METABOLIC ENZYME ACTIVITIES OF BENTHIC ZOARCIDS OFF THE COAST OF CALIFORNIA

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

THESIS ADVISOR

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ACKNOWLEDGEMENTS

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ABSTRACT

Our knowledge of deep-sea ecosystems is poor. Specifically, knowledge is lacking concerning deep-sea benthic communities and their metabolic rates. There has been much debate concerning what controls metabolic rates in animals, whether it is size and temperature or declining light levels and the selective pressure for locomotory capacity. Zoarcids are widely distributed and speciose, therefore, a great model for this research. The rates of four enzymes in twelve species of zoarcids were examined as a biochemical proxy for metabolic activity. Enzyme assays were performed on two anaerobic enzymes, citrate synthase and malate dehydrogenase, and two aerobic enzymes, pyruvate kinase and lactate dehydrogenase. Both benthic and benthopelagic species were analyzed, over a broad depth range and size gradient. No significant decline in enzymatic activities with increasing median depth of occurrence for both benthic and benthopelagic zoarcids was found. Only one benthic species, *Lyconema barbatum* exhibited changes in aerobic enzymatic activity with increasing mass, however only between the shallower specimens. The results from this research show that variations in enzyme activities exist among the different species of benthic and benthopelagic zoarcids, but is not accounted for by depth of occurrence or size. It is likely that the differences between species in enzymatic rates reflect the feeding and swimming lifestyles of these fishes. Further study is needed to understand the factors affecting the variation in enzyme activity.

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CHAPTER 1
INTRODUCTION

There is a significant lack of knowledge concerning the deep-sea ecosystem, which is one of the largest ecosystems on the planet. As commercial fisheries move to depths beyond continental shelves, and the ocean continues to be affected by anthropogenic activities, it becomes essential to understand how marine organisms will respond to these disturbances (Glover and Smith, 2003). Much of the deep-sea remains to be studied in terms of its biodiversity, biological productivity, species physiological strategies, and processes that are linked with surface ocean productivity (Rex and Etter, 2010). The benthic (on the seafloor) and benthopelagic (typically found near, but not on, the seafloor) communities, which make up a large proportion of this understudied realm of the ocean, are important for studying the energy requirements of organisms in the deep-sea ecosystem and need to be studied in more detail.

Zoarcids are a family of perciform fishes, which are a dominant taxa among fishes throughout the oceans (Mecklenburg et al., 2002). Popularly known as eelpouts, there are over 200 species, and they are found from shallow temperate waters to the polar seas, though species living in the tropics are found only in deep waters (Eschmeyer and Herald, 1983; Hildebrandt et al., 2011). Many eelpouts are deep-sea forms, and the limited information available on their biology comes from only a few specimens. Most species live associated with the seafloor between depths of 200-3000m, however there are some intertidal,

meso/bathy pelagic, and abyssal species. For the very few species which have been studied, they are poorly equipped for speed, having reduced muscle mass, lower protein content, and lower activities of aerobic and anaerobic enzymes. It is been suggested that this is due in part because they are visually limited tactile predators with an eel-like body form (Seibel and Drazen, 2007).

Metabolism is the most fundamental biological rate (Drazen and Seibel, 2007). It is the rate at which organisms acquire, transform, and use energy and materials. Metabolism provides the foundation with which to link the biology of individual organisms to the ecology of ecosystems and the roles that the organisms play (Brown et al., 2004). Most of the research to date related to deep-sea metabolism has been conducted on pelagic species (Drazen and Seibel, 2007). There are many challenges associated with laboratory-based and *in situ* measurements of deep-sea organisms' oxygen consumption. Enzyme assays can be used as a substitute for the standard procedure of measuring oxygen consumption and are a biochemical proxy of metabolic rate (Drazen and Seibel, 2007). For this study, we are primarily interested in examining the enzymatic rates in white muscle tissue, which comprises the bulk of the body mass in these benthic and benthopelagic fishes and is the power source of anaerobic burst swimming for prey capture and predator avoidance (Somero and Childress, 1980; Sullivan and Somero, 1980). We will be looking at the enzyme activities of two anaerobic enzymes associated with glycolysis, lactate dehydrogenase (LDH) and pyruvate kinase (PK), and two aerobic enzymes associated with the citric acid cycle, citrate synthase (CS) and malate dehydrogenase (MDH).

Currently there are two opposing theories about the differences in the metabolism between shallow and deep-water living species. These differing schools of thought explain the variations in metabolic rate to either be attributed to anatomical or environmental constraints or the diversity of ecological roles and the energy demands associated with those roles (Seibel and Drazen, 2007). Specifically, one of those theories that is used to predict metabolic rates is the Metabolic Theory of Ecology (Gillooly et al., 2001; Brown et al., 2004). These authors provide a model that characterizes the general effects of temperature and body mass on metabolic rate. They found that resting metabolic rates of a wide-range of organisms studied were correlated with temperature and body size. Therefore these two parameters were thought to be the major determinants of metabolic rates of any species of interest (Gillooly et al., 2001). However, there is a considerable amount of variability not explained by their relationship; more than a 300-fold variation between the fastest and the slowest marine animals after mass and temperature adjustments have been made (Seibel and Drazen, 2007).

An alternative hypothesis, the Visual Interactions Hypothesis (VIH) attributes the decline in metabolic rates with depth not to resource limitation or temperature and pressure constraints, but to a correlation with the reduction of light with depth, which contributes to reduced predator-prey interactions for visually interacting taxa (Childress and Mickel, 1985; Seibel and Drazen, 2007). The reduction in predator-prey interactions results in a relaxation of the selective pressure for locomotory capacity, and therefore a reduction in metabolism (Drazen and Seibel, 2007). Evidence supporting the VIH was found by Drazen

and Seibel (2007) who analyzed metabolic rates in a variety of marine taxa and observed a decline in metabolic rate with depth to be most pronounced in pelagic species, with less variation for benthic species. This is a result of the fact that the sea floor offers more chances for crypsis than does the open ocean. This relaxes predator-prey interactions and the need for long-range locomotory capacity. Benthopelagic species, which are more mobile than benthic species, tend to show a decline in metabolic rate with depth in between that of pelagic and benthic species. In further support, Hand and Somero (1983) found that the enzymatic activities of animals from the hydrothermal vent habitat were similar when compared to the activities of shallow-water species. With a high potential for energy metabolism, the hydrothermal vent species' enzyme activities are evidence that the low temperatures and higher hydrostatic pressures of the deep-sea are not important factors selecting for reduced metabolic rates in deep-sea organisms. Lastly, evidence was found from studies of animals inhabiting the isothermal Antarctic water column that didn't show declines in metabolic rates related to depth (Seibel and Drazen, 2007).

This study will evaluate the metabolism of benthic and benthopelagic zoarcids off the coast of California over a broad depth gradient and size range. Zoarcids are a good model to use for this research because they are widely distributed and speciose. In addition, zoarcids are visually limited predators, which also makes them a good model to use to test the VIH, particularly because there should be little or no decline in metabolic rate with depth. To test this hypothesis, the enzymatic rates of both aerobic and anaerobic enzymes in white

muscle tissue as a proxy for metabolic rate will be determined. The proposed research will contribute significantly to the understanding of the metabolism and ecology of zoarcids, a widely distributed group of fishes. In addition, it will supplement our lack of knowledge concerning the deep-sea ecosystem and energy transfer with depth. Lastly, the proposed research will help to implement sustainable fisheries management policies and assess how deep-sea fish will respond to future anthropogenic threats.

The null hypotheses that will be addressed in this study are:

H1: There is no change in metabolic enzyme activities of benthic and benthopelagic zoarcids with increasing depth.

My first objective will be to analyze biochemical indicators (CS, PK, MDH, and LDH) for metabolism in white muscle tissue in benthic and benthopelagic zoarcid teleosts caught off the coast of California between 100-3000m.

H2: There is no difference in metabolic enzyme activities between benthic and benthopelagic species of zoarcids.

My second objective will be to analyze biochemical indicators (CS, PK, MDH, and LDH) for metabolism in white muscle tissue between groups of zoarcid teleosts (benthic vs. benthopelagic) caught off the coast of California between 100-3000m.

H3: There is no difference in metabolic enzyme activities of benthic and benthopelagic zoarcids with increasing size.

My third objective will be to analyze biochemical indicators (CS, PK, MDH, and LDH) for metabolism in white muscle tissue in benthic and benthopelagic zoarcid teleosts over a broad size range intraspecifically.

CHAPTER 2
METHODS

2.1 Sample Collection

Stratified trawls were conducted in April and October of 2009, off the coast of California in Monterey Bay. Specimens were collected across a broad depth range, between 100-3000m. In April, otter trawls were deployed to 1000m, and beam trawls were used at 2000m and 3000m. In October, otter trawls were deployed at all depths. Specimens were placed on ice immediately after being sorted from the trawl, then weighed and measured. White muscle tissue was sampled below the first dorsal fin within hours of capture, and then transferred to liquid nitrogen for storage during the cruise. Once brought back to the laboratory, the samples were stored in a -80°C freezer until enzyme assays were performed.

2.2 Enzyme assays

Two aerobic enzymes, CS and MDH, and two anaerobic enzymes, PK and LDH, were assayed using standard protocols described in the literature (Childress and Somero, 1979; Somero and Childress, 1980; Hand and Somero, 1983). Our enzyme activity values reflect the highest potential activities possible in the tissues, which is the V_{max} of enzymatic rates (protocol established so that there is no rate limitation) (Somero and Childress, 1980). Lactate dehydrogenase was selected because it is the terminal enzyme in anaerobic glycolysis in vertebrate tissues and along with pyruvate kinase, provides a good measure of a tissue's

capacity for anaerobic metabolism (Somero and Childress, 1980). LDH activity can be analyzed to determine a fish's ability for burst locomotor capacity (Hand and Somero, 1983; Somero and Childress, 1980). Malate dehydrogenase is an important component of the citric acid cycle, involved in shuttling electrons between the cytosol and the mitochondria as well as, the maintenance of redox balance in the cell (Sullivan and Somero, 1980; Thuesen and Childress, 1994). It is also an indicator of intermediary aerobic metabolism (Somero and Childress, 1980). Citrate synthase plays a key regulatory role in the citric acid cycle and serves as a quantitative measure of a tissue's aerobic activity potential (Somero and Childress, 1980). Also, CS activity can be scaled to body size and represent whole body metabolism (Somero and Childress, 1980).

For each fish, two homogenates of white muscle were prepared. The frozen muscle tissue was weighed then an ice-cold Tris HCl buffer was added as a homogenizing medium (10mM, pH 7.55 at 10°C). The tissues were always kept on ice, and homogenized at 10°C using a motorized Dual-Kontes ground glass homogenizer. Any tissue not sufficiently homogenized using the motorized homogenizer was homogenized by hand.

Assays were run in a total volume of 2.0 ml at 10°C using a temperature controlled Shimadzu spectrophotometer with a temperature controlled water-jacketed 12-cell cuvette changer and a temperature controlled water bath. To account for temperature related metabolic variation, 10°C was chosen; a temperature that is within the range that all of the species included in this study can tolerate. Additionally, it is below the temperature known to cause the

denaturation of proteins, which results in the inactivation of enzymes. The CS assays were run at 412 nm, and PK, LDH, and MDH were run at 340 nm (International Units (IU)). After the CS assays, the homogenates were centrifuged (5000 g) for 5 minutes. The supernatant was then removed carefully to avoid the lipid layer. The supernatant was used for the remaining PK, LDH, and MDH assays.

To assure that there was no rate limitation during the enzyme assays, the chemical cocktails were prepared at the following saturating substrate conditions. For the CS cocktail, the following chemicals and amounts were used: 0.1 mM dithiobis-nitrobenzoic acid, 0.1 mM acetyl CoA, 2 mM $MgCl_2$, and 50 mM Imidazol HCl (pH 8 at 10°C). The reaction was initiated by 0.5 mM oxaloacetate. For the PK cocktail we mixed: 0.1 mM fructose 1,6 bisphosphate, 5.0 mM ADP, 0.15 mM NADH, 10 U of LDH, 10 mM $MgSO_4$, 100 mM KCl, and 80 mM Tris HCl (pH 7.8 at 10°C). The reaction was initiated by 1.0 mM phosphoenol pyruvate. For the MDH cocktail we mixed: 0.15 mM NADH, 0.5 mM oxaloacetate, 20 mM $MgCl_2$, and 100 mM Tris HCl (pH 8.1 at 10°C). Finally, for the LDH cocktail we mixed: 0.15 mM NADH, 2 mM sodium pyruvate, 100 mM KCl, and 80 mM Imidazole HCl (pH 7.8 at 10°C). Enzyme activity is expressed in units (μmol substrate converted to product per min) per gram wet weight of tissue. For all assays, the homogenates were done in duplicate and the average value was used in further statistics.

2.3 Median depth of occurrence

To analyze the relationship between metabolism and depth, the median depth of occurrence (MDO) was used. In previous studies, the minimum depth of occurrence has been used to analyze depth related trends in metabolism (Childress and Somero, 1979; Sullivan and Somero, 1980; Drazen and Seibel, 2007). The minimum depth of occurrence is defined as the depth below which 90% of the adult individuals of a species are caught (Seibel and Drazen, 2007). The minimum depth of occurrence takes into account the fact that a fish may not always occupy a certain depth, due to possible diel vertical and ontogenetic migrations (Collins et al., 2005). However, diel vertical migration has not been documented in demersal fishes off the coast of California. Therefore, the MDO is believed to better represent the given species as a whole. The minimum and maximum depths of occurrence were obtained from the literature and from the trawl data for all species, and the median was calculated from the two values (Anderson, 1989; Anderson, 1995; Andriashev, 1986; Eschmeyer and Herald, 1983; Lauth, 1999; Mecklenburg et al., 2002; Miller and Lea, 1972).

2.4 Statistical Analysis

The overall mean values for each species' enzyme activities were calculated. Power regressions were applied to explore relationships between enzyme activities, depth, and body mass. A log-log transformation was conducted prior to the regression analysis, when necessary. A Mann-Whitney U test was used to test for differences in enzyme activities between benthic and

benthopelagic species. Analyses were performed with Statistica, version 7.1 (StatSoft, Inc., www.statsoft.com).

CHAPTER 3
RESULTS

Twelve species of zoarcids were captured from 100 to 3000m off the California coast. One of them, a previously unknown species of eelpout, designated *Pachycara n. sp. A* by Dr. Eric Anderson (SAIAB), was new to science and is being described elsewhere.

3.1 Enzyme activities

The highest potential enzyme activities of CS, MDH, PK, and LDH in WM tissue were measured for 53 individuals of 12 species and are presented in Table 1. Activities are expressed in units (μmol substrate converted to product per min) per gram wet weight of tissue. For each species, the activity value is an average for all individuals studied. Overall, anaerobic enzyme activities (PK and LDH) were higher than aerobic enzyme activities (CS and MDH) for all species. Specifically, *L. barbatum* exhibited the highest enzyme activities for both aerobic and anaerobic enzymes (CS 1.88 units g^{-1}, MDH 28.40 units g^{-1}, PK 74.87 units g^{-1}, LDH 331.83 units g^{-1}). *B. brunneum* exhibited the lowest CS and MDH activity (CS 0.13 units g^{-1}, MDH 4.86 units g^{-1}), while *B. molle* exhibited the lowest PK and LDH activity (PK 9.33 units g^{-1}, LDH 18.87 units g^{-1}). One species of the deepest dwelling zoarcid included in this study (median depth of capture 3050.5 m), *Lycenchelys sp. B*, had relatively high aerobic enzyme activities (PK 54.76 units g^{-1}, LDH 134.44 units g^{-1}).

3.2 Enzyme activities versus MDO

There is no significant decline ($p>0.05$) in enzymatic activities (CS, PK, MDH, and LDH) with increasing median depth of occurrence for both benthic and benthopelagic zoarcids (Figure 1). The trend of a slight decline in enzymatic activity with MDO is driven by the species, *L. barbatum*. Removing the data point for this species reveals the complete lack of a relationship between enzyme activity and depth (Figure 2). *L. barbatum* is the shallowest dwelling species included in this study, with a MDO of 227.5 m. It is interesting that *L. cortezianus*, another shallower dwelling species with an MDO of 358.5 m did not have comparable enzymatic activities (CS 1.11 units g^{-1}, MDH 16.38 units g^{-1}, PK 60.29 units g^{-1}, LDH 118.11 units g^{-1}).

3.3 The influence of body mass

Table 1 summarizes the activities of CS, PK, MDH, and LDH in WM tissue for all species, presented with each species' mass ranges. *B. brunneum*, the largest zoarcid included in this study (606.5 – 890.1 g) exhibited the lowest CS and MDH activities (CS 0.13 units g^{-1}, MDH 4.86 units g^{-1}). Surprisingly, the next lowest MDH activity was shown by *B. molle*, represented by relatively small specimens (15.01 – 72.12 g). However, the next lowest CS activity was exhibited by *P. lepinium* at 490.7 g, but only represented by one individual in this study. The smallest individuals included in this study, *L. barbatum*, with a weight range of 2.46 – 12.27 g, did exhibit the highest enzyme activities across all four enzymes. The activities of CS, PK, LDH, and MDH in WM tissue of the different

species are plotted as functions of the species' body mass (g) in Figure 3. Figure 3a shows one significant decreasing linear trend in intraspecific CS activity with increasing mass in *L. barbatum* ($y=3.829 - 0.2432*x$, $R^2=0.7489$, p-value=0.0260). *L. barbatum* also exhibits a significant decreasing linear trend in MDH activity with increasing mass, shown in Figure 3d ($y=50.2908 - 2.7335*x$, $R^2=0.7432$, p-value=0.0272. CS and MDH activities showed no significant trends for all other species. In addition, both PK and LDH activities showed no significant trends for all species.

3.4 The influence of lifestyle

A comparison of the enzymatic activities between benthic and benthopelagic species was performed using a Mann-Whitney U test. CS enzyme activities showed no significant difference between benthic and benthopelagic species. However, a significant difference in enzyme activities was found for PK ($p<0.05$), LDH ($p<0.05$), and MDH ($p<0.05$).

Table 1. Summary of the highest potential white muscle enzyme activities for both aerobic (CS and MDH), and anaerobic (PK and LDH) enzymes at 10°C ± 1 standard deviation where applicable, presented with species' mass ranges and depths of occurrence and capture. Median depths of occurrence were obtained from Miller and Lea (1972), Eschmeyer and Herald (1983), Anderson (1995), Lauth (1999), and Mecklenburg (2002). Lifestyle BP refers to benthopelagic and B refers to benthic.

										Enzymatic Activity (units g-1 wet weight tissue)			
Species	**n**	**Lifestyle**	**Fish Mass Range (g)**	**Min Depth Occurance (m)**	**Max Depth Occurance (m)**	**MDO (m)**	**Min Depth Capture (m)**	**Max Depth Capture (m)**	**Median Depth Capture (m)**	**CS**	**MDH**	**PK**	**LDH**
Bothrocara brunneum	5	BP	606.5 - 890.1	199	1829	1014	997	2303	1650	0.13 ± 0.02	4.86 ± 1.27	9.95 ± 1.85	20.93 ± 5.84
Bothrocara molle	11	BP	15.01 - 72.12	400	2688	1544	2100	2233	2166.5	0.28 ± 0.14	5.85 ± 1.52	9.33 ± 0.75	18.87 ± 5.95
Lycenchelys sp B	5	B	22.9 - 158.2	-	-	-	3050.5	3050.5	3050.5	0.56 ± 0.36	11.09 ± 7.94	54.76 ± 36.96	134.44 ± 100.16
Lycenchelys micropora	3	B	55.11 - 124.49	2377	3512	2944.5	2105	2233	2169	0.55 ± 0.40	9.31 ± 2.14	27.74 ± 14.50	102.13 ± 30.86
Lycenchelys sp A	2	B	35.8 - 106.74	-	-	-	2188	2188	2188	0.24 ± 0.03	10.92 ± 4.57	25.91 ± 10.28	101.20 ± 27.57
Lycodes cortezianus	5	B	58.33 - 243.8	73	644	358.5	86.7	543	314.85	1.11 ± 0.15	16.38 ± 3.23	60.29 ± 12.47	118.11 ± 14.94
Lycodes diapterus	5	B	7.21 - 81.16	13	1300	656.5	309.5	1300.5	805	0.69 ± 0.27	17.03 ± 6.56	52.97 ± 19.84	142.90 ± 51.76
Lyconema barbatum	6	B	2.46 - 12.27	82	373	227.5	208.25	257.75	233	1.88 ± 1.11	28.40 ± 12.47	74.87 ± 19.55	331.83 ± 76.95
Pachycara n. sp A	5	B	376.7 - 728.01	-	-	-	2972.5	2972.5	2972.5	0.27 ± 0.10	8.20 ± 2.76	23.24 ± 17.34	38.16 ± 28.42
Pachycara bulbiceps	2	B	47.55 - 91.51	2400	4780	3590	2972.5	2972.5	2972.5	1.10 ± 0.21	8.70 ± 0.24	24.25 ± 6.51	67.81 ± 14.12
Pachycara gymninium	3	B	139.82 - 900.9	1829	3225	2527	2870.5	3095	2982.75	0.61 ± 0.26	19.74 ± 11.36	28.80 ± 9.73	117.32 ± 54.76
Pachycara lepinium	1	B	490.7	1728	2907	2317.5	2188	2188	2188	0.23	10.29	30.61	78.30
Total	53												

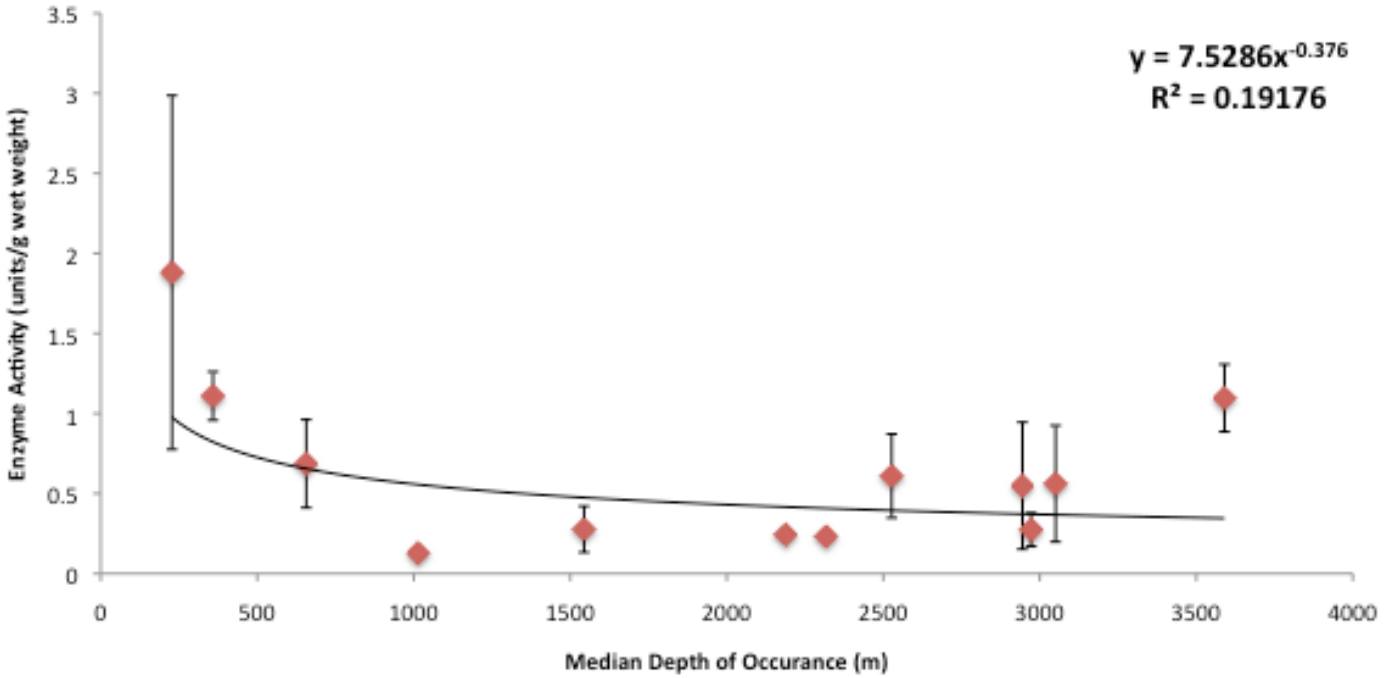

Figure 1. Plot of mean white muscle CS enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m).

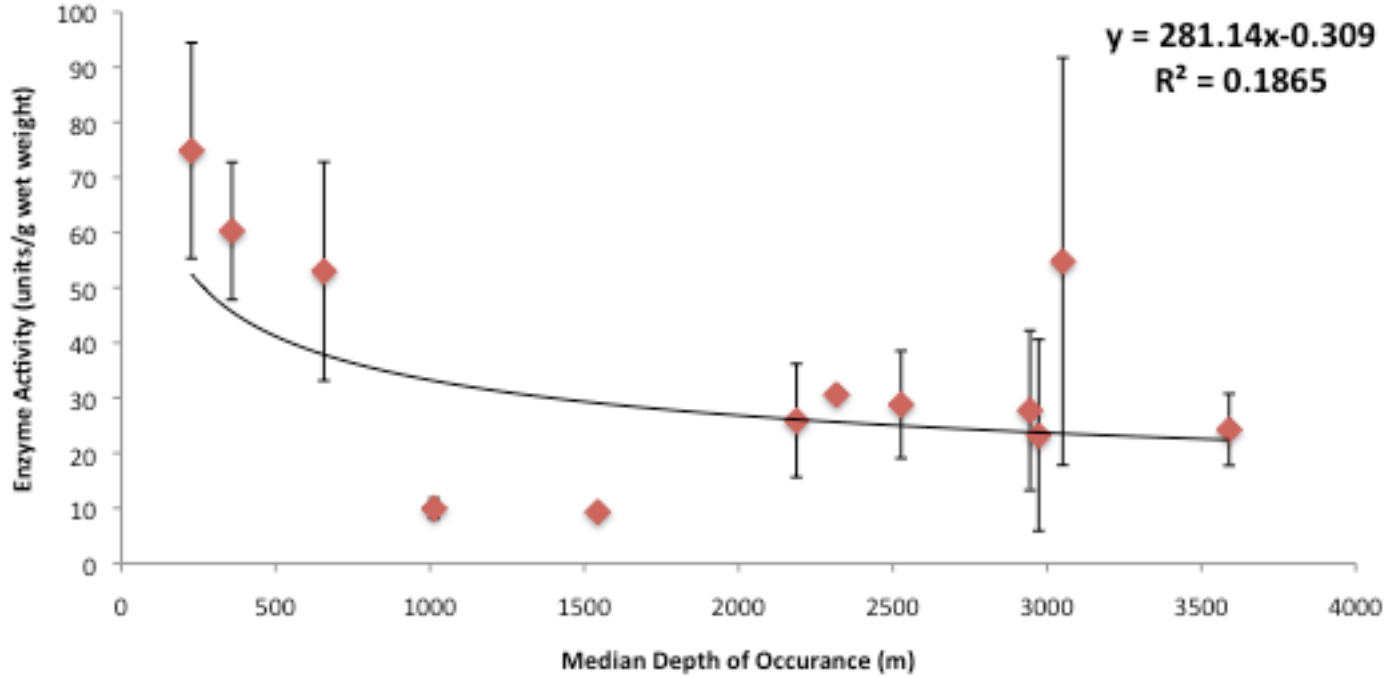

Figure 2. Plot of mean white muscle PK enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m).

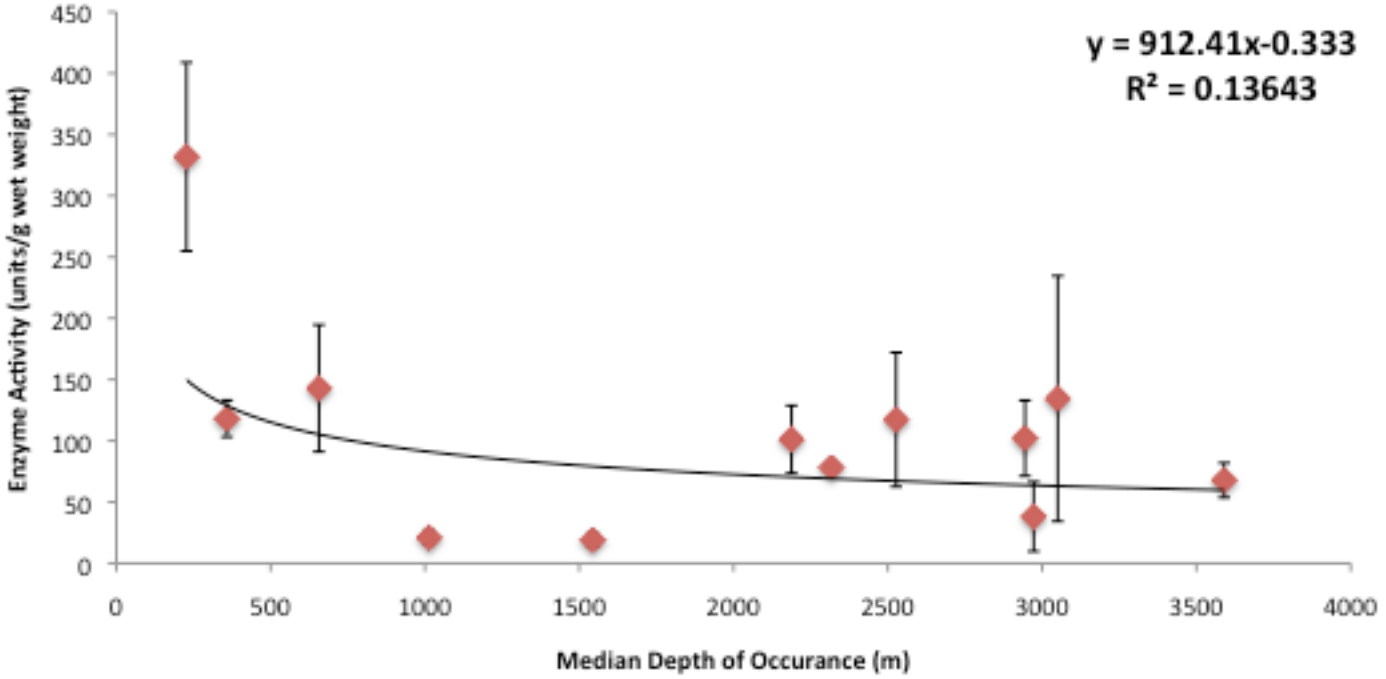

Figure 3. Plot of mean white muscle LDH enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m).

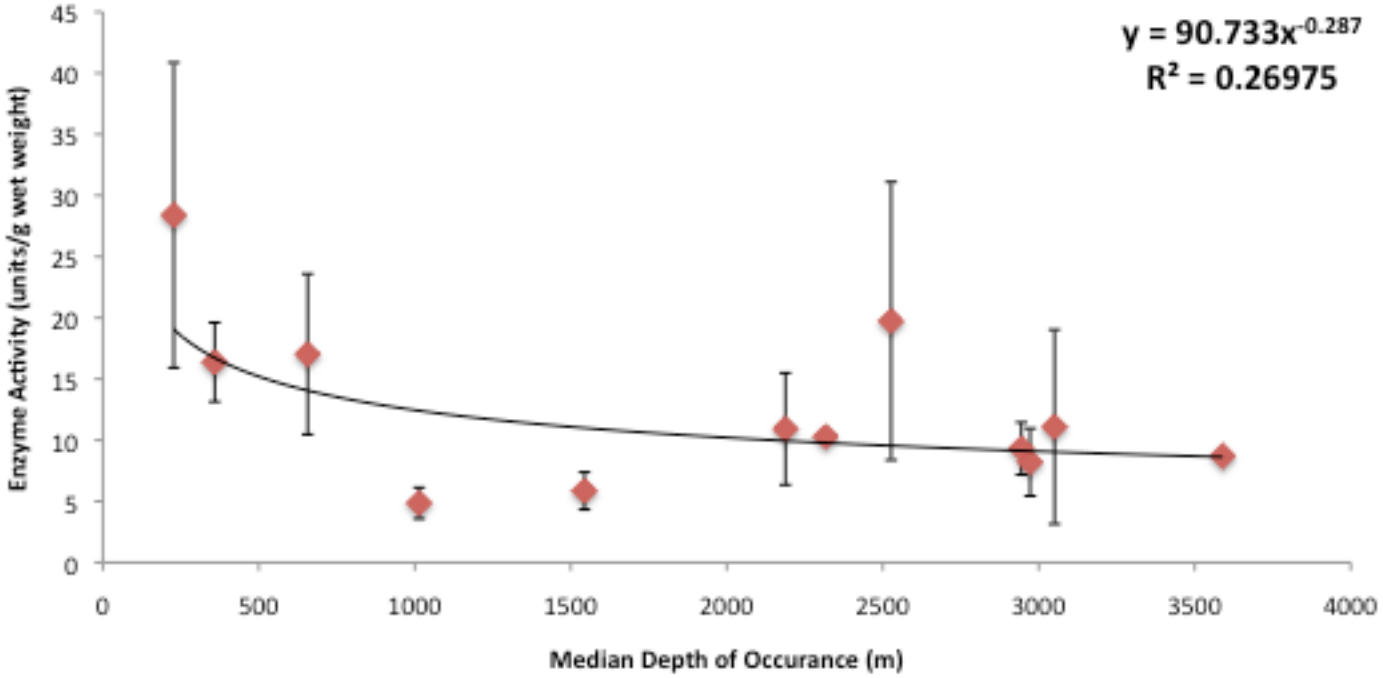

Figure 4. Plot of mean white muscle MDH enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m).

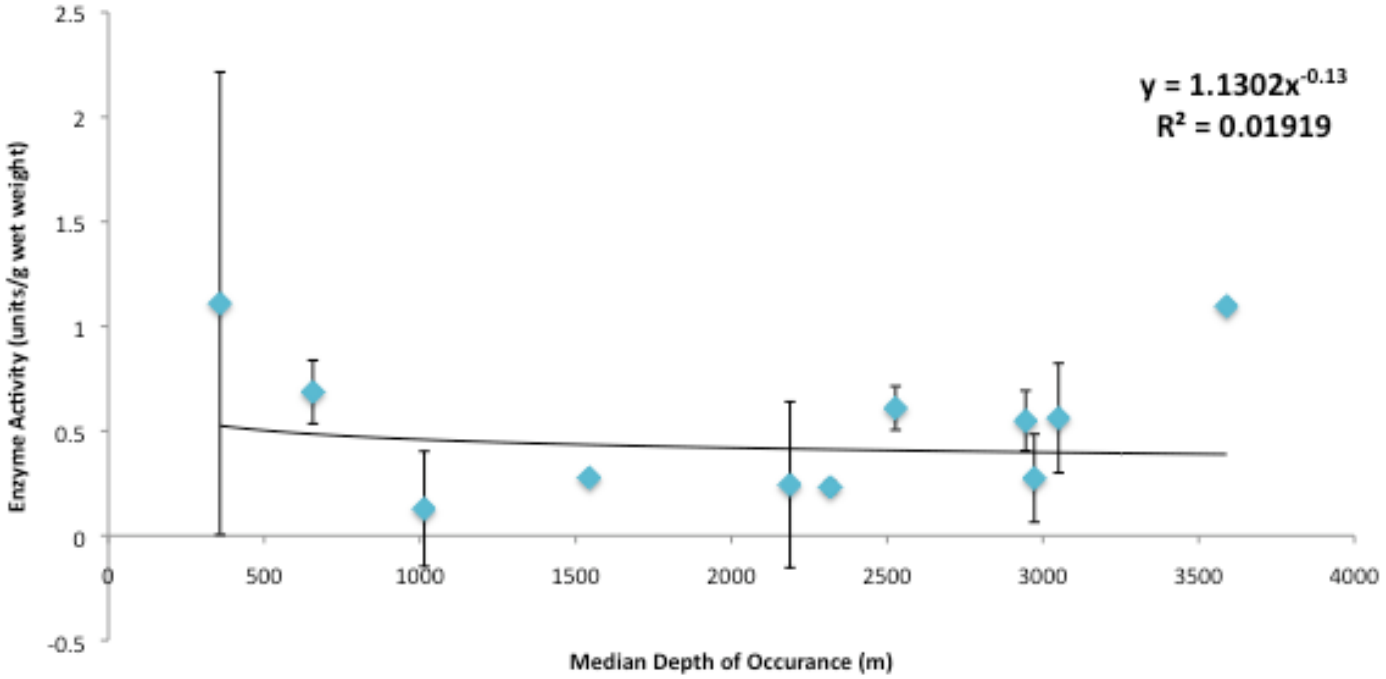

Figure 5. Plot of mean white muscle CS enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m), after removing *Lyconema barbatum*.

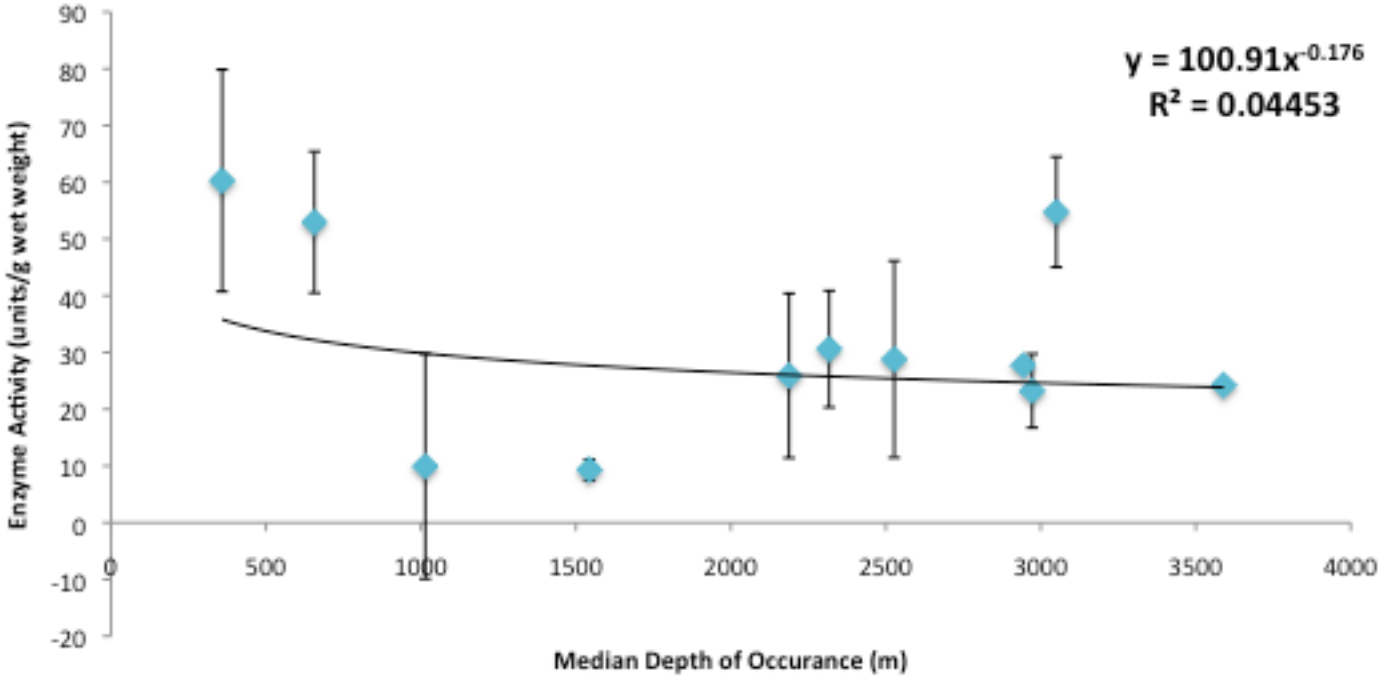

Figure 6. Plot of mean white muscle PK enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m), after removing *Lyconema barbatum*.

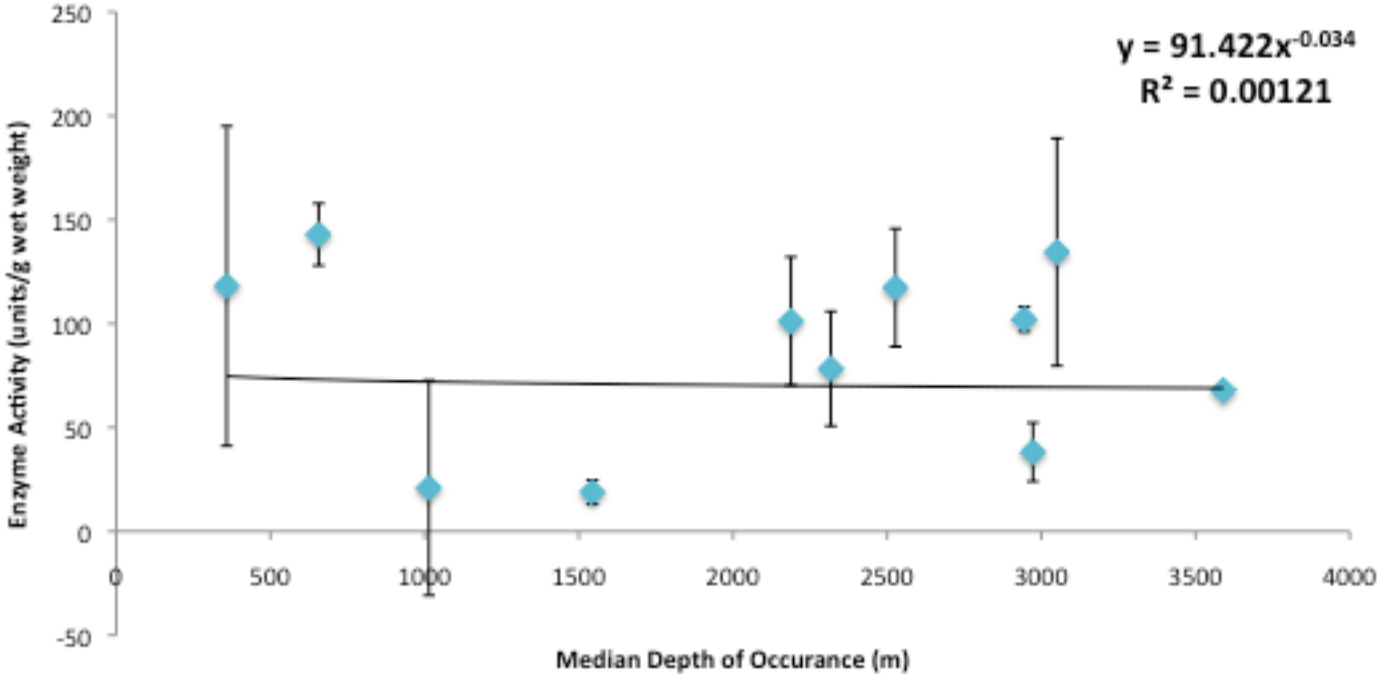

Figure 7. Plot of mean white muscle LDH enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m), after removing *Lyconema barbatum*.

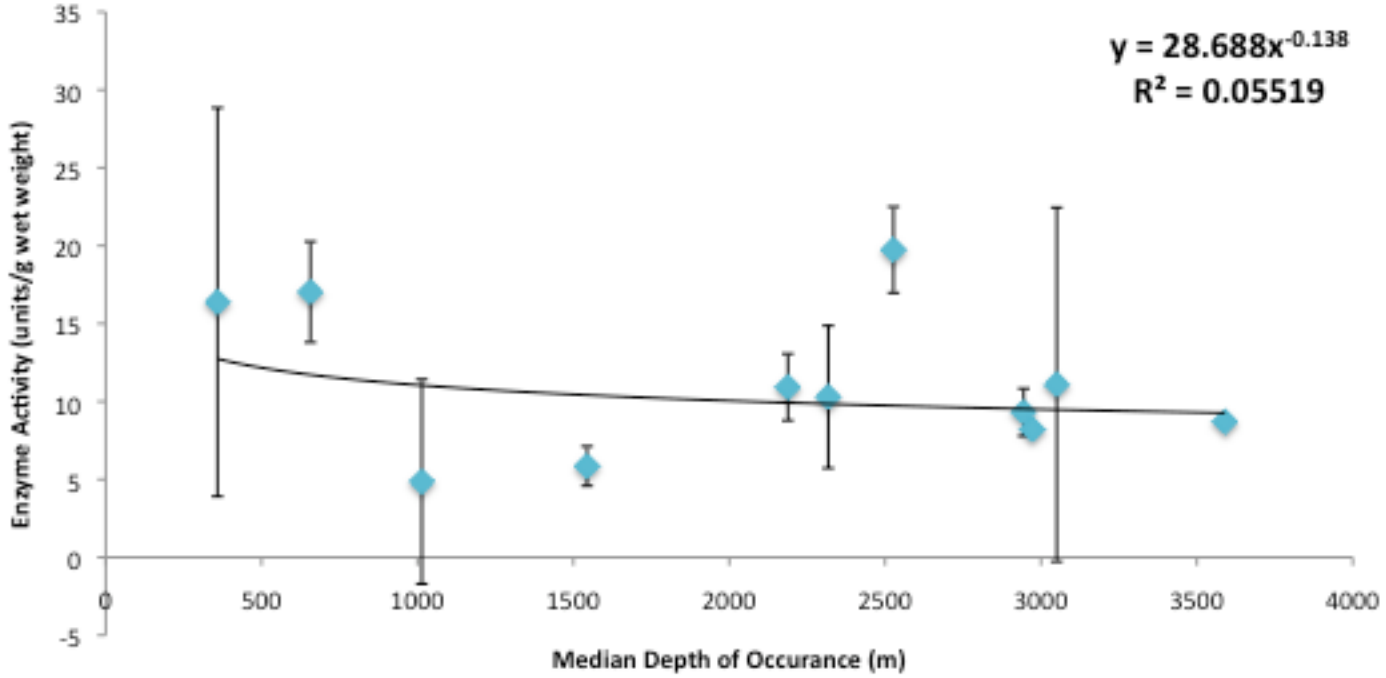

Figure 8. Plot of mean white muscle MDH enzyme activities (IU/g wet weight) ± 1 standard deviation where applicable for all species at 10°C plotted versus MDO (m), after removing *Lyconema barbatum*.

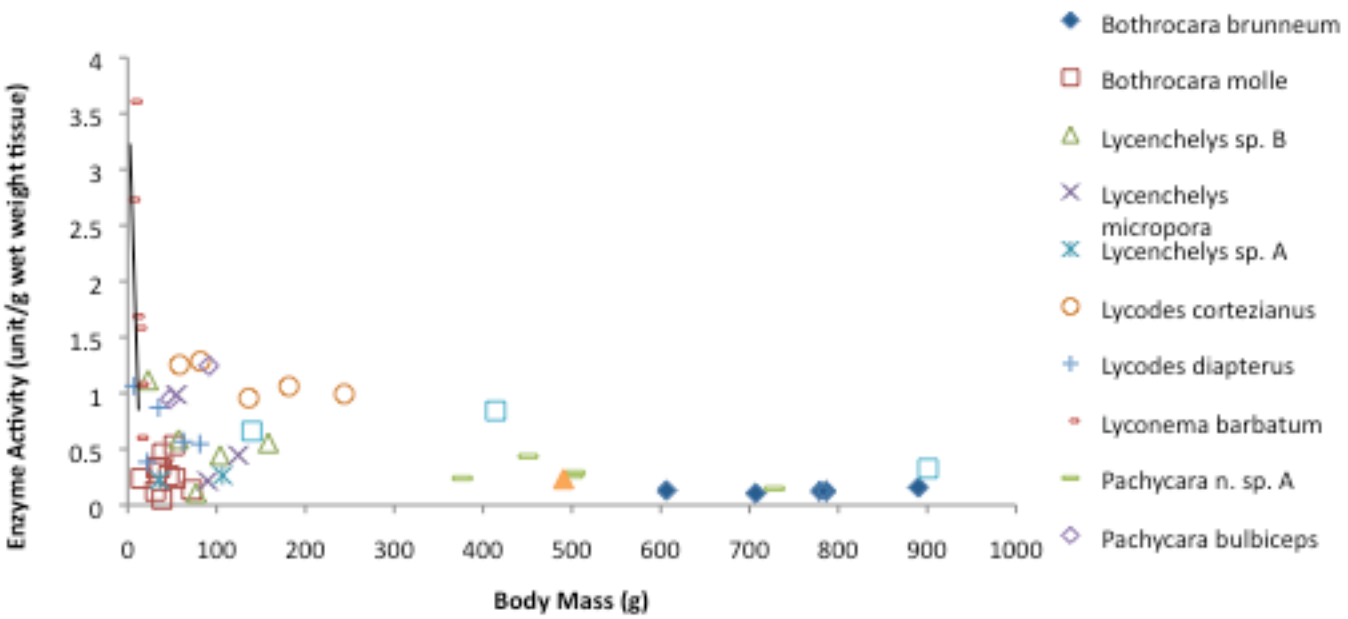

Figure 9. Plot of white muscle CS enzyme activities (IU/g wet weight) for all species at 10°C plotted versus body mass (g). Shows a significant decreasing linear trend with increasing mass in *Lyconema barbatum* ($y=3.829 - 0.2432*x$, $R^2=0.7489$, p-value=0.0260).

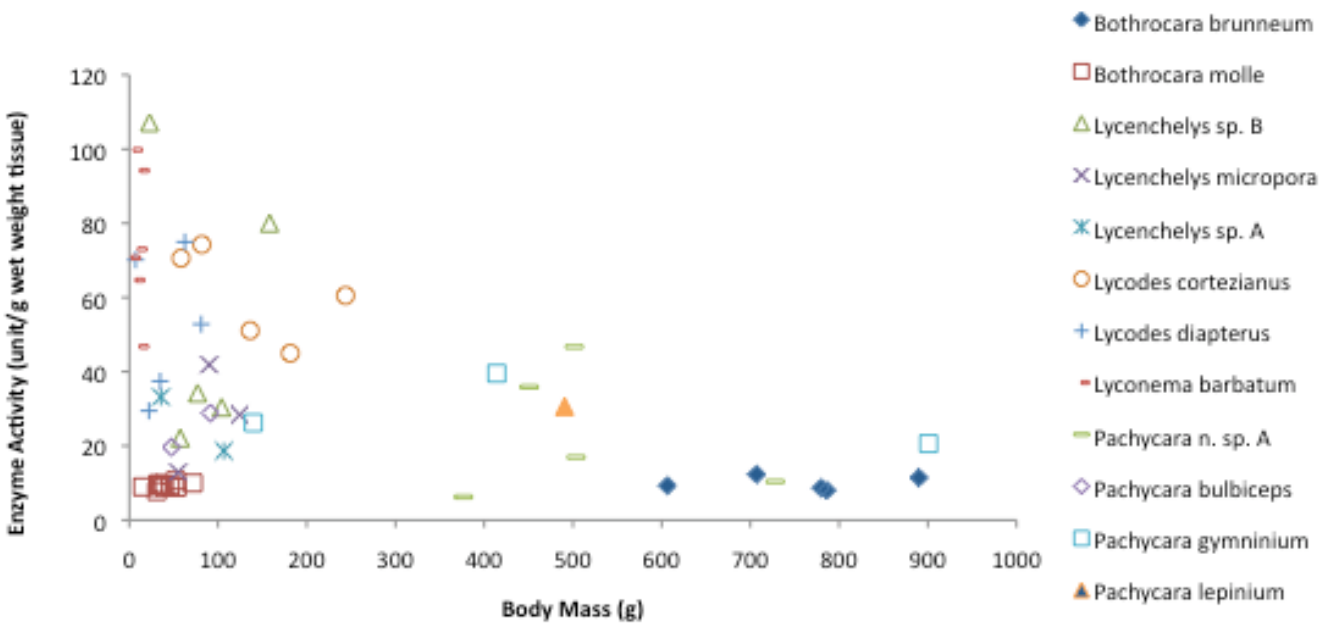

Figure 10. Plot of white muscle PK enzyme activities (IU/g wet weight) for all species at 10°C plotted versus body mass (g).

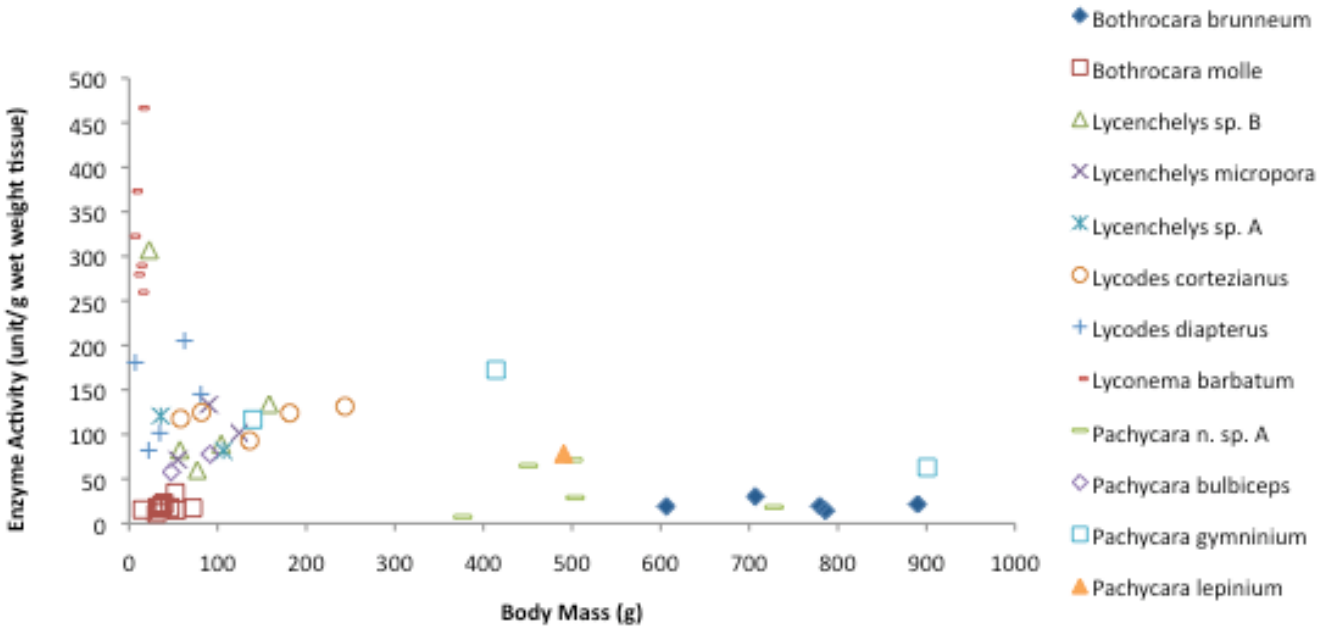

Figure 11. Plot of white muscle LDH enzyme activities (IU/g wet weight) for all species at 10°C plotted versus body mass (g).

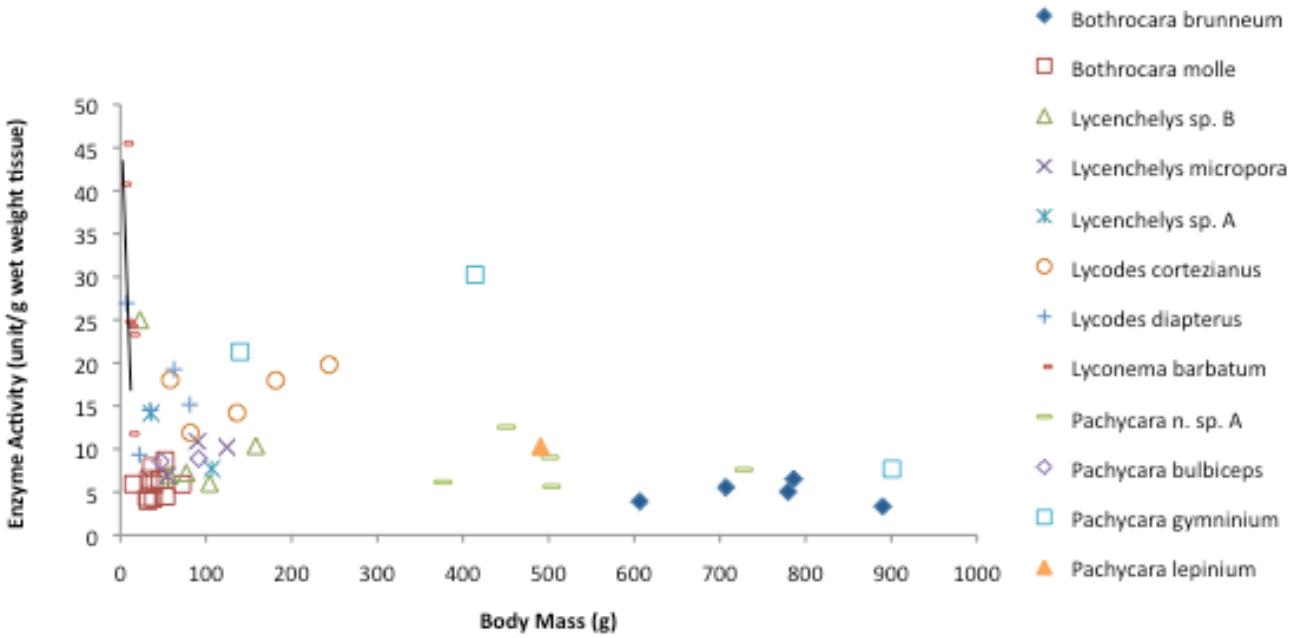

Figure 12. Plot of white muscle MDH enzyme activities (IU/g wet weight) for all species at 10°C plotted versus body mass (g). Shows a significant decreasing linear trend with increasing mass in *Lyconema barbatum* (y=50.2908 – 2.7335*x, R^2=0.7432, p-value=0.0272).

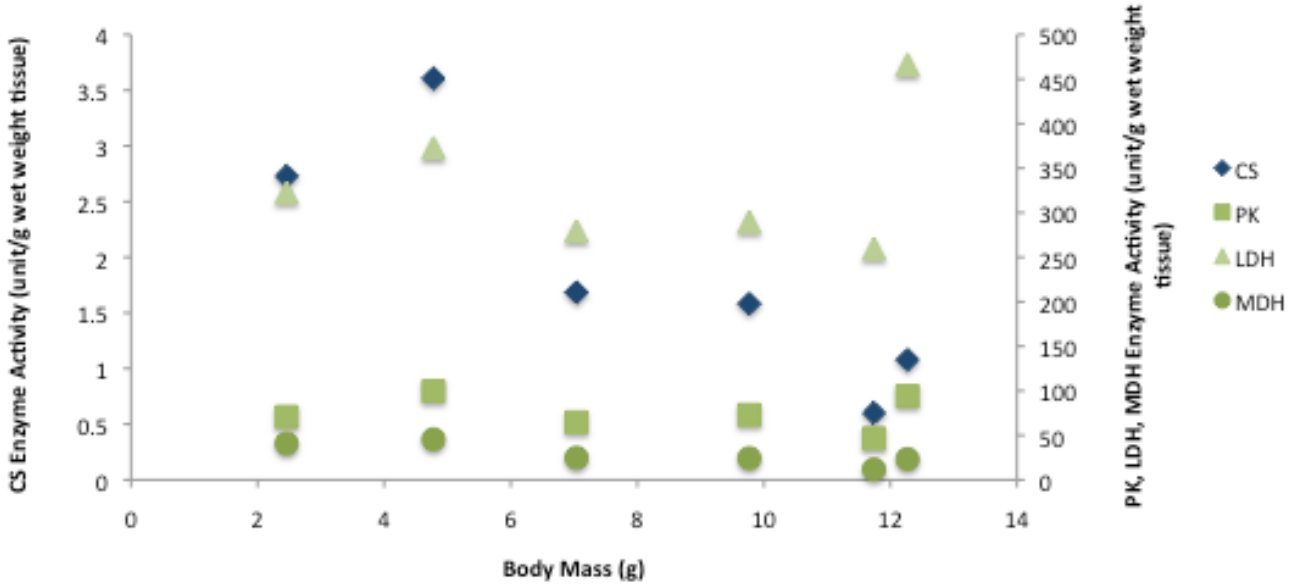

Figure 13. Plot of white muscle enzyme activities (IU/g wet weight) for *Lyconema barbatum* at 10°C plotted versus body mass (g). CS enzyme activity is plotted on the left y-axis and PK, LDH, and MDH are plotted on the right y-axis. Plot shows the variability of CS enzyme activity in the smaller sized specimens, compared to the larger sized specimen.

CHAPTER 4
DISCUSSION

This is the first study to evaluate the metabolism of zoarcids over a broad depth gradient and size range. The results from this study contribute greatly to our understanding of the biology and ecology of this widespread and speciose group of fishes.

4.1 Effects of size on enzyme activities

It continues to be a mystery as to which species will show allometric scaling and which ones will not. In this study, *L. barbatum* was the only species that exhibited variation in enzyme activities with changes in body mass. Both CS and MDH activities decreased with increasing mass, a trend that has been noted before for more active fishes, such as pelagic fishes (Figures 3-7; Somero and Childress, 1980). While depth doesn't explain this variation in enzyme activity across all specimens, there is variation that exists between the shallower specimens of *L. barbatum* (Figure 7). However, Drazen and Seibel (2007) found body mass to not fully explain the variability in metabolic rate exhibited by benthic and benthopelagic fishes.

4.2 Visual interactions hypothesis

Our results for benthic zoarcids support the visual interactions hypothesis, which states that deeper living benthic organisms will show no decline in

metabolism with increasing depth when compared to their shallower living counterparts (Figure 1; Drazen and Seibel, 2007). The reasoning behind this, is that the sea floor offers more chances for crypsis than does the open ocean, which relaxes the need for long-range locomotory capacity for benthic organisms in response to predator-prey interactions. Other studies have found similar results for other benthic animal groups. For example, Childress et al. (1990) found that benthic crustaceans do not exhibit a decline in metabolism with increasing depth other than what can be explained by the effects of temperature and size. In addition, benthic hagfishes show variation in metabolic rates but this variation doesn't correlate with depth (Drazen et al., 2011). Interestingly, Drazen and Seibel (2007) found sedentary benthic species to show a decline in enzymatic activity with increasing depth. The caveat is that they only compared benthic species at 400-500 m MDO to much shallower species at 10-40 m MDO. In contrast our study uses zoarcids, which are visually limited tactile predators, across a very broad depth range. Our results are similar to those for other taxa that do not rely heavily on vision, such as copepods, medusae, and chaetognaths (Seibel and Drazen, 2007).

The results from this study are comparable to the enzymatic rates of benthic and benthopelagic rockfishes from southern California in a study by Ombres et al. (2011). LDH (excluding *Lyconema barbatum*), PK, and CS values were very similar for the rockfishes and the zoarcids. The only values that were considerably higher for the rockfishes were for MDH. Interestingly, Ombres et al. (2011) used LDH activity as a tool for indentifying species that are

morphologically very similar. For CS activities, Ombres et al. (2011) had behavioral observations and oxygen consumption data from the literature to compare to. They found the enzymatic activities to correlate well with observed swimming activities (higher CS values for species that have been observed to have more active swimming behavior), in addition to the oxygen consumption data correlating with the enzyme activity results. They concluded that the species with lower CS values were more sedentary in habit, and relied on burst swimming for fight or flight situations. The species with the higher CS values were benthopelagic species, and were more active swimming species. Aside from the results and conclusions made for the benthopelagic species, we can draw similar conclusions for the benthic species about their lifestyle and predation strategies, which will be discussed in more detail.

The enzyme activities of hagfishes off the coast of California were analyzed by Drazen et al. (2011). They found interspecific variation in enzyme activities as well as, some of the lowest rates known for all fishes. Hagfishes are benthic, characterized by sluggish behavior, and poor burst swimming abilities, so the results were not surprising. Comparing the enzyme activities of the hagfishes to the activities for the zoarcids, in general there were much lower aerobic enzyme activities and much higher anaerobic enzyme activities for the zoarcids (*Bothrocara* spp. exhibited similar anaerobic enzyme activities). From this, it is probable that zoarcids have better burst swimming abilities and may be more active in general.

Curiously, the two benthopelagic fishes included in this study, *B. brunneum* and *B. molle* exhibited the lowest enzyme values for all species studied (Table 1). Both species were deep living so there is no comparison across depths that can be made. This is inconsistent with the VIH, which predicts that benthopelagic fishes would have higher enzymatic activities than benthic species because of greater locomotory requirements (Drazen and Seibel, 2007). These results are very interesting, because both species spend time in the water column and are thought to be more active than the other ten species. It is possible that our results may be due to sampling size, and further study should include specimens from the shallower end of their depth range in order to make a comparison across depth.

A comparison of the two benthopelagic species included in this study to other deep living benthopelagic species reveals interesting insights into how the results can be interpreted for *Bothrocara*. Past studies including species of macrourids and a synaphobranchid eel with MDOs greater than 1,500 m have exhibited relatively high enzyme activities for deep living fishes (Drazen and Seibel, 2007). Siebenaller et al. (1982) also studied various macrourid species and looked at the same four enzymes across a depth distribution. They found large interspecific differences in enzyme activities, which they concluded wasn't related to depth, but to size and predation strategies. Comparing Siebenaller et al.'s results to this study, it is notable that there was considerable interspecific variation between the macrourids, while the *Bothrocara* species had similar enzymatic activities. Looking at the actual enzymatic values for the different

enzymes, CS activities were much higher for the macrourids, and Siebenaller et al. (1982) did mention that their CS values were comparable to the shallower living species included in their study. MDH was higher for the macrourids as well. On the other hand, both PK and LDH (except for one macrourid's LDH value) were higher for both *Bothrocara* species (Table 1). Siebenaller et al. (1982) concluded based on the macrourids' low LDH enzyme activities, that they possibly have some of the lowest metabolic rates of any fishes. With this in mind, considering the low enzymatic values exhibited by the two *Bothrocara* species, these zoarcids may be included with the macrourids in the group of fishes with some of the lowest metabolic rates known.

4.3 Food limitation hypothesis

The deep sea is characterized by reduced productivity and is generally a food poor environment, with exceptions such as hydrothermal vents (Seibel and Drazen, 2007). The food limitation hypothesis has often been cited as the reason for declines in metabolic rates with increasing depth (Drazen and Seibel, 2007). Our results do not support the food limitation hypothesis, as there was no decline in enzymatic activity with depth. If food were the factor affecting metabolic rates, then we would have expected the enzyme activities to mirror the decrease in food availability with depth. Past studies have looked at regional differences in productivity and not found food to be the factor affecting variation in metabolic rates (Drazen and Seibel, 2007). Specifically, for benthic and benthopelagic fishes, water and protein contents of white muscle tissue have been analyzed to

address the food limitation hypothesis, as muscle composition relates to locomotory capacity and correlates well with metabolic rate (Drazen and Seibel, 2007; Seibel and Drazen, 2007). While there was a lack of clear trends in the muscle composition of benthic fishes, it was suggested that the ecologies of the fishes played a stronger role than food supply (Drazen, 2007).

4.4 Effects of lifestyle and predation strategy

The results from this research show that variations in enzyme activities exist among different species of benthic and benthopelagic zoarcids that are not accounted for by depth of occurrence or size. It is likely that the differences between species' enzymatic rates reflect the lifestyles and predation strategies of these fishes. In past studies, certain deeper dwelling species have shown high levels of enzyme activity and are thought to most likely be active predators like their shallow water counterparts. Whereas species with lower enzyme activities have most likely evolved sit-and-wait predator strategies (Sullivan and Somero, 1980). Sit-and-wait predators would exhibit higher LDH and PK activities than aerobic enzymes such as CS, in order to meet their glycolytic needs during burst swimming locomotion, which was exhibited by all species included in this study.

Relatively high enzyme activities have been shown for deeper-living scavengers, which may be a result of competition for limited available carrion (Drazen and Seibel, 2007), and our data for zoarcids supports this contention. Of the species examined, *Pachycara* spp. are likely facultative scavengers. *P. lepinium* was found to be attracted to baited traps by Cailliet et al. (1999), and

Pachycara spp. were found to be attracted to baited cameras in several studies (Drazen, 2007; Gutowska et al., 2004; Yeh and Drazen, 2011). Also, Jones et al. (1998) found *P. bulbiceps* to be scavengers, as they were present at dolphin carcasses. High CS activity, an indicator of aerobic potential, implies that an organism is a more active swimming species (Ombres et al., 2011). *P. bulbiceps* exhibited a relatively high CS value (1.10 units g^{-1}). The MDH and LDH values for *P. gymninium* were also relatively high, at 19.74 units g^{-1} and 117.32 units g^{-1}, respectively. However, both *Pachycara n. sp. A* and *P. lepinium* (n=1) exhibited relatively low enzyme activities for all enzymes. The *Pachycara n. sp. A* specimens had a noticeably large gelatinous layer under their skin. White muscle was also observed to float in cold seawater and was very watery (Yancey and Drazen, unpub data). Moreover, all specimens were considerably larger than the other *Pachycara* species. An increase in the water content and a change in buoyancy partially explains the lower enzyme activities of this species. From these results and observations, it is possible that *P. bulbiceps* and *P. gyminium* are both more active scavengers than the other *Pachycara* species included in study.

Few have studied the food habits of zoarcids directly, and the dietary information available for zoarcids is sparse (Ferry, 1997; Stevenson and Hibpshman, 2010). Ferry (1997) studied the food habits of *B. brunneum* and found the species to prey on a narrow range of benthopelagic fauna, which consisted primarily of shrimp-like crustaceans and small zoarcid fishes. Stevenson and Hibpshman (2010) also analyzed the diet of *B. brunneum*, but concluded that they are dietary generalists, feeding on a broad range of benthic

fauna. However, they similarly found the majority of their prey items to consist of various crustaceans, such as shrimps and mysids, in addition to small fishes, such as myctophids, zoarcids, and snailfishes. With fairly mobile prey, it was interesting to find that *B. brunneum* from this study had such low enzyme activities. *L. cortezianus* and other *Lycodes* spp. have also been found to prey on mostly benthic species by Ferry (1997), and their enzyme activities were found to be relatively high in this study. Polar *Lycodes* species' feeding habits were examined by Hildebrandt et al. (2011), and they found similar results. They found their prey items to consist mostly of benthic fauna, such as polychaetes, crustaceans, and molluscs, in addition to other fishes. Moller and King (2007) noted that the genus *Lycenchelys*, which includes 59 species, is more diverse with a wider vertical range than the genus *Pachycara*, which includes only 21 species. Similarly, the genus *Lycodes* includes 60 species, and is considered one of the most diverse and widespread among the eelpouts (Stevenson and Sheiko, 2009). Keeping this in mind, one could speculate that *Lycenchelys* and *Lycodes* species fill various niches, and may employ a wide range of predation habits and prey preferences. In general, literature concerning the food habits of *Lycodes*, *Lyconema*, and *Lyncenchelys* is unfortunately scant. A direction for future research would be to analyze the head morphology of these eelpouts, which is abundant in the literature, to gain insight into the feeding habits of these fishes. This has been done in the past on macrourid fishes (McLellan 1977), and would help to fill in the knowledge gaps that exist for eelpouts (Siebenaller et al., 1982).

CHAPTER 5
CONCLUSION

This research emphasizes the fact that enzymes are a useful tool in evaluating an organisms locomotory behaviors, based on the relationship of the expression of aerobic and anaerobic enzymes in their tissues. Therefore, metabolic enzymes activities can be used in combination with observational data to infer the lifestyles of fishes that are difficult to observe and monitor (Ombres et al., 2011).

As a result of this study, it is understood that temperature and body mass do not fully explain the variations in enzyme activities for benthic and benthopelagic fishes. It is likely that the differences between species' enzymatic rates reflect the lifestyles and predation strategies of these fishes. Further study is needed to compare the enzymatic rates of the zoarcids included in this study with the protein and water content of their tissues. Proximate composition (water, lipid, and protein) is a useful tool in indicating locomotory habits and energetic adaptations, and will help to make more definite conclusions (Drazen, 2007). In addition, studies focusing on the enzymatic activities of other tissues, such as heart and liver are needed.

Eelpouts do inhabit a wide range of habitats, as they are found in all oceans in the world, at all depths, and a range of lifestyles. This should be reflected in the variation of their enzymatic activities. Since we only examined 12 of the over 200 species of zoarcids, it remains open as to how these results apply to the zoarcid family as a whole.

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