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## Gut length and mass in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects

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**Abstract** Relative gut length, Zihler's index, and relative gut mass were measured in four species of prickleback fishes and the effects of ontogeny, diet, and phylogeny on these gut dimensions were determined. Of the four species, *Cebidichthys violaceus* and *Xiphister mucosus* shift to herbivory with growth (>45 mm SL), whereas *X. atropurpureus* and *Anoplarchus purpureus* remain carnivores. *A. purpureus* belongs to a carnivorous clade, and the three other species belong to an adjacent, herbivorous clade. Gut dimensions were compared in three feeding categories of the four species: (1) small, wild-caught juveniles representing the carnivorous condition before two species shift to herbivory; (2) larger, wild-caught juveniles representing the natural diet condition of the two carnivores and the two species that have shifted to herbivory; and (3) larger, laboratory-raised juveniles produced by feeding a high-protein artificial diet to small juveniles until they have reached the size of the larger, wild-caught juveniles. Comparisons of gut dimensions in categories (1) versus (2) tested for an ontogenetic effect, in (2) versus (3) for a dietary effect, and within each category for a phylogenetic effect. *C. violaceus* and *X. mucosus* increased gut dimensions with increase in body size and did not change ontogenetic trajectory in gut dimensions on the high-protein artificial diet, suggesting that they are genetically programmed to develop relatively large guts associated with herbivory. *X. atropurpureus* increased its gut dimensions with increase in size similar to its sister taxon, *X. mucosus*, suggesting a phylogenetic influence, but decreased

gut dimensions on the high-protein artificial diet, suggesting phenotypic plasticity. Nevertheless, *X. atropurpureus* displayed a larger gut than *A. purpureus*, further evidence that it evolved in an herbivorous clade. *A. purpureus* possessed a relatively small gut that was little affected by ontogeny or diet. Ontogeny and phylogeny more than diet appear to influence gut dimensions in the four species, thus favoring genetic adaptation over phenotypic plasticity as the major force acting on digestive system features in the two prickleback clades.

### Introduction

One of the most widely recognized anatomical features of vertebrates is that herbivores exhibit longer digestive tracts than do carnivores, and this pattern appears to be consistent among mammals (Korn 1992; Ellis et al. 1994; Stevens and Hume 1995), birds (Ricklefs 1996; Battley and Piersma 2005), reptiles and amphibians (Stevens and Hume 1995) and fishes (Al-Hussaini 1947; Kapoor et al. 1975; Horn 1989; Kramer and Bryant 1995b). Herbivores ingest food items that are often morphologically and chemically defended, encased in largely indigestible (at least by endogenous enzymes) fibrous cell walls, and that are considered nutrient poor (Horn 1989). The longer digestive tracts observed in herbivorous vertebrates are thought to increase the volume of food that can be ingested per feeding bout and lead to lengthier retention times of refractory compounds in the alimentary canal, thereby increasing exposure of ingesta to the battery of digestive processes in the gut (Ribble and Smith 1983; Sibley and Calow 1986; Horn 1989; Starck 2005). Such an increase in exposure can increase the efficiency by which an herbivorous diet can be digested (Starck 2005). Relatively long digestive tracts have been documented in both freshwater (Zihler 1982; Ribble and Smith 1983; Kramer and Bryant 1995b; Drewe et al. 2004) and marine (Al-Hussaini 1947; Kapoor et al. 1975; Horn 1989; Benavides et al. 1994; Horn and Ojeda 1999)

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herbivorous fishes. Even atherinopsids, which have relatively short, stomachless digestive tracts (Logothetis et al. 2001), exhibit increased gut length with increased degree of herbivory (Horn et al. 2005).

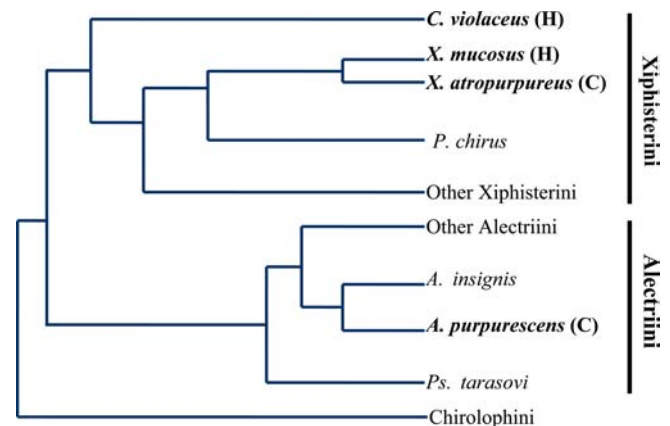
Many herbivorous fish species appear to begin life as carnivores or omnivores and shift to a more herbivorous diet as they grow (White 1985), and their gut lengths usually increase accordingly (Montgomery 1977; Stoner and Livingston 1984; Gallagher et al. 2001; Drewe et al. 2004). In fact, many fishes, regardless of diet, show an ontogenetic increase in their gut length relative to their body length, but those that consume more plant material as they increase in size can show disproportionate ontogenetic increases in gut length (Kramer and Bryant 1995a). Whether such an ontogenetic increase in gut length is genetically fixed or phenotypically plastic remains poorly understood. That is, would fishes that shift to a more herbivorous diet as they grow display the same ontogenetic trajectory and increase their gut lengths if they were to be raised on a high-protein, low-fiber diet, or would their gut lengths decrease in response to the nutrient-rich, easily digestible food?

Gut length can also vary among fish species with different body shapes (e.g., elongate versus deep-bodied), as deeper-bodied fishes generally have more space in their peritoneal cavities to accommodate longer, more highly coiled guts (Montgomery 1977; Barton 1982; Kramer and Bryant 1995b). Differences in body mass can also produce misleading results in comparisons of gut length because fishes that grow at faster rates tend to be heavier and exhibit longer guts than those that grow more slowly (Kramer and Bryant 1995a). Clearly, body mass must be taken into account in comparisons of gut length between small and large fishes of the same species, between similarly-shaped fishes with different body masses, or between species with different body plans altogether (Montgomery 1977; Zihler 1982; Kramer and Bryant 1995a, b). Relative gut length (RGL = gut length [mm]/standard length [mm]) is the gut length index probably used most commonly in comparisons among fishes with different diets (e.g., Al-Hussaini 1947; Kapoor et al. 1975; Drewe et al. 2004), but this index ignores differences in body mass. Zihler's Index {Zihler 1982;  $ZI = \text{gut length (mm)} / 10[\text{body mass (g)}^{1/3}]$ }, which relates gut length to body mass rather than standard length, offers a potentially powerful approach that takes into account differences in body mass.

Although closely related fish species may resemble each other in size, shape, and mass, comparisons of gut length in such taxa with different diets are limited (e.g., Zihler 1982; Cleveland and Montgomery 2003; Elliott and Bellwood 2003). Examination of gut morphology in closely related taxa would allow not only for comparisons of species that are more similar in shape and size, but also would allow for gut morphology data to be analyzed in a phylogenetic context. Elliott and Bellwood (2003) have shown the importance of taking evolutionary history into account when comparing gut length among fish species with different diets.

Gut length is not the only important morphometric character of the digestive tract relating to diet in fishes because gut surface area (Montgomery 1977; Frierson and Foltz 1992; Horn et al. 2005), and gut mass (Ribble and Smith 1983; Fuentes and Cancino 1990; King et al. 1994; Lloret and Planes 2003) are also important. Relative gut mass (RGM = gut mass [g]/body mass [g]) takes body mass into account and may be an informative method for determining the relative quantity of tissue dedicated to the gut in different species of fish with different diets. Fuentes and Cancino (1990) found that the RGM was higher in individuals of *Girella laevis* consuming a 50% algae diet than in individuals of this species consuming a carnivorous diet. RGM also has been found to increase in tadpoles fed an herbivorous diet in comparison to those fed a carnivorous diet (Tolosa and Diamond 1990). Comparisons of RGM between herbivorous and carnivorous fishes appear to be lacking in the literature. When used together with gut length, RGM ought to provide a useful measure for comparing total gut size in herbivores and carnivores.

In the present study, the effects of ontogeny, diet, and phylogeny on three gut dimensions were examined in four related species of pricklyback fishes (Family Stichaeidae), all of which occur in the same rocky intertidal habitat on the central California coast. Three of the species, *Cebidichthys violaceus*, *Xiphister mucosus*, and *X. atropurpureus*, are members of the Xiphisterini, whereas the fourth species, *Anoplarchus purpureus*, is a member of the Alectriini (Fig. 1; Stoddard 1985; K.M. Stoddard and M.H. Horn, unpublished data). Follett and Anderson (1990) expressed support for the



**Fig. 1** Partial phylogenetic hypothesis for two tribes in the subfamily Xiphisterinae, family Stichaeidae, based on analysis of 49 morphological characters (Stoddard 1985). Characters were analyzed and phylogenetic relationships assessed using PHYSYS software (consistency index 58.197); the relationships were corroborated using PAUP software (K.M. Stoddard and M.H. Horn, unpublished data). Species studied are denoted in *bold* with (H) for herbivore and (C) for carnivore. Other Xiphisterini represents four species, other Alectriini represents four species and Chirolophini represents eight species

Stoddard phylogenetic hypothesis in their paper describing two new prickleback species in the xiphisterine clade. The Xiphisterini appears to be the clade in which herbivory evolved in the Stichaeidae (Chan et al. 2004; German et al. 2004), whereas the Aletriini apparently comprises only carnivorous species (D.P. German and M.H. Horn, unpublished data). *A. purpurascens* and *X. atropurpureus* remain 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 (Horn et al. 1982; Setran and Behrens 1993). Because these pricklebacks are closely related, co-occur in rocky shore habitats, and can be obtained at similar ontogenetic stages, they represent ideal fish species in which to compare the effects of ontogeny, diet, and phylogeny on gut dimensions.

To investigate these effects on gut length and mass, the three gut dimension parameters (RGL, ZI, and RGM) were compared in three feeding 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-raised juveniles (abbreviated as  $L_{60-75}$ ) representing a subset of  $w_{30-40}$  juveniles grown on a high-protein artificial 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 gut dimensions in  $w_{30-40}$  versus  $W_{60-75}$  fish allowed us to test for an ontogenetic effect, in  $W_{60-75}$  versus  $L_{60-75}$  fishes for a dietary effect, and among species within each feeding category for a phylogenetic effect. This design thus allowed us to distinguish the effects of ontogeny from the effects of diet on the measured gut dimension parameters within species and to detect any phylogenetic influences among species on these gut dimensions. Gut contents were analysed in  $w_{30-40}$  and  $W_{60-75}$  fishes to confirm that they, at the time of the study, were consuming the diets reported in the literature.

We hypothesized the following outcomes relating to ontogeny, diet, and phylogeny: (1) both *C. violaceus* and *X. mucosus* will increase their RGL, ZI, and RGM with increase in size as they shift from a carnivorous diet to a more herbivorous diet; (2)  $L_{60-75}$  fish of *C. violaceus* and *X. mucosus* will show the same gut dimensions as  $W_{60-75}$  fish suggesting a genetic predisposition for an ontogenetic increase in gut dimensions and facilitation of digestion of an algal diet in these two species; (3) although carnivorous, *X. atropurpureus* will show gut dimensions that match those in *X. mucosus* in all feeding categories because these two fish species share a common ancestor, and because they also display similar digestive enzyme activities despite differences in diet (Chan et al. 2004; German et al. 2004); and (4) the three members of the Xiphisterini will display gut dimension parameters more similar to each other and different from *A. purpurascens*, the alecrtiine, which will exhibit smaller RGL, ZI, and RGM in all feeding categories and

show no ontogenetic change because of its animal diet and membership in a carnivorous clade.

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## Materials and methods

### Fish collection, maintenance and feeding experiment

Juveniles of each of the four prickleback species were collected by hand and dipnet at low tide from August 2000 to June 2001 from rocky intertidal habitat on the central California coast near Piedras Blancas (35°40'N, 121°15'W) and, for *X. mucosus*, also from Diablo Canyon (Stillwater Cove, 35°12'N, 120°51'W). Forty small juveniles of each species were transported, as in the 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 at the laboratory, 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 near-shore habitat (Horn et al. 1983). Twenty individuals of each species were selected randomly for feeding a high-protein artificial diet and placed individually into numbered 4.1-l compartments with nylon screen walls submerged inside 235-l tanks (ten compartments/tank) under a 12L:12D cycle as per Fris and Horn (1993). The remaining 20 fishes of each species were used for gut dimension analysis. All fish were held without food for 1 to 2 days to ensure hunger at the onset of the feeding experiment or to ensure a similar nutritional state among fishes being used for gut dimension analysis. Fishes have been shown to change gut lengths in response to nutritional state (e.g., full versus empty guts; Fuentes and Cancino 1990; Cleveland and Montgomery 2003). 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 fishes were fed the same high-protein artificial diet designed for marine fish larvae (BioKyowa, Cape Girardeau, MO, USA; ≥55% protein [mainly from marine animal sources], ≤10% lipid, and <4% crude fiber) three to four times a day to satiation until they reached 65–75 mm SL (*X. atropurpureus*, *X. mucosus*, and *C. violaceus*) or 60–70 mm SL (*A. purpurascens*), sizes well beyond that (45 mm SL) at which *C. violaceus* and *X. mucosus* shift to herbivory (Horn et al. 1982). Upon reaching the desired size ( $L_{60-75}$ ), 20 individuals of each species were used for analysis of gut dimensions. 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, predictably, the corresponding gut dimensions. Fishes grew at approximately the same rate in the laboratory feeding experiment as they did in the wild, taking 3–4 months to grow from  $w_{30-40}$  size to  $L_{60-75}$  or  $W_{60-75}$  size.

All fishes used in this study were juveniles, as no swollen ovaries or testes 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 and *X. atropurpureus* at 110–115 mm SL (Wingert 1974) and *A. purpurascens* at 85–110 mm SL (Coleman 1992). The fishes used in this study also were used for biochemical analyses of digestive enzyme activities (German et al. 2004) and histochemical and enzyme histochemical analyses of storage and absorption in the digestive system (Gawlicka and Horn 2005).

Handling of fish from capture to euthanization was conducted under approved protocol 99-R-10 of the Institutional Animal Care and Use Committee at California State University, Fullerton.

#### Fish dissection and measurements

Fishes were euthanized with an overdose of MS-222 ( $1 \text{ g l}^{-1}$  seawater), weighed (body mass  $\pm 0.001 \text{ g}$ ), measured (SL  $\pm 1 \text{ mm}$ ) and dissected on a chilled cutting board ( $\sim 4^\circ\text{C}$ ). The gut was removed by cutting just anterior to the stomach and at the anus. The gut was then uncoiled, without stretching, and the total gut length (GL  $\pm 0.1 \text{ mm}$ ) and gut mass (GM  $\pm 0.001 \text{ g}$ ) were measured. If present, the stomach and intestinal contents were pushed out prior to weighing the gut to allow for a more accurate measurement of gut mass. Then RGL (RGL = gut length [mm]/SL [mm]) and relative gut mass (RGM = gut mass [g]/body mass [g]) were calculated. The different species varied in body mass, and *C. violaceus* was significantly heavier than the three other species in the  $W_{60-75}$  and  $L_{60-75}$  categories (Table 3). Therefore, Zihler's index was used to account for differences in body mass among the species. All four species, however, are similar in shape because xiphisterine and alectriine stichaeids are elongate, eel-like fishes.

#### Gut content analysis

Ten  $w_{30-40}$  and ten  $W_{60-75}$  fishes of each species were euthanized with MS-222 ( $1 \text{ g l}^{-1}$  seawater) and frozen on dry ice within 3 h of capture and used for gut content analysis. The fishes were defrosted and dissected, and the gut removed as described earlier. The stomach and intestine contents were pushed out with the blunt side of a razorblade into a Petri dish. These contents were then suspended uniformly in water and analyzed under a dissecting microscope (equipped with

a net reticle,  $10 \times 10 \mu\text{m}^2$ ) using 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 each species and feeding category ( $w_{30-40}$  or  $W_{60-75}$ ).

#### Statistical analysis

The significance of ontogenetic, dietary and phylogenetic effects was tested for (1) each gut dimension parameter, and (2) all three measurements combined in each of the four species. The gut dimension parameters were compared individually between  $w_{30-40}$  and  $W_{60-75}$  fishes within each species to test for an ontogenetic effect and between  $W_{60-75}$  and  $L_{60-75}$  fishes in each species 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 body mass were made within each feeding category using one-way ANOVA. Interspecific comparisons of the gut dimension parameters were made within each feeding category using ANCOVA with body mass as a covariate, and, when body mass was not found to be a significant covariate, one-way ANOVA was used to analyze the data for that gut dimension. ANOVA and ANCOVA were followed by Tukey's HSD multiple-comparisons test with a family error rate set at  $P = 0.05$  using Minitab statistical software (version 13, State College, PA, USA). Prior to the analyses, Levene's test for equal variances was used to determine whether the data were suitable for the *t*-tests, ANOVA, and ANCOVA. Zihler's index for  $W_{60-75}$  and  $L_{60-75}$  fishes did not pass Levene's test, so the data were log-transformed before performing the ANOVA and ANCOVA, respectively.

The combined data for gut dimension parameters were analyzed in each species using non-metric multi-dimensional scaling (MDS) to display graphically the overall patterns of gut dimensions for ontogenetic ( $w_{30-40}$  versus  $W_{60-75}$ ) and dietary ( $W_{60-75}$  versus  $L_{60-75}$ ) effects. Multivariate analyses of similarity (ANOSIM) were used to test the ontogenetic ( $w_{30-40}$  versus  $W_{60-75}$ ), dietary ( $W_{60-75}$  versus  $L_{60-75}$ ) and phylogenetic (*X. mucosus* vs. *X. atropurpureus*; xiphisterine species versus the alectriine *A. purpurascens*) hypotheses of the combined gut dimension data. The outputs from ANOSIM, called *R* statistics, are based on the differences in mean ranks between groups and within groups (Clarke 1993). *R* values range from 0, no difference, to 1, very different. Statistical significance was examined by permutations ( $> 1,000$ ) of the grouping vector and the distribution under the null model; differences were considered significant at  $P \leq 0.05$ . MDS and ANOSIM were performed using PRIMER statistical software (version 5, Plymouth, UK).



## Results

### Ontogenetic effects

In this section ontogenetic comparisons ( $w_{30-40}$  vs.  $W_{60-75}$ ) of gut contents (Table 1, 2) and of each gut dimension parameter for each of the four species (Table 3) are reported. In addition, the combined gut dimension data in the  $w_{30-40}$  and  $W_{60-75}$  feeding categories of the four species (Table 4 and Fig. 2) are presented.

The  $w_{30-40}$  fish of all four species were carnivorous, feeding on a variety of small invertebrates, dominated by harpacticoid copepods in *C. violaceus*, *X. atropurpureus*,

and *A. purpureus*, and gammarid amphipods in *X. mucosus*. The predicted shift in diet was clear for *C. violaceus* and *X. mucosus*, as algae comprised 0.0 and 2.2% in  $w_{30-40}$  fish, but 70.9 and 53.7%, respectively, in  $W_{60-75}$  fish. The  $W_{60-75}$  fish of *X. atropurpureus* and *A. purpureus* were carnivorous, with animal material comprising 90.3 and 89.1%, respectively, of gut contents and gammarid amphipods as the dominant item (70.1 and 31.6%, respectively).

*Cebidichthys violaceus*, *X. mucosus*, and *X. atropurpureus* all showed significant ontogenetic increases in RGL and ZI, whereas *A. purpureus* showed no ontogenetic change in RGL and actually decreased ZI with increase in size. Both *C. violaceus* and *X. atropurpureus*

**Table 1** Gut contents of *Cebidichthys violaceus* (Cv) and *Xiphister mucosus* (Xm) in both the small-wild ( $w_{30-40}$ ) and large-wild ( $W_{60-75}$ ) categories ( $n = 10$  for all groups)

Gut item	Cv $w_{30-40}$	N/10	Cv $W_{65-75}$	N/10	Xm $w_{30-40}$	N/10	Xm $W_{65-75}$	N/10
Polychaetes	16.0 ± 9.8	3	–	–	0.9	1	3.9	1
Crustaceans								
Gammarid amphipods	11.8 ± 5.5	5	24.5 ± 8.1	9	91.5 ± 4.4	10	38.2 ± 9.97	10
Harpacticoid copepods	64.6 ± 13.8	8	0.2	1	–	–	0.2	1
Isopods	–	–	–	–	–	–	0.1	1
Ostracods	0.9	1	–	–	–	–	1.4	1
Other crustacean parts	1.8	1	2.5	1	5.5	2	–	–
Algae								
Rhodophyta	–	–	68.0 ± 9.2	9	2.2	1	20.9 ± 8.1	6
Chlorophyta	–	–	2.9	1	–	–	32.8 ± 10.7	6

Values are mean(%) ± SEM from a point-contact method of quantifying gut content. N/10 indicates the number of fish out of ten that ingested a particular item

**Table 2** Gut contents of *X. atropurpureus* (Xa) and *A. purpureus* (Ap) in both the small-wild ( $w_{30-40}$ ) and large-wild ( $W_{60-75}$ ) categories ( $n = 10$  for all groups)

Gut item	Xa $w_{30-40}$	N/10	Xa $W_{60-75}$	N/10	Ap $w_{30-40}$	N/10	Ap $W_{60-75}$	N/10
Nemertean worms	–	–	–	–	–	–	17.5 ± 10.1	3
Molluscs								
Littorine snails	10.0	1	–	–	–	–	–	–
Tegula snails	–	–	–	–	9.5	2	–	–
Bivalves	–	–	0.8	1	–	–	–	–
Limpets	–	–	–	–	–	–	0.2	1
Polychaetes	2.1	1	4.5	1	15.6 ± 7.9	3	16.8	2
Crustaceans								
Gammarid amphipods	13.4 ± 8.0	3	70.1 ± 10.4	3	19.9 ± 10.8	4	31.6 ± 10.7	8
Harpacticoid copepods	48.5 ± 13.9	7	7.0	2	44.3 ± 10.0	10	7.3	2
Isopods	3.33	1	–	–	–	–	–	–
Ostracods	6.19	3	–	–	–	–	0.61	1
Porcellanid crabs	–	–	7.1	1	–	–	–	–
Hermit crabs	1.3	1	–	–	–	–	–	–
Other crustacean parts	9.5	2	0.83	1	1.7	1	14.6 ± 9.2	4
Echinoderms								
Urchin test	–	–	–	–	–	–	0.2	1
Fish								
Fin rays	–	–	–	–	–	–	0.4	1
Algae								
Rhodophyta	–	–	9.71 ± 5.72	3	7.0	1	2.6	2
Chlorophyta	1.0	1	–	–	0.8	1	–	–
Angiosperms								
Surfgrass	–	–	–	–	–	–	8.3	1

Values are mean percent ± SEM from a point-contact method of quantifying gut content. N/10 indicates the number of fish out of ten that ingested a particular item

**Table 3** Results of two-tailed *t* tests for RGL, ZI and RGM from the ontogenetic ( $w_{30-40}$  versus  $W_{60-75}$ ) and dietary ( $W_{60-75}$  versus  $L_{60-75}$ ) perspectives in *C. violaceus*, *X. mucosus*, *X. atropurpureus* and *A. purpureus*

Fish species	RGL	ZI	RGM
Ontogenetic comparison $w_{30-40}$ versus $W_{60-75}$			
<i>C. violaceus</i>	$t = -9.86$ $P < 0.001$	$t = -10.02$ $P < 0.001$	$t = 2.58$ $P = 0.014$
<i>X. mucosus</i>	$t = -6.01$ $P < 0.001$	$t = -6.64$ $P < 0.001$	$t = -0.17$ $P = 0.865$
<i>X. atropurpureus</i>	$t = -10.97$ $P < 0.001$	$t = -19.80$ $P < 0.001$	$t = 3.49$ $P = 0.001$
<i>A. purpureus</i>	$t = 1.76$ $P = 0.090$	$t = 2.23$ $P = 0.032$	$t = 1.08$ $P = 0.287$
Dietary comparison $W_{60-75}$ versus $L_{60-75}$			
<i>C. violaceus</i>	$t = 1.99$ $P = 0.050$	$t = 2.60$ $P = 0.015$	$t = -2.91$ $P < 0.001$
<i>X. mucosus</i>	$t = -1.73$ $P = 0.090$	$t = -2.05$ $P = 0.048$	$t = 1.42$ $P = 0.170$
<i>X. atropurpureus</i>	$t = 8.12$ $P < 0.001$	$t = 8.37$ $P < 0.001$	$t = -2.25$ $P = 0.040$
<i>A. purpureus</i>	$t = -2.21$ $P = 0.030$	$t = -2.62$ $P = 0.012$	$t = 0.63$ $P = 0.530$

$df = 18$  for all comparisons. For actual RGL, ZI and RGM values, see Table 5

*purpureus* showed significant ontogenetic increases in RGM, whereas *X. mucosus* and *A. purpureus* did not. From a multivariate perspective, significant ontogenetic changes in gut dimensions were observed in *C. violaceus*, *X. mucosus* and *X. atropurpureus*, but not in *A. purpureus*.

As depicted in the MDS plot, the gut dimension parameters in *A. purpureus* differed markedly from those in *C. violaceus*, *X. mucosus*, and *X. atropurpureus* (Fig. 2). Little ontogenetic change was apparent in

**Table 4** Results of ANOSIM comparisons (*R* statistics) of combined gut dimension data from the ontogenetic ( $w_{30-40}$  versus  $W_{60-75}$ ) and dietary ( $W_{60-75}$  versus  $L_{60-75}$ ) perspectives in four species of prickleback fishes

Fish species	<i>R</i>	<i>P</i>
Ontogenetic comparison $w_{30-40}$ versus $W_{60-75}$		
<i>Cebidichthys violaceus</i>	0.81	< 0.01
<i>Xiphister mucosus</i>	0.51	< 0.01
<i>Xiphister atropurpureus</i>	0.99	< 0.01
<i>Anoplarchus purpureus</i>	0.06	0.06
Dietary comparison $W_{60-75}$ versus $L_{60-75}$		
<i>C. violaceus</i>	0.12	< 0.01
<i>X. mucosus</i>	0.02	0.24
<i>X. atropurpureus</i>	0.68	< 0.01
<i>A. purpureus</i>	0.07	0.07
Phylogenetic effects $W_{60-75}$		
<i>X. mucosus</i> versus <i>X. atropurpureus</i>	0.05	0.07
Xiphisterine species versus <i>A. purpureus</i>	0.98	< 0.01
Phylogenetic effects $L_{60-75}$		
<i>X. mucosus</i> versus <i>X. atropurpureus</i>	0.72	< 0.01
Xiphisterine species versus <i>A. purpureus</i>	0.65	< 0.01

Phylogenetic effects were assessed comparing *X. mucosus* and *X. atropurpureus*, as well as the three xiphisterine species (*C. violaceus*, *X. mucosus* and *X. atropurpureus*) versus the alectriine, *A. purpureus*. Differences are considered significant at  $P \leq 0.05$

*A. purpureus* compared to the three members of the Xiphisterini, in which the combined gut dimensions changed considerably with increase in size. The  $w_{30-40}$  fish of *C. violaceus* displayed similar gut dimensions to  $W_{60-75}$  fish of *X. mucosus* and *X. atropurpureus*, but shifted away in  $W_{60-75}$  fish, reflecting an apparent difference between *C. violaceus* and the two species of *Xiphister* in combined gut dimension parameters for larger, wild-caught juveniles.

### Dietary effects

In this section the effects of a high-protein artificial diet ( $W_{60-75}$  versus  $L_{60-75}$  fish) on each of the gut dimension parameters are reported in each of the four species (Table 3). In addition, the results of the combined gut dimension data in the  $W_{60-75}$  and  $L_{60-75}$  feeding categories of the four species (Table 4; Fig. 3) are presented.

Significant effects of diet on RGL were seen in *C. violaceus*, *X. atropurpureus*, and *A. purpureus*, whereas *X. mucosus* did not change its RGL in  $L_{60-75}$  fish relative to  $W_{60-75}$  fish. Both *C. violaceus* and *X. atropurpureus* significantly decreased their RGL in  $L_{60-75}$  fish relative to  $W_{60-75}$  fish, whereas *A. purpureus* increased its RGL on the high-protein artificial diet. Significant decreases in ZI were observed in  $L_{60-75}$  fish relative to  $W_{60-75}$  fish of *C. violaceus* and *X. atropurpureus*, whereas *X. mucosus* and *A. purpureus* increased their ZI on the high-protein artificial diet.  $L_{60-75}$  fish of *C. violaceus* and *X. atropurpureus* significantly decreased their RGM in comparison to  $W_{60-75}$  fish, whereas *X. mucosus* and *A. purpureus* showed no change in their RGM on this diet. From a multivariate perspective, significant effects of diet on gut dimensions were shown for *C. violaceus* and *X. atropurpureus*, but not for *X. mucosus* and *A. purpureus*. This pattern is less apparent in the MDS plot as all four species showed some changes in gut dimensions on the high-protein artificial diet (Fig. 3).

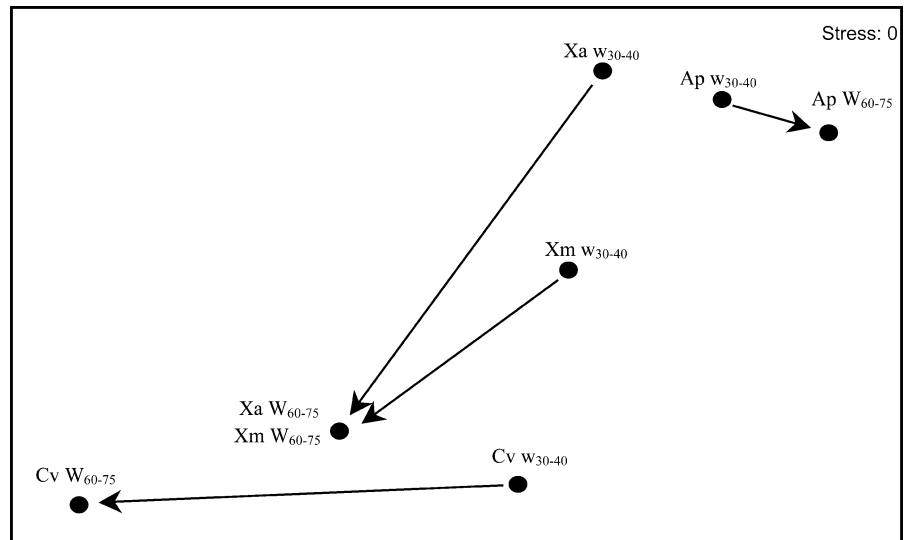
### Phylogenetic effects

In this section comparisons of each of the three gut dimension parameters among the four species in each of the three feeding categories ( $w_{30-40}$ ,  $W_{60-75}$ ,  $L_{60-75}$ ; Table 5) are reported. In addition, the MDS plots from the ontogenetic (Fig. 2) and dietary (Fig. 3) perspectives described earlier are used to compare species trajectories on the plots and to emphasize the phylogenetic relationships among the four species.

### $w_{30-40}$ fish

The  $w_{30-40}$  fish of *C. violaceus* and *A. purpureus* were the heaviest of the four species in this size category, followed by *X. atropurpureus* and *X. mucosus*. The  $w_{30-40}$  fish of *C. violaceus* displayed significantly larger RGL

**Fig. 2** Non-metric multidimensional scaling plot of all three gut dimension parameters combined for each species as a function of ontogeny ( $w_{30-40}$  and  $W_{60-75}$  categories) in *C. violaceus* (*Cv*), *X. mucosus* (*Xm*), *X. atropurpureus* (*Xa*) and *A. purpureus* (*Ap*). Arrows indicate magnitude of ontogenetic shifts in gut dimensions. The stress value indicates that the plot fits well (i.e., values  $<0.1$ ) into two-dimensional space. See Materials and methods for further details



than the three other species, which did not differ from one another, and body mass was not a significant covariate. The  $w_{30-40}$  fish of *C. violaceus* exhibited the largest ZI and RGM, followed by *X. mucosus*, which displayed significantly larger ZI and RGM than *X. atropurpureus* and *A. purpureus*, which, in turn, were not different from one another in either of these gut dimensions. Body mass was a significant covariate for RGM but not for ZI. RGM was negatively correlated with body mass in  $w_{30-40}$  fish ( $r = -0.505$ ,  $P < 0.001$ ), as *X. mucosus* displayed the smallest body mass but largest RGM, and *A. purpureus* the heaviest body mass and smallest RGM.

#### *W*<sub>60-75</sub> fish

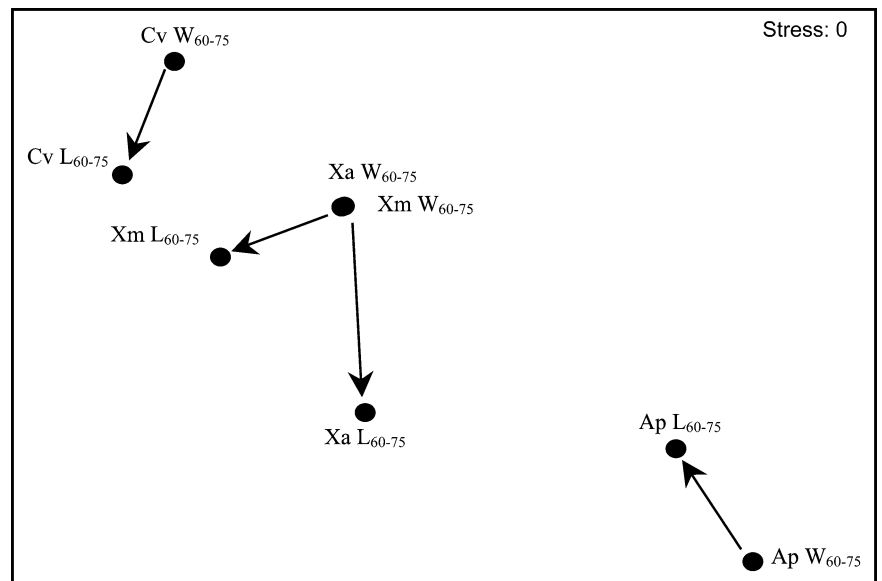
The  $W_{60-75}$  fish of *C. violaceus* showed significantly larger body mass than *A. purpureus*, which in turn exhibited significantly greater body mass than the two

*Xiphister* species, which were not different from one another. The  $W_{60-75}$  fish of *C. violaceus* exhibited the largest RGL, followed by the two species of *Xiphister*, and *A. purpureus* displayed the smallest RGL. The pattern of interspecific differences in  $W_{60-75}$  fish for ZI was identical to those for RGL. The  $W_{60-75}$  fish of *C. violaceus* and *X. mucosus* showed the largest RGM, and both possessed significantly greater RGM than that of *X. atropurpureus*, which, in turn, exhibited significantly greater RGM than that of *A. purpureus*. Body mass was not a significant covariate for any of the gut dimension parameters in  $W_{60-75}$  fish, thus allowing for comparisons of the different species based on diet and phylogeny.

#### *L*<sub>60-75</sub> fish

The  $L_{60-75}$  fish of *C. violaceus* were the heaviest of the four species, and the three other species were not sig-

**Fig. 3** Non-metric multidimensional scaling plot of all three gut dimension parameters combined for each species as a function of diet ( $W_{60-75}$  and  $L_{60-75}$  categories) in *C. violaceus* (*Cv*), *X. mucosus* (*Xm*), *X. atropurpureus* (*Xa*) and *A. purpureus* (*Ap*). Arrows indicate magnitude of diet-related changes in gut dimensions. The stress value indicates that the plot fits well (i.e., values  $<0.1$ ) into two-dimensional space



**Table 5** Interspecific comparisons of body mass (BM), RGL, ZI and RGM in three feeding categories ( $w_{30-40}$ ,  $W_{60-75}$  and  $L_{60-75}$ ) of *C. violaceus* (Cv), *X. mucosus* (Xm), *X. atropurpureus* (Xa) and *A. purpurescens* (Ap)

Fish species	SL	BM	RGL	ZI	RGM
Small wild-caught ( $w_{30-40}$ )					
Cv	37.90 ± 0.35	0.321 ± 0.010 c	0.77 ± 0.01 b	4.27 ± 0.06 c	0.056 ± 0.005 b
Xm	34.90 ± 0.47	0.169 ± 0.009 a	0.59 ± 0.01 a	3.75 ± 0.10 b	0.062 ± 0.003 c
Xa	38.60 ± 0.35	0.266 ± 0.007 b	0.55 ± 0.01 a	3.33 ± 0.04 a	0.043 ± 0.004 a
Ap	37.60 ± 0.41	0.323 ± 0.011 c	0.57 ± 0.01 a	3.11 ± 0.05 a	0.035 ± 0.002 a
Species		$F_{3, 76} = 59.57$ $P < 0.001$	$F_{3, 76} = 61.47$ $P < 0.001$	$F_{3, 76} = 56.49$ $P < 0.001$	$F_{3, 75} = 9.13$ $P < 0.001$
Body mass		–	$F_{1, 75} = 0.04$ $P = 0.835$ NS	$F_{1, 75} = 3.53$ $P = 0.064$ NS	$F_{1, 75} = 4.41$ $P = 0.039$ S
Large wild-caught ( $W_{60-75}$ )					
Cv	69.93 ± 0.75	1.982 ± 0.064 c	0.98 ± 0.02 c	5.46 ± 0.10 c	0.065 ± 0.003 c
Xm	69.73 ± 0.61	1.308 ± 0.034 a	0.73 ± 0.01 b	4.63 ± 0.09 b	0.064 ± 0.003 c
Xa	71.78 ± 0.86	1.337 ± 0.052 a	0.71 ± 0.01 b	4.66 ± 0.05 b	0.050 ± 0.002 b
Ap	66.30 ± 1.07	1.690 ± 0.087 b	0.53 ± 0.01 a	2.95 ± 0.05 a	0.037 ± 0.002 a
Species		$F_{3, 76} = 26.43$ $P < 0.001$	$F_{3, 76} = 168.65$ $P < 0.001$	$F_{3, 76} = 191.26$ $P < 0.001$	$F_{3, 76} = 36.77$ $P < 0.001$
Body mass		–	$F_{1, 75} = 1.14$ $P = 0.288$ NS	$F_{1, 75} = 0.64$ $P = 0.426$ NS	$F_{1, 75} = 3.37$ $P = 0.070$ NS
Large laboratory-fed ( $L_{60-75}$ )					
Cv	68.70 ± 0.41	2.016 ± 0.050 b	0.95 ± 0.01 c	5.16 ± 0.05 d	0.054 ± 0.004 c
Xm	67.25 ± 0.48	1.401 ± 0.030 a	0.77 ± 0.02 b	4.89 ± 0.09 c	0.066 ± 0.002 d
Xa	70.23 ± 0.41	1.137 ± 0.027 a	0.60 ± 0.01 a	4.04 ± 0.05 b	0.043 ± 0.002 b
Ap	60.50 ± 0.82	1.388 ± 0.141 a	0.58 ± 0.02 a	3.20 ± 0.08 a	0.039 ± 0.003 a
Species		$F_{3, 76} = 23.16$ $P < 0.001$	$F_{3, 75} = 36.82$ $P < 0.001$	$F_{3, 75} = 106.45$ $P < 0.001$	$F_{3, 76} = 42.58$ $P < 0.001$
Body mass		–	$F_{1, 75} = 11.74$ $P = 0.001$ S	$F_{1, 75} = 8.19$ $P = 0.005$ S	$F_{1, 75} = 1.14$ $P = 0.290$ NS

Values are mean ( $\pm$  SEM,  $n = 20$ ). Interspecific comparisons of BM were analyzed with one-way ANOVA and Tukey's HSD with a family error rate of  $P = 0.05$ . Interspecific comparisons of gut dimension parameters within a feeding category were analyzed with ANCOVA (using body mass as a covariate) and Tukey's HSD with a family error rate of  $P = 0.05$ . ANOVA was used for analyses in which body mass was not a significant covariate. Values for a parameter and feeding category that share a letter are not significantly different. SL is provided for reference and was not analyzed statistically

nificantly different from each other with respect to body mass in this feeding category. The  $L_{60-75}$  fish of *C. violaceus* still exhibited the largest RGL, followed by *X. mucosus*. The  $L_{60-75}$  fish of *X. atropurpureus* and *A. purpurescens* displayed RGLs that were not significantly different from one another, and both RGLs were significantly lower than those in *C. violaceus* and *X. mucosus*. Body mass was a significant covariate for RGL, and this gut dimension was positively correlated with body mass in  $L_{60-75}$  fish ( $r = 0.797$ ,  $P < 0.001$ ). Significant interspecific differences for ZI were detected among all species in  $L_{60-75}$  fish, with *C. violaceus* exhibiting the largest ZI, followed by *X. mucosus*, *X. atropurpureus*, and *A. purpurescens*, in that order. Body mass was a significant covariate for ZI, and a positive correlation was detected for body mass and ZI in this feeding category ( $r = 0.593$ ,  $P < 0.001$ ). Significant interspecific differences for RGM were detected among all species in  $L_{60-75}$  fish, with *X. mucosus* exhibiting the highest RGM, followed by *C. violaceus*, *X. atropurpureus*, and *A. purpurescens*, in that order. Body mass was not a significant covariate for RGM.

#### Overall patterns

Despite differences in diet, the two species of *Xiphister* showed similar ontogenetic increases in RGL and ZI and shared similar values of these two gut dimension parameters in  $W_{60-75}$  fish (Table 5). The ANOSIM and MDS plots also showed that  $W_{60-75}$  fish of these sister taxa were not different from one another from the multivariate perspective (Table 4; Figs. 2, 3). Further, all three xiphisterine species showed significant ontogenetic changes in their gut dimensions from the multivariate perspective, whereas *A. purpurescens* did not (Table 4). Differences also were found between *A. purpurescens* and the members of the Xiphisterini when the latter were combined into one statistical group (Table 4). These results suggest a phylogenetic signal affecting the gut dimensions in these species. *Xiphister mucosus* and *X. atropurpureus*, however, responded differently when fed the same high-protein artificial diet, and, as a result, were significantly different in  $L_{60-75}$  fish according to the ANOSIM (Table 4). Thus, support for a phylogenetic effect on gut dimensions in *X. atro-*



*purpureus* in this feeding category was somewhat diminished. Nevertheless,  $L_{60-75}$  fish of the three xiphisterine species formed a group that was significantly different from *A. purpureus* (Table 5).

## Discussion

Overall, the results of this study provided evidence for strong ontogenetic and phylogenetic influences on gut dimensions in the four prickleback species. Dietary effects were less important, but still reflected some phenotypic plasticity in these herbivorous and carnivorous fishes. Some, but not all, of the hypotheses were supported. First, as expected, both *C. violaceus* and *X. mucosus* increased their consumption of algae with increase in size and showed corresponding ontogenetic increases in RGL and ZI. Second, also as predicted, *C. violaceus* and *X. mucosus* showed the same ontogenetic trajectory in RGL and ZI after eating the high-protein artificial diet, indicating that their gut lengths are relatively fixed and do not change even if raised on a nutrient-rich, low-fiber diet that is different from the food they consume in nature. Third, as expected, *X. atropurpureus* remained carnivorous with increase in size and matched *X. mucosus* in RGL and ZI from the ontogenetic perspective. Contrary to the predictions, however, *X. atropurpureus* differed from *X. mucosus* in response to the high-protein artificial diet, challenging the hypothesis that their shared common ancestry may constrain anatomical changes in the gut of *X. atropurpureus*. Fourth, as hypothesized, *A. purpureus* exhibited the smallest RGL, ZI and RGM of the four prickleback species and exhibited no ontogenetic changes in gut dimensions, corroborating the carnivorous diet and different clade membership of this species. Finally, RGM varied appreciably both within and among species and is discussed in more detail below.

Ontogenetic increases in gut length are well known in marine and freshwater herbivorous fishes (Montgomery 1977; Zihler 1982; Ribble and Smith 1983; Stoner and Livingston 1984; Benavides et al. 1994; Kramer and Bryant 1995a; Gallagher et al. 2001; Drewe et al. 2004). Even carnivorous fishes can increase their gut lengths with increase in standard length, but herbivores tend to show a more rapid increase (Kramer and Bryant 1995a). The ontogenetic increase in gut length seen in *C. violaceus* and *X. mucosus* match this tendency, especially when compared to the carnivorous *A. purpureus*, which actually decreased its gut length with increase in size. The magnitude of the ontogenetic increase of RGL and ZI observed in *X. atropurpureus*, despite this species not exhibiting an ontogenetic dietary shift, suggests that *X. atropurpureus* may be phylogenetically constrained to increase its gut length because it evolved from the same herbivorous ancestor as *X. mucosus*. Both species of *Xiphister* are similar in morphology, and it is therefore not surprising to find that they have similar allometric growth patterns of the gut. In further support of their

shared evolutionary history, German et al. (2004) found that *X. mucosus* and *X. atropurpureus* were most similar to each other among the four prickleback species with respect to the activities of eight different digestive enzymes in the same specimens of these two species as used in the present study. *Xiphister atropurpureus* displayed larger RGL, ZI, and RGM than *A. purpureus* in both  $W_{60-75}$  and  $L_{60-75}$  fish. If diet was the only factor influencing gut dimensions in these fishes, then the carnivorous *X. atropurpureus* and *A. purpureus*, which both feature gammarid amphipods as their primary food item, would be most similar in their gut dimensions. Instead, *X. atropurpureus* exhibited the same “larger” RGL, ZI and RGM as *X. mucosus* in  $W_{60-75}$  fish and gut dimensions that were intermediate to the values found for *X. mucosus* and *A. purpureus* in  $L_{60-75}$  fish.

The gut dimension parameters reported here and digestive enzyme activities reported by German et al. (2004) suggest that *X. atropurpureus* may represent a recent divergence toward carnivory in a primarily herbivorous clade. In support of this contention, members of the tribe Xiphisterini for which dietary information exists (five of nine species) become increasingly herbivorous with age (Cross 1981; Barton 1982; Horn et al. 1982; DPG unpublished data on *Dictyosoma burgeri*, a northwestern Pacific species). Moreover, in the present study, all three xiphisterine species increased their gut dimensions ontogenetically. *X. atropurpureus*, therefore, appears to have retained at least some gut dimension and digestive enzyme features characteristic of its herbivorous ancestry.

The similarities between *X. atropurpureus* and *X. mucosus* in this study did not hold up when these fish were fed the high-protein artificial diet, which contradicts the prediction that these species would be the most similar under all measured circumstances. Because *X. atropurpureus* consumes a carnivorous diet in nature, this fish was expected to show little change in the measured gut dimensions on the high-protein artificial diet, because this diet should be similar in biochemical composition to its natural diet. Crustaceans, such as gammarid amphipods, can be composed of as much as 50% protein and 25–40% lipid (Lehtonen 1996; Lemos and Phan 2001), a diet that may be as nutrient-rich as that administered in the feeding experiment. It remains unknown as to why *X. atropurpureus* altered its gut dimensions on the high-protein artificial diet, but the decrease in gut dimensions in this species may be related to a change in intake.

Differences in growth rates observed between *X. mucosus* and *X. atropurpureus* on the high-protein artificial diet may provide an explanation for their differences in gut dimensions because fishes with faster growth rates have been shown to exhibit a larger RGL at a given size than fishes with slower growth rates (Kramer and Bryant 1995a). Our unpublished data on growth rates obtained during the feeding experiment showed that *X. mucosus* grew faster than *X. atropurpureus* in terms of body mass (*X. mucosus*

0.014 g day<sup>-1</sup>, *X. atropurpureus* 0.009 g day<sup>-1</sup>;  $t = 12.63$ ,  $P < 0.01$ ,  $df = 79$ ) and length (*X. mucosus* 0.41 mm day<sup>-1</sup>, *X. atropurpureus* 0.29 mm day<sup>-1</sup>;  $t = 10.48$ ,  $P < 0.01$ ,  $df = 79$ ). *X. mucosus* also attains a greater maximum size (580 mm SL) than *X. atropurpureus* (300 mm SL; Eschmeyer et al. 1983), and may have evolved to grow more rapidly to achieve the greater size that facilitates a larger gut and an herbivorous diet.

Physiological and morphological traits of the digestive system may be under similar selective pressures in vertebrates (Sabat et al. 1999). In this study, however, *X. atropurpureus* showed plasticity in its gut dimensions on the high-protein artificial diet, whereas *A. purpureus* did not. The opposite was true for these two species with regards to their digestive enzyme activities in the same specimens as used in this study (German et al. 2004). The conflicting responses of gut dimensions and digestive enzyme activities in these two species suggest that the physiological and morphological traits of their digestive systems may be under different selective pressures or are affected on different time scales. Clearly, diet is not the only selective force acting on digestive physiology and morphology in the pricklebacks, because, if it was, *X. atropurpureus* and *A. purpureus* would be more similar in their gut dimensions and digestive enzyme activities.

The relatively small changes in gut length in *C. violaceus* and *X. mucosus* after feeding ad libitum on a high-protein artificial diet strongly supports the hypothesis that these two species are genetically predisposed towards having relatively long digestive tracts. Experiments similar to this one are lacking in other fishes, but several bird species can increase their gut lengths in response to increased dietary fiber content and increased intake (Battley and Piersma 2005). Generally, an increase in intake is cited as the main cause of an increase in gut length in birds (Battley and Piersma 2005). Although intake was not estimated in wild-caught individuals of the pricklebacks in this study (making comparisons of intake between  $L_{60-75}$  and  $W_{60-75}$  fishes impossible), it can be confidently concluded that there were strong differences in dietary fiber content between the high-protein artificial diet (<4% crude fiber) and the red and green algae consumed by *C. violaceus* and *X. mucosus* in nature. Red and green algae of the same genera (*Porphyra* and *Ulva*) as those naturally eaten by *C. violaceus* and *X. mucosus* have fiber contents ranging from 20 to 50% (Wong and Cheung 2000; Bocanegra et al. 2003). In this case, dietary fiber content and any unknown changes in intake appeared to have little effect on gut length in *C. violaceus* and *X. mucosus*. The marked changes in gut dimensions observed in *X. atropurpureus* could be a result of changes in intake, but feeding experiments are necessary to test this hypothesis.

*A. purpureus* was the most carnivorous fish in this study and also exhibited the smallest gut dimensions of the four prickleback species. This species apparently does not compete well with *C. violaceus*, *X. mucosus* or

*X. atropurpureus* for space in the rocky intertidal habitat based on laboratory experiments, as they each force *A. purpureus* to settle on "less desirable" silty substrata (Jones 1981) and, perhaps, to consume a broader range of food items representing whatever prey resources are available. *A. purpureus* only slightly increased its gut dimensions on the high-protein artificial diet, indicating that it is already dedicating enough tissue to its gut to attain the nutrients that it needs.

RGL and ZI have been explored as potential indices to identify the dietary strategy of a fish based on its gut length, but they have limitations for such use. Based on RGL, Al-Hussaini (1947) recognized the feeding guilds of Red Sea fishes as follows: plankton feeders (0.5–0.7), carnivores (0.6–2.4), omnivores (1.3–4.2) and herbivores (3.7–6.0). Kramer and Bryant (1995b) categorized small fishes (50–100 mm SL) by RGL as carnivores (RGL = 0.6–0.8), omnivores (0.8–1.0) or as herbivores (2.5–16.4). Kramer and Bryant (1995b) also classified fishes of small body mass (0.3–3.0 g) according to their ZI as carnivores (ZI = 2.3–3.2), omnivores (2.4–5.8) or as herbivores (11.6–55.0). The RGL of marine herbivorous fishes, however, ranges from 0.5 to greater than 10 (Horn 1989), and use of these indices (RGL and ZI) while ignoring phylogenetic relationships of the fishes being compared can produce misleading results. Elliott and Bellwood (2003) reported that evolutionary history has a strong influence on RGL in chaetodontids, labrids and pomacentrids. Within these families, the authors showed that gut length varies predictably with diet: carnivores < omnivores < herbivores < corallivores. Comparisons of fishes among families, however, proved difficult, as chaetodontids generally possess longer guts than labrids and pomacentrids. For example, a carnivorous chaetodontid has a gut length more similar to an herbivorous pomacentrid.

The pricklebacks had much smaller RGL and ZI than the herbivores studied by Al-Hussaini (1947) or Kramer and Bryant (1995b). These differences are not surprising considering the elongate, eel-like shape of the pricklebacks. The body shape of the pricklebacks led previous authors to caution against making comparisons of gut size in the pricklebacks with those of other fish species (Montgomery 1977; Barton 1982). Furthermore, RGL and ZI are crude measures of gut morphology, and comparisons of these gut measures among fishes with different diets, especially those with completely different evolutionary histories, should be treated with caution.

RGM was used as an estimate of the amount of tissue each prickleback species was dedicating to its digestive tract. RGM (also called digestive somatic index) has been used to determine the feeding states of fishes, as individuals that are actively consuming food have heavier guts than those that are starved (King et al. 1994; Lloret and Planes 2003). Fuentes and Cancino (1990) suggested that RGM is a better indicator of diet than is gut length in fishes after they showed that *G. laevis* altered its RGM, but not RGL, in response to diets of

varying biochemical composition. In the present study, *C. violaceus* and *X. mucosus* consistently exhibited the largest RGM of the four prickleback species in all of the examined feeding categories. Why herbivorous fishes possess heavier guts than carnivorous fishes remains unknown, and further investigations into the advantages of a heavy gut in herbivorous fishes are warranted.

Whereas gut length can vary in response to intake, RGM appears to be more responsive to the biochemical composition of ingested dietary items (Fuentes and Cancino 1990; Starck 1999). Thus, the decrease in RGM observed in  $L_{60-75}$  fish of *C. violaceus* and *X. atropurpureus* may have been a direct reaction in these two species to the relatively high-nutrient load of the high-protein artificial diet. *X. mucosus*, however, actually displayed the largest RGM in all feeding categories and showed no change in RGM in response to the high-protein artificial diet. Perhaps its slender body and lighter mass equate to a minimum threshold RGM under which they cannot fall and still be able to digest algal material. In contrast, *C. violaceus*, with its larger body mass and longer gut, can afford to decrease its gut mass in response to foods of varying biochemical composition.

To our knowledge, this study presents the first attempt to compare gut dimensions in laboratory-fed fish with wild-caught fish of the same species, and it is also one of the few investigations to compare gut lengths of fishes in a phylogenetic context. The members of the prickleback clade in which herbivory appears to have evolved were shown to increase their gut dimensions with increase in body size regardless of diet, whereas a species from the adjacent carnivorous clade showed no such ontogenetic change. Gut dimension parameters can vary widely among different species, especially those of different body shapes and masses, and this study shows the importance of comparing closely related species of similar shape and mass to infer differences in gut dimensions as related to differences in diet.

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