



Evolution of ontogenetic dietary shifts and associated gut features in prickleback fishes (Teleostei: Stichaeidae)

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ARTICLE INFO

Article history:

Received 6 June 2013

Received in revised form 1 November 2013

Accepted 13 November 2013

Available online 21 November 2013

Keywords:

Herbivory

Gut contents

Relative gut length

Digestive biochemistry

Phylogenetic relationships

ABSTRACT

We tested the hypothesis that an ontogenetic dietary shift from carnivory to herbivory or omnivory, and concomitant changes in the gut facilitating digestion of algae, are synapomorphies of the tribes Xiphisterini and Esselenichthyini in the family Stichaeidae (pricklebacks). Previous investigations have revealed that two xiphisterine pricklebacks—*Xiphister mucosus* and *Xiphister atropurpureus*—become herbivorous or omnivorous, respectively, as their bodies grow larger, and that their guts show related changes in length and function. In this study we found that, with increase in size, the basal member of the Xiphisterini, *Phytichthys chirus*, showed an increased proportion of algae in its diet, increased activity of α -amylase and decreased activity of aminopeptidase, all of which support the synapomorphy hypothesis. *Cebidichthys violaceus*, a herbivore in the Esselenichthyini, shows similar ontogenetic changes in diet and digestive tract length and physiology, but these features were not observed in two derived carnivores, *Dictyosoma burgeri* and *Dictyosoma rubrimaculatum*, within the clade. These results suggest that herbivory is isolated to *C. violaceus* within the Esselenichthyini. Allometric relationships of gut length as a function of body size generally follow diet within the Xiphisterini and Esselenichthyini, with herbivores having the longest guts, which become disproportionately longer than body size as the fishes grow, omnivores intermediate gut lengths, and carnivores the shortest. A carnivore from an adjacent clade, *Anoplarchus purpureus*, had the shortest gut, which did not change in length relative to body length as the fish grew. Overall, our results clarify the patterns of dietary evolution within the Stichaeidae and lay the foundation for more detailed studies of dietary and digestive specialization in fishes in the family.

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1. Introduction

The evolution of fishes has been complicated (Near et al., 2013), with many adaptive radiations relating to feeding and locomotion. Indeed, much of fish diversification has something to do with dietary habits (Wainwright et al., 2012), and this dietary focus has led to detailed research on fish movement (Norton and Brainerd, 1993; Giacomini et al., 2013), cranial morphology (Wainwright et al., 2004; Cooper and Westneat, 2009), and jaw kinematics (Hernandez et al., 2008; Ferry-Graham et al., 2010; Wainwright et al., 2012). However, comparatively less attention has been paid to adaptations of the digestive tract for different diets, even though an animal's digestive strategy affects its resource acquisition, behavior, and trophic interactions (Karasov and Martínez del Río, 2007; Barboza et al., 2010). Although a handful of fish species cultured for human consumption (e.g., Gao et al., 2011), or laboratory research (e.g., Roeselers et al., 2011; Ulloa et al., 2011), are well-studied, such investigations are not necessarily focused on discerning how fishes function in the natural world. This lack of focus on fish digestion has led to a gap in the knowledge of fish nutritional ecology

in comparison to that of terrestrial vertebrates (Choat and Clements, 1998; Clements et al., 2009).

One fish family in particular, the family Stichaeidae (pricklebacks), has become a valuable system in which to study responses of the vertebrate digestive tract to dietary variations (Fig. 1; German et al., 2004; Gawlicka and Horn, 2006; Kim et al., in press). This family is characterized by dietary diversity, ontogenetic dietary shifts, convergent evolution of herbivory, and sister taxa with different food habits. Therefore, tracing the evolutionary path of digestive adaptations within the Stichaeidae could provide a model for understanding digestive adaptations in other fish or vertebrate groups. For example, in the Xiphisterini clade of the Stichaeidae (Fig. 1), *Xiphister mucosus* is herbivorous, whereas its sister taxon, *Xiphister atropurpureus*, is omnivorous (Saba, 2004; Boyle and Horn, 2006; German and Horn, 2006). Both species increase their gut length and gut mass in association with an ontogenetic shift from carnivory to herbivory or omnivory that occurs with increase in body size (German and Horn, 2006). An herbivore in a separate stichaeid clade, *Cebidichthys violaceus*, also shows ontogenetic shifts in diet and concomitant increases in gut length and mass, whereas a carnivore from a different stichaeid clade (*Anoplarchus purpureus*) does not (Fig. 1). These examples show that within a fish family, gut length changes with diet (Wagner et al., 2009).

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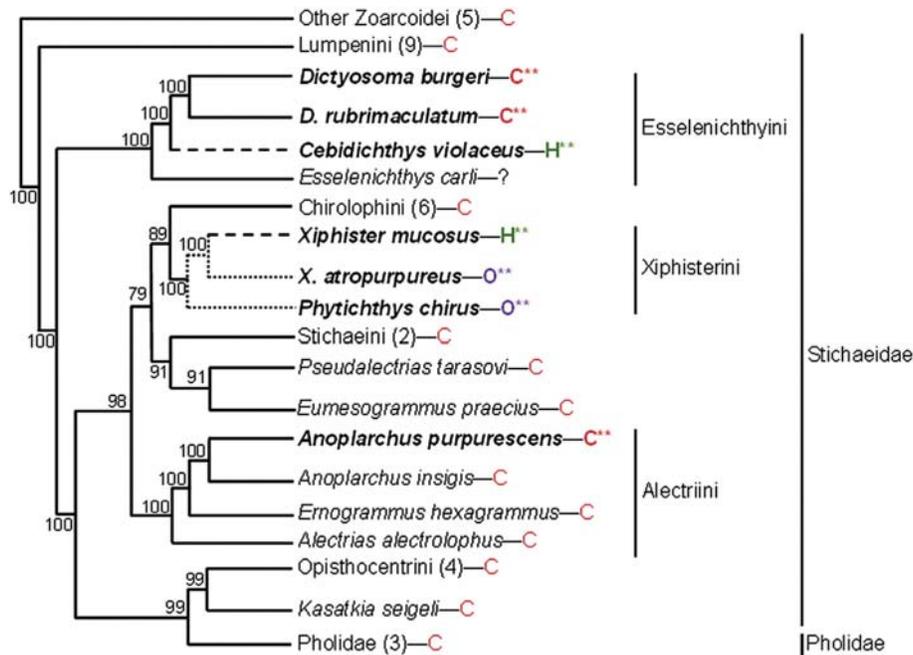


Fig. 1. Phylogenetic relationships of the family Stichaeidae based on 2100 bp of *cytb*, *16s*, and *tomo4c4* genes (Kim et al., in press). Bayesian posterior probabilities are indicated on nodes. Species used in this study bolded and indicated with **. H = herbivory, O = omnivory, C = carnivory. Evolution of herbivory (---) and omnivory (.....) are shown. Numbers in parentheses show number of taxa evaluated at that branch.

Ontogenetic increases in gut length occur in the herbivorous *X. mucosus* and *C. violaceus*, even when these species are raised in the laboratory on a high-quality diet (55% protein, <4% carbohydrate; German et al., 2004). The omnivorous *X. atropurpureus*, however, significantly decreases its gut length when consuming a high-quality diet in the laboratory, whereas *A. purpureus* shows no change. Thus, a spectrum of gut lengths occurs in the Stichaeidae: herbivores with longer guts that do not shorten in response to high-quality food, omnivores that show plasticity of gut length with dietary changes, and carnivores with shorter guts that do not lengthen in response to dietary shifts. These patterns support the hypothesis that generalists show more digestive plasticity than do specialists (Barboza et al., 2010).

Digestive enzyme activities among the four prickleback species also show dietary differences that largely parallel the patterns observed for gut dimensions. In particular, the activity of α -amylase, a carbohydrase, is higher and increases with body size more strongly in the two species of *Xiphister* and *C. violaceus* than in *A. purpureus* in both nature and when raised on the high-quality diet in the laboratory (German et al., 2004; Kim et al., in press). Despite their different natural diets (Saba, 2004; Boyle and Horn, 2006), the two *Xiphister* species are indistinguishable in α -amylase activity and in the activities of most other digestive enzymes (German et al., 2004). On the other hand, *A. purpureus*, shows higher activity of the protease aminopeptidase than do the other species, in the wild and in the laboratory (German et al., 2004).

In this study we examined patterns of adaptive diversification relating to diet by testing the hypothesis that ontogenetic dietary shifts towards omnivory/herbivory and associated changes of the gut facilitating algal digestion in larger fish evolved basally in the Xiphisterini and Esselenichthyini (German et al., 2004; Kim et al., in press). In this regard, *Phytichthys chirus* and the two species of *Dictyosoma* occupy strategic positions in the stichaeid tree (Fig. 1). Hence, we determined whether *P. chirus* and the two species of *Dictyosoma* show ontogenetic changes in diet and gut structure and function that are observed in other members of their respective clades. In particular, if *P. chirus* shows ontogenetic changes in gut structure and function, like the two *Xiphister* species, then the ontogenetic dietary change is a synapomorphy of the Xiphisterini. Similarly, if *Dictyosoma burgeri* and *Dictyosoma rubrimaculatum* show ontogenetic changes in

diet and gut length, then these changes are not unique to *C. violaceus* and at least evolved at the *C. violaceus*–*Dictyosoma* split. If, on the other hand, *P. chirus* and the two *Dictyosoma* species show no such gut features, then xiphisterine herbivory/omnivory evolved in the *Xiphister* species, and herbivory only in *C. violaceus* within the Esselenichthyini (Fig. 1).

Our study had three components. First, we analyzed the gut contents of *P. chirus*, *D. burgeri*, and *D. rubrimaculatum* to determine the proportion of algae in their diets. Second, we measured the activities of the same eight digestive enzymes in *P. chirus* as those studied by German et al. (2004) in *X. mucosus*, *X. atropurpureus*, *C. violaceus*, and *A. purpureus*. Third, we measured gut length in *X. mucosus*, *X. atropurpureus*, *P. chirus*, *C. violaceus*, *D. burgeri*, *D. rubrimaculatum*, and *A. purpureus* to determine digestive tract allometry in these species. These analyses were carried out to reveal the evolutionary path of ontogenetic dietary shifts and related gut changes in the prickleback fishes of the two tribes.

2. Materials and methods

2.1. Fish collections

Fifteen specimens of *P. chirus* (size range 42–156 mm SL) were collected by hand and dipnet from rocky intertidal habitats at low tide on San Juan Island (Dead Man Bay; 48°30.7'N, 123°8.7'W) and Lopez Island (Iceberg Point; 48°25'N, 122°53'W), Washington, in July 2002. An additional 21 specimens (size range 50–150 mm SL) were collected from Dean Man Bay in August 2012 for use in measurements of gut allometry. Following collection, the fish were transported live in aerated seawater in 48-L coolers to Friday Harbor Marine Laboratory and placed in a flow-through wet-table aquarium with circulating seawater at 11 °C pumped directly from Friday Harbor. The fish were held until field identification was verified, and usually less than 4 h. Individuals of *C. violaceus* (n = 72), *X. mucosus* (n = 68), *X. atropurpureus* (n = 73), and *A. purpureus* (n = 84) were collected by hand and dipnet variously from August 2000 to August 2012 at low tide from San Juan Island, Washington, and from rocky intertidal habitats on the central California coast near Piedras Blancas (35°40'N, 121°17'W). *X. mucosus* was also

collected at Diablo Canyon (Stillwater Cove, 35°12'N, 120°51'W). These fishes were used in previous investigations of digestive physiology in pricklebacks (German et al., 2004; German and Horn, 2006; Kim et al., in press), but for this study were used solely for measurements of gut allometry. The fish were either euthanized and dissected immediately in the field, or were transported live to the laboratory at California State University, Fullerton, where they were euthanized and dissected.

Each fish was euthanized with an overdose of MS-222 (1 g L⁻¹ seawater), measured (SL ± 0.5 mm), weighed (body mass, BM ± 0.1 g) and dissected on a cutting board kept on ice (4 °C). Each digestive system was removed by cutting just anterior to the stomach and at the anus. The guts were gently uncoiled, measured (gut length, GL), emptied of their contents by pushing with the blunt side of a razorblade, and weighed. Relative gut length was calculated as RGL = GL (mm) / SL (mm), and Zihler's Index (ZI = GL (mm) / 10[BM (g)]^{1/3}; Zihler, 1982) was used to account for the broad range of body masses among the fish studied (see German and Horn, 2006, for more detail on ZI).

Formalin-fixed specimens of *D. burgeri* (n = 42) and *D. rubrimaculatum* (n = 40) ranging in size from 34 mm to 200 mm SL were obtained from the Fisheries Research Laboratory at Mie University (FRLM), Shima, Japan, and from the National Museum of Nature and Science (NSMT), Tokyo, Japan, for analyses of gut contents and gut allometry (Appendix 1). These fishes were collected from rocky intertidal habitat on the southern (Mie Prefecture) and northern (Ishikawa Prefecture) coasts of Japan between 1970 and 2001. The fish were dissected as described above for the other species, except that they were not dissected on a chilled cutting board. Although some tissue shrinkage is observed in fixed tissues (Hsia et al., 2001; Yan et al., 2003; Jonmarker et al., 2006), the loss can be less than 5% (Jonmarker et al., 2006). For the intent of this study, shrinkage was assumed to be negligible for the determination of the allometric relationships between gut length and body size in the species of *Dictyosoma*. Because only the slopes of these allometric relationships, and not gut lengths alone, were compared among species, we are confident that the measured allometric relationships are legitimate, especially because the differences among species far exceed a 5% threshold.

2.2. Gut content analyses

Gut contents from *P. chirus*, *D. burgeri*, and *D. rubrimaculatum* were suspended uniformly in water and analyzed under a dissecting microscope (equipped with a net reticle, 10 μm × 10 μm, or for the two species of *Dictyosoma*, 13 μm × 13 μm) using a point-contact method as described by German et al. (2004). Gut contents were divided into the following categories: red algae, green algae, the seagrass *Phyllospadix* sp., limpets, polychaetes, amphipods, isopods, crabs, and unknown crustacean parts. The percentage of each item in the gut was determined for each individual fish. The data were then summarized into the categories of algal vs. animal, and the quantities of algal (including *Phyllospadix*) and animal material expressed as percentages of total point-contacts. Gut content analyses for *X. mucosus*, *X. atropurpureus*, *C. violaceus*, and *A. purpureus* were reported in a previous investigation (German and Horn, 2006).

2.3. Tissue homogenates

Immediately following dissection at Friday Harbor Laboratory, whole digestive tracts, including liver, pancreas and gall bladder, from the 15 *P. chirus* collected in 2002 were homogenized individually in ice-cold 50 mM Tris-HCl pH 7.4 [30 volumes (v/w) for individuals >75 mm SL; 60 volumes for individuals <75 mm SL] using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) with a 7-mm generator at a setting of 3.5 for 3 × 30 s. The homogenates then were centrifuged at 9300 g for 2 min at 4 °C and supernatants collected and stored in small aliquots (100–200 μl) at –80 °C. The supernatants

were transported on dry ice to the laboratory at California State University, Fullerton, and stored at –80 °C until used (within three months).

2.4. Assays of digestive enzyme activities

Biochemical assays of eight digestive enzymes—pepsin, trypsin, aminopeptidase, α-amylase, maltase, isomaltase, lipase, and alkaline phosphatase—were conducted on tissue homogenates from *P. chirus* as described for other prickleback species (German et al., 2004). Assays were carried out at 15 °C in duplicate for fish <75 mm SL and in triplicate for those >75 mm SL. Each reaction was run at saturating substrate concentrations as determined for each enzyme with gut tissues from *P. chirus* and read against a blank appropriate for each assay. Enzyme activities were expressed in U (1 μmol of substrate converted to product per min) per g of wet gut tissue.

2.5. Statistical analyses

Correlation analysis was used to determine the degree to which percent algae in the diet and the activity of each of the eight digestive enzymes varied with SL in *P. chirus*. Correlations (Spearman's rank correlation coefficient, ρ) were calculated using SPSS statistical software version 20 (IBM, Armonk, NY, USA), and 95% confidence intervals were determined for the correlation coefficients following Agresti and Finlay (1997). To avoid committing a type I error, the significance level for the individual correlations was adjusted with a Bonferroni correction (Agresti and Finlay, 1997). Gut allometry was examined in *X. mucosus*, *X. atropurpureus*, *P. chirus*, *C. violaceus*, *D. burgeri*, *D. rubrimaculatum*, and *A. purpureus* using linear regression contrasting RGL and ZI with SL. Slopes of regression lines were compared using ANCOVA (Zar, 1999) followed by Tukey's multiple comparisons test with *P* = 0.05 (equation 18.32; Zar, 1999). The digestive enzyme activities of *P. chirus* were compared qualitatively with those measured in the two species of *Xiphister*, *C. violaceus*, and *A. purpureus* (German et al., 2004). Because of differences in study design between the current investigation and the previous ones, no statistical comparisons were made between them, but, rather, general observations made of the magnitude of the measured digestive characteristics. Phylogenetically independent contrasts were avoided because of the low power that would be achieved by comparing only seven species (Blomberg and Garland, 2002).

3. Results

The results of each of the three sets of analyses varied across the range of fish sizes examined, and both significant and non-significant correlations were found. Of the 15 specimens of *P. chirus* examined, 11 individuals (64%) contained algal material in their digestive tracts. The percent algal material in these guts varied from 3.7% to 94.8% of total contents and was significantly and positively correlated with SL (Table 1). No specimens of either *D. burgeri* or *D. rubrimaculatum* had any algal material in their digestive tracts, and so no correlation analyses of percent algal material and body length were performed for these two species. Their diets were dominated by amphipods, unknown crustacean parts, and polychaetes. Activities of two of the eight digestive enzymes varied significantly with SL in *P. chirus*: aminopeptidase showed a negative correlation, and α-amylase a positive correlation (Table 1). The activities of the six other digestive enzymes were not significantly correlated with body size in this species. The correlations for diet, aminopeptidase, and α-amylase were strong based on the 95% confidence intervals for the coefficients.

The three members of the Xiphisterini and the three members of the Esselenichthyini each showed significant positive relationships of gut length and body length (Fig. 2; Table 2). The magnitude of these relationships (i.e., slopes) varied according to diet more so than any phylogenetic relationship (Table 2). The two herbivores, *C. violaceus* and *X. mucosus*, had the steepest slopes between gut length (RGL and ZI) and

Table 1Diet (% algal material in gut content) and digestive enzyme activities (U g gut tissue⁻¹) and the correlation of each with standard length (SL) in *Phytichthys chirus*.

Gut feature	Mean ± SD	Range	Correlation with SL			
			ρ	Lower 95% CI on ρ	Upper 95% CI on ρ	P
% algal material	28.50 ± 34.74	0.00–94.80	0.716	0.203	0.748	0.013
Pepsin	9.58 ± 2.60	6.24–17.72	0.209	−0.340	0.263	0.456
Trypsin	0.36 ± 0.18	0.11–0.74	−0.163	−0.623	−0.107	0.562
Aminopeptidase	0.29 ± 0.09	0.17–0.51	−0.648	−0.872	−0.614	0.009
α-Amylase	4.99 ± 2.74	0.79–8.75	0.750	0.386	0.774	0.001
Maltase	0.54 ± 0.16	0.30–0.80	−0.034	−0.537	0.023	0.906
Isomaltase	0.31 ± 0.15	0.08–0.59	0.236	−0.314	0.289	0.397
Lipase	0.53 ± 0.24	0.19–1.06	0.362	−0.185	0.410	0.185
Alkaline phosphatase	0.83 ± 0.47	0.11–2.00	−0.232	−0.665	0.177	0.406

Size range: 42–156 mm SL, mean 106.5 ± 36.0 mm, n = 15 in each case, except for diet: 77–156 mm SL, mean 114.1 ± 27.9 mm, n = 11. ρ, the Spearman Rank correlation coefficient; α = 0.15, with significance of individual tests at P = 0.017 after a Bonferroni correction. Significant P-values are in bold.

body length (SL), and their slopes were not significantly different from one another. The omnivorous *X. atropurpureus* had an intermediate slope between gut length and SL, and one that was significantly greater than those of *P. chirus*, *D. burgeri*, and *D. rubrimaculatum*, which had the weakest, albeit significant, slopes of gut length and body size among the pricklebacks measured. The aletriini, *A. purpureus*, showed no significant relationship between gut length and body size (Fig. 2; Table 2). Only the carnivorous *D. burgeri* and *D. rubrimaculatum* had similar RGL and ZI to *A. purpureus* at sizes greater than 60 mm SL (Fig. 2).

The scaling relationships of gut length as a function of standard length for each of the seven species were as follows: *X. mucosus* (SL^{1.51}), *X. atropurpureus* (SL^{1.32}), *P. chirus* (SL^{1.20}), *C. violaceus* (SL^{1.48}), *D. burgeri* (SL^{1.19}), *D. rubrimaculatum* (SL^{1.12}), and *A. purpureus* (SL^{1.00}). Thus, even though *D. burgeri* and *D. rubrimaculatum* are

carnivorous and do not shift their diet with ontogeny, they have guts that are generally longer than that of *A. purpureus*, and also increase in size with increase in body length.

4. Discussion

The results of this study support the hypothesis that an ontogenetic increase in gut length and digestive enzyme activities are diet-related synapomorphies of the Xiphisterini. However, within the Esselenichthyini, ontogenetic changes in diet and concomitant changes in gut structure and function are observed only in *C. violaceus*. Our three main findings are as follows. First, *P. chirus* consumed a higher proportion of algae as it increased in size, but *D. burgeri* and *D. rubrimaculatum* were carnivorous, showing no ontogenetic dietary change. Second, *P. chirus* showed an ontogenetic increase in α-amylase activity but a decrease in aminopeptidase

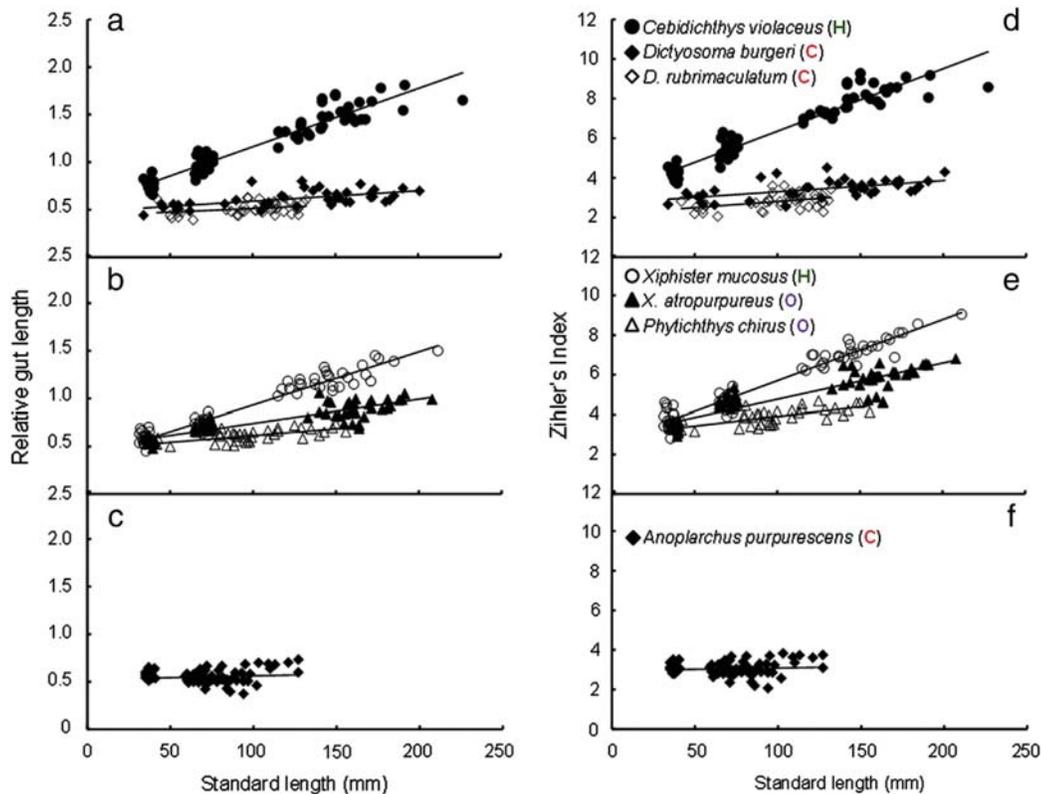


Fig. 2. Regressions of relative gut length [gut length (mm) × standard length (mm)⁻¹] as a function of standard length (left column, plots a–c) and Zihler's index [gut length × [10 × (body mass (g)^{1/3})]⁻¹] as a function of standard length (right column, plots d–f). This study included seven species of prickleback fishes with different diets from three different clades: Esselenichthyini (plots a and d), Xiphisterini (plots b and e), and the Aletriini (plots c and f). Phylogenetic relationships shown in Fig. 1. Regression statistics can be found in Table 2. H = herbivory, O = omnivory, and C = carnivory.

Table 2

Regression statistics of relative gut length (RGL) and Zihler's index (ZI) contrasted with SL for seven species of pricklebacks.

Species	Equation of the line	Standard error of slope	r ²	P	Slope comparison
<i>RGL vs. SL</i>					
<i>X. mucosus</i>	$y = 0.3765 + 0.0056x$	0.0002	0.9358	<0.001	d
<i>X. atropurpureus</i>	$y = 0.4902 + 0.0025x$	0.0002	0.8044	<0.001	c
<i>P. chirus</i>	$y = 0.4737 + 0.0014x$	0.0002	0.3939	<0.001	b
<i>C. violaceus</i>	$y = 0.5490 + 0.0061x$	0.0003	0.9169	<0.001	d
<i>D. burgeri</i>	$y = 0.4808 + 0.0011x$	0.0001	0.3519	<0.001	b
<i>D. rubrimaculatum</i>	$y = 0.4352 + 0.0008x$	0.0001	0.1011	0.046	b
<i>A. purpurescens</i>	$y = 0.5262 + 0.0004x$	0.0001	0.0165	0.245	a
<i>ZI vs. SL</i>					
<i>X. mucosus</i>	$y = 2.6161 + 0.0308x$	0.0014	0.9381	<0.001	d
<i>X. atropurpureus</i>	$y = 2.9446 + 0.0182x$	0.0013	0.7024	<0.001	c
<i>P. chirus</i>	$y = 2.9443 + 0.0096x$	0.0018	0.4304	0.001	b
<i>C. violaceus</i>	$y = 3.2068 + 0.0315x$	0.0014	0.9195	<0.001	d
<i>D. burgeri</i>	$y = 2.7365 + 0.0056x$	0.0007	0.3309	<0.001	b
<i>D. rubrimaculatum</i>	$y = 2.1916 + 0.0062x$	0.0009	0.1814	0.006	b
<i>A. purpurescens</i>	$y = 2.9568 + 0.0014x$	0.0006	0.0092	0.385	a

P values indicate the significance of the relationship between gut size and body size. Slopes were compared among species for each contrast with ANCOVA followed by a Tukey's multiple comparisons test. Significance was set at $P = 0.05$. Slopes that share a letter are not significantly different. Sample sizes are as follows, *X. mucosus* ($n = 68$), *X. atropurpureus* ($n = 73$), *P. chirus* ($n = 36$), *C. violaceus* ($n = 72$), *D. burgeri* ($n = 42$), *D. rubrimaculatum* ($n = 40$), and *A. purpurescens* ($n = 84$).

activity, consistent with its shift towards a more omnivorous diet. Finally, gut allometry was more aligned with diet than any one phylogenetic relationship, confirming the dietary affinities of *P. chirus*, *D. burgeri*, and *D. rubrimaculatum*. Nevertheless, each of the species in the Xiphisterini and Esselenichthyini showed a significant positive relationship of gut length and body size, whereas the carnivorous *A. purpurescens* showed no relationship between these variables.

Numerous fish species that consume macroalgae increase the algal proportion of their diet as they increase in size (Horn, 1989; Horn and Ojeda, 1999; Day et al., 2011b). This increase is a logical expectation because many herbivorous fish species are carnivores early in life (Horn, 1989), as are vertebrates in general (White, 1985). Cross (1981) found that *P. chirus* consumes gammarid amphipods, algae, isopods and polychaetes, and that algae increase in frequency of occurrence in its diet with increasing fish size (100% in fish 150–170 mm total length). His results were matched by those of the present study and are consistent with the ontogenetic increases in algal consumption shown by the three other herbivorous and omnivorous pricklebacks (Barton, 1982; Horn et al., 1982; Setran and Behrens, 1993; German et al., 2004; Saba, 2004; Boyle and Horn, 2006; German and Horn, 2006).

Digestive enzyme activities often reflect the diets of herbivorous and carnivorous fish species (German et al., 2010a). For example, fishes that consume at least some algae tend to have higher carbohydrase activities than carnivorous fishes, and some carnivores possess higher protease activities than herbivores (Hidalgo et al., 1999; German et al., 2004; Gawlicka and Horn, 2006; Horn et al., 2006; German et al., 2010a). The activities of the eight digestive enzymes determined in the present study for *P. chirus* broadly overlapped the relevant values in the four other prickleback species studied by German et al. (2004). Furthermore, aminopeptidase activities were negatively correlated with fish size in *P. chirus*, whereas α -amylase activity was positively correlated with fish size. Both of these changes are expected in a species that changes from a protein-rich, carnivorous diet to a carbohydrate-rich, herbivorous/omnivorous diet (German et al., 2004; Kim et al., in press). Generally, the activities of both aminopeptidase and α -amylase in *P. chirus* (Table 1) were more similar to the xiphisterine species than to the carnivorous *A. purpurescens* (see German et al., 2004 for activity levels).

P. chirus possessed relatively high trypsin and pepsin activities compared to the other xiphisterine species (German et al., 2004), but such higher protease activity may be expected in omnivorous taxa that consume animal material (Hidalgo et al., 1999; German et al., 2004; Horn et al., 2006; German et al., 2010a). Comparison of α -amylase to trypsin ratios (sensu Hidalgo et al., 1999) in the pricklebacks, however, indicates that the ratio in xiphisterine species (plus *C. violaceus*) ranges

from 13 to 91, whereas in *A. purpurescens* it is only 0.9. Thus, the "higher" protease activity in *P. chirus* is swamped by its elevated α -amylase activity, which suggests that soluble carbohydrates are an abundant energy source and are digested efficiently in *P. chirus*, as in the other omnivorous or herbivorous pricklebacks (German et al., 2004; Kim et al., in press). Indeed, this pattern is common among herbivores, as soluble storage polysaccharides are digested efficiently by algae-eating fishes (Krogdahl et al., 2005; Skea et al., 2007; German, 2009a; German and Bittong, 2009; German et al., 2010a; Day et al., 2011a; Kim et al., in press). This fact is further supported by the activities of the brushborder (membrane-bound) enzymes maltase and isomaltase in *P. chirus*, which align more closely with the other xiphisterine taxa, showing that the potential to hydrolyze, and presumably absorb (Krogdahl et al., 2005), glucose from disaccharides is higher in the members of the Xiphisterini and in *C. violaceus* than in *A. purpurescens*. Lipase activity in *P. chirus* is qualitatively more similar to the activity levels of this enzyme in the other xiphisterine taxa and *C. violaceus* (0.61 ± 0.14 U g tissue⁻¹), a result we hypothesized at the outset (see German et al., 2004).

Herbivorous fishes possess longer guts than carnivorous species (Al-Hussaini, 1947; Kapoor et al., 1975; Horn, 1989; Kramer and Bryant, 1995b; Elliott and Bellwood, 2003; Wagner et al., 2009), and gut length in algal consumers increases disproportionately with increase in fish size (Montgomery, 1977; Stoner and Livingston, 1984; Horn, 1989; Kramer and Bryant, 1995a; Horn and Ojeda, 1999). Gut lengths tend to be longer in herbivores to accommodate increased intake, thereby increasing total surface area, and maintaining some minimum transit time of food through the gut (Horn et al., 2006; German, 2009b; German et al., 2010a, 2010b). Thus, we observed the steepest slopes and scaling components of gut length versus body size in the herbivorous *C. violaceus* and *X. mucosus*. However, this relationship is related not only to diet, but also with maximum reported fish size. *C. violaceus* and *X. mucosus* have the largest body sizes at 760 mm and 580 mm SL, respectively, followed by *X. atropurpureus* at 300 mm SL (Eschmeyer et al., 1983). *P. chirus* reaches a maximum size of 200 mm (Eschmeyer et al., 1983), and *D. burgeri* and *D. rubrimaculatum* reach sizes of 250 mm SL (Masuda et al., 1984) and 179 mm SL (Shinohara et al., 2000), respectively. So a relationship appears to exist in pricklebacks between diet, gut allometry, and maximum reported body size (Horn, 1989). Indeed, across all animal taxa, a positive relationship exists between body size and intake, and food intake increases with decreasing food quality (Hillebrand et al., 2009). So, an increase in body size would be expected in animals eating copious amounts of low-quality food. A stark increase in body size clearly

emerged at the ancestor leading to the *Xiphister* species, and in the Esselenichthyini leading to *C. violaceus*. The evolution of larger body size and gut allometry in the pricklebacks was likely the result of high intake of low-quality food (i.e., algae).

Unfortunately, we do not have dietary or gut length data for *Esselenichthys carli* or *Esselenichthys laurae*, the basal taxa of the Esselenichthyini, as these species appear to be rare in nature, and therefore, in museum collections. In support of this contention, only a single tissue was available from *E. carli* (and none from *E. laurae*) for our previous phylogenetic analysis (Kim et al., in press). Thus, we were unable to obtain an adequate number of specimens of these species to dissect and analyze their diet and gut length. We are, therefore, left to speculate whether these species undergo ontogenetic changes in gut length. If our data provide any predictive power, the maximum reported sizes of *E. carli* and *E. laurae* (171 mm and 97 mm SL, respectively; Follett and Anderson, 1990) suggest that unlike *C. violaceus*, they are not herbivorous and have gut lengths on par with those of the carnivorous *D. burgeri* and *D. rubrimaculatum* within the same clade.

Our focus on gut length is different than an emphasis on total gut size, which would include gut volume. Total gut mass, volume, and intake tend to scale isometrically with body mass in terrestrial animals (Parra, 1978), and gut volume tends to increase in herbivores that are reliant upon microbial fermentation of plant or algal material in their guts (Clauss et al., 2007; Clements et al., 2009). A voluminous gut allows for slower digesta transit, giving microbes enough time to ferment available substrates (Pryor and Bjorndal, 2005; Karasov and Martínez del Río, 2007). Non-voluminous but still long and thin-walled guts are more indicative of high intake, rapid gut transit, and little reliance on microbial fermentation (Horn, 1989; Choat and Clements, 1998; German, 2009a,b, 2011). Fermentation seems to be only moderately developed in the pricklebacks (Kandel et al., 1994), which leads us back to gut length as an important metric of intake in these species.

Overall, the present study supports the contention that, in addition to external features related to feeding (e.g., cranial morphology, jaw kinematics), gut structure and function are useful metrics of adaptive diversification relating to diet. For example, *P. chirus* possesses features that its gut structure and function usually associated with the feeding and digestion in omnivorous or herbivorous prickleback fishes. As such, these features support the hypothesis expressed here and in previous work that ontogenetic changes in gut length and digestive enzyme activity facilitating digestion of an algal diet are synapomorphies of the xiphisterine clade of prickleback fishes. Herbivory and associated changes in gut structure and function in *C. violaceus* appear to be unique within the Esselenichthyini, confirming the convergent evolution of herbivory within the Stichaeidae.

Examining the evolutionary origins of dietary specialization and potential consequences on animal physiology is a key component of nutritional ecology and physiology (Karasov and Martínez del Río, 2007; Karasov and Douglas, 2013), and important for understanding how specializations arise (Axelsson et al., 2013). In this regard, the Stichaeidae is emerging as an important system for understanding dietary specialization in vertebrates (Kim et al., in press), and the findings of the present study lay a clear foundation for more detailed investigations of dietary and digestive specializations within this fish family and beyond.

Acknowledgments

We thank J. Gregg, T. Edwards, A.E. Sosa, and J. Horton for help in the field and B. Miller and A. Summers for logistical support at Friday Harbor Marine Laboratory. We also thank M. Saba for help in collecting fish at San Simeon. Financial support was provided by a grant (OCE-9906857) from the National Science Foundation (M. H. Horn, principal investigator), laboratory startup funds from UC Irvine (to D.P. German), and two Undergraduate Research Opportunity Program grants from UC Irvine (to A.E. Sosa). All handling of fish from capture to euthanization was conducted under approved protocol 99-R-10 of the Institutional

Animal Care and Use Committee (IACUC) at California State University, Fullerton, and IACUC protocol 2011-2989 of the University of California, Irvine.

Appendix 1. Museum lot numbers for the specimens of *Dictyosoma* examined in this study. Fisheries Research Laboratory at Mie University (FRLM), and National Museum of Nature and Science (NSMT)

FRLM—*Dictyosoma burgeri* 00017-00019, 00175, 00432, 00446, 00449, 00477, 00502, 00515, 00526-527, 00537, 00552, 00556, 00560, 00563, 00566, 00709, 00712, 02195, 02391, 10969, 11061.

FRLM—*Dictyosoma rubrimaculatum* 00020, 00022, 00031-32, 00164-165, 00169, 00180, 00447-448, 00456, 00460, 00462, 00469-470, 00474, 00496, 00499, 00506, 00516, 00528, 00543-544, 00551, 00553.

NSMT—*D. burgeri* 41759 (n = 11), 56768 (n = 5), 44668 (n = 2).

NSMT—*D. rubrimaculatum* 35562 (n = 2), 62081 (n = 1), 41760 (n = 12).

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