Determining the Thermal Tolerance of the Stage V Larvae of the *Homarus americanus*

by

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Abstract:

In response to warming water temperatures in the Gulf of Maine, this experiment attempts to define the thermal tolerance of Stage IV and Stage V *Homarus americanus* in order to test for an ontogenetic shift between the tolerances. I measured oxygen consumption in active and resting trials of Stage IV and Stage V *Homarus americanus* larvae to determine the average aerobic scope of each stage from 4℃ to 32℃. The Stage V larvae had twice the aerobic scope between 6℃ and 16℃ compared to that of the Stage IV's. This supports the hypothesis that the larvae of *H. americanus* are more cold tolerant in Stage V.

Introduction:

Increasing ocean temperatures have continued to diminish marine habitats and fishing industries. As ocean temperatures across the globe continues to rise the need to determine marine responses to these changes has increased. Water temperatures in the Gulf of Maine are rising faster than 98% of the world's oceans (Pershing et al. 2015). The New England coastlines warming in water temperatures has outpaced that of the North American region (Pershing et al. 2015), with potentially detrimental effects upon the local lobstering industry. Though it is expected that marine organisms in this region are either adapting or dying off, there is still little evidence that links physiological phenomenon and ecosystem level changes (Pörtner 2012). Marine organisms have very specific critical temperatures (T_c) (Frederich & Pörtner 2000) which define thermal tolerance windows for each organism. The thermal tolerance windows are species specific and the upper and lower T_c, and are signaled by the onset of anerobic metabolism or death of the organism (Frederich & Pörtner 2000). Prior to the critical temperatures are pejus (meaning turning for the worst) temperatures (T_p) which occur prior to the lethal temperatures but are the

onset of aerobic stress (Pörtner 2012). Changing water temperatures, or inconsistent temperatures can lead to thermal stress on marine organisms. Aerobic scope allows for the determination of thermal stress of marine organisms; scope is the difference between the active and resting oxygen consumption of the organisms. As you approach the higher and lower critical temperatures, scope values will drastically decrease. In order to maintain a steady aerobic scope, the organisms must remain within their respective T_p (Pörtner 2012). When this is not possible, due to warming temperatures, marine organisms must use more energy to compensate and protect themselves from thermal stress. Stages of lower aerobic scope do not allow for growth or reproduction, thus the overall scope decreases as the organism must accommodate to overall performance losses as they exposed to pejus temperatures or T_c (Pörtner 2012). The relationship between temperature and aerobic scope allows for the understanding of thermal adaptation in marine organisms (Fitzgibbon et al. 2014).

As water temperatures moves from ambient temperatures towards critical temperatures, there is an increased exposure of marine organisms to the pejus and critical temperatures (Quinn 2017). Corresponding to increasing water temperatures in the Gulf of Maine, the number of larvae of *Homarus americanus* settling in nurseries has decreased in both density and distribution (Wahle et al. 2015). *H. americanus* has several larval stages and a post-larvae stage, with Stages I-III as larvae, and IV are post-larvae. Stages I-IV are planktonic and they molt shortly after settling on the bottom and are considered early benthic phase in Stages V and VI (Lawton & Karl. L. Lavalli 1995). The post-larvae stage are highly temperature dependent; they have the ability to sense and respond to temperature changes within the environment and will avoid depths where the water temperatures are less than 12℃ (Annis 2005). Stage IV larvae experience thermal stress when exposed to temperatures below 12℃ for extended periods of time (Quinn 2017).

This study focused on short term exposure to extreme temperatures as we know that short term exposure can exert sub-lethal stress upon *H. americanus* (Quinn 2017) but the exact point of stress for each stage of development has not been fully mapped out yet. We hypothesized that the Stage V larvae will have a higher tolerance at cold stress than Stage IV, due to their settling on the seafloor where water temperatures are colder. However, the existence these sensitivities are well not established; there is still a lack of research into the effects of the warming of water temperatures upon pejus temperatures and thermal tolerance windows.

The goal of my research was to determine the aerobic scope of Stage IV and Stage V larvae as a function of temperature. I measured oxygen consumption, over a range of experimental temperatures both at rest and actively swimming. Aerobic scope and oxygen consumption values were used to determine the thermal tolerance of the larvae in each stage, as well as upper and lower pejus temperatures and the significance in the ontogenetic shift of the thermal tolerance between Stage IV and Stage V larva.

Methodology:

Oxygen consumption

The methodology used to determine oxygen consumption was derived from Waller et. al 2016. We used closed-cell micro-respirometry for the trials. We did not use the electrode microsensors form Waller et. al 2016 and instead used optical microsensors.

Stage IV larvae (236 in total) were captured near Boothbay Harbor, ME using a neuston net. They were then overnight shipped to Maryland where they were kept in dark conditions at 18°C, using 32 PSU *Instant Ocean* seawater (all experiments were also run in this artificial seawater).

The larvae were fed fresh hatched brine shrimp until they molted to Stage V or were used in oxygen consumption trials.

Oxygen consumption of individuals was measured using micro-respirometry as rest as well as active over a temperature range of 4℃ to 32℃. The difference between the two consumptions values were then used to determine the aerobic scope of each individual. Control chambers were run for each trial with identical screen/no screen set-ups within the chambers but without larvae. The *Unisense MicroOptode* optical oxygen sensors were calibrated at each treatment temperature, with a two-point system (0% and 100%) for each experimental run. The 0% chamber contained 0.1 M NaOH/Ascorbic acid and 32 PSU seawater and the 100% was purely 32 PSU seawater. The two calibration chambers were placed into the experimental temperature bath and used to allow the oxygen signal to stabilize and then calibrated to this value. Each larva was run in either an active or a resting trial, not both. Each chamber contained a stir bar. In the resting trials, a metal screen was placed in the bottom of the chamber and allowed for the protection of the larva from the stir bar. The active trials did not contain the metal screen, and the stir bar stimulated larvae to swim (if they managed to escape the stir bar the chamber was tapped against the wall of the chamber to get the larva back into the path of the stir bar).

Data were logged using *Rate* software. For each trial both (2 per trial) larvae were put into respiration chambers (approximately 40 ml of artificial seawater) and placed into the temperature bath for acclimation for 10 minutes. Oxygen saturation for each trial was than 80%. Two other chambers were aerated for 10 minutes in the same temperature bath (to prevent over/under saturation of oxygen during the trials). All 4 chambers were placed in the bath simultaneously. The larvae were transferred from the acclimation chambers to the trail chambers, and immediately returned to the water baths and covered by aluminum foil (to minimized light and

external visual stimuli). The oxygen consumption in each chamber was measured for 20 minutes and *Rate* software was used to calculate the slopes of the oxygen consumption over the last 10 minutes.

Length-Weight Relationship

The oxygen consumption values were adjusted to per mass unit for the individual larvae to normalize them and the aerobic scope slopes were averaged to create a standardized scope value for each experimental temperature, for each stage of larvae. We determined dry weight using the following methods. Aluminum weight boats were pre-weighed. Each sample was thawed and then placed in a drying oven at 40°C for forty-eight hours. The larvae and the weigh boats were then weighed again, and subtracted from the original weight to determine the weight of each sample. We also determined the carapace length of each larvae after oxygen consumption trials using photographs of each larvae. ImageJ software was used to determine the length of the carapace of each larva.

Statistical analysis

To determine whether or not there was a significant difference between the aerobic scope of the Stage IV and Stage V larvae, from 6°C to 16°C, we performed a two-tailed t-test, assuming unequal variance.

Results:

Oxygen consumption in the Stage IV larvae peaked at 26°C for the active trials and at approximately 32°C in the resting trials (Figure 1). For the lower temperatures (4°C to 10°C) the oxygen consumption of the resting trials was similar to that of the active trials. Over this period

the active trials had an average consumption of approximately 20 mmol/mg/h and the resting trials had an average of 10 mmol/mg/h (Figure 1). After 12°C the average oxygen consumption between the active and resting trials deviated and varied until 30°C. At this point, consumption for active trials had peaked and began to fall, whereas the consumption in the resting trials was peaking. The average consumption at 32°C for the active and resting trials were also similar as they had been at the lower temperature extremes.

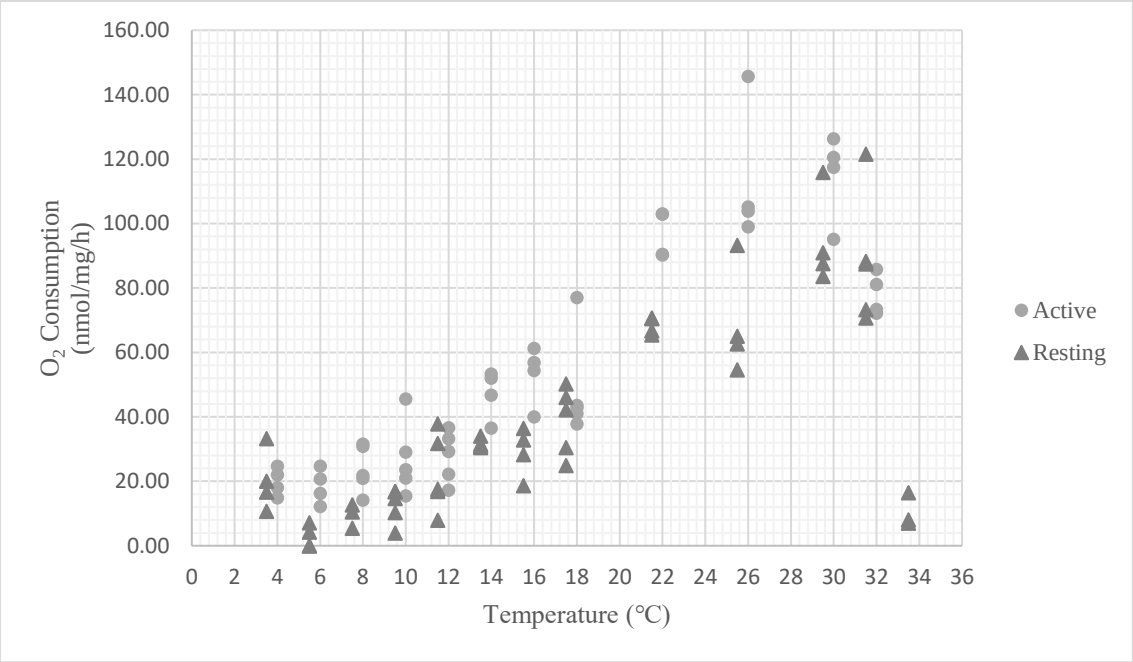

Figure 1. Oxygen consumption in stage IV *Homarus americanus* larvae. Each data point is representative of one larva, and each point was standardized to the dry weight of individuals. Resting data points are offset by -0.5°C to avoid overlap. n=3.5

Oxygen consumption in the Stage V larvae peaked at 30°C for the active trials and at 26°C and 34°C for the resting trials (Figure 2). There was more separation between the consumption of the active and resting trials at both the lower and higher temperature ranges. The active consumption in the Stage V larvae in the lower temperature range (4°C to 10°C) is higher than that of the Stage IV larvae as well as the Stage V larvae in the 18°C to 32°C trials. Oxygen consumption of

individuals in the active Stage V trials varied more between 22°C and 30°C than that of the resting trial individuals (Figure 2). The average consumption for the active Stage V's is approximately 50 mmol/mg/h were as for the Stage IV's it was 20 mmol/mg/h (Figures 1 and 2).

The average dry weight of the Stage IV larvae was 23 mg ±0.03 (S.D.) and the Stage V larvae was 30 mg ±0.04 (S.D.). There was no significant trend in the length weight relationship for either the Stage IV or Stage V larvae.

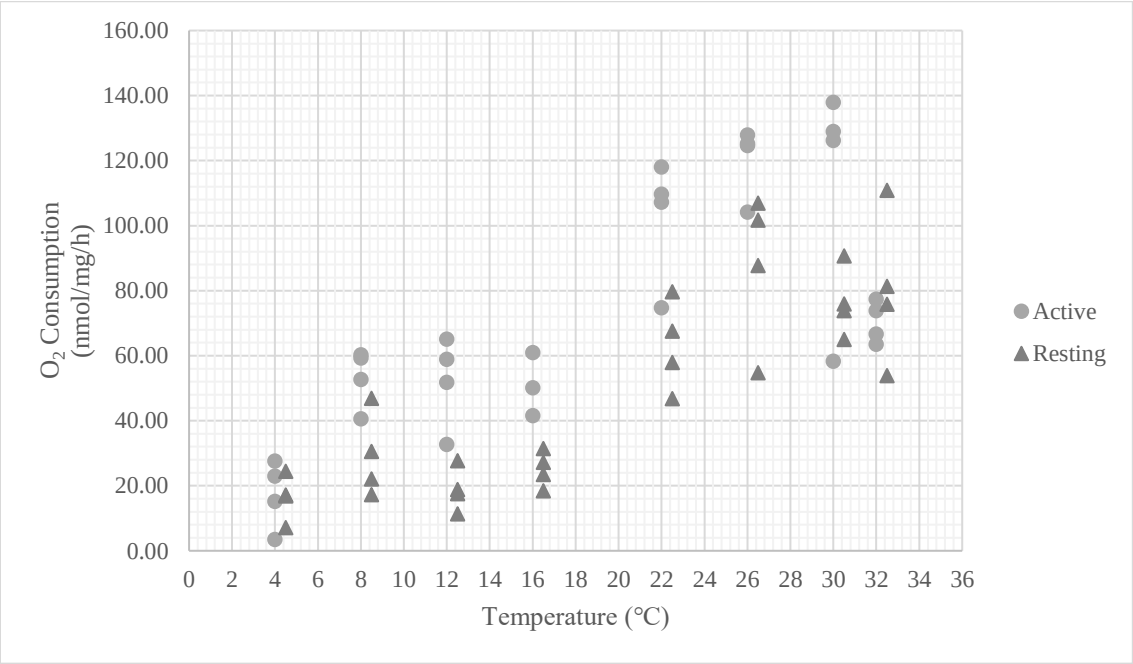

Figure 2. Oxygen consumption in stage V *Homarus americanus* larvae. Each data point is representative of one larva, and each point was standardized to the dry weight of individuals. Resting data points are offset by -0.5°C to avoid overlap. n=3.5

The overall aerobic scope of the Stage V larvae was consistently higher than that of the Stage IV larvae (Figure 3). For the colder temperatures, Stage IV's average scope went below 0 mmol/mg/h at 4°C (Figure 3). This is consistent with the lower oxygen consumption of the resting and active Stage IV's at and below 8°C. The Stage V average scope went to zero at 4°C, which was a 25 mmol/mg/h drop from the average scope at 8°C (Figure 3). At 8°C, the scope for

the Stage V (25 mmol/mg/h) was the same as that at 16°C (Figure 3) whereas the Stage IV at 8°C has an average scope of 10 mmol/mg/h but at 16°C had an average scope of 25 mmol/mg/h. For the higher temperatures, the Stage IV and Stage V average scope went below zero at 32°C (Figure 3). Between 12°C and 30°C, the Stage V larvae maintained an average scope between 30 to 40 mmol/mg/h whereas the Stage IV larvae average scope varied between ~15 mmol/mg/h and 45 mmol/mg/h. In the middle of the experimental temperatures, from 6°C to 16°C, the average scope of the Stage V larvae is approximately two times higher than that of the Stage IV larvae. The T-test yielded a p-value of 0.02, so we reject the null hypothesis and can determine that this two-fold scope in the Stage V larvae is not due to chance but rather to a higher thermal tolerance at lower temperatures of the Stage V larvae.

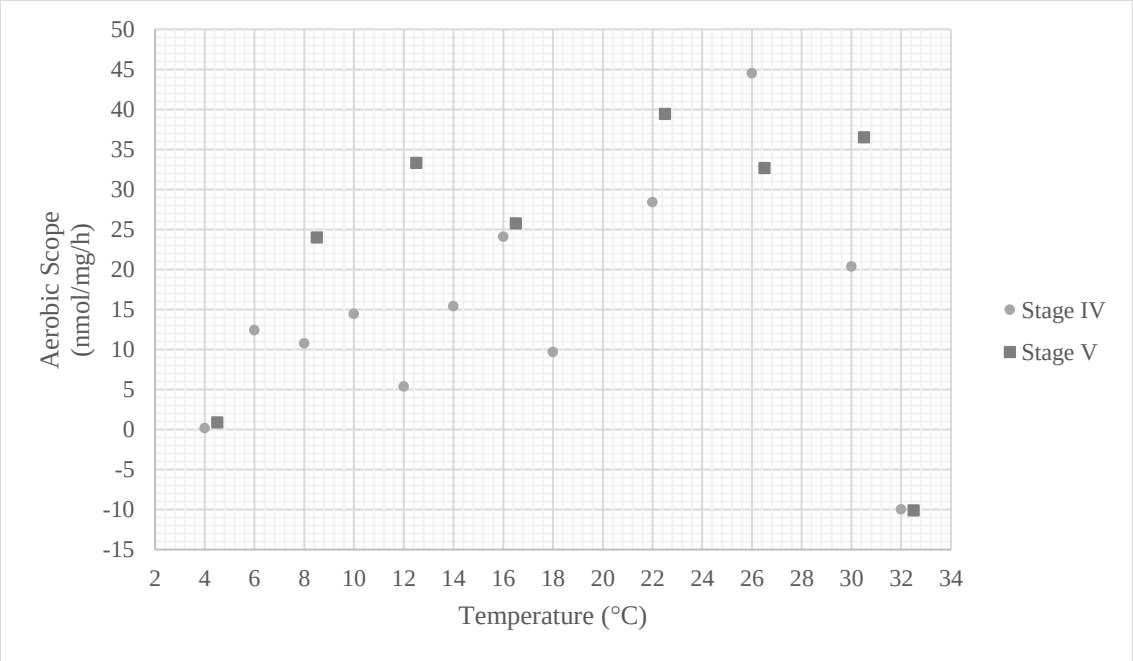

Figure 3. Aerobic scope of stage IV and stage V *Homarus americanus* larvae. The stage V data points are offset by -0.5°C to prevent overlap. n=3.5

Discussion:

There was an ontogenetic shift between the Stage IV and Stage V with the Stage V larvae having twice the aerobic scope at low temperatures than the Stage IV larvae. These results support the hypothesis that Stage V larvae have a higher tolerance for colder temperatures. The higher the aerobic scope of a larva, the greater its metabolic capacity. If an individual has a greater metabolic capacity, then they likely have higher tolerance to thermal stress. This tolerance allows for adaptation to short-term changes in ambient water temperatures.

Stage IV settle in late July to late September (Cobb et al. 1983) and are found in the top 1 meter of the water column (Annis 2005). They are therefore exposed to warmer temperatures before settling, and often are in ambient temperatures of approximately 12°C. Shortly after settling, the Stage IV molt to Stage V as the bottom temperatures continue to decrease into the fall. Thus, the observed ontogenetic shift is what is expected as *H. americanus* exhibits this transition from warmer to colder temperatures as the summer changes to fall.

The scope for both Stage IV and V either reached zero or went negative at the 4°C and 32°C indicating likely lower and upper T_c respectively (Figure 3). Stage V larvae survive in temperatures colder than 4°C in the winter (Quinn 2017) however when being taken from the 18°C ambient environment to the 4°C treatment, the scope drops to zero. MacKenzie 1988 previously demonstrated that the survival of Stage IV larvae is significantly lower at 10°C than at 12, 14 and 17°C. The scope for Stage IV drastically drops around 14°C (Figure 3) whereas the decline is much steadier in the Stage V larvae indicating higher tolerance of lower temperatures in the Stage V's. The average scope at the higher temperatures was higher for that of the Stage IV then the Stage V. This could suggest a possible higher pejus threshold in warmer temperatures, that is lost during molting to Stage V.

Despite the shared critical temperatures, the Stage IV and Stage V larva appeared to have difference in their pejus temperatures. Quinn 2017 previously determined that the optimal temperature range for the Stage IV larvae is 6.7℃ to 23.8℃. Both Stage IV and V seem to hit the "turning for the worse point" between 26℃ and 30℃ (Figure 3). For the lower temperatures turning points, the decline in scope was between 14℃ and 16℃ for the Stage IV's and 4℃ and 8℃ for the Stage V's. This implies that though the critical temperatures are the same, the tolerance as temperatures grow closer to the critical points are not consistent within the two stages.

The most likely source of error is due to larva swimming during the resting oxygen consumption trials or avoiding swimming by avoiding the stir bar during the active trials such as the Stage IV larvae at 18℃ (Figure 3).

In future research, this experiment would need to be performed with chronic exposures to extreme temperatures, rather than just acute temperature treatments. As well as this, the connection between chronic exposure to pejus temperatures/critical temperatures and growth and mortality of the different stages needs to be established. This will give a baseline of the complete metabolic performance of the larvae. It is expected that if the scope declines at the pejus temperatures then there would be longer temperature impairment in metabolic performance when continually exposed to pejus and critical temperatures.

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