The Effects of Chronic Hypoxia on *Hemigrapsus oregonensis* in Shallow and Deep Water

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Introduction

Marine heat waves and hypoxic events are becoming more common in oceans (Rabalais, et al., 2010), and there are devastating impacts on marine species, including those that fisheries rely on. Between 2018 and 2021, more than 10 billion snow crabs (*Chionoecetes opilio*) disappeared from the Bering Sea (Szuwalski, et al., 2023). The Dungeness crab (*Metacarcinus magister*) fishery in Washington state has also seen massive declines in the last decade (Marquis, 2022). As mass mortality events like these become more frequent, it is crucial to determine the causes and develop possible mitigation strategies.

One likely contributing factor to crab mortality is hypoxia. Marine hypoxic zones have become increasingly common in the Northeast Pacific (Barth, et al., 2024). These hypoxic zones are typically caused by photosynthetic organisms producing carbon, followed by microbial respiration (Rabalais, et al., 2010). These conditions are exacerbated by increased water stratification, longer water residence time, and increased primary production (Rabalais, et al., 2010). Anthropogenic influences are contributing to these factors by (i) increasing nutrient availability (fertilizer run-off and waste water production) for primary producers, (ii) increasing water stratification from rising surface temperatures due to global warming, and (iii) extended water residence time due to the slow down of the Atlantic Meridional Overturning Circulation (Jackson, et al., 2015).

Previous studies started investigating the effects of low dissolved oxygen on crabs. A 2019 paper found that acute hypoxia for six hours mimicking a tidal cycle induced brachycardia in *Hemigrapsus crenulatus* and *Hemigrapsus sexdentatus* (Falconer, 2019), and field experiments have found that crabs will move to shallower waters to escape low oxygen zones (Froehlich, et al., 2013). Work to develop mitigation strategies has also begun by evaluating the movement patterns of *Metacarcinus magister* to avoid hypoxic zones and how fisheries can use environmental data to develop management strategies (Froehlich, et al., 2017). However, a knowledge gap remains: what are the effects of long long-term hypoxia on crabs, and is there a difference in the severity of the impacts of hypoxia in deep water, compared to in shallow water?

Determining the effects of chronic hypoxia on crabs in shallow and deep water is important. This is because the crab fishery is a large industry currently in peril due to the decline in crabs in the Northeast Pacific. An experimental approach was used in this study to compare the physiology of crabs living in deep water and shallow water hypoxia, compared to a control environment. At one and two weeks, assays were run to determine the effects on the crabs.

Methods

Specimen Collection

The *Hemigrapsus oregonensis* crabs used in this study were collected from Lion's Park Boat Launch, Bremerton, WA 98310, 47 35'07" N 122 38'42" W between 11:30 and 13:34 at a -1.68 feet tide.

Experimental Setup

Three groups of crabs were exposed to three different treatment tanks. All three tanks had a salinity of 35 ppt, temperature of 35 °C, and oyster shells for cover. The first tank was a control with an airstone constantly running to provide high DO2 levels. The second tank simulated a shallow hypoxic environment with no airstone, so as crabs respired, a hypoxic environment

would develop. Additionally, this tank had a plastic mesh ramp from the bottom of the tank to the surface of the water that allowed crabs to respire at the surface and allow oxygen diffusion from the air to simulate shallow water. The final treatment was a deep hypoxia tank and had no airstone or mesh ramp. This tank's lid was sealed at the edges with duct tape, and the air between the water and the lid was suctioned out with a vacuum before sealing. This method was meant to prevent oxygen diffusion from the air to simulate deep water hypoxia surrounded by other hypoxic water. The two hypoxic tanks had nine crabs each, and the control tank (which was larger) had more than 20 crabs.

Experimental Timeline

At the end of the first week of the trial, half of the crabs in both hypoxic tanks were tested with non-lethal tests and marked with nail polish. Only half of the crabs were tested at the end of week one, so the untested crabs would serve as a control within the hypoxic tanks in case the week one tests caused a response in the week two tests. At the end of two weeks, all of the surviving crabs in the hypoxic tanks were tested with non-lethal tests, and lethal tests. Any mortality was recorded at the week one and two crab tests. Control crabs were also given the non-lethal and lethal tests to compare against the hypoxic crab test results.

Non-Lethal Tests

Respiration Rate was tested using the following resazurin assay protocol. A resazurin stock solution was prepared: 0.5 g resazurin salt, 10 mL DI water, and 10 µL DMSO. Next, a working solution was prepared: 148 mL seawater, 333 µL resazurin stock solution, 150 µL DMSO, 1.5 mL antibiotic solution (100x Penn/Strepp & 100x Fungizone). 35 mL of working solution was added to air-tight 100 mL testing chambers. Each crab was weighed before placing it in its testing chamber, and time was started. At 30-minute intervals, a 200 µL sample was pipetted from each testing chamber into a well of a 96-well plate. After three samples were taken, the crabs were rinsed and returned to their tanks. Plates were then run in a spectrophotometer plate reader (Agilent BioTek Synergy HTX with software version 5) at Excitation 530, Emission 590 to record fluorescence values. The resulting fluorescence values were divided by crab weight (g) to normalize for differences in crab size. Then, two-factor ANOVA tests followed by post hoc tests were performed to compare the difference in average respiration after 90 minutes between groups.

Lethal Tests

(i) **Hemolymph Lactate Levels** were determined by first extracting hemolymph from live crabs by inserting a hypodermic needle in the soft gap between the base of the claw and the abdomen. Hemolymph sample lactate content could then be analysed using the L-Lactate Assay kit (Caymen Chemical item no. 700510). A one-way ANOVA was used to test for significant differences in the average lactate concentrations between treatment groups. (ii) **Gill Tissue Analysis** was performed after hemolymph extraction. Crabs were euthanized by cutting 1 cm deep along the sagittal plane from the anterior side. Next, the carapace was removed using forceps, exposing the gill tissue (Figure 1). Gill tissue was qualitatively analyzed using the following categories: Good (orange/yellow and full), Partial (grey with some orange), Bad (black and deflated), Unusable (crushed, dyed by resazurin, or otherwise unidentifiable).

Results

There was no mortality at the end of week one, but by week two, 11% of the crabs in the shallow hypoxia treatment had died, and 100% of the crabs in the deep hypoxia treatment had died. The 100% mortality of the deep hypoxia treatment meant that respiration rate and

hemolymph lactate concentration could not be assessed for this treatment. For the deep hypoxia treatment, only the gill condition could be assessed during week two.

Gill Condition was worse in all of the crabs exposed to hypoxia compared to the control crabs. The crabs in the deep water hypoxia had the worst gill condition, with 88.9% of crab gills categorized as bad and 11.1% as partial. The crabs in the shallow water hypoxia treatment had better gill condition than the deep water treatment. Of the identifiable gills from crabs in the shallow treatment, 75% were categorized as bad and 25% as partial (Fig. 2).

Data regarding hemolymph lactate concentration were only available for control crabs and shallow hypoxia crabs from week two. The average lactate concentration for the control group was 170 μM higher than the shallow hypoxia treatment (Fig. 3). A one-way ANOVA test gave a p-value of 0.016, indicating that the difference in averages was significant.

The respiration rate of crabs in the shallow and deep water hypoxia treatments at the end of week one were similar, and both were higher than the control group (Fig. 4). A two-factor ANOVA test followed by a post-hoc test showed that the two hypoxic treatments had significantly higher respiration rates than the control after one week. Finally, the respiration rates of the shallow treatments were compared between week one and two against the control. Week one had the highest, followed by week two, and the control had the lowest (Fig. 5). A two-factor ANOVA test, followed by a post-hoc test, was used to compare the average respiration that had occurred by 90 minutes. These analyses showed there was no significant difference between the average respiration of crabs from the shallow hypoxic tank between weeks one and two. However, there was a significant difference between the shallow hypoxic tank's crabs from both weeks compared to the control crabs.

Discussion

To determine the effects of chronic hypoxia on *Hemigrapsus oregonensis*, crabs were kept in simulated deep water and shallow water hypoxic environments for two weeks. These crabs were compared to control crabs kept in a normoxic control environment. Determining the chronic effect of hypoxia on crabs has the potential to inform management strategies of commercial crab stocks that are currently declining, including *Chionoecetes opilio* (Szuwalski, et al., 2023) and *Metacarcinus magister* (Marquis, 2022) in the Northeast Pacific.

Perhaps the most notable finding is that crabs exposed to chronic hypoxia in deep water cannot survive. This conclusion is supported by a 100% mortality rate observed in the deep water hypoxia group after two weeks. The lethality of deep water hypoxia on *Hemigrapsus oregonensis* might explain why previous studies observed crabs migrating to shallow water when experiencing deep water hypoxia (Froehlich, et al., 2013). In this study, it remains unclear which provided more relief from hypoxia in the shallow tank, access to the surface to respire via the ramp or diffusion of air into the water.

The impact of hypoxia on gill atrophy was also a notable finding. In both hypoxic treatments, none of the crabs' gills were categorized as "Good" at the end of two weeks, and most were categorized as "Bad". This implies that even after a period of chronic hypoxia ends, there are likely lasting consequences due to damaged gills.

A surprising result was the significant decrease in hemolymph lactate concentration in crabs that experienced hypoxia. Typically, hypoxia causes an increase in lactate in crabs when exposed to hypoxia. However, previous studies have shown this increase as a response to a period of hypoxia lasting eight hours (Maciel, et al., 2008). A possible explanation for the decrease in lactate as a response to chronic hypoxia is the lactate paradox; the decrease in lactate

concentration as a response to acclimation to a hypoxic environment, which has been observed in humans (Lundby, et al., 2000) but could also apply to crabs.

The significant increase in respiration rate of crabs due to chronic hypoxia was another important finding. Previous research concluded that the increase in respiration rate of crustaceans is to compensate for the low oxygen, which likely applies to this scenario (McMahon, 2001). This increase in respiration rate also likely increases the metabolic rate, meaning that crabs would require more food and have less energy to maintain their immune system, growth, and reproduction. These impacts could explain the declines of other crab species populations (Szuwalski, et al., 2023) (Marquis, 2022).

Future research could build off of this study to monitor the effects of hypoxia on crabs after they are returned to a normoxic environment to determine how long gill atrophy lasts. Future work could also address some of the methodological shortcomings of this study. Some of these limitations include, (i) the limited sample size of the treatments, (ii) multiple manipulated variables between the two hypoxic treatments: ramp access to air and surface diffusion, (iii) more crabs in the control tank than the treatment tanks, and finally, (iv) the 50% of crabs that were tested with non-lethal tests after week one were marked with nail polish to see if the tests influenced week 2 tests, but the nail polish rubbed off, so the effect of week one tests was unverifiable.

The harmful effects of chronic hypoxia on *Hemigrapsus oregonensis* observed during this study provide evidence that the increasingly common hypoxic events in the Northeast Pacific are damaging crab populations. Based on this conclusion, the development of future crab population management strategies should consider hypoxic events, especially when they occur in deep water and for long periods of time.

Figures

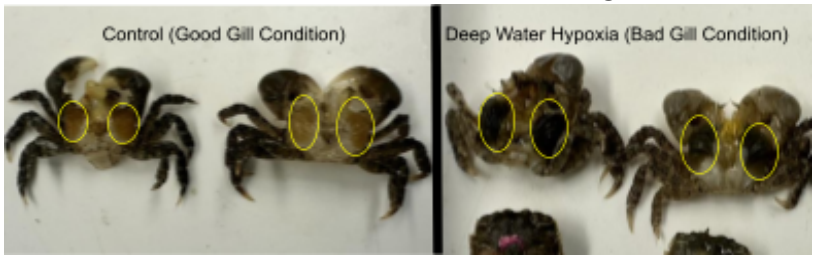

Figure 1. After the removal of the carapace from *Hemigrapsus oregonensis*, the gill tissue is exposed, highlighted by yellow circles. The two crabs on the left, from the control tank, both have healthy gills with an orange color and a full shape. The two crabs on the right, from the deep water hypoxia tank, both have atrophied, blackened gills.

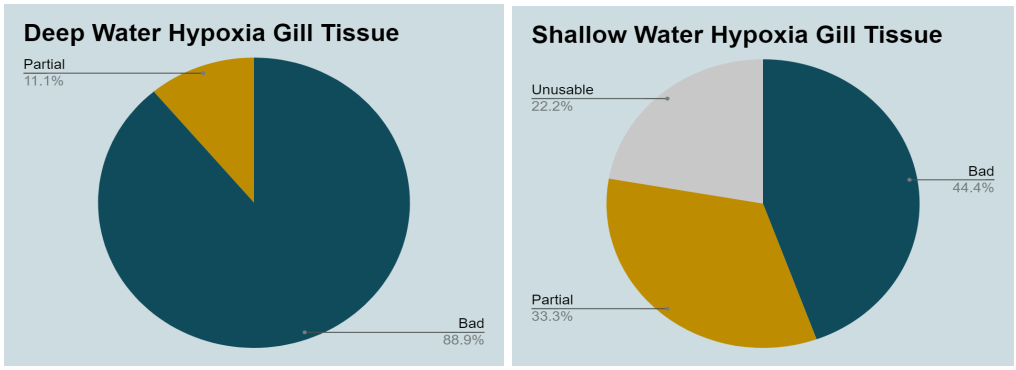

Figure 2. Percentage of crabs of each gill tissue condition from the two hypoxic treatments. The condition categories are: Good (orange/yellow and full gills), Partial (grey with some orange gills), Bad (black and deflated gills), Unusable (crushed, dyed by resazurin, or otherwise unidentifiable gills).

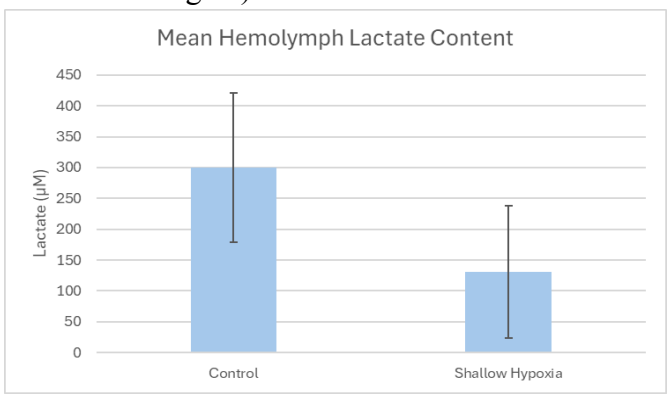

Figure 3. Mean lactate molarity in the extracted hemolymph of crabs from the control and shallow hypoxia treatments. The control group had a mean lactate concentration 170 µM higher than the shallow hypoxia treatment.

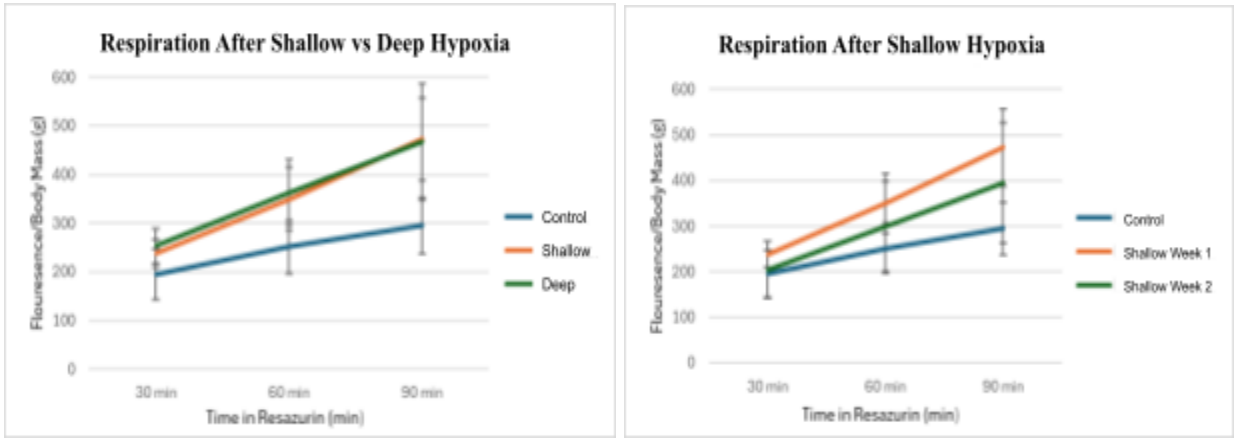

Figure 4. The average fluorescence of resazurin normalized for size using body mass is plotted over time. A higher fluorescence value of resazurin means more respiration has occurred. The "Respiration after Shallow vs Deep Hypoxia" graph on the left shows that the shallow and deep treatment crabs had similar respiration rates, and both were higher than the control. The "Respiration After Shallow Hypoxia" graph on the right shows that shallow week 1 treatment crabs had the highest respiration rate, followed by shallow week 1, and the control group had the lowest respiration rate.

Sources

Barth, J. A., Pierce, S. D., Carter, B. R., Chan, F., Erofeev, A. Y., Fisher, J. L., Feely, R. A., Jacobson, K. C., Keller, A. A., Morgan, C. A., Pohl, J. E., Rasmuson, L. K., & Simon, V. (2024). Widespread and increasing near-bottom hypoxia in the coastal ocean off the United States Pacific Northwest. Scientific Reports, 14(1). https://doi.org/10.1038/s41598-024-54476-0

Falconer, T. R. L., Marsden, I. D., Hill, J. V., & Glover, C. N. (2019). Does physiological tolerance to acute hypoxia and salinity change explain ecological niche in two intertidal crab species? Conservation Physiology, 7(1), coz086.https://doi.org/10.1093/conphys/coz086

Froehlich, H. E., Essington, T. E., Beaudreau, A. H., & Levin, P. S. (2013). Movement patterns and distributional shifts of Dungeness crab (Metacarcinus magister) and English sole (Parophrys vetulus) during seasonal hypoxia. Estuaries and Coasts, 37(2), 449-460.

Froehlich, H.E., Essington, T.E., McDonald, P.S. (2017). When does hypoxia affect management performance of a fishery? A management strategy evaluation of Dungeness crab (Metacarcinus magister) fisheries in Hood Canal, Washington, USA. Canadian Journal of Fisheries and Aquatic Sciences, 74(6), 922-932. https://cdnsciencepub.com/doi/full/10.1139/cjfas-2016-0269

Jackson, L. C., Kahana, R., Graham, T., Ringer, M. A., Woollings, T., Mecking, J. V., & Wood, R. A. (2015). Global and European climate impacts of a slowdown of the AMOC in a high resolution GCM. Climate Dynamics, 45(11-12), 3299-3316. https://doi.org/10.1007/s00382-015-2540-2

Lundby, C., Saltin, B., & Van Hall, G. (2000). The 'lactate paradox', evidence for a transient change in the course of acclimatization to severe hypoxia in lowlanders. Acta Physiologica Scandinavica, 170(4), 265-269.ttps://doi.org/10.1046/j.1365-201x.2000.00785.

Maciel, J. E., Souza, F., Valle, S., Kucharski, L. C., & Da Silva, R. S. (2008). Lactate metabolism in the muscle of the crab Chasmagnathus granulatus during hypoxia and post-hypoxia recovery. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 151(1), 61-65. https://doi.org/10.1016/j.cbpa.2008.05.178

Marquis, S. (2022, November). Stressed out: Dungeness crabs off the Pacific Northwest coast. NOAA Office of National Marine Sanctuaries. https://sanctuaries.noaa.gov/news/nov22/stressed-out.html

McMahon, B. R. (2001). Respiratory and circulatory compensation to hypoxia in crustaceans. Respiration Physiology, 128(3), 349-364. https://doi.org/10.1016/s0034-5687(01)00311-5

Rabalais, N.N., Diaz, R.J., Levin, L.A., Turner, R.E., Gilber, D., Zhang, J. (2010). Dynamics of distribution of natural and human-caused hypoxia. Biogeosciences, 7(2), 585-619. https://bg.copernicus.org/articles/7/585/2010/

Szuwalski, C. S., Aydin, K., Fedewa, E. J., Garber-Yonts, B., & Litzow, M. A. (2023). The collapse of eastern Bering Sea snow crab. Science, 382(6668), 306-310. https://doi.org/10.1126/science.adf6035