Behavioral ecophysiology of dungeness crabs during feeding and digestion in hypoxia

Jennifer Lynn Bernatis
University of Nevada, Las Vegas
BEHAVIORAL ECOPHYSIOLOGY OF DUNGENESS CRABS
DURING FEEDING AND DIGESTION IN HYPOXIA

by

Jennifer Lynn Bernatis

Master of Science
Fort Hays State University, 1996
Bachelor of Science
Fort Hays State University, 1994

A thesis submitted in partial fulfilment
of the requirements for the

Master of Science Degree in Biological Sciences
Department of Biological Sciences
College of Sciences

Graduate College
University of Nevada, Las Vegas
August 2005
The Thesis prepared by

Jennifer Lynn Bernatis

Entitled

Behavioral Ecophysiology of the Dungeness Crab During Feeding and Digestion in Hypoxia

is approved in partial fulfillment of the requirements for the degree of

Master of Science in Biological Sciences

Examination Committee Co-Chair

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Examination Committee Member

Graduate College Faculty Representative
ABSTRACT

Behavioral Ecophysiology of Dungeness Crabs During Feeding and Digestion in Hypoxia

by

Jennifer Lynn Bernatis

Dr. Shawn Gerstenberger, Examination Committee Chair
Associate Professor of Environmental and Occupational Health
University of Nevada, Las Vegas

Dr. Paul Schulte, Examination Committee Co-Chair
Associate Professor of Biology
University of Nevada, Las Vegas

_Cancer magister_ inhabits areas frequently subject to periods of hypoxia. In hypoxia, digestive processes pose an additional physiological burden to crabs already attempting to maintain adequate oxygen uptake. Crabs tended to cease feeding below 3.2 kPa, resuming feeding when dissolved oxygen levels were rapidly raised to 6 kPa. In a 10.5 - 21 kPa DO gradient starved and fed crabs showed no preference for any area of the apparatus. In a 2.5 - 10.5 kPa DO gradient, starved and fed crabs showed significant preference for the highest oxygen concentration. Starved crabs were less likely to enter hypoxic waters to feed; those that did moved food to higher oxygen concentrations prior to feeding. In the field, starved crabs traveled up to 1.3 km within 6 hours, while postprandial crabs, moved very little during the first 48 hours. The present study suggests
Cancer magister may use behavior to minimize the use of physiological mechanisms, and maximize foraging and digestive processes.
# TABLE OF CONTENTS

ABSTRACT ..............................................................................................................................iii

LIST OF FIGURES ..................................................................................................................vi

ACKNOWLEDGEMENTS ....................................................................................................vii

CHAPTER 1 INTRODUCTION .............................................................................................1

CHAPTER 2 MATERIALS AND METHODS .....................................................................7
  Collection and Maintenance ........................................................................................7
  Feeding in Hypoxia .......................................................................................................8
  Postprandial Behavior in Hypoxia ............................................................................10
  Foraging Behavior ......................................................................................................11
  Fieldwork ....................................................................................................................12
  Statistical methods .................................................................................................13

CHAPTER 3 RESULTS .........................................................................................................14
  Feeding in Hypoxia .....................................................................................................14
  Postprandial Behavior in Hypoxia ............................................................................15
  Foraging Behavior ......................................................................................................16
  Fieldwork ....................................................................................................................16

CHAPTER 4 DISCUSSION ...................................................................................................18
  Feeding in Hypoxia .....................................................................................................18
  Postprandial Behavior in Hypoxia ............................................................................23
  Fieldwork ....................................................................................................................26
  Conclusions .................................................................................................................28

REFERENCES ............................................................................................................... 31

APPENDIX ..............................................................................................................................36

VITA ....................................................................................  50
LIST OF FIGURES

Figure 1  Diagram of apparatus used for oxygen gradient experiments ......................34
Figure 2a  Feeding behavior of 25 C. magister in dissolved oxygen concentrations of 1.5kPa to 21kPa. Wet mass of food (g) consumed ........................................35
Figure 2b  Feeding behavior of 25 C. magister in dissolved oxygen concentrations of 1.5kPa to 21kPa. Time spent feeding (min) ..................................................36
Figure 3  Feeding behavior of Cancer magister as determined by crab mass ...........37
Figure 4a  Behavior of starved crabs (hatched bars) and postprandial crabs (open bars) in a high oxygen gradient of 10.5 kPa to 21 kPa ..........................38
Figure 4b  Behavior of starved crabs (hatched bars) and postprandial crabs (open bars) in a low oxygen gradient of 2.5 kPa to 10.5 kPa ...............................39
Figure 5a  Activity of starved crabs (hatched bars) and postprandial crabs (open bars) in a high oxygen gradient of 10.5 kPa to 21 kPa .................................40
Figure 5b  Activity of starved crabs (hatched bars) and postprandial crabs (open bars) in a low oxygen gradient of 2.3 kPa to 10.5 kPa .................................41
Figure 6a  Dissolved oxygen levels (kPa) as a function of depth (m) in the Bamfield Inlet, British Columbia .................................................................42
Figure 6b  Temperature (°C) as a function of depth (m) in the Bamfield Inlet, British Columbia .................................................................43
Figure 7a  Movement of C. magister in the Bamfield Inlet of starved crabs ............44
Figure 7b  Movement of C. magister in the Bamfield Inlet of postprandial crabs ......45

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
ACKNOWLEDGEMENTS

First, and foremost I would like to thank Dr. Iain McGaw for talking me out of doing a second bachelors and jumping right into this project. You had faith in my ideas and pretty well let me figure this project out on my own. I wish I had a stronger biology background coming in to this endeavour, as we could have gone much further with this project and found numerous other discoveries important to your interests in physiology and my interests in the environment. The 5 months conducting research in Bamfield were greatly appreciated and as I look to my future work, it is nice to know you will not be chasing me around the lab with a crab. Thank you for having the faith in me, encouraging me and for helping me realize where my professional abilities will be best utilized.

I can never express my gratitude enough to Dr. Shawn Gerstenberger and Dr. Chad Cross. The two of you have provided more professional support and encouragement then I ever expected as neither of you knew anything about me and took a chance. I hope I have met your expectations. Both of you have helped me truly understand the importance of being a good educator and mentor. As I am planning on teaching in my future, your sincerity, fairness, and enthusiasm will be remembered as I interact with my students. I only hope I inspire at least one student the way you have inspired me, thank you.

Next, I would like thank Dr. Paul Schulte for stepping into a role that allowed the
successful completion of my program. Without you I would not be writing this at this time. You were the only one willing to step into the position and although it was not your area of expertise, your comments and ideas were more than appreciated and will be remembered as I go forth to create more gradient tanks. I can never thank you enough either; you have been there since Day 1 serving many roles often within the same conversation. Thank you for being able to balance your roles of professor, mentor, and being a very good friend; I have learned a lot thanks to you.

I would also like to thank Dr. Steve deBelle and Dr. Dan Allen for taking the time to be a part of my thesis committee. Your comments and suggestions were very helpful towards the completion of the project. I would also like to thank the Director and Staff of the Bamfield Marine Sciences Centre. I would also like thank R.D. Anderson for endless hours of support.

Finally, I would like to thank my parents for their understanding in my desire to be a professional student. I promise one day I might finish, maybe. Until then I hope you realize how important your support and understanding have been to me through my academic and professional changes. And to my grandfather, Melvin Bailey, I am sorry you will never get to read this, I hope you knew how instrumental you were in my decision to continue on in my education and I only hope I can be half the educator you were to your students. Thank you.
CHAPTER 1

INTRODUCTION

The Dungeness crab, *Cancer magister*, ranges along the western seaboard of North America from the Bering Sea to S California (ADF&G 1994). Its preferred habitat ranges from shallow muddy bays, inlets, and estuaries at depths of 22 m or less, but extends out on the continental shelf to a depth of 290 m (DFO, 1988). It is largely absent on rocky bottoms, generally residing on sandy sea floors and eelgrass beds (DFO, 1988). *Cancer magister* is also known to migrate diurnally and seasonally with migratory movements over 400 km (Stevens, *et al.* 1984). This species is the largest of the cancrid crabs reaching about 230 mm carapace width and weights of around 2.5 kg. The crabs decrease molt cycles with age, but molt at least annually between June and October; and have an estimated life span between eight and thirteen years (ADF&G 1994). As predator and prey, Dungeness crabs play an important role in marine ecosystems. Its diet primarily consists of bivalves, small fish and crustaceans, including cannibalistic feeding during the molting months; Dungeness crabs serve as prey to numerous fish, other invertebrates, and marine mammals (PSMFC, 1996; DFO, 1988, 2002).

The Dungeness crab is commercially the most important crab species on the west coast. In 2000 approximately 37.7 million pounds of Dungeness crab were sold in the
U.S. market. This fishery is approximately a $500 million industry with domestic fisheries supplying 99% of the total product. The majority of crabs are trapped within three miles of shore (NMFS, 1999). Fisheries management practice has not included surveying the populations off the California, Oregon, and Washington coasts, and decisions are thus made from trapping data supplied by the commercial fisheries. Populations can vary year to year, however recent data suggest the Dungeness crab abundance appears to be increasing (NMFS, 1999). Although not considered overfished, all fisheries are considered fully utilized, except for Oregon which is considered underutilized (Bishop et al., 2001). Fisheries are only closed if early catch yields indicate a minimum catch will not be obtained.

As a commercially important species a plethora of information exists on the physiology and ecology of this species, and has enabled the species to be used as a bioindicator species for environmental perturbations. The Dungeness are sensitive to aquatic pollution and have been used for monitoring hydrogen sulfide levels in California resulting from dredging activities. Additionally, these crabs are sensitive to insecticides, heavy metals, PCB, and hydrocarbons (PSMFC, 1996). All of these activities directly contribute to the reduction of available oxygen in an ecosystem, making the Dungeness a particularly good species for monitoring habitats.

In the open ocean the water is normally saturated with oxygen and 100% saturated or normoxic (21 kPa), but may vary from anoxic to hyperoxic concentrations. Hypoxia, is species dependent, but is generally considered as any drop in oxygen below normoxic levels. Anoxia is defined as very low or absence of oxygen (<1 kPa). Coastal zones are
increasingly subject to episodes of hypoxia, and are accelerated by human-induced deleterious impacts (Rabalais, 2004). Patterns of hypoxia tend to follow seasonal trends directly related to the influx of freshwater and the increased nutrient input from human activities. (Rabalais, 2004). These activities include such actions as increased nutrients from rivers, dredging activities, and insufficient sewage treatment and threatens estuaries worldwide (Diaz and Rosenberg, 1995). These impacts have created habitats that are subjected to prolonged periods of hypoxia which disrupts benthic communities and have created detrimental consequences to coastal commercial fisheries (Diaz and Rosenberg, 1995; Baden et al. 1990; Rabalais, 2004).

Hypoxic zones are becoming more widespread and are one of the most deleterious human-induced impacts on marine ecosystems (Rabalais, 2004). The ecological effects of hypoxia are dependent, in part, on the severity and duration of the event (Sagasti et al., 2001); second, they can disrupt benthic and demersal communities as well as causing mass mortality of aquatic life (Diaz and Rosenberg, 1995). Generally, tolerance levels are higher for organisms residing in the sediments, whereas, mobile organisms such as fish and crustaceans may exhibit behavioral responses to avoid hypoxic areas (Hagerman, 1998; Diaz and Rosenberg, 1995). However, most organisms encountering hypoxia have some physiological means of adapting to short-term hypoxic exposure.

The ability to tolerate hypoxia depends on the species ranging from complete tolerance to intolerance. Generally, those species with greater motility (fish) are the less tolerant of hypoxia. The exposure to chronic hypoxia can cause widespread mortality of fish and invertebrates. As marine environments are constantly fluctuating many of the
organisms have developed physiological mechanisms to cope with brief periods of hypoxia. However, these mechanisms may result in the decreased capacity of other physiological activities.

Various species of decapod crustaceans reside in areas with fluctuating oxygen regimes. Physiological mechanisms enhance the ability of these crustaceans to cope with acute episodes of hypoxia. Many decapod crustaceans are able to maintain oxygen uptake during hypoxia by increasing the ventilation of the branchial chambers (Airriess and McMahon, 1994; McMahon, 2001). However, at a critical oxygen tension ($P_{crit}$), increases in ventilation rate are unable to compensate for the decreased oxygen availability; thereafter, the ventilation frequency decreases along with oxygen uptake (Airriess and McMahon, 1994). In hypoxic environments, most crustacean species also respond by exhibiting a bradycardia, thus limiting the amount of energy expenditure via cardiovascular mechanisms (McMahon and Wilkens, 1975; Airriess and McMahon, 1994; Reiber, 1994; Reiber and McMahon, 1998; McMahon, 2001). Additionally, crabs can alter blood flow during hypoxic exposure, redirecting blood to tissues requiring higher levels of oxygen (Airriess and McMahon, 1994; Reiber and McMahon, 1998; McGaw and McMahon, 2003). All the previous research was carried out on starved animals; this protocol is adopted because the stimulatory effects of digestion on metabolism (specific dynamic action) are well known (Wang, 2001). If the specific dynamic action is high, it may have significant effects on other systems. Recent research demonstrates these effects; the adaptive physiological mechanisms of Cancer magister in hypoxia may be compromised during and after feeding (McGaw, 2005). If the crabs are unable to balance
the demands of these physiological competing systems, the question then arises, do they exhibit behavioral responses to minimize the use of physiological mechanisms?

Behavioral responses are documented in terms of large scale changes in distribution and abundance; however, less information is available on fine-scale behavioral responses of mobile species to dynamic hypoxia (Bell et al., 2003a). Apart from migration, other behavioral responses have been noted in crustaceans. Specifically, crustaceans may limit activity as hypoxia increases thereby decreasing metabolic demands and the need for physiological mechanisms (Johansson, 1997). Some intertidal crustaceans, such as the green crab (*Carcinus maenas*) may break the water-air interface in an attempt to breath aerial oxygen (Hill et al., 1991), while others, such as *C. magister* may not (McMahon and Hankinson, 1993). Crustaceans residing in sediment, such as *Saduria entomon*, move to the sediment surface in an effort to gain additional oxygen (Johansson, 1997). The foraging responses of crabs in hypoxic water are diverse. *Callinectes sapidus* tend to feed less frequently and decrease the amount consumed during mild and severe hypoxia (Bell, et al., 2003b). While for other crabs, such as *Carcinus maenas*, hypoxia may not have as great an impact on foraging (Brante and Hughes, 2001).

The Dungeness crab, *Cancer magister*, is a commercially important species along the Pacific coast of North America. *C. magister* inhabits sandy and muddy bays and estuaries, where it can encounter hypoxic waters as low as 1.25 kPa (Airriess and McMahon, 1994; Bernatis and McGaw, 2004). Its physiological responses to hypoxia have been extensively studied. These alterations in physiological processes are thought to
enhance the ability of *Cancer magister* to cope with hypoxia (Airriess and McMahon, 1994; McGaw and McMahon, 2003). However, these experiments were performed on starved animals. Digestive processes place an additional burden on animals already attempting to supply tissues with adequate oxygen (McGaw, 2005a,b). Since these animals are highly mobile it follows that they may exhibit some behavioral mechanism, either moving to areas with higher oxygen tensions or reducing food intake during hypoxia, before resorting to more costly physiological mechanisms. Therefore, the aim of the present study was twofold: 1) determine if feeding varies under hypoxic conditions and, 2) investigate behavioral modification of postprandial *C. magister* in response to hypoxic conditions.
CHAPTER 2

MATERIALS AND METHODS

Adult male intermoult Dungeness crabs, *Cancer magister*, between 550-850 g were collected in Barkley Sound, British Columbia (Latitude 48°49" and Longitude 125°08"). The crabs were transferred to Bamfield Marine Sciences Centre and held in running sea water (31-32%) at a temperature of 10-12° C. Oxygen levels in the holding tanks were maintained at 16.8 kPa ± 1kPa. Crabs were acclimatized for one week prior to experiments and fed fish *ad lib* (*Lepidopsetta bilineata*) every other day. Animals selected for experiments were separated from the general population and starved for 2-3 days prior to experiments. This non-feeding period ensured that there was no food residue in the gut, but avoided the effects associated with long-term starvation (Ansell, 1973; Wallace, 1973). The water in the experimental tanks was acquired from the same source as the holding tanks. Nitrogen bubbled with ambient air was used to create hypoxic and gradient environments, and oxygen was bubbled with ambient air for higher oxygen regimes. Hydrographic measurements (dissolved oxygen, temperature, salinity) were taken using a YSI-85 DO-conductivity meter, prior to the start, and following completion of each experiment. During experiments each apparatus was surrounded with black plastic sheeting to minimize external disturbance, and all experiments were carried out in constant
dim light. All statistical analysis were carried out using SPSS statistical software. All data was tested for normality and in cases where data was non-normally distributed non-parametric tests were utilized.

The laboratory experiments were designed to serve two purposes: 1) to examine behavioural responses during hypoxia; this will be demonstrated in the feeding and gradient trials where presumably the crabs are able to choose their response; and 2) examine physiological responses to hypoxia, via egestion experiment in various oxygen tensions. Together, these experiments should provide greater insight into how *C. magister* regulates costly metabolic processes when exposed to hypoxic episodes.

The field experiments serve several purposes. First, as hypoxic waters become a greater threat to the marine environment, this mapping of DO and temperature in the benthic environment will be the most extensive completed in Barkley Sound to date of the sediment/water interface. Additionally, the field work will provide a natural response comparison to the laboratory gradient trials. Finally, the tracking of the crabs will provide information that confirms the frequent encountering of hypoxic environments by *C. magister* and support prior studies as to the mobility of the animal, while providing the short-term data that is currently missing from the literature.

Feeding in Hypoxia

This series of experiments examines the amount food consumption and time spent feeding by *C. magister* in altered oxygen levels. The hypothesis is that as oxygen decreases the amount of food consumed and the time spent feeding will also decrease. Crabs (n = 25 treatment) were held in individual chambers (45 cm x 45 cm x 20 cm depth)
in running aerated seawater and allowed to acclimatise for 4 h before experimentation. The oxygen level was changed during a 4h period from 16.8 kPa normoxic condition to required test treatments. This time period of 4 h was sufficient to allow changes in internal physiological parameters (McMahon, 2001). The crabs were then fed a fish meal (muscle only) of pre-determined mass. They were given one hour to commence eating, and allowed to continue eating *ad lib* until caseation of eating. When the crabs ceased feeding for 15 min, the food was removed and weighed for post-consumption mass. If a crab did not eat within 1 hour, the food was removed and the trial recorded as 0 g consumed. The amount of time spent eating was also recorded, with eating defined as the act of inserting food into the mouthparts (Johansson, 1997). Trials were carried out in seawater with dissolved oxygen levels of 21 kPa (100%), 15.75 kPa (75%), 10.5 kPa (50%), 5.25 kPa (25%), 3.15 kPa (15%), and 1.47 kPa (7%). All conditions were maintained with ±0.5 kPa.

Crabs that did not feed in low oxygen concentrations were rapidly returned to normoxic conditions by aerating the water and feeding activity was subsequently monitored. This allowed determination if feeding was in direct response to oxygen level (i.e. animals would not feed in hypoxia, but would feed in normoxia), versus any other cause (i.e. decrease in activity levels).

Crab mass varied by 300 g; therefore, to remove possible effects of animal mass (Stevens *et al*, 1982), the data was transformed by dividing the mass of food consumed by the mass of the animal. The amount of food consumed as well as the time spent feeding in each oxygen regime was analysed using a Kruskal-Wallis non-parametric ANOVA. Data
showing a significant effect were further compared using a Dunn's pair-wise comparison post hoc analysis.

**Postprandial Behavior in Hypoxia**

This series of experiments examines behavior associated with postprandial animals in the laboratory. As digestion places an additional demand on physiological systems, it is predicted postprandial crabs will seek higher oxygen levels than starved crabs; and secondly postprandial crabs will be less active than starved crabs.

A tank of 240 cm x 40 cm x 40 cm was used to monitor oxygen preferences of food deprived and postprandial crabs (n = 20). Bricks located along the length of the tank providing shelter for the crabs (McGaw, 2001), and defined areas for oxygen measurements. The tank was filled with running seawater (approx. 2.5 litres.min⁻¹) to a depth of 30cm. Air curtains were placed along the length of the tank (Fig. 1), and divided into sections. By manipulating the amount of nitrogen, ambient air and oxygen flowing into each air curtain, a gradient of dissolved oxygen of approximately 10 kPa difference could be maintained in the tank for several hours. The first set of trials used a gradient of 21 kPa (± 1 kPa) to 10.5 kPa (± 1 kPa). The second set of experiments used oxygen ranges from 10.5 kPa (± 1 kPa) to 2.5 kPa (± 1 kPa). Oxygen concentrations and the temperature of each area of the tank were recorded at the start and finish of each experiment (YSI-85 meter).

Twenty postprandial crabs (3 h post-feeding) were placed individually in random areas of the tank. The animals were allowed to acclimate in the apparatus for 15 min before the experiment began. Time spent in each area of the tank and activity patterns

10
were recorded for 1 hour using a time lapse video system (Panasonic AG-RT600AS VCR and WV-BP120 camera). The experiment was then repeated with 20 starved crabs (2-3 day starvation). The high and low oxygen areas were reversed between trials, to avoid any bias for specific areas of the tank. A control group of 20 crabs (starved 2-3 day) were tested in the tank at constant oxygen levels of 16.8 kPa. General linear model univariate analysis of variance test was used to compare the proportion of time the crabs spent in each oxygen concentration. Data showing significant effects were further tested using Tukey pair-wise comparison post hoc analyses.

Activity levels of starved and postprandial crabs were also analysed. If a crab remained motionless for more than 10 sec (time taken to walk through an area of tank) this was counted as inactivity. If the crab was motionless for less than 10 sec it was labelled as incidental data and was not used in the analysis. All other movement was counted as activity. Differences in activity level of starved and postprandial crabs in the high and low oxygen gradients were analysed using Mann-Whitney rank sum tests.

**Foraging Behavior**

This experiment is examining the foraging behavior of crabs when given a choice of oxygen concentrations. The hypothesis is that crabs will enter low areas of oxygen and feed, subsequently leaving the area for higher oxygen concentrations for digestive activity. Foraging behavior of starved crabs (2-3 days) was examined in the oxygen gradient apparatus using oxygen levels of 10.5 kPa to 2.5 kPa. Individual crabs (n = 32) were randomly placed in an area of the gradient and were given 15 minutes to acclimatize. Food (fish muscle) was then introduced into the tank by lowering it from the outside of
the plastic sheeting. Food was placed in the higher oxygen area (10.5 kPa) of the chamber for 16 trials. The experiment was then repeated with the food in the low oxygen area (2.5 kPa). Food handling and consumption and movement within the chamber were recorded for 1h using the time lapse video system. A control group of 16 crabs was tested using constant oxygen levels of 16.8 ± 1 kPa. For the control trials eight trials had food placed in one end, and then food was placed in the opposite end for another eight trials to ensure there was no preference for a certain area of the tank. The amount of time that crabs spent in each area when food was present and when no food was present in the gradient tank was analysed using the general linear model univariate analysis of variance test. Tukey’s HSD was used for post-hoc comparisons.

**Fieldwork**

The field work serves two aspects of the study: first it will allow tracking of starved and fed animals in their natural environments; second, it will provide hydrographic data for Bamfield Inlet, which has not been collected previously. Regarding the crab activity, it is predicted that starved crabs will not show a preference for oxygen concentrations and will exhibit greater activity levels than the postprandial crabs. Postprandial crabs will seek higher oxygen tensions and remain inactive for longer periods of time. Dissolved oxygen, temperature and salinity measurements were recorded during a number of tidal cycles in the Bamfield Inlet, British Columbia (Canadian Hydrographic, Chart 3671). The YSI-85 DO/salinity probe was attached to a circular weighted plate (0.5 m diameter) and lowered to the sediment. This ensured the probe was approximately 2 cm above the sediment surface, but did not sink into the sediment. Depth was recorded at
each location using a depth sounder (Lowrance 3500) and a position readings using a GPS were also taken.

The movements of fed and starved crabs (n=3 each) were also tracked in the field. Ultrasonic coded tags (IT85-2, Sonotronics Inc, Tucson, AZ) were attached to the carapace of each crab with epoxy glue. The animals were then allowed to settle in the holding tanks for 1 day prior to experiments. Three postprandial crabs (3 h post-feeding) and three starved crabs (2 d) were then released into Bamfield Inlet. The time between removal from the experimental tanks and release in the Inlet was less than 15 min. The crabs were located from a small boat using a DR-4 directional hydrophone and tracking receiver (Sonotronics Inc. Tucson, AZ). When maximal signal from the ultrasonic tag was obtained a GPS position was taken, as were dissolved oxygen, temperature and depth readings using the YSI-85 DO/salinity meter. The crabs were located at hourly intervals for 6 h, then at 24 h, 48 h and 72 h after release. The hydrographic parameters that starved and postprandial crabs experienced were compared using Repeated Measures ANOVA.

Statistical Methods

This data collected was then analysed using SPSS. All data was tested for normality, using the Shapiro - Wilk’s test at P = 0.05. When data were not normally distributed, the data was transformed using Arcsine transformation (Zar, 1999). If data could not be conformed to normality by transformation, original data were then analysed with the appropriate non-parametric test and post-hoc (see above). Data that was normal was analysed with the appropriate parametric test and post-hoc (see above).
CHAPTER 3

RESULTS

Feeding in hypoxia

Data reported here reflect a specific crab class, adult intermolt males, ranging from 550-850 g, and caution should be taken when extrapolating these results to other classes. Oxygen concentration had a significant effect on the amount of food that the crabs consumed (Kruskal-Wallis, P = 0.0001, Fig 2a). The greatest amount of food was eaten in 15.8 kPa, here crabs consumed 17.95 ± 1.94g of food. There was a steady decrease in food consumption at lower oxygen concentrations; with only 1.98 ± 0.6g of food consumed in 1.5 kPa. This level was significantly lower than the amount of food consumed by crabs in 5.3, 10.5, 15.8, 21 kPa (Dunn’s test, P < 0.05). Crabs in 3.2 kPa only consumed 6.37 ± 1.12g food. This was significantly less food than that consumed in 10.5 and 15.8 kPa.

The time spent feeding was also affected by oxygen concentration (Kruskal-Wallis, P = 0.0001, Fig. 2). Maximum feeding times of 89.4 ± 10.6 min were observed in 15.8 kPa; this time was significantly greater than that recorded in all other oxygen regimes, except 10.5 kPa. There was a trend towards a decrease in feeding time with decreasing oxygen levels. The lowest feeding time of 2.19 ± 0.52 min was recorded in 1.5 kPa. This
time was significantly less than feeding times recorded in 5.3, 10.5, 15.8 and 21 kPa (Dunn's test P < 0.05). Crabs in 3.2 kPa water, only fed for 14.35 ± 2.21 min, this was significantly less time than those observed in 10.5 and 21 kPa.

Not all the crabs fed in the lower oxygen regimes: 5 crabs in 5.3 kPa, 5 crabs in 3.2 kPa and 11 crabs in 1.5 kPa did not feed. However, 100% of these crabs fed within 30 sec of raising oxygen levels to 5.4 - 6.3 kPa. Additionally, as crab mass increased food consumption decreased proportional to crab mass (Fig. 3).

**Postprandial behavior in hypoxia**

In the high oxygen gradient (21 kPa - 10.5 kPa) fed and starved crabs exhibited similar behavior ($F_{3,1} = 0.67, P = 0.42, \text{Fig. 4a}$). Both fed and starved crabs, showed no significant preference for any area of the tank ($F_{3,1} = 2.1, P = 0.11$) spending mean times of 9.5 min to 23 min (Fed, SE ± 2.9-5 min) and 10 min to 21.5 min (Starved, SE ± 4.3-5.3 min) within each area of the tank. In the low oxygen gradient (10.5 kPa - 2.5 kPa) there was also no difference in preference between fed and starved crabs ($F_{3,1} = 0.19, P = 0.66, \text{Fig. 4b}$). However, both fed and starved crabs did show a preference for the highest oxygenated area of the tank ($F_{3,1} = 12.37, P = 0.0001$). The crabs moved to the highest oxygen area of the tank 10.1-11 kPa (Tukey test, $P < 0.05$) spending between 34.5 min and 29.3 min (SE ± 4.75 min and 5.25 min, fed and starved respectively) of the hour in this area.

There was no difference in activity levels of fed or starved crabs in the high oxygen gradient (Mann-Whitney, $U = 237.5, P = 0.85, \text{Fig. 5a}$) or low oxygen gradient (Mann-Whitney, $U = 432, P = 0.56, \text{Fig. 5b}$). In control conditions when oxygen levels were
maintained at $16.8 \pm 0.5$ kPa. Crabs did not show any significant preference for an area of the tank ($F_{3,1} = 0.55, P = 0.65$). The crabs would move around the tank during the first 15 min of the acclimation period, before settling in an area and exhibiting very little movement thereafter.

**Foraging behavior**

When feeding behavior was observed in an oxygen gradient (2.3-10.5 kPa), 22 of the 32 crabs fed in the oxygen gradient. When food was placed in the higher oxygen end (10.1-10.5 kPa) 13 of the 16 crabs entered into this area of the chamber and all of the crabs that entered into 10.1-10.5 kPa oxygen fed. Twelve of the crabs consumed the food in the area, only one crab moved the food to a lower oxygen regime to feed. In contrast, only 9 crabs entered the lower oxygen end of the gradient (2.3-2.7 kPa). Of these 9 crabs, only 6 actually fed. Two crabs remained in the 2.3-2.7 kPa to feed, while 4 crabs picked up the food and moved it to higher oxygen regimes to feed. At the end of the experiment 23 of the 32 crabs had moved to the highest oxygen area of the tank. In control conditions (15.8-16.8 kPa) when no gradient was present, 15 of the 16 crabs fed during the experiment and all fed in the area where the food was located. This presence of food did not alter the time that the crabs spent in each area of the gradient compared to when no food was present in the gradient ($F_{3,1} = 2.53, P = 0.114$).

**Fieldwork**

Oxygen, temperature, and salinity measurements were taken approximately 2-3cm above the sediment. The shallow water (< 3m) close to the shoreline was hyperoxic (Fig. 6a), with values as high as 28.5 kPa. Normoxic levels were reached at about 3m,
nevertheless several pockets of hyperoxic water (22 kPa - 23 kPa) were observed as deep as 5m. As depth increased there was a decrease in levels of dissolved oxygen. In the deeper areas (> 7m) water was hypoxic (< 13 kPa) with several areas in which water was nearly anoxic (< 1.5 kPa). Temperature varied from 21.1°C at < 1 m to a stable 12.1 °C at approximately 4 m (Fig 6b), while salinity was constant at 32 ppt ± 1 ppt at any given location.

Postprandial crabs exhibited very little movement during the first 48h after release (Fig 7a). Each of the 3 individuals was re-located within 10 m of the original release site. In contrast, the three starved crabs all moved away from their release site (Fig. 7b). The approximate distance covered by each of the three starved crabs within the first 6 h was 2,280 m, 130 m and 3,100 m, and assumed to be straight line movement. At the hourly intervals when crab position was tracked, starved crabs had moved into shallower, warmer (5.4 m ± 2.1 m SD, 13.1 °C ± 0.7 °C) water than postprandial crabs (7.1 m ± 1.8 m, 12.6 °C ± 0.7 °C) (RMANOVA P=0.005 and P=0.015 respectively). However, there was no significant difference in the oxygen regimes that starved (12.1 kPa ± 3.3 kPa SD) and postprandial crabs (11.9 kPa ± 2.5 kPa) experienced at each of the hourly measurement intervals (RMANOVA, P = 0.88). Starved crabs had moved from their previous locations when tracked at 24 h, 48 h and 72 h. Postprandial crabs were re-located in the vicinity of release site at 24 h and 48 h; it was only when tracked at 72 h that each of the 3 postprandial crabs showed significant movement (> 100 m) away from the release site.
DISCUSSION

Feeding in Hypoxia

Previous studies using *C. magister* have investigated food consumption with extensive focus on juvenile size classes or prey preference (Stevens *et al.*, 1982 and 1984, Juanes and Hartwick, 1990; Holsman, *et al.*, 2003). Numerous studies investigate predator-prey dynamics in hypoxia, examining variation in feeding behavior in relation to size of prey consumed or the rate of consumption (Mistri, 2004; Taylor and Eggleston, 2000; Hughes and Seed, 1995). Several studies have suggested that as crab size increases the amount of food consumed decreases proportionally (Holmsman, *et al.*, 2003; Stevens *et al.*, 1982). Results from the present study indicate the trend of a proportional reduction of food consumption continues through the lifecycle; the crabs in this study decreased their food intake proportional to an increase in crab size. This weak relationship may have resulted from a limited subject size in this study. Specifically we only examined ranging from 550 to 850 g and did not have an equal distribution of all crab sizes. This may partially be explained by changes in prey selection that occur with age. Nutrient densities of prey have been evaluated for juvenile *C. magister* (reviewed in Holmsman *et al.*, 2003), but there is no data available for adult prey selections. As *C. magister* is considered an
important predator species it follows that as they increase in size the prey available becomes more nutrient dense (Wardlaw and Kessel, 2002). As prey species become more complex, the level of vitamins, minerals, carbohydrates, lipids and proteins also increase; suggesting an adult crab is not required to consume vast amounts of prey but are able to obtain the required nutrients from smaller quantities but richer nutrient content prey.

Regardless of the size of the crabs, the decrease in oxygen concentration has significant effect on consumption quantities and the time spent feeding. Crabs in the current study were allowed to feed freely following a four hour acclimation time. McMahon and Wilkens (1975) showed the physiological parameters altered by exposure to oxygen stabilize in this time period and is ecological relevant in terms of the tidal cycle in Barkley Sound. McGaw (2005) found that following feeding heart rate increased significantly and remained elevated for 5-6 hours, returning to resting levels 10 hours after feeding. In addition there was a temporary increase in cardiac output and a prolonged increase in ventilation rate. When crabs were fed and exposed to hypoxia, animals showed a significant decrease in heart rate and stroke volume, but this drop was not as great as occurs in starved crabs. All parameters resumed to normal levels within one hour of being returned to normoxic conditions. Prior physiological experiments have forced meals of the same quantity but in a fraction of the time compared to free consumption rates into crabs to elicit responses. Because the effects of SDA are well documented (Wang et al., 1995), this practice of force feeding may elicit responses above the natural effects of a postprandial animal. By decreasing the amount of food ingested or slowing the rate of feeding may therefore limit the effects of SDA (Wang et al. 1995) ultimately decreasing
the physiological demands of the competing systems suggested by McGaw (2005). Future physiological studies may therefore need to consider the true impact of forced feeding and consider protocols similar to the natural environment.

The crabs used in this study showed a decrease in food consumption as oxygen levels decreased, and cessation of feeding at 1.5 kPa. The crabs that did not feed were rapidly returned to higher oxygen concentrations and in all cases began eating at approximately 5.4 kPa, which was reached within 30 seconds. While previous authors suggest a reduction in available energy as the reason for feeding decrease (Johansson, 1997), this study may suggest otherwise. The ability to return to feeding within 30 seconds following hypoxia may indicate the presence of external oxygen sensors allowing the animal to respond to sudden environmental changes without waiting for internal physiology, i.e. blood pH and oxygen concentrations, to return to normal. This external mechanism was suggested by McMahon and Wilkens (1975); the lobster Homarus americanus responded within 30 seconds to the introduction of oxygenated water across the gills with an increase in scaphognathite and heart beat and volume of water pumped through the branchial cavities, with internal mechanisms requiring several hours to return to normal. However, Legeay and Massabaum (1999) that found crabs maintain low blood oxygen levels regardless of external environment, again suggesting that some regulatory mechanisms are influenced by oxygen detected across the gill surface without regard to internal physiological status. While the possibility of a mechanism that responds quickly to environmental conditions exists this remains to be tested. As decreased activity has been attributed to changes in internal physiology (Johansson, 1997), this ability to return
to full activity levels and feeding may suggest that animals are not completely regulated by internal physiology but are responding to immediate environmental fluctuations. As tolerance to hypoxia is affected by a variety of perturbations, i.e. temperature, activity, molt stage (Herreid, 1980), the precise mechanism for long term adaptation will vary among species. *C. magister* is able to tolerate short term exposure to hypoxia (6 h) through a decrease in heart rate, maintaining aerobic metabolism and altering haemolymph flow (Airriess and McMahon, 1994). The ability to respond quickly to changes in oxygen, may support earlier work (reviewed in Diaz and Rosenberg, 1995) indicating that crustaceans are able to exploit food sources that move up and down the sediment layer in response to oxygen. Animals capable of a quick response would increase the time frame available to forage on susceptible prey, although this remains to be investigated.

During the feeding trials different food handling behaviors were noted as the oxygen levels decreased. In the lowest oxygen levels (1.5 kPa and 3.2 kPa) the crabs would hold the food under the thorax and handle the food with small motions; crabs also paused during feeding up to 12 minutes, several times during the hour long trial. In the higher concentrations the crabs extended the food in front of the body, used large tearing actions and discarded the food as feeding finished by tossing the food away. The pausing behavior in low oxygen has also been observed in *C. aestuarii* (Mistri, 2004), but the purpose of this behavior, i.e. protection from other predators or conservation of energy, remains unclear. While protection may be a possibility it seems unlikely, as *C. magister*’s tolerance is greater than conspecifics and are therefore less likely to be confronted by competitors in these environments. Unlike *C. sapidus* and *C. similis* that exhibit a
decrease in feeding at 15.8 kPa (Das and Stickle, 1993), *C. magister* exhibits the slowest feeding rate at 15.8 kPa (0.07 g/min) while all other concentrations are significantly faster (0.2 g/min). *C. magister* is also able to consume food at much lower oxygen concentrations as well as remain active. *C. sapidus* experienced mortality at 5.0 kPa (Das and Stickle, 1993). This may be explained by the difference in tolerance levels and the ability of Dungeness crabs to control digestion initiation (McGaw 2005). With this greater tolerance, *C. magister* would be able to increase their foraging time and locations by exploiting hypoxic waters.

Although the total amount of time spent in low oxygen concentrations by starved and foraging crabs was not significantly different, their behavior upon entering was noticeably different. Starved crabs in the low gradient (without the presence of food) tended to move around the perimeter of the area and exit or remain. However, crabs stimulated by the presence of food entered into the low gradient, procured the food and left with the food. There was one exception to this behavior as one of the crabs entering the low oxygen area fed in this location before exiting the area immediately following consumption. Prior studies indicate foraging tactics change with oxygen levels (Mistri, 2004; Brante, 2001), generally reducing the amount of prey consumed or decreasing the size of the prey consumed. However, in these studies the animals were not given the option to move the food to higher oxygen regimes. Hypoxic waters cause sediment dwelling organisms, such as molluscs and annelids, to migrate towards the water interface (reviewed in Diaz and Rosenberg, 1995), thereby increasing the amount of available prey for crabs. While a reduction of prey consumption may occur, it is also plausible to suggest
the animals may enter the hypoxic waters and move the prey to better feeding conditions in order to consume adequate amounts of energy (Pihl et al., 1992; Pearson et al., 1979). The food search gradient trails provided additional support to behavioral mechanisms when feeding in hypoxia. The ability to move food enables the animals to utilize their environment optimally; forage in locally hypoxic waters, and feed and digest in more suitable conditions. This behavior of moving prey to higher oxygenated locations prior to consumption lessens the risks, i.e. reduced motility, associated with lower energy availability. Data collected within Bamfield inlet confirms the presence of mobile localized hypoxic micro-pockets in which the crabs move through, providing a rationale for the ability to move food prior to consumption.

Postprandial Behavior in Hypoxia

There was no difference in activity levels between starved and postprandial crabs in either the low or high oxygen gradients, with an average of less than 50% of their time spent moving in the apparatus. The crabs were able to move the entire length of the tank multiple times within 1 minute (3 - 4 times) and therefore the position of the crab within the chamber was not dependent on its prior position and behavior was affected by changes in dissolved oxygen (Das and Stickle, 1994). Similarly, when H. vulgaris was exposed to long term hypoxia it did not exhibit a change in activity levels (Butler et al., 1978). This tolerance and ability to remain active may be partially explained through a re-prioritization of arterial blood flow. C. magister is able to adjust blood flow during hypoxia away from higher energy systems, i.e. digestive, and ambulatory, and divert to regulatory systems such as nervous tissue (McGaw, 2005). Therefore, although
movement activity is reduced, the increase in energy required to increase ventilation may suggest a transfer of energy not a reduction of activity.

Although the time of movement activity was not different between starved and postprandial crabs, the behavior observed was different. Starved crabs entering the low oxygen area of the tank moved through the area exploring the corners before leaving the area or choosing to remain in the area. All of the postprandial crabs exhibiting activity in the low oxygen concentrations frequently tried to break the air/water interface presumably attempting to increase oxygen intake (Taylor et al., 1977). The subsequent behavior consisted of longer periods in higher oxygen concentrations before moving back into the low concentrations, if they moved back in at all.

Hypoxic environments are generally avoided by fish and crustaceans (Renaud, 1986; Peterson and Peterson, 1990; Pihl et al. 1991). Behavior in an oxygen gradient with C. sapidus demonstrated similar results to the high oxygen gradient experiments in the present study: C. sapidus did not show an avoidance of hypoxia (Das and Stickle, 1994). C. sapidus showed no preference for any level of oxygen and but demonstrated similar erratic movements suggesting escape behavior (Das and Stickle, 1994) as was noticed by postprandial C. magister in the low oxygen gradient trials of this study. While such environments can be tolerated and are often not avoided by the crabs, behavior does change presumably in an attempt to increase the uptake of available oxygen. This suggests one of two explanations: 1) that physiological compensatory mechanisms may have limitations, or 2) behavior modification reduces the necessity for physiological modifications.
Feeding and digestion places additional energy demands on animals, moving to areas of higher oxygen would decrease the demands placed on the respiratory and circulatory systems thereby reducing the overall metabolic stress. Physiological changes associated with digestion vary among species (Ansell, 1973; Legeay, and Massabuau 1999; McGaw and Reiber, 2000). In *C. magister* the increase in heart rate is observed almost immediately and reaches a max at 5-6 hours after feeding (McGaw, 2005). In *Carcinus maenas* and *Callinectes sapidus* oxygen uptake increases by a factor of 2 after feeding. Heart rate and ventilation remain elevated for 16-18 hours and oxygen uptake remained elevated for 40-50 hours (Legeay and Massabuau, 2000; McGaw and Reiber, 2000). McMahon and Wilkens (1975) suggested a period of oxygen debt repayment after prolonged exposure to hypoxia. In the event the crab is digesting during the hypoxic exposure, the oxygen debt may become even greater, extending recovery time and reducing energy available for activities. However, the effect of SDA has yet to determined for adult *C. magister* but has been estimated to elicit a 20% increase of metabolic rate in subadult crabs (Holmsman, 2003). Determining the precise metabolic effect of feeding and digestion will demonstrate the role behavior plays in coping with these competing systems in less than adequate environments.

Although the focus of this study was hypoxia, unique behaviors were also noted in the highest oxygen levels. Crabs exposed to 100% oxygen tended to ignore the food, or eat for short periods during the experiment, coupled with excessive movement generally in an attempt to climb out of the test chamber or constant pacing of the chamber, and were more aggressive throughout the trials assuming an offensive posture and chelae.
movements. During all other trials, the crabs were observed to move through the testing area for several minutes, and then settle in one location until the introduction of food at which time they may have moved slightly, or when when finished feeding the food would be discarded and the animal would move to a new location to settle. This behavior is different from that expressed by *Carcinus maenas* which showed increased motor activity when exposed to a gradual changes in hypoxia (Taylor *et al.*, 1977). This difference in behavior may occur due to the congregating tendencies of *C. magister* during feeding in which cannibalism is a common occurrence (Stevens, *et al.*, 1984).

**Fieldwork**

In estuary environments the shallow waters are often hyperoxic, due to algal blooms and vascular plants releasing oxygen, and surface water run-off entering the system. (Rabalais, 2004). These shallow waters, with higher oxygen concentrations increase predator and prey distribution as organisms seek refuge from hypoxic waters (Bell and Eggleston 2005; Pihl *et al.* 1992). This change in distribution may lead to choices of feeding or defending from other predators, resulting in erratic behavior and decreased foraging. This change in behavior has been evaluated in fish, determining that if adequate energy for consumption is available, the risk to feed outweighs the risk of predation (Abrahams and Dill, 1989; Gilliam and Fraser, 1987). The behavior observed during the feeding trials of 21kPa saturated oxygen concentrations may be normal for *C. magister* in these locations as they attempt to feed as well as maintain a defensive posture. The layer of hyperoxic waters in Bamfield Inlet extends 2-3 m, with occasional pockets down to 5 m; however most crabs were captured in deeper water (8 m) which was usually
12 - 15 kPa and therefore will probably not come into contact with hyperoxic water that frequently.

Although a portion of the *C. magister* habitat in Bamfield Inlet has high oxygen levels, the crabs are predominately found at greater depths in the inlet which in turn indicates lower oxygen levels. The field measurements were taken 2-3cm above the sediment-water interface which is approximately the same height as the Milne-Edwards openings in which the crabs take in water (personal observation). The oxygen concentrations 2-3 cm above the sediment varied across the inlet from 15.8kPa to 1.5kPa, and fluctuated throughout the tidal cycle. As *C. magister* is a crab that routinely buries in the sediment, measurements were also taken at the sediment/water interface and the water was found to be anoxic. This range and dispersion of oxygen requires the animals living here to be tolerant and opportunistic which *C. magister* is both.

Hypoxic and anoxic waters are becoming more prevalent and are influenced by human activity (Rabalais, 2004). In Bamfield Inlet the animals are subjected to waste disposal by the community (solid waste removal only) as sewage is dumped into the environment. Personal observation of the area has noted a much lower number of organisms residing in areas adjacent to sewage pipes emptying into to inlet, a process known to deplete oxygen concentrations (Rabalais, 2004). *C. magister* habitats in Washington are subjected to dredging activities increasing the frequency and severity of hypoxic conditions (Stevens et al., 1984). Barkley Sound is the source water for Bamfield Inlet, and it contains logging transfer facilities, another source of increased hypoxia and hydrogen sulfide (DFO, 2002). Therefore animals in this are will frequently encounter
Crabs moved throughout the inlet and starved crabs covering distances up to 1-3 km during the first 6 hours, while fed crabs remained relatively motionless for the first 48 hours. The method used for measuring distance travelled is only an approximation as it assumes straight line movement, and relocation with a hydrophone was not 100% accurate. However, it is of interest to note that the fed crabs and starved crabs exhibited different results than obtained in the laboratory experiments where no significant difference in activity levels was observed. Part of this difference may be explained by the confines of the tank apparatus or the fact that postprandial crabs will bury (McGaw, 2004) and the crabs in the tank may have remained active because they were searching for an adequate location for digestive processes.

Although there was no significant difference in oxygen readings in the field for which fed and starved crabs came into contact with, oxygen data acquired over time indicates the presence of hypoxic zones. Therefore during their movement starved crabs undoubtably encounter hypoxic environments. The starved crabs were also found in shallower waters under fishermans’ docks, presumably searching for food, and movement was tracked through numerous eel grass beds in the inlet, known to be the preferred habitat (Butler, 1984), making exposure to these low oxygen zones probable.

Conclusions

*C. magister* may be one of the more hypoxia tolerant species. This is not unexpected as *C. magister* remains buried in the sediment for up to 48 hours where anoxic conditions are common (McGaw, 2004). Nevertheless feeding in hypoxia may pose an
additional burden for animals trying to maintain adequate oxygen uptake. Crabs may use physiological mechanisms to alleviate this problem. For example *C. magister* has the advantage of not fully committing to digestion after feeding by altering arterial haemolymph flow to digestive structures and possibly conserving oxygen to be used by other systems (McGaw, 2005). However the present study as shown that behavioral responses may be the first mechanism used to balance the demands of these simultaneously competing systems.

While *C. magister* may be highly tolerant of hypoxia, ideal oxygen conditions for this population have been determined and should be considered during future work. As feeding status is often a factor in physiological studies, providing appropriate oxygen conditions during maintenance of the animals and during experimentation. The results of this study provided a range of 10.5 to 15.8 kPa for optimal foraging activity. While this is appropriate for this population, it is important to determine this value for crabs in any habitat.

Fisheries are beginning to struggle on numerous coasts as a direct result of hypoxia. The value of the *C. magister* industry lends itself to the need of a full understanding of the organism. With the increasing destruction of their habitat understanding the mechanics from a genetic to whole organism should become a focus of future research. Specifically, mapping the genome would provide additional insight into population differences among habitats as well as providing information into the basic understanding of the physiological mechanisms allowing the animal to survive such diverse conditions.
Hypoxic zones are becoming more widespread and lasting for longer periods of time. Although the crabs may be able to survive in these environments, the behavior exhibited also suggests a possible failure to thrive. The crabs consume less food which ultimately results in less energy available for biological functions, such as growth and reproduction. As a key species acting as both predator and prey, protection and understanding of *C. magister* ranges is crucial to ensure the maintenance of these systems. A loss of the species would not only create a disruption in the habitats but also an economic crisis for those relying on the crab fishery. Clearly further research is needed to understand the mechanisms and behavior by which *C. magister* is able to tolerate hypoxic environments as many organisms rely on their presence to maintain healthy ecosystems.
REFERENCES


APPENDIX I

FIGURES

36
Figure 1: Diagram of apparatus used for oxygen gradient experiments. A constant flow of seawater (2.5 litres/min) maintained the salinity at 31-32% and the temperature at 10-12°C. Separate air curtains placed along the length of the tank allowed varying mixes of nitrogen, ambient air and oxygen to be infused into the tank. Bricks divided the tank into compartments, but also provided corners into which the crabs could retreat.
Figure 2a: Feeding behavior of 25 Cancer magister in dissolved oxygen concentrations of 1.5kPa to 21kPa, wet mass of food (g) consumed. Values are means ± SEM.

<table>
<thead>
<tr>
<th>kPa</th>
<th>1.5</th>
<th>3.2</th>
<th>5.3</th>
<th>10.5</th>
<th>15.8</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>q = 2.33</td>
<td>p &gt; 0.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>q = 4.13</td>
<td>p &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>q = 4.13</td>
<td>p &lt; 0.001</td>
<td>q = 1.83</td>
<td>p &gt; 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>q = 5.75</td>
<td>p &lt; 0.001</td>
<td>q = 1.60</td>
<td>p &gt; 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.8</td>
<td>q = 6.54</td>
<td>p &lt; 0.001</td>
<td>q = 2.39</td>
<td>q = 0.79</td>
<td>p &gt; 0.20</td>
<td>p &gt; 0.50</td>
</tr>
<tr>
<td>21</td>
<td>q = 4.65</td>
<td>p &lt; 0.001</td>
<td>q = 2.33</td>
<td>q = 0.48</td>
<td>q = 1.14</td>
<td>q = 1.94</td>
</tr>
</tbody>
</table>

Table 1a: Level of significance in feeding consumption of crabs. Matrices indicating significant differences in food consumed. Q values and p-values are given; bold indicate significance.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Figure 2b: Feeding behavior of 25 *Cancer magister* in dissolved oxygen concentrations of 1.5 kPa to 21 kPa, time spent feeding (min). Values are means ± SEM.

<table>
<thead>
<tr>
<th>kPa</th>
<th>1.5</th>
<th>3.2</th>
<th>5.3</th>
<th>10.5</th>
<th>15.8</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>q = 2.93</td>
<td>p &gt; 0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>q = 3.76</td>
<td>p &lt; 0.005</td>
<td>q = 1.17</td>
<td>p &gt; 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>q = 6.31</td>
<td>p &lt; 0.001</td>
<td>q = 3.71</td>
<td>p &lt; 0.001</td>
<td>q = 2.53</td>
<td>p &gt; 0.10</td>
</tr>
<tr>
<td>15.8</td>
<td>q = 8.21</td>
<td>p &lt; 0.001</td>
<td>q = 5.62</td>
<td>p &lt; 0.001</td>
<td>q = 4.41</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>21</td>
<td>q = 4.30</td>
<td>p &lt; 0.001</td>
<td>q = 1.68</td>
<td>p &gt; 0.50</td>
<td>q = 0.50</td>
<td>p &gt; 0.50</td>
</tr>
</tbody>
</table>

Table 1b: Level of significance in feeding time of crabs. Matrices indicating significant differences in time feeding. Q values and p-values are given; bold indicate significance.
Figure 3: Feeding behavior of *Cancer magister* as determined by crab mass. Data represents the mass of the crab versus the standardized proportion of food consumed (n = 146).
Figure 4a: Behavior of starved crabs (hatched bars) and postprandial crabs (open bars) in a dissolved oxygen gradient. Data represents mean times (± SEM) that 20 crabs spent in each area of the gradient; high oxygen gradient of 10.5 kPa to 21 kPa. There was no significant preference of an area between fed and starved crabs.
Figure 4b: Behavior of starved crabs (hatched bars) and postprandial crabs (open bars) in a dissolved oxygen gradient. Data represents mean times (+ SEM) that 20 crabs spent in each area of the gradient; low oxygen gradient of 2.5 kPa to 10.5 kPa. Both fed and starved crabs preferred the 10.1 - 10.5 kPa area ($F_{3,1} = 12.37$, $P = 0.0001$), otherwise no preference was found.
Figure 5a: Activity of starved crabs (hatched bars) and postprandial crabs (open bars) in a dissolved oxygen gradient. Data represents mean time moving (± SEM) 16 crabs spent in each area of a high oxygen gradient 10.5 - 21 kPa. There was no significant difference in movement activity between fed and starved crabs.
Figure 5b: Activity of starved crabs (hatched bars) and postprandial (open bars) in a dissolved oxygen gradient. Data represents mean time moving (± SEM) that 20 crabs spent in each area of a low oxygen gradient of 2.5 - 10.5 kPa. There was no significant preference between fed and starved crabs.
Figure 6a: Hydrographic measurements as a function of depth (m) in Bamfield Inlet, British Columbia. Dissolved oxygen (kPa) measurements taken at approximately 2 cm above the sediment surface at varying states of the tidal cycle during July - August 2004 (n = 177).
Figure 6b: Hydrographic measurements as a function of depth (m) in Bamfield Inlet, British Columbia. Temperature (°C) measurements taken at approximately 2 cm above the sediment surface at varying states of the tidal cycle during July - August 2004 (n = 177).
Figure 7a: Movement of *Cancer magister* in the Bamfield Inlet, British Columbia; crabs were fitted with ultrasonic tags and tracked at hourly intervals for 6 h, 24 h and at 48 h after release. Data shows the position of each crab after set time intervals (each number denotes time in h after release). Movement of starved crabs D-F, (n = 3) during 6 h after release. If a certain time is not shown on figures that individual crab could not be located at that time interval.
Figure 7b: Movement of Cancer magister in the Bamfield Inlet, British Columbia; crabs were fitted with ultrasonic tags and tracked at hourly intervals for 6 h, 24 h and at 48 h after release. Data shows the position of each crab after set time intervals (each number denotes time in h after release). Postprandial crabs A - C (n = 3), rings around release site show that the crabs were located in the same area, movement away from the release site is shown at 48 h. If a certain time is not shown on figures that individual crab could not be located at that time interval.
### Table 2: Average hydrographic measurements, and distance traveled by the starved and fed crabs during the first 6 hours of field behavior tracking. Starved crabs experienced significantly shallow, warmer water \((5.4 \text{ m} \pm 2.1 \text{ m SD}, 13.1 ^\circ \text{C} \pm 0.7 ^\circ \text{C})\) than postprandial \((7.1 \text{ m} \pm 1.8 \text{ m}, 12.6 ^\circ \text{C} \pm 0.7 ^\circ \text{C})\) (RMANOVA \(P=0.005\) and \(P=0.015\) respectively). There was no significant difference in the oxygen regimes that starved \((12.1 \text{kPa} \pm 3.3 \text{kPa SD})\) and postprandial crabs \((11.9 \text{kPa} \pm 2.5 \text{kPa})\) (RMANOVA, \(P = 0.88\)).

<table>
<thead>
<tr>
<th></th>
<th>Oxygen</th>
<th>Temperature</th>
<th>Depth</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved 1</td>
<td>9.9 kPa</td>
<td>12.6 °C</td>
<td>7.5 m</td>
<td>2.3 km</td>
</tr>
<tr>
<td>Starved 2</td>
<td>13.3 kPa</td>
<td>13.1 °C</td>
<td>5.4 m</td>
<td>.13 km</td>
</tr>
<tr>
<td>Starved 3</td>
<td>14.8 kPa</td>
<td>13.8 °C</td>
<td>3.3 m</td>
<td>3.1 km</td>
</tr>
<tr>
<td>Fed 1</td>
<td>10.6 kPa</td>
<td>12.6 °C</td>
<td>7.3 m</td>
<td>&lt; .01 km</td>
</tr>
<tr>
<td>Fed 2</td>
<td>11.3 kPa</td>
<td>11.7 °C</td>
<td>7.4 m</td>
<td>&lt; .01 km</td>
</tr>
<tr>
<td>Fed 3</td>
<td>13.4 kPa</td>
<td>13.1 °C</td>
<td>6.8 m</td>
<td>&lt; .01 km</td>
</tr>
</tbody>
</table>
VITA

Graduate College
University of Nevada, Las Vegas

Jennifer Lynn Bernatis

Home Address:
7611 French Springs Street
Las Vegas, Nevada 89139

Degrees:
Bachelor of Science, Exercise Science, 1994
Fort Hays State University

Master of Science, Exercise Physiology and Nutrition, 1996
Fort Hays State University

Master of Science, Biology, 2005
University of Nevada, Las Vegas

Publications:
Bernatis, J.L. and McGaw, I.J. (2005). Dungeness crabs use behavior to minimize the physiological costs of feeding and digestion in hypoxia. Society for Integrative and Comparative Biology S35.3
Thesis Title: Behavioral Ecophysiology of the Dungeness Crab During Feeding and Digestion in Hypoxia

Thesis Examination Committee:
Chairperson, Dr. Shawn Gerstenberger, Ph. D.
Co-Chairperson, Dr. Paul Schulte, Ph. D.
Committee Member, Dr. Chad Cross, Ph. D.
Committee Member, Dr. Steve deBelle, Ph.D.
Graduate Faculty Representative, Dr. Dan Allen, Ph. D.