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Feast to famine: The effects of food quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae)

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ABSTRACT

The gastrointestinal (GI) tract and associated organs are some of the most metabolically active tissues in an animal. Hence, when facing food shortages or poor food quality, an animal may reduce the size and function of their GI tract to conserve energy. We investigated the effects of prolonged starvation and varying food quality on the structure and function of the GI tract in a detritivorous catfish, *Pterygoplichthys disjunctivus*, native to the Amazonian basin, which experiences seasonal variation in food availability. After 150 days of starvation or consumption of a wood-diet too low in quality to meet their energetic needs, the fish reduced the surface area of their intestines by 70 and 78%, respectively, and reduced the microvilli surface area by 52 and 27%, respectively, in comparison to wild-caught fish consuming their natural diet and those raised in the laboratory on a high-quality algal diet. Intake and dietary quality did not affect the patterns of digestive enzyme activity along the guts of the fish, and the fish on the low-quality diet had similar mass-specific digestive enzyme activities to wild-caught fish, but lower summed activity when considering the mass of the gut. Overall, *P. disjunctivus* can endure prolonged starvation and low food quality by down-regulating the size of its GI tract.

1. Introduction

Many Amazonian fish species experience extremes in food availability—food is abundant during the wet season when they venture into flooded forests to feed, whereas little or no food is available during the dry season when many fishes are confined to main river channels or small temporary ponds (Fink and Fink, 1979). Some South American fish species go up to 6 months without eating at all (Rios et al., 2004), and in warmer conditions (e.g., 25 °C) than many higher-latitude fish species that fast during cool winter months (e.g., *Pseudopleuronectes americanus* in 5 °C water; McLeese and Moon, 1989). Because the gastrointestinal (GI) tract and its associated organs can account for up to 40% of an animal's metabolic rate (Cant et al., 1996), one would have the *a priori* expectation for the digestive tract to atrophy during periods of food deprivation and flourish during food abundance (Theilacker, 1978; Bogé et al., 1981; Karasov and Diamond, 1983; McLeese and Moon, 1989; Diamond and Hammond, 1992; Wang et al., 2006). Indeed, fishes enduring food deprivation have been observed to decrease their gut length (Rios et al., 2004), intestinal fold and microvilli length (Gas and Noailliac-Depeyre, 1976), and digestive enzyme activities (Krogdahl and Bakke-McKellep, 2005; Chan et al., 2008; Furné et al., 2008).

Parallel to long periods of starvation is the consumption of food (e.g., nutrient-poor detritus) that fails to meet the nutritional demands of the fish (Bowen, 1979; Kim et al., 2007). Generally, animals eating lower-quality food increase intake to meet their energetic needs (Karasov and Martínez del Rio, 2007), which in turn causes an increase in gut and organ size (Battley and Piersma, 2005; Leenhouwers et al., 2006), if increased intake of the food ultimately allows the animal to meet their energetic needs. It is unclear, however, how food too low in quality to meet the nutritional demands of a fish affects their GI tract.

The catfish family Loricariidae is diverse—nearly 700 described species in 80 genera (Armbruster, 2004)—and compose a large proportion of the ichthyofauna in the Amazonian basin (Winemiller, 1990; Flecker, 1992). Many loricariids are detritivorous (Delariva and Agostinho, 2001; Pouilly et al., 2003; de Melo et al., 2004; German, 2009b), and detritus varies in biochemical composition in space and time, and can include large amounts of inorganic or indigestible components (Bowen, 1979; Bowen et al., 1995; Wilson et al., 2003). Such a diet requires high levels of intake (Sibly and Calow, 1986), which has resulted in some loricariids having the longest GI tracts (11–30× their body lengths) known among fishes (Kramer and Bryant, 1995; German, 2009b). Despite an apparent need for continuous feeding, some loricariid catfishes endure starvation or low-quality food during the dry season (Armbruster, 1998), and as a result, may experience significant changes in their GI tracts.

In this study we examined how the GI tract of a detritivorous loricariid, *Pterygoplichthys disjunctivus*, responds to variations in food

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quality and quantity. Recent investigations of digestion in *P. disjunctivus* (among other loricariids) revealed that these fishes are geared for high intake, rapid gut transit, assimilation of soluble components of their diet, and little reliance on microbial endosymbiotic digestion (German, 2009b; German and Bittong, 2009). How do these patterns change when *P. disjunctivus* is consuming diets with different biochemical composition, especially a low-quality diet that fails to meet their energetic needs?

This study had four components. First, we examined gut and liver masses and intestinal surface areas in wild-caught *P. disjunctivus*, individuals of this species fed a high-quality algal diet in the laboratory, individuals fed a low-quality wood diet in the laboratory, and in individuals that were starved for 150 days. We hypothesized that low-quality food and starvation would elicit a reduction in digestive tract size on all levels as an energy conservation mechanism. Second, we measured the activity levels of 10 digestive enzymes that hydrolyze substrates present in the different diets offered to the fish (Table 1). Following the methodology of Skea et al. (2005), we measured enzyme activities along the intestine and determined whether the enzymes were produced endogenously (host-produced) or exogenously (produced by microorganisms or are inherent in the food). We did this by collecting three fractions from the gut sections: gut wall tissue (endogenous), gut fluid (enzymes secreted either by the fish or exogenous sources), and gut contents (exogenous). Because we observed little evidence of endosymbiotic digestion in wild-caught *P. disjunctivus* (German and Bittong, 2009), we hypothesized that there would be little change in the patterns of digestive enzyme activities among fish on the different diets, however, we expected the fish on the low-quality diet to qualitatively reduce their enzymatic activities in comparison to the other feeding groups. Third, we compared the Michaelis–Menten constants (K_m) of three disaccharidases (maltase, β-glucosidase, N-acetyl-β-D-glucosaminidase) active along the intestinal brushborder of the fish. Because diet can affect digestive enzyme activities (Levey et al., 1999; German et al., 2004), we hypothesized that the K_m values for an enzyme would vary with diet in *P. disjunctivus*, suggesting that different enzymatic isoforms can be expressed depending on substrate intake; however, we did not have expectations for directionality of changes in K_m with diet. Lastly, we measured the concentrations of Short-Chain Fatty Acids (SCFAs) in the intestinal fluids of the fish consuming the different foods. SCFA concentrations can indicate levels of microbial fermentation occurring in different regions of the fishes' GI tracts (Crossman et al., 2005). We hypothesized there would be little change in the patterns and concentrations of SCFAs in the fish on the different diets (Table 1) because of low SCFA concentrations (3.50 mM in the distal intestine) observed in wild-caught *P. disjunctivus* (German and Bittong, 2009). Overall, this study was designed to examine GI tract plasticity in a loricariid catfish to better understand how tropical detritivorous fishes survive the dry season and low-quality foods.

2. Materials and methods

2.1. Fish collection and feeding experiments

Twenty-four individuals of *P. disjunctivus* were captured by hand while snorkeling from the Wekiva Springs complex in north central Florida (28°41.321′ N, 81°23.464′ W) in May 2005. This population of *P. disjunctivus* is exotic in Florida, but has been living there for nearly two decades (Nico et al., 2009). Upon capture, fishes were placed in 128-L coolers of aerated river water and transported alive back to the University of Florida. Upon arrival, fish were randomly assigned, in pairs, to 75.6-L aquaria equipped with mechanical filtration, containing naturally dechloronated, aged tap water, and under a 12 L:12D light cycle. The thermostat in the aquarium laboratory was set at 25 °C for the duration of the experiment and the temperature and chemical conditions (pH, ammonia concentrations) of each tank was monitored daily to confirm that they did not vary during the experimental period. The tanks were scrubbed, debris and feces siphoned out, and 95% of the water changed every 5 to 7 days to limit algal and microbial growth in the tanks as possible confounding food sources. These fish were part of a study of stable isotopic incorporation rates (German, 2008), and hence, were fed an algal diet for 240 days before being switched to their respective diets.

After this initial laboratory feeding period, the fish were randomly assigned to one of two feeding groups: 16 individuals were fed an artificial wood-detritus diet (low-quality diet), and nine individuals were fed the same algal diet they had already been eating (high-quality diet; Table 2). Fish were fed either the wood or algae diets each evening across 150 days, and all fish were weighed every 30 days to record their performance on the different diets. Five fish were captured from the wild in February 2006 to act as negative controls; these fish were brought into the laboratory, housed individually in

Table 1
Hypothesized patterns of gastrointestinal tract characteristics in *P. disjunctivus*.

Characteristic	Location[a]	Substrate	Dietary source	Fractions assayed[b]	Expected pattern[c]	>fraction[d]
Enzyme activities						
Amylolytic	Lum., cont.	Starch, α-glucans	Algae, detritus	Fluid, contents	Decrease	Equal
Laminarinase	Lum., cont.	Laminarin	Diatoms	Fluid, contents	Decrease	Equal
Cellulase	Lum., cont.	Cellulose	Wood, algae, detritus	Fluid, contents	Decrease	Equal
Xylanase	Lum., cont.	Xylan	Wood, detritus	Fluid, contents	Decrease	Equal
Trypsin	Lum., cont.	Protein	Algae, detritus animals	Fluid, contents	Decrease	Equal
Lipase	Lum., cont.	Lipid	Algae, detritus animals	Fluid, contents	Increase	Equal
Maltase	BB, cont.	Maltose	Algae, detritus	Contents, gut wall	Decrease	Contents
β-glucosidase	BB, cont.	β-glucosides	Algae, wood, detritus	Contents, gut wall	Decrease	Contents
N-acetyl-β-D-glucos[e]	BB, cont.	N-acetyl-β-D-glucoam	Fungi, insects, detritus	Contents, gut wall	Increase	BB (gut wall)
Aminopeptidase	BB, cont.	Dipeptides	Algae, detritus animals	Contents, gut wall	Increase	BB (gut wall)
GI fermentation						
SCFA concentrations	–	(multiple)	–	Fluid	No pattern	–
Soluble carbohydrates						
Luminal concentrations	–	Mono-oligosaccharides	–	Fluid	Decrease	–

[a] Indicates area of the intestine in which an enzyme is active. Lum = lumen of the intestine; cont. = intestinal contents (ingesta); BB = brush border of the intestine.
[b] The portions of gut content or intestinal tissue in which the activity of an enzyme was assayed.
[c] This column shows the hypothesized patterns of the measured parameter along the fishes' GI tracts based on data from wild-caught fish (German and Bittong, 2009). For example, "decrease" means that enzyme activity, SCFA concentration, or carbohydrate concentration should decrease toward the distal intestine of the fish.
[d] Predictions of which assayed fractions will have higher activity of a particular enzyme. For example, "equal" means that the activity of that enzyme is expected to be equal in the fluid and contents of a given intestinal region.
[e] Complete name of the enzyme is N-acetyl-β-D-glucosaminidase, and the substrates are N-acetyl-β-D-glucoaminides.

Table 2
Proportions of nutrients in the two diets fed to *Pterygoplichthys disjunctivus* in the laboratory.

Dietary component	Wood diet	Algae diet
Soluble components (%)	37.49 ± 0.98	73.18 ± 0.71
NDF — total fiber (%)	62.51 ± 0.98	26.81 ± 0.01
ADF — cellulose + lignin (%)	49.01 ± 0.88	7.28 ± 0.01
Lignin + cutin (%)	23.16 ± 1.10	1.22 ± 0.13
Protein (%)	7.75 ± 0.07	31.84 ± 0.32
Lipid (%)	1.90 ± 0.20	11.28 ± 0.11
Ash (%)	2.60 ± 0.03	4.48 ± 0.05
Energy ($kJ \cdot g^{-1}$)	16.50	17.04

Values are mean ± SEM for soluble components, NDF, ADF, and lignin ($n = 4$–6). NDF = neutral detergent fiber; ADF = acid detergent fiber (Goering and Van Soest, 1970).

aquaria, and were not fed for the 150 day duration of the feeding experiment. The starving fish were monitored daily to ensure they were not in poor condition, and, as with the wood and algae-fed fish, were weighed and measured every 30 days.

The wood diet was largely composed of degraded wood of water oak (*Quercus nigra*) collected from the Sampson River, FL (29°51.37′ N, 82°13.16′ W). We chose submerged, degraded wood of a riparian tree because this is reflective of wood-detritus naturally consumed by the better known wood-eating relatives of *P. disjunctivus* in the genera *Hypostomus* and *Panaque* (German, 2009b). The wood diet contained the following ingredients: 80% *Q. nigra* wood, 9% corn gluten meal, 6% xanthan gum (binding agent), 2.1% corn meal, 1% L-lysine, 1% vitamin premix, 0.5% trace mineral mix, and 0.4% water-stable vitamin C (stay C). The corn gluten meal, corn meal, L-lysine, vitamins, and minerals were gifts from Hartz-Mountain Corporation (Secaucus, NJ), and intended specifically for use in fish food. Many of these ingredients are also contained in the algae diet (described below).

The wood was chopped into smaller pieces and ground to particle sizes of 0.25–1 mm, consistent with the particle sizes of wood found in the digestive tracts of wood-eating catfishes (German, 2009b). The wood was then autoclaved and dried at 60 °C for 24 h. The other ingredients were then added to the wood, along with a small amount of water (20 mL/100 g dry mass), and the mixture was homogenized vigorously by hand with a stirring rod. The artificial wood-detritus was then pressed into 4 × 2 mm (0.45 g) circular pellets in a hand press (Parr Instruments – Moline, IL). Because dried wood floats and the fish feed on the benthos, the pellets were adhered to a piece of PVC pipe with a small drop of superglue (Loctite, Avon, Ohio), and sunk in the aquaria where the fish actively fed on the pellets during the evening hours (i.e., in the dark). Because some of each pellet was permanently polymerized in the superglue, a small portion (~0.05 g) of each pellet was inedible by the fish. Superglue (2-Octyl Cyanoacrylate) is not toxic and has been approved by the Food and Drug Administration of the United States of America for use in human wound care (Schwade, 2008). Thus, as an inert compound, the superglue did not represent an additional variable in the wood diet, nor did the fish actually consume polymerized glue. Individual fish were offered, and consumed, 10 pellets per night, equating to approximately 8% of their body mass (wet mass basis) per day.

Fish on the algae diet were fed Wardley® Premium Algae Discs (Hartz-Mountain Corporation), which contain the same corn gluten meal, corn meal, vitamins, and minerals as the artificial wood-detritus diet, and are similar in size (6 × 1 mm, 0.30 g, per disc). The wood and algal diets were nearly isocaloric, making nutrient and fiber concentrations the main differences among the food types (Table 2). Fishes on the algae diet were fed 7 discs per night, equating to approximately 6% of their body mass, on a wet mass basis, per day. We considered the feeding levels of the wood and algal diets to be *ad libitum* because previous observations showed that *P. disjunctivus* consumed 2–5% of their body mass per evening in wood (German, 2009b).

An additional 10 fish were captured from the wild in March 2006 to serve as "wild controls", consuming their natural diet of algae, detritus, diatoms, animal material, and sediment (German, 2009b). Thus, in this study we examined the affects of diet on gut structure and function among four feeding groups: wild-caught fish, fish consuming a low-quality wood-diet in the laboratory, fish consuming a high-quality algal diet in the laboratory, and fish that had been starved.

The fish were fed their respective diets (or starved) for 150 days after which they were euthanized in buffered water containing $1 \cdot g\,L^{-1}$ tricaine methanesulfonate (MS-222, Argent Chemicals Laboratory, Inc., Redmond, WA, USA), measured [standard length (SL) ± 1 mm], weighed [body mass (BM) ± 0.5 g], and dissected on a chilled (~4 °C) cutting board. All fish were sampled in the morning (7:00–10:00 h) to ensure they had food in their guts. Whole GI tracts were removed by cutting at the esophagus and at the anus and processed in a manner appropriate for specific analyses (see below). For each fish, the whole (empty) GI tract and liver were weighed, and the digestive-somatic index [(DSI = GI tract mass · body mass^{-1}) × 100] and hepato-somatic index [(HSI = liver mass · body mass^{-1}) × 100] determined. To further assess differences in body size among the feeding groups, condition factors [CF = (100,000 · body mass)/(standard length^3)] of the fish were also calculated. All handling of fish from capture to euthanasia was conducted under approved protocols D995 and E822 of the Institutional Animal Care and Use Committee of the University of Florida.

2.2. Dietary composition

The proportions of nutrients in the diets fed to *P. disjunctivus* in this study are presented in Table 2. Proximate analyses were performed following the methods of the Association of Official Analytical Chemists (AOAC International, 2006). Total fat was determined by acid hydrolysis followed by extraction in petroleum ether, and total protein was determined by Kjeldahl extraction. Ash was determined by drying the diets at 105 °C (dry matter), and then combusting them at 550 °C for 3 h. The remaining content was ash. Soluble carbohydrate was calculated as the nitrogen-free extract, or the proportion of the diet that wasn't analytically determined as moisture, protein, fat, crude fiber, or ash. Energetic content was calculated based on the fat, protein, and total carbohydrate (soluble carbohydrate + crude fiber) contents of the diets. Although determined to calculate energy content, crude fiber and nitrogen-free extract values are not reported. Instead, the more meaningful (Karasov and Martínez del Rio, 2007) neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions were determined by refluxing dietary samples with neutral detergent and acid detergent solutions (Goering and Van Soest, 1970) as described by German (2009b). Lignin content was measured by refluxing samples in 72% sulfuric acid for 3 h at room temperature (24 °C; German, 2009b). Soluble components (i.e., soluble carbohydrates and proteins) represent the fraction lost after refluxing the samples in neutral detergent solution (Goering and Van Soest, 1970).

2.3. Histological and TEM analyses

Upon removal from the body, the digestive tracts of two individuals of each feeding group (and three wild-caught fish) were immediately placed in ice-cold Trump's fixative [4% formaldehyde, 1% glutaraldehyde, in 10 mM sodium phosphate (monobasic) and 6.75 mM sodium hydroxide; (McDowell and Trump, 1976)], pH 7.5, to prevent any degradation of the gut ultrastructure. The guts were gently uncoiled while submerged in the fixative, the length of the intestine was measured [IL (mm)], and six 1-mm sections were excised from each of the proximal, mid, and distal intestine (Frierson and Foltz, 1992) and placed in their own individual vials containing fresh Trump's fixative. These tissues were then allowed to fix over night (12 h) at 4 °C. Three of the sections were designated for analysis

with transmission electron microscopy (TEM), whereas the other three were designated for use in histological analyses.

Following fixation, the tissues were removed from the fixative and rinsed in 0.1 M phosphate buffered saline (PBS), pH 7.5, for 3×20 min, and a final rinse overnight at 4 °C. Following rinsing in PBS, the tissues designated for histological analyses were rinsed for 40 min in running DI water, and prepared following German (2009b). Intestinal tissues were serially sectioned at 7 μm, stained in a modified Masson's trichrome (Presnell and Schreibman, 1997), and photographed at 40×, 60×, and 120× with a Hitachi KP-D50 digital camera attached to an Olympus BX60 bright-field light microscope. Images ($n=5$ per intestinal region, per individual fish; 30 images per feeding group) were used to quantify the intestinal surface area of the fish on the different diets. The circumference of the intestinal sections [IC (mm)] was measured along the serosa using Image J analytical software (Abramoff et al., 2004). We then measured the length of the mucosal lining of the intestine (ML), and calculated the mucosal area as the ratio of ML to IC ($ML \cdot IC^{-1}$; McLeese and Moon, 1989; Hall and Bellwood, 1995). This mucosal area multiplier allows one to observe how much the mucosal folds increase the inner surface area of the intestine. The surface area of each region of the intestine was calculated as IL/3×regional IC×the mucosal area (Frierson and Foltz, 1992). Because we have defined the proximal, mid, and distal intestine as equal length sections (German, 2009b), the length of each intestinal region was estimated as IL/3. The sum of these surface areas provided an estimate of total intestinal surface area for each feeding group.

Tissue sections designated for TEM were prepared following German (2009b) and photographed using a transmission electron microscope (H-7000, Hitachi, Japan). Images ($n=5$ per intestinal region per individual fish; 30 images per feeding group) were used for measurements of microvilli surface area per length of the intestinal epithelium following a two-dimensional model developed by Frierson and Foltz (1992). In this model each microvillus is represented by a rectangle topped with a semicircle whose diameter (D) equals the width of its base and whose height (H) equals the distance between the base and the top point below the glycocalyx. For each image, individual width and height of 15–70 microvilli and the length of the intestinal epithelium (IEL) that these microvilli occupied were measured using Image J analytical software. Microvilli surface area was calculated as MVSA (μm^2) = $(H\pi D)+(\pi R^2)$, where $R=0.5D$. For each fish, the sum of MVSA (μm^2) for each region was divided by IEL (μm) and averaged for each of the proximal, mid, and distal intestine. MVSA was reported as the mean MVSA per μm of IEL for each gut region (Horn et al., 2006). The MVSA per μm of IEL provides a metric of how microvilli increase intestinal surface area in the feeding groups.

All intestinal tissue from the fish consuming the artificial wood-detritus in the laboratory were inadvertently postfixed in osmium tetroxide, which turns the tissue black in color. Osmium tetroxide is used to provide contrast to tissues for electron microscopy (Bozolla and Russell, 1999), but is not typically used prior to histological analyses. Therefore, we reversed the effects of the osmium tetroxide from a subset of this tissue by submerging them in 1 M sodium metaperiodate for 3×1 h, followed by a graded ethanol series and preparation for histology as described above. The osmium tetroxide did not change the integrity of the tissue for histology (i.e., the structure was unaffected), but these tissues did not stain as clearly as those from the other feeding groups. This absolutely did not affect our analyses, however, because we were interested in the folding patterns of the intestinal tissue, not the different tissue layers that can be revealed with trichrome staining (Presnell and Schreibman, 1997). Thus, although the tissues from the fish in this feeding group stained darkly (Fig. 1), this did not interfere with our measurements of intestinal surface area.

We recognize that the sample sizes used for the analyses of gut surface area are low ($n=2$–3) compared to other studies of gross gut structure (e.g., Kramer and Bryant, 1995; German and Horn, 2006). However, histological and electron microscopic analyses are considerably more time consuming and expensive than analyses of gross gut structure, and for this reason, previous analyses of fish intestinal surface area have also used low sample sizes (Frierson and Foltz, 1992, $n=2$; Horn et al., 2006, $n=3$). Furthermore, it was difficult to capture enough fish on which to perform all of the desired analyses. Even though we had low samples sizes, the data on gut surface area still provide useful insight into how these fishes modulate their gut structure in response to changes in dietary biochemical composition and intake. Nevertheless, future analyses should use larger sample sizes for better statistical power.

2.4. Tissue preparation for digestive enzyme analyses

For fishes designated for digestive enzyme analyses (wild-caught fish, $n=10$; laboratory wood diet, $n=7$; laboratory algae diet, $n=6$), the guts were dissected out, placed on a sterilized, chilled (~4 °C) cutting board, and uncoiled. The stomachs were excised, and the intestines divided into three sections of equal length representing the proximal, mid, and distal intestine. The gut contents were gently squeezed from each of the three intestinal regions with forceps and the blunt side of a razorblade into sterile centrifuge vials. These vials (with their contents) were then centrifuged at 10,000g for 5 min at 4 °C (Skea et al., 2005). Following centrifugation, the supernatants (heretofore called "intestinal fluid") were gently pipetted into separate sterile centrifuge vials, and the pelleted gut contents and intestinal fluid were frozen (in their separate vials) in liquid nitrogen. Gut wall sections were collected from each intestinal region of each specimen by excising an approximately 30 mm piece each of the proximal, mid, and distal intestine. These intestinal pieces were then cut longitudinally, and rinsed with ice-cold 0.05 M Tris–HCl buffer, pH 7.5, to remove any trace of intestinal contents. The gut wall sections were placed in separate sterile centrifuge vials and frozen in liquid nitrogen. All of the samples were then stored at −80 °C until prepared for analysis.

The intestinal fluids and pelleted gut contents were homogenized on ice following Skea et al. (2005), as described by German and Bittong (2009). The protein content of the homogenates was measured using bicinchoninic acid (Smith et al., 1985). Digestive enzyme activities were not measured in the starving fish because they had very little intestinal fluid, no gut contents, and we had a limited sample size of these fish ($n=5$). Thus, only gut size and surface area measurements were taken from the starving fish.

2.5. Assays of digestive enzyme activity

All assays were carried out at 25 °C in triplicate using the BioRad Benchmark Plus microplate spectrophotomer and Falcon flat-bottom 96-well microplates (Fisher Scientific) as described by German and Bittong (2009). All pH values listed for buffers were measured at room temperature (22 °C), and all reagents were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). All reactions were run at saturating substrate concentrations as determined for each enzyme with gut tissues from the four species. Each enzyme activity was measured in each gut region of each individual fish, and blanks consisting of substrate only and homogenate only (in buffer) were conducted simultaneously to account for endogenous substrate and/or product in the tissue homogenates and substrate solutions (Skea et al., 2005; German and Bittong, 2009). All activities were calculated with extinction coefficients determined for each product (e.g., glucose or xylose for polysaccharidases and maltase; p-nitroaniline for trypsin and aminopeptidase; p-nitrophenol for lipase and disaccharidases), and all activities are reported in U (1 μmol product liberated per min) per gram wet mass of fluid, tissue, or content.

Polysaccharidase activities (i.e., activities against starch, laminarin, cellulose, and xylan) were measured in intestinal fluid and pelleted

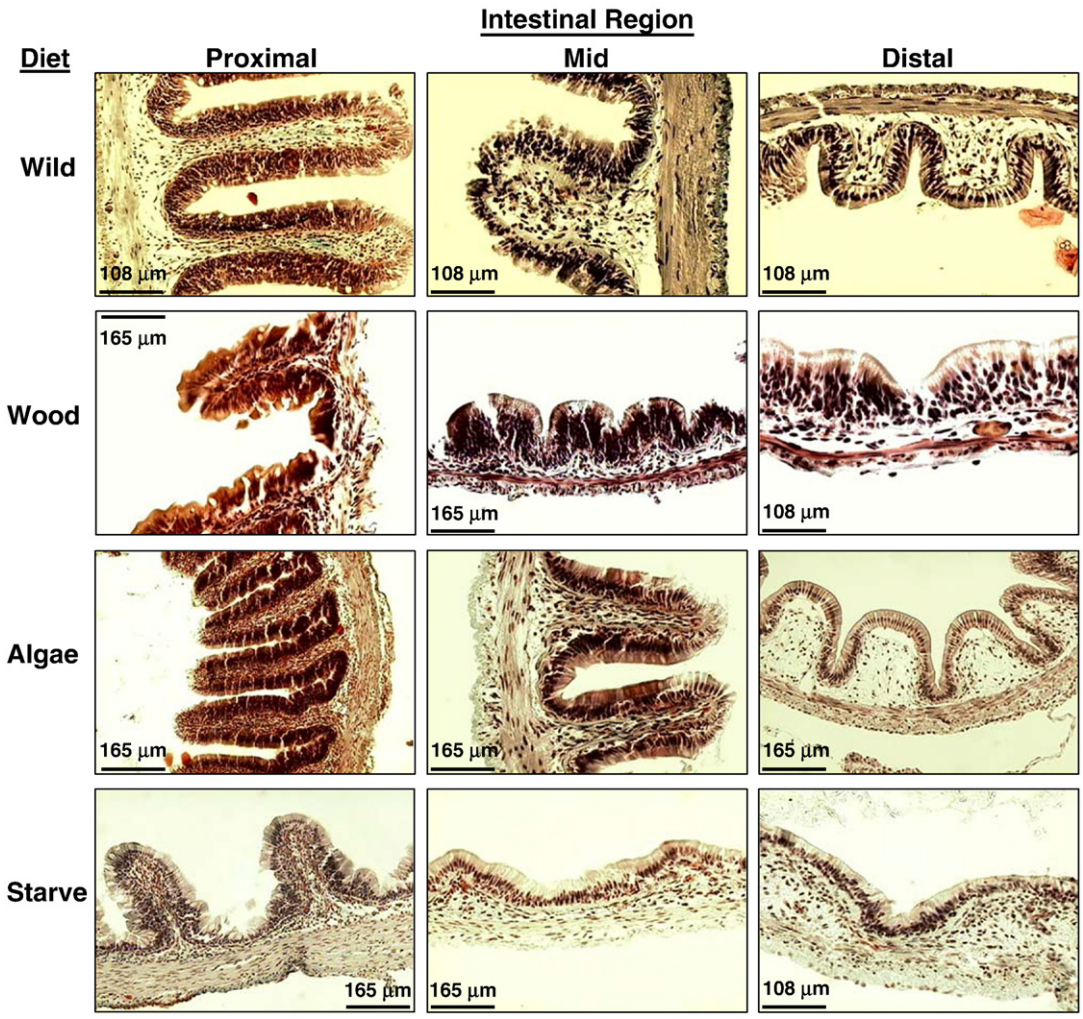

Fig. 1. Cross-sections of proximal, mid, and distal intestinal tissue of *P. disjunctivus* consuming different diets. Tissues were stained with a modified Masson's trichrome. Scale bars are labeled on each photograph.

gut contents according to the Somogyi-Nelson reducing sugar assay (Nelson, 1944; Somogyi, 1952).

Trypsin activity was assayed in the intestinal fluid and pelleted gut contents using a modified version of the method designed by Erlanger et al. (1961) and the synthetic substrate Nα-benzoyl-L-arginine-*p*-nitroanilide hydrochloride (BAPNA).

Aminopeptidase activity was measured in gut wall tissues and pelleted gut contents according to Roncari and Zuber (1969) using the synthetic substrate alanine-*p*-nitroanilide.

Lipase (non-specific bile-salt activated E.C. 3.1.1.—) activities were assayed in the intestinal fluids and pelleted gut contents using a modified version of the method designed by Iijima et al. (1998) and the synthetic substrate *p*-nitrophenyl-myristate.

Previous work showed no differences in the activities of the polysaccharide degrading enzymes, trypsin, and lipase between the intestinal fluids and gut contents of *P. disjunctivus* (German and Bittong, 2009), and none were observed in this study. Thus, only total activities (gut fluid + gut contents) are reported for these enzymes.

2.6. Assays of disaccharidase activity

Maltase activity was measured in gut wall tissues and pelleted gut contents following Dahlqvist (1968), as described by German and Bittong (2009) and German et al. (2004). The Michaelis–Menten constant (K_m) for maltase was determined for gut wall samples with substrate concentrations ranging from 0.56 mM to 112 mM.

The activities of the disaccharidases β-glucosidase and N-acetyl-β-D-glucosaminidase (NAG) were measured in gut wall tissues and pelleted gut contents using *p*-nitrophenol conjugated substrates. The K_m was determined for these enzymes in the gut wall samples using substrate concentrations ranging from 0.2 mM to 12 mM and 0.04 to 1.2 mM, respectively.

2.7. Gut fluid preparation, gastrointestinal fermentation, and luminal carbohydrate profiles

Measurements of symbiotic fermentation activity were indicated by relative concentrations of Short-Chain Fatty Acids (SCFA) in the fluid contents of the guts of the fishes at the time of death (Pryor and Bjorndal, 2005; German and Bittong, 2009; German et al., 2010). Concentrations of SCFA in the intestinal fluid samples from each gut region in each species were measured using gas chromatography as described by Pryor et al. (2006).

To examine the presence of reducing sugars of various sizes in the intestinal fluids of the fish, 1 µL of filtered intestinal fluid was spotted on to pre-coated silica gel plates (Whatman, PE SIL G) together with standards of glucose, maltose, and tri- to penta-oligosaccharides of glucose. The thin-layer chromatogram (TLC) was developed with

ascending solvent [isopropanol/acetic acid/water, 7:2:1 (v/v)] and stained with thymol reagent (Adachi, 1965; Skea et al., 2005; German and Bittong, 2009). The glucose concentration in the intestinal fluid of the fish was measured following German (2009a).

2.8. Statistical analyses

Comparisons of body mass and total SCFA concentrations were made among the different feeding groups with ANOVA followed by a Tukey's HSD with a family error rate of $P=0.05$. The fish in the different feeding groups had different condition factors and body masses (Table 3) at the beginning and end of the experiment. Body mass is, therefore, an important variable to consider in comparisons made throughout the analyses, and thus, condition factors, digestive-somatic indices, total gut surface areas, and MVSA were compared among feeding groups with ANCOVA (using body mass as a covariate), followed by Tukey's HSD. The daily rate of body mass change was determined with an exponential model. Digestive enzyme activities and MVSA were compared among gut regions within each feeding group with ANOVA, followed by Tukey's HSD. Because of inherent differences in intake and, therefore, in digesta retention time among the different feeding groups, inter-feeding group comparisons of digestive enzyme activities were not made. Instead, only qualitative differences among the feeding groups, and differences in enzymatic activity patterns along the gut will be discussed. Maltase, β-glucosidase, NAG, and aminopeptidase activities values were compared among the gut walls and gut contents of each gut region in each feeding group with *t*-test. K_m values of maltase, β-glucosidase, and NAG were compared among fish in the different feeding groups with ANOVA, followed by Tukey's HSD. Prior to all significance tests, a Levene's test for equal variance was performed to ensure the appropriateness of the data for parametric analyses. If the data were not normal, they were log transformed, and normality confirmed prior to analysis. All tests were run using SPSS (12.0) statistical software.

3. Results

3.1. Body mass, gut mass and gut structure

All of the fish lost weight on the artificial wood-detritus diet, with the mean daily loss being approximately 0.06% per day, whereas the fish on the algae diet gained about 0.16% of their body mass per day (Table 3). The starving fish lost approximately 0.03% of their body mass per day over the course of the experiment. No differences in condition factor were detected among the fish in the different feeding groups at the end of the experiment, although this varies as a function of body mass (Table 3). The digestive-somatic index was significantly greater in the wild-caught and algae-fed fish than in the wood-fed and starved fish, and these differences were not the result of differences in body mass among the feeding groups (Table 3). The hepato-somatic index was significantly greater in the algae-fed fish than the other fish, which did not differ from one another. Body mass played more of a role in HSI comparisons, likely because the starvation group had the largest mean body mass and the lowest HSI (Table 3).

Diet did not just affect the overall mass of the gut, but the ultrastructure as well. The wild-caught fish and those that ate algae in the laboratory had larger intestinal folding patterns than the fish consuming wood or those that were starved in the laboratory (Fig. 1). These effects were also observed in the TEM images, as the wild-caught and algae-fed fish had longer and denser microvilli than the wood-fed or starved fish (Fig. 2). Some of the starved fish had no discernable microvilli remaining in the distal intestine.

The algae-fed fish possessed significantly greater mucosal area in their proximal intestine than the other feeding groups (Fig. 3; ANCOVA—feeding group: $F_{3,7}=95.68$, $P=0.002$; body mass: $F_{1,3}=1.08$, $P=0.375$), and the wild-caught fish had greater mucosal area than the wood-fed or starving fish. The same pattern was evident in the mid intestine (ANCOVA—feeding group: $F_{3,7}=84.92$, $P=0.002$; body mass: $F_{1,3}=0.59$, $P=0.497$). The wild-caught and algae-fed fish had equally large mucosal areas in their distal intestines, and both were significantly larger than the other feeding groups (ANCOVA—feeding group: $F_{3,7}=76.01$, $P=0.003$; body mass: $F_{1,3}=1.36$, $P=0.328$). All four feeding groups had significantly greater mucosal area in their proximal intestines than in the other gut regions (Fig. 3; all ANOVA stats $F>30$, and $P<0.008$). The algae-fed fish exhibited significantly greater mucosal area in their mid intestine than their distal intestine.

The wild-caught fish had larger proximal intestine microvilli surface area (MVSA) than those fish consuming the low-quality wood diet or those starved in the laboratory, but not greater than the algae-fed fish (Fig. 3; ANCOVA—feeding group: $F_{3,7}=22.27$, $P=0.015$; body mass: $F_{1,3}=24.06$, $P=0.016$). However, body mass significantly varied among the fish and accounted for some of the variation in MVSA among the feeding groups, likely because the starvation group was the heaviest, but had the lowest MVSA. No significant differences were detected for MVSA of the mid intestine (Fig. 3; ANCOVA—feeding group: $F_{3,7}=2.03$, $P=0.287$; body mass: $F_{1,3}=0.92$, $P=0.408$) or the distal intestine (Fig. 3; ANCOVA—feeding group: $F_{3,7}=2.50$, $P=0.236$; body mass: $F_{1,3}=0.11$, $P=0.760$) among the different feeding groups, likely due to low sample size. Neither the wild-caught nor the wood-fed fish showed significant changes in MVSA along the intestine (ANOVA: $F<2$, $P>0.11$), whereas the algae-fed and starved fish had significantly greater MVSA in the proximal intestine than in the distal intestine (ANOVA: $F>8$, $P<0.002$). The starved fish also had significantly greater MVSA in the mid intestine than the distal intestine (Fig. 3).

The wild-caught and algae-fed fishes had similar total gut surface areas (wild-caught: 386.13 ± 11.36 cm^2; algae-fed: 439.95 ± 26.11 cm^2),

Table 3
Daily rate of body mass (BM) change, final body mass, final condition factor [CF = (10,000 · body mass)/standard length^3] digestive-somatic index [DSI = (GI tract mass/body mass) × 100], and hepato-somatic index [HSI = (liver mass/body mass) × 100] in *Pterygoplichthys disjunctivus* consuming different diets.

Feeding group (*n*)	Daily rate of BM change (%)	Final BM (g)	CF	DSI	HSI
Wild fish (11)	N/A	171.29 ± 25.32^b	2.08 ± 0.02^a	12.09 ± 0.59^b	0.55 ± 0.04^a
Laboratory wood diet (14)	-0.057 ± 0.005	75.53 ± 4.14^a	2.23 ± 0.08^a	8.23 ± 0.39^a	0.55 ± 0.06^a
Laboratory algae diet (9)	0.160 ± 0.024	123.52 ± 18.94^b	2.21 ± 0.05^a	14.59 ± 0.73^b	0.96 ± 0.11^b
Laboratory starvation (5)	-0.026 ± 0.011	298.71 ± 60.80^c	1.72 ± 0.08^a	6.77 ± 0.39^a	0.38 ± 0.05^a
Feeding group	–	$F_{3,38}=23.95$	$F_{3,38}=1.88$	$F_{3,38}=33.46$	$F_{3,38}=9.33$
	–	$P<0.001$	$P=0.151$	$P<0.001$	$P<0.001$
Body mass	–	–	$F_{1,34}=3.22$	$F_{1,34}=0.03$	$F_{1,34}=3.37$
	–	–	$P=0.082$	$P=0.857$	$P=0.075$

Values are mean (±SEM). Fish were fed their respective diets for 150 days. All biometric characteristics were measured at the end of the experiment with the exception of body mass, which was measured at 30-day intervals throughout the experiment. Final body mass was compared among feeding groups with ANOVA. CF, DSI, and HSI were analyzed with ANCOVA using body mass as a covariate. Tests were followed by Tukey's HSD with a family error rate of $P=0.05$. Values for BM, CF, DSI or HSI that share a superscript letter are not significantly different. Because wild fish were caught at a single time point there are no data for the daily rate of BM change for this feeding group.

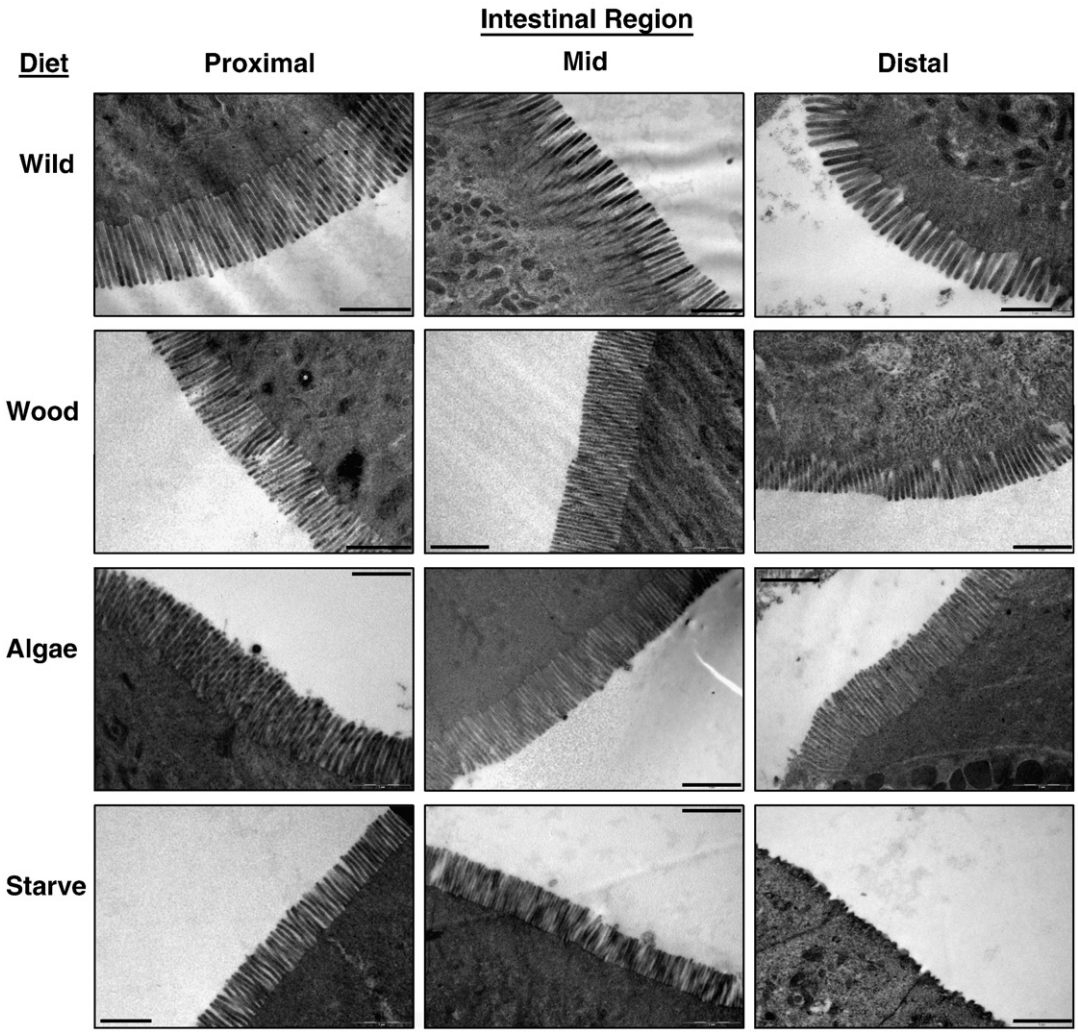

Fig. 2. Transmission electron microscope (TEM) micrographs of the proximal, mid, and distal intestine of *P. disjunctivus* consuming different diets. Black scale bar = 1 μm on all images.

and both were significantly greater than the wood-fed (85.76 ± 1.90 cm^2) or starved fish (119.25 ± 5.20 cm^2), which didn't differ from one another (ANCOVA — feeding group: $F_{3,7} = 112.99$, $P<0.001$; body mass: $F_{1,3} = 0.48$, $P = 0.54$).

3.2. Digestive enzyme activities

Total amylase and laminarinase activities were significantly greater in the proximal and mid intestines than in the distal intestines of the fish in all of the feeding groups (Supplemental Table S1, Fig. 4). Cellulase activity was significantly greater in the proximal intestines of the wild-caught and wood-fed fish than in their distal intestines; no difference was detected among the gut regions in the fish that ate algae in the laboratory (Supplemental Table S1, Fig. 4). Like cellulase, xylanase activity was significantly greater in the proximal intestine of the wild-caught fish than in their distal intestine. No xylanase activity was detected in the wood-fed fish.

All of the feeding groups showed significantly greater trypsin activity in their proximal and mid intestines than in their distal intestines (Supplemental Table S1, Fig. 5). The wild-caught fish possessed significantly greater aminopeptidase activity in the gut walls of their mid intestine than in the contents of this gut region, but no differences were detected in the other intestinal regions (Table 4). The algae-fed fish showed significantly greater aminopeptidase activity in the contents of their proximal intestine than in the gut wall of this intestinal region, and greater activity in the gut wall of their mid intestine in comparison to gut contents from this intestinal region. The wild-caught fish significantly increased the aminopeptidase activity in their mid intestine gut wall in comparison to the gut walls of the other gut regions (ANOVA $F_{2,17} = 16.92$, $P<0.001$). The wood- and algae-fed fish showed no statistical difference in gut wall aminopeptidase activity among the gut regions (Table 4). The algae-fed fish showed significantly greater gut content aminopeptidase activity in their proximal intestine than the other gut regions (ANOVA $F_{2,17} = 12.53$, $P = 0.001$), but no regional differences were observed in the gut contents of the other feeding groups.

The patterns of lipase activity were distinctly different between the feeding groups. Although the fish consuming wood or algae in the laboratory showed significant decreases in lipase activity distally in their intestines, no differences were detected in the intestines of the wild-caught fish (Supplemental Table S1, Fig. 5).

3.3. Disaccharidase activities

Maltase activities in the gut contents were significantly higher than the activities of this enzyme in the gut walls in the entire intestine of all the feeding groups, except the distal intestine of wood-fed fish (Fig. 6). All feeding groups showed decreasing maltase activities in their gut contents distally in their intestines (ANOVA $P<0.05$ for each feeding group). The wild-caught fish showed an

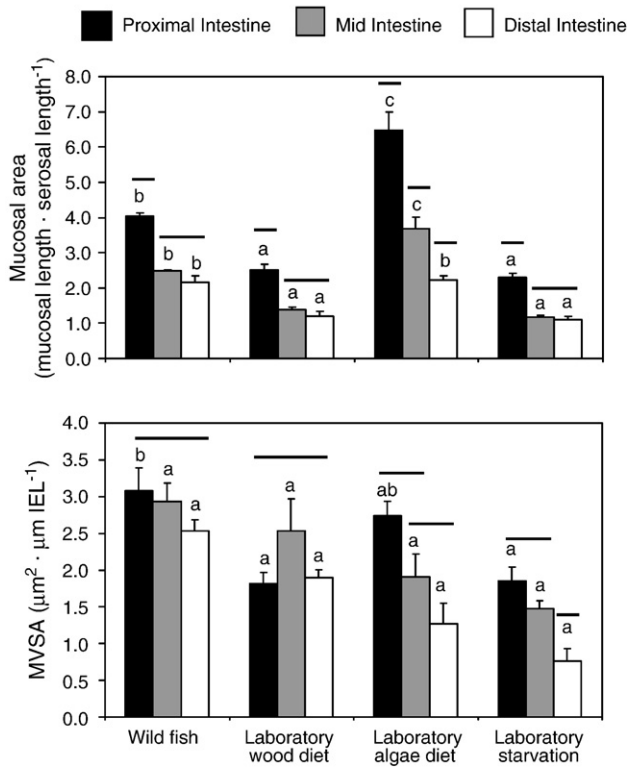

Fig. 3. Mucosal area and microvilli surface area (MVSA) per length of intestinal epithelium (IEL) in *P. disjunctivus* consuming different diets. Values are means and error bars represent SEM. Inter-feeding group comparisons of mucosal area and MVSA in each gut region were made with ANCOVA (using body mass as a covariate) followed by a Tukey's HSD with a family error rate of $P=0.05$. Bars for a specific gut region sharing a letter are not significantly different among the feeding groups. Intra-feeding group comparisons of mucosal area and MVSA among gut regions were made with ANOVA followed by a Tukey's HSD with a family error rate of $P=0.05$. Lines of a different elevation over two bars indicate a significant difference ($P<0.01$) among those gut regions within that feeding group. Data for wild-caught fish re-drawn from German (2009b).

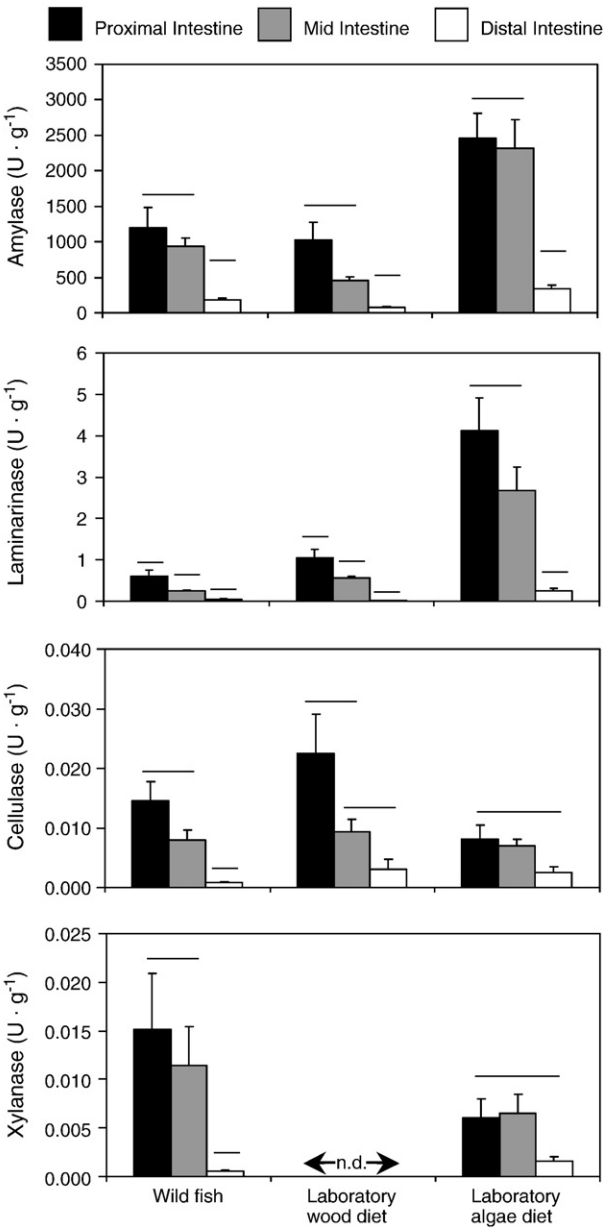

Fig. 4. Total activities (intestinal fluid + microbial extract) of amylase, laminarinase, cellulase, and xylanase in three regions of the intestine of *P. disjunctivus* consuming different diets. Values are means and error bars represent SEM. Intra-feeding group comparisons of each enzyme among gut regions were made with ANOVA followed by a Tukey's HSD with a family error rate of $P=0.05$. Lines of a different elevation passing over two bars indicate a significant difference in enzyme activity ($P<0.01$) among those gut regions within that feeding group. n.d. = not detectable. Inter-feeding group comparisons of enzyme activities were not made. Data on wild-caught fish re-drawn from German and Bittong (2009).

increase in maltase activity in the gut walls of their mid intestine (ANOVA $F_{2,17}=28.75$, $P<0.001$), whereas the wood-fed fish showed significant (ANOVA $F_{2,20}=7.73$, $P=0.004$) decreasing activity distally in their gut walls (Fig. 6).

The pattern of β-glucosidase activity oscillated distally in the intestine of the wild-caught fish, with the gut contents showing higher activity of this enzyme in the proximal ($t=2.84$, $P=0.018$, $d.f.=10$) and distal ($t=2.07$, $P=0.058$, $d.f.=14$) intestine, and the gut wall showing higher activity in the mid intestine ($t=2.39$, $P=0.031$, $d.f.=14$; Fig. 6). No differences in β-glucosidase activity were detected among the gut walls and gut contents in any gut region of the wood-fed fish ($P>0.235$), and the algae-fed fish only showed a significant difference in β-glucosidase activity between the gut wall and content of the proximal intestine ($t=10.94$, $P<0.001$, $d.f.=10$). All feeding groups showed decreasing β-glucosidase activity in their gut contents distally in their intestines (ANOVA $P<0.05$ for each feeding group). The wild-caught fish showed an increase in β-glucosidase activity in the gut walls of their mid intestine (ANOVA $F_{2,17}=33.72$, $P<0.001$), whereas the wood-fed fish showed significant (ANOVA $F_{2,20}=21.78$, $P<0.001$) decreasing activity distally in their guts (Fig. 6). The algae-fed fish had significantly greater β-glucosidase activity in their proximal and mid intestine gut walls than in their distal intestine gut wall (ANOVA $F_{2,17}=4.96$, $P=0.022$).

The N-acetyl-β-D-glucosaminidase (NAG) activity was generally not different between the pelleted gut contents and the gut walls of the fish, with the exceptions of the distal intestine of the wild-caught fish (gut wall activity>gut content activity), and the proximal intestine of the algae-fed fish (gut wall activity<gut content activity; Fig. 6). The wild-caught fish exhibited significantly greater NAG activity in their distal intestine gut wall than in the gut walls of other intestinal regions (ANOVA $F_{2,17}=14.92$, $P<0.001$), whereas the other feeding groups showed no difference in gut wall NAG activity among the different intestinal regions. The gut content NAG activity did not significantly change among the gut regions of the wild-caught and wood-fed fish, however, it decreased significantly in the distal intestines of the algae-fed fish (ANOVA $F_{2,17}=8.23$, $P=0.004$).

The algae-fed fish possessed significantly lower maltase K_m values in their proximal intestines than the wild-caught fish, but the wood-fed fish were not different from any feeding group (Supplemental Table S2). The wild-caught and wood-fed fish possessed significantly

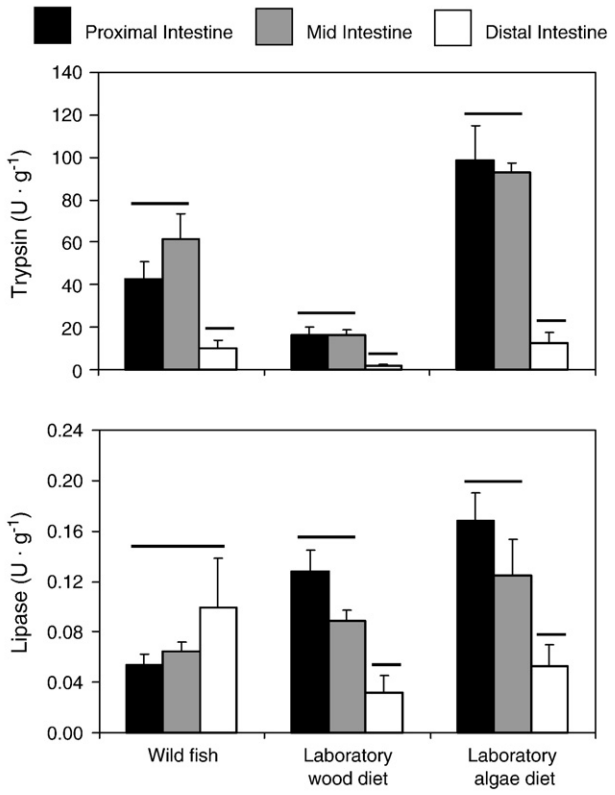

Fig. 5. Total activities (intestinal fluid + gut contents) of trypsin and lipase in three regions of the intestine of *P. disjunctivus* consuming different diets. Values are means and error bars represent SEM. Intra-feeding group comparisons of each enzyme among gut regions were made with ANOVA followed by a Tukey's HSD with a family error rate of $P = 0.05$. Lines of a different elevation passing over two bars indicate a significant difference in enzyme activity ($P < 0.01$) among those gut regions within that feeding group. Inter-feeding group comparisons of enzyme activities were not made. Data on wild-caught fish re-drawn from German and Bittong (2009).

Table 4
Aminopeptidase activities ($U \cdot g^{-1}$) in the gut walls and gut contents of *Pterygoplichthys disjunctivus* consuming different diets.

	Aminopeptidase activity		
Feeding group (n)	PI	MI	DI
Wild fish†			
Gut wall (6)	0.358 ± 0.029^{a}	0.712 ± 0.089^{b}	0.217 ± 0.052^{a}
Gut contents (10)	0.254 ± 0.045^{a}	0.262 ± 0.030^{a}	0.237 ± 0.053^{a}
t	1.64	5.78	0.25
P	0.123	**<0.001**	0.805
Laboratory — wood diet (7)			
Gut wall	0.461 ± 0.044^{a}	0.484 ± 0.149^{a}	0.440 ± 0.102^{a}
Gut contents	0.373 ± 0.081^{a}	0.192 ± 0.060^{a}	0.270 ± 0.061^{a}
t	0.96	1.82	1.43
P	0.357	0.094	0.177
Laboratory — algae diet (6)			
Gut wall	0.989 ± 0.163^{a}	1.546 ± 0.406^{a}	0.914 ± 0.404^{a}
Gut contents	1.825 ± 0.184^{b}	0.798 ± 0.123^{a}	0.671 ± 0.217^{a}
t	3.40	2.73	0.53
P	**0.007**	**0.021**	0.607

Note: Values are mean (±SEM). Aminopeptidase activity was assayed with the colorimetric substrate Alanine-*p*-nitroanilide. Comparisons were made between the activities of the gut wall and gut contents of each gut region in each feeding group with *t*-test; values considered significantly different at $P \leq 0.05$ (in **bold**). For each feeding group, the aminopeptidase activities of the gut wall and gut content fractions were individually compared among the gut regions with ANOVA followed by Tukey's HSD. For a particular feeding group, the gut wall or gut content activities (in rows) in different intestinal regions that have different superscript letters are significantly different. PI = proximal intestine; MI = mid intestine; DI = distal intestine.

† data from German and Bittong (2009).

lower β-glucosidase K_m values than the algae-fed fish. No differences in NAG K_m were detected among the feeding groups (Supplemental Table S2).

3.4. Gastrointestinal fermentation and luminal carbohydrate profiles

The wild-caught and wood-fed fish showed no pattern of SCFA concentrations (increasing or decreasing) distally in their intestines, whereas the algae-fed fish exhibited significantly higher SCFA concentrations in their distal intestines than in their proximal or mid intestine (Table 5). Although the SCFA concentrations were low for all feeding groups, the ratios of acetate:propionate:butyrate varied with diet, with the wild-caught and algae-fed fish showing a predominance of acetate in all gut regions, whereas the wood-fed fish contained 29% propionate in all gut regions.

The thin-layer chromatograms illustrated that soluble oligo- and disaccharides were present in the proximal and mid intestine but were absent in the distal intestine of the wild-caught and wood-fed fishes (Supplemental Fig. S1). Some sugars were detectable in the distal intestine of the algae-fed fish. No glucose was detectable in the intestinal fluid of any gut region of fish from any feeding group, suggesting that glucose is rapidly absorbed in *P. disjunctivus*.

4. Discussion

The results of this study generally supported our hypotheses. First, *P. disjunctivus* in all feeding groups showed decreasing intestinal surface areas towards their distal intestines, and fish consuming the low-quality wood diet decreased the size of their GI tracts on gross and ultrastructural levels, similar to starving fish. With few exceptions, digestive enzyme activities followed the same general patterns along the GI tracts of the fish from all feeding groups, although the algae-fed fish had qualitatively higher activities of most enzymes. Despite reducing the size of their gut, the wood-fed fish maintained mass-specific digestive enzyme activities similar to wild-caught fish. Because *P. disjunctivus* can endure prolonged starvation and/or chronic low-quality food in nature, we expected this fish species to down-regulate the structure and function of its gut to conserve energy in these situations. Although the wood-fed fish did reduce the size of their GI tracts, the alimentary canals of these fish continued to function and may provide insight into how *P. disjunctivus* and other detritivorous loricariids endure long periods of low-quality food during the Amazonian dry season.

4.1. Gut mass and gut structure

Phenotypic flexibility of organ size and function allows one to view how an animal copes with a changing environment (Piersma and Drent, 2003; Blier et al., 2007). For this reason, many studies have examined changes in gut structure and function in response to food availability in invertebrates (e.g., Gao et al., 2008) and in fishes (e.g., Gas and Noailliac-Depeyre, 1976; McLeese and Moon, 1989; Rios et al., 2004). When faced with starvation or poor food quality *P. disjunctivus* decreases the size of its gut, and cytoplasmic staining was reduced in all regions of the gut of starved fish, indicating a reduction in cell function. Similar results have been observed in starving *Salmo salar* (Baeverfjord and Krogdahl, 1996) and *C. carpio* (Gas and Noailliac-Depeyre, 1976), perhaps as an energy conservation mechanism. *Lota lota* and *Rutilus rutilus* each lowered their metabolic rates by nearly 50% after 28 days of starvation at 20 °C (Binner et al., 2008). Because the GI tract is so metabolically active (Cant et al., 1996), it appears to be a likely candidate tissue to down-regulate in the absence of food. We did notice a decline in movement by the starved fish in the laboratory, and Nelson (2002) observed slight decreases in the metabolic rates of loricariids fasted for up to 1 week.

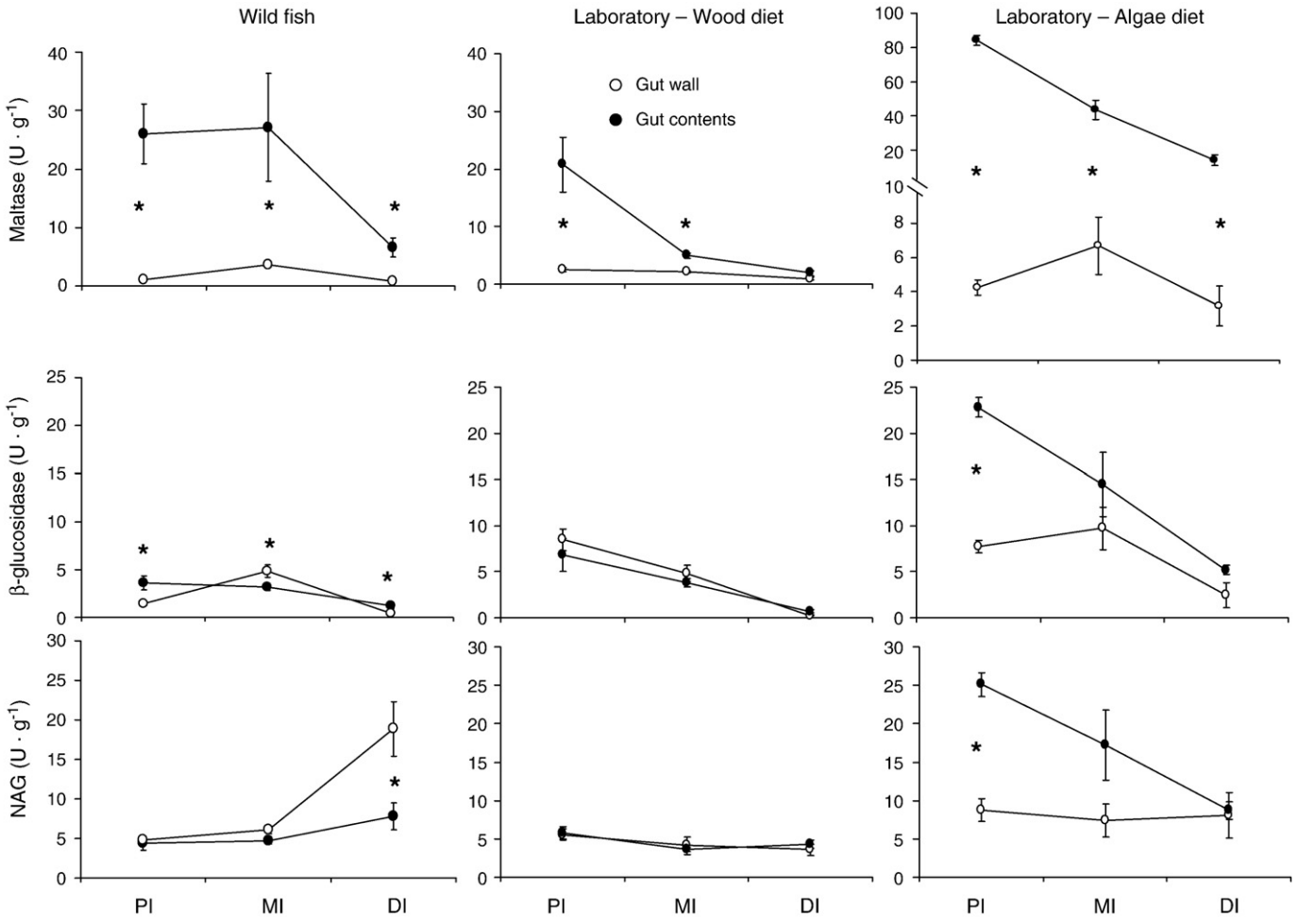

Fig. 6. Maltase, β-glucosidase, and N-acetyl-β-D-glucosaminidase (NAG) activities in the gut walls and gut contents of the proximal intestine (PI), mid intestine (MI), and distal intestine (DI) of *P. disjunctivus* consuming different diets. Comparisons were made of the activities of each enzyme between the gut walls and microbial extracts of each gut region with *t*-test. Significant differences ($P \leq 0.01$) indicated with an asterisk (*). Data from wild-caught fish are re-drawn from German and Bittong (2009).

To our knowledge, this study is the first to quantify changes in intestinal surface area that can occur in a single fish species consuming different foods (or in the absence of food). *P. disjunctivus* altered the surface area of their intestines in two ways: first by changing the size of the intestinal folds (mucosal area), and second, by changing microvilli surface area. For example the algae-fed fish had approximately 13% greater intestinal area than the wild-caught fish (because of larger intestinal folds), but the wild-caught fish had 45% greater microvilli surface area (MVSA) than the algae-fed fish when comparing summed MVSA for the entire gut. Thus, the wild-caught fish had a larger actual intestinal surface area than the algae-fed fish, and this was achieved through increased MVSA. Horn et al. (2006) observed greater MVSA in an herbivorous population of *Atherinops affinis* than in a carnivorous population of this same species, and they attributed these differences to variation in dietary composition and intake. The same may have occurred in *P. disjunctivus* in this study. Despite consuming 33% more food per day than was consumed by the algae-fed fish, the wood-fed fish decreased their intestinal surface area by decreasing the mucosal and microvilli surface area, similar to the starved fish. Furthermore, similar to Gas and Noailliac-Depeyre (1976), we observed a disappearance of microvilli from some enterocytes in the distal intestines of starved fish. Future studies of fish GI tract size should recognize that histological and ultrastructural changes may be more informative than gross changes, as the former are correlated with absorptive capacity (Secor et al., 2000).

Table 5
Total short-chain fatty acid concentrations (mM) and ratios of acetate:propionate: butyrate in three intestinal regions of *Pterygoplichthys disjunctivus* eating their natural diet, a wood diet, or an algal diet.

Intestinal region	Wild diet†	Ratio	Wood diet	Ratio	Algae diet	Ratio
Proximal	2.44 ± 0.41^{a}	64:18:18	1.77 ± 0.61^{a}	48:29:23	1.08 ± 0.19^{a}	64:19:17
Mid	2.40 ± 0.44^{a}	70:16:14	1.37 ± 0.50^{a}	52:29:18	2.24 ± 0.55^{a}	65:22:13
Distal	3.50 ± 0.68^{a}	75:13:12	2.17 ± 0.41^{a}	60:29:11	6.91 ± 1.12^{b}	78:16: 6
	$F_{2,17} = 1.28$		$F_{2,17} = 0.62$		$F_{2,17} = 28.26$	
	$P = 0.31$		$P = 0.55$		$P < 0.01$	

Note. Values are mean ± SEM. Comparisons of SCFA concentrations among gut regions within a feeding group were made with ANOVA followed by Tukey's HSD with a family error rate of $P = 0.05$. SCFA values for a feeding group that share a superscript letter are not significantly different. $N = 6$ for each feeding group.
† Data for wild-caught fish from German and Bittong (2009).

The intestinal surface areas we measured in wild-caught and algae-fed *P. disjunctivus* at the level of mucosal area rival those of the "villi area" of mammals of similar size (100–200 g; Karasov and Hume, 1997). Because mammals are endothermic, it is commonly argued that they require higher intake than ectothermic animals, and hence, have larger overall intestinal surface area to meet increased absorptive demands (Karasov and Hume, 1997; Karasov and Martínez del Rio, 2007). However, if an ectothermic animal is eating food that is sufficiently low-quality (i.e., contains a large proportion of inorganic material), they, too, have high levels of intake, and would also require an expanded absorptive surface area to ensure assimilation of nutrients. Thus, the loricariids have the longest gut lengths relative to their body size among fishes (Kramer and Bryant, 1995; German,

2009b), and intestinal surface areas that are relatively exaggerated for an ectothermic animal of their size. The MVSA of the loricariids may also match that of mammals, but methodological differences among studies makes it difficult to directly compare MVSA among the fish and mammals of similar size.

4.2. Digestive enzyme activities

As in other fishes (Harpaz and Uni, 1999; Mommsen et al., 2003; German, 2009; German and Bittong, 2009), there appears to be a clear zonation along the intestine of *P. disjunctivus*, with most enzymatic activities elevated in the proximal (amylase, laminarinase, trypsin, β-glucosidase) or mid (maltase, aminopeptidase) intestine, concomitant with MVSA and luminal carbohydrate concentrations in these gut regions. All of the polysaccharide degrading enzymes decreased in activity towards the fishes' distal intestines. Typically, enzymes of endogenous origin (e.g., amylase, trypsin) decrease in activity towards the distal intestines of fish (Skea et al., 2005, 2007; German, 2009; German and Bittong, 2009), likely because they are secreted from the pancreas into the proximal intestine. Thus, this general pattern is not affected by diet or intake in *P. disjunctivus*. Cellulase and xylanase, however, are not endogenous enzymes produced by fish (Krogdahl et al., 2005). It appears that *P. disjunctivus* and other detritivorous loricariids consume microbes and enzymes of microbial origin (e.g., cellulases) with their detrital diet, and thus, cellulolytic and xylanolytic activities are highest in the proximal intestine and decrease in the distal intestine as the microbes and their enzymes are degraded (German and Bittong, 2009). Animals with endosymbiotic microbes in their GI tracts typically have enzymatic activities against structural polysaccharides that increase in the hindgut, where the microbes are most densely populated (Potts and Hewitt, 1973; Nakashima et al., 2002; Mo et al., 2004; Skea et al., 2005, 2007). Interestingly, wild-caught *P. disjunctivus* showed increasing N-acetyl-β-D-glucosaminidase (NAG) and lipase activities in their distal intestines. Both lipase (Murray et al., 2003) and NAG (Gutowska et al., 2004) are produced endogenously in fishes, and thus, the activity patterns of these enzymes may reflect nitrogen and lipid scavenging mechanisms in the distal intestine of healthy fish. The wood-fed fish, however, consumed a low-lipid (1.90%) diet, and thus, we expected them to show a similar pattern of increasing lipolytic activity in their distal intestines. Why this pattern was not replicated in the wood-fed fish is not clear, but may have to do with the fact that these fish cannot be considered "healthy" after consuming an insufficient diet over 150 days.

The wood-fed fish, with their reduced intestinal masses, maintained similar patterns and magnitudes of mass-specific enzymatic activities as wild-caught fish, especially in the proximal and mid intestine. When a fish is starving, or at least in negative energy balance, they can rapidly mobilize protein reserves from their GI tract over the first few days of starvation (Krogdahl and Bakke-McKellep, 2005), but slow this process as starvation continues (Theilacker, 1978; Houlihan et al., 1988). This suggests that *P. disjunctivus* on the low-quality wood diet may have reduced the size of their guts relatively early on in the experiment, but maintained gut function as intake of this diet continued.

From a stable isotopic standpoint, the wood-fed fish were obtaining carbon from their diet, from the corn meal and corn gluten meal fractions in particular (German, 2008), and thus, it was beneficial for these fish to maintain digestive enzyme activities at levels similar to wild-caught fish, even with lower absorptive area. Lower absorptive surface area can correlate with lower nutrient uptake in snakes (Secor et al., 2000), and because the overall mass of the gut was reduced in *P. disjunctivus* consuming the wood diet, they had reduced summed digestive enzyme capacity (when compared to wild-caught and algae-fed fish) for their entire gut. This finding is the take-home message of this study: *P. disjunctivus* does not lower mass-specific digestive enzyme activities when consuming a diet too low in quality to meet their energetic needs, but lowers its gut mass and surface area, potentially decreasing the metabolic cost of maintaining GI tract tissue. This pattern may be reflective of fish in nature consuming low-quality detritus, although there would likely be more exogenous input of enzymes from the food itself (see below). Unfortunately, limited sample size prohibited us from measuring enzymatic activities in the starving fish to observe how they differed from the wood-fed fish. Conversely, the algae-fed fish had qualitatively higher activity levels of most digestive enzymes in comparison to the wild-caught and wood-fed fish, especially in their proximal intestines. This likely represents a non-specific increase in enzymatic activity, concomitant with increases in tissue mass and function, as even enzymes (e.g., laminarinase, which digests laminarin) for substrates not present in the algal diet increased in activity.

The wood-fed fish in this study consumed sterilized wood, and yet, exhibited detectable cellulase activity, primarily in the proximal intestine. These results could suggest that the fish have a resident microbial community producing cellulolytic enzymes in their GI tract (Nelson et al., 1999), and in the proximal intestine in particular. Das and Tripathy (1991) claimed that grass carp (*Ctenopharyngodon idella*) have an endogenous cellulase that is inducible by an increase in cellulose in the diet, even in fish that had been treated with the antibiotic tetracycline. The problem with the study performed by Das and Tripathy (1991) and the current investigation is that the substrate used to assay for cellulase, carboxy-methyl cellulose, is also hydrolysable by β-glucosidase (Clements and Raubenheimer, 2006). Thus, cellulase activity from a "sterile" diet or gut may reflect the action of β-glucosidase, the activity of which showed similar patterns along the gut as cellulase in *P. disjunctivus*. We also attempted to assay cellulase in *P. disjunctivus* with crystalline cellulose, which is not hydrolysable by β-glucosidase, and did not detect activity. Xylanase, which digests components of hemicellulose, disappeared in *P. disjunctivus* consuming the sterilized wood diet, suggesting that this enzyme is solely ingested with food.

4.3. Disaccharidase activities

For the disaccharidases, it is apparent that there was greater exogenous enzymatic input in the wild-caught and algae-fed fish (especially for β-glucosidase and N-acetyl-β-D-glucosaminidase) than in the wood-fed fish. This is evident in the lack of differences in disaccharidase activity levels between the gut wall and gut contents of the wood-fed fish. The role of digestive enzymes consumed with food in the digestive process is incompletely understood, although the field of "probiotics" suggests that ingested enzymes, especially enzymes that fishes do not synthesize (e.g., phytase), are beneficial to the fish (Rao et al., 2009). Detritivorous fishes consume digestive enzymes with their food; microbial enzymes are inherent in soils (Allison and Jastrow, 2006), biofilm (Sinsabaugh et al., 1991), and degrading wood (Sinsabaugh et al., 1992). Thus, our low-quality diet may not have been a suitable proxy for natural wood-detritus, in that it lacked exogenous enzymatic activity, with the exception of maltase, which was likely inherent in the corn meal and corn gluten meal.

We predicted that the different diets would affect the K_m values of disaccharidases in the proximal intestine gut walls of the fish. K_m values have been observed to be different among prickleback fish species (German et al., 2004) and loricariid catfish species (German, 2008) with different diets, suggesting that fishes with different diets may express different isoforms of digestive enzymes according to diet. The algae diet in this study had more soluble carbohydrates in it than the wood diet, and likely more than the natural detrital diet of *P. disjunctivus* (German, 2009b). Hence, the maltase expressed by the algae-fed fish exhibited a lower K_m, and thus, was more efficient than the maltase in the fish in the other feeding groups. The opposite was true for β-glucosidase, as the wood diet and natural detrital diet of *P. disjunctivus* likely contain more of the substrates for this enzyme—β-

glucosides, like cellobiose. Thus, in *P. disjunctivus*, an increase in a substrate concentration in a food can elicit differences in enzyme activities and in an enzyme's binding affinity for that substrate, likely through expression of different isoforms. K_m is best examined with isolated enzymes, but our analyses suggest that future studies of digestive enzymes in response to diet, disaccharidases in particular, should examine K_m in addition to activity level. Lower β-glucosidase K_m in the wood-fed and wild-caught fishes could indicate efficient digestion of β-glucosides, which may provide an important energy source for these fishes (German, 2008; German and Bittong, 2009), especially when they are enduring poor food quality during the dry season.

4.4. Gastrointestinal fermentation

The algae-fed fishes were the only feeding group that exhibited greater SCFA concentrations in their distal intestines than in the proximal or mid intestines. Endosymbiotic fermentation in fishes may often be the result of microbial utilization of soluble components in the hindgut rather than fibrous ones (Mountfort et al., 2002). Furthermore, Nelson et al. (1999) found that anaerobic microbes isolated from the guts of *Panaque maccus*, a wood-eating loricariid, were only able to grow on a glucose substrate. The algae-fed fish were the only feeding group in this study with soluble oligo- and disaccharides remaining in their distal intestines. Thus, these soluble carbohydrates may have provided the substrates for fermentation in the distal intestines of these fish. Nevertheless, the concentrations of SCFAs in *P. disjunctivus* are low by fish standards (e.g., <20 mM in the hindgut; Choat and Clements, 1998), suggesting that this species does not meet significant amounts of their energy needs through microbial fermentation and SCFAs, regardless of diet.

4. Conclusion

The results of this study indicate that *P. disjunctivus* down-regulates the size of its GI tract when faced with starvation or a diet too low to meet its energetic needs. However, GI tract function may continue if the fish are actually consuming food, no matter how low the quality. Secor et al. (2002) showed that a complex mixture of nutrients, proteins in particular, stimulate the intestine of starving pythons, causing intestinal enlargement and an up-regulation in nutrient transport. The wood-diet in this study may have been low enough in protein content to contribute to a reduction in gut size, but not a reduction in mass-specific digestive enzyme activities. Because loricariids experience seasonal food deprivation or low-quality food, the plasticity in gut size is likely important from an energetic standpoint (Wang et al., 2006), but enzymatic digestion must continue, no matter how low the quality of food. The next step in this investigation is the recovery—on what time scale does the gut recover when the fish are allowed to eat food sufficient to meet their energetic needs following chronic exposure to starvation or poor nutrition (e.g., Blier et al., 2007)? Clearly, *P. disjunctivus* is capable of non-specific increases in digestive enzyme activity and overall digestive machinery when consuming a high-quality diet, as observed in the algae-fed fish. This may show the ability of *P. disjunctivus* to match digestive function with metabolic load (Diamond and Hammond, 1992), a necessary characteristic during the recovery phase. Nevertheless, as ectotherms, fishes can endure harsh conditions and food deprivation over longer time scales than endothermic animals. This may provide an advantage within the aquatic systems of the tropics, which can vary in food availability across space and time, but have relatively consistent warm temperatures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cbpa.2009.10.018.

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