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Determining the Standard metabolic Rate of the Mantis Shrimp, Squilla empusa: the First Step in Calculating the Heat Increment of Feeding

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Determining the Standard Metabolic Rate of the Mantis Shrimp, Squilla empusa: *The First Step in Calculating the Heat Increment of Feeding*

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Table Of Contents

List of Figures

Abstract

The heat increment of feeding ($HI_{F)}$ is the metabolic response following a feeding period. There is an increase in metabolism following a meal. The heat increment of feeding of the mantis shrimp, *Squilla empusa*, has not been studied before, although almost half of the published invertebrate HI_F studies have been on crustaceans.

The standard metabolic rate is the minimum metabolic rate of a postabsorptive ectotherm, at rest, during its non-active period, at a defined temperature. The standard metabolic rate (SMR) of the mantis shrimp was measured using indirect calorimetry, measuring the dissolved oxygen concentrations of the inflowing water and the outflowing water of a closed system over a 24 hour interval. From there, the oxygen consumption rate (mg/O_2/hr/g tissue) was calculated and compared to the time of day to determine the standard metabolic rate of each individual.

A circadian rhythm associated with the SMR was observed where it starts at an average low consumption rate of 0.125 mg/O_2/hr/g tissue at around 19:00, reaches an average peak of 0.175 mg/O_2/hr/g tissue at about 02:30 and sunsequently declines to an average of 0.125 mg/O_2/hr/g tissue again until about 09:00. The shrimp consumed the most at night, which is consistent with the fact that they are nocturnal animals. In future studies, the next step would be to measure the oxygen consumption directly following a meal and calculating the difference between the postprandial oxygen consumption and the SMR to determine the HI_F

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1. Introduction

The metabolic response following a feeding period is a phenomenon that has been studied for more than 200 years (Secor, 2009). Heat increment of feeding (HI_F) represents an increase in metabolism following a meal. A few possible reasons for this increase in metabolism have been suggested throughout the years. Among them are the ideas of the organism warming the food up to core body temperature, the active uptake of nutrients across the small intestine, and the organs, especially the liver expending energy to process the food (Overgaard *et al.*, 2002). Many studies have shown that the latter reasoning, organs utilizing energy to process the food, is the biggest source of the increase in metabolism, although the magnitude is determined by the type of food, size of the meal and how often the organism feeds (Hill *et al.*, 2012).

HI_F has been quantified for many species, both vertebrates and invertebrates. In general, there is a quick increase in metabolism following a meal that peaks and subsequently declines to the preprandial level (Secor, 2009). There is a huge range of changes in metabolic rate associated with digestion, from up to 697% increase in snakes to a mere 25% increase in humans (Secor, 2009). As briefly mentioned before, many aspects affect the HI_F such as the type and size of the meal as well as body size and composition of the subject. Environmental factors such as temperature and oxygen concentrations affect the HI_F as well (Secor, 2009). Heat increment of feeding research is a growing field. Many taxa have been studied thus far but unstudied species, sources, determinants and control of HI_F are open areas of study (Secor, 2009).

Many of the invertebrate species studied for their metabolic response to feeding are aquatic or semi-aquatic with most of them marine (Secor, 2009). Marine invertebrates have the greatest variation in body size, composition and meal size compared to other major taxa (Secor, 2009). Almost half of the species that have been studied are of the subphylum Crustacea, of which the mantis shrimp is a member. A study was done on the HI_F of the shore crab, *Carcinus maenas*, and the relationship with temperature (Robertson *et al.*, 2002). It was found that following fasting, oxygen consumption varied directly with the temperature to which the crabs were acclimated. It was also discovered that HI_F was higher at the ambient temperature (15 °C) than at the lower (7 °C) and higher (22 °C) temperatures “despite similar ration sizes in all groups” (Robertson *et al.*, 2002). The same study also discovered that the time to peak and the heat increment of feeding response length varied inversely with temperature (Robertson *et al.*, 2002). A second experiment investigating *Carcinus maenas* was looking at protein synthesis and oxygen consumption after a meal (Houlihan *et al*., 1990) where it was concluded that the amount of food eaten is an important factor influencing postprandial oxygen consumption. Closed respirometry was used to measure the SMR and then the heat increment of feeding over a thirty minute time period post-feeding (Houlihan *et al.*, 1990). It was discovered that there was a relationship between the amount of food consumed and the HI_F measured via oxygen consumption (Houlihan *et al.*, 1990). The duration of the postprandial metabolic response was linearly-dependent on the size of the meal consumed. The maximum increase in oxygen consumption after a meal was found to be 2.3 times greater than the resting metabolic rate and 5.4 times greater during periods of activity (Houlihan *et al.*, 1990). Similar experiments have been performed on the spiny lobster, *Panulirus argus* (Perera *et al*., 2007) and have shown that metabolism increased linearly with water temperature and the relationship was found to be statistically significant (Perera *et al.*, 2007). Similarly, feeding affected the metabolism, with oxygen consumption increasing after a meal (Perera *et al.*, 2007). A final experiment was a study of the heat increment of feeding and heat loss related to growth in Chinese shrimp, *Fenneropenaeus chinensis* (Huang *et* al., 2008). It was found that the heat increment of feeding was much greater than in fasting shrimp, as expected.

Heat increment of feeding can be measured in a multitude of ways but with aquatic animals, indirect calorimetry is usually used, measuring oxygen consumption (Secor, 2009). The first, and arguably most important step in determining the heat increment of feeding is to establish a baseline metabolic rate for comparison. In general, this baseline metabolic rate is determined for the individual subjects with the preferred baseline being the standard metabolic rate for ectotherms and the basal metabolic rate for endotherms (Secor, 2009). The SMR is the minimum metabolic rate of a postabsorptive ectotherm at rest during its non-active period at a defined temperature, while BMR is essentially the same measurement but for endotherms, at a neutral temperature (Hill *et al.,* 2012). As previously stated, one of the more common methods is utilizing indirect calorimetry to quantify the expended energy via oxygen consumption coupled with carbon dioxide production rates (Secor, 2009). Basically, the subject is fed after a baseline is measured. The meal is usually a targeted percentile of the animal's body mass, and metabolic rates are measured afterwards either continuously or at assigned time intervals until the baseline is reached again (Secor, 2009). Without this baseline, there is not metabolic rate for comparison against the postprandial metabolic rate.

Mantis shrimp are stomatopods, related to shrimp, crabs, and lobster (ref). They are highly territorial and violent, nocturnal animals (Dingle and Caldwell, 1968). Mantis shrimp are often studied for their fast-moving claws and incredible eyes. Their claws produce one of the fastest movements in the animal kingdom (Patek *et al.*, 2004). Their eyes have dozens of photoreceptors than can see a wide range of colors, as well as ultraviolet light , unlike humans who only have three photoreceptors (Cronin and Marshall, 1989). While mantis shrimp are fascinating flagship organisms for speed and vision, there has been little to no research on their oxygen consumption levels, metabolism, or circadian rhythms. In this study, the standard metabolic rate of the mantis shrimp, *Squilla empusa*, was determined as the first step to defining the heat increment associated with feeding in the species. The indirect calorimetry method of measuring ingoing and outgoing dissolved oxygen concentrations and subsequently calculating the consumption was used in this experiment as a means of accurately measuring the oxygen consumption.

2. Methods

This experiment was conducted in the Roger Williams University CEED Marine Laboratory. Seawater was used in the system at the normal laboratory temperature of ~18 °C. A closed-chamber open flow system was designed and built to measure the dissolved oxygen concentrations going in and coming out of the system along with the rate of flow of seawater through the system. Oxygen consumption was measured for six individual shrimp and compared to time of day and body size.

2.1. System Design:

A system was built that allowed one to contain the shrimp in a flow-through chamber and to measure the ingoing and outgoing dissolved oxygen concentrations from the chamber (Appendix 1: Diagram 1, Figure 10). The seawater supply started at a head tank and flowed through the respirometer via gravity with flow rate adjusted with a valve. The water flowed through an oxygen probe to determine the inflowing dissolved oxygen concentration. It then flowed through the animal chamber where the shrimp was contained and then out of the chamber, through a second sensor attached to a second oxygen probe, measuring the outflowing dissolved oxygen consumption. The water then flowed through a calibrated rotameter flow meter (outflow was set to ~70% inflow) (Appendix 1: Figure 9) and then out as waste. The temperature throughout the system was maintained at the normal laboratory temperature of approximately 18 °C.

2.2. Care of Mantis Shrimp

The shrimp, *Squilla empusa*, were collected from local fisherman in Mount Hope Bay. The six shrimp used in this study were housed individually in small, plastic tanks with flow-through seawater. The water flow was constant through each tank and the temperature was maintained at the normal laboratory temperature of 18°C. The shrimp were each given a PVC pipe in their tank to simulate burrowing conditions. The mantis shrimp were fed one shrimp, regularly.

2.3. Data Collection and Analysis

One shrimp was measured at each time and there were six shrimp measured total. The shrimp was selected and fed one small shrimp meal. There was a subsequent waiting period of twenty-four hours to allow for complete digestion of the meal. The oxygen probes were calibrated at zero and 100% saturation using 100% saturated seawater. After calibration, water was allowed to flow through the system and fill the chamber. The shrimp was placed into the chamber and the water flow was adjusted such that the differential between the inflow and outflow was 30% saturation, i.e. outflow was 70% of the oxygen concentration of the inflow. Flow was measured by a calibrated flow meter. Data collection occurred for twenty-four hours. After the data collection period, the shrimp was weighed and returned to its housing.

Data was collected using a GoPro ™ Hero 3 Silver Edition Camera and a GoPro ™ Scorpion mount. The camera took a picture of the oxygen probe screens once every sixty seconds for the entire measurement period (Appendix 2: Figure 11). The measurement period lasted about twenty-four hours. The concentrations on each picture were manually entered into Microsoft Excel, along with time of day, in five minute intervals. Notes were made about activity levels, as the shrimp were visible in the chamber. Once all of the data were entered, the difference between the oxygen concentrations into and out of the system was calculated. From there, oxygen consumption (mg/O_2/hr/g tissue) was calculated according to the Fick principle (Steffensen, 1989; Fick, 1870) as:

$$Mo_2 = Vw\,(Cwo_2,\text{ in} - Cwo_2,\text{ out}) / bw \qquad (1)$$

Where Vw is the water flow rate through the respirometer, Cwo_2, in and Cwo_2, out are the concentrations of oxygen flowing into the system and out of it, respectively, and finally bw is the weight of the shrimp in grams. ~~Oxygen~~ consumption was then plotted against time of day and a 6th order polynomial trend line was determined for each graph. The average consumption (mg/O_2/hr/g) over the twenty-four hour period of all of the shrimp (n=6) was calculated and plotted against time of day. Finally, average consumption (mg/O_2/hr) was calculated for each shrimp and compared relative to body weight (g).

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3. Results

3.1. Standard Metabolic Rates

The oxygen consumption showed a general trend for each of the six shrimp in the experiment. There were three peaks in the consumption of each shrimp (Figures 1-6). There was a peak at the beginning of the measurement period, one more in the middle of the night, and one towards the end of the period. The initial peak is an acclimation period. All of the mantis shrimp start at an average low consumption of 0.125 mg/O_2/hr/g tissue at around 19:00, reach an average peak of 0.175 mg/O_2/hr/g tissue at about 02:30 and continue to decline to an average of 0.125 mg/O_2/hr/g tissue again until about 09:00. Although some of the data had weaker peaks (Figures 4 and 5), the same general pattern was present for all six test subjects, regardless of gender or size. All of the shrimp had oxygen consumptions throughout the measuring period close to 0.2 mg/O_2/hr/g tissue or below with the exception of Shrimp 3 which had a consumption that was slightly elevated, compared to the rest. The average consumption of all shrimp was calculated for each time point throughout the day (Figure 7) and showed the same pattern.

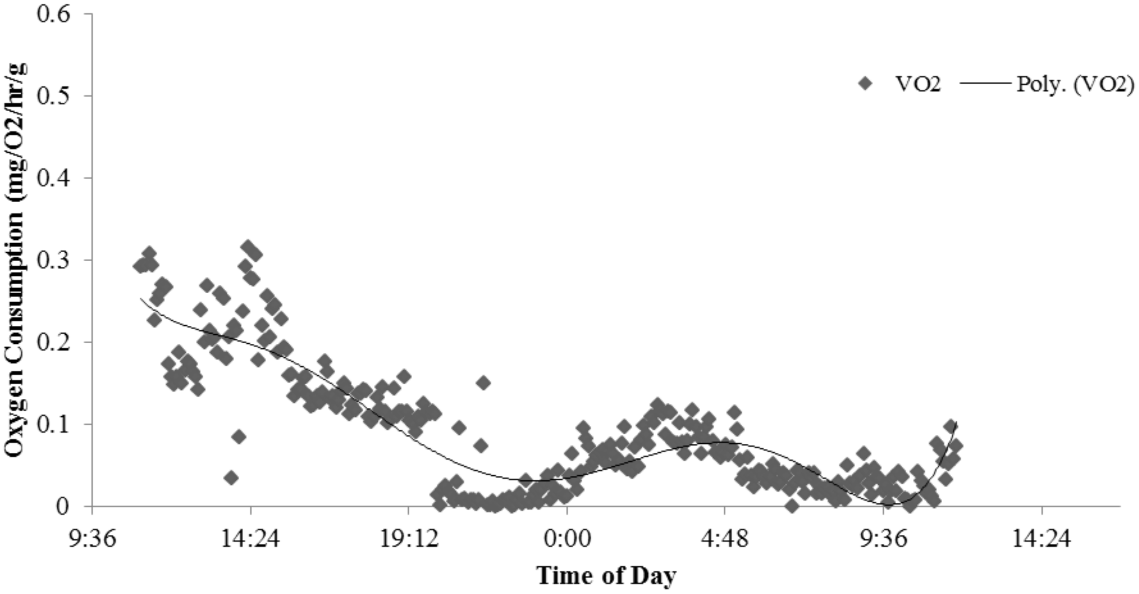

Figure 1: Oxygen consumption (mg/O_2/hour/g tissue) calculated for Shrimp 1 from 11:05, February 28, 2014 to 11:50, March 1, 2014. Shrimp 1 weighs 55.7g and is of undetermined gender.

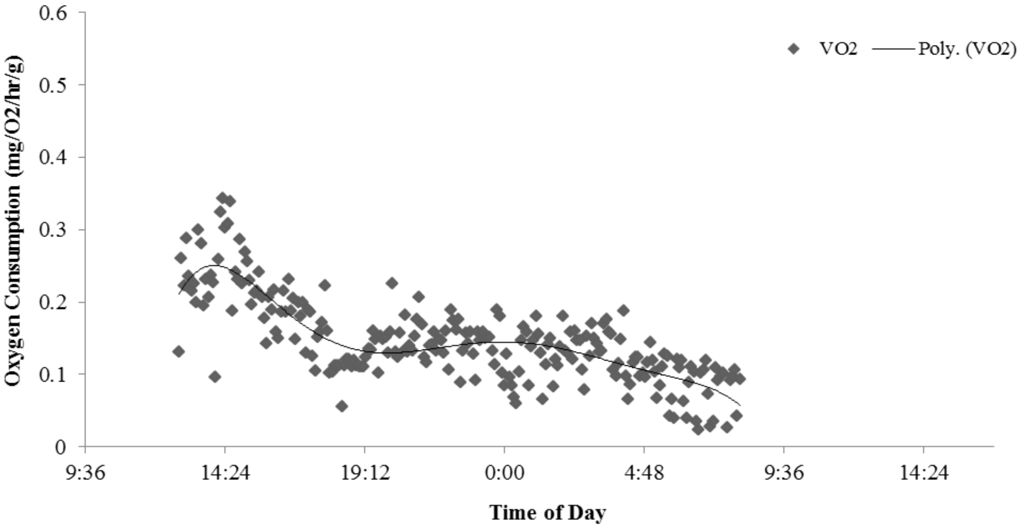

Figure 2: Oxygen consumption (mg/O_2/hour/g tissue) calculated for Shrimp 2 from 12:50, March 5, 2014 to 8:05, March 6, 2014. Shrimp 2 is female, weighing 51.1g.

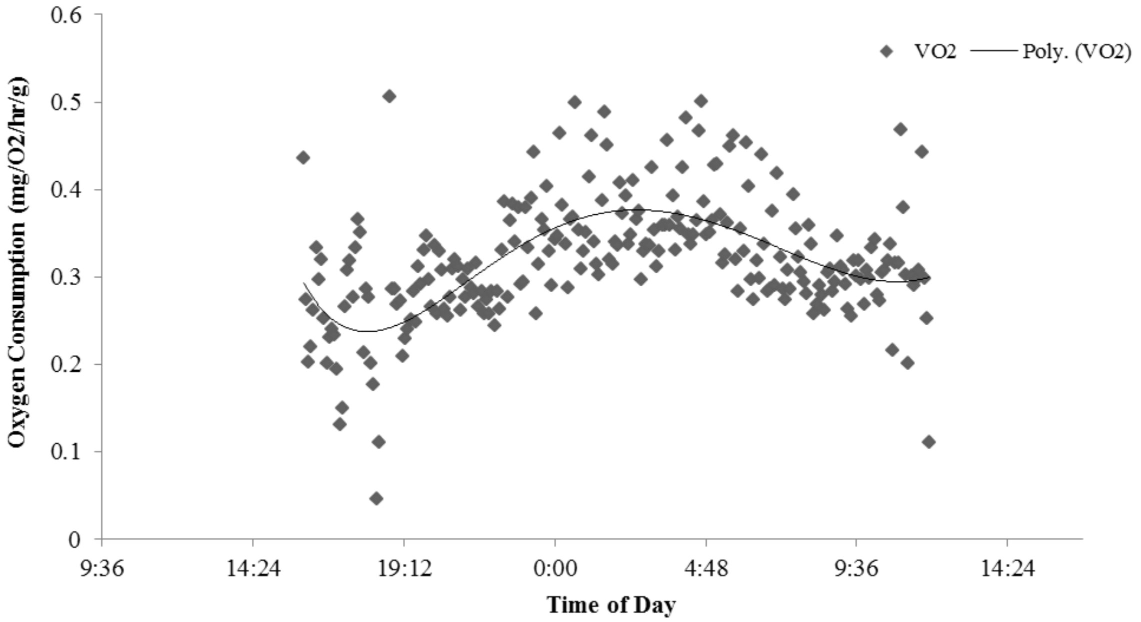

Figure 3: Oxygen consumption (mg/O$_2$/hour/g tissue) calculated for Shrimp 3 from 16:00, March 6, 2014 to 11:55, March 7, 2014. Shrimp 3 is female, weighing 40.4g.

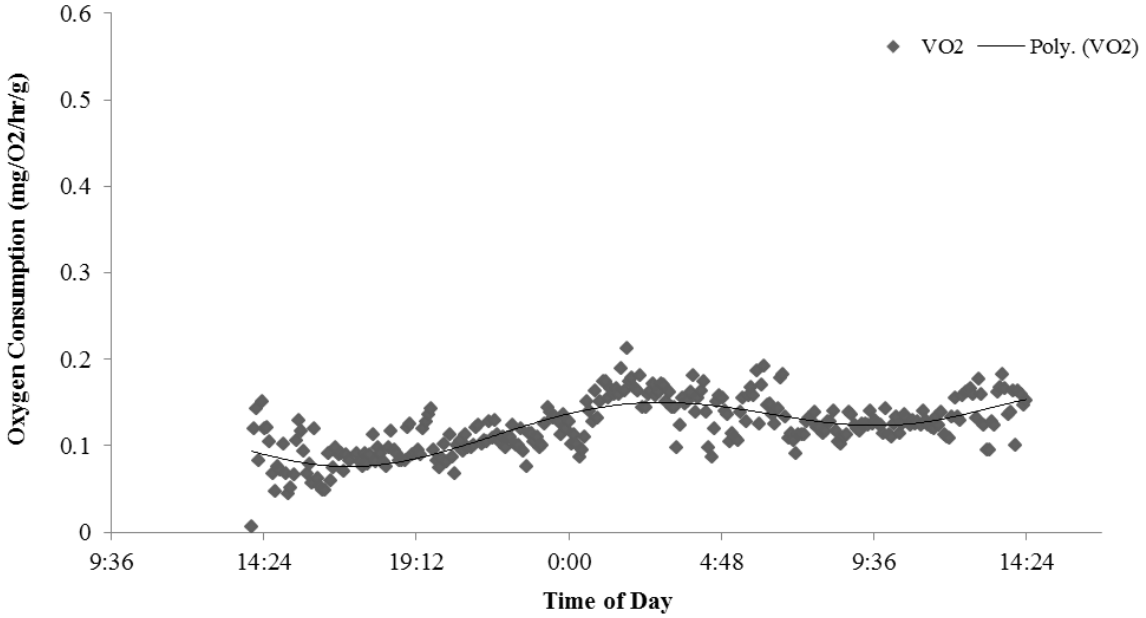

Figure 4: Oxygen consumption (mg/O$_2$/hour/g tissue) calculated for Shrimp 4 from 14:00, March 19, 2014 to 14:25, March 20, 2014. Shrimp 4 weighs 41.4g and if of undetermined gender.

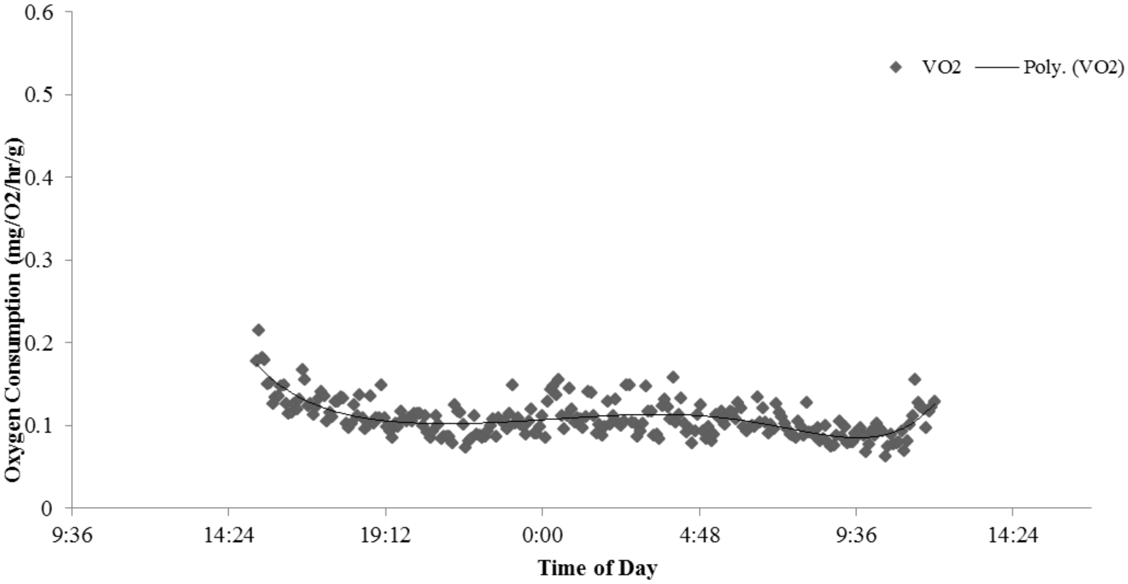

Figure 5: Oxygen consumption (mg/O_2/hour/g tissue) calculated for Shrimp 5 from 15:15, March 20, 2014 to 12:00, March 21, 2014. Shrimp 5 is female, weighing 59.1g.

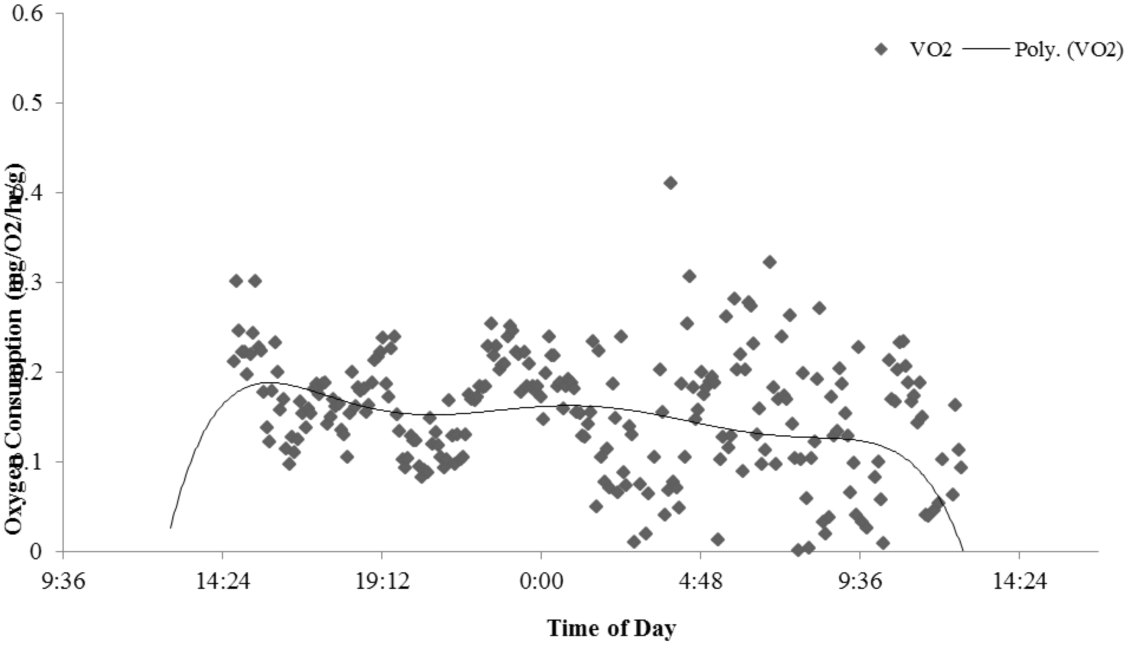

Figure 6: Oxygen consumption (mg/O_2/hour/g tissue) calculated for Shrimp 6 from 14:45, March 21, 2014 to 12:50, March 22, 2014. Shrimp 6 weighs 49.2g and is of undetermined gender.

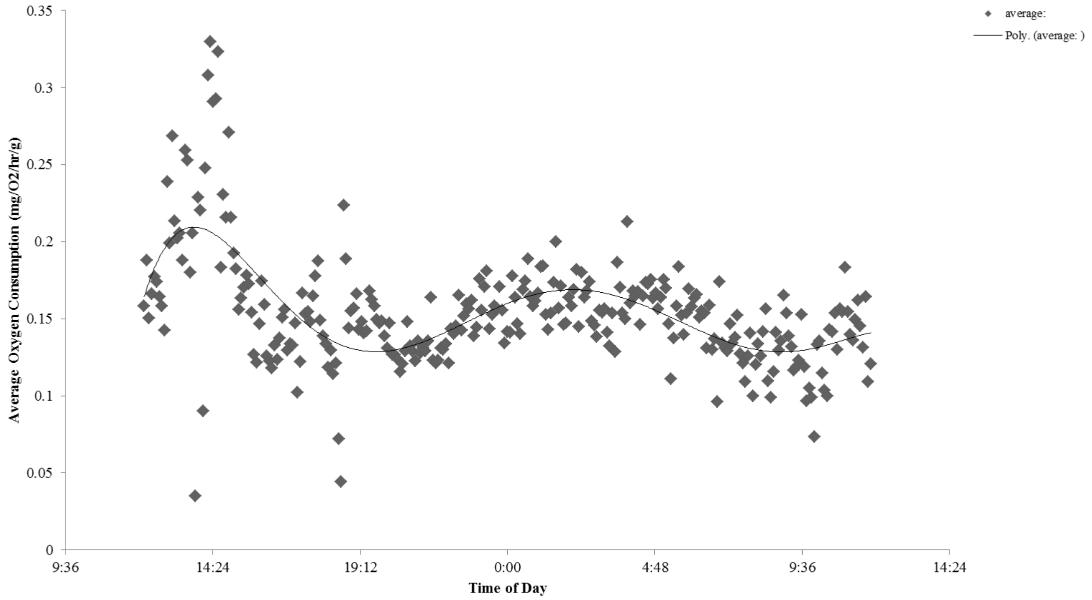

Figure 7: Average oxygen consumption rate (mg/O_2/hr/g tissue) of all shrimp compared to time of day (n=6).

3.2. Consumption Compared to Size

The data initially appeared to be scattered with a negative correlation between average oxygen consumption (mg/O_2/hr) and body weight (g). The slope is -0.1549 which shows the negative correlation. The R^2 value is 0.205: the increase in weight of the shrimp accounts for about 20.5% of the decline in oxygen consumption. As body weight increased, the average oxygen consumption decreased.

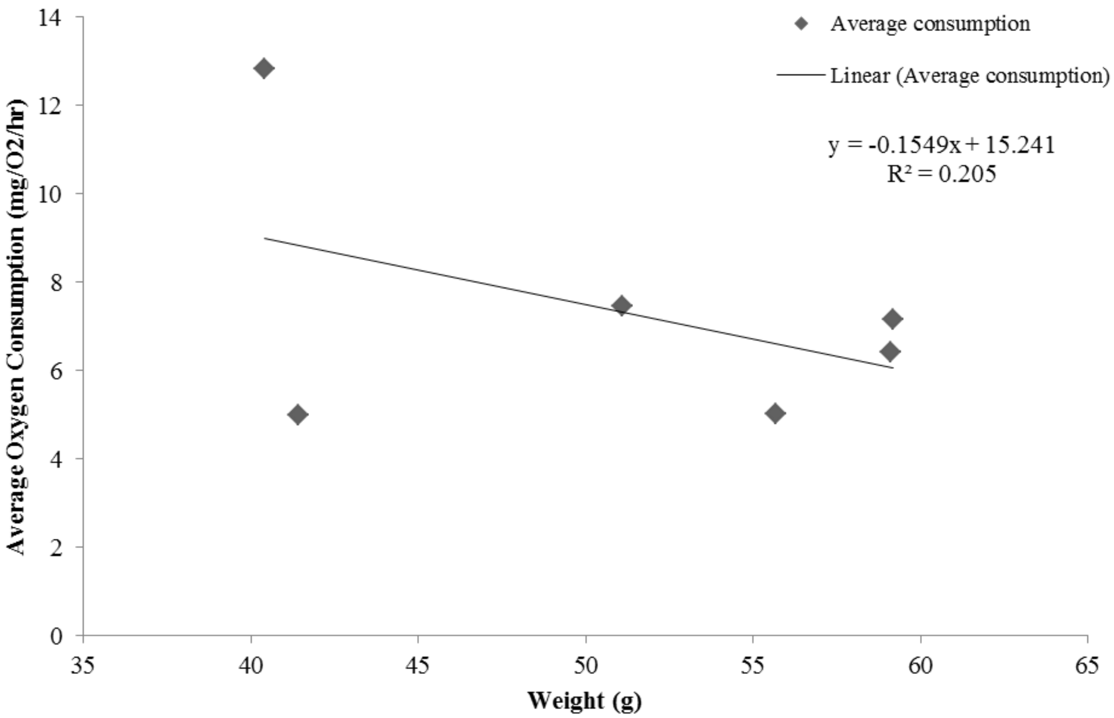

Figure 8: Average oxygen consumption (mg/O_2/hr) in comparison to body size (weight in grams) for all shrimp (n=6).

4. Discussion & Conclusions

This study on the heat increment of feeding of the mantis shrimp, *Squilla empusa*, determined the first part necessary for measuring HI_F, which is the standard metabolic rate of the species. From the standard metabolic rate, light was shed on the circadian rhythms of this species as well.

Although the individual shrimp had different oxygen consumption levels, there was a distinct pattern in each as well as in the combined average oxygen consumption of all six shrimp over the measuring period. There was a peak at the beginning of each time period. There was regarded as the acclimation period of the shrimp adjusting to the new enclosure. Therefore, the acclimation period is ignored and the start was at a lower consumption for each shrimp around 19:00 which then rose towards the middle of the night, peaking at about 02:30, and then declining towards the morning hour of 09:00. This corresponds with the knowledge that mantis shrimp are nocturnal organisms (Goldsmith and Cronin, 1993) and most active during the night. This phenomenon of circadian rhythms in terms of oxygen consumption been observed in other crustaceans as well. Both *Callinectes sapidus* and *Astacus leptodactylus* have been shown to have rhythms to their oxygen consumption in order to compensate for natural increases in muscular activity and oxygen needs (Massabuau, 2001).

As previously stated, the individual shrimp's oxygen consumption levels are different for each individual, although they do show the same pattern. It is widely known that temperature and salinity have an effect on the standard metabolic rate and consequently, the heat increment of feeding in crustaceans, and shrimp are no exception (Allan *et al.*, 2006) but the discrepancies in the oxygen consumption here is not from those parameters because temperature and salinity were constant throughout the experiment and therefore, each shrimp experienced the same environmental conditions. Seasonal variation in the heat increment of feeding has been shown in some species of lobster (Radford *et al.,* 2004) as well but that would not be the case here either, because all shrimp were measured within about a month of each other The differences are then possibly due to gender, activity levels, or body size.

In terms of differences in oxygen consumption and therefore standard metabolic rate due to gender, there have been studies that show that female blue crabs have a lower respiration rate than males (Engel and Eggert, 1974) while later studies show no difference in oxygen consumption among genders for the same species of blue crab (Laird and Haefner Jr, 1976). In this study, it is inconclusive as to whether gender affected the oxygen consumption or not. One known female had the highest oxygen consumption while another known female had the lowest. Of the six mantis shrimp, three were known to be female because they had laid eggs, yet the other three were considered undetermined. This was due to the fact that they had not laid eggs but were not confirmed to be male because sexing mantis shrimp is difficult, especially while alive. The physiological research was not conducted to determine the gender of the undetermined individuals or to determine stages of sexual maturity in any of the shrimp either.

There were different levels of activity among the shrimp throughout their measuring periods. Active animals have been shown to have a higher consumption rate than inactive animals and that difference in consumption becomes amplified with increasing body size (Wallace, 1972). Shrimp 3 was one of the most active shrimp, moving constantly in the chamber, and had the highest oxygen consumption while shrimp

4, of similar body size, had one of the lowest and was not active throughout the measurement period. Shrimp 3 also happened to be the lightest shrimp of the six, weighing 40.1 grams. If shrimp 4 is disregarded, the general trend is that with increased body size, there is less activity and less oxygen consumption. Shrimp 5, for example was the largest, weighing 59.1 grams and the least active, not moving at all throughout the time period. It is unclear if shrimp 5 didn't move due to size in the small chamber or due to some other factor. Looking at body size compared to oxygen consumption, there is a negative correlation: as body size increases, average oxygen consumption over the time period decreases. Many studies have shown that there is an increase in oxygen uptake with larger body size (Zeuthen, 1953) but more recent observations have shown that resting weight-specific oxygen consumption in crustaceans decreased with increasing weight (Bridges and Brand, 1980). In this study, with increased body size, there is a decrease in average oxygen consumption. Larger animals have lower mass-specific body rates than smaller ones (Hill *et al.,* 2012) but in this experiment there was a very small range of body sizes so that trend doesn't necessarily apply and with an R^2 value of 0.205, only about 20.5% of the variability in oxygen consumption is due to body size. There is no definitive reason for the differences in average oxygen consumption for the individual measurements of the shrimp. The differences in the individual oxygen consumption are minimized when the consumptions of the shrimp are averaged for each time of the day. If the first peak in consumption is, again, considered to be an acclimation period, the shrimp all start at an average low consumption of 0.125 mg/O_2/hr/g tissue at around 19:00, reach an average peak of 0.175 mg/O_2/hr/g tissue at about 02:30 and continue to decline to an average of 0.125 mg/O_2/hr/g tissue again until about 09:00. This is the same pattern with every individual and the average overall consumption which shows that this is the standard metabolic rate of the shrimp and their circadian rhythm of oxygen consumption.

This determination of the standard metabolic rate and associated circadian rhythms is conclusive for the six individuals that were tested in this study. There was variability in the data of individual shrimp. If more shrimp had been tested, the variability may have had less of an effect on the average consumption throughout the measuring period and the standard metabolic rate would be more robust. This research was the first step in determining the heat increment of feeding of the species, *Squilla empusa*. After measuring the oxygen consumption of more shrimp, the next step would be to measure the oxygen consumption of the same shrimp directly after consumption of a meal and then calculating the HI_{F} from there.

Knowing the HI_{F} with this particular species not only adds to the general knowledge of heat increment of feeding and metabolism of different species as a whole but also have more local implications. Mantis shrimp are not a widely popular item for human consumption. This could change once the metabolism of the shrimp is determined. It has been shown that beef quality can be improved with optimization of the cow’s nutritional plan (Warren *et al.*, 2008). Here, something similar could be implemented. Understanding the metabolism and nutritional requirements allows for a better nutritional plan to be constructed as mantis shrimp are raised in a laboratory setting. Having an optimal nutritional plan could allow the shrimp to reach market size more quickly. This addition to seafood variety available could then perhaps put less fishing pressure on comparable species that are less available or less sustainable.

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6. Appendices

6.1. Appendix 1: System Schematics

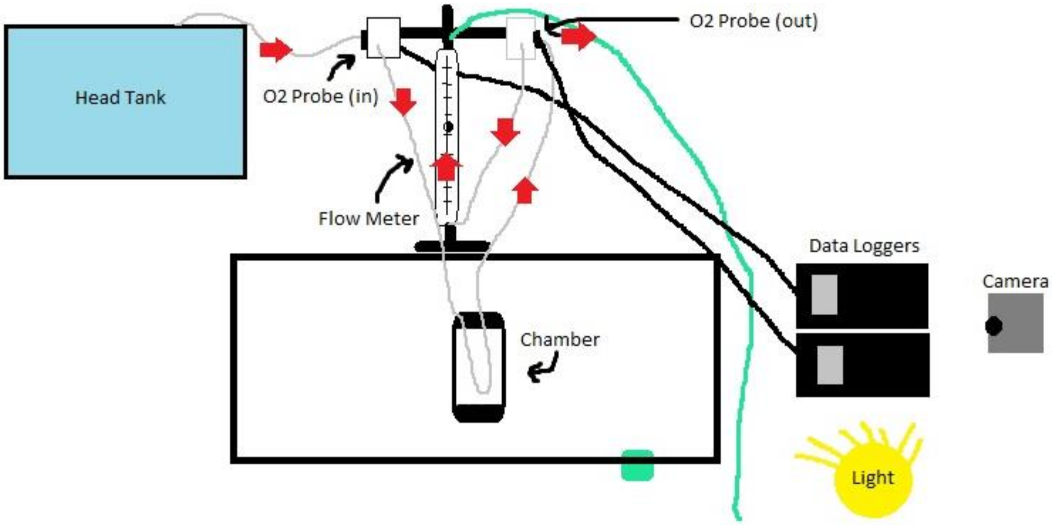

Figure 9: Schematic for system designed to measure dissolved oxygen concentrations of the inflow and outflow of a closed chamber. Water flowed in the direction of the red arrows.

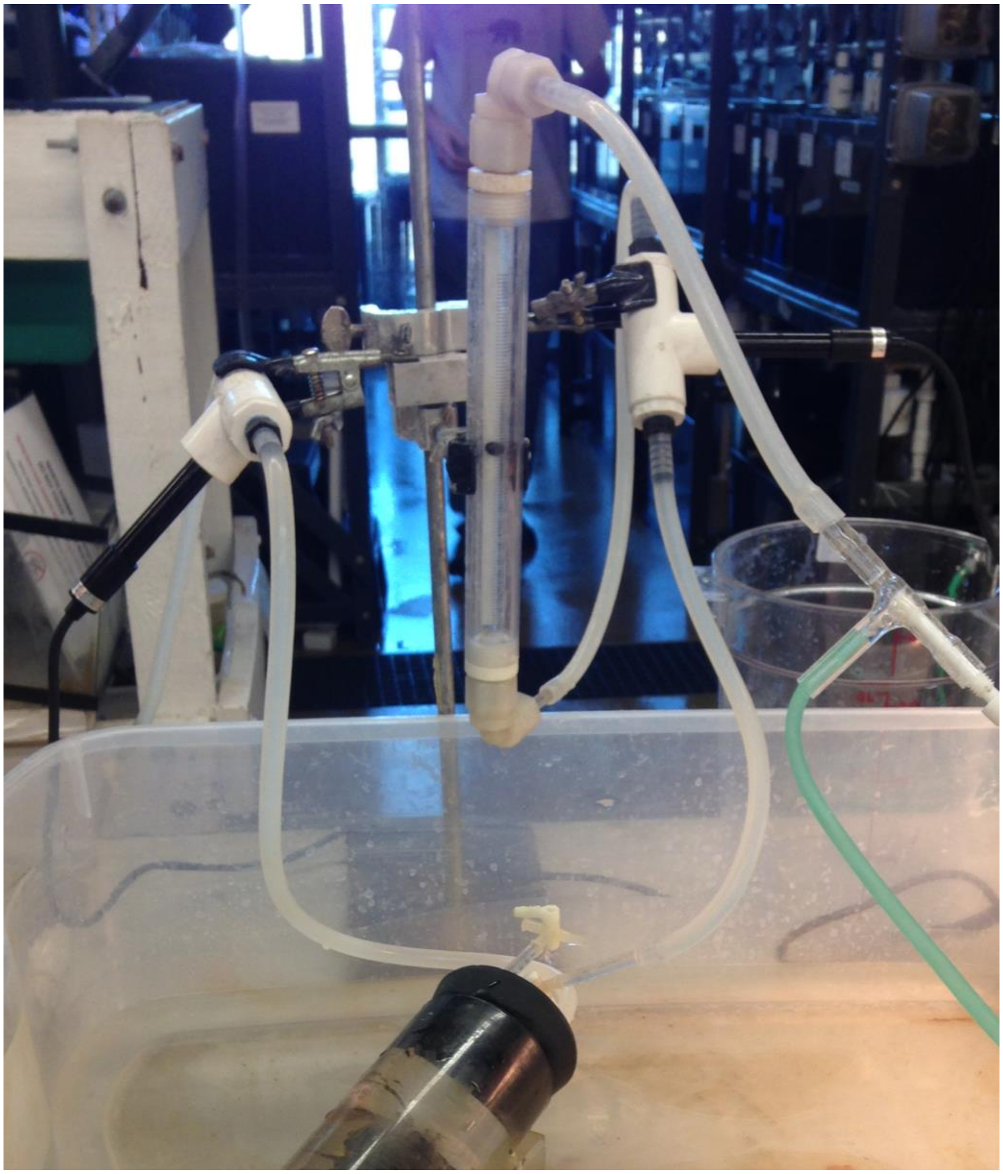

Figure 10: View of the actual flow meter and dissolved oxygen sensors.

Figure 11: View of system from GoPro™ camera angle

6.2. Appendix 2: Data Sample

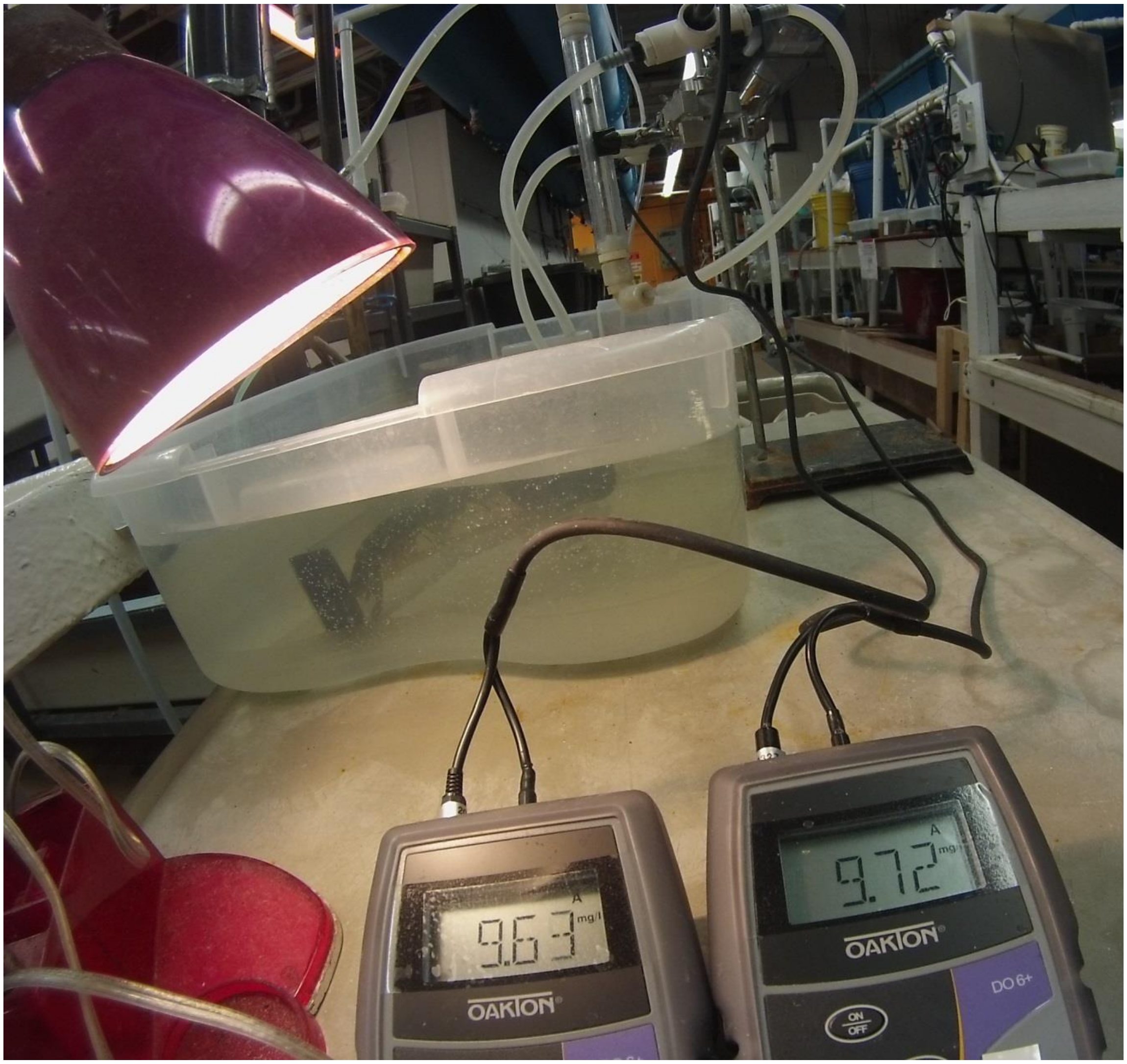

Figure 12: Sample of data collected by GoPro™ camera