

# Mitigation of Hypoxic Ecosystems Using Hemolymph Analysis of *Callinectes sapidus* and *Procambarus clarkii* in Relation to *Spartina* Grasses

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Received 10 April 2016; accepted 18 July 2016; published 21 July 2016

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## Abstract

In the past 80 years, Louisiana has lost over one million acres in land, affecting plants and crustaceans that live in these environments. The first objective of this research was to determine the relationship between diffused oxygen in water and time with the behavioral and physiological health of *Callinectes sapidus* (blue crab) and *Procambarus clarkii* (crawfish). After 24 hours, manual dexterity, or the amount of time it takes for a crustacean to flip itself over increased under hypoxic conditions. Hemolymph was extracted and tested for lactate, glucose, and protein. Crawfish were also exposed to this same procedure. An additional crawfish study comparing *Spartina* plants to assimilate oxygen for aeration compared to mechanical aeration was conducted. The hypoxic groups for all three studies at the 0.05 level had a significantly greater manual dexterity time and greater amounts of lactate and glucose. The protein was significantly higher in hypoxia-exposed crawfish. Time and aeration affect the health of crustaceans, and plants were shown to effectively provide normoxic oxygen levels at a similar level as mechanical aeration. These studies support the importance of marsh grasses for the coast and crustacean viability.

## Keywords

Coastal Restoration, Barrier Island Mitigation, Marine Ecology

### 1. Introduction

Coastal erosion is a serious problem in Louisiana causing significant economic and land loss. Louisiana's coastal infrastructure is worth more than \$150 billion in value. The commercial fisheries in Louisiana provide 25% to 35% of the nation's entire catch. These southern fishermen are first in the yearly harvest of shrimp (*Penaeus sp.*), crabs (*Callinectes sapidus*), oysters (*Crassostrea virginica*), red snapper (*Lutjanus campechanus*), crawfish (*Procambarus clarkii*), wild catfish (*Siluriformes*), mullet (*Mugilidae*), and sea trout (*Salmo trutta*). Fisheries account for an estimated 3.1 billion dollar impact to Louisiana in 2009. By 2050, it is predicted that the commercial fish industry will decrease by \$550 million and the loss for recreational fisheries will be about \$200 million a year [1]. Twenty-five percent of the energy supply in the United States is dependent on facilities in Louisiana. The oil and gas companies in Louisiana have a net value over \$16 billion a year. Twenty thousand miles of pipes run through federal lands offshore and many more are inland. The wetlands serve as protection for pipes from waves and secure the pipelines in place. As wave action deteriorates land, more pipelines are exposed increasing the risk of shipping traffic being disrupted. Louisiana is first in the nation for total shipping weight, so channeling occurs often in order to allow the large cargo to pass through successfully. Saltwater intrusion as a result of channelization destroys wetland plants, contributing to the loss of habitats for wildlife. It is estimated that at the current rate of land loss more than 155 miles of waterways and several of the ports will be exposed to open water within fifty years [1]. Erosion is defined as land loss caused by natural forces including water, waves, ice, wind, or tide [2]. If erosion continues at the current rate, approximately 640,000 more acres of Louisiana's land will be submerged by the year 2050 [3]. The impacted land area is approximately the size of the state of Rhode Island. Land subsidence, erosion, and sea level rise are the major contributing factors to land loss. The coast supports important infrastructure like ports, pipelines, navigational waterways, and highways, which all hold financial significance. The importance of Louisiana's coast has worldwide ecological significance affecting the abundance of fisheries, wildlife and waterfowl, and impacting a critical migratory flyway [3]. If current rates continue, the wetland loss in Louisiana alone could cost the nation \$36 billion in the next 50 years [3]. This is mostly the result of erosion and subsidence [3]. Land subsidence occurs naturally or as a result of human activities. Decomposing organic substrates without replenishment occurs throughout Louisiana in coastal and developed areas. Subsidence impacted 17,000 square miles in the United States. Land subsidence is the result of a gradual lowering of the soil surface elevation of an organic soil, or a reduction in the thickness of organic matter [4]. Oxidation is one major cause of subsidence because in this process, a plant tissue's organic carbon is converted to water and carbon dioxide gas. The loss of organic matter causes the land to collapse or sink [4]. Much of the national land subsidence occurs due to the exploitation of groundwater. With the rate of subsidence increasing, land elevations decreased becoming fully submerged by water and unable to sustain terrestrial life. Subsidence has become a major issue in Louisiana, causing a continuous loss of land [5].

Louisiana's coastal management requires a deep knowledge of factors that influence land change over time [6]. Quantifying coastal land loss is essential for historical documentation and restoration of eroding barrier islands. The irregular shape of deposited barrier islands and marshlands is frequently in the shape of complex polygons. Pick's Theorem is a simple mathematical method used to determine the areas of complex and non-convex polygons. Pick's Theorem is defined as  $A = I + B/2 - 1$ , where  $A$  is the total area,  $I$  is the number of intersection points on the interior, and  $B$  is the number of intersection points on the boundary. A plane is tiled with  $1 \times 1$  square grid, and if shifted one unit north, south, east, or west, it will finish its position on another square. Each intersection point is used in the formula whether included on the border or interior of a polygon [7]. A land owner in Oregon used Pick's Theorem to calculate the approximate size of his forest. To approximate the area, dots in the interior of the polygon are added to half the amount of dots on the boundary and the sum is multiplied by the scale factor [7].

A barrier island is defined as a long, narrow coastal island, separated from the mainland by a shallow water lagoon or marsh [2]. Barrier islands are areas of sandy land located by a shore that consists of environments in-

cluding: dunes, marshes, coastal flats, and swales. Barrier islands support diverse marine and plant life and buffer wave energy from the storms; however, the barrier islands are typically low-lying, making them susceptible to flooding. These islands are incessantly changing position and location, moved by currents, waves, and tides [8]. The Louisiana barrier islands are created by the Mississippi River's delta. Settlements on the islands have been abandoned due to erosion and exposure to storms. Grand Isle is the only Louisiana barrier island in Louisiana that still contains a settlement. These front-line islands endure the strongest impact from any storm, buffering the damaging forces from populated areas [8]. Barrier islands serve as a crucial ecosystem for wildlife and serve as nesting habitats for birds. The aquatic environments adjacent to land provide sustenance and socioeconomic benefits. Barrier islands are essential for the protection of Louisiana's resources and communities [8]. The barrier islands and wetlands serve as protection from hurricanes and heavy winds. Every 14,256 feet of wetlands will be inundated by 1 foot of storm surge. The loss of each one-mile strip of land on the coast costs an estimated \$5,752,816 more every year. About 65% of Louisiana's population dwells on the coast or within fifty miles. These 2,000,000 people are left exposed to dangerous winds and hurricanes without the barrier islands and marshes [1].

The Isle Derniere islands (29°02'19"N90°48'15"W), also known as Last Islands (English translation), are a specific group of barrier islands located in the Gulf of Mexico between Caillou Bay and Lake Pelto and are made up of the following islands: Whiskey, Trinity, East, Raccoon, and Wine (or Vine). The closest village to the islands is Cocodrie, located thirteen miles northeast of Trinity Island. This island served as the "first line of defense" for the land behind it. It is estimated that in 1300 AD, Last Island formed from soft mud flats and high grasses [9]. The Mississippi River gradually deposited sediment until it was not an efficient path. Then, erosion began which released sand and mud into the Gulf. The geology of Louisiana's coastal zone is intrinsically linked to the history of the Mississippi River [10]. The sandy region formed into a long arc over time, and formed a sandy area known as Isle Derniere (French) in the 1800's. Island Derniere was originally a resort island with white beaches, clearer water than the marshy mainland, and constant breezes [9]. A hurricane on August 10, 1856 severely damaged Isle Derniere and completely inundated the island with its storm surge. Sallenger [9] remarked that after the 1856 hurricane, Isle Derniere had been pushed beyond a tipping point from which it could not recover. The hurricane killed two-hundred people, around half the population on the island. Every structure on the island was destroyed, and the name changed from a singular 24 mile island, to plural, Isles Dernieres, since the island had separated into two smaller islands. Today, after additional storms, eroding, and subsidence, the once whole island is now segmented. The massive hurricane denuded the island vegetation which was essential for soil stabilization and eliminated shelter that provided food for organisms. Wetland plants also provide habitat and oxygen, another necessity for organisms. Today the fragmented Isle Derniere is a haven for seabirds like pelicans and other animals and plants [9]. The plants on barrier islands have adapted to the constantly changing environment with extensive root systems, photosynthesizing and releasing oxygen, and these tough leaves are able to withstand wind and salt. Several species of *Spartina* are commonly used on the coast due to their strong roots, adaptability to hypoxia, and high salt tolerance [8].

A major problem in the Gulf of Mexico is dangerously low oxygen levels that occur each summer since oxygen diffusion is less in warm water. Biological indicators including algal blooms, fish swimming at the top of water for air or fish kills serve as a clue to dead zones. This hypoxic region is called the dead zone. The dead zone is predicted to be 5840 miles in 2016. The dead zone is caused when nutrients from fertilizer runoff and soil erosion flow into the Gulf proliferating small organisms. After dissolved oxygen levels become anoxic organisms begin to die resulting in a dead zone. The decomposition of organisms requires increased oxygen demand [11]. Wastewater treatment facilities use tertiary water treatment to reduce the nutrient loads. Wastewater is passed through man-made marshes so that plants can assimilate available nutrients. Another cause of Gulf of Mexico dead zones is a lack of storm activity or windy weather which would blend normoxic and hypoxic water. Hypoxic water eventually becomes anoxic when oxygen demand is increased, where it cannot support marine life. Reducing the dead zone could be achieved by using water diversions sent from the Mississippi River through marsh areas which could decrease the waste nutrients that are typically deposited into the Gulf of Mexico [11]. Any damage done to wetland habitats can greatly decrease the quantity and diversity of living organisms that are dependent on these areas for shelter and sustenance. Louisiana marshes commonly serve as nurseries for organisms that are financially significant for the economy and essential to the aquatic food chain, like blue crabs and crawfish. Barrier islands are gradually segmented causing a spike in shrimp populations. More area on the shoreline, also called land-water interface, is available for organisms to sustain themselves. Although

it seems counter-intuitive, an increase in shrimp harvests could actually be a warning sign of past and future land loss. This concept is clearly demonstrated in an activity developed by Blanchard which explains the importance of coastlines to shrimp populations. Eventually, the size of the marshes decreases as a result of erosion and subsidence, until the island cannot sustain the food web.

Shrimp move into estuarine nursery areas and feed at the vegetation (marsh grass, mangrove, or seagrass) water interface [12]. Post larvae shrimp from 25 to 44 mm indiscriminately ingest the top layer of sediment, which is primarily comprised of *Spartina*, algae, and microorganisms. The health of a marsh impacts the productivity of Louisiana's estuaries and bays [13]. As living organisms decline, the perimeter increases as erosion begins, until it is fully eroded at which point it is too late to reverse [13].

The genus *Spartina* (S.) (*S. alterniflora*, *S. spartinae* and *S. patens*) is uniquely adapted to live in the harsh Louisiana Gulf of Mexico coastline. *Spartina alterniflora*, also known as smooth cordgrass or oystergrass, is used along salt marshes particularly for coastal restoration of levees, shores, canal banks, and other places where soil and water meet. This grass stabilizes soil effectively in places that are loosely packed and can tolerate up to 33 ppt salinity. *Spartina alterniflora* are typically 2 to 7 feet in height with a typical leaf blade. They are long-lived perennials that thrive in the warm seasons. Smooth cordgrass normally grows continuously and parallel to shorelines. *Spartina alterniflora* is primarily propagated using division. The circulation of water around this grass impacts its strength as well as the amount of debris or multitude of herbivores in the area [14]. *Spartina spartinae* grass, commonly known as gulf cordgrass, work effectively with projects that pertain to coastal restoration. These plants can serve as habitats for birds and a source for the forage of cattle and geese. Plant leaves are stout, thickly clumped, and flowered in spring and summer. *Spartina spartinae* grows on the Gulf Coast from Texas to Florida and also grows into Eastern Mexico. These grasses require very little maintenance and are fairly tolerant to drought. The durability of this grass makes it a practical grass to aid coastal restoration [15]. *Spartina patens* grasses, also referred to as saltmeadow cordgrass, are used for coastal restoration to stabilize levees and dunes by beaches or on barrier islands. They provide food and shelter to many species of wildlife and are commonly used in areas with loose soil. These grasses normally grow from one to four feet tall and have shiny, dark green leaves. *Spartina patens* are found growing within marshes containing salt or brackish conditions, dunes of low elevation, sandy beaches, and tidal flats. They commonly grow along the coast of the Gulf of Mexico and on the Great Lakes' shores. These marsh grasses have a high tolerance for salinity [16].

Land subsidence, erosion, sea level rise, and human activities are major contributing factors to land loss. Saltwater intrusion as a result of channelization kills large areas of plants contributing to the loss of habitats for wildlife. In addition to high salinity, hypoxic and anoxic areas are deadly to organisms but can be aided by an increase in vegetation [17]. Determining the species of *Spartina* that diffuses the most oxygen into water can assist with management of negative hypoxic and anoxic effects. When oxygen levels are increased, organisms are able to survive and preserve habitats. Erosion can still occur, but marine organisms along the shoreline can lessen their effects [8]. Louisiana's marshes and barrier islands serve as part of our storm defenses, a vital and productive ecosystem that buffers from the impact of tropical storms and hurricanes [1]. These marshes are critically endangered by subsidence, erosion, salt water intrusion and pollution. Without restoration efforts, the Isle Derniere Islands are predicted to disappear in the year 2033 [1].

The objectives of these two studies were as follows: Study 1: To determine the effect of oxygen levels on *Callinectes sapidus* to establish the relationship between aeration and lactate, glucose, and protein concentration; also, to determine the effect of hypoxia on blue crab behavior in relation to manual dexterity (time it takes to correct position). Study 2: To determine the effect of oxygen levels on *Procambarus clarkia* to determine the influence of aeration on lactate, glucose, and protein concentration. Additionally, an objective was to determine the relationship between *Spartina* species and the health of crawfish exposed to hypoxia on crawfish behavior in relation to manual dexterity (time it takes to correct position).

## 2. Materials and Methods

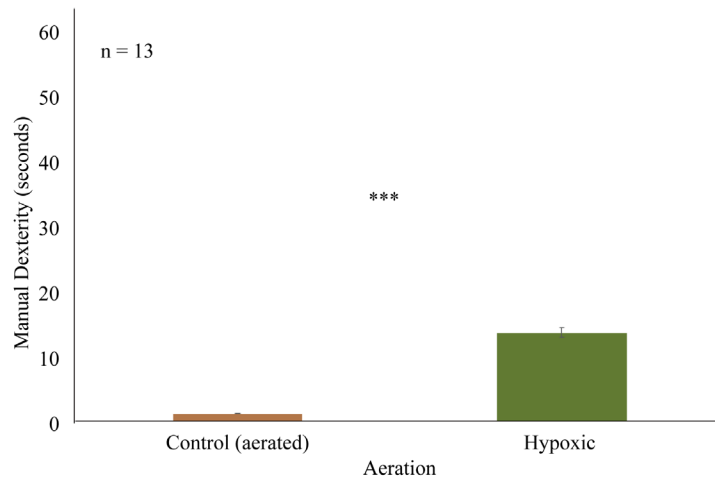
**Study 1:** 32 blue crabs were collected off the coast of Grand Chenier, Louisiana and transported to the laboratory. Thirty-two fifteen-gallon buckets with 1 crab in each, 16 hypoxic with plastic covers, the other 16 oxygenated by a water well (control) were used to establish oxygen treatments. An aged dechlorinated water solution and instant ocean mixture of 15 parts per thousand salinity was used in each container for blue crabs. The duration of the experiment was for 24 hours, measuring dexterity and hemolymph samples were extracted at 4 hours

and 24 hours. Dissolved oxygen measurements were taken to ensure that the parameters of 1.0 - 2.0 mg/L as hypoxic and <1.0 mg/L as anoxic were met. The meter used was a Hanna HI 98186 dissolved oxygen/BOD/OUR/SOUR Meter hydrolyzed using ten drops of HI 7041S Dissolved Oxygen Probe Filling Solution. To measure the behavior of crabs in each group, each crab was flipped over and time to correct itself was measured. In seconds from 0 - 60. If the crab was limp, but not dead, its time was recorded a value of 60. Dead animals were not counted in this part of the procedure. Hemolymph of crabs was measured with syringes after wiping the crab properly with Kimtech wipes to eliminate contamination. These syringes have a needle with of 20G. The samples are placed in closed tubes, labeled, mixed with a pestle, and centrifuged for five minutes at 3000g in an IEC HN-SII Centrifuge at 24°C. Protein concentration was measured with a Reichert VET 360 protein refractometer, lactic acid with a NOVA Biomedical Lactate Plus lactate meter, and glucose with a TELECARE™ Blood Glucose Monitoring System. The data was then analyzed using the Statistical Analysis System. The Statistical Analysis System was used to conduct ANOVA tests on collected data in order to measure the statistical significance at the 0.05 level between each treatment.

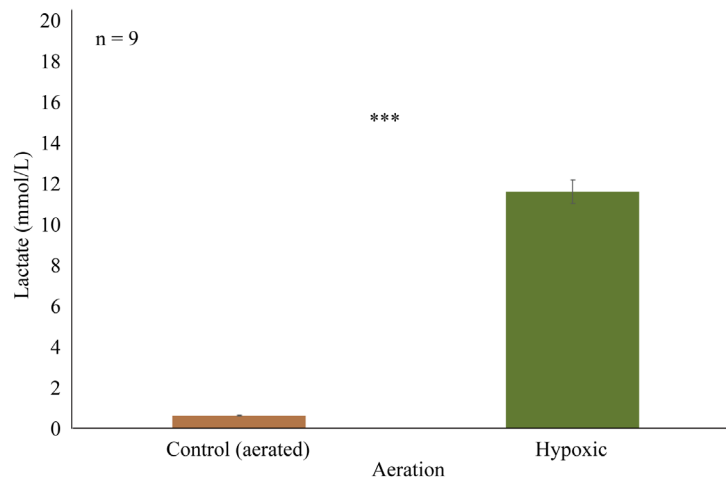
**Study 2:** 28 crawfish were collected from the LSU Aquaculture Research Station's ponds. Fifteen-gallon buckets with 14 crawfish in each, one hypoxic with no aeration, the other oxygenated by a water well (control). The experiment was for a duration of 24 hours, measuring one group at 4 hours and the other at 24 hours. Dissolved oxygen in water was measured to ensure that the parameters of 1.0 - 2.0 mg/L as hypoxic and <1.0 mg/L as anoxic are met. The meter used is a Hanna HI 98186 dissolved oxygen/BOD/OUR/SOUR Meter hydrolyzed using ten drops of HI 7041S Dissolved Oxygen Probe Filling Solution. To measure the behavior of crawfish in each group crabs were flipped over and a timer was used to measure the number of seconds from 0 - 60 for the crawfish to correct itself. If the crawfish was limp, but not dead, its time is given a value of 60. Dead animals were not counted in this part of the procedure. Hemolymph of crawfish was extracted with 18G 1.5" needles after wiping the crawfish properly with a Kimtech wipe to eliminate contamination. To make the extraction easier, microhematocrit capillary tubes should be used. The samples are placed in closed tubes, labeled, mixed with a pestle, and centrifuged for five minutes at 3000 g in an IEC HN-SII Centrifuge at 24°C. Protein concentration was measured with a Reichert VET 360 protein refractometer, lactic acid with a NOVA Biomedical Lactate Plus lactate meter, and glucose with a TELECARE™ Blood Glucose Monitoring System. Repeat this process for the 24-hour group. Next, crawfish were added to fifteen-gallons of water. *Spartina patens* plants, *Spartina spartinae* plants, and control containers with no vegetation were established to determine how the plants affect the dissolved oxygen and therefore the behavior of crawfish, and if vegetation is a valid substitution for water wells. There are 12 total containers, 3 within each treatment, and 5 crawfish in each pot. The experiment was established for 24 hours, measuring one group at 4 hours and the other group at 24 hours. Dissolved oxygen was measured as discussed above to ensure the parameters of anoxia and hypoxia are met before extracting the hemolymph of the crawfish. To measure the behavior of crawfish in each group, crawfish were flipped over and timed to correct themselves in seconds from 0 - 60. If the crawfish was limp, but not dead, its time is given a value of 60. Dead animals were not counted in this part of the procedure. Hemolymph was extracted from crawfish with 18G 1.5" needles after wiping the crawfish properly with a Kimtech wipe to eliminate contamination. To make the extraction easier, microhematocrit capillary tubes were used. The samples were placed in closed tubes, labeled, mixed with a pestle, and centrifuged for five minutes at 3000 g in an IEC HN-SII Centrifuge at 24°C. Protein concentration was measured using a Reichert VET 360 protein refractometer, lactic acid with a NOVA Biomedical Lactate Plus lactate meter, and glucose with a TELECARE™ Blood Glucose Monitoring System. Repeat for the 24-hour group. The data was then analyzed using the Statistical Analysis System. Data was collected and means statistically compared using a Duncan's Multiple Range Test.

### 3. Results and Discussion

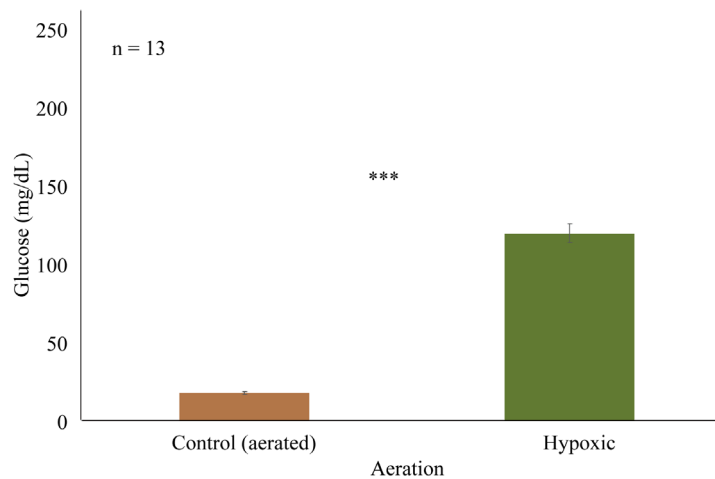
The blue crabs exposed to hypoxic conditions required significantly more time to flip themselves over from their backs than aerated crabs (Figure 1). This increased time to right self could indicate decreases in predation avoidance or reduced ability to feed. The average lactate in the hemolymph of crabs subjected to hypoxia was significantly higher than the average lactate in the hemolymph of crabs that were exposed to oxygen (Figure 2). The blue crabs that were not oxygenated also contained significantly more glucose than crabs that were oxygenated (Figure 3). There was no statistical difference found between the protein concentrations of blue crabs that were kept in hypoxia and those that had oxygenation (Figure 4).



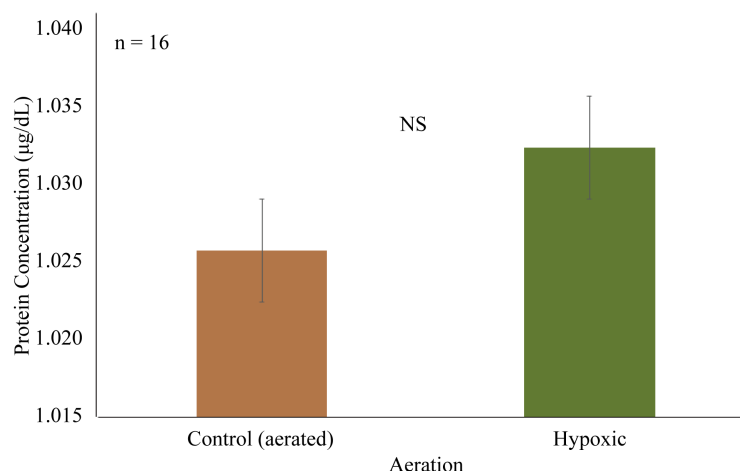
**Figure 1.** The effect of aeration on manual dexterity of *Callinectes sapidus*. Significance: NS = Not significant; \* = 0.05; \*\* = 0.01; \*\*\* =  $\leq 0.001$ ; Vertical bars represent standard error.



**Figure 2.** The effect of aeration on lactate in *Callinectes sapidus*. Significance: NS = Not significant; \* = 0.05; \*\* = 0.01; \*\*\* =  $\leq 0.001$ ; Vertical bars represent standard error.



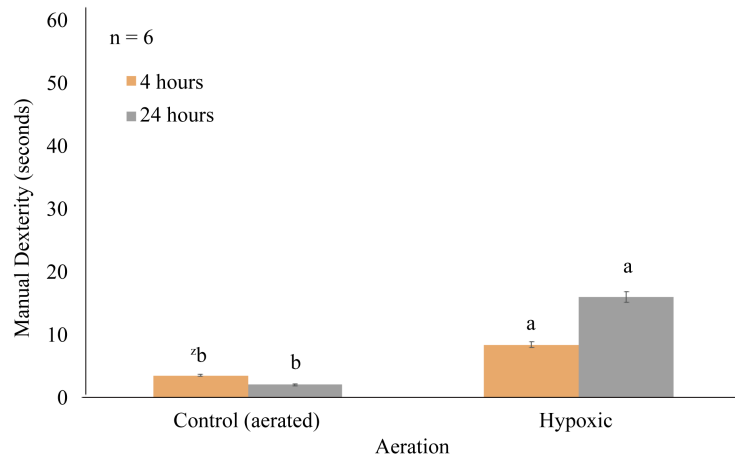
**Figure 3.** The effect of aeration on glucose in *Callinectes sapidus*. Significance: NS = Not significant; \* = 0.05; \*\* = 0.01; \*\*\* =  $\leq 0.001$ ; Vertical bars represent standard error.



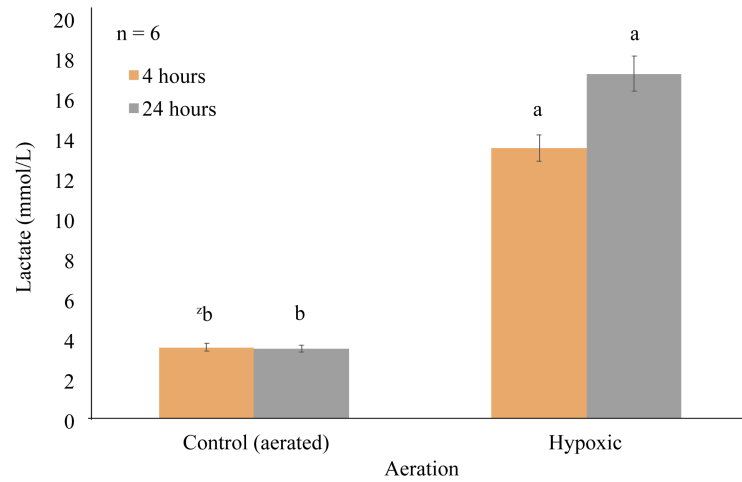
**Figure 4.** The effect of aeration on protein concentration in *Callinectes sapidus*. Significance: NS = Not significant; \* = 0.05; \*\* = 0.01; \*\*\* =  $\leq 0.001$ ; Vertical bars represent standard error.

The crawfish exposed to hypoxic conditions at 4 hours had a significantly longer period of recovery from overturning than the crawfish at 4 hours that had normoxic aeration, with the p value at 0.05 throughout the analyses. At 24 hours, the average length of time for the control to recover itself at 24 hours was significantly greater than the time taken by crawfish that received oxygen in the 24 hour group (Figure 5). At both 4 and 24 hours, the crawfish that had sufficient access to oxygen had significantly lower amounts of lactate in their hemolymph (Figure 6); however, the crawfish exposed to oxygen had significantly lower amounts of glucose in their hemolymph after 24 hours. At 4 hours, the amount of glucose in both groups was not significantly different, only 34.4 mg/dL compared to the 24-hour difference of 131 mg/dL (Figure 7). The same result for the crawfish's protein concentration because the 4-hour group did not show significant differences, while the 24-hour group did show a statistical difference (Figure 8). When *Spartina* (*S.*) grasses replaced the air wells, the crawfish that were exposed to hypoxic conditions had a significantly higher manual dexterity recovery period than the crawfish exposed to *S. patens* and *S. spartinae* under both time allotments (Figure 9). The lactate in the hemolymph of the crawfish exposed to *S. species* was significantly lower than the lactate of the crawfish in the control for 4 hours and 24 hours (Figure 10). Like lactate, the glucose in the hemolymph of crawfish that had adequate amounts of oxygen was significantly less in mg/dL than that of the crustaceans exposed to DO levels <1.0 mg/L at both 4 and 24 hours. Within the species, the glucose measurements for *S. patens* were larger than the glucose measurements for *S. spartinae*, but not significantly different (Figure 11). As for protein concentration in the hemolymph of crawfish, there was no significance in the difference of their µg/dL of protein between the control and experimental group at either time (Figure 12).

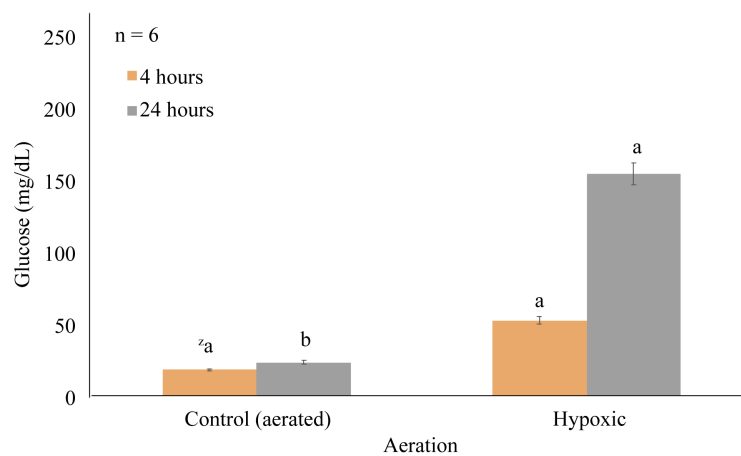
Determining the amelioration of different *Spartina* grasses or the absence of vegetation on marshes provides an improved understanding of marsh grass selection and the overall coastal environment. Erosion, subsidence, and salt-water intrusion are serious problems not only in Louisiana, but across the globe, continuously taking a toll on land, vegetation, and habitats. With erosion comes loss of land and with land-loss comes habitat damage and economic decline. Marsh grasses and trees continuously protect vulnerable islands from dangerous storms and inauspicious salt water intrusion. These grasses can lessen the force of strong hits and slow salt water intrusion by tolerating some salinity. Without these crucial plants, environments continue to decline due to lack of protection. Even with plants, many marshes are deteriorating due to hypoxia, or lack of oxygen. An environment without oxygen cannot support life which is needed to prolong the lives of marshes. No oxygen means vegetation and habitat loss which then leads to vulnerability and later open-water areas. An effective way to combat these noxious forces is marsh grasses because they provide shelter, protect inward land against the daily ebb and flow of water, commonly reduce salt water intrusion, prevent subsidence, and produce oxygen. This oxygen supplies organisms with necessary nutrients, and plants produce it as a bi-product of photosynthesis. With the data collected in the experiment, it does show a slight relationship between species and dissolved oxygen in water, as well as penetration force of the soil surface and subsidence. This valuable information can be used to effectively select marsh grasses that resist erosion and sinking and protect the marshes as well as possible. The



**Figure 5.** The effect of aeration and time on manual dexterity of *Procambarus clarkii*. <sup>z</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.

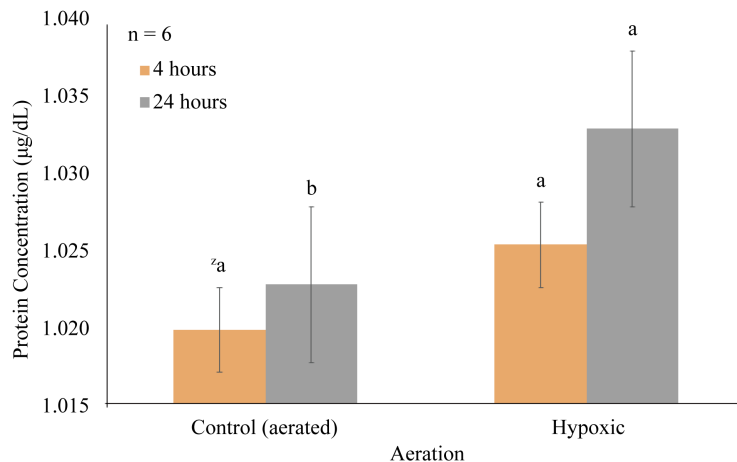


**Figure 6.** The effect of aeration and time on lactate in *Procambarus clarkii*. <sup>z</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.

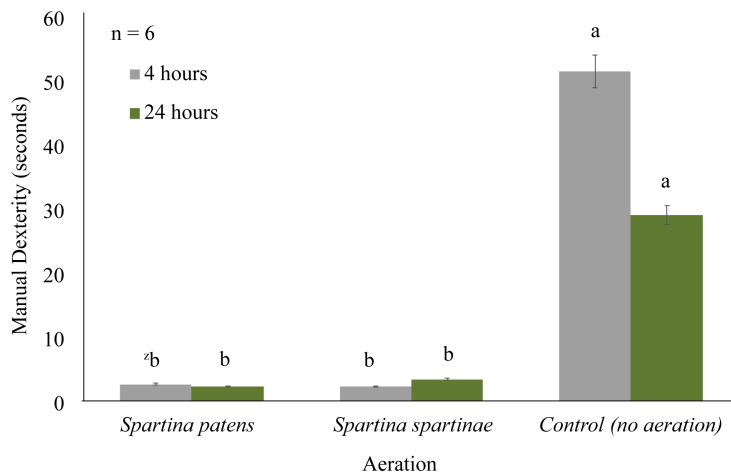


**Figure 7.** The effect of aeration and time on glucose in *Procambarus clarkii*. <sup>z</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.

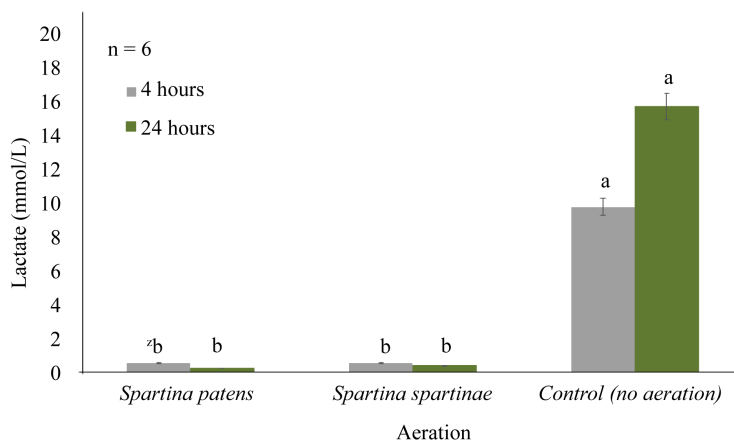




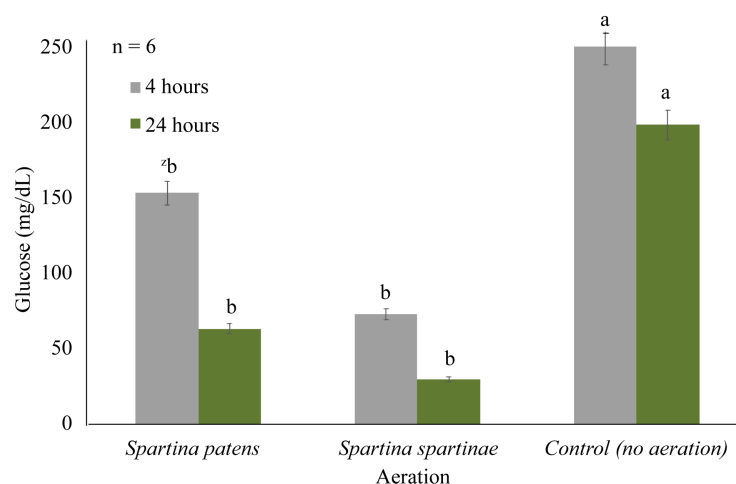
**Figure 8.** The effect of aeration and time on protein concentration in *Procamburus clarkii*. <sup>z</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.



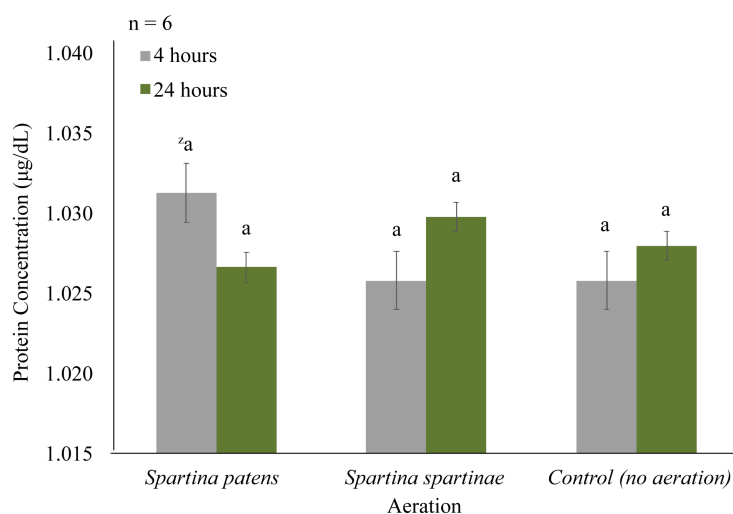
**Figure 9.** The effect of plant species and time on manual dexterity of *Procamburus clarkii*. <sup>z</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.



**Figure 10.** The effect of plant species and time on lactate in *Procamburus clarkii*. <sup>z</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.



**Figure 11.** The effect of plant species and time on glucose in *Procamburus clarkii*. <sup>2</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.



**Figure 12.** The effect of plant species and time on protein concentration in *Procamburus clarkii*. <sup>2</sup>Means with the same letter are not significantly different at the 0.05 level; Vertical bars represent standard error.

barrier islands and marshes are essential to main-shore protection. Vegetation can slow forces of erosion, subsidence, and hypoxia on the Louisiana coast. The management of Louisiana's coastline will require a balance between economic demands and preservation of sensitive coastal environmental resources.

This research establishes the significance of water and vegetation health in relation to the health of the organisms living in hypoxic ecosystems. The ability to identify the health of the water by an indicator species like Louisiana crawfish demonstrates the connection between the environment and its fisheries. Coastal cultures thrive when the environments that make up the ecosystem thrive as well, and such marsh health can only be true with adequate oxygen levels, much animal life, normal metal concentrations, and other crucial stabilizing factors. These variables support the cycles that make up a healthy marsh and allow environments to naturally succeed. Also, humans utilize aquatic animals for food sources, economical benefactors, and recreational purposes. The health of each living animal reflects the health of the environment, and the ability to see this connection by one organism makes this procedure a probable option for detecting hypoxia or other problems with the water health. In addition to healthy hemolymph analysis, the crustaceans' behaviors, such as their activeness and ability to correct their position, are essential for healthy marshlands. Finally, this plan for restoration clearly shows the ability of humans to use plants for oxygen production that can effectively replace water wells; soil, air, and wa-

ter filters; natural habitats; buffers for fatal storms; lifelines for aquatic organisms; and most importantly, highly successful tools to rebuild barrier islands.

#### 4. Conclusion

Manual dexterity, lactate, glucose, and protein concentration were analyzed in the hemolymph of *Callinectes sapidus* and *Procambarus clarkia*. There was an influence of time and aeration on manual dexterity, lactate, glucose, and protein concentration, indicating the physiological and external effects of hypoxia and anoxia on aquatic wildlife. In both crabs and crawfish, the hypoxic conditions hindered manual dexterity, demonstrated by the increase in time to correct themselves compared with the control organisms. The lactate, as well, was significantly greater in the hypoxic groups, showing the relationship between hypoxia and increased lactate. Glucose measured in the hemolymph of organisms was higher in the hypoxic crab and crawfish/species groups, but with the crawfish alone, the glucose at 4 hours was statistically similar across the two groups; however, at 24 hours, there were significantly higher levels of glucose in the hypoxic groups of both studies. Similarly, protein concentration was not significantly different between the hypoxic and oxygenated groups for crabs in either time group, but the crawfish protein concentration was statistically different at the 24-hour mark indicated by the elevated protein of the hypoxic crawfish. These studies prove that time of exposure to hypoxic and normoxic oxygen levels has an effect on the contents in hemolymph. *Spartina patens* and *Spartina spartinae* were sufficient substitutes to mechanical aerators by maintaining healthy levels of lactate, glucose, and protein in the crawfish's hemolymph.

#### 5. Application to Society

Determining the amelioration of different *Spartina* grasses or the absence of vegetation on marshes will provide an improved understanding of the role of marsh grass selection and the overall coastal environment. Erosion, subsidence, and salt-water intrusion, serious problems not only in Louisiana, but across the globe, continuously take a toll on land, vegetation, and habitats. With erosion comes loss of land and with land-loss comes habitat damage and economic decline. Marsh grasses and trees continuously protect vulnerable islands from dangerous storms and inauspicious salt water intrusion. These grasses can lessen the force of strong hits and slow salt water intrusion by tolerating salinity. Without these crucial plants, environments continue to decline due to lack of protection. Even with plants, many marshes are deteriorating due to hypoxia, or lack of oxygen. An environment without oxygen cannot support life which is needed to prolong the longevity of marshes. No oxygen means vegetation and habitat loss which then leads to vulnerability and later open-water areas. An effective way to combat these noxious forces is marsh grasses because they provide shelter, protect inward land against the daily ebb and flow of water, commonly reduce salt water intrusion, prevent subsidence, filter nutrient run-off and produce oxygen. This oxygen supplies organisms with necessary nutrients. With the data collected in the experiment, it does show a relationship between species and dissolved oxygen in water. This information can be used to effectively select marsh grasses that resist erosion and sinking and protect the marshes. The barrier islands and marshes are essential to main-shore protection. Vegetation can slow forces of erosion, subsidence, and hypoxia on the Louisiana coast. The management of Louisiana's coastline will require a balance between economic demands and preservation of sensitive coastal environmental resources. Coastal populations utilize aquatic animals for food sources, economical benefactors, and recreational purposes. The health of each living animal reflects the health of the environment, and the ability to see this connection by one organism makes this procedure a probable option for detecting hypoxia or other problems with the water health. In addition to healthy hemolymph analysis, the crustaceans' behaviors, such as their activeness and ability to correct their position, are essential for healthy marshlands. Finally, this plan for restoration clearly shows the ability of humans to use plants for oxygen production that can effectively replace water wells; soil, air, and water filters; natural habitats; buffers for fatal storms; lifelines for aquatic organisms; and most importantly, highly successful tools to rebuild barrier islands.

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