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Sub-Lethal Effects of Hypoxia/Hypercapnia on Callinectes Sapidus in the York River Estuary, Virginia

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SUB-LETHAL EFFECTS OF HYPOXIA/HYPERCAPNIA ON CALLINECTES SAPIDUS IN THE YORK RIVER ESTUARY, VIRGINIA

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

By

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Richmond, Virginia
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ABSTRACT

SUB-LETHAL EFFECTS OF HYPOXIA/HYPERCAPNIA ON CALLINECTES SAPIDUS
IN THE YORK RIVER ESTUARY, VIRGINIA

By SANDRA R. HYPES, Masters of Interdisciplinary Science

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Interdisciplinary Science at Virginia Commonwealth University. Virginia Commonwealth University, 1999.

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

This research examined effects of hypoxic environments on blue crabs, Callinectes sapidus in an estuarine environment. Hypoxic conditions were treated as a multiple stressor involving low dissolved oxygen (D.O.), increased carbon dioxide (hypercapnia), and low pH concurrently. The objectives were to: 1) identify hypoxia/hypercapnia by monitoring D.O. and pH as an indicator of hypercapnia in shallow regions of the York River, 2) measure blue crab abundance, and 3) describe blue crab responses to hypoxia/hypercapnia via field work at Taskinas Creek and lab measurements of respiration. Ambient D.O. and pH were positively correlated in the Taskinas Creek and York River sites (r = .73). Crab abundance (CPUE) was not significantly different among D.O. and pH ranges. It was concluded that hemolymph blood lactate concentration was not considered a good in situ biomarker for exposure to hypoxic/hypercapnic conditions. Oxygen uptake was not significantly different between normoxic and hypoxic conditions but was significantly affected by pH.
**Introduction**

Hypoxia, depletion of dissolved oxygen, is an environmental problem occurring on a global basis with increasing occurrences in shallow coastal and estuarine habitats (Diaz and Rosenberg, 1995). Hypoxia has been defined as concentrations of \( O_2 < 2.0 \) mg l\(^{-1}\), and anoxia is \( O_2 < 0.2 \) mg l\(^{-1}\) (Newcombe and Horne, 1938). Hypoxia is assessed as a dose-response stressor with LD\(_{50}\) values for different ecological and commercial aquatic species such as blue crabs, finfish, and mollusks. However, in physiological terms hypoxia is defined, as the point when compensatory effectiveness fails to meet oxygen needs within the organism, often below 50% saturation or \( PO_2 < 80 \) mmHg (at sea level).

Diaz and Rosenberg (1995) identified over twelve major rivers worldwide to be hypoxic and both U.S. coasts, as well as the Gulf of Mexico, that have seasonal hypoxic events on an annual basis. In the past thirty years, hypoxic areas have tripled in size and volume (Diaz and Rosenberg, 1995). In 1983, low dissolved oxygen was originally identified as the most pressing environmental problem in the Chesapeake Bay (deFur, 1997). In 1992, the Chesapeake Bay dissolved oxygen goal for restoration of living resources habitat was established as a monthly average of 5.0 mg/l for aquatic species. Recently, the 1998 Congress passed legislation earmarking federal funding for research and assessment of the hypoxia problem in the Gulf of Mexico (P.L. 105-383 “Harmful Algal Bloom and Hypoxia Research and Control Act of 1998”).
The basic cause of coastal hypoxia is the over enrichment from nutrients, termed eutrophication, which increases biotic oxygen demand. Eutrophication linked to anthropogenic activities also has adversely effected oxygen budgets of major coastal ecosystems (Diaz and Rosenberg, 1995). Shallow estuarine environments often experience hypoxic conditions in the summer due to high temperatures and low mixing of stratified waters (Officer et al., 1984).

In most coastal hypoxic areas such as the Gulf of Mexico and the Chesapeake Bay, hypoxia begins in early spring, and peaks in mid-August and disappears by late fall. Elevated rainfall runoff also increases nutrient input (N, P) into the estuarine habitat, stimulating algal growth that results in eutrophication. Algal biomass eventually dies off, and algal decomposition reduces dissolved oxygen levels in the bottom waters.

Hypoxia has significant ecological effects at both the lethal and sub-lethal levels for many aquatic organisms. Hypoxia is treated in ecological risk assessment (US Environmental Protection Agency 1992; Suter 1993) as a single stressor with low dissolved oxygen levels. The response is viewed toxicologically with LD_{50} values for many aquatic organisms including finfish, mollusks, and crustaceans. As with a toxic chemical, a lethal dose of dissolved oxygen is one that results in the mortality of 50% of the total number of animals. Miller and Wise (1996) observed juveniles of aquatic species such as winter flounder and mud crabs have increased lethality at less than 2.0 mg/l. Sub-lethal effects in terms of reduced growth and disease resistance for American lobster and grass shrimp occur at ranges of 3.5-4.5 mg/l (Miller and Wise, 1996). These effects occur at both the molecular and organismal levels with responses linked to
populations and community dynamics within the estuarine environment (Pihl et al., 1991; deFur, 1997). Diaz and Rosenberg (1995) demonstrated that increased severity and duration of hypoxic events reduced diversity and abundance of benthic invertebrate populations.

Aquatic hypoxia is the result of aerobic respiratory activity with O₂ consumption and CO₂ excretion by aquatic organisms, principally microorganisms. In nutrient enriched systems, metabolism is fueled by nutrients, elevating oxidative metabolism and thus both O₂ consumption and CO₂ production. Ambient pH decreases as a result of the reaction of CO₂ with the water: CO₂ + H₂O → H₂CO₃ → H⁺ + HCO₃⁻. During the daylight hours, photosynthetic rates are high, which results in high D.O. and low CO₂. However at night, respiration is the dominant process in the estuary with O₂ consumption and increased CO₂ production (hypercapnia). Therefore, during night-time and early morning hours, aquatic organisms face hypoxia, hypercapnia, and a decreased pH. This phenomenon normally occurs during low tide due to lower water volumes resulting in overall lower concentrations of D.O. and higher CO₂ concentrations compared to high tide.

**Literature Review**

**Blue Crab**

The blue crab, *C. sapidus*, is a dominant opportunistic benthic predator in estuaries, lagoons and coastal habitats of the Western Atlantic, Caribbean, and Gulf of
Mexico (Williams, 1984). Blue crabs are economically important throughout their range and over the last ten years have supported the largest single-species crab fishery worldwide (FAO, 1990). The decline of spawning females in the Chesapeake Bay over the past six years and decline in overall dredge numbers of the winter of 1998 indicate crab populations have reached or are above fishing mortality threshold levels (CBSAC, 1998). The blue crab is an important natural resource requiring sound management to protect its long-term health and associated ecological, social, and economic benefits.

Blue crab distribution in an estuary in the summer months is dependent upon molt stage, among other factors (Hines et al., 1987; Wolcott and Hines, 1990). Shirley et al. (1990) found that males approaching ecdysis migrate into tidal marsh low salinity areas to escape high predation pressures present in the river basin. Abiotic factors including salinity, temperature, dissolved oxygen, pH (as a measure of carbon dioxide) are all components determining optimal blue crab habitat during the developmental period. However, during seasonal hypoxic (low dissolved oxygen) /hypercapnic (high CO₂) events, blue crab abundance may be reduced in comparison to normoxic conditions in tidal creeks due to high sensitivity to low oxygen at the molt and reduced available habitat (deFur, 1990).

**Hypoxia/Hypercapnia**

Hypoxia has been defined as \(O_2 < 2 \text{ mg l}^{-1}\) and anoxia is \(O_2 < 0.2 \text{ mg l}^{-1}\) and both conditions are assessed as a dose-response stressor with LD₅₀ values for
different aquatic species. In physiological measurements, hypoxia is defined in partial
pressure (40 mmHg and below) or saturation (25% and below). Normoxic conditions are
considered in the range 130-160 mmHg and 75%-100% saturation (Fig. 1). The following
measures of O₂ are all equivalent but only under the conditions given, T=25°C, salinity=
20 ppt, 760 mmHg: 1 ml/l = 1.4 mg/l = 1.4 ppm = 23.9 mmHg = 23.9 torr = 3.18
Kilopas = 45.7 mM = 89.3 mg at/l = 4.3% O₂ vol. = 14% air saturation (Diaz and
Rosenberg, 1995). Selection of dissolved oxygen units is dependent on the scale of
observation. For example ecologists measure D.O. in concentration (mg/l), and
physiologists measure D.O. in partial pressure (mmHg). Dissolved oxygen threshold
levels vary among organisms depending on the life stage in the overall life cycle of the
organism.

This toxicological approach to treating low dissolved oxygen in isolation has
been questioned because as oxygen decreases, CO₂ increases due to biological
respiration (Burnett, 1997). This effect is even more evident at night when
photosynthesis does not occur (O₂ production stops), and CO₂ production is highest
during this period. CO₂ produced metabolically reacts with H₂O, resulting in pH
decrease as CO₂ forms carbonic acid (CO₂ + H₂O → H₂CO₃ → H⁺ + HCO₃⁻). During
daytime hours, when photosynthetic organisms produce O₂ and consume CO₂, pH
increases. Therefore, there is a positive correlation between oxygen saturation and pH
in the estuarine environments (Fig. 2 and 3) (Truchot and Duhamel-Jouve, 1980;
Christmas and Jordan, 1987; Dustan, 1993). Burnett (1997) noted that animals facing
hypoxic conditions also face increases in carbon dioxide (hypercapnia) and reduced pH (acidosis). Therefore, hypoxia cannot be viewed as a single toxic stressor with defined LD₅₀ values but as a multiple stressor challenging the adaptive ability of estuarine organisms.

In the field of ecological risk assessment, conditions such as toxic chemicals, heat or disease are termed stressors, and are often considered singly. Multiple stressors are defined as the combined effects of multiple and diverse stresses on an ecological system. Multiple stressors effects could be additive, nonadditive with defined thresholds, or have complex interactions (Suter, 1993). In the present case, low dissolved oxygen, high CO₂, low pH represent multiple stressors having an additive effect on aquatic organisms.

Several environmental surveys have reported increased carbon dioxide and low pH occurring simultaneously. In Gunston Cove, a tidal freshwater tributary of the Potomac River, large variations in water pH and oxygenation occurred throughout the summer (Jones et al., 1986). D.O. was positively correlated with pH in daytime hours with D.O. over 200% with high pH, and at nighttime D.O. fell below <50% saturation with decreased pH. Christmas and Jordan (1988) observed a positive correlation between dissolved oxygen and pH over oyster bars (Fig.2) in the Choptank. D.O. and pH values over the oyster bars were highly variable. Increased D.O. and pH were attributed to photosynthesis by phytoplankton and decreased D.O. and pH to respiration by microorganisms within the water column and the sediment. A similar
The correlation between the variables suggested that metabolic processes were the causative agents for low ambient pH (Fig. 3) in Charleston Harbor Estuary (Dustan, 1993). Cochran and Burnett (1996) measured dissolved CO₂ directly in the same estuary with values of >1mmHg occurring during the day and increasing to 5 mmHg at night. Thus, these studies support Burnett’s (1997) conclusion that hypoxia is actually a multiple stressor condition that combines low dissolved oxygen, increased carbon dioxide (CO₂), and low pH occurring concurrently.

Seasonal hypoxia in coastal areas has increased three fold globally over the last thirty-years. The determination of hypoxia/hypercapnia as a simultaneous event will help determine whether the present toxic ecological approach is appropriate for hypoxia alone for the identification, restoration or reclamation of these hypoxic areas or whether a new multiple stressor approach is needed. The identification of aquatic species’s (i.e blue crab) responses to hypoxia/hypercapnia will provide greater insight into the habitat requirements for these organisms and overall understanding of an important ecological species.

**Physiological and Behavior Responses to Hypoxia**

Using physiological adaptations, many aquatic crustaceans can tolerate moderate hypoxia and maintain oxygen uptake. An increase in heart rate and ventilation across respiratory surfaces increases oxygen extraction and maintains uptake in low oxygen concentrations (McMahon and Wilkens, 1983; Cameron, 1989). In several decapods, including blue crab (*C. sapidus*), sand fiddler crab (*Uca pugilator*), Norway lobster
(Nephrops norvegicus), enhanced oxygen transport by respiratory pigments may substantially increase the efficiency of oxygen uptake and transport oxygen during hypoxia (Herreid, 1980; Mangum and Burnett, 1986; Baden et al., 1990; Mangum, 1993). Production and alteration of these pigments such as structurally changing hemocyanin in C. sapidus is stimulated by chronic hypoxic environmental conditions (deFur et al., 1990). The species cited above, hemocyanin is sensitive to hemolymph pH, an important element in increasing oxygen affinity.

However, in some freshwater decapods such as Procambarus clarki, hemocyanin oxygen affinity is not sensitive to H+ (Mangum 1983). Other organisms can maintain uptake levels until certain critical oxygen pressures are reached, and then a sharp decline in oxygen uptake occurs with a decrease in oxygen pressure (Cameron, 1989). The ability of animals to regulate oxygen uptake under declining oxygen conditions is characterized by whether oxygen uptake remains constant (regulators) or changes with ambient oxygen (conformers). Notwithstanding more than twenty-five years of data showing responses ranging between strict regulation and conformity (Mangum and Van Winkle, 1973), many aquatic crustaceans, including blue crabs, display quite characteristic responses to ambient hypoxia. At the critical oxygen pressure (P_{crit}), regulators shift from aerobic to anaerobic respiration for energy needs. The anaerobic pathway produces less energy and results in lactate build-up contributing to an oxygen debt. Additionally, some invertebrates such as annelids and bivalves can lower overall biochemical rates by metabolic shunts or shut-downs, requiring less energy. For example, oyster larvae can respond to hypoxia by lowering overall metabolism (Widdows et al., 1989).

Hypercapnia, can also provoke physiological responses in some crustaceans. External hypercapnia produces an internal acidosis by increasing P_{CO2} in the
hemolymph. In freshwater crayfish (*Astacus leptodactylus*), living in hyperoxic water (PO$_2$ ≥ 600 Torr), hemolymph pH was 0.3-0.4 standard units lower than animals in hypercapnic waters (PO$_2$ ≥ 40 Torr) (Dejours and Armand, 1980). Some organisms compensate for hypercapnia by increasing hemolymph buffers such as bicarbonate and calcium ions (Cameron, 1978; Cameron and Batterton, 1978; Booth et al., 1984; Lindinger et al., 1984; Dwyer and Burnett, 1996). Red rock crab, *Cancer productus* demonstrated this buffering ability by the addition of carbonate or bicarbonate to the hemolymph instead of elevated CO$_2$ excretion (deFur et al., 1980). The compensation process requires many hours in various crab species but may take several weeks in crayfish species such as *A. leptodactylus* (Dejours and Beekenkamp, 1977). This compensation does not totally maintain normal hemolymph pH, and animals faced with this environmental condition are exposed to stress for the duration of the hypoxic event.

Behavioral responses to hypoxic conditions vary among different organisms. For mobile organisms, the first response may be avoidance of the hypoxic area. This may be demonstrated in avoidance behavior seen in juvenile spot (*Leiostomus xanthurus*) and blue crab (*C. sapidus*) moving from hypoxic to normoxic water (Phil et al., 1991). Other organisms such as hogchoker (*Trinectes maculatus*), take advantage of hypoxic events by exploiting moribund or recovering benthos affected by hypoxia (Phil et al., 1992). Some organisms avoid hypoxic environments by practically leaping out of the water into the rich oxygen air environment (Welsh, 1975; Cochran and Burnett, 1996). As aquatic animals avoid hypoxic areas, there may be an increase in predation as animals crowd together in smaller areas. Blue crabs show an increase in cannibalism among congeners due to fatigue from elevated oxygen consumption (MO$_2$) from muscular activity. Movement out of hypoxic areas may also decrease access to preferred feeding grounds or spawning habitats for some
organisms (Miller and Wise, 1996). Another behavioral modification during hypoxic events is to reduce activity and, therefore, oxygen demands. Some decapod species such as American Lobster (*Homarus americanus*) and the shore crab (*C. maenas*) pause respiratory behavior or unilaterally pump the scaphognathites during hypoxic conditions (McMahon and Wilkens, 1975; Jouve-Duhamel and Truchot, 1983). The mud snail (*Nassarius obsoletus*) and the brackish water clam (*Rangia cuneata*) experience aerobic shutdown during hypoxic events (Mangum and Van Winkle, 1973). In low oxygen concentrations, Norway lobsters (*Nephrops norvegicus*), suffered from hypoxia-induced starvation rather than lack of food (Baden et al., 1990). This behavior can place the organism at a disadvantage by decreasing the ability to escape predation.

**Blue Crab Responses**

Initial blue crab response to hypoxia in the York River is to migrate from the area and return when conditions improve (Pihl et al., 1991). Crabs held in hypoxic conditions resulted in a physiological response with oxygen supply being maintained by increased scaphognathite beat frequency, as oxygen declined in the water (Batterton and Cameron, 1978; Cameron, 1989). The increased flow of water over the gills from this elevated beat frequency, results in an increased oxygen supply to the tissues. Also, frequency and duration of ventilatory reversals increase during hypoxia. Adaptive responses were observed when blue crabs were exposed to hypoxic water for 7-25 days. However, hemocyanin concentrations and structural changes to the hemocyanin molecule increased oxygen affinity (deFur et al. 1990; deFur and Burnett, 1995).
Cameron and Batterton (1975) exposed blue crabs to acute hypercapnia (Pco₂ = 15 Torr) conditions, lowering hemolymph pH 0.3 to 0.5 units within a two hour period. A gradual return toward normal pH brought about by bicarbonate (HCO₃⁻) buffering occurred over the next 24 to 48 hours (Cameron, 1978). Exposure to hypoxia/hypercapnia simultaneously has been investigated in the blue crab, *C. sapidus* (deFur and Burnett, 1995). Natural environmental conditions were simulated in the investigation by allowing the oxygen to drop as oxygen uptake by crabs increased and water Pco₂ increased. The *C. sapidus* response to moderate hypoxia (58% air saturation) and hypercapnia of (Pco₂ = 2.6 torr) was an increase in venous Pco₂ (2.8 to 5.0 torr) but hemolymph Po₂ and pH remained unchanged. In more severe hypoxia (12% air saturation) and greater hypercapnia (Pco₂ = 3.7 torr) Pco₂ remained constant (5.0 torr) however hemolymph pH increased (pH = 7.76), total hemolymph CO₂ increased (6.4 to 13.7) and lactate concentration increased. In the normal physiological pH range, blue crab hemocyanin has a normal Bohr shift, i.e. the affinity is decreased by increasing Pco₂ and decreasing pH (Cameron, 1983). However, hemocyanin oxygen affinity increases with pH as an adaptive measure (Dejours 1975; Truchot, 1980; Booth et al., 1982). The hemocyanin molecule of *C. sapidus* and sand fiddler crab *Uca pugilator* is adapted for hypoxia/hypercapnia conditions due to the increased oxygen affinity affect caused by CO₂ and lactate increases (Mangum, 1994; Mangum, 1993).
**Research Objectives**

The objectives of the present research were: 1) to determine if hypoxia and hypercapnia occur concurrently in shallow hypoxic regions of the York River by monitoring D.O. and pH, 2) describe physiological responses of blue crabs, *C. sapidus* to hypoxia/hypercapnia via measurements of hemolymph and oxygen uptake by blue crabs, and 3) assess population responses to hypoxia/hypercapnia by monitoring blue crab abundance in shallow seasonal hypoxia/hypercapnia areas of the York River before, during, and after these events.

**Study Site**

Two study sites were located in York River State Park in Croaker, Virginia at the Taskinas Creek component of the Chesapeake Bay National Estuarine Research Reserve in Virginia (CBNERRVA) site (Fig. 4). The beach site was on the York River and Taskinas Creek, a saltmarsh creek, was a tributary to the York River. The beach site was located directly on the York River with variation in salinity ranging from 10 ppt-15 ppt during the summer months. Taskinas Creek is a tributary tidal creek to the York River with depth ranging from 1-3m during tidal cycles. Salinity ranged from 14 ppt at the mouth to 5 ppt upstream in the tidal creek. A water quality monitoring station installed and monitored by CBNERRVA is located at the first meander nearest the mouth of the creek (Fig. 4).
Methods

Field Methods

In order to identify the relationship between hypoxia and hypercapnia, ambient water quality data (1998) were obtained regularly from the water quality monitoring station maintained by CBNERRVA and portable field meters. The monitoring station measured ambient pH, D.O., salinity, and temperature, recorded every 15 minutes and stored electronically. Additionally, hand held YSI (55B oxygen, S-C-T 33, and Orion 210 pH) meters were used to record physiochemical conditions upstream of the monitoring station in the tidal creek and at the beach site. pH \([H^+]\) was used as an indirect measure of CO\(_2\).

Crab abundance was estimated by two different sampling methods. At the beach site on the York River, a beach seine of 9 m with a bag was pulled for a reach of 55 m in the mudflat area and adjacent right and left marsh areas. In the tidal creek, due to the depth and muddy conditions, crab pots were used to sample crab abundance. Four crab pots were fished from the mouth of the tidal creek to upstream fork every three days for the months of July-Oct (Fig. 4). Crab abundance was estimated in Catch per Unit Effort (CPUE) and total number of crabs. Molt stages were determined according to the scheme of Drach (1939) as described by Mangum (1985). The molt cycle is divided into: Intermolt (Stage C), premolt (stages D\(_1\)-D\(_4\)), ecydysis (stage E) and postmolt (stages A and B).
Laboratory Methods

Lactate levels were assessed in crabs collected and sampled in the field. 0.2 ml of venous crab hemolymph was withdrawn from the intersegmental membrane between the carapace and swimming leg by using chilled (to slow hemolymph clotting) 1cc hypodermic syringes. Crabs were sampled while in the crab pots by restraining them against the wire mesh; air exposure time was recorded. A control laboratory experiment was conducted to determine the effects of air exposure on hemolymph lactate levels with air exposure. Each crab was held in a wire mesh enclosure for time ranging from one minute to eleven minutes of air exposure.

The crab hemolymph was placed in 0.4 ml of perchloric acid for deproteination, placed on ice, was analyzed according to Sigma Technical Bulletin No. 826-UV with modifications according to (Graham et al., 1983) to eliminate interference from Cu in hemocyanin. A Beckman 40 spectrophotometer was used to read absorption at a wavelength 340nm.

Oxygen uptake was measured by an open flow respirometer system for both normoxic and hypoxic/hypercapnic conditions (McMahon and Wilkens, 1975). Six adult, intermolt, male C. sapidus (106-134 g) were obtained locally and held in an aquarium for two months at experimental salinity and temperature. Crabs were weighed, sized, and molt stage was determined before being placed in a 1944 ml plexiglas respirometer following the basic design of deFur, 1990. Crabs were allowed to become accustom to the respirometer overnight before measuring respiration (McMahon and Wilkens, 1975).
Flow rates were adjusted to yield an oxygen tension of 30-50 mmHg in the out-flowing water; this rate was usually between 150-250 ml/min. Approximately 2-3 cm of sand was placed in the bottom of the respirometer and $\text{Mo}_2$ of the respirometer with substrate without the crab was determined and subtracted from the measurements with the crab (deFur, 1990).

Normoxic water ($\text{D.O.}=130-150$ mmHg and $\text{pH} = 7.6-8.0$) was pumped into the respirometer for a thirty minute period, and oxygen uptake was measured. Hypoxic/hypercapnic conditions were obtained by turning off the air flow in the water supply tanks, allowing D.O. and pH to decrease naturally by respiration processes of other blue crabs. After a 15 min flow through period, measurements of outflowing water were taken for 30 min with hypoxic/hypercapnic (30-40 mmHg/6.6-6.8) conditions. $\text{Mo}_2$ (mol $\text{O}_2$/kg-min) was calculated from (flowing inspired and outflowing water) $\text{Po}_2$; the difference used to calculate oxygen uptake according to the equation: $\text{Mo}_2 = \text{flow (ml/min)} \cdot (\Delta \text{Po}_2) \cdot \text{solubility coefficient} / \text{mass in Kg}$. Oxygen partial pressure was measured with polarographic electrodes and meters (IL). Electrodes were calibrated with air saturated water and N$_2$ gas saturated water. O$_2$ extraction defined as the amount of O$_2$ removed from the water as it passes over the respiratory surface was determined by:

$$E(\text{Extraction Efficiency}) = \text{P}_i\text{O}_2(\text{inhalent O}_2) - \text{P}_e\text{O}_2(\text{exhalent O}_2)$$

and percent efficiency ($\%\text{U}$) by ($\%\text{U} = \frac{E}{\text{P}_i\text{O}_2} \times 100$) (Herreid, 1980).
**GIS Methods**

Crab abundance and water quality data collected during the 1998 sampling season were used to create coverages in geographic information system (GIS). Metadata were recorded for the sampling period by CBNERRVA staff. A digital elevation model (DEM) and topography maps were used as base maps for the coverages. These maps were imported from the USGS website (ftp://greenwood.cr.usgs.gov/pub/quad-indexes/) and DEM 1x2 quad (Gressitt) CD, respectively. Crab sampling station points along the beach and tidal creek study sites were captured using a Trimble Geographical Positioning System (GPS). These points were downloaded using Pathfinder software and corrected using base station coordinates at the Virginia Institute of Marine Science. Data were first converted into shape files; these shapefiles were then imported into ArcView (3.1). The GPS points were converted from Datum 83 to Datum 27 in ARC/INFO due to the base map projection in Universal Transverse Mercator (UTM) Datum 27 (ArcView datum conversion extension does not support 83 to 27 conversions).

Coverages were created by importing converted text files with station ID for water quality and crab abundance data. The (View.XYcoordinateftab) add avenue script imported from the Environmental Systems Research Institute (ESRI 1999) website was used to determine and add x,y coordinates for the sampling stations. The avenue text file was imported, compiled in the script view and run within the view window. The attribute sampling point table was then joined (one to many) using the Station ID as a common
field with the water quality and crab abundance tables. These joined tables were then added to the view by the use of add event theme using X,Y coordinates.

The shapefiles were then interpolated using the IDW method with hydrology as a barrier for temperature, salinity, pH, D.O., and crab number (Fig. 5). A clipped area within the hydrology shape was used as a mask for analysis. Each table was renamed and legends modified for color display. Map queries were then used to create a coverage for hypoxic (<4.5mg/l) and hypercapnic (~7.0 pH) water quality conditions in the tidal creek. Blue crab abundance coverage was overlaid with hypoxic and hypercapnic coverage. Hypoxic and hypercapnic conditions did not occur at the York River beach sampling site. Map queries were then used to create an optimal water quality habitat coverage for temperature, salinity, D.O., and pH for blue crabs in both the tidal creek and beach site. Optimal abiotic conditions identified from the literature (Table 1) were used to create an estuarine multiple habitat layer for crab abundance using the single habitat coverages (Ruiter, 1998). The optimal conditions for the tidal creek and the beach site were different for each parameter due to juveniles utilizing the beach site with higher sensitivity to abiotic factors. The actual blue crab abundance coverages were then overlaid with the optimal habitats at each site and compared using descriptive statistics.

**Statistical Analysis**

Non-parametric Chi-square tests were conducted on crab abundance data to identify significant differences among sampling stations. A Pearson’s correlation was
also used to identify the abiotic factors related to crab abundance. Step-wise regression was used to identify the best indicators for predicting crab abundance. Parametric ANOVA were conducted on the hemolymph and oxygen extraction efficiency (ext. %) measurements to determine significant differences among different pH and D.O. conditions. Paired t-tests were conducted on oxygen uptake measurements before and after exposure to hypoxic/hypercapnic conditions.

**Results**

Water quality monitoring data for July, August, September when hypoxia was anticipated, showed a correlation between D.O. and pH [H+] (Fig. 6). Hypoxia and low pH (=Hypercapnia) showed a correlation r=-.73, p=.000. In the month of August, this same relationship between D.O. and pH [H+] was observed with a higher correlation (r=-.80, p=.000) (Fig. 7). The highest frequency of hypoxic events in Taskinas Creek occurred in the month of August.

Three dataloggers that recorded pH, salinity, temperature, and dissolved oxygen were placed upstream from the permanent water quality monitoring station in the tidal creek for a single 24 hour period in September 1998 (Fig. 8). D.O. and pH [H+] showed the highest correlation during the 24 hour sampling with a r=-.96 at Taskinas 1. Low D.O. and low pH occurred during the night and early morning hours in Taskinas Creek (Fig. 9 and Fig. 10), the same diel relationship seen in Gunston Cove (Jones et al., 1986) and Charleston Harbor (Dustan, 1993). Low pH and low D.O. occurring in the late night,
early morning hours and increased oxygen and pH during daylight hours. Other data
collected at the beach and upstream tidal creek sites showed the same correlation between
D.O. and pH but with a lower r value (Fig. 11). This lower correlation could be
attributed to low sampling numbers for upstream and down stream stations compared to
the data collected by the permanent water quality monitoring station at Taskinas 5.

A three to one ratio of male to females crabs was observed in crab collections at
both sampling sites. There was a significant difference at the beach site between crab
abundance (CPUE) at the marsh areas (York 1 and York 3) compared to the mudflat
(York 2) (Fig. 12). Crab abundance (CPUE) in the tidal creek was variable with the
highest CPUE at Taskinas 1 and Taskinas 5, however was not significant among different
among sampling stations (Fig. 13). Crab abundance was not significantly different
among the D.O./pH regimes. Crabs were collected within a D.O. range of 3.0 mg/l-10.0
mg/l with highest abundance collected at the beach site in the D.O. range of 6.0-10.0
mg/l and in the tidal creek between 4.0-8.0 mg/l. (Fig.14). Crab abundance was not
significantly different among pH values in both the beach site and Taskinas Creek
(Fig.15). Salinity was another significant factor affecting crab abundance. The lower
salinity upstream sampling areas had the highest crab abundance (Table 2). The beach
site had 16.7 CPUE per haul with size range (0.2-13.5cm) compared to 5.6 crabs per day
pot fished with size range number (7.0-15cm) for Taskinas Creek. The abundance
sampling at the beach sites resulted in larger number of small crabs verses fewer larger
crabs in the tidal creek (t=16.73, p.000). Crabs in the tidal creek were primarily pre-molt
stages (D1-D3) and the small juveniles collected at the beach between 0.2-2cm in length were difficult to identify in terms of molt stage.

**Hypoxia/Hypercapnia**

Hypoxia/Hypercapnia occurred at all crab abundance monitoring stations during the sampling period, except for stations Taskinas 1 and Taskinas 5 (Fig.16). Hypoxia/hypercapnia events did not occur at the beach site during July-September sampling period. Crab abundance was not significantly correlated with normoxic areas; Taskinas 4 showed one of the highest CPUE, for all the tidal creek stations but was located in the hypoxic/hypercapnic areas. However, Taskinas 1 showed the same CPUE and was not located in an hypoxic/hypercapnic area but at the highest upstream station. Crab abundance was significantly correlated with salinity in both the tidal creek and the York River beach site respectively (r =-.577, p=.031; r = .403, p=.025; Pearson’s Correlation) (Table 3); abundance was inversely related to salinity in the tidal creek.

**Laboratory Measurements**

Hemolymph lactate levels varied considerably in animals sampled in the field, ranging from 0.1 to 6.0 mmol/l (Fig.17) and showed no significant difference among D.O. and pH conditions (f=.84, p=.62: one-way ANOVA). Laboratory air exposure results varied within the ranges 0.001-0.297 mmol/L with no correlation between exposure time (1-9 minutes) and hemolymph lactate. However, after the nine minute exposure time, consistent elevated lactate amounts were observed. Two crabs exposed
for 10 and 11 minutes had the highest lactate levels 0.203 and 0.297 mmol/L. These highly variable field (x=.1121) and laboratory (x=.0944) lactate results could be attributed to different molt stages as well as pre-exposure conditions in the tidal creek before hypoxic events.

Oxygen uptake was significantly higher in normoxic conditions (140-160 mmHg and pH=7.1-8.0) than in hypoxic/hypercapnic (30-50 mmHg and pH=6-7) (f= 15.163, p=.000; paired t-tests) (Fig. 18). Oxygen extraction efficiency was not significantly different for the blue crab for low pH/low dissolved O₂. Yet, when individual stressors (low D.O. and low pH) were analyzed among different pH values there were significant differences (f=10.750, p=.000; paired t-tests). Decreased oxygen extraction efficiency at low and high ambient pH values and increased extraction efficiency at mid-range pH values of 7.0-7.2 (Fig. 19).

**Discussion & Conclusion**

Hypoxia and low pH were identified in this study as multiple stressors in the Taskinas Creek study sites. Assuming that pH is a function of CO₂ and thus, hypercapnia is considered an additional stressor in this system. At least three other investigations also observed low pH and hypoxic in freshwater (Jones et al., 1987), mesohaline (Cochran and Burnett, 1996), and marine (Christmas and Jordan, 1988) waters. The present work demonstrates this same phenomenon in a smaller system with greater salinity fluctuations. This conclusion is important in understanding restoration and
management of hypoxic areas. Aquatic animals not only face low dissolved oxygen but face increased CO₂ and low pH. Hypoxia is not a single stressor with defined toxic values but a multiple stressor condition challenging aquatic organisms in an estuarine environment.

Crab abundance did not exhibit a significant difference among D.O./pH regimes, indicating that blue crabs respond to a combination of abiotic and biotic conditions including salinity, temperature, and tidal cycle, molt stage, during growth and development. Optimal crab habitat values for temperature, salinity, dissolved oxygen, and pH (Van Heukelem, 1991) occurred at both sampling sites (Table 1). Taskinas 1 and Taskinas 5 showed optimal conditions in the tidal creek (Fig. 20). Higher optimal condition criteria for dissolved oxygen and pH at the beach site was due to increased sensitivity of juveniles to hypoxia/hypercapnia compared to adults in the tidal creek. Optimal conditions at the beach site were the emergent marsh areas York 1 and York 3 (Fig. 21). A step-wise regression analysis showed that all four physicochemical factors influenced blue crab habitat, however the low r-squared value suggests that factors are confounding in predicting optimal habitat or that other unidentified factors are involved (Table 4). However, as hypoxic/hypercapnic areas continue to increase in size, frequency, and duration in coastal regions, the optimal conditions in terms of D.O. and pH are reduced which, in turn, increases mortality and competition among blue crabs. Salinity was an important factor affecting crab distribution, with one of the highest CPUE stations (Taskinas 1) located upstream in the low salinity part of the tidal creek. Larger
juveniles grow and segregate habitat with large males generally occupying the upper reaches of small tributaries (Hines et al., 1987). Blue crabs gain a developmental advantage molting in low salinity areas due to a nearly 100% increase in size compared to crabs molting in higher salinity water (deFur et al., 1988). There was not a correlation between hypoxic/hypercapnic areas and crab abundance. Another possible explanation for low correlation among factors is that blue crabs move out of the tidal creek into the York River channel using it as a refuge during these hypoxic/hypercapnic events and move back in after the events with no overall effect on abundance.

In addition to the physicochemical factors, structure provided by emergent marsh areas was important for optimal blue crab habitat at the beach site. The present study supports Ryer et al. (1990) observations that blue crabs may utilize marsh areas similar to sea grass beds as a molting refuge because of reduced predation pressure relative to other shallow water habitats. The marsh areas (York 1 and York 3) at the beach site showed an overall higher crab abundance than the mudflats area. These emergent marsh areas provided shelter and protection for juvenile and pre-molt crabs from predators.

Blue crab hemolymph lactate was not significantly different among D.O./pH regimes in this study, and therefore hemolymph lactate was not considered a good in situ biomarker for exposure to hypoxia and hypercapnia. The hemolymph sampling protocol, duration of exposure, and individual variations among crabs may have caused the overall variation in results. Other possible biomarkers may be hemolymph pH and hemocyanin subunits for long-term exposure to hypoxia/hypercapnia (deFur, 1988; Mangum, 1994; Mangum, 1993). Lactate raises the in vitro and in vivo (Booth et al., 1982) affinity of
oxygen and opposes the Bohr effect in *C. sapidus* (Truchot, 1980) and Dungeness crab (*Cancer magister*) Graham et al. (1983).

Blue crab oxygen uptake followed a linear relationship with declining uptake during hypoxia conditions due to a decrease in ambient oxygen availability. Blue crabs are partial regulators, with oxygen consumption maintained down to about 40% saturation (2mg/l) (Cameron, 1978). Lobsters (*Homarus vulgaris*) show this same ability to maintain % extraction after 72h exposure to hypoxia (Butler et al., 1978). This ability to regulate oxygen uptake may decline at higher temperatures, after exercise or after disturbance (McMahon and Wilkens, 1983).

Oxygen extraction efficiency, though not uniform, was maintained over a wide range of O2 and pH conditions with the organism at rest. Oxygen extraction efficiency was significantly different in crabs among different pH ranges (Fig. 15). A low extraction efficiency at low ambient pH may be caused by a decrease in the oxygen affinity of the respiratory pigment, hemocyanin. A lower affinity may be directly caused by an internal acidosis resulting in a Bohr Shift that reduces the hemocyanin affinity for oxygen at the gills. Again, optimal conditions for pH appear to be in the middle pH range with highest extraction % rates at a pH range of 7.0-7.2. It is less clear why oxygen extraction decreased at alkaline pH. Alkaline water may raise hemolymph pH, raising hemocyanin oxygen affinity so that at a given venous Po2 in the tissues, hemocyanin returning to the gill will have a higher O2 saturation and will thus extract less oxygen from the branchial
water. Other organisms, such as freshwater fish displayed no avoidance response to high pH when oxygen conditions were supersaturated (Serafy and Harrell, 1993).

The restoration of estuarine habitats is directly linked to evaluating criteria for ecological conditions such as hypoxia. The typical approach to low oxygen conditions is to treat it as a single stressor, not considering CO₂ or pH fluctuations (Burnett, 1997). The present research includes these important environmental conditions in addition to low dissolved oxygen and uniquely addresses the sub-lethal effects on an estuarine organism both in a field and laboratory setting. The identification of hypoxia/hypercapnia and acidosis as a multiple stressor has implications for worldwide coastal management approaches in terms of anthropogenic activities. At the present time, hypoxia is addressed toxicologically; Chesapeake Bay Dissolved Oxygen Goal for Restoration of Living Resources Habitats (1992) identified target goals for dissolved oxygen at 5 mg/l concentration or higher. The criteria of these goals infer that restoration of hypoxic coastal habitats will be reached at this concentration level with no reference to other concurrent stressors such as pH. Presumably, the factors causing hypoxia also lead to low pH and hypercapnia. Thus restoration of normoxic conditions should address both conditions, but the present approach fails in two respects.

First, 5mg/l is not the correct measure of dissolved oxygen in reference to organisms living in the estuarine ecosystem. The unit of measure mg/l for oxygen is the concentration of oxygen within the aquatic system whereas sub-lethal physiological effects are determined by partial pressure gradients in blue crabs and other aquatic
species. Therefore, percent saturation or measurements in torr or mmHg would be a more accurate measure of oxygen for the functioning of aquatic organisms.

Secondly, optimal conditions for the blue crab in the present study were 2.0 mg/l higher than the defined goal of 5.0 mg/l. Therefore, the criteria set by the Chesapeake Bay Dissolved Oxygen Goal for Restoration of Living Resources is too low. At O₂ levels between 2.0 and 5.0 mg/l, systems still undergo hypercapnia and low pH resulting in adverse effects on aquatic animals.

In summary, this study examined hypoxia and hypercapnia as concurrent multiple seasonal stressor in a shallow water tidal creek. Sub-lethal effects of low dissolved oxygen and high CO₂ (measured indirectly by pH) on blue crabs were observed on both the organismal and population levels. Optimal dissolved oxygen and pH conditions are important in determining blue crab distribution along with other physicochemical factors. Physiological responses in blue crabs show oxygen uptake decreasing with hypoxic/hypercapnic conditions and extraction efficiency varying among ambient PO₂/pH. Hemolymph lactate does not correlate with hypoxic and hypercapnic conditions in this study. Further investigation is needed to fully understand responses to ambient PO₂/pH in the aquatic systems and adaptive mechanisms for animals living in this environment.
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Appendix
Appendix

Table 1. Physicochemical factors used for modeling optimal habitat. The beach sampling site had a higher criteria for juvenile development.

<table>
<thead>
<tr>
<th>Taskinas Creek</th>
<th>York River Beach Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (&gt;7.0ppt)</td>
<td>Salinity (&gt;9.9 ppt)</td>
</tr>
<tr>
<td>Temperature (&gt;21.8 °C)</td>
<td>Temperature (&gt;18.3 °C)</td>
</tr>
<tr>
<td>Dissolved Oxygen (&gt;4.5 mg/l)</td>
<td>Dissolved oxygen (&gt;5.0 mg/l)</td>
</tr>
<tr>
<td>pH(&gt; 7.0)</td>
<td>pH(&gt;7.4)</td>
</tr>
</tbody>
</table>

(Van Heukelem, 1991)

Table 2. Chi-square analysis of parameters vs. crab abundance. All factors were showed a significant differences at the alpha ≤ .05 for crab abundance.

<table>
<thead>
<tr>
<th>Water Quality Characteristics</th>
<th>Chi square</th>
<th>d.f.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved Oxygen</td>
<td>377.0</td>
<td>7</td>
<td>.0000*</td>
</tr>
<tr>
<td>pH</td>
<td>502.7</td>
<td>4</td>
<td>.0000*</td>
</tr>
<tr>
<td>Salinity</td>
<td>413.9</td>
<td>18</td>
<td>.0000*</td>
</tr>
<tr>
<td>Temperature</td>
<td>293.0</td>
<td>14</td>
<td>.0000*</td>
</tr>
<tr>
<td>Tide</td>
<td>205.0</td>
<td>2</td>
<td>.0000*</td>
</tr>
</tbody>
</table>

* significant at .05 ≤
Table 3. Pearson’s correlation of abiotic conditions vs. number. Crab abundance was significantly influenced by salinity at the tidal creek and beach site. Dissolved oxygen and pH are not significantly correlated.

<table>
<thead>
<tr>
<th>Abiotic Factors</th>
<th>Beach Site-Number</th>
<th>Taskinas Creek -Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.O.</td>
<td>Pearson Correlation</td>
<td>-.143</td>
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<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.627</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>pH</td>
<td>Pearson Correlation</td>
<td>.018</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.952</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>Number</td>
<td>Pearson Correlation</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>Temp</td>
<td>Pearson Correlation</td>
<td>-.113</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.700</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>Salinity</td>
<td>Pearson Correlation</td>
<td>-.577*</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.031</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>14</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed)
Table 4. A stepwise regression for four abiotic parameters. All parameters were important but were not good predictors of crab abundance with an $r^2$ of .451 for the beach site and .233 for the tidal creek.

<table>
<thead>
<tr>
<th>Beach Model</th>
<th>R</th>
<th>R square</th>
<th>Adjusted R square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.577$^a$</td>
<td>.333</td>
<td>.277</td>
<td>10.6729</td>
</tr>
<tr>
<td>2</td>
<td>.639$^b$</td>
<td>.408</td>
<td>.300</td>
<td>10.4986</td>
</tr>
<tr>
<td>3</td>
<td>.669$^c$</td>
<td>.448</td>
<td>.282</td>
<td>10.6328</td>
</tr>
<tr>
<td>4</td>
<td>.672$^d$</td>
<td>.451</td>
<td>.208</td>
<td>11.1744</td>
</tr>
</tbody>
</table>

Creek Model

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R square</th>
<th>Adjusted R square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.080$^a$</td>
<td>.006</td>
<td>-.028</td>
<td>1.5672</td>
</tr>
<tr>
<td>2</td>
<td>.168$^b$</td>
<td>.028</td>
<td>-.041</td>
<td>1.5672</td>
</tr>
<tr>
<td>3</td>
<td>.419$^c$</td>
<td>.175</td>
<td>.084</td>
<td>1.4704</td>
</tr>
<tr>
<td>4</td>
<td>.472$^d$</td>
<td>.233</td>
<td>.104</td>
<td>1.4542</td>
</tr>
</tbody>
</table>

Beach Model

a. Predictors: (Constant), Salinity
b. Predictors: (Constant), Salinity, D.O.
c. Predictors: (Constant), Salinity, D.O., pH
d. Predictors: (Constant), Salinity, D.O., pH, Temp
e. Creek Model
f. Predictors: (Constant), D.O.
g. Predictors: (Constant), D.O., pH
h. Predictors: (Constant), D.O., pH, Salinity
i. Predictors: (Constant), D.O., pH, Salinity, Temp
Figure 1- Comparison of oxygen units in terms of saturation, concentration, and hypoxia.
Figure 2- Dissolved oxygen versus pH over four Choptank River Oyster Bars (Christmas and Jordan, 1988).

Figure 3- Oxygen and pH in water samples collected in the James Island Creek located in the Charleston Harbor Estuary in March 1993.
Figure 4- Sampling stations for the beach site located on the York River and Taskinas Creek. Monitoring station is located at Taskinas 5 sampling site.
Figure 5- Flow chart describing geographic spatial analysis methods.

Figure 6- Monitoring station pH [H'] and dissolved oxygen data for the months of July, August, and September (1998). Dissolved oxygen is correlated with pH [H'].
Figure 7- Monitoring station pH [$[H^+]]$ and dissolved oxygen data for the month of August showing the highest correlation for the 1998 sampling period.

Figure 8- Upstream tidal creek dissolved oxygen and pH [$[H^+]]$ for a 24 hour sampling period. A diel pattern was observed with the lowest pH [$[H^+]]$ and dissolved oxygen occurring at night and early morning.
Figure 9 - pH and dissolved oxygen variation over a 24 hr period at Taskinas 1 in September of 1998. Low D.O. and pH was observed in late night and early morning hours.

Figure 10 - pH and dissolved oxygen variations over a 24 hr period at Taskinas 5 in April of 1999. Overall pH and D.O. values were higher when compared to September of 1998 measurements, however low pH and D.O. values were also observed in the late night early morning hours.
Figure 11- Upstream dissolved oxygen and pH \([H^+]\) data for Taskinas Creek and York River beach site. A lower \(r\) value was correlated with smaller sample size.

Figure 12- Crab abundance (CPUE) at the beach site with significant differences between marsh areas and the mudflat.
Beach site and the tidal creek abundance was highly variable and was not significantly among different DO. Values for both the

Figure 14. Crab abundance (CPUE) for dissolved oxygen for beach site and Tashkans Creek. Crab

![Graph showing CPUE (days) for different sites.](image)

Figure 13. CPUE for crab abundance in Tashkans Creek. Highest CPUE was at Tashkans 1 and

![Graph showing Mean CPUE (Days) for different sites.](image)
Figure 15-Crab abundance vs. pH at the beach site and Taskinas Creek. No significant difference was observed among pH values with values ranging from 7.2-8.0 at the beach site and 6.5-7.8 in the tidal creek.

Figure 16-Hypoxia/Hypercapnia areas vs. crab abundance in Taskinas creek. Abundance was highest at Taskinas 1 outside identified hypoxic/hypercapnic areas.
Figure 17—Hemolymph lactate levels for range of 3mg/l-10mg/l.

Figure 18—Oxygen uptake under normoxic and hypoxic/hypercapnic conditions.
Figure 19- Oxygen extraction efficiency at different pH values. Low efficiency at low and high pH values and high extraction efficiency at mid-range pH (7.0-7.2).

Figure 20- Optimal habitat for the York River beach site. Optimal sites were correlated with marsh areas.
Figure 21-Optimal habitat areas within Taskinas Creek vs. crab abundance. Taskinas 1 and Taskinas 4 identified as optimal habitat.
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