Introduction

In recent years a trend of hypoxic water events have been documented along the west coast of the United States of America, posing a threat to the crab industry (Foden-Vencil, 2020). Increased hypoxia in these coastal waters has been linked to declines in overall catch for crabbing companies, decreasing their revenue. The source of the increased hypoxia is caused by the effects of climate change, specifically changes in water transport and eutrophication. Changes in temperature has resulted in wind pattern changes, impacting upwelling. When the upwelling of cold water occurs it brings hypoxic water from the deep to more shallow waters. With changing wind patterns there has been a trend of increased upwelling of hypoxic waters; a pattern that has been occurring for decades (Barth et al, 2024). Climate change has also led to the growth of algal blooms during the spring and summer. Growth and the subsequent die-off of algae leads to organic matter sinking in the water column. The process of the algae being decomposed consumes oxygen, decreasing dissolved oxygen in the water (Anderson et al, 2021). Together these changes to the coastal waters are leading to more hypoxic waters, threatening viability of organisms in these waters.

Through observation and input from those in the crab industry, the decline in crabs seems to be linked to the timing of increased hypoxia (Foden-Vencil, 2020). To test this possible link we used *Hemigrapsus oregonensis* (hairy shore crabs) as a model organism to determine if hypoxic waters have an impact on respiration, energy levels, stress, and mortality (Holland & Leonard, 2020). Finding out how these physiological factors respond to hypoxia can provide insight to population dynamics which could inform management strategies for the crab industry.

Methods

Hypoxia can occur in varying intensities. We wanted to test how the hairy shore crabs are affected by mild hypoxia in their natural environment of the intertidal and in the more extreme hypoxic deepwaters. To simulate these conditions we had two polycarbonate food storage containers filled with water and placed in a water table set to 13°C. The water in the containers themselves were maintained at the same temperature as the table and had a salinity of 35ppt and a starting O_2 concentration of about 7-8ppm. In both containers shells were placed to mimic their natural environment. There was also a control tank with all the previously listed variables except it contained an airstone, where the experimental containers did not. In the intertidal and deepwater treatment, nine crabs were placed in each.

The deepwater container was sealed with masking tape and a plug was placed inside a hole on the side of the container to prevent oxygen from entering. Before sealing completely we left one hole on the lid exposed and used a tube connected to a vacuum nozzle to suck air out for 30 seconds, then quickly removed the tube and taped the hole shut. As a note, after one week half of the crabs were removed for tests, then placed back into the containers. The air removal step was done again, but this time we sealed the container with duct tape for a stronger seal.

The intertidal container was not sealed and all the holes in both the lid and the container were left open. This container also had a mesh plastic ramp to allow the crabs to access the air above the water like they would have in the intertidal. The experiment lasted two weeks with non-lethal tests being performed on four crabs from each container after one week. The water and contents of the containers remained the same other than the tape on the deepwater container as noted before.

After the first week we performed righting and resazurin tests on the four individuals from each container and labeled them with nail polish on their carapace. These two non-lethal tests were to perform as a baseline to see if the experience of going through these tests was the cause of physiological changes and stress behavior or if it was the hypoxia. In other words we wanted to test if

the crabs tested one week in had dramatically different results compared to those only tested on week 2.

On week one the two tests performed were a righting test and resazurin assay. For the righting test we placed a crab in an empty container, flipped it to have its carapace touching the bottom of the container (on its back) and timed how long it took the crab to flip back over.

The resazurin assay was done by first drying off and weighting each individual. They were then placed in individual beakers containing 35mL of resazurin working solution. The working solution was made in a large batch, so for more simple ratios the process is as follows: to make 10mL of resazurin stock solution 0.5g of resazurin salt, 10mL DI water, and 10µL DMSO are combined. This stock solution is then used to make the working solution that is placed in the beakers. To make 150mL of working solution, 333µL of resazurin stock solution is combined with 148mL of seawater (23-25 ppt), 150µL DMSO, and 1.5mL of antibiotic solution 100x Penn/Strep and 100x Fungizone. Once the crabs were placed in the beaker with the resazurin working solution the beaker was covered with tin foil and a timer was started. After 30, 60, and 90 minutes 200ul of the solution was drawn out of the beaker using a micropipette. This was placed into wells on a plate to be then run on a leader at Excitation 530 and Emission 590 to get fluorescence values.

After two weeks all living crabs were put through the righting test and resazurin assay as well as two lethal tests, examination of gill tissue and hemolymph extraction. The hemolymph extraction was done first on any living crabs. A fine needle syringe was inserted at the base of a leg between the carapace and the leg. Only a small, unmeasured amount was extracted; enough to make the leg go stiff but not enough where it's visible above the tip of the syringe. The hemolymph was then frozen and used to get lactate concentrations. Before the dissections for gill tissues any crabs that were alive were quickly euthanized by snipping with scissors directly between its eyes. The carapace was then removed with tweezers to expose the gill tissue. After examining every crab there were five conditions of the tissue seen. Each crab was either labeled good, bad, partial, pink, or unusable. We determined these categorizations based on appearance and consultation with a crab expert, Andy Nutzhorn. Bad gill tissue was very dark and showing atrophy, good was yellow and orange in color, partial was tissue that had both yellow and some dark sections, pink was tissue that was dyed due to resazurin, and unusable was for crabs that had tissue that we could not examine due to damage. Figure 1 provides visual examples of the different gill tissue conditions.

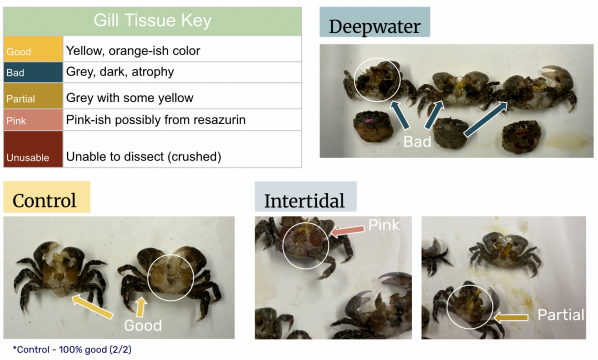


Figure 1. Key to gill tissue condition categorization and example photos of these conditions. The key on the top left gives descriptors of what each condition looks like and correlates to fig. 5a and fig. 5b. The key has a 'good' category that wasn't seen in fig. 5 due to there being two control crabs both with good tissue (100% good). Circles on photos indicate where the gill tissue is located and arrows are colored according to their condition and labeled for further clarification of each example.

Results

Reighting Time

After one week there was no significant difference between the righting of the intertidal and deepwater group (fig.2). The control group data available to us was from week zero which also did not have a significant difference compared to the experimental groups. After week two there was 100% mortality of the deepwater group, so the only data collected was from the intertidal group.

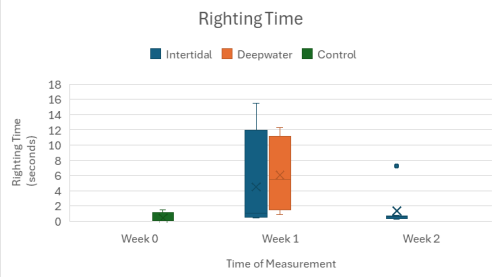


Figure 2. Graph of righting times in seconds from week 1 compared to week 2. No significant difference between treatments in week one. Complete mortality in deepwater week 2 led to no data.

Resazurin

After receiving the fluorescence data back from the resazurin tests we divided it by body mass to normalize the data. Compared to the control group both hypoxia, or experimental groups had higher rates of respiration (fluorescence/body mass (g)). Relative to each other the intertidal group and the deepwater had relatively similar rates of respiration (fig. 3a). In Figure 3a the respiration rates were the average of the group which is the combined average of the individuals across both weeks. In Figure 3b, to look more closely at differences in respiration from week one compared to week two we can see in week two the intertidal group had lower respiration rates than in week one. Due to the 100% mortality of the deepwater group we do not have the data to compare changes in week one to week 2. Another important note is that the control group data is only from week 2.

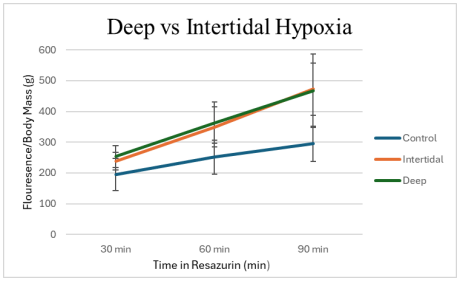


Figure 3a. Graph of fluorescence to body mass at different time lengths in resazurin. Blue is control crab treatment, orange is intertidal, and green is deepwater. Higher respiration rates in intertidal and deep compared to control, but no significant difference between intertidal and deep.

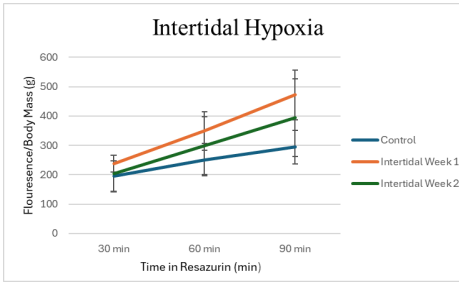


Figure 3b. Graph of fluorescence to body mass at different time lengths spent in resazurin for intertidal treatment crabs from week 1 compared to week 2 and control. Orange is intertidal week 1, green is intertidal week 2, and blue is control.

Gill Tissue

In the intertidal group we found that 44.4% of the total crabs in that condition had bad, atrophic tissue, 33.3% were partial, 11.1% pink, and 11.1% unusable (fig. 4a). For the deepwater group 88.9% of crabs in the higher hypoxic treatment

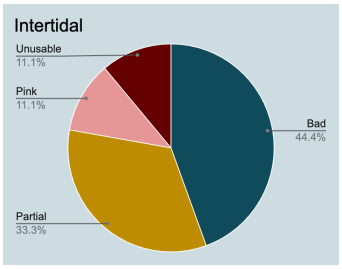


Figure 4a. Pie chart of gill tissue condition of intertidal treatment after two weeks. Dark red indicates an individual that was unable to be dissected due to degradation. Teal represents atrophic gills, dark yellow is showing partial atrophy, pink is a result of resazurin dye.

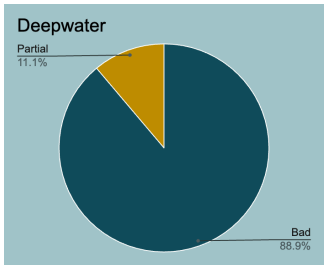
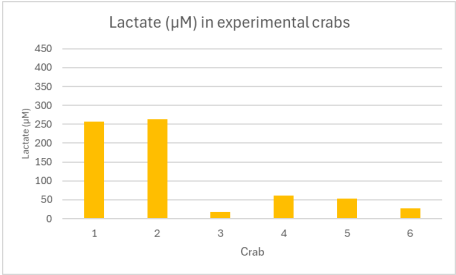


Figure 4b. Pie chart of gill tissue condition of deepwater treatment after two weeks. Teal represents individuals with atrophic gill tissue and dark yellow represents partial atrophy.

had bad tissue and 11.1% were partial (fig.4b). To limit the number of individuals that were euthanized we only looked at two control individuals that both had good gill tissue.

Hemolymph - Lactate

Compared to the control crabs the crabs in the intertidal conditions had much lower lactate levels. Figure 5a shows the intertidal crab lactate levels for each individual and the control crabs which visually display this large difference in levels.



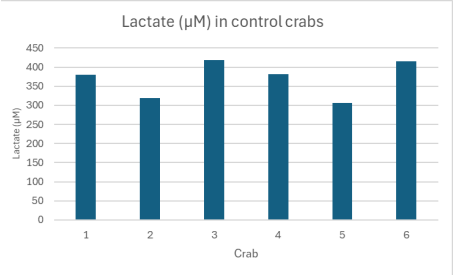


Figure 5a. Lactate levels in intertidal and control crabs. The x-axis represents crab individuals and y-axis lactate levels in (uM). Levels are relatively low for intertidal compared to control.

Discussion

The different tests chosen for this experiment correlate with important physiological changes that could be a result of hypoxia. Understanding this data can aid in predicting population changes for the crab industry to inform potential management strategies.

For the resazurin the expected result was a decrease in respiration rate in the experimental groups due to a shift from aerobic to anaerobic respiration. We expected this because a decrease in oxygen should force this shift, but instead we saw an increase in respiration. After week 2 the respiration of the intertidal group did begin to decrease compared to week 1. This could mean with longer exposure to hypoxia respiration could continue to decrease. The increase in respiration could also be a stress response of increased heart rate attempting to deliver more oxygen to the body.

From the hemolymph extraction and lactate levels we also saw results that did not turn out as expected. In low oxygen conditions the expected shift to anaerobic respiration should cause increased lactate production. With the large difference between levels in the intertidal crabs and the control crabs there is likely an effect from hypoxia, but maybe not as seen traditionally with anaerobic respiration. The lactate paradox is a phenomenon that has been observed in hypoxic conditions. Essentially the exposure to sudden hypoxia can cause a spike in lactate accumulation followed by a large decrease even though the hypoxia is still present. In a paper by Lundby et al (2000), they performed a study on humans hiking at high altitudes for six weeks and found that lactate levels had an initial spike and by the end of the six weeks lactate had returned to normal. Their research suggests that severe hypoxia for long periods of time may return lactate levels back to normal. Although this was done on humans and the lactate levels of the intertidal crabs were even lower than the control, it is something to consider when evaluating effects of hypoxia.

One point of data that is not very accurate in assessing response to hypoxia was the righting time. Overall the righting times for each crab varied significantly and there appeared to be no significant difference between each of the groups. If there were significant differences we would've expected to make an argument for hypoxia causing more lethargy, but considering the data we did collect we cannot make this conclusion.

The most convincing data to support hypoxia is impacting the viability of crabs is the gill tissue dissections and mortality. In the deepwater group that was exposed to the extreme hypoxic

conditions every individual died and 88.9% had bad tissue, meaning all but one had atrophic gills and the ninth showed signs of some atrophy. The intertidal group only experienced one fatality and the majority had either bad or partial gill tissue atrophy, and none had good tissue. The gradual progression from the control having good tissue to the intertidal having partial and bad, and the deepwater having almost entirely bad tissue correlates with the increasing levels of hypoxia.

The results gathered from the gill tissue dissections and the abnormalities in respiration rate and lactate levels show that hypoxia is impacting crabs to some extent. The gill tissue implies severe hypoxia could be fatal due to atrophy of the gills. A paper by Li et al (2022) noted that when exposed to hypoxia the gills are usually the first tissue to be impacted which supports our results in the context to the length of this experiment. Less severe atrophy in the intertidal groups implies that having access to air, as they did, may be important in combating hypoxia. With further research and improvements to the experimental design this data could be used by the commercial crab industry to predict changes in crab populations. Having the ability to predict population change could prove useful in being prepared for hypoxic events or even lead to solutions to mitigate the impacts these events have on revenue.

Citations

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