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FISH 460AB

Final Paper

1. Introduction

Hypoxic water columns have become an acute threat to the productivity of west coast Dungeness crab fisheries. Seasonal occurrences of hypoxic and anoxic conditions during summer months have been observed with increasing intensity over the last 70 years, particularly along the Washington and Oregon coasts (Barth et al., 2024). Consequently, fisheries have experienced a loss of revenue (Holland & Leonard, 2020) and have struggled to implement effective management in response to hypoxic events (Froehlich et al., 2017).

The appearance of hypoxic conditions is associated with the occurrence of harmful algal blooms (HABs), which have become increasingly common with the progression of climate change (Barth et al., 2024). Nutrient runoff is responsible for excess productivity by phytoplankton as light levels increase in spring (Wallace & Gobler, 2021), and while dissolved oxygen (DO2) levels are initially high at the surface, death and decomposition of sinking phytoplankton result in unbalanced restoration at depth. As nutrients and light diminish in the fall, and the algal bloom collapses, surface and coastal waters may become hypoxic as well.

Understanding how exposure to hypoxic conditions affects physiology is critical to predicting population dynamics, behavior, and dispersal of Dungeness crabs. Previous studies have been conducted on the effect of acute hypoxia on the cardiovascular system in crabs and found evidence of induced bradycardia (Falconer et al., 2019) as well as elevated hemolymph pH and lactate levels (Lallier & Truchot, 1989). These findings indicate physical stress and anaerobic metabolism in crabs under conditions similar to those left by HABs. Less is known, however, about the impact of emersion breathing as a mitigator of stress under hypoxic conditions. Crabs living in near-shore and intertidal habitats with access to the surface may be able to improve their metabolic ability and reduce damage to their bodies by engaging in this behavior. *Hemigrapsus oregonensis* was selected as a model organism for the purposes of this study for its commonness, ease of handling, and relatedness to Dungeness crabs. This study seeks to explore the difference in physiological stress responses between *H. oregonensis* with and without the ability to use emersion breathing in order to predict the impact of habitat distribution on survivability under hypoxic conditions.

2. Methods

Three treatments were used to assess the physiological impacts of hypoxia. Nine *Hemigrapsus oregonensis* individuals were assigned to each of the control, deepwater, and intertidal tanks. In the control tank, crabs were given hides of natural shells and concrete as well as an airstone to maintain a DO2 concentration between 6 and 7 ppm. The deepwater tank was made to replicate the conditions of the control tank without the presence of an airstone. It was also sealed with duct tape and vacuumed prior to sealing. The intertidal tank was also designed to replicate the control tank but lacked an airstone and had a square of mesh placed inside to allow crabs access to the surface.

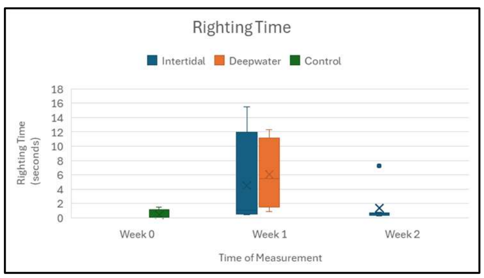
After being allowed to sit for a period of one week, four individuals were selected from each treatment tank and administered non-lethal tests including righting tests and resazurin assays. Resazurin assays were conducted by loading 35mL of working solution into crab chambers. Every 30 minutes for 90 minutes, 200uL were withdrawn from each crab chamber and placed in a 96-well plate. The well plate was then run by a plate reader at Excitation 530; Emission 590 values to obtain fluorescence. Crabs were patted dry and weighed to the nearest one-hundredth of a gram before being placed in a chamber. To control for stress induced by handling, each individual was numbered with nail polish before the tests were administered and replaced in their respective tanks.

After two weeks, non-lethal tests were administered again as well as lethal tests. Each crab had 0.5mL of hemolymph drawn and was terminated for gill tissue dissection. The hemolymph was then tested using the Cayman Chemical L-Lactate Assay Kit per the manufacturer's instructions. Gill tissue was graded using a visual scale: good, bad, partial, pink, and unusable. Pink and unusable were excluded from interpretation.

3. Results

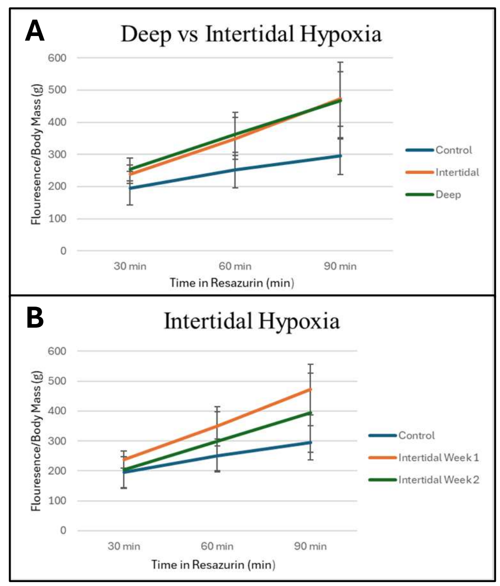
Over the course of two weeks, DO2 levels in the intertidal treatment dropped from 6-7 ppm to 3.9 ppm and then to 3.2 ppm. In the deepwater treatment, DO2 dropped to 3.6 ppm and then to 3.4 ppm. Additionally, total mortality was observed in the deepwater tank by the end of week 2, which could explain why the final DO2 concentration was higher if respiration ceased earlier. Crabs in the intertidal tank displayed preferential behavior for the surface by the end of the second week, confirming the difference between treatments.

Fig. 1.



Average righting time in seconds plotted against the week of data collection. Control crabs were tested only in the first week but can be assumed to be uniform across the two weeks of testing. No significant difference was observed between intertidal and deepwater treatments in week 1 or between intertidal treatments of week 1 and week 2. No data was collected for the deepwater treatment in week 2 due to 100% mortality.

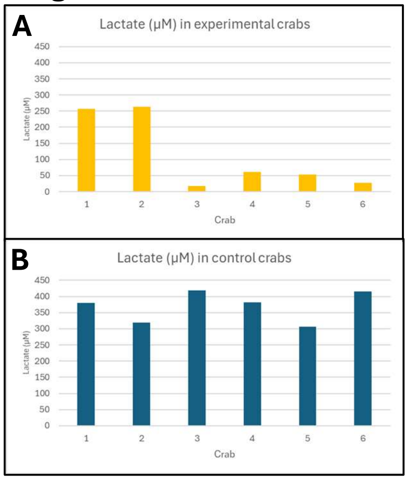
Fig. 2.



Fluorescence of resazurin product was normalized to crab body weight and plotted against the duration after which samples were collected from the crab chambers. (A) Results from week 1 and (B) intertidal treatments of weeks 1 and 2 are plotted against the same group of controls.

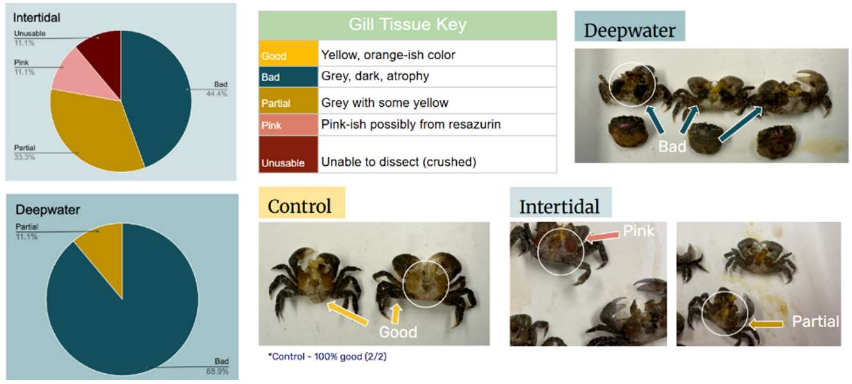
Little variation was observed between righting times across each of the treatments. Intertidal and control righting times had extremely low median values, but intertidal results were skewed by a few data points. A one-way ANOVA test between week one results yielded a p-value of 0.329. Mortality of the deepwater treatment crabs in the second week prevented full analysis of resazurin and

Fig. 3.



Total lactate was measured from hemolymph extracts. (A) Of the nine intertidal treatment crabs only 6 produced usable hemolymph samples. (B) These were compared against six randomly selected samples from control crabs.

Fig. 4.



Gill tissue dissections were performed on all crabs from both deepwater and intertidal treatments with the exception of a single intertidal crab which had rotted and was crushed during dissections. Two dissections were performed on control crabs for reference of healthy tissue, Gill tissue was graded between good, partial wasting, and bad, with additional demarcation for tissue that appeared stained by resazurin or was unusable.

lactate assays, but intertidal and control crabs were still tested fully. Intertidal crabs demonstrated a lower production of lactate than that of control crabs, averaging just 130 micromoles relative to the control's average of 300 micromoles. A two-sample T-test yielded a p-value of 0.016. In the first week, intertidal and deepwater treatments displayed a similar rate of respiration during resazurin assays, both above that of the control. Between week 1 and week 2, the respiration rate of the intertidal treatment decreased.

Gill tissue dissections were graded on the coloration and texture of gill tissues. Some results were denoted as uninformative but not discarded from the dataset – gills suspected of being stained by resazurin or rotted were separately labeled. Two dissections of control crabs were performed to establish a baseline of healthy gill tissue to compare against. Nearly half of the usable intertidal crabs' gill tissues exhibited only partial wasting, whereas 88% of the deepwater treatment group were fully wasted.

4. Discussion

This study proved that access to air limits the physiological stress experienced by *H. oregonensis*, but the biological mechanisms at work remain unclear. After two weeks, the nail polish applied to the crabs tested in week 1 became mostly dissolved in the water such that the numbers became indistinguishable. Therefore, attempts to control for the stress of testing were abandoned. Righting time showed little variation across treatments throughout the duration of the experiment and was not a reliable indicator of behavioral stress. However, Gill tissue dissection after exposure to hypoxic conditions showed that all crabs of both treatment groups were degraded by the lack of DO2. The crabs of the deepwater treatment with no access to the surface not only showed a greater degree of wasting but all experienced mortality. By contrast, although many had equally wasted gill tissue, the intertidal treatment group only had a single mortality by the end of two weeks. Emersion breathing was not directly observed during the experiment, but the increased survivability and observation of preferential behavior toward the surface after two weeks indicated that it could have played a role in limiting physiological stress.

Lactate was investigated as a potential indicator of anaerobic respiration, but the results of physiological assays were inverse to expectation. Crabs of the intertidal treatment group displayed a significantly lower average lactate production than the control group. If taken at face value, this result states that crabs experiencing hypoxia performed less anaerobic respiration than the controls, but the truth may be more complex. Examples of the “lactate paradox” have been documented since its description in 1981, where lactate levels appear much lower than expected under hypoxic conditions (Bartlett & Lehnhard, 2010). Leading hypotheses point to a reduction of lactate production by working tissue as a result of acclimatization to low-oxygen environments. While most research on the lactate paradox has investigated its occurrence in humans, it is possible that similar effects are present across organisms that utilize fermentation in the production of ATP.

Resazurin assays may provide better evidence of respiratory stress. In both treatments, the respiration rate was raised above the control group after the first week. After the second week, the respiration rates of the treatment group decreased but remained above the control group. It is possible that heightened respiration in crabs exposed to hypoxia is due to opportunistic breathing. The crab chambers for resazurin assay were filled with aerated water and may have provided the crabs with a chance to perform aerobic respiration. The decrease in respiration after the second week could be due to the wasting of gill tissues.

Future investigations should closely monitor the physiology of *H. oregonensis* crabs to create a more complete picture of stress response. DO2 concentration and lactate production should be tested on a daily timescale in order to track the progress of acclimatization. Furthermore, emersion breathing behavior needs to be directly observed under experimental conditions before conclusions can be drawn about its effect on crab physiology during hypoxic exposure.

The results of this study demonstrate that near-shore habitats could provide some refuge for crabs during a hypoxic event. Blue crabs, *Callinectes sapidus*, have been shown to perform some shoreward migration during the upwelling of hypoxic waters as an avoidance behavior (Bell et al., 2009). However, the orientation mechanism of these crabs is attributed to current movement and may be only used to escape hypoxic waters rather than seek shallower water. Furthermore, it might not be possible for crabs to orient in nearshore environments, and therefore they would be vulnerable to gradual hypoxia by algal respiration. Dungeness crab fisheries should adjust management strategies to reflect nearshore habitat as critical to the survival of stocks during hypoxic events and avoid harvesting those areas. Additionally, it can be expected that crab populations without access to emersion breathing or the ability to escape hypoxic events will experience mortality within a period of two weeks, while those with access to the surface may have a prolonged but not indefinite period of survival.

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