



VCU

Virginia Commonwealth University
VCU Scholars Compass

Theses and Dissertations

Graduate School

2009

The Responses of Blue Crabs (*Callinectes sapidus*) to Hypoxia/ Hypercapnia in Freshwater

James Martin
Virginia Commonwealth University

Follow this and additional works at: <https://scholarscompass.vcu.edu/etd>



Part of the [Environmental Sciences Commons](#)

© The Author

Downloaded from

<https://scholarscompass.vcu.edu/etd/1847>

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

**RESPONSES OF BLUE CRABS (CALLINECTES SAPIDUS) TO
HYPOXIA/HYPERCAPNIA IN FRESHWATER**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University.

By

James Thomas Martin
Bachelor's of Science-Virginia Commonwealth University

Thesis Director: Dr. Peter L. deFur, Affiliate Associate Professor, Center for Environmental
Studies

Virginia Commonwealth University
Richmond, Virginia
April 2009

Acknowledgments

The author wishes to thank the following people and organizations: VCU Rice Center for funding and use of facilities; Dr. Peter deFur my thesis advisor for guidance and providing equipment; Dr. Michael Fine, Dr. Greg Garman, Dr. Jennifer Stewart, and Dr. Brian Barnstein for providing additional access to equipment and facilities; Kyle Newman and Anna Kushner, my friends, for various assistance throughout the study. Finally, I would like to thank my parents for their love and support. The research was supported by a graduate student research grant from the VCU Rice Center.

Table of Contents

Acknowledgements.....	2
List of Tables.....	5
List of Figures.....	6
Abstract.....	8
Introduction/Literature Review.....	9
Methods.....	15
Collection.....	15
Experimental Animals and Conditions.....	16
Ambient pH Changes.....	16
Hemolymph Sampling and Analysis.....	17
Statistical Analysis.....	18
Results.....	19
Behavior.....	19
PO ₂	19
PCO ₂ and pH.....	20
Lactate.....	20
Hemocyanin.....	20
Mortality.....	21
Discussion and Conclusions.....	21
PCO ₂ and pH.....	22
Lactate.....	24
Hemocyanin.....	27

Mortality.....	28
Conclusions.....	30
Literature Cited.....	32
Appendix.....	37

List of Tables

Table 1- Mean crab size and mass for all experiments.....	37
Table 2- Postbranchial hemolymph variables of blue crabs exposed to moderate hypoxia (75-85 mmHg), 23 C, Freshwater for 21 days. Trial 3.....	37
Table 3- Postbranchial hemolymph variables of blue crabs exposed to moderate hypoxia (50-60 mmHg), 23 C, freshwater for 3 days. Trial 1.....	37
Table 4- Postbranchial hemolymph variables of blue crabs exposed to moderate hypoxia (50-60 mmHg), 23 C, freshwater for 11 days. Trial 2.....	37
Table 5- Mean hemolymph pH and sample size (#) for control and exposure data in trials 1 and 2.....	37
Table 6- Mean lactate and sample size (#) for control and exposure data in trials 1, 2, and 3.....	38
Table 7- Mean hemocyanin and sample size (#) for control and exposure data in trials 1, 2, and 3.....	38

List of Figures

Fig. 1- Sex composition of blue crabs captured during past studies in Chesapeake Bay tributaries.....	39
Fig. 2- Male (top) and mature female (bottom) molt stages of blue crabs during capture by Hines et al. (1987).....	40
Fig. 3- Conceptual model of hypothesized positive responses to exposure.....	41
Fig. 4- Conceptual model of hypothesized negative responses to exposure.....	41
Fig. 5- Location of VCU Rice Center collection area.....	42
Fig. 6- Blue crab after being prepared for study.....	43
Fig. 7- Ambient pH decline during exposure for trials 1, 2, and 3.....	43
Fig. 8- Ambient oxygen and pH during trials 1, 2, and 3.....	44
Fig. 9- Postbranchial hemolymph PO ₂ of blue crabs in hypoxia/hypercapnia (50-60 mmHg) for 8 days, 23 C, freshwater.....	44
Fig. 10- Postbranchial hemolymph PCO ₂ of blue crabs in hypoxia/hypercapnia (75-85 mmHg) for 21 days, 23 C, freshwater.....	45
Fig. 11- Postbranchial hemolymph pH of blue crabs in hypoxia/hypercapnia (50-60 mmHg) for 3 and 11 days, 23 C, freshwater.....	45
Fig. 12- Postbranchial hemolymph lactate of blue crabs in hypoxia/hypercapnia for 3 and 11 days (50-60 mmHg), and 21 days (75-85 mmHg), 23 C, freshwater.....	46
Fig. 13- Postbranchial hemolymph hemocyanin of blue crabs in hypoxia/hypercapnia for 3 and 11 days (50-60 mmHg), and 21 days (75-85 mmHg), 23 C, freshwater.....	47
Fig. 14- Percent mortality of blue crabs in hypoxia (50-60 mmHg) for 4 days, 23 C, freshwater, pH between 7.14-7.20. Trial 1.....	48
Fig. 15- Percent mortality of blue crabs in hypoxia (50-60 mmHg) for 13 days, 23 C, freshwater, pH between 6.52-6.83. Trial 2.....	48
Fig. 16- Ambient pH and hemolymph pH during trial 1 (hypoxia (50-60 mmHg) for 13 days, 23 C, freshwater).....	49

Fig. 17- Ambient pH and hemolymph pH during trial 2 (hypoxia (50-60 mmHg) for 13 days, 23 C, freshwater).....	49
Fig. 18- Conceptual model of results and possible physiological consequences.....	50

ABSTRACT

RESPONSES OF BLUE CRABS (CALLINECTES SAPIDUS) TO HYPOXIA/HYPERCAPNIA IN FRESHWATER By JAMES T. MARTIN, Masters of Science

A thesis submitted in partial fulfillment of the requirements for the degree Master of Science in Environmental Science at Virginia Commonwealth University, 2009.

Major Director: Dr. Peter L. deFur, Affiliate Associate Professor, Center for Environmental Studies

The present research examined respiratory responses of blue crabs to long term (4, 13, and 21 days) hypercapnic hypoxia in freshwater at 23 C. Hypoxic conditions (50-60 & 75-85 mmHg O₂) were induced by allowing the crabs to consume their oxygen supply, resulting in a hypercapnic induced decrease in pH that remained through the exposure. Postbranchial hemolymph responses to hypoxia/hypercapnia in freshwater demonstrate decreases in PO₂, increases in PCO₂, and decreases in pH. Lactate levels decreased over time, but hemocyanin concentration was highly variable with no trends. PH, lactate, and hemocyanin observations also demonstrated high variability and a variety of different responses in individual crabs. There was no evidence of improving oxygen transport abilities. Despite varying responses high mortality rates were observed. The high mortality rate suggests blue crabs are not able to survive the multiple stress of hypoxia/hypercapnia along with the stress of living in freshwater. The mortality rates observed are much greater than previous blue crab hypoxic studies in saltwater. Elevated mortality may result from a failure of oxygen transport, acid-base balance or ion regulation.

Introduction and Literature Review

Aquatic Hypoxia or low dissolved oxygen has been recognized as harmful to living systems except for a few anaerobic bacteria. Newcombe and Horne (1938) decades ago defined hypoxia on the basis of concentrations, less than 2.0 mg/l, but partial pressure is a more appropriate physiological measure of O₂ saturation. Most aquatic organisms cannot tolerate hypoxia less than 25% saturation and will be physiologically stressed (Diaz, 2001). Hypoxia becomes a physiological stress when O₂ pressure is lower than oxygen demand or O₂ supply limits the scope for activity or growth. Not meeting biological oxygen demand results in various physiological stresses, as aerobic respiration is the primary process of producing energy for most organisms. In addition to hypoxia, hypercapnia is a condition of high CO₂, it becomes a physiological stress when an organism's blood is overburdened with CO₂. When hypoxia and hypercapnia occur simultaneously an organism's respiratory system, as well as other physiological functions, will be severely stressed (Burnett, 1997).

Hypoxia and hypercapnia commonly co-occur in estuaries as the respiratory consumption of O₂ and production of CO₂ in a hypoxic environment leads to low O₂ and high CO₂. The elevated levels of CO₂ during hypoxia lead to a decrease in pH and an additional acidic stress. The decrease in pH is dependent on the production of CO₂ and increase in PCO₂. When CO₂ is added to water, the following reaction takes place $[\text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3 = \text{HCO}_3^- + \text{H}^+]$. This reaction leads to elevated bicarbonate and hydrogen levels in the aquatic environment and in tissues (Burnett, 1997). Decreased pH becomes a serious secondary stressor during hypoxia/hypercapnia because acidosis of the blood causes a decrease in the oxygen carrying capacity of most respiratory pigments (Dejours, 1981).

The most common cause of hypoxia in the Chesapeake Bay and many other waterways is high nutrient loads or eutrophication (Diaz, 2001; Kemp, 2005;). Eutrophication is the process of increased organic enrichment of an ecosystem, generally through increased nutrient inputs (Nixon, 1995). Eutrophication causes hypoxia through increased primary production, resulting in algal blooms and increased organic carbon (Diaz, 2001; Kemp et al., 2005). These algal blooms cause hypoxia by blocking sunlight, which decreases photosynthesis in aquatic vegetation, and through the bacterial breakdown of dead algal blooms or organic carbon. Bacterial decomposition of the algal blooms results in increased O₂ consumption and a decrease in ambient O₂ (Cooper and Brush, 1991). Current research and historical data from the Chesapeake Bay and water bodies around the world demonstrate eutrophication, and the resulting ecological effects present hypoxic stress for many forms of aquatic life (Diaz, 2001; Hagy et al., 2004; Kemp et al., 2005).

Freshwater tributaries of the Chesapeake Bay are important for the migration and development of blue crabs, yet also pose physiological stress to the crabs that make the journey to freshwater. During summer months blue crabs frequently migrate up freshwater rivers and creeks to molt (Hines et al., 1987). The migration of blue crabs demonstrates that sex and molt stage play a role in migration behavior, as Hines et al. (1987) and Shirley et al. (1990) found higher numbers of molting male blue crabs in a tidal creek compared to a river basin. Figures 1 and 2 give some geographic locations of blue crab sex and molt proportions collected in previous studies. Male blue crabs moving up tributaries to molt are presented with the stress of osmoregulation and molting. During molting, blue crabs are already in a vulnerable life stage where they are more vulnerable to hypoxia (Mangum et al., 1985; deFur, 1990). As the migration into dilute waters presents several physiological risks, the migration also provides

important benefits to development. These developmental benefits include decreased predation (Shirley et al., 1990) and greater body size gains in molting, due to facilitation of water uptake resulting from the large osmotic gradient in freshwater (deFur et al., 1988).

As blue crabs migrate throughout estuaries and tributaries, they are constantly under the possible stress of hypoxia/hypercapnia, along with the stress of osmoregulation. This multiple stress scenario can present itself as hypoxia/hypercapnia in freshwater and in low salinity estuarine environments; salt marshes and estuarine habitat have highly dramatic changes in pH and salinity (Cochran and Burnett, 1996). This multiple stress scenario can play out in many of the Bay blue crab habitats during all of their life stages.

Hypoxia has been observed in the Chesapeake Bay, and many of the Bay tributaries (Kuo and Neilson, 1987; Diaz, 2001). Throughout the Chesapeake Bay area, creeks, rivers, and bays have experienced hypoxic conditions at varying salinities and freshwater (Summers et al., 1997). In Virginia there have been observations of hypoxia in the lower reaches of the Rappahannock (Kuo and Neilson, 1987; Kuo et al., 1991; Park et al., 1996; Diaz, 2001) York (Kuo and Neilson, 1987; Diaz, 2001) and James Rivers (Kuo and Neilson, 1987). The tributaries display periodic hypoxia, and the bay displays seasonal summer hypoxia (Diaz, 2001).

Hypoxia/hypercapnia has also been observed in different bay and tributary environments. Hypercapnic pH decline has been observed during oxygen decline at James Island Creek in the Charleston Harbor Estuary (Burnett, 1997), shallow salt marshes (Cochran and Burnett, 1996), the Choptank River (Christmas and Jordan, 1988), and the York River and one of its tidal creeks (Hypes, 1999). The correlation between O₂ and pH was strongest at night and the early morning.

Blue crabs are strong osmoregulators, they maintain their hemolymph Na⁺ at 280 mmol/l in freshwater (Mantel, 1967). Increases in energy demand in dilute waters have been

demonstrated by increases in Na^+/K^+ ATPase activity at the gills (Towle et al., 1976; Li et al., 2006). Increased energy demand can present a major physiological problem for blue crabs exposed to hypoxia in freshwater. Due to the limited O_2 supply, osmoregulation could be compromised.

Different salinities affect blood pH in many organisms. Estuarine crabs exposed to freshwater have demonstrated a hemolymph alkosis: *C. sapidus* (Weiland and Mangum, 1975), *C. meanes* (Truchot, 1981), and *Eriocheir sinensis* (Truchot, 1992). These studies also demonstrate physiological adjustments to hemolymph pH are also necessary in freshwater.

Blue crabs survive hypoxic conditions in saltwater with varying hypoxic exposure times; 2-4 hours (Lowery and Tate, 1986), 7 days (deFur and Pease, 1988), and 25 days (deFur et al., 1990). To compensate, blue crabs maintain or increase hemolymph pH, increase hemocyanin concentration, and use anaerobic respiration (Lowery and Tate, 1986; deFur and Pease, 1988; deFur et al., 1990). Responses of other crustaceans to long-term hypoxia (5-21 days) have been studied in lobster (McMahon et al., 1978; Butler et al., 1978) and crayfish (McMahon et al., 1974; Wilkes and McMahon, 1982A; Wilkes and McMahon, 1982B). These studies demonstrated maintenance of the oxygen transport by increased pH and increased hemocyanin oxygen affinity in response to hypoxia.

Respiratory function changes in crabs exposed to hypoxia; several studies have found variable responses in different crabs (deFur and Pease, 1988; Henry et al., 1990; Rantin et al., 1996). Blue crabs hyperventilate in response to hypoxic conditions (Batterton and Cameron, 1978; deFur and Pease, 1988). Despite the increase in ventilation, postbranchial hemolymph PO_2 decreases by approximately 80% in 50 mmHg hypoxia (deFur and Pease, 1988; deFur et al.,

1990). These studies suggest that blue crabs will respond to hypoxia with hyperventilation and a large decrease in postbranchial hemolymph O_2 during hypoxic exposure.

Blue crab postbranchial hemolymph CCO_2 (total carbon dioxide) becomes elevated in response to hypoxia (deFur and Pease, 1988), likely due to an internal hypercapnia produced during hypoxia. A hypercapnic environment would only contribute to internal hypercapnia during hypoxia. Aquatic animal blood PCO_2 is sensitive to changes in ambient PCO_2 , causing changes in gill CO_2 exchange (Truchot and Forge, 1998). Crustaceans and fishes reactions to increased PCO_2 include partially compensating by elevating blood bicarbonate levels (Burnett, 1997) and hyperventilation, which may increase excretion of CO_2 (Mangum, 1997). deFur and Pease (1988) observed that blue crabs can maintain and elevate hemolymph pH in the presence of increased postbranchial CCO_2 during hypoxia, suggesting an increase in HCO_3^- to maintain hemolymph pH.

Previous studies on blue crabs demonstrated maintained or increased hemolymph pH during hypoxia (deFur and Pease, 1988; deFur et al., 1990). This response has also been observed in lobsters and crayfish in long-term hypoxia (McMahon et al., 1974; McMahon et al., 1978; Butler et al., 1978; Wilkes and McMahon, 1982A; Wilkes and McMahon, 1982B). None of these previous investigations included changes in ambient pH, which was nearly held constant. The hemolymph pH response is an important factor for survival during hypoxia because it prevents hemolymph acidosis and maintains or increases hemocyanin oxygen affinity, improving the animal's oxygen transport capabilities (Mangum, 1980). This result has been demonstrated in the crabs *Carcinus maenas*, *Cancer pagurus*, (Truchot, 1980) *Libinia emarginata*, *Ocypode quadrata*, (Burnett, 1979) and *C. sapidus* (deFur et al., 1990).

Blue crabs can use anaerobic respiration in response to hypoxia, indicated by increases in lactate (Lowery and Tate, 1986; deFur et al., 1990), the end product of anaerobic metabolism in crustaceans (Phillips et al., 1977). Severe hypoxia studies (<0.2 mg/l O_2) on crabs have demonstrated the highest lactate levels (Albert and Ellington, 1985; Lowery and Tate, 1986). Long-term moderate hypoxic exposure (50 mmHg for 25 days) increased lactate several fold by the 23-25 day (deFur et al., 1990). These studies suggest the extent of anaerobic respiration is dependent on the severity and duration of the hypoxic exposure.

Lactate also influences hemocyanin oxygen affinity. Increased L-lactate, the most common form, raises the oxygen affinity of hemocyanin in the crabs *Cancer magister* (Graham et al., 1983), *Carcinus maenes*, and *Cancer pagurus* (Truchot, 1980), and lobster (Bouchet and Truchot, 1985). During hypoxia lactate plays an important role in alternative energy utilization as well as the improvement of the oxygen transport system.

Long-term adaptations to hypoxia cause an increase in hemocyanin concentration and alteration of hemocyanin subunit composition (deFur et al., 1990). Blue crabs can increase hemolymph hemocyanin concentrations by 40% in hypoxic conditions, increasing its oxygen carrying capacity of the hemolymph (deFur et al., 1990). Under moderate hypoxia, blue crab hemocyanin concentration decreased by day 7 but increased more than 3 times by day 25 (deFur et al., 1990). This study suggests that in hypoxic conditions, increases in the oxygen carrying capacity of the hemolymph are utilized to endure hypoxia.

Most previous work with blue crab hemocyanin and its role in hypoxia has focused on studying the changes in hemocyanin subunit composition and its changes in oxygen affinity (Burnett, 1979; Mangum and Rainer, 1988; deFur et al., 1990; Mangum, 1997). Little study has been put into hemocyanin concentration and its effect on the oxygen transport system. deFur et

al. (1990) observed hemocyanin concentration, subunit composition, and oxygen affinity in blue crabs exposed to long-term hypoxia. In this study, the rise in hemocyanin concentration did not occur until there had been a change in subunit composition, the authors suggested that the hemocyanin adaptation may be expedited by an increase in hemocyanin concentration.

To understand the risks posed to blue crab physiology from hypoxia/hypercapnia in freshwater, physiological functions essential to surviving hypoxia were monitored during exposure to hypoxia/hypercapnia in freshwater (Figures 3 and 4). The present research investigated physiological changes in postbranchial hemolymph PO_2 , PCO_2 , pH, lactate, and hemocyanin.

Methods

Collection

Adult intermolt male blue crabs were captured at the VCU Rice Center in late summer (August-Early October). The VCU Rice Center is located in the tidal freshwater James River, approximately 75 river miles upriver from the Chesapeake Bay (Figure 5). The crab pots were baited with chicken or turkey wings and checked every 24 hours. Crabs were immediately transported to the VCU Life Sciences building, an approximate 45-minute trip, in a storage container filled with approximately 1 inch of water from the collection site.

Ambient conditions at the collection site were: temperature = ~20-30 C, pH = ~7.00-9.25, O_2 = 70%-205%, salinity = ~0.20-0.60 ppt.

Experimental Animals and Conditions

Crabs were held in a freshwater tank at 140-160 mmHg O₂, 23 C, and pH > 7.5 maintained by addition of bicarbonate. The crabs were fed frozen fish twice a week during the holding and exposure conditions. Crabs were fed at least 24 hours before hemolymph extraction. Crabs' claws were restrained using vinyl tubing and cyanoacrylate. To extract postbranchial hemolymph, holes were drilled in the carapace over the heart using a dremel tool and were covered with latex rubber affixed with cyanoacrylate (Figure 6). The crabs were given 2 days to recover from these procedures before hemolymph samples were taken.

The experiment was conducted three times to increase the sample size and in an attempt to increased survival. Hypoxic conditions were induced by decreasing the number of air stones, and allowing the crabs to consume their oxygen supply until the tank O₂ level reached 50-60 mmHg O₂ (for trial 3, 75-85 mmHg O₂). PO₂ levels in the tank were monitored with a polarographic O₂ electrode and pH/blood gas analyzer (Instrumentation Laboratory 113), and pH levels with a pH electrode and pH meter (Radiometer PHC3001 w/ Radiometer pHM82).

Ambient pH Changes

Once hypoxic conditions were initiated, pH was allowed to decline along with declining O₂; pH did not decrease identically in the three trials. In trial 1 pH declined from 7.79 to 7.36 as O₂ pressure decreased from 140 mmHg to 48 mmHg. For the remainder of trial 1, pH ranged from 7.14 to 7.20 (Figures 7 and 8). In trial 2 pH declined from 7.64 to 6.94 as O₂ pressure decreased from 141 mmHg to 55 mmHg. For the remainder of trial 2, pH ranged from 6.52 to 6.83 (Figures 7 and 8). In trial 3 pH declined from 7.72 to 7.17 as O₂ pressure decreased from

135 mmHg to 81 mmHg. For the remainder of trial 3, pH ranged from 6.65 to 7.09 (Figures 7 and 8).

Hemolymph Sampling and Analysis

Crabs were given at least 5 days to acclimate to the holding tank and recover from handling before hemolymph extraction. Postbranchial hemolymph samples for trial 1 were taken 3 times throughout a 4-day exposure; days 0 (control), 1, and 3. Samples for trial 2 were taken 5 times throughout a 13-day exposure; days 0, 1, 5, 8, and 11. Samples for trial 3 were taken 7 times throughout a 21-day exposure; days 0, 1, 5, 9, 12, 15, 18, and 21. Hemolymph samples of 600 μ l were extracted from holes drilled in the carapace using iced glass syringes with 23-gauge needles (McDonald et al., 1977; deFur et al., 1990).

PO₂: 200 μ l of the hemolymph sample was injected into a thermostatted cuvette containing an oxygen electrode (Radiometer E5047). A pH blood gas analyzer (Corning 165) was used to determine the PO₂. The PO₂ electrode was calibrated using gas cylinders containing 0% O₂, and ambient air.

PCO₂: 200 μ l of the hemolymph sample was injected into a thermostatted cuvette containing a carbon dioxide electrode (Radiometer E5037). A pH monitor (Radiometer pHM82) was used to determine the PCO₂. The PCO₂ electrode was calibrated using gas cylinders containing known CO₂ percentages of 0.1% and 1%.

pH: 200 μ l of the hemolymph sample was injected into a thermostatted capillary pH electrode (Radiometer G299A). A pH blood gas analyzer was used to determine pH (Corning 165). The pH electrode was calibrated using Radiometer precision buffers.

Lactate: Lactate concentration was determined using a lactate assay kit (A-108S Biomedical Research Services, University at Buffalo). 20 μ l of the hemolymph sample was added to 80 μ l of DI water. Proper dilution was determined with an optimal dilution test. 20 μ l of diluted sample was added to a 96-well plate. Next 50 μ l of lactate assay solution was added to the well, and the plate was incubated in a humidified 37 C incubator for 30-60 minutes. Finally 50 μ l of 3% acetic acid was added to each well and the plate was analyzed with a micro plate reader at 492 nm absorbance.

Hemocyanin: 20 μ l of hemolymph sample was added to 400 μ l of DI water (Hagerman, 1986). The diluted sample solution was analyzed with a spectrophotometer at 338 nm absorbance. The concentration was determined with the extinction coefficient 2.33 determined for *Portunidae* hemocyanin (Nickerson and Van Holde, 1971).

Statistical Methods

I used a randomized block design ANOVA test with Fisher's LSD test to test for significant differences ($\alpha=0.05$) between all control and exposure day means. For each variable tested in more than one trial (pH, lactate, and hemocyanin), those control data and exposure data were compared to other trials using a randomized block design ANOVA test with Fisher's LSD test ($\alpha=0.05$). For this test the mean control for each variable (if exposure = 0) was compared to the mean controls of the other experiments and the mean of all exposure data for each variable (if exposure > 0) was compared to the mean exposure data of the other experiments. All variables collected in a trial (except PO_2) were tested for any correlations between variables (PCO_2 , pH, lactate, hemocyanin). Crab size and mass was tested for significant correlations ($\alpha=0.05$) with

time of mortality. PCO₂, pH, lactate, and hemocyanin during exposure were compared with time of mortality for significant correlations ($\alpha=0.05$).

Results

Twenty-one intermolt crabs used in the study ranged from 104.5-187.5 grams, and carapace length ranged from 12.5-15.5 cm. The mass of the crabs in trials 1 and 3 were significantly lower ($\alpha=0.05$) than in trial 2 (Table 1), and the carapace length was significantly different ($\alpha=0.05$) in all trials (Table 1), with trial 2 having the longest carapace length followed by trials 1 and 3.

Behavior

Hypoxic conditions in trials 1 and 2 caused most crabs to become active and attempt to climb out of the tank. After 24 hr exposure all crabs became quiescent for the duration of the trial. Similar behavior has been demonstrated in *C. sapidus* at 50 mmHg O₂ (deFur et al., 1990), and <0.2 mg/l O₂ (Lowery and Tate, 1986). In trial 3 the quiescent behavior was less prominent, and most crabs still remained slightly active throughout hypoxia.

PO₂

PO₂ control data taken from trial 2 (PO₂ was only taken during trial 2) ranged from 84.0-96.4 mmHg. Within 24 hours of hypoxia, postbranchial hemolymph PO₂ dropped approximately 80% to 14.0-17.0 mmHg. The PO₂ for the remainder of the experiment ranged from 16.0-19.0 mmHg (Figure 9). These controls and responses have been observed in similar hypoxic

conditions (50.0 mmHg) with blue crabs at 500 mOsM (deFur et al., 1990) and 16 ppt (deFur and Pease, 1988).

PCO₂ and pH

Hemolymph PCO₂ showed an increase after 24 hours of exposure, but this response and all others were not significant ($\alpha=0.05$) (PCO₂ only measured in trial 3). Control values and exposure values were high (Table 2 and Figure 10). No changes in hemolymph pH were significant ($\alpha=0.05$) in trials 1 and 2 (Tables 3 and 4). Yet trial 2 demonstrated a noticeable decline in pH by day 5, as mean pH declined from a control of 7.719 ± 0.017 to 7.657 ± 0.011 (Figure 11). No significant differences ($\alpha=0.05$) between pH controls were observed between trials 1 and 2. pH exposure data in trial 1 was significantly ($\alpha=0.05$) lower than trial 2 (Table 5).

Lactate

No significant changes ($\alpha=0.05$) were observed in hemolymph lactate for trials 1, 2, and 3 (Tables 2, 3, and 4). Yet trials 1 and 2 demonstrated noticeable decreases in mean lactate and standard error after 24 hours of exposure, as lactate in trial 1 decreased from $1323 \pm 240 \mu\text{M}$ to $588 \pm 130 \mu\text{M}$ and trial 2 decreased from $1158 \pm 453 \mu\text{M}$ to $601 \pm 92 \mu\text{M}$ (Figure 12). No significant differences ($\alpha=0.05$) were found between lactate control data for any trials. The exposure data in trial 3 was significantly lower than trials 1 and 2 (Table 6).

Hemocyanin

Trials 1, 2, and 3 showed no significant changes ($\alpha=0.05$) in hemolymph hemocyanin, and all trials demonstrated consistently high variability (Tables 2, 3, 4, and Figure 13). Trial 1

hemocyanin control data was found to be significantly ($\alpha=0.05$) lower than trials 2 and 3 (Table 7). No significant differences ($\alpha=0.05$) were found between trials for hemocyanin exposure data (Table 7).

Mortality

Trials 1 and 2 yielded high mortality rates, 100% mortality was reached before the end of the experimental goal of 21 days exposure. In trial 3 only one crab died during 21 days of hypoxia. In trial 1, 100% mortality was reached by day 4 (Figure 14). In trial 2, 100% mortality was reached by day 13 (Figure 15). No significant ($\alpha=0.05$) correlations were found between mortality and any of the hemolymph controls or exposure variables observed. Trial 1 demonstrated a significant correlation between mortality and mass ($r = .90$, $p\text{-value} = .0060$). In trial 2 this correlation was not repeated and no significant correlations were found between mass or carapace length. Multiple regression tests were also conducted on all variables with mortality, with no significant models found.

Discussion and Conclusions

The control data of the current study demonstrates that the blue crabs display similar hemolymph control results with past blue crab literature. The similarities of pH, and lactate with previous research demonstrate that the blue crabs are similar to crabs obtained in previous studies.

PCO₂ and pH

Mean hemolymph PCO₂ control data of the current study is higher than PCO₂ levels reported in other estuarine crab studies in saltwater (Figure 10) (PCO₂ was only measured in trial 3). The current study's control mean of PCO₂ was 4.64 ± 0.36 mmHg (Figure 10), while Truchot (1981) and Truchot (1992) report mean controls in saltwater of ~1.5 mmHg for *Carcinus maenas* and ~2 mmHg for *Eriocheir sinensis* respectively. *Eriocheir sinensis* and *Carcinus maenas* also demonstrate that hemolymph PCO₂ will increase after being transferred to freshwater (Truchot, 1981; Truchot, 1992). The high hemolymph PCO₂ in blue crabs held in freshwater demonstrates an internal hypercapnia. The high PCO₂ in freshwater will increase the vulnerability of blue crabs in freshwater exposed to hypoxia/hypercapnia as an increase in internal PCO₂ is already present.

The increase in hemolymph PCO₂ after 24 hours of hypoxia/hypercapnia demonstrates an additional compounding hypercapnic stress (Figure 10). The increase in mean PCO₂ levels after 24 hours of hypoxia/hypercapnia is not surprising as previous research demonstrates an increase in hemolymph CCO₂ in response to hypoxia (deFur and Pease, 1988). The increase in internal PCO₂ of the current study is due to the increase in ambient PCO₂, indicated by a decline in ambient pH. Together the ambient and internal hypercapnic stress presents a cumulative hypercapnic stress that the blue crabs will have to compensate for to maintain hemolymph pH, as increases in ambient PCO₂ drives declining ambient and hemolymph pH.

Decreases in hemolymph PCO₂ at day 5 and day 9 were demonstrated during the exposure of trial 3 (Figure 10). It has been suggested that estuarine organisms may deal with internal hypercapnia by increasing CO₂ excretion through hyperventilation (Mangum, 1997). Blue crabs respond to hypoxia with hyperventilation and an increase in CCO₂ (deFur and Pease,

1988). The decrease in internal PCO_2 is unexpected and suggests that ambient PCO_2 declined during the exposure.

In the present case, adjusting to a freshwater environment does not affect hemolymph pH in blue crabs in contrast to hemolymph pH control data that are commonly found in many blue crab studies. This range of pH controls has been observed in various salinities; 0 ppt (deFur, et al 1988), 16 ppt (deFur and Pease, 1988), 500 mOsM (deFur, et al 1990), and 30 ppt (Mangum, et al 1985). The control data for trials 1 and 2 along with previous work by deFur et al. (1988) demonstrate that freshwater does not cause any major changes in hemolymph pH. These comparisons are contrary to salinity and hemolymph pH studies with *Eriocheir sinensis* (Truchot, 1992), *Carcinus maenas* (Truchot, 1981), and *Callinectes sapidus* (Weiland and Mangum, 1975); as all demonstrated an increase in hemolymph pH when transferred to dilute seawater.

Hemolymph pH response to hypoxia/hypercapnia resulted in mean decreases (Figure 11). The mean hemolymph pH decrease demonstrated in trials 1 and 2 are lower and more dramatic than mean hemolymph pH changes seen in previous hypoxia experiments with maintained ambient pH (deFur and Pease 1988; deFur et al., 1990). After 6 days of hypoxia (45-55 mmHg) pH had remained stable with a mean of ~8, along with a slight mean increase by the last day of hypoxia (deFur and Pease, 1988). Similar values were also obtained in hypoxia for 6 and 25 days (deFur et al., 1990). A stronger decline in hemolymph pH is not surprising due to hypercapnia and the resulting decline in ambient pH, along with the already present internal hypercapnia during the freshwater holding conditions (Figures 10 and 11).

Despite declining hemolymph pH, it is most likely that some acid base balance mechanism was used during the exposure. Blue crabs exposed to hypercapnia use ion transport

to balance pH with HCO_3^- (Cameron, 1978). Since blue crabs have demonstrated maintained pH during internal hypercapnia (Cameron, 1978), this suggests that H^+ and HCO_3^- ion transport rather than CO_2 transport are the primary mechanisms for pH compensation in blue crabs. The current study also suggests other metabolic mechanisms, such as ion exchange were used to prevent hemolymph acidosis, as hemolymph pH was maintained relative to ambient pH (Figures 16 and 17). Throughout the trials increased HCO_3^- through ion gill transfer is likely to be a contributor to preventing larger declines in hemolymph pH.

Hemolymph pH and PCO_2 levels indicate hypercapnic and acidic stresses due to hypoxia/hypercapnia in freshwater. High hemolymph PCO_2 (Figure 10) and decreases in hemolymph pH (Figure 11) both caused an internal stress that is known to decrease hemocyanin oxygen affinity. This relationship between CO_2 , pH, and oxygen affinity is a respiratory response known as the Bohr shift (Dejours, 1981). The Bohr shift has been demonstrated in numerous decapods, including *Callinectes sapidus* (Mangum and Rainer, 1988), *Libinia emarginata*, and *Ocypode quadrata* (Burnett, 1979). The ability to improve oxygen affinity is essential to the survival of organisms exposed to hypoxia. The inability to improve the oxygen transport system and maintain hemolymph pH demonstrates that hypoxia/hypercapnia in freshwater is an exposure scenario that can severely stress blue crabs and decrease their oxygen transport abilities.

Lactate

The mean control lactate levels were similar to other control values observed in different salinities; 11 ppt (deFur, 1990) and 500 mOsM (deFur et al., 1990), thus salinity does not seem to affect lactate levels. This result is not surprising because there are no O_2 deficits and no

metabolic changes. The comparison of current research and past research demonstrates that lactate does not change due to changing salinity.

Mean lactate levels decreased during hypoxia/hypercapnia exposure, unlike other hypoxic studies that demonstrate blue crabs responding to hypoxia with an increase in lactate (deFur et al., 1990; Lowery and Tate, 1986). There are several differences between these studies that may account for the different responses of lactate. Lowery and Tate (1986) had a more severe hypoxic exposure (<0.2 mg/l O_2) than the current study. Also, both deFur et al. (1990) and Lowery and Tate (1986) did not have an additional hypercapnic stress during exposures. These comparisons suggest that exposure time, hypoxic severity, and hypercapnic stress could influence the lack of anaerobic respiration response in the current study.

The lactate results of trials 1, 2, and 3 suggest that anaerobic respiration is being used conservatively throughout the exposures, possibly to prevent further acidic stress. This result is demonstrated in trials 1 and 2, as mean control values are the highest and have high variability, then after the exposure begins mean lactate levels decrease and variability decreases (Figure 12). The decreases in mean lactate and variability presents the possibility that blue crabs are using anaerobic respiration to a limited capacity, keeping lactate levels low. Lower lactate levels could benefit blue crabs during hypoxia/hypercapnia as lower lactate levels would prevent further hemolymph acidic stress.

Lack of anaerobic respiration has been observed in estuarine organisms during hypoxia/hypercapnia (Hypes, 1991; Cochran and Burnett, 1997). The lactate levels observed in the current study are similar to the lactate results found by Hypes (1991) working on blue crabs from the nearby York River, as varying hypoxia/hypercapnia environments demonstrate higher values and variability at higher O_2 levels and lower values and variability at lower O_2 levels. An

absence of anaerobic respiration in response to hypercapnia is observed in three estuarine organisms (two fish and a shrimp) with lactate levels increasing in response to hypoxia but not increasing further in response to an additional hypercapnic stress (Cochran and Burnett, 1996).

Hypercapnia and acidic stress during the exposures is a factor that may have affected the use of anaerobic respiration. Reviews of anaerobic respiration and lactate studies found increases in H^+ ions and decreases in intracellular pH inhibit glycolysis (Myers and Ashley, 1997; Prampero and Ferretti, 1999). The pH induced effect on glycolysis is possible in the current experiment unlike deFur et al. (1990), Lowery and Tate (1986), and deFur and Pease (1988), where pH was nearly held constant. In a hypoxic/hypercapnic environment not only would the build up of lactate increase internal acidic stress, the presence of hypoxia would limit the ability of the blue crabs to remove lactate. Since lactate has to be removed aerobically, it is probable that the removal of lactate in long-term hypoxia would be severely hindered, thus limiting anaerobic respiration.

The duration of the exposures may have also affected the lactate responses. deFur et al. (1990) demonstrated that blue crabs will increase lactate after exposure to long-term hypoxia in saltwater after 25 days of exposure. Since 100% mortality was reached (Figures 14 and 15) days before day 21 in the current study, trials 1 and 2 did not have enough time to repeat the results observed by deFur et al. (1990). In trial 3, by day 21 mean lactate levels remained lower than deFur et al. (1990). At this point, there were only two crabs still being sampled, with both crabs demonstrating increased levels of lactate, but these increases were not significant and far from the mean of 1.79 mM reported on day 25 by deFur et al. (1990). The current study presents no evidence of increased anaerobic respiration during hypoxia/hypercapnia in freshwater for up to 21 days.

It is important to note that lactate responses to oxygen levels have been debated, as lactate does not always respond to oxygen availability (Myers and Ashley, 1997). Increased lactate responses of blue crabs during decreased oxygen are not consistently demonstrated as well, with several studies observing different results (Lowery and Tate, 1986; deFur and Pease, 1988; deFur et al., 1990; Hypes, 1999). The results of the current study demonstrate a lack of anaerobic respiration response. This lack of response also coincides with the blue crabs quiescent behavior, suggesting that conservation of energy played an important part in the responses to the exposure.

Hemocyanin

The control data for hemocyanin are lower than previous blue crab studies covering the full range of salinities: 0 ppt (Mason et al., 1985), 500 mOsM (deFur et al., 1990), and 21.1 & 31.1 ppt (deFur et al., 1985; Mangum et al., 1985). All of these previous studies demonstrate mean hemocyanin concentrations ranging from 3.1-6.1 g/100ml. The results of the present study suggest that freshwater conditions result in lower hemocyanin concentrations, but this assumption conflicts with previous work that demonstrates no major differences between freshwater and saltwater blue crab hemocyanin concentrations (Mason et al., 1983).

There were no trends observed in hemocyanin concentrations in any of the trials in response to hypoxia/hypercapnia in freshwater (Figure 13), suggesting that hemocyanin concentrations do not change in response to hypoxia/hypercapnia in freshwater. Similar mean hemocyanin concentrations observed throughout the current study have been reported by deFur et al. (1990), on day 7 of hypoxic exposure. This decrease in the middle of a 25 day hypoxic

exposure coincided with a change in subunit composition (deFur et al., 1990). Hemocyanin concentrations may have been low in the current study due to changing subunit composition.

Exposure time may have played a role as well, as increases in hemocyanin concentration were not observed until day 25 of hypoxia (deFur et al., 1990). Since 100% mortality was reached several days before day 21 of trials 1 and 2, the blue crabs of these trials may have not been given enough time to utilize increased hemocyanin concentrations to assist in their survival. In trial 3 two crabs were sampled on day 21 of the exposure with one crab demonstrating high increases in concentration and the other demonstrating low concentration. These data in trial 3 provide no evidence that hemocyanin concentration is increased during long-term freshwater hypoxia/hypercapnia.

The mean hemocyanin concentrations observed in all control and exposure data were possibly influenced by molt stage and not any of the control or exposure conditions. The same mean values observed in this study have been observed in blue crab molting studies during molt and post molt stages E-B2 (Mangum et al., 1985; deFur et al., 1985). Molting cycle is a likely factor as most blue crabs that move up tidal creeks and rivers into freshwater molt (deFur, 1990; Shirley et al., 1990). If hemocyanin concentrations are due to post molt stages, this suggests that during molting periods hemocyanin concentration is heavily dependent on the molt cycle. It is also plausible that capture and experimentation after molt made it difficult for the crabs to rebuild their hemocyanin concentrations.

Mortality

Mortality rates are much lower in trial 3 where oxygen was increased. In trial 3 only 1 crab out of 5 died throughout a 21-day exposure. Trial 3 had 75-85 mmHg O₂, which is almost

double the oxygen levels used in trials 1 and 2 at 50-60 mmHg O₂ (in terms of percent oxygen saturation). Trial 3 had a similar decrease of pH, and therefore a similar acidic stress. The survival of the crabs in trial 3 suggest that there was enough oxygen to supply compensation abilities in the blue crabs, helping to maintain internal pH and salinity as well as provide more ATP for all physiological functions.

The high rates of mortality demonstrate that blue crabs do not survive long-term hypoxia/hypercapnia exposure in freshwater, with 100% mortality by day 4 in trial 1 (Figure 14) and 100% mortality by day 11 in trial 2 (Figure 15). deFur et al. (1990), reported mortality levels of 20% after 25 days of hypoxic exposure (50 mmHg) in saltwater (500 mOsM) with controlled pH. The crabs in deFur et al. (1990), also demonstrated much better responses that assisted the crabs in survival to the exposure, which included maintained and slightly increased hemolymph pH, the use of anaerobic respiration, increased hemocyanin concentration, and increased hemocyanin oxygen affinity. None of these physiological responses were demonstrated in the current study and the absence of these responses most likely played a major role in the mortality results through decreased ATP supply and decreased oxygen transport abilities (Figure 18). The additional stresses of freshwater and hypercapnia in the current study's exposure appear to be the factors that resulted in the high mortality rates and lack of positive physiological responses.

Of all the variables observed, hemolymph PCO₂ and pH indicated that the hypercapnic/acidic stress played an important role in the mortality of the crabs. Salinity more than likely played an important role in the mortality as well. Internal osmolality and pH balance are both maintained primarily by the gills via active ion transport (Cameron, 1978; Towle, 1997). A failure in gill function or shortage of oxygen supplies to the gills would result in a decreased

ability to maintain internal pH and salinity balance. Since hemolymph pH values were lower than other previous studies, this suggests that gill function was stressed. Declines in oxygen affinity and failure to improve the oxygen carrying capacity of the hemolymph possibly contributed to mortality as well. The pH and hemocyanin data demonstrate no signs of increasing the oxygen transport capacity of the hemolymph. Decreases in hemolymph pH induce decreases of hemocyanin oxygen affinity, and the low levels of hemocyanin also limit the carrying capacity of the hemolymph. Even though gill failure and decreased oxygen transport abilities are highly possible to have accounted for the high mortality there is no definitive evidence to determine which one or combination of physiological failures led to the high mortality rates.

Due to the high mortality rates it is also possible that the crabs from the Rice Center site where exposed to various environmental contaminants. Mercury, TBT, DBT, DDT and its metabolites, PCBs, and chlordanes were detected in blue catfish captured in nearby areas of the tidal James River (Garman et al., 1998). Without pathological examination any of these environmental stressors more than likely would go undetected and could be influencing physiological functions of blue crabs.

Conclusions

The current study demonstrates that blue crabs are not capable of surviving hypoxia/hypercapnia in freshwater at 50 mmHg O₂. Ambient acidosis due to hypoxia/hypercapnia demonstrates a strong acidic stress on the blue crabs. The effects of this stress were demonstrated by high CO₂ and low pH values observed in the hemolymph during the exposures. Blue crabs do not appear to be utilizing any of the physiological responses that were

hypothesized to assist them in freshwater hypoxia/hypercapnia survival (Figure 3). The reaction that most likely helped the crabs survive as long as they did was the quiescent behavior, as this response conserved energy. It appears there was just not enough energy to maintain all physiological functions needed to survive the multiple stress environment.

The findings of this study are important to note, as this exposure scenario has the ability to occur in bays, their estuaries and tributaries. In a natural environment the exposure scenario used in this study would be extremely harsh and limit the probability of survival greatly. Blue crabs under such stresses would be more vulnerable to prey and would have difficulty performing essential tasks such as foraging and molting. Basic functions such as exercise, fighting, and eating in a hypoxic environment would also exacerbate the hypoxia/hypercapnia conditions even more. Blue crabs that migrate to lower salinities to molt would be put in an extremely high stress condition if a hypoxic/hypercapnic exposure occurred during the additional stresses of osmoregulation and/or molting.

The current study demonstrated that blue crabs cannot survive hypoxia/hypercapnia in freshwater at 50 mmHg O₂. Although highly possible theories were developed to explain mortality, this study cannot determine definitively all the exact physiological failures that took place and which ones caused mortality. Further research is needed to answer those questions and to understand all physiological responses. To better understand the physiological responses of blue crabs to multiple stress hypoxia, other variables need to be observed during the exposures. Future research should include the analysis of postbranchial and prebranchial hemolymph osmolality, hemocyanin oxygen affinity, hemocyanin subunit composition, bicarbonate, and total CO₂. Future research would also include the study of these variables in juveniles and different stages of molt during multiple stress hypoxias.

Literature Cited

- Albert, J. L. and W. R. Ellington. 1985. Patterns of energy metabolism in the stone crab, *Menippe mercenaria*, during severe hypoxia and subsequent recovery. The Journal of Experimental Zoology. 234:175-183.
- Batterton, C. V. and J. N. Cameron. 1978. Characteristics of resting ventilation and response to hypoxia, hypercapnia, and emersion in the blue crab *Callinectes sapidus* (rathbun). The Journal of Experimental Zoology. 203:403-418.
- Bouchet, J. Y. and J. P. Truchot. 1985. Effects of hypoxia and L-lactate on the haemocyanin-oxygen affinity of the lobster, *Homarus vulgaris*. Comp. Biochem. Physiol. 80A(1):69-73.
- Burnett, L. E. 1979. The effects of environmental oxygen levels on the respiratory function of hemocyanin in the crabs, *Libinia emarginata* and *Ocypode quadrata*. Journal of Experimental Zoology. 210:289-300.
- Burnett, L. E. 1997. The challenges of living in hypoxic and hypercapnic aquatic environments. American Zoologist. 37(6):633-640.
- Butler, P. J., E. W. Taylor, and B. R. McMahon. 1978. Respiratory and circulatory changes in the lobster (*Homarus vulgaris*) during long term exposure to moderate hypoxia. J. Exp. Biol. 73:131-146.
- Cameron, J. N. 1978. Effects of hypercapnia on blood acid-base status, NaCl fluxes, and trans-gill potential in fresh water blue crabs, *Callinectes sapidus*. Journal of Comparative Physiology 123:137-141.
- Christmas, J. F. and S. J. Jordan. 1987. Choptank River oyster bed mortality study: An analysis of biological and physiochemical data associated with a study of four lower Choptank River oyster bars. p. 1-51. Maryland department of Natural Resources, Coastal Resources Division, Monitoring and Data Management Program.
- Cochran, R. E. and L. E. Burnett. 1996. Respiratory responses of the salt marsh animals, *Fundulus heteroclitus*, *Leiostomus xanthurus*, and *Palaemonetes pugio* to environmental hypoxia and hypercapnia and to the organophosphate pesticide, azinphomethyl. Journal of Experimental Marine Biology and Ecology. 195:125-144.
- Cooper, S. R. and G. S. Brush. 1991. Long-term history of Chesapeake Bay anoxia. Science. 254(5034):992-996.
- deFur, P. L., C. P. Mangum, and B. R. McMahon. 1985. Cardiovascular and ventilatory changes during ecdysis in the blue crab, *Callinectes sapidus* rathbun. Journal of Crustacean Biology. 5(2):207-215.

- deFur, P. L. and A. L. Pease. 1988. Metabolic and respiratory compensation during long-term hypoxia in blue crabs, *Callinectes sapidus*, in Understanding the Estuary: Advances in Chesapeake Bay Research. Chesapeake Research Consortium Publication, pp. 608-616.
- deFur, P.L., Nusbaumer, D. and Lewis, R. J. 1988. Physiological aspects of molting in blue crabs from the tidal fresh-water Potomac River, Virginia. *Journal of Crustacean Biology*. 8(1):12-19.
- deFur, P. L. 1990. Respiration during ecdysis at low salinity in blue crabs, *Callinectes sapidus* rathbun. *Bulletin of Marine Science*. 46(1):48-54.
- deFur, P. L., C. P. Mangum, and J. E. Reese. 1990. Respiratory responses of the blue crab, *Callinectes sapidus*, to long-term hypoxia. *Biol. Bull.* 178:46-54.
- Dejours, P. 1981. Principles of comparative respiratory physiology. Elsevier/North-Holland Biomedical Press.
- Diaz, R. J. 2001. Overview of hypoxia around the world. *Journal of Environmental Quality*. 30(2):275-281.
- Garman, G., R. Hale, M. Unger, and G. Rice. 1998. Fish tissue analysis for chlordecone (kepone) and other contaminants in the tidal James River, Virginia: A report to the United States Protection Agency. Center for Environmental Studies at Virginia Commonwealth University, The Virginia Institute of Marine Science, and College of William and Mary.
- Graham, R. A., C. P. Mangum, R. C. Terwilliger, and N. B. Terwilliger. 1983. The effect of organic acids on oxygen binding of hemocyanin from the crab *Cancer magister*. *Comp. Biochem. Physiol.* 74A:45-50.
- Hagerman, L. 1986. Haemocyanin concentration in the shrimp *Crangon crangon* (L.) after exposure to moderate hypoxia. *Comp. Biochem. Physiol.* 85A(4):721-724.
- Hagy, J. D., W. R. Boynton, C. W. Keefe, and K. V. Wood. 2004. Hypoxia in Chesapeake Bay, 1950-2001: Long-term change in relation to nutrient loading and river flow. *Estuaries* 27(4):634-658.
- Henry, R. P., H. L. Handley, A. Krarup, and H. M. Perry. 1990. Respiratory and cardiovascular responses of two species of Deep-sea crabs, *Chaceon fenneri* and *C. quinque-dens*, in normoxia and hypoxia. *Journal of Crustacean Biology*. 10(3):413-422.
- Hines, A. H., R. N. Lipcius and A. M. Haddon. 1987. Population dynamics and habitat partitioning by size, sex and molt stage of blue crabs, *Callinectes sapidus* in a subestuary of central Chesapeake. *Bay. Mar. Ecol. Prog. Ser.* 36:55-64.

- Hypes, S. R. 1999. Sub-lethal effects of hypoxia/hypercapnia on *Callinectes sapidus* in the York River estuary, Virginia. Virginia Commonwealth University, Richmond, Virginia.
- Kemp, W. M., W. R. Boynton, J. E. Adolf, D. F. Boesch, W. C. Boicourt, G. Brush, J. C. Cornwell, T. R. Fisher, P. M. Glibert, J. D. Hagy, L. W. Harding, E. D. Houde, D. G. Kimmel, W. D. Miller, R. I. E. Newell, M. R. Roman, E. M. Smith, and J. C. Stevenson. 2005. Eutrophication of Chesapeake Bay: Historical trends and ecological interactions. Marine Ecology Progress Series. 303:1-29.
- Kuo, A. Y. and B. J. Neilson. 1987. Hypoxia and salinity in Virginia estuaries. Estuaries. 10(4):277-283.
- Kuo, A. Y., K. Park, and M. Z. Moustafa. 1991. Spatial and temporal variabilities of hypoxia in the Rappahannock River, Virginia. Estuaries. 14(2):113-121.
- Li, T., R. Roar, M. Vana, S. Pate, and J. Check. 2006. Gill area permeability and Na^+ , K^+ -ATPase activity as a function of size and salinity in the blue crab *Callinectes sapidus*. The Journal of Experimental Zoology. 305A:233-245.
- Lowery, T. A. and L. G. Tate. 1986. Effects of hypoxia on hemolymph lactate and behavior of the blue crab *Callinectes sapidus* rathbun in the laboratory and field. Comp. Biochem. Physiol. 85A(4):689-692.
- Mangum, C. P. 1980. Respiratory function of the hemocyanins. American Zoologist. 20:19-38.
- Mangum, C. P., B. R. McMahon, P. L. deFur, and M. G. Wheatly. 1985. Gas exchange, acid-base balance, and oxygen supply to the tissues during a molt of the blue crab *Callinectes sapidus*. Journal of Crustacean Biology. 5(2):188-206.
- Mangum, C. P. and J. S. Rainer. 1988. The relationship between subunit composition and O_2 binding of blue crab hemocyanin. Biol. Bull. 174:77-82.
- Mangum, C. P. 1997. Adaptation of the oxygen transport system to hypoxia in the blue crab, *Callinectes Sapidus*. American Zoologist. 37:604-611.
- Mantel, L. H. 1967. Gill potential and ion regulation in *Callinectes*. Comp. Biochem. Physiol. 20:743-753.
- Mason, R. P., C. P. Mangum, and G. Godette. 1983. The influence of inorganic ions and acclimation salinity on hemocyanin-oxygen binding in the blue crab, *Callinectes sapidus*. Biological Bulletin. 164(1):104-123.
- McDonald, D. G., B. R. McMahon, and C. M. Wood. 1977. Patterns of heart and scaphognathite activity in the crab, *Cancer magister*. J. Exp. Zool. 202:33-44.

- McMahon, B. R., W. W. Burggren, and J. L. Wilkens. 1974. Respiratory responses to long-term hypoxic stress in the crayfish *Orconectes virilis*. J. Exp. Biol. 60:195-206.
- McMahon, B. R., P. J. Butler, and E. W. Taylor. 1978. Acid base changes during recovery from disturbance and during long term hypoxic exposure in the lobster *Homarus vulgaris*. J. Exp. Zool. 205:361-370.
- Myers, J. and Ashley E. 1997. Dangerous curves: A perspective on exercise, lactate and the anaerobic threshold. Chest. 111:787-795.
- Newcombe, C. L. and W. A. Horne. 1938. Oxygen-poor waters of the Chesapeake Bay. Science. 88(2273):80-81.
- Nickerson, K. W. and K. E. Van Holde. 1971. A comparison of molluscan and arthropod hemocyanin-I. Circular dichroism and absorption spectra. Comp. Biochem. Physiol. 39(B):855-872.
- Nixon, S. W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia. 41:199-219.
- Park, K., A. Y. Kuo, and B. J. Neilson. 1996. A numerical model study of hypoxia in the tidal Rappahannock River of Chesapeake Bay. Estuarine, Coastal and Shelf Science. 42:563-581.
- Phillips, J. W., R. J. W. McKinney, F. J. R. Hird, and D. L. MacMillan. 1977. Lactic acid formations in crustaceans and the liver function of the midgut gland questioned. Comp. Biochem. Physiol. 56B:427-433.
- Prampero, P. E. and Ferretti, G. 1999. The energetics of anaerobic muscle metabolism: a reappraisal of older and current concepts. Respiration Physiology. 118:103-115.
- Rantin, F. T., A. L. Kalinin, and J. C. Freitas. 1996. Cardio-respiratory function of swimming blue crab *Callinectes danae* smith, during normoxia and graded hypoxia. Journal of Experimental Marine Biology and Ecology. 198:1-10.
- Shirley, M. A., A. H. Hines, and T. G. Wolcott. 1990. Adaptive significance of habitat selection by molting adult blue crabs *Callinectes sapidus* (Rathbun) within a subestuary of the Chesapeake Bay. Journal of Experimental Marine Biology and Ecology. 140:107-119.
- Summers, K. J., S. B. Weisberg, A. F. Holland, J. Koa, V. D. Engel, D. L. Breitburg, and R. J. Diaz. 1997. Characterizing dissolved oxygen conditions estuarine environments. Environmental Monitoring and Assessment. 45:319-328.
- Towle, D.W., G. E. Palmer, and J. L. Harris. 1976. Role of gill Na⁺K-dependent ATPase in acclimation of blue crabs (*Callinectes sapidus*) to low salinity. The Journal of Experimental Zoology. 196:315-322.

- Towle, D. W. 1997. Molecular approaches to understanding salinity adaptation of estuarine organisms. *Amer. Zool.* 37:575-584.
- Truchot, J. P. 1971. Fixation de l'e oxygene par le serum de *Carcinus maenas*. *Acad. Sci. (paris)* 272:984-987.
- Truchot, J. P. 1980. Lactate increases the oxygen affinity of crab hemocyanin. *The Journal of Experimental Zoology.* 214:205-208.
- Truchot, J. P. 1981. The affect of water salinity and acid-base state on the blood acid-base balance in the euryhaline crab, *Carcinus maenas*. *Comp. Biochem. Physiol.* 68A:555-562.
- Truchot, J. P. 1992. Acid-base changes on transfer between sea- and freshwater in the Chinese crab, *Eriocheir sinensis*. *Respiration Physiology.* 87:419-427.
- Truchot, J. P. and J. Forgue. 1998. Effect of water alkalinity on gill CO₂ exchange and internal PCO₂ in aquatic animals. *Comp. Biochm. Physiol.* 119A(1):131-136.
- Weiland, A. L. and C. P. Mangum. 1975. The influence of environmental salinity on hemocyanin function in the blue crab, *Callinectes sapidus*. *The Journal of Experimental Zoology.* 193:265-274.
- Wilkes, P. R. H., and B. R. McMahon. 1982A. Effect of maintained hypoxic exposure on the crayfish *Orconectes rusticus*. *J. Exp. Biol.* 98:119-137.
- Wilkes, P. R. H., and B. R. McMahon. 1982B. Effect of maintained hypoxic exposure on the crayfish *Orconectes rusticus*. *J. Exp. Biol.* 98:139-149.

Appendix

Table 1. Mean crab size and mass for all experiments. * = Mean significantly different ($\alpha=0.05$) then results in same column.

Trial	Mass (g)	Carapace Length (cm)
1	134	13.4*
2	162.5*	14.9*
3	130.5	14.1*

Table 2. Postbranchial hemolymph variables of blue crabs exposed to moderate hypoxia (75-85 mmHg), 23 C, Freshwater for 21 days. Trial 3.

Exposure (Days)	n	CO ₂ (mmHg)		Lactate (uM)		Hemocyanin (g/100ml)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	5	4.64	0.36	354	223	1.458	0.251
1	5	5.49	0.24	251	148	1.579	0.38
5	5	4.7	0.38	194	151	0.867	0.088
9	4	3.21	0.74	178	81	1.052	0.364
12	3	5.71	0.96	471	347	0.897	0.347
15	2	5.45	0.13	281	332	2.001	1.343
18	2	5.76	0	256	29	1.374	0.644
21	2	4.79	0.4	406	129	1.92	1.262

Table 3. Postbranchial hemolymph variables of blue crabs exposed to moderate hypoxia (50-60 mmHg), 23 C, freshwater for 3 days. Trial 1.

Exposure (Days)	n	pH		Lactate (uM)		Hemocyanin (g/100ml)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	8	7.625	0.059	1323	240	0.660	0.129
1	5	7.591	0.098	588	130	0.961	0.441
3	1	7.676		490		0.667	

Table 4. Postbranchial hemolymph variables of blue crabs exposed to moderate hypoxia (50-60 mmHg), 23 C, freshwater for 11 days. Trial 2.

Exposure (Days)	n	O ₂ (mmHg)		pH		Lactate (uM)		Hemocyanin (g/100ml)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	8	92	4	7.719	0.017	1153	458	1.459	0.2
1	8	16	0.53	7.735	0.015	601	92	1.586	0.247
5	5	17	0.45	7.657	0.011	579	208	1.494	0.338
8	4	18	0.41	7.690	0.022	386	63	1.383	0.296
11	3			7.736	0.064	505	230	0.847	0.402

Table 5. Mean hemolymph pH and sample size (#) for control and exposure data in trials 1 and 2. * = Mean significantly different ($\alpha=0.05$) then results in same column.

Trial	Control	Exposure
1	7.625 (8)	7.605* (6)
2	7.719 (8)	7.706* (20)

Table 6. Mean lactate and sample size (#) for control and exposure data in trials 1, 2, and 3. * = Significantly different ($\alpha=0.05$) then results in same column.

Trial	Control	Exposure
1	1323 (8)	572 (6)
2	1152 (8)	538 (20)
3	354 (5)	271* (23)

Table 7. Mean hemocyanin and sample size (#) for control and exposure data in trials 1, 2, and 3. * = Significantly different ($\alpha=0.05$) then results in same column.

Trial	Control	Exposure
1	0.66* (8)	0.912 (6)
2	1.459 (8)	1.412 (20)
3	1.458 (8)	1.228 (23)

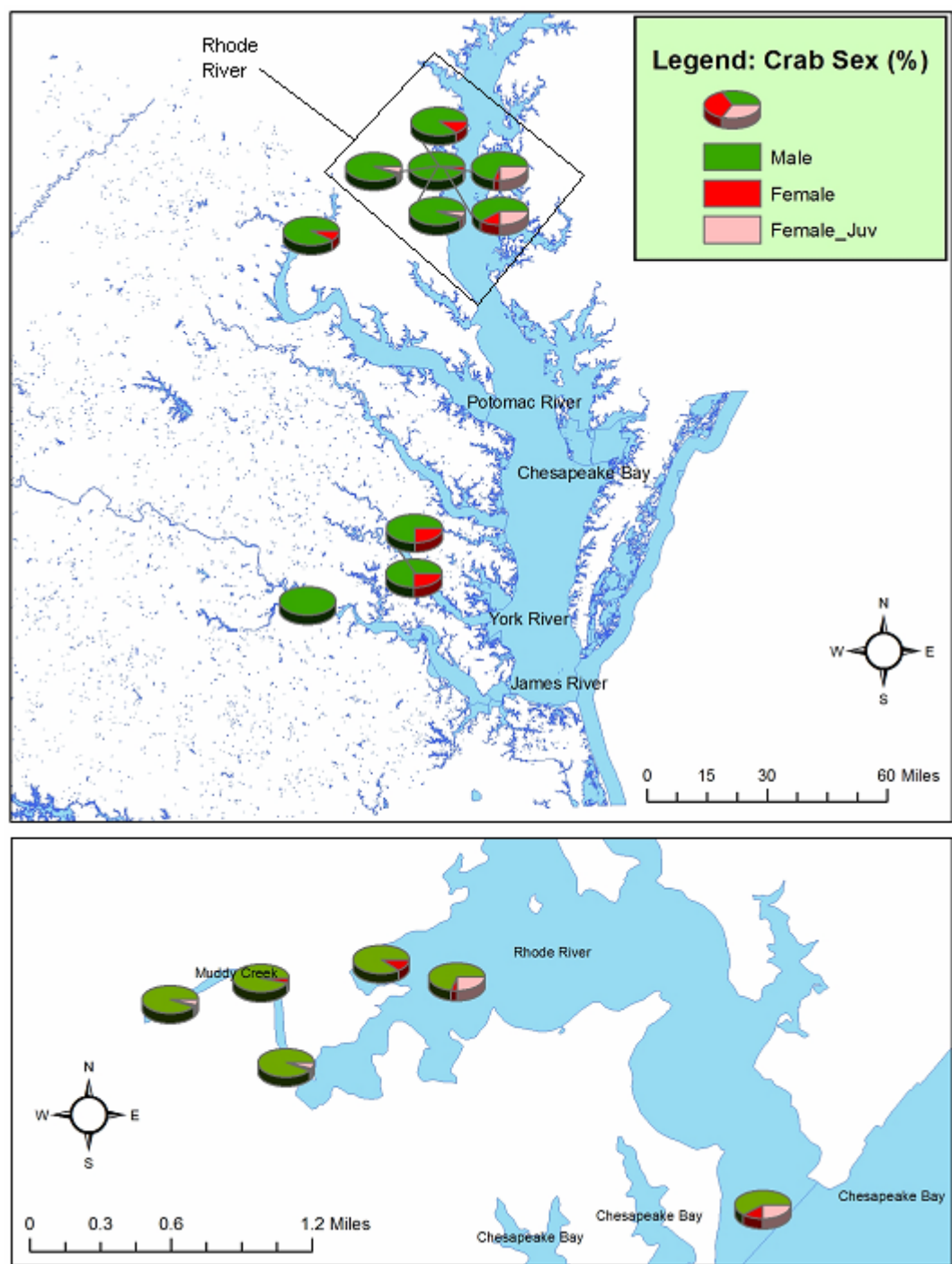


Figure 1. Sex composition of blue crabs captured during past studies in Chesapeake Bay tributaries. Data obtained from Hines et al., 1987; Shirley et al., 1990; deFur et al., 1988; Hypes, 1999 (Top). Close up of Rhode River study site (Bottom).

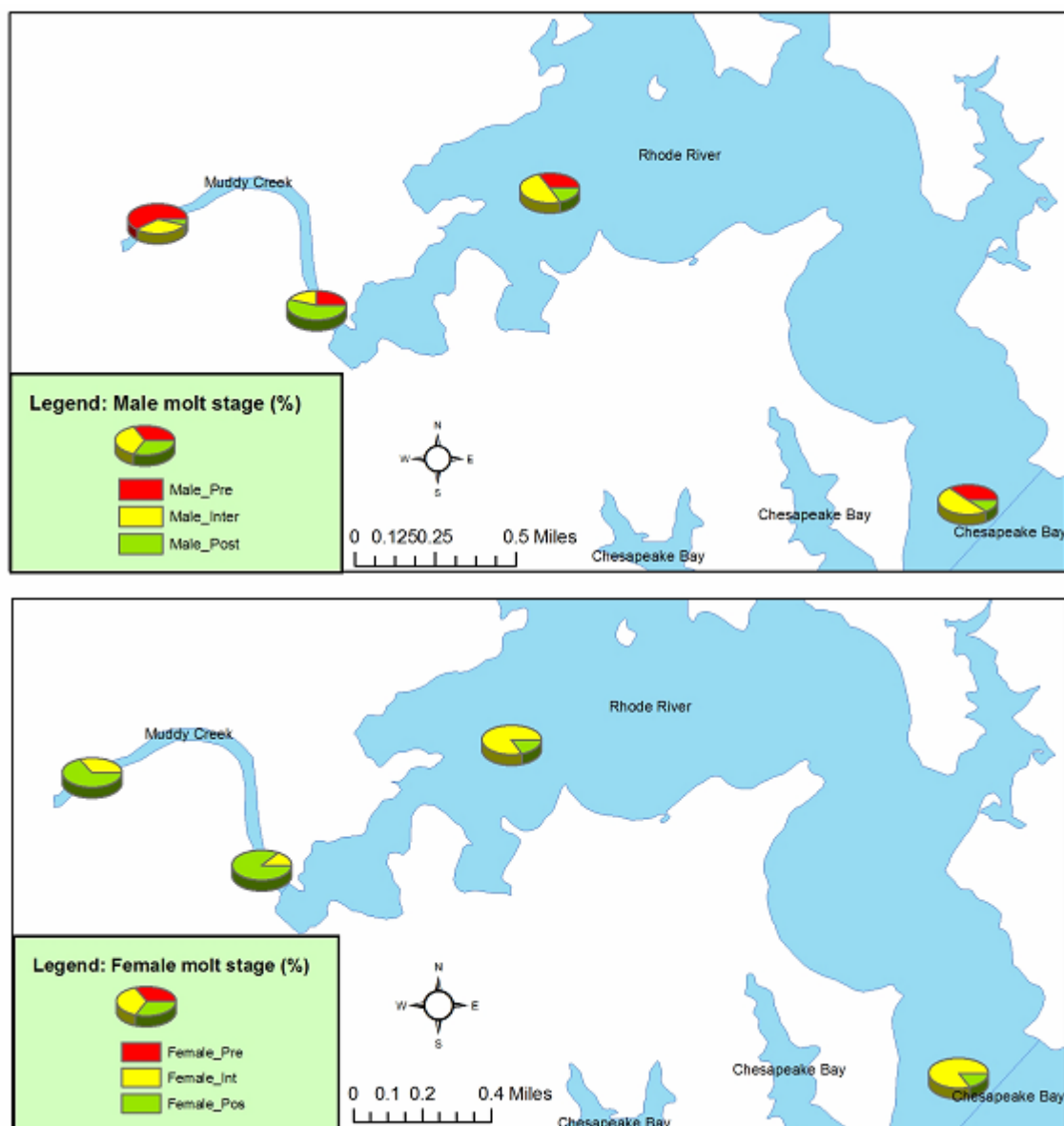


Figure 2. Male (top) and mature female (bottom) molt stages of blue crabs during capture by Hines et al. (1987). Note that transition to molt stage (d_0) is included in the Pre-molt category.

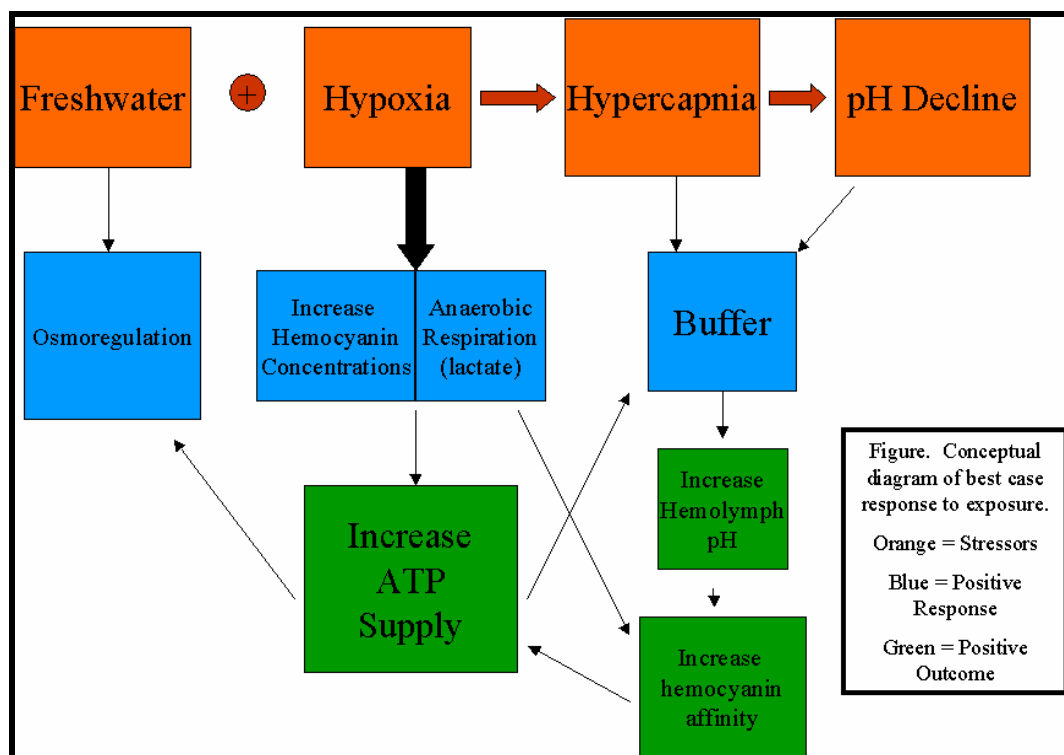


Figure 3. Conceptual model of hypothesized positive responses to exposure.

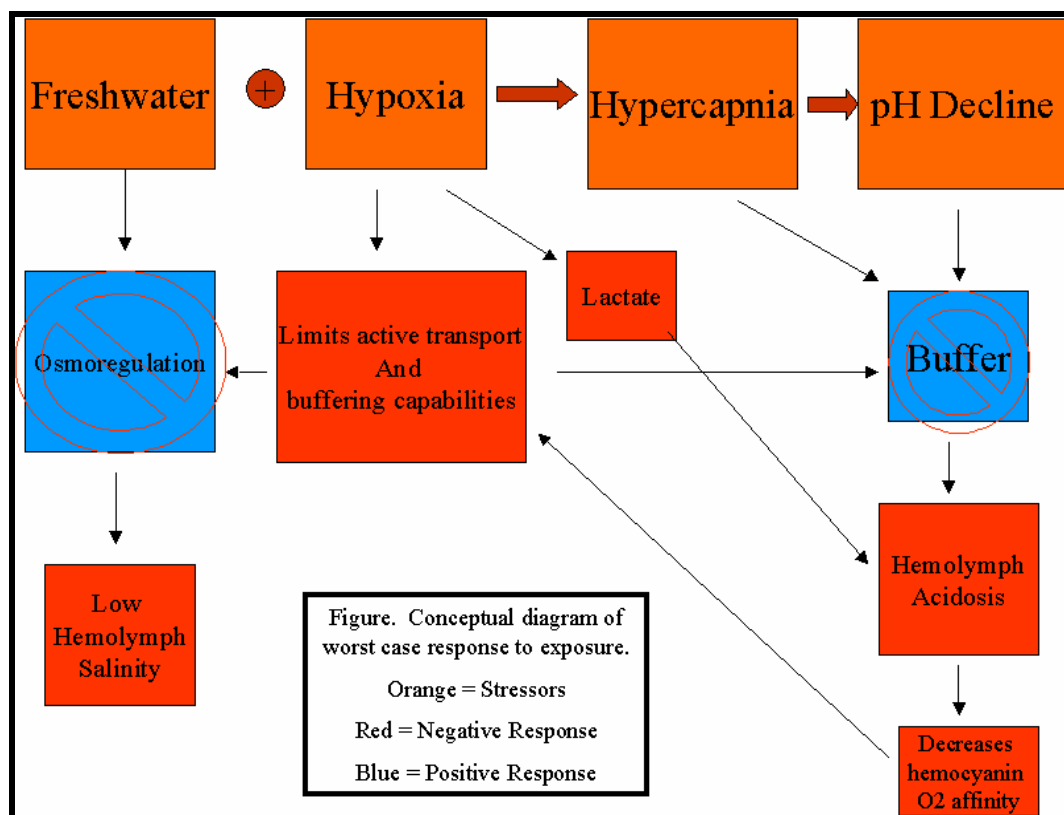


Figure 4. Conceptual model of hypothesized negative responses to exposure.

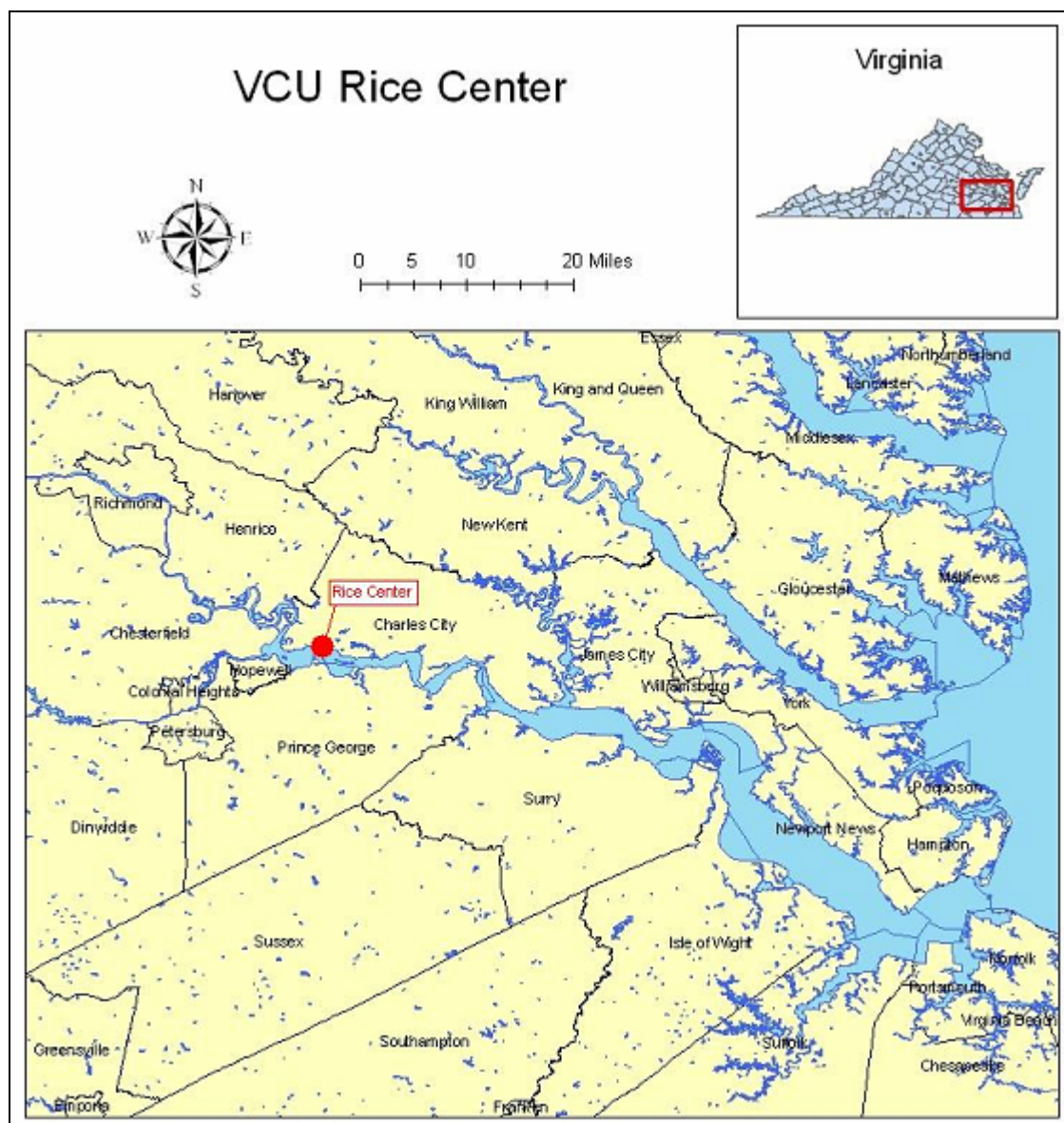


Figure 5. Location of VCU Rice Center collection area.

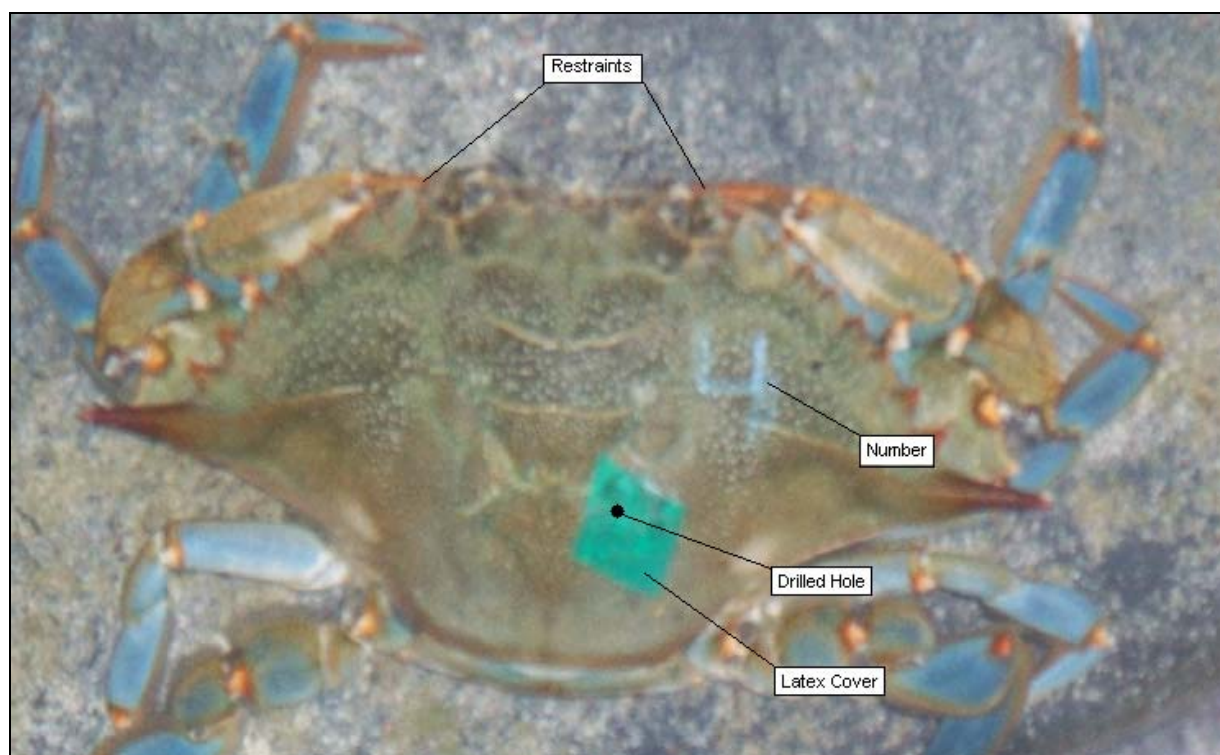


Figure 6. Blue crab after being prepared for study.

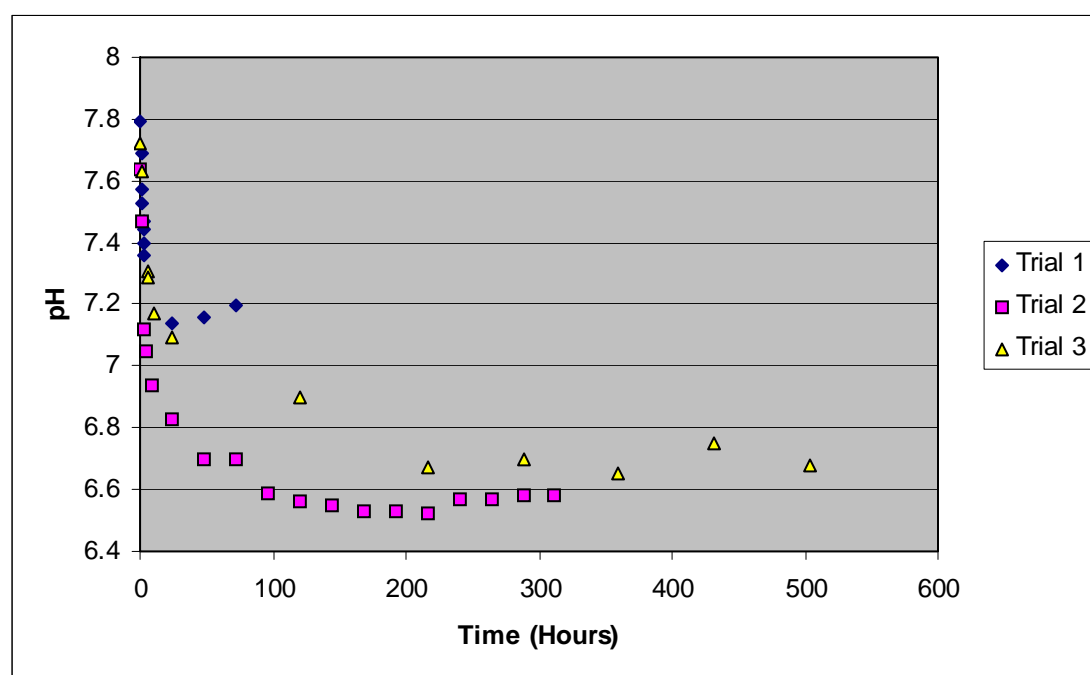


Figure 7. Ambient pH decline during exposure for trials 1, 2, and 3.

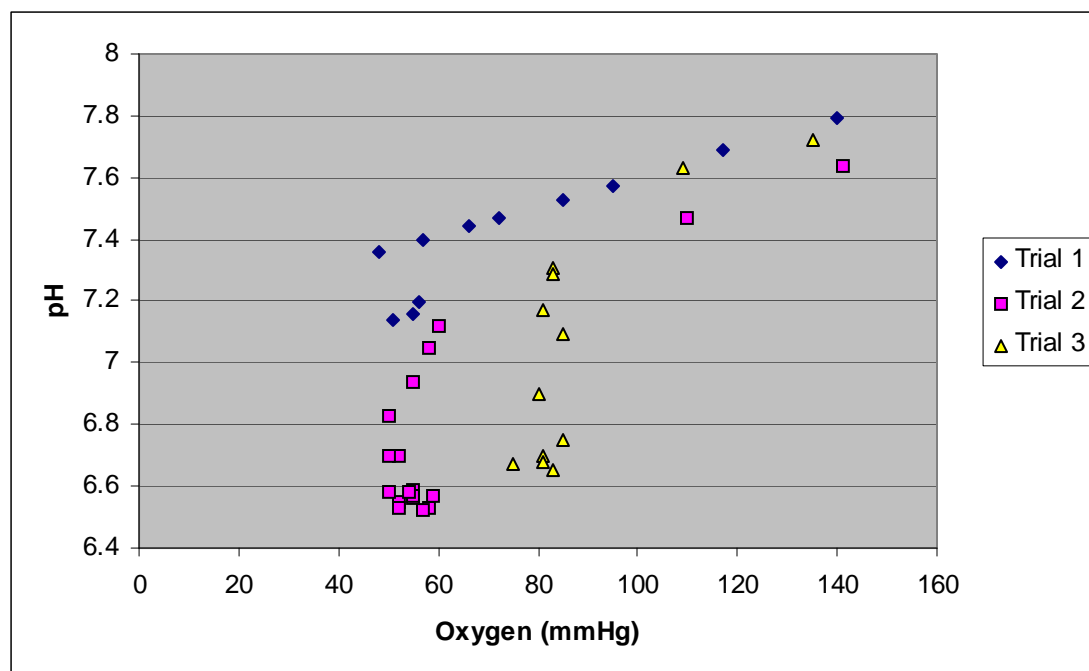


Figure 8. Ambient oxygen and pH during trials 1, 2, and 3.

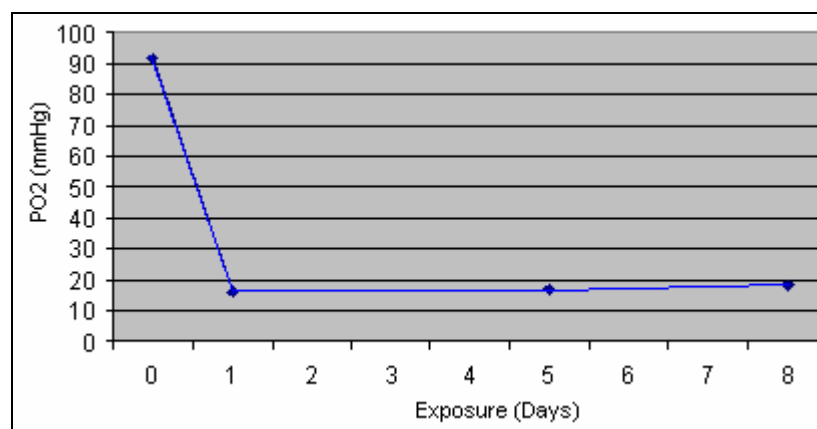


Figure 9. Postbranchial hemolymph PO₂ of blue crabs in hypoxia/hypercapnia (50-60 mmHg) for 8 days, 23 C, freshwater. Data are mean \pm S.E. for trial 2. Sample sizes given in Table 4.

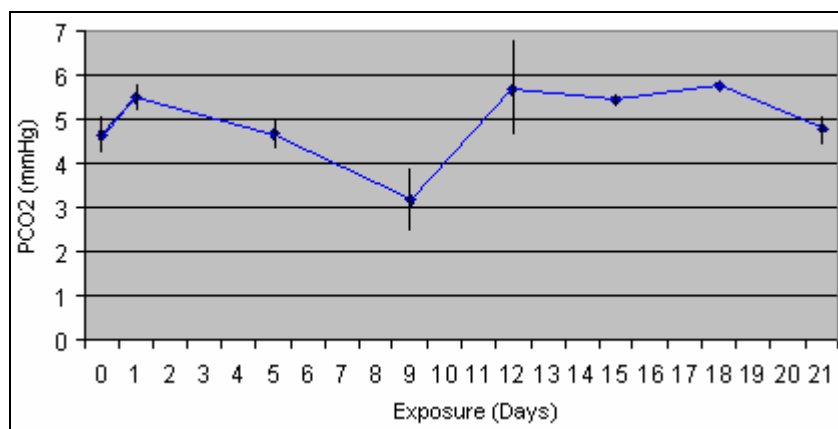


Figure 10. Postbranchial hemolymph PCO₂ of blue crabs in hypoxia/hypercapnia (75-85 mmHg) for 21 days, 23 C, freshwater. Data are mean \pm S.E. for trial 3. Sample sizes given in Table 2.

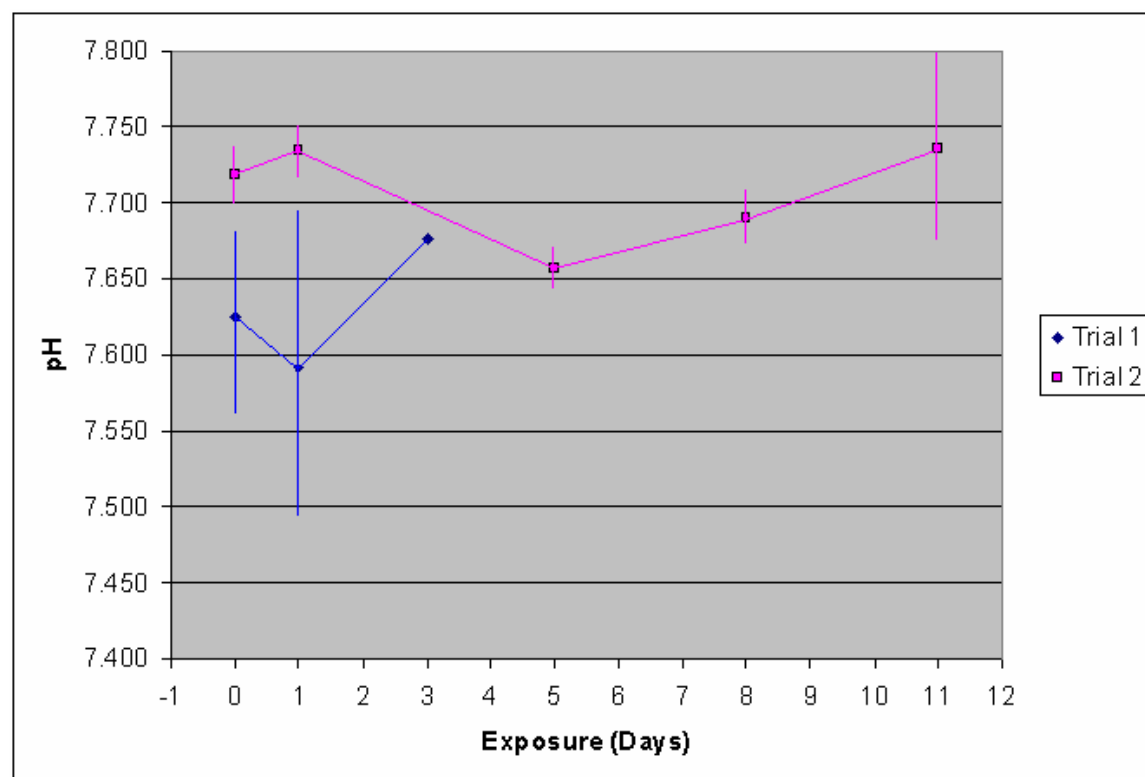


Figure 11. Postbranchial hemolymph pH of blue crabs in hypoxia/hypercapnia (50-60 mmHg) for 3 and 11 days, 23 C, freshwater. Data are mean \pm S.E. for trials 1 and 2. Sample sizes given in Tables 3 and 4.

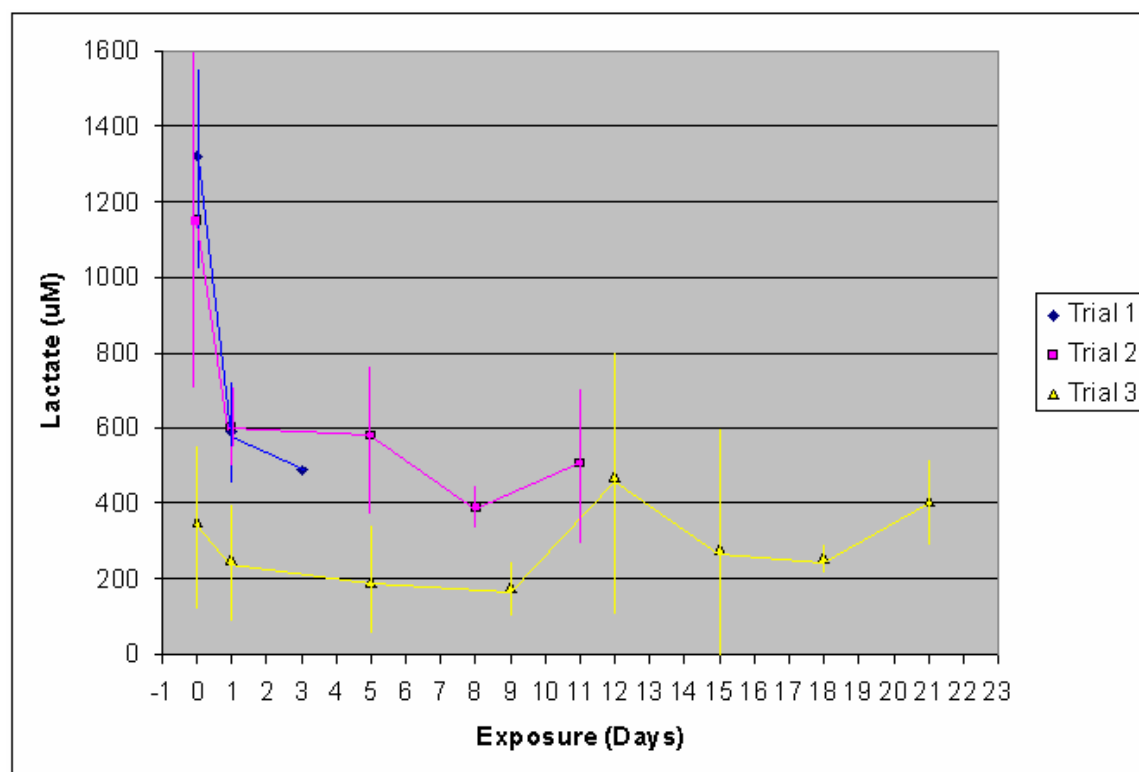


Figure 12. Postbranchial hemolymph lactate of blue crabs in hypoxia/hypercapnia for 3 and 11 days (50-60 mmHg), and 21 days (75-85 mmHg), 23 C, freshwater. Data are mean \pm S.E. for trials 1, 2, and 3. Sample sizes given in Tables 2-4.

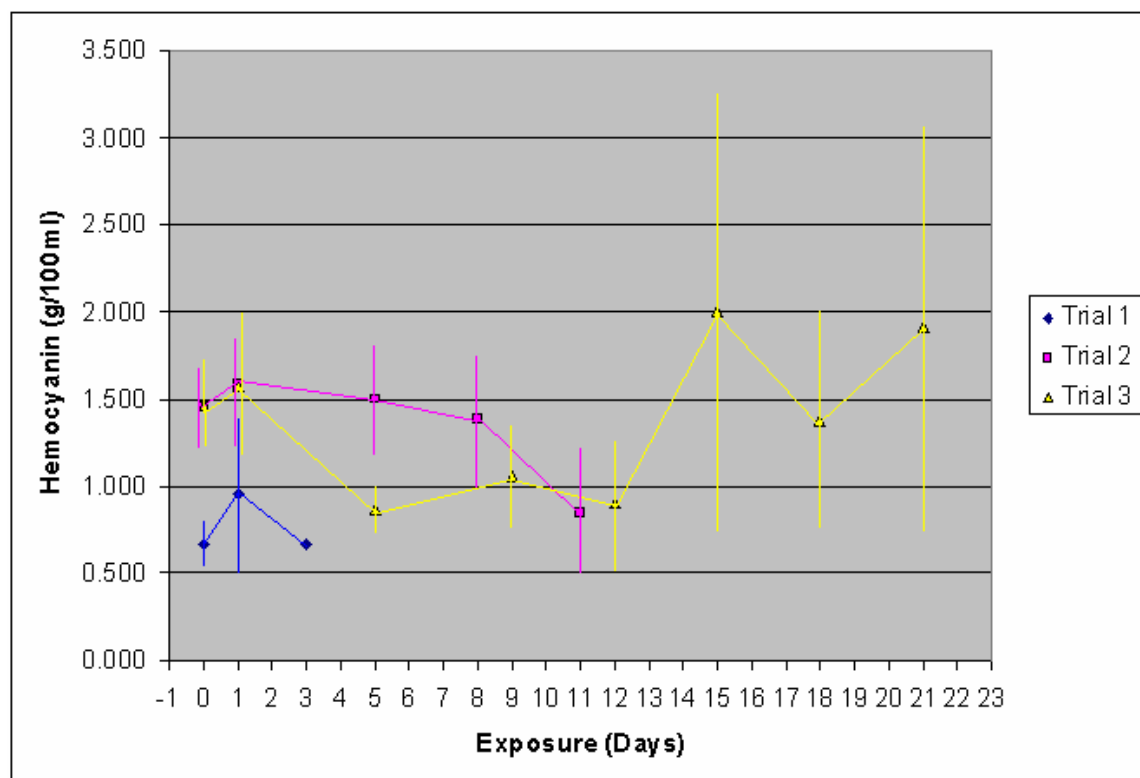


Figure 13. Postbranchial hemolymph hemocyanin of blue crabs in hypoxia/hypercapnia for 3 and 11 days (50-60 mmHg), and 21 days (75-85 mmHg), 23 C, freshwater. Data are mean \pm S.E. for trials 1, 2, and 3. Sample sizes given in Tables 2-4.

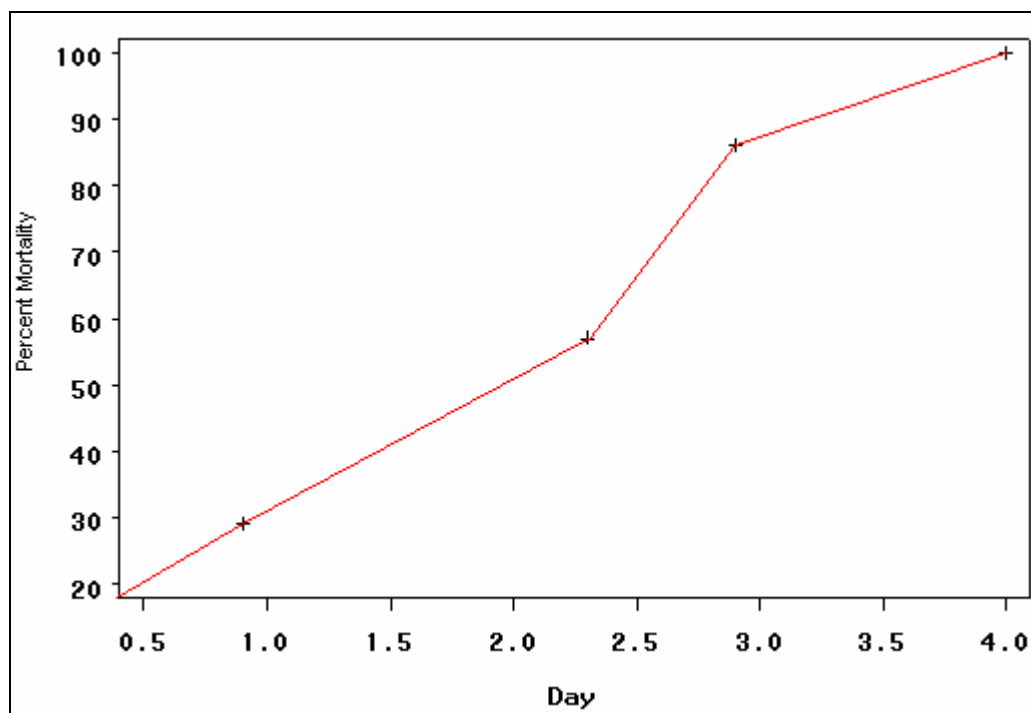


Figure 14. Percent mortality of blue crabs in hypoxia (50-60 mmHg) for 4 days, 23 C, freshwater, pH between 7.14-7.20. Trial 1.

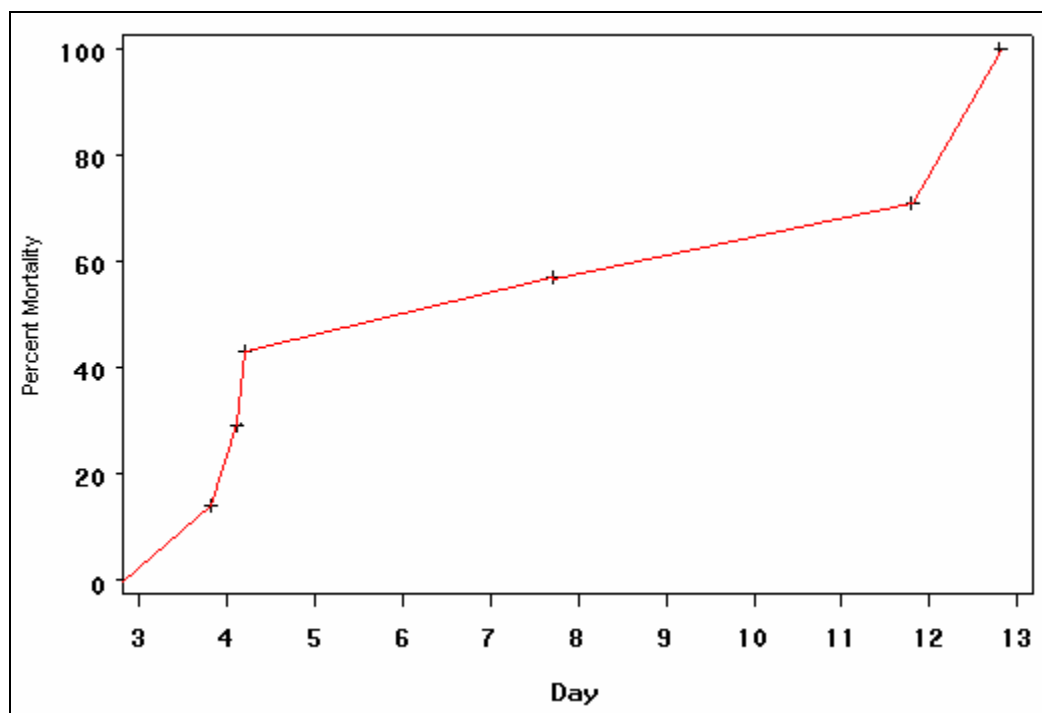


Figure 15. Percent mortality of blue crabs in hypoxia (50-60 mmHg) for 13 days, 23 C, freshwater, pH between 6.52-6.83. Trial 2.

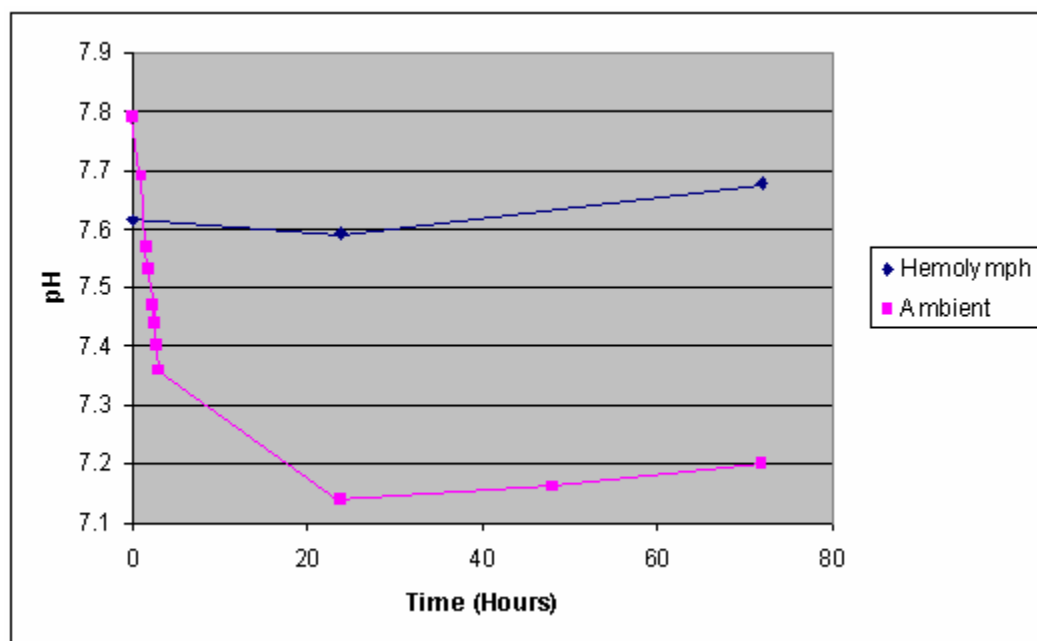


Figure 16. Ambient pH and hemolymph pH during trial 1 (hypoxia (50-60 mmHg) for 13 days, 23 C, freshwater).

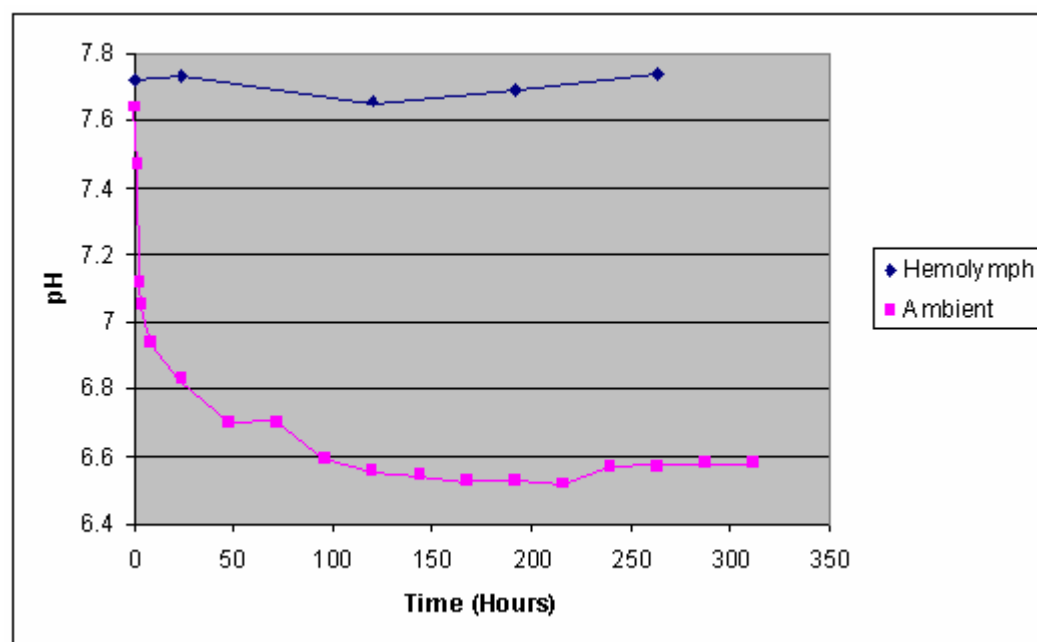


Figure 17. Ambient pH and hemolymph pH during trial 2 (hypoxia (50-60 mmHg) for 13 days, 23 C, freshwater).

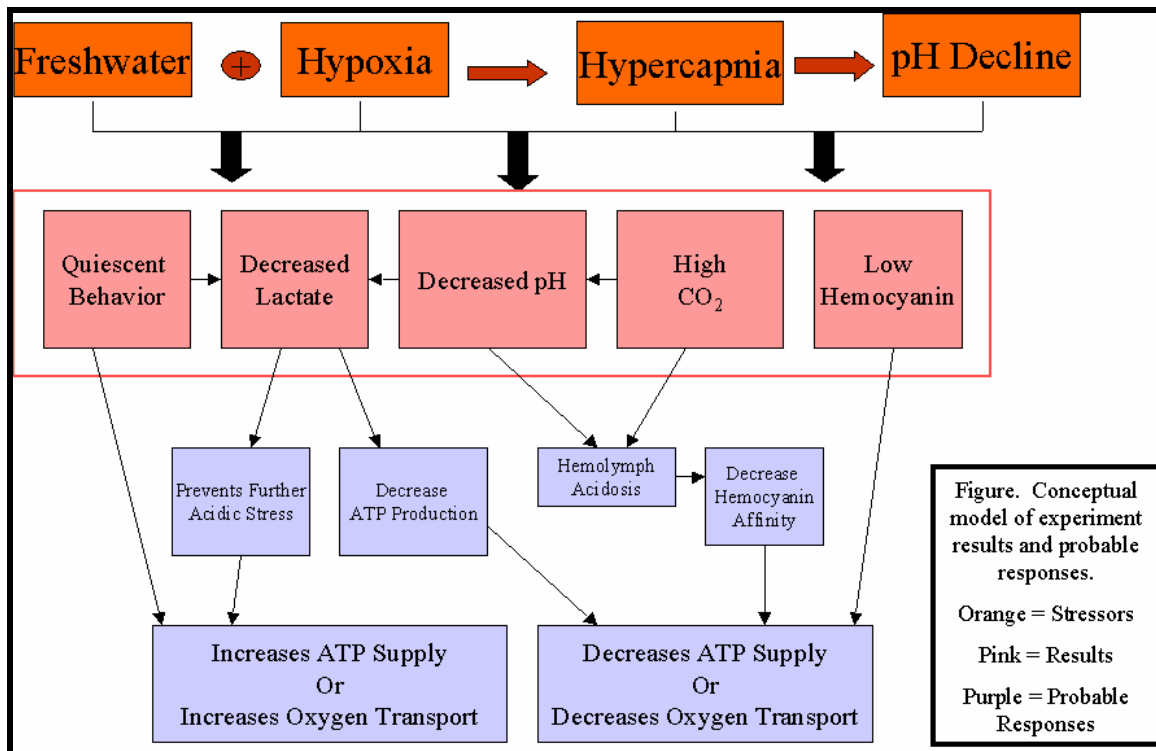


Figure 18. Conceptual model of results and possible physiological consequences.

Vita

James T. Martin

School Address: Center of Environmental Studies
Virginia Commonwealth University
P.O. Box 843050
Richmond, VA 23284-3050
804-828-7202

Fax: 804-225-3559
Ces_web@vcu.edu

Education:

- Culpeper County High School, Culpeper, VA-2000
- B.S., Virginia Commonwealth University, Richmond, VA, Education- Environmental Science

Professional Experience:

- GIS Technician, World View Solutions, 2009-Present
- Research Assistant, Environmental Stewardship Concepts, 2008-Present
- Environmental Science Assistant, Water Well Solutions, 2006

Professional Skills:

Computer Literate-(Mac/PC, Word, Excel, Power Point, Access), Geographic Information Systems (GIS), Global Positioning Systems (GPS), Statistical Analysis (SAS, JMP, SPSS), Water Quality Monitoring, Ecological Risk Assessment, Hydrogeology, Physiology, Toxicology, Ecosystem Restoration, Delineations, Blood Gas Analysis, Grant Writing, Grant Management, Lab Analysis (Plate Reader and Spectrophotometer), Installation and Repair of Submersible Pumps, Aquifer Monitoring and Testing

Awards/Honors:

- VCU Rice Center Graduate Student Research Grant- 2008
- VCU Dean's List- 2005
- National Dean's List- 2004

Presentations:

- VCU Rice Center Symposium-Poster Presentation, 2009
- SICB Conference-Boston Poster Presentation, 2009
- AERS Conference-Oral Presentation, 2008

