Variations in Thermal Tolerance of *Acartia clausi* Nauplii: Implications for Marine Heatwave Resilience

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Abstract

Ocean warming is expected to disproportionately impact early life stages of marine organisms, yet the thermal tolerance of larval populations remains poorly understood. This study tested whether *Acartia clausi* nauplii acclimated to warm (20 °C) versus cool (16 °C) temperatures differed in survival across a range of heat exposures. While LT_{50} did not differ significantly between treatments, warm-acclimated nauplii exhibited a significantly more gradual mortality slope, indicating phenotypic plasticity in thermal response. The variability in plasticity among individuals suggests differing capacities to cope with warming, which could influence population resilience during marine heatwaves. These findings highlight the potential role of plasticity in buffering larval populations against short-term temperature extremes. Although *Acartia* was the focus of this study, other sensitive larval populations face similar risks, making it essential to understand naupliar thermal responses for predicting Gulf of Maine food web stability under global climate change.

Introduction

Global climate change is impacting the distribution and spread of species (Pinsky et al., 2013). While taxa are not uniformly affected across the globe, research suggests that there is little to no lag in response to climate for marine species, indicating a capacity to either adapt to changing ocean temperatures or shift their historical geographic range (Pinsky et al., 2013). As ocean temperatures warm, sensitive larval life stages may especially be at risk as they rely on ocean currents for dispersal and tend to occupy surface waters where temperatures are elevated (Bashevkin et al., 2020). For many marine species, the larval phase represents the only opportunity for individuals to disperse away from parental populations, providing connectivity for spatially isolated populations (O'Connor et al., 2007). Despite this, larval stages are often overlooked in physiological research due to the difficulty of working with such small organisms and the brief duration of these early developmental stages. Instead, studies focus on identifying responses of adult populations to warming ocean temperatures. There is a weak understanding of how larvae are impacted by rising temperatures, even though these stages are considered less robust than their juvenile and adult counterparts (Franke et al., 2024). It is therefore critical to research the adaptability of larval organisms to elevated sea surface temperatures.

The Gulf of Maine is home to many larval-bearing species, forming the basis of productive fisheries that contribute over $3.2 billion annually to Maine's economy and provide more than 33,000 jobs statewide (Wallace et al., 2023). However, the region is warming three times faster than the rest of the ocean (Pershing et al., 2015), making

native species especially vulnerable to climate impacts. Many important fishery species like lobster, herring, and cod have larval stages. Larvae of these species rely on plentiful zooplankton prey to survive, such as copepods, which have their own larval stages known as nauplii. Recent projections suggest that warming waters could cause substantial decreases in copepod populations due to climate change effects (Grieve et al., 2017). If larvae of prey species cannot survive climate impacts, it could disrupt the whole food web. Warming oceans are often hypothesized to contribute to this decline, but it is unclear whether warming has a consistently negative effect across all life stages.

Copepods are small crustaceans found in nearly all aquatic environments, forming the base of many marine food webs. Their nauplii may be especially vulnerable to temperature stress. The implications of a sharp decrease in the abundance of these foundational prey species are vast, as important fish stocks, such as haddock and cod, rely on copepods as a lipid-rich nutritional source (Grieve et al., 2017). The Northern Right Whale, a critically endangered species native to the Gulf of Maine, also relies on large quantities of copepods as a base of their diet (Grieve et al., 2017). Among the Gulf's diverse copepod community, *Acartia clausi* is both abundant and ecologically important. Like other copepods, it produces nauplii, and if these early stages cannot survive warming waters, adult resilience alone will not be enough to sustain populations, as recruitment failure would halt generational replacement. Additionally, the effects of climate change, such as temperature and ocean acidification, have been shown to

impact larval survivorship and the dispersal of planktonic eggs and larvae, ultimately influencing adult spawning population size (Llopiz et al., 2014).

But as water temperatures gradually increase, will dispersive larval species and the foundations of the marine ecosystem have the ability to change with their new surroundings? Studies observing the critical temperature maxima and upper lethal temperature of *Acartia spp*. have reported that tolerance to higher temperatures increases with increasing acclimation temperature (Gonzalez, 1974). Additionally, researchers have found that the temperature at which 50% mortality is observed (LT_{50}) of warmer-acclimated copepods was higher than those acclimated to lower temperatures, specifically using seasonal water temperature variation (Jiang et al., 2008). These results suggest that *Acartia spp.* exhibit physiological plasticity, which may allow them to adapt to changing conditions.

To understand how nauplii respond to warming, we compared the lethal thermal limits of nauplii acclimated to warm (20 °C) and cool (16 °C) treatments. The interest of this study is to determine whether nauplii have the innate ability to withstand warming through genetics or hold the capabilities to adapt quickly through acclimation (plasticity). Plasticity is the ability of an organism to exhibit different traits or behaviors from the same underlying genetic code when exposed to different environmental conditions. If *Acartia clausi* nauplii have sufficient genetic or phenotypic plasticity to adapt to warmer temperatures, the nauplii acclimated to warmer waters should survive at higher rates than cold-acclimated nauplii when subjected to increasingly warm test conditions. Any

observed differences in survivorship may have implications for the potential of copepod larval stages to adapt to a changing environment, increasing population resilience.

Methods

Collecting test subjects

The *Acartia clausi* for the experiment were collected using a horizontal plankton tow with a mesh size of 200 microns by boat in Quahog Bay (Harpswell, ME). In the lab, the plankton were examined and used to create a culture of mature adult *Acartia clausi*. They were cultured in 1 L of artificial seawater with low aeration using airstones to provide constant bubbling. The salinity of the cultures was kept at 30 ppt, and this was monitored daily to ensure minimal fluctuation. The culture was fed to satiation using equal parts of prepared *Rhodomonas* and *Tetraselmis* phytoplankton cultures, which have been demonstrated to satisfy *Acartia* nutritional needs, to a food saturating level of 800 µg C/L (Feinberg & Dam, 1998). Copepods were acclimated to two different temperature treatments: 16 °C, representing ambient/cool conditions, and 20 °C, representing marine heatwave/warming conditions.

Preparing test subjects

The test subjects were acclimated to their temperature treatment for at least 24 hours before a trial was run. Approximately 1 hour before the trial, test subjects were transferred into a dish for ease of transport when setting up the experiment.

Experimental Setup

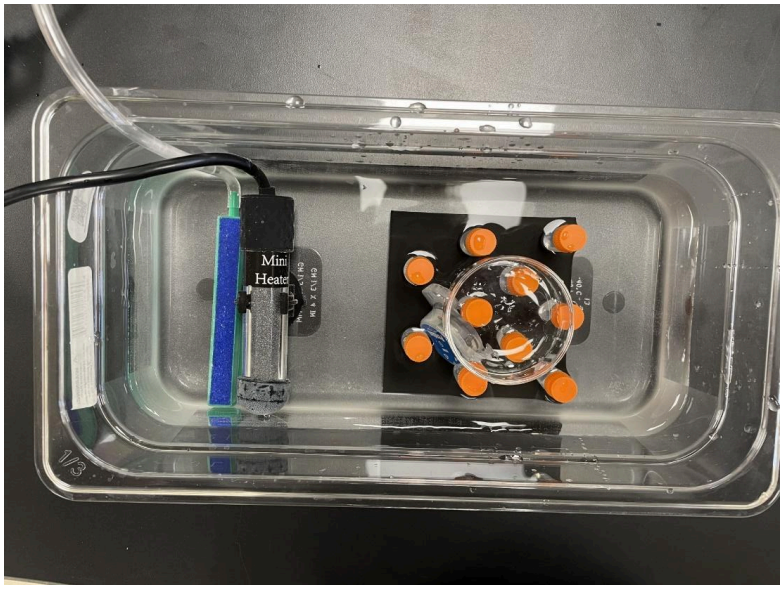

Figure 1. The arrangement of each bin, excluding the tinfoil covering

The basic setup for the experiment consisted of five plastic bins containing a heater, an air stone, five tubes with 1 test subject each, and a tray that holds ten tubes (Figure 1). A HOBO temperature logger was present in each bin to record temperature fluctuation and ramp speed of the heaters. The heaters were secured to the plastic bins and placed in a standardized location, with the air stones slightly underneath the pump to ensure good water flow and even heating. Each bin had 1.5 L of water, so the heater was fully submerged and the orange caps of the tubs were just barely out of the water. The bins were set up at least an hour before the trial began so the water could reach equilibrium with the incubator temperature. While the trays were acclimating, the air stones were plugged in to allow for balanced air flow and adjustment before the trial began. The lowest temperature treatments shared air inflow, while the other three treatments each had an individual air pump.

Loading test subjects

HOBO (ONSET MX2201) loggers were prepared to record temperature data for each temperature treatment. The heaters were then set to 18 °C as an intermediate temperature between the two treatments. 2 mL tubes were placed into the custom tray inside the bin. They were filled to the 1.8 mL line with fresh seawater, and a micropipette was used to put one nauplius into each tube. The micropipette was set to 50 µL to ensure standardization, and the tubes were capped tightly and placed into the tray. For each trial, there were at least 5 nauplii per temperature. The HOBO logger was placed between the tubes, about 2 inches from the heater. A small glass dish was placed on top of the tubes to weigh them down and ensure they stayed submerged during the trial. Each bin was then covered in tinfoil to decrease evaporation loss. The nauplii acclimated for 30 minutes while the heaters were set to ~18 °C. After the 30 minutes had elapsed, the heaters were set at 17.78 (64), 23.89 (75), 28.33 (83), 30 (86), and 33.89 (93 °F) °C. The lowest temperature was selected to fall between the ambient condition (16 °C) and the marine heatwave condition (20 °C) to provide a standardized intermediate temperature. The highest temperature was chosen to produce 100% mortality based on previous studies and LT_{50} data for the closely related congener *Acartia tonsa* (Holmes-Hackerd et al., 2023). The three middle temperatures were chosen to represent both sublethal and near-lethal conditions based on the known limits of this species. Each trial was left to run overnight, starting at approximately 3:30 PM and running until 7 AM.

Data Collection and Analysis

At approximately 7 am, the tubes were checked for surviving nauplii, starting with the warmest temperature and proceeding to the coolest. Each heater was unplugged in correspondence with the test subjects being removed. Thus, nauplii in the warmest temperature trial were subjected to experimental temperatures for the lowest amount of time, and those in the lowest temperature trial for the longest amount of time. Each tube was rinsed into a dish and checked under a microscope (ZEISS STEMI 305). Once the subject was located, the survival status was recorded, or it was marked as missing and removed from further analysis. After collection of various parameters, including survival, elapsed time, and data recorded from the HOBO logger, like time to temp and actual temp, the data was analyzed in R to produce a logistic regression and estimate the slope and LT_{50}. The package used to analyze the results was the package 'drc' (Ritz et al., 2015). Two models were created to fit the data. The first was a simple model that did not include acclimation temperature as an effect. In contrast, the second model included an additional variable, representing the temperature conditions under which the nauplii were cultured. An ANOVA was used to compare these two models to determine whether acclimation temperature had a significant impact on the survivorship curve. The compparm() function was used to determine whether the LT50 and slope of each treatment were significantly different from one another.

Results

The simpler model details the proportion of surviving nauplii over each temperature they were exposed to (Figure 2). The data indicate a gradual rise in mortality with increasing temperature, with a slope of -7.88 and an LT_{50} of 23.49. The line approaches 1 as

temperature increases; thus, there is a higher likelihood of mortality as temperature increases. The more complex model (Figure 3) showcases the data when acclimation temperature is included as an additional parameter. Though the LT50 of each treatment were similar (cold: 23.96, warm: 23.18), the slope of the warm acclimated nauplii was much more gradual compared to the steep curve of the cold acclimated nauplii (cold: -13.78, warm: -5.93).

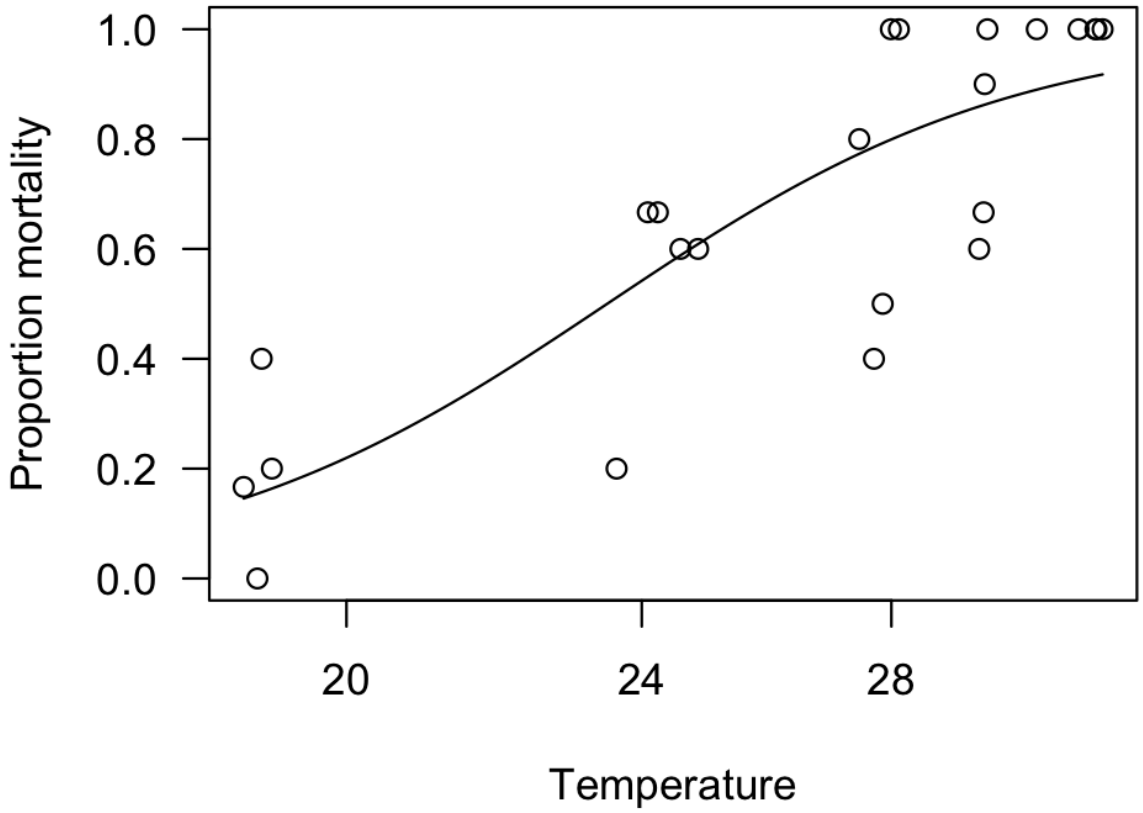

Fig 2. Model 1 that includes temperature and the mortality of nauplii

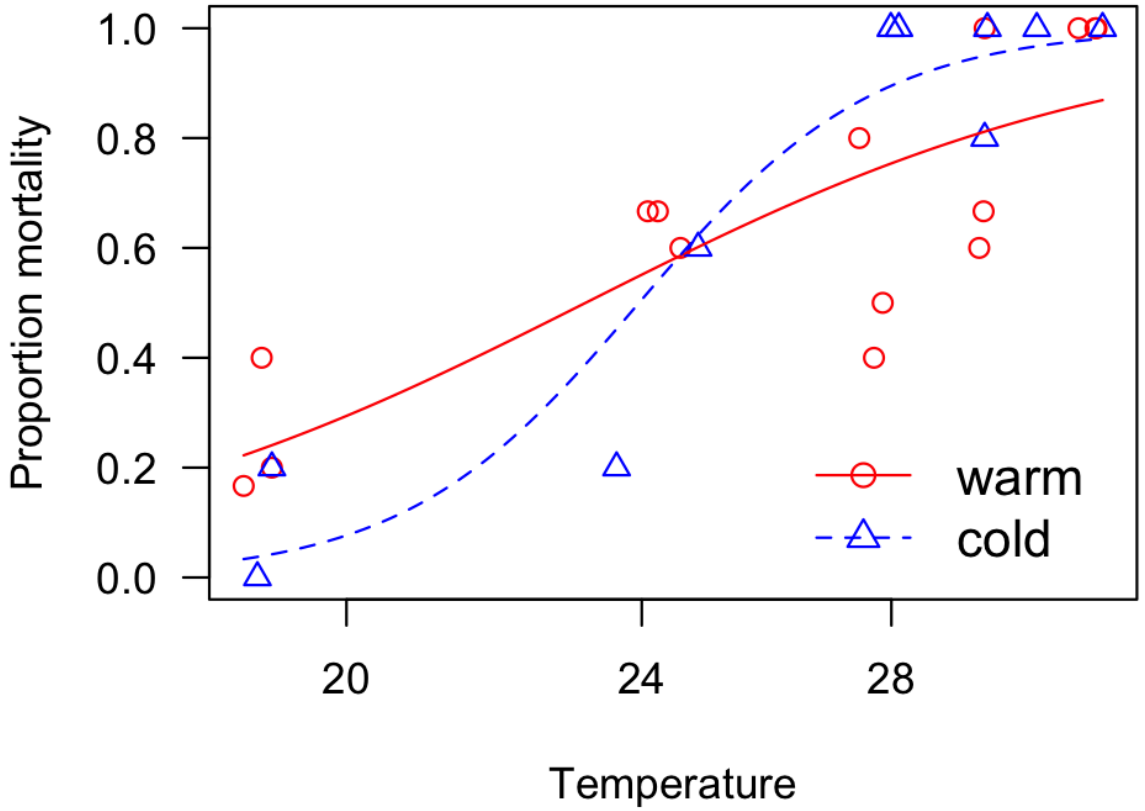

Fig 3. Model 2 survivorship curve displaying the different temperature treatments

An ANOVA comparing the two models indicated that Model 2 (Figure 3) had a higher log-likelihood than Model 1 (Figure 2), suggesting a better fit. However, the likelihood ratio test yielded marginal significance (LR(2) = 5.40, p = 0.067), meaning there was a 6.73% probability that the apparent improved fit of Model 2 was due to chance or an untested variable. The slope under warm conditions was significantly lower than under cold conditions (p-value<0.001) (Figure 3). No significant difference was found in LT_{50} values between the warm and cold treatments (t=-0.523, p=0.601).

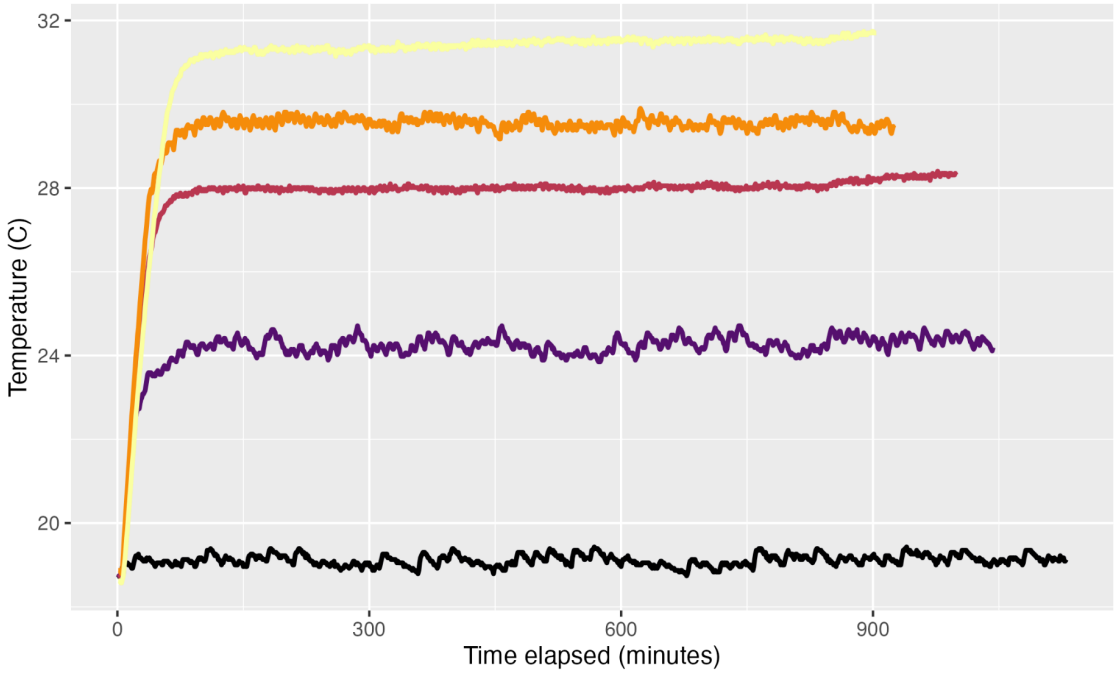

Fig 4. Temperature ramp graph displaying HOBO logger data and the temperature fluctuations that occurred in an average trial

Figure 4 displays HOBO data from one trial to showcase the ramp speed (rate of heating) of the heaters after the trial begins. When the heater was turned on, the trial was set to begin; however, ramp speeds were not consistent between temperatures (Figure 4; Table 1). Additionally, the set temperature of the heater was not always the actual temperature that was recorded by the HOBO logger. On average, the set temperature varied from the actual temperature of the treatment by <2 °C.

Table 1. Time exposed (in minutes) at each temperature level across five trials from nauplii acclimated to cold and warm temperatures. Ramp speed (°C/min) is displayed for each heater in parentheses to show the gradual increase in temperature over time.

Temperature	Trial 1 (Cold)	Trial 2 (Cold)	Trial 3 (Warm)	Trial 4 (Warm)	Trial 5 (Warm)
17.78°C (lowest)	847 (0.01)	959 (-0.01)	1052 (-0.021)	1107 (0.02)	1016 (-0.002)
23.89°C	869 (0.07)	912 (0.09)	903 (0.095)	961 (0.06)	935 (0.072)
28.33°C	844 (0.11)	839 (0.1)	902 (0.08)	935 (0.17)	803 (0.037)
30°C	752 (0.04)	835 (0.13)	778 (0.057)	830 (0.11)	870 (0.15)
33.89°C (highest)	827 (0.12)	787 (0.12)	706 (0.06)	567 (0.02)	826 (0.21)

Table 1 indicates the time each nauplius was exposed to the average temperature in each trial for the 5 set temperatures. While the exposure times vary between trials, on average, the nauplii placed in the lowest temperature treatment were exposed for the longest time interval, and time decreased as temperature increased. Ramp speeds are also included (Table 1) as the heaters took time to reach their maximum temperatures (Figure 4).

Discussion

The results indicate that acclimation to a warmer temperature yields a different thermal survivorship curve for nauplii. As the p-value exceeded the conventional 0.05 threshold, Model 1 was considered the more appropriate model for further interpretation. However, the marginally significant p-value, along with prior information, tells us that temperature treatment has an impact on thermal survivorship. This may suggest there is a relationship between acclimation temperature and thermal tolerance, as the value lies just outside the typical 5% accepted error. Notably, the test subjects used in this experiment were not from the same parental generations, and the resulting genetic variation may have introduced uncontrolled variability to the experiment. This limitation arose partly due to time and logistical constraints, as trials were conducted on different dates and ran overnight rather than in parallel to one another. Start and end times were kept largely consistent to account for metabolism and circadian rhythm; however, the trials occurred over one month. Considering these factors, it is reasonable to expect a p-value >0.05 for this type of experiment (Gonzalez, 1974).

In Model 2 (Figure 3), the slope of the warm acclimated treatment was more gradual than that of the cold acclimated treatment, indicating lower relative mortality at higher temperatures, but higher mortality at lower temperatures. This pattern suggests that warm acclimated nauplii exhibited phenotypic plasticity. Plasticity allows organisms to adjust their physiology or behavior within their lifetime, providing for a rapid buffer against environmental stressors. The ability of nauplii to express phenotypic plasticity is very important as marine heatwaves become more common, as it may allow nauplii to

adapt to changing conditions and survive to adulthood. In contrast, the cold treatment had a steep slope, suggesting the nauplii had a reduced capacity to tolerate temperature increases.

Interestingly, the shift in slope without a marked change in LT50 implies that not all nauplii responded equally to warming. If all nauplii had plastic responses, the entire curve would shift to the right and increase LT50. Yet the observed variability suggests differences in individual capacity to cope with increased temperature. This may be explained by genetic variation, maternal effects, or previous environmental exposure. There was greater survival of warm acclimated nauplii at the warmer temperatures, but lower survival at the cold (Figure 3). Lower survival at lower temperatures could be a tradeoff for adapting to survive warmer temperatures. Variability in the population is important as it influences the resilience of a population. If certain groups of the population have a higher thermal tolerance that allows them to persist during heatwaves, the population die-off likelihood would decrease, and the ecosystem may be able to maintain function.

The warm treatment in this study was designed to simulate marine heatwave conditions in the Gulf of Maine, a region with rapid ocean warming. *Acartia sp.* are key primary consumers in this ecosystem; thus, a widespread mortality event could trigger bottom-up trophic effects. This disruption to the food web would be catastrophic for species that rely on *Acartia* as a food source. Therefore, plasticity may be critical for buffering populations against short-term thermal extremes while natural selection acts

over longer timescales. Gaining a greater understanding of the limits and variability of this response is essential for predicting the fate of *Acartia* and other larval populations under climate change. This insight will be especially important for anticipating broader ecosystem consequences as average water temperatures continue to rise.

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