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Steady heartbeat: Field and laboratory studies indicate unexpected resilience to high temperatures for the ribbed mussel *Geukensia demissa*

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology.

By
Ashlyn Smith
Under the mentorship of Dr. Sophie George

ABSTRACT

Salt marshes are important ecosystems found along the coast of Georgia. Salt marshes are hosts to diverse organisms that interact with each other to promote many ecosystem services, such as storm buffering and flooding, and absorption of excess nutrients. Among these diverse organisms is the ribbed mussel, *Geukensia demissa*. Mussels are a foundation species in this intertidal landscape, and without them the whole salt marsh would be negatively affected. The purpose of this investigation was to explore the thermal stress response of *G. demissa* to rising temperatures. Mussels were collected from three locations that were landlocked, close to a road, and far away from a road on Tybee Island, Georgia. The presence of this road has greatly increased the temperature in the salt marsh. Thus, we hypothesize that mussels will have an increased heart rate with increased temperature. To test this hypothesis, the heartbeat of *G. demissa* were recorded using an IR sensor in laboratory and field experiments at reduced and elevated temperatures. Results showed that mussels from locations regularly experiencing elevated temperatures do not have an increased heart rate. However, mussels from locations that experience lower temperatures did. These results indicate that mussels are more resilient to higher temperatures than previously expected. This study is significant for marsh conservationists and scientists wishing to preserve and maintain the salt marsh, its ecosystem services.

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Introduction

Salt marshes provide a variety of ecosystem services to people, including erosion control, water filtration, and storm buffering (Barbier et al. 2011, Leonardi et al. 2018). One of the most important benefits salt marshes hold to other ecosystems and humans is the filtration of water runoff, which diminishes nitrogen input to estuaries and the ocean. Excess nitrogen can lead to toxic algal blooms and marine dead zones (Gedan et al. 2009). However, these ecosystem services are reduced due to anthropogenic effects and the salt marsh's sensitive location. There has been a large decline in the vital salt marsh ecosystem due to climate change, sea level rise, erosion, tidal submergence, and urban development (Bilkovic et al. 2017, Derksen-Hooijberg et al. 2017). This is mainly due to large ships eroding away the sea shore with their waves, the high real estate potential of these locations, and development by the tourism industry. Among these anthropogenic factors affecting the salt marsh, increased shoreline armoring due to rising seas and growing development are the greatest factors contributing to decreased salt marsh habitat (Bilkovic et al. 2017). Other anthropogenic factors affecting the salt marsh include the creation of salt ponds for salt production, the introduction of invasive plant and animal species, and the conversion of marsh to fertile farmland. These human impacts have caused consumer control to dominate the salt marsh habitat, detrimentally affecting the salt marsh ecosystem (Ainsworth & Long 2005, Gedan et al. 2009). This means that the predators in the marsh habitat are predominant and tilt the delicate balance and relationship between marsh organisms.

Among the factors putting the salt marsh at great risk, rising temperatures and climate change are the most predominant. The mean ocean temperature has increased by .11°C per decade since 1970 and ocean levels have risen .19 m between 1900 and 2010. Both temperature and ocean level are expected to continue to rise. These rising temperatures have led to shifts in plant species distributions, which have caused negative effects of resident and migratory species. Heat waves have also shown negative changes in seagrass species (IPCC report 2019). Rising temperatures have been shown to increase the physiological stress response of intertidal animals and lead to tens of thousands of dead mussels along the California coast (Fields et al. 2016). Many studies have measured the physiological stress response in intertidal species by recording their heart rate. These studies indicated that high temperatures do affect the cardiac performance in intertidal species. For instance, the limpet species *Cellena toreuma* and *Lottia digitalis* and two lineages of the brown mussel *Perna perna* experienced higher heart rate at high temperatures (Bjelde and Todgham 2013, Huang et al. 2015, Tagliarolo & McQuaid 2016).

Intertidal organisms are already experiencing increased thermal stress due to the emersion/immersion cycle. The Santini (2000) and Tagliarolo & McQuaid (2016) studies noted higher cardiac activity during daytime immersion. However, during emersion, mollusks e.g. mussels, limpets and gastropods, face significant increases in temperature. It is suspected that these intertidal organisms may already experience their thermal tolerance limit during low tide. This may explain the mass mussel die-off in California when temperatures got to over 38°C at low tide in the middle of the day (Kreeger et al. 2015, Fields et al. 2016). Exposure to high temperatures during low tide can be further

exacerbated by building roads through the salt marsh. Roads interrupt tidal flow and thus subject salt marsh organisms to longer periods of emersion and high temperatures. Exposure to very high temperatures for long periods of time can lead to extremely high heart rates and death. The ribbed mussel *Geukensia demissa* is quite common in salt marshes along the east coast of the United States (Bertness 1984) with densities of over 2000 individuals/m^2 in New England salt marshes (Honig et al. 2015). However, no studies of *G. demissa* have addressed the effects of extreme temperatures on cardiac activity of this foundation species in southeastern Georgia's salt marshes.

This study examined the effects of increased temperatures on heart rate of *G. demissa* in laboratory and field experiments. *G. demissa* was chosen because it is an ecosystem engineer and at risk from anthropogenic effects. *G. demissa* helps stabilize the marsh by filtering organic material and producing biodeposits (Bilkovic et al. 2017). These biodeposits promote cordgrass (*Spartina alterniflora*) growth which then aids the binding of sediments and reduces erosion. The binding of sediment and aggregates of mussels also helps stabilize the growth of the cordgrass. *G. demissa* also directly influences the diversity and ecosystem multifunctionality of the salt marsh as a secondary foundation species (Angelini et al. 2016). It has been found that larger aggregates of mussels increase the number and diversity of other invertebrate inhabitants of the marsh including mud crabs, marsh crabs, snails, and fiddler crabs. Increased aggregation of mussels has also been observed to increase water infiltration, cordgrass and invertebrate biomass, and soil accretion (Angelini et al. 2016). This has been experimented only on a patch scale but indicate the vast importance and role ribbed mussels play in the salt marsh.

For example, the mutualistic relationship between *G. demissa* and *S. alterniflora* improves the overall health of the salt marsh (Angelini et al. 2016, Derksen-Hooijberg et al. 2017). Cordgrass are taller and found at higher densities in the presence of ribbed mussels (Bertness 1984, Angelini et al. 2016). In turn mussel abundance increases. Angelini et al. (2016) predicted that loss of *G. demissa* could increase the time it takes for salt marshes to recover from drought by up to 100 years. The benefits and ecosystem services that *G. demissa* provide may be diminished by roads that cut through salt marshes. The resulting interruption of tidal flow may subject the mussels to higher temperatures for longer periods of time and cause heart rates to increase to dangerous levels.

To understand the effects of temperature on the heart rate of *G. demissa*, heart rate measurements were made with a non-invasive infrared technique developed by Burnett et al. (2013) in the laboratory and field in 2018 and 2019. Three locations were chosen on Tybee Island, Georgia's salt marshes. One location was landlocked with roads on either side of the marsh. This location only received tidal flow through an underground culvert, has very short *S. alterniflora* and was expected to have the highest temperatures. The second location was the high salt marsh. This location is in close proximity to a road, but still receives regular tidal flow. The third location is the mid salt marsh which was not too far from a small creek with regular tidal flow. The mid marsh was expected to experience lower temperatures due to the taller *S. alterniflora*. Based on the results of previous studies, it is hypothesized that the heart rates of *G. demissa* will increase as temperatures increase in the laboratory and field. By studying the relationship between rising

temperatures and *G. demissa*, it will be possible to implement policies that will increase preservation of Georgia's salt marshes.

Methods

Description of locations

G. demissa from Tybee Island (32.000517 N, -80.845767 W) were collected in September and October of 2018, and April and September of 2019 for three laboratory and three field experiments. Three locations were chosen (Supplementary Table 1 appendix). At each of these locations, four meter by meter plots were randomly chosen (Figure 1A and 1B, Supplementary Table 1 appendix). Common to these three locations were mounds composed of mussels and cordgrass. Mounds are raised portion of the substrate. Mussels are found on these mounds and can form aggregates of up to 200 mussels.

Location one (Landlocked) was chosen because it is characterized by roads on either side of the marsh (Old Tybee road and Highway 80), interrupted tidal flow, lengthened high tides, increased temperatures and lower densities of mussels. Location one only receives tidal flow from a culvert connecting the main salt marsh to the landlocked region of the salt marsh. As a result, location one experiences lengthened high and low tides. This made location one an ideal location to measure the physiological stress response of higher temperatures on *G. demissa.* Location two (High marsh) was chosen because it is close to Old Tybee road. Close proximity to a road interrupts tidal flow, but to a lesser degree than the landlocked location. Location three (Mid marsh) was chosen because it is most exemplary of the natural marsh environment, with normal tidal flow and temperatures. (Figure 1A and 1B, supplemental Table 1 appendix). Tide level

varied for each field experiment between -0.2 and 0.03m (Supplementary Table 2 appendix).

The temperature at high and mid marsh were, on average, between 37°C and 43°C during the day. The temperature at the landlocked location was, on average, between 37°C and 53°C. The average height of the cordgrass was shorter at the landlocked location and high marsh (38.8 and 44.4 cm), and taller (61 cm) at the mid marsh location. There was lower density coverage of *S. alterniflora* at the landlocked and high marsh locations, with higher density in the mid marsh (Table 1).

Table 1. Average height and percent cover of *Spartina alterniflora* at three locations (landlocked, high marsh and mid marsh) off old Tybee Road, Tybee Island, Georgia. There were four plots per location, and ten cordgrass stems were measured in each plot.

	number of plots (n)	Average height (cm)	Standard Deviation	Average density coverage (%)	Standard Deviation
Land-locked	4	38.8	5.9	45	8.1
High marsh	4	44.4	9.4	48	11.9
Mid marsh	4	61.0	2.0	84	2.5

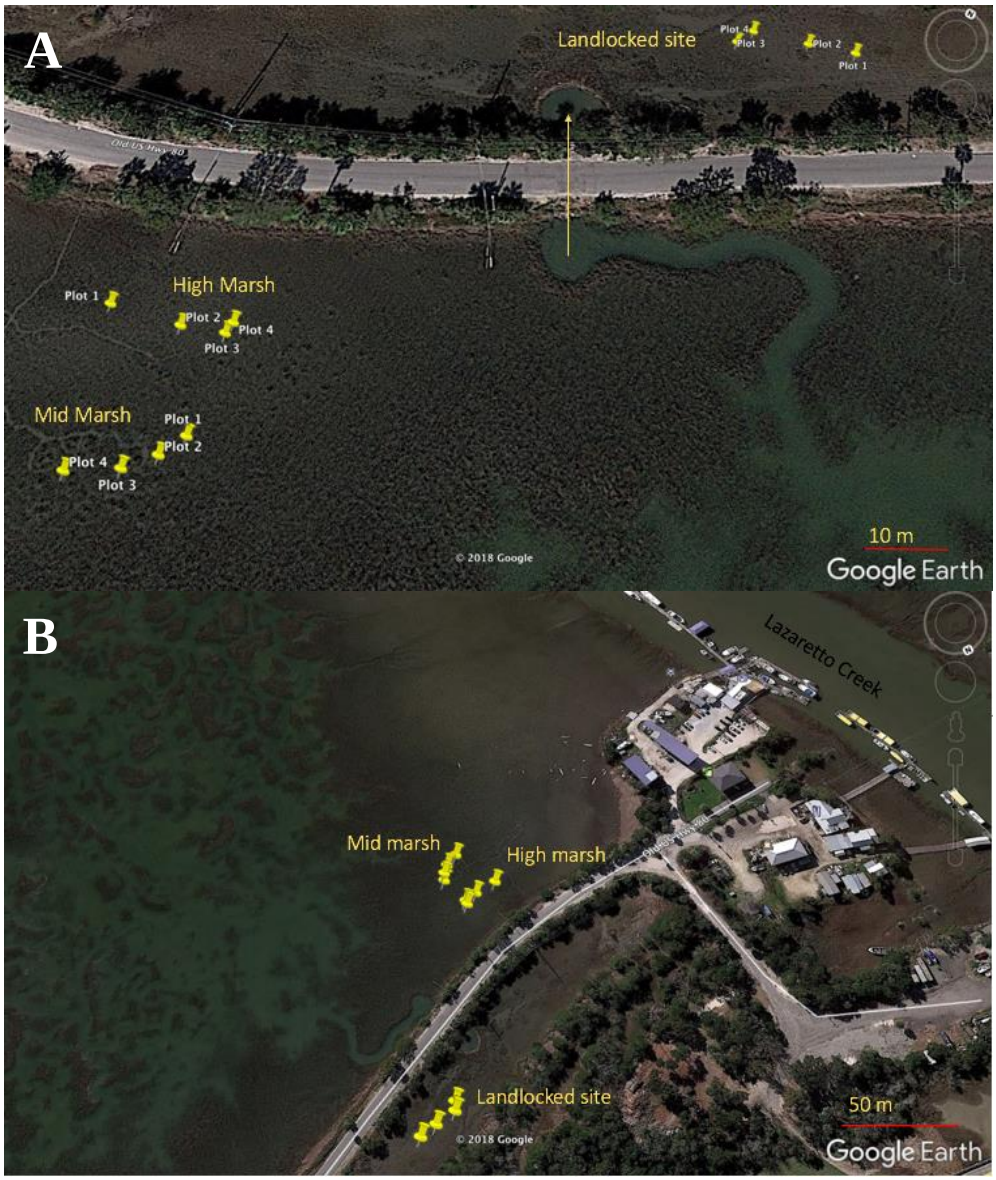

Figure 1A and B. Plots at the landlocked, high marsh, and mid marsh locations near Lazaretto Creek and Old Tybee Road, Tybee Island, Georgia. A yellow arrow indicates the position of the culvert under Old Tybee Road, leading to the landlocked location.

Mussel Collection and Lab Maintenance

At each location, four plots were selected, totaling 12 plots. To measure the heart rate of mussels, 2-3 mussels were collected from each plot for a total of 10 mussels from each location in September and October of 2018, and April and September of 2019.

These mussels were collected to measure heart rate in the laboratory, and then returned to the field for in situ measurement of heartbeat.

In the laboratory, mussels were kept in three containers (45 x 30 x 30 cm) for two-four weeks (10 mussels per container per location). Each tank was aerated with two air stones and the salinity was kept between 30 to 33 %, which is the salinity in the natural habitat (Supplemental Table 3). The tanks were cleaned twice a week. The mussels were fed either mud containing algae from the salt marsh through a pipette, or with an algal shellfish diet (Shellfish Diet 1800 Batch #19229) three times a week. Mussels collected from all three locations were 89-116 mm in length (Supplemental Table 4). There was no statistical difference in mussel size from the three locations ($p = 0.0697$).

Method used to measure heartbeat of mussels

The heart rate was collected using a non-invasive technique developed by Burnett et al. (2013) This method has successfully been used to measure the heart rate of limpets, mudcrabs, and mussels. This technique consists of the following components: PicoScope 2207B, Burnett heartbeat monitor amplifier, IR sensor, USB-6009, and PC or Mac computer. All components were purchased from Digikey and picotech.com.

The heart rate sensors are made of an IR emitter and IR detector in a small package that creates an image of the heart and detects movement in the circulatory system, thus detecting a heartbeat. This is accomplished by IR light passing through the shell and reflecting off of the heart and circulatory system. As the heart contracts, a different amount of IR light is reflected and this is received and recorded by the IR detector. The signal is then translated into an electrical signal that is picked up by an

attached circuit board and converted from an analog to a digital signal by Pico software on a laptop (Burnett et al. 2013).

The IR sensor was physically placed on the mussel's shell, above the heart (Figure 2A and 2B). Placement of the IR sensor on the mussel can greatly affect the signal of the heartbeat, as analyzed by the Pico-software. Interference with a signal can cause an unreadable signal (Figure 3A). This interference can be caused by water, movement, or light. Placement of the IR sensor close to the heart or not on it may result in a weak signal, but with no interference (Figure 3B). Placement of the IR sensor directly on top of the heart will result in a very strong signal that can be easily analyzed for heartbeat (Figure 3C).

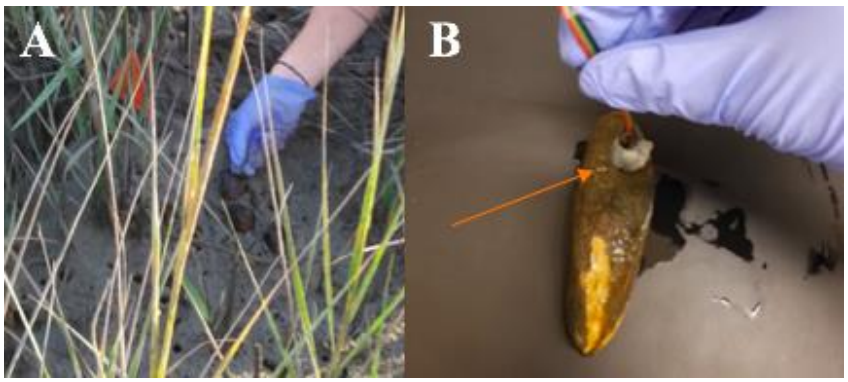

Figure 2A and B. Placement and orientation of mussels and the IR sensor on *Geukensia demissa* in the field (A) and in the lab (B).

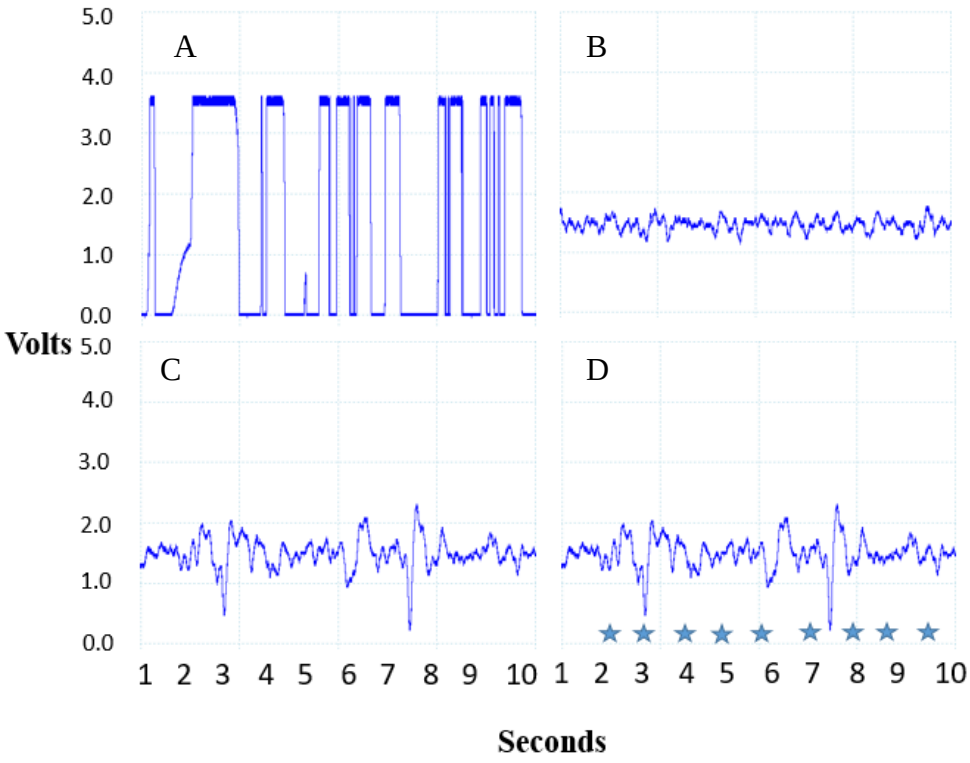

Figure 3A-D. Variation in heartbeat signal in *Geukensia demissa*. When the sensor is moved it produces interference in the heartbeat signal (A). Wrong placement of the IR sensor or placed not above the heart can result in a signal that is difficult to read (B). Placement of the IR sensor directly above the heart indicates a strong and regular signal (C). Each star represents a heartbeat, and shows how the number of heartbeats/min was estimated (D).

Laboratory Experiments

To simulate the elevated and reduced temperatures that mussels experience throughout the day in the salt marsh environment, the heart rate of mussels was recorded at a reduced temperature (~ 20°C), at an intermediate temperature (~30°C), and at an elevated temperature environment (~36°C). This laboratory experiment was first conducted in October of 2018 and then repeated in November 2018. The intermediate temperature experiment was conducted in April 2019.

For the low temperature experiment, the water in each tank was siphoned out and the mussels heart rate was recorded at ~20°C. For the elevated temperature experiment, the mussels were placed in a waterbath (30 x 15 x 15 cm). This waterbath was separated into three levels by Rubbermaid sink mats that were cut and fit to make three levels.

Mussels from the landlocked location were placed on the lowest level of the water bath, mussels from the high marsh were placed on the middle level, and mussels from mid marsh were placed on the topmost level. They were placed in an orientation that allowed the heartbeat to be collected with very little disturbance. The water was increased gradually from 20 to 36 degrees Celsius over the course of an hour and maintained for 30 minutes. After an hour and 30 minutes, the water level was siphoned out and lowered to expose the first 10 mussels to the air so the heart rate could be recorded. The heart rate was recorded for 1 minute/mussel and saved to a computer as a Picoscope file. This was then repeated for the mussels in the lower levels. All heartbeats were collected within 45 minutes.

Field Experiments

Heartbeat was measured in the field to compare the physiological stress response in situ with laboratory experiments. This process of collecting the mussel's heartbeat was emulated in the field in October of 2018 (21°C), April 2019 (20.5°C) and September 2019 (40°C)

Mussels were returned to the location they originated from, in plots previously marked at each location. Mussels from the high marsh, mid marsh, and the landlocked locations were placed in the substrate and left alone for 30 minutes to one hour. The mussels were placed upright in the mud, leaving up to three centimeters of the top of the

mussel visible to allow for placement of the IR sensor (Figure 2A). The heart rate was then individually recorded for all 30 mussels for one minute. The mussels from the high marsh were measured first, and the landlocked location was measured last because mussels remained submerged for a longer period of time at this location even when the tide has gone out.

During the collection of the heartbeat, the materials were housed in a waterproof container. The Picoscope and amplifier were connected to each other, and the cords and IR sensor were fed out of the container and linked to a laptop computer or placed on the mussel for in situ recording. There were two observers. One observer held the waterproof container and placed the sensor on each mussel. The second observer held the computer and ran the Picoscope program and saved each Picoscope file per mussel.

At the high marsh, mid marsh, and landlocked locations, the temperature was recorded using HOBO pendant MX Temperature/Light Data Logger and HOBO TidbiT MX 2203 Temperature 400' Data Logger.

Statistical analysis

The lab data was analyzed with a 2-way ANOVA with interaction. Temperature (elevated and low) and location (high, mid, and landlocked salt marsh) were fixed factors. To compare the field and lab data, a mixed model 3-way nested ANOVA was used. The fixed factors were the location (high, mid, and landlocked salt marsh), the temperature (elevated and low for laboratory and field experiments) and the type of experiment (lab and field). Mussels were a random factor nested within location. When differences were significant a Tukey HSD test was used to determine which treatments differed. Data were

initially screened for equality of variances and normality. All data were analyzed using JMP Pro 13.

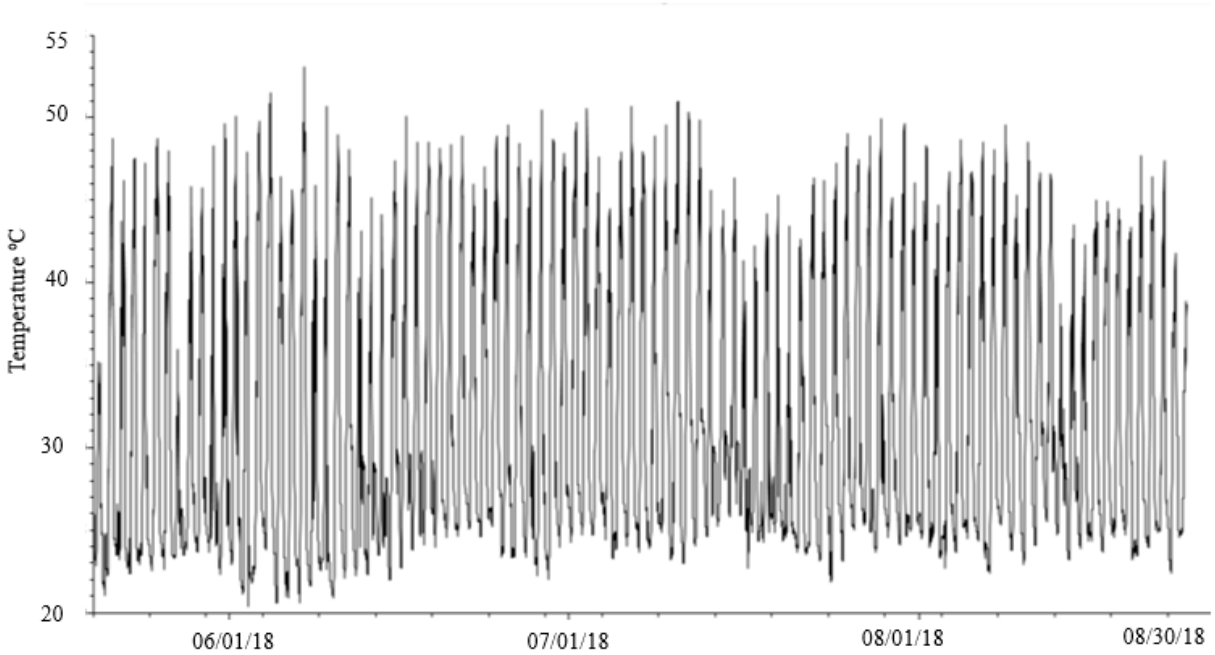

Figure 4A. Continuous recording of temperature from dataloggers installed close to the landlocked location in 2018. The highest air temperature recorded at this location was 54°C.

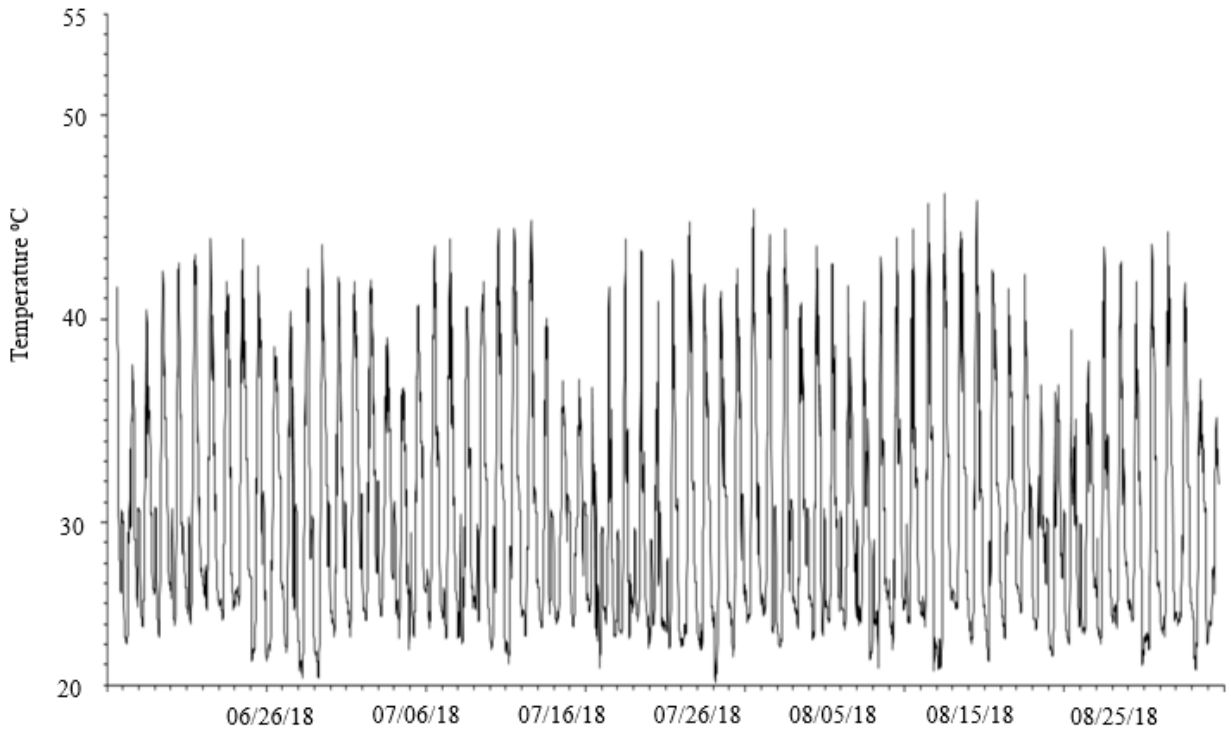

Figure 4B. Continuous recording of temperature from dataloggers installed close to the mid marsh location in 2018. The highest air temperature recorded at this location was 46°C.

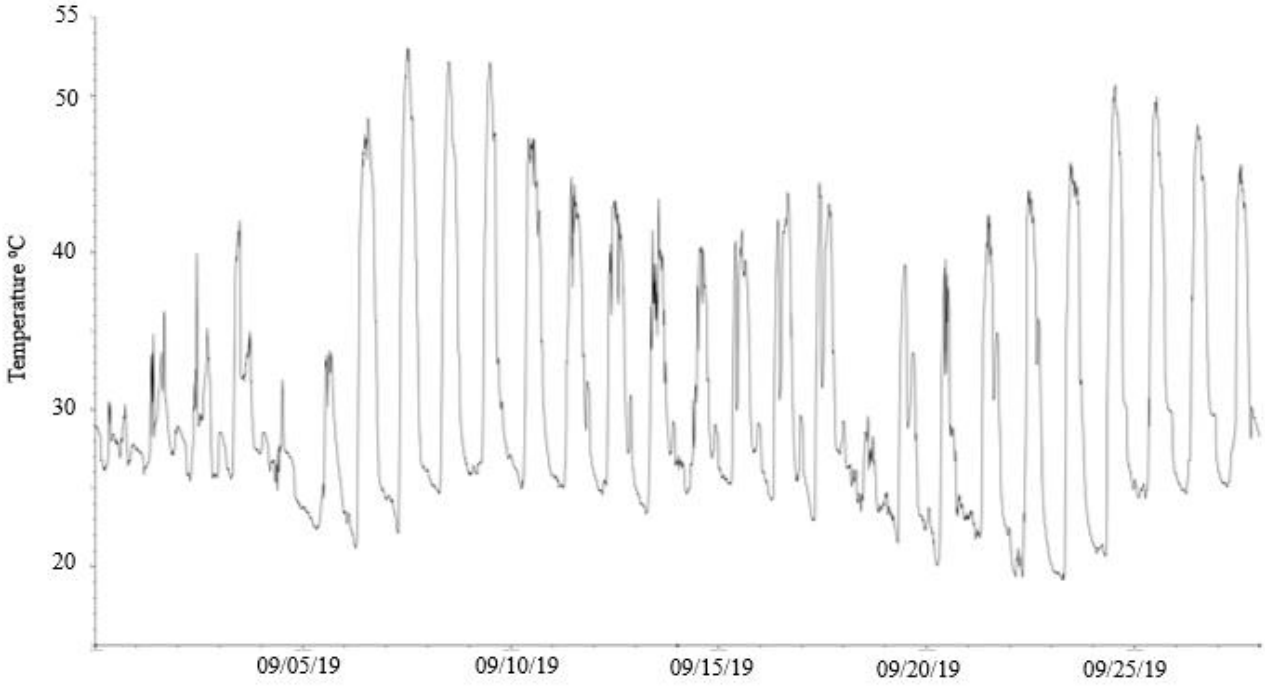

Figure 4C. Continuous recording of temperature from dataloggers installed close to the landlocked location in 2019. The highest air temperature was 54°C.

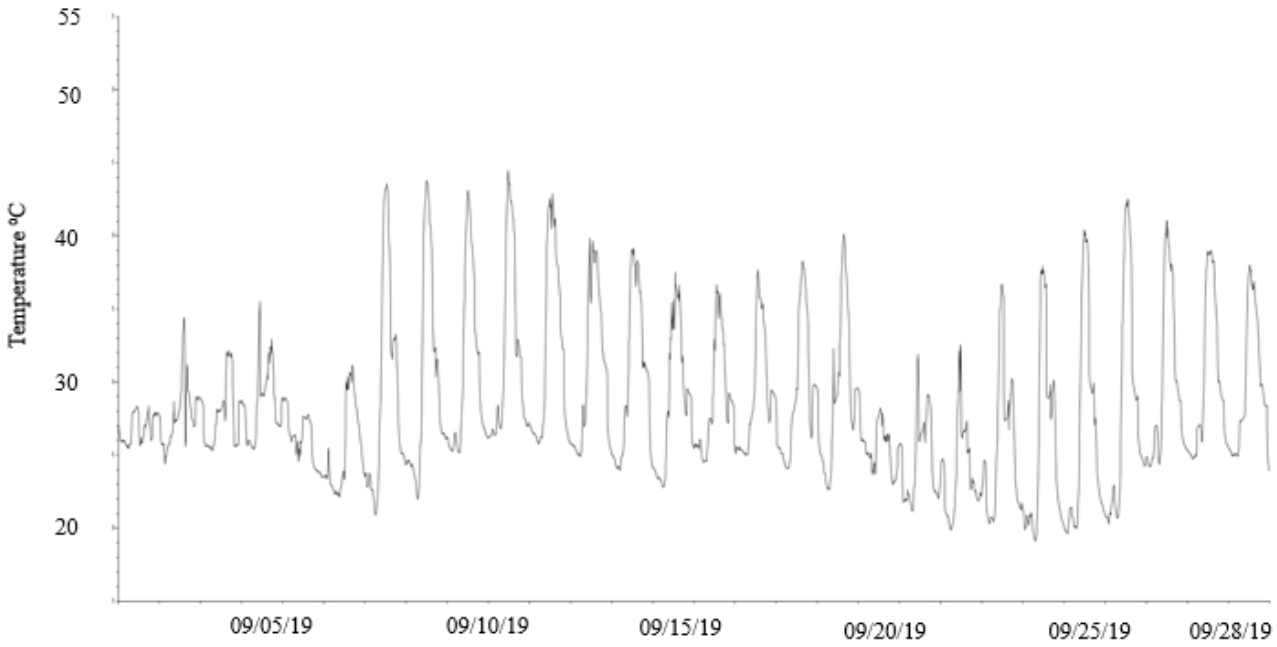

Figure 4D. Continuous recording of temperature from dataloggers installed close to the mid marsh location in 2019. The highest air temperature was 45°C.

Results

Temperature Measurements

The temperatures in the field fluctuated between 22°C and 43°C in the high and mid salt marsh and 22°C and 48°C for the landlocked location (Figure 4A to 4D). This >20°C fluctuation of temperature indicates a stressful environment for *G. demissa*. For the first two field experiments on October 26, 2018 and April 20, 2019, the temperature was ~21°C and ~20.5°C, respectively. On September 28, 2019, the temperature during field experiments was 40°C. In 2018 and September 2019, maximum day temperatures fell below 40°C during storms in 2019 (hurricane Dorian with maximum winds of 185mph September 5, 2019, and tropical storms mid-September with maximum sustained

winds of 50mph, NOAA). Maximum temperatures during this time fell below 30°C near the mid marsh and below 35°C at the landlocked location.

Laboratory experiments

Significant differences were observed among mussels from the high marsh, mid marsh, and landlocked location. Mussels from these locations were exposed to room (20°C), intermediate (30°C) and elevated (36°C) temperatures in the laboratory. As expected, the mean heart rates of the mussels in the high salt marsh and mid salt marsh showed a significant increase when exposed to an elevated temperature of 36°C (high marsh, reduced temperatures 51.3 beats/min, elevated temperatures 70.0 beats/min; mid marsh, reduced temperatures 49.3 beats/min, elevated temperatures 68.4 beats/min; $P < .0001$, Figure 5A). However, the mussels from the landlocked location did not show a significant change in mean heart rate at different temperatures (low temperature 54.8 beats/min, elevated temperature 55.4 beats/min, Figure 5A). When exposed to 20 or 30°C in the lab, mean heart rate did not differ significantly among mussels from the landlocked, high and mid marsh locations. Interestingly, heart rates of mussels were lowest at 30°C varying between 46.5-49.8 beats/min at all three locations. The largest variation in heart rate was observed for mussels from the landlocked location exposed to 20°C in the lab.

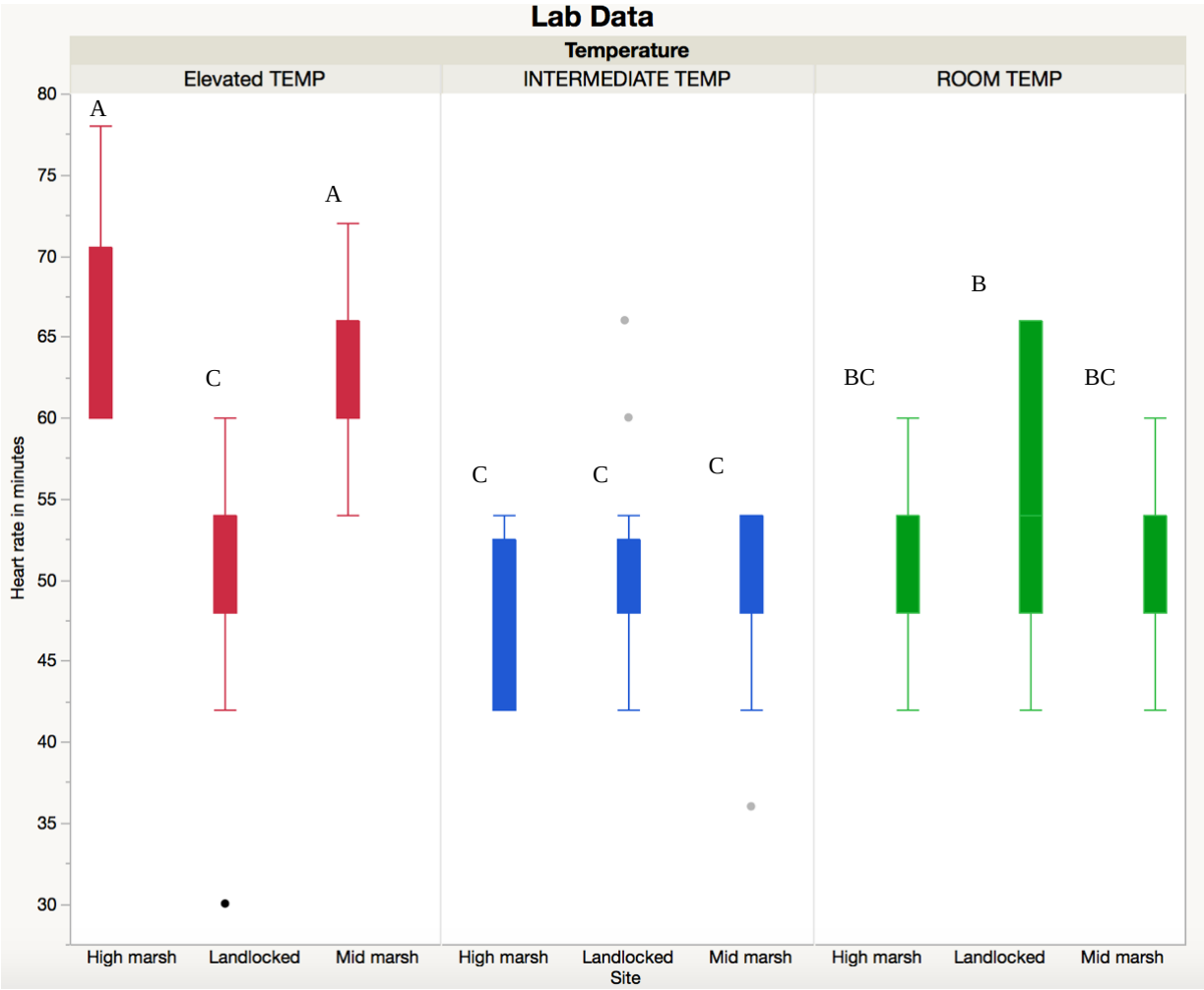

Figure 5A. Laboratory experiment: Box plots of heart rate per minute for ribbed mussels, *Geukensia demissa* from the landlocked, high marsh, and mid marsh locations off of old Tybee road, Tybee Island, Georgia. The box represents the interquartile range. If visible a line in the middle of the box is the median. Outliers outside the box are represented by closed circles and minimum and maximum values are shown. Ten mussels were used per location for a total of 30 mussels for the elevated temperature, 30 for the intermediate temperature, and 30 for room temperature. Different letters indicate significant differences among locations and temperatures (p = 0.05).

Field Experiments

As with the laboratory data, significant differences were observed among mussels from the landlocked, high marsh and mid marsh locations exposed to low (20°C) and elevated (40°C) temperatures. Overall, mussels from the landlocked location had significantly lower mean heart rate (47.6 beats/min) followed by mussels in the high

marsh (54.0 beats/min) and mussels in the mid marsh (68.5 beats/min, Figure 5B, $p = 0.05$). Mussels from the mid marsh had the highest mean heart rate at the elevated temperature (68.5 beats/min) and the lowest heart rate at the low temperature (46.2 beat/min). Mean heart rate for mussels from the high marsh and landlocked did not differ significantly. There was no difference in mean heart rate at elevated and low temperatures for mussels from the high marsh (elevated 54.0 beats/min, low 58.8 beats/min). The heart rate of mussels from the landlocked location (at elevated temperatures) was similar to those from the mid marsh at low temperatures (Figure 5B).

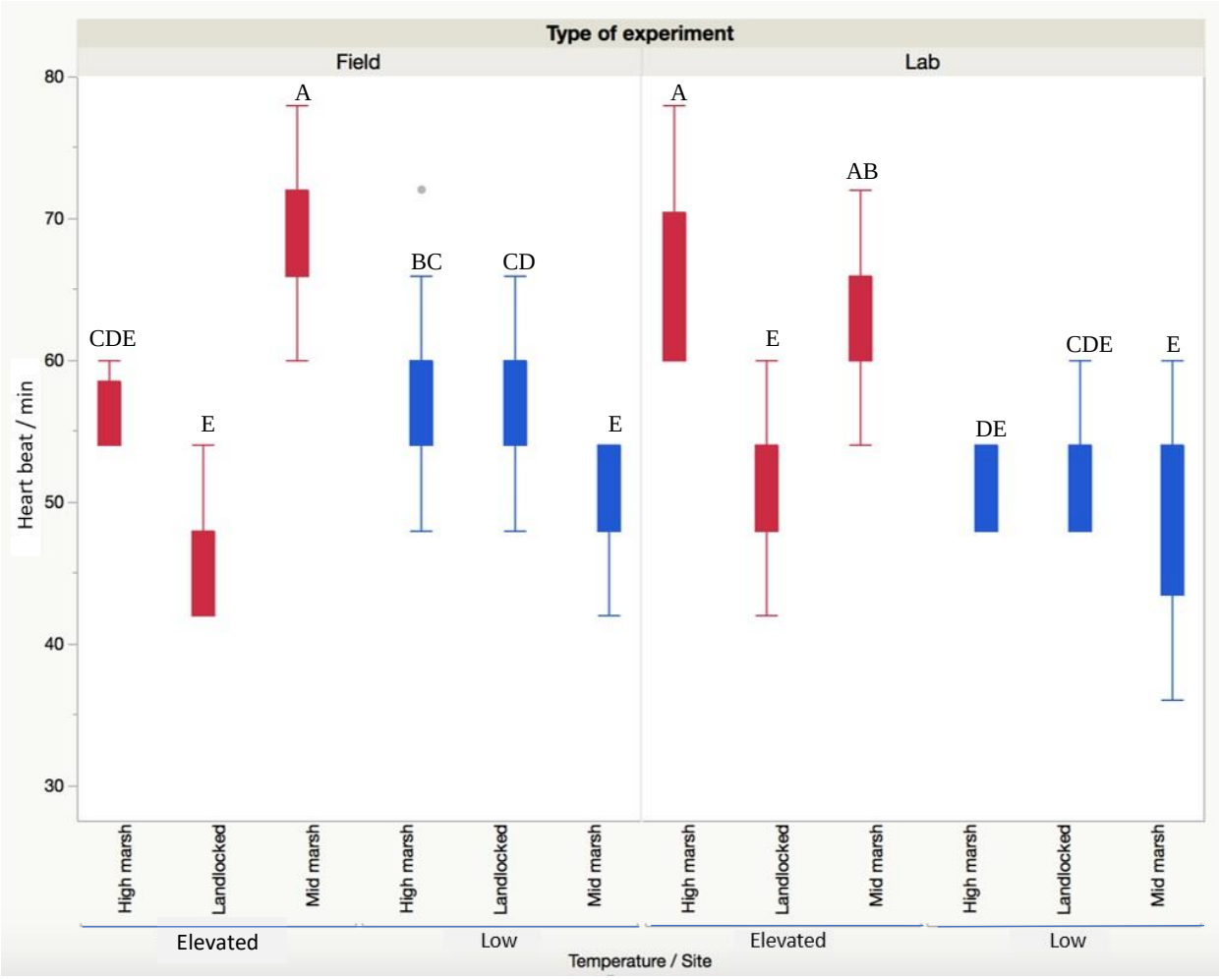

Figure 5B. Comparison between laboratory and field experiments: Box plots of heart rate per minute for ribbed mussels *Geukensia demissa* from the landlocked, high marsh, and mid marsh at Tybee Island, GA at elevated and low temperatures. The box represents the interquartile range. If visible a line in the middle of the box is the median. Outliers outside the box are represented by closed circles and minimum and maximum values are shown. Ten mussels per location were used, 30 for elevated and 30 for low temperature treatments. Different letters indicate significant differences among locations and temperatures (p = 0.05).

Laboratory and Field comparison

When field (experiments 1 and 3, 21 and 40°C respectively) and lab (experiment 2, 20 and 36°C respectively) data were compared, a three-way ANOVA showed no significant difference in the mean heart rate of mussels from field and lab experiments (p = 0.5455). There was significant interaction between location, temperature, and type of experiment (P < 0.0001. Figure 5B, Supplementary Table 6 appendix).

Mussels from the high and mid marsh had higher heart rates at elevated temperatures in the laboratory and in the field especially those from the mid marsh (lab: high marsh 65.7 beats/min, mid marsh 64.8; field: high marsh 54.0, mid marsh 68.5; Figure 5B). In the field, the high marsh and landlocked mussels responded to elevated and reduced temperatures in the same way as the high marsh, mid marsh, and landlocked mussels did to low temperatures in the laboratory (lab: landlocked mean heart rate: 53.4 beats/min, mid marsh 47.7 beats/min, high marsh 51.0 beats/min; field: reduced: landlocked 56.4 beats/min, high marsh 58.8 beats/min; field: elevated: landlocked 47.6, high marsh 54.0; Figure 5B). Mussels from the high marsh and mid marsh responded similarly to elevated temperatures in the laboratory (high marsh, 65.7 beats/min, mid marsh 64.8 beats/min). There was a significant interaction between location and temperature ($P < .0001$).

Interestingly, when field (experiments 1 and 3, 21 and 40°C respectively) and lab (experiments 2 and 3, 20 and 30°C respectively) data were compared, mean mussel heart rates did not vary among locations ($p = 0.1013$, Supplemental Figure 1, and Table 7). But there were significant differences between type of experiment (field or laboratory, Supplemental Figure 1, and Table 7). and between elevated and low temperatures ($p = 0.4960$, Figure 5B). There was a significant interaction between type of experiment (lab or field) location (landlocked, mid and high marsh) and temperature (elevated and low, $P < 0.0001$, Supplemental Figure 1, Table 7). The highest mean heart rate was recorded in situ for mussels in the mid marsh (68.5±1.6 beats per minute). The heart rate for these mussels was significantly higher than for mussels in all other treatments. Mean heart rate for mussels in the laboratory with the exception of a few cases tend to be significantly

lower than in the field ($P < 0.0001$, Supplemental Figure 1). In the field, values ranged from 46.5-49.2 beats per minute for mussels at elevated temperatures and 47.7 to 53.4 beats per minute for mussels at low temperatures. In the laboratory values ranged from 49.2 to 68.5 beats for mussels at intermediate temperatures and from 49.9 to 58.8 for field elevated temperatures. The lowest mean heart rates were recorded for mussels at elevated temperatures in the landlocked location and for mussels from the high marsh (46.5 and 47.7, respectively) (Supplemental Figure 1).

Discussion

Global temperatures are continuing to rise. Currently, temperatures have risen 1.5°C above pre-industrial levels (IPCC 2019). Salt marshes are one of the ecosystems susceptible to rising temperatures. Specifically, temperature and salinity are one of the main drivers of species distribution of intertidal species (Braby and Somero 2006). Thus, increasing temperatures is having a significant effect on the distribution, density, and interspecies relationships within the marsh. One of these organisms is the ribbed mussel *Geukensia demissa. G. demissa* is an intertidal and foundation species that experiences a wide range of temperatures from the daily immersion/emersion cycle as observed in the present study. The present study observed the physiological stress response in *G. demissa* from increasing aerial temperatures.

The locations chosen were relatively close in proximity. However, they were characterized by differing tidal flow, *Spartina alterniflora* heights and percent cover, temperature and density of mussel aggregations. Temperatures reached up to 48°C in the main marsh, and up to 54°C in the landlocked location. The landlocked location experienced the most consistent high temperatures, with the main marsh only

experiencing these high temperatures periodically. Due to the temperatures recorded at the three locations, we hypothesized that mussels from all locations will have an increased heart rate with increased temperature. Our results partially support this hypothesis. Our data indicated that mussels from the landlocked region had lower heart rates at high temperatures. Yet, mussels from the mid and high marsh had higher heart rates at elevated temperatures for laboratory experiments. In situ experiments showed that only mussels from the mid marsh had higher mean heart rates at elevated temperatures. The laboratory and in situ results of heart rate in response to higher temperatures in mussels from the mid and high marsh is indicative of a thermal stress response. On the other hand, our experiments never reached the maximum temperatures recorded by data loggers in the field. Thus, it is possible that mean heart rates for mussels from the mid and high marsh may have even higher heart rates when exposed to higher temperatures than observed in this study.

Variation of heart rate in situ

During the day, temperatures in the salt marsh fluctuate to values >20°C. This is due to the normal tidal regime and increasing temperatures as the sun rises. This is a stressful environment for the ribbed mussels, which is further exacerbated by the presence of a road.

Our data indicates that the heart rate of ribbed mussels from the mid marsh increased significantly at elevated temperatures. The mid marsh experiences the lowest temperatures, has the most consistent tidal flow, and is furthest from the road of the three locations. Thus, it is possible the mussels from the mid marsh had higher heart rates at elevated temperatures because they are the least adapted to these stressful conditions.

Other studies have found that the heart rate of mussels is strongly affected by the tidal regime and temperature, and that mussels either increase or decrease their heart rate dependent on their previous acclimation/acclimatization (Tagliarolo & McQuaid 2016, Huang 2015).

In the present study, the mussels in the high marsh and landlocked location had significantly lower heart rates than those from the mid marsh at elevated temperatures. Furthermore, heart rates for these mussels did not differ significantly when measured at elevated and low temperatures. Studies have shown that heart rates for some molluscs remain low when temperatures increase. For example, under heat wave conditions, the heart rate of *Mytilus galloprovincialis* decreased significantly (Olabarria et al. 2016). Likewise, the heart rate of the bay mussel *Mytilus trossulus* (Braby and Somero 2006) and that of the intertidal snail *Echinolittorina malaccana* (Marshall et al. 2011) decreased in response to increasing temperature. Olabarria et al. (2016) noted that the intertidal snail *E. malaccana* has a remarkable capacity for metabolic depression especially when temperatures are between 30 and 45°C. They noted that this might be a strategy aimed at lowering the metabolic rate, conserving energy and enhancing survival when exposed to warm air for prolonged periods. Furthermore, mussels that were previously adapted to lower temperatures were the ones that had the highest heart rates when exposed to higher temperatures (Braby and Somero 2006). Thus, *G. demissa* from the landlocked and high marsh locations may be using a similar strategy to cope with heat stress. This implies that the mussels from the high marsh and landlocked location must be physiologically adapted to higher temperatures, much like *M. galloprovincialis* in the Braby and Somero (2006)

study. This may indicate that *G. demissa* has the ability to evolve to higher temperatures from climate change.

Variation of heart rate in laboratory studies

Surprisingly, our data indicates that mussels from the landlocked location are the only ones that had lower heart rates in response to elevated temperatures in the laboratory. Interestingly, the landlocked location experiences the highest temperatures and most interrupted tidal flow. Thus, the mussels from this location experience the most stressful conditions, yet still have lower heart rates. Lower heart rates when exposed to higher temperatures has also been observed in the intertidal snail *E. malaccana* and the mussel *M. galloprovincialis* (Braby and Somero 2006, Olabarria et al. 2016). This aligns with the study by Braby and Somero (2006) in which *M. galloprovincialis* had a lower heart rate in response to higher temperatures, likely because it has historically been adapted to higher temperatures. Ribbed mussels from both the mid and high marsh had higher heart rates at 36°C. This implies that these mussels from the mid and high marsh may have not yet adapted a strategy to combat thermal stress like *E. malaccana* and *M. galloprvincialis* have.

Mussels from all three locations had similar heart rates at an intermediate temperature as they did at lower temperatures. This is suggestive that mussels can withstand temperatures up to 30°C. However, in this study only mussels from the high marsh and landlocked locations can deal with elevated temperatures by decreasing heart rates which may lead to lower metabolic rates and increased survival.

Higher heart rates in response to elevated temperatures is suggestive that mussels in the mid and high marsh cannot withstand elevated temperatures. However, a lower

heart rate from the mussels from the landlocked location is indicative that mussels have the ability to adapt to rising temperatures. Over time, mussels from all regions in the salt marsh may adapt to the increasing temperatures happening around the globe.

Comparison of in situ and laboratory studies

When comparing the laboratory and in situ data, the mid marsh had an increased heart rate at an elevated temperature for both field and laboratory experiments. However, mussels from the high marsh responded to elevated temperatures only in the laboratory. Recent studies have yielded results indicating that laboratory studies are not always reliable as they underestimate the heart rates of animals when compared to field experiments. Surprisingly, mussels exposed to high temperatures in the laboratory have a higher mortality rate than in the field when exposed to the same temperature (Tagliarolo & McQuaid 2016). Furthermore, other studies with Fire Island scallops observed higher heart rates in the lab than in the field at the same temperature (Gurr et al. 2018). This indicates that the higher heart rates observed in laboratory experiments in this study may be underestimating the true resilience to temperature of ribbed mussels in the field. Thus, it is a plausible explanation that mussels from the high marsh can withstand higher temperatures than expected in the lab. On the other hand, the mussels from the landlocked location had lower heart rate in response to higher temperatures in either experiment. Therefore, it can be predicted that landlocked mussels may withstand temperatures as high as 45°C. More studies are needed to determine whether they can withstand temperatures as high as 54°C. However, results for mussels from the high marsh at elevated and low temperatures were inconsistent when comparing laboratory and field studies. One explanation for this would be that the mussels measured in the lab

was a different group of mussels than the ones measured in the field. However, this does not explain why the mussels from the mid marsh and landlocked locations were consistent when comparing laboratory and field studies. More research is needed to explore these differences.

Further research

Our laboratory experiments confirmed that the Burnett et al. (2013) technique is an effective method to record cardiac activity in *G. demissa*. The measurement of heart rate in response to temperature for *G. demissa* is the first study of its kind in Georgia. However, there is more to be explored about the anthropogenic effects on the salt marsh and its organisms.

Firstly, in this study mussels were placed back in the salt marsh where they were easily accessible for collection of their heartbeat. However, there may be a difference in heart rate for mussels that are placed near the center of the mussel aggregate versus close to the edge where they are isolated from other individuals. Helmuth (1998) and Jurgens and Gaylord (2016) notes that mussels living in beds/large aggregates can experience lower temperatures than solitary mussels living close to the fringes when exposed to high rates of solar flux. Helmuth (1998) noted that the reverse could occur under low solar flux. Thus, further research should explore the differences of heart rate of ribbed mussels in the middle of the aggregate and on its fringes.

Secondly, a method needs to be developed where the IR sensor is glued to the outside of the mussel shell to collect the heartbeat. This would minimize human interference with the signal and yield a result that would be less influenced by handling.

Thirdly, it has been noted in other studies that air temperature is a poor predictor of an ectothermic animal's body temperature (Zippay and Helmuth 2012). In this study, only air temperature was recorded. Thus, the specific body temperature at which *G. demissa* may respond or lower their heart rate is still unknown. It could also be further explored as to why there are lower density aggregations of mussels in the landlocked and high marsh locations when compared to the mid marsh. While the mussels from the landlocked location and high marsh had a lower heart rate at higher temperatures, the number of mussels in the aggregates was very low. This could be due to the temperature being a primary driver in setting a species distribution (Zippay and Helmuth 2012). It is possible that this is why mussel densities have remained low in the locations regularly experiencing high temperatures.

Overall, this research could help quantify how rising temperatures will affect this delicate ecosystem. Increased number of culverts could help reduce negative effects on the marsh for mussels not yet adapted to higher temperatures.

Conclusions

It is important to understand how temperature, and more broadly climate change, is affecting salt marsh ecosystems. This study investigated how the cardiac activity of *Geukensia demissa* would change with reduced and elevated temperatures in both laboratory and in situ experiments. Our results were interesting and supported previous studies in that some intertidal species decrease their heart rate in response to higher temperatures. Results also indicated that laboratory studies may not be sufficient to predict the resilience of mussels to higher temperatures. Understanding how temperatures

affect heart rate could help quantify how these stressors are affecting the intertidal ecosystem.

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Appendix

Supplemental Table 1. Coordinates of 1m x 1m plots in the landlocked, high marsh, and mid marsh locations at Tybee Island, Georgia.

Coordinates of Plots (1m x 1m)								
Location	Plot 1		Plot 2		Plot 3		Plot 4	
	Latitude	Longitude	Latitude	Longitude	Latitude	Longitude	Latitude	Longitude
Landlocked	32.01475	-80.880117	32.014733	-80.8802	32.014717	-80.8803	32.014683	-80.880317
High marsh	32.013867	-80.880967	32.013883	-80.88085	32.0139	-80.880783	32.013917	-80.880783
Mid marsh	32.013767	-80.880733	32.013733	-80.88075	32.0137	-80.880783	32.013667	-80.88085

Supplemental Table 2. Tidal height in feet, time of low and high tides, and the moon rise and set at Old Tybee Rd., Tybee Island, GA for the three field experiments.

	Day			Tide height (ft)	Time		
October 2018	F	26	Low	3:54 AM	-0.1	7:35 AM	Set 9:22 AM
		26	High	9:33 AM	8.3	6:39 PM	Rise 8:26 PM
		26	Low	4:24 PM	0.2		
		26	High	9:57 PM	7.3		
April 2019	Sa	20	Low	3:49 AM	-1.1	6:49 AM	Set 7:51 AM
		20	High	9:23 AM	7.6	7:56 PM	Rise 9:27 PM
		20	Low	3:59 PM	-0.9		
		20	High	9:52 PM	8.3		
Setember 2019	Sa	28	Low	2:20 AM	-0.5	7:15 AM	Rise 6:55 AM
		28	High	8:05 AM	8.5	7:13 PM	Set 7:35 PM
		28	Low	2:46 PM	-0.8		

Supplemental Table 3. Average salinity (%) for the landlocked, high marsh, and mid marsh locations at Tybee Island, Georgia for 2018 and 2019.

Location	Average Salinity (%)
Landlocked	33
High Marsh	33
Mid marsh	34

Supplemental Table 4A. Size of collected mussels (mm) from the landlocked, high marsh, and mid marsh locations at Tybee Island, Georgia for the first laboratory and field experiments.

	Mussel Size (mm)		
	Landlocked	High Marsh	Mid Marsh
Mussel 1	95	103	105
Mussel 2	95	100	110
Mussel 3	102	105	100
Mussel 4	95	100	110
Mussel 5	101	105	95
Mussel 6	94	97	114
Mussel 7	100	105	105
Mussel 8	100	98	100
Mussel 9	95	104	98
Mussel 10	95	95	100

Supplemental Table 4B. Size of collected mussels (mm) from the landlocked, high marsh, and mid marsh locations at Tybee Island, Georgia for the second laboratory experiment.

	Mussel Size (mm)		
	Landlocked	High Marsh	Mid Marsh
Mussel 1	105	100	98
Mussel 2	98	97	95
Mussel 3	90	105	105
Mussel 4	95	105	106
Mussel 5	100	98	10
Mussel 6	95	104	102
Mussel 7	92	105	105
Mussel 8	100	100	99
Mussel 9	94	95	109
Mussel 10	95	95	10

Supplemental Table 4C. Size of collected mussels (mm) from the landlocked, high marsh, and mid marsh locations at Tybee Island, Georgia for the second and third laboratory experiment and the second field experiment.

	Mussel Size (mm)		
	Landlocked	High Marsh	Mid Marsh
Mussel 1	96	100	112
Mussel 2	94	95	110
Mussel 3	90	100	115
Mussel 4	91	115	102
Mussel 5	85	115	96
Mussel 6	90	93	99
Mussel 7	90	106	98
Mussel 8	96	110	109
Mussel 9	104	104	107
Mussel 10	96	102	105

Supplemental Table 4D. Size of collected mussels (mm) from the landlocked, high marsh, and mid marsh locations for the third field experiment.

	Mussel Size (mm)		
	Landlocked	High Marsh	Mid Marsh
Mussel 1	94	103	100
Mussel 2	98	112	100
Mussel 3	96	96	103
Mussel 4	98	100	105
Mussel 5	100	100	109
Mussel 6	90	103	112
Mussel 7	98	93	102
Mussel 8	97	96	107
Mussel 9	96	96	102
Mussel 10	96	108	110

Supplemental Table 5. Air temperature at Old Tybee Rd., Tybee Island, Georgia for the three separate field experiments.

Experiment	Temperature (°C)
Field 1	21
Field 2	20.5
Field 3	40

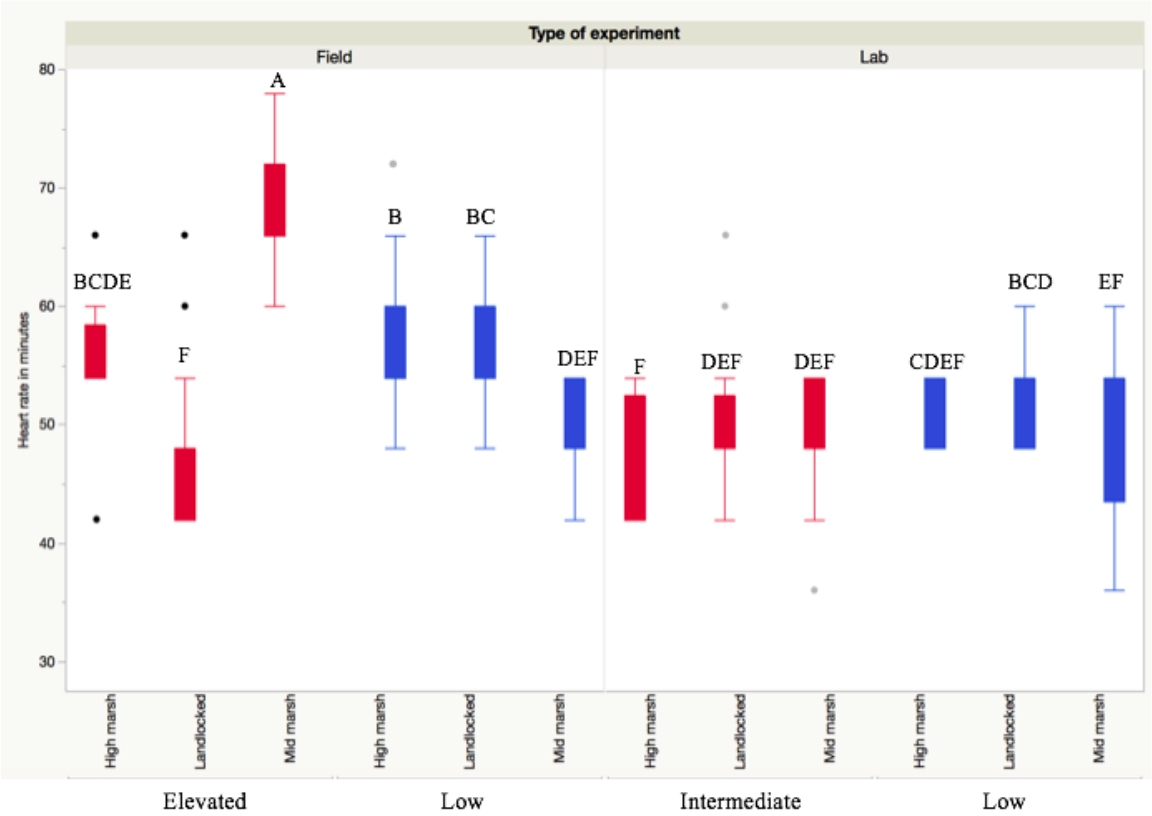
Supplemental Table 6. Three-way ANOVA of the heart rate of the ribbed mussel *Geukensia demissa* collected from three locations (landlocked, mid marsh, and high marsh) and exposed to low and elevated temperatures in the laboratory and the field at Tybee Island, Georgia. N=221. Data used were from experiments 2 (20°C) and 3 (30°C) in the lab and experiments 1 (21°C) and 3 (40°C) in the field.

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Type of experiment	1	1	12.1325	0.3667	0.5455
Temperature	1	1	1640.3869	49.5837	<.0001*
Site	2	2	1632.1294	24.6670	<.0001*
Type of experiment*Temperature	1	1	792.6274	23.9586	<.0001*
Type of experiment*Site	2	2	204.4592	3.0901	0.0476*
Type of experiment*Site*Temperature	2	2	987.8354	14.9296	<.0001*
Site*Temperature	2	2	5226.7771	78.9944	<.0001*

Supplemental Table 7. Three-way ANOVA of the heart rate of the ribbed mussel *Geukensia demissa* collected from three locations (landlocked, mid marsh and high marsh) exposed to low and elevated temperatures in the laboratory and the field, at Tybee Island, Georgia. N=221. Data used were from experiments 2 (20 and 36°C respectively) in the lab and experiments 1 (21°C) and 3 (40°C) in the field.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Type of experiment	1	1	2272.5152	77.7827	<.0001*
Temperature	1	1	13.5870	0.4650	0.4960
Site	2	2	135.2705	2.3150	0.1013
Type of experiment*Temperature	1	1	257.0923	8.7996	0.0034*
Type of experiment*Site	2	2	1040.7335	17.8109	<.0001*
Type of experiment*Site*Temperature	2	2	1264.7222	21.6442	<.0001*
Site*Temperature	2	2	2713.3857	46.4363	<.0001*



Supplemental Figure 1. Comparison between second laboratory and first and third field experiments: Box plots of heart rate per minute for ribbed mussels *Geukensia demissa* form the landlocked, high marsh, and mid marsh at Tybee Island, Georgia at elevated and low temperatures. The box represents the interquartile range. If visible a line in the middle of the box is the median. Outliers outside the box are represented by closed circles and minimum and maximum values are shown. Ten mussels per location were used, 30 for elevated and 30 for low temperature treatments. Different letters indicate significant differences among locations and temperatures (p= 0.05).