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Effect of thermal variation on the cardiac thermal limits of a eurythermal marine teleost (*Girella nigricans*)★

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A R T I C L E I N F O

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A B S T R A C T

Although most animals live in complex, thermally variable environments, the impact of this variability on specific physiological systems is still unresolved. The ectotherm heart is known to change in both structure and function to ensure appropriate oxygen delivery under different thermal regimes, but the plasticity of the upper thermal limits of the heart under stable or variable thermal acclimation conditions remains unknown. To investigate the role of thermal variability on cardiac acclimation potential, we acclimated a eurythermal fish, opaleye (*Girella nigricans*), to three static temperature treatments (13, 16, and 19 °C) as well as two oscillating treatments which cycled between maximum and minimum temperatures every 12 h (13–19 °C and 16–22 °C). These temperatures and daily thermal ranges were chosen to mimic the conditions observed in the rocky intertidal environments in Santa Barbara, CA, USA where the fish were collected. We hypothesized that increasing temperature would increase upper thermal limits of the heart, and that variable acclimations would result in broader acute thermal performance curves (TPCs) compared to static acclimations. We measured maximum heart rate during acute warming to determine cardiac thermal performance (i.e., the temperature corresponding to the onset of cardiac arrythmia, the temperature at maximum heart rate, absolute maximum heart rate, and the Arrhenius breakpoint temperature) and construct acute TPCs. Rising static acclimation temperatures increased upper thermal limits but had no impact on peak maximum heart rate. The warmest static temperature did, however, cause a narrowing of the acute TPC. Fish acclimated to variable conditions had the same upper thermal limits compared to fish acclimated to static conditions with the same mean temperature in all metrics of thermal performance. Further, there was no significant broadening of the acute TPC. This study suggests that cardiac plasticity is robust to thermal variation in this eurythermal fish.

1. Introduction

Temperature is an important driver of the biological rates of ectotherms, influencing metabolic rate, heart rate (f_H), and growth (Currie et al., 2014; Eliason and Anttila, 2017; Fry, 1947). To survive and thrive under changing thermal conditions, individuals must move (i.e., behavioral thermal regulation; Reynolds and Casterlin, 1979), acclimate (Johnston and Dunn, 1987), or do some combination of these to meet their physiological needs (Crawshaw, 1977). Here, thermal acclimation refers to the reversible modification of an organism's physiology to maintain performance (da Silva et al., 2019; Rome et al., 1992; Schulte et al., 2011). Acclimation can be measured through changes in thermal performance curves (TPCs), which describe the impact of temperature on an organism's performance, including thermal optima and upper and lower thermal limits (Rohr et al., 2018). TPCs can be shifted right or left, changed in height, or altered in breadth in response to different acclimation conditions (Schulte et al., 2011). Indeed, fish's thermal limits are plastic traits which are directly related to their acclimation conditions (Badr et al., 2016). To ensure experimental results accurately represent natural phenomena, it is essential that assumptions regarding simplifications (e.g., the use of static acclimation conditions) to a study system are thoroughly tested. The extent to which TPCs and thermal limits of specific organs, such as the heart, are impacted by static vs. variable acclimation conditions is, however, largely unknown.

Thermal variation on tidal, diel, seasonal, and decadal time scales is intrinsic to almost all habitats on earth. Understanding how thermal

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variability may affect the acclimation capacity and upper thermal limits of an individual is an important knowledge gap that has implications for both climate change and basic research (Farrell et al., 2009; Sinclair et al., 2016; Stevens, 1989; Sunday et al., 2011). Significant work has investigated thermal acclimation capacity under different static temperatures; however, the impact of incorporating thermal variability into acclimation conditions is still largely unknown. Responses to thermally variable acclimation varies across levels of biological organization and ontogeny (Dowd et al., 2015; Morash et al., 2018; Morissette et al., 2020; Pisano et al., 2019; Rossi et al., 2020). At the organismal level, thermal variability has inconsistent effects on marine ectotherms, with some studies reporting decreases or no effect of thermal variation on the metabolic rate of Atlantic salmon (*Salmo salar*) (Morash et al., 2018; Morissette et al., 2020). In contrast, brook trout (*Salvelinus fontinalis*) survival increased after incorporating thermal variability into the acclimation conditions, while growth was negatively impacted (Pisano et al., 2019). At the cellular level, thermal variability has been shown to have no impact on plasma cortisol levels in bluntnose minnow (*Pimephales notatus*), spotfin shiner (*Cyprinella spiloptera*), white sucker (*Catostomus commersonii*), and smallmouth bass (*Micropterus dolomieu*) (Eldridge et al., 2015), but plasma cortisol did increase in juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) (Mesa et al., 2002). Heat shock proteins, a common indicator of thermal stress, generally increase under variable conditions (Mesa et al., 2002; Narum et al., 2013), but this may depend on the duration and magnitude of the thermal cycling (Tunnah et al., 2017). The lack of consistent responses to thermal variation across species and studies limits the ability to assess individual and population level responses to increasingly variable marine environments. Further, little to no work has been to determine how the incorporation of thermal variability impacts specific organs systems.

As the driver of the cardiorespiratory system, the heart must respond to both acute and long-term changes in temperature to facilitate appropriate cardiac output (Eliason and Anttila, 2017). At acute time scales, fish almost exclusively increase heart rate (f_{H}) to improve oxygen delivery to the tissues in response to rising temperatures (Farrell et al., 2009; Fry, 1947; Mendonca and Gamperl, 2010). Heart rate cannot increase indefinitely though. Under intense acute thermal stress, f_{H} will peak or plateau, then decline into arrythmia (Casselman et al., 2012). On longer times scales (i.e., weeks to months), acclimation to warm temperatures can increase maximum f_{H}, thus improving performance under acute thermal stress. This is accomplished through morphological changes in the heart, including changes in ventricle mass, the proportion of collogen, and the percent of spongy myocardium (reviewed by Keen et al., 2017). At the subcellular level, cardiac remodeling includes changes in troponin I and C (Alderman et al., 2012; Genge et al., 2016), contractile protein phosphorylation state (Keen et al., 2017; Klaiman et al., 2011), and expression level of sarcoendoplasmic reticulum Ca-ATPase (SERCA) (Korajoki and Vornanen, 2013; Vornanen, 2021). These changes help to maintain cardiac output through increases in stroke volume, f_{H}, or some combination thereof, thus meeting the increased metabolic demand of the tissues associated with rising temperatures. Given the importance of cardiac function in maintaining physiological performance at all biological levels, the upper thermal limits of the heart have been used to better understand whole organism thermal tolerance in an ecological context (Drost et al., 2016; Eliason and Anttila, 2017; Farrell et al., 2009). However, it is unknown how thermal variation during acclimation impacts cardiac remodeling and upper thermal limits.

The objective of this study was, therefore, to evaluate how variable thermal acclimation impacts the cardiac thermal performance of a eurythermal marine teleost fish, opaleye (*Girella nigricans)*. We were specifically interested in assessing ecologically relevant, naturally occurring temperature regimes typically encountered by opaleye (i.e., no climate change extreme scenarios were included). Opaleye are native to the west coast of North America ranging from Santa Barbara, California to Baja California, Mexico. They inhabit shallow, near-shore areas and intertidal zones, and are regularly subjected to thermal variation on tidal, diel, and seasonal time scales, with temperatures ranging down to 11 °C and in excess of 24 °C (Fig. 1; LTER SBC and Washburn, 2020). To accomplish our objective, we used the Arrhenius Breakpoint Temperature (ABT) Test. The ABT Test on the heart has been correlated with whole organism metrics of thermal tolerance (Casselman et al., 2012; Gilbert and Farrell, 2021). This test can provide insight into the acclimation capacity of the heart through the construction of acute TPCs, from which multiple metrics of thermal tolerance can be calculated (Fig. 2a), including the Arrhenius breakpoint temperature (T_{ABT}), peak f_{Hmax}, the temperature at which peak f_{Hmax} occurs (T_{PEAK}), and the temperature of the onset of arrythmias (T_{ARR}). By comparing these metrics and curves across individuals acclimated to different thermal conditions, we can elucidate the impacts of acclimation on the heart's thermal plasticity. We hypothesized that increasing temperature acclimation would increase cardiac thermal tolerance in opaleye (i.e., right shift the acute TPCs for f_{Hmax}, Fig. 2b). We also hypothesized that variable acclimations would result in a trade-off with higher cardiac thermal tolerance but lower peak f_{Hmax} (i.e., a broader and flatter acute TPC for f_{Hmax}, Fig. 2c) compared to a static acclimation.

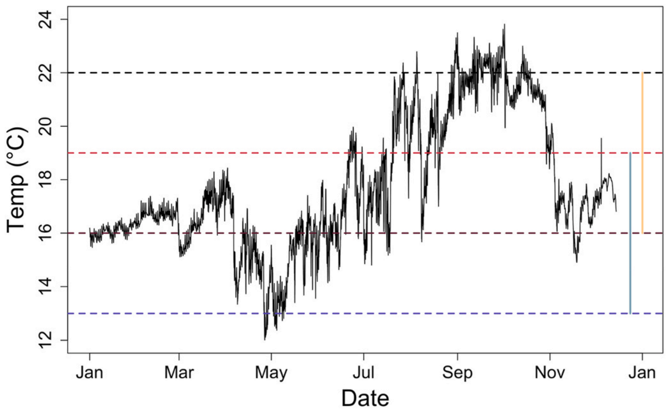

Fig. 1. Temperature (°C) in Santa Barbara Harbor for all of 2015. The two vertical lines on the right side show the temperature range of the variable acclimation treatments, while the horizonal lines show the three static acclimation temperatures used in this study, and the upper temperature experienced by the warm variable treatment. Harbor temperature data were obtained from Santa Barbara Coastal LTER from a moored instrument at Santa Barbara Harbor/Stearns Wharf (LTER 2020).

2. Methods

All work was approved by the University of California, Santa Barbara Institutional Animal Care and Use Committee. Opaleye (N = 54; mass = 44.6 ± 2.6 g, total length = 12.8 ± 2.0 cm, mean ± SE) were collected via rod and reel from the Santa Barbara Harbor, CA, USA during Autumn 2020. Individuals were acclimated for a minimum of 3 weeks in 25-gallon flow-through seawater tanks at the University of Santa Barbara. One tank (12–13 fish per tank) was randomly assigned to each temperature treatment. There were three static temperature treatments (13, 16, and 19 °C), and two diel oscillating temperature treatments (13–19 °C and 16–22 °C; Fig. 3). These temperatures and variabilities were chosen to mimic naturally occurring temperatures and daily fluctuations from the collection site (Fig. 1). Temperature was continuously monitored via iButton temperature loggers (Maximum Integrated, San Jose, CA) and a custom-built remote monitoring system using raspberry pi (Cambridge, UK). Tanks were plumbed with two sources of temperature-controlled seawater (10 °C chilled, and 20 °C heated) and temperature fluctuations were controlled via aquarium dip heaters and controllers (Inkbird, London, England). Fish were fed a mixed diet of brine shrimp and red ogo (*Gracilaria pacifica*) *ad libitum* and were held under a 14:10 light: dark cycle to mimic natural conditions (Table 1).

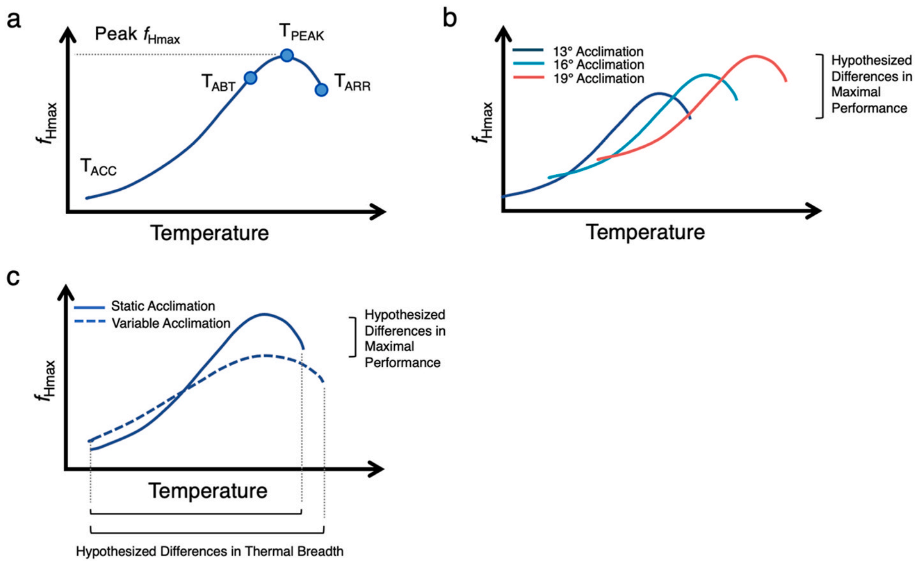

Fig. 2. Acute thermal performance curves and the associated metrics we can extract from these curves (A): acclimation temperature (T_{acc}), Arrhenius Breakpoint Temperature (T_{ABT}), peak maximum heart rate (peak f_{Hmax}), temperature of peak f_{Hmax} (T_{PEAK}), and the temperature at which the heart became arrhythmic (T_{ARR}). Hypothesized differences in performance at three different static acclimation temperatures (B). Hypothesized differences in acute TPCs between variable and static acclimation temperatures with the same mean temperature (C).

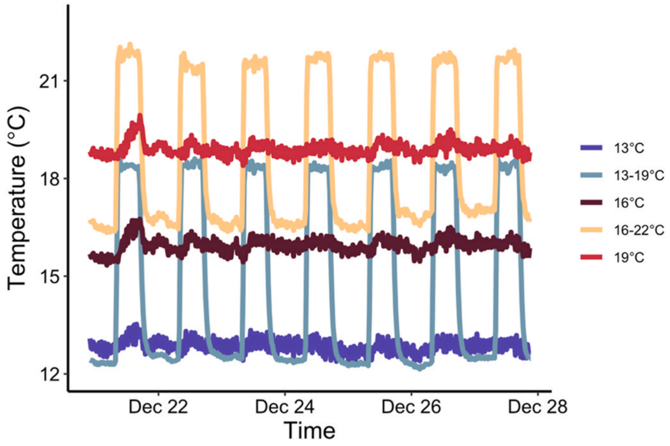

Fig. 3. Data from temperature loggers (ibuttons, Maximum Integrated, San Jose, CA) placed in tanks where fish were acclimated to one of three static temperatures, or one of two variable temperatures.

Table 1
Sample size (N), total length (cm), body mass (g), and relative ventricular mass (RVM; %) in opaleye acclimated to five different temperature treatments. There were no significant differences in fish size metrics among acclimation treatments as determined through an analysis of variance (ANOVA; α = 0.05). Data are presented as mean ± SE.

Acclimation Treatment	13 °C	16 °C	13–19 °C	19 °C	16–22 °C
Tank Temperature	13.4 ± 0.0	16.3 ± 0.0	15.2 ± 0.0	19.1 ± 0.0	18.6 ± 0.0
Sample Size	13	11	9	11	10
Total Length (mm)	13.2 ± 0.8[b]	11.9 ± 0.5[a]	13.1 ± 0.5	13.4 ± 0.4	12.6 ± 0.8
Body Mass (g)	46.7 ± 7.4	35.4 ± 4.6	47.9 ± 5.1	49.9 ± 3.8	43.1 ± 6.7
Relative Ventricular Mass (RVM; %)	0.03 ± 0.01[c]	0.02 ± 0.00[c]	0.03 ± 0.00[a]	0.03 ± 0.01	0.03 ± 0.02

[a] Sample size is lower by one.
[b] Sample size is lower by two.
[c] Sample size is lower by three.

Arrhenius breakpoint tests (ABT tests) were conducted following Casselman et al. (2012) and Gilbert and Farrell (2021). Briefly, fish were anesthetized in seawater from their acclimation tank with 80 mg L^{-1} MS-222 buffered with 1g L^{-1} $NaHCO_3^-$. Individuals were weighed and placed ventral side up in the test tank which contained a maintenance dose of anesthetic (65 mg L^{-1} MS-222 buffered with 1 g L^{-1} $NaHCO_3^-$). Aerated seawater was continuously circulated past the gills, and fish were fitted with stainless steel Needle Tip Electrodes (AD Instruments INC, Colorado Springs, CO, USA) just under the skin to detect an ECG signal. This signal was amplified and filtered (60hz Notch filter; Mains filter; Low-Pass: 2Kz; High Pass: 10hz; Range: 2 mV) using a Dual Bio Amp and Powerlab data acquisition system (AD Instruments INC, Colorado Springs, CO, USA).

Following a 30 min equilibration period at the acclimation temperature (Ferreira et al., 2014; Hansen et al., 2017), atropine sulfate (1.2 mg kg^{-1} in 0.9% NaCl) was injected intraperitonially to block vagal tone. Fifteen minutes later, isoproterenol (4 μg kg^{-1} in 0.9% NaCl) was also injected intraperitonially to maximally stimulate β-adrenoreceptors (Hardison et al., 2021). Fifteen minutes after isoproterenol injection, water temperature was heated at 1 °C every 6 min using a Polystat recirculating heater/chiller (Cole-Palmer, Vernon Hills, IL, USA). At each 1 °C interval, f_H and temperature were allowed to stabilize to record f_H. Temperature was increased until the onset of cardiac arrythmia (T_{ARR}), as indicated by a transition from rhythmic to arrhythmic beating or a missed QRS peak resulting in a precipitous drop in heart rate (Casselman et al., 2012). All fish were immediately euthanized at the end of the test, and both total length and ventricle mass were recorded.

2.1. Data analysis for Arrhenius breakpoint test

All ECG analyses were performed in LabChart software (AD Instruments, Dunedin, New Zealand). f_H was calculated for each temperature increment from 15 continuous seconds of measurements using automated ECG analysis software in lab chart (Gradil et al., 2016). Arrhenius breakpoint tests were performed using the "segmented" package in R (version 1.1-0), and the temperature corresponding to the breakpoint in f_H was defined as T_{ABT}. Maximum heart rate (f_{Hmax}) was defined as the highest f_H recorded over a 15 s measurement. Peak temperature (T_{PEAK}) was the temperature corresponding to f_{Hmax}.

2.2. Statistical analysis

All data were statistically analyzed using R (version 3.5.1). Data were analyzed using a 1-way ANOVA (Car package version 3.0-2; significance level $\alpha = 0.05$) with post-hoc Tukey HSD. Polynomial curves were fit to f_H data and compared using Bayes information criterion (BIC). BIC showed strongest support for a third order polynomial model which included acclimation treatment and acute test temperature (Supplemental Table 1). We also conducted a principal component analysis using the R package "factoextra" (Kassambara and Mundt, 2020) to visualize levels of overlap among different treatment responses.

3. Results

Fish body size (i.e., total length, fish mass) and RVM did not differ across treatments following the three-week acclimation period (total length $p = 0.44$, fish mass $p = 0.45$, and RVM $p = 0.63$; One-Way ANOVA, Table 1).

All fish displayed similar TPCs with acute warming, where f_H increased and reached a peak before declining and becoming arrhythmic (Fig. 4). Thermal performance increased with warm acclimation. The 13 °C static treatment had significantly lower T_{ABT} and T_{PEAK} than the 16–22 °C variable treatment ($p < 0.01$ and $p = 0.01$, respectively; Fig. 5, Table 2). T_{ARR} in fish acclimated to 13 °C was significantly lower compared with fish acclimated to 13–19 °C ($p = 0.02$), 19 °C ($p < 0.01$), and 16–22 °C ($p < 0.01$). Fish acclimated to 16 °C also exhibited significantly lower T_{ARR} than fish acclimated to 16–22 °C ($p = 0.04$). However, most notably, for all three metrics, fish acclimated to variable conditions did not exhibit any significant differences from their mean static counterpart based on Tukey's post-hoc test (i.e., 13–19 °C did not differ from 16 °C [T_{ABT} $p = 0.98$, T_{PEAK} $p = 0.95$, T_{ARR} $p = 0.98$, peak f_{Hmax} $p = 0.91$] and 16–22 °C did not differ from 19 °C [T_{ABT} $p = 0.47$, T_{PEAK} $p = 0.83$, T_{ARR} $p = 0.48$, peak f_{Hmax} $p = 0.99$]; Fig. 5). In the case of the 13–19 °C treatment, there also were no significant differences with the maximum stable temperature experienced (19 °C; T_{ABT} $p = 1.0$, T_{PEAK} $p = 0.99$, T_{ARR} $p = 0.96$, peak f_{Hmax} $p = 0.99$).

Table 2
Metrics calculated from each individual acute thermal performance curves. All data are presented as mean ± SE, and abbreviations are as follows: Arrhenius breakpoint temperature (T_{ABT}), temperature of peak heart rate (T_{PEAK}), temperature at which arrythmias began (T_{ARR}), maximum heart rate (f_{Hmax}).

Acclimation Treatment	13 °C	16 °C	13–19 °C	19 °C	16–22 °C
Sample Size	13	11	9	11	10
T_{ABT} (°C)	23.6 ± 0.5	24.8 ± 0.5	25.3 ± 0.5	25.2 ± 0.3	26.3 ± 0.5[a]
T_{PEAK} (°C)	27.3 ± 0.4	28.1 ± 0.5	28.6 ± 0.4	28.9 ± 0.7	29.7 ± 0.5
T_{ARR} (°C)	28.6 ± 0.4	30.0 ± 0.4	30.4 ± 0.4	30.8 ± 0.5	31.7 ± 0.4
Peak f_{Hmax} (bpm)	169.1 ± 4.0	171.8 ± 5.3	179.7 ± 4.9	175.6 ± 9.1	177.0 ± 6.6

[a] Sample size is lower by one.

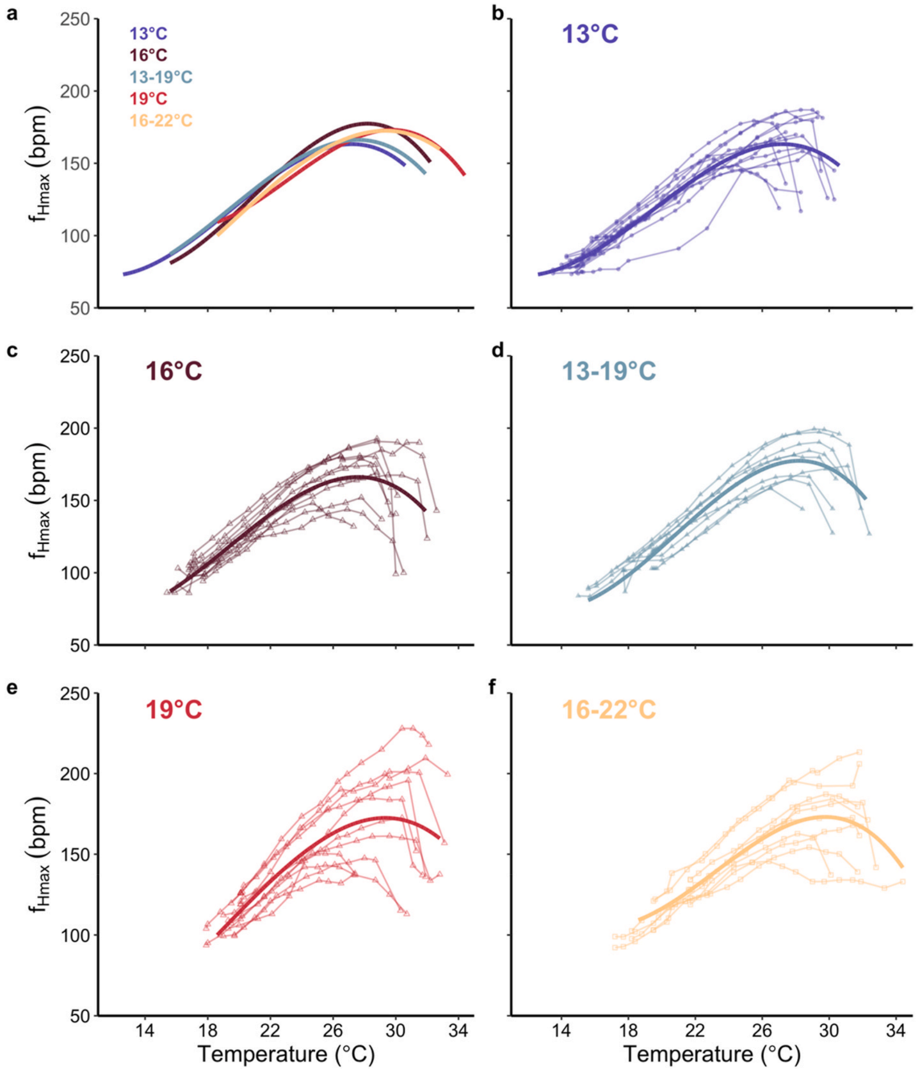

Fig. 4. Acute thermal performance curves for maximum heart rate (f_{Hmax}) in opaleye fish acclimated to one of five temperature regimes. Warming started at the acclimation temperature and increased until the onset of arrythmias. For variable temperature treatments, warming began at the maximum temperature experienced due to the timing of trials (i.e., trials occurred during the day). Panel a shows the best fitting model for all treatments. This model is a third order polynomial including an interaction between acclimation treatment and acute temperature, as well as individual fish as a random effect to account for repeated measure on the same individual. Panels b–f show individual opaleye (pale line and dots), as well as model predicted values (thick solid lines).

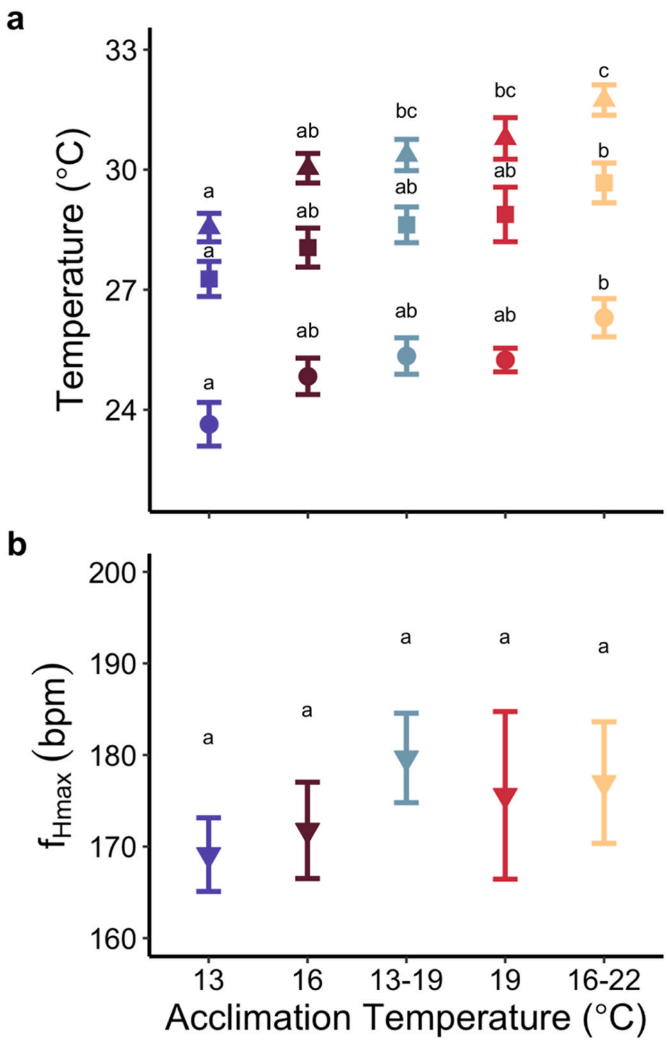

Fig. 5. The effect of thermal acclimation on transition, critical temperatures, and peak maximum heart rate (f_{Hmax}) during acute warming in opaleye. Mean (±SE) temperatures at the onset of cardiac arrhythmic (T_{ARR}; Triangles), the occurrence of peak maximum heart rate (T_{PEAK}; squares), and at the Arrhenius breakpoint (T_{ABT}; circles) are shown, along with peak f_{Hmax} (upside-down triangles). Lowercase letters indicate significant differences among acclimation temperatures (Bonferroni-adjusted multiple comparisons, $\alpha = 0.05$).

Fish exposed to variable thermal conditions did not have broader acute TPCs compared to their static counterparts (Fig. 6). We did, however, see a significantly narrower acute TPC at 19 °C compared to 13 °C and 16 °C ($p < 0.01$ and $p = 0.01$, respectively; Fig. 5).

The principal component analysis was performed using a correlation matrix, and the first two components explained 69.7% of the variation in the data. Remaining components explained less the 15% of the variation in the data and were excluded from further analysis. The plot grouping each individual (Fig. 7) shows separation in the area of mean density for the 13 °C treatment and the 16–22 °C treatment, with high amount of overlap with all other acclimation treatments.

4. Discussion

Here, we sought to investigate the role of ecologically relevant static and variable thermal acclimations on maximum heart rate and the upper thermal limits of the heart of a eurythermal marine teleost. We found that opaleye did increase their upper thermal limits under increased acclimation temperatures, but there was no impact of thermal variability compared to static thermal acclimation treatments on cardiac performance. We demonstrated that this eurythermal fish possesses the ability to maintain cardiac function across a broad array of thermal conditions that mimic the seasonal and diel fluctuations in temperature that occur in their native habitats. As global climate change increases both the mean temperature and thermal variability in aquatic environments, understanding the ability of ectotherm cardiorespiratory systems to respond to different thermal conditions is essential (Drost et al., 2016; Ekstrom et al., 2017).

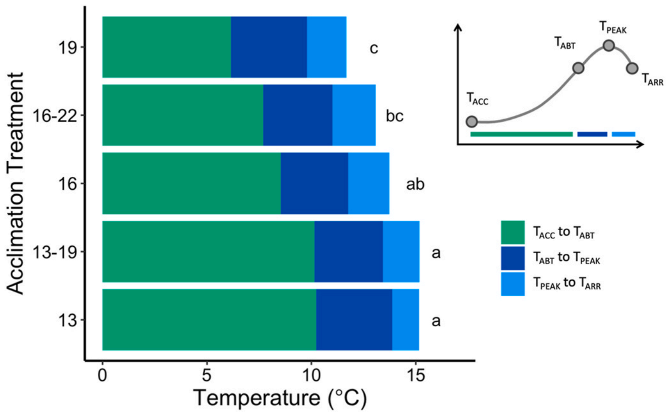

Fig. 6. Mean thermal distance between thermal tolerance metrics derived from acute thermal performance curve in opaleye acclimated to one of five different acclimation conditions. Dissimilar letters indicate significant differences between the mean temperature of acclimation (T_{ACC}) and the temperature of the onset of arrythmia (T_{ARR}). Remaining abbreviations are as follows: Arrhenius Breakpoint Temperature, T_{ABT}; Temperature of peak maximum heart rate, T_{PEAK}.

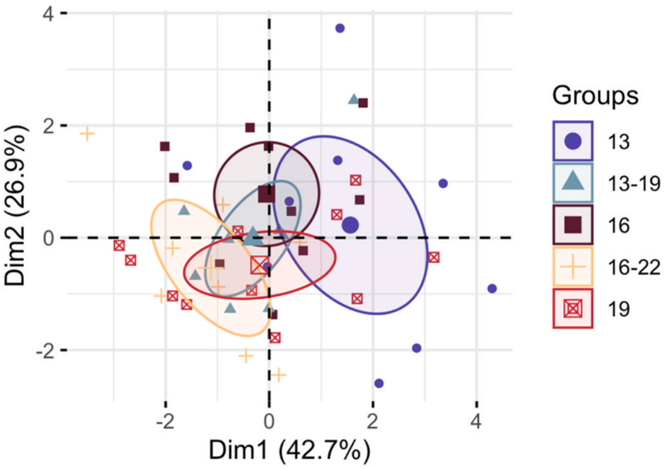

Fig. 7. Principle component analysis (PCA) for all individuals (points), as well as means (large symbols). Together, these two dimensions explain 69.7% of total variance. Areas of mean density are shown in shaded ovals. Increased overlap in shaded circles is indicative of increased similarity in physiological performance among acclimation treatments.

4.1. Increases in thermal tolerance under static acclimation conditions

Fish are known to increase their thermal tolerance with warm acclimation as a mechanism to maintain physiological performance under the seasonal changes that occur in their thermal environment (da Silva et al., 2019; Schulte et al., 2011). We, therefore, hypothesized that increases in static acclimation temperature would increase the upper thermal limits and peak f_{Hmax} without concurrent changes in the breadth of the acute TPCs. We did see increases in T_{ARR} in fish acclimated to warmer temperatures, though this did not hold for other measures of upper thermal limits for the static treatments. While increases in T_{ARR} with increases in acclimation temperature are common in teleost fishes (Anttila et al., 2014; Ferreira et al., 2014; Gilbert and Farrell, 2021) the utility of this metric is still debated. In all cases, T_{ARR} occurs below the critical thermal limit (CT_{max}) at which a fish loses equilibrium. However, T_{ARR} has the benefit of mechanistically describing a deficit in cardiac function which would rapidly lead to insufficient oxygen delivery, decreased metabolism and functional death, whereas CT_{max} lacks a mechanistic link to true function (Lefevre et al., 2021).

We did not find any changes in peak f_{Hmax} across the three static treatment temperatures (13, 16, 19 °C), although there was higher interindividual variability in the 19 °C treatment which suggests some level of phenotypic variability on which selection can act (Bennett et al., 2019; Collins et al., 2016). Generally, eurythermal fishes are predicted to have evolved broad TPCs to facilitate life in thermally variable environments (Healy and Schulte, 2012; Janzen, 1967; Rohr et al., 2018), and opaleye adhere to this model. Similarly, goldfish (*Carassius auratus*) and Atlantic herring (*Clupea harengus*) saw no significant changes in peak f_{Hmax} with acclimation temperature increases of 8 and 7 °C respectively (Ferreira et al., 2014; Moyano et al., 2020). In contrast, the common killifish (*Fundulus heteroclitus*) showed a 32% increase in peak f_{Hmax} following acclimation to 5 and 33 °C (Safi et al., 2019), suggesting that different eurythermal fishes have different capacities and/or strategies for cardiac thermal acclimation.

It is worth noting that our most extreme test temperatures (i.e., 13 °C and 19 °C for static) were within the range of temperatures experienced during seasonal thermal variation in their native habitat, and thus may be within the thermal range for which cardiac performance is optimized. Indeed, Hardison et al. (2021) found a significant difference in f_{Hmax} between opaleye acclimated to 12 and 20 °C, temperatures which may represent thermal extremes and, at least for 12 °C, be outside the optimal thermal range for cardiac performance. Future work looking to examine the thermal limits of eurythermal fishes may consider exposing fish to temperatures exceeding the mean annual maximum and minimums, and should also consider examining lower levels of biological organization (e.g., thermal acclimation capacity of cardiac ion channels, pacemaker cells, B-adrenoreceptors, or sarcoendoplasmic reticulum Ca^{2+-}ATPase) to elucidate mechanisms of tolerance (Castilho et al., 2007; da Silva et al., 2011; Graham and Farrell, 1989; Keen et al., 2017; Korajoki and Vornanen, 2013; Landeira-Fernandez et al., 2004; Shiels et al. 2015; Shiels et al., 2000; Vornanen, 2021).

We did see significant narrowing of the thermal breadth of fish acclimated to 19 °C compared to those acclimated to 16 °C or 13 °C. We did not predict to see narrowing at these temperatures, as opaleye seasonally experience temperatures up to 23 °C in the Santa Barbara harbor (where these fish were collected), and even warmer temperatures in the southernmost part of their range (Baja California, Mexico). This 19 °C treatment may have approached a functional upper thermal limit in this northernmost population and could be indicative of population differences in thermal tolerance (Donelson et al., 2019; Eliason et al., 2013; Hampe and Petit, 2005; Sunday et al., 2011), though the genetic structure and differentiation of opaleye has not been determined. This apparent approaching of the upper thermal limit in this study may not hold true for other populations, or for fish collected during the late summer months, after they have experienced intermittent, seasonal exposure to high temperatures. Future work should investigate the potential for seasonal acclimatization and local adaptation of populations to their specific environmental conditions.

4.2. Impacts of thermal variability during acclimation

Most studies examining the impact of static vs. variable thermal acclimation conditions have focused either on the whole-animal, looking at metrics such as growth (Eldridge et al., 2015; Flodmark et al., 2004; Imholt et al., 2011; Meeuwing et al., 2004), metabolism (Beauregard et al., 2013; Lyytikainen and Jobling, 1998; Morash et al., 2018; Morissette et al., 2020; Oligny-Hébert et al., 2015), critical thermal limits (Cooper et al., 2021; Rodgers et al., 2018), or on molecular indicators of stress, such as heat shock proteins (Mesa et al., 2002; Narum et al., 2013; Tunnah et al., 2017). We specifically sought to examine the impact of different acclimation conditions on cardiac function, as the large body of work examining cardiac plasticity has almost exclusively used static temperature treatments (Eliason and Anttila, 2017).

The lack of differences in T_{ARR} between fish acclimated to static or variable thermal regimes with the same mean temperature suggests that incorporating thermal variation has no impact on opaleye upper thermal limits. To our knowledge, there are no studies to date examining the impact of thermal variability on the upper thermal limits of the teleost heart. Because cardiac thermal tolerance is thought to be a limiting factor driving organismal thermal tolerance (Eliason and Anttila, 2017), however, we sought to examine our findings within the larger context of upper critical thermal limits (CTmax). When looking at the impact of thermal variation on the upper thermal limits of eurythermal fishes, there are instances of variation increasing thermal tolerance (e.g., fathead minnows, *Pimephales promelas*; zebrafish, *Danio rerio*) (Salinas et al., 2019; Schaefer and Ryan, 2006), and having no impact on upper thermal limits (e.g., convict cichlid, *Archocentrus nigrofasciatus*; channel catfish, *Ictalurus punctatus;* largemouthbass, *Micropterus salmoides*; squaretail mullet, *Liza vaigiensis;* crescent terapon, *Terapon jarbua*) (Cooper et al., 2021; Currie et al., 2004; Eme et al., 2011). These differences across studies may be due to the length of acclimation to a thermocyle (Threader and Houston, 1983), the magnitude and regularity of the thermal variability (Rodgers et al., 2018), as well as the position of the acclimation temperature(s) on the thermal performance curve of the species (Hernández-Rodríguez and Bückle-Ramirez, 2010).

Although opaleye did not display significant differences in measures of cardiac thermal tolerances between static and variable acclimation conditions, for every metric, it appeared that the fish acclimated to variable conditions outperformed their static counterpart. For example, the variable treatment 16–22 °C had a mean T_{ABT} of 26.3 ± 0.5 °C (mean ± SE), while the static 19 °C treatment had a mean T_{ABT} of 25.2 ± 0.3 °C. We also saw some separation of treatments in the PCA, which could suggest the variable temperature treatments performed well compared to fish exposed to a constant temperature. This trend has also been observed in the cardiac function of other ectotherms. For example, cardiac upper thermal limits of snails (genera *Tegula*), acclimated to variable conditions (14–22 °C) were more similar to those acclimated to 22 °C than those acclimated to 14 °C (Stenseng et al., 2005). Mechanistically, the daily reprieves from thermal stress may be a thermal refugia of sorts, during which fish are able to remove reactive oxygen species (ROS) which accumulated during the warm periods (Vornanen, 2020; Vornanen et al., 2002). It is also possible that fish are physiologically primed to handle the worse conditions they experience, rather than the mean temperature of their environment as stated in the *Climate Extremes Hypothesis* (Pither, 2003; Stevens, 1989; Sunday et al., 2019). Indeed, Brett (1956) asserts that increases in thermal tolerance occur quickly (e.g., less than 24 h), while the loss of this increased thermal tolerance occurs much more slowly taking weeks. Further testing regarding the impact of episodic thermal stress, particularly regarding thermal variation or different magnitudes and positions across a species' TPC, would help elucidate the mechanisms driving this observed phenomenon.

Our second hypothesis was that fish exposed to variable thermal conditions would have broader acute TPCs. We did not detect any statistically significant differences in thermal breadth between acclimation treatments with the same mean temperature, however there was a trend for variable treatments to have a broader thermal margin before becoming arrhythmic compared to their static acclimation counterparts. While it is possible that acclimation processes were incomplete after the 3-week acclimation period, it is likely that the fish were approaching steady state conditions in this study (Ekstrom et al., 2016). Instead, we suggest that this lack of statistically significant variation may be due to the eurythermal nature of our study species. *Janzen's Rule* states that tropical environments (i.e., habitats with low thermal variability) will select for species with narrow thermal tolerance ranges (Janzen, 1967). A corollary would be that fish native to habitats that experience high levels of thermal variability will have broad thermal ranges (Sunday et al., 2019). As eurythermal fishes are physiologically equipped to handle a broad range of temperatures (Ferreira et al., 2014; Logan and Buckley, 2015; Safi et al., 2019), opaleye may largely have the cardiorespiratory mechanisms to withstand thermal variability. In other words, opaleye in this study may not have experienced thermal conditions extreme enough to drive measurable physiological specializations (da Silva et al., 2019).

We also must consider that fish in this study were measured during the warm portion of their variable acclimations, and some physiological characteristics can be altered on timescales of minutes to hours (e.g., hematocrit, lipid membrane fluidity; Seebacher et al., 2014). Indeed, others have posited that differences in outcomes between similar studies may be due to the starting test temperature (Morash et al., 2018; Morissette et al., 2020). Cooper et al. (2021), however, found that measuring critical upper and lower temperatures (CT_{max} and CT_{min}) during the high and low peaks of their thermally variable acclimations resulted in significant differences only with CT_{min}, supporting the concrete ceilings hypothesis regarding upper thermal tolerance (Gaston et al., 2009; Sandblom et al., 2016).

Overall, the lack of differences between variable and static thermal acclimation conditions suggests that, at least for some eurythermal species, incorporating natural thermal variability into lab-based experimental designs may have little impact. More work is needed to fully understand the mechanisms enabling the maintenance of cardiac function over diverse thermal regimes seen here.

5. Conclusions

Ectotherms possess a suite of cardiorespiratory responses to meet the challenges of changes in temperature and the associated changes in metabolic demand (Jutfelt, 2020; Seebacher et al., 2014). Eurythermal fishes who are regularly exposed to frequent thermal variations on tidal, diel, and seasonal time scales, may not experience large impacts of thermal variability on their cardiac performance. Indeed, the lack of statistical difference between static and variable thermal regimes on the cardiac thermal limits of eurythermal juvenile opaleye suggest this population is not affected by natural diel thermal fluctuations. We did, however, see a narrowing of the acute TPC as static temperatures increased, suggesting this may be a good metric to determine population-specific upper thermal limits, although more work is necessary to connect these lab-based metrics to ecological function. Future work should explore a broader suite of physiological performance metrics, as well as how different variation patterns (e.g., stochastic vs. regular) may impact the cardiac thermal limits in ectotherms.

CRediT authorship contribution statement

Gail D. Schwieterman: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Emily A. Hardison:** Methodology, Resources, Writing – review & editing. **Erika J. Eliason:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crphys.2022.02.002.

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