Temperature Effects on Respiratory Rate and Hatching Success of *Acartia spp.* (Copepoda: Calanoida) in Quahog Bay, Maine

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Abstract

Increasing ocean temperatures within the Gulf of Maine are a major concern for marine ectotherms, such as copepods. The copepod genus *Acartia* spp. plays an essential role in the ecosystem as a primary consumer and environmental indicator. They play a key role as prey for forage fish species and also indirectly impact the economy in the Gulf of Maine. Two key biological processes for copepods, hatching success and respiratory rate, are influenced by temperature. This study observed the hatching success and respiratory rate of *Acartia* spp. at a low temperature treatment representing current average summer temperatures (16°C), and a high temperature treatment representing a marine heat wave scenario (20°C). There did not appear to be any impact of temperature on hatching success, although this analysis may have been limited by small sample size. Respiratory rate, however, was found to be significantly impacted by temperature. Nauplii within the high temperature treatment had significantly higher respiration rates than those in the lower temperature treatment. Overall, warming temperatures do have significant effects on some biological processes that are essential to marine ectotherms such as *Acartia* spp. Further research should be conducted to understand the degree to which increasing temperatures could be detrimental to complementary species.

Introduction

The Northern Atlantic, especially the Gulf of Maine, is anticipated to be impacted by climate change through sea level rise, increasing water temperature, ocean acidification, increasing abnormal and extreme weather patterns, and decreasing dissolved oxygen levels (Fernandez et al. 2020; Pinsky et al. 2013). Additionally, overfishing, pollution, and introduction of invasive species are further detrimental anthropogenic impacts upon the marine ecosystem, which directly influence native biological communities across all trophic levels (Harris and Tyrrell, 2001).

One of the biggest concerns for population densities of marine organisms within the Gulf of Maine is increasing water temperature (Fernandez et al. 2020). The Gulf of Maine is warming at a rate of approximately 0.03°C each year, a rate three times higher than the global average (Pershing et al. 2015). This is alarming because the majority of the marine wildlife native to the Gulf of Maine are ectotherms. These organisms rely on the temperature within their environment to regulate their body temperature, as well as other physiological and biological processes. Marine ectotherms such as copepods have a specific temperature range at which their performance is optimal. The copepod genus *Acartia* spp., which is found globally, has an optimal thermal range of -1°C to 30°C (Leandro et al. 2006;Yoshida et al. 2012). They can be exposed to temperatures outside of this range, but only for a limited duration before mortality occurs (Pinsky et al. 2013). Additionally, copepods can acclimatize to their environment and expand their thermal tolerance window over a significant period of time, but the rate at which the climate is changing might be too fast. This is concerning for copepods, such as *Acartia* spp., which play a crucial role in the Gulf of Maine ecosystems as both environmental indicators and as the foundation of the marine food web as primary consumers (Dipper, 2022).

Within the North American and European Atlantic coasts, *Acartia* spp. is one of the most abundant copepod genera. *Acartia* spp. typically range from 0.05 mm to 1.5 mm in length with females being larger than males. They can be divided into three segments: prosome (head and essential organs), metasome (legs), and urosome (reproductive organs). Multi-purpose antennae are also found on all *Acartia* spp. and aid in reproduction, feeding, and fleeing. Similar to other copepod species, *Acartia* spp. have six naupliar stages, followed by six copepodid stages before they are mature adults. *Acartia* spp. are omnivores and feed on a variety of microorganisms such as phytoplankton and ciliates. Studies suggest that environmental conditions such as temperature and salinity have considerable effects on seasonal patterns, hatching success, egg development, and respiratory rate of *Acartia* spp. (Leandro et al. 2006; Gaudy et al. 1999; Longoria, 2003; Yoshida et al. 2012; Uye, 1980; Breteler and Schogt, 1994). This is due to copepods' high sensitivity and vulnerability to short-term environmental fluctuations, which have been linked with hydrological patterns, seasonal fluctuations, trophic status, and pollution (Marques et al. 2012). *Acartia* spp. play an essential role within the Gulf of Maine ecosystem as a primary forage species for fish, lobster larvae, and other meroplankton, providing vital fatty acids that aid in larval development (Ascher, 2023). Furthering our understanding of their thermal sensitivity will give insight into the vulnerability of similar species to the effects of climate change (Somero, 2010).

This study aims to observe *Acartia spp.* hatching success and respiratory rate at different environmentally-plausible temperatures, due to their abundance in the estuaries and coastal waters within the Gulf of Maine (Gaudy et al. 1999). Treatment temperatures of 16°C and 20°C were chosen due to their relevance to sea surface temperatures observed within the Gulf of Maine. 16°C is the average summer temperature from 1982-2016, while 20°C was the summer temperature observed during the marine heatwaves in 2012 and 2016 (Pershing et al. 2018).

Measuring hatching success in response to higher temperatures allows for inferences of similar zooplankton species' hatching success. Respiratory rate is closely tied to temperature in marine ectotherms. As environmental temperature increases the demand for oxygen also increases, but oxygen solubility within the water decreases. There are already observations of low hypoxia tolerance in *Acartia* spp., and frequency, intensity, and duration of hypoxic events may increase with increased average sea surface temperatures (Ruz-Moreno et al. 2023; Richmond et al 2003). The hypothesis tested here is that hatching success and respiratory rate in the 20°C treatment would be significantly higher than in the 16°C due to the temperature sensitivity of the two biological processes.

Methods

Collecting Copepods

Copepod sampling was primarily achieved through 4 horizontal boat tows that were taken in Quahog Bay in July 2024. The horizontal tows were taken for a duration of about 10-15 minutes using a 100-micron mesh net. Roughly a half-dozen 1-quart jars were partially filled with artificial seawater with a 25 ppt salinity, which was tested using a refractometer and placed in coolers with ice packs. After the tow was completed, the plankton were then apportioned into the jars and placed back into the coolers until they reached the lab. Once they reached the lab they were placed in ambient room temperature. In addition to the boat tows, 2 horizontal dock tows were also conducted at times when the boat was not available. The same methodology and process was applied to the dock tows.

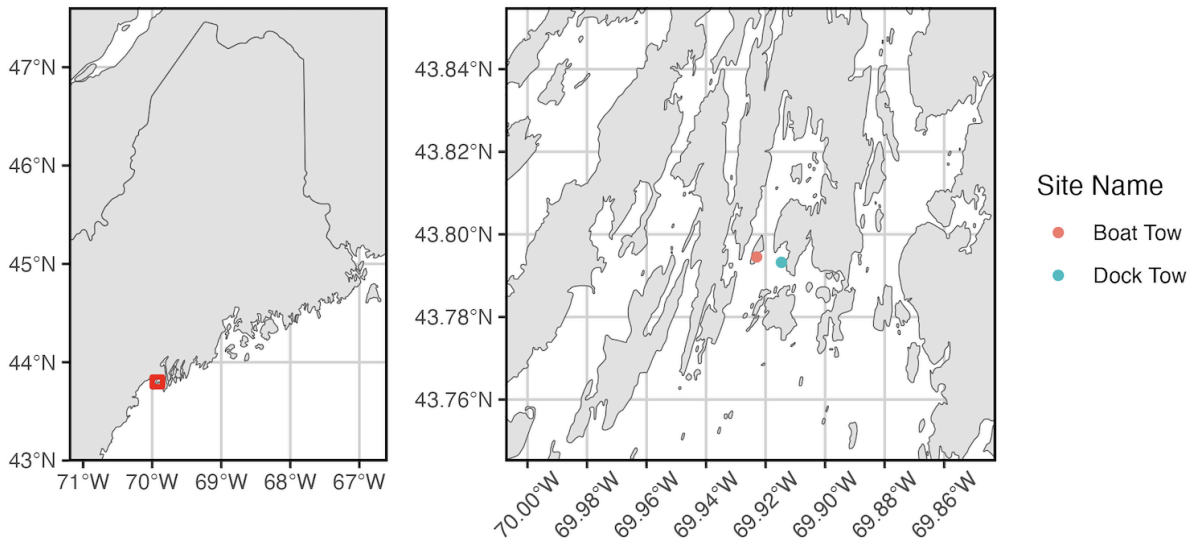

Figure 1: Map of boat and dock tows conducted at Quahog Bay in Harpswell, Maine.

Filtering Copepods

Filtering of copepods, nauplii, and eggs was done as soon as possible to limit mortality and allow acclimation to their respective temperatures. A separate 1-quart jar of artificial seawater was created with the same salinity of 25 ppt, and was used to fill 4 petri dishes and spray bottles. The copepods were then filtered through 180 µm and 53 µm sieves separating the adult copepods from the eggs and nauplii and removing any debris. Using a 100 µl micropipette and microscope, 40 live eggs were placed into two petri dishes representing either the high (20°C) or low (16°C) temperature treatments. Eggs that were cloudy were considered viable, and any clear eggs were indications of dead or already hatched eggs and were not collected (Drillet et al. 2005). Petri dishes were then placed into their incubators and maintained at their respective temperature treatments with a 12:12 light:dark cycle.

Hatching Success

Hatching success was calculated as the number of hatched eggs divided by the initial number of eggs in each temperature treatment. Any eggs missing at the time of the counts were considered to have hatched (Drillet et al. 2005). Both dishes were observed *ad libitum*. The 16°C treatment was always observed first and had a maximum elapsed time between observations of 25 hours and 13 minutes, a minimum of 12 hours and 10 minutes, and a median of 20 hours. The 20°C treatment had a maximum elapsed time between observations of 25 hours and 10 minutes, a minimum of 12 hours and 12 minutes, and a median of 20 hours and 16 min. The average monitoring and recording time of each dish for both 16°C and 20°C was approximately 14 minutes. The eggs were observed for a total of 4 days (07/25/2024 – 07/29/2024).

Respiratory Rate

Using the Loligo© Microplate system, the respiratory rate was recorded for nauplii at both treatment temperatures. The microplate consists of 24 80 µL wells in a 4x6 grid. The microplate and reader were placed in the respective incubator prior to starting the experiment to allow both to acclimate to the proper temperature. Prior to the experiment, 1 quart of water was brought to oxygen equilibrium within the respective temperature, which was later used to fill the wells. All wells were filled carefully with a squirt bottle to not damage the sensors and reduce bubbles that would affect the data. A thorough check was conducted under the microscope, and any bubbles or debris were removed with a 100 µL micropipette. There were 14 controls that contained only water and were slightly overfilled to ensure that no bubbles formed when the plate was sealed, and 10 treatment wells that contained one nauplius per well. Nauplii were placed into wells using a micropipette, and their presence was visually confirmed using a microscope. Once all controls and treatments were completed and predominantly free from bubbles and debris, a sealing film was pressed over the wells so no bubbles would form. After the microplate was sealed, it was aligned with the reader in the incubator and placed in an area with ambient/low light to limit influences upon the photosensitive sensors. Data was collected every 15 seconds over a 18-hour period and was not disturbed until fully completed. Calibrations for both 16° and 20°C were performed prior to the experiment. We calculated respiratory rate as

the change in oxygen saturation over time for the same time period (10 pm - 4am), allowing the controls to level off and nauplii to acclimate. Changes in oxygen saturation during those selected time periods were then converted into nanomoles per hour.

Statistical analyses

Hatching success was visualized through a stepwise plot. Visualization prior to modeling and statistical tests allowed for any inaccuracies to be addressed. A generalized linear model (GLM) was then run to test for differences in hatching rate between the two temperatures. Relevant statistical assumptions were also tested and all were met. A power analysis was run to determine the number of eggs needed to detect a significant difference with a confidence of 95% between the two temperatures. Respiratory rate was visualized through a box plot and checked for outliers. A *Welch Two Sample t-test* was performed to test for significant differences in respiration rate between the two temperature treatments. All coding and visualizations were done with R software (R Core Team 2024).

Results

Hatching Success

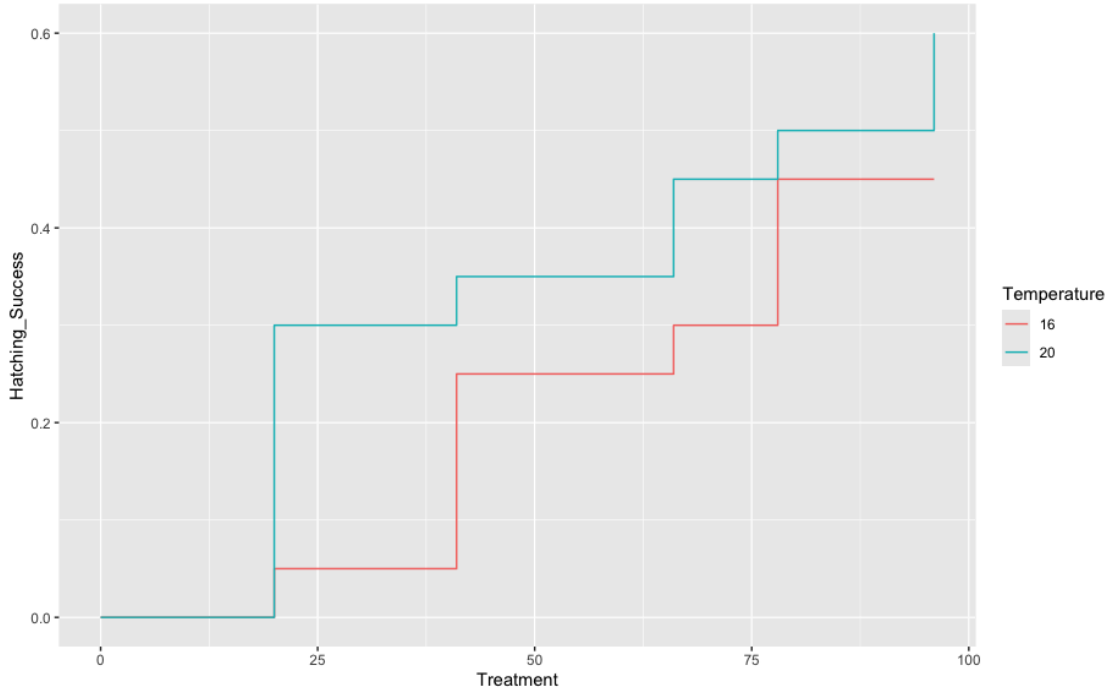

Figure 2: Stepwise plot displaying hatching success over 96 hours at 16°C (red) and 20°C (blue)

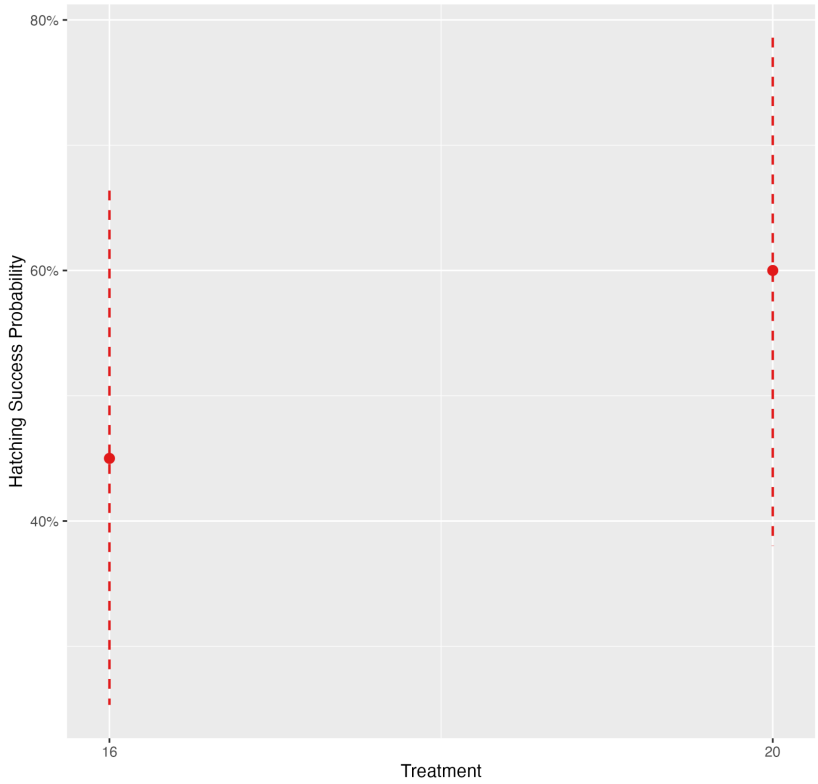

Figure 3: Plot displaying the predicted probability of hatching success at 16°C and 20°C with a 95% confidence interval

Both treatments followed a very similar positive trend *(Figure 2).* There was only one point of overlap at the 78 hour mark between the two treatments (*Figure 2).* Also, the 20°C treatment had two considerable increases of hatching success at the 24 and 65 hour mark in comparison to 16°C (*Figure 2).* Assessment of the temperature treatment on hatching success was accomplished through a GLM and the modeled means were plotted with their 95% confidence intervals. The data showed no significant differences (p=0.344) and there were clear overlaps between the 95% confidence intervals in the predicted probability of hatching success (*Figure 3).* To further understand the differences behind the data, a power analysis was run. Using a two sided power analysis, we found that a sample size of 286 eggs per treatment would be needed to observe significant differences between treatments with 95% confidence.

Respiratory Rate

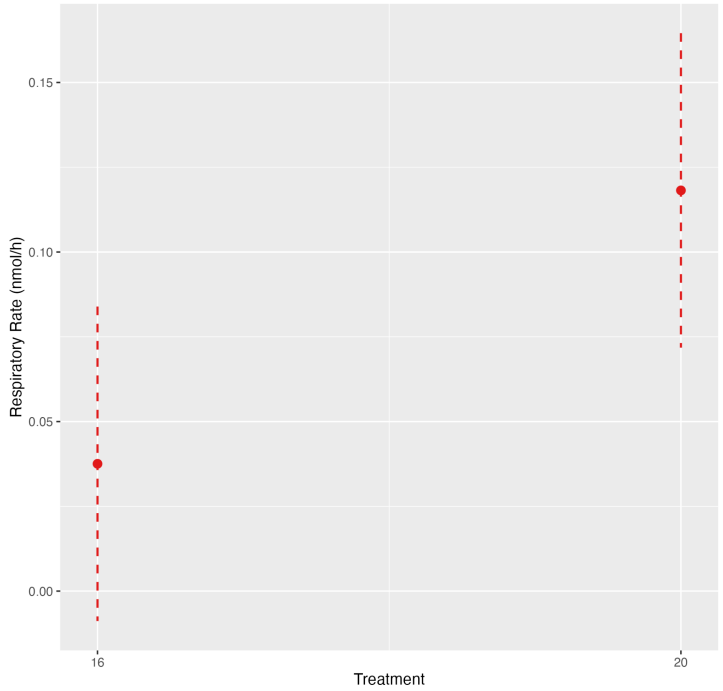

Figure 4: Plot displaying modeled respiratory rate (nmol/h) at 16°C and 20°C with a 95% confidence interval

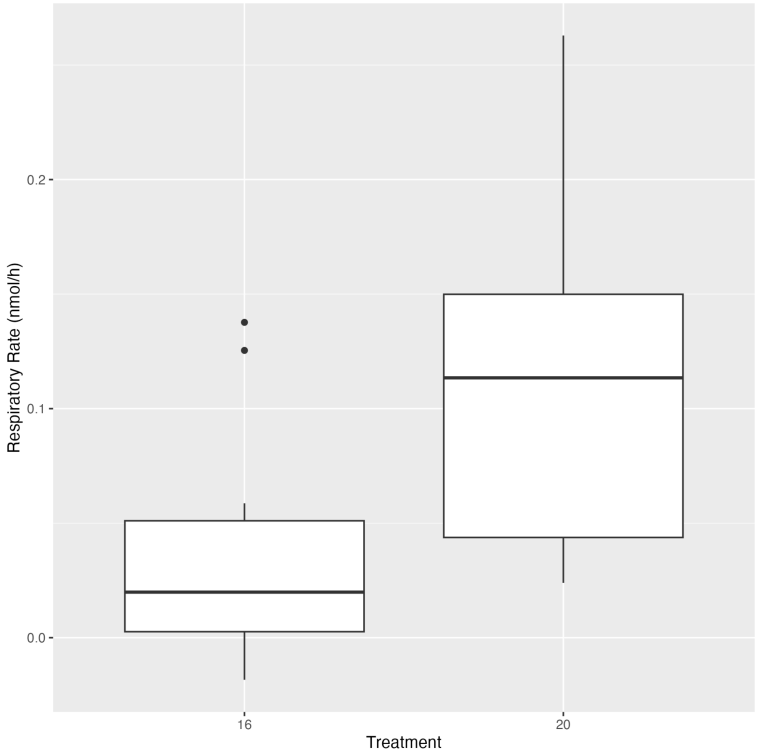

Figure 5: Boxplot displaying observed respiratory rate (nmol/h) at 16°C and 20°C

Observing the boxplot, there are differences between the 16°C and 20°C treatment. The median respiration rate of the 16°C treatment is 0.0199 nmol/hr, compared to 20°C that has a median of 0.113 nmol/hr (*Figure 5).* There is low overlap of interquartile ranges between the two treatments and the variability within the 20°C treatment is greater (*Figure 5)*. Additionally, there are two observable outliers in the 16°C treatment that were not considered extreme (*Figure 5).* A t-test was run to check for significance between the two treatments, we found that the difference in respiration rates between treatments was significant (p=0.0188) (*Figure* 4).

Discussion

As sea surface temperatures within the Gulf of Maine continue to increase, copepod biological processes such as hatching success and respiratory rate are expected to increase as a result (Leandro et al. 2006; Gaudy et al. 1999; Longoria, 2003). Within the first 24 hours of observations, the 20°C treatment had a greater increase in initial hatching success than the 16°C (*Figure 2).* However, we found no significant difference in the hatching success of *Acartia spp.* at 16°C and 20°C at the end of the experiment. Furthermore, there was considerable overlap between the two treatments at a 95% confidence interval (*Figure 3).* Numerous factors may have played a role in why there was no significant difference between the two treatments. This was a very small sample size of 40 eggs total with only one trial, where according to the power analysis 286 eggs per treatment would have been needed to detect a significant difference. Additionally, *Acartia* spp. have been observed hatching successfully from temperatures ranging between 12°C-25°C (Yoshida et al. 2012). This, in conjunction with the wide temperature tolerance range that has been exhibited by *Acartia* spp. (Yoshida et al. 2012; Leandro et al. 2006), would explain the insignificant difference in hatching success between the two treatments.

Unlike hatching success, there was a significant difference in respiratory rate (nmol/h) between the two treatments (*Figure 4,5)*. Nauplii in the higher treatment had significantly greater respiration rates than those in the lower treatment, suggesting that the initial stages of *Acartia* spp. may be sensitive to warming temperatures within their environment. With the sensitivity of naupliar respiration rates to increasing temperatures, we might expect more energy to be devoted to metabolism rather than somatic growth under continued warming conditions (Hackerd et al.2023; Roman and Pierson, 2022). If *Acartia* spp. are unable to attain greater sizes this could negatively impact populations of primary forage fish species that feed upon *Acartia* spp. Moreover, increasing water temperatures results in reduced oxygen solubility, but increased oxygen demand for marine ectotherms such as *Acartia* spp. (Roman and Pierson, 2022). This could result in ecosystems becoming hypoxic, which can have adverse effects on the biological processes of *Acartia* spp. (Richmond et al. 2005). Though there were significant differences between the treatments, there were still some aspects that could have impacted the data. Only the first 2 naupliar stages were observed, so it is uncertain whether this trend would continue for

later developmental stages. Lastly, there were two outliers within the 16°C treatment that could have impacted the distribution of the data (*Figure 5)*.

Although only the respiratory rate displayed significant differences between temperature treatments, there are still inferences and conclusions that can be drawn. In future experiments, a greater number of eggs should be used to observe hatching success and at time intervals that more accurately capture the data. Mitigating the air bubbles through a different technique to seal the film could help minimize the larger variability in the respiration rate data. Naupliar survival could also be taken into account to expand future datasets. More trials would be beneficial to both experiments, especially the respiratory rate. Observing the respiratory rate for *Acartia* spp. at different developmental stages, both larval and adult, would help strengthen our understanding of the impacts of increasing temperature on *Acartia* spp. and other copepods. Furthermore, studying how other essential biological processes are impacted by warming temperatures in a model species such as *Acartia* spp. allows us to draw important inferences about how warming may impact other marine ectotherms.

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