RESEARCH ARTICLE

Diet mediates thermal performance traits: implications for marine ectotherms

Emily A. Hardison*, Krista Kraskura, Jacey Van Wert, Tina Nguyen and Erika J. Eliason

ABSTRACT

Thermal acclimation is a key process enabling ectotherms to cope with temperature change. To undergo a successful acclimation response, ectotherms require energy and nutritional building blocks obtained from their diet. However, diet is often overlooked as a factor that can alter acclimation responses. Using a temperate omnivorous fish, opaleye (*Girella nigricans*), as a model system, we tested the hypotheses that (1) diet can impact the magnitude of thermal acclimation responses and (2) traits vary in their sensitivity to both temperature acclimation and diet. We fed opaleye a simple omnivorous diet (*ad libitum Artemia* sp. and *Ulva* sp.) or a carnivorous diet (*ad libitum Artemia* sp.) at two ecologically relevant temperatures (12 and 20°C) and measured a suite of whole-animal (growth, sprint speed, metabolism), organ (cardiac thermal tolerance) and cellular-level traits (oxidative stress, glycolytic capacity). When opaleye were offered two diet options compared with one, they had reduced cardiovascular thermal performance and higher standard metabolic rate under conditions representative of the maximal seasonal temperature the population experiences (20°C). Further, sprint speed and absolute aerobic scope were insensitive to diet and temperature, while growth was highly sensitive to temperature but not diet, and standard metabolic rate and maximum heart rate were sensitive to both diet and temperature. Our results reveal that diet influences thermal performance in trait-specific ways, which could create diet trade-offs for generalist ectotherms living in thermally variable environments. Ectotherms that alter their diet may be able to regulate their performance at different environmental temperatures.

KEY WORDS: Temperature, Thermal acclimation, Fish, Thermal limits, *Girella nigricans*, Omnivore

INTRODUCTION

Understanding the full range and maximum capacity of ectotherm physiological responses to environmental change is essential to predict species' vulnerability to global climate change (Huey et al., 2012; Somero, 2011; Stillman, 2003). Temperature is a critical environmental factor governing ectotherm physiology, behavior and ecology (Brett, 1971). The current paradigm suggests that ectotherms have three options when faced with unfavorable temperatures: they can move to a more suitable habitat, adapt

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over multiple generations or acclimate to the new conditions (Daufresne et al., 2009; Glanville and Seebacher, 2006; Hofmann and Todgham, 2010; Somero, 2011). Thermal acclimation is an essential survival mechanism for ectotherms living in variable environments and a critical coping mechanism against global climate change (Bernhardt and Leslie, 2013; Jackson et al., 2021; Seebacher et al., 2015). During thermal acclimation, ectotherms undergo reversible phenotypic changes that improve their performance at a given temperature (Fig. 1; e.g. enzyme activity, membrane composition, mitochondrial density, oxygen transport, organ morphology and function; Anttila et al., 2014; Chung and Schulte, 2020; Ekström et al., 2016; Little et al., 2020a; Seebacher et al., 2015). It is often assumed that ectotherms will achieve the same level of performance after repeated exposures to a temperature, as long as all other environmental conditions (i.e. salinity, pH, dissolved oxygen) are held the same (Sinclair et al., 2016). To undergo a successful acclimation response, ectotherms require energy and nutritional building blocks obtained from their diet. Diets vary considerably in nutritional and energetic content, which suggests that different diets may mediate distinct thermal acclimation responses (Fig. 1).

Food quality and availability can change seasonally, with global climate change and across habitats (Alton et al., 2020; Arnold et al., 2010; Birnie-Gauvin et al., 2017; Ho et al., 2010). Many ectotherms are also generalists and vary their diet to meet their nutritional requirements or maximize energy-use efficiency (Jobling, 2016; Johnson et al., 2017; Kaiser and Hughes, 1993; Raubenheimer et al., 2005; Rubio et al., 2003, 2009; Sánchez-Vázquez et al., 1998). Some ectotherms also change their diet with temperature (Boersma et al., 2016a; Carreira et al., 2016; Jang et al., 2015; Rho and Lee, 2017; Schmitz and Rosenblatt, 2017; Vejříková et al., 2016). For example, multiple omnivorous fishes consume higher proportions of algae as water temperatures increase (e.g. Behrens and Lafferty, 2012; Emde et al., 2016; González-Bergonzoni et al., 2016; Guinan et al., 2015; Prejs, 1984). The exact reasons for these diet shifts are unknown, with some suggesting that the optimal dietary protein to carbohydrate ratio for ectotherms differs across temperatures (Lee et al., 2015; Rho and Lee, 2017; Zhang et al., 2020), or that cold temperature constrains the digestive physiology of herbivores and omnivores (Floeter et al., 2005; González-Bergonzoni et al., 2012). These proposed explanations hint at a broader hypothesis: that omnivores consume different proportions of plants and animals to regulate their physiological responses to changing temperatures. More broadly, any changes in an ectotherm's diet that coincide with a change in environmental temperature (through differences in dietary preference, availability or nutrient composition) might alter its thermal performance.

To understand the interaction between diet and temperature, we must consider how traits critical to survival may be differentially affected (Fig. 1). Measuring thermal limits in conjunction with vital biological rates provides comprehensive insight into ectotherm

List of symbols and abbreviations

ABT	Arrhenius breakpoint temperature
CT_{max}	critical thermal maxima
CT_{min}	critical thermal minima
f_H	heart rate
$f_{H,max}$	maximum overall heart rate
$f_{H,min}$	minimum overall heart rate
MMR	maximum metabolic rate
SMR	standard metabolic rate
T_{AB}	breakpoint temperature on the heart
T_{arr}	temperature at the first cardiac arrhythmia
T_{peak}	temperature corresponding to maximum heart rate
TL	total length
TPC	thermal performance curve

thermal biology in variable and changing environments (Magozzi and Calosi, 2015). A common assumption in thermal biology is that biological rates have the same thermal sensitivity and that aerobic capacity and baseline metabolism can be used as proxies for many performance traits (Fry, 1947; Brett, 1971; Claireaux and Lefrançois, 2007; Pörtner, 2001, 2010; for a critique, see Clark et al., 2013; Schulte, 2015). However, there is growing support for a multiple-performance, multiple-optima model, which states that thermal sensitivity differs across biological rates (i.e. absolute aerobic scope, standard metabolic rate, growth rate, sprint speed) and is not always predictable based on aerobic capacity or baseline metabolism (Clark et al., 2013; Dell et al., 2011; Kellermann et al., 2019; Seebacher et al., 2015). For example, Healy and Schulte (2012) demonstrated that specific growth rate was negative at temperatures where absolute aerobic scope (i.e. maximum−standard metabolic rate) was maximal in killifish. This model has been challenging to test empirically as few performance traits are usually

measured per study and these traits are often considered separately from upper and lower thermal limits (Magozzi and Calosi, 2015). If diet and temperature together influence biological rates and thermal limits, ectotherms may be incentivized to make diet choices that improve their thermal responses. However, if fitness-enhancing traits are differentially affected by diet and temperature, there could be important performance consequences associated with an ectotherm's ultimate diet choices.

Opaleye (*Girella nigricans*) are temperate omnivorous fish that consume a greater proportion of algae in warmer water across their geographic range (Behrens and Lafferty, 2012), which makes them an ideal model for exploring diet effects on thermal acclimation responses. Here, we tested the hypothesis that when offered a simple choice omnivorous diet (*ad libitum Artemia* sp. and *Ulva* sp.) versus a carnivorous diet (*ad libitum Artemia* sp. only) at two ecologically relevant temperatures (12 and 20°C), opaleye would make diet choices that altered their thermal acclimation responses in trait-specific ways. As juveniles, opaleye live in the intertidal zone where they face many challenges, including escaping predators, maintaining growth rates and dealing with high daily thermal variation (Somero, 2010). Therefore, we adopted an integrative approach and assessed the opaleye's thermal acclimation responses at the whole-animal (growth, sprint speed, metabolism, critical thermal limits), organ (cardiac thermal limits) and cellular (glycolytic capacity, oxidative stress) levels to compare ecologically and physiologically relevant performance traits and thermal limits for the fish in their juvenile life stage and identify any trade-offs associated with the treatment diets. We hypothesized that biological rates would increase with temperature but have different thermal sensitivities depending on the diet treatment. Specifically, we predicted that there would be costs to consuming the omnivorous diet (e.g. higher digestive infrastructure costs resulting in higher maintenance metabolism or reduced growth from lower protein diet) that would be offset by increases in the performance of other traits (e.g.
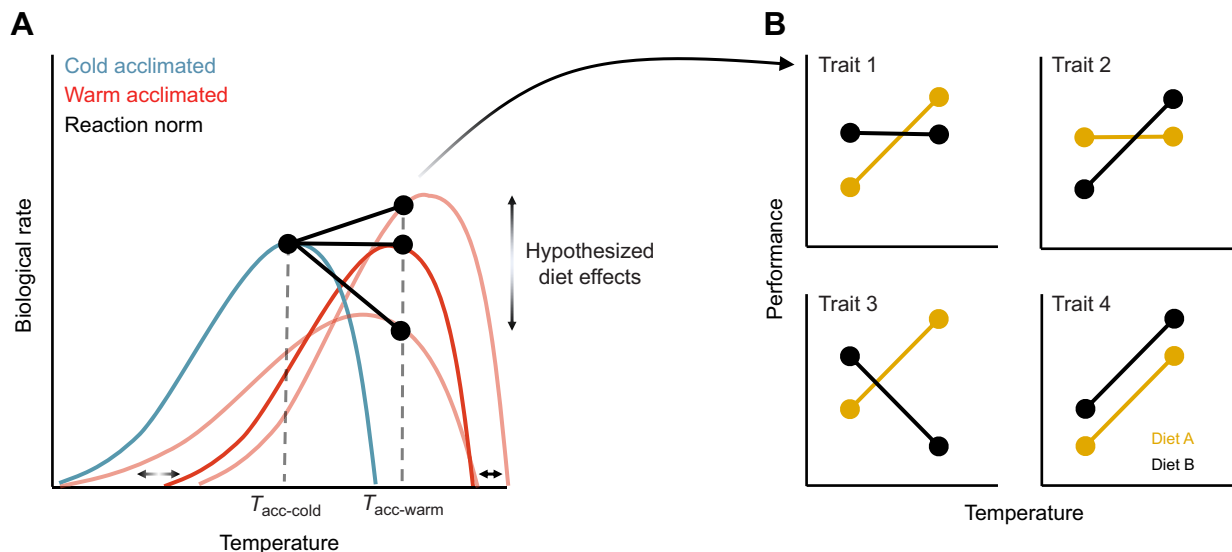

Fig. 1. Conceptual graphs illustrating how diet may affect thermal acclimation responses and how those effects could be trait specific. (A) Diet can affect thermal performance. The graph shows how acute thermal performance curves (TPC) shift towards the acclimation temperature (T_{acc}), where blue is the acute TPC after acclimation to cold and red is the acute TPC after acclimation to warm conditions. Note that both cold and warm acclimation may be affected by diet, but only potential effects of warm acclimation are displayed for simplicity. Diet may influence the shape (height and breadth) of those acute TPC (indicated by pink curves) or the location of the curve along the *x*-axis (i.e. temperature of peak performance). These effects could influence the slope of the line between reaction norms (performance at acclimation conditions, indicated by black lines). (B) Diet and temperature may interact and have trait-specific effects. The graphs are a series of hypothetical reaction norm plots for various traits. These traits can have different diet and temperature sensitivities, which could create performance trade-offs for ectotherms consuming different diets.

thermal limits, sprint performance, glycolytic capacity, oxidative stress).

MATERIALS AND METHODS
Fish collection
Juvenile opaleye, *Girella nigricans* (Ayres 1860), were collected in spring 2019 [experiment 1: respirometry, sprint, growth, critical thermal maxima (CT_{max}/CT_{min}); $N=144$, mean±s.d. body mass (BM) 14.75±3.53 g and total length (TL) 9.42±0.76 cm] and in winter 2020 (experiment 2: Arrhenius breakpoint test; $N=126$, BM 19.5±6.1 g and TL 10.5±1.1 cm) by seine or hook and line from Santa Barbara Harbor, CA, 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 (9–12 fish per tank). Prior to the start of acclimation, fish were held at ambient conditions (mean±s.d. experiment 1: 13.9±1.1°C and experiment 2: 16.2±0.6°C) and fed *ad libitum* omnivorous diets (*Ulva* sp. and *Artemia* sp.). All protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara.

Acclimation and diet treatments
Fish were randomly assigned to one of two ecologically relevant temperatures (12 and 20°C, representative of the low and high seasonal temperatures experienced in Santa Barbara, CA, USA; Fig. 2) and fed one of two *ad libitum* diets (omnivorous: *Artemia* sp. and *Ulva* sp.; and carnivorous: *Artemia* sp.) in a factorial design with 3–4 replicate tanks per treatment. *Ulva* sp. (collected by hand from Goleta Beach, Goleta, CA, USA) and *Artemia* sp. (brineshrimpdirect.com) were replaced every morning. Diets were selected based on stomach content information from Barry and Ehret (1993), which demonstrated that both *Ulva* sp. and small crustaceans constitute a significant portion of wild opaleye diets, and Behrens and Lafferty (2007), which used *Ulva* sp. as a representative herbivorous diet for a lab study on opaleye. Preliminary data demonstrated that *Ulva* sp.-only diets were not nutritionally sufficient. Dietary proximate analysis is provided in Table S1.

Food consumption rates were assessed during preliminary trials. All tanks were fasted for 24 h, and then the fish were offered their
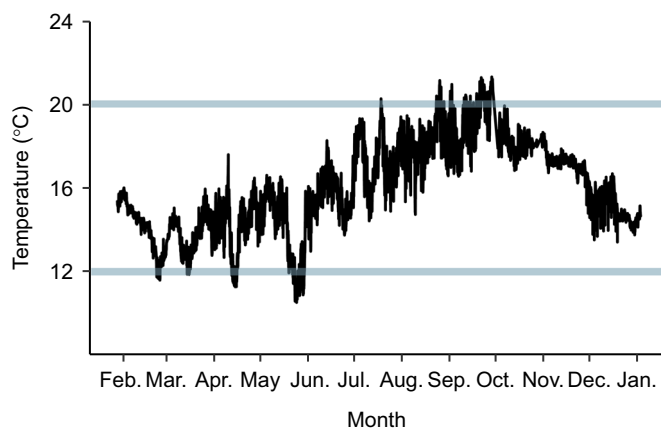

Fig. 2. Temperature data collected in 2019 at Stearns Wharf in Santa Barbara, CA, USA (located next to Santa Barbara Harbor, where fish were collected). The temperature sensor was mounted at 2 m depth and set to record every 4 min. Blue lines indicate treatment temperatures (12 and 20°C). Data source: Washburn, 2021 and Southern California Coastal Ocean Observing System (SCCOOS; https://www.sccoos.org/data/autoss/timeline/?main=single&station=stearns_wharf).

pre-weighed diet treatment for 1 h. The remaining *Artemia* sp. and *Ulva* sp. were removed and weighed. However, we were unable to obtain any measurable estimates of *Ulva* sp. consumption during these brief 1 h trials. At 20°C, *Artemia* sp. consumption was ~23% lower in the omnivorous treatment (7.39% body mass) than in the carnivorous treatment (9.58% body mass), suggesting that the fish in the omnivorous treatment were supplementing their diet with *Ulva* sp. This is consistent with our visual observations that the fish readily consumed *Ulva* sp. in the warm treatment (though they consistently ate more *Artemia* sp. compared with *Ulva* sp.). In contrast, at 12°C, *Artemia* sp. consumption was only ~10% lower in the omnivorous treatment (3.65% body mass) compared with the carnivorous (4.07% body mass) and we did not observe the fish consuming *Ulva* sp. at this temperature. This suggests that the fish either were not consuming *Ulva* sp. or were doing so in small amounts at the cold acclimation treatment. Further, opaleye ate less at 12°C compared to 20°C. Overall, *Artemia* sp. consumption was ~55% lower in the cold treatment compared with the warm.

Temperature and dissolved oxygen content were monitored 1–2 times daily by hand using a Digi-sense Traceable Singe RTD thermometer (Cole Palmer, IL, USA) and an OxyGuard handy Polaris 2 dissolved oxygen meter (OxyGuard International A/S, Farum, Denmark). Oxygen was maintained at >80% air saturation throughout the study. The average temperature per treatment was 20.0±0.4 and 12.2±0.4°C in the two experiments (mean±s.d.; determined from in-tank Thermochron 4 K iButtons programmed to record every 20 min). Fish were acclimated to treatment conditions for 3 weeks prior to experimentation (14 h:10 h light:dark cycle). All individuals were fasted for 36–40 h prior to respirometry, thermal limit and sprint testing. All tests were performed at acclimation temperatures unless otherwise noted.

Intermittent flow respirometry
Respirometry was conducted using 12 respirometers in one of three sizes: 349, 579 and 711 ml. Water was flushed and recirculated through the chamber at a rate of 2.5 l min^{-1} (Eheim Universal 300 pumps, Eheim, Germany). Dissolved oxygen was measured continuously in each respirometer using a robust oxygen probe and Firesting optical oxygen meter (Pyroscience, Germany).

Fish were transferred with minimal air exposure (<10 s) to a cylindrical chase tank (20 l), where they were chased by hand for 5 min and then immediately placed in the respirometers to obtain an estimate of their maximum metabolic rate (MMR) (this chase protocol was the most effective at eliciting MMR in opaleye; data not shown). Chases occurred between 10:30 h and 14:30 h and were followed by ~20 h of automated measurement cycles (15 min total flush/recirculation cycle). Tanks were covered in shade cloth to minimize potential disturbance. Fish were held at either 20.1±0.6 or 11.8±0.4°C (mean±s.d.) across all tests. Background respiration was measured before and after each test for ≥3 full measurement cycles.

After 20 h in the respirometers, fish were removed and anesthetized in 80 mg l^{-1} MS-222 buffered with 80 mg l^{-1} NaHCO$_3^-$ (Sigma Aldrich Co., St Louis, MO, USA). Each fish was weighed (mass in g), measured for TL, and tagged with a unique color code using Visible Implant Elastomer Tags (Northwest Marine Fisheries Inc., Seattle, WA, USA).

Data analysis for respirometry data
All oxygen consumption data were analyzed in R (version 3.5.1) using best practices as outlined in Rosewarne et al. (2016) and Chabot et al. (2016) (http://www.R-project.org/). The data were used to

calculate four metabolic rate metrics which define an individual's aerobic energy budget. Standard metabolic rate (SMR) is the baseline metabolic rate needed to survive, and MMR is the maximum rate of energy expenditure. The difference between these two metabolic rates is the absolute aerobic scope (AAS=MMR−SMR), which is representative of the aerobic energy budget a fish has to perform all critical biological processes (Clark et al., 2013). Factorial aerobic scope (FAS=MMR/SMR) is another estimate of the aerobic energy budget and represents the scope for increasing metabolic rate proportional to SMR (Clark et al., 2013). Only fish for which >75% of measurements followed a linear decrease with R^2>0.9 were included in SMR analysis (i.e. >60 $\dot{M}_{O_2}$ measurements total per fish; N=6–10 per treatment). SMR was calculated as the lowest 15% quantile of all recorded measurement cycles (Chabot et al., 2016). MMR was calculated as the steepest 120 s slope during the first measurement period (Little et al., 2020b; N=6–11 per treatment). All presented metabolic rate measurements are body mass specific (i.e. presented as mg O_2 kg^{-1} min^{-1}). We tested for the need to account for body size scaling but given the small size range in the study (8–39 g), we did not find evidence of a scaling relationship when the data were plotted on log–log plots (data not shown). Given the lack of knowledge on a species-specific scaling exponent, we did not scale the metabolism to a common body size using an allometric scaling slope. Background respiration was assessed and, when applicable, a linear regression was fitted between the pre- and post-background measurements and subtracted from the corresponding $\dot{M}_{O_2}$ values (Rodgers et al., 2016; Rosewarne et al., 2016).

Sprint speed
We used a modified protocol based on Kraskura and Nelson (2018). The setup included a custom-built acrylic sprint chamber (128 cm×30 cm×30 cm, L×W×H; water height 10 cm; water volume 41.6 l) with a camera (Canon EOS Rebel T4i) positioned above the sprint chamber to ensure full view of the work area. Each sprint trial was recorded at 60 frames s^{-1} and saved for digital analysis. Fish were placed behind a gated and shaded area in a sprint chamber and habituated to the chamber for 10 min. The gate was removed and the fish were motivated to sprint by manual chasing. Each fish performed ≤7 trials with 5 min of recovery between trials. Only trial videos where the fish had undergone a sprint with (1) a straight path of >20 cm, (2) an unobstructed view of 50 cm of the chamber, (3) active and continued bursts, and (4) no back and forth swimming around the chamber were used in subsequent analysis. Temperature was monitored throughout all trials and remained within ±1°C of the test temperature. All videos used in analysis were visualized and validated by ≥2 researchers. Videos were tracked in ImageJ/MtrackJ and subsequently analyzed in R to determine the fastest 5 frames of sprinting (~10 cm) per trial; 2–3 trials were analyzed per fish and averaged to determine each fish's sprint performance (cm s^{-1}) (Figs S1 and S2; N=8–10 per treatment). All fish were given at least 3 weeks of recovery between respirometry or thermal limit experiments and the sprint test.

Thermal limits: CT$_{max}$ and CT$_{min}$ testing
We used a standardized critical thermal maxima and minima tests (CT$_{max}$ and CT$_{min}$, respectively) as described in Beitinger and Lutterschmidt (2011). CT$_{max}$ and CT$_{min}$ represent the most extreme temperatures a fish can survive at for a short period of time. Briefly, 3–4 fish per tank were transferred to the testing tank (78 cm×61 cm×18 cm, L×W×H; water height 12 cm, water volume 57 l) at their treatment temperature with minimal air exposure (<10 s; total N=8–12 per treatment). After a brief

habituation period (~5 min), the test tank was heated (CT$_{max}$; 1500 W immersion heater, Process Technology, Willoughby, OH, USA) or cooled (CT$_{min}$; 1 HP AquaEuro chiller, AquaEuroUSA, Los Angeles, CA, USA) at a rate of ~0.3°C min^{-1} until fish lost the ability to maintain their righting response. Temperature was recorded at the loss of equilibrium and the fish were immediately placed in a recovery tank at an intermediate temperature before being returned to their treatment tanks. All fish fully recovered following the critical thermal tests.

Growth
All fish were weighed and measured for length prior to acclimation and again after 8 weeks under treatment conditions (note that some had been randomly removed for tissue sample collection, see below) to estimate growth across tanks (i.e. average weight gain per week).

Dissections and frozen tissue assays
Fish from each treatment (N=6 per treatment) were euthanized by cerebral concussion followed by severing of the spinal cord on day 27–34 of acclimation after the aerobic scope trials were complete. Morphometrics (body mass and TL) were measured, and a white muscle and liver sample were flash frozen in liquid nitrogen and stored at −80°C for future analysis. Stomach and remaining gut contents were emptied and leftover fish remains were stored at −20°C for proximate analysis (see Supplementary Materials and Methods and Table S1).

Lactate dehydrogenase assays
Lactate dehydrogenase activity assays were performed as outlined in Little et al. (2020a) as a proxy for glycolytic capacity in white muscle, where high levels of lactate dehydrogenase activity indicate that the animal has a high capacity to support anaerobic ATP production to fuel burst swimming or hypoxia tolerance (Little et al., 2020a). Briefly, white muscle samples were homogenized in homogenization buffer (0.1% Triton, 50 mmol l^{-1} Hepes, 1 mmol l^{-1} EDTA, pH 7.4 buffer) before being run in triplicate (intra-assay coefficient of variation, CV <15%) on a SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices) using a wavelength of 340 nm to measure the disappearance of NADH. The assay was repeated at 8, 12, 20, 26 and 32°C.

Lipid peroxidation assays
Thiobarbituric acid reactive substances were quantified in the liver to estimate lipid peroxidation as a proxy for oxidative stress using a commercially available fluorometric assay kit (Cayman Chemical). Here, higher levels of lipid peroxidation are indicative of greater oxidative damage to cellular components (Castro et al., 2012). Samples were homogenized in RIPA buffer and treated according to the manufacturer's instructions before being run in duplicate at an excitation wavelength of 544 nm and emission wavelength of 590 nm.

Thermal limits: Arrhenius breakpoint temperature test
Arrhenius breakpoint temperature (ABT) tests on the heart were conducted (N=6–14 per test and per treatment) as outlined in Casselman et al. (2012) and Gilbert et al. (2020). Briefly, fish were anesthetized in seawater containing 80 mg l^{-1} MS-222 buffered with 1 g l^{-1} NaHCO$_3^-$ before being placed ventral side up in an experimental sling in the test tank (10 l seawater containing buffered 65 mg l^{-1} MS-222). Water was circulated past the gills to irrigate them. Stainless Steel Needle Tip Electrodes (ADInstruments Inc., Colorado Springs, CO, USA) were inserted just under the skin to

detect an ECG signal, which was amplified using a Dual Bio Amp amplifier (ADInstruments Inc.) and filtered (filters: 60 Hz Notch filter; mains filter; low pass: 2 kHz; high pass: 10 Hz; range: 2 mV).

After a 30 min equilibration period at the acclimation temperature (Ferreira et al., 2014; Hansen et al., 2017), atropine sulfate was injected intraperitoneally (1.2 mg kg^{-1} in 0.9% NaCl) to block vagal tone followed by isoproterenol (4 µg kg^{-1} in 0.9% NaCl) to maximally stimulate β-adrenoreceptors. Any fish that did not respond to the drug injections or for which experimental error occurred (e.g. water pump failure) were removed from the analysis and not considered further. These drug concentrations were tested prior to experimentation to ensure doubling the concentration did not further increase heart rate (f_H; beats min^{-1}). Fifteen minutes after isoproterenol injection, water temperature was heated (warm ABT test) or cooled (cold ABT test) at 1°C every 6 min (Polystat recirculating heater/chiller; Cole-Palmer, Vernon Hills, IL, USA). At each 1°C interval, f_H and temperature were stabilized to record a value for f_H. This procedure was repeated 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 f_H (Casselman et al., 2012) or until the known average CT$_{max}$ for the species (generally 0–5°C higher than T_{arr}; Chen et al., 2015; Muñoz et al., 2014; Safi et al., 2019), to ensure that curves could later be fitted to the data for comparisons of acute thermal performance curves across treatments. All fish were immediately euthanized at the end of the test.

Data analysis for ABT tests
All ECG analyses were performed in LabChart software (www.adinstruments.com). f_H was calculated for each temperature increment from 15 continuous seconds of measurements using automated ECG analysis software in LabChart (Gradil et al., 2016). The heart may set upper thermal tolerance temperatures in fish (Anttila et al., 2014; Muñoz et al., 2014). The warm ABT test measures three sublethal thermal limits on cardiac function (T_{AB}, T_{peak}, T_{arr}). Each of these limits indicates transition temperatures where the heart's capacity to transport oxygen, nutrients and immune cells becomes compromised (Anttila et al., 2014; Muñoz et al., 2014). The first thermal limit (T_{AB}) is highly correlated with the thermal optimum for aerobic scope in other teleosts (Anttila et al., 2013; Ferreira et al., 2014). T_{AB} was calculated by performing ABT tests on the rising phase of the thermal performance curve for f_H using *segmented* (v1.1-0; Muggeo, 2008) in R. The temperature corresponding to the breakpoint in f_H was defined as T_{AB} (warm ABT test) or $T_{AB-cold}$ (cold ABT test). Overall maximum heart rate ($f_{H,max}$) was defined as the highest f_H recorded during the 15 s measurement phases in the warm ABT test and minimum heart rate ($f_{H,min}$) was defined as the lowest f_H recorded during the 15 s measurement phases during the cold ABT test. Peak temperature (T_{peak}) was the temperature corresponding to $f_{H,max}$.

Statistical analysis
All data were statistically analyzed using R (version 3.5.1). All metrics were investigated for normality using Shapiro–Wilk tests and quantile–quantile plots, and for heteroscedasticity using Levene's test. Data that were not normally distributed were log-transformed before statistical analysis (only FAS). Data are displayed with untransformed values. All data were statistically analyzed (significance level α=0.05) using a 2-way ANOVA (*Car* v3.0-2; Fox and Weisberg, 2011) with *post hoc* Tukey HSD, except for the thermal limits from the cold ABT, which were analyzed using a *t*-test. Differences between treatments were also assessed

using Cohen's *D*-tests (*effsize*; https://CRAN.R-project.org/package=effsize). Note that 12°C fish were not tested for the cold ABT because of complications surrounding COVID-19 forcing the early shutdown of the experiment. In all 2-way ANOVA tests, significance of interaction between diet and temperature was tested for and excluded when non-significant. Polynomial curves were fitted to f_H data and compared using Akaike information criterion (AIC), where the fit with the lowest AIC score was assigned the best fit model, but all models with ΔAIC<2 were considered (Burnham and Anderson, 2002). Measures of thermal sensitivity for all biological rates were calculated for each diet treatment using Q_{10} values, where:

$$Q_{10} = \frac{R_2}{R_1}^{(10/(T_2-T_1))}, \qquad (1)$$

R_1 is the treatment mean at 12°C, R_2 is the treatment mean at 20°C, T_1 is 12°C and T_2 is 20°C.

RESULTS
Metabolic rates
Metabolism was influenced by diet and temperature, but each metabolic rate responded differently (Figs 3 and 4). SMR was 28% higher in the 20°C omnivorous diet treatment compared with the carnivorous diet treatment, resulting in a significant interaction between diet and temperature (Fig. 3A, Table 1). This was further supported by a large effect size between the 20°C treatments (Cohen's *D*-test). In contrast, MMR significantly increased with acclimation temperature, but did not differ across diet treatments (Fig. 3B,C, Table 1). There was a marginal, but not significant diet and temperature effect on AAS (Fig. 3C, Table 1). This was likely driven by the high individual variability in MMR, as the effect of diet on AAS had a *P*-value of 0.087 and medium effect sizes between diet treatments at 20°C. In contrast, FAS in the 20°C omnivorous diet treatment was significantly lower than that in the carnivorous diet treatment (diet: d.f.=1, *F*=5.796, *P*=0.023), which was largely driven by a 44% higher FAS (with a large effect size) in the carnivorous versus omnivorous diet (Fig. 3D, Table 1).

Other biological rates and traits
Growth rate, sprint speed, lipid peroxidation and lactate dehydrogenase activity were inconsistently affected by diet and temperature (Fig. 4). Growth was significantly higher at 20°C but did not differ across diets (Fig. 4, Table 1; Fig. S2). Unexpectedly, the Q_{10} value for growth was higher than that for all other rates (28.05) and not close to any of the Q_{10} values for metabolic rate (range 1.28–4.12; Fig. 4, Table 1; Fig. S2). Proximate analyses for whole tissue did not differ between treatment groups (Table S1).

In contrast with growth, maximum sprint speed did not differ in response to thermal acclimation (Q_{10}=1.04) or diet treatment (Fig. 4, Table 1; Figs S1 and S2). Similarly, lipid peroxidation in liver tissue did not differ in response to temperature and showed a marginal but insignificant effect of diet (Fig. 4; Fig. S2; temperature: d.f.=1, *F*=0.318, *P*=0.579; diet: d.f.=1, *F*=3.260, *P*=0.085). Lactate dehydrogenase activity in white muscle was moderately affected by temperature acclimation (Q_{10}=2.10), being higher at 20°C compared with 12°C, but did not differ across diets (Fig. 4; Fig. S3). Lactate dehydrogenase activity also increased with acute temperature exposure (Fig. S3). Overall, Q_{10} values for the reaction norms differed dramatically across biological rates, with sprint speed having a Q_{10} of 1.04 (insensitive to temperature), while growth rate had a Q_{10} of 28.05 (highly sensitive to temperature) (Fig. 4).
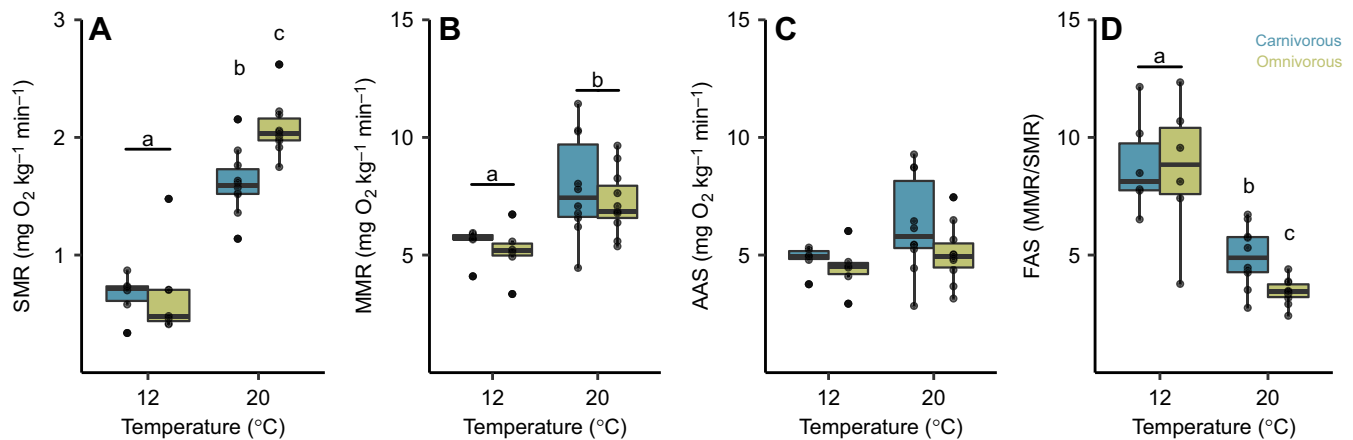
Fig. 3. Oxygen uptake rate (mg O$_2$ kg^{-1} min^{-1}) after an exhaustive chase protocol in opaleye acclimated to 12 or 20°C and fed either a carnivorous or omnivorous diet. (A) Standard metabolic rate (SMR), (B) maximum metabolic rate (MMR), (C) absolute aerobic scope (AAS; MMR−RMR) and (D) factorial aerobic scope (FAS; MMR/SMR). Blue, carnivorous diet (*Artemia* sp.); green, omnivorous diet (*Artemia* sp. and *Ulva* sp.). Lowercase letters indicate significant differences ($P<0.05$) between treatment groups where applicable (see Table S1 for 2-way ANOVA outputs). Boxplots represent interquartile ranges (boxes and whiskers), median values (solid lines) and outliers (>1.5 beyond interquartile range) plotted as data points outside the whiskers.

Thermal tolerance

All thermal limits increased with acclimation to 20°C (Fig. 5, Table 2). Upper thermal limits (CT$_{max}$, T_{AB}, T_{peak}, T_{arr}) increased by 2.6–5.3°C and CT$_{min}$ increased by 2.1–2.3°C with warm acclimation (Fig. 5, Table 2). Surprisingly, $f_{H,max}$ was significantly lower in the omnivorous treatments, which was driven by a 10% lower $f_{H,max}$ in the 20°C omnivorous treatment relative to the carnivorous treatment (Fig. 5, Table 2). As expected, f_H followed the

shape of an acute thermal performance curve (TPC), where it increased with an acute temperature increase until T_{peak}, at which point f_H began declining with temperature until the onset of cardiac arrhythmia (T_{arr}; Fig. 5A). Thermal acclimation to 20°C shifted the acute TPC for f_H to the right of the TPC at 12°C (Fig. 5A). There was evidence to support an effect of diet on model selection for f_H in the warm ABT test, where the best fit model by AIC was a third-order polynomial curve that incorporated an interaction of acclimation
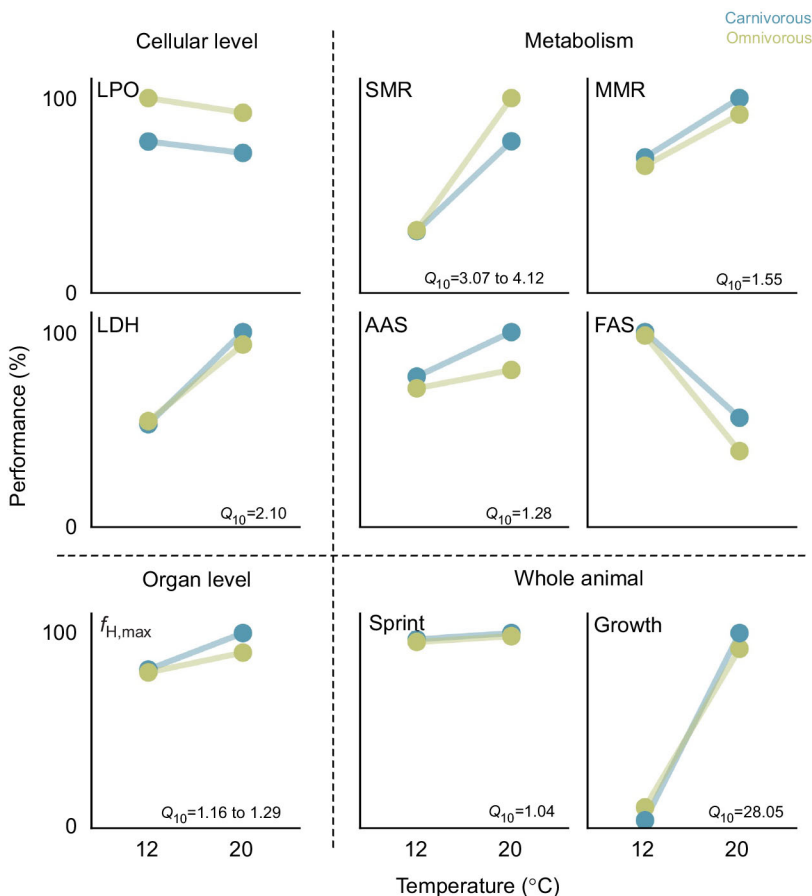

Fig. 4. Reaction norms plotted across all measured biological rates and traits. Average data from each treatment, scaled to the maximum average treatment value (i.e. the maximum treatment value is equal to 100%). All values are from fish tested at their acclimation temperature. Graphs are arranged by level of biological organization (cellular, organ, whole animal) and labelled by trait: SMR, MMR, AAS (MMR−SMR), FAS (MMR/SMR), maximum overall heart rate ($f_{H,max}$), maximum sprint speed, growth rate, lipid peroxidation (LPO) and lactate dehydrogenase activity (LDH). Q_{10} values are listed for all biological rate measurements (i.e. everything except LPO and FAS), as a range when the diet treatments were statistically different and as individual values when the diet treatments were not statistically different. Lines and circles indicate reaction norms and colors indicate diet treatment (blue, carnivorous diet; green, omnivorous diet).

Table 1. Summary statistics for biological rates

Biological rate	Temp. (°C)	Carnivorous		Omnivorous		Diet			Temperature			Diet×Temperature		
		n	Mean±s.e.m.	n	Mean±s.e.m.	d.f.	F	P	d.f.	F	P	d.f.	F	P
SMR (mg O_2 kg^{-1} min^{-1})	12	6	0.66±0.07	7	0.67±0.14	1	8.577	0.007	1	147.093	<0.001	1	5.282	0.029
	20	10	1.62±0.09	10	2.08±0.07									
MMR (mg O_2 kg^{-1} min^{-1})	12	6	5.49±0.28	6	5.15±0.45	1	1.061	0.311	1	16.542	<0.001			
	20	10	7.89±0.69	11	7.23±0.40									
AAS (mg O_2 kg^{-1} min^{-1})	12	6	4.84±0.23	6	4.46±0.41	1	3.131	0.087	1	3.656	0.066			
	20	10	6.27±0.66	10	5.05±0.40									
FAS (MMR/SMR)	12	6	8.81±0.83	6	8.65±1.21	1	5.796	0.023	1	52.242	<0.001			
	20	10	4.94±0.41	10	3.43±0.17									
Growth (g week^{-1})	12	3	0.03±0.19	3	0.08±0.01	1	0.003	0.958	1	23.498	<0.001			
	20	3	0.75±0.15	3	0.69±0.16									
Sprint speed (cm s^{-1})	12	10	112.36±4.88	9	110.85±5.26	1	0.099	0.756	1	0.456	0.504			
	20	8	116.10±5.46	8	114.26±6.01									

Means±s.e.m. for each test group and ANOVA results are presented. SMR, standard metabolic rate; MMR, maximum metabolic rate; AAS, absolute aerobic scope; FAS, factorial aerobic scope; Temp., temperature, d.f., degrees of freedom.

temperature, acute test temperature and diet (Fig. 5A; Table S2). These models demonstrated that the acute TPC for f_H was lower across temperatures in the 20°C choice treatment compared with 20°C carnivorous treatment, which was consistent with the observed differences in $f_{H,max}$ (Fig. 5A). Altogether, these results demonstrate that for the 20°C acclimated fish, the omnivorous diet reduced overall cardiac function across temperatures but did not affect the upper thermal tolerance limits on the heart.

The results of the cold ABT test showed similar evidence of an effect of diet on f_H and $f_{H,min}$. Here, diet did not have a significant effect on any cold thermal limits (CT$_{min}$, $T_{AB-cold}$, $T_{arr-cold}$), but $f_{H,min}$ was lower (16%) in the omnivorous treatment compared with the carnivorous treatment at 20°C. There was also evidence to support an effect of diet on model selection, where the best fit model by AIC was a fourth order polynomial curve that incorporated an effect of acute test temperature and diet (Fig. 5C; Table S2).

DISCUSSION

Using an omnivorous fish, opaleye, as a model species, we found evidence that diet can influence thermal performance in ectotherms and does so in trait-specific ways. This has critical implications for our understanding of species responses to temperature change. Specifically, we examined three concepts outlined in Fig. 1: (1) whether diet and temperature acclimation can affect thermal performance and limits, (2) whether ecologically important traits vary in their diet and temperature sensitivity (integrating multiple levels of biological organization) and (3) whether trait-specific variation in diet and temperature responses creates performance trade-offs for opaleye fed an omnivorous diet compared with a carnivorous diet.

Thermal limits increased with temperature but did not differ across diet treatments

We assessed thermal limits using a standard and commonly used critical thermal (CT) test, as well as an ABT test, which measures thermal limits of the heart (Casselman et al., 2012). The heart may be a primary regulator of functional thermal tolerance in fishes. It is responsible for oxygen, immune cell, metabolite and waste transport around the body and is thought to be the first organ system to fail at extreme temperatures (Christen et al., 2018; Eliason and Anttila, 2017). The heart starts showing declines in performance (at T_{AB}) ~10–20°C lower than CT$_{max}$ and fails (T_{arr}) at temperatures ~0–5°C lower than CT$_{max}$ in fishes (Chen et al., 2015; Muñoz et al., 2014; Safi et al., 2019). As expected, all thermal limits increased with

temperature acclimation and CT$_{max}$ was 2.7–3.8°C higher than T_{arr}. Opaleye showed a highly plastic acclimation response across all thermal limits, consistent with other temperate marine ectotherms (Vinagre et al., 2016). However, their thermal limits did not differ between diets.

While we did not observe a diet difference here, diet quality and quantity can alter thermal limits in ectotherms (fishes: Hoar and Cottle, 1952; Craig et al., 1995; Abdel-Ghany et al., 2019; Gomez Isaza et al., 2019; Lee et al., 2016; Turko et al., 2020; Woiwode and Adelman, 1992). Most previous studies used formulated diets varying in lipid composition; thus, dietary lipid composition may be a primary factor affecting thermal limits. This is critical to consider in the context of aquaculture, where animal feeds should be designed to ensure farmed animals have adequate thermal performance and resistance to suboptimal temperatures. To our knowledge, this is the first study to test the effect of quasi-natural diets on the thermal acclimation of critical and cardiac thermal limits in ectotherms. Research with natural diets is ecologically relevant for ectotherms that consume broad diets and especially for fish such as opaleye that seem to change their diet in response to temperature (Behrens and Lafferty, 2007, 2012; Emde et al., 2016; González-Bergonzoni et al., 2016; Guinan et al., 2015; Vejříková et al., 2016). Climate change is altering the nutritional landscape for many aquatic ectotherms (Birnie-Gauvin et al., 2017; Huey and Kingsolver, 2019). Managers and biologists should consider the effects of this on ectotherm thermal limits. Thus, future research should explore whether other natural diets can influence thermal limits in ectotherms, as this has critical implications for how they may respond to global climate change.

Thermal acclimation responses did not scale with temperature and diet equally across biological rates

The biological rates assessed here did not scale with diet and temperature equally. Thermal sensitivity differed dramatically across biological rates, where sprint speed did not differ with diet and temperature acclimation ($Q_{10}=1.04$), lactate dehydrogenase activity was moderately affected by temperature ($Q_{10}=2.10$), but insensitive to diet, and growth was highly temperature sensitive ($Q_{10}=28.05$), but also not sensitive to the diet treatments used in this study. These results are consistent with large scale meta-analyses, which have revealed that Q_{10} values differ across biological rates (Dell et al., 2011; Seebacher et al., 2015). Diet sensitivity was also inconsistent across rates, where SMR and $f_{H,max}$ were sensitive to diet,
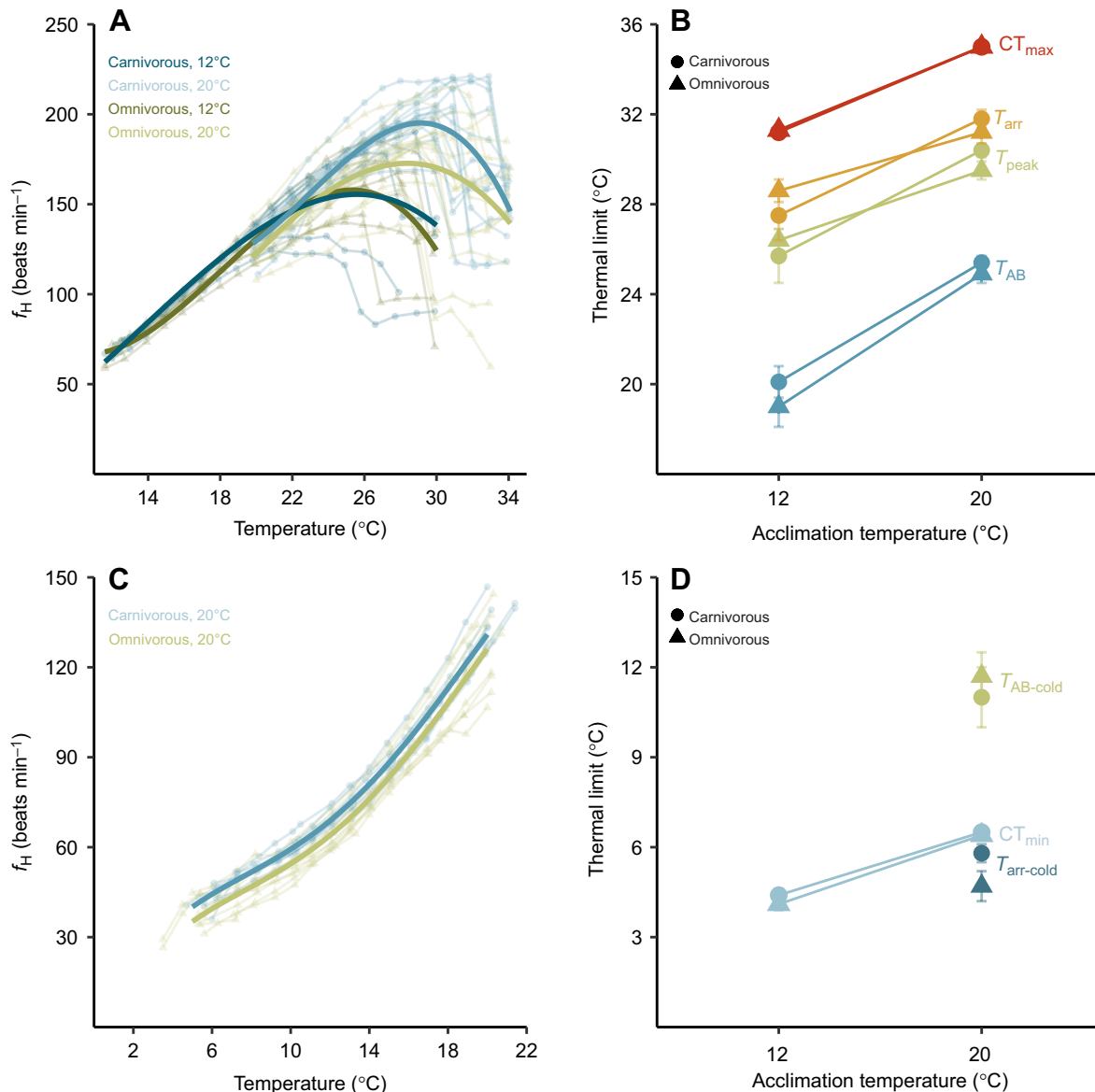
Fig. 5. Cardiac thermal performance. (A) Individual- and treatment-level responses of heart rate (f_H) of opaleye during acute warming from 12°C (dark blue, carnivorous diet, $N=6$; and dark green, omnivorous diet, $N=7$) or 20°C (light blue, carnivorous diet, $N=13$; and light green, omnivorous diet, $N=14$). Curves are third-order polynomials that account for the interaction diet×acclimation temperature×acute temperature. This was determined to be the best fit model by AIC (Table S2). (B) Upper thermal limits across diet and temperature treatments in opaleye. Circles and triangles indicate mean (±s.e.m.) values for the carnivorous and omnivorous diet treatments, respectively. (C) Individual- and treatment-level responses in f_H from opaleye during acute cooling from 20°C (light blue, carnivorous diet, $N=9$; and light green, omnivorous diet, $N=11$). Curves are fourth-order polynomials that account for a fixed effect of diet and acute temperature. This was determined to be the best fit model by AIC (Table S2). (D) Lower thermal limits across diet and temperature treatments in opaleye. Circles indicate mean (±s.e.m.) values for the carnivorous diet treatment and triangles indicate mean (±s.e.m.) values for the omnivorous diet treatments. Note that f_H limits are missing from the 12°C treatments because of issues surrounding COVID-19. T_{arr}, temperature at the onset of cardiac arrhythmia; T_{AB}, breakpoint temperature of the heart; T_{peak}, temperature corresponding to maximum heart rate; CT_{max}, critical thermal maximum; CT_{min}, critical thermal minimum.

but all other rates were not. This was surprising for rates that are known to be impacted by diet, such as growth rate. Diet has been shown to have interactive effects with temperature on growth in other ectotherms (Sengalese sole: Guerreiro et al., 2012; rohu: Mishra and Samantaray, 2004; yellowtail kingfish: Ilham and Fotedar, 2016; crustaceans: Malzahn et al., 2016; Persson et al., 2011; Ruiz et al., 2020; Starke et al., 2020; insects: Kingsolver et al., 2006; Lee and Roh, 2010). However, *Ulva* sp. supplementation has had mixed effects on growth in aquaculture fish, with some studies showing modest amounts of *Ulva* sp. reducing growth, while others finding positive or no effect of *Ulva* sp. supplementation on growth (Wan

et al., 2019). Future work should explore how broader diet differences affect growth in relation to other important biological rates.

The effects of temperature on sprint speed in comparison to lactate dehydrogenase activity were similarly unexpected. Sprinting in fish is primarily driven by glycolytic fast twitch white muscle (McDonald et al., 1998; Kraskura and Nelson, 2018). Lactate dehydrogenase is a critical enzyme in lactic acid fermentation during glycolysis. Thus, we expected that sprint performance would have a comparable thermal sensitivity to lactate dehydrogenase activity. Lactate dehydrogenase activity increased with temperature acclimation, suggesting the opaleye had a greater anaerobic capacity

Table 2. Summary statistics for all thermal limits

| Thermal limit | Temp. (°C) | Results | | | | Statistical parameters | | | | | |
| | | Carnivorous | | Omnivorous | | Diet | | | Temperature | | |
		n	Mean±s.e.m.	n	Mean±s.e.m.	d.f.	F (or t*)	P	d.f.	F	P
CT_{min}	12	9	4.4±0.2	10	4.1±0.2	1	1.984	0.168	1	226.206	<0.001
	20	12	6.5±0.1	8	6.4±0.1						
T_{AB}	12	6	20.1±0.7	7	19.0±0.9	1	2.127	0.153	1	110.743	<0.001
	20	13	25.4±0.3	14	24.9±0.4						
T_{peak}	12	6	25.7±1.2	7	26.4±0.5	1	0.612	0.439	1	46.990	<0.001
	20	13	30.4±0.3	14	29.5±0.4						
T_{arr}	12	6	27.5±1.1	7	28.6±0.5	1	0.021	0.886	1	33.766	<0.001
	20	13	31.8±0.4	14	31.2±0.5						
CT_{max}	12	10	31.2±0.2	10	31.3±0.2	1	0.547	0.465	1	500.078	<0.001
	20	8	35.0±0.2	10	35.0±0.1						
$f_{H,max}$	12	6	166.6±12.5	7	163.5±6.2	1	5.243	0.028	1	17.669	<0.001
	20	13	204.8±4.4	14	184.4±5.4						
$T_{arr-cold}$	12	NA	NA	NA	NA	18	1.790*	0.090			
	20	9	5.8±0.3	11	4.7±0.5						
$T_{AB-cold}$	12	NA	NA	NA	NA	14	−0.493*	0.630			
	20	8	11.0±1.0	8	11.7±0.8						
$f_{H,min}$	12	NA	NA	NA	NA	18	2.407*	0.027			
	20	9	44.5±1.9	11	37.4±2.2						

Means±s.e.m. for each test group and ANOVA results are presented. *Note that t-test results are reported on the results of the cold ABT test, as this test was only run on 20°C acclimation fish because of complications surrounding COVID-19. CT_{min}, critical thermal minimum; T_{AB}, breakpoint temperature of the heart; T_{peak}, temperature corresponding to maximum heart rate; T_{arr}, temperature at the onset of cardiac arrhythmia; CT_{max}, critical thermal maximum; $f_{H,max}$, maximum heart rate across entire warm ABT test; $f_{H,min}$, minimum heart rate during cold ABT test.

at 20°C (McDonald et al., 1998). However, this did not translate to increases in sprint performance. Other enzymes in glycolysis could be rate-limiting steps (e.g. phosphofructokinase; McDonald et al., 1998) and more highly correlated with sprint speed. Sprint speed has been examined in one other diet×temperature study in fishes, which measured macronutrient selection and temperature effects on damselfish (Rowe et al., 2018). Macronutrient selection did not change with temperature, but sprint speed was highest in the colder temperature treatment (Rowe et al., 2018). Fish use two different swimming modes: anaerobically powered burst swimming and sustained aerobically powered swimming. These swimming modes may be differentially affected by diet and temperature. The effect of diet and temperature on aerobically powered swimming and maximum swim speeds (i.e. maximum swimming speed, U_{max}, and critical swimming speed, U_{crit}) have been assessed in grey mullet (Vagner et al., 2015, 2019), with no observed effect of diet, but an effect of temperature on U_{max} and no effect of diet or temperature on U_{crit}. Given that two metabolic traits, SMR and FAS, were affected by diet and temperature in this study, future work should explore how aerobically powered swimming is impacted by different levels of omnivory.

The diverse diet and temperature responses that we observed across biological rates resulted in no consistent pattern in the thermal sensitivity of any metabolic rates or levels of biological organization. The small number of papers that have measured the interactive effects of diet and temperature on metabolic rates in other ectotherms have found mixed results (i.e. insects: Alton et al., 2020; Schmitz and Rosenblatt, 2017; fish: Pérez-Casanova et al., 2010; Vagner et al., 2015). For example, Vagner et al. (2015) found an interactive effect of dietary fatty acid composition and temperature on the AAS of juvenile grey mullet. In contrast, Pérez-Casanova et al. (2010) did not find any effects of macronutrient ratios on metabolism in juvenile Atlantic cod and haddock. Our results were especially surprising for SMR, as it is often assumed that maintenance metabolism is coupled to all other biological rates and should have the same thermal sensitivity (Gillooly et al., 2001;

Brown et al., 2004). Aerobic scope is also considered a master physiological factor that determines the capacity for all other aerobically powered functions (Fry, 1947; Brett, 1971; Claireaux and Lefrançois, 2007; Pörtner, 2010), although this idea has been heavily debated (Clark et al., 2013; Schulte, 2015). Here, AAS did not differ in response to diet or temperature (although FAS did) and there was also no observed pattern between AAS, SMR and other biological rates. These results indicate that caution should be taken when using AAS and SMR as indicators of overall performance or proxies for other biological rates, especially when predicting the effects of multiple factors, such as diet and temperature.

Performance costs and benefits for diet choice at different acclimation temperatures

Contrary to our hypothesis, we did not find evidence of a performance trade-off to the omnivorous diet used in this study. Instead, opaleye that consumed the omnivorous diet displayed several higher costs: lower f_H across a thermal gradient, higher SMR and reduced FAS when acclimated to 20°C. Given that the thermal limits of the heart did not change with diet, it was remarkable that diet downshifted the thermal performance curve for $f_{H,max}$. A reduction in $f_{H,max}$ indicates a reduced capacity to transport oxygen, metabolites, immune cells and waste around the body. There are several potential mechanisms that could have driven this reduction in $f_{H,max}$ across acute temperatures. For example, differences in the lipid composition of the diet can impact membrane remodeling, which can affect cardiac function in fish (Chatelier et al., 2006; McKenzie, 2001). Further, although *Ulva* sp. contains no known herbivore deterrents, our results suggest some sort of anti-nutrient effect of *Ulva* sp. supplementation, which could have caused the observed reductions in cardiac thermal performance.

The higher SMR observed in the 20°C omnivorous treatment was expected as omnivorous and herbivorous animals generally have higher digestive infrastructure costs than carnivores (e.g. broader digestive enzymes, higher gut surface area; Caruso and Sheridan, 2011; Clements et al., 2009; Horn, 1989). Consistent with the higher

SMR at 20°C, the opaleye in the omnivorous treatment had a reduced FAS (3.43) that was less than half that at 12°C (8.65). In contrast, the carnivorous diet maintained a 44% higher FAS at 20°C (4.94) compared with the omnivorous diet. FAS is indicative of the amount of scope available to perform critical biological functions that scale proportional to SMR (Farrell, 2016). Digestion generally requires at least a doubling of SMR in many fishes (i.e. FAS>2; Chabot et al., 2016; Farrell, 2016; McCue, 2006). This suggests that the opaleye in the omnivorous treatment at 20°C may have been on the threshold of having limited scope for activities beyond digestion. As climate change increases the seasonal extreme temperatures and the frequency of marine heatwaves in the rocky intertidal zone (IPCC, 2019), the opaleye's FAS will decrease further, which could limit other important biological functions, such as digestion, and exacerbate energetic tradeoffs associated with different diets. Thus, another avenue for future research will be to untangle diet×temperature effects on digestive costs (i.e. specific dynamic action) in relation to AAS and digestion efficiency (Jutfelt et al., 2021).

Given that opaleye and other omnivorous fishes eat more plants in warmer water in the wild, we expected there to be a performance advantage to the omnivorous diet. We measured a suite of traits to test for any performance benefits that may offset the costs of the omnivorous treatment. Specifically, we explored how diet and temperature affected (1) thermal tolerance, (2) maximum sprint speed, (3) glycolytic capacity and (4) oxidative stress. We predicted that because opaleye eat more plants in warmer water (Behrens and Lafferty, 2012), the omnivorous diet would result in higher thermal limits than the carnivorous diet at 20°C. However, we did not find evidence of any benefits to the omnivorous diet. We also expected that the higher lipid and protein content of the carnivorous treatment would raise oxidative stress relative to the omnivorous diet. Analysis of lipid peroxidation in liver tissue revealed a marginal trend of higher oxidative stress in the omnivorous diet treatment. However, antioxidant capacity could still have been higher in the omnivorous treatment. For example, Coggins et al. (2017) examined the effect of dietary glutathione supplementation on thermal limits, antioxidant capacity and lipid peroxidation in *Daphnia* sp. As the glutathione concentration increased, so did total antioxidant capacity, but glutathione supplementation did not alter lipid peroxidation or thermal limits (Coggins et al., 2017). In contrast, Castro et al. (2012) examined the effect of macronutrient ratios (45% and 55% protein) and temperature acclimation (12 and 18°C) on multiple antioxidant enzymes and lipid peroxidation in juvenile Senegalese sole. Lipid peroxidation differed across temperature treatments and was highest in the 55% protein diet (Castro et al., 2012). The limited amount of research on these interactions and inconclusiveness across studies indicate that more research is needed to elucidate the role of dietary antioxidants and macronutrient ratios in regulating oxidative stress across temperatures and taxa.

While it was not within the scope of this study, other important performance traits may have differed between the diet treatments. These include, but are not limited to, differences in microbiome diversity and function, visual acuity, cognitive ability, digestion efficiency and digestive costs relative to aerobic scope, immune function, aerobic swimming performance and cardiac stroke volume (Glencross and Rutherford, 2010; Koven et al., 2018; Vagner et al., 2014, 2019). It remains unclear whether opaleye and other omnivorous ectotherms consume different proportions of plant to animal to regulate their thermal responses. Here, the opaleye's omnivorous diet did not maximize their performance compared with a more specialized carnivorous diet. However, many other factors govern diet choice in the wild, including life history (Zhang et al.,

2020), competition (Pfenning, 1990), predation (Schmitz et al., 2016), food availability (MacArthur and Pianka, 1966) and habitat structure (Behrens and Lafferty, 2007). Therefore, the ecological benefits of consuming an omnivorous diet may outweigh the physiological costs.

Many ectotherms change their diets with temperature; either directly because their diet preference changes in response to temperature (Boersma et al., 2016b; Carreira et al., 2016; Devries and Appel, 2014; Lee et al., 2015; Lemoine et al., 2014; Rho and Lee, 2017; Schmitz et al., 2016; Vejříková et al., 2016) or indirectly because food availability or the nutritional content of a diet item changes with temperature (Alton et al., 2020; Boersma et al., 2016b; Cross et al., 2015; Ho et al., 2010). In either scenario, generalist ectotherms that have the capacity to adjust their diet may be at an advantage compared with those with more specialized inflexible diets. However, the ultimate diet choices that ectotherms make may not always be 'better' or 'worse' because diet and temperature can interact and have trait-specific effects. Not all diet choices are necessarily adaptive. Irrespective of the reasons why an ectotherm eats what it eats, our work here demonstrates that diet choices have consequences. These consequences have far-reaching implications, including whether diet choice can facilitate geographic range expansion, or colonization of warmer or cooler habitats; and further, whether specialist diets constrain thermal niches or whether diet can facilitate differences in acclimation rates or performance under fluctuating temperatures. Overall, diet should be treated as an interacting factor that has the capacity to modify the thermal responses of ectotherms.

Concluding remarks

Thermal acclimation is a key mechanism that ectotherms employ to maintain performance across a range of temperatures. Acclimation requires energy and nutritional building blocks that ectotherms obtain from their diet. Here, we explored whether different diets mediated distinct thermal acclimation responses in an omnivorous fish, opaleye. We found clear evidence that diet influences thermal acclimation responses. However, there was no consistent pattern in how different biological rates responded to the temperature and diet treatments, with Q_{10} values ranging from 1 to 28. When confronted with a seasonal warm temperature (20°C), the opaleye in the omnivorous diet treatment had inferior performance (higher SMR, lower FAS and lower cardiac performance) relative to the opaleye fed a carnivorous diet treatment. Global climate change is already changing the average and extreme temperatures that marine ectotherms experience as well as their nutritional landscape (IPCC, 2019; Birnie-Gauvin et al., 2017). These environmental changes are likely to interact and alter many ectotherms' thermal performance in the wild. Incorporating multiple interacting factors into our understanding of species' responses to global climate change is the next step in ensuring that researchers capture the resilience of different species and populations (Jackson et al., 2021). Accordingly, diet is essential to consider when predicting ectotherm performance in variable environments and in response to global climate change.

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