EFFECTS OF LOW OXYGEN ON BEHAVIOR OF THE MANTIS SHRIMP *HEMISQUILLA CALIFORNIENSIS*

by

Tyler Scott James Shelton

A THESIS

submitted to

WALLA WALLA UNIVERSITY

in partial fulfillment
of the requirements for the
degree of

MASTER OF SCIENCE

30 July 2008

This thesis for the Master of Science degree
has been approved by the Department of Biological Sciences
and the Office of Graduate Studies
Walla Walla University

David L. Cowles
Major Professor

Committee Member

Committee Member

Committee Member

Dean of Graduate Studies

Observer of the Process - Graduate Representative

Candidate

Oct 9, 2008
Date

ABSTRACT

EFFECTS OF LOW OXYGEN ON BEHAVIOR OF THE MANTIS SHRIMP *HEMISQUILLA CALIFORNIENSIS*

by

Tyler Scott James Shelton

Within marine habitats certain of organisms seem capable of surviving in the face of what with many other species would be extreme environmental conditions. One example in particular is the Stomatopod *Hemisquilla californiensis* which routinely lives in burrows which are blind-ended and often sealed, leading to hypoxia (Basch and Engle, 1989; Richter, 1998). Generally, marine animals respond to hypoxia through physiological adaptations, use alternative metabolic pathways, or through specific behavioral changes. *H. californiensis* demonstrates neither clear physiological adaptation nor possesses obvious anaerobic capacity (Peters, 1997; McFadden, 2004). In this study the behavior(s) of eleven *H. californiensis* individuals were observed and compared during periods of high and low oxygen conditions. In hypoxia the pleopod beat rate of *H. californiensis* increased significantly though beat amplitude decreased. There were

consistent trends toward fewer and shorter pauses in pleopod beating, though only one such trend was significant. Conversely, there was no significant change in general activity levels during hypoxia. This research suggests that *H. californiensis* changes its activity patterns in respiration-related behaviors during hypoxia but that the hypoxic conditions used in this experiment may not have been extreme enough to elicit significant changes in whole-animal activity patterns.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

INTRODUCTION

The marine environment is one of constant challenges with respect to the survival of its inhabitants. Organisms dwelling in dynamic ocean areas may encounter highly stressful conditions such as insufficient oxygen levels to sustain normal oxidative metabolism (Zebe, 1982; Peters, 1997; Flück *et al*, 2007). In response to such conditions, many intertidal organisms employ a variety of mechanisms including anaerobic metabolic pathways (De Zwaan and Putzer, 1985; Hervant *et al*, 1998; Yaikin *et al*, 2002) and behavioral adaptations (Torres *et al*, 1977; Cassista, 1995; Richter, 1998).

The mantis shrimp *Hemisquilla californiensis* is known to inhabit burrows in mud or sand substratum at depths of 4 to 90 meters off the southern coast of California. *H. californiensis* spends much of its time inside its burrow which typically extends 0.5 meters downward at a 60° angle then turns and extends a meter horizontally (Basch and Engle, 1989). As this burrow is a blind-ended structure having only one entrance, very little oxygenated water would be expected to circulate deep within the burrow. Oxygen concentration may further be affected by biological oxygen demand from the benthic microorganisms within the burrow (Hervant *et al*, 1998). During inactive periods, this species may cap its burrow entrance with a secretion/sediment mix, thereby inhibiting water circulation even further (Dingle and Caldwell, 1978; Basch and Engle, 1989). An environment of strongly hypoxic conditions (O_2 level < 10 mm Hg, 6% saturation) in such a burrow has indeed been observed (Cassista, 1995; Richter, 1998).

In a burrow with low oxygen levels *H. californiensis*, an oxyconformer (Peters, 1997), might be expected to display reduced activity in order to decrease oxygen

consumption. However, studies of this species indicate that it remains active during time spent deep within its burrow where oxygen levels would be expected to be the lowest (Basch and Engle, 1989; Cassista, 1995; Richter, 1998). Furthermore, *Hemisquilla* can survive in anoxic conditions (O_2 < 1 mm Hg) and may remain active under these conditions for up to 48 hours (McFadden, 2004).

How then is *H. californiensis* able to remain persistently active in what should be a strongly oxygen limited environment? In general, crustaceans possess only moderate anaerobic capacity with low anaerobic energy production efficiency and an eventual buildup of toxic levels of lactate (Zebe, 1991; Hervant *et al*, 1998). *H. californiensis* itself has shown a relatively large tolerance to lactate buildup within tissues and hemolymph, but no indications of any large pH buffering capacity (McFadden, 2004). Furthermore, little information has been found to date to indicate that *H. californiensis* relies on any other alternative anaerobic pathways (McFadden, 2004).

Due to a lack of effective anaerobic ability *H. californiensis* must either employ other adaptive physiological mechanisms or behaviorally adapt to hypoxic conditions in order to survive. One such behavior in the might be burrow ventilation. Richter (1998) found a correlation between periodic high speed flow events which were seen to occur within the burrow (6.96 cm/s peak flow speed for 42 s) and rising oxygen levels in the burrow. These flow events did not seem to occur when the animal was actively moving inside the burrow or when it was present at the burrow entrance. However, Richter observed that there was little noticeable change in behavior during periods of high and low oxygen concentration.

In consideration of Richter's observations, it seems likely that this species displays subtle but important behavioral responses to hypoxic conditions. Changes are also apt to be found within physical patterns associated with respiration that Richter did not quantify (e.g. – pleopod beating). The goal of this research was to visually record and quantify differences in whole-animal behavior and respiration-related activity by *Hemisquilla californiensis* during low and high oxygen conditions.

MATERIALS AND METHODS

Specimen Capture and Acclimation

Eleven *H. californiensis,* 3 male and 8 female, were collected by otter trawl off the coast of southern California by commercial fishermen (see Appendix). They were transported to College Place by car in insulated, temperature-controlled containers, isolated from one another to prevent fighting. The containers were continuously aerated by battery powered air pumps. All shrimp were acclimated 2-3 days in lab conditions prior to experimental trials. Lab conditions were similar to those in the southern California collection site and included: ~17° C water temperature, water oxygenation with 32-35 ppt salinity, a pH of 8.0-8.2., and a 14:10 daylight cycle.

Experimental Design

To simulate a natural burrow, a test chamber was constructed using clear acrylic pipe, approximately 1 meter in length, 8 cm in diameter, with a ¼ in wall thickness (Figure 1). A perforated cap was used to create a one-ended burrow, while a barrier of meshed nylon fishing line confined the subject to a particular area of the burrow. The test chamber spanned two rectangular water tanks and was marked with a series of vertical lines, each spaced 1 cm apart, in order to provide a visual reference of the animal's position and movement during each trial.

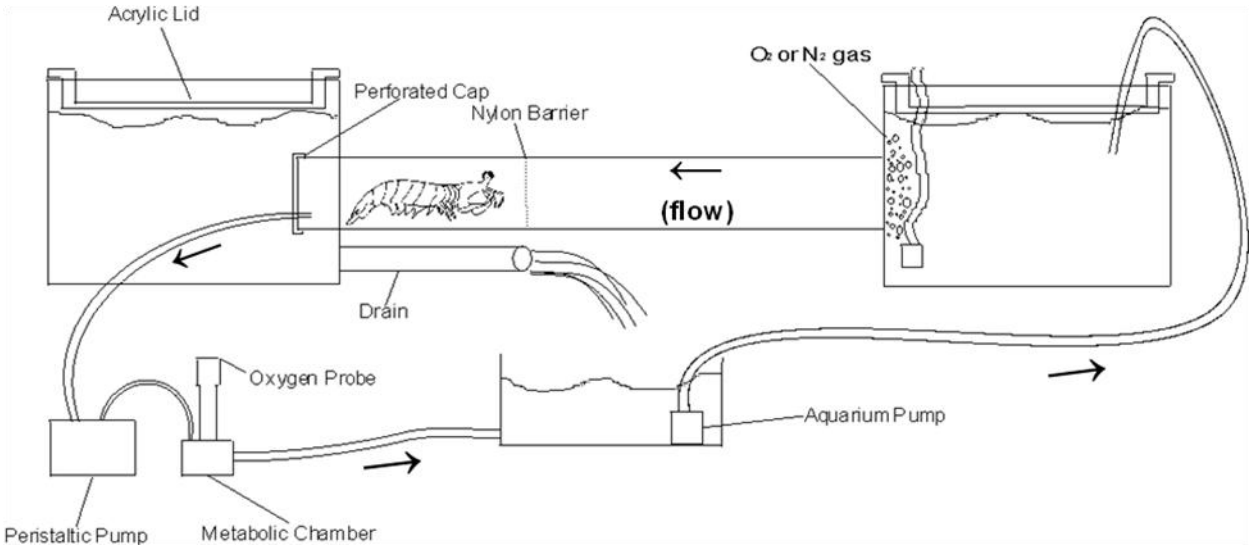

Figure 1. Experimental chamber. Circulation achieved via an aquarium pump.

Once a test individual was loaded into the chamber a gently circulating current (approximately 1.6 cm/s) was created using a ViaAqua VA1300 aquarium pump (Figure 1) resulting in a constant mixing of water within the burrow and consequent control of oxygenation. Conditions of full oxygen saturation (> 90%) and low oxygen saturation (< 20%) were simulated by bubbling air and nitrogen gas respectively into the inflow tank in order to test the differences in the subject's responses to these different conditions. To minimize surface gas exchange and spontaneous re-oxygenation of water during low oxygen periods lids were placed over the exposed portions of the chambers. Oxygen saturation was monitored with a polarigraphic oxygen electrode (Nester 8500) which sampled water directly from the end of the burrow (behind the shrimp) (Figure 1).

Nitrate levels of water within the experimental chamber were monitored with the Hagen Nitrate Test Kit and the entire water volume was replaced if nitrate levels approached 20 mg/L in order to avoid exceeding the recommended safe range of 0-50 mg/L stated by Hagen Inc. Saltwater for the laboratory and experimental chamber was made using the BIO-SEA Marinemix formula from Aquacraft.

Data Sampling

Shrimp behavior was observed during three sequential conditions: an initial period of high oxygen saturation (> 90%), a period of low oxygen saturation (< 20%), and a "recovery" period in which oxygen saturation increased back to above 90%. Each condition period lasted approximately 30 minutes (Figure 2). The room containing the

experimental chamber was kept at subdued light. Initial high oxygen sampling occurred only after a 20 minute period in which the animal was allowed to

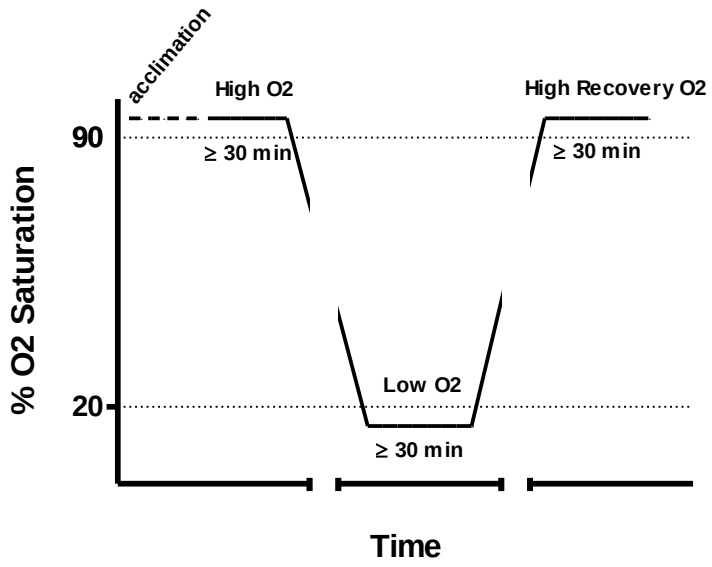

Figure 2. Timeline of data sampling according to oxygen condition. Horizontal dashed lines are present to provide as a visual reference for each oxygen condition; High oxygen conditions were above 90% oxygen saturation whereas, low oxygen conditions were above 20% saturation. Slanted portions and separations in the timeline represent transition periods where oxygen saturation was being adjusted and no data was being sampled. Transitions from High to Low O2 conditions lasted 40-50 minutes, while transitions from Low to High Recovery O2 lasted 30-40 minutes. The acclimation phase of the timeline was 20 minutes.

acclimate to the burrow. During the experimental periods shrimp behavior was recorded using a tripod-mounted Sony digital camcorder (DCR-SR30) in night vision mode and

simultaneously observed the behavior from an adjacent room using a video monitor in order to avoid disturbance to the animal. Quantified behaviors included the rate (bpm, beats per minute) and amplitude of pleopod beating, frequency and length of pauses between bouts of pleopod beating, and occurrences of whole-animal behavior (i.e. – flipping over, handling the barrier and cap, and linear movement) which could indicate agitated or searching behavior. Oxygen levels in the chamber were recorded simultaneously with the behavioral recordings. Both the behavioral video and the oxygen recordings were time-stamped by recording individual events into an activity logger program in order to aid further analysis in concordance with video recordings.

To record the shrimp's activity using the activity logger program, I viewed the experimental session on video during either live recording or a replay. A digital clock in the activity logger program was first synchronized with the video time stamp. As I watched the video I pressed a specific key for each type of activity I observed. This created an activity data file which digitally recorded the time at which each activity occurred. Precision of the program was to the nearest tenth of a second, but with my added response time my estimated accuracy was within 0.5 second. All pleopod beats, flips, and times spent handling the mesh or cap were recorded in this fashion for each trial.

After activity was logged using the activity logger program, an activity analysis program was used. Inputs to the analysis program included the digitized activity log along with a second digital file of the oxygen levels recorded throughout the experiment. Mean pleopod beat rate at each oxygen condition was measured in the following manner:

first the program scanned through the activity log to find a sequence of 30 uninterrupted beats. Thirty beats was chosen to represent a protracted sequence of uninterrupted beats while not reaching a beat sequence so long that not enough sequences could be sampled within the allotted time period. While counting up to the 30 beats, the average interval between all beats in the group was calculated after each new beat. If the last interval was greater than 2x the average of the previous beats the interval was assumed to be a pause and the count was started over at the next beat. Once a sequence of 30 beats without a pause was found the activity analysis program recorded it as a genuine beat sequence along with the oxygen level that was present at the time of the sequence. The program then continued searching through the file for other genuine beat sequences, which it defined as any sequence of 30 beats which included no beat interval greater than 2x the length of the average interval for the last genuine sequence and separated from the last genuine sequence by at least 10 seconds. Any interval which exceeded 2x the average interval in the last genuine beat sequence was recorded as a pause. The activity analysis also placed all records of time spent handling the mesh barrier or cap into a separate list which was then used to manually calculate cumulative totals of time spent handling the barrier.

For measurement of pleopod beat amplitude I reviewed the video taken during pleopod beat rate sampling, pausing playback every 3 minutes in order to make the appropriate measurements. The amplitude of pleopod beats was quantified by measuring the peak angle of the forward and backward movement of pleopod #3 (found through frame by frame analysis) using a protractor. If a pause in playback occurred during an

observable stop in pleopod beating video was forwarded until natural pleopod noticeable pleopod movement resumed. Amplitude was sampled for a total of 10 data samples during each oxygen condition period and was non-dependent on genuine beat sequences.

Whole-animal activity was observed by reviewing the video during the three different oxygen conditions and counting individual events. Such events included flipping over (turning to face the opposite direction) and the number of lines crossed as a consequence of forward or backward movement within the chamber during each oxygen condition. These behaviors, in addition to time spent handling the barriers, were taken to represent indices of animal activity which could indicate agitation or searching behavior. Each shrimp underwent up to three trials, separated by at least 24 hours, unless mortality occurred prior to subsequent trials.

Data Analysis

First tested parameters (i.e. – amplitude and rates of pleopod beats, number and duration of pauses, number of lines crossed and flips, and cumulative time spent handling a barrier) of all trials for each animal were compared to make sure there were no consistent time-dependent changes in activity, meaning that the animal's behavior did not change in a systematic way with the time in captivity. This was evaluated with the aid of ANOVA (or of chi-square for data consisting of counts). After verifying that there were no consistent time-dependent changes in animal behavior during the series of trials, all data from the trials of each animal were averaged before ANOVA so that only the

average of each parameter tested for each animal was used in the final analysis. This was done to maintain independence of the data. In addition, prior to ANOVA the trimmed mean was used by discarding the highest and lowest value recorded under each parameter in order to smooth any outliers which may occur and to normalize the data (Klipp *et al*, 2005). The Newman-Keuls test was used as a post-test. For non-parametrically distributed data such as pause length, number of pauses, and time spent handling the mesh barrier or cap Kruskal-Wallis analysis with Dunn's Multiple Comparison post test was used.

RESULTS

Initial checking for time-dependent confounders

Considering the sample size of eleven animals, multiple parameters being tested, and multiple trials per animal, it is not surprising that a small number of significant differences were found among some of the individual animals' responses during trials. However, none of these differences showed any consistent trend from early to late trials so the data from the three trials of each animal were averaged and only the mean value from each animal was used for ANOVA analysis to preserve the independence of the data.

Changes in respiration-related activity during different oxygen conditions

Although pleopod beat rate varied among subjects (Figure 3), it averaged about 22 -23 beat per minute (bpm) during both the initial and recovery oxygen conditions, with no significant difference found between them (Figure 3, Table 1). However, pleopod beat rate during low oxygen conditions was approximately 25% faster at 28 bpm, a highly significant difference (Table 1). Similarly, beat amplitude did not differ significantly between the initial and recovery high oxygen conditions (Figure 4, Table 1), but both of these had significantly higher amplitude than during low oxygen. The 33 degree beat amplitude during low oxygen conditions was about 10% lower than during initial high oxygen conditions, while upon recovery amplitude increased to the previous level or slightly exceeded initial amplitude, though the difference was not significant.

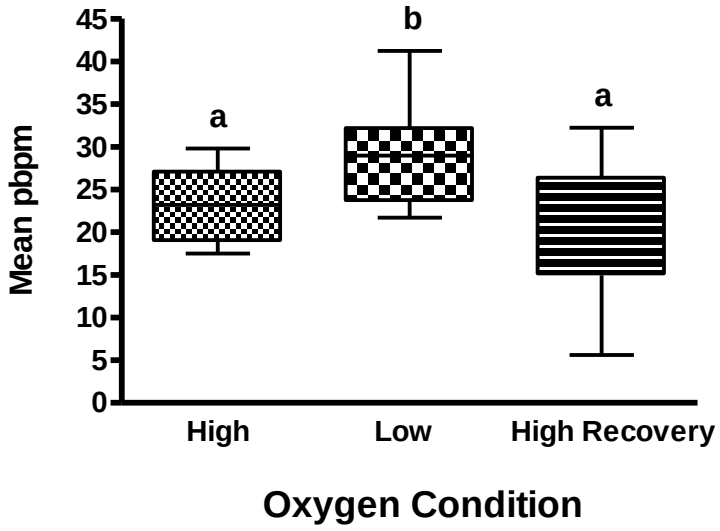

Figure 3. Comparison of mean pleopod beats per minute (pbpm) exhibited by *H. californiensis* during three different oxygen conditions. The figure above is a box plot: central bar = median, box = 25th to 75th percentile, and bars = full range of data. Groups marked **a** were significantly different from group **b** (ANOVA, $p = 0.0109$, $n = 9$ for each condition).

Table 1. Summary of trimmed means and corresponding P values for tested parameters. All measurements are means except for values with * which are medians because of nonparametrically distributed data; ° = degrees, s = seconds. Bold P values are significant.

Parameter	units	High O2	Low O2	High Recovery O2	P value
Pleopod Beats Per Minute	bpm	23.07	28.47	22.18	**0.0109**
Amplitude	°	36.41	32.82	38.61	**0.0025**
Pause Duration	s	42.00	28.31	64.29	**0.0173**
Number of Pauses	(count)	18.33*	12.33*	17.33*	0.0842
Handling Time	s	359.00*	58.00*	214.00*	0.3019
Number of lines crossed	(count)	36.00*	26.00*	37.67*	0.1120
Number of flips	(count)	4.67*	2.67*	3.33*	0.2856

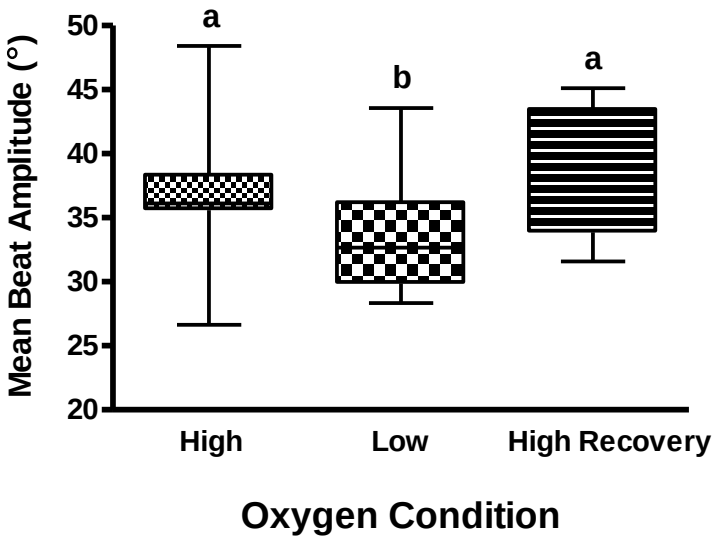

Figure 4. Comparison of mean pleopod beat amplitudes exhibited by *H. californiensis* during three different oxygen conditions. The figure above is a box plot: central bar = median, box = 25th to 75th percentile, and bars = full range of data. Groups marked **a** were significantly different from group **b** (ANOVA, p = 0.0025, n = 9 for each condition).

The number and duration of pauses between pleopod beats also showed trends toward differences in low versus high oxygen conditions, though only one such difference was significant (Figure 5). The average length and number of pauses dropped by about 1/3 in the low oxygen conditions as compared to those in the initial high oxygen conditions, though because of individual variability this trend was not significant (Figure 5 and 6, Table 1). Similarly, upon recovery the number and length of pauses increased again. Indeed, pause duration more than doubled in the high oxygen levels upon recovery from low pO_2, a change which was significant (Table 1).

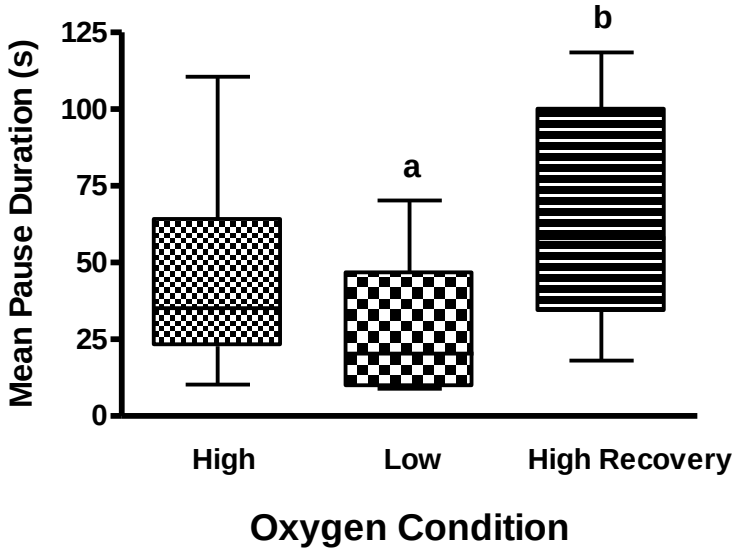

Figure 5. Comparison of mean pause durations observed in *H. californiensis* during three different oxygen conditions. The figure above is a box plot: central bar = median, box = 25th to 75th percentile, and bars = full range of data. Group marked **a** was significantly different from group **b** (ANOVA, p = 0.0173, n = 9 for each condition).

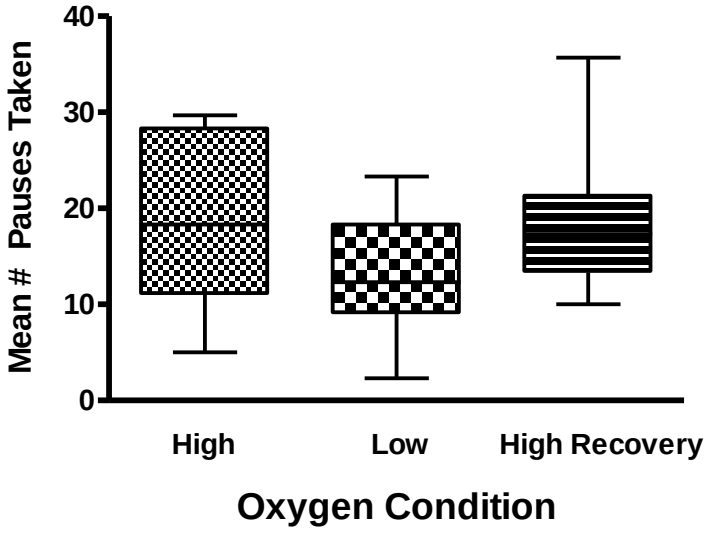

Figure 6. Comparison of the mean number of pauses taken by *H. californiensis* during three different oxygen conditions. The figure above is a box plot: central bar = median, box = 25th to 75th percentile, and bars = full range of data. There was no significant difference among groups (n = 9 for each condition).

Changes in overall activity

Unlike the activities directly related to respiration, there were no significant changes in whole-animal activity between high and low oxygen conditions (Figures 7-9, Table 1). Although the number of lines crossed, the number of flips the subject made, and the amount of time spent handling the cap and mesh barriers all appeared to be higher in high oxygen conditions, there was much individual variability, great overlap in the ranges, and consequently, none of these changes were significant.

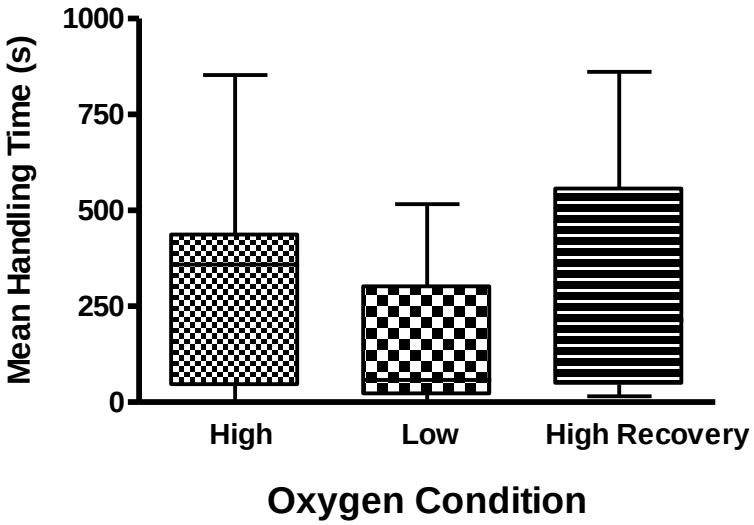

Figure 7. Comparison of the mean time spent handling the barrier or cap by *H. californiensis* during three different oxygen conditions. The figure above is a box plot: central bar = median, box = 25th to 75th percentile, and bars = full range of data. There was no significant difference among groups (n = 9 for each condition).

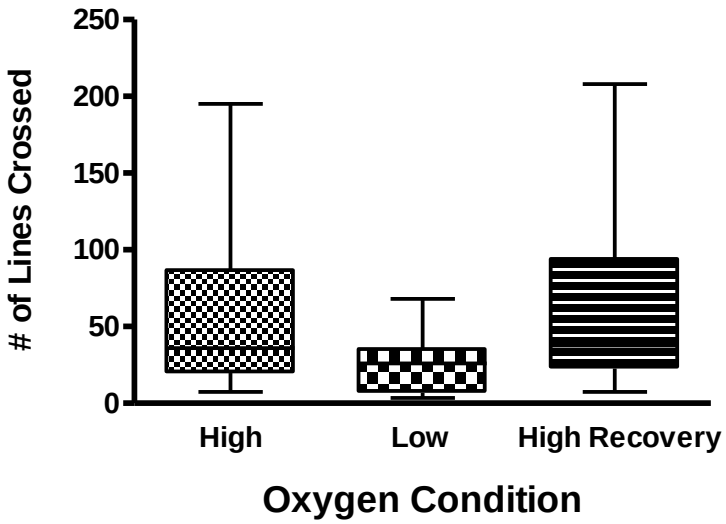

Figure 8. Comparison of mean movement within the burrow by *H. californiensis* during three different oxygen conditions. The figure above is a box plot: central bar = median, box = 25th to 75th percentile, and bars = full range of data. There was no significant difference among groups (n = 9 for each condition).

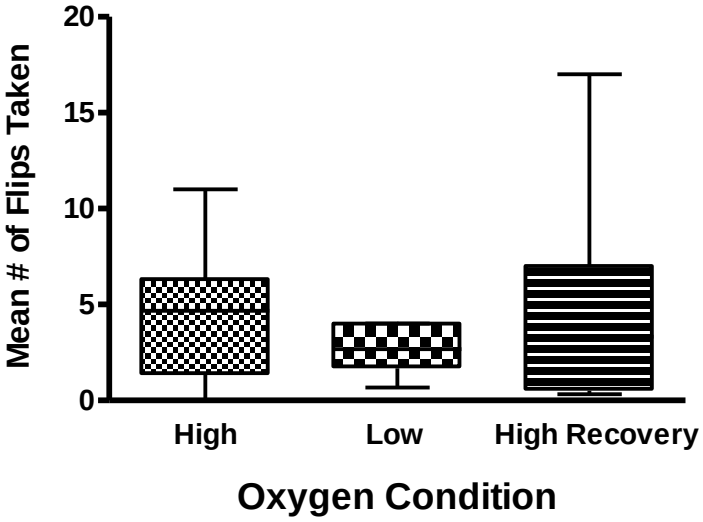

Figure 9. Comparison of the mean number of flips taken by *H. californiensis* during three different oxygen conditions. The figure above is a box plot: central bar = median, box = 25th to 75th percentile, and bars = full range of data. There was no significant difference among groups (n = 9 for each condition).

DISCUSSION

This study showed that *Hemisquilla californiensis* displays clear behavioral changes at low oxygen conditions compared to high oxygen conditions (Table 1). It has previously been established that *H. californiensis* does not appear to possess a large capacity for anaerobic metabolism or use alternative metabolic pathways to increase metabolic efficiency (McFadden, 2004; Peters, 1997). It is likely then that the findings of this research represent the central adaptive response of this species to low oxygen conditions.

Changes in Respiratory-Related Behavior

Upon investigation of behavioral adaptations that *H. californiensis* may employ it was found that the rate of pleopod beating increases significantly during low oxygen, as compared to the observed rates during high oxygen conditions before and after a drop in oxygen (Table 1 and Figure 3). Characteristic of all stomatopods, the pleopods of *H. californiensis* are the main site of biramous gill attachment, although lesser epipodal gills are also attached at the base of the thoracopods. It is likely therefore that an increase or decrease in the movement of the pleopods can influence the rate at which oxygen is exchanged across the epithelial surface of the gills and thus directly affect the rate of aerobic metabolism. The faster pleopod beat rate observed at low oxygen would seem to indicate that *H. californiensis* is increasing the rate at which its gill epithelium is exposed to oxygen to compensate for limited local oxygen availability. Active changes in pleopod beat rate in response to dropping oxygen have been previously demonstrated in the

burrow dwelling shrimp *Neotrypaea* (*Callianassa) californiensis*, although this response was also thought to involve pleopod movement as a means of burrow ventilation (Torres *et al*, 1977).

Opposite the trend displayed in the rate of pleopod movement, the amplitude of *H. californiensis*'s pleopod movement was observed to significantly decrease at lower oxygen conditions and increase with higher oxygen conditions (Table 1 and Figure 4). Such behavior indicates that while *H. californiensis* is actively responding to low oxygen with increased pleopod beat rates, it may be conserving the amount of energy expended in their operation by using shorter or perhaps more efficient strokes. This behavior may be somewhat analogous to that of canine panting where energy is conserved by maintaining short, consistent breaths during a series of panting (Reece, 2004).

There were also noticeable trends seen in pauses of pleopod beating with lower numbers and shorter durations at low oxygen conditions, although significance was only seen between the low and high recovery oxygen conditions for pause duration (Figures 5 and 6). This behavior suggests that *H. californiensis* focuses more closely on respiration at low oxygen conditions (i.e. – more consistent pleopod beating) than at higher oxygen conditions. During conditions where oxygen is a limiting factor, it would seem advantageous for this shrimp to maintain activity contributed to respiration rather than a reduce this activity (i.e. – frequent or longer pausing of pleopod beating). It is important to note that the frequency of pauses did not increase during low oxygen conditions, a behavioral pattern that could indicate incipient respiratory failure. Animals under extreme respiratory limitation and nearing collapse may not be able to obtain enough

oxygen to fully support even critical respiration activities such as pleopod beating. Furthermore, the frequency of pauses also did not decrease in recovery, a pattern that could signify more intense respiratory exchange in support of repaying an oxygen debt. The fact that these behavioral patterns were not present supports the notion that *H. californiensis* is adapted or possesses broad respiratory plasticity towards low oxygen conditions and was not strongly stressed by the experimental conditions.

Changes in Non-Respiratory Behavior

During this study there were no significant changes in behavior not directly relating to respiration (i.e. – changing of body orientation, horizontal movement, or handling of barriers). The findings are consistent with those of past studies (Cassista, 1995; Peters, 1997; Richter, 1998) which showed no significant decrease in whole-animal activities under low oxygen conditions. The apparent trends of higher activity during higher oxygen conditions observed in the parameters mentioned above, although not significant, suggests that *H. californiensis* is consolidating its energy to respiratory-related behavior (Figures 7-9). In addition, the lack of a significant drop in whole-animal activity during low oxygen conditions further supports suggestion that this species is not experiencing a noticeable amount of stress as a consequence of low oxygen (if the parameters of whole-animal activity used in this experiment are indeed representative of agitated behavior). An animal not experiencing noticeable stress during such conditions would again be considered well adapted towards low oxygen conditions (Flück *et al*, 2007).

Implications of Adaptive Behavior

When confronted with low oxygen conditions an organism may compensate by utilizing physiological adaptation, mechanisms of anaerobic metabolism, or behavioral adaptation. *H. californiensis* appears to compensate for low oxygen conditions at least in part through changes in respiratory-related behavior.

The use of physiological adaptations to low oxygen has been demonstrated in Crustaceans. *Daphnia magna* increases the gene expression of hemoglobin subunits with higher oxygen affinity during hypoxia and consequential transport efficiency (Paul *et al*, 2004; Bavis *et al* 2007). Such an adaptation would seem to be within the more highly specialized portion (that is, more keenly attuned towards survival) of physiological adaptation for crustaceans frequently dwelling in hypoxia. Adaptations of species accustomed to living in stable low oxygen, such as those found in the oceanic oxygen minima, include high circulatory capacity, high gill surface area, and thin blood-to-water diffusion distance across the gills (Childress and Seibel, 1998).

Unlike organisms living at stable low oxygen conditions, those living in unstable hypoxic or occasional anoxic environments often rely more on anaerobiosis and metabolic suppression. According to Yaikin *et al* (2002) the Anomuran crab *Petrolisthes laevigatus* resorts to anaerobic activity via the octopine dehydrogenase and lactate dehydrogenase pathways when subjected to oxygen limitation. This response seems to represent a short-term adaptation at best however, as *P. laevigatus* cannot survive more than three hours under hypoxic conditions. Similarly, two Thalassinidean examples, *Neotrypaea* (*Callianassa) californiensis* and *Upogebia pugettensis*, switch to anaerobic

metabolism via lactate formation and also utilize decreased metabolic activity while in hypoxia (Zebe, 1982). Such adaptations seem most directly useful for survival during short periods of hypoxia/anoxia (e.g. - low tides). Anaerobiosis typically has much lower energy yields than those of aerobic metabolism and is seldom used to sustain long-term activity. However, species capable of anaerobiosis are typically found in unstable, dynamic environments which do not remain permanently hypoxic and thus do not require long-term survival in low oxygen conditions.

So then where do these findings place *H. californiensis* within the spectrum of respiratory adaptations of the Crustacea? Conditions of reduced oxygen availability seem to create a drive to emerge from metabolic stress (Flück *et al*, 2007), yet *H. californiensis* consistently lives in a relatively stable low oxygen environment, capping its burrow and spending up to nine uninterrupted hours inside under conditions that should become hypoxic within a short time (Basch and Engle, 1989; Cassista, 1995). This species does not use the classical adaptations of anaerobiosis or lowered metabolic rate such as would be found among species from more rapidly-changing, unstable environments (Peters, 1997; Hervant *et al*, 1998; McFadden, 2004). In contrast, *H. californiensis* appears to align more closely with those animals that have adapted to life in consistent and stable low oxygen conditions which display a primary reliance on aerobic metabolism (Childress and Seibel, 1998); displaying clear changes in respiratory-related behavior at low oxygen conditions. It must be noted, however, that unlike this portion of the respiratory spectrum *H. californiensis* often experiences exposure to normoxic conditions

likely during foraging, cleaning, and hunting activities and thus is somewhat unique in its apparent responses to low oxygen.

ACKNOWLEDGEMENTS

I would like to thank Walla Walla University Technical Services for their contributions valuable time and expertise. I would like to express appreciation to Dr. Galusha and Dr. Ligman for their avid support and encouragement throughout the research process. Thank you very much to Scott and Gail Shelton whose dedicated support and encouragement were crucial to my persistence during this research. Finally I would like to thank Dr. Cowles whose dedication, advice, and many hours of contributing effort were invaluable throughout the research and writing process.

LITERATURE CITED

Basch L. and J.M. Engle. 1989. Aspects of ecology and behavior of the stomatopod *Hemisquilla ensigera californiensis* (Gonodactyloidea: Hemisquillidae). Selected Symposia and Monographs U.Z.I. 199-212.

Bavis R.W., F.L. Powell, A. Bradford, C.C.W. Hsia, J.E. Peltonen, J. Soliz, B. Zeis, E.K. Fergusson, Z. Fu, M. Gassmann, C.B. Kim, J. Maurer, M. McGuire, B.M. Miller, K.D. O'Halloran, R.J. Paul, S.G. Reid, H.K. Rusko, H.O. Tikkanen, and K.A. Wilkinson. 2007. Respiratory plasticity in response to changes in oxygen supply and demand. Integr. Comp. Biol. 47(4): 532-551.

Cassista J.J. 1995. The aerobic metabolism and behavior of the burrow-dwelling mantis shrimp *Hemisquilla ensigera californiensis*. Masters Thesis. Loma Linda University, Loma Linda, CA.

Childress J.J. and B.A. Seibel. 1998. Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. J. Exp. Biol. 201: 1223-1232.

De Zwaan A. and V. Putzer. 1985. Metabolic adaptations of intertidal invertebrates to environmental hypoxia (a comparison of environmental anoxia to exercise anoxia). Symp. Soc. Exp. Biol. 39: 33-62.

Dingle H. and R.L. Caldwell. 1978. Ecology and morphology of feeding and agonistic behavior in mudflat Stomapods (Squillidae). Biol. Bull. 155: 134-149.

Flück M., K.A. Webster, J. Graham, F. Giomi, F. Gerlach, and A. Schmitz. 2007. Coping with cyclic oxygen availability: evolutionary aspects. Integr. Comp. Biol. 47(4): 324-331.

Hervant F., J. Mathieu, and G. Messana. 1998. Oxygen coonsumption and ventilation in declining oxygen tension and posthypoxic recovery in epigean and hypogean Crustaceans. Journal of Crustacean Biology. 18(4): 717-727.

Klipp, E., R. Herwig, A. Kowald, C. Wieling, and H. Lehrach. 2005. Systems biology in practice: concepts, implementation and application. 87. Wiley-VCH, Weinheim, Germany.

McFadden M.A. 2004. Living without oxygen: the anaerobic metabolism of *Hemisquilla californiensis*. Masters Thesis. Walla Walla College, College Place, WA.

Paul R.J., B. Zeis, T. Lamkemeyer, M. Seidl, and R. Pirow. 2004. Control of oxygen transport in the microcrustacean *Dauphnia*: regulation of haemoglobin expression as central mechanism of adaptation to different oxygen and temperature conditions. Acta Physiol. Scand. 182: 259-275.

Peters L.P. 1997. Oxyconformity in burrow-dwelling Crustaceans: aerobic metabolism of *Hemisquilla ensigera californiensis*. Masters Thesis. Loma Linda University, Loma Linda, CA.

Reece W.O. 2004. Functional anatomy and physiology of domestic animals (3rd ed). 258-263. Wiley-Blackwell, Oxford.

Richter T.L. 1998. Ventilation behavior of the mantis shrimp *Hemisquilla ensigera californiensis* in hypoxic burrows. Master's Thesis. Loma Linda Univeristy, Loma Linda, CA.

Torres J.J., D.L. Gluck, and J.J. Childress. 1977. Activity and physiological significance of the pleopods in the respiration of *Callianasa californiensis* (Dana) (Crustacea: Thalassinidea). Abstract. Biol. Bull. 152: 134-146.

Yaikin J., R.A. Quinones, and R.R. Gonzalez. 2002. Aerobic respiration rate and anearobic enzymatic activity of *Petrolisthes laevigatus* (Anomura, Porcellanidae) under laboratory conditions. J. Crustac. Biol. 22(2): 345-352.

Zebe E. 1982. Anaerobic metabolism in *Upogebia pugettensis* and *Callianassa californiensis* (Crustacea, Thalassinidea). Comp. Biochem. Physiol. 72B(4) 613-617.

Zebe E. 1991. Arthropods. 218-237 in Bryant, Christopher (ed.) Metazoan Life Without Oxygen. Chapman and Hall, N.Y.

APPENDIX

Additional information for *H. californiensis* individuals tested. Individuals with less than 3 trials experienced mortality prior to the end of the study.

Animal i.d.	Sex	Weight (g)	Capture Date	# Trials
0702	female	118	9/27/07	3
0801	female	132	3/27/08	3
0803	female	122	3/27/08	3
0804	male	123	3/27/08	1
0805	male	135	3/27/08	3
0806	female	122	3/27/08	2
0807	female	119	3/27/08	3
0808	female	71	3/27/08	3
0809	female	124	3/27/08	3
0810	female	128	3/27/08	1
0811	male	216	3/27/08	3