10-22-2008

Determining the Usefulness of Aerobic and Anaerobic Enzyme Assays as Proxies for Rockfish Ecological Data.

Erica M. Hudson
University of South Florida

Determining the Usefulness of Aerobic and Anaerobic Enzyme Assays as Proxies for Rockfish Ecological Data.

by

Erica M. Hudson

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
College of Marine Science
University of South Florida

Major professor: Joseph J. Torres, Ph.D.
David Mann, Ph.D.
Susan Bell, Ph.D.

Date of Approval:
October 22, 2008

Keywords: Metabolic Poise, Lactate dehydrogenase, Citrate Synthase, proximate composition, *Sebastes*

ACKNOWLEDGEMENTS

I am extremly grateful for the support and guidance I have received throughout the course of my degree from Dr. Jose Torres, my major professor. Funding for this work was provided by NSF grant # 052332 and a NOAA grant #AB133F06SE2766. I would also like to thank the members of my committee for their advice, expertise and guidance throughout this research and especially in the revision process of my thesis.

Special thanks to Joe Donnelly for all of his help in the lab. Joe started this project and worked most of the kinks out before I picked it up. Without his knowledge of techniques and the lab in general this process might have taken a whole lot longer. Also thanks to Waldo Wakefield from NMFS for his insight and coordination of the sampling effort.

Also thanks to all my friends and family who have supported me in this effort over the past few years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

DETERMINING THE USEFULNESS OF AEROBIC AND ANAEROBIC ENZYME ASSAYS AS PROXIES FOR ROCKFISH ECOLOGICAL DATA

Erica M. Hudson

ABSTRACT

Rockfish are commercially and recreationally important, yet due to the in habitat depths at which rockfish inhabit, little is known about their ecology. As a consequence, management of rockfish population as a fishery resource is a work in progress. In particular, changes in physiological condition aver the course of the year is poorly described. This study examined 19 different species of *Sebastes* from the Southern California Bight over four seasons (late summer, fall, winter, and spring) using metabolic enzyme assays. Enzymes used were lactate dehydrogenase (LDH), malate dehydrogenase (MDH), pyruvate kinase (PK), and citrate synthase (CS). Some muscle composition data (percent water, percent protein, percent lipid, and protein as a percentage of wet mass) were also used to help interpret the enzyme data. Enzyme activity was lowest in the summer when expressed as activity per gram wet weight but when it was expressed per gram protein the trend was reversed. We found that the rockfish tend to have the highest protein as a percentage of wet mass (P%WM) in the spring right before the upwelling period begins and have the lowest P%WM in late summer after the peak of upwelling. Their metabolic poise (represented as CS/LDH)

grouped according to locomotory habit (benthic or bentho-pelagic). A mass and oxygen consumption plot also showed that the species group according to locomotory habit. With those known to be benthic grouped together and those species that are known to more actively swimming had higher values. This knowledge could be used to infer whether a rockfish that hasn't been well studied is benthic or bentho-pelagic.

INTRODUCTION

Rockfishes of the genus *Sebastes* (family Scorpaenidae) are an abundant and diverse group of fishes occupying the coastal waters of North America. There are over 102 species worldwide, with most of them (~ 96 of those species) found along the Pacific coast of North America and in the Gulf of California (Love, Yoklavich, Thorsteinson, Butler, 2002), with the greatest diversity (~ 56 species) found in the southern California bight. (See Figure 1 to the left) The fish collected during this study occupy a fairly narrow depth range (30m-200m) within an extensive North-South distribution. Rockfishes occupy a variety of habitats ranging from deep benthic and benthopelagic to kelp forests in the nearshore. Not surprisingly, they also exhibit many

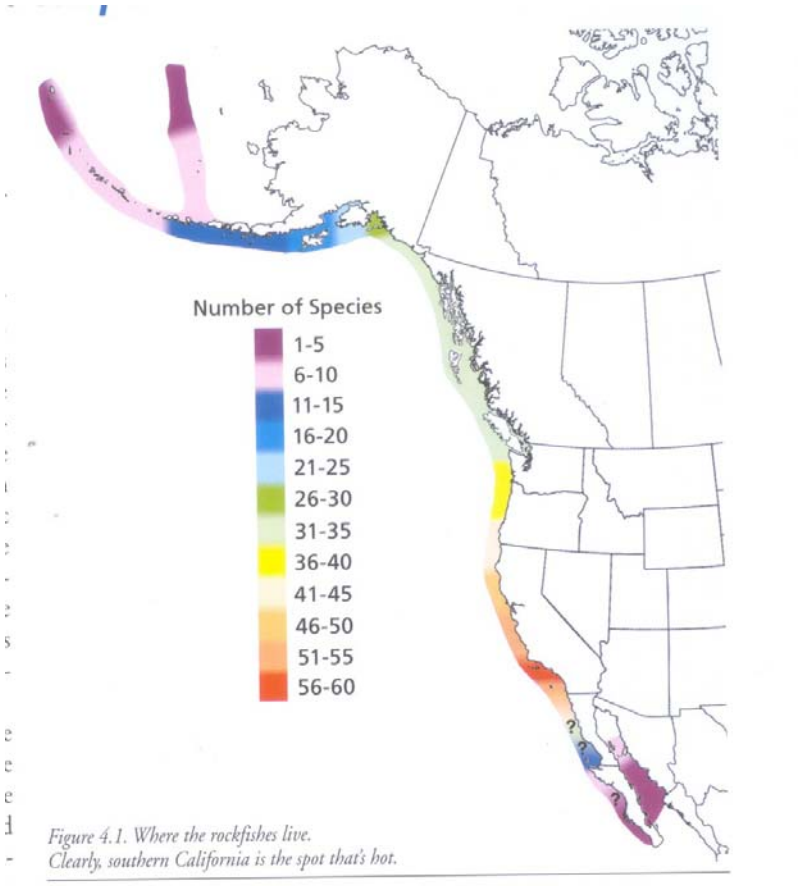

Figure 1: Diversity of rockfish species along the Pacific coast of North America (Love, Yoklavich, Thorsteinson, Butler, 2002)

different feeding strategies such as sit and wait, schooling to concentrate prey, and opportunistic feeding.

Rockfishes prefer hard bottom areas with some habitat complexity such as boulder fields, reefs, oil platforms and kelp forests. Behavioral observations suggest that while different species may use the same outcrop and even school together, there is habitat partitioning, with some species being more benthic and others more bentho-pelagic. Almost all rockfishes are highly valued as food-fishes and are exploited by commercial and recreational fishermen.

Rockfish are very long lived (20-100yrs), reaching sexual maturity at 4-10 yrs of age. They have internal fertilization and brood their young until their release as hatchlings. Most species reproduce once per year but some species are believed to reproduce year round; in Southern CA most rockfish release their young in early spring to summer after the coastal upwelling has begun. Their larvae spend up to a year in the pelagic realm and juveniles spend their first year or more in kelp forests and other shallow habitats. As they grow, they descend to greater depths with increasing age and size. Rockfish size varies considerably with species but the average adult weight for the group is one kg. Larger species can reach up to 20 kg (Love, Yoklavich, Thorsteinson, Butler, 2002).

The southern California bight undergoes seasonal variation in upwelling strength. When averaged bi-monthly, the months of January and February show the slowest coastal current velocities of the year. Current speed picks up in March-April and continues to speed up in May-June. The current velocities peak in July-August and begin to decrease

in September-October and November-December. Faster current velocities are correlated with increased upwelling strength. (Winant, Dever, Hendershott, 2003)

Proximate composition (protein, lipid, water, carbohydrate, and ash content) of nine species of rockfish off the coast of Oregon, showed a slight variation between species but found no differences in composition throughout the year (Thurston, 1960) suggesting that seasonal change in condition may not be evident in these fish. Siebenaller and Yancey (1984) explored the relationship of protein content in white muscle tissue in meso-pelagic fishes from different depths. Their findings suggested that differences in enzyme activity were not due to the general dilution of muscle protein but due to the differences in species' depth of occurrence. They attributed these differences in enzyme activity with depth of occurrence to the lower levels of light and food availability at depth which in turn affects metabolism and enzyme activity. In a study on the chemical composition of midwater fishes of the coast of southern California Childress and Nygaard (1973) found that water content increased with depth while protein, lipid, and ash content decreased. Their results suggested that fishes occupying different depths had compositions that scaled with their different needs in locomotion, buoyancy control, and burst swim capability.

The enzymes chosen for examination in this present study were L-lactate dehydrogenase (LDH), L-malate dehydrogenase (MDH), Pyruvate kinase (PK), and Citrate synthase (CS). LDH is the terminal enzyme in the anaerobic glycolysis in vertebrate tissues, and therefore is good indicator of anaerobic capacity and overall condition. MDH plays several roles in energy metabolism. The mitochondrial isozyme

(m-mdh) is a component of the citric acid cycle and along with the cytoplasmic isozyme (s-mdh), functions in shuttling reducing equivalents between the mitochondria and cytoplasm but its main role is in aerobic metabolism. PK is a good indicator of glycolytic capacity and along with LDH is a good indicator of anaerobic metabolism. CS is found within the mitochondrion and is positioned at the beginning of the citric acid cycle. CS is therefore an important regulatory site in the citric acid cycle and can be used as a quantitative index of citric acid cycle activity and therefore aerobic activity.

The enzymes described above have been used extensively for investigating a variety of metabolic questions in rockfishes and other fish taxa. For example, metabolic activity of deep and shallow living teleosts was compared using enzyme activities in shallow and deep living rockfishes *Sebastes* and S*ebastolobus* (Childress, Somero, 1979; Siebenaller, 1983; Siebenaller, Somero, 1982; Sullivan, Somero, 1980; Vetter, Lynn, 1997; Yang, Lai, Graham, Somero, 1992). Further studies examined nutritional state (Sullivan, Somero, 1983) habitat, feeding, and locomotory strategy (Somero, Childress, 1990; Sullivan, Somero, 1980; Yang, Somero, 1993) the relationship of activity with size (Somero, Childress, 1980), changes in enzyme activity with growth rate (Pelletier, Guderley, Dutil, 1993), changes due to temperature effects (Kawall, Torres, Sidell, Somero, 2002; Torres, Somero, 1988a; Wilson, Somero, Prosser, 1974), and adaptations of enzyme activity to living in an oxygen minimum zone (Yang, Lai, Graham, Somero, 1992).

Size and depth have been shown to affect enzyme activity in separate studies by multiple researchers. Childress and Somero (1979) studied the scaling effects of the

enzymes due to minimum depth of occurrence and found that enzyme activity decreased with increasing depth of occurrence. Somero and Childress (1990) showed that fish with different locomotory strategies, benthic vs pelagic, had markedly different enzyme activities. They found that in the pelagic fish there was a higher enzyme activity, presumably due to their greater need for a well developed locomotory ability relative to their benthic counterparts. In a similar study, the effects of nutritional state were studied (fasted vs well-fed) and the enzyme activity in the two fish was significantly affected by the nutritional condition (Yang, Somero, 1993). In fasted fishes the enzyme activities were much lower than in the well fed fishes and fasted fishes had comparable enzyme activities to field caught fishes. Therefore, in order to properly interpret the enzyme activities, size, depth and behavior of the individual fish need to be taken into consideration.

Many studies have correlated oxygen consumption rates with enzyme activities (Donnelly, Torres, 1988; Seibel, 2007; Torres, Belman, Childress, 1979; Vetter, Lynn, 1997; Yang, Somero, 1993). CS activity correlated well with oxygen consumption rates in rockfish off the coast of southern California (Yang, Somero, 1993). The regression equation generated by their work is applicable to other rockfish found in the same region with similar temperature and depth regimes.

The aim of the present study was to describe the diversity in enzyme activities and muscle proximate composition within and between closely related species of rockfish, to use those differences to determine metabolic poise and condition and to deduce the

underlying causes for differences in enzyme activity and determine if they would be useful in interpreting species seasonal cycles and overall ecology.

METHODS

Sample Collection

Fishes were collected by National Marine Fisheries Service hook-and-line surveys off the coast of Southern California during four separate cruises (see Figure 2 below for sample locations). For each experimental fish, a wedge of white muscle from directly behind the head was removed immediately after capture and frozen in liquid nitrogen. Muscle specimens were kept in liquid nitrogen or at -80 ^{o}C in a cryogenic deep-freeze until used for the enzyme assays described below. Specimen breakdown for the four cruises was as follows. The November cruise of 2004 collected 52 samples from 4 different species, the April '05 cruise collected 66 samples from 6 species, the August-September '05 cruise collected 110 samples from 17 different species and in the September-October '05 cruise 35 samples from 5 species were collected. The total sample size was 263 samples from 19 different species (see Table 1 below). Samples were shipped to Florida in a Dewar containing liquid nitrogen which kept samples at approximately -195°C. Samples were then stored in a -80° C freezer until analyzed

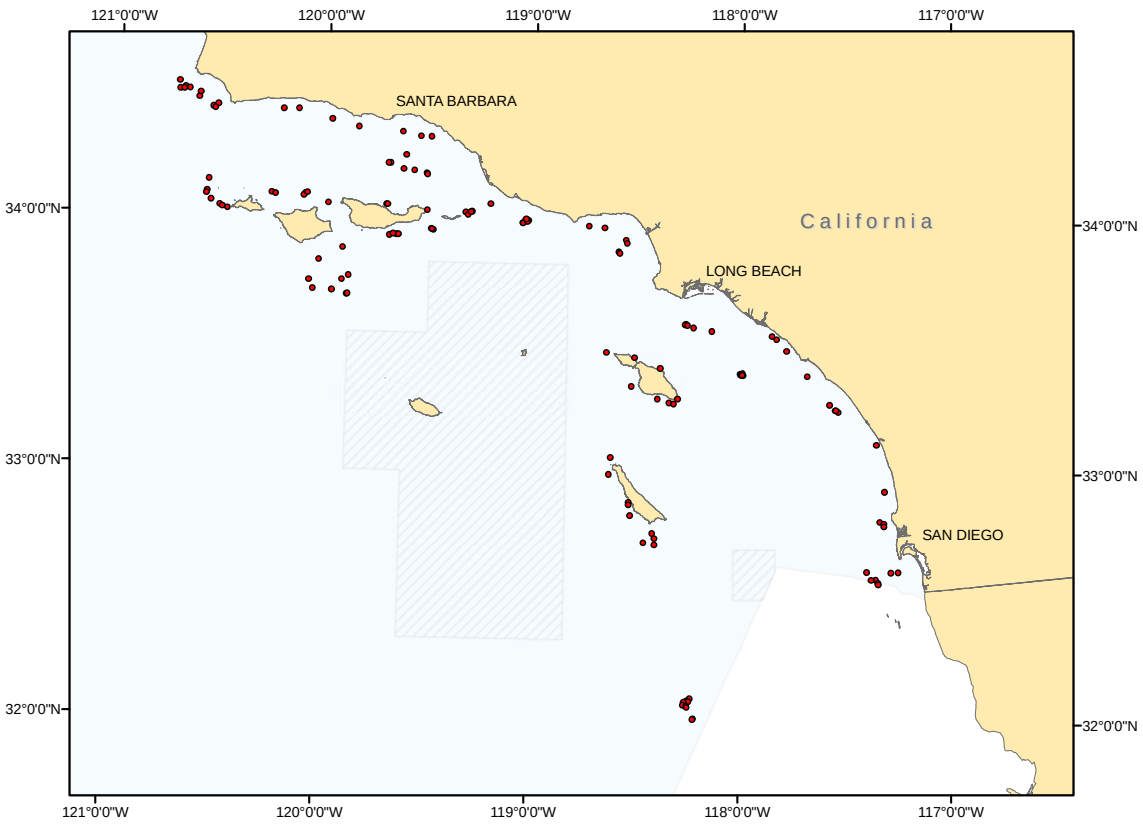

Figure 2: Sample sites in the southern California Bight

Table 1: Captured size and depth ranges for each species and location and season each species was captured in.

	Season				location where samples were caught	captured size range (cm)	captured depth range (m)
	Nov '04	Apr '05	Aug '05	Sept '05			
species	**n**	**n**	**n**	**n**			
Bank	0	0	3	3	3	35-46	134-238
Blackgill	0	0	2	0	3	52-55	238
Boccaccio	19	14	15	0	1, 2, 3, 6, 8, 9, 10, 13	36-70	83-238
Canary	0	0	1	5	7, 8	35-54	97-109
Chilipepper	0	10	6	1	2, 4, 6	25-43	128-170
Copper	0	0	1	0	8	47	96
Cowcod	0	0	0	16	3, 4, 5, 11, 13	49-79	105-183
Flag	0	0	0	5	4, 5, 7, 11	22-38	40-165
Greenblotched	5	0	6	0	2, 6, 8, 14	24.5-43	78-147
Greenspotted	10	2	5	0	6, 8, 9, 10	23-39	85-128
Greenstriped	0	0	1	0	3	34	187
Mexican	0	0	2	0	6	42-48	143-147

Olive	0	0	6	0	8, 9	33-46	41-97
Speckled	0	0	7	0	9	32-35	86-95
Starry	0	0	20	0	2, 3, 8, 9	18-40	41-138
Swordspine	0	0	1	0	6	22	145
Vermilion	18	11	25	0	1, 2, 3, 8, 9, 10, 12, 13, 14	27-58	46-139
Widow	0	9	5	0	8	36-45	94.7-117
Yellowtail	0	6	4	0	8	36-51	94-115

Enzyme analyses

Fish white muscle samples were homogenized in 50mM Imadazole/HCl buffer (pH 7.2 @ 20°C) using a ground glass homogenizer. Samples were kept at ice-bath temperature for the duration of the assays. Homogenates were centrifuged at 4500 rpm for 10 minutes at 10°C. Samples were placed on ice and the supernatant was used within three hours to measure enzyme activity. Activities were measured at 10°C±0.2°C using a thermostatted CARY 1E UV/Visible spectrophotometer with data analysis software. Enzyme activity was expressed in units (µmol substrate converted to product min^{-1}) per gram wet weight of tissue and also in units (µmol substrate converted to product min^{-1}) per gram protein. All enzyme assays followed the procedure of Childress and Somero (1979) with the slight modifications listed below.

The activity of LDH was measured by adding 10µl of the supernatant to 1.0 ml of assay cocktail which consisted of 80mM Imadazole buffer, 5.0mM sodium pyruvate, and 0.15mM of NADH. The reaction was followed by recording the decrease in absorbance at 340nm resulting from oxidation of NADH. The slope of the initial portion of the tracing was used as the reaction rate.

The activity of MDH was measured by adding 30µl of the supernatant to 1.0 ml of assay cocktail containing 40mM Lesley's special buffer (0.2M Imadazole, 0.2M $MgCl_2$), 0.4mM oxaloacetate, and 0.15mM NADH. The reaction was followed by recording the decrease in absorbance at 340nm resulting from oxidation of NADH. The slope of the initial portion of the tracing was used as the reaction rate.

The activity of PK was measured by adding 20µl of the supernatant to 1.0 ml of assay consisting of PK 'cocktail' (160mM Imadazole, 200mM KCl, 0.2mM D-Fructose-1,6-bisphosphate, 20mM $MgSO_4$), 0.15mM NADH, 1.0mM phospoenolpyruvate, 5.0 mM Adenosine 5'-diphosphate, and LDH coupling enzyme from rabbit muscle solution. The reaction was followed by recording the decrease in absorbance at 340nm resulting from oxidation of NADH. The slope of the initial portion of the tracing was used as the reaction rate.

The activity of CS was measured in an assay medium containing 60µl of the supernatant, 50mM Imadazole, 0.4mM 5,5-dithio-bis(2-nitrobezoic acid) (DTNB), 0.1mM Acetyl-Coenzyme A. The reaction was followed by recording the increase in absorbance at 412nm due to the reaction of the reduced coenzyme-A liberated from the enzymic reaction with DTNB. The rate of absorbance increase was first recorded in the absence of oxaloacetate and then after addition of oxaloacetate to compute the true CS activity. The blank (no oxaloacetate) was subtracted from the total activity to compute true CS activity.

Protein Analysis

For protein, homogenate was diluted by a factor of 20 using distilled water. Homogenate was then air-evacuated using nitrogen and placed in the freezer until protein analysis was conducted. Protein composition in white muscle tissue was measured using the method established in Lowry et al. (1951). Absorbance was measured at 750nm. Values were then compared to a standard curve to obtain values for protein content (Lowry, Rosebrough, Farr, Randall, 1951).

Lipid Analysis

Lipid levels were determined on 200µl of homogenate using the methods of Donnelly et al (1990). Breifly, lipids were extracted using a mixture of methanol, and chloroform and filtered to removed particulates. Concentrations were determined using the charring method of Marsh and Weinstein (1966) with stearic acid as a standard. (Bligh, Dyer, 1959; Marsh, Weinstein, 1966; Reisenbichler, Bailey, 1991)

Dry and Ash weight measurements

One ml aliquots of homogenate were dispensed into pre-combusted, pre-weighed crucibles and dried to a constant weight in a 60ºC oven. Water level (%WM) was estimated from a calculated homogenate dry mass concentration (i.e., DM concentration = total sample DM / total homogenate water volume; where water volume = water added for homogenation + water in tissue; and assuming 1g water ≈ 1ml water). Ash content (% DM) was measured following combustion of the dried crucibles at 500ºC for 3-4 hours.

Oxygen Consumption

Oxygen consumption ml h^{-1} (VO2) was calculated using CS activity values (M) in field caught specimens in this regression equation generated by Yang and Somero (1993).

$\log VO_2 = -2.217+1.042\log M$ ($r^2 = 0.900$)

Statistical Analysis

Statistics were used to examine the significance of the species specific differences in enzyme activities and composition using ANCOVA's to account for the effect of mass. Seasonal differences in enzyme activities and composition within species were examined with nested ANOVA's. All statistical analyses were conducted using Statistica (Statsoft Inc.) with a significance level of $p< 0.05$.

RESULTS

Enzyme activities

Mean enzyme activities for all species are shown in Appendix A. Activities are expressed as μmol substrate converted to product per minute (U or units) per gram wet mass (U g^{-1} wet mass) and also units per gram protein (U g^{-1} protein). The overall mean values for each species were calculated, as well as the mean values for each species during each season (Appendix A). Species differences in enzyme activity were examined using only the samples from the August/September cruise to eliminate seasonal effects. ANCOVAs and nested ANOVAs were calculated with all data values. Duncan's multiple range test enabled discrimination between homogenous groups.

Overall, Widow exhibited the highest LDH activity (expressed in WM) and Bank exhibited the lowest LDH activity both of which were significantly different from the rest of the species. Chilipepper had significantly higher MDH values (when expressed in WM) than the rest of the rockfish species. Chilipepper, Bocaccio and Canary exhibited significantly higher PK activity per gram WM. Bank, Canary, Chilipepper, Vermillion, Widow and Yellowtail exhibited significantly higher CS activity (see Figures 3 and 4 below).

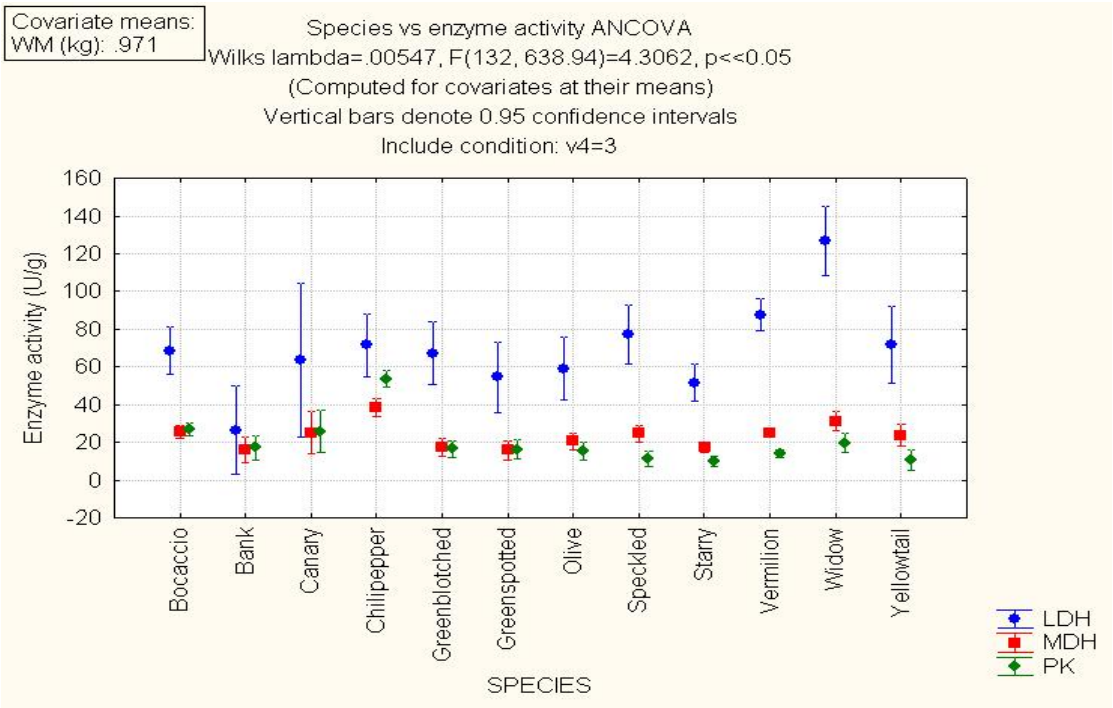

Figure 3: This is an ANCOVA representing the relationships between all the species averaged over all seasons with the effect of mass removed and the different enzyme activities (expressed per gram wet mass). Note that LDH is the most variable.

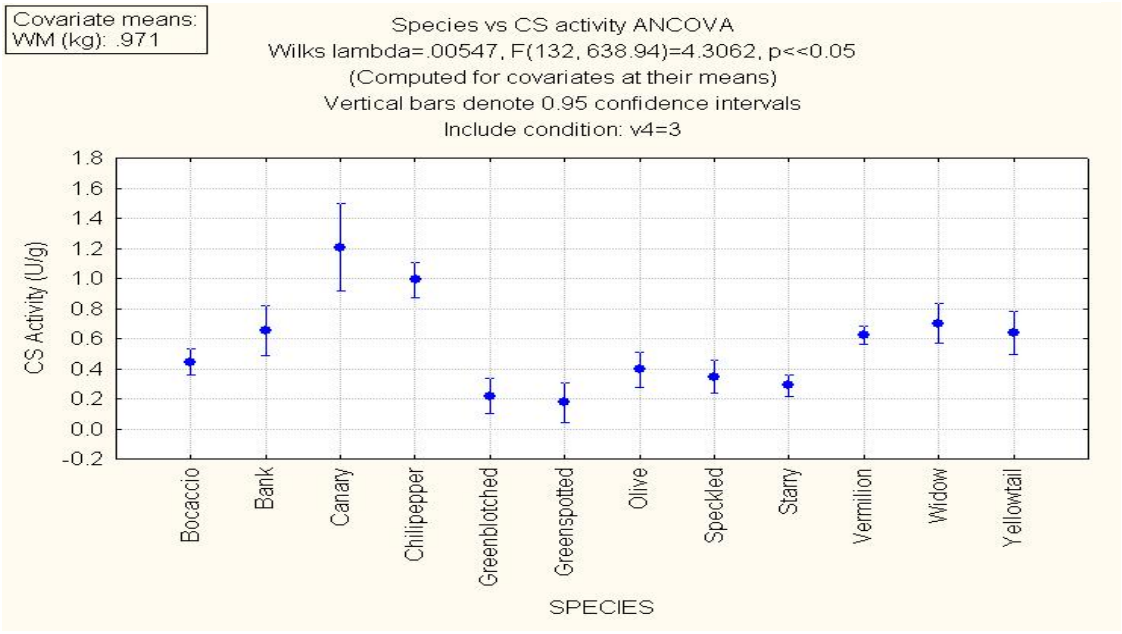

Figure 4: CS activity versus species ANCOVA with mass effects removed. Note that Bank, Canary, Chilipepper, Vermillion, Widow and Yellowtail have significantly higher enzyme activities.

Enough specimens of Boccacio, Greenspotted, Greenblotched, Chilipepper, Vermillion, Widow, and Yellowtail were caught in each season to compare enzymes across season and species. Most of those species showed a marked seasonality with November showing the highest enzyme activity per gram WM and August and September containing the lowest activity values. Activity values expressed as activity per gram protein showed the exact opposite trend with August and September values being the highest and November and April being the lowest (see Figures 5 and 6 below).

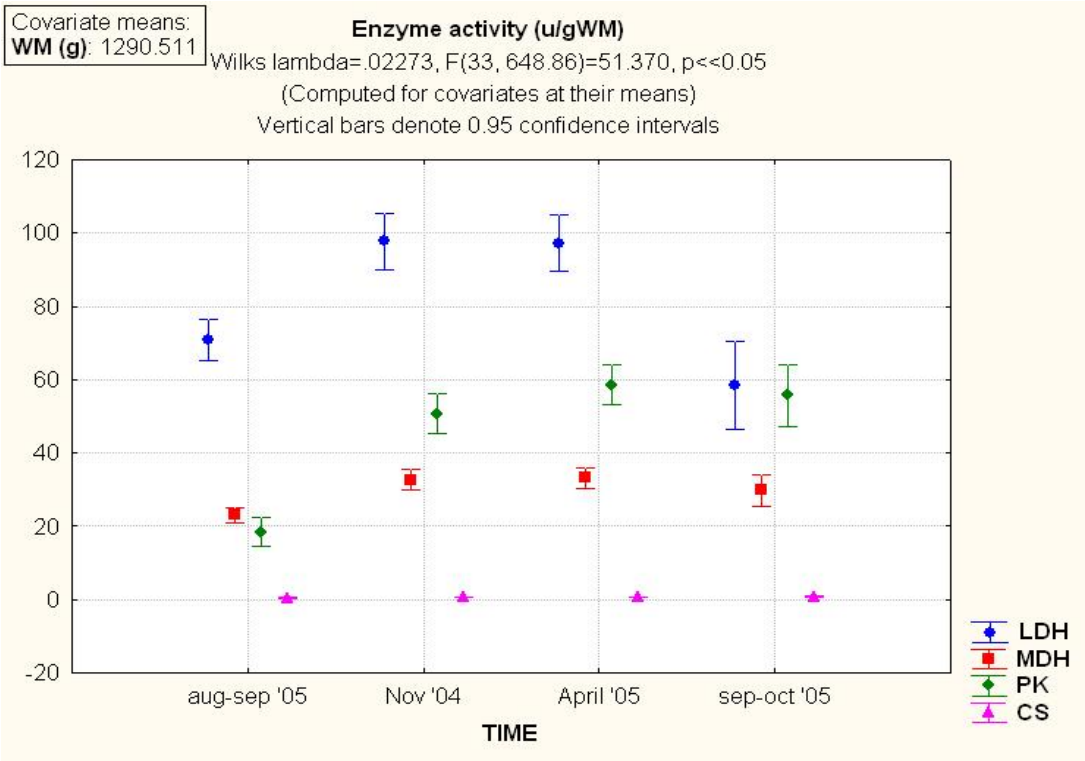

Figure 5: Enzyme activity (U/g) VS season

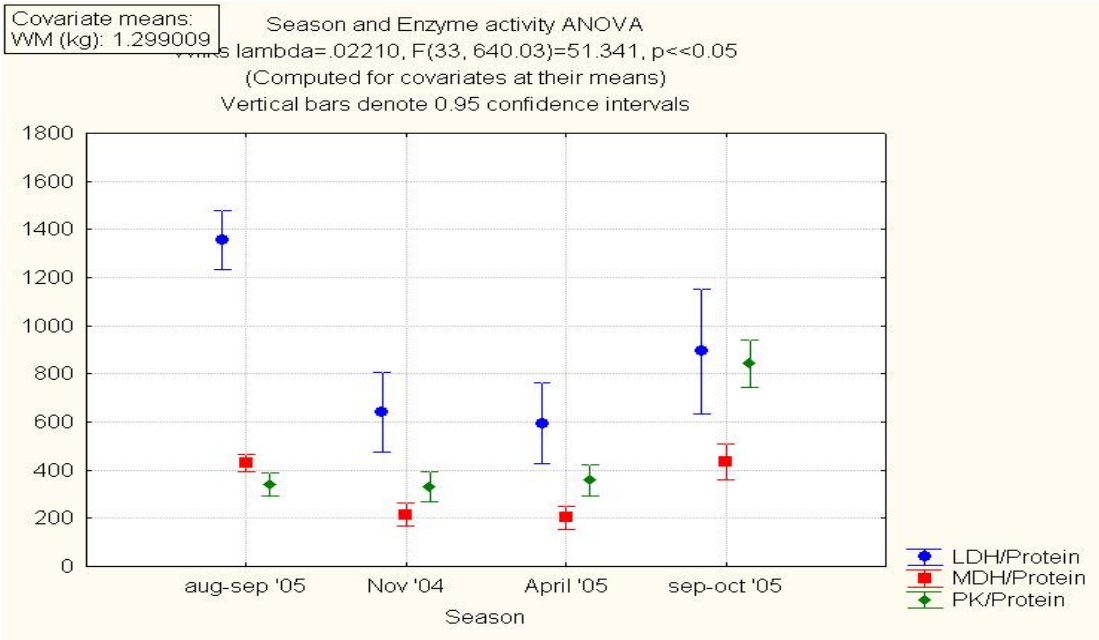

Figure 6: Enzyme activity (u/g protein) VS season

Muscle Proximate composition

Percent protein, lipid, water and ash mean values are listed for each species as well as a mean value for each species during each season in Appendix B. In general, Canary and Widow had the highest percent water and Speckled and Bocaccio had the lowest. Yellowtail had the highest protein as a percentage of ash-free dry mass (AFDM) and Bocaccio had the lowest. None of these differences were statistically different significant. (See Figure 7 below)

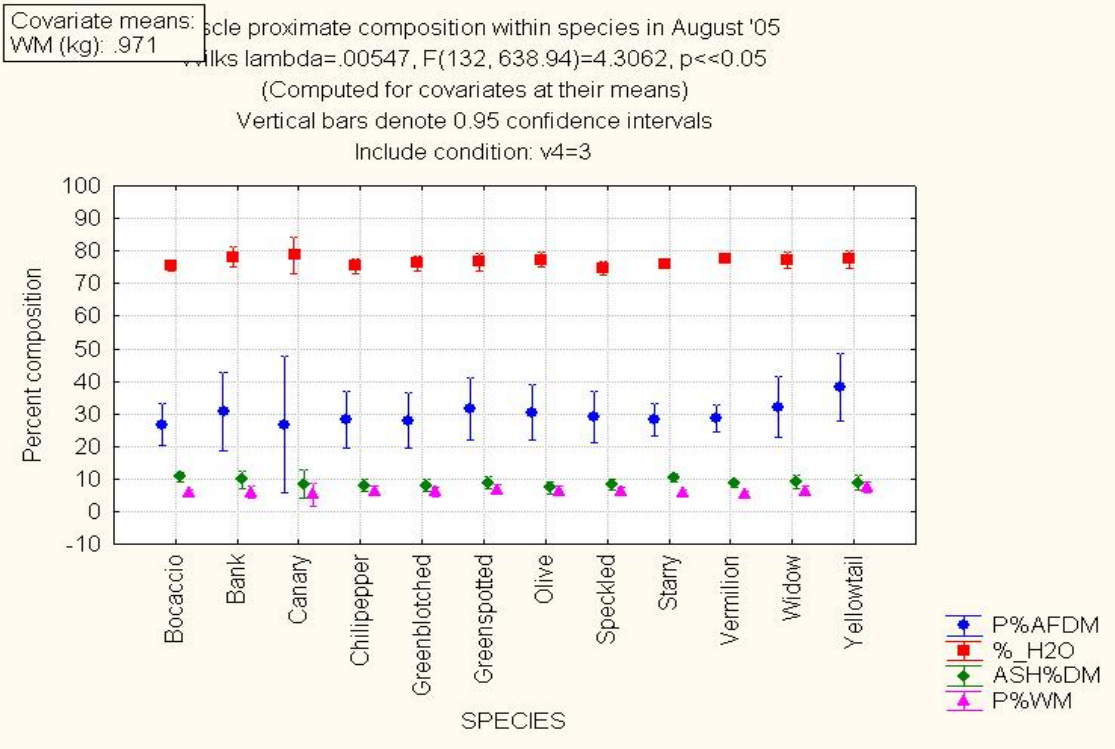

Figure 7: Muscle proximate composition among all species caught in the upwelling periods (August and September)

Mass and enzyme activity

Cowcod outweighed all the other species by 4kg on average with a mean weight of 4.6kg, and Flag was the smallest of all the species with a mean weight of 0.39kg. Overall enzyme activity was more linked with season than with mass (probably due to the fact that mass was also associated with season).

Seasonal Trends

For seven species enough samples were obtained to compare across different seasons. On average November and April showed higher enzyme activity values when presented as activity per gram wet weight than the August and September samples. When the data were represented as activity per gram protein the opposite trend was

observed with August and September values being much higher than those from November and April. Nested ANOVA's showed that in all species captured in multiple seasons enzyme activity (when expressed per gram protein) was significantly higher in August and September than November and April. (See Figure 8 below)

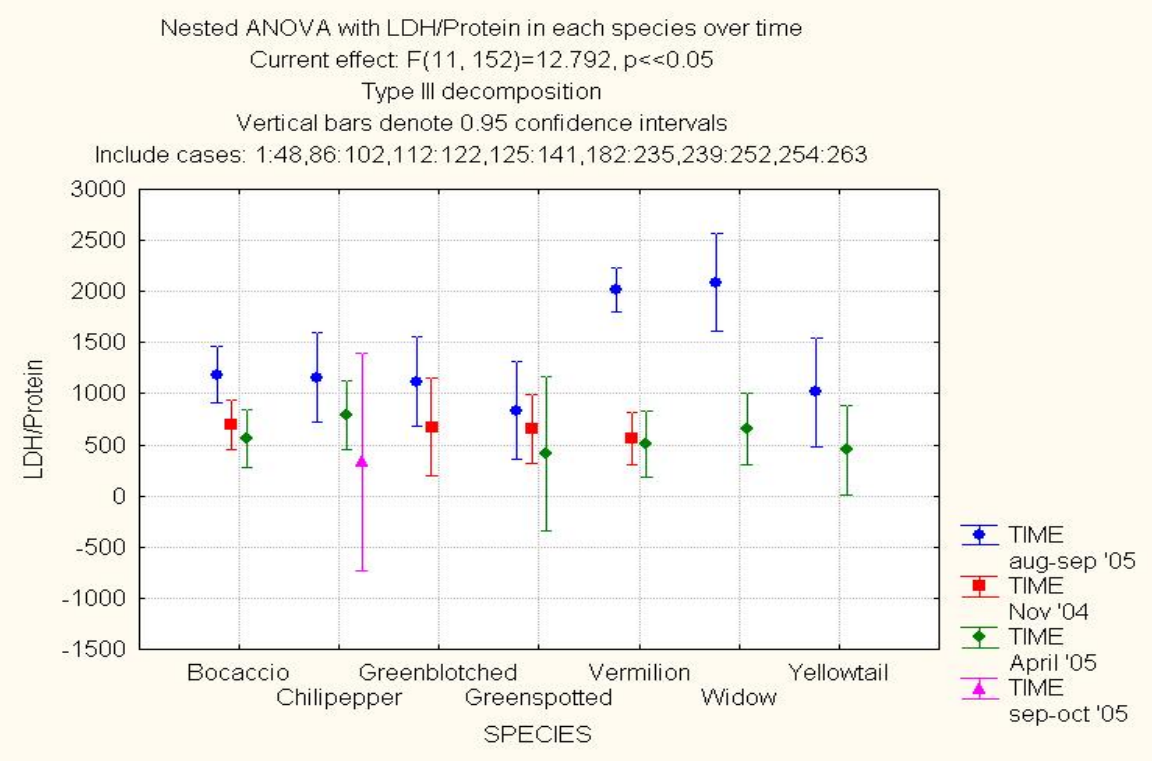

Figure 8: Nested ANOVA with LDH activity (U/g protein) in each species in each time period

Protein expressed either as a percentage of wet weight (P%WM) or as a percentage of ash free dry mass (P%AFDM) was significantly higher in the November and April samples than in the August and September samples. The percent water didn't change significantly with season. In each species the same trends were observed with protein concentration, though significance varied directly with sample size.

In Bocaccio and Vermilion lipid concentrations were determined for November and August samples. The August lipid concentrations were much higher in both species than the November lipid values ($p<0.05$, ANOVA) (see Figures 9-11 below).

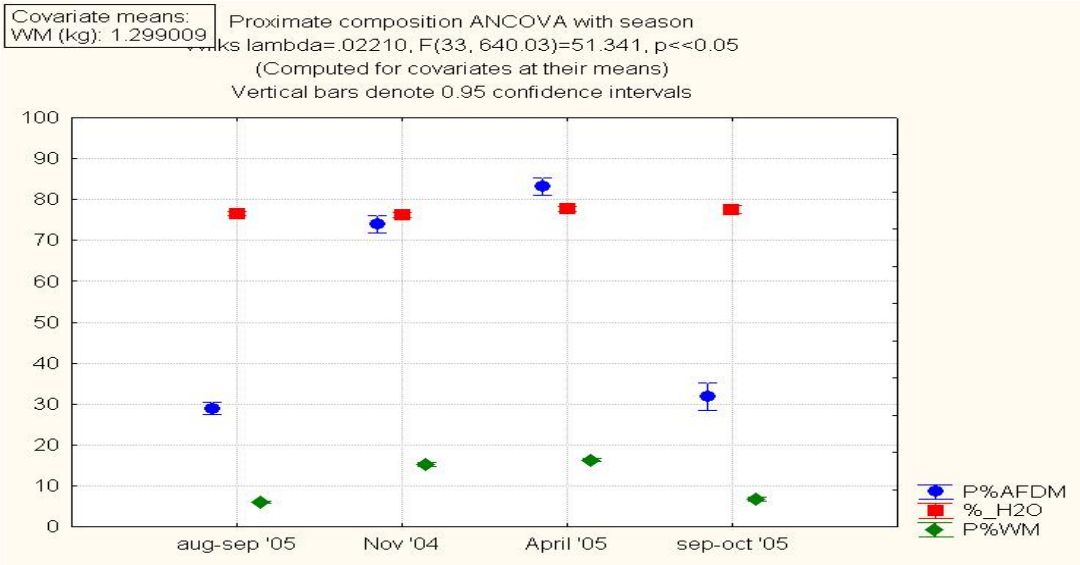

Figure 9: Muscle proximate composition with season ANOVA

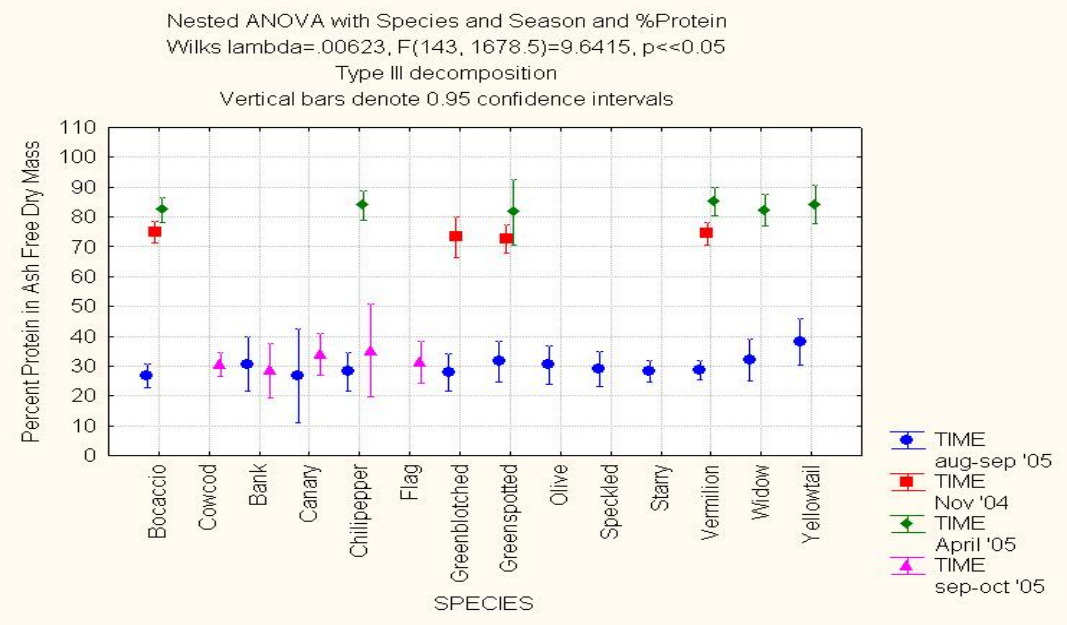

Figure 10: Nested ANOVA with season and muscle percent protein ash free dry weight in each species

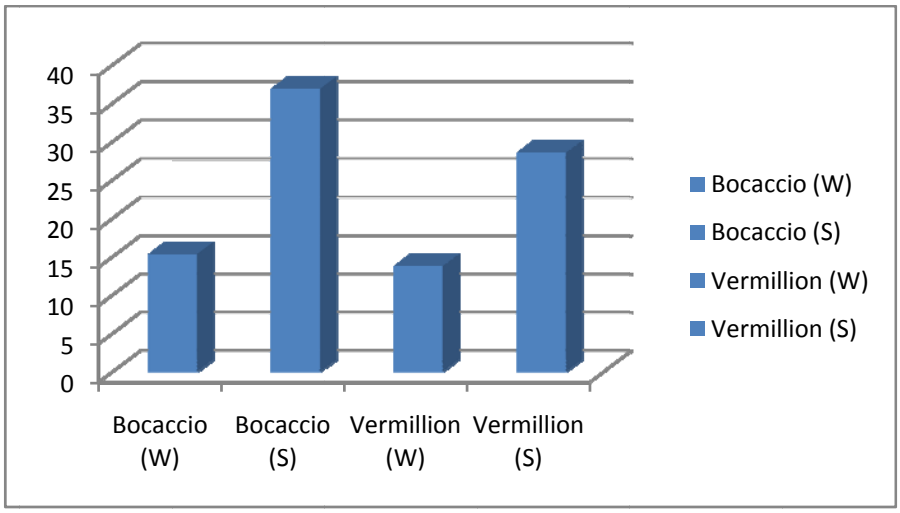

Figure 11: Lipid concentration in two species in different seasons

CS/LDH ratio

The CS/LDH ratio was calculated for all species and averaged over all seasons. The CS/LDH ratio shows the importance of aerobic versus anaerobic metabolism. Species fell into two groups depending on locomotory habit. The benthopelagic Cowcod, Bank, Canary, Chilipepper and Flag exhibited a significantly higher CS/ LDH ratio than their benthic relatives: Greenblotched, Greenspotted, Olive, Speckled and Starry. (see Figure 12 below)

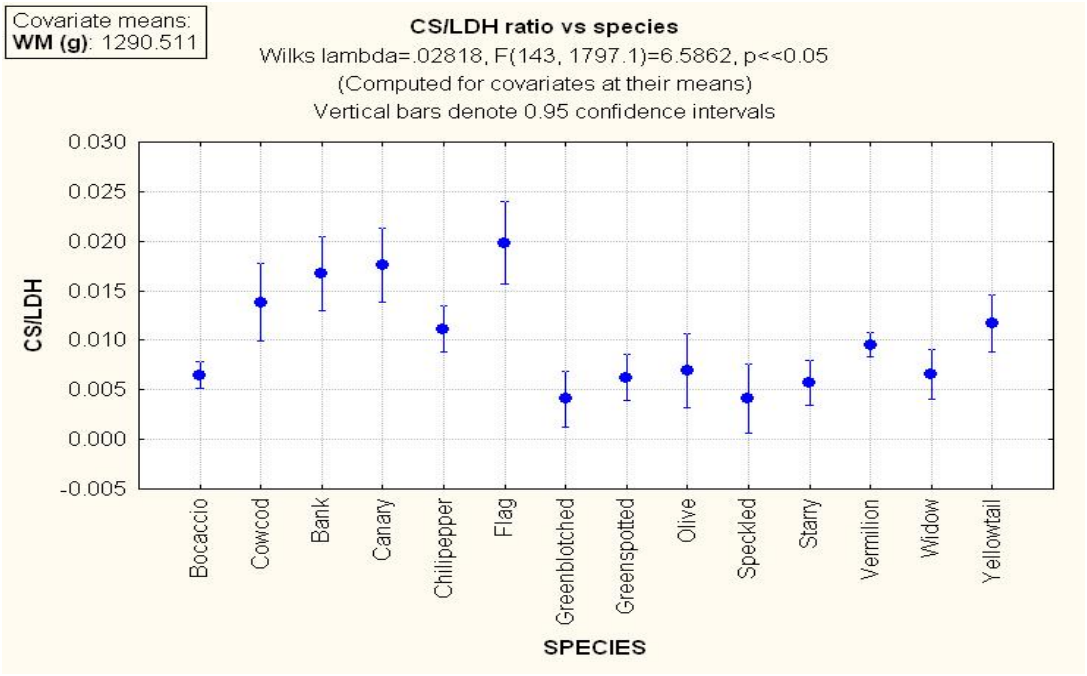

Figure 12: This ANCOVA shows the relationship between the CS/LDH ratio (which shows the relative importance of aerobic metabolism vs anerobic metabolism) and species. Some species have a much higher CS/LDH ratio showing that they are more active compared to the other species.

Oxygen Consumption

Oxygen consumption rates were calculated with the regression equations generated by Yang and Somero (1993) on fed and fasted scorpenaids. Species with a higher oxygen consumption rate per gram mass were assumed to be more active based on their metabolic demands. Interestingly, in this analysis as in the CS/LDH ratio, species grouped together according to locomotory strategy with the more benthic species toward the bottom and the more bentho-pelagic species toward the top of the plot (see Figure 13 below).

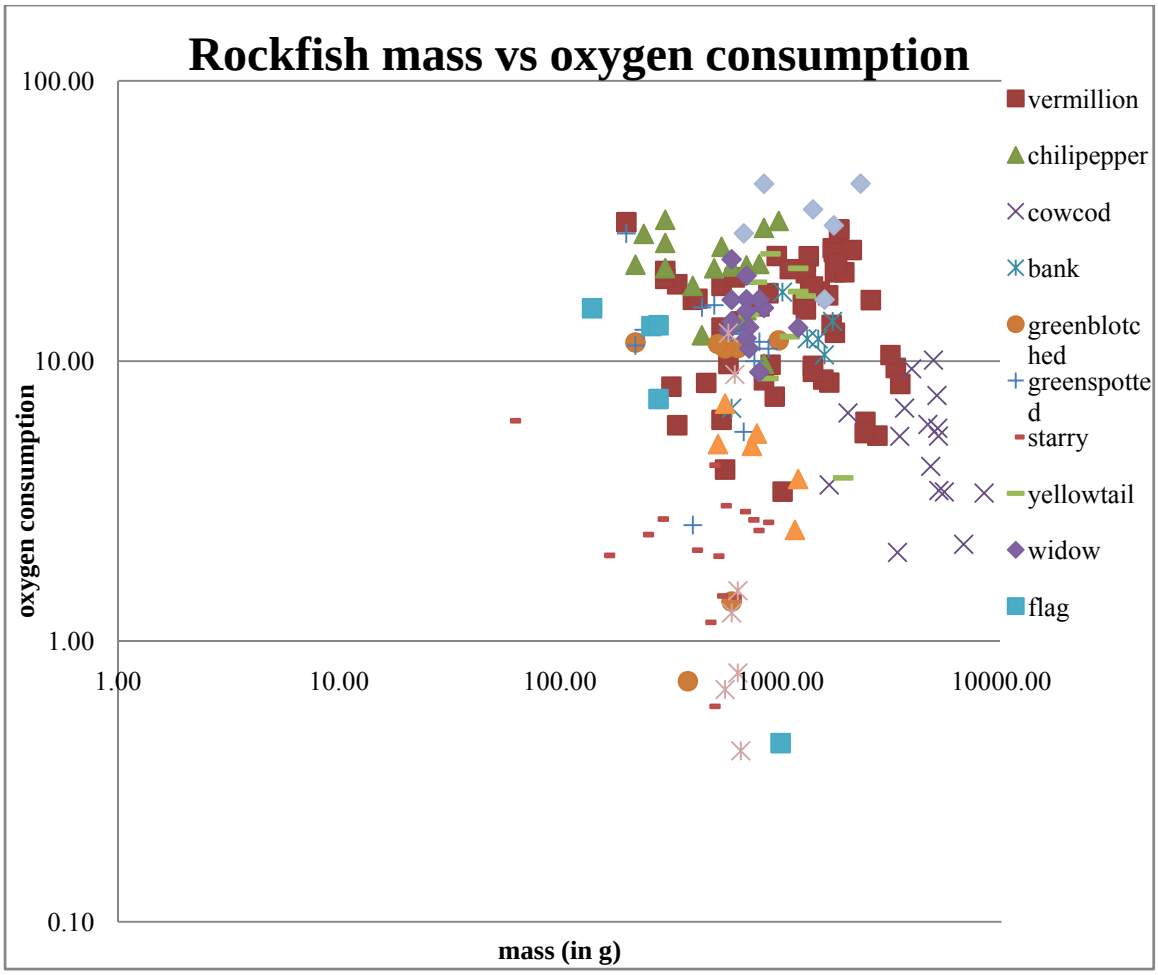

Figure 13: The above figure shows mass vs oxygen consumption with each species represented. Some group together more strongly than others. For example Cowcod is a much larger fish than all the others and therefore groups further to the right. Canary is more active (bentho-pelagic) and is grouped toward the top. Speckled is grouped towards the bottom and tends to be more sedentary, or benthic.

DISCUSSION

Enzyme assays

Enzyme assays have been used in both fish and invertebrates to determine species' locomotory habits, depth regimes, metabolism, and condition (Bishop, Torres, 2001; Bishop, Torres, Crabtree, 2000; Castellini, Somero, 1981; Childress, 1995; Childress, Nygaard, 1973; Childress, Somero, 1979; 1990; Childress, Taylor, Cailliet, Price, 1980; D'Aoust, 1970; Donnelly, Torres, 1988; Donnelly, Torres, Hopkins, Lancraft, 1990; Drazen, Seibel, 2007; Goolish, 1991; 1995; Goolish, Adelman, 1987; 1988; Ikeda, Torres, Hernandez-Leon, Geiger, 2000; Kawall, Torres, Sidell, Somero, 2002; Low, Somero; Pelletier, Guderley, Dutil, 1993; Seibel, 2007; Seibel, Drazen, 2007; Siebenaller, 1983; Siebenaller, 1984; Siebenaller, Somero, 1978; 1982; Siebenaller, Yancey, 1984; Siebenaller, Somero, 1989; Somero, 1992; Somero, Siebenaller, 1979; Somero, Childress, 1980; 1985; 1990; Sullivan, Somero, 1980; 1983; Torres, Somero, 1988a; b; Torres, Belman, Childress, 1979; Torres, Aarset, Donnelly, Hopkins, Lancraft, Ainley, 1994; Vetter, Lynn, 1997; Webb, 1976; Wilson, Somero, Prosser, 1974; Yang, Somero, 1993; Yang, Lai, Graham, Somero, 1992). In the present study, enzyme activities were useful in elucidating the seasonal change in condition of nineteen species of rockfish due either to reproductive effects, seasonal food availability due to upwelling or some combination of both.

Not surprisingly, when looking at the relative importance of aerobic versus anaerobic metabolism (the CS/LSH ratio) in the rockfishes, they group according to locomotory habit. Those species that have a higher CS/LDH are more aerobically poised suggesting a lifestyle that involves a greater need for aerobic metabolism and therefore more active swimming. Those who have a lower CS/LDH ratio rely more on burst swimming and are more sedentary or benthic and rely more on a sit and wait or ambush prey acquisition strategy. (Love, Yoklavich, Thorsteinson, Butler, 2002)

As in previous studies (i.e. Yang and Somero (1993)) LDH activity as a standalone value proved to be a good proxy for condition. The high values of LDH/gram protein showed that the fish were in better condition (i.e. well fed) during the summer months. The lipid contents in Bocaccio and Vermillion also confirmed this with high lipid contents in the summer and lower contents in the winter. Later in the year due to growth, reproduction or a less abundant food supply their lipid reserves became depleted and percent protein increased causing them to be leaner.

MDH performs two functions in the cell. The first is as an intermediate in the Krebs' cycle. The second is as a shuttle to allow entry into the mitochondrion of the electrons produced by glycolytic activity during periods when sufficient oxygen is available for aerobic processes. The mitochondrial membrane is impermeable to cytosolic NADH. Cytosolic MDH regenerates the oxidized co-factor NAD^+ for use in the glycolytic pathway, in turn producing malate from oxalo-acetic acid that can then pass through the mitochondrial membrane and be re-oxidized as a Kreb's Cycle intermediate (Lehninger, 1970). Since our assay does not discriminate between the cytosolic and

mitochondrial forms of the enzyme, a high activity of MDH suggests high activity in both compartments, suggesting in turn a high activity of the glycolytic pathway. The activity of MDH mirrors that of LDH for most species throughout all seasons.

CS is an aerobic indicator that also is a good proxy for oxygen consumption. The respiration studies and enzymatic correlations by Yang and Somero (1993) on rockfish provided the regression equations from which the oxygen consumption data were generated. In

Figure **13** Figure 13 you can see that some species, such as Starry and Speckled are much lower than the overall average. Those species have been observed to be more benthic in habit, relying on burst swimming for fight or flight situations. Other species, such as Canary and Chilipepper are higher than the overall average implying that they are a more active swimming species. Behavioral observations reported in Love et al. (2002) suggest that both Canary and CHilipepper school in groups as benthopelagic species, corroborating the results of the enzyme analyses.

The CS/LDH ratio represents the importance of aerobic versus anaerobic capability of the fish. Organisms that rely mainly on aerobic activity for locomotion tend to be animals which spend most of the time actively swimming or otherwise maintaining their position in the water column. Those fish that are more sedentary rely on anaerobic pathways to provide burst swimming in predator and prey interactions. This enzyme data combined with previous behavioral studies shows that there is indeed a noticeable difference between the benthic and the bentho-pelagic species.

Seasonal trends

The difference in enzyme activity when expressed as activity per gram wet weight vs. when expressed as activity per gram protein can be explained by the change in percent protein observed in the samples.

The changes in body composition are consistent with what one might expect during those times of year. In the summer when there is upwelling and increased nutrients which cascade down the food web, the fish are eating and storing excess energy as fat, thus decreasing their muscle water content. Throughout the year as the seasons change and upwelling decreases, primary production and zooplankton biomass also decrease (Dailey, Reish, Anderson, 1993). This translates down the food web and food becomes scarcer for *Sebastes.* The fish in turn become leaner and their percent protein increases. Energetic expenditure might also play a role in the seasonal change in body composition. Most rockfish reproduce in the spring and summer months so the changes observed in body composition could be due to reproductive effects.

CONCLUSIONS

Enzyme activity along with muscle proximate composition can be a very useful tool in evaluating a species physical condition. Increases in protein concentration during the winter months, coupled with decreased lipid and a constant or slightly increasing water level show that the fish are losing weight and energy stores. The obvious reasons for different physical conditions throughout the year, like upwelling, and reproduction are the most likely causes but such a drastic change over the course of a year was not expected. Enzymes can also be very useful in helping a researcher determine what sort of locomotory behaviors an animal is most likely to rely on due to the expression of aerobic versus anaerobic enzymes found in their tissue. A low expression of aerobic enzymes indicates that the animal most likely relies on anaerobic burst responses whereas a high expression of aerobic enzymes implies that the animal is more active. These findings show that enzymes and muscle proximate composition can be used along with limited observational data on related species to deduce condition and life habits in species that are difficult to observe and monitor.

REFERENCES

Bishop, R.E., Torres, J.J., 2001. Leptocephalus energetics: assembly of the energetics equation. Marine Biology 138, 1093-1098.

Bishop, R.E., Torres, J.J., Crabtree, R.E., 2000. Chemical composition and growth indices in leptocephalus larvae. Marine Biology 137, 205-214.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian journal of Biochemistry adn Physiology 37, 911-917.

Castellini, M.A., Somero, G.N., 1981. Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. Journal of Comparative Physiology B 143, 191-198.

Childress, J.J., 1995. Are there physiological and biochemical adaptations of metabolism in deep-sea animals? TREE 10, 30-36.

Childress, J.J., Nygaard, M.H., 1973. The chemical composition of midwater fishes as a function of depth of occurrence off southern California. Deep-Sea Research 20, 1093-1109.

Childress, J.J., Somero, G.N., 1979. Depth related enzymic activities in muscle brain and heart of deep living pelagic marine teleosts. Marine Biology 52, 273-283.

Childress, J.J., Somero, G.N., 1990. Metabolic scaling: a new perspective based on sclaing of glycolytic enzyme activities. American Zoology 30, 161-173.

Childress, J.J., Taylor, S.M., Cailliet, G.M., Price, M.H., 1980. Patterns of growth, energy utilization and reproduction in some meso and bathypelagic fishes off southern California. Marine Biology 61, 27-40.

D'Aoust, B.G., 1970. The role of lactic acid in gas secretion in the teleost swimbladder. Comp. Biochem. Physiol. 32, 637-668.

Dailey, M.D., Reish, D.J., Anderson, J.W., 1993. Ecology of the Southern California Bight. University of California Press, 926 pp.

Donnelly, J., Torres, J.J., 1988. Oxygen Consumption of midwater fishes and crustaceans from the eastern Gulf of Mexico. Marine Biology 97, 483-494.

Donnelly, J., Torres, J.J., Hopkins, T.L., Lancraft, T.M., 1990. Proximate composition of Antarctic meso pelagic fishes. Marine Biology 106, 12-23.

Drazen, J.C., Seibel, B.A., 2007. Depth-related trends in metabolism of benthic and benthopelagic deep-sea fishes. Limnol. Oceanogr. 52, 2306-2316.

Goolish, E.M., 1991. Aerobic and Anaerobic scaling in fish. Biol. Rev. 66, 33-56.

Goolish, E.M., 1995. The metabolic consequences of body size. In: Mommsen, H.a. (Ed.), Biochemistry and Molecular biology of fishes. Elsevier Science, pp. 335-366.

Goolish, E.M., Adelman, I.R., 1987. Tissue-specific cytochrome oxidase activity in largemouth bass: the metabolic coast of feeding and growth. Physiol. Zool. 60, 454-464.

Goolish, E.M., Adelman, I.R., 1988. Tissue-specific allometry of an aerobic respirotory enzyme in a large and a small species of cyprinid (Teleostei). Canadian Journal of Zoology 66, 2199-2208.

Ikeda, T., Torres, J.J., Hernandez-Leon, S., Geiger, S.P., 2000. Metabolism, ICES Zooplankton Methodology Manual. Academic Press, pp. 455-532.

Kawall, H.G., Torres, J.J., Sidell, B.D., Somero, G.N., 2002. Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. Marine Biology 140, 279-286.

Lehninger, A.L., 1970. Biochemistry. Worth Publishers Inc., New York, NY.

Love, M.S., Yoklavich, M., Thorsteinson, L., Butler, J., 2002. The Rockfishes of the Northeast Pacific. University of California Press, Berkeley and Los Angeles, California

London, 405 pp.

Low, P.S., Somero, G.N., Adaptation of muscle pyruvate kinases to environmental temperatures and pressures. J. Exp. Zool. 198, 1-12.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Marsh, J.B., Weinstein, D.B., 1966. Simple charring method for determination of lipids. Journal of Lipid Research 7, 574-576.

Pelletier, D., Guderley, H., Dutil, J.-D., 1993. Does the aerobic capacity of fish muscle change with growth rates? Fish Physiology and Biochemistry 12, 83-93.

Reisenbichler, K.R., Bailey, T.R., 1991. Microextraction of total lipid from mesopelagic animals. Deep-Sea Research 38, 1331-1339.

Seibel, B.A., 2007. On the depth and scale of metabolic rate variation: scaling of oxygen consumption rates and enzymatic activity in the Class Cephalopoda (Mollusca). The Journal of Experimental Biology 210, 1-11.

Seibel, B.A., Drazen, J.C., 2007. The rate of metabolism in marine animals: environmental constraints, ecological demands and energetic opportunites. Philosophic transactions of the Royal Society B, 1-18.

Siebenaller, J.F., 1983. The pH-dependence of the effects of hydrostatic pressure on the M4-lactate dehydrogenase homologs of Scorpaenid fishes. Marine Biology Letters 4, 233-243.

Siebenaller, J.F., 1984. Analysis of the biochemical consequencse of ontogenetic vertical migration in a deep living teleost fish. Physiol. Zool. 57, 598-608.

Siebenaller, J.F., Somero, G.N., 1978. Pressure-adaptive differences in LDHs of congeneric fishes living at different depths. Science 201, 255-257.

Siebenaller, J.f., Somero, G.N., 1982. The maintnenece of different enzyme activity levels in congeneric fishes living at different depths. Physiol. Zool. 55, 171-179.

Siebenaller, J.F., Yancey, P.H., 1984. Protein compositino of white skeletal muscle from mesopelagic fishes having different water and protein contents. Marine Biology 78, 129-137.

Siebenaller, J.F., Somero, G.N., 1989. Biochemical Adaptation to the deep sea. Reviews in Aquatic Science 1, 1-25.

Somero, G.N., 1992. Adaptations to high hydrostatic pressure. Annual Review Physiology 54, 557-577.

Somero, G.N., Siebenaller, J.F., 1979. Inefficient lactate dehydrogenases of deep-sea fishes. Nature 282, 100-102.

Somero, G.N., Childress, J.J., 1980. A violation of the metabolism-size scaling paradigm: activites of glycolytic enzymes in muscle increase in larger-size fish. Physiol. Zool. 53, 322-337.

Somero, G.N., Childress, J.J., 1985. Scaling of oxidative and glycolytic enzyme activies in fish muscle. In: Gilles, R. (Ed.), Circulation Respiration and Metabolism. Springer-Verlag, Berlin Heidelberg, pp. 250-262.

Somero, G.N., Childress, J.J., 1990. Scaling of ATP-supplying enzymes, myofibrillar proteins and buffering capacity in fish muscle: relationship to locomotory habitat. J. Exp. Biol. 149, 319-333.

Sullivan, K.M., Somero, G.N., 1980. Enzyme Activites of fish skeletal muscles and brain as influenced by depth of occurrence and habits of feeding and locomotion. Marine Biology 60, 91-99.

Sullivan, K.M., Somero, G.N., 1983. Size and diet-related variations in enzymic activity and tissue composition in the Sablefish, Anoplopoma fimbria. Biol. Bull. 164, 315-326.

Thurston, C.E., 1960. Proximate composition of nine species of rockfish. 38-42.

Torres, J.J., Somero, G.N., 1988a. metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. Marine Biology 98, 169-180.

Torres, J.J., Somero, G.N., 1988b. Vertical distribution and metabolism in antarctic mesopelagic fishes. Comp. Biochem. Physiol. 90B, 521-528.

Torres, J.J., Belman, B.W., Childress, J.J., 1979. Oxygen consumption rates of midwater fishes as a function of depth of occurrence. Deep-Sea Research 26A, 185-197.

Torres, J.J., Aarset, A.V., Donnelly, J., Hopkins, T.L., Lancraft, T.M., Ainley, D.G., 1994. Metabolism of Antarctic micronektonic Crustacea as a function of depth of occurrence and season. Marine Ecology Progress Series 113, 207-219.

Vetter, R.D., Lynn, E.A., 1997. Bathymetric demography, enzyme activity patterns and bioenergetics of deep-living scorpaenid fishes (genera Sebastes and sebastolobus): paradigms revisited. Marine Ecology Progress Series 155, 173-188.

Webb, P.W., 1976. Chaptor 20: Effects of size on performance and energetics of fish, pp. 315-331.

Wilson, F.R., Somero, G.N., Prosser, C.L., 1974. Temp-metabolism relations of two species of Sebastes from different thermal environments. Comp. Biochem. Physiol. 47B, 485-491.

Winant, C.D., Dever, E.P., Hendershott, M.C., 2003. Characteristic patterns of shelf circulation at the boundary between central and southern California. Journal of Geophysical Research 108, 3021-3034.

Yang, T.-H., Somero, G.N., 1993. Effects of feeding and food deprivation on oxygen consumption, muscle protein conc. And activities of energy metabolism enzymes in muslce and brain of shallow-living (scorpaena guttata) and deep-living (sebastolobus alascanus) scorpaenid fishes. J. Exp. Biol. 181, 213-232.

Yang, T.H., Lai, N.C., Graham, J.B., Somero, G.N., 1992. Respiratory, Blood, and heart enzymatic adaptations of Sebastolobus alascanus (scorpaenidae; Teleostei) to the oxygen minimum zone: A comparative study. Biol. Bull. 183, 490-499.

Appendices

Appendix A: Mean enzyme activities plus or minus the standard deviation for all species

Table 2a: The average of all enzyme values expressed (U/g wet mass) for all species

Species	LDH	MDH	PK	CS
Bocaccio	91.87±4.00	32.38±1.35	50.55±3.21	0.53±0.024
Cowcod	50.10±5.13	21.27±1.96	56.67±4.37	0.41±0.021
Bank	52.44±12.68	15.88±2.17	44.19±15.33	0.65±0.05
Canary	93.27±17.63	45.30±10.42	77.10±15.41	1.36±0.14
Chilipepper	107.97±11.07	45.62±2.58	79.61±6.76	0.99±0.055
Flag	29.23±4.94	27.42±4.74	19.55±2.38	0.57±0.095
Greenblotched	82.86±6.82	24.03±2.89	30.38±5.87	0.40±0.064
Greenspotted	80.73±7.93	24.46±2.09	34.37±4.8	0.55±0.069
Olive	59.25±9.17	20.28±1.91	15.25±1.25	0.39±0.022
Speckled	77.60±8.75	24.12±1.41	11.11±1.07	0.36±0.064
Starry	53.30±4.27	16.74±1.12	9.93±0.84	0.31±0.013
Vermillion	85.76±2.62	28.51±0.97	33.09±2.55	0.75±0.034
Widow	108.18±6.94	28.32±1.44	36.07±4.24	0.74±0.033
Yellowtail	71.29±7.34	27.29±1.80	27.31±4.96	0.79±0.074

Table 2b: The average of all enzyme values expressed (U/g protein) for all species

Species	LDH/Protein	MDH/Protein	PK/Protein	CS/Protein
Bocaccio	810.99±46.87	299.17±22.55	409.25±18.62	4.77±0.31
Cowcod	796.39±87.24	318.53±25.54	865.13±65.72	6.49±0.42
Bank	871.43±196.00	269.55±41.18	721.30±232.32	11.04±1.13
Canary	1434.27±265.66	686.73±145.59	1176.00±243.28	21.06±2.30
Chilipepper	897.58±92.65	428.19±52.24	675.09±44.26	9.73±1.41
Flag	465.50±76.89	427.46±55.16	325.48±59.37	8.95±1.11
Greenblotched	915.99±89.99	251.20±20.79	291.90±26.79	3.93±0.16
Greenspotted	678.03±70.97	198.96±12.63	263.26±24.81	4.14±0.35
Olive	970.98±191.20	330.83±48.77	244.55±29.00	6.41±0.61
Speckled	1357.18±212.99	425.11±57.14	185.76±15.17	5.78±0.51
Starry	1073.76±157.25	324.06±42.23	209.73±42.23	6.00±0.64
Vermillion	1209.74±153.56	363.88±38.51	313.75±27.37	9.08±0.75
Widow	1167.57±217.86	293.08±45.19	305.14±19.56	7.42±.94
Yellowtail	676.82±148.68	243.43±32.69	202.65±18.90	6.95±0.90

Appendix A (Continued)

Table 2c: The average of all enzyme values expressed (U/g wet mass) for all species in November '04

Species	LDH	MDH	PK	CS
Bocaccio	110.32±26.40	37.00±10.43	62.93±18.57	0.61±0.17
Greenblotched	101.07±17.21	32.23±6.65	47.17±16.95	0.63±.01
Greenspotted	95.81±31.9	28.72±7.29	41.57±20.29	0.70±0.21
Vermillion	84.71±17.95	30.78±6.49	43.94±13.82	0.86±0.26

Table 2d: The average of all enzyme values expressed (U/g protein) for all species in November '04

Species	LDH/Protien	MDH/Protein	PK/Protein	CS/Protein
Bocaccio	699.78±141.04	234.43±55.15	400.83±118.51	3.95±1.24
Greenblotched	671.09±95.70	213.59±35.58	313.23±107.39	4.19±0.23
Greenspotted	653.20±254.03	194.39±54.86	275.80±126.89	4.74±1.47
Vermillion	562.95±104.86	204.08±35.62	289.87±84.25	5.75±1.75

Table 2e: The average of all enzyme values expressed (U/g wet mass) for all species in April '05

Species	LDH	MDH	PK	CS
Bocaccio	93.51±21.44	31.28±6.71	58.41±17.94	0.58±0.13
Chilipepper	137.58±26.42	46.98±4.79	97.13±23.07	0.98±0.18
Greenspotted	68.14±6.87	27.33±2.70	44.81±6.19	0.68±0.01
Vermillion	82.52±19.59	31.66±6.45	44.95±13.47	0.89±0.23
Widow	97.79±19.45	27.02±6.28	45.27±11.10	0.77±0.15
Yellowtail	70.94±14.68	29.63±4.51	38.23±9.13	0.91±0.17

Table 2f: The average of all enzyme values expressed (U/g protein) for all species in April '05

Species	LDH/Protien	MDH/Protein	PK/Protein	CS/Protein
Bocaccio	561.55±115.48	187.87±35.63	350.49±102.88	3.51±0.76
Chilipepper	797.46±130.69	273.87±31.44	564.38±126.72	5.66±0.93
Greenspotted	412.27±41.29	165.39±16.43	271.14±37.28	4.11±0.07
Vermillion	504.09±115.41	192.78±31.65	273.54±75.39	5.46±1.31
Widow	654.64±106.87	180.85±35.87	304.27±74.48	5.15±0.83
Yellowtail	449.73±84.21	187.56±22.43	240.80±43.15	5.79±1.13

Appendix A (Continued)

Table 2g: The average of all enzyme values expressed (U/g wet mass) for all species in August '05

Species	LDH	MDH	PK	CS
Bocaccio	66.97±11.37	27.57±7.55	27.56±8.96	0.40±0.13
Bank	26.46±7.55	16.12±6.09	17.27±1.87	0.65±0.19
Canary*	64.14	24.55	25.62	1.22
Chilipepper	72.16±28.40	37.92±5.66	53.47±5.41	1.01±0.32
Greenblotched	67.69±13.39	17.21±4.98	16.40±3.24	0.23±0.04
Greenspotted	55.62±23.30	14.81±3.15	15.82±4.19	0.20±0.07
Olive	59.25±9.17	20.28±1.91	15.25±1.25	0.39±0.022
Speckled	77.60±8.75	24.12±1.41	11.11±1.07	0.36±0.064
Starry	53.30±4.27	16.74±1.12	9.93±0.84	0.31±0.013
Vermillion	87.25±20.25	25.32±6.70	14.04±5.82	0.62±0.17
Widow	126.91±27.48	30.66±2.28	19.53±6.41	0.71±0.08
Yellowtail	71.84±35.42	23.80±6.04	10.94±1.84	0.63±0.23

*only one specimen caught in this season

Table 2h: The average of all enzyme values expressed (U/g protein) for all species in August '05

Species	LDH/Protien	MDH/Protein	PK/Protein	CS/Protein
Bocaccio	1184.68±295.62	485.09±147.56	474.78±140.91	7.02±2.28
Bank	453.40±158.54	276.26±118.91	293.11±54.30	11.15±3.91
Canary*	1229.91	470.81	491.30	23.36
Chilipepper	1158.10±521.14	593.43±73.29	844.24±130.80	15.81±5.04
Greenblotched	1120.08±246.69	282.54±76.83	274.14±75.69	3.73±0.64
Greenspotted	834.03±360.07	221.54±52.53	235.05±62.08	2.97±0.99
Olive	970.98±191.20	330.83±48.77	244.55±29.00	6.41±0.61
Speckled	1357.18±212.99	425.11±57.14	185.76±15.17	5.78±0.51
Starry	1073.76±157.25	324.06±42.23	209.73±42.23	6.00±0.64
Vermillion	2018.27±1250.65	562.15±318.36	350.11±281.91	13.24±5.55
Widow	2090.87±692.19	495.09±104.83	306.73±79.39	11.51±2.52
Yellowtail	1017.47±627.28	327.24±124.96	145.43±18.43	8.71±3.93

*only one specimen caught in this season

Appendix A (Continued)

Table 2i: The average of all enzyme values expressed (U/g wet mass) for all species in September '05

Species	LDH	MDH	PK	CS
Cowcod	50.10±5.13	21.27±1.96	56.67±4.37	0.41±0.021
Bank	78.43±18.17	15.65±5.75	71.11±36.71	0.65±0.06
Canary	99.10±45.64	49.45±26.16	87.41±31.39	1.39±0.38
Chilipepper*	26.84	78.36	61.36	1.12
Flag	29.24±4.94	27.43±4.74	19.56±2.38	0.58±0.095

*only one specimen caught in this season

Table 2j: The average of all enzyme values expressed (U/g protein) for all species in September '05

Species	LDH/Protien	MDH/Protein	PK/Protein	CS/Protein
Cowcod	796.39±87.24	318.53±25.54	865.13±65.72	6.49±0.42
Bank	1289.48±163.91	262.85±105.67	1149.50±506.52	10.93±1.97
Canary	1475.14±718.87	729.91±380.78	1312.94±550.68	20.61±6.16
Chilipepper*	335.62	979.96	767.30	13.96
Flag	465.50±76.89	427.46±55.16	325.48±59.37	8.95±1.11

*only one specimen caught in this season

Appendix B: Mean proximate composition values for all species in all seasons

Table 3a: Mean proximate composition values for all species averaged over all seasons

Species	P%AFDM	%water	P%WM	Ash%DM
Bocaccio	62.02±3.57	75.85±0.33	13.83±0.71	12.82±0.41
Cowcod	30.41±1.13	76.48±0.40	7.17±0.31	6.66±0.64
Bank	29.59±1.64	77.87±0.81	7.64±0.21	5.99±1.41
Canary	32.80±1.56	78.56±0.47	7.2±0.36	6.52±1.13
Chilipepper	61.41±6.84	76.02±0.43	10.52±1.31	12.83±1.03
Flag	31.19±1.78	77.92±0.61	8.89±0.37	6.27±1.11
Greenblotched	48.49±7.26	76.91±0.46	8.44±1.42	10.15±0.55
Greenspotted	61.59±5.06	76.74±0.31	11.27±0.99	12.67±0.83
Olive	30.41±1.83	77.27±0.56	7.23±0.42	6.40±0.54
Speckled	29.08±5.72	74.89±1.5	7.77±0.89	6.28±0.42
Starry	28.17±2.78	76.20±0.60	9.83±0.55	5.94±0.34
Vermillion	55.88±3.74	77.65±0.35	10.48±0.73	10.95±0.39
Widow	64.33±6.97	78.41±0.74	11.83±1.16	11.71±1.13
Yellowtail	65.79±7.92	77.74±0.94	10.42±1.37	12.48±0.52

Table 3b: Mean proximate composition values for all species in November '04

Species	P%AFDM	%water	P%WM	Ash%DM
Bocaccio	74.80±6.58	75.14±2.72	17.07±1.33	15.67±4.99
Greenblotched	73.22±6.67	77.62±1.14	9.24±0.68	15.03±2.12
Greenspotted	72.64±6.38	76.61±1.30	13.12±1.08	14.88±3.24
Vermillion	74.35±5.21	76.98±1.37	12.95±1.02	15.02±2.96

Table 3c: Mean proximate composition values for all species in April '05

Species	P%AFDM	%water	P%WM	Ash%DM
Bocaccio	82.45±3.39	77.32±1.35	11.56±0.97	16.51±2.47
Chilipepper	84.00±5.52	76.5±2.04	12.43±0.82	17.19±4.61
Greenspotted	81.59±6.11	77.5±1.56	9.85±0.02	16.52±0.42
Vermillion	85.12±3.71	78.56±1.21	10.36±0.87	16.34±1.56
Widow	82.25±5.95	79.01±2.18	13.29±0.83	14.87±4.57
Yellowtail	84.15±6.13	77.97±2.34	11.36±0.91	15.77±1.27

Appendix B (continued)

Table 3d: Mean proximate composition values for all species in August '05

Species	P%AFDM	%water	P%WM	Ash%DM
Bocaccio	26.78±3.54	75.39±1.73	11.86±0.71	5.77±4.85
Bank	30.69±4.78	78.20±2.84	9.85±0.52	5.96±3.81
Canary*	26.7225978	78.7754938	8.05	5.215164381
Chilipepper	28.12±2.21	75.42±1.19	7.5±0.54	6.39±0.85
Greenblotched	27.89±1.53	76.32±1.67	7.78±0.53	6.09±1.34
Greenspotted	31.52±1.82	76.73±1.30	8.14±0.34	6.73±1.16
Olive	30.41±1.83	77.27±0.56	7.23±0.42	6.40±0.54
Speckled	29.08±5.72	74.89±1.5	7.77±0.89	6.28±0.42
Starry	28.17±2.78	76.20±0.60	9.83±0.55	5.94±0.34
Vermillion	28.63±14.23	77.74±3.41	8.69±2.10	5.43±1.65
Widow	32.08±10.94	77.33±3.63	8.87±1.18	6.37±0.55
Yellowtail	38.25±11.47	77.42±4.17	9.03±1.14	7.56±1.08

*only one specimen caught in this season

Table 3e: Mean proximate composition values for all species in September '05

Species	P%AFDM	%water	P%WM	Ash%DM
Cowcod	30.41±1.13	76.48±0.40	7.17±0.31	6.66±0.64
Bank	28.50±3.70	77.55±1.25	5.43±0.61	6.03±0.89
Canary	34.02±2.68	78.52±1.29	7.03±0.66	6.79±3.05
Chilipepper*	35.21659652	74.8537437	9.7	7.996656999
Flag	31.19±1.78	77.92±0.61	8.89±0.37	6.27±1.11

*only one specimen caught in this season