# PERFORMANCE PROCESSES IN *MINUCA PUGNAX* ACROSS TEMPERATURES AND IMPLICATIONS FOR NORTHERN RANGE EXPANSION

By

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# ABSTRACT

The Atlantic Marsh fiddler crab, *Minuca pugnax*, has migrated north of its historic range edge in Cape Cod, Massachusetts, and can be found in central Maine (Johnson pers comm.). The migration of *M. pugnax* leads toward the Gulf of Maine, a climate change hotspot. We studied the possible influences of expanded range temperatures on performance by investigating the effects of temperature on the respiration rate, running speed, and heart rate of *M. pugnax*. Both the respiration rate and running speed experiments were conducted in temperature-controlled chambers that were held at a single temperature for each trial. The temperature in the chamber ranged from 15°C to 35°C in increments of 5°C. Before the respiration rate experiment, each crab was placed in an individual glass beaker with a scannable sticker on the inside of the glass. Respiration rates were then measured using a PreSens Fibox 4, which recorded the oxygen consumption as a proxy for respiration rate over the course of an hour. Female *M. pugnax* did not show a significant change in respiration from 20°C to 35°C, while males had a peak respiration rate at 25°C. Running speed was measured using an entirely linear "racetrack" made of acrylic plexiglass, the surface of which was covered by a thin layer of damp sand to mirror the intertidal environment of *M. pugnax*. One crab was placed on a racetrack at a time to get individual running speeds and was encouraged to run by being "chased" using a popsicle stick. Female *M. pugnax* had a significantly higher running speed at 20°C through 35°C. Males had a peak running speed at 30°C. For both the running speed and respiration rate experiments, both male and female *M. pugnax* had significantly depressed performance at 15°C. Heart rates of *M. pugnax* were measured using voltage signal sensors connected to a Newshift AMP03 Heartrate Monitor over a 15°C to 35°C ramp. Both female and male *M. pugnax* showed no significant differences in heart rate. Our data implies that although the heart rate of *M. pugnax* may not be affected by the colder expanded range, issues for performance may arise in running speed and respiration rate.

# INTRODUCTION

Climate change is pervasive enough to affect every level of biodiversity, starting from individual organisms all the way through large biomes (Bellard et al. 2012). This pervasiveness has been extremely clear within marine environments. Globally, sea surface temperatures have increased on average by 0.67°C just over the last century (Martinez-Soto 2023). Although this number is seemingly small, its impacts are massive and have led to negative ecosystem-scale changes. For example, increases in sea surface temperature have led to ocean acidification and decreases in net primary productivity (Steinacher et al. 2010). The effects of climate change have also extended towards salt marshes, another type of marine ecosystem. Changes in land use, such as the method of draining salt marshes in order for urbanization, have negatively impacted their global distribution (Thorne et al. 2012). Even without the effects of climate change, salt marshes have low wildlife habitat diversity due to physiological conditions of the ecosystems such as tidal flooding and low plant diversity; global salt marsh habitat destruction only continues to perpetuate this at a higher level, which may result in significant biodiversity loss (Thorne et al. 2012). *Minuca pugnax*, also known as the Atlantic marsh fiddler crab, is an intertidal organism most commonly found in salt marshes along the East Coast of the United States (Johnson et al. 2019). Currently, its range stretches all the way from northern Florida to central Maine (Johnson et al. 2020; Johnson pers. comm). *M. pugnax* is a burrowing crab, and the density of its burrows can be used to estimate the population density (Martinez-Soto & Johnson 2020). Currently, burrow densities along the range can be up to 300 burrows $m^{-2}$, which although has been shown to somewhat overestimate fiddler crab density, implies that they are extremely dense across the entire range (Martinez-Soto & Johnson 2020). Therefore, the way it responds to climate change related stressors in saltmarsh ecosystems is of great importance.

*M. pugnax* is just one of many species that have been affected by climate change (Zajac et al. 2022). Although *M. pugnax* is a smaller species of crustacean- measuring anywhere from 6-23mm in carapace width- its impact on the ecosystem it inhabits is quite large (Johnson et al. 2019, 2020; Moore 2019). Within these salt marsh ecosystems, *M. pugnax* plays the role of an ecosystem engineer (Johnson 2020). When an organism is an ecosystem engineer, this refers to its ability to either directly or indirectly adjust the availability of environmental resources to other organisms in the ecosystem through causing changes in the physical state of biotic or abiotic materials; ultimately, this plays a huge role in structuring the ecosystem they live in (Jones et al. 1994). One of the ways that *M. pugnax* acts as an ecosystem engineer is through its ability to impact saltmarsh functioning through its effects on benthic microalgal biomass through consuming diatoms and cyanobacteria (Johnson et al. 2020). A recent study (Johnson et al. 2020) found that plots of saltmarsh that contained *M. pugnax* had around 74% lower diatom biomass and

Figure 1. *Minuca pugnax* from Yale Peabody Museum (Gall & Lazo-Wasem 2024)

77% lower cyanobacteria biomass than plots that did not contain *M. pugnax*. Cyanobacteria and diatoms are able to create biofilms on the surface of sediment in salt marshes, which helps to reduce tidal erosion (Fagherazzi et al. 2013). Additionally, other saltmarsh organisms such as gastropods and polychaetes also feed on diatoms and cyanobacteria (Johnson et al. 2020). The implications of this are that *M. pugnax* has huge effects on the food webs in salt marshes through competing with these other organisms for microalgal food, while also physically affecting the saltmarsh through largely reducing the amount of sediment biofilm, and therefore increasing erosion in the ecosystem. In this way, *M. pugnax* has a large influence in creating the structure of salt marshes. Therefore, the way that climate change affects it is also crucial to understanding how salt marsh ecosystems as a whole may look in the future. If *M. pugnax* is unable to indirectly affect the amount of tidal erosion in the ecosystem due to the effects of climate change, the extent to which they influence their ecosystem will also change. Therefore, it would be important to understand if and how their behaviors change due to climate change.

The behaviors of *M. pugnax*, for example, the efficiency at which it consumes diatoms and cyanobacteria, could be thought of as a mode of performance. Generally speaking, performance in an organism refers to its ability to execute tasks or functions that are important for survival as well as reproduction (Kingsolver & Huey 2003). This means that performance can be both an outward behavior, such as foraging, or an internal process, such as

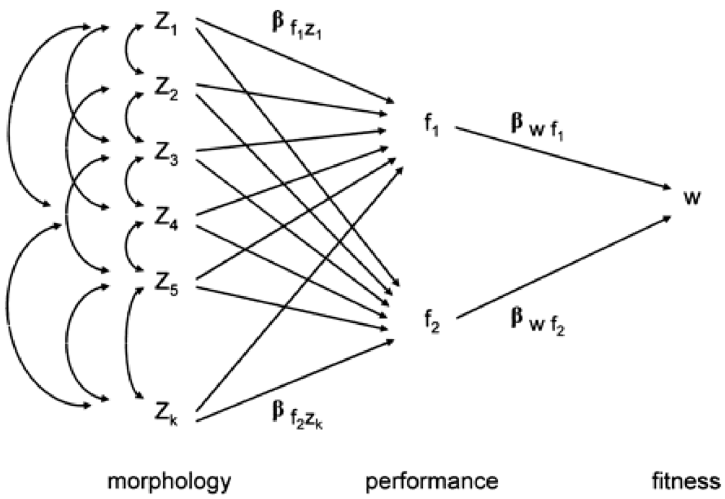

Figure 2. The complex pathway and relationships between morphology, performance, and general fitness (Arnold 1983)

respiration. The continued allocation of energy towards the execution of these tasks and functions can be considered a type of sustained, directional selection (Kingsolver & Huey 2003). Performance fits in the middle of a complex pathway between morphology and fitness (Figure 2) (Arnold 1983). In this figure, z represents differences in a morphological trait, such as body size, which ultimately lead to differences in performance, for example, foraging efficiency, which here is represented as f1 and f2 (Arnold 1983). The performance of foraging efficiency, which differs based on morphology, ultimately leads to W, a measurement of relative fitness (Arnold 1983).

The performance of an organism does not exist in a vacuum, in the sense that morphology is not the only deciding factor on how performance looks, as environment is also a factor. Increases in global temperature affect organisms through altering the kinetic energy of their inner biochemical reactions, which can lead to larger scale changes in physiology and behavior (Madeira et al. 2012). An example of the connection between molecular and behavioral impacts of temperature increases can be seen with *Pachygrapsus marmoratus*, another species of crab. Reproducing female *P. marmoratus* have increasingly variable quantities of a heat shock protein (HSP70), a protein associated with adaptation to changes in environmental temperature, when the temperature of their environment is increased (Madiera et al. 2012b; Hu et al. 2022). The varying quantities of HSP70 in reproducing females may be because large amounts of energy need to be allocated for reproduction, and therefore the energy to produce HSP70 to adapt to environmental temperatures is not available (Madiera et al. 2012b). What this tells us is that environmental temperature can affect molecular-level functioning of an organism, which in this case led to impacts on reproduction. Similarly to the results of the study by Ghalambor et al. (2004), this change in performance- an increase in variability of reproductive output in response to an environmental stressor- shows that changes in temperature are a valid stressor for causing changes in performance. Additionally, the compromise between the two modes of performance, reproductive output and protein production, shows that environmental stressors do cause real change to how organisms perform within their environment, which can lead to large implications about their survival if the stressor persists. Understanding how performance changes with certain environmental stressors, such as temperature, may point to how well organisms function in their changing environments. Therefore, the impacts of temperature on the performance of *M. pugnax* directly relate to their ability to act as ecosystem engineers, and consequently the structure of saltmarsh ecosystems.

Regardless of the capacity for environmental adaptation, each organism has thermal limits, the measurements of which can vary based on methodology of the actual measurements and the conditions under which it is measured (Kingsolver 2018). Critical temperatures, namely the critical thermal maximum ($CT_{max}$) and the critical thermal minimum ($CT_{min}$), refer to the temperatures at which physiological failure of the organism occurs (Kingsolver 2018). $CT_{max}$ refers to the highest temperature an organism can reach before failure, and $CT_{min}$ refers to the lowest. This failure is mainly defined by the mode of performance. However, this critical temperature isn't always absolute. Organisms also have a thermal safety margin (TSM) which can be thought of as excess thermal tolerance that allows an organism to survive in an area where the maximum environmental temperature exceeds its critical thermal temperature (Sunday et al. 2014). In addition to critical thermal temperatures at which physiological failure occurs, there is an optimal temperature at which the organism performs best; this is called the optimal temperature ($T_{opt}$) (Krenek et al. 2012). Similarly to critical thermal temperatures, this optimal temperature also varies between modes of

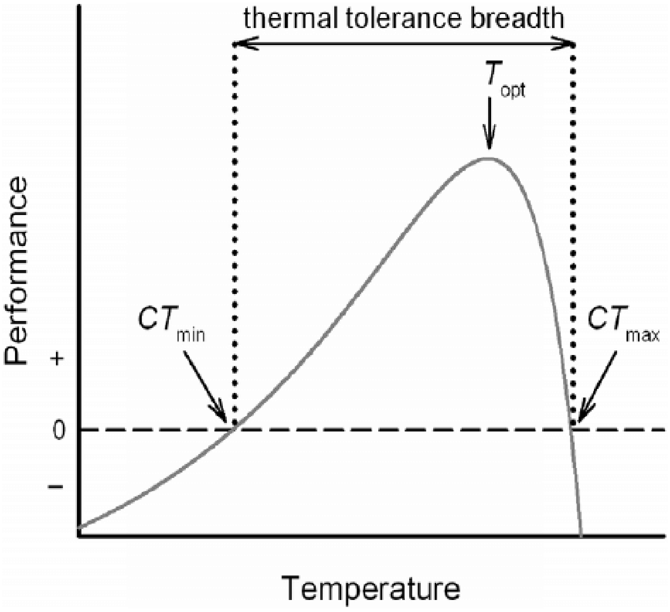

Figure 3. Traditional performance curve of an ectotherm. $CT_{min}$ and $CT_{max}$ act as bounds to the curve, while the peak of the curve is at $T_{opt}$ (Krenek 2012)

performance (Angilletta et al. 2002). In this way, thermal tolerance within the context of performance can be thought of as a bell curve, with the critical thermal temperatures setting bounds for a breadth of tolerance with a peak performance at $T_{opt}$ (Figure 3) (Krenek et al. 2012; Angilletta et.al 2002).

In aquatic environments, the variation in critical thermal temperatures is mostly based on geographic location and the type of ecosystem the organism lives in. Previous studies on aquatic organisms, in particular aquatic invertebrates, have found these differences. One review in 2020 (Cereja 2020) looked at differences in critical thermal temperatures and thermal safety margins across 92 papers on marine invertebrates. This review found that temperate aquatic organisms, as opposed to tropical aquatic organisms, had higher thermal tolerance (Cereja 2020). Additionally, marine organisms in temperate environments have lower $CT_{max}$ values than those living in temperate freshwater environments (Cereja 2020). *M. pugnax*, as we know, is an intertidal organism that lives in temperate environments (Johnson et al. 2019). Therefore, we know that its temperate geographic location may allow for higher thermal tolerance.

In a similar way to how critical thermal temperatures vary across location, performance, and the resulting implications for fitness, are not unchanging, but rather also are dependent on external factors (Ghalambor et al. 2004). Adaptation to environmental stressors can cause tradeoffs between different kinds of performance within the same species. Tradeoffs refer to the compromises that an organism makes between competing functions or modes of performance

(Ghalambor et al. 2004). For example, a 2004 case study on Trinidadian guppies found that increased predation from fish caused a reallocation of energy towards reproduction (Ghalambor et al. 2004). However, this allocation of energy competes with the allocation of energy towards swimming performance for evading predators (Ghalambor et al. 2004). A lab based experiment found that swimming velocity and distance- both of which can be a measure of swimming performance- declined over the course of the pregnancy for pregnant female guppies (Ghalambor et al. 2004). This can be considered a tradeoff, as an increase in reproductive function and performance compromised swimming performance. What this tells us is that performance is not only dynamic, in the sense that it changes in response to stressors, but also that its measurability is variable based on the task or function being focused on. This is important in the context of *M. pugnax*, as its ability to perform functions that provide the structure of saltmarsh ecosystems needs to stay consistent.

The idea that critical thermal temperatures can be affected by physical environment or geographic area also poses the question of how the performance of specific species varies between different environments and their unique climatic conditions. However, we cannot begin to answer this question without first considering that organisms have different systems of thermoregulation. Most organisms fit into one of two broad categories of thermoregulation. The first type of thermoregulator is an endotherm. Endotherms are organisms that have internal feedback processes that are able to produce and reduce the amount of heat, and therefore the internal temperature, of the organism (Legendre & Davesne 2020). These processes happen at multiple levels and therefore allow those organisms to adapt to environmental changes in their environment (Legendre & Davesne 2020). On the other hand, considering how performance looks across temperatures is especially important for the second type of thermoregulator: ectotherms. Ectotherms are organisms with body temperatures that change with the temperature of their environment, meaning that performance on all levels is affected by changes in their environment (Huey & Kingsolver 1989; Zuo et al. 2012). This poses an issue when environmental temperatures reach or begin to reach critical thermal maxima (Kingsolver 2018; Zuo et al. 2012). Not only does the general body temperature of an ectotherm fluctuate with its environment, but the rates of biochemical reactions and biological processes also increase exponentially with increases in temperature (Zuo et al. 2012). Without any internal feedback processes to regulate temperature, this would be very deadly.

Due to this, ectotherms, like *M. pugnax*, must rely on behavioral strategies to reduce or increase their body temperature (Ramalho et al. 2023). Ectotherms can therefore be thought of as behavioral thermoregulators, as their ability to change their body temperature relies on behavioral performance. For example, recent studies have shown that burrowing behavior in *M. pugnax* can be thought of as a method of behavioral thermoregulation (Brodie et al. 2023). One function of *M. pugnax* burrows is to escape into the sediment, and therefore cooler temperatures, during the hot summer months (Brodie et al. 2023). However, when the temperature continually rises, the amount of time that *M. pugnax* stays in the burrow increases as well (Brodie et al. 2023). This longer “move” to the burrow allows *M. pugnax* to better regulate its internal temperature by engaging in behavior that would put it in a lower-temperature environment. However, they cannot stay down there forever, and therefore this thermoregulatory behavior comes at the cost of spending time doing other important tasks, such as foraging or reproducing. In this sense, foraging and reproductive tasks could be seen as competing modes of performance to thermoregulation. When *M. pugnax* chooses to go into its burrows at higher temperatures and stay for longer, its performance for thermoregulation improves, however, this comes at the cost of performance of reproduction and foraging. This is a performance tradeoff in *M. pugnax*, and tells us that its overall fitness is dependent on its ability to balance these tradeoffs. The fact that *M. pugnax* is a behavioral regulator is important when considering its role in saltmarsh ecosystems. As an ecosystem engineer, if environmental stressors such as temperature increase dramatically, *M. pugnax* may have to make important tradeoffs that would cause ecosystem-level shifts. For example, we know that *M. pugnax* consumes diatoms and cyanobacteria, which indirectly causes increased tidal erosion in salt marshes (Johnson et al. 2020). If the temperature of the salt marsh ecosystem increased dramatically, and *M. pugnax* spent most of its time in its burrow to thermoregulate, its performance of consuming diatoms and cyanobacteria would then be compromised (Brodie et al. 2023; Johnson et al. 2020). As an indirect consequence of this tradeoff, tidal erosion would decrease in the salt marsh ecosystem, which may restructure the environment that depends on *M. pugnax* as a consumer. Additionally, the increased temperature of the salt marsh, and therefore less time that *M. pugnax* may be consuming, would make the ecosystem overall less suitable for it to live in, which could have implications for its overall fitness and survival.

One option for improving fitness in ectotherms is to migrate to a different environment with a more suitable temperature for many modes of performance. The current level of global species redistribution due to climate change is the highest in 21,500 years (Martinez-Soto 2023). One review on range shifts found that less than half of all reviewed papers concluded that species ranges were shifting as expected ($n_{species}$=12,009) (Rubenstein et al. 2023). This would imply that external processes are responsible for the current global redistribution of biodiversity (Rubenstein et al. 2023). These external processes could be climate-driven, such as increases in temperature or precipitation, or non-climate-driven, such as changes in land use change or wildfires (Rubenstein et al. 2023). This review also specifically referenced crustaceans as an entire taxonomic group and concluded that there have been significant latitudinal shifts toward the poles (Rubenstein et al. 2023). The organisms that migrate to different environments because of climate-driven processes are known as climate migrants (Martinez-Soto 2023). The existence of climate migrants has huge implications for their historic and expanded ranges. Some climate trajectory models have shown that climate migrants may not be replaced in their historic ranges, which would lead to a decrease in species richness (Burrows et al. 2014). Not only does this lack of replacement have implications for the ecosystems that the climate migrants originally inhabited, but also how the ecosystems in their newly expanded range will change with their presence. Additionally, how the migrating organism will perform in their expanded range compared to their historic range is of question as well.

*M. pugnax* is an example of an ectothermic climate migrant. The critical thermal maximum for *M. pugnax* is around 40°C, which allows it to live in a wide variety of saltmarsh environments (Hews 2021). *M. pugnax* is most active during the summertime, which also contains the season for its reproduction (Hews 2021). Within that time range, the median body temperatures ($T_b$) are the highest during the reproduction season, and the lowest right before the reproduction season starts (Hews 2021). Most studies agree that this reproduction season is consistent across its geographic range (Johnson et al. 2014; Hews et al. 2021). The historic range of *M. pugnax* stretches all the way from northern Florida to Cape Cod, MA, USA, and was established until 2003 (Johnson 2024). Recently, in July 2023, it was

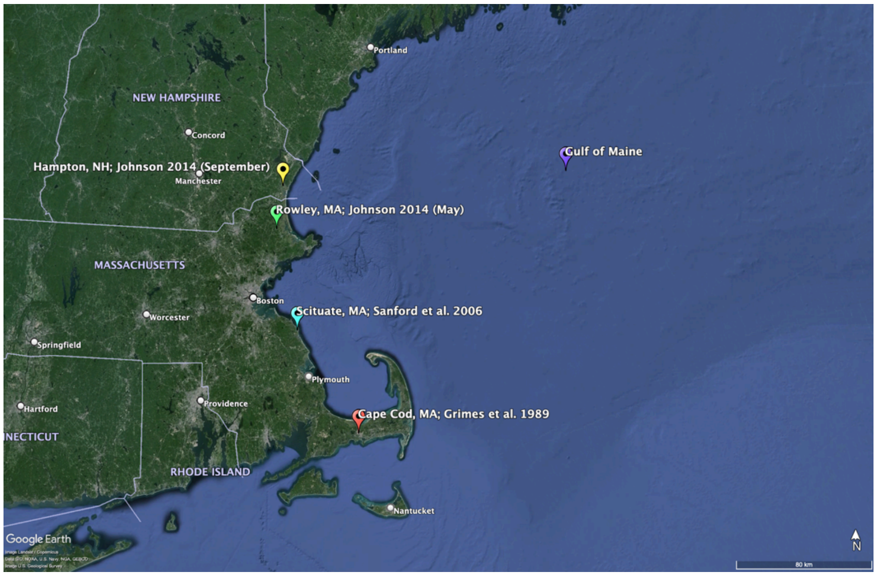

Figure 4. Map of established northern range edges of *M. pugnax* over time (Johnson 2014; Sanford et al. 2006; Grimes et al. 1989)

recorded by NOAA that ocean temperatures in Florida reached a high of 101.1°F which is approximately 38°C (NOAA 2023). This is very close to the $CT_{max}$ of *M. pugnax* (40°C), and would imply that the organisms in that area would be under intense heat stress. Continued global warming, which based on the recent NOAA data is intense in the southern range edge, may be prompting *M. pugnax* to expand its range in the northern direction. In 2006, the northern range edge was expanded from Cape Cod, MA to Scituate MA, and then to Rowley, MA in 2014 (Johnson 2014). The most recently published study on the northern range edge of *M. pugnax* determined that it had expanded its range up to Hampton, NH (Johnson 2014). This range edge was published ten years ago, and combined with anecdotal sightings of *M. pugnax* in central Maine, would imply that the range of *M. pugnax* is continually expanding North (Johnson pers. comm.).

As *M. pugnax* travels farther north, scientists are focused on one area along the coast: the Gulf of Maine. The Gulf of Maine is of current interest to scientists because it is a climate change hotspot. Over the past decade, the surface water temperatures of the Gulf have increased 99% faster than any other ocean on the planet (Pershing et al. 2015). The trend of increased warming isn't stopping either, as the Gulf recently experienced its hottest five-year period on record from 2015-2020 (Pershing et al. 2021). With these changes in temperature, large ecosystem changes have been occurring in the Gulf as well. For example, small crustaceans that serve as important prey species in the Gulf have also started to move northward, creating empty niches in the ecosystem (Pershing et al. 2021).

With *M. pugnax* continuously migrating north, there is a question of how they might perform in these ecosystems, and what the implications of that may be.

The northern range expansion of *M. pugnax* in the direction of the Gulf of Maine, along with their historic large-scale ecosystem impacts needs to be more thoroughly investigated. Despite a plethora of existing literature on range expansion, there has not been enough research on the performance processes of *M. pugnax* through the lens of its northern range expansion. Understanding how the performance of *M. pugnax* may look in their newly expanded northern range could provide a solid foundation of how they will fit into these new ecosystems. This study aims to fill that gap by understanding how multiple types of performance look over a range of temperatures, including those that are common in the expanded northern range. Comparing the measures of multiple modes of performance in *M. pugnax* can better visualize how they may fit into these new ecosystems. In order to gain a holistic view of performance differences across temperature ranges for *M. pugnax*, this study focuses on three modes of performance: respiration rate, heart rate, and running speed. These modes of performance were chosen based on the usefulness of their direct measurements and the large implications they provide for overall fitness. Respiration rate was measured due to what it tells us about metabolism and allocation of energy. Generally speaking, a decrease in respiration rate due to stress conserves metabolic functioning (Newell 1973). Studying respiration rate differences across temperatures could point to not only the metabolism of *M. pugnax*, but also its ability to allocate energy to any other mode of performance. Running speed is also a very important mode of performance for *M. pugnax*. Not only can running speed give implications for how *M. pugnax* might evade predators, but running is also used in guarding burrows to ensure a space for reproduction (Mautz et al. 2011).

The implications of changes in running speed may tell us how well *M. pugnax* can evade predators as well as give insight into changes in reproductive success in its expanded range. Finally, we chose to measure heart rate in order to get a general idea of how *M. pugnax* responds to stressors (Burnett et al. 2013). This understanding may give a mechanism or better foundation for why other modes of performance, such as respiration or running speed, may change due to differences in temperatures.

Based on our information on *M. pugnax*, along with knowledge of its movement across the eastern United States coast, we believe that *M. pugnax* has variable performance based on temperature. We hypothesize that respiration rate, running speed, and heart rate will increase with increased temperature. Within the ranges of measured performance for *M. pugnax*, we expect to see an optimal temperature range at which *M, pugnax* performs best. However, we do not expect that this range will be the same between modes of performance, or between sexes. Through understanding the performance of *M. pugnax* across temperatures, this study aims to propose possible mechanisms for the expanding range of *M. pugnax* by analyzing how temperature-based performance differences relate to the seasonal temperatures in the current range and the proposed expanded range.

# METHODS

## COLLECTION SITES

The experiments discussed in this thesis took place from October 2022 through September of 2023. The running speed and respiration rate experiments were conducted in October through December of 2022, and the *M. pugnax* used in these experiments were collected both from Wareham, Massachusetts, and Flax Pond Marine Laboratory, Long Island, New York in October 2022. *M. pugnax* used in the heart rate experiments from August through September 2023 were also collected from Flax Pond Marine Laboratory in Long Island, New York. All specimens were used within one week of collection so that lab-based experimental results would closely mirror those conducted in a field environment. The experiments were conducted by the members of the Brodie lab.

## RUNNING SPEED

The running speed of *M. pugnax* was assessed using an entirely linear “racetrack” in a temperature-controlled chamber. The temperature of the chamber ranged from 15°C-35°C in increments of 5°C. To prepare the crabs for racing, each testing group of *M. pugnax* was acclimated to the temperature of the chamber for 24 hours prior in an incubator. It is important to note that testing groups were separated by sex to avoid confusion when recording the data. About 30 minutes before being placed on the track to run, each crab was individually placed in a glass beaker covered with a thin layer of seawater to minimize stress during transportation to the racetrack.

The racetrack was constructed of acrylic plexiglass, the surface of which was covered by a thin layer of damp sand to mirror their intertidal environment. The walls of the racetrack were covered with black paper to minimize possible distractions for the crab. Additionally, two sets of infrared (IR) sensors were placed at each end of the racetrack, which measured the time it took for the crab to get from one side of the racetrack to the other. When crossing the first set of sensors, a timer would start recording until the second set of sensors were crossed. These sensors were programmed using a Python-coded Arduino board.

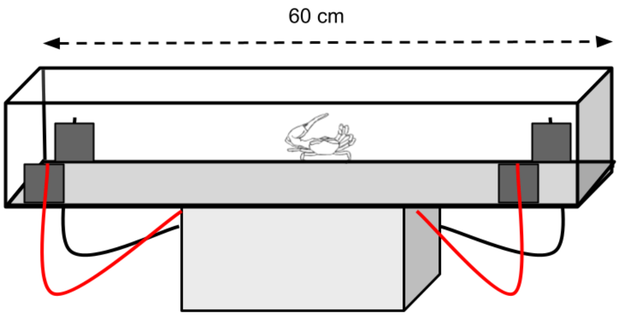

Figure 5. Experimental set up for crab racetrack and speed measurements (length=60cm). Crabs were divided by sex.

One crab was placed on the racetrack at a time to get individual running speeds. It is important to note that the handler for the crabs remained consistent for each testing group and that the orientation in which the crabs were placed on the track was also consistent. For male *M. pugnax* specifically, each crab was placed with the large claw in front of their body and toward the end of the track. Each crab was placed in front of the first set of IR sensors and was encouraged to run by being “chased” using a wooden dowel. This choice was made in order to simulate how *M. pugnax* may run in response to a predator. After passing the second set of IR sensors, the speed for the trial was calculated by dividing the distance between the two sets of IR sensors by the time taken to cross through the second set. Each crab was also tested three times at a single temperature, the fastest speed of which was recorded and used in the analysis.

The data was analyzed using a one-way ANOVA test, and crabs were analyzed separately by sex. In order to meet normality standards, data was transformed using a logarithmic function. After the initial ANOVA test, post-hoc tests were conducted.

## RESPIRATION RATE

We assessed the respiration rate of *M. pugnax* over a range of 15°C to 35°C (in 5°C increments) in a temperature controlled chamber. Prior to the experiment, crabs were placed in an incubator for 24 hours at the test temperature in order for them to acclimate. This made sure that stress was minimized and that respiration rates did not reflect the potential effects of both temperature and stress. Testing groups were again separated by sex. Before being brought to the chamber, crabs were placed in individual glass vials, which were closed tightly to ensure that air could not enter or leave. Each vial had a light-sensitive sticker placed on the inside of the glass. These stickers were scanned using PreSens Fibox 4 at a salinity of 0 ppm, which was able to record

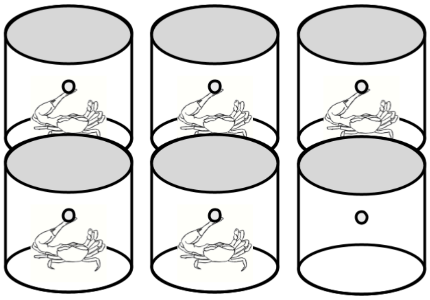

Figure 6. Experimental set-up for respiration measurements of M. pugnax using PreSens Fibox 4. Crabs were divided by sex.

the oxygen consumption in the vial as a proxy for the respiration rate of *M. pugnax*.

Crabs were tested in groups of five with a sixth, empty vial serving as a control group. Each testing group was brought to the chamber in their individual vials in a covered box to minimize distractions or stress before the experiment. Once the crabs were brought to the chamber, they were given ten minutes to acclimate before the experiment began. After acclimating, each vial was scanned using the PreSens Fibox 4 every ten minutes over the course of an hour. Each scan of the oxygen consumption was recorded and used for analysis.

This data was analyzed using a one-way ANOVA test, and crabs were separated by sex for analysis. To meet normality standards, this data was also transformed using a logarithmic function. Following the initial ANOVA test, a post-hoc Tukey test was used to further analyze data.

## HEART RATE

Heart rate was measured using a voltage sensor connected to a Newshift AMP03 Heartrate Monitor. Nine males and nine females were used in this experiment. Each *M. pugnax* was attached to their own sensor using cyanoacrylate glue at the center of its carapace. Each crab was placed

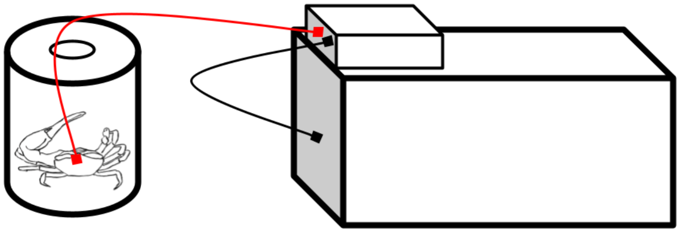

Figure 7. Diagram of heart rate experiment set up for M. pugnax using LabChart Powerlab connected to Newshift AMP03 Heartrate Monitor. Crabs were divided by sex.

in their own container in a temperature-controlled incubator, with the sensor still attached and the monitor connected to a PowerLab box, the hardware component of LabChart. LabChart software was able to record the voltage signals each crab gave to their sensors throughout the experiment. The temperature of the incubator was raised 5°C every 30 minutes, with a ten-minute transition period between each temperature. We started the temperature of the incubator at 15°C, and using the temperature ramp we tested voltage signals up to 35℃. The voltage signals from the sensors were interpreted as cyclic measurements, and the number of cyclic peaks was recorded in LabChart. We used this as a proxy for heart rate (measured in beats per minute) and took an average every ten minutes, excluding the transition periods, for each crab for the duration of the experiment.

Due to the repeated measures in this experiment, we used a generalized least squares model (GLS) in order to analyze the data. The model that fit the data best was the unstructured covariance matrix with symmetrical correlation structure (AIC= 4.42±0.01). Additionally, the data needed a square root transformation in order to meet normality standards.

## RANGE TEMPERATURE MODELING

To fit performance within the context of the expanded range of *M. pugnax*, we looked at open-source air temperature data from the National Oceanic and Atmospheric Association (NOAA). Air temperature can be used as a proxy for surface temperature, which would best describe the temperature conditions that *M. pugnax* would be experiencing (Brodie pers. comm.). Sampling locations were randomly chosen based on coastal areas along the eastern United States coast that were within the bounds of the extended range. Air temperature measurements were average monthly temperatures from April through September, and were recorded for 2003, 2008, 2013, 2018, and 2023. This data was compiled, monthly averages were converted to degrees Celsius (°C), and yearly summer averages were calculated. In order to visualize changes in temperature, yearly summer air temperature averages were graphed based on location over time.

# RESULTS

Table 1. Collection summary data across the three performance experiments, including date, location, number and sex distribution of crabs, and average carapace width (mm)

| Experiment | Collection Date | Location | Number of Crabs Collected | Number of Crabs by Sex | Average Carapace Width (mm) |
|---|---|---|---|---|---|
| Running Speed | September-October 2022 | Wareham, Massachusetts<br>*41.7625°N 70.722222°W* | 94 | Female: 44<br>Male: 50 | 15.81±0.01 |
| Respiration Rate | September-October 2022 | Wareham, Massachusetts<br>*41.7625°N 70.722222°W* | 70 | Female: 35<br>Male: 35 | *N.D.* |
| Heart Rate | August-September 2023 | Flax Pond, Long Island, New York<br>*40.960778°N, 73.138744°W* | 27 | Female: 12<br>Male: 15 | 15.66±0.01 |

We visualized the results of the running speed experiment through a split-box plot separated by sex. The results of the experiment showed that temperature did have a significant effect on running speed, and that for both sexes, running speed increased with temperature. Additionally, both sexes had significantly depressed running speeds at 15°C.

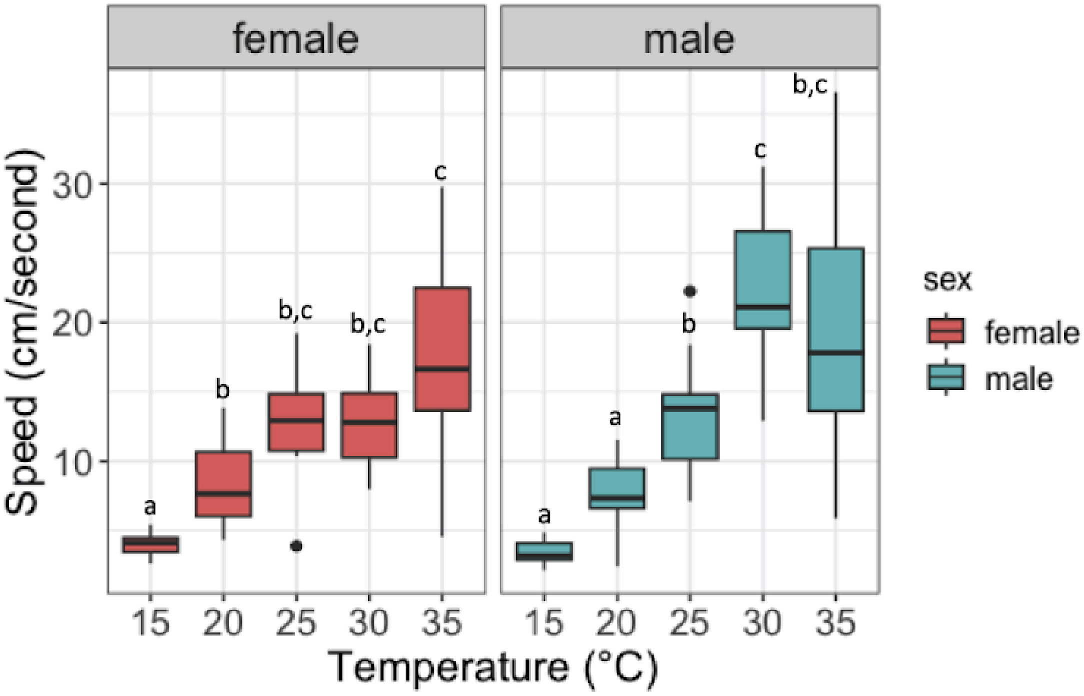

Figure 8. Performance curves demonstrated by boxplots of male and female *M. pugnax* based on running speeds (cm/s) from temperatures of 15°C to 35°C.

The results of the one-way ANOVA test showed that temperature did have a significant effect on the running speed of *M. pugnax*. Both male and female crabs had significantly higher running speeds at higher temperatures.

Table 2. Results of ANOVA tests of the effect of temperature on running speed in *M. pugnax*

| Sex | F value | p value (ANOVA) |
|---|---|---|
| Male | 60.293 | $3.38 \times 10^{-11}$ |
| Female | 30.672 | $1.394 \times 10^{-6}$ |

Respiration rates were visualized using a split boxplot that separated measurements based on sex. Unfortunately, ANOVA stats from this experiment were unable to be recovered, however, significance was previously marked on the boxplots and was designated using letters a, b, and c. We found that running speed was significantly depressed for both sexes at 15°C ($p<0.05$). For female *M. pugnax*, there were no significant changes between 20°C and 35°C. For males, however, there was a $T_{opt}$ at 25°C. Rates at 20°C were significantly higher than at 15°C and lower than at 25°C, but not significantly different when compared to those at 30°C and 35°C.

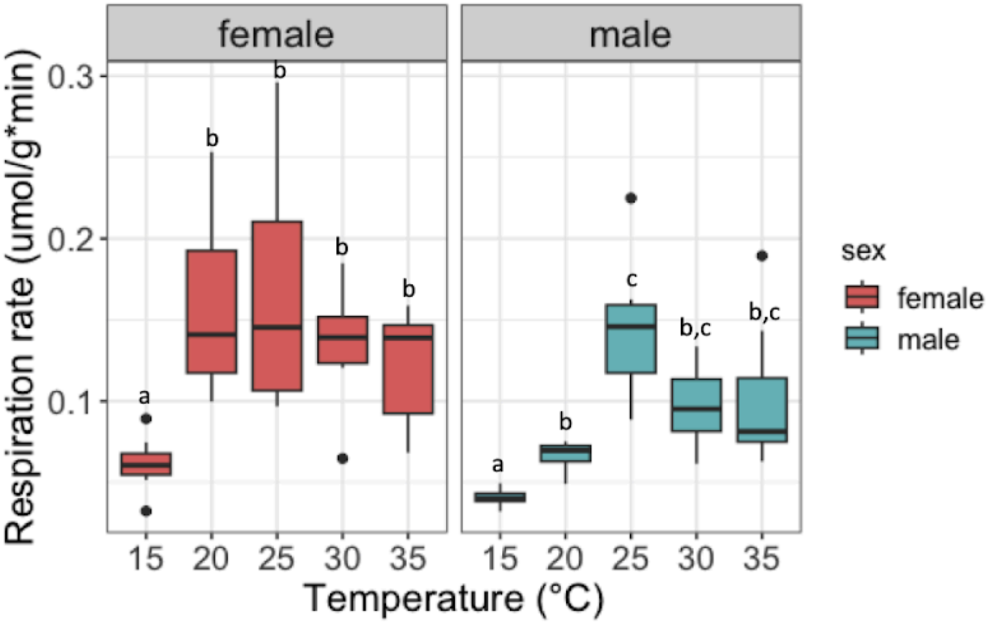

Figure 9. Performance curves demonstrated by boxplots of male and female *M. pugnax* based on respiration rates (umol/g*min) from temperatures of 15°C to 35°C.

Heart rates were visualized using a split boxplot that was separated by sex category. We found that there was no impact of temperature on heart rate for male or female crabs (Pr=0.4946). However, sex was a significant factor, with male *M. pugnax* having significantly higher heart rates than females (Pr=0.025). Additionally, there was a significant interactive effect between size and carapace width, showing that larger crabs, within their respective sex categories, had lower heart rates than those with smaller carapace widths (Pr=0.0306).

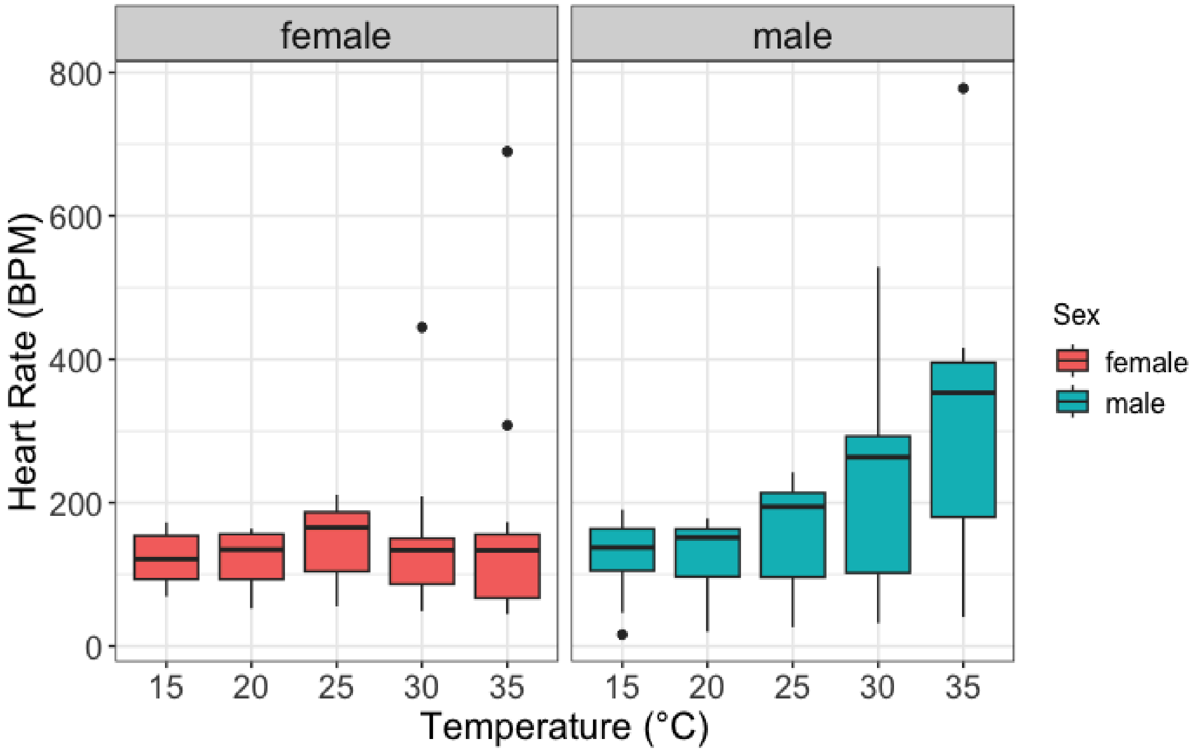

Figure 10. Performance curves demonstrated by boxplots of male and female *M. pugnax* based on heart rates (BPM) over a temperature gradient of 15°C to 35°C.

This is a table of the results of the generalized least squares (GLS) model with an unstructured covariance matrix with symmetrical correlation structure. The model found that temperature did not have a significant effect on heart rate (Pr=0.4946). However, both sex and the interactive effect between sex and carapace width were significant in changing heart rate (Pr=0.0250, 0.0306 respectively). Males had significantly higher heart rates than females, and larger crabs within their sex category had lower heart rates.

Table 3. Statistics summary table of GLS model with unstructured covariance matrix with symmetrical correlation structure for heart rate experiment (AIC= 4.42±0.01)

| Condition | Degrees of Freedom | X^2 | Pr (>X^2) (±0.0001) |
|---|---|---|---|
| Intercept | 1 | 12.0725 | 0.0005 |
| Temperature (°C) | 4 | 3.3914 | 0.4946 |
| Sex | 1 | 5.0305 | 0.0250 |
| Carapace Width (mm) | 1 | 0.0393 | 0.8428 |
| Temperature:Sex | 4 | 2.6082 | 0.6254 |
| Temperature:Carapace Width | 4 | 2.8781 | 0.5784 |
| Sex:Carapace Width | 1 | 4.6726 | 0.0306 |
| Temperature:Sex:Carapace Width | 4 | 2.1802 | 0.7027 |

Summer temperature averages across the range of *M. pugnax* were calculated using NOAA open source weather data. Monthly average temperatures for April-September were compiled for the years 2003, 2008, 2013, 2018, and 2023, and averages were calculated based on those values. Visually speaking, locations higher in latitude had lower average temperatures during the summer months.

Table 4. Summary table of summer temperature averages (°C) across the range of *M. pugnax* from 2003-2023

| Location | Summer Temp. Average, 2003 (°C) | Summer Temp. Average, 2008 (°C) | Summer Temp. Average, 2013 (°C) | Summer Temp. Average, 2018 (°C) | Summer Temp. Average, 2023 (°C) |
|---|---|---|---|---|---|
| Portland Area, ME | 15.0 | 15.6 | 15.8 | 16.4 | 16.0 |
| Chatham, MA | 15.7 | 17.1 | 17.0 | 17.3 | 18.9 |
| Islip Area, NY | 17.7 | 18.8 | 18.4 | 19.1 | 19.2 |
| Dover, DE | 20.0 | 21.1 | 20.5 | 21.7 | 21.3 |
| Myrtle Beach Area, SC | 23.2 | 23.3 | 23.0 | 24.1 | 23.3 |
| Sapelo Island, GA | 24.9 | N.D. | 25.3 | 26.1 | 24.9 |
| St. Augustine, FL | 25.2 | 25.7 | 25.5 | N.D. | 27.3 |

Average summer (April-September) range temperature across randomly selected points on the range of *M. pugnax* were visualized using a line chart. Areas higher in latitude, especially Portland, ME, had a visually lower average summer temperature than those in the historic range, especially those at the southern range edge.

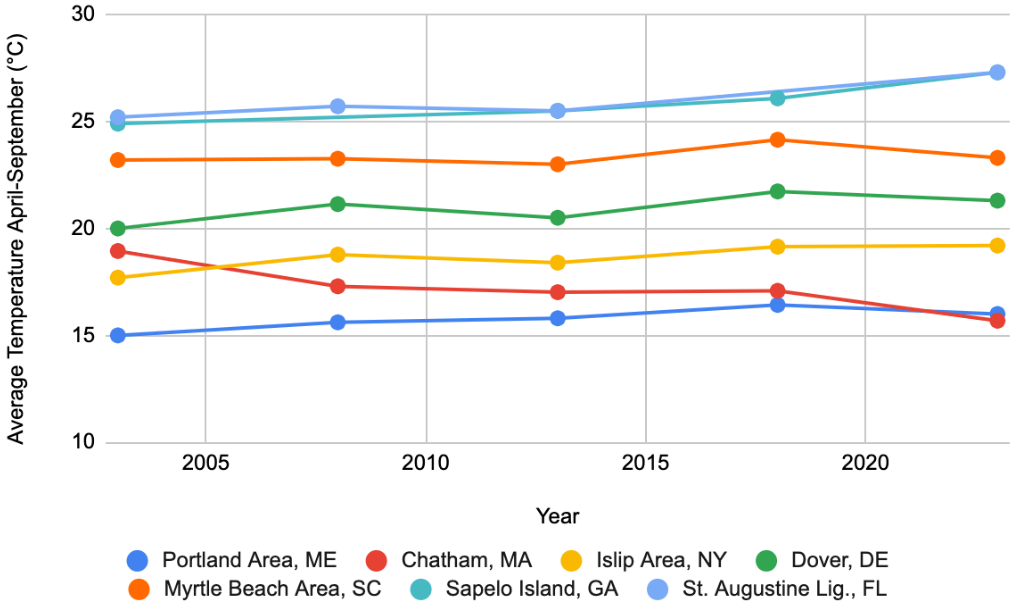

Figure 11. Average summer temperatures (°C) over the course of 20 years (2003, 2008, 2013, 2018, 2023) from randomly selected locations across the range of *M. pugnax*

# DISCUSSION

The results of our three experiments show a complex picture of performance for *M. pugnax*, and therefore similarly complex implications for their performance in the expanded northern range. We tested how respiration rate and running speed varied in different, constant temperature settings from 15°C to 35°C along with how heart rate changed over a temperature gradient from 15°C-35°C. The results of these performance tests have large implications for how *M. pugnax* may function in its expanded northern range based on temperature modeling of its entire range. Putting temperature-based performance in the context of the temperature of its environment gives a clearer picture of its ability to be an effective ecosystem engineer.

The results of the running speed experiment showed that temperature does have an effect on both male and female *M. pugnax*, with higher running speeds at increased temperatures ($p=3.38 \times 10^{-11}$, $1.394 \times 10^{-6}$ respectively) (Table 2). Additionally, the one-way test showed that the variation in running speed based on temperature was stronger for males than females ($F_{Males}=60.293$, $F_{Females}=30.672$)(Table 2). Running speed was significantly depressed at 15°C for both sexes (Figure 8). This decrease in running speed would suggest that at 15°C, it is harder for both male and female crabs to run. At 20°C, female crabs have a significantly higher running speed, but males do not. However, males generally ran faster than females (Figure 8). Additionally, males had a more classic shaped performance curve, with peak running speeds at 30°C (Figure 8). At 35°C, there was a non significant decrease in running speed. This suggests that their $T_{opt}$ is

30°C. For females, running speed continuously increased up to 35°C, which does not show a clear peak in running speed and shows a nontraditional performance curve (Figure 8). Seeing as though the $CT_{max}$ for *M. pugnax* is 40°C, perhaps if we tested them at temperatures past 40°C, we would see a true peak for females at 35°C, and a continued decrease in running speed for males (Hews et al. 2021)

Recently published literature has found similar results. Levinton et al. 2020 studied sprinting speeds from 5°C to 45°C in a similar species of fiddler crab, *Leptuca pugilator*. They studied sprinting speeds in male crabs, and found a $T_{opt}$ at 35°C at about 25 cm/sec, which is similar to *M. pugnax* at 20.07cm/sec (Appendix Table 1)(Levinton et al. 2020). After 35°C, the running speed decreases until fatigue between 40°C and 45°C (Levinton et al. 2020). If we had continued our experiment at higher temperatures past the $CT_{max}$ of *M. pugnax*, we may have also seen a clear decrease after our $T_{opt}$ at 30°C, as Levinton et al. (2020) did with 35°C (Figure 9). Although this prior literature only worked with male crabs, the female crabs in our experiment also had a $T_{opt}$ at 35°C, with a continual increase beforehand (Figure 9). Therefore, if the experiment were extended we may also see a clearer $T_{opt}$ and a decrease toward fatigue as the temperature reaches, and then surpasses, the $CT_{max}$ of *M. pugnax*. However, the methodology of running speed differed between our experiment and past research. The same 2020 study tested running speed by putting *L. pugilator* on a treadmill, which in essence forces it to run (Levinton et al. 2020). Not only did we use a different species of fiddler crab, but the only encouragement we gave the crabs to run was the chasing via popsicle stick. Therefore, Levinton et al. (2020) may have found running speeds that are slightly higher than what would be naturally occurring. If this is true, then our results are even more valid, as they were just slightly below that of the study.

In the northern expanded range, the average summer temperatures are visually and quantifiably much lower than those at lower latitudes (Figure 11). Since both male and female *M. pugnax* have significantly depressed running speeds at 15°C, which is a similar temperature to that of Portland, ME, this would imply that running speed performance would not be ideal. This might be because of energy allocation issues. If running speed is not entirely essential to survival, running speed may be one of the first modes of performance to have its energy allocated elsewhere. This may mean that *M. pugnax* will not be able to evade predators as well if their running speed is compromised by the lower temperature. Additionally, decreased running speed may have implications for tasks that require movement, such as foraging. If *M. pugnax* is not able to run as fast as it does in the historic range, it may not be able to forage for food as well, which may lead to larger consequences for its overall fitness and survival.

The results of the respiration rate experiment showed that there was an increase in respiration rate based on temperature along with some clear differences between the sex categories. Interestingly enough, respiration rates for both males and females were significantly depressed at 15°C, which matches the results of the running speed experiment as well (Figure 8,9). Additionally, there were some clear differences between the sex categories in respiration rate. Male

*M. pugnax* had significantly lower respiration rates than that of females, which would imply that as temperature increases in their environment, males will have lower respiration, and possibly lower fitness, than females (Figure 9). Despite the fact that females had higher respiration rates, the respiration rate of male *M. pugnax* followed a more traditional performance curve with a clear $T_{opt}$ at 25°C (Figure 9). After 25°C, respiration rates began to decrease, although not significantly. This was not seen in females, as their respiration rate increased significantly at 20°C, but had no more significant changes at higher temperatures (Figure 2).

The results of our experiment somewhat match those of prior literature as well. Respiration rate experiments conducted on similar species of fiddler crab found that respiration rate increased with temperature (Jiminez & Bennett 2005). However, the respiration rates in this 2005 study almost doubled from 26°C to 32°C (Jiminez & Bennett 2005). This is unlike our results, which show a clear $T_{opt}$ for males at 25°C and a non-significant difference at 35°C (Figure 9). Additionally, the increase in respiration rate for females was also non-significant between 25°C and 35°C. This may be entirely due to different species of fiddler crab being the focus of the two studies. Additionally, the methodology was different. Jiminez & Bennett (2005) used a respirometer flask with manometer fluid to measure respiration, while we used the PreSens Fibox 4 scanner. Due to the fact that this prior literature was in 2005, the scanner method we used may be more accurate in measuring changes in respiration rates.

In the expanded range, more specifically in Portland, Maine, the average summer temperature in 2023 was 16.0°C (Table 3). This, along with the data showing significantly depressed respiration rates for both sexes at 15°C, would imply that respiration would be greatly affected in the expanded range. *M. pugnax* will most likely have a lower respiration rate in the expanded range, regardless of sex. However, in order to adapt to these colder temperatures long term, *M. pugnax* may need to allocate more energy towards respiration in order to survive, and may have lower performance in other modes of performance, such as running speed or those associated with reproduction. This would then imply that *M. pugnax* may spend more time on adapting to respiration as opposed to other modes of performance, so they may not be as active so as to adapt to those colder temperatures.

The results of the heart rate experiment did not match the hypothesis that temperature would have a significant effect on heart rate. This can be seen in the results of the GLS model, which shows that temperature, and any interaction involving temperature and another factor, were not significantly correlated with changes in heart rate ($Pr_{Temp}$=0.4946, $Pr_{Temp:Sex}$=0.6254, $Pr_{Temp:CW}$= 0.5784, $Pr_{Temp:Sex:CW}$=0.7027) (Table 2). However, the GLS model did determine that sex was a significant driver in changes in heart rate, with males having a significantly higher heart rate than females ($Pr_{Sex}$= 0.0250) (Table 2). Additionally, the interaction between carapace width and sex were significant, showing that specifically larger crabs had significantly lower heart rates compared to that of smaller crabs within their sex category ($Pr_{Sex:CW}$=0.0306) (Table 2). These results make some sense, and give insight into how performance may look in the expanded range. The lack of significant change in heart rate due to temperature is surprising, as we did see significant changes in respiration rate and running speed due to temperature. However, the significant difference in heart rate between sex categories would imply that there is still a pattern among modes of performance in *M. pugnax* to be significantly different between sexes (Table 2).

The specific non-invasive methodology we used for measuring the heart rate of *M. pugnax* has not been replicated and published by another group; therefore, the results of our experiment can only be compared to those of a similar species. Prior literature studied how *Leptuca pugliator*, a similar species of fiddler crab on the eastern coast, responded to a temperature gradient through measuring its heart rate in air (Levinton et al. 2020). Their results showed that heart rate did increase, with a $T_{opt}$ of 35°C and a gentle decline down to 45°C (Levinton 2020). Our results may be different than this due to the extent to which they ramped their temperature gradient and the fact that their study only focused on male crabs. The temperature ramp of this experiment ranged from 20°C to 45°C, the upper limit of which exceeds the $CT_{max}$ of *M. pugnax* (Hews 202; Levinton et al. 2020). We may have seen the same kind of curve if our temperature ramp was similar, however, we were not planning on bringing *M. pugnax* to its $CT_{max}$ and over for our experiment. Therefore, our data could only be showing the first part of the curve up to $T_{opt}$, and perhaps we would have seen a similar decrease in heart rate if *M. pugnax* was taken past 35°C. Additionally, the use of only male crabs means that this study is not entirely comparable to that of the results for the females in ours. However, we did see that sex was a significant factor, with males having a significantly higher heart rate than females (Table 2). If Levinton et al. (2020) were to redo their experiment with females, perhaps they might have seen that the performance curve with a $T_{opt}$ of 35°C was not present in females. The performance curves of our heart rate experiment show a non-significant increase in heart rate up to 35°C for males, and a mostly visually constant heart rate for females (Figure 10). In this way, sex differences are definitely a huge factor in heart rate over the gradient, so more replicates of our experiment would need to be done in order to confirm that they are consistent in order to viably compare our results to this prior study.

In the expanded range, the results of our heart rate experiment would imply that the performance of heart rate may not be affected by the colder temperatures in the same way that running speed and respiration rates would. Given the non-significant increases in temperature for males, and mostly constant heart rates for females, lower temperatures would not produce a significantly depressed or increased heart rate (Figure 10). Additionally, prior literature has shown in a similar species that heart rate only begins to decrease after 35°C, which is very far from the average temperatures of ~16°C in the Gulf of Maine (Table 3) (Levinton 2020). Therefore, this mode of performance most likely does not affect the fitness of *M. pugnax* in the same way that respiration rates and running speeds do at lower temperatures due to the lack of significant change. However, our results do show that sex is a significant factor, so this would imply that males would still have a significantly higher heart rate than females at these lower temperatures, despite the gradient itself not producing significant change of heart rate both overall and within sex categories (Figure 10) (Table 2).

Despite generally adequate data and clear conclusions, there is definitely room for improvement. The results of this series of experiments clearly showed differences in performance between sex categories, and the fact that neither males or females is consistently performing at a higher rate than the other suggests that they may make different tradeoffs in performance. In the future, research could look at more modes of performance, such as foraging or burrowing behavior, over the same temperature range in order to get a larger picture of what performance differences may look like. To an extent, the implication that females choose to allocate energy towards respiration at the cost of running speed in order to maintain a steady heart rate is reductive. Running speed may not be the only mode of performance that females allocate energy from, respiration may not be the only mode of performance that it allocates to, and the end goal may not be to only maintain heart rate- there may be another reason or mode of performance that is even more important. This line of reasoning is the same with males. Their implied allocation of energy towards running speed over respiration in order to maintain heart rate may not be the case, but we won't understand the larger picture of tradeoffs in performance unless more modes are explored. In addition to testing modes of performance by themselves, it would be interesting to design an experiment where *M. pugnax* would have to make a tradeoff between modes of performance. If this was conducted, we could better see which modes of performance are connected and perhaps see clearer differences in tradeoffs between sex categories.

Even despite a smaller and perhaps reductive picture of performance, the result of two out of three modes of performance being significantly depressed in temperatures similar to that of the expanded range does not bode well for *M. pugnax*. The implications of lower performance may cause lower reproduction rates, foraging ability, and a higher mortality rate. Over time, this may transform populations of *M. pugnax* into sink populations that cannot be self-supported (Furrer & Pasinelli 2015). The temperatures of the locations along the current range, outside of Portland, ME, have annual summer temperatures that generally match higher performance values in our experiments (Table 3; Figure 11). Even if heart rate is not significantly affected by temperature, that doesn't rule out the possibility of *M. pugnax* having lower overall fitness and survival in its expanded range, as the two are inextricably linked (Table 2) (Arnold 1983). Current research efforts should focus on looking at the larger picture of performance differences based on temperature and continued sampling events to determine how fast and where the northern range edge of *M. pugnax* is. *M. pugnax* is an ecosystem engineer who is extremely vital to maintaining salt marsh ecosystems (Johnson et al. 2020). Its decrease in performance, therefore, may ultimately restructure salt marsh ecosystems in ways that will continue to perpetuate and exacerbate the effects of climate change. In order to maintain and conserve this major ecosystem along the East Coast, *M. pugnax* needs to be centered in our conversations and research about performance and the resulting ecological impact.

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# APPENDIX

Table 1. Median and mean running speeds for *M. pugnax* from 15°C-35°C, separated by sex

| Temperature | Sex | Median Speed (cm/s) | Mean Speed (cm/s) |
|---|---|---|---|
| 15°C | Combined Female and Male | 3.71 | 3.70 |
| 15°C | Female | 4.13 | 3.97 |
| 15°C | Male | 3.18 | 3.42 |
| 20°C | Combined Female and Male | 7.65 | 8.07 |
| 20°C | Female | 7.65 | 8.49 |
| 20°C | Male | 7.35 | 7.65 |
| 25°C | Combined Female and Male | 13.39 | 13.00 |
| 25°C | Female | 12.93 | 12.46 |
| 25°C | Male | 13.80 | 13.45 |
| 30°C | Combined Female and Male | 16.99 | 17.63 |
| 30°C | Female | 12.79 | 12.86 |
| 30°C | Male | 21.11 | 22.39 |
| 35°C | Combined Female and Male | 16.63 | 19.04 |
| 35°C | Female | 16.63 | 17.61 |
| 35°C | Male | 17.80 | 20.04 |

Table 2. Median and mean respiration rates for *M. pugnax* from 15°C-35°C, separated by sex

| Temperature | Sex | Median Respiration Rate (umol/g*min) | Mean Respiration Rate (umol/g*min) |
|---|---|---|---|
| 15°C | Combined Female and Male | 0.047 | 0.051 |
| 15°C | Female | 0.060 | 0.061 |
| 15°C | Male | 0.040 | 0.041 |
| 20°C | Combined Female and Male | 0.087 | 0.113 |
| 20°C | Female | 0.141 | 0.159 |
| 20°C | Male | 0.070 | 0.066 |
| 25°C | Combined Female and Male | 0.146 | 0.156 |
| 25°C | Female | 0.145 | 0.167 |
| 25°C | Male | 0.146 | 0.145 |
| 30°C | Combined Female and Male | 0.122 | 0.116 |
| 30°C | Female | 0.139 | 0.134 |
| 30°C | Male | 0.095 | 0.097 |
| 35°C | Combined Female and Male | 0.093 | 0.108 |
| 35°C | Female | 0.139 | 0.121 |
| 35°C | Male | 0.081 | 0.102 |

Table 3. Median and mean heart rates for *M. pugnax* from a 15°C-35°C gradient, separated by sex

| Temperature | Sex | Median Heart Rate (BPM) | Mean Heart Rate (BPM) |
|---|---|---|---|
| 15°C | Combined Female and Male | 134.7 | 125.9 |
| 15°C | Female | 121.5 | 121.5 |
| 15°C | Male | 136.1 | 127.2 |
| 20°C | Combined Female and Male | 137.3 | 125.2 |
| 20°C | Female | 134.5 | 122.9 |
| 20°C | Male | 151.5 | 127.0 |
| 25°C | Combined Female and Male | 171.7 | 154.6 |
| 25°C | Female | 165.4 | 146.2 |
| 25°C | Male | 194.4 | 161.3 |
| 30°C | Combined Female and Male | 147.1 | 194.9 |
| 30°C | Female | 133.7 | 146.8 |
| 30°C | Male | 263.6 | 233.4 |
| 35°C | Combined Female and Male | 173.0 | 245.8 |
| 35°C | Female | 133.5 | 169.3 |
| 35°C | Male | 353.2 | 307.0 |