Physiological and Biochemical Responses to Hypoxia in the Blue Crab, Callinectes Sapidus Rathbun, the Lesser Blue Crab, Callinectes Similis Williams, and the Southern Oyster Drill, Stramonita Haemastoma Linnaeus.

Tapash Das
Louisiana State University and Agricultural & Mechanical College

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Physiological and biochemical responses to hypoxia in the blue crab, *Callinectes sapidus* Rathbun, the lesser blue crab, *Callinectes similis* Williams, and the southern oyster drill, *Stramonita haemastoma* Linnaeus

Das, Tapash, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1993
PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES TO HYPOXIA IN THE BLUE CRAB, CALLINECTES SAPIDUS RATHBUN, THE LESSER BLUE CRAB, C. SIMILIS WILLIAMS, AND THE SOUTHERN OYSTER DRILL, STRAMONITA HAEMASTOMA LINNAEUS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Zoology and Physiology

by

Tapash Das
B.S., Burdwan University, India, 1981
M.S., Kalyani University, India, 1983
December 1993
DEDICATION

I am dedicating this dissertation to my beloved parents and sister whose sacrifice and inspiration is a constant source of support in the every walk of my life.
ACKNOWLEDGMENTS

The completion of this dissertation would not have been possible without the help of many others. I would like to extend my deepest gratitude and thanks to my advisor, Dr. William B. Stickle jr. for his guidance, counselling and constant support over the last four and a half years. I am also indebted for all the help I have received from the members of my committee, Dr. J. F. Siebenaller, Dr. J. W. Fleeger, Dr. K. M. Brown and Dr. D. W. Foltz.

Many thanks go to Dr. Marianne Hollay for her invaluable help with statistical analyses, Mr. Jeff Tamplin for his help in some of the illustrations and Mr. Ron Bouchard for his continuous help with photography.

Special thanks are extended to Melanie Morgan, Phillip Corey Jackson, John L. Guerin, Dr. Shiao Wang and Dr. John McCall for their help and support.

I wish to express my heartfelt gratitude to my parents and sister for their patience, love and understanding during these trying years.
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ABSTRACT

This study measured the physiological and biochemical changes associated with exposure of the juvenile blue crab, Callinectes sapidus Rathbun and the lesser blue crab, C. similis Williams, to long term (28 d) hypoxia, short term (10 d) transfer from hypoxia to normoxia and a diurnally fluctuating oxygen regime for 28 days. The southern oyster drill, Stramonita haemastoma Linnaeus, was also exposed to 28 days of constant hypoxia to compare the responses of a tolerant species with the two species of Callinectes. The 28 day LC$_{50}$ estimates for C. sapidus, C. similis and S. haemastoma were respectively 106, 43 and 11.5 Torr under constant hypoxic exposure for 28 d. Feeding rates for the crabs of both species exposed to 50 and 25 Torr oxygen were significantly lower than for crabs exposed to higher levels of oxygen. Growth and molting rates of crabs exposed to constant hypoxia were always lower than for crabs exposed to normoxia. Feeding rate in S. haemastoma declined linearly with declining oxygen under constant exposure to hypoxia. Oxygen consumption rates of the two crab species under various hypoxic levels were significantly different. Mean oxygen consumption in C. similis exposed to hypoxia was higher than for crabs exposed to normoxia. Rate of adaptation for blue crabs transferred from hypoxia to normoxia was faster than when transferred from normoxia to hypoxia. Detection and avoidance of hypoxic water by the two species of crabs was also observed under laboratory conditions. Both species of crabs were able to detect and avoid hypoxic water and stay at an intermediate oxygen tension. Crabs were found to be more active at higher oxygen tensions. Behavioral responses, when crabs were exposed to hypoxic treatments, were clearly due to hypoxic stress. The feeding rate of C. sapidus exposed to diurnally varying oxygen tension was significantly higher than for the crabs exposed to normoxia while the feeding rate of C. similis exposed to diurnally varying oxygen tension was significantly lower than the ones exposed to normoxia. RNA and DNA
concentration decreased over time in both species of crabs exposed to diurnal variation in oxygen tension. Concentration of the individual nucleic acids were found to be a reliable measure of hypoxia stress than the RNA:DNA ratio.
CHAPTER 1

GENERAL INTRODUCTION
Seasonal depletion of dissolved oxygen in subpycnocline waters is often observed in estuaries and nearshore waters of continental shelves (Sanford et al. 1990). Occurrence of hypoxic water masses has been reported by a number of researchers from a variety of locations worldwide, particularly in Europe and America (Bedinger et al. 1981, Turner and Allen 1982, Boesch 1983, Officer et al. 1984, Stachowitsch 1984, Renaud 1985, 1986a, Rabalais et al. 1986a, b, Rosenberg 1986, Parker and O'Reilly 1991, Portnoy 1991). The occurrence of hypoxic bottom water off the Louisiana coast west of the Mississippi Delta is a common and recurrent phenomenon (Bedinger et al. 1981, Stuntz et al. 1982, Boesch 1983, Leming and Stuntz 1984, Renaud 1985, 1986a, Rabalais et al. 1986a, b) locally known as the occurrence of dead water (Turner and Allen 1982, Boesch 1983, Renaud 1985, 1986a). Hypoxic water masses may persist for weeks and are usually associated with stratification of the water column, phytoplankton blooms, and stable high pressure weather systems (Ragan et al. 1978, Bedinger et al. 1981, Harper et al. 1981, Boesch 1983, Renaud 1985, Rabalais et al. 1991a, 1992b). On the Louisiana continental shelf, input from the Mississippi and the Atchafalaya rivers are important contributors of both fresh water and nutrients which can cause a strong halocline and stimulate the production of a large phytoplankton biomass (Boesch and Rabalais 1991). Decomposition of the sinking phytoplankton contributes to oxygen depletion in the isolated bottom water. Diel variation in the dissolved oxygen tension of estuarine water along the Louisiana Gulf coast occurs frequently, especially during the summer months, with water becoming very hypoxic during the early morning hours (Das and Stickle, unpublished observation).

Hypoxia can be tolerated by all organisms for a variable amount of time although this condition is ultimately incompatible with survival of most organisms which use oxygen for the production of energy. Mass mortality of marine and estuarine organisms has been widely reported from areas affected by low dissolved oxygen in the water.
Commercial crab-pot fishermen have frequently reported dead blue crabs in pots (Carpenter and Cargo 1957, Tatum 1979, Van Engel 1982). Hypoxia is a major environmental variable affecting the performance and ultimately survival of marine organisms which respire aerobically.

The blue crab *Callinectes sapidus* Rathbun occurs in the lower reaches of rivers, estuaries, and coastal waters along the Atlantic seaboard and Gulf of Mexico (Churchill 1919, Gunter 1938, Odum 1953, Cameron 1978). Juveniles and adults occupy habitats ranging from freshwater to hypersaline waters of as high as 117°/oo S (Williams 1984) but larvae are relatively stenohaline (Costlow and Bookhout 1959). Tolerances may vary with life stages and hypoxia may also be stressful to these species. Their high abundance, diverse feeding habits and importance as prey species for a variety of organisms make them an integral part of the coastal ecosystem. *C. similis* Williams is an offshore congener of the *C. sapidus* and occurs in the oceanic littoral zone in salinities above 15°/oo and at a depth of almost 100 meters (Williams 1984). The distributional patterns and niche characteristics of these two species overlap quite frequently (Engel 1977). They primarily utilize the same prey groups (Hsueh et al. 1992). The high overlap in diet and niche characteristics of these two crab species suggests a competition for common resources (Engel 1977). Norse and Fox-Norse (1979) suggested a common pattern of occurrence among various species within the genus *Callinectes* along all three ocean borders (eastern Pacific, western Atlantic and eastern Atlantic) where blue crabs occur. Based on the evolutionary relationship of these species there are large inshore species which belong to *bocourtii* group and small offshore species which belong to *danae* and *marginatus* group. Based on their ecological and geographical patterns, Norse (1977) suggested different abilities to withstand physical and chemical conditions for these species in the spectrum of biotopes they inhabit. In the northern Gulf of Mexico, *C.*
Callinectes sapidus is the large estuarine species and C. similis is the small offshore species (Perry 1975). Therefore, studying the effect of hypoxia on these two species would provide us with valuable information about their susceptibility to this environmental variable (hypoxia) and their differential responses to cope up with the changes in the dissolved oxygen tension in the ambient water.

The southern oyster drill, Stramonita (=Thais) haemastoma, is an important predator on oyster reefs and other hard substrates in the northern Gulf of Mexico. It is responsible for losses of up to half the yearly oyster crop in Louisiana (Cake 1983). S. haemastoma is exposed to diurnal fluxes of dissolved oxygen, ranging from 16 to 120% of saturation over a 24-h period and is very tolerant of chronic hypoxia (Kapper and Stickle 1987, Stickle et al. 1989). Use of this species provided baseline data required to compare the responses of the two crab species with a very hypoxia tolerant species from the same habitat.

The overall aim of this study is to measure and compare the responses of the two crab species and the gastropod, Stramonita haemastoma, to hypoxia and anoxia. My null hypothesis is, responses to hypoxia in the two species of crabs, Callinectes sapidus and C. similis, are similar as are the responses of S. haemastoma. The alternative hypothesis is, responses to hypoxia differs in the two species of crabs as well as in the oyster drill. The specific objectives of this study are to: (1) compare the hypoxia tolerance of juvenile C. sapidus and C. similis and adult S. haemastoma, (2) quantify the effects of long-term exposure (28 d) to hypoxia on the feeding rate, growth, molting and respiration of the juvenile C. sapidus and C. similis as well as on the feeding rates of adult S. haemastoma, (3) determine the effects of abrupt changes from different levels of hypoxia to normoxia on the survival, feeding rate, growth and molting of C. sapidus, (4) determine whether juvenile C. sapidus and C. similis can detect and avoid specific levels of dissolved oxygen under laboratory conditions, (5) determine the behavioral responses associated with short
term hypoxic exposure, (6) compare the responses of juveniles of the two crab species to
diurnally varying oxygen tension, and (7) determine the difference in response to chronic
hypoxia and diel variation in oxygen tension using feeding rate, survival, growth, molting
and changes in the nucleic acid concentration as indices of sublethal stress.

These objectives are addressed in 3 separate chapters. In the second chapter of
this dissertation I have investigated the physiological responses of the two species of
krabs, *Callinectes sapidus* and *C. similis*, and the gastropod, *Stramonita haemastoma*,
following exposure to long-term (28 d) hypoxia and abrupt changes from hypoxia to
normoxia. Therefore, the second chapter compared the effects of constant hypoxia on the
survival and feeding rate of all three species as well as the growth, molting and oxygen
consumption rate of *C. sapidus* and *C. similis*. This chapter has been published in

The effect of hypoxia on the movement patterns of the blue crab, *Callinectes
sapidus* and the lesser blue crab, *C. similis*, to and from the estuarine region is unknown.
Mature female *C. sapidus* hatch their eggs in high salinity water (Milliken and Williams
1984). Egg-bearing female *C. sapidus* may occur and spawn in the coastal Gulf and
estuarine waters year-round (Gunter 1950, Daugherty 1952, More 1969, Adkins 1972a,
Perry 1975). Egg-bearing *C. similis* also spawn in high salinity waters (Hsueh *et al.* in
press). Stuck and Perry (1981) reported a year-round abundance of *C. similis* magalopae
in the waters of Mississippi Sound with a peak during February and March. Thus, an
extensive occurrence of hypoxia in nearshore and offshore water could cause a potential
problem for the spawning female population of the blue crabs as well as the larval and
juvenile stages. Low dissolved oxygen may also act as a barrier to the migration of
juvenile blue crabs from high salinity to low salinity waters (Van Engel 1982). Chapter
three of this dissertation probes into the capacity of the juveniles of these 2 species of
crabs to detect and avoid hypoxic water under laboratory conditions. This chapter has been submitted to Marine Biology and is currently undergoing revision.

The fourth chapter of this dissertation addresses physiological and biochemical responses of juvenile *Callinectes sapidus* and *C. similis* exposed to a fluctuating oxygen regime. The objective of this chapter is to compare the responses of the two crab species exposed to diel variation in oxygen tension with that of long-term exposure to constant hypoxia.

In order to quantify the responses of the three species to constant and diel variation in oxygen tension, survival was used as a measure of resistance adaptation. LC$_{50}$ was used as a quantifiable measure of survival. It is a very sensitive indicator of hypoxia tolerance because the determination of a LC$_{50}$ value requires data about both survival and mortality from a range of treatments tested. Feeding rate, growth, molting, concentration of RNA and DNA and RNA:DNA ratio were used as a measure of capacity adaptation. Feeding rate is the primary determinant of an animal's energy budget. Feeding rate has been shown to vary 5-9 times more than metabolic rate along environmental factor gradients (Stickle 1985). Therefore, a measure of the feeding rate provides a direct measure of stress involved with exposure to hypoxia. Growth and molting are direct consequences of changes in feeding rate. Measurement of the oxygen consumption rate provides an estimate of metabolic costs of adaptation to the hypoxia gradient. Changes in nucleic acid concentration and the RNA:DNA ratio have been studied in relation to various biological processes in both laboratory experiments and field studies. Since DNA content per cell nucleus remains constant in somatic cells within a given species (Leslie 1955) and changes in cellular RNA content reflect changes in cellular activity (Brachet 1960) the ratio of RNA to DNA is used as a reliable indicator of protein synthetic activity within cells. RNA:DNA ratios have been shown to be correlated with growth (see review by Bulow 1987) and growth is affected by
environmental stressors. Therefore, measurement of the RNA:DNA ratio should serve as an useful indicator of stress.
CHAPTER 2

RESPONSES OF CRABS CALLINECTES SAPIDUS AND C. SIMILIS AND THE
GASTROPOD STRAMONITA HAEMASTOMA TO HYPOXIA AND ANOXIA
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Tapash Das
Zoology & Physiology
Louisiana State University
Baton Rouge, LA 70803-1725
U.S.A
Phone (504) 388-1739
Fax (504) 388-1763

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INTRODUCTION

Seasonal depletion of dissolved oxygen in subpycnocline waters is often observed in estuaries and nearshore waters of continental shelves (Sanford et al. 1990). Occurrence of hypoxic water masses have been reported by a number of researchers during the last decade in the Chesapeake Bay, New York harbor, New England estuaries and on the Louisiana continental shelf (Bedinger et al. 1981, Turner and Allen 1982, Boesch 1983, Officer et al. 1984, Renaud 1985, 1986a, Rabalais et al. 1986a, b, Parker and O'Reilly 1991, Portnoy 1991). Mass mortalities or massive shoreward migration of demersal fishes and crustaceans to shallow water have been reported when the dissolved oxygen level in the water was very low (Loesch 1960, May 1973, Garlo et al. 1979, Pavela et al. 1983, Pihl et al. 1991). The occurrence of hypoxic water masses off the Louisiana coast is almost an annual phenomena and may persist for weeks (Bedinger et al. 1981, Turner and Allen 1982, Boesch 1983, Renaud 1985, 1986a, and Rabalais et al. 1986a, b). This creates a threat to marine fauna, including the blue crab, Callinectes sapidus, and the lesser blue crab, Callinectes similis.

The blue crab Callinectes sapidus Rathbun occurs in lower reaches of rivers, estuaries, and coastal waters along the Atlantic seaboard and Gulf of Mexico coast of USA (Churchill 1919, Gunter 1938, Odum 1953, Cameron 1978). Juveniles and adults occupy habitats ranging from freshwater to hypersaline waters of as high as 117°/oo S (Williams 1984) although larvae are relatively stenohaline (Costlow and Bookhout 1959). Salinity, water temperature, food availability, predation, substratum, available habitats and pollutants affect growth and survival of blue crabs (Gulf Coast Research Laboratory, Ocean Springs, 1986; Georgia Cooperative Fishery and Wildlife Research Unit, Athens, 1989). Tolerances may vary with life stages and hypoxia may also be stressful to these species. Blue crabs do not conform to specific trophic levels and are characterized as
opportunistic benthic omnivores. Their high abundance, diverse feeding habits and importance as prey species for a variety of organisms make them an integral part of the coastal ecosystem.

*Callinectes similis* Williams, is an offshore congener of the *C. sapidus* and occurs in the oceanic littoral zone in salinities above 15°/oo and at a depth of almost 100 meters (Williams 1984). The distributional patterns and niche characteristics of these two species overlap quite frequently and in some cases the crabs compete for food and other resources (Engel 1977). They primarily utilize the same prey groups (Hsueh et al. 1991). This high overlap in the diet of these two crab species suggests a competition for common resources.

The southern oyster drill, *Stramonita (=Thais) haemastoma*, is an important predator on oyster reefs and other hard substrates in the northern Gulf of Mexico. It is responsible for losses of up to half the yearly oyster crop in Louisiana (Cake 1983). *S. haemastoma* is exposed to diurnal fluxes of dissolved oxygen, ranging from 25 to 120% of saturation over a 24-h period and is very tolerant of hypoxia (Kapper and Stickle 1987, Stickle et al. 1989).

The specific objectives of this study are to: (i) compare the hypoxia tolerance of the juvenile *Callinectes sapidus*, and *Callinectes similis* and adult *Stramonita haemastoma*; (ii) quantify the effects of long term exposure (28 days) to hypoxia on the feeding rate, growth, molting, and respiration of the juvenile *C. sapidus* and *C. similis* and feeding rates of adult *S. haemastoma*; (iii) determine the effects of abrupt changes from different levels of hypoxia to normoxia on the survival, feeding rate, growth and molting of *C. sapidus*.
MATERIALS AND METHODS

**Collection and maintenance**

*Callinectes sapidus* were collected nearshore Ocean Springs, Mississippi (Longitude 88°49.9’W; Latitude 30°24.4’N); *C. similis* were collected nearshore Port Fourchon, Louisiana (Longitude 90°11.4’W; Latitude 29°6.7’N); and *Stramonita haemastoma* were collected from bulkheads and jetties onshore Caminada Pass, Louisiana (Longitude 90°3.0’W; Latitude 29°12.6’N). All animals were collected between August and September 1991, when the water temperatures at Ocean Springs, Port Fourchon, and Caminada Pass was 28, 30, and 30°C and the salinity was 22, 29, and 30°/oo respectively. The animals were brought back to the laboratory and placed into artificial sea water made from Instant Ocean, at 24°C and salinities matching field conditions. All animals were kept under constant illumination. After a few days all animals were acclimated in a step wise fashion to the experimental salinity (30°/oo). All three species were held for two weeks at the final temperature-salinity combination before being used in experiments. Blue crabs and lesser blue crabs were individually isolated as described below and were fed frozen abdominal portions of the grass shrimp, *Palaemonetes pugio*, and the oyster drills were fed live mussels, *Ischadium recurvum*.

**Hypoxia exposure system**

The hypoxia exposure system consisted of three sets of 12 flow-through aquaria. Two aquaria were assigned for each experimental oxygen tension for each species. Each experimental aquarium (38 liter) consisted of an undergravel filter overlaid with oyster chips. Water was pumped from a large filtration unit into each aquarium by a peristaltic pump at a rate sufficient to ensure the water was completely exchanged several times.
each day. The filtration units were large (76 liter) partitioned plywood boxes lined with non-toxic fiberglass resin. A separate filtration unit was used for each species.

Target oxygen tensions of 119, 73, 50, 25 and 0 Torr (77%, 47%, 32%, 16% and 0% of air saturation and 5.57, 3.39, 2.31, 1.16, and 0 ppm dissolved oxygen) were created and maintained by mixing bottled nitrogen and oxygen with Matheson gas mixers (model 7402T) along with 0.03% carbon dioxide to maintain the pH at approximately 7.8. Each gas mixer was connected to an outlet manifold and the air mixture was passed through the undergravel filter in each aquarium. One gas mixer was used for each oxygen level and for each species. Ambient air was used to drive the undergravel filters of the normoxic tanks (155 Torr). PO$_2$ was always maintained within 10% of the target value in the two higher levels, and within 5% of the target value for the 50 and 25 Torr O$_2$ tanks. The zero Torr tanks were maintained within 5% of the target value for the first 6 days and then within 1% for the remaining 22 days. A plexiglas lid was used to cover each aquarium and then covered with a clear plastic wrapping to minimize air exchange. The water level was maintained in each aquarium by a constant-level siphon which drained water from the aquaria into one of the filter boxes at a rate equivalent to the inflow rate. Experimental conditions (temperature, salinity, PO$_2$, pH, ammonia) were checked daily. Ammonium concentration never exceeded 25µM.

The number of individuals used at each PO$_2$ were 16 divided into two groups of eight in each of the two aquaria designated for that PO$_2$ for the blue crabs and the lesser blue crabs and 12 divided into two groups of 6 for the oyster drills. Within each aquarium each crab was kept in a chamber (chambers were made from two pairs of PVC tubing of 21 cm in diameter and were 28 cm long with nylon meshed screens on both ends) to prevent cannibalism. Animals were chosen carefully for each PO$_2$ to minimize the weight and size differences within and among the treatments.
**Hypoxia bioassay**

For the 28 day constant hypoxic exposure experiment, individuals of all three species were transferred directly from normoxic water (155 Torr O₂) to different hypoxic levels (119, 73, 50, 25, and 0 Torr O₂) and were maintained throughout the experiment. In the transfer experiment, the blue crabs were exposed to three levels of constant hypoxia (73, 50, and 25 Torr O₂) for 10 days and then transferred directly to normoxic water (155 Torr O₂) and were maintained for another ten days. This experiment was performed to investigate the amount of physiological stress involved when the oxygen concentration in water drops suddenly and returns to normal after staying at that level for several days.

Survival at each PO₂ was determined daily for all three species throughout the experimental period. A crab was considered dead when it did not show any sign of movement after stimulation and by a color change to straw color once it was dead for more than a few hours. A snail was considered dead when both the siphon and the foot failed to respond to tactile stimulation. LC₅₀ values were calculated for each day using the Spearman-Karber technique (Hamilton et al. 1977).

**Determination of food consumption, molting, and growth rate**

Both crab species were fed weighed abdominal portions of frozen grass shrimp (*Palaemonetes pugio*) *ad libitum* so some tissue remained at the end of the day. Uneaten portions of the food were removed, weighed and replaced with a new ration daily. Oyster drills were fed live mussels (*Ischadium recurvum*) *ad libitum*. The amount of dry tissue consumed daily by the drills was calculated from a dry tissue weight to length regression equation of the mussels (Least square method). Crabs and drills that died during the hypoxic exposure were excluded from the feeding rate analysis the week they died.
Growth rate was measured for the crabs only since growth of oyster drills include an increase in body tissue weight and incorporation of CaCO₃ in the shell making it impossible to measure the increase in soft tissue weight. Each time a crab molted it was reweighed and the difference between the initial and final weight was converted into percent increase in weight.

**Measurement of the scope for growth**

Scope for growth for both blue crabs and oyster drills was calculated (Table 2.2). Stickle et al. (1989) provided an estimate of heat dissipation in the blue crabs and the oyster drills when exposed to hypoxia and anoxia. Assuming a linear decline in the heat dissipation rate from normoxia to anoxia, a measure of respiratory energy loss can be estimated. The caloric concentration of grass shrimp (*Palaemonetes pugio*) abdominal tissue was taken from Guerin and Stickle (1992). Scope for growth was determined using the balanced energy equation of Winberg (1960). The equation is \( P = (C-F) - (R+U) = Ab - (R+U) \), where, \( P \) = scope for growth; \( Ab \) = energy absorbed from food consumed = (C, energy absorbed from food - F, energy lost as feces); \( R \) = energy lost as respiration; \( U \) = energy lost as excretion and was not estimated but typically only amounts to 4.5 - 11.9 % of energy losses at 25°C and 35‰/o S (Guerin & Stickle 1992). Scope for growth for *C. similis* could not be calculated because heat dissipation rate under various hypoxic levels were not available.

**Starvation experiment**

Sixteen blue crabs were maintained under normoxia for five days and monitored for daily feeding rate, then starved for 10 days and monitored for daily food consumption for another 10 days to determine if starvation for a period of time results in an enhanced feeding rate after food is again made available.
Measurement of oxygen consumption

Oxygen consumption rates at constant Po2's were measured using the flow-through system described by Stickle et al. (1985). Incurrent water was bubbled with a gas mixture of appropriate Po2 from a gas mixer identical to those used in the hypoxia exposure system. Water was pumped via a submersible pump from the appropriate dosing compartment through a manifold, with excess water returned to a separate reservoir via a return tube. Ten side ports from the manifold were connected to separate flow-through respiration chambers (250 ml.) so that a blank (control) chamber was placed at each end. Water of appropriate oxygen tension was pumped from the reservoir into the distribution manifold at a low flow rate (~20ml/min). Two hours were allowed between loading crabs and the initiation of the respiration determinations to allow for crab adaptation to the chambers. Oxygen consumption in each chamber was calculated according to the following equation:

\[ \mu l \text{O}_2 \text{hr}^{-1} = \% \text{oxygen used by the crab} \times \text{flow rate (l. hr}^{-1}) \times 1000 \times \text{oxygen content in water at that partial pressure}. \]

Percentage oxygen used by each crab was calculated from the difference between the mean oxygen partial pressure of the water flowing out of the control chambers and the water flowing out of the experimental chamber and dividing that by the experimental oxygen tension. Oxygen content in the water at a particular experimental oxygen tension and salinity-temperature combination was calculated using the following equation:

\[ A^* (\text{Experimental oxygen tension in Torr. 155}^{-1}) \]

where, A is the oxygen content in the normoxic water at a particular salinity-temperature combination. Water samples from the chambers were drawn anaerobically and were injected into a StrathKelvin oxygen meter (model 781) connected to a flow-through water jacketed oxygen electrode.
Statistical analysis

All rate functions were determined by either one-way or two-way analysis of variance (ANOVA). Differences among treatment means were determined by Tukey's range test or by Students-t-test. Repeated measure analysis was performed to determine differences in mean weekly food consumption between different levels and within each level of all three species (SAS Inst., Inc. 1989). To remove effects of body weight on the measured rate functions, all rate functions were standardized to a 1 g unit body weight. Oxygen consumption in the two crab species was measured per unit ash-free dry weight (AFDW). Different regressions were used to convert wet weight to AFDW. Respiration rates were measured by a three way factorial analysis. Since oxygen consumption rate of different species rarely assumes either of the two ideal shapes (oxconformity or oxyregulation) that describe perfect conformity or regulation (Mangum and Van Winkle, 1973), mean oxygen consumption at different days and within each level and species was compared by polynomial contrasts.

RESULTS

Response to constant hypoxic exposure

Response of Callinectes sapidus:

Callinectes sapidus was very sensitive to hypoxia (Fig. 2.1), with a 28 day LC₅₀ of 106 Torr O₂. All C. sapidus exposed to anoxia and 25 Torr O₂ died within 6 days of exposure. 100% mortality did not occur in any other hypoxic level.

Feeding rates in Callinectes sapidus exposed to normoxia, 119 and 73 Torr O₂ were not significantly different from each other (Tukey's range test) but were significantly different from the rates of crabs exposed to 50 Torr O₂ (Fig. 2.2a). Crabs exposed to 25
Figure 2.1  LC$_{50}$ of the blue crab, *Callinectes sapidus*, the lesser blue crab, *C. similis*, and the oyster drill, *Stramonita haemastoma*, as a function of hypoxia and anoxia at 30°/oo S and 24°C. The shaded portions indicate the period during which mortality rate was too low for LC$_{50}$ calculation.
Figure 2.2  *Callinectes sapidus* and *C. similis*. Average feeding rate (±SE) of crabs exposed to various levels of hypoxia for a period of 28 d. Letter's represent Tukey's range test for differences between treatment means. Means with the same letter are not significantly different from each other. (a) Feeding rate of *C. sapidus* expressed per unit body weight, (b) Mean feeding rate per crab in *C. sapidus*, (c) Feeding rate of *C. similis* expressed per unit body weight, (d) Mean feeding rate per crab in *C. similis*. 
mg wet food • day •
mg wet body, wt • day •
and 10 Torr O₂ were not included in the feeding rate analysis since 100% mortality occurred in those two levels within the first 6 days of exposure.

A significant linear decline in the feeding rate with increasing hypoxia was noticed when the feeding rate was measured on a per crab basis (Fig. 2.2b). The crabs exposed to normoxia grew faster than those exposed to different hypoxic levels, and their overall feeding rate was higher though the rate per unit body weight remained unchanged (Fig. 2.2a). Repeated measure analysis of the weekly feeding rate did not show any significant trend in the feeding rate (Fig. 2.3a).

Growth was measured in the blue crabs as percent increase in wet weight with respect to the initial weight of the crab. Significant differences in the growth of the crabs exposed to normoxia and those exposed to hypoxia were observed (Table 2.1). Only one crab molted in the 25 Torr O₂. Molting rate of the crabs exposed to normoxia was faster than in the crabs exposed to different hypoxic levels (Fig. 2.4a). Conversely, internoult period was shorter in the crabs exposed to normoxia than the crabs exposed to different hypoxic levels (Table 2.1).

**Response of *Callinectes similis***:

*Callinectes similis* exhibited was more tolerant to hypoxia than *C. sapidus* with a 28 day LC₅₀ of 43 Torr O₂ (Fig. 2.1). All the crabs exposed to anoxic water died within 3 days of exposure. 100% mortality did not occur in any other hypoxic levels.

Feeding rates of *Callinectes similis* exposed to normoxia (155 Torr) and 119 Torr O₂ were not significantly different from each other (Tukey’s range test) with a mean feeding rate of 401 and 447 mg wet food. gm wet body wt.⁻¹ day⁻¹ respectively but were significantly different from crabs exposed to 50 and 25 Torr O₂ (Fig. 2.2c) with a mean feeding rate of 338 and 330 mg wet food. gm wet body wt.⁻¹ day⁻¹ respectively. Crabs exposed to anoxia were not included in the feeding rate analysis because of 100% mortality in that level within 3 days.
Figure 2.3  Mean weekly feeding rate (±SE) of (a) *Callinectes sapidus* and (b) *C. similis*, exposed to various levels of hypoxia for a period of 28 d at 30°/oo S and 24°C.
Figure 2.4  Molting rate of (a) *Callinectes sapidus* (b) *C. similis* exposed to various levels of hypoxia for 28 d.
The figure depicts the number of molts per crab per day at different torrs of oxygen.

**Figure a**
- X-axis: Torr of oxygen (0, 25, 50, 73, 119, 155)
- Y-axis: No. of molts crab⁻¹ day⁻¹
- Data points show an increasing trend with increasing torr of oxygen.

**Figure b**
- X-axis: Torr of oxygen (0, 25, 50, 119, 155)
- Y-axis: No. of molts crab⁻¹ day⁻¹
- Data points show a similar trend as Figure a, with a peak at 119 Torr.
A significant linear decline in the feeding rate with increasing hypoxia was noticed when the feeding rate was measured on a per crab basis (Fig. 2.2d). The crabs exposed to normoxia grew faster than those exposed to different hypoxic levels, and their overall feeding rate was higher though the rate per unit body weight was the same for 155 and 119 Torr O\textsubscript{2} (Fig. 2.2c). Repeated measure analysis of the weekly feeding rate showed a significant linear decline over time (Fig. 2.3b).

Significant differences occurred in the growth rate of *Callinectes similis* exposed to normoxia (155 Torr) and those exposed to different levels of hypoxia (Table 2.1). Molting rate in the crabs decreased linearly with the severity of hypoxia with the only exception of 119 Torr (Fig. 2.4b). The intermolt period in the crabs exposed to normoxia was shorter than that of crabs exposed to hypoxia (Table 2.1).

**Response of *Stramonita haemastoma***:

*Stramonita haemastoma* was very resistant to hypoxia with a 28 day LC\textsubscript{50} of 11.5 Torr O\textsubscript{2} (Fig. 2.1). 100% mortality occurred only under total anoxia. The first death occurred after day 11, and all the oyster drills exposed to anoxia were dead by day 22.

Feeding rates in all oyster drills exposed to hypoxic water were significantly different from those exposed to normoxia. Feeding rate of *Stramonita haemastoma* exposed to 119 and 73 Torr O\textsubscript{2} were not significantly different from each other (Tukey's range test) but were significantly different from the drills exposed to 50 and 25 Torr O\textsubscript{2} (Fig. 2.5a). The lowest feeding rate was recorded in the oyster drills exposed to anoxia. Repeated measure analysis of feeding rate by week showed a strong interaction with time within each level (prob. < .01; Fig. 2.5b).

Scope for growth in the blue crabs exposed to the two higher levels of hypoxia was not much different from the normoxic scope for growth but, it declined linearly in the
Table 2.1. Growth and molting phenomena in *Callinectes sapidus* and *C. similis* exposed to 28 days of hypoxia at 30°/∞ S and 24°C. Crabs exposed to 0 Torr O₂ died within a few days and did not molt or grow. 16 crabs were used at each PO₂ for each species. SAP = *Callinectes sapidus*, SIM = *Callinectes similis*.

<table>
<thead>
<tr>
<th>Torr of O₂</th>
<th>155</th>
<th>115</th>
<th>73</th>
<th>50</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAP</td>
<td>SIM</td>
<td>SAP</td>
<td>SIM</td>
<td>SAP</td>
</tr>
<tr>
<td>Average</td>
<td>686</td>
<td>735</td>
<td>713</td>
<td>691</td>
<td>651</td>
</tr>
<tr>
<td>initial</td>
<td>±43</td>
<td>±71</td>
<td>±58</td>
<td>±68</td>
<td>±36</td>
</tr>
<tr>
<td>wet wt (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Increase in growth</td>
<td>501</td>
<td>186</td>
<td>305</td>
<td>113</td>
<td>246</td>
</tr>
<tr>
<td>Intermolt period (days)</td>
<td>12.4</td>
<td>13.6</td>
<td>14</td>
<td>7.8</td>
<td>14.5</td>
</tr>
<tr>
<td>Crabs molted 3 times</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Crabs molted twice</td>
<td>13</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Crabs molted once</td>
<td>1</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total molts</td>
<td>33</td>
<td>18</td>
<td>16</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>
Figure 2.5 *Stramonita haemastoma.* (a) Average feeding rate (±SE) per unit dry body weight as a function of hypoxia. Letters represent Tukey's range test for differences between treatment means. Means with the same letter are not significantly different from each other. (b) Mean weekly feeding rate (±SE) as a function of hypoxia.
oyster drills with the severity of hypoxia from 92% in 119 Torr O₂ to 64% in 25 Torr O₂ (Table 2.2).

Response to direct transfer from constant hypoxic exposure to normoxia

Feeding rates in the crabs exposed to various levels of hypoxia were not significantly different from those exposed to normoxia during the pre-transfer period. Feeding rate of blue crabs transferred directly from various hypoxic levels to normoxia increased significantly (P < .01) during the post-transfer period compared to their feeding rate when exposed to hypoxia (pre-transfer period, Fig. 2.6a). During the pre-transfer period molting rate was faster in the crabs exposed to normoxia compared to those exposed to various hypoxic levels. But, during the post-transfer period molting rate was much faster in the crabs previously exposed to hypoxia than in those exposed to normoxia (Fig. 2.6b).

Response of Callinectes sapidus to starvation

After a period of 10 days starvation, feeding rate of the blue crabs increased sharply above the pre-starvation feeding rate. The mean increase over the pre-starvation feeding rate was 78 ± 7 mg wet food. gm wet body wt.⁻¹ day⁻¹ which equals to 123% increase and was found to be highly significant (student's t-test, P < .01). This huge increase is attributable to the first two days of the 10 day post-starvation period (Fig. 2.6c). However, this elevated feeding rate of the blue crabs during the 10 days post-starvation period accounted for only 31% of the calculated deficit incurred during the starvation period.
### Table 2.2. Scope for growth of \textit{Callinectes sapidus}, and \textit{Stramonita haemastoma}, under normoxic, different levels of hypoxic, and anoxic conditions. All energy budgets are given in Kilo Joules. gm dry body wt.\textsuperscript{1} day\textsuperscript{-1}

<table>
<thead>
<tr>
<th>Oxygen Concentration (Torr)</th>
<th>N</th>
<th>Energy Consumed (C)</th>
<th>% of normoxic</th>
<th>Energy Absorbed (Ab)</th>
<th>Energy Lost (R)</th>
<th>% of normoxic</th>
<th>Scope for growth (P)</th>
<th>% of normoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Callinectes sapidus}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>16</td>
<td>6.15</td>
<td>100</td>
<td>2.74</td>
<td>0.44</td>
<td>100</td>
<td>2.3</td>
<td>100</td>
</tr>
<tr>
<td>119</td>
<td>2</td>
<td>6.5</td>
<td>106</td>
<td>2.89</td>
<td>0.35</td>
<td>80</td>
<td>2.54</td>
<td>110</td>
</tr>
<tr>
<td>73</td>
<td>6</td>
<td>6.2</td>
<td>101</td>
<td>2.76</td>
<td>0.23</td>
<td>52</td>
<td>2.53</td>
<td>110</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>4.53</td>
<td>74</td>
<td>2.02</td>
<td>0.17</td>
<td>39</td>
<td>1.9</td>
<td>83</td>
</tr>
<tr>
<td>\textit{Stramonita haemastoma}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>12</td>
<td>1.19</td>
<td>100</td>
<td>0.97</td>
<td>0.21</td>
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<td>1.07</td>
<td>90</td>
<td>0.87</td>
<td>0.17</td>
<td>81</td>
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</tr>
<tr>
<td>73</td>
<td>12</td>
<td>1.04</td>
<td>88</td>
<td>0.85</td>
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<td>52</td>
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<td>97</td>
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<tr>
<td>50</td>
<td>12</td>
<td>0.89</td>
<td>75</td>
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<td>0.08</td>
<td>38</td>
<td>0.64</td>
<td>84</td>
</tr>
<tr>
<td>25</td>
<td>12</td>
<td>0.67</td>
<td>56</td>
<td>0.54</td>
<td>0.05</td>
<td>24</td>
<td>0.49</td>
<td>64</td>
</tr>
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</table>
**Figure 2.6** *Callinectes sapidus*. (a) Increase in feeding rate during the post-transfer period in the transfer experiment when maintained at 3 levels of hypoxia for 10 d and then transferred to normoxia for 10 d. Increase is shown by the mean difference (±SE) in feeding rate before and after transfer to normoxia and plotted on the Y-axis against various oxygen concentrations on the X-axis. Letters represent Tukey’s range test. Means with the same letter are not significantly different from each other. (b) Molting rate during the transfer experiment. Dotted bars represent the pre-transfer period and hatched bars represent the post-transfer period. (c) Mean daily feeding rate during the starvation experiment. A clear and sharp increase in the feeding rate can be noticed after day 15 during the post-starvation period.
Oxygen consumption in Callinectes sapidus and Callinectes similis

Respiration was measured on day 0 upon the direct transfer from normoxic water to various levels of hypoxic water to obtain the acute response. Respiration was also measured on crabs exposed to various levels of hypoxia for days 1, 7, 14, 21, and 28. A significant (P < .001) difference in the weight specific oxygen consumption rate between the two species of crabs was observed with increasing duration of hypoxic exposure. The acute response of Callinectes sapidus on day 0 was a lower rate of oxygen consumption at 25 and 50 Torr O₂ compared to the normoxic consumption and a relatively higher rate at 115 Torr (Fig. 2.7a). The increase continued at 50 and 115 Torr on day 1 and the 25 Torr remained at the same level. Oxygen consumption remained depressed or the same as the normoxic crabs during the rest of the hypoxic exposure at all the hypoxic levels. Oxygen consumption in C. sapidus varied between days within a level and between levels within a day. A significant (P < .001) day by level interaction was found to exist. Blue crabs exposed to 25 and 50 Torr O₂ exhibited a lower oxygen consumption rate than the crabs exposed to normoxia (Fig. 2.7).

The ratio of mean oxygen consumption in normoxic crabs over the period of 28 days to mean oxygen consumption within a hypoxic level over 28 days was used as an index to compare the integrated rate of oxygen consumption in hypoxic crabs to the normoxic ones. Values > 1 indicate a higher level of oxygen consumption and values < 1 indicate a possible oxygen debt (Fig. 2.7c). Mean oxygen consumption in C. sapidus exposed to various hypoxic levels was less than the crabs exposed to normoxic water. The acute response of C. similis was similar to C. sapidus on day 0 but, the oxygen consumption rate in C. similis exposed to hypoxia remained elevated for the rest of the 28 day period. Significant (P < .01) differences in the oxygen consumption rate between levels as well as a significant day by level interaction was found in both C. sapidus and
Figure 2.7  Mean daily respiration (±SE) at various levels of hypoxia of (a) Callinectes sapidus and (b) C. similis. (c) Ratio of mean hypoxic oxygen consumption of the 2 crab species within a level over 28 d to mean normoxic oxygen consumption over 28 d plotted against various oxygen concentrations. Normoxic oxygen consumption rate was fixed at 1 and is represented by the heavy line. A ratio of greater than 1 indicates a higher level of oxygen consumption compared to the normoxic consumption and a ratio of less than 1 indicates a possible oxygen debt.
(a) Callinectes sapidus

(b) Callinectes similis

(c) Ratio of Callinectes similis to Callinectes sapidus
C. similis (Fig. 2.7b). Mean oxygen consumption over 28 days in all hypoxic levels was > 1 compared to the normoxic consumption in C. similis. Three way factorial analysis showed significant differences (P < .001) in the oxygen consumption rate of the two species of crabs. All the lesser blue crabs exposed to hypoxia consumed more oxygen on an average compared to the blue crabs exposed to the same levels of hypoxia.

**DISCUSSION**

*Callinectes sapidus* was very sensitive to hypoxia with a 28 day LC₅₀ of 106 Torr O₂. *C. similis* was able to tolerate moderate hypoxia with a 28 day LC₅₀ of 43 Torr O₂, and *Stramonita haemastoma* was very resistant to hypoxia with a 28 day LC₅₀ of 11.5 Torr O₂. Stickle *et al.* (1989) found the 28 day LC₅₀ value for *C. sapidus* to be 111 Torr and for *S. haemastoma* to be 19 Torr O₂. Kapper and Stickle (1987) also reported *S. haemastoma* to be extremely tolerant of mild hypoxia. From the above data it seems clear that juvenile *C. similis* can tolerate hypoxia better than juvenile *C. sapidus*. These two species, especially the juvenile stages, compete for food and other resources most often (Engel 1977). DeFur *et al.* (1990) qualitatively demonstrated only 20% death of adult *C. sapidus* at moderate (50 Torr) hypoxia during 25 days exposure. Findings of this study are different for the juvenile blue crabs. 81% mortality was observed at 50 Torr over the 28 day period. All the crabs maintained at 25 and zero Torr O₂ died within a short period. This may be due to the higher oxygen demand per unit body mass in the juvenile blue crabs.

The feeding rate of neither *Callinectes sapidus* nor *C. similis* varied directly with the severity of hypoxia. Feeding rate of *C. sapidus* exposed to 50 Torr O₂ was 74% of the normoxic feeding rate. Feeding rate of *C. similis* exposed to 50 and 25 Torr O₂ was 84 and 82% of the normoxic feeding rate respectively. Metabolic rates of both species of
crabs are not depressed in a comparable manner to that of the oyster drills and thus their metabolic demand for O$_2$, when exposed to moderate hypoxia, remains close to the normoxic level and they die quickly when exposed to anoxia (Stickel et al. 1989). Blue crabs are not very good anaerobes (Carpenter and Cargo 1957) and they are more dependent on their O$_2$ transport system than most other groups of animals. Limited anaerobic metabolism exists in crustaceans involving pathways alternative to classical glycolysis with the production of L-lactate during exuviation and brief exposure to hypoxia (Fields 1985 and Mangum et al. 1985).

Weekly weight specific feeding rate of both species of crabs exhibited a steady decline over time with a steeper decline in C. similis. Since this effect was consistent for hypoxic as well as normoxic crabs, the decline in feeding rate may not be due to hypoxic exposure, rather it may be due to increased body size or prolonged captivity. But the feeding rate per unit body weight was always lower in crabs exposed to hypoxia than those exposed to normoxia.

The importance of glucose in metabolic processes has been widely recognized. Because aerobic metabolic rate decreases in hypoxia-sensitive cells under oxygen limiting conditions, the demand for glucose for anaerobic glycolysis may rise dramatically to compensate for the energetic shortfall (Hochachka 1986). Glucose concentration in the blood depends on the rate of metabolism. The main source of metabolic energy in the crustaceans are glucose derivatives (Poolsangunan and Uglow 1974, Ramos and Fernandez 1981). Oxygen consumed by crustaceans is used as the last acceptor of electrons in the respiratory process which usually begins with the degradation of carbohydrates (Rosas et al. 1992). Thus the feeding rate seems to be intimately related to the oxygen consumption. I found in both species of crabs the metabolic rate remains at a very high level when exposed to various levels of hypoxia to cater to the greater need
for glucose in the hypoxia sensitive cells. This creates a higher oxygen demand when the ambient oxygen tension is low.

Molting rate in both species of crabs was considerably higher for crabs exposed to normoxia (155 Torr O₂) than those exposed to hypoxia though the overall molting rate of *Callinectes similis* was much lower than *C. sapidus*. Frequent mortality while molting was observed in *C. sapidus* exposed to hypoxia. This was not observed in *C. similis*. Mangum *et al.* (1985) showed that the hemocyanin in the hemolymph of a blue crab transports more than 95% of its O₂ requirements and almost none is carried in the free form. They also reported that this system does not meet respiratory demand during molting when the metabolic demand is greater than usual. Due to increased uptake of water during molting, the hemocyanin in the blood is diluted to about one-fifth of the intermolt concentration which in turn reduces the O₂ carrying capacity of the blood. This reduction in oxygen delivery is compounded by hypoxia and results in increased mortality in *C. sapidus* exposed to hypoxia while molting. In *C. similis* we failed to observe death during molting which probably suggests either an evolutionary adaptation to lengthen the intermolt period when exposed to hypoxia, possibly to avoid extra stress during an already existing environmental stressor, or a slower growth rate compared to *C. sapidus*. Glucose is necessary in the chitin synthesis upon molting (Rosas *et al.* 1992). Increasing the intermolt period and thus reducing the molting frequency is indeed an optimal adaptive strategy to reduce stress and thus the need for more glucose for chitin synthesis in *C. similis*. Because of this high demand for glucose, feeding rate did not decline directly with the severity of hypoxia in *C. sapidus* and the rate of oxygen consumption remained quite high.

Feeding rate in the oyster drills *Stramonita haemastoma* varied directly with the severity of hypoxia. Suppression of the metabolic rate of *S. haemastoma* at low oxygen tension may be responsible for the linear decline in their feeding rate with declining
oxygen tension of the water (Stickle et al. 1989). The heat dissipation rate in *S. haemastoma* has been reported to be very low, only 9% of normoxia, when exposed to anoxia and oyster drills exhibit an oxygen debt upon return to normoxic water (Stickle et al. 1989). Metabolic rate depression in mollusks is possible because, instead of the classical and less efficient glycolysis system, they switch to relatively more efficient succinate and propionate pathways during exposure to hypoxia and anoxia (Gade 1983, Gnaiger 1983a, 1987, deZwaan and Thillart 1985).

Weekly food consumption of the oyster drills within each treatment level showed a strong interaction with time. Oyster drills exposed to various treatment levels exhibited an increase in food consumption during the second week of the experiment (oyster drills exposed to 50 Torr O₂ also exhibited increase during the third week); the rate of food consumption then gradually declined over time in all levels. The initial shock of transfer from normoxia to hypoxia was probably responsible for the very low feeding rate during the first week after which they became acclimated and consumed more food. But with prolonged exposure their feeding rate could not be maintained and the rate of food consumption declined.

An estimate of the scope for growth under normoxia and different levels of hypoxia and anoxia in *Callinectes sapidus* and *Stramonita haemastoma* shows no significant difference in the scope for growth at the two higher levels of hypoxia in *C. sapidus*. But in *S. haemastoma* a linear decline in the scope for growth occurs with the severity of hypoxia. *S. haemastoma* has a lower feeding and metabolic rate that results in a lower scope for growth which appears to be an adaptive advantage. When oyster drills are exposed to lower levels of dissolved oxygen in the water they restrict their metabolic demands for maintenance. As is true with exposure of marine invertebrate carnivores to other environmental stressor gradients (Stickle 1985), variation in energy consumption is much greater than variation in metabolic rate; 6:1 in juvenile *C. sapidus* and 3.3:1 in
S. haemastoma. After a lower threshold limit of metabolic rate is reached in S. haemastoma at intermediate water temperature (24°C), this metabolic rate reduction becomes futile as when oyster drills were exposed to anoxic water.

Crustaceans compensate for short term hypoxia by increasing ventilation (Batterton and Cameron 1978, Taylor 1982, Pease and DeFur 1987), increased extraction efficiency (Hagerman and Uglow 1985), and/or oxygen affinity of the hemocyanin (Hagerman and Uglow 1985, DeFur et al. 1990). An increase in oxygen consumption was observed at all hypoxic levels in Callinectes similis at least by day 1 compared to the normoxic crabs. Oxygen consumption remained at this elevated rate for the rest of the 28 day hypoxic exposure, which demonstrates a clear over compensation in this species. In C. sapidus, oxygen consumption in the hypoxic crabs varied within a narrow limit around the normoxic consumption showing a partial compensatory acclimation over the 28 days hypoxic exposure. The increased oxygen consumption in C. similis may be due to both increased ventilation and an increased extraction efficiency. Sanchez et al. (1991) demonstrated higher extraction efficiency in C. similis per unit of metabolically active biomass among six different crustacean species studied.

In decapod crustaceans, hemocyanin in the blood is responsible for a very large fraction of the oxygen supplied to metabolizing tissue. The transport mechanism is perturbed during and immediately following a molt (Mangum et al. 1985). During the pre-molt period a decrease in blood calcium, which acts as an allosteric effector may increase oxygen affinity of hemocyanin, that accompanies hardening of the new shell lowers the hemocyanin oxygen affinity (Mangum et al. 1985). Thus an increased supply of oxygen is necessary at this stage of the molt cycle. In our experiment under hypoxic exposure, oxygen consumption in Callinectes sapidus showed no increase with extreme of hypoxia. Average oxygen consumption was lower than normoxic consumption, but the feeding rate remained unchanged. Thus, the metabolic demand during molting was higher and the
supply of oxygen was low. This probably was the main cause of death during molting in *C. sapidus*. In *C. similis* the opposite is true. They maintained a higher rate of oxygen consumption throughout the hypoxic exposure and the frequency of molting was less compared to *C. sapidus*. The extra amount of oxygen was probably used for metabolic maintenance, increasing the likelihood of survival.

In the direct transfer experiment no significant difference in the rate of food consumption existed with hypoxia over 10 days though the hypoxic crabs always exhibited lower food consumption rates compared with the crabs exposed to normoxia. But when the crabs were transferred back to normoxia (post-transfer period), the food consumption rate of the crabs previously exposed to hypoxia increased sharply. It is probable that the rate of adaptation from hypoxia to normoxia is much faster than from normoxia to hypoxia since the feeding adaptations in the blue crabs associated with hypoxic exposure seem to be very minor. The starvation experiment created a similar type of response as was noticed after the transfer of the crabs from hypoxic to normoxic water in the transfer experiment. The food consumption rate increased very sharply and stayed at that elevated level.

Tolerance to hypoxia is possibly also related to the developmental history of the blue crab. Complete acclimation to hypoxia has not been reported for either of the two species in the laboratory. But partial acclimation to frequently occurring patchy hypoxia in the Gulf of Mexico may be possible. Occurrence of widespread hypoxia is a regular phenomenon in the northern Gulf of Mexico during the summer months. Crustaceans can avoid hypoxia by swimming away from deeper to shallower water (Loesch 1960, May 1973, Pavela *et al.* 1983, Renaud 1986b and Pihl *et al.* 1991), but some exposure to hypoxia may be unavoidable when the hypoxic water mass is very wide spread. It is reasonable that some acclimation occurs in blue crabs and the lesser blue crabs to these frequently occurring events which probably helps them to resist short term hypoxia.
Depending on the acclimation history of the crab population, hypoxia tolerance will vary and so will other responses related to it.
CHAPTER 3

DETECTION AND AVOIDANCE OF HYPOXIC WATER BY JUVENILE
CALLINECTES SAPIDUS AND C. SIMILIS
INTRODUCTION


The effect of hypoxia on the movement patterns of the blue crab, Callinectes sapidus Rathbun and the lesser blue crab, C. similis Williams, to and from the estuarine region is unknown. Mature female C. sapidus hatch their eggs in high salinity water (Millikin and Williams 1984). Egg-bearing female C. sapidus may occur and spawn in the coastal Gulf and estuarine waters year-round (Gunter 1950, Daugherty 1952, More 1969.
Adkins 1972a, Perry 1975). Egg-bearing *C. similis* also spawn in high salinity waters (Hsueh *et al.* in press). Stuck and Perry (1981) reported a year-round abundance of *C. similis* magalopae in the waters of Mississippi Sound with a peak during February and March. Thus, an extensive occurrence of hypoxia in nearshore and offshore water could cause a potential problem for the spawning female population of blue crabs as well as the larval and juvenile stages. Low dissolved oxygen may also act as a barrier to the migration of juvenile blue crabs from high salinity to low salinity waters (Van Engel 1982). Juvenile blue crabs are estuarine-dependent and regularly utilize the marsh creek habitat (Orth and van Montfrans 1987, Williams *et al.* 1990, van Montfrans *et al.* 1991, McClintock *et al.* 1993). Diurnal variation in the dissolved oxygen tension of water in the marshes of the Louisiana Gulf coast is frequent, with water becoming almost anoxic during the early morning period in summer (Das and Stickle, unpublished observation). We think short duration of hypoxic exposure may also stress the blue crabs and the lesser blue crabs and may be equally important to their distribution and survival.

The objectives of this study are (i) to determine whether juvenile *C. sapidus* and *C. similis* can detect and avoid specific levels of dissolved oxygen under laboratory conditions, (ii) to observe the behavioral responses associated with short term hypoxic exposure and (iii) to consider how the behavioral responses may affect field populations of the two species of blue crabs.

**MATERIALS AND METHODS**

*Collection and maintenance*

*Callinectes sapidus* and *C. similis* were collected nearshore at Port Fourchon, Louisiana (90°11.4'W, 29°6.7'N) between June and September, 1992, when the water temperature was 28 - 30°C and the salinity was 28 °/oo. The animals were brought back
to the laboratory and placed into artificial sea water made from Instant Ocean® at room temperature (23°C) and salinity matching field conditions. All the crabs were kept under constant illumination and acclimated to the laboratory conditions for two weeks before being used in the experiments. Crabs were individually isolated in chambers made from a pair of PVC pipe sections that fit together (one piece with 21 cm and the other piece with 23 cm inside diameter and both 28 cm in length) with nylon meshed screens on the free end of each piece to prevent cannibalism. Animals were chosen to minimize weight and size differences within and among the treatments (average weight 750 - 850 mg; average carapace width 20 - 25 mm). Crabs were fed frozen abdominal portions of the grass shrimp *Palaemonetes pugio*. Avoidance was not analyzed with respect to sex because it is difficult to sex the juvenile blue crabs unequivocally.

**Experimental design and procedure**

The experiment was conducted in an avoidance apparatus modified from Renaud (1986b; Fig. 3.1), consisting of a glass chamber 100 cm long and 10 cm in diameter marked at 1 cm intervals. A raised floor of transparent white plastic mesh was placed across the center of the chamber to facilitate movement of the crabs within the tube. There were two water inlets, one at each end of the tube, and a centrally located outlet at the top of the chamber. Two animal entry ports at each end of the chamber near the inlet tubes, allowed introduction of the crabs into the chamber. The inlets were connected to both the control and experimental water tanks and a gravity flow system controlled water flow into the chamber. Water flow from either water source could be directed to either end of the chamber with in-line valves. Two flow meters, one on each side, were installed before the inlets were connected to the chamber to maintain a constant flow rate. Water from the outlet was controlled by a valve and thus required flow rates could be achieved by altering the outflow. The outlet opened into a bio-filter from
**Figure 3.1** Schematic diagram of the avoidance behavior apparatus and the experimental setup. Figures are not drawn according to scale.
To Gas Mixer

Intermediate Saturation Tank (Hypoxic Water)

Hypoxic Water Tank

Inlet Valves

Flow Meter

Animal Entry Port

Test Chamber

Outlet Valve

Sampling Ports

Biofilter

Air

Hypoxic Water Tank

Control Water Tank
which water was pumped back to the control and experimental tanks. There were nine sampling ports located along the length of the apparatus for monitoring dissolved oxygen tension. Water samples from the sampling ports were drawn anaerobically and injected into a Strathkelvin oxygen meter (model 781) connected to a flow-through water jacketed oxygen electrode.

Target oxygen tensions of 90, 75, 50, 25, and 0 Torr (60%, 50%, 35%, 16%, and 0% of air saturation or 4.2, 3.5, 2.3, 1.2, and 0 ppm of dissolved oxygen) were created and maintained by mixing bottled nitrogen and oxygen with a Matheson gas mixer (model 7402T) along with 0.03% carbon dioxide to maintain the pH at approximately 7.8. A total of 76 crabs (41 Callinectes sapidus and 35 C. similis) of both species were tested at various PO$_2$s. Because of technical difficulties, only C. sapidus was exposed to the 75 Torr oxygen tension. Each crab was used only once in an experiment.

Crabs were initially introduced from one end of the avoidance chamber and allowed to acclimate inside the chamber for one hour while normoxic water (155 Torr at 23°C and 30 °/o S) flowed through the chamber from both ends. Entry of the crabs into the chamber, either through the right or left animal entry port, was switched for each experiment to avoid any possibility of starting preference. Following one hour of acclimation, crabs were allowed to stay inside the chamber for another 30 min, which served as a control period. The position of the crabs within the chamber during the control period was noted every 3 min. Upon completion of the control period, treatment water of desired dissolved oxygen tension was introduced into the end of the chamber nearest to the crab while normoxic water flow was maintained from the other end of the chamber. This created an oxygen gradient inside the chamber so that the side of hypoxic water entry was most hypoxic and oxygen tension gradually increased towards the middle of the chamber, while the other side was completely normoxic. The middle region of the chamber (50 cm mark) served as a hypothetical boundary line between the hypoxic
and normoxic (control) zone. This boundary line was not very sharply demarcated and there was always some mixing of hypoxic and normoxic water in this region. The oxygen gradient was measured for each experiment. The test period continued for 60 min and the position of the crab within the chamber was noted every minute along with their behavioral responses. Behavior was observed through slits in curtain to prevent outside movements and shadows from influencing crab behavior. Laminar water flow, as determined with dye tests, of 450 ± 50 ml/min was maintained by the inline valves. After every experiment the chamber was flushed with normoxic water.

The following response variables were measured for each crab:

1) **Total Time** - The amount of time spent by each crab at the hypoxic end of the chamber during the final 50 min of each 60 min treatment. The first 10 min of each experiment were allowed to establish a stable oxygen gradient inside the chamber. Movement of crabs inside the chamber was monitored constantly to measure the amount of time spent by each crab at the hypoxic end of the chamber.

2) **Response Time** - The time in minutes before a crab's initial entry into the control (normoxic) side of the chamber after the introduction of hypoxic water.

3) **Location** - Location was scaled from 0 to 100, based on the side of hypoxic water entry being 0, for the position of each crab within the chamber.

4) **Activity Index** - Activity index was calculated based on the total distance (cm) travelled by a crab during the final 50 min of the 60 min experimental period. This index was calculated for each crab within each treatment and the mean activities at various oxygen tensions were compared.

Tests for significant differences between treatments for the above four response variables were determined by either one-way or two-way analysis of variance (ANOVA; SAS Inst., Inc. 1989) and split plot design. Levene's test was used to determine the homogeneity of variances within treatments. All analysis were performed on both
untransformed and log transformed data. Differences among treatment means were determined by Waller-Duncan multiple range test.

5) Preferred Oxygen Tension - Oxygen tension most preferred by a crab or the oxygen tension at which a crab spent most of its time was measured. Movement of crabs was monitored constantly to measure the amount of time spent by each crab at the hypoxic or normoxic (control) side of the chamber. Actual oxygen tensions were measured from the 9 sampling ports located along the length of the avoidance chamber for each experiment and a predicted oxygen value was generated from the actual gradient for each location of a crab used in a particular experiment. The actual oxygen gradient in the chamber followed a sigmoidal pattern (See results). The sigmoidal function was made linear using a natural log transformation:

\[ Y_i = \ln \left( \frac{\text{oxy} + 1}{155} \right) \]

where, \( \text{oxy} \) = actual oxygen tension measurement from a particular sampling port, and 155 is the asymptotic normoxic oxygen tension at 23°C temperature and 30 °/oo S. A value of 1 was added to each oxygen value because the natural log of "0" does not exist.

A simple linear regression was then run between these transformed oxygen values \( Y_i \) and the actual locations obtained for each crab during the final 50 min of a 60 min experimental period using PROC REG (SAS Inst., Inc., 1989) to obtain predicted oxygen values for each location of a crab. The linear regression model used is as follows:

\[ Y_i = \alpha + \beta \cdot \text{location}_i + \epsilon_i \]

where, \( Y_i \) = transformed oxygen tension, \( \alpha \) = estimated intercept, \( \beta \) = estimated slope, Location\( i \) = observed location in cm for each crab, and \( \epsilon_i \) = random error term for each crab. The average \( R^2 \) value, which indicates the proportion of variability observed in \( Y_i \) that is explained by the simple linear regression model, was 0.8847 (n = 76) indicating a good fit of the data to the linear model. The natural log of these transformed values were then back transformed into actual oxygen values. A mean preferred oxygen value,
the oxygen tension at which a crab spent most of its time, was calculated for each crab from these transformed oxygen values.

Percent avoidance of the hypoxic side was also used to calculate avoidance for each crab within each treatment. Percent avoidance was calculated based on a crab’s position within the avoidance chamber with respect to the 50 cm mark, *i.e.* hypoxic side or normoxic (control) side. Significant percent avoidance was determined with analysis of variance.

Our null hypothesis was that blue crabs cannot detect hypoxia, and will spend about the same amount of time at the control and treatment side of the chamber based on the assumption that there is no side preference. The alternative hypothesis was that blue crabs can detect and avoid hypoxia and will spend more time at the control side of the chamber through avoidance of hypoxic water.

**RESULTS**

The average positions of *Callinectes sapidus* and *C. similis* were equally distributed between the left and right side of the chamber during the control periods, indicating that there was no preference for either side (*P* < 0.7). Since a crab was capable of travelling the entire distance within the chamber several times in one minute, the position of a crab within the chamber at a particular instant was not dependent on its previous position. Therefore, crab behavior during the treatments was only affected by changes in dissolved oxygen concentration. Fig. 3.2 shows the typical avoidance and non-avoidance responses of two crabs within the chamber during two different experiments, one at a low *P*O$_2$ (0 Torr, Fig. 3.2a) and another at a higher *P*O$_2$ (90 Torr, Fig. 3.2b).

When average crab position, treatment versus control side of the chamber, was used to determine avoidance, *Callinectes similis* was found to detect and avoid hypoxia
Figure 3.2  (a) A typical avoidance response of *Callinectes similis* when the dissolved oxygen level was very low. The crab spent most of its time at the normoxic end of the chamber, (b) A typical non-avoidance response of *C. sapidus* when the dissolved oxygen level was higher. The crab movement has no detectable pattern and is fairly random.
at zero (72 ± 2.2 percent avoidance) and 25 Torr oxygen (70 ± 4.4 percent avoidance) tension (P < 0.004). No significant avoidance was detected at 50 Torr (57 ± 5.1 percent avoidance) and 90 Torr (52 ± 3.9 percent avoidance) oxygen tension in C. similis. Avoidance was not detected in any treatment (62 ± 2.3 percent avoidance at 0 Torr; 49 ± 7.3 percent avoidance at 25 Torr; 60 ± 6.3 percent avoidance at 50 Torr; 52 ± 6.4 percent avoidance at 75 Torr; and 46 ± 7.5 percent avoidance at 90 Torr) in C. sapidus.

A linear decline in response time with increasing oxygen tension was observed in C. similis (Fig. 3.3). Response time for C. similis exposed to 0 and 25 Torr oxygen was significantly (P < 0.05) longer than the response time of crabs exposed to 50 and 90 Torr oxygen (Waller-Duncan multiple range test). No definite trend was noticed in C. sapidus (Fig. 3.3). Response time of C. sapidus exposed to 75 Torr oxygen tension was shorter than all other treatments. This sudden drop can not be explained.

A significant (P < 0.001) linear increase in total time, the amount of time spent at the hypoxic end, with increasing oxygen tension was observed in C. similis (Fig. 3.4). The amount of time spent at the hypoxic end by C. similis exposed to 0, 25, and 50 Torr oxygen tension was significantly less than the ones exposed to 90 Torr oxygen tension (Waller-Duncan multiple range test). No significant trend was noticed in the amount of time spent at the hypoxic end by C. sapidus (Fig. 3.4).

Both species of crabs were found to be more active at the higher oxygen tensions (75 and 90 Torr) and were significantly different (P < 0.005) from the more hypoxic levels (0, 25, and 50 Torr) where the crabs were found to be much less active (Waller-Duncan multiple range test; Fig. 3.5).

The preferred oxygen tension for both species increased with increasing oxygen tension (Table 3.6). The mean oxygen gradient at each treatment and the preferred oxygen level of the two species of crabs at those treatments are plotted in Fig. 3.2. Mean preferred oxygen tension for C. similis ranged from 82 - 121 Torr with a mean of 108 Torr.
Figure 3.3 *Callinectes sapidus* and *C. similis*. Response time (±SE), the time in minutes before a crab's initial entry into the control side of the chamber after the introduction of hypoxic water plotted against various levels of oxygen tension. Letter's represent Waller-Duncan multiple range test for differences between treatment means.
Figure 3.4  *Callinectes sapidus* and *C. similis*. Total time (±SE), the amount of time spent by each crab at the treatment side of the chamber plotted against various levels of oxygen tension. Letter's represent Waller-duncan multiple range test for differences between treatment means.
Figure 3.5  *Callinectes sapidus* and *C. similis*. Activity (±SE), an index of the distance (cm) travelled by a crab during the final 50 minutes of a 60 minute experimental period, plotted against various levels of oxygen tension. Letter's represent Waller-Duncan multiple range test for differences between treatment means.
oxygen. Mean preferred oxygen tension for *C. sapidus* ranged from 98 - 125 Torr with a mean of 112 Torr oxygen.

Noted behavioral responses of both crab species during the treatments were: (1) an initial increase in their activity with the introduction of hypoxic water into the chamber. This initial burst in activity was more prominent in *C. sapidus* compared to *C. similis*, (2) frequent movement of the eye-stalk, (3) avoidance of hypoxic zone by slowly crawling out of the area (when avoidance was present), (4) restless and erratic movements showing clear signs of escape behavior, (5) rapid movement of their antennae. These behaviors were a clear sign of stress due to low oxygen tension in the water. These behaviors were not present at the 90 Torr oxygen tension in either species.

**DISCUSSION**

We have used different response variables in this study to analyze avoidance. Justification for using these variables is to avoid the limitation imposed on the experiment by a continuous oxygen gradient. We found that none of the three variables, location, response time, and total time can be used as a robust measure of avoidance because of the nature of the oxygen gradient within the chamber as discussed below.

Calculation of the average crab position (percent avoidance), treatment versus control side of the chamber showed clear avoidance of hypoxic water by *Callinectes similis* at 0 and 25 Torr oxygen tension. Significant avoidance was not detected at any other levels by either species. Renaud (1986b) reported significant avoidance of hypoxic water by *Penaeus aztecus* and *P. setiferus* when the dissolved oxygen concentration was ≤1.5 ppm (~ 32 Torr at 23°C and 30 °/oo S). Petersen and Petersen (1990) reported a linear relationship between avoidance and oxygen concentration in the sand goby, *Pomatoschistus minutus* with significant avoidance at 30% oxygen saturation (~ 46 Torr
Figure 3.6  The mean oxygen tension recorded from the nine sampling ports for each treatment are plotted against location of those sampling ports. Mean preferred oxygen tensions, oxygen tension most preferred by a crab or the oxygen tension at which a crab spent most of its time, are superimposed on the oxygen gradient to show the oxygen preference of crabs at various treatments. Mean preferred oxygen tension for *C. similis* is 108 Torr and for *C. sapidus* 112 Torr oxygen.
OXYGEN TENSION (Torr)

TREATMENT = 0 TORR

TREATMENT = 25 TORR

TREATMENT = 50 TORR

TREATMENT = 75 TORR

TREATMENT = 90 TORR

\[ \triangle = \text{C. sapidus} \]

\[ \bigcirc = \text{C. similis} \]
Table 3.1. Mean preferred oxygen tension of *Callinectes sapidus* and *C. similis* at various treatment levels. S.E. = Standard error.

<table>
<thead>
<tr>
<th>Oxygen Tension (Torr)</th>
<th>Preferred oxygen (Torr)</th>
<th>C. sapidus</th>
<th>S.E.</th>
<th>C. similis</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>98.16</td>
<td>2.41</td>
<td>82.32</td>
<td>2.87</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>100.40</td>
<td>2.46</td>
<td>116.30</td>
<td>1.92</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>121.95</td>
<td>1.76</td>
<td>111.48</td>
<td>2.20</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>116.21</td>
<td>1.24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>125.58</td>
<td>1.27</td>
<td>120.82</td>
<td>1.05</td>
</tr>
</tbody>
</table>
at 23°C and 30 °/oo S). Analysis of total time, amount of time spent by each crab at the hypoxic end, however revealed significant avoidance by *C. similis* at 0, 25, and 50 Torr oxygen tension. When the total time was calculated for *C. sapidus* no significant avoidance was detected. We conclude that total time is a more sensitive measurement of hypoxia avoidance since it gives an exact estimation of time spent by each crab at the hypoxic end (time spent by each crab at the hypoxic and normoxic end for each experiment was monitored constantly) in comparison with location. Location of each crab within the chamber was noted every minute during each experiment but a crab was capable of travelling the entire length of the chamber several times within one minute. Thus the position of a crab between two successive measurements remained undefined whereas the total time gave a complete measurement of the amount of time spent at the hypoxic end of the chamber during an experiment.

Measurement of response time, the time in minutes before a crab's initial entry into the control side of the chamber after the introduction of hypoxic water, showed a significant linear decline with increasing oxygen tension in *Callinectes similis* but no such trend was obvious in *C. sapidus*. Contrary to our expectation, the response time of *C. similis* exposed to 0 and 25 Torr oxygen was significantly longer than in crabs exposed to 50 and 90 Torr oxygen. After the initial introduction of hypoxic water into the chamber to the achievement of a final stable gradient there was always a time lag of about 10 min. This was the time required for the mixing of hypoxic and normoxic water to occur. Therefore when anoxic or acutely hypoxic water was introduced from one end of the chamber, which was initially filled up with normoxic water, the dissolved oxygen level did not drop immediately at that end. I think the response time is a reflection of the time required to achieve a stable gradient and for the crab to detect and then avoid the hypoxic water zone. However, at less stressful higher oxygen tension (50 and 90 Torr oxygen), which does not induce immediate avoidance, response time may not be a
reflection of avoidance but rather the random movement pattern of the crabs. Response time can be a good indicator of avoidance when hypoxia is very acute but, at moderate hypoxia it is not a very dependable measure of avoidance because of variability in individual crab to crab behavior.

Use of average location, total time, and response time have demonstrated clearly that C. similis is able to detect and avoid hypoxia at zero and 25 Torr oxygen tension and C. sapidus is not as sensitive in initiating an avoidance behavior even at the acute hypoxic condition. In using the above three variables as a measure of avoidance we assumed the following: (i) the avoidance chamber was hypothetically divided into two sides - treatment side and control side, (ii) the treatment side was completely hypoxic and the control side was completely normoxic and there was a sharp boundary between the hypoxic and normoxic zone which was the mid point of the chamber where the outlet was located. Based on these assumptions we inferred that if a crab stayed at the treatment side it showed non-avoidance and if it stayed at the control side it showed avoidance. But, the picture was not so simple when we measured oxygen gradients from the nine sampling ports located along the length of the chamber. Oxygen gradient in the chamber followed a sigmoidal pattern gradually increasing from the hypoxic side to the normoxic side (See results). Therefore, the assumption of a hypothetical sharp boundary between the hypoxic and normoxic zone was invalid. As a result the crab location and time spent at the treatment side (total time) could not be used as a completely reliable measure of avoidance because in both cases this distinction would have been biased. Instead, I used the crab locations for each experiment to predict an oxygen value for each location and from those oxygen values calculated the most preferred oxygen value for each crab or the oxygen tension at which a crab spent most of its time. The preferred mean oxygen value for C. similis was 108 Torr and for C. sapidus was 112 Torr. Thus use of mean preferred oxygen tension, which is an unbiased measure of avoidance, proved that both species of
crabs were able to detect hypoxic water and choose an optimum oxygen tension which helped them to conserve the extra energy required for hyperventilation.

Activity, as measured by the distance travelled by a crab during the final 50 min of a 60 min experiment, of the crabs was used as a measure of stress during the experiment. Both species of crabs were found to be significantly less active at the severe levels of hypoxia (0, 25, and 50 Torr oxygen). Crustaceans compensate for short term hypoxia by increasing ventilation (Batterton and Cameron 1978, Taylor 1982, Pease and DeFur 1987). This requires an extra supply of energy which is obtained from the energy available for locomotion, food gathering etc. Therefore at severe hypoxic levels (≤25 Torr) the crabs become less active because they are energy limited compared to the crabs exposed to the higher levels of oxygen where the activities are higher too.

Minimum dissolved oxygen requirement for various aquatic species varies widely and the effect of hypoxia depends on their tolerance limit and duration of exposure. Survival of an organism exposed to stressfully low dissolved oxygen depends on the animal's capability of detecting the event, directly or indirectly, and physiologically acclimating or altering their behavioral pattern. When the degree of hypoxia is stressfully low, behavioral alterations, such as avoiding the hypoxic water mass or moving around the spatially and temporally isolated hypoxic water mass, are of more importance than physiological tolerance. This is especially true for Callinectes sapidus because of their inefficient anaerobic capacity (Carpenter and Cargo 1957). The inability to detect severe hypoxia or anoxia may limit the distribution of the species by reducing the energy available for locomotion, growth, and reproduction (Brett and Groves 1979). The ability to detect and avoid hypoxia in the field has been reported in many species including the Kumura prawn, Penaeus japonicus (Egusa and Yamamoto 1961), European brown shrimp, Crangon crangon (Hagerman and Uglov 1982), adult blue crabs, Callinectes sapidus and several unidentified species of crustaceans and fish (Loesch 1960, May
1973, and Pihl et al. 1991). Das and Stickle (1993) reported that *C. sapidus* is more sensitive to hypoxia, with a 28 day LC$_{50}$ of 106 Torr oxygen, than *C. similis* with a 28 day LC$_{50}$ of 43 Torr oxygen. They also reported that severe hypoxia retards the growth rate of both species of juvenile blue crabs. Thus the ability to detect and avoid hypoxia in these two species of blue crabs is important for their survival, sustained growth and distribution.
CHAPTER 4

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF JUVENILE BLUE CRABS, *CALLINECTES SAPIIDUS* AND *C. SIMILIS*, TO DIURNALLY VARYING DISSOLVED OXYGEN TENSION
INTRODUCTION

Hypoxia is one of the major environmental variables affecting the performance and ultimately survival of estuarine dependent blue crabs (Das and Stickle 1993). Previous investigations were mainly concerned with the physiological and biochemical responses following exposure to long term hypoxia (Pease and DeFur 1987, DeFur et al. 1990, Phil et al. 1991, Das and Stickle 1993) of the blue crabs *Callinectes sapidus* and *C. similis*. In the present study, however, we measured several physiological and biochemical responses of these two species exposed to a fluctuating oxygen regime. Published observations documenting diel variation in dissolved oxygen tension are lacking. Diel variation in the dissolved oxygen tension in estuarine water along the Louisiana Gulf coast occurs frequently, especially during the summer months, with water becoming extremely hypoxic during the early morning period (see results). Both *C. sapidus* and *C. similis* spend a portion of their life cycle in the vegetated habitat of the coastal Gulf and estuarine waters (Gunter 1950, Daugherty 1952, More 1969, Adkins 1972a, Perry 1975, Orth and van Montfrans 1987, Williams et al. 1990, Hsueh et al. in press) and are thus exposed to diurnally varying levels of dissolved oxygen.

Das and Stickle (1993) used survival, feeding rate, growth and molting to measure responses of *Callinectes sapidus* and *C. similis* when exposed to chronic hypoxia. Survival is a very sensitive indicator of hypoxia tolerance as is feeding rate, which varies 5-9 times more than metabolic rate along environmental factor gradients (Stickle 1985). Deoxyribonucleic acid (DNA) is mainly found in the cell nucleus associated with chromosomal material and the total quantity of DNA remains constant in the somatic cells within a given species (Leslie 1955). On the other hand, ribonucleic acid (RNA) is produced during gene transcription and correlates positively with the rate of protein synthesis and thus reflects changes in the cellular activity (Brachet 1960). Since the
amount of DNA remains constant for a given number of cells, the ratio of RNA to DNA is used as a reliable indicator of the protein synthetic activity within cells (see review by Bulow 1987). Nucleic acid concentration and the ratio of RNA to DNA have been used to study cell growth and development in crustaceans (Sulkin et al. 1975), molluscs (e.g. Collier 1976) and fishes (see review by Bulow 1987). It has also been used to measure the effects of various stressors and toxicant on fishes (e.g. Barron and Adelman 1984, Lowery and Somero 1990, Wang et al. 1993) and the blue crabs (Wang and Stickle 1986, Wang and Stickle 1988). In this study we have used the change in concentrations of nucleic acids and RNA:DNA ratio to measure the amount of stress resulting from exposure to constant and diurnally fluctuating dissolved oxygen.

The objectives of this study are to (i) compare the responses of juvenile Callinectes sapidus and C. similis to diurnally varying oxygen tension, and (ii) determine the difference in response to chronic hypoxia and diel variation in oxygen tension using the following indices: survival, feeding rate, growth, molting and changes in the RNA and DNA concentration as well as in the RNA:DNA ratio.

MATERIALS AND METHODS

Collection and maintenance

Callinectes sapidus and C. similis were collected nearshore at Port Fourchon, Louisiana (90°11.4' W, 29°6.7' N) or near Caminada Pass, Louisiana, USA (90°3.0' W, 29°12.6' N) during June, 1992, when the water temperature was 28 - 30°C and the salinity was 28 °/oo. The crabs were brought back to the laboratory and placed into artificial sea water made from Instant Ocean® at room temperature (23 - 25°C) and salinity matching field condition. All the crabs were kept under constant illumination and were acclimated in a step wise fashion to the experimental salinity (30°/oo). Crabs were
individually isolated in chambers made from a pair of PVC pipe sections (one with 21 cm inside diameter and the other piece with 23 cm inside diameter and both were 28 cm in length) that fit together with nylon meshed screens on the free ends of each piece to restrain the crabs. Crabs were chosen carefully to minimize weight and size difference. All the crabs were held for 2 wk at the final salinity before being used in the experiment. Crabs were fed frozen abdominal portions of the grass shrimp *Palaemonetes pugio*.

**Field Observation**

Diel variation in oxygen tension was monitored using a multiparameter water quality monitoring instrument (DataSonde 3, Hydrolab Corp., Austin, Texas) at Port Fourchon and Caminada Pass, Louisiana, at the beginning and end of June, 1992, for a period of 7 d each when the animals were collected. Data were recorded every 15 minutes during each collection period. The oxygen probes were installed at a depth of about 6 ft and 1.5 ft at Caminada Pass and Port Fourchon respectively.

**Experimental design**

**Constant Hypoxia Exposure System:**

Crabs of both species were maintained at 155 (normoxic water) and 50 Torr oxygen tension throughout the 28 d experimental period as control treatments. In an earlier study (Das and Stickle 1993) we found that 100% mortality occurred in *Callinectes sapidus* exposed to 25 Torr oxygen tension within 6 days of exposure. 100% mortality did not occur at any higher levels of dissolved oxygen tension. I have used 50 Torr as a control exposure so I can have representative samples from this level throughout the experimental period. Two aquaria were assigned for each control treatment. Each aquarium (76 l) consisted of an undergravel filter overlaid with crushed oyster shells. Water from a large filtration unit was pumped constantly into each
aquarium with peristaltic pumps at a rate sufficient to recycle the water several times a day.

An oxygen tension of 50 Torr was created and maintained by mixing bottled nitrogen and oxygen with a Matheson gas mixer (model 7402T) along with 0.03% carbon dioxide to maintain the pH at approximately 7.8. The gas mixer was connected to an outlet manifold and the air mixture was passed through the undergravel filter in each of the two aquaria assigned for the 50 Torr control treatment. Ambient air was used to drive the undergravel filters of the normoxic tanks (155 Torr). Each aquarium was covered with a plexiglas lid and then with a clear plastic wrapping to minimize air exchange. The water level in each tank was maintained with a constant level siphon which drained water from the aquarium into the filtration unit. Experimental conditions (temperature, salinity, PO$_2$, ammonia) were checked daily. The ammonium concentration in the tanks never exceeded 25 μM. A group of 15 normoxia acclimated crabs of each species were transferred to each of the replicate aquaria for each oxygen tension (a total of 30 crabs of each species) and were monitored for survival, feeding rate, growth and molting.

**Diurnally Fluctuating Oxygen System:**

Diel fluctuation of dissolved oxygen similar to the fluctuations noticed in the field was simulated in the laboratory with a computer program that controlled a mass flow gas mixer (Model GF-3, Cameron Instrument Company, Port Aransas, Texas) and a multiparameter water quality monitoring device (Scout 3, Hydrolab Corp., Austin, Texas). This fully automated system used a simple feedback control algorithm to sinusoidally vary the oxygen tension in the water. Oxygen tension varied from 25 Torr (16% air saturation) to 155 Torr (100% air saturation) at 23 - 25°C and 30°/oo S (Fig. 4.1). All other experimental conditions were maintained as in the constant hypoxia exposure system. A group of 30 normoxia acclimated crabs of each species were transferred separately.
Figure 4.1 Plot of the diurnal variation in oxygen tension in the laboratory for a period of 28 d.
replicates of 15 each, to the fluctuating oxygen system and monitored for 28 d for survival, feeding rate, growth and molting.

**Determination of food consumption, molting and growth rate**

Both crab species were fed weighed abdominal portions of frozen grass shrimp, *Palaemonetes pugio*, ad libitum so some tissue remained at the end of the day. Uneaten food was removed, weighed and replaced with a new ration daily. Crabs that died during the experiment were removed from the feeding rate analysis from the week they died. Each time a crab molted it was reweighed and the difference between the initial and final weight was converted into percent increase in weight.

**Measurement of DNA and RNA**

DNA and RNA analyses were performed according to the procedure developed by Munro and Fleck (1966). All analyses were performed on whole crabs rather than on specific tissues because of the small size of the crabs. Six crabs of each species from each of all 3 treatments (155, 50 Torr and Fluctuating O₂) were sacrificed after 0, 7, 14, 21 and 28 d exposure. However, some samples were lost due to crab deaths in the hypoxic treatment, in the fluctuating O₂ treatment or during sample preparation. The minimum sample size was four. Crabs were sacrificed and frozen in liquid nitrogen on each sampling date and then stored at -80°C. Individual frozen crabs were pulverized in a mortar and pestle and homogenized in 10 volumes of ice-cold distilled water using a Tekmar homogenizer. 200 µl of homogenate was dried at 80°C in pre-weighed aluminum pans for 24 hrs to determine the dry weight of each sample. 200 µl of homogenate was used separately for DNA and RNA analysis. Macromolecules including nucleic acids were precipitated from the homogenate by adding 100 µl of 0.6 N and 500 µl of 0.2 N perchloric acid and incubating the mixture on ice for at least 10 minutes. The samples
were then centrifuged at 5600 X g at 4°C for 15 min. The supernatant was discarded and
the pellet was washed twice in 750 µl of ice-cold 0.2 N perchloric acid. The pellet was
saved for DNA and RNA analysis. DNA content of the pellet was determined using the
diphenylamine procedure (Burton 1956) according to Shatkin (1969). Two vol of
diphenylamine reagent and 1 vol of 0.5 N perchloric acid were added to the pellet, mixed
and incubated at 25°C for 20 hrs. DNA content of each sample was measured at an
absorbance of 600 nm and compared with the standard prepared from calf thymus DNA
(Sigma Chemical Co.). Analyses were performed in duplicate.

RNA content of the pellet was determined using the modified Schmidt-
Thannhauser procedure recommended by Munro and Fleck (1966). Homogenates were
hydrolyzed by adding 500 µl of 0.3 N potassium hydroxide and the mixture was
incubated at 37°C for 30 min. After cooling the digest on ice macromolecules were
precipitated by adding 250 µl of 1.5 N perchloric acid and the mixture was incubated on
ice for at least 10 min. The samples were then centrifuged at 5600 X g at 4°C for 15 min.
The supernatant was saved and the pellet was washed twice with 150 µl 0.2 N perchloric
acid. RNA content of the pooled supernatant was measured at 260 nm and compared
with the standard prepared from yeast RNA (Sigma Chemical Co.). Analyses were
performed in duplicates.

Statistical analysis

To remove the effects of body weight, the feeding rates were standardized to a 1
g unit wet body weight. Feeding rate was analyzed with a repeated measure analysis.
Differences in feeding rate within and among treatments were determined with
polynomial contrasts. Nucleic acid concentration was standardized for each crab within
each treatment for 1 g unit dry body weight. Total nucleic acid in each crab was also
measured. Nucleic acid concentration and RNA:DNA ratio were analyzed as factorial
design. Differences among treatment means were determined by using least square means. Regression analysis was used to determine the effect of crab body size on the nucleic acid content and RNA:DNA ratio for the crabs exposed to normoxia.

RESULTS

The diurnal trend in dissolved oxygen tension along the collection sites of coastal Louisiana is plotted in Fig. 4.2. Data presented in the figure are the traces over each 7 days at each location (Caminada Pass and Port Fourchon) and the average of the two locations over the 14 d period.

The overall feeding rate (feeding rate per day over the 28 days of exposure) in the two species of crabs followed distinct trends (Table 4.1). The overall feeding rate of Callinectes sapidus exposed to diurnally varying oxygen tension was higher (p < 0.001) than the treatment groups maintained at 155 and 50 Torr oxygen. Overall feeding rate of C. sapidus exposed to 50 Torr oxygen tension was lower (p < 0.001) than the crabs exposed to normoxic water. In contrast, the overall feeding rate of C. similis exposed to diurnally varying oxygen tension was lower (p < 0.001) than in the crabs exposed to 155 and 50 Torr oxygen. Overall feeding rate of C. similis exposed to normoxic water was higher (p < 0.001) than the crabs exposed to 50 Torr oxygen tension.

Feeding rate of both species of crabs exposed to the diurnally varying oxygen tension dropped (p < 0.001) from the first week to the second week and then stayed at the same level for the rest of the 28 d experimental period when it was analyzed on weekly basis (Fig. 4.3).

Growth was measured in both species of crabs as percent increase in wet weight with respect to the initial weight of the crab. Growth rate in the crabs exposed to the diurnally varying oxygen tension was significantly higher than the crabs exposed to 50
**Figure 4.2** Diurnal trend in oxygen tension along the collection sites of coastal Louisiana. Data presented in the figure are the recordings at the two collection sites (Port Fourchon and Caminada Pass) and the average of the two collection sites over the 14 days of recording period.
Table 4.1. *Callinectes sapidus* and *C. similis*. Overall feeding rate (expressed as food consumed / day over the 28 d exposure) of the two species of crabs. 30 crabs were used at each PO₂ for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatments</th>
<th>Overall food consumption (mg)</th>
<th>Std. error</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Callinectes sapidus</em></td>
<td>Fluctuating</td>
<td>492.5</td>
<td>30.1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>155 Torr</td>
<td>388.4</td>
<td>16.9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>50 Torr</td>
<td>286.2</td>
<td>16.2</td>
<td>0.001</td>
</tr>
<tr>
<td><em>Callinectes similis</em></td>
<td>Fluctuating</td>
<td>275.4</td>
<td>17.2</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>155 Torr</td>
<td>400.5</td>
<td>23.8</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>50 Torr</td>
<td>337.5</td>
<td>29.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4.2. *Callinectes sapidus* and *C. similis*. Growth and molting phenomena the two species of crabs exposed to various hypoxia regime for 28 days. 30 crabs were used at each PO₂ for each species. Sap = *C. sapidus*; Sim = *C. similis*.

<table>
<thead>
<tr>
<th>Oxygen (Torr) Tension</th>
<th>155</th>
<th>50</th>
<th>Fluctuating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sap</td>
<td>Sim</td>
<td>Sap</td>
</tr>
<tr>
<td>Average initial wet wt (mg)</td>
<td>655 ± 80</td>
<td>574 ± 52</td>
<td>635 ± 52</td>
</tr>
<tr>
<td>Increase in wet weight (%)</td>
<td>98</td>
<td>76</td>
<td>67</td>
</tr>
<tr>
<td>No. of crabs molted once</td>
<td>10</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>
Figure 4.3 *Callinectes sapidus* and *C. similis*. Mean daily feeding rate (± SE) of the two species of crabs exposed to the fluctuating oxygen regime and 155 and 50 Torr oxygen tension during each week.
Torr oxygen tension (Table 4.2). Growth rate of *C. sapidus* exposed to diurnally varying oxygen tension was higher than the growth rate of *C. similis*. No significant differences were noticed in growth rate of crabs exposed to normoxia and diurnally varying oxygen tension.

Only 3% mortality in *Callinectes sapidus* and 11% mortality in *C. similis* occurred in the crabs exposed to the fluctuating oxygen tension. Mortality in the crabs exposed to 50 Torr oxygen tension was 82% and 50% for *C. sapidus* and *C. similis* respectively. There was no mortality in the crabs exposed to 155 Torr oxygen tension.

The ratio of RNA to DNA of both species of crabs exposed to 155 Torr oxygen tension increased with increasing body size over the 28 days (*p* < 0.01, Fig. 4.4). Slope of the regression for either species did not differ significantly from each other (*p* = 0.43).

Diurnally varying oxygen tension strongly affected the nucleic acid concentration of both species of crabs. The RNA concentration of *Callinectes sapidus* and *C. similis* decreased by 36% and 35% respectively on day 28 in the crabs exposed to diurnal oxygen variation compared to the control crabs of each species from day 0 (Table 4.3). The DNA concentration of *C. sapidus* and *C. similis* decreased by 40% and 21% respectively on day 28 in the crabs exposed to diurnal oxygen variation compared to the control crabs of each species from day 0 (Table 4.3). In comparison, the RNA concentration of *C. sapidus* and *C. similis* decreased by 27% in both species on day 28 in the crabs exposed to the 50 Torr oxygen tension compared to the control crabs of each species from day 0 (Table 4.3). The DNA concentration of *C. sapidus* and *C. similis* decreased by 31% and 23% respectively on day 28 in the crabs exposed to the 50 Torr oxygen tension compared to the control crabs of each species from day 0 (Table 4.3). There was a significant effect of time (*p* < 0.01) on the RNA and DNA concentration of both species of crabs.

The RNA:DNA ratio of *C. sapidus* increased by 9% while that of *C. similis* decreased by 18% on day 28 in the crabs exposed to diurnal oxygen variation compared
Figure 4.4  Relationship between the RNA:DNA ratio and body weight in juvenile *Callinectes sapidus* and *C. similis*. N = 22 in *C. sapidus* (filled circles) and N = 19 in *C. similis* (empty triangles).
**Callinectes similis**

\[ Y = 1.7386903 + 0.0081678 \times \]

**Callinectes sapidus**

\[ Y = 1.1212448 + 0.0081678 \times \]
Table 4.3. *Callinectes sapidus* and *C. similis*. Changes in the RNA and DNA concentration and RNA:DNA ratio in the two species of crabs at the end of the experiment (day 28) as compared to the control crabs from day 0. Nucleic acid concentration is given in µg/mg dry body wt.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>Treatment</th>
<th>Day</th>
<th>RNA % of day 0</th>
<th>DNA % of day 0</th>
<th>RNA:DNA Ratio % of day 0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Callinectes sapidus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155 Torr</td>
<td>0</td>
<td>155 Torr</td>
<td>0</td>
<td>1296 ± 71</td>
<td>696 ± 30</td>
<td>1.93 ± 0.128</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
<td>28</td>
<td>1091 ± 70</td>
<td>697 ± 67</td>
<td>1.64 ± 0.105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluctuating</td>
<td>28</td>
<td>825 ± 112</td>
<td>419 ± 74</td>
<td>2.11 ± 0.130</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 Torr</td>
<td>941 ± 110</td>
<td>481 ± 58</td>
<td>1.97 ± 0.057</td>
</tr>
<tr>
<td><strong>Callinectes similis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155 Torr</td>
<td>0</td>
<td>155 Torr</td>
<td>0</td>
<td>1111 ± 109</td>
<td>456 ± 56</td>
<td>2.56 ± 0.192</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
<td>28</td>
<td>1142 ± 86</td>
<td>785 ± 58</td>
<td>1.49 ± 0.145</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluctuating</td>
<td>28</td>
<td>719 ± 23</td>
<td>361 ± 25</td>
<td>2.09 ± 0.145</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 Torr</td>
<td>714 ± 48</td>
<td>353 ± 34</td>
<td>2.02 ± 0.100</td>
</tr>
</tbody>
</table>
to the control crabs of each species from day 0 (Table 4.3). The RNA:DNA ratio of C. *sapidus* increased by 2% and of *C. similis* decreased by 21% on day 28 in the crabs exposed to 50 Torr oxygen tension compared to the control crabs of each species from day 0 (Table 4.3). The RNA:DNA ratio of both species changed significantly (p < 0.001) over time and was also significant (p < 0.001) at the species level (Fig. 4.5, 4.6).

Both species of crabs maintained at 155 Torr oxygen tension showed an overall increase in DNA and RNA synthesis over time. There was a 72% increase in DNA concentration in *Callinectes similis* when it was compared to the control crabs from day 0. Increase in the DNA concentration in *C. sapidus* was negligible.

**DISCUSSION**

Food consumption of *Callinectes sapidus* over the 28 d period of exposure was significantly higher in the diurnally varying oxygen tension than the two control (155 and 50 Torr oxygen tension) treatments. On the other hand, *C. similis* exposed to the diurnally varying oxygen tension consumed significantly less food as compared to the two control treatments. Das and Stickle (1993) reported a 123% increase in feeding rate in *C. sapidus* after 10 days of starvation. In the present study *C. sapidus* was exposed to oxygen tensions high enough to allow efficient feeding for a sufficient period of time during the diurnal variation in dissolved oxygen. However, a lower feeding rate of *C. similis* may be due to a variable metabolic rate over the diurnal cycle due to hypoxic stress. The metabolic rate of *C. similis* exposed to chronic hypoxia was elevated over the rate of crabs exposed to normoxia indicating overcompensation (Das and Stickle 1993). The growth rate, measured as percent increase in wet weight, of both species of crabs exposed to the fluctuating oxygen regime was comparable to the crabs of both species exposed to 155 Torr. Wang (1986) reported a lower tissue content with growth in
Figure 4.5 *Callinectes sapidus*. Concentration of RNA, DNA and RNA:DNA ratio (± SE) of the blue crabs exposed to fluctuating oxygen regime and 155 and 50 Torr oxygen during the four sampling dates. The mean concentration of the nucleic acids and the RNA:DNA ratio on day 0 was used as a standard starting point.
Callinectes sapidus

a

RNA Conc. (log gm dry wt)

Sampling Period (days)

50 Torr

b

DNA Conc. (log gm dry wt)

Sampling Period (days)

155 Torr

Fluctuating

c

RNA/DNA Ratio

Sampling Period (days)
Figure 4.6 *Callinectes similis*. Concentration of RNA, DNA and RNA:DNA ratio (± SE) of the lesser blue crabs exposed to fluctuating oxygen regime and 155 and 50 Torr oxygen during the four sampling dates. The mean concentration of the nucleic acids and the RNA:DNA ratio on day 0 was used as a standard starting point.
Callinectes similis

(a) RNA Conc. (log10 g/cm)

(b) DNA Conc. (log10 g/cm)

(c) RNA/DNA Ratio

Sampling Period (days)

50 Torr
155 Torr
Fluctuating
juvenile blue crabs exposed to water soluble fraction of crude oil compared to the control crabs and has suggested size increase under stressful environmental condition is a possible anti-predation adaptation. In the present experiment, growth was measured as increase in wet weight. Therefore, a similar growth rate in the two species of crabs exposed to fluctuating and 155 Torr oxygen may be due to the extra amount of water present in the crabs exposed to diel oxygen variation and the size increase may essentially an anti-predation strategy. The increase in size was not as great for either species of crabs exposed to 50 Torr oxygen tension because of the stress involved with constant hypoxic exposure.

Wang and Stickle (1986, 1988) reported the ratio of RNA to DNA as a reliable and sensitive index of stress in juvenile Callinectes sapidus. Wang et al. (1993) used both RNA:DNA ratio and RNA content as a reliable measure of fish growth fed crude oil contaminated food. They have also indicated that RNA content may be a more useful indicator of fish growth than the ratio of RNA to DNA. Barron and Adelman (1984) used RNA, DNA and RNA:DNA ratio to measure the growth of larval fish exposed to sublethal concentrations of toxicant. RNA, DNA concentration and the RNA:DNA ratio have been shown to be a measure of growth rate in fishes (Buckley 1984, Bulow 1987).

Growth rate in Callinectes similis exposed to diurnal oxygen variation was comparable to the crabs exposed to 155 Torr oxygen tension but, the feeding rate was lower in the crabs exposed to diel oxygen variation. A high growth rate and low intake of food might cause a situation similar to starvation which would result in decreased RNA synthesis leading to a selective cell catabolism as suggested by Wang and Stickle 1986. As a direct result of a decrease in cell number the DNA concentration per unit mass of crab also decreased. On the other hand, a poor nutritional status can not be inferred from the apparent decrease in the RNA and DNA concentration in C. sapidus. Since nucleic acid concentrations were measured as µg nucleic acid per unit body weight, a
decrease in the nucleic acid concentration per unit body weight with increasing body size was possible especially if the crabs were growing by increasing the cell size or relative carapace size and not cell number. Mustafa (1978) proposed growth by increase in size of cells rather than cell number in the catfish, *Heteropneustes fossilis*. A response similar to *C. similis* exposed to the diurnally varying oxygen tension has been observed in both species exposed to the 50 Torr oxygen tension. Crabs exposed to 50 Torr oxygen consumed significantly less food compared to the crabs exposed to normoxic water and there was a significant decrease in both RNA and DNA concentration by 28 d. In addition the growth rate of the crabs of both species exposed to 50 Torr oxygen tension was significantly lower than the crabs exposed to normoxia. Therefore, the explanation for the apparent decrease in RNA and DNA concentration in *C. similis* exposed to diurnal oxygen fluctuations may not hold for the crabs exposed to 50 Torr oxygen tension. The fluctuating oxygen regime had a similar effect on the nucleic acid concentration of both species of crabs but, in *C. similis* the effect was probably compounded by a low consumption of food which was a direct effect of hypoxia. The trend of decreasing nucleic acid concentration in crabs exposed to the 50 Torr oxygen tension was similar to the response of the crabs exposed to the fluctuating oxygen regime. Wang and Stickle (1986) found a 73% decrease in RNA concentration and 31% decrease in DNA concentration in the blue crabs starved for 30 days. In the present study RNA concentration in neither species decreased more than 35% of the concentration in crabs at 155 Torr on day 0.

Sulkin et al. (1975) reported a cyclic activity pattern in the RNA:DNA ratio during larval development of the Xanthid crab *Rithropanopeus harrisii*. They have reported a peak of protein synthesis during the intermolt period followed by a drop before molt. In the present study the RNA:DNA ratio for both species of crabs was variable. The fluctuating nature of the RNA:DNA ratio was mainly due to the fluctuation in the RNA and DNA concentration in all the treatments over time. This apparent fluctuation in the
nucleic acid concentration may be due to the molting activity in the crabs. However, the changes in the nucleic acid concentration in relation to the molt cycle was not measured in this study. When the effects of environmental and biological factors on the nucleic acid synthesis are unknown for a natural population the utility of RNA:DNA ratio becomes limited (Dagg and Littlepage 1972, Ota and Landry 1984). Changes in the nucleic acid concentration in both species of crabs due to hypoxic stress were not of the same magnitude as reported by Wang and Stickle (1986) for juvenile blue crabs starved for 30 d. The RNA:DNA ratio in their study decreased from 2.92 for the control crabs to 1.15 in the experimental crabs after 30 days of starvation, a 61% decline from the control level. The ratio increased by 51% after 5 days of feeding which demonstrates the sensitivity of the RNA:DNA ratio to changes in food availability. In this study the magnitude of decrease in the concentration of either RNA or DNA and the RNA:DNA ratio is much less compared to those mentioned by Wang and Stickle (1986). This probably indicates that the diurnal variation in oxygen tension did not stress the crabs enough to cause an equivalent amount of change in the concentration of RNA and DNA or the RNA:DNA ratio. Mortality in the blue crabs due to hypoxic stress is not as much due to bio-energetic constraints as it is because of the stress during molting when demand for oxygen goes up by several folds (Perry et al. 1979).
CHAPTER 5

SUMMARY AND CONCLUSIONS
The Louisiana continental shelf is the largest, most severe, and persistent zone of hypoxia in the United States coastal waters (Rabalais and Harper 1992a). Hypoxia is a major environmental factor which can cause tremendous stress to the abundant demersal fauna (fish, penaeid shrimp and swimming crabs) which characterize the Gulf of Mexico shelf environment (Boesch and Rabalais 1991). Reports suggest that low levels of dissolved oxygen cause mortality of _Callinectes sapidus_ and can impede their migration (Tatum 1982, Van Engel 1982). The distributional pattern of _C. similis_, an offshore congener of _C. sapidus_ in the Gulf of Mexico region overlaps frequently. Spawning habits of _C. sapidus_ and _C. similis_ overlap temporally and spatially (Perry 1975). Thus some parts of the life cycle of these two species are exposed to the same set of environmental variables. Occurrence of diel variation in dissolved oxygen has not been reported before in the coastal Gulf of Mexico. In the present study diel variation in dissolved oxygen has been shown to occur at the two collection sites where the juveniles of these two species co-occur. Migration to higher salinity waters that are favorable for larval development has been reported for both _C. sapidus_ and _C. similis_ (Millikin and Williams 1984, Williams 1984, Hines _et al._ 1987). Therefore, both of these species are exposed to low dissolved oxygen during different parts of their life cycle.

Tolerance to hypoxia is probably related to the developmental history of the organism. Changes in the resistance and capacity adaptation of an organism over its lifetime is related to the degree of exposure to the stressor gradient as well as to its evolutionary history. _C. similis_ is exposed to constant hypoxia in the offshore water for a major part of its life cycle whereas, _C. sapidus_ is more often exposed to the diel variation in dissolved oxygen in the coastal vegetated habitat. Only the ovigerous female _C. sapidus_ migrate to the higher salinity water when they are exposed to the constant hypoxia for only a short period. A direct consequence of this may be a greater tolerance to constant hypoxia in _C. similis_ compared to _C. sapidus_.

Since hypoxia is a common and recurrent phenomenon along the Louisiana Gulf coast, an ability to properly detect and avoid hypoxia is of importance in the survival to these estuarine-dependent species. Diel variation in the dissolved oxygen tension in estuarine waters along the Louisiana Gulf coast has also been shown to be of frequent occurrence, especially during the summer months with water becoming almost anoxic during the early morning period of the day. Therefore, ability to avoid hypoxia becomes even more important in daily activities like foraging, diel migration, etc. The inefficient anaerobic capacity of the blue crabs make them vulnerable to hypoxic exposure and timely detection becomes necessary. This study demonstrated that although both species of crabs can detect and avoid hypoxic water, Callinectes similis is more efficient in detecting and avoiding hypoxia than C. sapidus. Avoidance of hypoxic water by larger motile invertebrates was documented by Stachowitsch (1984). Rabalais et al. (1991b) documented similar behavior among motile marine invertebrates and vertebrates in the Gulf of Mexico continental shelf off Louisiana. Tolerance to hypoxia and ability to detect and avoid the phenomenon in a timely manner may also be related to resource partitioning within the environment shared by these two species. Such differences in tolerance and avoidance ensure their survival and coexistence in a common habitat, at least for a portion of their life cycle, without excluding each other by competitive interaction.
LITERATURE CITED


Tapash Das was born in Bihar, India, on July 3rd, 1961. He graduated from Burdwan University, India, in 1981 with a B.S. degree in Zoology. He also received his M.S. in Zoology from Kalyani University, India, in 1983. He entered the Ph.D. program in the Department of Zoology and Physiology at the Louisiana State University in 1989. His research publications include a study on the responses of blue crabs and southern oyster drill to hypoxia and anoxia (Marine Ecology Progress Series 98: 263-274, 1993).
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Tapash Das

Major Field: Zoology

Title of Dissertation: Physiological and Biochemical Responses to Hypoxia in the Blue Crab, Callinectes sapidus Rathbun, the Lesser Blue Crab, C. similis Williams, and the Southern Oyster Drill, Stramonita haemastoma Linnaeus

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signature]
Joseph T. Libralato

[Signature]
Ken Brown

David W. Folky

[Signature]
John Peto

James T. James

Date of Examination:

October 29, 1993