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Aerobic and anaerobic enzyme assays in Southern California Rockfish: Proxies for physiological and ecological data

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ABSTRACT

Rockfish are commercially and recreationally important, yet due to the depths they inhabit (3–500 m), little is known about their ecology. The present study examined 19 different species of *Sebastes* from the Southern California Bight over four seasons (late summer, fall, early winter, and spring) using metabolic enzyme assays. Enzymes used were lactate dehydrogenase (LDH), malate dehydrogenase (MDH), pyruvate kinase (PK), and citrate synthase (CS). Muscle proximate composition data (protein, water and lipid content) 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. The rockfish have the highest protein as a percentage of wet mass (P%WM) in the spring, right before the upwelling period begins and the lowest P%WM in late summer after the peak of upwelling. Lipid content also showed a seasonal change with the highest lipid content in the late summer and the lowest in winter. The enzyme profiles have been broken into three groups which fit with their locomotory habit and their prey selection. These findings show that enzymes and muscle proximate composition can be used along with limited observational data on related species to deduce condition, seasonal impacts and ecologically important life habits in species that are difficult to observe and monitor.

1. 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 (~96) being found along the Pacific coast of North America (Love et al., 2002). The greatest diversity (~56 species) is found in the southern California bight.

Rockfish are very long lived (10–200 years), reaching sexual maturity at 4–10 years of age. They have internal fertilization and brood their young until their release as larvae. Most species reproduce once per year. In southern California, most rockfish release their young after the onset of coastal upwelling, in early spring to summer. Their larvae spend up to a year in the pelagic realm and, after settling, juveniles spend their first year in nearshore habitats. As they grow, many descend to greater depths with increasing age and size (Cailliet et al., 2001). Rockfish size varies considerably with species but the average adult weight for the group is one kilogram. Larger species can reach up to 20 kg (Love et al., 2002).

Rockfishes occur in a fairly narrow depth range (30–500 m) within an extensive North–South distribution, occupying a variety of habitats ranging from deep benthic and benthopelagic to kelp forests nearshore. Not surprisingly, they also exhibit many different feeding strategies including ambush predation, schooling to concentrate prey, and opportunistic feeding (Love et al., 2002). Because most rockfish inhabit depths difficult to reach with SCUBA and other methods of visually acquiring behavioral data, limited data exist on rockfish ecology and behavior. In cases such as this, the use of biochemical physiological techniques may be useful for deducing differences in behavior by bridging the gap between data acquired from gut contents, tagging studies, and line sampling and more expensive in-situ camera-based evaluations of behavior. Techniques such as intermediary metabolic enzyme activity provide an integrated index of fish condition as well as on general activity levels that can be used to deduce the basics of a fish's ecology (e.g. benthic sluggish vs. benthopelagic).

The enzymes chosen for examination in the 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 a 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

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with the cytoplasmic isozyme (s-mdh), functions in shuttling reducing equivalents between the mitochondria and cytoplasm. 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 (Childress and Somero, 1979). The enzymes described above have been used extensively for investigating a variety of metabolic questions in rockfishes and other fish taxa.

The Southern California Bight undergoes pronounced seasonal variation in upwelling strength (Winant et al., 2003) resulting in large swings in primary and secondary production. Since the oceanographic seasons affect food availability, some variability would be expected in the overall condition of rockfish at different seasons throughout the year. However, Thurston (1960) found that proximate composition (protein, lipid, water, carbohydrate, and ash content) of nine species of rockfish off the coast of Oregon showed only slight variation between species and found no differences in composition throughout the year, suggesting little seasonal change in condition.

The purpose of the present study was to apply a different suite of indicators to see if seasonal change and species specific differences in lifestyle could be detected with a more sensitive set of condition measures: intermediary metabolic enzymes. Past studies have shown the usefulness of intermediary metabolic enzymes in elucidating physical condition and other physiologic parameters. We postulate that there will be enough of a difference in metabolic indicators between these 19 closely related species to determine their locomotory habit (benthic vs. benthopelagic).

2. Methods

2.1. Sample collection

Fishes were collected by National Marine Fisheries Service hook-and-line surveys off the coast of Southern California during four separate cruises (Fig. 1, Table 1). 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 °C in a cryogenic deep-freeze until used for the enzyme assays described below. The total sample size was 263 samples from 19 different species (Table 1).

2.2. Enzyme analyses

Fish white muscle samples were homogenized in 50 mM Imidazole/HCl buffer (pH 7.2 at 20 °C) using a ground glass homogenizer. Samples were kept at ice-bath temperature for the duration of the assays. Activities were measured at 10 ± 0.2 °C using a thermostatted 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 per gram protein (Tables 2 and 3). All assays were done in triplicate and the average value was used in further statistics. All enzyme assays followed the procedure of Childress and Somero (1979).

2.3. Protein analysis

For protein, homogenate was diluted by a factor of 20 using distilled water. The air above the homogenate was displaced with

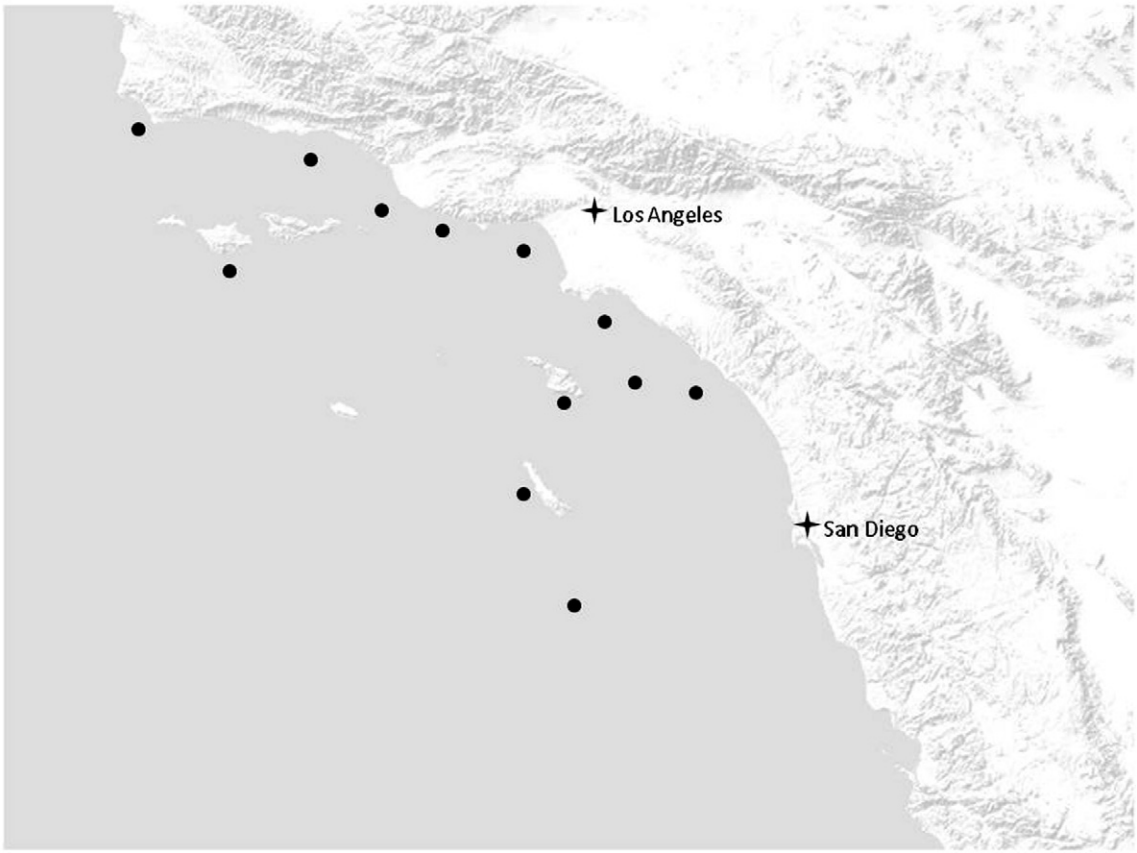

Fig. 1. Sample sites in the Southern CA Bight.

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

Species	Season				Location where samples were caught	Captured size range (cm)	Captured depth range (m)	Total depth range (m)	Maximum size (cm)
	Nov '04	Apr '05	Aug '05	Sept '05					
	n	*n*	*n*	*n*					
S. caurinus	0	0	1	0	7	47	96	183	66
S. chlorostictus	10	2	5	0	5, 7, 8, 9	23–39	85–128	363	47.2
S. constellatus	0	0	20	0	1, 2, 7, 8	18–40	41–138	274	46
S. elongatus	0	0	1	0	2	34	187	495	43
S. ensifer	0	0	1	0	5	22	145	433	25
S. entomelas	0	9	5	0	7	36–45	94.7–117	549	59
S. flavidus	0	6	4	0	7	36–51	94–115	549	66
S. goodei	0	10	6	1	1, 3, 5	25–43	128–170	491	59
S. levis	0	0	0	16	2, 3, 4, 10, 12	49–79	105–183	491	94
S. macdonaldi	0	0	2	0	5	42–48	143–147	238	66
S. melanostomus	0	0	2	0	2	52–55	238	768	61
S. miniatus	18	11	25	0	1, 2, 7, 8, 9, 11, 12	27–58	46–139	436	76
S. ovalis	0	0	7	0	8	32–35	86–95	366	56
S. paucispinis	19	14	15	0	1, 2, 4, 6, 7, 8, 9, 12	36–70	83–238	478	91
S. pinniger	0	0	1	5	6, 7	35–54	97–109	838 (439)	76
S. rosenblatti	5	0	6	0	1, 5, 7	24.5–43	78–147	491	48
S. rubrivinctus	0	0	0	5	3, 4, 6, 10	22–38	40–165	418	44
S. rufus	0	0	3	3	2	35–46	134–238	454	55.2
S. serranoides	0	0	6	0	7, 8	33–46	41–97	172	61

Location legend: 1, 14 mile bank; 2, 60 mile bank; 3, Anacapa Island; 4, Catalina; 5, Central coast; 6, Harrison reef; 7, Pt Conception; 8, San Clemente Island; 9, San Pedro Bay; 10, Santa Barbara Channel; 11, Santa Monica Bay; 12, Santa Rosa flats.

nitrogen before placing it in a conventional deep freeze (−20 °C) for a maximum of 96 h until protein analysis was conducted. Protein composition in white muscle tissue was measured using the method of Donnelly et al. (1990). Absorbance was measured at 750 nm. Values were then compared to a standard curve to obtain values for protein content.

2.4. Lipid analysis

Lipid levels were determined on 200 µl of homogenate using the methods of Donnelly et al. (1990). Briefly, lipids were extracted using a 3:1:1 mixture of methanol, chloroform and water followed by filtration to remove particulates. Concentrations were determined using the charring method of Marsh and Weinstein (1966) with stearic acid as a standard (Bligh and Dyer, 1959; Marsh and Weinstein, 1966; Reisenbichler and Bailey, 1991).

2.5. Dry and ash weight measurements

One-milliliter aliquots of homogenate were dispensed into pre-combusted, pre-weighed crucibles and dried to a constant weight in a 60 °C oven. Ash content (% DM) was measured following combustion of the dried crucibles at 500 °C for 3–4 h.

2.6. Oxygen consumption

Oxygen consumption ml h^{-1} (VO_2) was calculated using CS activity values (M) in field caught specimens using the regression equation generated by Yang and Somero (1993).

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

2.7. Statistical analysis

The overall mean values for each species were calculated, as well as the mean values for each species during each season. Differences in enzyme activity between species were examined using only the samples from the August/September cruise to eliminate seasonal effects. Species-specific differences in enzyme activities and composition were analyzed using ANCOVAs with species as the categorical variable and mass as the continuous covariate. Seasonal differences in enzyme activities and composition within species were examined with nested ANOVAs. Duncan's multiple range test enabled discrimination between homogenous groups. All statistical analyses were conducted using Statistica (Statsoft Inc.) with a significance level of $p<0.05$.

3. Results

3.1. Muscle proximate composition

Mean values for percent protein, lipid, water, and ash in each species as well as seasonal means are listed in Table 4. 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 did not change significantly with season. In each species the same trends were observed with protein concentration, though significance varied directly with sample size. Fish are mainly composed of water, protein and lipid; since the protein concentration was changing and the water content was remaining relatively constant, lipid concentrations in *S. paucispinis* and *S. miniatus* were determined for November and August samples (Table 6). The August lipid concentrations were much higher in both species than the November lipid values ($p<0.05$, ANOVA) suggesting strongly that the drop in protein level was a direct consequence of increased lipid.

3.2. Enzyme activities

Mean enzyme activities for all species are shown in Tables 2 and 3. Activities are expressed as µmol substrate converted to product per minute (U or units) per gram wet mass (U g^{-1} wet mass, WM) or units per gram protein (U g^{-1} protein). Overall, *S. entomelas* exhibited the highest LDH activity (expressed in WM) and *S. rufus* exhibited the lowest. Both were significantly different from the remaining species. *S. goodei* had significantly higher MDH values (when expressed in WM) than the rest of the rockfish species. *S. goodei*, *S. paucispinis* and *S. pinniger* exhibited significantly higher PK activity per gram WM.

Table 2
Mean enzyme activity expressed per gram wet weight for each species in each season with standard deviation in parentheses. An (*) is used to represent those species with only one sample in that time period.

Species	LDH				MDH			
	Nov '04	April '05	Aug '05	Oct '05	Nov '04	April '05	Aug '05	Oct '05
S. paucispinis	110.32 (±26.40)	93.51 (±21.44)	66.97 (±11.37)		37.00 (±10.43)	31.28 (±6.71)	27.57 (±7.55)	
S. levis				50.10 (±5.13)				21.27 (±1.96)
S. rufus			26.46 (±7.55)	78.43 (±18.17)			16.12 (±6.09)	15.65 (±5.75)
S. pinniger			64.14*	99.10 (±45.64)			24.55*	49.45 (±26.16)
S. goodei		137.58 (±26.42)	72.16 (±28.40)	26.84*		46.98 (±4.79)	37.92 (±5.66)	78.36*
S. rubrivinctus				29.24 (±4.94)				27.43 (±4.74)
S. rosenblatti	101.07 (±17.21)		67.69 (±13.39)		32.23 (±6.65)		17.21 (±4.98)	
S. chlorostictus	95.81 (±31.9)	68.14 (±6.87)	55.62 (±23.30)		28.72 (±7.29)	27.33 (±2.70)	14.81 (±3.15)	
S. serranoides			59.25 (±9.17)				20.28 (±1.91)	
S. ovalis			77.60 (±8.75)				24.12 (±1.41)	
S. constellatus			53.30 (±4.27)				16.74 (±1.12)	
S. miniatus	84.71 (±17.95)	82.52 (±19.59)	87.25 (±20.25)		30.78 (±6.49)	31.66 (±6.45)	25.32 (±6.70)	
S. entomelas		97.79 (±19.45)	126.91 (±27.48)			27.02 (±6.28)	30.66 (±2.28)	
S. flavidus		70.94 (±14.68)	71.84 (±35.42)			29.63 (±4.51)	23.80 (±6.04)	

Species	PK				CS			
	Nov '04	April '05	Aug '05	Oct '05	Nov '04	April '05	Aug '05	Oct '05
S. paucispinis	62.93 (±18.57)	58.41 (±17.94)	27.56 (±8.96)		0.61 (±0.17)	0.58 (±0.13)	0.40 (±0.13)	
S. levis				56.67 (±4.37)				0.41 (±0.021)
S. rufus			17.27 (±1.87)	71.11 (±36.71)			0.65 (±0.19)	0.65 (±0.06)
S. pinniger			25.62*	87.41 (±31.39)			1.22*	1.39 (±0.38)
S. goodei		97.13 (±23.07)	53.47 (±5.41)	61.36*		0.68 (±0.01)	1.01 (±0.32)	1.12*
S. rubrivinctus				19.56 (±2.38)				0.58 (±0.095)
S. rosenblatti	47.17 (±16.95)		16.40 (±3.24)		0.63 (±.01)		0.23 (±0.04)	
S. chlorostictus	41.57 (±20.29)	44.81 (±6.19)	15.82 (±4.19)		0.70 (±0.21)	0.68 (±0.01)	0.20 (±0.07)	
S. serranoides			15.25 (±1.25)				0.39 (±0.022)	
S. ovalis			11.11 (±1.07)				0.36 (±0.064)	
S. constellatus			9.93 (±0.84)				0.31 (±0.013)	
S. miniatus	43.94 (±13.82)	44.95 (±13.47)	14.04 (±5.82)		0.86 (±0.26)	0.89 (±0.23)	0.62 (±0.17)	
S. entomelas		45.27 (±11.10)	19.53 (±6.41)			0.77 (±0.15)	0.71 (±0.08)	
S. flavidus		38.23 (±9.13)	10.94 (±1.84)			0.91 (±0.17)	0.63 (±0.23)	

S. rufus, *S. pinniger*, *S. goodei*, *S. miniatus*, *S. entomelas* and *S. flavidus* exhibited significantly higher CS activity (see Fig. 2).

Enough specimens of *S. paucispinis*, *S. chlorostictus*, *S. rosenblatti*, *S. goodei*, *S. miniatus*, *S. entomelas*, and *S. flavidus* were caught in each season to compare enzymes across season and species (Tables 2 and 3). 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 expressed as units per gram protein showed precisely the opposite, with August and September values being highest and November and April being lowest. 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 (Table 3). The flip-flop in enzyme activity when expressed as a function of wet mass versus that when expressed as a function of protein clearly implied that protein levels in the muscle tissue were changing with season.

LDH enzyme values were plotted against CS values for each species (Fig. 2). The plots revealed three behavioral groups that corresponded well to observed activity patterns for the study species, active swimmers, opportunistic predators, and ambush predators.

4. Discussion

Enzyme assays have been used in both fish and invertebrates to determine species' locomotory habits, depth regimes, metabolism, and condition (Childress and Somero, 1979, 1990; Donnelly and Torres, 1988; Drazen and Seibel, 2007; Seibel, 2007; Seibel and Drazen, 2007; Siebenaller and Somero, 1982; Siebenaller and Yancey, 1984; Somero and Childress, 1980, 1985; Sullivan and Somero, 1980, 1983; Torres and Somero, 1988; Torres et al., 1979; Torres et al., 1994; Vetter and Lynn, 1997; Wilson et al., 1974; Yang and Somero, 1993; Yang et al., 1992). In the present study, enzyme activities were useful in elucidating locomotory habits and seasonal changes in condition of nineteen species of rockfish.

4.1. 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 were consistent with what one might expect given the oceanographic seasons of the California borderland. In the early spring to summer there is upwelling and increased production which cascades down the food web. During this period fish are eating and storing excess energy as lipid in a variety of energy depots including muscle decreasing their muscle water content. As the seasons change and upwelling decreases, primary production and zooplankton biomass also decrease (Dailey et al., 1993). This translates down the food web and the quality of available food decreases for most *Sebastes* species. The fish in turn become leaner and the percentage of their body mass that is composed of protein increases as lipid is combusted for fuel. 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 also be due to a reproductive drain on lipid levels.

Table 3
Mean enzyme activity expressed per gram protein for each species in each season with standard deviation in parentheses. An (*) was used to represent those species that only had one sample for that time period.

Species	LDH/Protein				MDH/Protein			
	Nov '04	April '05	Aug '05	Oct '05	Nov '04	April '05	Aug '05	Oct '05
S. paucispinis	699.78 (±141.04)	561.55 (±115.48)	1184.68 (±295.62)		234.43 (±55.15)	187.87 (±35.63)	485.09 (±147.56)	
S. levis				796.39 (±87.24)				318.53 (±25.54)
S. rufus			453.40 (±158.54)	1289.48 (±163.91)			276.26 (±118.91)	262.85 (±105.67)
S. pinniger			1229.91*	1475.14 (±718.87)			470.81*	729.91 (±380.78)
S. goodei		797.46 (±130.69)	1158.10 (±521.14)	335.62*		273.87 (±31.44)	593.43 (±73.29)	979.96*
S. rubrivinctus				465.50 (±76.89)				427.46 (±55.16)
S. rosenblatti	671.09 (±95.70)		1120.08 (±246.69)		213.59 (±35.58)		282.54 (±76.83)	
S. chlorostictus	653.20 (±254.03)	412.27 (±41.29)	834.03 (±360.07)		194.39 (±54.86)	165.39 (±16.43)	221.54 (±52.53)	
S. serranoides			970.98 (±191.20)				330.83 (±48.77)	
S. ovalis			1357.18 (±212.99)				425.11 (±57.14)	
S. constellatus			1073.76 (±157.25)				324.06 (±42.23)	
S. miniatus	562.95 (±104.86)	504.09 (±115.41)	2018.27 (±1250.65)		204.08 (±35.62)	192.78 (±31.65)	562.15 (±318.36)	
S. entomelas		654.64 (±106.87)	2090.87 (±692.19)			180.85 (±35.87)	495.09 (±104.83)	
S. flavidus		449.73 (±84.21)	1017.47 (±627.28)			187.56 (±22.43)	327.24 (±124.96)	

Species	PK/Protein				CS/Protein			
	Nov '04	April '05	Aug '05	Oct '05	Nov '04	April '05	Aug '05	Oct '05
S. paucispinis	400.83 (±118.51)	350.49 (±102.88)	474.78 (±140.91)		3.95 (±1.24)	3.51 (±0.76)	7.02 (±2.28)	
S. levis				865.13 (±65.72)				6.49 (±0.42)
S. rufus			293.11 (±54.30)	1149.50 (±506.52)			11.15 (±3.91)	10.93 (±1.97)
S. pinniger			491.30*	1312.94 (±550.68)			23.36*	20.61 (±6.16)
S. goodei		564.38 (±126.72)	844.24 (±130.80)	767.30*		5.66 (±0.93)	15.81 (±5.04)	13.96*
S. rubrivinctus				325.48 (±59.37)				8.95 (±1.11)
S. rosenblatti	313.23 (±107.39)		274.14 (±75.69)		4.19 (±0.23)		3.73 (±0.64)	
S. chlorostictus	275.80 (±126.89)	271.14 (±37.28)	235.05 (±62.08)		4.74 (±1.47)	4.11 (±0.07)	2.97 (±0.99)	
S. serranoides			244.55 (±29.00)				6.41 (±0.61)	
S. ovalis			185.76 (±15.17)				5.78 (±0.51)	
S. constellatus			209.73 (±42.23)				6.00 (±0.64)	
S. miniatus	289.87 (±84.25)	273.54 (±75.39)	350.11 (±281.91)		5.75 (±1.75)	5.46 (±1.31)	13.24 (±5.55)	
S. entomelas		304.27 (±74.48)	306.73 (±79.39)			5.15 (±0.83)	11.51 (±2.52)	
S. flavidus		240.80 (±43.15)	145.43 (±18.43)			5.79 (±1.13)	8.71 (±3.93)	

4.2. Enzyme assays

As in previous studies (i.e. Yang and Somero, 1993) LDH activity as a stand-alone value proved to be a good proxy for condition. The high values of LDH/gram protein suggested that the fish were in better condition (i.e. well fed) during the summer months. The lipid contents in *S. paucispinis* and *S. miniatus* confirmed this theory by exhibiting high lipid content in the summer and lower lipid content in the winter. Later in the year, due to growth, reproduction, or a less abundant food supply, their lipid reserves became depleted, causing them to become leaner and thus increasing their percent protein as a function of wet mass. LDH as a measure of anaerobic capacity showed that *S. entomelas* and *S. rufus* (two fish that are morphologically very similar) have very different enzyme activity profiles showing that enzyme activity can be a useful tool in identification.

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 oxaloacetic 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 activity is useful as an aerobic indicator and can also be 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 in Table 5 were generated. In Fig. 3 you can see that some species, such as *S. rosenblatti* and *S. chlorostictus* have a much lower CS activity than the other species. Those species have been observed to be more sedentary in habit, relying on burst swimming for fight or flight situations. Other species, such as *S. pinniger* and *S. goodei* have higher CS activity levels implying that they are a more active swimming species. Behavioral observations reported in Love et al. (2002) suggest that both *S. pinniger* and *S. goodei* school in groups as benthopelagic species, corroborating the results of the enzyme analyses.

Fig. 2 is a plot of LDH vs. CS activity for the species included in this study. The data broke naturally into three groups coinciding with reported diets of the species (Love et al., 2002). Group I showed both high LDH activity and high CS activity. All are highly active species that are found schooling in the upper water column; past diet studies have listed them as mainly piscivorous predators. Group II were opportunistic feeders with prey ranging from gelatinous organisms to benthic crustaceans. Group III had the lowest CS activity and it is characterized as mainly comprised of sit and wait, or ambush type predators, with very little continuous swimming activity but who are capable of large bursts of speed when catching their prey. For some species within each group where there was little information on diet. For those species we looked to the species that their enzyme profile most closely resembled and assumed a similar prey spectrum.

Table 4
Mean chemical composition data for each species in each season with standard deviation in parentheses. An (*) was used to represent species that only had one sample for that time period.

Species	P%AFDM				%water			
	Nov '04	April '05	Aug '05	Oct '05	Nov '04	April '05	Aug '05	Oct '05
S. paucispinis	74.80 (±6.58)	82.45 (±3.39)	26.78 (±3.54)		75.14 (±2.72)	77.32 (±1.35)	75.39 (±1.73)	
S. levis				30.41 (±1.13)				76.48 (±0.40)
S. rufus			30.69 (±4.78)	28.50 (±3.70)			78.20 (±2.84)	77.55 (±1.25)
S. pinniger			26.72*	34.02 (±2.68)			78.78*	78.52 (±1.29)
S. goodei		84.00 (±5.52)	28.12 (±2.21)	35.22*		76.50 (±2.04)	75.42 (±1.19)	74.85*
S. rubrivinctus				31.19 (±1.78)				77.92 (±0.61)
S. rosenblatti	73.22 (±6.67)		27.89 (±1.53)		77.62 (±1.14)		76.32 (±1.67)	
S. chlorostictus	72.64 (±6.38)	81.59 (±6.11)	31.52 (±1.82)		76.61 (±1.08)	77.50 (±1.56)	76.73 (±1.30)	
S. serranoides			30.41 (±1.83)				7.23 (±0.42)	
S. ovalis			29.08 (±5.72)				74.89 (±1.5)	
S. constellatus			28.17 (±2.78)				76.20 (±0.60)	
S. miniatus	74.35 (±5.21)	85.12 (±3.71)	28.63 (±14.23)		76.98 (±1.37)	78.56 (±1.21)	77.74 (±3.41)	
S. entomelas		82.25 (±5.95)	32.08 (±10.94)			79.01 (±2.18)	77.33 (±3.63)	
S. flavidus		84.15 (±6.13)	38.25 (±11.47)			77.97 (±2.34)	77.42 (±4.17)	

Species	P%WM				Ash%DM			
	Nov '04	April '05	Aug '05	Oct '05	Nov '04	April '05	Aug '05	Oct '05
S. paucispinis	17.07 (±1.33)	11.56 (±0.97)	11.86 (±0.71)		15.67 (±4.99)	16.51 (±2.47)	5.77 (±4.85)	
S. levis				7.17 (±0.31)				6.66 (±0.64)
S. rufus			9.85 (±0.52)	5.43 (±0.61)			5.96 (±3.81)	6.03 (±0.89)
S. pinniger			8.05*	7.03 (±0.66)			5.22*	6.79 (±3.05)
S. goodei		12.43 (±0.82)	7.50 (±0.54)	9.70*		17.19 (±4.61)	6.39 (±0.85)	8.00*
S. rubrivinctus				8.89 (±0.37)				6.27 (±1.11)
S. rosenblatti	9.24 (±0.68)		7.78 (±0.53)		15.03 (±2.12)		6.09 (±1.34)	
S. chlorostictus	13.12 (±0.08)	9.85 (±0.02)	8.14 (±0.34)		14.88 (±3.24)	16.52 (±0.42)	6.73 (±1.16)	
S. serranoides			7.23 (±0.42)				6.40 (±0.54)	
S. ovalis			7.77 (±0.89)				6.28 (±0.42)	
S. constellatus			9.83 (±0.55)				5.94 (±0.34)	
S. miniatus	12.95 (±1.02)	10.36 (±0.87)	8.69 (±2.10)		15.02 (±2.96)	16.34 (±1.56)	5.43 (±1.65)	
S. entomelas		13.29 (±0.83)	8.87 (±1.18)			14.87 (±4.57)	6.37 (±0.55)	
S. flavidus		11.36 (±0.91)	9.03 (±1.14)			15.77 (±1.27)	7.56 (±1.08)	

5. 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 rich lipid stores. Upwelling and reproduction are the most likely causes for the different physical conditions throughout the year, but such a drastic change over the course of a year was not anticipated. 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 relies on anaerobic burst responses whereas a high expression of aerobic enzymes implies that the animal is continuously active. Enzyme assays also proved to be a way to distinguish between two similar looking species. These

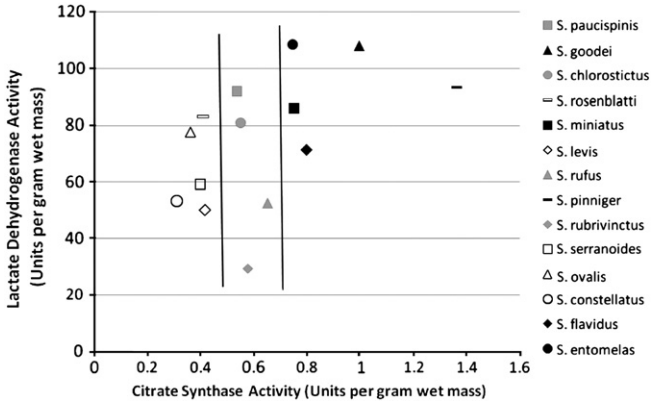

Fig. 2. Enzyme profiles with LDH activity on the *y*-axis and CS activity on the *x*-axis. This broke into three groups according to comparison with past diet analysis. Group I is represented in black and is primarily piscivorous, group II is represented in gray and represents opportunistic species, group III is represented in white with a black outline and represents ambush predators.

Table 5
Calculated oxygen consumption using CS activity and the equation generated by Yang and Somero (1993).

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

Species	Calculated VO_2
S. chlorostictus	9.20
S. constellatus	2.26
S. entomelas	14.93
S. flavidus	16.42
S. goodei	22.06
S. levis	5.33
S. miniatus	15.23
S. ovalis	3.74
S. paucispinis	8.83
S. pinniger	32.71
S. rosenblatti	5.09
S. rubrivinctus	9.99
S. rufus	12.14
S. serranoides	4.82

Table 6
T-test results for lipid data on *S. miniatus* in winter and summer (vw, vs) and *S. paucispinis* in winter and summer (bw, bs).

t-Test: two-sample assuming equal variances.

	vw	*vs*
Mean	13.8186	28.60562
Variance	268.928	136.6079
Observations	6	6
Pooled variance	202.7679	
Hypothesized mean difference	0	
df	10	
t Stat	−1.79863	
$P(T<=t)$ one-tail	0.05114	
t Critical one-tail	1.812461	
$P(T<=t)$ two-tail	0.10228	
t Critical two-tail	2.228139	
	bw	*bs*
Mean	15.37522	36.91852
Variance	121.7824	122.6902
Observations	6	7
Pooled variance	122.2775	
Hypothesized mean difference	0	
df	11	
t Stat	−3.5018	
$P(T<=t)$ one-tail	0.002477	
t Critical one-tail	1.795885	
$P(T<=t)$ two-tail	0.004955	
t Critical two-tail	2.200985	

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 (Table 6).

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