Digestive Enzyme Activities in Herbivorous and Carnivorous Prickleback Fishes (Teleostei: Stichaeidae): Ontogenetic, Dietary, and Phylogenetic Effects

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Accepted 3/10/04

ABSTRACT

We measured the activities of eight digestive enzymes in four species of herbivorous and carnivorous prickleback fishes and determined the effects of ontogeny, diet, and phylogeny on these enzyme activities. Of the four species, *Cebidichthys violaceus* and *Xiphister mucosus* shift to a more herbivorous diet as they grow (≥45 mm SL [standard length]), whereas *Xiphister atropurpureus* and *Anoplarchus purpurescens* remain carnivores throughout life. Digestive enzyme activities of small (30–40 mm SL) carnivorous juveniles were compared with those of larger (60–75 mm SL) wild-caught juveniles that had consumed a natural diet and larger (60–75 mm SL) juveniles raised on a high-protein animal diet. *Cebidichthys violaceus* and both species of *Xiphister* showed ontogenetic changes in digestive enzyme activities, whereas *A. purpurescens* did not. Despite dietary differences between *X. atropurpureus* and *X. mucosus*, these sister taxa displayed the most similar digestive enzyme activities from ontogenetic and dietary perspectives (high α-amylase and lipase and low trypsin and aminopeptidase activities), and both were more similar to *C. violaceus*, a member of the same largely herbivorous clade, than either was to *A. purpurescens*, a member of an adjacent, carnivorous clade. The results support the hypothesis that phylogeny influences digestive enzyme activities in these fishes. *Anoplarchus purpurescens*, a carnivore with a diverse diet, showed great plasticity in enzyme activity, especially trypsin and aminopeptidase, which were elevated in this species to the highest level among the four species after consuming the high-protein diet. These results support the hypothesis that fishes with relatively broad diets can modulate digestive enzyme activities in response to changes in dietary composition.

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Physiological and Biochemical Zoology 77(5):789–804. 2004. 1522-2152/2004/7705-3116$15.00

Introduction

Although the array of digestive enzymes in bony fishes is the same as that in other vertebrates (Stevens and Hume 1995; Hidalgo et al. 1999), fish digestive enzymes are less well-studied. The prevailing paradigm is that digestive enzyme activities in fishes are indicative of feeding ecology, correlating well with diet (Kapoor et al. 1975; Fernández et al. 2001). Animals are thought to be plastic in their digestive enzyme production in response to diet, because the metabolic expense of producing large amounts of digestive enzymes would be wasted by animals ingesting low levels of the substrates for those enzymes (Karasov 1992; Caviedes-Vidal et al. 2000). Accordingly, herbivorous fishes often exhibit higher carbohydrase activities, apparently to digest the storage carbohydrates of macroalgae, which can contain up to 50% carbohydrate (Horn et al. 1986), whereas carnivorous fishes frequently show higher proteolytic enzyme activities, to digest their high-protein animal diets (Fish 1960; Cockson and Bourne 1972; Reimer 1982; Sabapathy and Teo 1993; Cahu et al. 1998; Hidalgo et al. 1999). Digestive enzyme activities in fishes, however, vary among species and can be influenced by age as well as by the quantity and composition of diet (Peres et al. 1998). The influence of age and diet on digestive enzyme activities can best be compared in species that undergo ontogenetic shifts in diet, thus eliminating interspecific differences in digestive enzyme activities as a confounding variable. For example, studies of both marine (Moran and Clements 2002) and freshwater (Drewe et al. 2004) fishes that undergo ontogenetic dietary shifts found changes in digestive enzyme activities that reflect the dietary changes of the fishes. However, Kuz'mina (1996) found some differences in digestive enzyme activities with age in four unrelated species of fish, all carnivores throughout their lives. The question remains, however, whether fishes are genetically programmed to undergo ontogenetic shifts in digestive enzyme activities, or whether these activities are modulated in response to ingested items. Would the enzyme activities change if a fish were raised on a diet different from that consumed in nature?

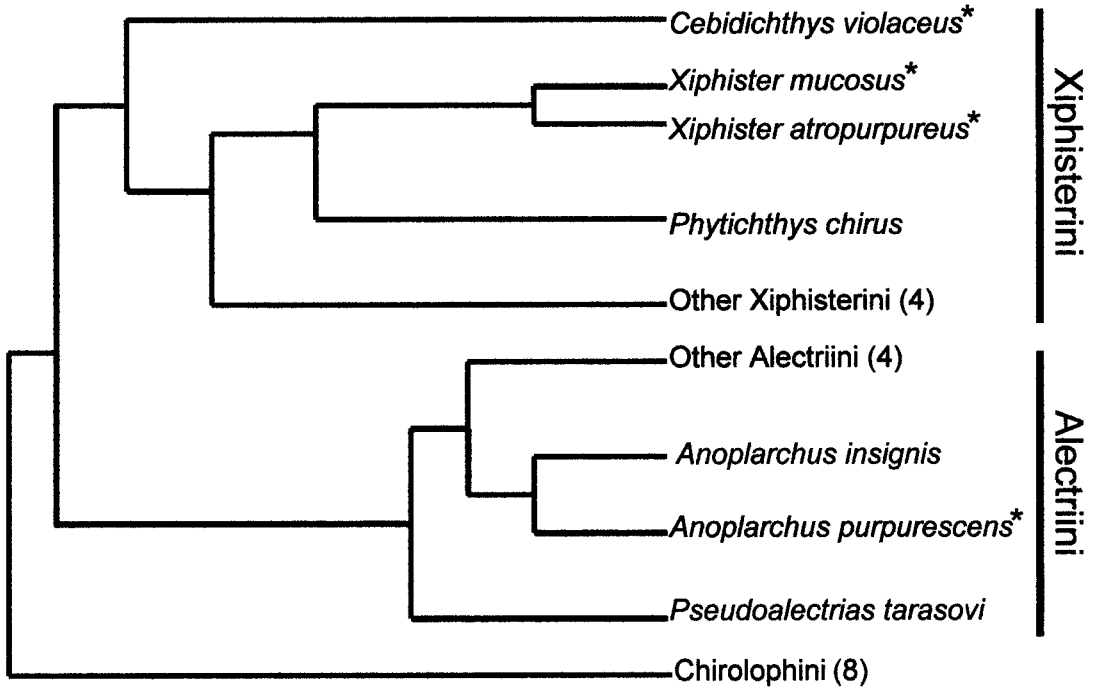

Figure 1. Partial phylogenetic hypothesis for two tribes in the subfamily Xiphisterinae, family Stichaeidae, based on analysis of 49 morphological characters (Stoddard 1985). We analyzed characters and assessed phylogenetic relationships with PHYSYS software (consistency index 58.197) and corroborated the relationships with PAUP software (K. M. Stoddard and M. H. Horn, unpublished data). Species studied are indicated by an asterisk, and numbers in parentheses next to groups indicate number of species not listed.

Although digestive enzyme activities in fishes appear to be strongly correlated with diet and perhaps change with ontogeny, little is known of the effects of phylogeny on digestive enzymes (Fernández et al. 2001). To understand the relationship of diet, digestive physiology, and an animal's evolutionary history, more than two related species need to be compared (Caviedes-Vidal et al. 2000). Digestive enzyme activities have been examined in at least two related species of fishes with different diets (Bitterlich 1985; Buddington et al. 1987; Chakrabarti et. al 1995; Hidalgo et. al 1999), but diet, not the phylogenetic relationships among the species, was the focus of these studies. Two recent studies using more than two related species offer contrasting results. Fernández et al. (2001) found that, among sparid fishes, a herbivore exhibited higher α-amylase activity than the carnivores examined. Conversely, Chan et al. (2004) found that the carnivorous stichaeid fish *Xiphister atropurpureus*, a member of an otherwise largely herbivorous clade (Stoddard 1985; K. M. Stoddard and M. H. Horn, unpublished data), exhibited higher α-amylase activity than *Cebidichthys violaceus*, a herbivore in the same clade. Chan and coworkers suggested that the former species may be phylogenetically constrained to show elevated α-amylase activity because of its relationship with herbivores in the clade. These examples leave open the question, What is more important in influencing digestive enzyme activities, diet or phylogenetic relationship?

To our knowledge, only two studies (Moran and Clements 2002; Drewe et al. 2004) have examined digestive enzyme activities at different postlarval stages in fish species that undergo an ontogenetic shift in diet; none has compared the digestive enzyme activities of such fishes with the same species raised on a controlled lab diet to observe whether changes in digestive enzyme activities occur with increase in size irrespective of diet. Previous investigators of digestive enzyme activities in cultured fishes grown on a formulated diet did not compare the data from these fishes with those from members of the same species on their natural diet (Kawai and Ikeda 1972; Reimer 1982; Lindner et al. 1995; García-Carreño et al. 2002). Also lacking are comparative studies that analyze digestive enzyme activities of more than two related fish species in a phylogenetic context.

In this study, we examined the effects of ontogeny, diet, and phylogeny on the activities of digestive enzymes in four species of prickleback fishes (family Stichaeidae), all of which occur in the same rocky intertidal habitat on the central California coast. Three of the species, *C. violaceus*, *Xiphister mucosus*, and *X. atropurpureus*, are members of the Xiphisterini, a largely herbivorous clade, whereas the fourth species, *Anoplarchus purpurescens*, is a member of the Alectriini, an adjacent carnivorous clade (Fig. 1; Stoddard 1985; K. M. Stoddard and M. H. Horn, unpublished data). Similar phylogenetic conclusions were reached for these pricklebacks by Yatsu (1986), although he

placed *C. violaceus* in a different subfamily with *Dictyosoma* (a western Pacific genus), which Stoddard (1985) placed in a derived position within the Xiphisterini. *Anoplarchus purpurescens* and *X. atropurpureus* are carnivores throughout life, whereas *C. violaceus* and *X. mucosus* begin life as carnivores but undergo an ontogenetic shift to herbivory and adopt an algal diet as they grow beyond 45 mm standard length (SL; Barton 1982; Horn et al. 1982; Setran and Behrens 1993). Because these fishes are closely related, live in the same habitat, and can be obtained at similar ontogenetic stages, they represent ideal species for comparative investigations of digestive enzyme activities in ontogenetic, dietary, and phylogenetic contexts.

To investigate the effects of ontogeny, diet, and phylogeny on digestive enzyme activities, we compared activities in three categories of the four target species: (1) small wild-caught carnivorous juveniles (30–40 mm SL; abbreviated as $w_{30–40}$), (2) larger wild-caught carnivorous or herbivorous juveniles (60–75 mm SL; abbreviated as $W_{60–75}$), and (3) larger laboratory-fed juveniles (abbreviated as $L_{60–75}$), representing a subset of the $w_{30–40}$ juveniles grown on a high-protein animal diet until they reached 60–75 mm SL, well past the size (45 mm SL) at which *C. violaceus* and *X. mucosus* shift to herbivory in nature. Comparisons of activities between $w_{30–40}$ and $W_{60–75}$ fish allowed us to test for an ontogenetic effect, those between $W_{60–75}$ and $L_{60–75}$ fish for a dietary effect, and those among species within categories for a phylogenetic effect. This design thus allowed us to distinguish the effects of ontogeny from the effects of diet on digestive enzyme activities within species and to detect any phylogenetic influences among species on these activities.

We chose to measure the activities of the following eight digestive enzymes that act on proteins, carbohydrates, or lipids in the gut lumen or at the brush border of the intestinal epithelium: the proteases pepsin, trypsin, and aminopeptidase; the carbohydrases α-amylase, maltase, and isomaltase; lipase; and alkaline phosphatase. We hypothesized the following outcomes relating to ontogeny, diet, and phylogeny: (1) Both *C. violaceus* and *X. mucosus* will increase their carbohydrase activities and decrease their protease, lipase, and alkaline phosphatase activities with increase in size as they shift to a more herbivorous diet; (2) $L_{60–75}$ fish of *C. violaceus* and *X. mucosus* will show the same enzyme activities as $W_{60–75}$ fish, suggesting a biochemical specialization of the gut toward preferential digestion of an macroalgal diet in these two species; (3) *X. atropurpureus* will exhibit the same profile of digestive enzyme activities expected for *X. mucosus*, despite their differences in diet, because of their close phylogenetic relationship; and (4) the three members of the Xiphisterini will display digestive enzyme activity profiles more similar to each other's, and different from those of *A. purpurescens*, which will exhibit high protease, lipase, and alkaline phosphatase activities and low carbohydrase activities in all categories because of its carnivorous diet.

Material and Methods

Fish Collection and Maintenance and Feeding Experiment

Juveniles of each of the four prickleback species were collected by hand and dip net from August 2000 to June 2001 at low tide from rocky intertidal habitat on the central California coast near Piedras Blancas (35°40′N, 121°17′W) and, for *Xiphister mucosus*, also from Diablo Canyon (Stillwater Cove, 35°12′N, 120°51′W). Ten small juveniles ($w_{30–40}$, 30–40 mm SL) of each species were killed with MS-222 (1 g L^{-1} seawater) and frozen on dry ice within 3 h of capture and used for gut content analysis. An additional 30 small juveniles of each species were transported, as in our previous studies of prickleback fishes (e.g., Fris and Horn 1993), live out of water to the laboratory at California State University, Fullerton, in 48-L coolers containing a small amount of the brown alga *Silvetia compressa* to provide cover and dampness. Bags of ice were suspended in the coolers to maintain a cool temperature.

Upon arrival, all fish were placed into 235-L tanks containing filtered, UV-sterilized, and circulating seawater held at 15°C, within the upper part of the temperature range (9°–17°C) of the central California nearshore habitat (Horn et al. 1983). Twenty individuals of each species were randomly selected for feeding a high-protein animal diet (see below) and placed individually into numbered 4.1-L compartments with nylon screen walls submerged inside 235-L tanks (10 compartments/tank) under a 12L : 12D cycle as per Fris and Horn (1993). The remaining 10 fish of each species were used for enzyme assays (see "Assays of Digestive Enzyme Activity"). All fish were held without food for 1–2 d to ensure hunger at the onset of the feeding experiment. The lighting over the tanks was not uniformly positioned, so the compartments were rotated within the tanks, and a different numbered compartment, with its fish, was rotated among tanks on a daily basis to eliminate potential "tank effects." All fish were fed the same commercial diet designed for marine fish (BioKyowa, Cape Girardeau, Mo.; 55% protein, 10% lipid, and <4% carbohydrate) three to four times a day to satiation until they reached 65–75 mm SL (*Xiphister atropurpureus*, *X. mucosus*, and *Cebidichthys violaceus*) or 60–70 mm SL (*Anoplarchus purpurescens*), sizes well beyond those at which *C. violaceus* and *X. mucosus* shift to herbivory in nature (Horn et al. 1982). Upon reaching the desired size ($L_{60–75}$), 10 individuals of each species were killed with MS-222 and used for analysis of digestive enzyme activity. Twenty juveniles of each of the four fish species that had reached this size range ($W_{60–75}$) in nature were captured between December 2000 and November 2001 to represent fish consuming a natural diet and showing the corresponding digestive enzyme activity. Ten $W_{60–75}$ fish were killed within 3 h of capture for gut content analysis to confirm that their diets were the same as those reported in the literature. The remaining 10 fish of each species were transported as described for $w_{30–40}$ fish and used for assays of digestive enzyme activity. All fish used in this study were juveniles,

Table 1: Michaelis-Menten constants (K_m) for trypsin, α-amylase, and lipase in large wild-caught ($W_{60–75}$) juveniles of four prickleback fishes

Species	Trypsin (mM BAPNA)	α-amylase (% Starch)	Lipase (mM p-NPM)
Cebidichthys violaceus	.44 ± .24[ab]	.36 ± .13[a]	.22 ± .12[ab]
Xiphister mucosus	.61 ± .37[ab]	.33 ± .15[a]	.23 ± .08[b]
Xiphister atropurpureus	.84 ± .15[b]	.29 ± .12[a]	.17 ± .09[ab]
Anoplarchus purpurescens	.26 ± .15[a]	.26 ± .10[a]	.06 ± .04[a]

Note. K_m values (mean ± SEM, $n = 5$) determined by nonlinear regression. Data were analyzed with ANOVA followed by Tukey's HSD with a family error rate of $P = 0.05$. Values sharing a letter are not significantly different. BAPNA = α-N-benzoyl-L-arginine-p-nitroanilide; p-NPM = p-nitrophenyl-myristate.

as no swollen testes or ovaries were observed in any dissected specimen. These observations were consistent with previous studies showing that *C. violaceus* reaches sexual maturity at ~350 mm SL (Marshall and Echeverria 1992), *X. mucosus* at 270–290 mm SL, *X. atropurpureus* at 110–115 mm SL (Wingert 1974), and *A. purpurescens* at 85–110 mm SL (Coleman 1992). All handling of fish from capture to killing was conducted under approved protocol 99-R-10 of the Institutional Animal Care and Use Committee at California State University, Fullerton.

Fish Dissection, Measurements, and Gut Content Analysis

Once killed with MS-222, all fish were weighed (BM ± 0.001 g), measured (SL ± 1 mm), and dissected on a chilled cutting board (~4°C). Gut contents were pushed out and gut mass (GM ± 0.001 g) then measured. The gut contents from the $w_{30–40}$ and $W_{60–75}$ fish ($n = 10$) specified for dietary analysis were suspended uniformly in water and analyzed under a dissecting microscope equipped with a net reticle (10 × 10 μm) according to a point-contact method similar to that of Jones (1968), as described by Smith (2002). If a gut item occupied an intersection of two reticle lines, it was counted as a contact. Contacts were totaled for gut content categories, and the percentage of each item was determined for each individual fish; the results were then totaled and expressed as a mean percentage for a species and category ($w_{30–40}$ or $W_{60–75}$). Data were summarized as proportions of animal and macroalgal material to provide a broad interspecific comparison of diet composition.

Assays of Digestive Enzyme Activity

Whole guts from 10 fish from each category were homogenized individually in ice-cold 50 mM tris-HCl buffer, pH 7.4 (30 volumes [v/w] for $W_{60–75}$ and $L_{60–75}$ fish; 60 volumes for $w_{30–40}$ fish), with a Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.) with a 7 mm generator at a setting of 3.5 for 3 × 30 s. The homogenates were then centrifuged at 9,300 *g* for 2 min at 4°C and the supernatants collected and stored in small aliquots (100–200 μL) at −80°C until just before use in spectrophotometric assays of the activities of the eight digestive enzymes.

All assays were carried out at 15°C in duplicate for $w_{30–40}$ and triplicate for $W_{60–75}$ and $L_{60–75}$ fish with the SPECTRAmax 190 microplate spectrophotometer (Molecular Devices, Sunnyvale, Calif.) and Falcon flat-bottom 96-well microplates (Fisher Scientific, Tustin, Calif.), except that for pepsin, which required a quartz microplate because of the acidic conditions of the assay and the UV wavelength at which this assay is read. All pH values listed for buffers were measured at room temperature (22°C), and all reagents were purchased from Sigma-Aldrich Chemical (St. Louis) unless specified otherwise. Each reaction was read against a blank appropriate for each assay, and all reactions were run at saturating substrate concentrations as determined for each enzyme with gut tissues from the four target species. Preliminary assays were performed on the homogenates of $W_{60–75}$ fish to determine the Michaelis-Menten constants (K_m) for trypsin, α-amylase, and lipase and to examine any possible differences among the species (Table 1). The K_m for trypsin was determined with Nα-benzoyl-L-arginine-p-nitroanilide hydrochloride (BAPNA) at concentrations ranging from 0.2 to 5.25 mM, for α-amylase with starch concentrations ranging from 0.25% to 3.75%, and for lipase with p-nitrophenol myristate at concentrations ranging from 0.05 to 1 mM. We determined K_m values with nonlinear regression using Kaleidograph (Synergy, Reading, Pa.).

Pepsin (EC 3.4.23.1) activity was measured according to Anson (1938); 100 μL of 2% hemoglobin in 60 mM HCl (pH 2) was incubated with 25 μL of homogenate in a microcentrifuge tube for 30 min. The reaction was stopped by adding 200 μL of 5% trichloroacetic acid, and the reaction mixture was centrifuged at 4,200 *g* at 4°C for 6 min. Because this procedure measures the amount of L-tyrosine released in the assay, the absorbance at 280 nm was read in a spectrophotometer in a quartz microplate, and pepsin activity was determined with an L-tyrosine standard curve. Pepsin activity was expressed in U (1 μmol of L-tyrosine liberated per minute) per gram wet weight of gut tissue.

Trypsin (EC 3.4.21.4) activity was measured with a modified

version of the Preiser et al. (1975) method. Because trypsin is secreted in the pancreas as a zymogen (Vonk and Western 1984), it must be activated before analysis. In a microcentrifuge tube, 15 μL of enterokinase (4 U mL^{-1} in 40 mM succinate buffer, pH 5.6) was combined with 100 μL of homogenate and incubated for 15 min at room temperature. The substrate (BAPNA) was dissolved in 100 mM tris-HCl buffer (pH 8.0) by heating to 95°C. After enterokinase activation, 25 μL of the activated homogenate was combined with 175 μL of 2 mM BAPNA in a microcentrifuge tube and incubated for 1 h at 15°C. The reaction was stopped by adding 200 μL of 0.2 M HCl. This step was followed by the Bratton-Marshall reaction, which entails the subsequent addition of 50 μL each of 0.1% sodium nitrite, 0.5% ammonium sulfamate, and 0.05% N-1-naphthylethylenediamine HCl at 3-min intervals. The absorbance was read at 550 nm, and activity was determined with a p-nitroaniline standard curve constructed with the Bratton-Marshall reagents. Trypsin activity was expressed in U (1 μmol p-nitroaniline liberated per minute) per gram wet weight of gut tissue.

Aminopeptidase (EC 3.4.11.2) activity was measured according to Roncari and Zuber (1969). In a microplate, 75 μL of 2.04 mM L-alanine-p-nitroanilide HCl dissolved in 200 mM sodium phosphate buffer (pH 7.0) was combined with 25 μL of homogenate. The increase in absorbance was read continuously at 410 nm for 15 min and activity determined with a p-nitroaniline standard curve. Aminopeptidase activity was expressed in U (1 μmol p-nitroaniline liberated per minute) per gram wet weight of gut tissue.

The α-amylase (EC 3.2.1.1) activity was measured according to the Somogyi-Nelson method (Nelson 1944; Somogyi 1952), as described by Gawlicka et al. (2000). Starch substrate was prepared by boiling 1% soluble starch in 0.8 M sodium citrate buffer (pH 7) for 5 min. In a microcentrifuge tube, 50 μL of the starch solution was combined with 25 μL of sodium citrate buffer and 25 μL of homogenate. The incubation was stopped after 2 h by adding 20 μL of 1 M NaOH and 200 μL of Somogyi-Nelson reagent A (0.2 M Na_2HPO_4, 0.1 M $C_4H_4KNaO_6 \cdot 4H_2O$, 0.1 M NaOH, 0.03 M $CuSO_4 \cdot 5H_2O$ [Fisher AC423610030], and 1.27 M Na_2SO_4 dissolved in nanopure H_2O). Somogyi-Nelson reagent B (0.04 M $H_{24}Mo_7N_6O_{24} \cdot 4H_2O$, 0.5 M H_2S, and 0.02 M $Na_2HAsO_4 \cdot 7H_2O$ dissolved in nanopure H_2O) was added after the assay solution was boiled for 10 min. The resulting solution was diluted in water and centrifuged at 6,000 *g* for 5 min. The glucose content of the solution was then determined spectrophotometrically at 650 nm. The α-amylase activity was determined from a glucose standard curve and expressed in U (1 μmol glucose liberated per minute) per gram wet weight of gut tissue.

Maltase (EC 3.2.1.20) and isomaltase (EC 3.2.1.10) activities were measured according to Dahlqvist (1968). In a microcentrifuge tube, 20 or 25 μL (maltase or isomaltase assay, respectively) of homogenate were incubated with 10 μL of 56 mM maltose or isomaltose solutions in 100 mM maleate buffer, pH 6.0. The reactions were stopped after 1 h by adding 300 μL of assay reagent (Sigma GAGO20) dissolved in 0.5 M tris-HCl (pH 7.0). The reaction mixture was then incubated at 37°C for 1 h to let color develop, and its absorbance was read at 450 nm. Maltase and isomaltase activities were determined with a glucose standard curve and expressed in U (1 μmol glucose liberated per minute) per gram wet weight of gut tissue.

Lipase (nonspecific bile salt–activated, EC 3.1.1.-) activity was measured according to Iijima et al. (1998). In a microcentrifuge tube, 287 μL of 5.2 mM sodium cholate dissolved in 250 mM tris-HCl (pH 9) was combined with 20 μL homogenate and 8 μL of 10 mM 2-methoxyethanol and incubated at room temperature for 15 min to allow for lipase activation by bile salts. The substrate p-nitrophenyl myristate (18 μL of 10 mM p-nitrophenyl myristate dissolved in 100% ethanol) was then added and the assay mixture incubated at 15°C for 2 h, after which time the reaction was stopped with 467 μL of acetone/heptane (5 : 2, v : v). The samples were then centrifuged at 6,100 *g* for 2 min. The absorbance of the resulting lower aqueous layer was read at 405 nm, and activity was determined with a p-nitrophenol standard curve. Lipase activity was expressed in U (1 μmol p-nitrophenol liberated per minute) per gram wet weight of gut tissue.

Alkaline phosphatase (EC 3.1.3.1) activity was measured according to the method of Walter and Schütt (1974), as described by Gawlicka et al. (2000). In a microplate, 55 μL of 100 mM ammonium bicarbonate buffer with 1 mM $MgCl_2$ (pH 7.8) was combined with 20 μL of 20 mM p-nitrophenyl phosphate (dissolved in 100 mM ammonium bicarbonate buffer, pH 7.8, with 1 mM $MgCl_2$) and 25 μL of homogenate. The increase in absorbance was read continuously at 405 nm for 15 min, and activity was determined with a p-nitrophenol standard curve. Alkaline phosphatase activity was expressed in U (1 μmol p-nitrophenol liberated per minute) per gram wet weight of gut tissue.

Statistical Analysis

The significance of ontogenetic, dietary, and phylogenetic effects was tested for (1) the activity of each enzyme and (2) the total activity [{[U (g gut tissue)$^{-1}$] × g GM} × (g BM)$^{-1}$] for all eight enzymes combined in each of the four species. We compared the activity of each enzyme between $W_{30–40}$ and $W_{60–75}$ fish to test for an ontogenetic effect and that between $W_{60–75}$ and $L_{60–75}$ fish to test for a dietary effect, using a two-tailed *t*-test with the significance level set at $P \leq 0.05$. Interspecific comparisons of enzyme activity were made within each feeding category to test for a phylogenetic effect with one-way ANOVA (Minitab, ver. 13, State College, Pa.) followed by a Tukey's HSD multiple-comparisons test with a family error rate set at $P = 0.05$. If the data for a particular enzyme did not

pass Levene's test for equal variances and did not display normality (as examined with residual vs. fits plots), the data were log-transformed before analysis. The isomaltase data did not pass Levene's test and did not display normality even after being log-transformed. The ANOVA output for isomaltase, therefore, was compared with a Kruskal-Wallis rank-mean test followed by a nonparametric multiple-comparisons test similar to Tukey's HSD (Zar 1999, eq. [12.23]).

We analyzed the data for total activity of all enzymes combined per species, using nonmetric multidimensional scaling (MDS) to display graphically the overall patterns of digestive enzyme activity for ontogenetic ($w_{30–40}$ vs. $W_{60–75}$) and dietary ($W_{60–75}$ vs. $L_{60–75}$) effects. Multivariate analysis of similarity (ANOSIM) was used to test statistically for ontogenetic ($w_{30–40}$ vs. $W_{60–75}$), dietary ($W_{60–75}$ vs. $L_{60–75}$), and phylogenetic (alectriine vs. xiphisterine clades; *X. atropurpureus* vs. *X. mucosus*) effects on total activity of all enzymes combined. The outputs from ANOSIM, called *R* statistics, are based on the differences in mean ranks between and within groups (Clarke 1993). *R* values range from 0 (no difference) to 1 (very different). Statistical significance is examined by permutations (usually >1,000) of the grouping vector and the distribution under the null model; differences are considered significant at $P \leq 0.05$. Because of the sheer number of comparisons in this study, and because all ANOSIM comparisons were significant, ANOSIM outputs, where appropriate, were condensed and reported as a summary of all tests performed for a particular hypothesis (e.g., $R \geq 0.40$, $P < 0.01$ represents four different within-species comparisons, all of which had *R* values of at least 0.40 and *P* values less than 0.01). The data for both the MDS and ANOSIM comparisons were fourth-root–transformed to balance the differences in activity among enzymes. We performed both analyses using PRIMER (ver. 5, Plymouth, U. K.).

Results

Ontogenetic Effects

In this section we report ontogenetic comparisons ($w_{30–40}$ vs. $W_{60–75}$) of gut contents (Fig. 2) and the activity of each of the digestive enzymes for each of the four species (Table 2). In addition, we present the total activities of all enzymes combined in the $w_{30–40}$ and $W_{60–75}$ feeding categories of the four species (Fig. 3).

The $w_{30–40}$ fish of all four species of pricklebacks were carnivorous, feeding on a variety of small invertebrates (for details of gut contents, see German 2003). The predicted shift in diet was clear for *Cebidichthys violaceus* and *Xiphister mucosus*, as macroalgae comprised, respectively, 0.0% and 2.2% of the gut contents in $w_{30–40}$ fish, but 70.9% and 53.7% in $W_{60–75}$ fish. The $W_{60–75}$ fish of *Xiphister atropurpureus* and *Anoplarchus purpurescens* remained carnivorous, as animal material comprised 90.3% and 89.1%, respectively, of their gut contents.

Cebidichthys violaceus significantly decreased its pepsin activity with increase in size, whereas the three other species did not show significant ontogenetic differences in pepsin activity. No ontogenetic change in trypsin activity was observed in any of the prickleback species. *Xiphister mucosus* significantly increased its aminopeptidase activity with increase in size, whereas the three other species showed no ontogenetic change. *Cebidichthys violaceus*, *X. mucosus*, and *X. atropurpureus* all exhibited significant increases in α-amylase activity with increase in size, whereas *A. purpurescens* did not. Both *C. violaceus* and *X. atropurpureus* exhibited ontogenetic increases in maltase activity, whereas *A. purpurescens* showed a significant decrease, and *X. mucosus* showed no change. *Cebidichthys violaceus*, *X. mucosus*, and *X. atropurpureus* all showed significant increases in isomaltase activity with increase in size, whereas *A. purpurescens* did not. *Cebidichthys violaceus*, *X. mucosus*, and *X. atropurpureus* all significantly increased their lipase activity with increase in size, whereas *A. purpurescens* did not. Both *C. violaceus* and *X. mucosus* significantly decreased their alkaline phosphatase activity with increase in size, whereas *X. atropurpureus* and *A. purpurescens* showed no change.

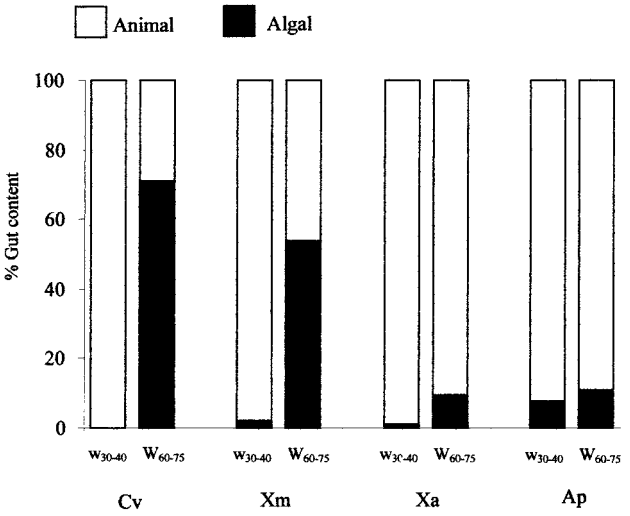

Figure 2. Percentage of animal and algal material in gut contents of two wild-caught size categories ($w_{30–40}$ and $W_{60–75}$) of *Cebidichthys violaceus* (*Cv*), *Xiphister mucosus* (*Xm*), *Xiphister atropurpureus* (*Xa*), and *Anoplarchus purpurescens* (*Ap*); $n = 10$ fish for each category.

Ontogenetic differences in the total activities of the eight digestive enzyme combined were revealed for all four prickleback species (ANOSIM: $R \geq 0.20$, $P < 0.01$). As depicted in the MDS plot, the enzymatic patterns in *A. purpurescens* differed markedly from those in *C. violaceus*, *X. mucosus*, and *X. atropurpureus* (Fig. 3). Little ontogenetic change was apparent in *A. purpurescens* compared with the three members of the Xiphisterini, in which the combined enzyme pattern changed considerably with increase in size. The digestive enzyme patterns of $w_{30–40}$ fish of *C. violaceus* were similar to those of $W_{60–75}$ fish of *X. mucosus* and *X. atropurpureus* but shifted away in $W_{60–75}$

Table 2: Within-species comparisons (two-tailed *t*-tests) of the activity of each digestive enzyme from the ontogenetic and dietary perspectives in four prickleback fishes

Species	Pepsin	Trypsin	Amino-peptidase	α-amylase	Maltase	Isomaltase	Lipase	Alkaline Phosphatase
Ontogenetic comparison ($w_{30–40}$ vs. $W_{60–75}$):								
Cebidichthys violaceus:								
t	2.18	−.98	−1.2	−2.54	−2.40	−3.17	−4.99	−5.24
P	.04	.34	.24	.02	.03	<.01	<.01	<.01
Xiphister mucosus:								
t	.79	−.65	−3.46	−6.00	.19	−2.25	−4.68	4.61
P	.44	.52	<.01	<.01	.85	.04	<.01	<.01
Xiphister atropurpureus:								
t	1.20	1.13	−1.91	−14.48	−2.18	−2.70	−5.12	.98
P	.24	.27	.07	<.01	.04	.02	<.01	.34
Anoplarchus purpurescens:								
t	−1.90	.98	−1.65	−1.82	2.11	1.66	.57	1.65
P	.07	.34	.12	.09	.05	.12	.58	.12
Dietary comparison ($W_{60–75}$ vs. $L_{60–75}$):								
C. violaceus:								
t	−5.45	.15	−2.91	−1.72	−3.50	−2.06	−.29	−6.53
P	<.01	.88	<.01	.10	<.01	.05	.78	<.01
X. mucosus:								
t	−2.80	−5.80	1.42	−1.42	−3.05	−1.21	−2.47	−2.31
P	.01	<.01	.17	.17	<.01	.24	.02	.03
X. atropurpureus:								
t	.06	−2.39	−2.25	.30	−5.37	−7.90	−.87	−6.28
P	.96	.03	.04	.77	<.01	<.01	.39	<.01
A. purpurescens:								
t	2.17	−2.92	−3.11	−3.97	−5.14	−2.96	−1.08	−7.47
P	.04	<.01	<.01	<.01	<.01	<.01	.29	<.01

Note. $n = 10$ fish for each category; df = 18 for all comparisons. See Table 3 for enzyme activity values.

fish, reflecting an apparent difference between *C. violaceus* and the two *Xiphister* species in total activity of the digestive enzymes combined for larger, wild-caught juveniles.

Dietary Effects

In this section we report the effects of a high-protein diet ($W_{60–75}$ vs. $L_{60–75}$ fish) on activities of each of the eight digestive enzymes in each of the four species (Table 2). In addition, we present the total activities of all enzymes combined in the $W_{60–75}$ and $L_{60–75}$ feeding categories of the four species (Fig. 4).

Both *C. violaceus* and *X. mucosus* increased their pepsin activities after eating the high-protein diet, whereas *X. atropurpureus* showed no change, and *A. purpurescens* decreased its pepsin activities. *Xiphister mucosus*, *X. atropurpureus*, and *A. purpurescens* all increased their trypsin activities on the high-protein diet, whereas *C. violaceus* did not. All fishes exhibited dietary effects in aminopeptidase activities, with *C. violaceus*, *X. atropurpureus*, and *A. purpurescens* increasing activities of this enzyme after eating the high-protein diet but *X. mucosus* showing a decrease. Only *A. purpurescens* exhibited a significant dietary effect in α-amylase activity, increasing its activity after eating the high-protein diet. All four species showed significant increases in maltase activity after consuming the high-protein diet. *Cebidichthys violaceus*, *X. atropurpureus*, and *A. purpurescens* all showed significant dietary effects for isomaltase, increasing their activities after eating the high-protein diet, whereas *X. mucosus* did not. All four species slightly increased their lipase activities after eating the high-protein animal diet, but the increase in activity was significant only for *X. mucosus*. All four species also increased their alkaline phosphatase activities after consuming the high-protein diet, but the magnitude of increase was about three times greater for *X. atropurpureus* and *A. purpurescens*, compared to less than two times for the other two species.

Significant effects of the high-protein diet on the total activity of all eight digestive enzymes combined were exhibited by all four prickleback species (ANOSIM: $R > 0.40$, $P < 0.01$). As depicted in the MDS plot, however, the magnitude of the dietary

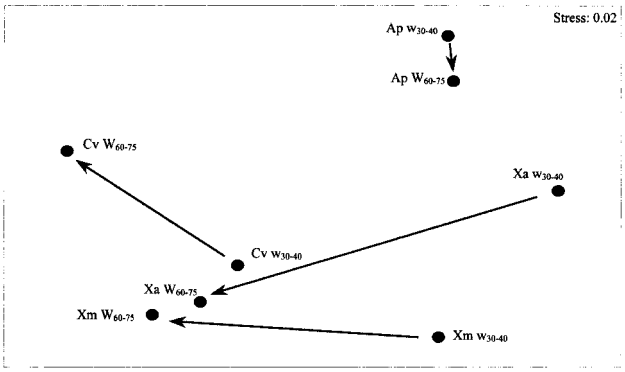

Figure 3. Nonmetric multidimensional scaling plot of the total activities of eight digestive enzymes combined for each species as a function of ontogeny ($w_{30–40}$ and $W_{60–75}$ categories) in *Cebidichthys violaceus* (*Cv*), *Xiphister mucosus* (*Xm*), *Xiphister atropurpureus* (*Xa*), and *Anoplarchus purpurescens* (*Ap*). Arrows indicate magnitude of ontogenetic shifts in digestive enzyme activity. The stress value indicates that the plot fits well (i.e., values of <0.1) into two-dimensional space. See "Material and Methods" for further details.

effects on enzyme activity was greater in *A. purpurescens* than in the three xiphisterine species (Fig. 4).

Phylogenetic Effects

In this section we report comparisons in activities of each of the eight digestive enzymes among the four species in each of the three feeding categories ($w_{30–40}$, $W_{60–75}$, and $L_{60–75}$; Table 3). In addition, we use the MDS plots from the ontogenetic (Fig. 3) and dietary (Fig. 4) perspectives described above to compare species trajectories on the plots and to emphasize the phylogenetic relationships among the four species.

$w_{30–40}$ Fish. Xiphister atropurpureus in this feeding category exhibited significantly higher pepsin activity, 1.5–2 times that of the three other species, which did not differ from each other in activity of this enzyme. Trypsin activity was significantly highest in *A. purpurescens*, about two to three times that in *C. violaceus* and *X. atropurpureus*, which did not differ from each other, and about seven times that in *X. mucosus*, which had the significantly lowest activity for this enzyme. *Anoplarchus purpurescens* showed significantly higher aminopeptidase activity, about two times that of the three other species, which did not differ from each other in activity of this enzyme. Although variable, α-amylase activity in *C. violaceus* was significantly higher than that in both *X. atropurpureus* and *A. purpurescens* but not that in *X. mucosus*, and these three species did not differ in activity of this enzyme. Neither maltase nor isomaltase activity differed significantly among the four species. Lipase activity was indistinguishable in *C. violaceus* and *A. purpurescens* but significantly higher (about two to three times) in these two species than in *X. atropurpureus* and *X. mucosus*, which did not differ from each other in activity of this enzyme. Alkaline phosphatase activity was statistically indistinguishable in *X. mucosus* and *A. purpurescens* but significantly higher in these two species than in either *C. violaceus* or *X. atropurpureus*, which did not differ from each other in activity of this enzyme.

$W_{60–75}$ Fish. Xiphister atropurpureus and *A. purpurescens* in this feeding category displayed significantly higher pepsin activity than *X. mucosus*, which, in turn, displayed significantly higher activity than *C. violaceus* for this enzyme. The highest trypsin activity was recorded for *A. purpurescens*, but it was not significantly greater than that in *C. violaceus*, whose trypsin activity was statistically higher than that in *X. mucosus* but not that in *X. atropurpureus*; the two species of *Xiphister* were not significantly different from each other in activity of this enzyme. As in $w_{30–40}$ fish, *A. purpurescens* in this larger wild-caught category exhibited significantly higher aminopeptidase activity than the three other species, which did not differ from each other. The α-amylase activity was statistically indistinguishable among *X. mucosus*, *X. atropurpureus*, and *C. violaceus*, but the activities displayed in these three species was 12–20 times higher than that in *A. purpurescens*. The highest mean maltase activity was shown by *C. violaceus*, but the value was not significantly different from that of *X. atropurpureus*; both of these species had higher activities of this enzyme than either *X. mucosus* or *A. purpurescens*, which did not differ from each other. *Cebidichthys violaceus* showed the significantly highest isomaltase activity, two to four times greater than that of the three other species, whereas the two species of *Xiphister* were statistically indistinguishable, and only *X. atropurpureus* was significantly higher in activity of this enzyme than *A. purpurescens*. *Cebidichthys violaceus* exhibited significantly higher lipase activity, about two times greater than that of the three other species, which did not differ from each other. In contrast, *C. violaceus* showed

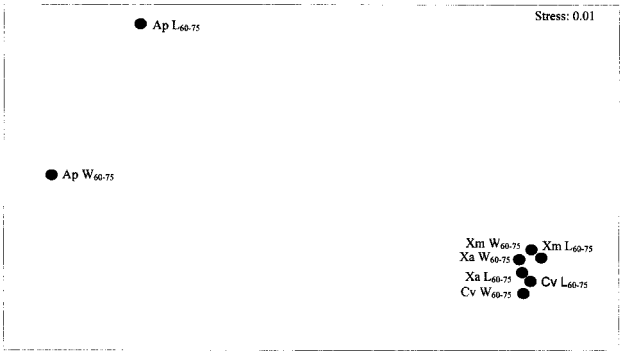

Figure 4. Nonmetric multidimensional scaling plot of the total activities of eight digestive enzymes combined for each species as a function of diet ($W_{60–75}$ and $L_{60–75}$ categories) in *Cebidichthys violaceus* (*Cv*), *Xiphister mucosus* (*Xm*), *Xiphister atropurpureus* (*Xa*), and *Anoplarchus purpurescens* (*Ap*). The stress value indicates that the plot fits well (i.e., values of <0.1) into two-dimensional space.

Table 3: Among-species comparisons of digestive enzyme activities from the phylogenetic perspective in three feeding categories of four prickleback fishes (U g gut tissue^{-1})

Species	Pepsin	Trypsin	Amino-peptidase	α-amylase	Maltase	Isomaltase	Lipase	Alkaline Phosphatase
Small wild-caught ($w_{30–40}$):								
Cebidichthys violaceus	9.41 ± 1.49[a]	.17 ± .02[b]	.29 ± .03[a]	2.71 ± .60[b]	.39 ± .03[a]	.25 ± .03[a]	.32 ± .04[b]	.24 ± .02[a]
Xiphister mucosus	9.70 ± 1.68[a]	.06 ± .01[a]	.22 ± .05[a]	.93 ± .23[ab]	.28 ± .04[a]	.15 ± .03[a]	.12 ± .03[a]	.60 ± .05[b]
Xiphister atropurpureus	16.02 ± 1.85[b]	.13 ± .02[b]	.27 ± .04[a]	.28 ± .04[a]	.30 ± .07[a]	.18 ± .03[a]	.09 ± .02[a]	.27 ± .06[a]
Anoplarchus purpurescens	8.51 ± 1.82[a]	.43 ± .06[c]	.56 ± .04[b]	.18 ± .04[a]	.38 ± .03[a]	.21 ± .03[a]	.29 ± .04[b]	.41 ± .06[b]
F[a]	4.02	26.89	9.11	16.86	2.14	1.76	13.79	9.89
P	.01	<.01	<.01	<.01	.11	<.17	<.01	<.01
Large wild-caught ($W_{60–75}$):								
C. violaceus	6.09 ± .30[a]	.21 ± .03[bc]	.34 ± .04[a]	4.28 ± .23[b]	.72 ± .11[b]	.58 ± .08[c]	.61 ± .05[b]	.12 ± .01[a]
X. mucosus	8.34 ± .40[b]	.08 ± .01[a]	.47 ± .05[a]	7.33 ± .93[b]	.32 ± .05[a]	.27 ± .04[ab]	.37 ± .05[a]	.32 ± .03[b]
X. atropurpureus	13.33 ± 1.25[c]	.10 ± .02[ab]	.36 ± .03[a]	5.47 ± .76[b]	.45 ± .04[ab]	.33 ± .03[b]	.33 ± .06[a]	.30 ± .04[b]
A. purpurescens	12.15 ± .60[c]	.39 ± .10[c]	.59 ± .09[b]	.35 ± .10[a]	.29 ± .04[a]	.14 ± .02[a]	.25 ± .02[a]	.32 ± .04[b]
F[a]	20.68	12.16	9.43	82.00	6.64	15.50	11.28	19.96
P	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
Large laboratory-fed ($L_{60–75}$):								
C. violaceus	10.65 ± .78[a]	.21 ± .03[a]	.52 ± .06[b]	5.22 ± .49[b]	1.48 ± .23[c]	.85 ± .13[b]	.63 ± .05[c]	.28 ± .03[a]
X. mucosus	9.87 ± .37[a]	.29 ± .03[a]	.35 ± .01[a]	8.77 ± .73[c]	.53 ± .03[a]	.34 ± .04[a]	.49 ± .04[bc]	.40 ± .02[b]
X. atropurpureus	13.25 ± .74[b]	.18 ± .03[a]	.48 ± .04[ab]	5.12 ± .71[b]	1.02 ± .11[bc]	.77 ± .05[b]	.37 ± .05[ab]	.82 ± .08[c]
A. purpurescens	8.52 ± 1.56[a]	.98 ± .18[b]	.91 ± .06[c]	.85 ± .10[a]	.75 ± .11[ab]	.46 ± .06[a]	.32 ± .05[a]	.94 ± .09[c]
F[a]	4.26	16.35	24.60	92.56	14.24	10.05	8.81	40.01
P	.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01

Note. Values are means (±SEM, $n = 10$). Within-species comparisons of the activities for each enzyme within a feeding category were analyzed with one-way ANOVA and Tukey's HSD with a family error rate of $P = 0.05$. Values for a specific enzyme and feeding category that share a letter are not significantly different.

[a] df = 3, 36.

significantly lower alkaline phosphatase activity, less than half that of the other three species, which did not differ from each other in the activity of this enzyme.

$L_{60–75}$ Fish. As in $w_{30–40}$ fish, *X. atropurpureus* displayed significantly higher pepsin activity than the three other species. *Anoplarchus purpurescens* exhibited significantly higher trypsin activity, about three to five times greater than the three other species, which did not differ from each other. *Anoplarchus purpurescens* also showed the significantly highest aminopeptidase activity, followed by *C. violaceus* and *X. atropurpureus*, which were statistically indistinguishable, as were the two species of *Xiphister*. For α-amylase activity, *X. mucosus* showed the significantly highest level, followed by *C. violaceus* and *X. atropurpureus*, which were statistically indistinguishable, and then *A. purpurescens*, with 6–10 times less activity of this enzyme than the other species. *Cebidichthys violaceus* and *X. atropurpureus* exhibited the highest, statistically indistinguishable maltase activity, but that of the latter species was not significantly different from that of *A. purpurescens*, which, in turn, was not statistically different from that of *X. mucosus*. Isomaltase activity was significantly higher in *C. violaceus* and *X. atropurpureus* than in *A. purpurescens* and *X. mucosus*, which were statistically indistinguishable in activity of this enzyme. *Cebidichthys violaceus* and *X. mucosus* displayed the highest, statistically indistinguishable lipase activity, but that of the latter species was not significantly different from that of *X. atropurpureus*, which, in turn, was not statistically different from that of *A. purpurescens*. Alkaline phosphatase activity was significantly highest in *A. purpurescens* and *X. atropurpureus*, which were statistically indistinguishable, followed by *X. mucosus* and then *C. violaceus*, with the significantly lowest activity for this enzyme.

Overall Patterns. Despite their dietary differences, *X. mucosus* and *X. atropurpureus* showed similar patterns of ontogenetic change in the total activities of all eight enzymes combined, converging on each other in the MDS plot in the $W_{60–75}$ category (Fig. 3). The three xiphisterine fishes in both the $W_{60–75}$ and $L_{60–75}$ categories clustered together closely (Fig. 4) and formed a group statistically distinct from $W_{60–75}$ and $L_{60–75}$ fish of *A. purpurescens* ($W_{60–75}$ ANOSIM: $R = 0.37$, $P < 0.01$; $L_{60–75}$ ANOSIM: $R = 0.94$, $P < 0.01$). Furthermore, the xiphisterine species all showed sizeable ontogenetic ($w_{30–40}$ vs. $W_{60–75}$) changes in digestive enzyme activity, whereas *A. purpurescens* did not. And the members of the Xiphisterini showed little, albeit significant, change in digestive enzyme activity in response to the high-protein diet, whereas *A. purpurescens* changed its digestive enzyme activity considerably. *Anoplarchus purpurescens* clearly had digestive enzyme activity profiles different from those of the three xiphisterine species. These results suggest a phylogenetic signal affecting digestive enzyme activities in the four prickleback species.

Discussion

The results of this study supported, for the most part, our hypotheses. First, *Cebidichthys violaceus* and *Xiphister mucosus* increased their carbohydrase activities with an ontogenetic shift to a macroalgal diet. Second, *C. violaceus*, *X. mucosus*, and *Xiphister atropurpureus* showed modest change in total activity of all digestive enzymes combined in response to consuming the high-protein diet, suggesting that the activities of their digestive enzymes are, to some extent, genetically programmed to undergo specific ontogenetic changes. This pattern was most evident in the carbohydrases, the activities of which increased ontogenetically despite the fishes being raised on a high-protein, low-carbohydrate diet. Third, the similarity between *X. mucosus* and *X. atropurpureus* in the activities of several enzymes, not just the carbohydrases, suggests that phylogeny, perhaps more than diet, influences digestive enzyme activities in these two fish species. Last, *Anoplarchus purpurescens*, in contrast, showed little ontogenetic change in digestive enzyme activity on its natural diet but exhibited sizeable changes in the suite of digestive enzyme activities after eating the high-protein diet, suggesting that this fish exhibits phenotypic plasticity in response to different ingested substrates. Thus, *A. purpurescens* has the potential to change the ontogenetic trajectory of its digestive enzyme activities but does not do so in nature because this species does not change its diet as it grows.

Phylogenetic relationships have been suggested as playing a role in influencing digestive enzyme activities in rodents (Sabat et al. 1999) and birds (Martínez del Rio 1990; Caviedes-Vidal et al. 2000), but more often diet has been stated to be the factor most important in influencing digestive enzyme activities in fishes (Fernández et al. 2001). In this study, *X. mucosus* and *X. atropurpureus* displayed the most similar patterns of digestive enzyme activities from both the ontogenetic and dietary perspectives, as *X. atropurpureus* matched *X. mucosus* in trypsin, α-amylase, and lipase activities. These results are similar to those of Chan et al. (2004), who found that larger individuals of *X. mucosus* and *X. atropurpureus* displayed similar α-amylase activities. Furthermore, we found that both species of *Xiphister* were similar to *C. violaceus* and that all three of these xiphisterine species were largely different from *A. purpurescens* from both the ontogenetic and the dietary perspectives, fully supporting the idea that phylogenetic constraints play a role in influencing digestive enzyme activities in these fishes. In addition, the results of this study corroborate the clade proposed by Stoddard (1985) and suggest that digestive enzyme activities may be used as characters to support the phylogeny. For example, high α-amylase activity may be a synapomorphy of the Xiphisterini, whereas high trypsin and aminopeptidase activities may be a synapomorphy of the Alectriini. *Phytichthys chirus*, the sister taxon to the two species of *Xiphister* in Stoddard's phylogenetic hypothesis, also displays high α-amylase activity and similar ontogenetic changes in the same suite of digestive

enzymes as the other xiphisterine species (D. P. German, unpublished data). Although *X. atropurpureus* is a carnivore, this species may represent a recent divergence toward carnivory in a primarily herbivorous clade, as all of the members of the tribe Xiphisterini for which the dietary information exists (five of nine species) become increasingly herbivorous with age (Cross 1981; Barton 1982; Horn et al. 1982; D. P. German and M. H. Horn, unpublished data on *Dictyosoma burgeri*, a northwestern Pacific species). *Xiphister atropurpureus*, therefore, appears to have retained the digestive enzyme activities characteristic of the herbivores in the clade, perhaps because not enough evolutionary time has transpired for the digestive enzyme activities of this fish to reflect its diet. We recognize that the phylogenetic breadth covered in this study is narrow; thus, we are cautious in stating that phylogenetic relationships influence digestive enzyme activities in any of the fishes other than the two sister taxa of *Xiphister*.

Cebidichthys violaceus, with its high α-amylase and lipase activities in $w_{30–40}$ fish, was distinct from the two *Xiphister* species in the $w_{30–40}$ category, according to the multivariate analysis. The significant ontogenetic decrease in pepsin activity and the higher lipase, maltase, isomaltase, and trypsin activities exhibited by $W_{60–75}$ fish of *C. violaceus* placed it in a different position than *X. mucosus* and *X. atropurpureus* in the ontogenetic MDS plot (Fig. 3), suggesting that it is more specialized, at the $W_{60–75}$ stage, to consume and digest an algal diet than are the other species studied. These results are consistent with the diet of *C. violaceus* in the $W_{60–75}$ category and with the positions of the three species in the xiphisterine clade, as *C. violaceus* is not the sister taxon of the two species of *Xiphister*.

Modulation of digestive enzyme activities in response to diet is common but not universal among vertebrates (Sabat et al. 1999). Diet-related plasticity of digestive enzyme activity can be explained by the "adaptive modulation hypothesis," which states that variation in diet should confer upon animals the ability to modulate their digestive enzyme activity accordingly, whereas species with narrower diets should show no such ability (Karasov 1992). This study appears to be unique from the adaptive modulation perspective for at least two reasons: (1) we investigated the effects of ontogeny on the activities of digestive enzymes in wild populations of fishes consuming their natural diets, and (2) we compared the results with the effects of a formulated diet on the same species after a 3–4 mo feeding trial, a period long enough to parallel the length of the time required for the ontogenetic dietary shift to occur in nature. Most previous studies testing the adaptive modulation model on various vertebrate species have been of relatively short duration and have not attempted to distinguish the effects of ontogeny from the effects of diet on digestive enzyme activity (Reimer 1982; Lindner et al. 1995; Sabat et al. 1998, 1999; Levey et al. 1999; Caviedes-Vidal et al. 2000; García-Carreño et al. 2002).

The three xiphisterine species significantly changed the activities of five of the eight digestive enzymes (but not the same five enzymes among species) in response to the high-protein diet and also showed significant modulation, according to the ANOSIM. The changes displayed by the xiphisterine species, however, were less marked than those observed in *A. purpurescens*, which modulated seven of the eight enzyme activities and showed a large change in the dietary MDS plot (Fig. 4). Two variable components of an animal's natural diet have been suggested as affecting digestive enzyme activities: (1) breadth of diet, or dietary flexibility, and (2) biochemical composition of dietary items, as even the food of a specialist can show periodic changes in composition (Martínez del Rio 1990; Sabat et al. 1999; Caviedes-Vidal et al. 2000). *Anoplarchus purpurescens*, albeit carnivorous, consumes the widest variety of food items of the four prickleback species examined (German 2003), and thus the large modulation in enzyme activity seen in *A. purpurescens* correlates with its broad invertebrate diet. From an evolutionary standpoint, the members of the Xiphisterini may show less dietary flexibility and modulate their digestive enzyme activities on a lesser scale. For example, adult *C. violaceus* and *X. mucosus* have been shown to consume macroalgae year-round, only changing somewhat the species composition of macroalgae taken based on seasonal abundance (Horn et al. 1982). Algal biochemical composition varies interspecifically and can change seasonally (Horn et al. 1986; Nelson et al. 2002), but apparently not on a scale that elicits the plasticity of digestive enzyme activity in the members of the Xiphisterini that is seen in *A. purpurescens*. Carbohydrates appear to be important nutrients for the xiphisterine species, regardless of diet. *Xiphister atropurpureus* has the second-broadest diet of these four prickleback species (German 2003) but shows less plasticity than *A. purpurescens* in the overall suite of enzyme activities in response to a high-protein diet. This smaller modulation of enzyme activities by *X. atropurpureus*, despite a broad diet, further supports the contention that this fish is phylogenetically constrained in its digestive physiology.

Comparing digestive enzyme activities between different studies is difficult because of differences in methodology, including differences in assay substrates, assay temperatures, instruments used for analysis, units of reported activity, and species and ontogenetic stages examined, as well as in the quantity and composition of diet (Peres et al. 1998; Logothetis et al. 2001; Chan et al. 2004). Therefore, the prickleback digestive enzyme activities measured in this study were compared only among each other, with broad references to similar patterns found in other fishes.

The hypotheses that the carnivorous species would display consistently high protease activities in all feeding categories and higher protease activities than the more herbivorous fishes were partially supported. Pepsin activities generally have been correlated with diet in fishes (Fish 1960; Sabapathy and Teo 1993; Chakrabarti et al. 1995; Drewe et al. 2004), and in this study, the carnivorous *X. atropurpureus* and *A. purpurescens* exhibited

higher pepsin activities than the herbivorous *C. violaceus* and *X. mucosus*. Conversely, Chan et al. (2004) reported that in larger individuals pepsin activity did not vary among these same four prickleback species. Each prickleback species in this study, however, showed species-specific responses toward diet in its pepsin activities, indicating that pepsin activities can be modulated with diet in these species and are not fixed at a given level. For example, an ontogenetic decrease in pepsin activity was observed for *C. violaceus*, similar to the pattern seen in the frugivorous characid fish *Brycon guatemalensis* (Drewe et al. 2004). *Xiphister mucosus*, however, did not decrease its pepsin activity significantly with the ontogenetic shift in diet. This fish, in contrast to *C. violaceus*, consumed a considerable amount of animal material at the size classes examined and thus may decrease pepsin activity only at a larger size, when this fish is more completely herbivorous. Both *C. violaceus* and *X. mucosus* exhibited an increase in pepsin activities on the high-protein diet, showing that pepsin activities in these two fishes can be elevated in response to an increase in protein content in the diet. The decrease in pepsin activity displayed by *A. purpurescens* on the high-protein diet indicates that this fish may rely more on trypsin and aminopeptidase for protein digestion under such circumstances.

The lack of ontogenetic changes in trypsin and aminopeptidase activities in *C. violaceus* and *X. mucosus* as they shifted to a more herbivorous diet suggests that protein remains an important nutrient for herbivorous, as well as carnivorous, juveniles. The members of the Xiphisterini, however, displayed lower trypsin and aminopeptidase activities than *A. purpurescens*. The high trypsin and aminopeptidase activities found in *A. purpurescens* in this study are consistent with reports that the activities of these two proteases are higher in carnivorous than in herbivorous fishes and birds (Fraisse et al. 1981; Hofer and Schiemer 1982; Sabapathy and Teo 1993; Caviedes-Vidal et al. 2000) and supports digestion of a higher protein diet by *A. purpurescens*. Modulation of trypsin and aminopeptidase has been reported for fishes and birds fed high- versus low-protein diets (Reimer 1982; Das and Tripathy 1991; Sabat et al. 1998; Levey et al. 1999; Caviedes-Vidal et al. 2000; García-Carreño et al. 2002), and indeed all four prickleback species changed the activities of at least one of these proteases in response to the high-protein diet in this study. *Anoplarchus purpurescens*, however, elevated its trypsin and aminopeptidase activities to a greater level than the three xiphisterine species after eating the high-protein diet. Whereas all four prickleback species appeared to be able to modulate their protease activities in response to varying protein levels, *A. purpurescens*, as the prickleback with the broadest diet, shows a higher degree of plasticity and may assimilate protein more efficiently than the three xiphisterine species. Carnivorous fishes generally have higher protein requirements than herbivores (35%–55% for carnivores vs. <30% for herbivores; Horn et al. 1995), and the protease activities reported here support the proposition that the members of the Xiphisterini have lower protein requirements than *A. purpurescens*. Based on the preliminary K_m values recorded in this study, *A. purpurescens* may be expressing a different trypsin isoform than the xiphisterine fishes, but this possibility requires further investigation.

Both *C. violaceus* and *X. mucosus* increased their α-amylase activities with increase in size and shift in diet; in contrast, *A. purpurescens* exhibited very low α-amylase activities that did not change ontogenetically. These results corroborate findings that α-amylase activities are higher in herbivorous than in carnivorous fishes (e.g., Sabapathy and Teo 1993; Chakrabarti et al. 1995; Hidalgo et al. 1999; Fernández et al. 2001; Chan et al. 2004) and higher in fishes consuming more starch at one ontogenetic stage than at another (Moran and Clements 2002; Drewe et al. 2004). The findings that *C. violaceus* exhibited the highest maltase and isomaltase activities also are consistent with reports that fishes and birds with mostly herbivorous diets possess high activities of these brush border disaccharidases (Gohar and Latif 1961; Chiu and Benitez 1981; Fraisse et al. 1981; Martínez del Rio 1990; Sabapathy and Teo 1993; Harpaz and Uni 1999). *Cebidichthys violaceus* may possess higher maltase and isomaltase activities than *X. mucosus*, however, because the latter fish is still consuming some animal material at the size classes examined in this study. *Xiphister mucosus* may, therefore, increase its activities of these two enzymes when larger and more herbivorous.

The high level of α-amylase activity we found in the xiphisterine species, despite their consuming a low-starch diet, corroborates the findings of another feeding study, which found that after being raised on a high-protein, low-carbohydrate diet, *C. violaceus* still exhibited intestinal transport that favored sugars over amino acids (Buddington et al. 1987). The results of this study and those of Buddington et al. (1987) suggest that carbohydrates are important to the xiphisterine species, regardless of diet, and that their α-amylase activities are fixed at levels appropriate to digest their natural food items. The surprising increase in maltase and isomaltase activities in the pricklebacks on the high-protein diet is consistent with a previous report (Sabat et al. 1998) that birds fed a high-protein, carbohydrate-free diet exhibit higher activities of these two enzymes than birds fed a 100% carbohydrate diet. Sabat and coworkers argued that the increase in activity of these two brush border carbohydrases represents a nonspecific response to the high-protein diet. Conversely, Quan and Gray (1993) found that rats decrease the amount of active sites per enzyme molecule for isomaltase, thereby decreasing their disaccharidase activity in the absence of substrate. Overall, the diverse patterns of maltase and isomaltase activities among the pricklebacks on the high-protein, low-starch diet are not different from those reported for birds and other fishes fed high- versus low-starch diets (Kawai and Ikeda 1972; Buddington and Hilton 1987; Levey et al. 1999; Caviedes-Vidal et al. 2000), but they expose our uncertainty about the mechanisms underlying why some

animals modulate digestive enzyme activities whereas others do not. Examination of the relationship between enzyme activities, diet, gut retention times, and nutrient transport is needed to elucidate why seemingly anomalous patterns of digestive enzyme activities occur in the pricklebacks and other vertebrates (Sabat et al. 1999).

Alkaline phosphatase showed the highest degree of phenotypic plasticity of all of the enzymes measured in this study. Activities of this brush border enzyme have been reported to be indicators of the intensity of nutrient absorption in the enterocytes of fishes (Harpaz and Uni 1999; Gawlicka et al. 2000). Both *C. violaceus* and *X. mucosus* decreased their alkaline phosphatase activities as they shifted toward an algal diet, a diet recognized as being low in protein (Horn 1989). *Xiphister atropurpureus* and *A. purpurescens* showed no ontogenetic changes in alkaline phosphatase activities, reflecting their carnivorous diets in both size categories examined. Alkaline phosphatase activity also has been reported to be an indicator of carbohydrate and lipid absorption (Fraisse et al. 1981; Calhau et al. 2000). The activity of this enzyme in pricklebacks, however, appears to be an indicator of overall nutrient transport, rather than of any particular dietary constituent, as the pattern of alkaline phosphatase activities did not match those observed for any of the other measured proteases, carbohydrases, or lipase.

All four prickleback species increased their alkaline phosphatase activities in response to the high-protein diet, apparently because of the increased nutrient load. As carnivores, however, both *X. atropurpureus* and *A. purpurescens* showed an ability to increase dramatically their alkaline phosphatase activities in response to the high-protein diet, whereas the more herbivorous fishes did not, reflecting the probability of a low nutrient load in their natural diets. Consistent with this result, the carnivorous *Morone saxatilis* and omnivorous *Oreochromis niloticus* displayed alkaline phosphatase activities similar to one another's, and both had greater alkaline phosphatase activities than the herbivorous *Hypophthalmichthys molitrix* after being fed a high-protein diet (Harpaz and Uni 1999). To our knowledge, this study is the first to compare alkaline phosphatase activities between fish raised on a high-protein diet with those of members of the same species consuming their natural diets.

Surprisingly, the highest level of lipase activity appears to be in the herbivorous species. Fishes on a high-lipid diet have been shown to possess higher lipase activities than those consuming a low-lipid diet (Reimer 1982; Das and Tripathy 1991). Therefore, we expected that lipase activities would follow trends similar to protease activities, because there are more lipids in a primarily crustacean diet (approximately 21%–44% lipid; Lehtonen 1996; Lemos and Phan 2001) than in an algal diet (<5% lipid; Horn et al. 1986; Nelson et al. 2002). In addition, lipids are an important protein-sparing energy source for carnivorous fishes, which ingest low amounts of carbohydrates (Watanabe 1982). We found, however, that lipase activity was elevated in herbivorous individuals of *C. violaceus*, *X. mucosus*, and, to some extent, *X. atropurpureus*, suggesting that lipids are a more important source of energy to the members of the Xiphisterini than they are to *A. purpurescens*. As with trypsin, the K_m data suggest that the xiphisterine fishes are expressing a different lipase than *A. purpurescens*, further setting them apart. Perhaps *C. violaceus* and *X. mucosus* have higher lipase activity to extract all the lipids available from their algal diet and whatever animal material they ingest, thus maximizing the use of lipids as an energy source. A similar explanation was offered for protease activities and protein use in the detritivorous *Dorosoma cepedianum*, which consumes a diet very low in protein (Smoot and Finlay 2000).

Cebidichthys violaceus has been reported to feed to meet energy (Fris and Horn 1993) and protein requirements (Horn et al. 1995), but in either case, *C. violaceus* converts dietary protein to body protein with equal or greater efficiency than most carnivorous fishes (Fris and Horn 1993). Because *C. violaceus* consumes a low-protein diet in the wild (≤10% dry weight; Fris and Horn 1993) and may have much greater protein requirements than the 10% in their algal diet (Horn et al. 1995), this fish may require all dietary protein for structural and metabolic purposes and therefore turns to lipids as an energy source secondary to carbohydrates. The same process may occur in the two species of *Xiphister*. These arguments, however, are offered with the caveat that *C. violaceus* has been reported to assimilate only 44% of lipid from an algal diet (Horn et al. 1986). Clearly, further investigation is needed into the assimilation of lipids and the contribution of lipids to the daily energy budget of the pricklebacks. The carnivorous *A. purpurescens*, with its high trypsin and aminopeptidase activities, may rely more on protein to meet its energy requirements, and its low lipase activity may be sufficient to obtain what lipid it needs from its higher lipid diet.

High lipase activities in herbivorous fishes are not limited to this study. The omnivorous *Atherinops affinis* shows dietary affinity depending on habitat; certain estuarine populations of *A. affinis* are herbivorous, whereas kelp-forest *A. affinis* are carnivorous (Smith 2002). Herbivorous *A. affinis* were found to have higher lipase activities than their kelp-forest representatives, and both *A. affinis* populations exhibited higher lipase activities than their close relatives *Atherinopsis californiensis* and *Leuresthes tenuis*, which are both strict carnivores (M. H. Horn, unpublished data). Another fish species, *B. guatemalensis*, does not change its lipase activities as it undergoes an ontogenetic dietary shift from a high-lipid, insectivorous diet to a low-lipid, frugivorous diet (Drewe et al. 2004). Furthermore, the herbivorous *Labeo rohita* exhibits the highest lipase activities when compared to two omnivores, *Sardinella longiceps* and *Liza subviridis*, and a carnivore, *Rastrelliger kanagurta* (Nayak et al. 2003). Overall, our cursory survey found that the pattern of highest lipase activities in herbivores is evident in at least 12 species of fishes representing seven families. Thus, this unex-

pected pattern of lipase activity is not unique to the pricklebacks and deserves further attention, including an analysis emphasizing the contribution of lipids to the daily energy budget of herbivorous versus carnivorous species.

To our knowledge, this study is the first to address the influences of ontogeny, diet, and phylogeny on digestive enzyme activities in fishes. We have shown that (1) digestive enzyme activity in xiphisterine pricklebacks is genetically programmed to match ontogenetic shifts in diet, (2) the adaptive modulation hypothesis has some support in prickleback fishes, as digestive enzyme activities can be somewhat fixed at levels necessary to digest a specific diet and can be modulated in a species with a variable diet, and (3) phylogenetic relationships can influence digestive enzyme activities, as seen in the similarities of the two species of *Xiphister* and in the pronounced differences between the members of the Xiphisterini and *A. purpurescens.*

Acknowledgments

We thank K. Boyle, M. Saba, K. Kim, D. Smith, K. Drewe, E. Cox, and S. Choi for assistance in the field and laboratory, J. Carroll for providing access to Diablo Canyon and helping to collect *Xiphister mucosus*, J. Degan for help in constructing the tank shelves and fish containers, K. Dickson and N. Ross for discussions on digestive enzymes, K. Messer and S. Murray for statistical advice, and L. Crummett for moral support. Financial support was provided by a grant (OCE-9906857) from the National Science Foundation (M. H. Horn, principal investigator), a Sigma Xi Grant-in-Aid of Research, and the Departmental Associations Council and Department of Biological Science at California State University, Fullerton.

Literature Cited

Anson M.L. 1938. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J Gen Physiol 22:79–89.

Barton M.G. 1982. Intertidal vertical distribution and diets of five species of central California stichaeoid fishes. Calif Fish Game 68:174–182.

Bitterlich G. 1985. Digestive enzyme pattern of two stomachless filter feeders, silver carp, *Hypophthalmichthys molitrix* Val., and bighead carp, *Aristichthys nobilis* Rich. J Fish Biol 27: 103–112.

Buddington R.K., J.W. Chen, and J. Diamond. 1987. Genetic and phenotypic adaptation of intestinal nutrient transport to diet in fish. J Physiol 393:261–281.

Buddington R.K. and J.W. Hilton. 1987. Intestinal adaptations of rainbow trout to changes in dietary carbohydrate. Am J Physiol 253:G489–G496.

Cahu C.L., J.L. Zambonino Infante, A. Peres, P. Quazuguel, and M.M. Le Gall. 1998. Algal addition in sea bass (*Dicentrarchus labrax*) larvae rearing: effect on digestive enzymes. Aquaculture 161:479–489.

Calhau C., F. Martel, C. Hipólito-Reis, and I. Azevedo. 2000. Differences between duodenal and jejunal rat alkaline phosphatase. Clin Biochem 33:571–577.

Caviedes-Vidal E., D. Afik, C. Martínez del Rio, and W.H. Karasov. 2000. Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. Comp Biochem Physiol A 125:11–24.

Chakrabarti I., M.A. Gani, K.K. Chaki, R. Sur, and K.K. Misra. 1995. Digestive enzymes in 11 freshwater teleost fish species in relation to food habit and niche segregation. Comp Biochem Physiol A 112:167–177.

Chan A.S., M.H. Horn, K.A. Dickson, and A. Gawlicka. 2004. Digestive enzyme activities in carnivores and herbivores: comparisons among four closely related prickleback fishes (Teleostei: Stichaeidae) from a California rocky intertidal habitat. J Fish Biol 65:848–858.

Chiu Y.N. and L.V. Benitez. 1981. Studies on the carbohydrases in the digestive tract of the milkfish *Chanos chanos.* Mar Biol 61:247–254.

Clarke K.R. 1993. Non-parametric multivariate analysis of changes in community structure. Aust J Ecol 18:117–143.

Cockson A. and D. Bourne. 1972. Enzymes in the digestive tract of two species of euryhaline fish. Comp Biochem Physiol A 41:715–718.

Coleman R.M. 1992. Reproductive biology and female parental care in the cockscomb prickleback, *Anoplarchus purpurescens* (Pisces: Stichaeidae). Environ Biol Fishes 35:177–186.

Cross J.N. 1981. Structure of a Rocky Intertidal Fish Assemblage. PhD diss. University of Washington.

Dahlqvist A. 1968. Assay of intestinal disaccharidases. Anal Biochem 22:99–107.

Das K.M. and S.D. Tripathy. 1991. Studies on the digestive enzymes of grass carp, *Ctenopharyngodon idella* (Val.). Aquaculture 92:21–32.

Drewe K.E., M.H. Horn, K.A. Dickson, and A. Gawlicka. 2004. Insectivore to frugivore: ontogenetic changes in gut morphology and digestive enzyme activity in the characid fish *Brycon guatemalensis* from Costa Rican rainforest streams. J Fish Biol 64:890–902.

Fernández I., F.J. Moyano, M. Díaz, and T. Martínez. 2001. Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). J Exp Mar Biol Ecol 262:1–12.

Fish G.R. 1960. The comparative activity of some digestive enzymes in the alimentary canal of tilapia and perch. Hydrobiologia 15:161–178.

Fraisse M., N.Y.S. Woo, J. Noaillac-Depeyre, and J.C. Murat. 1981. Distribution pattern of digestive enzyme activities in intestine of the catfish (*Ameiurus nebulosus* L.) and of the carp (*Cyprinus carpio* L.). Comp Biochem Physiol A 70:443–446.

Fris M.B. and M.H. Horn. 1993. Effects of diets of different protein content on food consumption, gut retention, protein conversion, and growth of *Cebidichthys violaceus* (Girard), an herbivorous fish of temperate zone marine waters. J Exp Mar Biol Ecol 166:185–202.

García-Carreño F.L., C. Albuquerque-Cavalcanti, M. Angeles Navarette del Toro, and E. Zaniboni-Filho. 2002. Digestive proteinases of *Brycon orbignyanus* (Characidae, Teleostei): characteristics and effects of protein quality. Comp Biochem Physiol B 132:343–352.

Gawlicka A., B. Parent, M.H. Horn, N. Ross, I. Opstad, and O.J. Torrissen. 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): indication of readiness for first feeding. Aquaculture 184:303–314.

German D.P. 2003. Digestive Enzyme Activities in Herbivorous and Carnivorous Prickleback Fishes (Teleostei: Stichaeidae): Ontogenetic, Dietary, and Phylogenetic Effects. MS thesis. California State University, Fullerton.

Gohar H.A.F. and A.F.A. Latif. 1961. The carbohydrases of some scarid and labrid fishes from the Red Sea. Pub Mar Biol Stn Al Ghardaqa 11:127–164.

Harpaz S. and Z. Uni. 1999. Activity of intestinal mucosal brush border membrane enzymes in relation to the feeding habits of three aquaculture fish species. Comp Biochem Physiol A 124:155–160.

Hidalgo M.C., E. Urea, and A. Sanz. 1999. Comparative study of digestive enzymes in fish with different nutritional habits: proteolytic and amylase activities. Aquaculture 170:267–283.

Hofer R. and F. Schiemer. 1982. Proteolytic activity in the digestive tract of several species of fish with different feeding habits. Oecologia 48:342–345.

Horn M.H. 1989. Biology of marine herbivorous fishes. Oceanogr Mar Biol Annu Rev 27:167–272.

Horn M.H., K.F. Mailhiot, M.B. Fris, and L.L. McClanahan. 1995. Growth, consumption, assimilation and excretion in the marine herbivorous fish *Cebidichthys violaceus* (Girard) fed natural and high protein diets. J Exp Mar Biol Ecol 190: 97–108.

Horn M.H., S.N. Murray, and T.W. Edwards. 1982. Dietary selectivity in the field and food preferences in the laboratory for two herbivorous fishes (*Cebidichthys violaceus* and *Xiphister mucosus*) from a temperate intertidal zone. Mar Biol 67: 237–246.

Horn M.H., S.N. Murray, and R.R. Seapy. 1983. Seasonal structure of a central California rocky intertidal community in relation to environmental variations. Bull South Calif Acad Sci 82:79–94.

Horn M.H., M.A. Neighbors, and S.N. Murray. 1986. Herbivore responses to a seasonally fluctuating food supply: growth potential of two temperate intertidal fishes based on the protein and energy assimilated from their macroalgal diets. J Exp Mar Biol Ecol 103:217–234.

Iijima N., S. Tanaka, and Y. Ota. 1998. Purification and characterization of bile salt-activated lipase from the hepatopancreas of red sea bream, *Pagrus major*. Fish Physiol Biochem 18:59–69.

Jones R.S. 1968. A suggested method for quantifying gut contents in herbivorous fishes. Micronesica 4:369–371.

Kapoor B.G., H. Smit, and I.A. Verighina. 1975. The alimentary canal and digestion in teleosts. Adv Mar Biol 13:109–239.

Karasov W.H. 1992. Test of the adaptive modulation hypothesis for dietary control of intestinal transport. Am J Physiol 267: R496–R502.

Kawai S. and S. Ikeda. 1972. Studies on digestive enzymes of fishes. II. Effect of dietary change on the activities of digestive enzymes in carp intestine. Bull Jpn Soc Sci Fish 38:265–270.

Kuz'mina V.V. 1996. Influence of age on digestive enzyme activity in some freshwater teleosts. Aquaculture 148:25–37.

Lehtonen K.K. 1996. Ecophysiology of the benthic amphipod *Monoporeia affinis* in an open-sea area of the northern Baltic Sea: seasonal variations in body composition, with bioenergetic considerations. Mar Ecol Prog Ser 143:87–98.

Lemos D. and V. Phan. 2001. Ontogenetic variation in metabolism, biochemical composition and energy content during the early life stages of *Farfantepenaeus paulensis* (Crustacea: Decapoda: Penaeidae). Mar Biol 138:985–997.

Levey D.J., A.R. Place, P.J. Rey, and C. Martínez del Rio. 1999. An experimental test of dietary enzyme modulation in pine warblers *Dendroica pinus*. Physiol Biochem Zool 72:576–587.

Lindner P., A. Eshel, S. Kolkovski, A. Tandler, and S. Harpaz. 1995. Proteolysis by juvenile sea bass (*Dicentrarchus labrax*) gastrointestinal enzymes as a method for the evaluation of feed proteins. Fish Physiol Biochem 14:399–407.

Logothetis E.A., M.H. Horn, and K.A. Dickson. 2001. Gut morphology and function in *Atherinops affinis* (Teleostei: Atherinopsidae), a stomachless omnivore feeding on macroalgae. J Fish Biol 59:1298–1312.

Marshall W.H. and T.W. Echeverria. 1992. Age, length, weight, reproductive cycle and fecundity of the monkeyface prickleback (*Cebidichthys violaceus*). Calif Fish Game 78:57–64.

Martínez del Rio C. 1990. Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. Physiol Zool 63:987–1011.

Moran D. and K.D. Clements. 2002. Diet and endogenous carbohydrases in the temperate marine herbivorous fish *Kyphosus sydneyanus* (Perciformes: Kyphosidae). J Fish Biol 60: 1190–1203.

Nayak J., P.G. Viswanathan Nair, K. Ammu, and S. Mathew. 2003. Lipase activity in different tissues of four species of fish: rohu (*Labeo rohita* Hamilton), oil sardine (*Sardinella longiceps* Linnaeus), mullet (*Liza subviridis* Valenciennes) and Indian mackerel (*Rastrelliger kanagurta* Cuvier). J Sci Food Agric 83:1139–1142.

Nelson M.M., C.F. Phleger, and P.D. Nichols. 2002. Seasonal

lipid composition in macroalgae of the northeastern Pacific Ocean. Bot Mar 45:58–65.

Nelson N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153: 375–380.

Peres A., J.L. Zambonino Infante, and C. Cahu. 1998. Dietary regulation of activities and mRNA levels of trypsin and amylase in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiol Biochem 19:145–152.

Preiser H., J. Schmitz, D. Maestracci, and R.K. Crane. 1975. Modification of an assay for trypsin and its application for the estimation of enteropeptidase. Clin Chim Acta 59:169–175.

Quan R. and G.M. Gray. 1993. Sucrase-α-dextrinase in the rat, postinsertional conversion to inactive molecular species by a carbohydrate free diet. J Clin Investig 91:2785–2790.

Reimer G. 1982. The influence of diet on the digestive enzymes of the Amazon fish Matrincha, *Brycon* cf. *melanopterus*. J Fish Biol 21:637–642.

Roncari G. and H. Zuber. 1969. Thermophilic aminopeptidases from *Bacillus stearothermophilus*. I. Isolation, specificity, and general properties of the thermostable aminopeptidase I. Int J Protein Res 1:45–61.

Sabapathy U. and L.H. Teo. 1993. A quantitative study of some digestive enzymes in the rabbitfish, *Siganus canaliculatus* and the sea bass, *Lates calcarifer*. J Fish Biol 42:595–602.

Sabat P., J.A. Lagos, and F. Bozinovic. 1999. Test of the adaptive modulation hypothesis in rodents: dietary flexibility and enzyme plasticity. Comp Biochem Physiol A 123:83–87.

Sabat P., F. Novoa, F. Bozinovic, and C. Martínez Del Rio. 1998. Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. Physiol Zool 71:226–236.

Setran A.C. and D.W. Behrens. 1993. Transitional ecological requirements for early juveniles of two sympatric stichaeid fishes, *Cebidichthys violaceus* and *Xiphister mucosus*. Environ Biol Fishes 37:381–395.

Smith D.R. 2002. Trophic Position of Estuarine and Kelp-Bed Populations of the Omnivorous Silverside Fish *Atherinops affinis* (Teleostei: Atherinopsidae) from Southern California: Analyses of Dietary Items and ^{15}N and ^{13}C Stable Isotopes. MS thesis. California State University, Fullerton.

Smoot J.C. and R.H. Finlay. 2000. Digestive enzyme and gut surfactant activity of detritivorous gizzard shad (*Dorosoma cepedianum*). Can J Fish Aquat Sci 57:1113–1119.

Somogyi M. 1952. Notes on sugar determination. J Biol Chem 195:19–23.

Stevens C.E. and I.D. Hume. 1995. Comparative Physiology of the Vertebrate Digestive System. 2d ed. Press Syndicate of the University of Cambridge, Melbourne.

Stoddard K.M. 1985. A Phylogenetic Analysis of Some Prickleback Fishes (Teleostei, Stichaeidae, Xiphisterinae) from the North Pacific Ocean, with a Discussion of Their Biogeography. MS thesis. California State University, Fullerton.

Vonk H.J. and J.R.H. Western. 1984. Comparative Biochemistry and Physiology of Enzymatic Digestion. Academic Press, London.

Walter K. and C. Schütt. 1974. Alkaline phosphatase in serum (continuous assay). Pp. 860–864 in H.U. Bergmeyer, ed. Methods of Enzymatic Analysis. Vol. 2. 2d ed. Academic Press, New York.

Watanabe T. 1982. Lipid nutrition in fish. Comp Biochem Physiol B 73:3–15.

Wingert R.C. 1974. Comparative Reproductive Cycles and Growth Histories of Two Species of *Xiphister* (Pisces: Stichaeidae) from San Simeon, California. MS thesis. California State University, Fullerton.

Yatsu A. 1986. Phylogeny and zoogeography of the subfamilies Xiphisterinae and Cebidichthyinae (Blennioidei: Stichaeidae). Pp. 663–678 in T. Uyeno, R. Arai, T. Taniuchi, and K. Matsuura, eds. Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes. Ichthyological Society of Japan, Tokyo.

Zar J.H. 1999. Biostatistical Analysis. 4th ed. Prentice-Hall, Englewood Cliffs, N.J.