Assessing the impact of heatwave exposure on the swimming performance, kinematics, and metabolism of a nearshore marine fish, *Cymatogaster aggregata*

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Abstract

The severity and frequency of marine heatwaves (MHWs) have increased drastically across the globe, with some of the most intense heatwaves happening within the last decade. The consequences of MHWs vary in severity and include range shifts and diet changes as well as mortality events in marine species. In fishes, elevated temperatures can lead to changes in whole-animal metabolism and performance metrics. However, the impact of temperature on a key performance metric, optimal swim speed (Uopt) is not fully understood. Here, we investigate how heatwave exposure (I.e., +2°C and +4°C) over a five-day period affects the metabolic rate and swimming performance of the shiner perch *(Cymatogaster aggregata)* using a swimming respirometer. Preliminary findings demonstrated that Uopt slightly increased following moderate MHW exposure (+2°C) and plateaued at higher MHW exposure (+4°C). However, metabolic costs and maximum swimming speed peaked following moderate MHW exposure and declined under more intense MHWs. Not only does this provide more information on the swimming energetics and performance of a common nearshore species, but it also provides insight into heat tolerance and how this species may respond to future projected marine heatwaves.

Introduction

Anthropogenic climate change has caused ocean temperatures to steadily increase across the globe. Within the long-term global warming trend, the frequency and intensity of discrete extreme warming events in our oceans have increased, with the most extreme marine heatwaves (MHWs) occurring in the past 40 years (Hobday et al., 2016; Holbrook et al., 2020; Smith et al., 2021). In particular, the Northeast Pacific Ocean has experienced severe and prolonged MHWs within the past 10 years (Barkhordarian et al., 2022; Cheung & Frölicher, 2020; Song et al., 2023). The biological impacts of MHWs are profound, including mass coral bleaching events, degraded seagrass beds and kelp forests, and redistribution of marine species (reviewed in Smith et al., 2022). Given the projected increases in MHWs as global warming continues, the mechanistic basis of the biological impacts of MHWs is increasingly necessary to understand consequences at the population and ecosystem level.

Temperature may be the single most important abiotic factor affecting fish physiology and behaviour (Angilletta et al., 2002; McKenzie & Claireaux, 2010). Ectotherms like fish are particularly sensitive to acute temperature changes (Fry, 1971; von Biela et al., 2019; Rogers et al., 2021), and the effect of temperature on aerobic metabolism as a measure of energetic demand has been studied extensively (Bullock, 1955; Norin & Gamperl, 2018). For ectotherms, the limits of aerobic metabolism are measured as maximum metabolic rate

(MMR), the oxygen consumption rate (MO2) at an organism's highest possible activity level, and standard metabolic rate (SMR), the baseline oxygen consumption needed for routine maintenance (Krogh, 1914; Brett, 1964; Killen, 2014; Chabot et al., 2016). Aerobic scope is the difference between these metabolic rate limits and indicates the total energy available to an organism to perform its activities (Pörtner & Farrell, 2008; Clark et al., 2013). Metabolic performance of ectotherms is greatest within an optimal temperature range, and at non-optimal temperatures, aerobic metabolic performance declines (Sandblom et al., 2014; Schulte, 2015). As temperatures increase beyond the optimum, the energy required for baseline activities increases exponentially while maximum metabolic rates initially increase, plateau, and decline at increasingly higher temperatures. These temperature effects ultimately constrain aerobic scope, and energetic demand is increasingly met via anaerobic metabolic processes. Importantly, organismal responses to warming depend on the intensity and duration of heat exposure and are generally more pronounced at higher temperatures and with acute exposure (McKenzie & Claireaux, 2010; Reid & Ricciardi, 2022; Stewart et al., 2023). Rapid changes in temperature, exemplified through MHWs, can outpace an individual's acclimation capacity, leaving them more vulnerable to detrimental thermal effects (Gunderson & Stillman, 2015; Alfonso et al., 2021).

Water temperature also affects fish locomotion, which is necessary for virtually all activities of fishes, including foraging and migration (Castro-Santos et al., 2022). Thermal effects on fish locomotion can be assessed using several different swimming performance metrics (Lee et al., 2003; Jones et al., 2008). One common approach is through using a swim tunnel respirometer which involves the measurement of specific swimming parameters during stepwise increases in water velocity (McKenzie & Claireaux, 2010). As flow speed increases fish will reach their gait transition speed (Upc), which is the speed at which fish switch from a steady swimming gait to an unsteady swimming gait (i.e., burst and coast), and indicates an increasing reliance on anaerobic metabolism to fuel locomotion (Videler, 1993). As water velocity continues increasing, fish eventually encounter a critical swimming speed (Ucrit), which is the speed at which fish can no longer continue swimming against a current (Brett, 1964; Plaut, 2001). Experimental determination of Ucrit in a swimming respirometer also allows for the measurement of MMR because Ucrit is considered the limit of a fish's swimming capacity, and thus when maximal oxygen consumption occurs (Farrell & Steffensen, 1987). When combined with measures of metabolic rate at multiple water velocities, the optimal swimming speed (Uopt) of a fish can be estimated from the cost of transport (COT) curve (i.e., oxygen consumption rates measured at precise speed increments) and is defined as the speed at which the COT is minimized (Tucker, 1970; Tudorache et al., 2011). These different swimming performance metrics are typically maximized at optimal temperatures as muscle function and aerobic metabolic performance improve at these temperatures (McKenzie & Claireaux, 2010). The relationships between swimming performance, metabolism, and temperature present unique challenges for the ecology and fitness of marine fishes in the context of climate change.

As oceans continue warming, the effects of temperature change on Uopt are of growing interest. The metabolic rates and locomotor performance of fishes both vary with temperature, and COT is expected to change with temperature for many fish species which predicts changes to Uopt (Dickson et al., 2002; Yin et al., 2021). The effects of temperature on locomotor performance can also be examined through kinematics analysis. For example, in a labriform swimmer, the bluegill sunfish (*Lepomis macrochirus*), the amplitude and beat frequency of the pectoral fins and tail varies with acute temperature changes (Jones et al., 2008). Similarly, studies on brown trout (*Salmo trutta*) and freshwater drum (*Aplodinotus grunniens*) found that acute warming increased fin beat frequencies and aerobic metabolism in both species (Lea et al., 2016; Laubach et al., 2023). While none of these studies addressed Uopt specifically, changes in Uopt should be expected given the increased metabolism and fin-beat frequencies observed during acute warming in these species. Such changes in the efficiency of swimming may impact the fitness of fishes since swimming is required for virtually all activities (e.g., foraging, seeking refuge, and migration). Given the projected increases in anthropogenic MHWs, establishing a clear relationship between Uopt and acute warming can provide valuable information on the fitness consequences of marine fishes as climate change progresses.

Here, we investigated the effects of present and future MHWs in the Pacific Northwest (PNW) on the swimming energetics, kinematics, and performance in the shiner perch (*Cymatogaster aggregata*). We hypothesized that the energetic efficiency of swimming would decrease (i.e., Uopt increases with temperature) with increasing temperature as a result of increased metabolic demand and increased pectoral fin- and tail-beat frequency. We also hypothesized that Ucrit would decrease with temperature due to the temperature-driven changes in energetic demand and swimming kinematics.

Methods

Fish Collection and Husbandry

Shiner perch (*Cymatogaster aggregata* Gibbons, 1854) were caught by beach seining at Jackson Beach on San Juan Island, Washington, USA (48°31'10.5" N 123°00'36.2" W). *Cymatogaster aggregata* is a non-migratory eastern Pacific fish species that lives in brackish and demersal water at various depths (1 - 146m), making them susceptible to acute water temperature variations such as MHWs and/or cold spells (Eschmeyer et al., 1983). They employ a labriform swimming mode, meaning they use their pectoral fins when steady swimming with a transition to body undulations at higher speeds (Mussi et al., 2002). All individuals used in experiments (n = 31) were caught between July 16 and August 12, 2023, with a standard length of 9.96 cm (9.04 - 11.21 ± 0.1 cm; range ± SE) and a mass of 22.82g (17.66 - 29.85 ± 0.6 g; range ± SE).

Fish were transported to the University of Washington Friday Harbor Laboratories facilities within 2 hours of capture and were held in 160 L unfiltered flow-through seawater tanks (90 l x 60 w x 30 h cm). Normal diurnal light cycles (14h light: 10h dark) were followed throughout their time in captivity, and temperatures were monitored daily (July 20 - August 16, 2023) and averaged 12.85°C (11.9-14.9 ± 0.67°C; range ± SE). Tanks were aerated with average dissolved oxygen levels of 7.55 ± 0.18 mg/L (range ± SE) and average salinity of 30.37 ± 0.07 PSU (range ± SE). Data for dissolved oxygen and salinity were obtained from the Friday Harbor Laboratories Ocean Observatory platform, which measures oceanographic variables every hour at 1.5 m depth within Friday Harbor (48°32'43" N 123°00'43" W), and data were averaged for the period July 18 - August 16 (NANOOS NVS Data Explorer, 2023). After collection, fish were initially fasted until they were acclimated to their holding tanks. Once acclimated, fish were fed with fresh shrimp pieces every three days. Animal care protocol was approved by the institutional animal care and use committee (IACUC) of the University of Washington (permit 4238-04).

Heatwave Determination

Here, we define MHWs following the definition of Hobday et al (2016), which is “a discrete, prolonged warm water anomaly above the climatological mean with a minimum duration of 5 days”. Temperature data for heatwave treatment determination were obtained online from the National Oceanic and Atmospheric Administration (NOAA) from Station No. 9449880 (NOAA, 2023) in Friday Harbor, WA (48°32'43" N 123°0'44" W). This station measures water temperatures at 1.7 m depth below mean lower low water (MLLW). Data from a 31-year span (1992 - 2023) were used to determine the climatology and analyzed to identify the heating rate, duration, and average maximum temperature (Fig. S3; Table S3).

Heatwave Treatments

Control fish were kept at a mean temperature of 13.5°C. For heatwave treatments, fish were held either in pairs or in groups of four in flow-through tanks to lower stress levels since *C. aggregata* is a social species (Eschmeyer et al., 1983). Group size was increased to four after some preliminary testing to ensure that fish were never left alone while a fish was being tested. Treatment temperatures were raised from 13.5°C to either 15.5°C (i.e., +2°C) or to 17.5°C (i.e., +4°C) at a rate of 0.5°C every 12 hours until treatment temperatures were reached. A MHW was designated from the first temperature increase and individuals were exposed to MHW conditions for five days (i.e., 120 h ± 12h) before swimming respirometry trials began. Temperatures in MHW tanks were regulated with a temperature controller (WILLHI WH1436A; ± 0.1°C) and monitored continuously (SONOFF THR316D). After each respirometry experiment, MHW-exposed fish were brought down to the control temperature at a rate of 0.5°C every 12 hours before being released).

Respirometry

A Steffensen-type acrylic swimming respirometer (5.31 L; see Fig. 1 and Table S1 for components and dimensions, respectively) was used to measure metabolic rate as mass-specific oxygen consumption rate (MO2) with a fiber optic oxygen meter (Fibox 3, Precision Sensing GmbH) with temperature compensation and AutoResp software version 1.6.0 ((Loligo® Systems, Viborg, Denmark)). The swim tunnel was connected to a sump (see Table S1 for dimensions) which supplied recirculated, filtered, and temperature-controlled saltwater (~30.4 PSU). Water temperature was held constant within 0.1°C using a cooling relay (Isotemp 4100) and an 800W aquarium heater (Finnex Inc.) controlled by a temperature regulator (Inkbird Thermostat ITC1000) in the sump, and water was aerated continuously. A UV sterilizing filter was used to reduce bacterial growth in the system. The swim tunnel was blocked off using tarps to prevent stress to test fish caused by human movement. Swim tunnel experiments were recorded from above and from the side using a a Canon VIXIA HV30 camera and a mirror held at a 45° angle with homogeneous lighting placed on either side of the swim tunnel to allow monitoring and data collection without interfering with the test fish.

Prior to their introduction to the swim tunnel, measurements were taken for standard length (cm), body depth (cm), and body width (cm), as well as weight (g). Fish were acclimated to the swim tunnel chamber at 0.5 body lengths per second (BL/s) until MO2 values plateaued (4 - 10h). Water velocity for each fish in the swim tunnel was determined based on their standard length and water velocity calculations made during preliminary testing. Values for MO2 were obtained through 10-minute duty cycles (measure time: 300 sec; wait: 90 sec; flush: 210 sec). Standard metabolic rate (SMR) was extrapolated from data collected during the acclimation period. Maximum metabolic rate (MMR) was taken as the metabolic rate measured at the maximum swimming speed obtained using a critical swimming speed (Ucrit) protocol (described below). Bacterial respiration was measured before and after each swim trial by running the O2 sensor for 36 minutes (measure time: 1800 sec; wait: 60 sec; flush: 300 sec) at 0.5 BL/s without a fish in the swim tunnel. Water was periodically changed in the sump and swim tunnel to reduce bacterial load.

Swimming Performance

Swimming performance was measured after flow calibration was performed using a vane-wheel flow probe (Höntzsch GmbH, Waiblingen, Germany) and digital flow meter (Höntzsch GMBH, Waiblingen, Germany, HFA Serial No. 172). Calibration was performed at three heights in the respirometry chamber (I.e., top, middle, bottom) at different motor speeds starting at 300 RPM and increasing up to 2700 RPM. The speed calibration obtained ranged from 1 to 77.5 cm/s. To measure Ucrit, water velocity was increased every 30 minutes by a precise speed increment for each treatment (control: 0.7 BL/s ; +2°C: 0.6 BL/s; +4°C: 0.5 BL/s) until the test fish rested its tail on the back grid for at least five seconds, demonstrating that it could not sustain swimming at that speed without resting (Brett, 1964; Plaut, 2001; Johansen & Jones, 2011). After the first few treatments (four and two trials for the +2°C and +4°C treatments, respectively), increments were changed to 0.7 BL/s for all treatments to ensure that approximately the same number of data points were collected among treatments until the expected Ucrit was observed. This change in speed increments was accounted for during subsequent data analysis. Increments were chosen to ensure at least 6 data points (i.e., 6 water velocities) for proper curve fitting and prevent exhaustion by not letting individuals acclimate to the current water velocity based on previous studies where Ucrit was estimated at 4.5 BL/s at normal seasonal temperatures for *C. aggregata* (van der Hoop et al., 2018). Gait transition speed (Upc) was estimated as the water velocity where the fish gait-transitioned more than once in a 5 second interval (Roche et al., 2014). After Ucrit was observed, water velocity was returned to 0.5 BL/s for a 15-minute recovery period, after which the fish was removed from the respirometer and placed in a recovery tank.

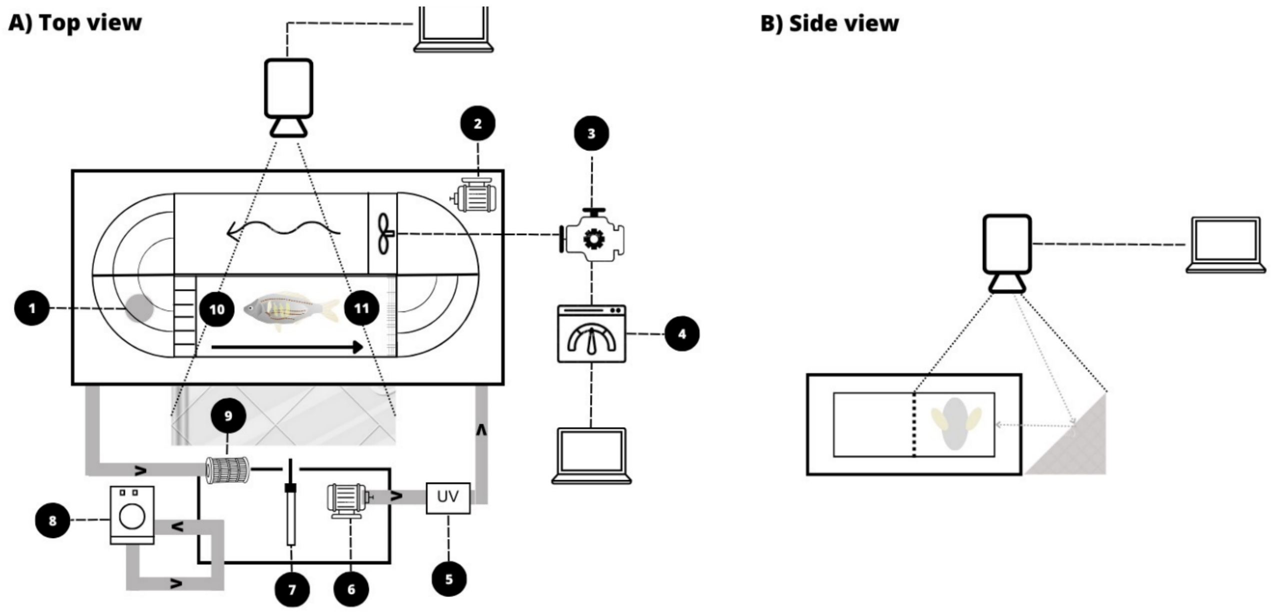

Figure 1. Schematic of swim tunnel setup. 1: Flush chimney and probe entrance; 2: Flush pump (EHEIM GmbH 1001.310); 3: Electric motor (AC-motor, DRS71, SEW Eurodrive); 4: Frequency inverter / motor controller (MOVITRAC LTE, SEW Eurodrive); 5: UV filter; 6: Circulation pump (EHEIM GmbH 1048.3F1); 7: Water heater (Finnex inc.); 8: Chiller (Isotemp 4100); 9: Filter bag; 10: Honeycomb panel; 11: Metal grid.

Kinematics

Videos were taken during the swimming trials by recording each fish simultaneously from above and the side using a mirror set at a 45° angle (Fig. 1). For each video, three 10-second sections per swimming speed will be selected where the fish is fully visible and using steady swimming at speeds before gait transition began. At higher swimming speeds when burst and coast swimming began, video sections with the fish fully visible will be selected. Pectoral fin beat frequency will be measured by counting the number of beats per 10 s and extrapolating over 1 minute, and a mean of the three values per speed will be used for analysis. Pectoral fin beat amplitude will be measured for each fin beat in the 10 s interval by measuring the total vertical movement of the pectoral fin using the side view. Means for each 10 s interval will be used for analysis. Tail beat frequency will be obtained by counting the number of completed tail beats in the 10 s interval to then calculate the mean at all speeds. Tail beat amplitude calculations will then be conducted the same way as the pectoral fin beat amplitude with horizontal movement instead of vertical. Kinematics will be analyzed manually using video analysis software (Kinovea).

Data Analysis

MO2 calculations with R^2 values ≥ 0.80 were kept for further analysis. Background respiration was accounted for using a linear regression of bacterial MO2 measured before and after each respirometry experiment. As mentioned above, the water in the respirometer and sump was changed periodically, depending on whether bacterial respiration levels went above a constant level. Predicted regression values were subtracted from MO2 during the respirometry experiments.

MO2 during the acclimation period was determined using the 20% quantile method. The following curves were fit to MO2 data measured at each swimming speed during respirometry experiments and extrapolated to 0 BL/s to estimate SMR: 1) a two-parameter exponential curve, denoted in Equation 1 (Brett, 1964; Webb, 1975; Korsmeyer et al., 2002; Roche et al., 2013); 2) a three-parameter exponential curve denoted by Equation 2 (Roche et al., 2013); and 3) a three-parameter hydrodynamics-based power curve denoted in Equation 3 (Wu, 1977; Videler, 1993; Korsmeyer et al., 2002; Roche et al., 2013). Previous studies have demonstrated that a two-parameter exponential and a hydrodynamics-based power curve fitting procedures can underestimate and overestimate SMR, respectively (Korsmeyer et al., 2002; Roche et al., 2013). Therefore, the three-parameter exponential curve was chosen to perform future analysis (see supplemental material for the other methods).

Equation 1 (2 Parameter exponential curve): $MO2 = a10^{bU}$

Equation 2 (3 Parameter exponential curve): $MO2 = a + b10^{cU}$

Equation 3 (3 Parameter power curve): $MO2 = a + bU^c$

Q10 coefficients were calculated to compare the effects of temperature on SMR between heating treatments. Q10 coefficients were calculated (Equation 4) by taking the quotient of the observed SMR values at two different temperatures (θ_1, θ_2) and raised to a power exponent of 10 divided by the difference in temperature (Vant't Hoff, 1884; Schmidt-Nielsen, 1997).

Equation 4: $Q10 = (R_2/R_1)^{10/(\theta 2-\theta 1)}$

The cost of transport (COT) was calculated by taking the quotient of MO2 over swimming speed in BL/s (Equation 5), and the optimum swimming speed (i.e., Uopt) was determined as the swimming speed with the least COT (Videler, 1993). Critical swimming speed (Ucrit) was calculated following Brett's method (Brett, 1964). Ucrit is measured by correcting the highest maintained swimming speed (Ui; cm/s) with the time elapsed at the highest speed (Ti; minutes), the speed increment (Uii; cm/s) and the interval time between increments (Tii; minutes) (Equation 6; Plaut, 2001). Maximum swimming speed (Umax) was defined as the highest speed where an individual could maintain swimming.

Equation 5: $COT = MO2/U$,

Equation 6: $Ucrit = U_i + U_{ii}(T_i/T_{ii})$

Data will be analyzed using linear models with temperature and water velocity as explanatory variables and body length as a covariate. Metabolic rates (SMR, MMR), aerobic metabolic scope, Uopt, Ucrit, Umax, gait transition speed, and pectoral fin- and tail-beat frequencies will be included as dependent variables. Visual inspection of quantile-quantile plots and Shapiro-Wilk's test of normality will be conducted to assess the assumption of normality, while visual inspection of residuals plots and Bartlett's test for homogeneity will be conducted to assess the assumption of homogeneity of variance. Post hoc multiple comparisons will be done using Student's t-tests on the estimated marginal means of the model.

Results

Metabolic rates

Based on our preliminary analysis (N = 16), SMR generally increased with temperature and swimming speed (Fig. S1 & 2). SMR peaked in the 15.5°C group (163.47 ± 19.35 mg O2/kg • hr) while the lowest mean SMR was estimated in the 13.5°C group (97.53 ± 40.22 mg O2/kg • hr). Mean SMR at 13.5 and 17.5°C were very similar and had overlapping standard errors. Between 15.5 and 17.5°C, Q10 of SMR decreased to 0.15 from 13.23 and averaged 6.69 across temperatures (Table S2). Maximum metabolic rate increased with temperature and was obtained at the highest swimming speeds for each individual (Fig. 2). Mean aerobic metabolic scope increased was highest in the 17.5°C group (605.60 ± 76.36 mg O2/kg • hr.) and was similar between the 13.5°C (448.33 ± 35.61 mg O2/kg • hr.) and 15.5°C (439.50 ± 75.20 mg O2/kg • hr.) groups.

Swimming performance

Relative to the control temperature (13.5°C), the mean optimum swimming speed (Uopt) increased slightly following MHW exposure (Fig. 2). Mean Uopt values were similar between the 15.5°C (2.56 ± 0.58 BL/s) and 17.5°C (2.74 ± 0.43 BL/s) treatment groups, and standard errors of mean Uopt overlapped across all temperature groups (Fig. 3). Maximum swimming speed (Umax) at each temperature showed some variance among groups (Fig. 4). Indeed, when comparing swimming speed in BL/s, fish at 13.5°C were able to reach 5.4 BL/s (3.3 – 6.1 ± 0.3) while the +2°C and +4°C treatments respectively reached 5.2 BL/s (4.0 – 6.5 ± 03) and 6.1 BL/s (4.7 – 7.3 ± 0.3). Fish at 15.5°C were therefore able to maintain swimming at lower maximal speed than the two other treatments, with the group at 17.5°C reaching the highest mean Umax.

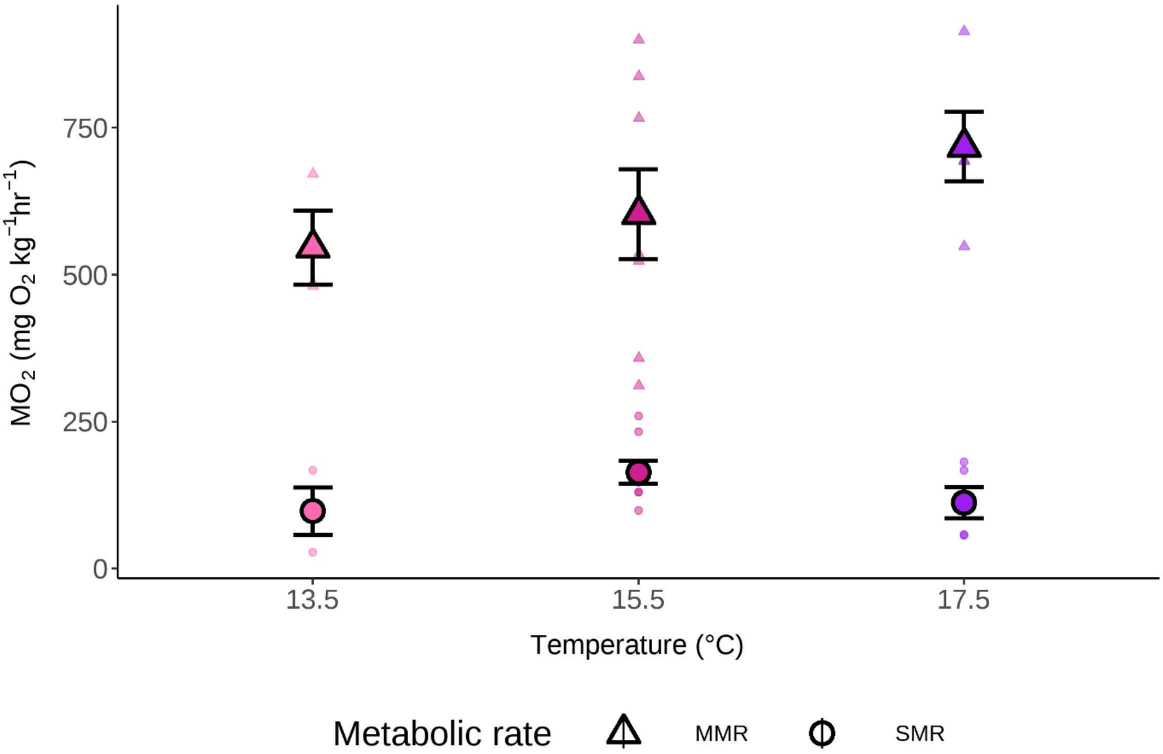

Figure 2. Preliminary metabolic rates for each temperature group ($n_{13.5}$ = 3, $n_{15.5}$ = 8, $n_{17.5}$ = 4). Maximum metabolic rates (MMR; triangles) was defined as the metabolic rate measured at the highest swimming speed where the fish could no longer swim. Standard metabolic rates (SMR; circles) were estimated by extrapolating a three-parameter exponential function to a swimming speed of 0 body lengths/s. Large triangles and circles represent mean values for SMR and MMR, respectively, with standard errors of the mean at each temperature. Small triangles and circles represent individual values for SMR and MMR, respectively.

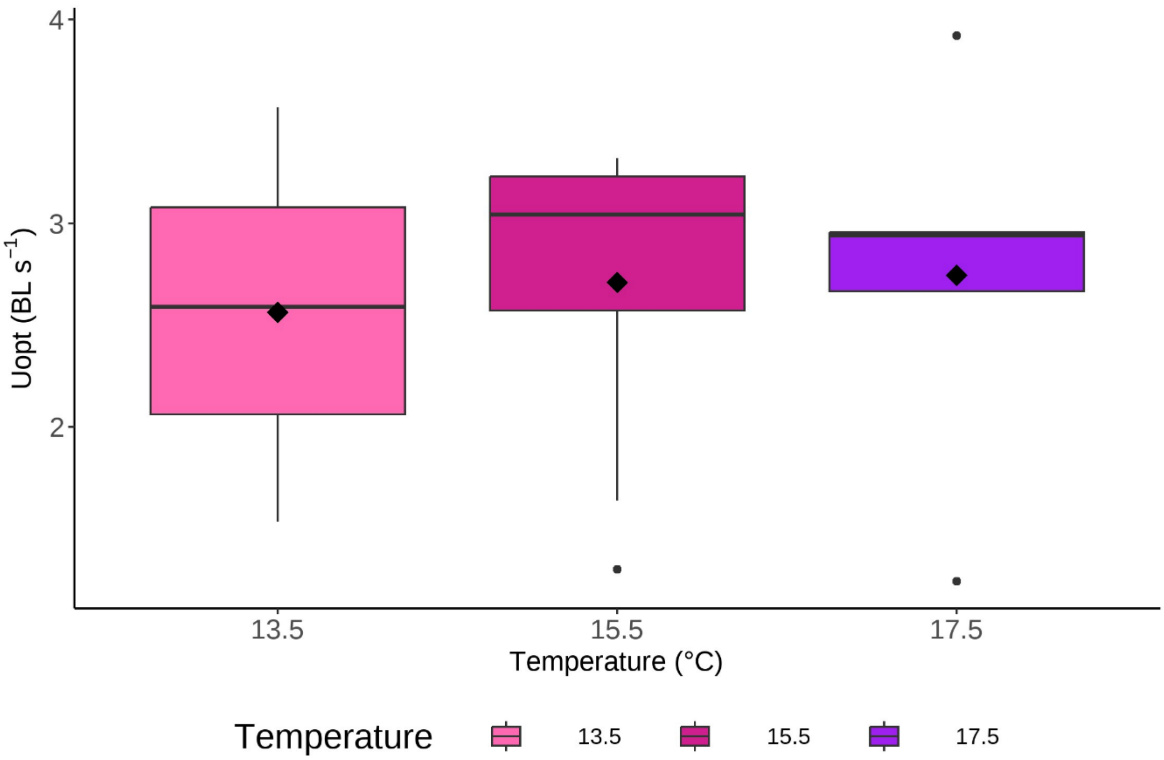

Figure 3. Preliminary optimal swimming speed (Uopt) for each temperature group ($n_{13.5}$ = 3, $n_{15.5}$ = 8, $n_{17.5}$ = 4), calculated as the swimming speed with the least cost of transport. Cost of transport was calculated by taking the first derivative of a three-parameter exponential function. Diamonds represent mean values at each temperature.

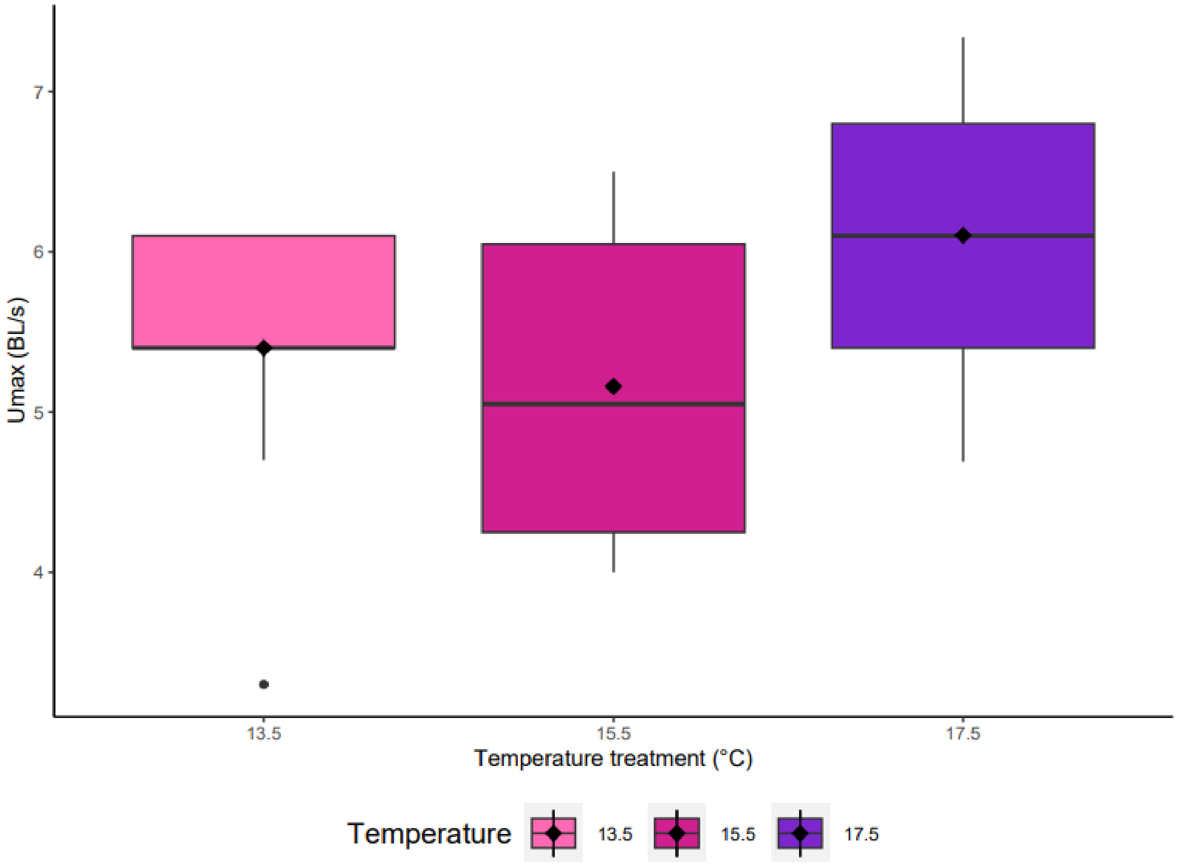

Figure 4. Preliminary maximum swimming speed (BL/s) at each temperature treatment of tested *C. aggregata* (n = 29). Diamonds represent mean values at each temperature.

Discussion

In our study we demonstrate that acute warming during MHWs alters the swimming energetics and performance of a temperate marine fish, *C. aggregata*. Within an animal's thermal window, SMR is expected to increase exponentially with temperature as the rate of biochemical reactions increases, necessitating increased oxygen consumption (Pörtner & Peck, 2010; Schulte et al., 2015). Predictably, SMR increased by 40.3% with moderate warming (+2°C) and increased another 14.7% after intense warming (+4°C; Fig. 2). Consequently, Q10 values were very high for moderate warming of +2°C and surprisingly low for extreme warming of +4°C (Table S2). In other temperate fishes, the effect of warming on SMR typically yields Q10 values of between 2-3, indicating an exponential increase in SMR (Schurmann & Steffensen, 1997; Dalla Via et al., 1998; Lefrançois & Claireaux, 2003). These surprising results are likely due in part to the small sample size of our preliminary analysis ($n_{13.5}$ = 3, $n_{15.5}$ = 8, $n_{17.5}$ = 4). As we analyze more data, we anticipate that an exponential increase in SMR with temperature will become more apparent and Q10 values will be more similar to those previously reported. However, alternative thermal effects on SMR in fish have been observed; for example, McDonnell & Chapman (2016) found that the SMR of a tropical cichlid (*Pseudocrenilabrus multicolor victoriae*) did not increase continuously between 24 and 34°C and decreased at the highest MHW temperature. Other studies have also found dramatic differences in Q10. For a Danish population of round goby (*Neogobius melanostomus*) acclimated to temperatures between 5 and 28°C, the calculated Q10 values of SMR were below 2 at intermediate acclimation temperatures and higher at the lowest (4.13) and highest (11.31) temperatures, respectively (Christensen et al., 2021).

We found that MMR progressively increased between 13.5 and 17.5°C, and aerobic metabolic scope was maximized at 17.5°C. Maximum metabolic rate generally increases with temperature before plateauing and subsequently decreasing as temperature continues increasing (Schulte, 2015). For *C. aggregata*, MMR may continue increasing beyond 17.5°C and reach its maximum at higher temperatures. This species is found in subtropical waters at the lower limit of its geographic distribution where summer temperatures can be greater than 20°C, and a southern Californian population has a reported thermal tolerance limit of 31°C when acclimated to 17°C (Shrode et al., 1982). These characteristics indicate that MMR may indeed continue increasing beyond 17.5°C, at least in warm-acclimated or subtropical populations. Additionally, changes in MMR predominantly account for temperature-induced changes in aerobic metabolic scope (Pörtner, 2010; Norin et al., 2016), meaning aerobic metabolic scope may also continue increasing at temperatures beyond 17.5°C. As we analyze more data, we expect that differences in MMR and aerobic scope across temperature groups will become more apparent. If the observed trend continues, *C. aggregata* may be able to maintain aerobic metabolic performance at higher temperatures than those tested here, therefore suggesting that temperate populations may be experiencing suboptimal temperatures for aerobic metabolic performance. Southern populations of temperate species can typically tolerate warmer temperatures than their northern counterparts as a result of differences in thermal history (Fangue et al., 2006; Stewart & Allen, 2014). The available data on thermal tolerance for *C. aggregata* primarily comes from subtropical populations and may differ from temperate populations like that tested here. Intraspecific variation at the individual level could also contribute to differences in aerobic metabolism. Each fish in this study was only tested at a single temperature, so future studies should employ a repeated measures design to capture this individual variation.

Despite the temperature-driven changes in metabolism detected in this study, our preliminary results do not show an appreciable change in Uopt across our treatment temperatures. Uopt increased by only 7% following a +4°C MHW; however, we found that Uopt across all temperatures far exceeded theoretical predictions (i.e., ~1 BL/s) up to 174% in the highest temperature group (Weihs, 1974; Trump & Leggett, 1980). While acute warming is expected to increase Uopt, the elevated Uopt of our lowest temperature group was unexpected. One possible explanation for this is that 13.5°C represents the warmest average temperatures for the San Juan Islands (Fig. S3A). The Uopt of *C. aggregata* at lower, more representative temperatures may more closely align with theoretical expectations. Yet, Uopt was still approximately one-half of Umax in each temperature group which aligns with theoretical expectations and suggests that the warmth experienced during the 2023 summer may have influenced Uopt. Maximum swimming speed differed across temperature treatments and was considerably higher at 17.5°C (Fig. 4). While our preliminary analysis revealed high realized optimal and maximum swimming speeds across temperature groups, we expect that average Uopt and Umax values will decrease, and temperature effects will become more apparent as we continue our analysis.

Conclusion

In conclusion, we were unable to accept or reject our hypothesis that increased temperature would be linked to lower swimming performance because of altered energetics. Although we did find differences in SMR and MMR values between each temperature treatment, these differences were not as expected. Metabolic rates did not follow the expected patterns, with neither an exponential increase in SMR nor a plateau of MMR values at increasing temperatures observed. Our preliminary sample size is low for some treatments, and we anticipate stronger patterns will emerge once the full dataset is analyzed. Following swimming performance experiments, we found that the optimal swimming speed only marginally increased with temperature. Surprisingly, our preliminary results show abnormally high values for optimal and maximum swimming speeds, which could potentially be linked to the high seasonal temperatures experienced during our experiments. As with the metabolic rates, our preliminary analysis is affected by the low sample size currently analyzed and we expect more defined relationships between temperature and swimming performance following complete analysis. Based on our preliminary trends and existing data on this species, we likely did not capture the upper thermal limits of this species' metabolic and swimming performance. With more temperature treatments, it may have been possible to better understand the limits of performance in *C. aggregata* and account for the high seasonal variances in temperature. We intend to collect kinematic measurements and Ucrit data and conduct our analyses with the full dataset to better understand the possible effects of marine heatwaves on the swimming performance and energetics of *C. aggregata*.

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Supplementary Information

Table S1. Dimensions of the swimming respirometer setup.

Setup	**Measurement**	**Dimension (cm)**
Swim tunnel	Length	77.6
	Width	32.5
	Height	21.3
	Water height	15
Respirometry chamber	Length	28
	Width	7.5
	Height	6.5
Sump	Length	54
	Width	48.5
	Water height	24.5

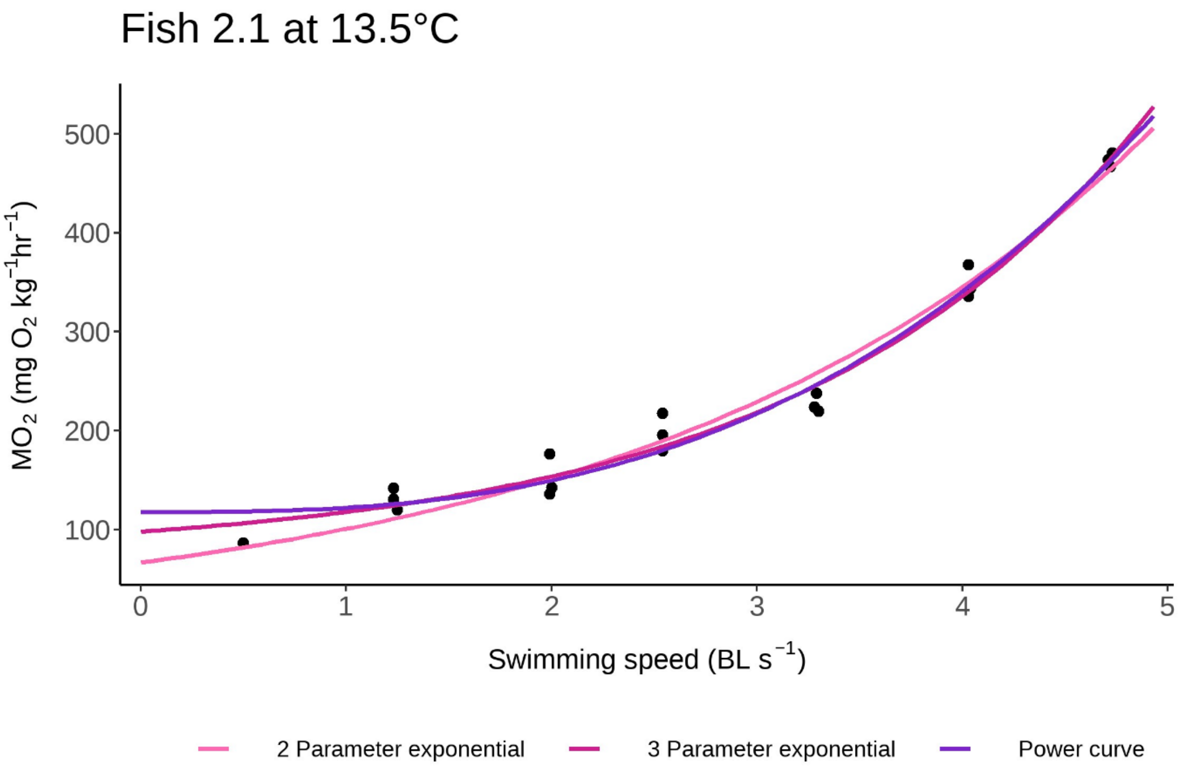

Figure S1. Example data from a swimming respirometry experiment for an individual fish where metabolic rate was measured three times at each swimming speed (body lengths/s; BL/s). Standard metabolic rate was estimated by extrapolating a two-parameter exponential function (light pink line), the three-parameter exponential function (dark pink line), and a hydrodynamics-based power function (purple line) to 0 BL/s.

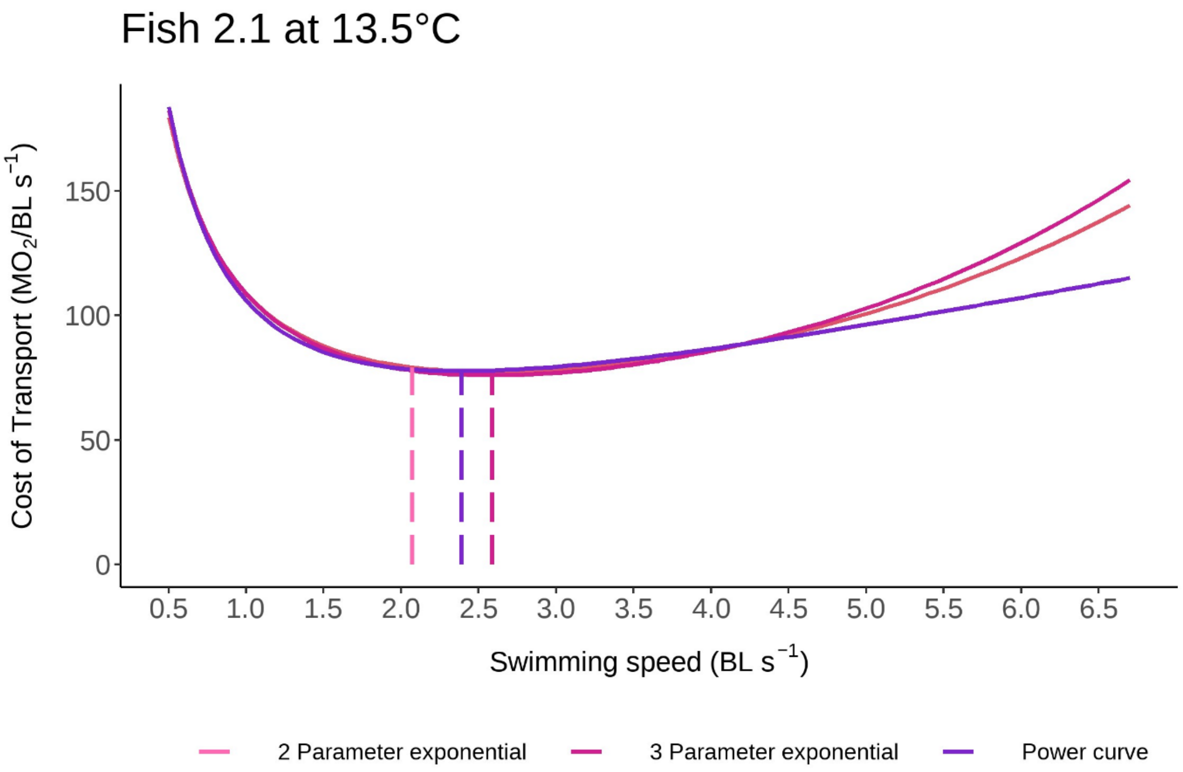

Figure S2. An example of the cost of transport (COT) across swimming speeds for an individual fish calculated by fitting the first derivative of a two-parameter exponential function (solid light pink line), a three-parameter exponential (solid dark pink line) function, and a hydrodynamics-based exponential function (solid purple line). The optimum swimming speed for each fitted function (dashed lines) was calculated as the swimming speed with the least COT.

Table S2. Q10 values calculated for each two degree Celcius temperature change depending on the method to estimate standard metabolic rate (SMR).

Temperatue 1 (°C)	**Temperature 2 (°C)**	**SMR Estimation Method**	**Q10**
13.5	15.5	2 parameter exponential	10.05
13.5	15.5	3 parameter exponential	13.23
13.5	15.5	Hydrodynamics-based power	8.55
15.5	17.5	2 parameter exponential	2.65
15.5	17.5	3 parameter exponential	0.15
15.5	17.5	Hydrodynamics-based power	0.11

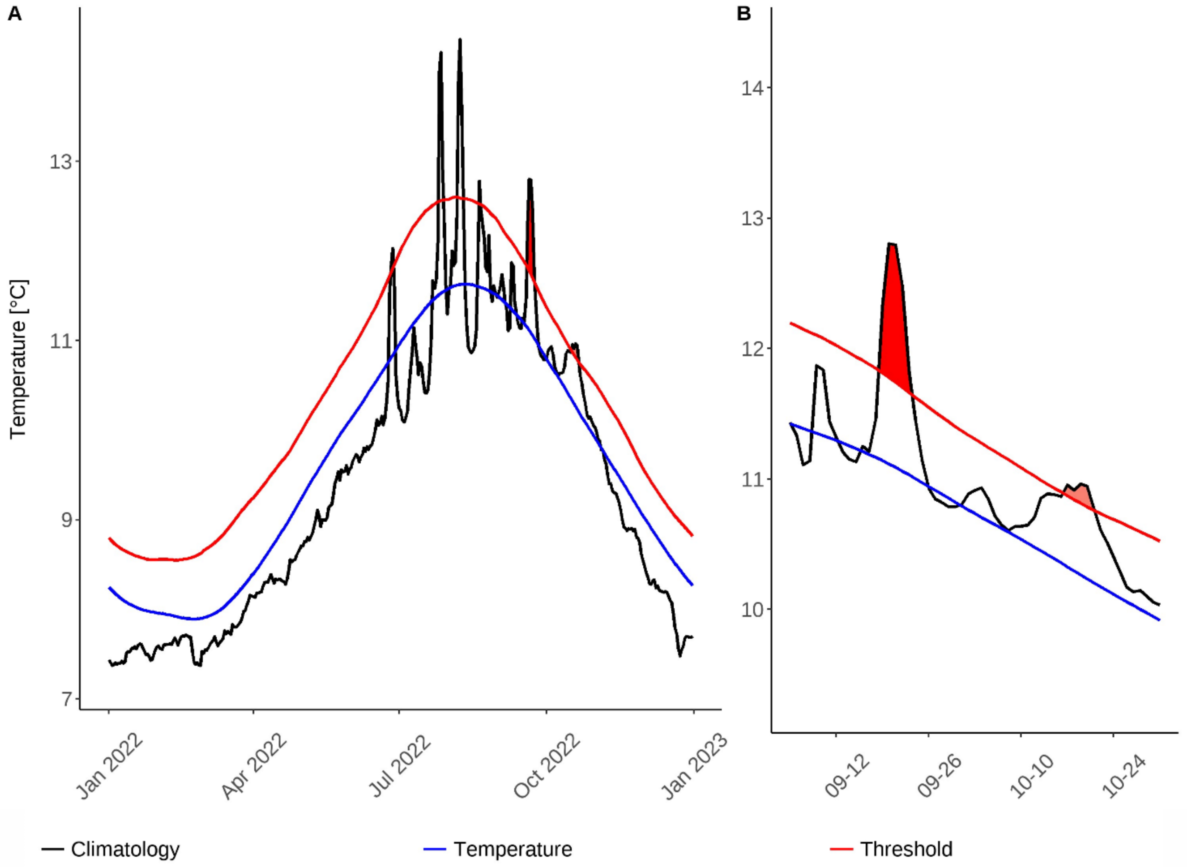

Figure S3. Example data from the marine heatwave (MHW) characterization, showing the climatology (black line), the weather station data (blue line), and the MHW threshold (red line), defined as the 90th percentile temperature for each day of the year across a 31 year time span. A) Data for the entire year of 2022. B) Zoomed in view of the two MHWs from the year 2022.

Table S3. Minimum, maximum, and mean ± standard error of the mean (SEM) for marine heatwave parameters identified from the National Oceanic & Atmospheric Administration (NOAA) weather station No. 9449880 at Friday Harbor Laboratories (N = 59). Mean and maximum intensities represent the average and maximum warming anomalies above the climatological mean. Cumulative intensity represents a daily heat stress index that incorporates the intensity and duration of warm anomalies in an area and is equivalent to the NOAA Coral Reef Watch program's degree heating weeks product (Liu et al., 2017).

Marine heatwave parameter	**Minimum**	**Maximum**	**Mean ± SEM**
Duration (days)	5	117	15.50 ± 2.75
Mean intensity (° C)	0.58	2.28	1.20 ± 0.065
Maximum intensity (° C)	0.63	4.06	1.58 ± 0.10
Cumulative intensity (° C • days)	3.22	106.18	15.84 ± 2.54
Heating rate (°C/day)	0.0085	1.40	0.30 ± 0.044