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Diet changes thermal acclimation capacity, but not acclimation rate, in a marine ectotherm (*Girella nigricans*) during warming

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Global climate change is increasing thermal variability in coastal marine environments and the frequency, intensity and duration of marine heatwaves. At the same time, food availability and quality are being altered by anthropogenic environmental changes. Marine ectotherms often cope with changes in temperature through physiological acclimation, which can take several weeks and is a nutritionally demanding process. Here, we tested the hypothesis that different ecologically relevant diets (omnivorous, herbivorous, carnivorous) impact thermal acclimation rate and capacity, using a temperate omnivorous fish as a model (opaleye, *Girella nigricans*). We measured acute thermal performance curves for maximum heart rate because cardiac function has been observed to set upper thermal limits in ectotherms. Opaleye acclimated rapidly after raising water temperatures, but their thermal limits and acclimation rate were not affected by their diet. However, the fish's acclimation capacity for maximum heart rate was sensitive to diet, with fish in the herbivorous treatment displaying the smallest change in heart rate throughout acclimation. Mechanistically, ventricle fatty acid composition differed with diet treatment and was related to cardiac performance in ways consistent with homoviscous adaptation. Our results suggest that diet is an important, but often overlooked, determinant of thermal performance in ectotherms on environmentally relevant time scales.

1. Introduction

Understanding the mechanisms driving thermal tolerance in ectotherms is a primary focus in ecological physiology. Temperature is one of the most important environmental factors governing the physiology, behaviour and ecology of ectotherms [1,2]. Temperature profiles vary substantially across environments, and thus endemic ectotherms are adapted to tolerate their local thermal regimes [3]. Temperate marine environments are especially thermally dynamic, with changes in temperature occurring on acute (diurnal), intermediate (seasonal upwelling and marine heatwaves) and long-term (annual–decadal) time scales [4–6]. Importantly, climate change is expected to increase the severity and duration of natural (i.e. upwelling, diurnal cycles) and extreme (i.e. heat waves) sources of temperature variation [6,7]. To survive in these dynamic thermal environments, ectotherms must be able to cope with changes in temperature across all relevant time scales [6,8,9]. As such, these ectotherms generally possess the capacity for high levels of thermal plasticity, otherwise known as thermal acclimation capacity.

Thermal acclimation occurs through a series of phenotypic changes across levels of biological organization that optimize biological rates, minimize waste production or cellular damage or conserve energy [8,10–12]. While these changes are occurring, the animal's performance changes as a function of how long they have been exposed to the new conditions. Thus, acclimating quickly may be just as, if not more important than full acclimation capacity [9,13–15], especially in the context of an increasingly variable environment. As the heart is essential for ensuring blood circulation, which is critical for transporting nutrients, waste, immune cells and oxygen, around the body, it is thought to govern thermal tolerance in several ectotherms [16] and has been shown to rapidly acclimate to high temperatures in some temperate fish [12,17]. Given that acclimation is a remodelling process, successful and efficient acclimation requires energy and nutrients. Thus, the rate of acclimation and overall acclimation capacity for cardiac thermal tolerance may be dependent on the quality and quantity of what an ectotherm has eaten.

Climate change is predicted to alter food availability, prey nutritional quality, and diet preference for several ectotherms [18–21], yet the interaction of diet and temperature on thermal performance remains largely unknown. Animals have a remarkable capacity to vary their diet to meet their nutritional requirements through changes in their consumption rate or diet selection [22–24]. Generalist ectotherms, like omnivores, can choose between foraging for plants or animals to meet their nutritional needs [20]. Interestingly, some omnivorous aquatic ectotherms increase the ratio of plant to animal in their diet as temperature increases, suggesting that the nutritional needs of these ectotherms are temperature dependent [20]. The physiological consequences of these diet shifts are not well understood but may be associated with altered performance and thermal tolerance [25–27]. An outstanding question is if the ratio of plant to animal in an omnivore's diet affects their thermal plasticity. More specifically, can diet affect thermal acclimation rates and capacity, and if so, what mechanisms underly these differences.

Diet quality varies dramatically across prey types. For omnivores, plants tend to be higher in mineral content, antioxidants and complex carbohydrates, while animal diets tend to have higher protein and lipid content. In general, fatty acid (FA) composition also differs between plants, algae and animals, although the exact composition is also dependent on the environment (i.e. terrestrial, marine, freshwater) and conditions (e.g. environmental oxygen, temperature) [28]. For example, the global availability of essential omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are expected to decline due to increasing temperatures associated with climate change [29]. FA composition can influence membrane fluidity, thermal limits [30,31], cardiac performance [32], swimming performance [33], growth [34], cognitive function [35] and diet preference [27], suggesting that variation in FA composition may be an important mechanism underlying thermal performance differences in ectotherms whose diet changes with temperature.

The objective of our study was to determine whether diet impacts the (1) rate and (2) capacity of cardiac thermal acclimation in a temperate omnivorous fish, opaleye (*Girella nigricans*), during a simulated warming event. We measured thermal performance of maximum heart rate (f_{Hmax}) as it is thought to limit thermal tolerance in fishes, and heart rate is governed by processes occurring across biological lipid membranes [16]. Opaleye were fed either a fully herbivorous (ad lib red algae, *Gracilaria pacifica*), carnivorous (ad lib brine shrimp, *Artemia* sp.), or omnivorous (free choice between algae and *Artemia* sp.) diet for two weeks at 12°C. We then increased temperature to 20°C, simulating an acute marine warming event and periodically measured cardiac thermal performance over the next two weeks while the fish were acclimating to 20°C. We predicted that the lower energy, lipid and protein content in the algae compared to *Artemia* sp. would result in lower acclimation capacity, slower acclimation rates, and reduced upper thermal limits compared to the carnivorous treatment. Further, that fish in the omnivorous treatment would have the highest performance due to the greater diversity of nutrients available, high energetic content of the diet, and flexibility to choose between the algae and *Artemia* sp. To examine the mechanisms underlying observed differences in cardiac performance, we measured the FA composition of the fish's ventricles and related FA composition to heart rate and thermal tolerance.

2. Methods

(a) Fish collection

Juvenile opaleye (estimated to be <1 year old) [36] were collected in May–August, 2021 by hook and line from Santa Barbara harbour, California, USA (34.40829, −119.691389). Fish were transported in coolers (>70% air saturation) to the University of California, Santa Barbara and held in 95 l fiberglass flow-through seawater tanks ($n = 224$ fish total, 14 fish per tank, 4× turnover h^{-1}). Prior to the start of acclimation, fish were held at ambient conditions (ranged approx. 12.5–17.5°C throughout the study) for <3 weeks and fed ad libitum omnivorous diets (*G. pacifica* from the cultured abalone farm in Goleta, CA and *Artemia* sp. from brineshrijmpdirect.com). All protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara (permit No. 935).

(b) Acclimation and diet treatments

Opaleye were held at 12°C and fed ad libitum one of the three diet treatments for two weeks. The diet treatments were (1) carnivorous (*Artemia* sp.), (2) herbivorous (red macro algae native to California called *G. pacifica*) and (3) choice omnivorous (*Artemia* sp. and algae). Consistent with other aquatic omnivorous ectotherms, opaleye increase algae relative to prey consumption in the warmer part of their geographic range [37]. After two weeks, fish were subjected to a cardiac thermal tolerance test called an Arrhenius breakpoint temperature test (ABT test; outlined below). One to two days later, temperature in the holding tanks was raised over approximately 10–12 h from 12 to 20°C (representative of the low and high seasonal temperatures experienced in Santa Barbara, CA). As temperate intertidal fish, this rate of temperature change is high for Santa Barbara, however not unheard of given the ecology of the species and a common rate of change in more southern parts of their range. Following the temperature change, individuals from each treatment were tested using an independent sampling design on day 1 (post-change), 3, 7 and 14–15 for their upper cardiac thermal limits using the same ABT test. The experiment was replicated 5 times to ensure adequate sample sizes for each time point (final $n = 7$–15 fish per diet treatment per time point). The 5th replicate was only run until day 3 post temperature change to increase the sample sizes at earlier time points. We also included one tank of fish that were acclimated to 20°C for 4 weeks and tested on week 2 and 4 of acclimation (electronic supplementary material, figure S1).

At the time that ABT tests were conducted, body mass and total length were 36.42 ± 16.12 g and 12.18 ± 1.87 cm (mean ± s.d.), respectively and did not differ with diet treatment. However, condition factor varied slightly across diet treatments (herbivorous = 1.79 ± 0.13, carnivorous = 1.87 ± 0.18 and omnivorous = 1.97 ± 0.26 g cm^{-3}; $p < 0.001$; $\chi^2 = 25.015$). Temperature and dissolved oxygen content were monitored daily by hand using an Omega Thermocouple (Omega Engineering, Norwalk, CT, USA) and an Oxyguard Handy Polaris 2 (OxyGuard International

A/S, Farum, Denmark). Oxygen was maintained at >80% air saturation throughout the study. Average temperature per treatment was 12.2 ± 0.5°C and 20.0 ± 0.6°C across replicates (mean ± s.d.; determined using Thermochron 4K iButtons programmed to record every 20 min; accuracy ± 1°C and resolution to 0.5°C). Replicate level temperature data is provided in electronic supplementary material, table S1. Fish were held under a 14 : 10 h light: dark cycle.

(c) Direct field sampling

We examined how our results compared to seasonally warm-acclimatized opaleye in the wild. Juvenile opaleye were collected in the August of 2021 (avg. temp for 2-weeks prior was 18.4 ± 1.0°C; mean ± s.d.; $n = 10$) from Santa Barbara harbour using hook and line. A maximum of 4 fish were caught per day for 3 days. Fish were immediately transported back to the University of California, Santa Barbara and an Arrhenius breakpoint temperature test followed by dissection (described below) was conducted within 3 h of being caught using water from the harbour.

(d) Dissections and stomach contents

After each ABT test, fish were euthanized and dissected. Liver and ventricle were weighed for hepatosomatic index (HSI) and relative ventricular mass (RVM). The ventricle was flash frozen for later analysis. Fish were not fasted before testing so that we could evaluate their stomach contents. Fish in the carnivorous treatment had similar results to fasted opaleye fed brine shrimp and acclimated to 12 and 20°C in Hardison *et al.* [26]. Thus, we do not anticipate that post-prandial increases in the oxygen uptake rate had a major impact on our findings. Stomach contents were weighed and sorted in fish from the omnivorous treatment to estimate the proportion of algae to brine shrimp (see electronic supplementary material, table S2).

(e) Proximate analysis

Frozen fish remains on day 14 (after 2 weeks at 20°C and 4 weeks on the treatment diet; stored at −20°C) were homogenized using a Fisher Brand Bead Mill 24 and subsamples of the homogenate were weighed and freeze dried (Labconco Lyophilizer). Subsamples of algae and *Artemia* sp. were also freeze dried for proximate analysis (see electronic supplementary material, table S3). Protein, lipid and ash content were estimated as described in Hardison *et al.* [26]. Total fatty acid composition was determined on ventricles ($n = 9$ per treatment and time point; stored at −80°C) from fish acclimated 2 weeks at 12°C and 2 weeks at 20°C and diet samples ($n = 6$ per diet) by gas chromatography/mass spectroscopy (GC-MS) at the University of California, San Diego Lipidomics Core using methods outlined in Quehenberger *et al.* [38] (see electronic supplementary material, table S4, table S5). Fatty acids that were 'not detected' in the sample were assumed to be 0.

(f) Thermal limits: Arrhenius breakpoint test

ABT tests were conducted as outlined in Hardison *et al.* [26] and Schwieterman *et al.* [39] (see electronic supplementary material, table S6 for specific values). Briefly, fish were anesthetized in seawater containing 80 mg l^{-1} buffered MS-222 and then placed in an experimental sling in a 10 l test tank which contained a maintenance dose of buffered 65 mg l^{-1} MS-222. Water was circulated continuously past the gills throughout the test and stainless-steel needle tip electrodes (AD Instruments, Colorado Springs, CO, USA) were shallowly inserted under the skin above the heart to detect an ECG signal. The signal was amplified using a Dual Bio Amp (AD Instruments, Colorado Springs, CO, USA) and filtered (Filters: 60 hz notch filter; mains filter; low-pass: 1 kz; high pass: 10 hz; range: 1–2 mV).

After a 15 min equilibration period at the acclimation temperature, atropine sulfate was injected intraperitonially (1.2 mg kg^{-1} in 0.9% NaCl) to block vagal tone. 15 min later, isoproterenol was injected intraperitonially (4 ug kg^{-1} in 0.9% NaCl) to maximally stimulate ß-adrenoreceptors. In 20°C acclimated fish, water temperature was cooled from 20 to 18°C before the start of the test to provide additional f_{Hmax} data for breakpoint temperature calculations. After isoproterenol injection, fish were given a 30-minute equilibration period. Then water temperature was heated (warm ABT test) at 10°C h^{-1} (Polystat recirculating heater/chiller; Cole-Palmer, Vernon Hills, IL, USA) while continuously recording an ECG trace. At each 1°C interval, f_{Hmax} and temperature were stabilized for 30 s to record an average value for heart rate at the temperature. This procedure was repeated until the onset of cardiac arrythmia (T_{ARR}). The fish were kept in the test until the next 1°C interval after T_{ARR} (<6 min) to ensure that the decrease in heart rate following cardiac arrythmia was captured for fitting thermal performance curves (TPC). Fish were omitted from the analysis when experimental error occurred (e.g. water pump failure, drug injection complications, or ECG signal was too noisy for software to interpret; 11% of individuals tested). Cold ABT tests were only performed on the last day of testing (i.e. day 14/15 post temperature switch; $n = 5–7$ per diet). The same rate of temperature change (decrease by 10°C h^{-1}) was targeted; however, below 11°C, the rate of decrease in temperature was harder to maintain and deviated occasionally from the desired rate (approx. 0.3–1°C every 6 min). We do not anticipate that this impacted the results, as Casselman *et al.* [40] found that slower rates of temperature change did not impact ABT test results.

(g) Data analysis for Arrhenius breakpoint test

At each 1°C temperature increment, f_{Hmax} was calculated from 15 continuous seconds of ECG recordings using automated ECG analysis software in LabChart (AD Instruments; www.adinstruments.com) [26]. Three thermal limits were calculated from the warm ABT test (T_{AB}, T_{PEAK}, T_{ARR}). The lowest thermal limit (T_{AB}) represents when the heart first starts showing signs of impairment due to temperature. For example, $\mathrm{T_{AB}}$ was comparable to the optimal temperature window for aerobic scope in Pacific salmon [40], rainbow trout [41], and goldfish [42]. T_{AB} was defined as the temperature corresponding to a breakpoint in a plot of log(f_{Hmax}) against the Arrhenius temperature. This breakpoint analysis was conducted using *segmented* package (v1.1-0) [43] in R. Note that $\mathrm{T_{AB}}$ may be artificially high in the early time points because the test started at a higher temperature (18 instead of 12°C). For the warm ABT test, the highest f_{Hmax} recorded during any 15 s measurement period was designated as the overall peak heart rate (peak f_{Hmax}). The temperature in which peak f_{Hmax} occurred was defined as the peak temperature (T_{PEAK}). This indicates an important transition temperature where cardiac arrythmia usually follows shortly thereafter. Finally, T_{ARR} indicates the temperature where the heart is no longer able to maintain rhythmic beating. At 12 and 20°C, T_{ARR} occurs at temperatures approximately 2.7–3.8°C lower than critical thermal maxima ($\mathrm{CT_{max}}$) in opaleye, and thus represents a more functional upper thermal limit for these fish [26].

(h) Statistical analysis

All data were statistically analysed using R (v. 3.5.1). All metrics were investigated for normality using Shapiro–Wilk tests and quantile–quantile plots, and for heteroscedasticity using Levene's test. All data were sufficiently normal with equal variance. Acclimation rate data were statistically analysed (significance level $\alpha = 0.05$) by fitting a linear mixed effect model where time point and diet were included as fixed effects and replicate number was

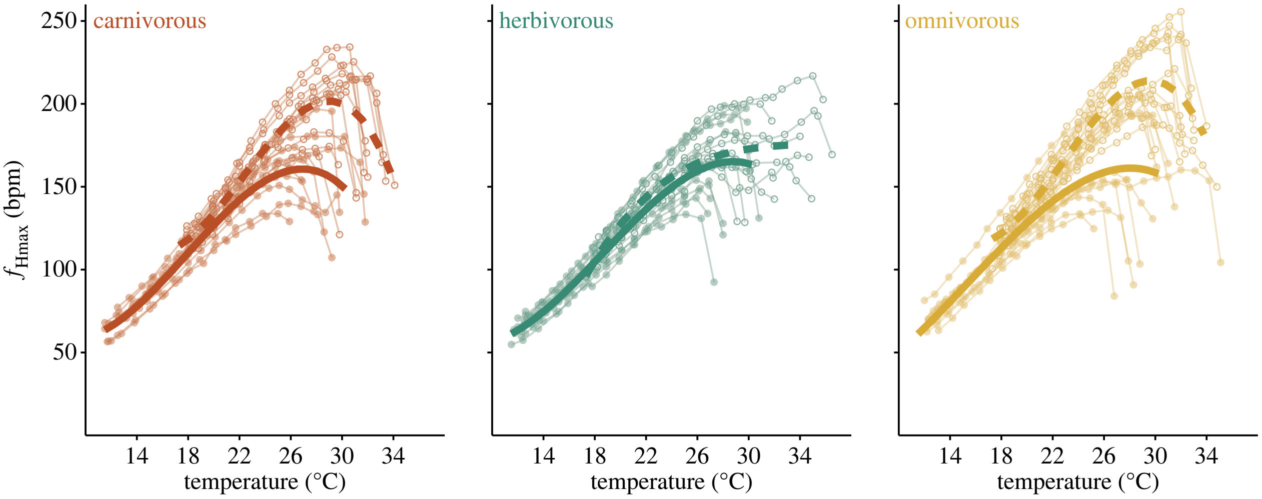


Figure 1. Dietary effects on cardiac thermal acclimation capacity. Individual- and treatment-level responses of max heart rate (f_{Hmax}) of opaleye during acute warming. Panels show fish from different diet treatments (from left to right: carnivorous, herbivorous, omnivorous) that were tested after a 2-week acclimation to 12°C (closed circles) and then 20°C (open circles). Curves indicate treatment level effects after 12°C (solid line) and 20°C (dashed line) acclimation and are third-order polynomials that account for the interaction diet × time point × acute temperature with random effects of individual fish id and replicate. This was determined to be the best fit model by SIC (electronic supplementary material, table S7).

included as a random effect (lme4 v. 1.1–19) [44]. We ran a 2-way ANOVA on each model followed by a post hoc Tukey test when significant main effects were detected. In all 2-way ANOVA tests, the interaction between diet and time point was tested for and excluded when non-significant. Polynomial curves were fitted to acclimation capacity f_{Hmax} data (i.e. data from fish held for 2 weeks at 12°C and fish then held for 2 weeks at 20°C) and compared using Schwarz information criterion (SIC), where the fit with the lowest SIC score was assigned the best fit model, but all models with $\Delta SIC < 7$ were considered (electronic supplementary material, table S7) [45]. Replicate number and individual were included as random effects.

To simplify fatty acid analysis, the double bond index (DBI) and average chain length were calculated for each sample. $DBI = \sum_1^n n \times$ (proportion of FA with n double bonds), where $n =$ the number of double bonds in a fatty acid chain. Average chain length $= \sum_1^L L \times$ (proportion of FA with L chain length), where L = the number of carbons in the fatty acid chain. Linear regressions between DBI or average chain length and all cardiac parameters (f_{Hmax}, peak f_{Hmax}, T_{ARR}, T_{PEAK}, T_{AB}) were assessed in fish acclimated for 2 weeks at 12°C and 2 weeks at 20°C. Residual plots of each linear regression model were visually assessed in R to investigate normality and heteroscedasticity. Complete dietary and tissue sample FA analysis (i.e. mean ± SEM for all FAs measured) is provided in the supplement (electronic supplementary material, table S4, table S5).

3. Results

(a) Acclimation capacity

The thermal acclimation capacity for f_{Hmax} was influenced by diet in opaleye (figure 1; electronic supplementary material, table S8). Model selection revealed strong evidence of an interactive effect of diet and temperature on the acute TPC for f_{Hmax} in the warm ABT test (electronic supplementary material, table S7). The best fit model by SIC was a third-order polynomial curve that incorporated an interaction of acclimation temperature (12 or 20°C), diet (herbivorous, omnivorous, carnivorous), and acute test temperature (electronic supplementary material, table S7). The acute TPC for f_{Hmax} followed a traditional shape with a negative skew, where f_{Hmax} increased with temperature until T_{AB}, at which point f_{Hmax} began leveling off until $\mathrm{T}_{\mathrm{PEAK}}$. T_{ARR} generally occurred shortly after T_{PEAK}.

Upper thermal limits (T_{AB}, T_{PEAK}, T_{ARR}) increased by an average of 3.3–3.9°C (irrespective of diet) when switched from 12 to 20°C (figure 2; electronic supplementary material, table S6). While diet did not affect any thermal limits (diet effect for T_{AB} $p = 0.1647$, T_{PEAK} $p = 0.2269$, T_{ARR} $p = 0.1998$, electronic supplementary material, tables S6 and S8), it did impact the height of the acute TPC (diet effect $p < 0.001$). For the omnivorous and carnivorous treatments, peak f_{Hmax} increased during acclimation from 12 to 20°C (figure 3). In contrast, the herbivorous treatment did not alter peak f_{Hmax} in response to warm acclimation ($p = 0.9856$ from Tukey HSD comparing herbivore treatment at 12°C to the herbivore treatment after 2 weeks at 20°C). Accordingly, the herbivorous treatment had a lower Δ peak f_{Hmax} (peak $f_{\mathrm{Hmax\ at\ 20°C}}$−peak $f_{\mathrm{Hmax\ at\ 12°C}}$) of 12.62 bpm compared to 55.10 and 43.96 bpm for the omnivorous and carnivorous treatments, respectively.

(b) Acclimation rate

The majority of the acclimation response for thermal limits occurred in the first 3 days post temperature change, with an average of 69.2% of the response for T_{AB}, 60.6% for T_{PEAK}, and 60% for T_{ARR} occurring in the first 3 days at 20°C, irrespective of diet (electronic supplementary material, table S9). Diet did not impact any thermal limit at any time point during acclimation from 12 to 20°C (figure 2; electronic supplementary material, table S8). However, there was an interactive effect of diet and time point on peak f_{Hmax} (diet × time point $p = 0.0128$), where it was lower in the herbivorous treatment compared to the omnivorous (23.3% higher) and carnivorous (16.5% higher) treatments after the 2-week acclimation to 20°C (figure 3; electronic supplementary material, table S8).

(c) Warm versus cold thermal limits and seasonally acclimatized fish

A group of carnivorous fish were tested after an extended acclimation period to 20°C (2 and 4 weeks) (electronic supplementary

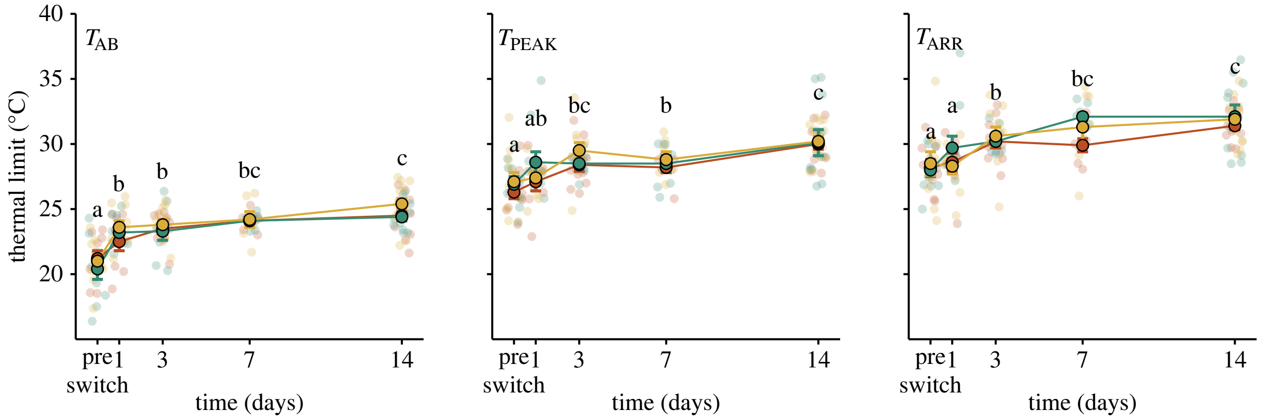


Figure 2. Dietary effects on thermal acclimation rate for cardiac thermal limits. Individual- and treatment-level acclimation rate responses for upper thermal limits (T_{AB}, T_{PEAK}, T_{ARR}) across diet treatments and time points in opaleye. Colours distinguish diet treatments (red: carnivorous, green: herbivorous, yellow: omnivorous). Individual responses are indicated by the translucent points, and treatment level mean (±SEM) are overlayed with connecting lines. Different letters indicate significant differences across time points ($p < 0.05$) by Tukey HSD. Each thermal limit was analysed independently. There were no significant effects of diet on any thermal limit.

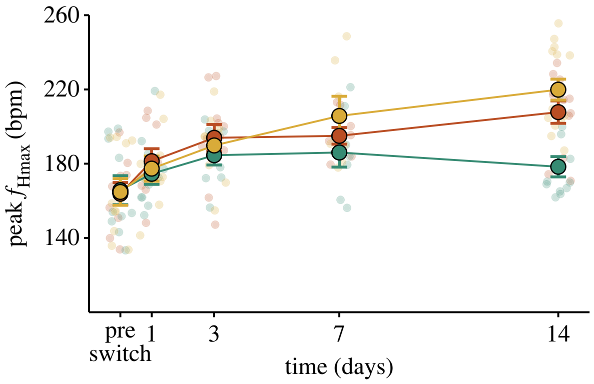


Figure 3. Dietary effects on thermal acclimation rate for peak f_{Hmax}. Individual- and treatment-level acclimation rate responses in peak f_{Hmax} across diet treatments and time points in opaleye. Colors distinguish diet treatments (red: carnivorous, green: herbivorous, yellow: omnivorous). Individual responses are indicated by the translucent points. Treatment level mean (± SEM) are overlayed with connecting lines. There was a significant interactive effect of diet and time point ($p = 0.0128$; electronic supplementary material, table S8) on peak f_{Hmax}.

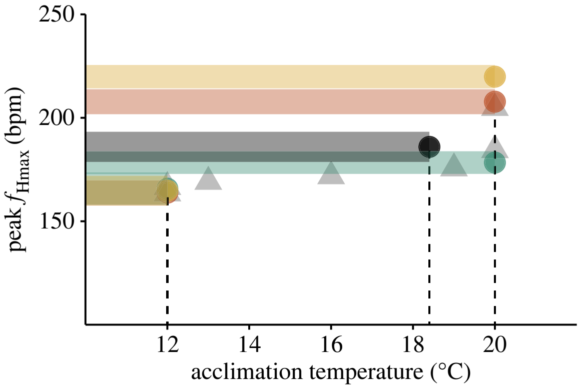


Figure 4. Comparison of treatment to opaleye literature values in peak f_{Hmax}. Comparing mean peak f_{Hmax} values from current treatments to seasonally acclimatized opaleye as well as all other available literature values on opaleye [26,39]. Light Gray filled triangles indicate mean values from other studies on opaleye. Colors distinguish diet treatments used in this study (red: carnivorous, green: herbivorous, yellow: omnivorous). Dark gray circle indicates the mean for seasonally acclimatized opaleye. Shaded rectangles indicate ± SEM for each treatment in this study. Dashed lines indicate acclimation or acclimatization temperature (from prior 2 weeks) in this study.

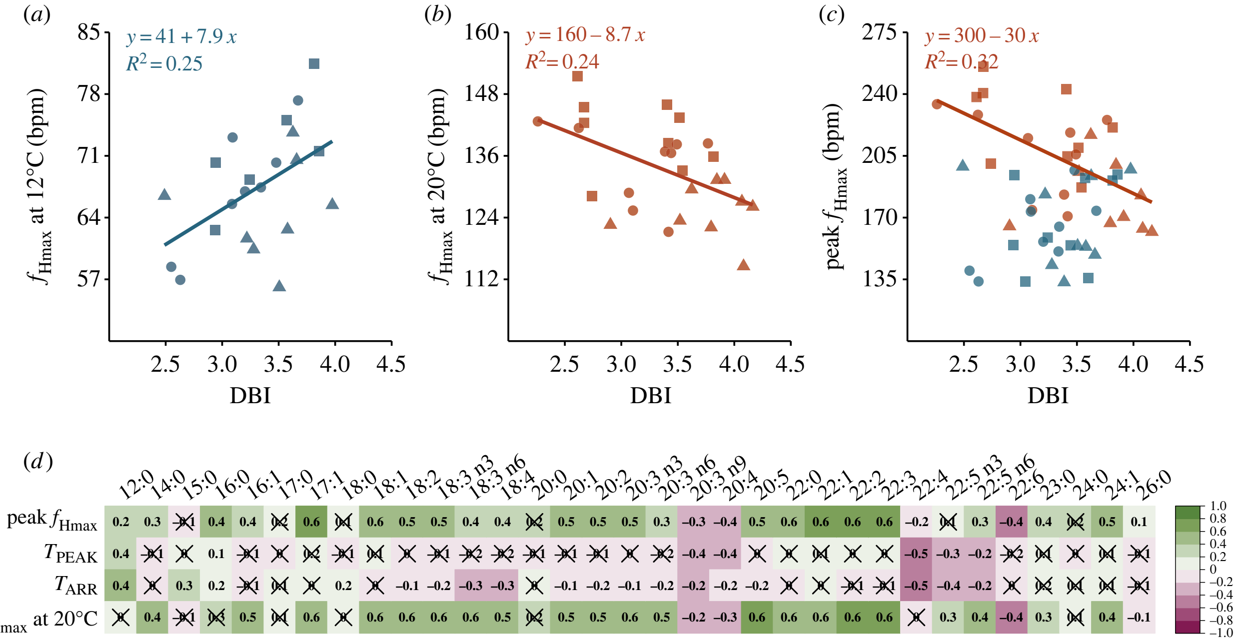
material, figure S1). Peak f_{Hmax} increased from 2 to 4 weeks while T_{PEAK} and T_{ARR} did not change, indicating the opaleye could have continued acclimating a bit more if the acclimation time had been extended to 4-weeks across treatments (electronic supplementary material, figure S1). The fish tested in the acclimation rate experiment had an average peak f_{Hmax} of 207.78 ± 6.04 bpm and 31.4 ± 0.3°C T_{ARR} after 14 days at 20°C compared to 213.20 ± 5.94 bpm and 32.7 ± 0.4°C from opaleye held 4 weeks at 20°C. Thus, the majority of the opaleye's acclimation response was complete by week 2 post-temperature change, which is a more representative time scale for what they experience in the wild.

On the last time point (day 14 at 20°C), both upper and lower thermal limits for f_{Hmax} were assessed. Diet did not affect either upper (see electronic supplementary material, table S8) or lower thermal limits (Diet effect on $T_{ARR-COLD}$ $\chi^2 = 0.660$, d.f. = 2, $pr > \chi^2 = 0.719$). For the cold ABT test, $T_{ARR-COLD}$ was 5.3 ± 0.2°C for the herbivorous, 5.5 ± 0.5°C for the omnivorous, and 5.8 ± 0.6°C for the carnivorous treatments.

Seasonally acclimatized opaleye (i.e. tested immediately following wild capture) experienced an average intertidal temperature of 18.4 ± 1.0°C (mean ± s.d.) in the 2 weeks prior to field sampling. Fish sampled in the late summer (August) had cardiac and morphometric performance that was closest to the herbivorous treatment at 20°C (figure 4), with an average peak f_{Hmax} of 185.96 ± 7.29 bpm and T_{AB} of 23.4 ± 0.3°C, T_{PEAK} of 28.9 ± 0.6°C, and T_{ARR} of 30.6 ± 0.7°C. Further, HSI was an average of 1.1% and RVM was 0.059%, where HSI was most similar to the herbivorous treatment at 12 and 20°C (1.1 and 0.8%, respectively).

(d) Cardiac fatty acid analysis

Diets and ventricles differed in their fatty acid composition (electronic supplementary material, table S4, table S5, figure S2). The algae had lower fatty acids overall and displayed higher proportions of saturated fats compared to brine shrimp (table S4). In the ventricle, however, the DBI was highest in the 20°C herbivorous treatment, which was largely driven by differences in DHA content at that time (electronic



	12:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3 n3	18:3 n6	18:4	20:0	20:1	20:2	20:3 n3	20:3 n6	20:3 n9	20:4	20:5	22:0	22:1	22:2	22:3	22:4	22:5 n3	22:5 n6	22:6	23:0	24:0	24:1	26:0
peak f_{Hmax}	0.2	0.3	~~−0.1~~	0.4	0.4	~~0.2~~	0.6	~~0.1~~	0.6	0.5	0.5	0.4	0.4	~~0.2~~	0.5	0.5	0.5	0.3	−0.3	−0.4	0.5	0.6	0.6	0.6	0.6	−0.2	~~0.1~~	0.3	−0.4	0.4	~~0.2~~	0.5	0.1
T_{PEAK}	0.4	~~−0.1~~	~~0~~	0.1	~~−0.1~~	~~0~~	~~0.2~~	~~−0.1~~	~~0.1~~	~~0~~	~~−0.1~~	~~−0.2~~	~~−0.2~~	~~−0.1~~	~~−0.1~~	~~−0.1~~	~~0~~	~~−0.2~~	−0.4	−0.4	~~0~~	~~0~~	~~0.1~~	~~0~~	~~0~~	−0.5	−0.3	−0.2	~~−0.2~~	~~0.1~~	~~0~~	~~0.1~~	~~−0.1~~
T_{ARR}	0.4	~~0~~	0.3	0.2	~~−0.1~~	~~0.1~~	~~0~~	0.2	~~0~~	−0.1	−0.2	−0.3	−0.3	~~0~~	−0.1	−0.2	−0.1	−0.2	−0.2	−0.2	−0.2	~~0~~	~~0~~	~~−0.1~~	~~−0.1~~	−0.5	−0.4	−0.2	~~0~~	~~0.2~~	~~0.1~~	~~0.1~~	~~−0.1~~
f_{Hmax} at 20°C	~~0~~	0.4	~~−0.1~~	~~0.3~~	0.5	~~0.1~~	0.6	~~0~~	0.6	0.6	0.6	0.6	0.5	~~0.2~~	0.5	0.5	0.6	0.5	−0.2	−0.3	0.6	0.6	0.6	0.6	0.6	~~0~~	0.3	0.4	−0.4	0.3	~~0.1~~	0.4	−0.1

Figure 5. Fatty acid analysis from opaleye ventricles. Plots a–c show linear regressions between DBI and f_{Hmax} from opaleye across all diet treatments, where shapes indicate different diet treatments (carnivorous: circle, herbivorous: triangle, omnivorous: square). (*a*) f_{Hmax} at 12°C from 12°C acclimated opaleye (*b*) f_{Hmax} at 20°C from 20°C acclimated opaleye (*c*) Absolute peak f_{Hmax} from 12°C (blue) and 20°C (red) acclimated opaleye. Equations are presented for significant linear regressions between parameters ($p < 0.05$). There was no significant relationship between peak f_{Hmax} and DBI for 12°C acclimated fish. (*d*) Correlations between cardiac parameters and individual fatty acids or fatty acid summary metrics from 20°C acclimated fish ventricles only (made using 'Corrplot' v. 0.84 in R). Numbers and colours indicate the correlation coefficient. Non-significant correlations are crossed out (alpha < 0.05).

supplementary material, table S5, figure S2). Fish in the herbivorous treatment also had lower amounts of fatty acids with 18 carbon chains (e.g. oleic, linoleic, γ-linolenic) compared to the carnivorous and omnivorous treatments (electronic supplementary material, table S5, figure S2).

Variation in f_{Hmax} could be partially explained by differences in ventricle DBI. For example, 12°C acclimated fish showed a significant positive relationship between DBI versus f_{Hmax} at 12°C ($p = 0.018$; $R^2 = 0.25$; figure 5*a*), while 20°C acclimated fish displayed a significant negative relationship between ventricle DBI versus f_{Hmax} at 20°C ($p = 0.009$; $R^2 = 0.24$; figure 5*b*) and ventricle DBI versus peak f_{Hmax} ($p = 0.002$; $R^2 = 0.32$; figure 5*c*). There was no relationship between DBI versus peak f_{Hmax} in 12°C acclimated fish ($p = 0.182$; $R^2 = 0.07$; figure 5*c*). DBI and average chain length were highly positively correlated, irrespective of diet ($p < 0.001$; $R^2 = 0.98$). Thus, similar trends in average chain length versus cardiac parameters were observed. Several other significant correlations existed between cardiac parameters and specific fatty acid content (figure 5*d*; electronic supplementary material, figure S3).

4. Discussion

Diet is an understudied factor that could impact the rate and capacity for thermal acclimation in ectotherms. Here, we measured how diet impacted the time course of cardiac thermal plasticity in a temperate omnivorous fish, opaleye, during a simulated warming event. We found that diet did influence the acclimation capacity for f_{Hmax} but not the rate of acclimation. We also examined how cardiac thermal performance related to ventricle fatty acid composition and found significant relationships between fatty acid composition and f_{Hmax}. This has important implications for our understanding of species vulnerability to rising temperatures.

(a) Thermal acclimation capacity was diet dependent

In the face of ongoing anthropogenic changes to temperature and food resources [7,19], it is critical to consider if nutrition can mediate ectotherm resilience. Here, diet clearly impacted cardiac remodeling for f_{Hmax}, but it did not alter the acclimation capacity of thermal limits (T_{AB}, T_{PEAK} or T_{ARR}). In all diet treatments, thermal limits increased with warm temperature acclimation, consistent with cardiac responses observed in opaleye and many other fishes [11,26]. For this generalist omnivorous species, a short-term shift to a lower energy, lipid, and protein diet did not constrain the fish's thermal limits or their ability to acclimate them to a higher temperature. Further, the more nutritionally diverse (omnivorous) diet treatment here did not result in higher thermal limits than the other diet treatments.

Instead, the height of the acute TPC for f_{Hmax}, but not the breadth of the TPC, was sensitive to diet. Consequently, diet determined thermal plasticity for f_{Hmax}, with fish in the herbivorous treatment undergoing the lowest change in peak f_{Hmax} between 12 and 20°C. Similar diet effects on TPCs for f_{Hmax} have been found in two other studies [26,46]. In Hardison *et al.* [26], *Ulva* sp. consumption lowered peak f_{Hmax}, but not T_{AB}, T_{PEAK} or T_{ARR} in opaleye at 20°C (figure 4). In Papadopoulou *et al.* [46], allicin supplementation reduced f_{Hmax} across temperatures in exercise trained trout. Our data add to growing evidence that the amplitude of the acute TPC is more plastic than the breadth of the curve. While our results are from measures of cardiac performance, other performance metrics may follow similar patterns. For example, in some cases aerobic capacity may be more sensitive in amplitude, rather than breadth [47,48]. Differences in the amplitude of acute TPCs can alter functional thermal tolerance. Even though thermal limits were not impaired by diet here, the reduced f_{Hmax} in the herbivorous treatment indicates that f_{Hscope} (max – resting heart rate) could have a narrower thermal breadth if there were not corresponding decreases in resting heart rate [49]. In this case, performance under realistic temperature scenarios, or functional thermal tolerance, may be impaired, while more commonly measured extreme thermal limits (e.g. $\mathrm{CT_{max}}$, $\mathrm{LT_{50}}$, T_{ARR}) remain the same. Notably, our findings on fed opaleye here were similar to results on opaleye that were fasted before testing, indicating that being fed did not have a major impact on f_{Hmax} [26]. However, metabolism (and thus heart rate) increase during digestion and the metabolic cost of digestion can differ depending on an animal's diet [50]. Thus, the scope for heart rate during digestion may also vary with diet and could limit functional thermal tolerance in opaleye.

Opaleye are generalists that feed on a mixture of macroalgae, and small invertebrates (e.g. small crustacea, hydroids), but are known to eat proportionally more animal prey in the colder part of their geographic range [37]. At least in the short term, algae consumption in this study was not associated with any decrease in cardiac thermal performance at cold temperatures (12°C). Notably, opaleye in the herbivorous treatment had lower HSI (1.1%) than the carnivorous (3.1%) and omnivorous (3.0%) treatments at 12°C, indicative of an energetic disadvantage. However, more research is needed to determine the energetic and performance costs associated with herbivory in active fishes at cold temperatures and how the ecological role of herbivorous and omnivorous fish will change with an increasingly warm and more variable thermal environment.

(b) Thermal acclimation rate did not change with diet

In temperate environments, temperature often changes on shorter time scales than it takes for full thermal acclimation to occur [5]. For the endemic ectotherms, plasticity within the first few days after a temperature shift may be more important than overall acclimation capacity. However, most thermal performance data is collected on ectotherms that are fully acclimated to treatment conditions (generally ≥2 weeks), leading to overestimations of real-world thermal limits and performance. If diet can alter the rate of acclimation, this could incentivize temperate fish to modify their diet with temperature.

As expected, thermal limits increased throughout the 2-week acclimation to 20°C, but surprisingly, there were no differences between diets. We did not observe any differences in acclimation rate despite HSI being higher in the carnivorous and omnivorous treatments (indicative of greater energy stores) after the initial 2-week acclimation to 12°C. All thermal limits showed a similar logarithmic relationship with time, consistent with acclimation rate studies using a critical thermal maxima test to estimate thermal limits [51,52]. These results add further evidence that consuming a lower quality diet (i.e. algae) did not impair thermal limits in opaleye, at least on time scales that are environmentally relevant.

Thermal limits were not affected by diet, but peak f_{Hmax} was, where fish from early time points had similar cardiac performance irrespective of diet, but performance diverged between the herbivorous and carnivorous/omnivorous treatments during the last week of acclimation to 20°C. Given the greater nutritional diversity (e.g. availability of micro and macronutrients), and flexibility in the omnivorous treatment and that opaleye, and some other aquatic omnivores [20], appear to consume greater proportions of algae in their diet in warmer water, we suspected the fish in the omnivorous treatment would have faster acclimation rates relative to those in the carnivorous and herbivorous treatments. However, we did not observe this. While fish in the omnivorous treatment had higher f_{Hmax} across temperatures than those in the herbivorous treatment after 2 weeks at 20°C, they had similar performance to the carnivorous treatment across all metrics and time points.

Notably, the fish at 12°C were much less likely to have food in their stomachs at the time of sampling, so it was not possible for us to estimate the exact ratio of algae to brine consumption at that time. However, we only started detecting small amounts of algae consumption on day 3 after raising the tank temperature to 20°C. The lack of differences between the carnivorous and omnivorous treatments may have resulted from fish not eating algae long enough for it to impact their heart rate. While not within the scope of this study, it would be interesting to extend out to longer time points and see if prolonged acclimation to the omnivorous treatment at 20°C results in even greater algal consumption and higher cardiac performance. In Hardison *et al.* [26] opaleye acclimated to three weeks on an omnivorous diet (consisting of *Ulva* sp and brine shrimp) had slightly reduced cardiac thermal performance relative to a carnivorous treatment, indicating that 3–4 weeks at a given temperature may be necessary to observe subtle diet effects. Consistent with this, Sandblom *et al.* [9] demonstrated that the metabolism during digestion (or specific dynamic action) and standard metabolic rate took between 4 to 8 weeks to fully acclimate after switching from 10 to 16°C in shorthorn sculpin (*Myoxocephalus scorpius*).

There are a small number of studies that have measured how quickly cardiac performance acclimates in fish, without a diet manipulation [12,17,53]. Interestingly, all studies found that temperate fish rapidly acclimate, with most of their response occurring within 3 days. Ekstrom *et al.* [12] measured f_{Hscope} in rainbow trout (*Oncorhynchus mykiss*) acclimated from 9 to 16°C and tested at 16°C. f_{Hmax} increased after 1 day at 16°C, and then began decreasing as acclimation occurred. These results were consistent with data from the herbivorous treatment, where f_{Hmax} at 20°C increased within 3 days of acclimation, but went back down by 2-weeks at 20°C. In contrast, the f_{Hmax} for the carnivorous and omnivorous treatments remained high throughout acclimation to 20°C. Gilbert *et al.* [17] used the ABT test to determine how f_{Hmax} changed during acclimation from 10 to 18°C in rainbow trout. In line with our results here, trout also underwent >50% of their acclimation response for f_{Hmax} in the first 72 h post temperature change.

The speed with which temperate fishes, like trout and opaleye, mount an acclimation response indicates that acclimation rate may be a conserved and selected upon factor, although this has yet to be tested. This work demonstrates how essential it is that we integrate time of exposure into our modelling of thermal tolerance and predictions of species responses to climate change. While these fish rapidly acclimate, they are still most vulnerable in the first few days after a temperature change, as their thermal tolerance has not fully compensated, and they are actively remodelling. It is logistically challenging to gather these data, but once in hand, they can be used to predict performance in complex environments, under variable, more realistic conditions.

(c) Fatty acid composition

Fatty acids serve many important functions, including as the phospholipid tails in biological membranes. Changes in fatty acids and membrane performance can impact cellular processes occurring across them, such as membrane-bound enzyme activities, membrane potential, and ion movement [1,28], which can have consequences for organ and whole-animal performance. Although membrane fatty acid composition is tightly regulated, composition and content differ greatly across diets and animal fatty acid composition can be related to fatty acid composition of the diet [54,55]. Consequently, dietary fatty acid composition can influence cardiac function, whole-animal metabolism and swimming performance in fish [32,33,55]. For example, in Chatelier *et al.* [55], seabass (*Dicentrarchus labrax*) had lower cardiac output and aerobic scope when fed a diet containing fish oil as opposed to canola oil or palm oil. The effect of dietary lipids on CT_{max} and lethal thermal limits has also been assessed in a handful of studies, with mixed results. Oil type did not influence upper thermal limits in juvenile angelfish [56], but total lipid composition (10 versus 20%) had a significant effect on CT_{max} in juvenile barramundi [31]. The effect of dietary fats on cardiac thermal plasticity in fish is relatively unknown. In our study, ventricle fatty acid composition was related to f_{Hmax} and acclimation temperature.

DBI is a measure of how 'unsaturated' a tissue sample is on average, with higher DBI indicating a greater presence of double bonds in the fatty acid tails [57]. Here, cold-acclimated fish had higher heart rates (improved cardiac performance) when their ventricle fatty acids had more double bonds (DBI), or potentially more fluid membranes (thus counteracting the cold). The opposite trend was observed for warm-acclimated fish: they had improved cardiac performance when their ventricles had fewer double bonds, or potentially reduced membrane fluidity. In particular, saturated fatty acid (SFA) content appeared to drive the relationship between f_{Hmax} and DBI in 12°C acclimated fish, while highly unsaturated fatty acids (HUFA) appeared to be the major driver of the negative relationship between DBI and f_{Hmax} /peak f_{Hmax} in 20°C acclimated fish. DBI and average chain length were also positively correlated, meaning that fish with more unsaturated fats in their ventricle also had longer FA chains. While adding double bonds lowers the melting point and increases membrane fluidity, lengthening the carbon chain raises the melting point and increases membrane stability [1]. Thus, the fish may have increased the carbon chain length to partially compensate for their higher DBI.

Even though the algal dietary fatty acid composition had a higher proportion of saturated fats, the herbivorous treatment at 20°C had the highest ventricle DBI, which was largely driven by differences in the HUFA, DHA. DHA is an essential fatty acid critical to cognitive function and development [58]. However, it has six double bonds, resulting in a melting point of −44°C. It is unclear why the herbivorous fish ventricles contained more HUFAs, like DHA, relative to SFAs at 20°C. One potential explanation is that the herbivorous fish were metabolizing the SFAs or prioritizing their deposition in other tissues, as SFA have higher energy density compared to HUFAs of the same length (although digestibility is lower). Given the lower lipid content of the herbivorous diet, the fish could have prioritized meeting their metabolic demand over optimizing their membrane fluidity.

Given the high degree to which cells can regulate membrane performance and the many other functions for lipids in the body, it is not surprising that only approximately 20–30% of the variation in f_{Hmax} could be explained by fatty acid composition. Christen *et al.* [59] found significant negative linear relationships between thermal limits (time to CT_{max}) and omega-3 fatty acid composition in *Salvelinus alpinus*, *S. fontinalis*, and their hybrids. Here, there were some significant correlations between specific fatty acids and cardiac thermal limits, although there was no significant linear regression found between overall DBI and cardiac thermal limits. While not evaluated here, other factors, such as sterol composition [60], phospholipid class [61] and phospholipid fatty acid composition [62], are known influencers of membrane performance that may have differed between the fish ventricles at the time of sampling and could explain more of the variation in heart rate across treatments.

The global availability of the essential omega-3 fatty acids, DHA and EPA, are expected to decrease with increasing temperatures associated with climate change [29]. While there is concern over the predicted reduction in the global availability of omega-3 FAs, the results here indicate that higher tissue SFA is associated with improved cardiac performance in warm for opaleye. In other words, shifts in global FA composition may be of concern for human nutrition and other measures of fish performance (e.g. fish cognitive health), but could also mediate cardiac upper thermal tolerance in fishes.

5. Conclusion

Our results demonstrate that for generalist species, like opaleye, variation in diet is an important modulator of thermal plasticity on environmentally relevant time scales. While diet did not affect the fish's thermal limits for f_{Hmax} or acclimation rate, it did impact the height of their acute TPC. Thus, ectotherm thermal performance changes as a function of exposure time, and the amplitude of acute TPCs can be influenced by the animal's nutrition. Mechanistically, fatty acids were related to heart performance, indicating that they may be a primary regulator of cardiac thermal performance in ectotherms. While temperate fish, like opaleye, have a remarkable capacity for rapid thermal plasticity, they are still most vulnerable in the first few days after a warming event, as they have yet to fully compensate for changes in temperature. These results highlight the importance of accounting for changes in nutrition, and thermal exposure time when assessing ectotherm thermal tolerance.

Ethics. All protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara (#935). Animal collections were performed under the California Department of Fish and WIldlife Scientific Collection Permit (S-190280004-19044-001).

Data accessibility. The data and code that support the findings of this study are openly available in Dryad at https://doi.org/10.25349/D9Q905 [63].

The data are provided in electronic supplementary material [64].

Authors' contributions. E.A.H.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; G.D.S.: investigation, methodology, validation, writing—review and editing; E.J.E.: conceptualization, funding acquisition, investigation, project

administration, resources, software, supervision, validation, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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