metcalfe, N. B., Pörtner, H.-O., Rummer, J. L., Seebacher, F., Speers-Roesch, B., Sundin, J., Valenzuela, J. A., & White, C. R. (2021). Intraspecific variation in tolerance of warming in fishes. *Journal of Fish Biology,* 98(6), 1536–1555. https://doi.org/10.1111/jfb.14620.

Medvick, P. A., & Miller, J. M. (1979). Behavioral thermoregulation in three Hawaiian reef fishes. *Environmental Biology of Fishes, 4*(1), 23–28. https://doi.org/10.1007/BF00005924.

Meeker, N. D., Hutchinson, S. A., Ho, L., & Trede, N. S. (2007) Method for isolation of PCR-ready genomic DNA from zebrafish tissues. *BioTechniques*, 43, 610–614.

Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CT_{max} is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports*, 8(1), 7099. https://doi.org/10.1038/s41598-018-25593-4.

Moyano, M., Candebat, C., Ruhbaum, Y., Alvarez-Fernandez, S., Claireaux, G., Zambonino-Infante, J.-L., & Peck, M.A. (2017). Effects of warming rate, acclimation temperature and ontogeny on the critical thermal maximum of temperate marine fish larvae. *PLoS One*, 12, e0179928.

Mundy, B. C. (2005). Checklist of the fishes of the Hawaiian Archipelago. *Bishop Museum Bulletin in Zoology,* 6. Bishop Museum Press, Honolulu.

Myrick, C. A., Folgner, D. K., & Cech, J. J. (2004). An annular chamber for aquatic animal preference studies. *American Fisheries Society*, 133: 427-433.

National Instruments. (2020). *LabVIEW (Version 2020)* [Software]. National Instruments Corporation. https://www.ni.com.

Nay, T. J., Johansen, J. L., Habary, A., Steffensen, J. F., & Rummer, J. L. (2015). Behavioural thermoregulation in a temperature-sensitive coral reef fish, the five-lined cardinalfish (Cheilodipterus quinquelineatus). *Coral Reefs*, 34(4), 1261-1265.

Nielsen, E. E., Bach, L. A., & Kotlicki, P. (2006). HYBRIDLAB (version 1.0): A program for generating simulated hybrids from population samples. *Molecular Ecology Notes, 6*(4), 971–973. https://doi.org/10.1111/j.1471-8286.2006.01433.x.

NOAA Integrated Ecosystem Assessment Program. (n.d.). Why was 2015 so hot in Hawai‘i? Retrieved July 10, 2025, from https://www.integratedecosystemassessment.noaa.gov/regions/hawaii/why-was-2015-so-hot-hawaii.

Norin, T., Malte, H., & Clark, T. D. (2014). Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *Journal of Experimental Biology*, 217(2), 244-251.

OMEGA Bio-tek. (n.d.). *E.Z.N.A.® Tissue DNA Kit* [Product No. D3396]. Norcross, GA, USA: OMEGA Bio-tek. Retrieved from https://www.omegabiotek.com.

Ospina, A.F., & Mora, C. (2004). Effect of body size on reef fish tolerance to extreme low and high temperatures. *Environmental Biology of Fishes*, 70, 339–343.

Ørsted, M., Jørgensen, L. B., & Overgaard, J. (2022). Finding the right thermal limit: A framework to reconcile ecological, physiological and methodological aspects of $\mathrm{CT}_{\square\mathrm{ax}}$ in ectotherms. *Journal of Experimental Biology, 225*(19), jeb244514. https://doi.org/10.1242/jeb.244514.

Pacific Islands Ocean Observing System (PacIOOS). (n.d.). Marine climate of the Central Pacific [Ocean Atlas]. *PacIOOS, University of Hawai‘i at Manoa*. Retrieved July 15, 2025, from https://www.pacioos.hawaii.edu/education/ocean-atlas/.

Payne, N. L., Morley, S. A., Halsey, L. G., Smith, J. A., Stuart-Smith, R., Waldock, C., & Bates, A. E. (2021). Fish heating tolerance scales similarly across individual physiology and populations. *Communications Biology*, 4(1), 1–5.

Pérez-Escudero, A., Vicente-Page, J., Hinz, R. C., Arganda, S., & Polavieja, G. G. de. (2014). idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nature Methods*, 11(7), 743–748. https://doi.org/10.1038%2Fnmeth.2994.

Pörtner, H. -O. (2010). Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, 213, 881–893.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945–959.

Raby, G. D., Morgan, R., Andreassen, A. H., Stewart, E. M. C., De Bonville, J., Hoots, E. C., Kuchenmüller, L., Metz, M., Rowsey, L. E., Green, L., Griffin, R. A., Martin, S., Bauer Reid, H., Ern, R., Åsheim, E. R., Cowan, Z., Leeuwis, R. H. J., Blewett, T. A., Speers-Roesch, B., Clark, T. D., Binning, S. A., Sundin, J., & Jutfelt, F. (2025). Measuring critical thermal maximum in aquatic ectotherms: a practical guide. *EcoEvoRxiv*. https://doi.org/10.6084/m9.figshare.28319774.v1.

Randall, J. E. (2007). Reef and Shore Fishes of the Hawaiian Islands. *Honolulu: Sea Grant College Program, University of Hawai*‘*i*.

Recsetar, M. S., Zeigler, M. P., Ward, D. L., Bonar, S. A., & Caldwell, C. A. (2012). Relationship between fish size and upper thermal tolerance. *Transactions of the American Fisheries Society*, 141, 1433–1438.

Reiser, S., Temming, A., Eckhardt, A., & Herrmann, J. P. (2013). Automation and critical evaluation of an annular chamber for aquatic ectotherm temperature preference experiments. *Methods in Ecology and Evolution*, 4, 531-541.

Reynolds, W. W., & Casterlin, M. E. (1979). Behavioral thermoregulation and the “Final Preferendum” paradigm. *American Zoologist*, 19(1), 211-224.

Rigg, A.L., Fowler, A.M., Nagelkerken, I., & Booth, D. J. (2025). Faster larval growth and shorter pelagic duration enhance the post-settlement persistence of a common range-extending coral-reef fish in a temperate ecosystem. *Coral Reefs*, https://doi.org/10.1007/s00338-025-02627-8.

Rowe, C. E., Figueira, W., Raubenheimer, D., Solon-Biet, S. M., & Machovsky-Capuska, G. E. (2018). Effects of temperature on macronutrient selection, metabolic and swimming performance of the Indo-Pacific damselfish (*Abudefduf vaigiensis*). *Marine Biology,* 11, 165-178. https://doi.org/10.1007/s00227-018-3435-7.

Rooney, J. J., Wessel, P., Hoeke, R., Weiss, J., Baker, J., Parrish, F., Fletcher, C. H., Chojnacki, J., Garcia, M., Brainard, R., & Vroom, P. (2008). Geology and geomorphology of coral reefs in the northwest Hawaiian Islands. In: Riegl BM, Dodge RE (eds) Coral Reefs of the USA. Coral Reefs of the World, Vol 1, *Springer*, 515-567.

RStudio Team. (2023). *RStudio: Integrated Development Environment for R*. RStudio, PBC. Retrieved from https://posit.co/products/open-source/rstudio/.

Schram, E., Bierman, S., Teal, L. R., Haenen, O., Van de Vis, H., & Rijnsdorp, A. D. (2013). Thermal preference of juvenile Dover Sole (*Solea solea*) in relation to thermal acclimation and optimal growth temperature. *PLoS One*, 8(4), e61357. doi:10.1371/journal.pone.0061357.

Schurmann, H., Steffensen, J. F., & Lomholt, J. P. (1991). The influence of hypoxia on the preferred temperature of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology, 157*(1), 75–86. https://doi.org/10.1242/jeb.157.1.75.

Song, C. B., Near, T. J., & Page, L. M. (1998). Phylogenetic relations among percid fishes as inferred from mitochondrial cyto- chrome b DNA sequence data. *Molecular Phylogenetics and Evolution* 10, 343–353.

Stephens, M., & Donnelly, P. (2003). A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics, 73*(5), 1162–1169. https://doi.org/10.1086/379378.

Stevens, E. D., & Sutterlin, A. M. (1976). Heat transfer between fish and ambient water. *Journal of Experimental Biology, 65*(1), 131–145. https://doi.org/10.1242/jeb.65.1.131.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change,* 2(9), 686–690. https://doi.org/10.1038/nclimate1539.

Taberlet, P., Meyer, A., Bouvet, J. (1992). Unusual mitochondrial DNA polymorphism in two local populations of blue tit. *Parus caeruleus*. *Molecular Ecology* 1, 27–36.

University of Hawai‘i Pacific Islands Ocean Observing System (PacIOOS). (n.d.). Sea surface temperature. *Ocean Atlas of Hawai‘i*. Retrieved July 20, 2025, from Pacific Islands Ocean Observing System.

van Hall, E. S. (2021). Temperature preference and aerobic scope in Zebrasoma flavescens and the response to rising sea temperatures (Master’s thesis, Hawai‘i Pacific University). *Hawai‘i Pacific University*.

van Hall, E. S., & Korsmeyer, K. E. (in press). Aerobic scope and temperature preference in yellow tang (*Zebrasoma flavescens*) at current and elevated sea temperatures. *Journal of Comparative Physiology B.*

Vaughan, G. O., Grimes, K. W., Brandl, S. J., Kuo, C. Y., Looby, A. E., Hussey, N. E., & Clark, T. D. (2025). Narrow margins: Aerobic performance and temperature tolerance of coral reef fishes facing extreme thermal variability. *Global Change Biology, 31*(3), e70100. https://doi.org/10.1111/gcb.170100.

Vinagre, C., Dias, M., Cereja, R., Abreu‐Afonso, F., Flores, A. A. V., & Mendonça, V. (2019). Upper thermal limits and warming safety margins of coastal marine species – Indicator baseline for future reference. *Ecological Indicators, 102*, 644–652. https://doi.org/10.1016/j.ecolind.2019.03.030.

Wedding, L. M., Lecky, J., Gove, J. M., Walecka, H. R., Donovan, M. K., Williams, G. J., Jouffray, J.-B., Crowder, L. B., Erickson, A., Falinski, K., Friedlander, A. M., Kappel, C. V., Kittinger, J. N., McCoy, K., Norström, A. V., Nyström, M., Oleson, K. L. L., Stamoulis, K. A., White, C., & Selkoe, K. A. (2018). Advancing the integration of spatial data to map human and natural drivers on coral reefs. *PLOS ONE, 13*(3), e0189792. https://doi.org/10.1371/journal.pone.0189792.

West, B. T., Welch, K. B., & Galecki, A. T. (2014). Linear Mixed Models. A Practical Guide Using Statistical Software (Second ed.). Boca Raton, FL: Chapman and Hall/CRC.

Winn, C., Chiswell, S., Firing, E., Karl, D., & Lukas, R. (1990). *Hawaii Ocean Time-series: Data report 2* (SOEST-92-1). *School of Ocean and Earth Science and Technology, University of Hawaii*. Retrieved from https://www.soest.hawaii.edu/HOT_WOCE/data_report.html.

Wren, J. L. K., Kobayashi, D. R., Jia, Y., & Toonen, R. J. (2016). Modeled population connectivity across the Hawaiian Archipelago. *PLOS ONE,* 11(12), e0167626. https://doi.org/10.1371/journal.pone.0167626.

Yao, Y.-L., & Wang, C. (2022). Marine heatwaves and cold‐spells in global coral reef zones. *Progress in Oceanography*, 209, Article 102920. https://doi.org/10.1016/j.pocean.2022.102920.

Zhu, Z., & Li, T. (2017). The record-breaking hot summer in 2015 over Hawaiʻi and its physical causes. *Journal of Climate*, 30, 4253–4266. https://doi.org/10.1175/JCLI-D-16-0438.1.

Tables and Figures

Table 1. Morphological results for both species groups and the hybrids [mean (min – max)].

	(*n*)	**Anal Fin Ray Count**	**Dorsal Fin Ray Count**	**Fork Length (cm)**	**Depth (cm)**	**Mass (g)**
Abudefduf abdominalis	1	12	13	8.2	2.4	11.9
Abudefduf vaigiensis	13	11.6 (10 - 12)	12.5 (11 - 13)	8.7 (7.5 - 10.4)	3.9 (2.8 – 5.3)	21.7 (12.2 – 38.3)
Hybrid	26	12 (10 - 14)	12.6 (12 - 13)	10.3 (7.4 – 16.2)	4.3 (3.0 - 7.3)	36.1 (8.7 - 124.6)

Table 2. Temperature preference (T_{pref}) results in the annular preference chamber with a range of 22 - 32°C: first quartile (Q1, lower avoidance temperature), median, third quartile (Q3, upper avoidance temperature), and interquartile range (IQR) for each species group and hybrids across all time periods: 'Available temperatures', 'no-gradient' usage, and '24 h'preference trial [mean ± s.d.]. *Abudefduf abdominalis n* = 1; *A. vaigiensis n* = 13; hybrids *n* = 26. During 'No-gradient' usage, temperatures were uniform at ambient temperature (~26.5°C), and values represent the eventual temperatures at the fish positions after the gradient had been established.

	Temperature (°C)											
	Abudefduf abdominalis				***Abudefduf vaigiensis***				**Hybrid**			
Period	**Q1**	**Median**	**Q3**	**IQR**	**Q1**	**Median**	**Q3**	**IQR**	**Q1**	**Median**	**Q3**	**IQR**
Available temperatures	24.1	26.8	29.5	5.4	23.7 ± 0.3	26.5 ± 0.3	29.3 ± 0.3	5.6 ± 0.2	23.9 ± 0.2	26.6 ± 0.2	29.4 ± 0.2	5.6 ± 0.2
No-gradient usage	23.7	26.3	29.3	5.6	24.7 ± 1.8	26.7 ± 1.5	28.9 ± 1.0	4.2 ± 1.4	24.9 ±1.6	27.2 ± 1.4	29.1 ± 1.1	4.1 ± 1.6
24 h preference	23.7	24.9	26.6	2.9	26.6 ± 0.8	27.4 ± 0.9	28.4 ± 1.0	1.7 ± 0.6	25.6 ± 1.8	27.0 ± 1.4	28.3 ± 1.2	2.7 ± 1.4

Table 3. Estimates of fixed effects on median temperature preference (T_{pref}) (℃) from a marginal linear model for *Abudefduf* spp.

Parameter	**Estimate**	**SE**	**df**	**t**	***p*-value**
Intercept	26.6	0.1	36.6	303	**< 0.001**
Structure Q_{Ava}	-0.0484	0.1263	36.6	-0.384	0.704
Mass	0.000289	0.001545	36.6	-0.187	0.853
Period					
‘No-Gradient’ usage	0.727	0.479	38.1	1.52	0.137
‘24 h’ preference	-0.280	0.431	38.4	-0.649	0.520
Interactions					
‘No-Gradient’ usage × Q_{Ava}	-0.485	0.690	38.1	-0.703	0.486
‘24 h’ × Q_{Ava}	1.29	0.62	38.4	2.08	**0.044**
‘No-Gradient’ usage × Mass	-0.00210	0.00844	38.1	-0.248	0.805
‘24 h’ × Mass	-0.00279	0.00758	38.4	-0.368	0.715

Time ‘period’ was included as repeated measures with individual fish as subjects. The period ‘available temperatures’ was used as the reference for comparison. Mass was centered at the average mass of 32.8 g. Structure Q_{Ava} was an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero). During ‘No-gradient’ usage, temperatures were uniform at ambient temperature (~26.5°C), and values represent the eventual temperatures at the fish positions after the gradient had been established.

Table 4. Estimates of fixed effects on the first quartile (Q1, lower avoidance temperature) of temperature preference (T_{pref}) (℃) from a marginal linear model for *Abudefduf* spp.

Parameter	**Estimate**	**SE**	**df**	**t**	***p*-value**
Intercept	23.9	0.1	37.3	283	**< 0.001**
Structure Q_{Ava}	-0.136	0.122	37.3	-1.12	0.271
Mass	-0.000343	0.001487	37.3	-0.231	0.819
Period					
‘No-Gradient’ usage	0.615	0.476	37.4	1.03	0.310
‘24 h’ preference	0.756	0.597	37.9	1.59	0.121
Interactions					
‘No-Gradient’ usage × Q_{Ava}	0.635	0.859	37.4	0.739	0.465
‘24 h’ × Q_{Ava}	2.155	0.685	37.9	3.14	**0.003**
‘No-Gradient’ usage × Mass	0.0100	0.0105	37.4	0.951	0.348
‘24 h’ × Mass	-0.0129	0.0084	37.9	-1.53	0.133

Time ‘period’ was included as repeated measures with individual fish as subjects. The period ‘available temperatures’ was used as the reference for comparison. Mass was centered at the average mass of 32.8 g. Structure Q_{Ava} was an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero). During ‘No-gradient’ usage, temperatures were uniform at ambient temperature (~26.5°C), and values represent the eventual temperatures at the fish positions after the gradient had been established.

Table 5. Estimates of fixed effects on the third quartile (Q3, upper avoidance temperature) of temperature preference (T_{pref}) (℃)from a marginal linear model for *Abudefduf* spp.

Parameter	**Estimate**	**SE**	**df**	**t**	***p*-value**
Intercept	29.3	0.1	35.0	401	**< 0.001**
Structure Q_{Ava}	0.0981	0.1054	35.0	0.931	0.358
Mass	0.00209	0.00129	35.0	1.63	0.113
Period					
‘No-Gradient’ usage	0.0470	0.3727	38.5	0.126	0.900
‘24 h’ preference	-1.49	0.38	37.2	-3.88	**< 0.001**
Interactions					
‘No-Gradient’ usage × Q_{Ava}	-0.658	0.536	38.5	-1.23	0.228
‘24 h’ × Q_{Ava}	0.656	0.553	37.2	1.19	0.243
‘No-Gradient’ usage × Mass	-0.0108	0.0066	38.5	-1.65	0.108
‘24 h’ × Mass	-0.000333	0.006764	37.2	-0.0493	0.961

Time ‘period’ was included as repeated measures with individual fish as subjects. The period ‘available temperatures’ was used as the reference for comparison. Mass was centered at the average mass of 32.8 g. Structure Q_{Ava} was an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero). During ‘No-gradient’ usage, temperatures were uniform at ambient temperature (~26.5°C), and values represent the eventual temperatures at the fish positions after the gradient had been established.

Table 6. Estimates of fixed effects on temperature preference (T_{pref}) interquartile range (IQR, ℃) from a marginal linear model for *Abudefduf* spp.

Parameter	**Estimate**	**SE**	**df**	**t**	***p*-value**
Intercept	5.43	0.06	37.7	89.4	**< 0.001**
Structure Q_{Ava}	0.234	0.087	37.7	2.68	**0.011**
Mass	0.00244	0.00107	37.7	2.28	**0.029**
Period					
‘No-Gradient’ usage	-0.568	0.520	36.9	-1.09	0.282
‘24 h’ preference	-2.25	0.34	38.1	-6.55	**< 0.001**
Interactions					
‘No-Gradient’ usage × Q_{Ava}	-1.29	0.75	36.9	-1.73	0.093
‘24 h’ × Q_{Ava}	-1.50	0.49	38.1	-3.04	**0.004**
‘No-Gradient’ usage × Mass	-0.0208	0.0092	36.9	-2.27	**0.029**
‘24 h’ × Mass	0.0125	0.0060	38.1	2.08	**0.045**

Time ‘period’ was included as repeated measures with individual fish as subjects. The period ‘available temperatures’ was used as the reference for comparison. Mass was centered at the average mass of 32.8 g. Structure Q_{Ava} was an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero). During ‘No-gradient’ usage, temperatures were uniform at ambient temperature (~26.5°C), and values represent the eventual temperatures at the fish positions after the gradient had been established.

Table 7. Summary of multiple linear regression analysis on critical thermal maximum (CT_{max}) for *Abudefduf* spp.

Parameter	**Coefficient**	**SE**	***p*-value**
Intercept	38.1	0.266	**<0.001**
Mass	-0.0118	0.0033	**0.001**
Structure Q_{Ava}	0.395	0.280	0.168

Structure Q_{Ava} was an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero).

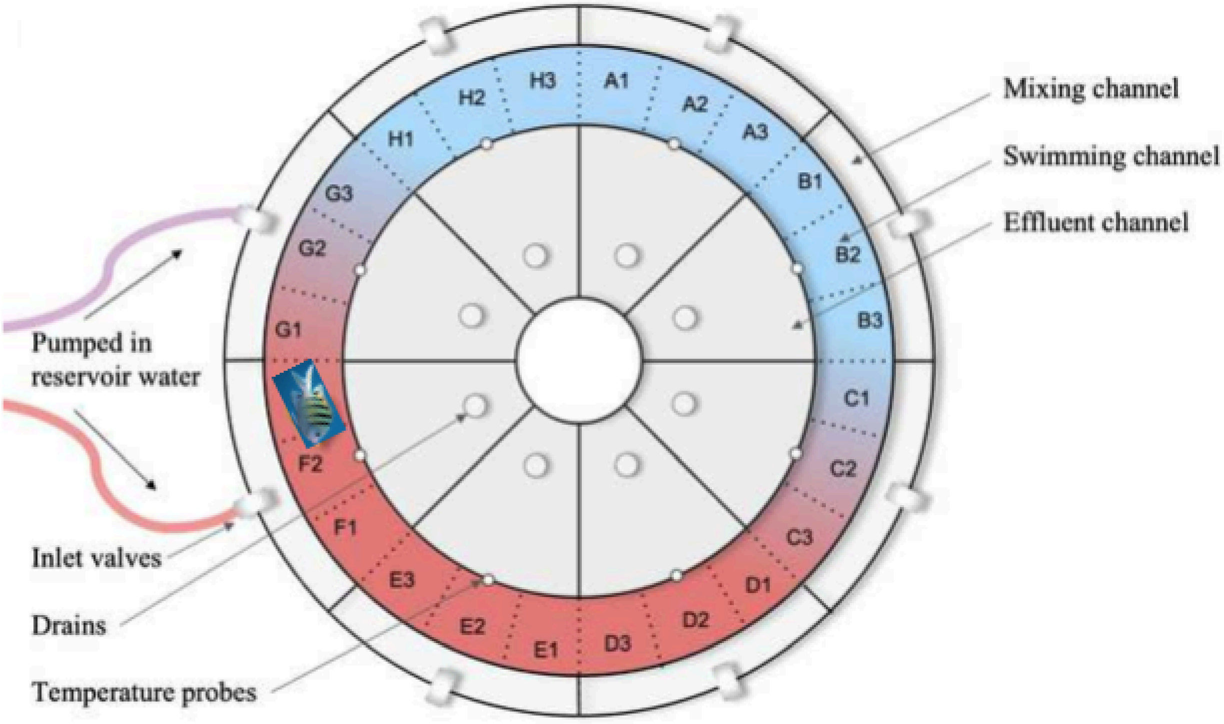

Figure 1. Temperature preference (T_{pref}) annular chamber schematic. Each of the eight primary temperature sections (A-H) included an inlet valve where water from one of the five reservoirs was pumped into the section via the mixing channel to create a temperature gradient. Each primary section also included a drain in the effluent channel and a temperature probe in the swimming channel. Modified from van Hall & Korsmeyer, in press.

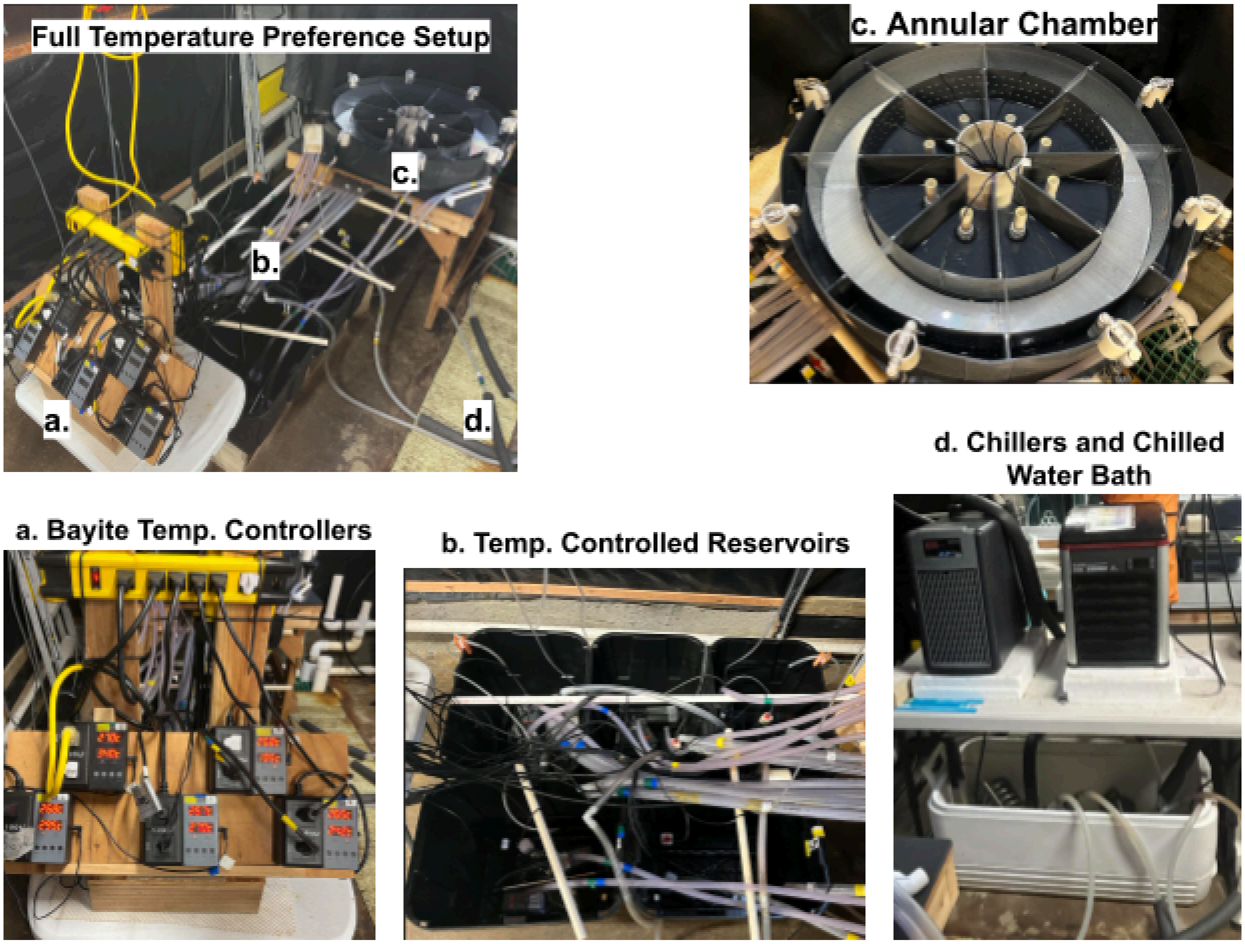

Figure 2. Temperature preference (T_{pref}) annular chamber in lab setup. The full T_{pref} set-up except for the chillers and water bath are displayed in the top left image with its components labeled: a. Baylite temperature controllers, b. Temperature controlled reservoirs, c. Annular chamber, d. Chillers and chilled water bath.

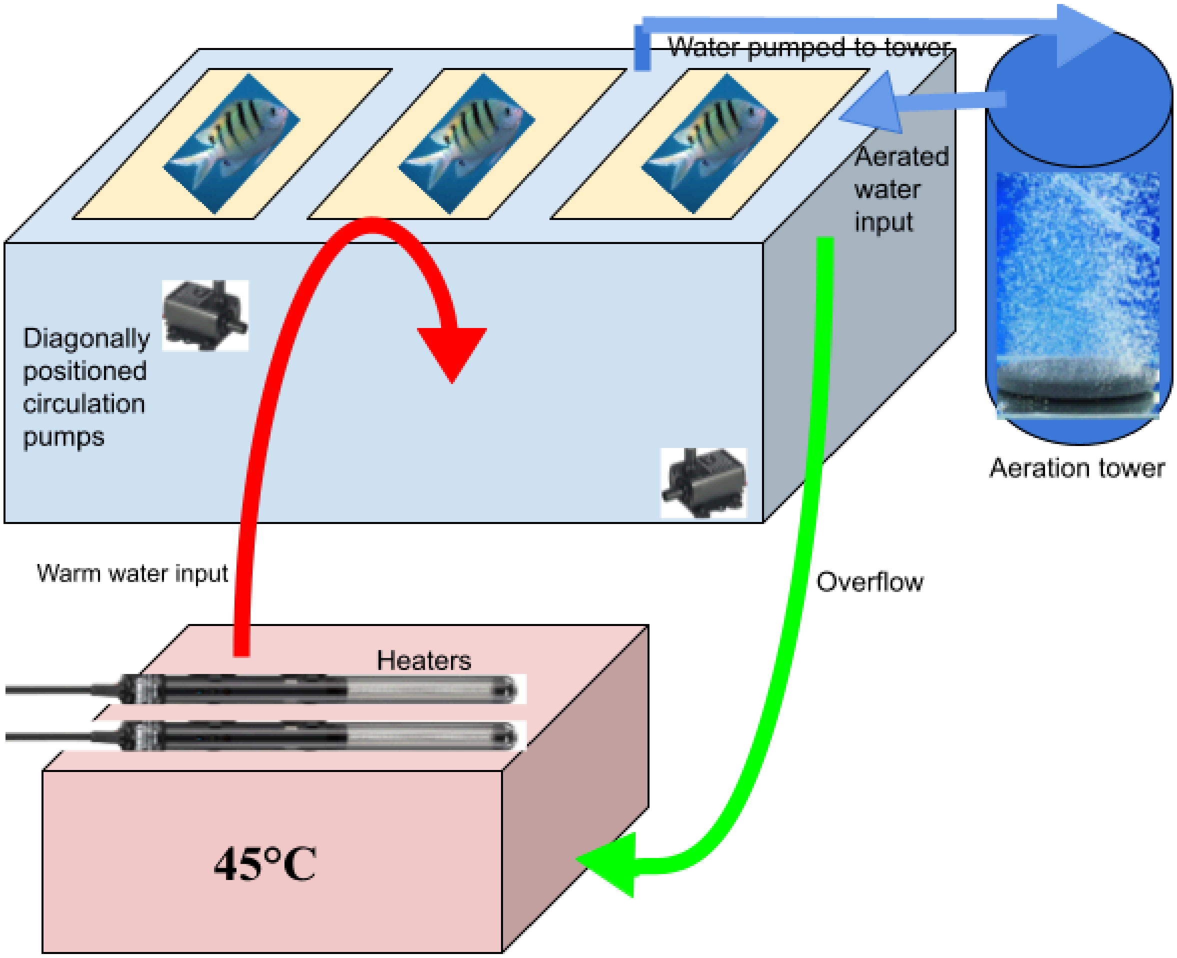

Figure 3. Critical thermal maximum (CT_{max}) experimental set-up schematic. The red box represents the warm water reservoir and the blue box represents the experimental tank. Yellow boxes represent the three perforated floating plastic baskets. Cylinder represents the aeration tower and bubble trap. The red arrow indicates the warm water pumped in from the warm water reservoir and the green arrow represents over-flow from the experimental tank to the warm water reservoir. The blue arrows represent water pumped to and from the aeration tower.

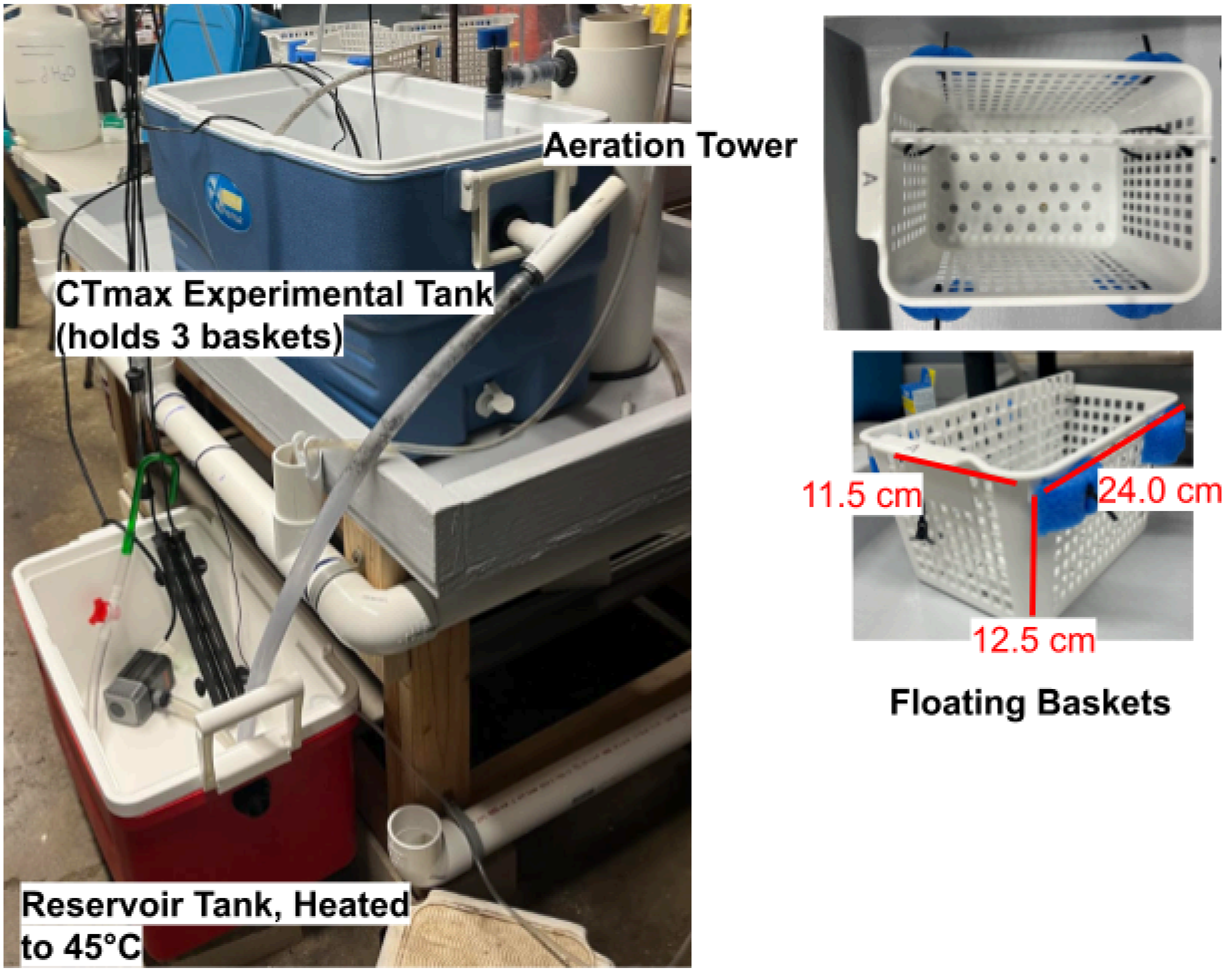

Figure 4. Critical thermal maximum (CT_{max}) in-lab experimental set-up. The red cooler was the warm water reservoir which included two heaters totaling to 1000 W and a pump, *EHEIM: 1048*. The blue cooler was the experimental tank where the three floating baskets contained the test subjects. There were two submersible water pumps positioned in diagonally opposite corners within the experimental tank; there was also a pump to the aeration tower. Floating baskets (pictured on the right) were 11.5 x 12.5 x 24.0 cm and each held a single fish.

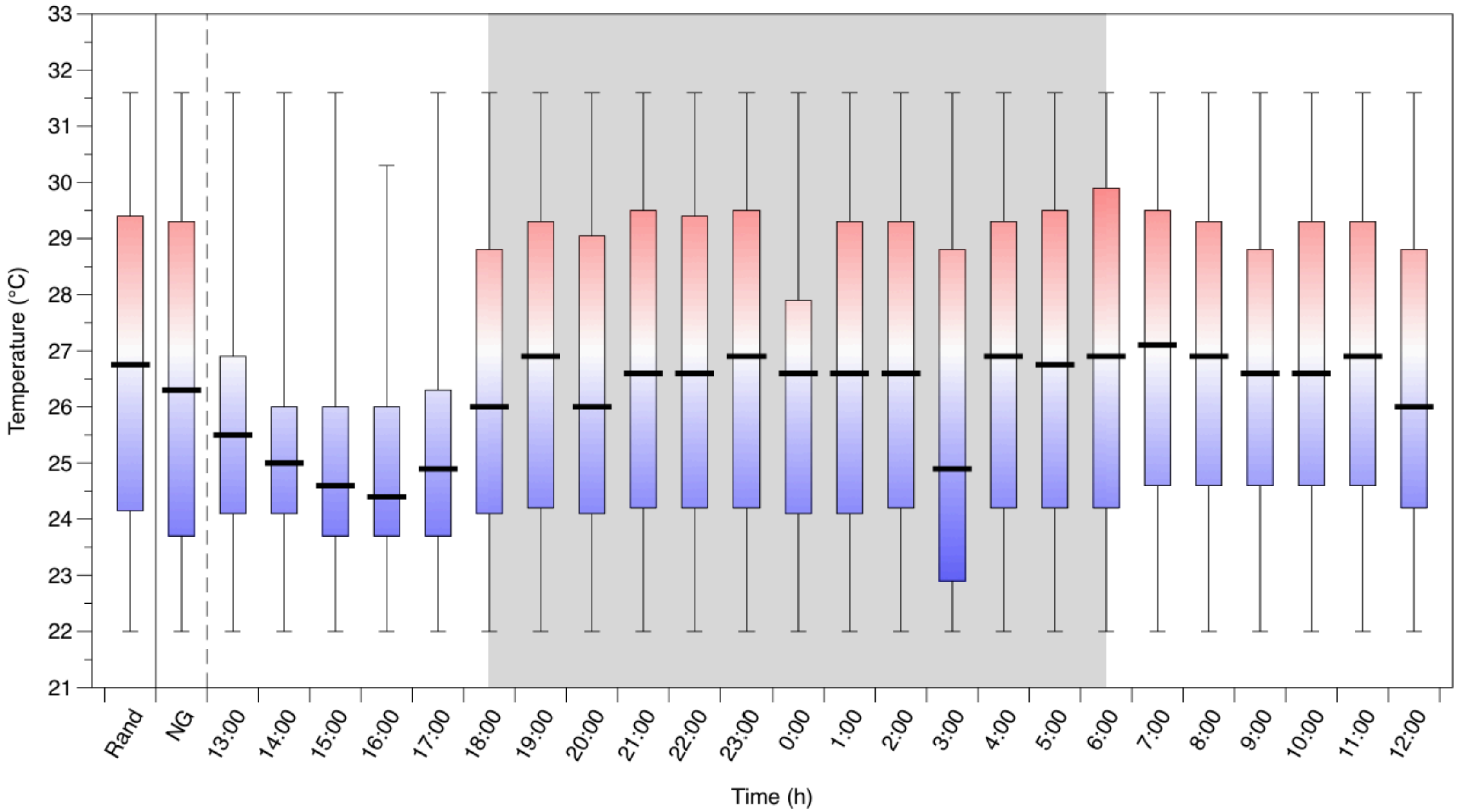

Figure 5. Temperature preferences (T_{pref}) of the single *A. abdominalis* for every hour of the '24 h' period. T_{pref} for every hour represented by the average median (bar), first quartile (Q1, lower avoidance temperature) and third quartile (Q3, upper avoidance temperature) (box), and temperature ranges (whiskers). Rand is the average available temperatures during the 'available temperatures' period and NG is the average of observed use of eventual temperatures during 'no-gradient' usage period (no temperature gradient present in the annular chamber). The shaded gray region denoted nighttime hours. 'Non-choosing' hours, when no temperature selection occurred: 18:00, 19:00, 20:00, 21:00, 22:00, 23:00, 1:00, 2:00, 4:00, 5:00, 6:00, 7:00, 8:00, 9:00, 10:00, 11:00, 12:00.

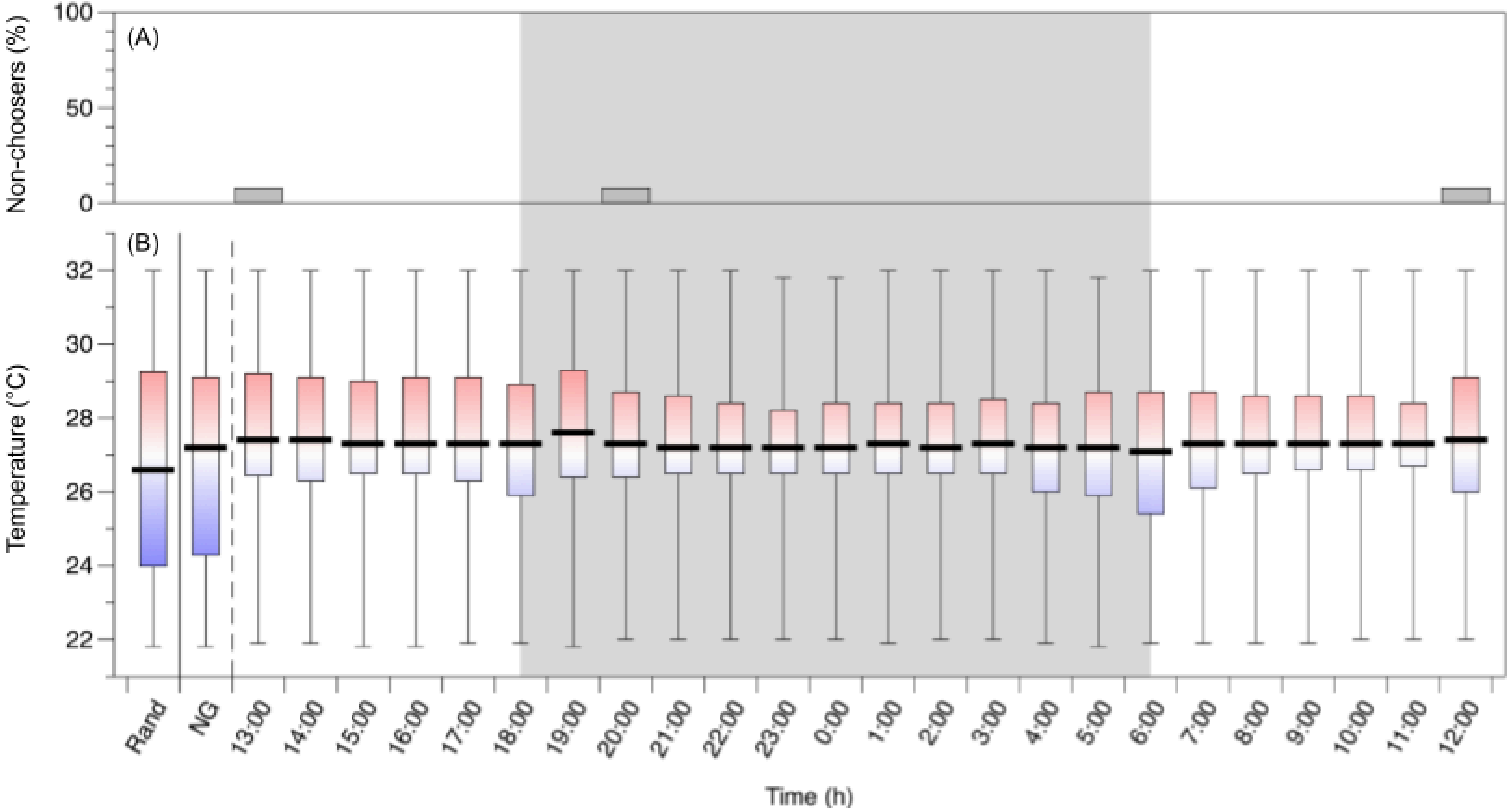

Figure 6. Temperature preferences (T_{pref}) of *Abudefduf vaigiensis* for every hour of the '24 h' period. (A) The percentage of individuals identified as 'non-choosers', those who showed no temperature selection, for each hour. (B) T_{pref} for every hour represented by the average median (bar), first quartile (Q1, lower avoidance temperature) and third quartile (Q3, upper avoidance temperature) (box), and temperature ranges (whiskers). Rand is the average available temperatures during the 'available temperatures' period and NG is the average of observed use of eventual temperatures during 'no-gradient' usage period (no temperature gradient present in the annular chamber). The shaded gray region denoted nighttime hours.

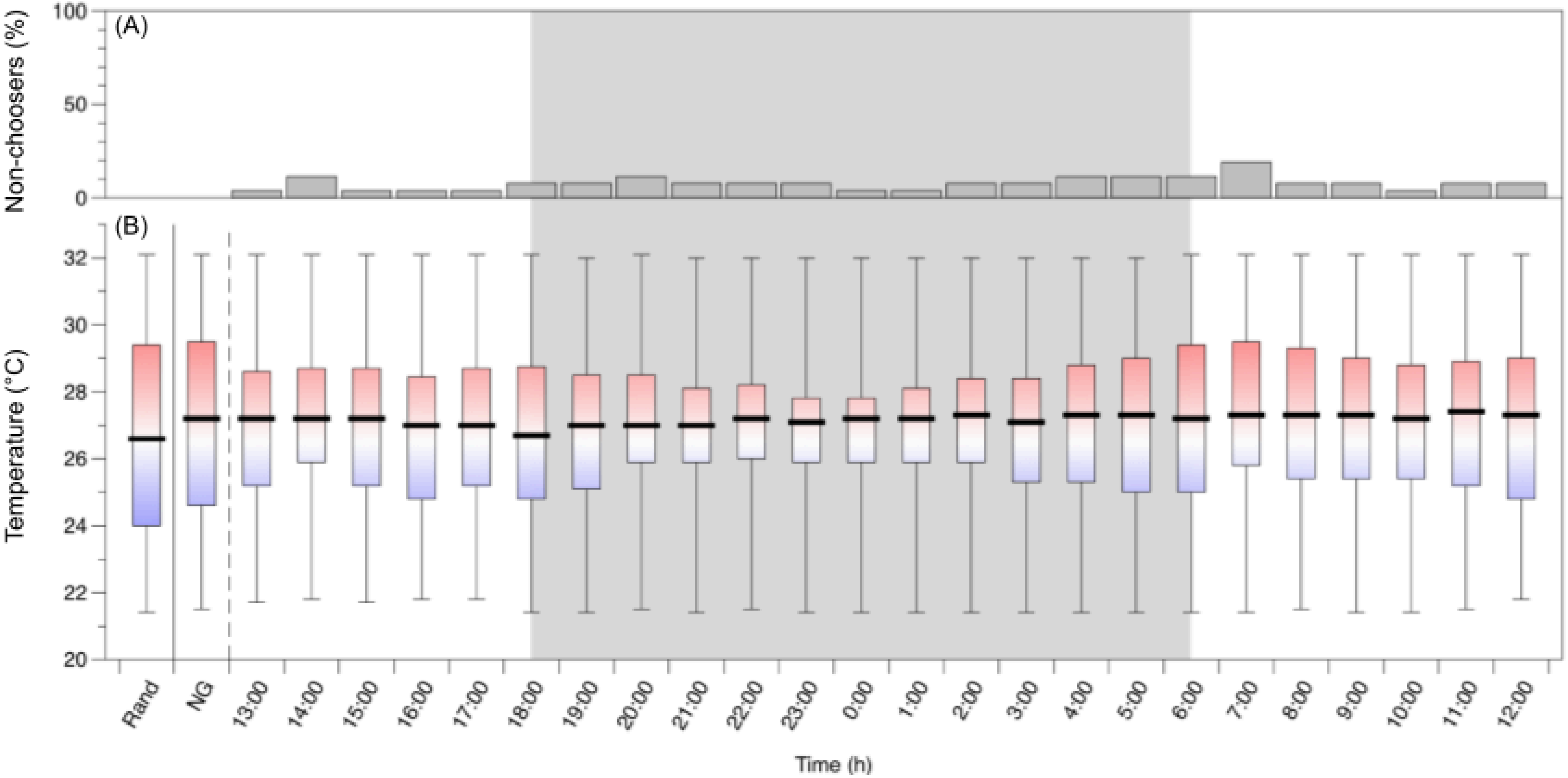

Figure 7. Temperature preferences (T_{pref}) of hybrids (*Abudefduf vaigiensis* and *A. abdominalis*) for every hour of the '24 h' period. (A) The percentage of individuals identified as 'non-choosers', those who showed no temperature selection, for each hour. (B) T_{pref} for every hour represented by the average median (bar), first quartile (Q1, lower avoidance temperature) and third quartile (Q3, upper avoidance temperature) (box), and temperature ranges (whiskers). Rand is the average available temperatures during the 'available temperatures' period and NG is the average of observed use of eventual temperatures during 'no-gradient' usage period (no temperature gradient present in the annular chamber). The shaded gray region denoted nighttime hours.

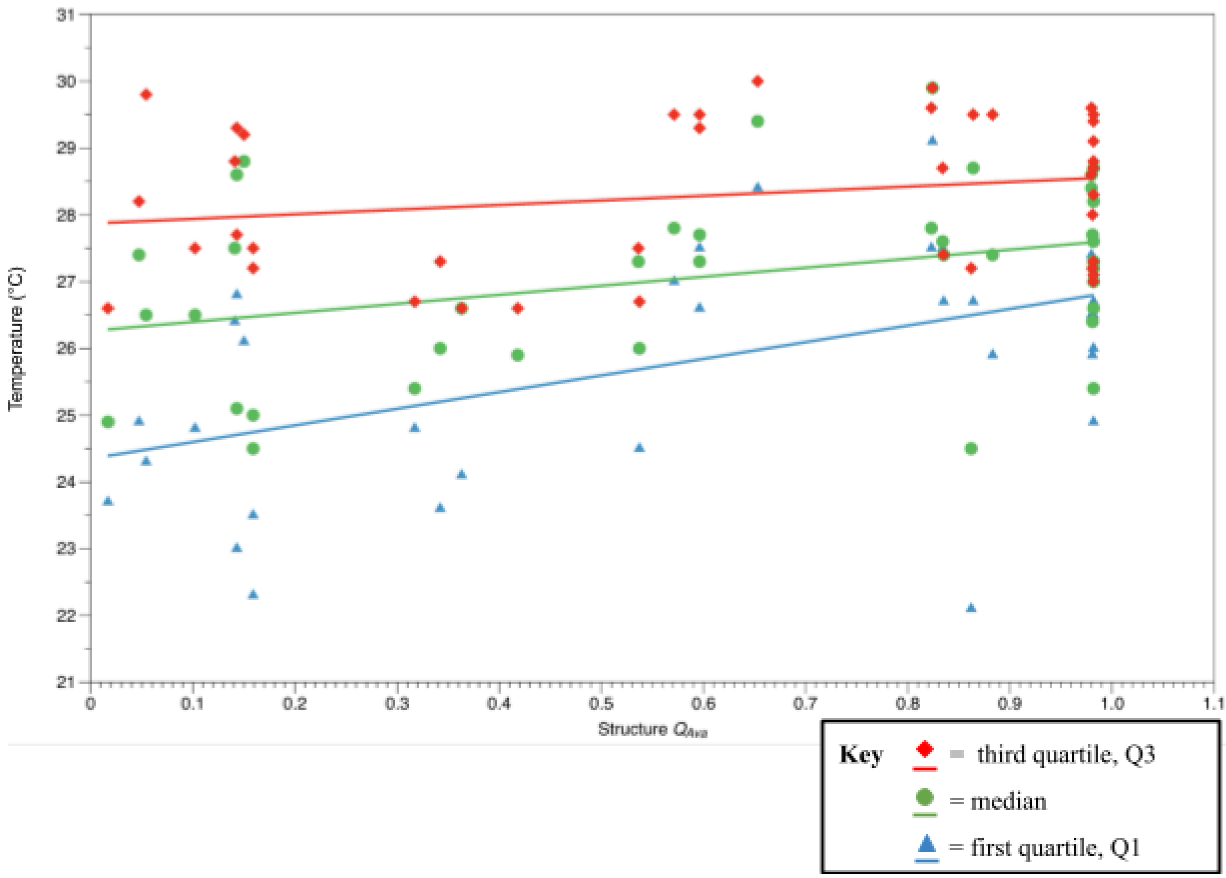

Figure 8. Temperature preference (T_{pref}) first quartile (Q1, lower avoidance temperature), median, and third quartile (Q3, upper avoidance temperature) as a function of Structure Q_{Ava} (an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero)). Q1 = 24.4 + 2.49X, $R^2 = 0.290$; Median = 26.3 + 1.35X, $R^2 = 0.131$; Q3 = 27.9 + 0.691X, $R^2 = 0.0479$.

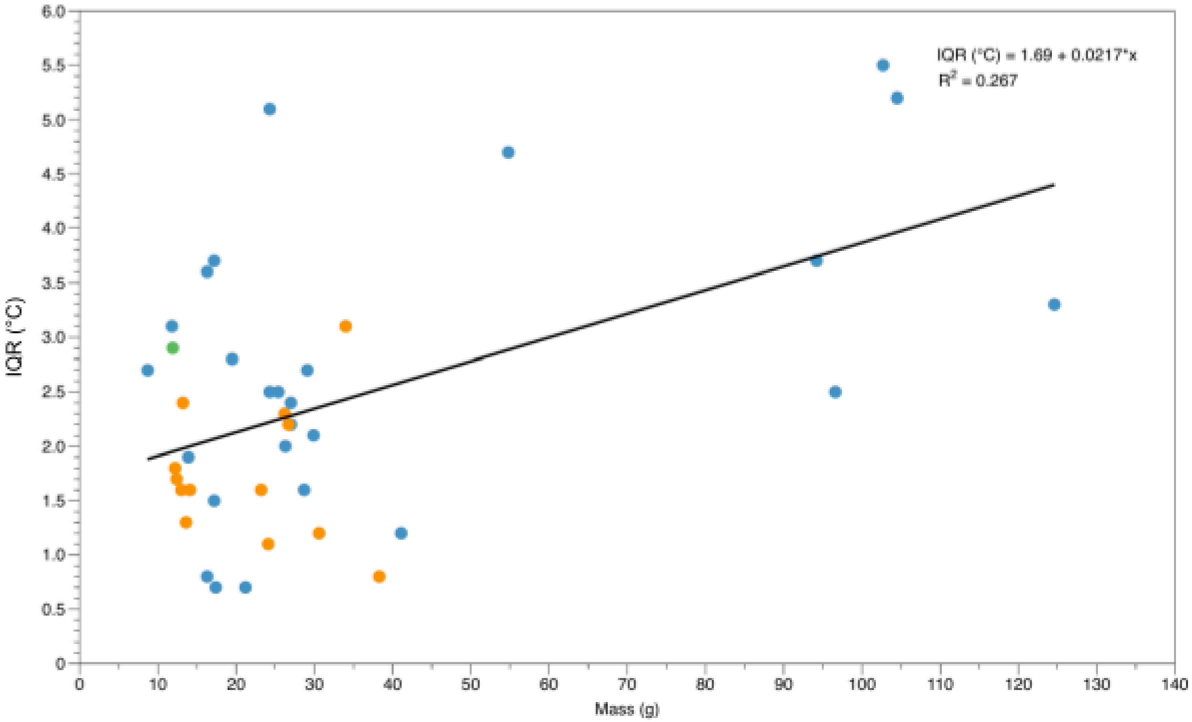

Figure 9. Relationship between interquartile range, IQR, and mass. Green points represent *A. abdominalis* individuals, blue points represent hybrids, and orange points represent *A. vaigiensis* individuals.

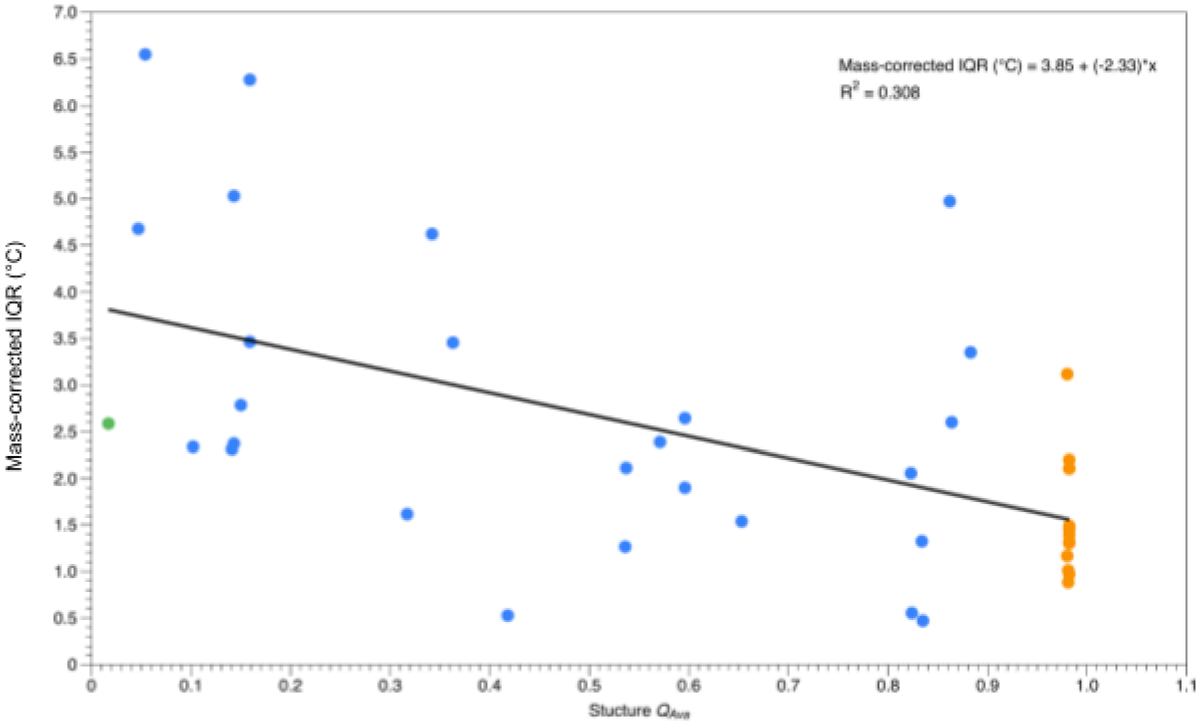

Figure 10. Mass-corrected IQR as a function of Structure Q_{Ava}(an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero)). Green points represent *A. abdominalis* individuals, blue points represent hybrids, and orange points represent *A. vaigiensis* individuals. IQR mass-corrected to 32.8 g [= IQR - 0.015 * (32.8 - mass)].

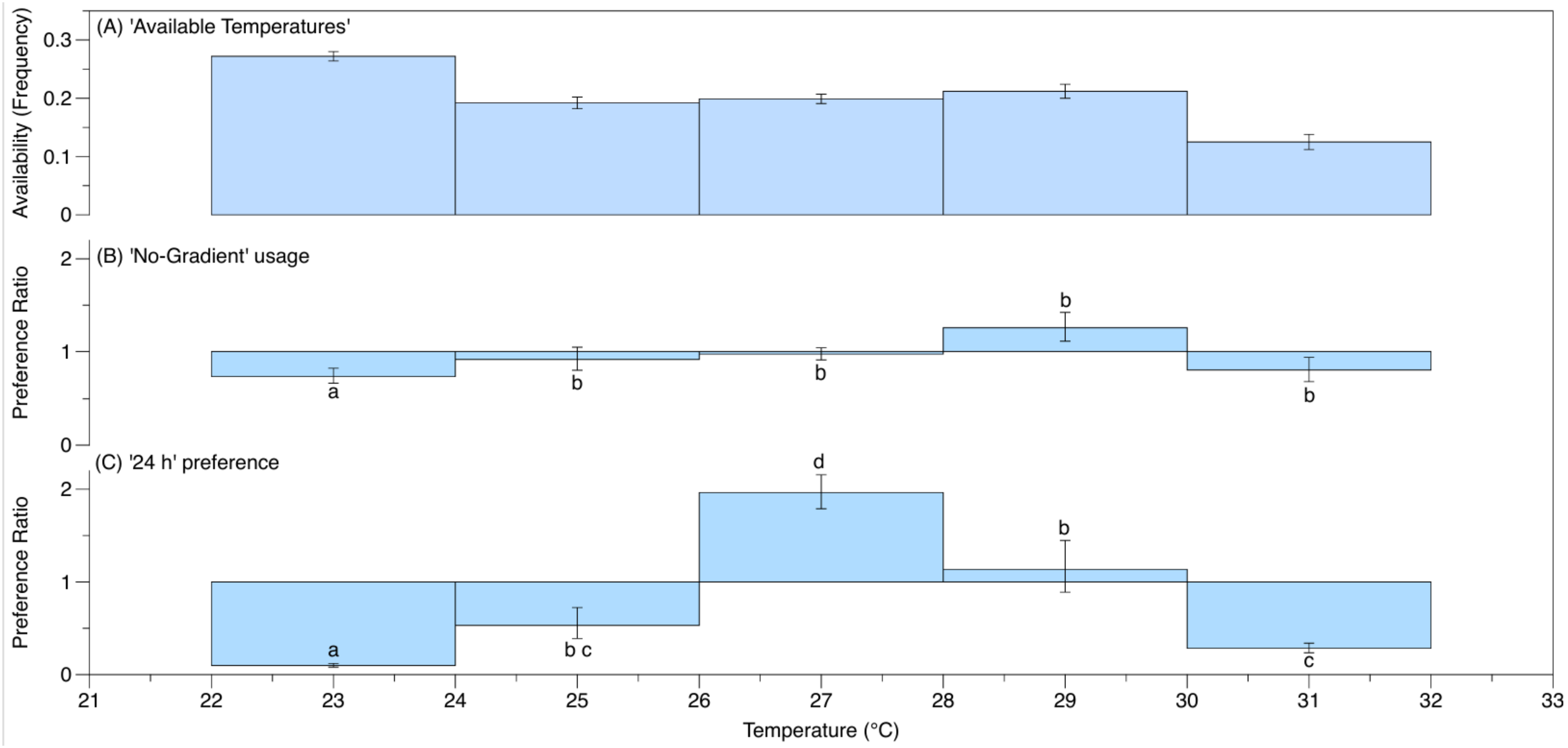

Figure 11. Temperature preference (T_{pref}) of *Abudefduf vaigiensis*. Error bars are S.E.M. Different letters represent significant differences among temperature bins based on rank compositional analysis, $p < 0.05$. (A) The average proportion (frequency) of available temperatures in the (T_{pref}) annular chamber during the 'available temperatures' period. (B) Geometric means of preference ratio (proportion used / proportion available) during the 'no-gradient' usage period in each eventual temperature zone before the temperature gradient from 22 - 32°C was established. (C) Geometric means of the preference ratio during the '24 h' preference period when the temperature gradient from 22 - 32°C was established.

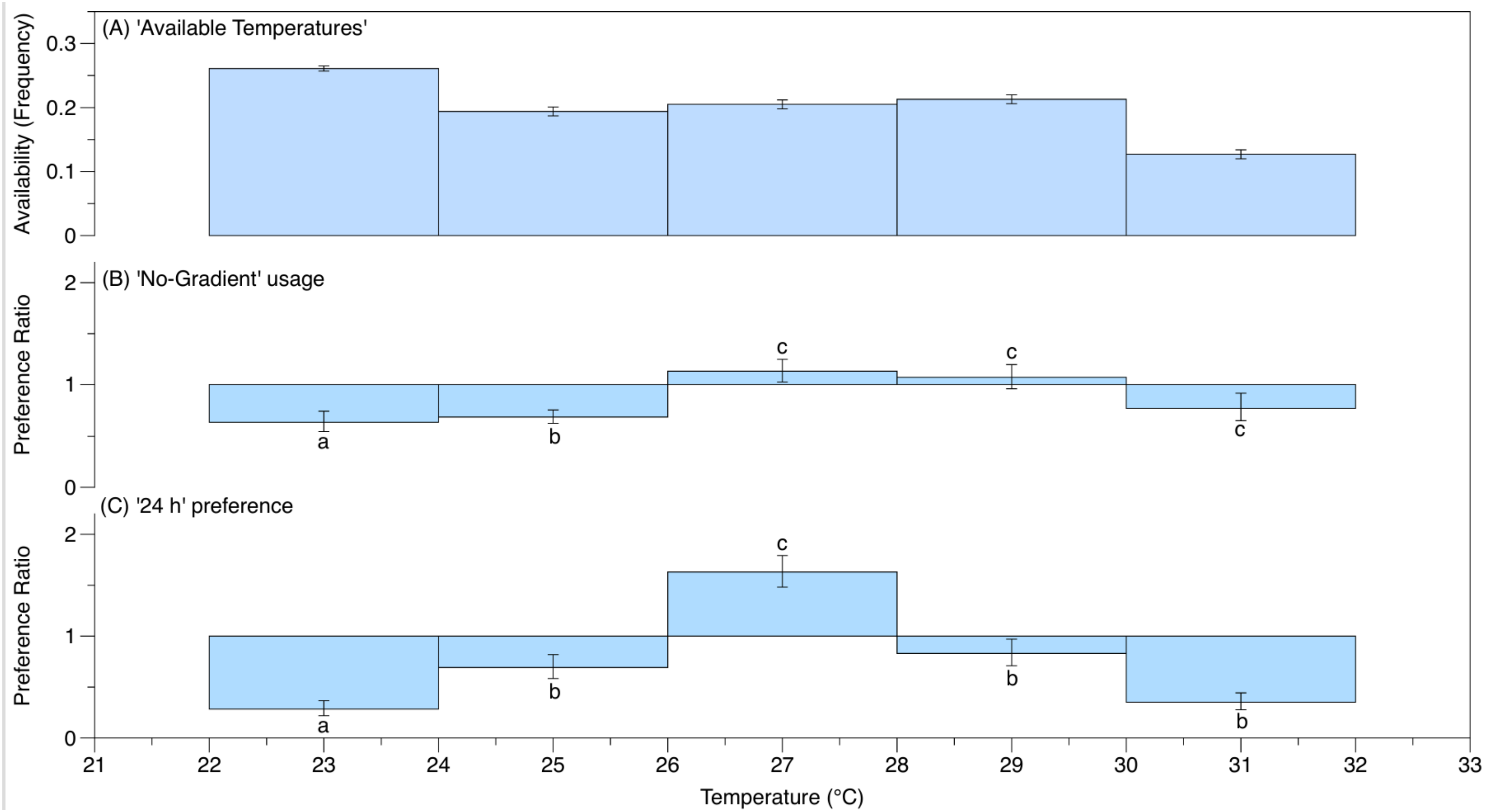

Figure 12. Temperature preference (T_{pref}) of hybrids (*Abudefduf vaigiensis* and *A. abdominalis*). Error bars are S.E.M. Different letters represent significant differences among temperature bins based on rank compositional analysis, $p < 0.05$. (A) The average proportion (frequency) of available temperatures in the T_{pref} annular chamber during the 'available temperatures' period. (B) Geometric means of preference ratio (proportion used / proportion available) during the 'no-gradient' usage period in each eventual temperature zone before the temperature gradient from 22 - 32°C was established. (C) Geometric means of the preference ratio during the '24 h' preference period when the temperature gradient from 22 - 32°C was established.

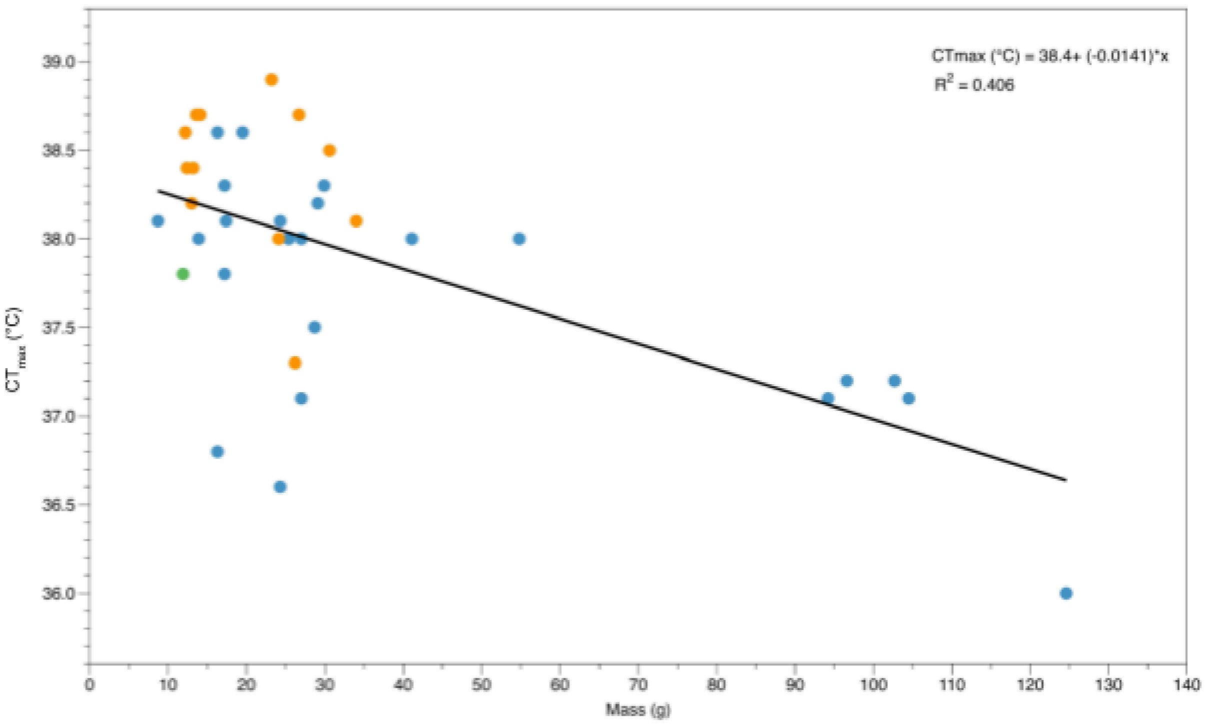

Figure 13. Critical temperature maximum (CT_{max}) as a function of body mass. Green points represent *A. abdominalis* individuals, blue points represent hybrids, and orange points represent *A. vaigiensis* individuals.

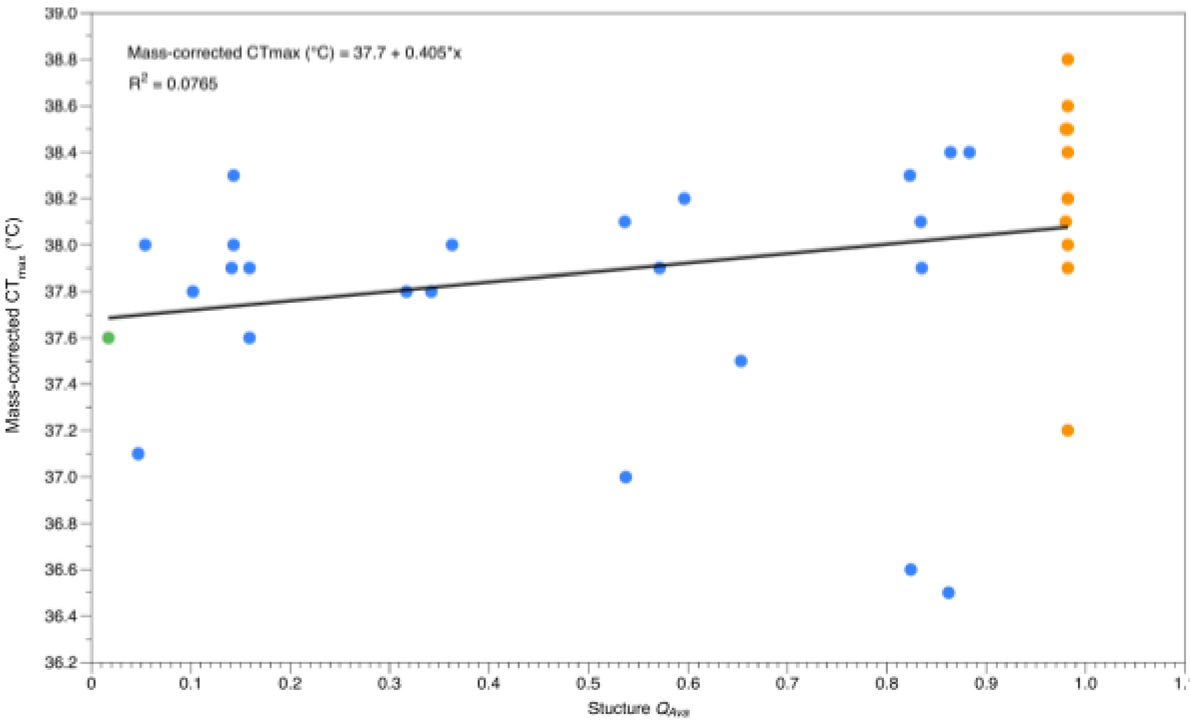

Figure 14. Relationship between mass-corrected critical temperature maximum (CT$_{max}$) and Structure Q_{Ava}(an estimate of genetic similarity to the parent species *A. vaigiensis* (Q_{Ava} close to 1.0) or *A. abdominalis* (Q_{Ava} close to zero)). Green points represent *A. abdominalis* individuals, blue points represent hybrids, and orange points represent *A. vaigiensis* individuals. CT$_{max}$ mass-corrected to 32.8 g [= CT$_{max}$ - 0.0118 * (32.8 - mass)].