THE BURYING BEHAVIOR AND ANAEROBIC RESPONSE OF TWO CANCRID CRAB SPECIES: *METACARCINUS MAGISTER* AND *CANCER PRODUCTUS*

by

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ABSTRACT

Metacarcinus magister and *Cancer productus* are two species of cancrid crabs which routinely bury into sediment and thus encounter hypoxic conditions for extended periods of time. Both species are known to utilize physiological and behavioral adaptations in order to cope with hypoxic environments. Hemolymph samples were taken from individuals exposed to experimental and *in situ* conditions and analyzed for lactate. Almost all the crabs had measureable amounts of lactate in their hemolymph in both normoxic and hypoxic conditions in field and lab experiments. The crabs also buried deeper into the sediment as oxygen levels fell, possibly in an attempt to save energy.

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INTRODUCTION

In all animals the energy needed for metabolic processes is obtained through catabolic oxidations. In catabolism high energy molecules are broken down into simpler molecules, releasing energy for use in all the other metabolic processes of the cell. Catabolism is typically a two-step process: glycolysis, followed by the Krebs cycle and the electron transport chain (ETC). Although glycolysis releases some metabolic energy anaerobically, the Krebs cycle and the ETC yield the majority of useful energy. Oxygen is vital to these processes as the Krebs cycle and ETC cannot proceed unless oxygen is available as the final electron acceptor (Horton et al., 2002). For this reason animals which remain active for more than brief bursts do so primarily by the use of aerobic metabolism.

Animals living in marine environments often encounter significant variations in oxygen availability. In some of these situations, adequate levels of oxygen are not available to support aerobic metabolism. Oxygen diffuses less readily through water than through air and regions with decreased oxygen levels are common in aquatic environments (Dobson and Frid, 2009). These regions include areas which have long been isolated from any oxygen sources, such as oxygen minimum zones and the stagnant waters at the bottoms of some fjords and ocean basins. Also, oxygen may become depleted in areas where it is rapidly used up and only slowly replaced. Such areas include sediments – especially fine sediments such as sand or mud, hydrothermal vents, and hypoxic zones below regions of high productivity and eutrophication (Diaz and

Rosenberg, 1995). Due to the slow rate of oxygen diffusion from water into sediment, sandy or muddy sediments are often anoxic (Nakamura and Stefan, 1994). Organisms inhabiting these areas must have mechanisms to generate the energy required for survival under low levels of oxygen.

Several metabolic compensatory mechanisms are utilized by animals which encounter low oxygen environments. Some species (e.g., mollusks and annelid worms) are quite adept at anaerobic metabolism. They are able to use alternative anaerobic pathways which result in the excretion or defecation of harmful byproducts and some species may even be capable of obtaining a little more energy anaerobically from food than is available through glycolysis alone (Schöttler and Bennet, 1991, Zwaan, 1991). However, there is still relatively little net energy gleaned through these pathways and these animals are relatively inactive.

Active, rapidly moving species tend to have a higher rate of metabolism than less active organisms of comparable size do (Vernberg, 1982). Such animals typically have little capacity for extended anaerobic metabolism and usually have no special metabolic pathways for detoxifying anaerobic byproducts nor for extracting extra energy anaerobically from food sources. As a result, these animals usually cannot live for more than a few minutes without oxygen. Being motile, these animals usually either avoid areas low in oxygen or die if trapped within them. Arthropods are among the most active and motile marine animals, potentially allowing them the opportunity to leave unfavorable environments when the supply of oxygen is inadequate. However, several bathypelagic crustaceans do inhabit water with very low oxygen concentrations and many

species of decapod crustaceans bury themselves in soft sediment (Bellwood, 2002). Because sediments usually range from hypoxic to anoxic, benthic crustaceans living on sediments often encounter low oxygen conditions and must adjust to them. If these species remain on the surface of the sediment they are in direct contact with the oxygenated water. If they burrow, they may pump water into the burrow to aerate it, maintaining a constant flow of oxygen-rich water.

In hypoxic conditions some crustaceans are able to produce enough energy by glycolysis and lactate formation to sustain low metabolic rates and survive at least for a time (Albert and Ellington, 1985). Some motile species, such as some *Cancer* crabs, have been shown to display an increase in motility in an apparent attempt to move to a normoxic area (Cook and Boyd, 1965; Bernatis et al., 2007). Others, however, regularly bury themselves in the sediment (Bellwood, 2002), which could potentially expose them to long-term hypoxia or anoxia.

Metacarcinus magister, the Dungeness crab (Figure 1), is a very common benthic crab in the Pacific Northwest and is commercially captured using traps set in deep water. Members of this species may grow to 20 cm wide at the carapace. The slightly smaller *Cancer productus*, the Red Rock crab, which may have a carapace up to 15 cm wide, is a common low intertidal to subtidal species (Figure 2). It is not commercially exploited like *M. magister* due to its heavy shell. This omnivorous crab feeds on barnacles, smaller crabs, and dead fish. Both these crab species are found in relatively shallow subtidal and even intertidal water in sandy or muddy bays especially where there is a good growth of eelgrass (Kozloff, 1993, Curtis and McGaw, 2008).

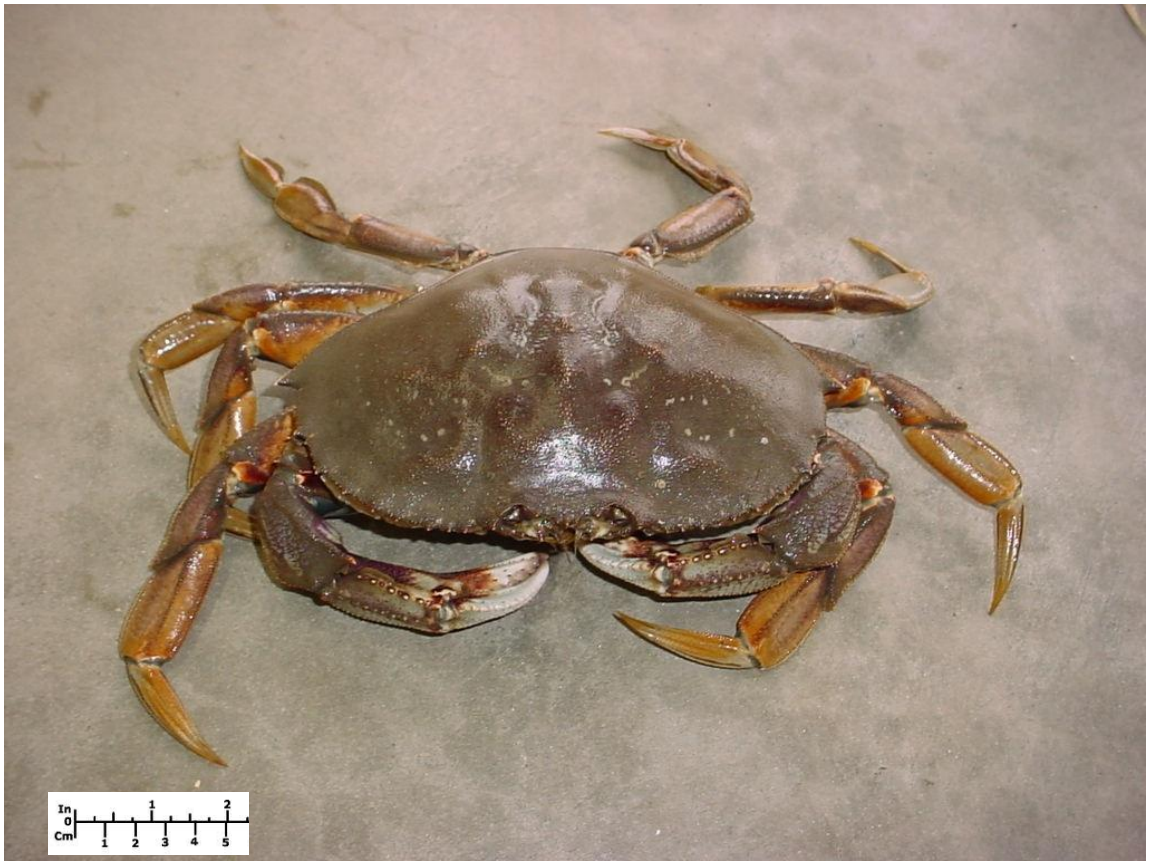

Figure 1. *Metacarcinus magister* is a large, edible crab which is abundant along the Northwest coast of the United States. (Photograph by David Cowles)

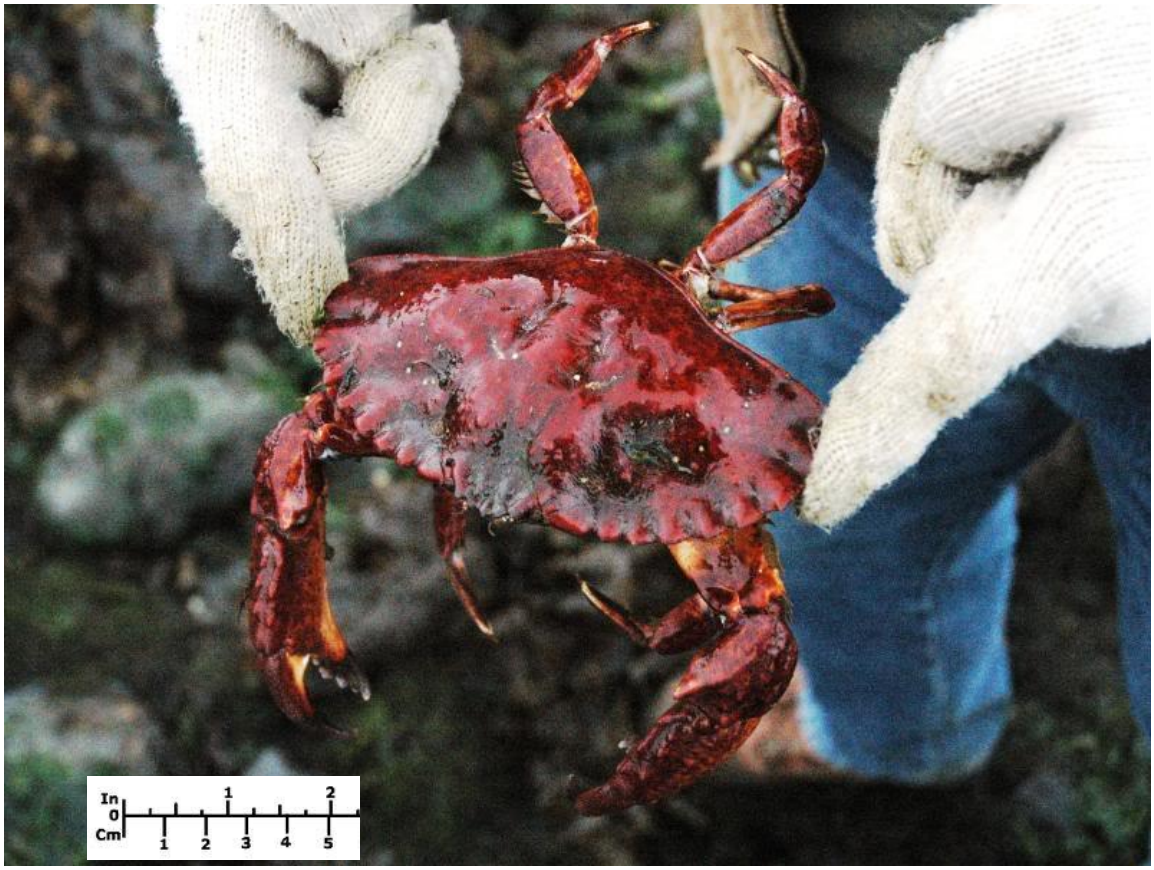

Figure 2. *Cancer productus* is a slightly smaller species of cancrid crab, also abundant in the Pacific Northwest. Individuals of this species can be quite aggressive when agitated. (Photograph by Melinda Kutzner)

To obtain oxygen, these species maintain ventilation through their branchial gill chamber by taking water in through the Milne-Edwards openings at the base of the chelae and legs (Figure 3). The water enters from below through the Milne-Edwards openings, passes over the gills, and out through the exhalant openings adjacent to the mouth parts, driven by the beating action of scaphognathites. *M. magister* and *C. productus*, however, are known to bury into the substratum with little more than their eyes and antennae showing (McGaw, 2004) (Figure 4). While buried, the Milne-Edwards openings at the base of the legs are buried well under the sediment, and due to the slow rate of water interchange through sand and general hypoxic conditions within sediment, these crabs should be subjected to low levels of oxygen during this time. Perhaps to compensate for this, shallow-burying species like *M. magister* and *C. productus* have been shown to fold their chelae close to their body when buried to form a slight open space in front of them known as the exostegal channel (Figure 4). This channel would likely only facilitate the expulsion of water, however, not intake of oxygenated water (McGaw, 2005).

Female crabs of both species may be subjected to further degrees of hypoxia. Several weeks before the female molts the male and female enter into the pre-mating embrace during which the female is tucked under the male and oriented upside-down so that their abdomens touch and their heads face the same direction. Males with females tucked underneath them can be found buried under the sediment together with the female fully covered with sand or nearly so (Figure 5). How she is able to obtain enough oxygen to support her metabolism while in this position are not clear.

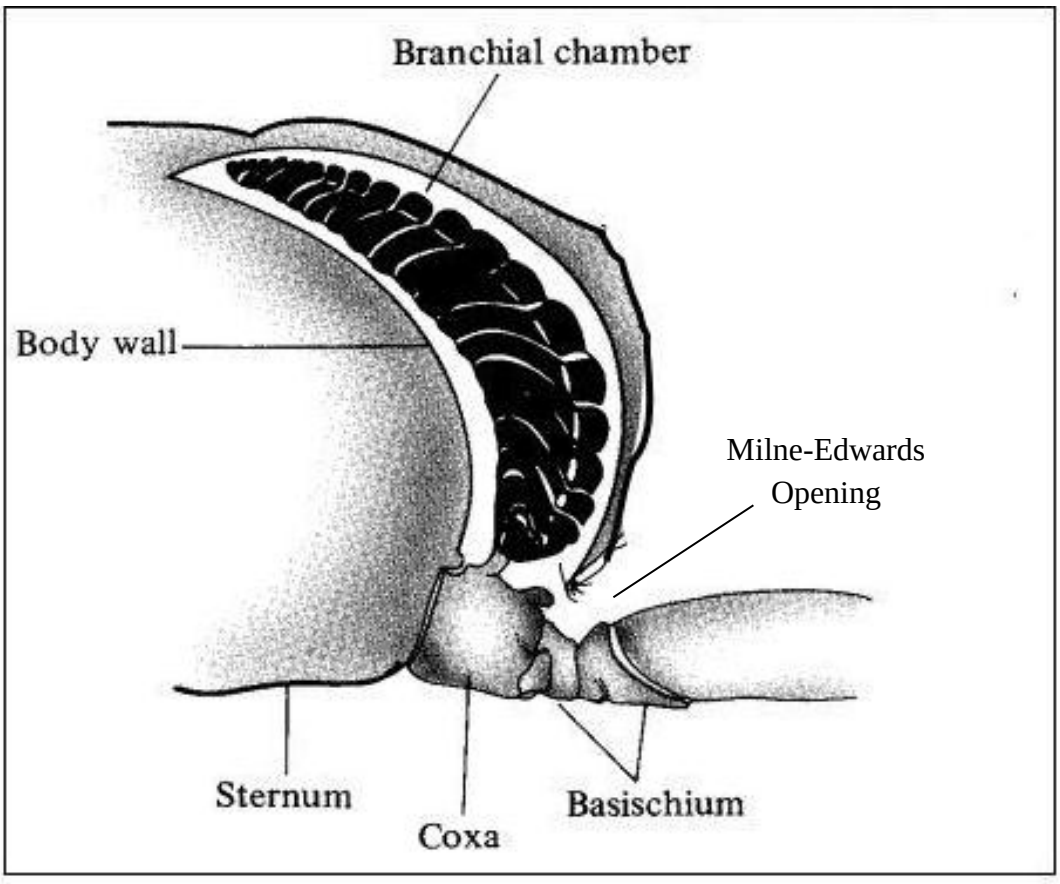

Figure 3. Cross section through the gill chamber of a typical crab. This image shows the gill extending from the coxa of the limb into the branchial chamber. Adapted from Cumberlidge (1999).

Figure 4. *Cancer productus* buried in the sand. The exostegal channels (EC) are visible. (Photograph by Melinda Kutzner)

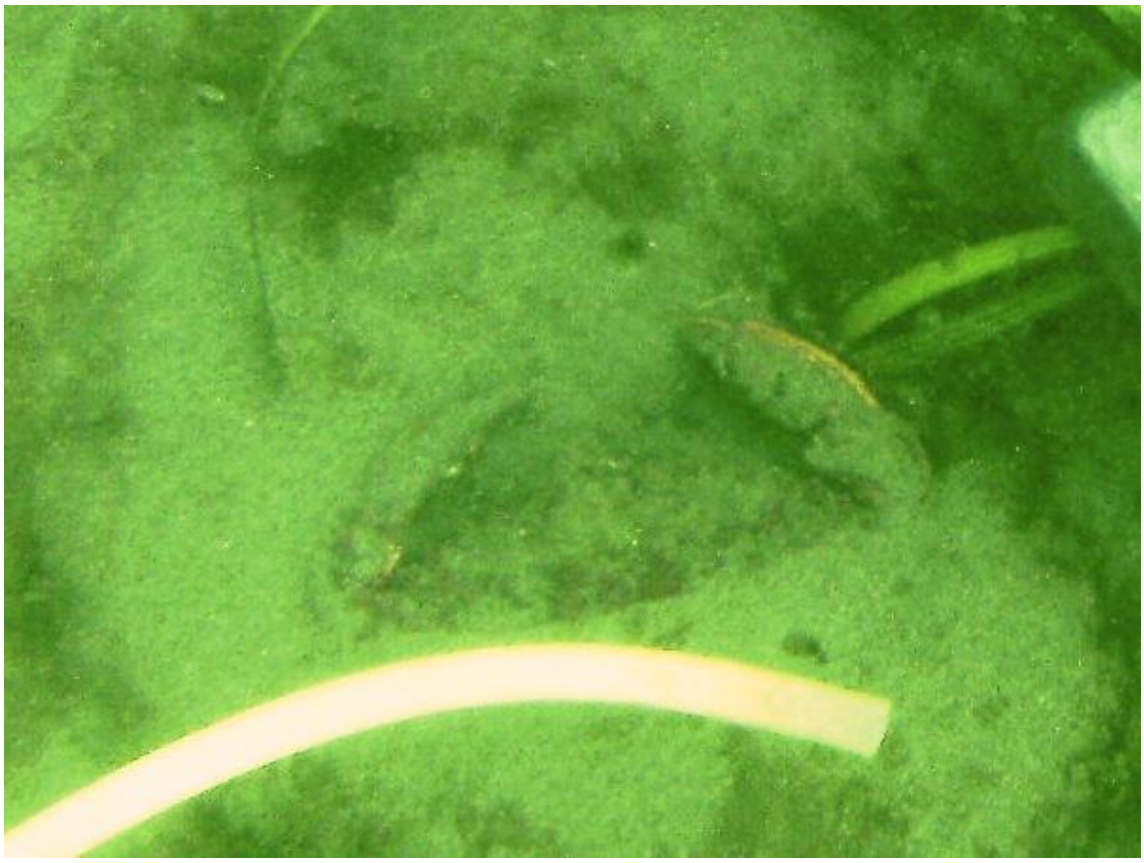

Figure 5. Male *Cancer magister* with a smaller female tucked underneath in the sediment. (Photograph of experiment in Rosario Bay by Melinda Kutzner)

The purpose of this research was 1.) to investigate whether *M. magister* and *C. productus* avoid conditions of anoxia or hypoxia, 2.) to measure the extent of buildup of toxic anaerobic metabolites in the blood during burial, and 3.) to test whether hypoxia limits the length of time the species can remain buried. It was my hypothesis that burying in the sediment blocks *M. magister*'s and *C. productus'* access to oxygenated water and leads to metabolic hypoxia or anoxia. Because of this, I hypothesized that lactate levels would accumulate in *M. magister*'s and *C. productus'* blood while they were buried, limiting the time they could remain so. Furthermore I predicted that the extent of burial of *M. magister* and *C. productus* would be reduced if oxygen became even less available.

MATERIALS AND METHODS

Field Tests

Seven *Metacarcinus magister* and nine *Cancer productus* were found on sandy substrate between depths of 6-9 meters by SCUBA divers during June-August of 2011. For each of these, a 46 cm^2 x 5 cm tall acrylic cover was placed over them on the sea bottom where they were found and the edges all around the cover were sealed by pressing sediment over them. This cover had an internal volume of 7.95 liters. Two different cover types were used. One was sealed to block all circulation of oxygenated water to the crab so that the crab's respiration would deplete the oxygen from the water. The second was identical except that it contained a number of holes to allow water circulation and maintain oxygen at higher levels. A digital camera (SeaLife® DC800 Underwater Camera) with time-lapse capabilities was mounted on a tripod over the acrylic box and recorded still photographs at a rate of one shot per minute for about an hour while the divers waited at the surface. A small buoy was attached to the acrylic box and the tripod in order to facilitate relocation of the equipment. At the end of the hour the divers returned to the experimental setup and noted whether the crab had remained buried or emerged from the sediment. Thirty ml of water was then extracted via a port from the water the crab was exposed to inside the cover to be tested for oxygen content. The crabs were captured and taken to the surface where the sex of the crab was determined and 0.4-0.8 ml of hemolymph was extracted from the base of the 5^{th} (rear) walking leg using a 3 ml syringe. The crab, water sample, and hemolymph sample were taken ashore.

The crabs were placed into a running seawater table in the lab at ambient temperature and salinity. Water samples were analyzed using a YSI® 550A oxygen electrode within 1 hour of collection. Hemolymph samples were placed in 1 ml microcentrifuge tubes and frozen within 15 minutes of collection at -18 ºC.

Lab Tests

A total of 37 crabs, 24 *M. magister* and 13 *C. productus*, including the crabs used in the field tests, were brought to the lab and held in running seawater tanks at ambient seawater temperature, salinity, and pH for laboratory tests. The tanks were 80 x 50 x 25 cm high and had 5 cm of Rosario Bay sediment on the bottom. No more than 8 crabs were kept a tank at a time. Crabs where given 24 hours to acclimate to the laboratory conditions before the lab experiment began. Data were not counted for a crab if it had been disturbed within the past 5 minutes, such as at the beginning of the experiment or if it had been seen engaging in an aggressive interaction with another crab.

Correlating Hemolymph Lactate Levels to Extent of Hypoxia

In order to calibrate hemolymph lactate levels with low environmental oxygen levels, nitrogen gas was bubbled through a column of water which was then pumped into one of the crab holding tanks to create a hypoxic condition inside the tank. An acrylic cover, cut to the dimensions of the holding tank, was placed on the surface of the water of this tank to eliminate oxygen diffusion from the air into the water in the tank. The second

tank served as an oxygenated control. The groups of crabs within each of these tanks were then subjected to differing degrees of oxygen saturation for a period of about 1 hour. The levels of oxygen saturation used were near full saturation (at least 90%), 50% saturation, and 17% saturation, as determined by a YSI® 550A oxygen electrode and meter. Approximately 0.5 ml of hemolymph was extracted from each of these 37 crabs at the end of the hour, placed in 1 ml microcentrifuge tubes, and frozen within 15 minutes at -18 °C.

Determination of Lactate Concentration

Frozen hemolymph samples were transported to Walla Walla University for analysis. Samples were transported in a large cooler filled with ice to prevent thawing and possible degradation of lactate. The presence of lactate in the hemolymph samples was determined by oxidation with lactate dehydrogenase (LDH) (Gutmann and Wahlfeld, 1974). NAD^+ was added to the assay to initiate the oxidation process as seen in the following equation:

$$\text{Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

Assuming complete reaction, the amount of lactate present in the sample was equal to the amount of NADH produced by the reaction. The formation of NADH was measured by an increase in absorption at 340 nm (Gutmann and Wahlfeld, 1974). For analysis, 0.5 ml of hemolymph was deproteinized in 1 ml of 1 N perchloric acid. In order

to neutralize the solution 0.6 ml of 5 M potassium carbonate and 2.5 ml hydrazine/glycine buffer (0.5 M glycine, 0.4 M hydrazine, pH 9.0) were added. 200 µl of NAD^+ solution (40 mM β-NAD^+) was added next. Initial absorbance (E_1) was measured and 0.02 ml of LDH suspension (5 mg of enzyme/ml) was added to initiate the oxidation reaction. Final absorbance (E_2) was measured after samples were incubated at 37° C for 2 hours (Gutmann and Wahlfeld, 1974). In an alternate assay, lactate levels were measured directly using the FaCT® Lactate Pro portable lactate analyzer, which was designed to measure lactate in athlete's blood. The two methods gave similar results and only the results from the LDH method outlined above are reported here.

Determination of the Extent of Burial

Experimentation on extent of burial took place at the Rosario Beach Marine Laboratory. The same 37 crabs mentioned earlier were used for this study in the same tanks. The crabs readily buried themselves into the tank sediment. Time lapse camera surveillance was used to monitor burial and emergence behavior and movements of these crabs during daylight hours, while the oxygen concentration of one of the tanks was reduced to 50% or 17% of air saturation. A digital camera (SeaLife® DC800) took pictures every minute of all the crabs in both tanks for a period of 0.5 to 5 hours or until the camera SD card filled up. Later these photographs were reviewed and the burial extent of each crab was recorded. Each of these burial extents for an individual crab constituted one lab record, so a single photograph contained as many records as it did crabs. For recording the burial extents I established a scale which ranged from 0 to 7

(Table 1). A 0 on the scale indicated a crab completely unburied or moving on the surface of the sediment. A crab sitting on the sediment was given a 1. A 2 was given to crabs with just their chelipeds partially buried. A 3 was specified for crabs with their legs mostly buried and sediment present on the carapace. Crabs that had their entire chelipeds buried and their carapace mostly buried were given a 4. Crabs that had their carapace completely buried under the sediment with grooves around the anterior and lateral edges of the carapace were given a 5. A 6 was given to crabs who were completely buried with an established exostegal channel around the anterior portion of the crab. A 7 was given to crabs who were completely buried and out of sight. At one minute intervals the number of crabs which had changed location since the last minute was also noted. These data were used to compare the relationship between the oxygen level in the tanks (90%, 25%, and 17%) and the average extent of burial and relative amount of movement by the crabs in the tanks.

Statistical Analyses

One-way ANOVA was used to compare species burial extent at different air saturations. Recently disturbed individuals or those in a premating embrace were excluded from the main analysis. In a second analysis the burial extent of clutching male and clutched female *C. productus* in a premating embrace were compared to one another and to single males by one-way ANOVA. If significant differences were found, a

Table 1. Summary of the scale of burial extent ranging from 0 to 7 assigned to *Metacarcinus magister* and *Cancer productus*.

Numeric Assignment	**Description**
0	Crabs moving on the surface of the sediment
1	Crabs sitting on the sediment
2	Crabs with cheliped partially buried
3	Crabs with legs mostly buried and sediment present on the carapace
4	Crabs with chelipeds completely buried and carapace mostly buried
5	Crabs with carapace completely buried and with grooves around the anterior and lateral edge of the carapace
6	Crabs are completely buried with an established exostegal channel around the anterior portion of the carapace
7	Crabs buried and completely out of sight

Tukey post hoc test was run among groups. In some of the comparisons between two groups a t-test was used instead. A significance level of $p \leq 0.05$ was used for all the statistical tests. An arcsine transformation was used to transform proportions before analysis. Standard deviations were indicated by the ± symbol.

RESULTS

Burial Extent and Hypoxia

In both lab and field tests, both species spent a substantial portion of their time partially buried in the sediment. In the field, burial extents ranged from 1-5 both at the beginning and at the end of the observations. In the 5293 lab records of undisturbed crabs there was also abundant evidence of crabs burying at least partly (to an extent of 2 or more), which would result in a decrease in the flow of oxygenated water to their gills. *Cancer productus* buried more often than *Metacarcinus magister* did (Chi-square = 487.7, df = 1, $p < 0.001$). *C. productus* buried at a score of 2 or greater in 605/667 lab records (91.7%) (Figure 6A), while *M. magister* buried at a 2 or greater in 2057/4563 records (45.1%) (Figure 6B).

The two species differed significantly in their extent of burial at three oxygen pressures tested, with *M. magister* consistently burying to a lesser extent than did *C. productus* (Figure 7). Both species buried to a greater degree under hypoxic conditions than under normoxic conditions (Figure 7). There were not enough data obtained from the field studies to determine significance, however data gathered in the lab at 90% oxygen saturation (normoxia) showed *C. productus* was buried at a mean extent of 3.4 (± 0.8) on the burial scale while *M. magister* buried at a mean of only 1.7 (± 1.5) (T-test, F = 46.4, df = 4039, $p < 0.001$). At 17% oxygen saturation (deep hypoxia) *C. productus* buried at a mean extent of 3.8 (± 1.6) and *M. magister* buried at a mean extent of 2.7 (± 1.4) (T-test, F =4.9, df = 857, $p < 0.001$) (Figure 7). For both species, the extent to which

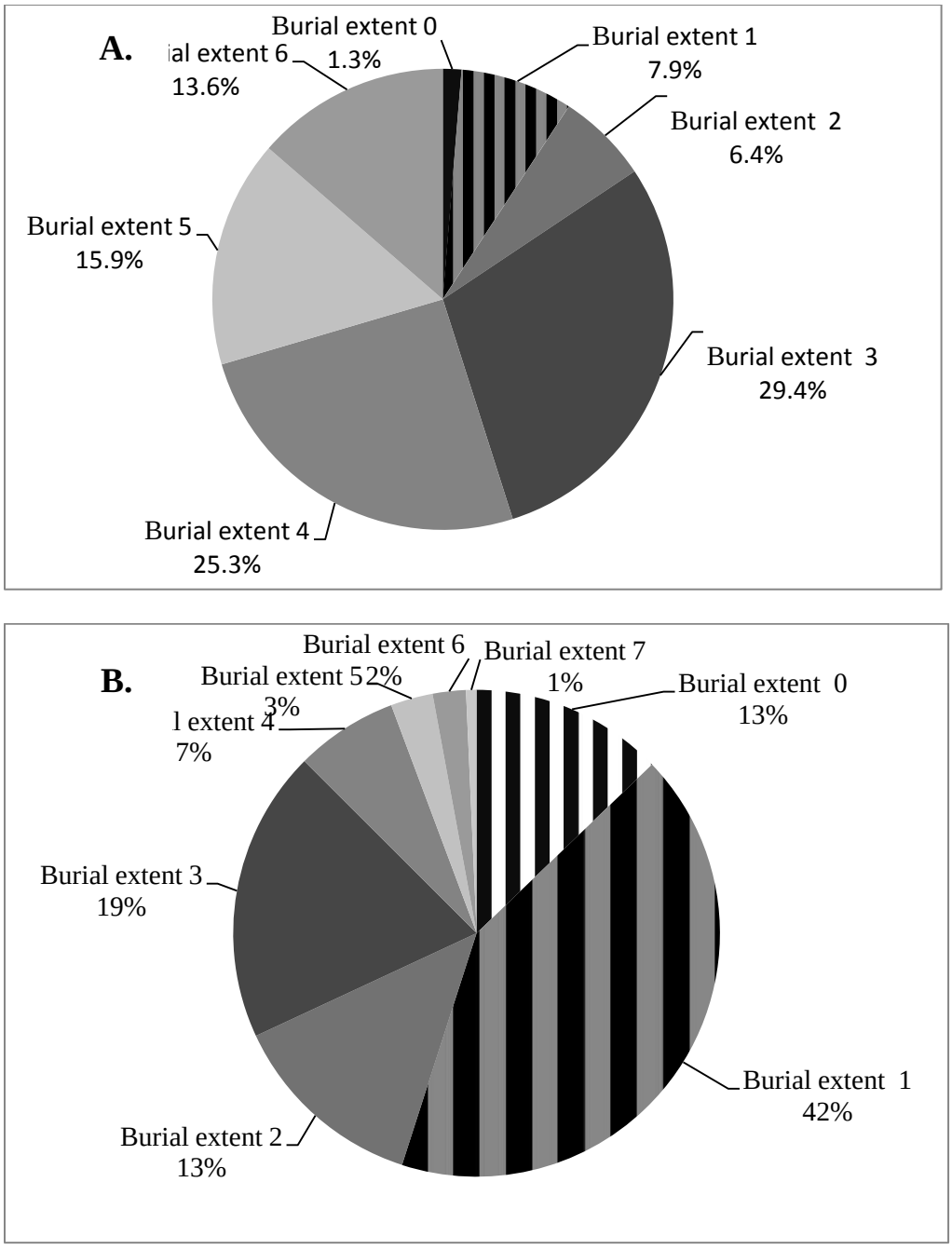

Figure 6. Percentage of time spent in the different stages of burial for crabs in the lab. A. *Cancer productus*, B. *Metacarcinus magister*. Striped areas indicate burial extents which do not restrict flow of oxygenated water through the gills, while areas without stripes represent burial degrees which likely restrict flow of water.

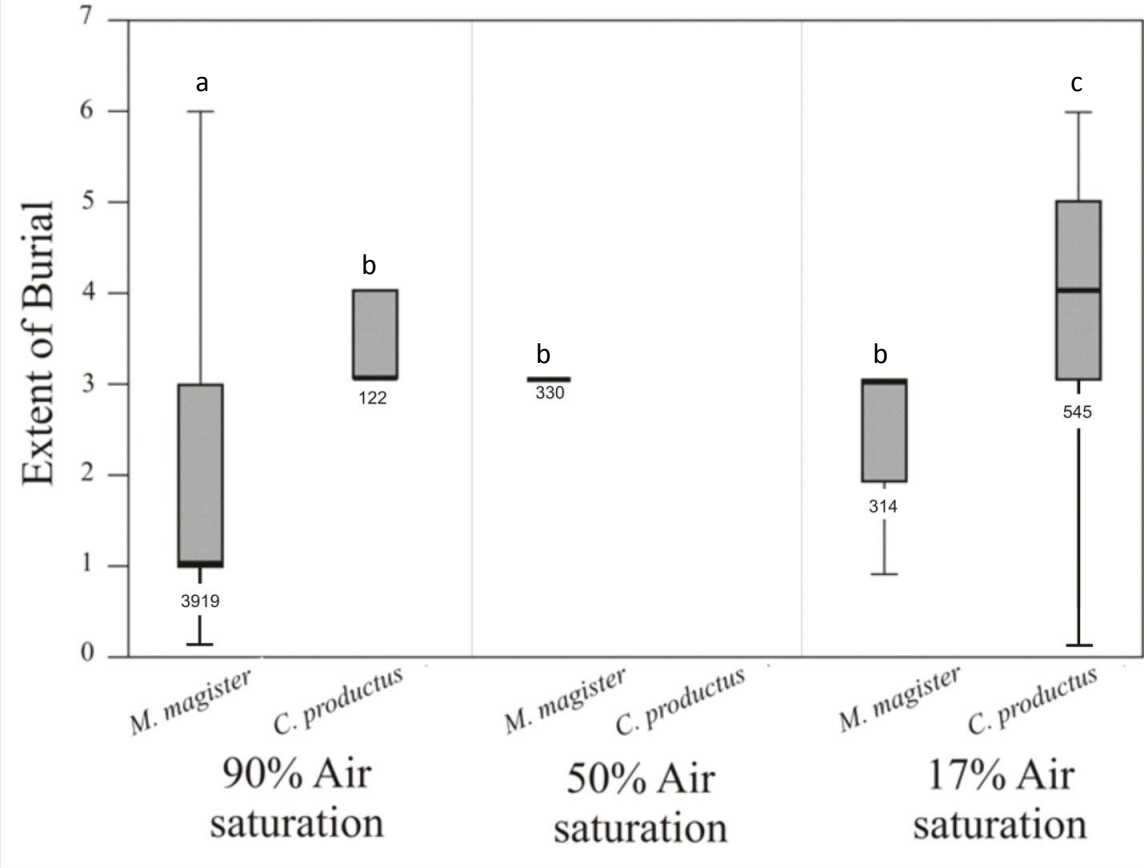

Figure 7. A comparison of burial extent between *Cancer productus* and *Metacarcinus magister* under differing degrees of hypoxia in the laboratory. In this experiment, 90% air saturation is considered normoxic, while lesser percentage saturations are hypoxic. Box height indicates the interquartile range while black bars indicate the median. Error bars designate the full range of the data. Different letters indicate groups that are significantly different from each other. The number below each boxplot represents the number of records for that measurement. See table 1 for definitions of burial extents.

they buried themselves also increased significantly with decreasing levels of ambient oxygen. For *C. productus*, the increase in burial extent during hypoxia of single crabs was significant at the 0.05 confidence level (ANOVA, $F = 8.2$, $df = 1, 665$). *C. productus* males clutching females in a mating embrace buried to a lesser extent than did single crabs both at normoxia (ANOVA, $F = 5.1$, $df = 1, 141$, $p < 0.05$) and in the deep hypoxia of 17% air saturation (ANOVA, $F = 88.6$, $df = 1, 661$, $p < 0.001$) (Figure 8). Females clutched in a mating embrace were buried to a greater extent than was the male clutching them, but they also were buried more deeply than single crabs were both at 90% air saturation and at 17% air saturation (Figure 8). For *M. magister*, the increase in burial extent during hypoxia of single crabs was significant at the 0.001% confidence level (ANOVA, $F = 178.3$, $df = 1, 4560$, Figure 7). No *M. magister* in a pre-mating embrace were found and measured.

Not only did the crabs bury to a greater degree under hypoxic conditions, they also became more quiescent resulting in less movement. One-way ANOVA was used to compare the proportion of crabs that changed positions in the tank per minute as a proportion of the total number of crabs present in the tank. Disturbed individual crabs and crabs engaged in a premating embrace were excluded from this analysis. Under normoxic conditions about 23% of crabs moved per minute. As oxygen levels decreased to 50% oxygen saturation or below the percentage of crabs moving in the tanks fell to 10% or less, a highly significant difference (ANOVA, $F = 56.6$, $df = 3, 1073$, $P < 0.001$) (Figure 9).

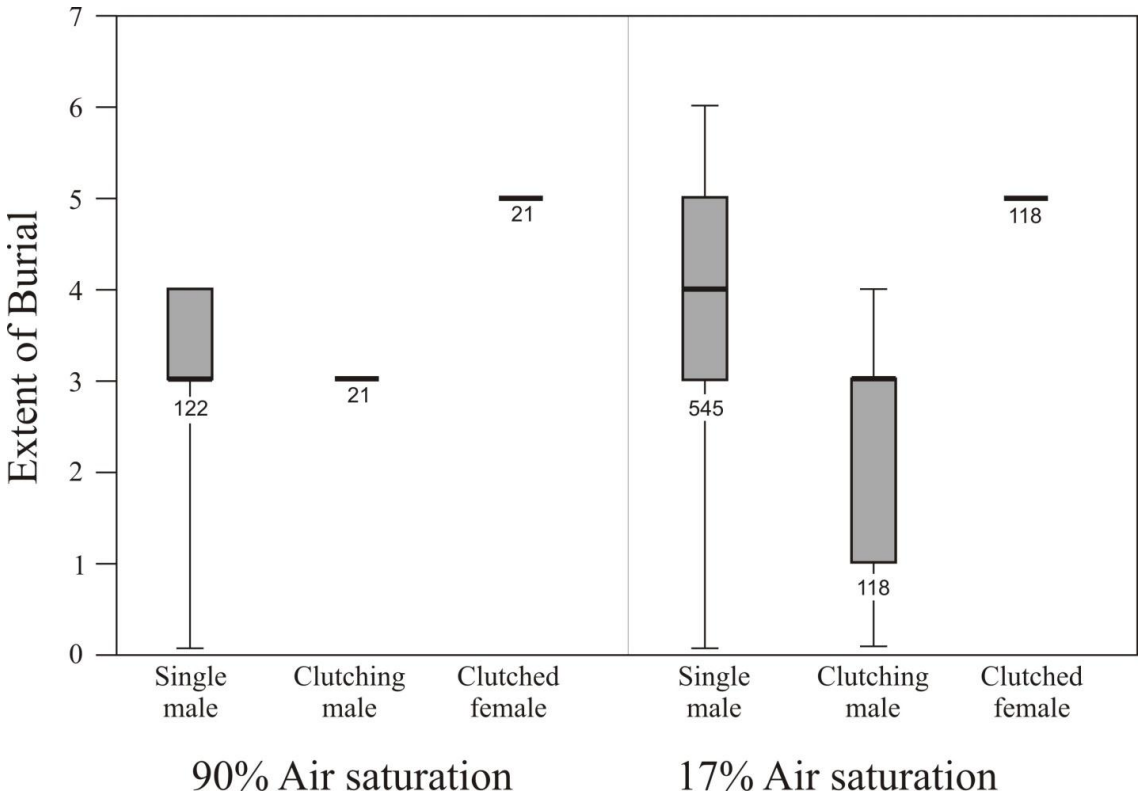

Figure 8. Comparison of single male, clutching male, and female *C. productus* under normoxic (90% air saturation) and hypoxic (17% air saturation) conditions. Box height indicates the interquartile range while black bars indicate the median. Error bars designate the full range of the data. Different letters indicate groups that are significantly different from each other. The number below each boxplot represents the number of records for that measurement.

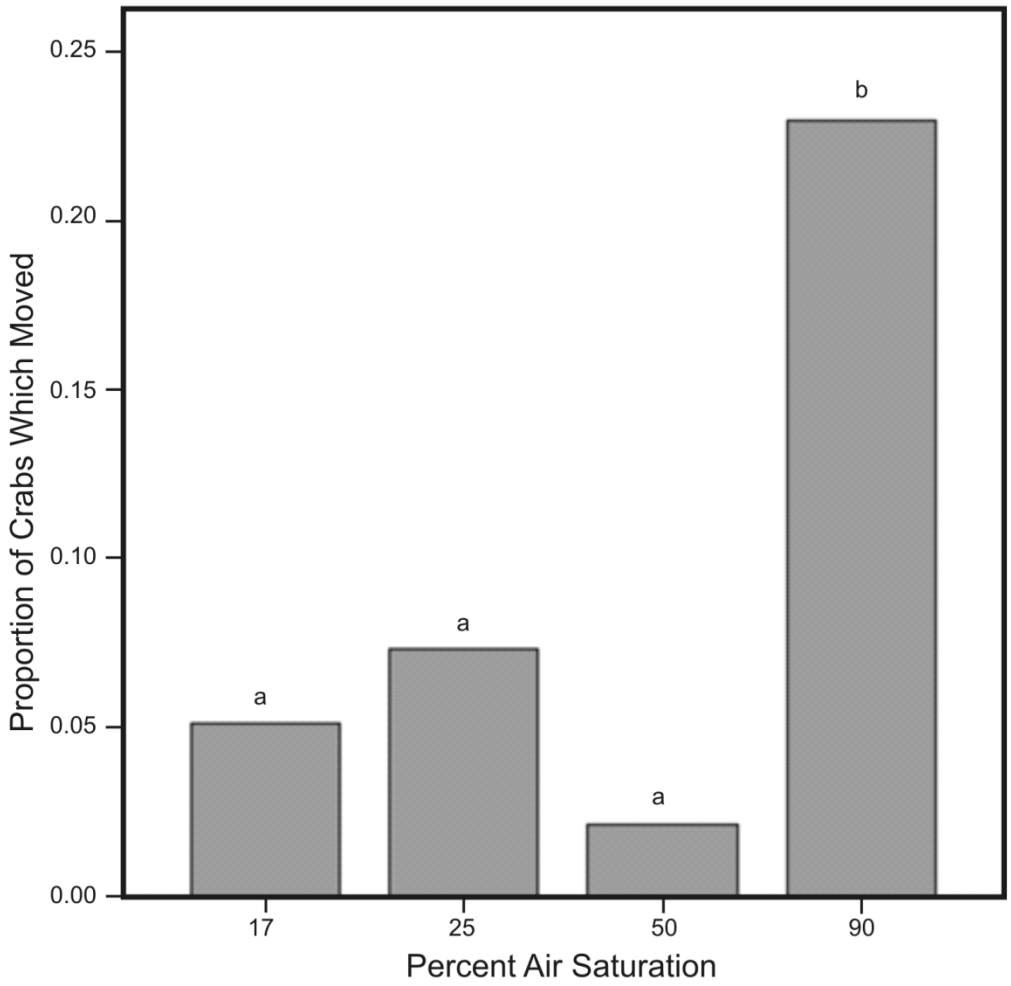

Figure 9. A comparison of the proportion of crabs which changed location versus ambient air saturation (ANOVA, $F = 56.6$, $df = 3, 1073$, $P < 0.001$). Different letters indicate groups which are significantly different from each other.

Hemolymph Lactate Levels and the Extent of Hypoxia

Lactate is the most quantitatively important and measureable product of anaerobic metabolism in most crustaceans (Zebe, 1991; McFadden, 2004). Consequently, an increase in hemolymph lactate concentrations as oxygen levels decreased was expected. The trend which actually occurred in the blood of the two experimental crab species, however, was more complex than this. Lab experiments showed that elevated lactate is a common feature for these crabs even in normoxic conditions. Most of the crabs, whether or not they were buried, had measurable amounts of lactate in their blood (Figure 10). The amount of lactate present ranged from 0.016036 to nearly 0.04 mg/ml (Figure 11), but was not significantly correlated with the extent of burial the crabs in the field had been at during the field experiment nor with the level of hypoxia to which they had been exposed in the lab. However, in crabs from both the lab and the field, blood lactate levels ranged slightly higher in crabs which had been exposed to hypoxic water during the experiment (Figure 10). This difference was not significant.

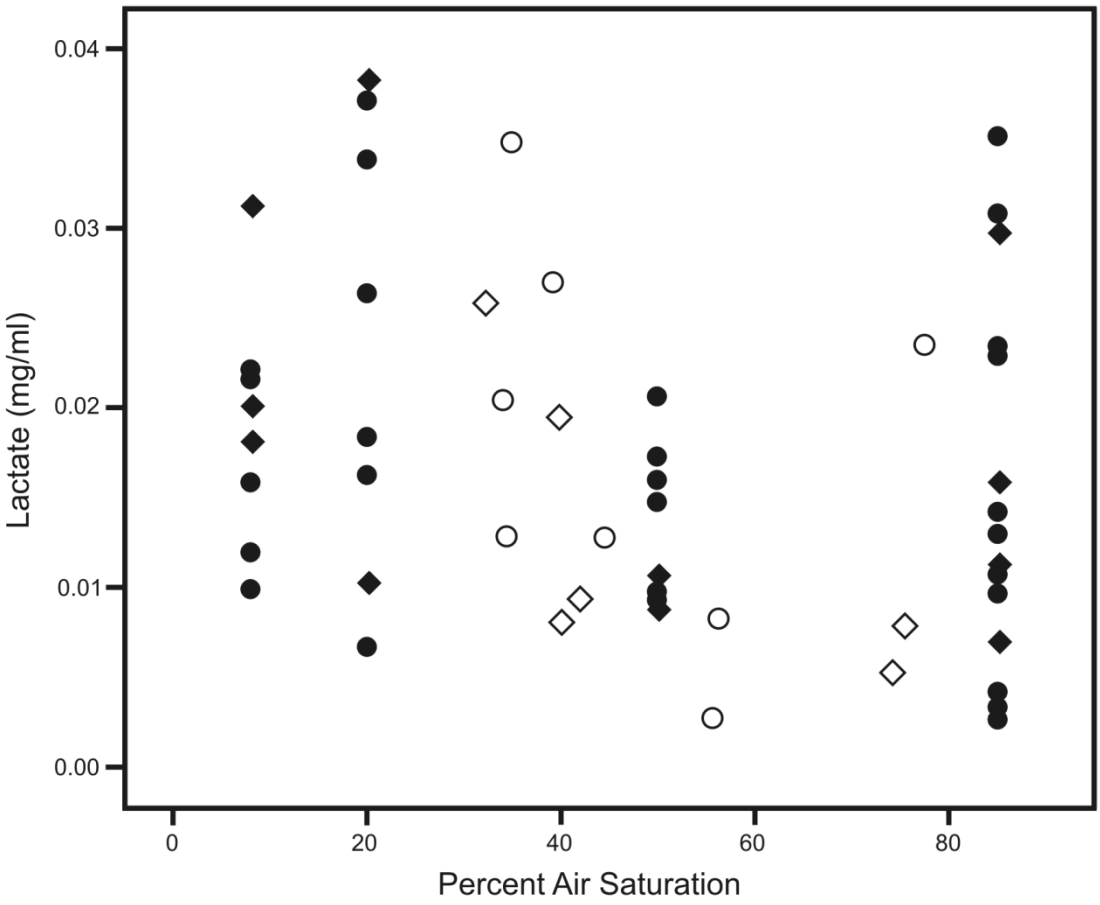

Figure 10. Hemolymph lactate levels as a function of percentage of air saturation the crabs were exposed to for *C. productus* (diamonds) and *M. magister* (circles) in the field and laboratory experiments. The highest range of lactate levels is seen in individuals exposed to hypoxic conditions of less than 90% air saturation, although the trend is not significant. Black symbols represent lab experiments while gray symbols indicate field experiments.

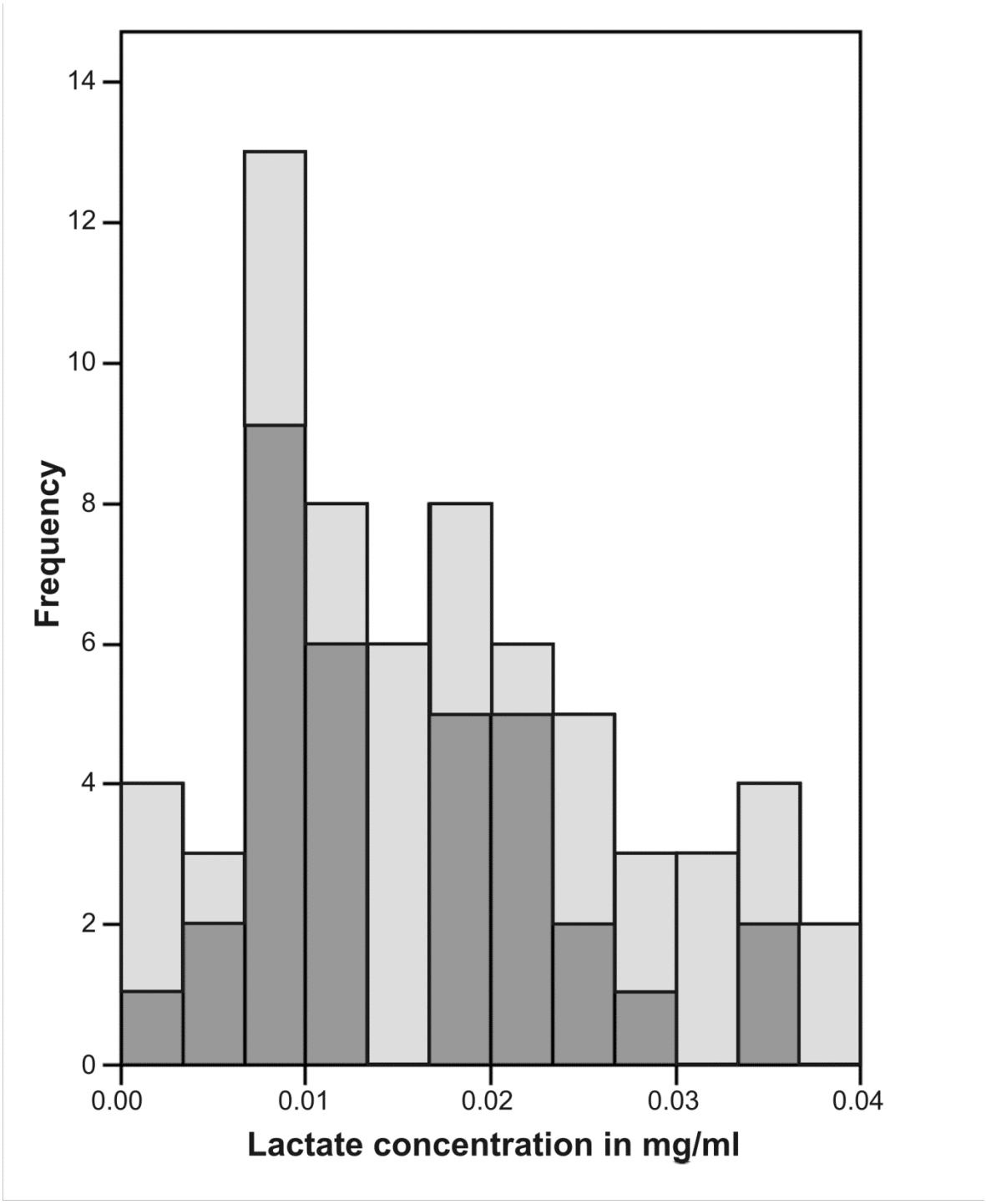

Figure 11. The frequencies of different lactate concentrations found in the hemolymph samples taken from crabs in laboratory conditions (dark gray) and field conditions (lighter gray). n = 65.

DISCUSSION

This study shows that *Cancer productus* and *Metacarcinus magister* spend a considerable amount of time at least partially buried in the sediment (Figure 6), and so are regularly exposed to hypoxic or anoxic conditions. Although the oxygen saturation in the sediment was not directly measured the mud was black, which is indicative of anoxia (Nakamura and Stefan, 1994). The exposure to anoxic conditions is reflected in the substantial levels of lactate found in the crabs hemolymph, with some crabs at all levels of ambient oxygen levels having elevated lactate levels (Figure 10). The high levels of lactate in some crabs at normoxia may be due to recent bouts of burial, even if the crabs were not buried during the experiment. Furthermore, the lack of significant difference in hemolymph lactate between crabs exposed to an hour-long session of normoxic and those in hypoxic environments suggests that these crabs routinely bury to at least the extent and length of time necessary to experience hypoxia comparable to what they were subjected to during this experiment. Some data suggest that my experimental conditions were beginning to produce a small increase in lactate level (Figure 10), however, in that the highest lactate levels were found in several crabs which were exposed to some of the lowest oxygen levels and the very lowest levels of lactate were found in several of the crabs which had been kept in normoxic conditions during the experiment. All the crabs in hypoxic conditions had at least some measureable amount of lactate present in their hemolymph (Figure 10). Although lab conditions of hypoxia were intended to expose the crabs to low levels of oxygen, it would appear that longer and/or more intense hypoxia

will be needed in order to press the limits past what these crabs normally experience and determine the full extent of their response when exposed to hypoxia.

Work done by Airriess and McMahon (1994) indicates that *M. magister* is capable of surviving at extremely low oxygen levels for prolonged periods of time. They showed that lactate levels only increased significantly when exposed to 14% air saturation for 6 hours, considerably longer than the 1 hour period used in this study (Airriess and McMahon, 1994). As a side note, I discovered that under some circumstances burial in the sediment may not always expose crabs to as low levels of oxygen as expected. During two field experiments crabs were found and studied among eelgrass (*Zostera marina*). Photosynthesis by the eelgrass has been shown to increase the amount of oxygen in the water around them, sometimes creating a hyperoxic environment (over 100% oxygen saturation) (Borum et al., 2006). Even though the crabs were covered in a sealed chamber, which should normally have induced hypoxia, photosynthesis by the eelgrass present in the acrylic box actually led to an increase in the ambient oxygen inside the box to the highest levels measured in the field tests. This high level of oxygen would likely facilitate the oxygen uptake by the crabs unless they were fully buried.

The significantly greater extent of burial and lower levels of activity exhibited by both crab species under hypoxic conditions was most unexpected (Figures 7 and 9). The hypothesis was that the crabs exposed to hypoxic conditions would tend to emerge from the sediment in an attempt to obtain more oxygen. However, these crabs did the opposite, burying themselves deeper and moving about to a lesser extent during hypoxia.

This does not appear to be the most adaptive response to low oxygen, since this would make it even more difficult to obtain enough oxygen to support normal metabolism. It is possible that these crabs rarely encounter water with oxygen levels of 50% air saturation or less, and so do not have an adaptive response to hypoxia in the water column. Recently, however, several widespread hypoxic or anoxic events have occurred along the northwest Pacific coast (Keller et al., 2010), resulting in extensive mortalities in these two crab species. If the crabs respond to low oxygen by burying into the sediment, this may well increase the mortality during these events. It is possible that crab burying behavior is a generalized response to any threat or distress and such a response may be adaptive when dealing with other stresses such as predation or competition. However, this response is likely not favorable when dealing with extensive bouts of hypoxia.

The significantly greater extent of burial exhibited by *C. productus* as compared to *M. magister* was also unexpected (Figure 7). McGaw (2005) has shown that *M. magister* may be better equipped anatomically, behaviorally, and physiologically for burial than is *C. productus*, and concluded that *M. magister* was the more proficient at burying of the two species. Using dye tracers he was able to show that *M. magister* is capable of reversing the flow of water through the ventilator channels, thus avoiding the problem of clogged Milne-Edwards openings while partly buried in the sediment. This species was also shown to increase the amount, magnitude and duration of these ventilatory reversals in order to access oxygenated water while buried. McGaw also noted that the abundant fringes of setae along the front margins and underside of the carapace in *M. magister* may serve as water channels, allowing the circulation of

oxygenated water to the Milne-Edwards openings while the crab is buried. These features were not observed in *C. productus*, which instead has features typical of non-burying crab species. McGaw observed that *C. productus* makes frequent shifts when buried in order to loosen the sand around its carapace and chelae in order to maintain the integrity of the exostegal channel. Dye tracers indicated that *C. productus* maintains a one-way flow of water through the brachial chambers even when buried (McGaw, 2005). These behavioral and physiological differences between these two species led McGaw to conclude that *M. magister* is better adapted for burying in the sediment than is *C. productus.* It is possible that *M. magister* does indeed bury to a greater extent than *C. productus* under some conditions, as postulated by McGaw (2005), but this was not observed in this experiment. Burial extent may vary seasonally and the two species may be on different phases of their burial cycle. It may also be that *C. productus* buries more deeply in the type of sediment found in Rosario Bay while *M. magister* buries to a greater extent in other substrates. *C. productus* may also emerge more quickly and completely at night than does *M. magister* which could decrease the amount of hypoxic exposure experienced by *C. productus.* At any rate, this study has confirmed that both species bury themselves into the sediment and are capable of spending substantial portions of time in these conditions, at least during the day. Therefore, both species can be expected to be routinely exposed to hypoxia during burial.

In summary, both *C. productus* and *M. magister* buried themselves in the sediment and were therefore almost certainly subjected to hypoxic or anoxic conditions during that time. Measurable amounts of lactate in their hemolymph showed that both

species were able to perform anaerobic metabolism (Figure 10). Also, both species buried deeper into the sediment as oxygen levels decreased, with *C. productus* burying to a significantly greater degree than did *M. magister* (Figure 7). Furthermore, both species of crabs displayed significantly lower levels of activity when exposed to hypoxic conditions at or below 50% air saturation than in normoxic conditions (Figure 9). These behaviors are not optimal for obtaining oxygen, and it is possible that they are generalized responses to threat or distress which do not help in this situation.

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