J Comp Physiol B (2014) 184:497–512
DOI 10.1007/s00360-014-0813-4

ORIGINAL PAPER

Physiological responses to short-term fasting among herbivorous, omnivorous, and carnivorous fishes

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Received: 13 October 2013 / Revised: 29 January 2014 / Accepted: 6 February 2014 / Published online: 7 March 2014

Abstract We explored the integrated role of dietary specialization and feeding periodicity on the response of the gastrointestinal tract of teleosts fishes to short-term (7–10 days) fasting and refeeding. Fasted and fed herbivorous grass carp (*Ctenopharyngodon idella*), omnivorous channel catfish (*Ictalurus punctatus*), and carnivorous largemouth bass (*Micropterus salmoides*) were compared for digestive organ masses, intestinal morphology, gastrointestinal pH, and the specific activities and total intestinal capacities of the intestinal hydrolases aminopeptidase (APN) and maltase and intestinal nutrient transporters. All three species experience intestinal hypertrophy with feeding as noted by significant increases in enterocyte dimensions. Of the three, only *I. punctatus* experienced a postprandial increase in intestinal length, and only *C. idella* experienced significant modulation of intestinal microvillus length. Feeding resulted in acidification of the stomachs of *I. punctatus* and *M. salmoides*. Predicted to exhibit a relatively modest set of postprandial responses because of their more frequent feeding habits, *C. idella* only experienced increases in APN and maltase activity with feeding and no significant regulation of nutrient uptake. Significant regulation of hydrolase activities and nutrient uptake were exhibited by *I. punctatus* and *M. salmoides*, with *I. punctatus* experiencing the most comprehensive set of responses. As predicted by food habits, there was an interspecific gradient in intestinal length and glucose uptake extending from longer intestines and greater glucose uptake for the herbivorous *C. idella*, intermediate lengths and glucose uptake for the omnivorous *I. punctatus*, and shorter intestines and reduced glucose uptake for the carnivorous *M. salmoides*. Among teleosts fishes, short episodes of fasting lead to significant alterations in intestinal form and function that are rapidly restored with feeding.

Keywords Fasting · Intestinal hydrolases · Intestinal morphology · Intestinal nutrient transport · Microvilli · pH · Refeeding · Teleosts · *Ctenopharyngodon idella* · *Ictalurus punctatus* · *Micropterus salmoides*

Introduction

Food and feeding habits play an important role in defining the form and function of the vertebrate gastrointestinal tract (Karasov and Martinez del Rio 2007). Vertebrates not only display a variety of dietary specializations, ranging from herbivory to omnivory to carnivory, but also vary in the size and frequency of meals (Stevens and Hume 1995). Regardless of diet and feeding habits, the gut possesses a suite of adaptive features tailored to the demands of meal digestion and absorption (McCauley and Bjorndal 1999; Raubenheimer and Simpson 1999; Karasov and Martinez del Rio 2007). This adaptation is particularly evident among the teleost fishes due to their varied diet and feeding

Communicated by I.D. Hume.

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habits, diverse phylogeny, and the number of distinct variations in the general plan of their gut (e.g., presence or absence of a stomach or pyloric ceca; Wilson and Castro 2010).

Among teleosts fishes, a match between intestinal morphology and diet has been recognized. Herbivorous fishes tend to possess relatively long narrow intestines that are coiled and fairly uniform in structure throughout, whereas the intestines of carnivorous species are much shorter, thicker, and straighter with a greater degree of mucosal folding (Kapoor et al. 1975; Ferraris and Ahearn 1984; Kramer and Bryant 1995). At the pinnacle of teleost digestive tract complexity are herbivores such as the surgeonfish (Acanthuridae) and the drummer (Kyphosidae), with particularly long intestines and ingesta retention times, enabling more effective digestion of large quantities of their low-quality, fibrous food by providing an increased duration of exposure to intestinal enzymes, transporters, and microbiota (Rimmer and Wiebe 1987; Polunin et al. 1995; Choat et al. 2004). The higher nutrient concentration (i.e. protein and fat content) of a carnivorous diet tends to allow fishes to rely on quality rather than quantity, resulting in a lower intake of more easily digested and absorbed food, thus explaining the shorter intestines of carnivores (Benavides et al. 1994; Stevens and Hume 1995; Karasov and Martinez del Rio 2007). Intestinal function is likewise correlated with natural diet among fishes: herbivorous fishes possess higher intestinal capacities to process and absorb glucose than carnivorous fishes (Hidalgo et al. 1999; German et al. 2004; Horn et al. 2006; Day et al. 2011a), while also exhibiting lower rates of intestinal proline uptake compared to carnivorous species (Buddington et al. 1987).

Diet and nutrient concentration also dictate a spectrum of frequencies with which fishes eat, and hence there exists a continuum of periodicity of feeding and fasting (Penry and Jumars 1987; Horn and Messer 1992). Exclusively herbivorous fishes (e.g. not detritivores or omnivores) tend to either graze continuously or browse frequently on algal and plant materials throughout daylight hours, and thus are perpetually digesting and absorbing food with very short episodes of digestive quiescence (Horn 1989; Horn and Messer 1992). Carnivorous fishes feed on prey that may be more mobile, harder to catch, less abundant or only available periodically and thus feed more intermittently with longer episodes between meals (Sibley and Calow 1986; Arrington et al. 2002). Possessing an inherently low metabolic rate (relative to endotherms), fishes can more easily tolerate prolonged periods of fasting. Extended fasting is natural for many fishes and is associated with ecological and life history events such as low food availability, migration, reproduction, and dormancy (Navarro and Gutiérrez 1995).

How long a fish can survive an extended fast is a function of the amount of stored energy (e.g., glycogen and fat) and structural energy (e.g. protein) available for maintenance and the rate at which these substrates are catabolized (Navarro and Gutiérrez 1995). Given a finite amount of endogenous resources for any individual, any mechanism that can reduce maintenance cost during extended fasting, and hence prolong survival, would be advantageous. Such a mechanism has been observed for infrequently feeding snakes which, upon the completion of digestion, downregulate intestinal structure and function as an adaptive step to reduce maintenance cost between meals (Secor and Diamond 2000; Ott and Secor 2007a). Feeding triggers the rapid upregulation of gastrointestinal performance to meet the demands of meal digestion and absorption (Secor and Diamond 2000; Ott and Secor 2007a). For relatively frequently feeding amphibians, snakes and other reptiles that experience short inter-meal durations, intestinal performance is modulated less markedly between feeding and fasting (Secor and Diamond 2000; Secor 2005; Cox and Secor 2010). Predictably, similar selective pressures would generate variation in the magnitude by which fish regulate intestinal performance and result in adaptive correlates between feeding habits and digestive physiology (Buddington et al. 1987; Secor 2005; German et al. 2010a).

To examine the potential adaptive interplay between feeding habits and gastrointestinal physiology for fishes, we investigated the effects of short-term fasting and subsequent refeeding on gastrointestinal form and function of the herbivorous grass carp (*Ctenopharyngodon idella*), the omnivorous channel catfish (*Ictalurus punctatus*) and the carnivorous largemouth bass (*Micropterus salmoides*). The differing feeding habits of these fish allowed us to explore the potential interactions between diet and feeding periodicity, and the morphological and functional flexibility of their gastrointestinal tract. We predicted that the herbivorous and frequently feeding *C. idella* would possess a longer intestine and greater carbohydrate processing potential and glucose uptake, and experience relative modest changes in gut form and function between fasting and feeding. In contrast, the carnivorous and more intermittent feeding *M. salmoides* would have a shorter intestine, reduced carbohydrase activity and uptake, and regulate intestinal function more dramatically between feeding and fasting. The expected postprandial responses of the omnivorous *I. punctatus* were predicted to be intermediate between *C. idella* and *M. salmoides*. We examined these a priori assumptions on feeding habits and gastrointestinal responses by measuring from fasted and fed fishes: (1) gastrointestinal pH, (2) organ wet and dry masses; (3) intestinal morphometrics at the gross and cellular level; (4) intestinal brush border hydrolase activities

and capacities; and (5) intestinal nutrient transport rates and summed capacities. The results of this study provide some insight as to how fishes with different feeding frequencies, as a function of their different diets, respond to the onset of fasting and how the regulation of their digestive function and morphology is prioritized.

Materials and methods

Fish and feeding habits

The grass carp (*C. idella*), native to rivers in China and Russia, has been introduced worldwide for aquaculture and as a biological control for aquatic vegetation (Pierce 1983). It is herbivorous and feeds frequently and relatively indiscriminately on available terrestrial and aquatic macrophytes (Pierce 1983). It lacks a stomach and relies largely on its pharyngeal jaws to mechanically grind a plant-heavy diet prior to chemical digestion in the intestine (Shireman and Smith 1983). The channel catfish (*I. punctatus*), native to eastern and central North America, has been introduced elsewhere in the United States largely through aquaculture (McMahon and Terrell 1982). It is an opportunistic omnivore with a varied diet that includes detritus, plants, insects, crustaceans, other fish and amphibians (Jackson 2004). It possesses a thin-walled, J-shaped stomach and a relatively thick-walled intestine that progressively thins distally until the ileo-rectal valve, followed by a similarly thick-walled rectum (Reifel and Travill 1979; Sis et al. 1979). The largemouth bass (*M. salmoides*) likewise is native to eastern North America and has also been introduced throughout central and western United States and northern Mexico. This fish transitions with age from a diet of zooplankton and insects to feeding less frequently on larger prey that include crustaceans, other fish, amphibians, and the occasional small reptile, bird or mammal (Cochran and Adelman 1982; Brown et al. 2009). Its large, thin-walled stomach is followed by 9–12 terminally bi- and trifurcated pyloric ceca that extend at the start of the intestine and aid in nutrient absorption (Reifel and Travill 1979; Buddington and Diamond 1987). The bass's intestine is short, thick walled and lacks a clearly defined ileo-rectal valve (Reifel and Travill 1979).

Fish maintenance, dissection and tissue preparation

Fish were obtained from commercial fish farms and were fasted for 7–10 days to insure that they were post-absorptive and had the opportunity to downregulate gastrointestinal performance. Individuals of each species were separated into two groups ($n = 5$–6 in each) with one group remaining fasted and the other group fed. *Ctenopharyngodon idella* was fed centipede grass (*Eremochloa ophiuroides*), *I. punctatus* was fed commercial catfish chow (Purina Mills; Gray Summit, MO), and *M. salmoides* was fed golden shiners (*Notemigonus crysoleucas*, 5–8 g). There was no significant difference (P values >0.28) in body mass between fasted and fed fish of each species. All fish care and experimentation were conducted under an approved protocol of the University of Alabama Institutional Animal Care and Use Committee.

Fasted and fed (24 h postfeeding) fish were transported to the laboratory and killed by severing their spinal cord immediately behind their head, followed by pithing of the brain. Each fish was then measured for standard length, total length, and body mass. A mid-ventral incision exposed the visceral organs and for *I. punctatus* and *M. salmoides* luminal pH of the stomach and 2–3 regions of the intestine were measured using a slender pH probe (Accument 13-620-95, Fisher Scientific; Pittsburgh, PA). We removed and weighed the stomach, liver, gall bladder, intestines, spleen, kidneys, and visceral fat. The stomach, gall bladder, intestines, and pyloric ceca (*M. salmoides* only) were then emptied of any contents and re-weighed (to obtain empty mass). For each fish, we measured the length of the intestine and for *M. salmoides* the length of each ceca. Organs, with the exception of the intestine and pyloric ceca which were used for nutrient uptake analysis, were dried to a constant mass at 55 °C to calculate organ dry masses.

Because these fish differ in intestinal morphology, we partitioned their intestines in slightly different ways. For *C. idella*, which lack both a stomach and an ileo-rectal value, we divided the intestine into two segments of equal length (designated proximal and distal). The intestine and rectum of *I. punctatus* were clearly demarcated by the ileo-rectal valve; therefore we divided the intestine into two segments of equal length and isolated the rectum. For *M. salmoides* which possesses pyloric ceca and lack a distinct ileo-rectal valve, we separated the pyloric ceca and divided the intestine into two segments of equal length. Immediately following their partitioning, intestinal segments and ceca were kept in ice-cold Ringer's (124 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 2 mM $MgSO_4$, 12 mM $NaHCO_3$, 2.8 mM NaH_2PO_4) that was aerated with 95 % O_2 and 5 % CO_2.

Morphological and histological examination

Two indices of intestinal length were calculated using the measurements taken during fish dissection. The length of the intestine was compared to body length to calculate relative intestinal length (RIL = intestinal length/total body length or intestinal length/standard length), and to body mass to calculate Zihler index [ZI = intestinal length $\times$ 10 (body mass$^{1/3}$)$^{-1}$; German and Horn (2006);

Day et al. (2011b)]. As the pyloric ceca of *M. salmoides* represent a substantial amount of absorptive area, both RIL and ZI were calculated for intestine proper and intestine plus pyloric ceca.

Histological examination of the proximal intestine of all three taxa and the ceca of *M. salmoides* were performed on 1-cm segments of tissue fixed in 10 % fish Ringers-buffered formalin. Segments were embedded in paraffin, transverse sectioned using a rotary microtome (6 μm), and the sections placed on glass slides, stained with hematoxylin and eosin and mounted using glass coverslips and DPX (Mass Histology Inc., Worcester, MA). From each cross section, we measured the width of the mucosa/submucosa layer, the muscularis/serosa layer, and the height and width of enterocytes using a light microscope and video camera linked to a computer with image-analysis software (Motic Image Plus; Richmond, British Columbia, Canada). We calculated for each fish the average width of the mucosa/submucosa and muscularis/serosa layers from ten measurements taken at different positions of the cross section. Similarly, we calculated the average enterocyte height (H), width (W), and volume from ten individual enterocytes measured at different positions of the cross section. Individual enterocyte volume was calculated based on a formula for a cylinder, volume $= (0.5\ W)^2 \pi H$.

Small samples (1 mm^3) of the proximal intestine of each fish were fixed in 2.5 % glutaraldehyde in fish Ringers solution for transmission electron microscopy. Samples were later post-fixed in 1 % osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Spurr resin. Ultra-thin sections ($\approx$90 nm) were placed on copper grids, stained with uranyl acetate and lead citrate, and examined on a Hitachi electron microscope. For each sample, we measured the height (H) and diameter (D) of 50–100 microvilli and calculated for each the microvillus surface area (MVSA, μm^2) using the equation $(H\pi D) + (\pi R^2)$, where $R = 0.5D$ (German 2009).

Intestinal brush border enzyme assays

The activities of the intestinal brush border enzymes aminopeptidase-N (APN) and maltase were examined from fasted and fed treatments of each of the three fish species. From each intestinal segment, a 1-cm section was immediately snap frozen in liquid nitrogen, stored at −80 °C, and later homogenized on ice in PBS buffer (pH 7.0) at a dilution of 1:100 (w/v) and aliquoted into cryovials and stored at −80 °C. We assayed APN (EC 3.4.11.2) following the procedure of Wojnarowska and Gray (1975) as described by Cox and Secor (2008). Intestinal homogenate was added to 0.34 mM leucyl-β-naphthylamide substrate and *p*-hydroxymercuribenzoic acid and incubated at 25 ± 1 °C for 30 min. The reaction was stopped with the addition of 40 % trichloroacetic acid and absorbance was measured at 560 nm using a spectrophotometer (DU 530, Beckman Coulter, Inc., Fullerton, CA, USA). APN activity was calculated using a β-napthylamide standard curve and expressed in U (1 μmol β-napthylamide liberated) min^{-1} g^{-1} wet mass of tissue.

Maltase (EC 3.2.1.20) was assayed using the Dahlqvist (1968) assay as described by Cox and Secor (2008). Tissue homogenate was incubated in 62.5 mM maltose at 25 °C for 30 min. Glucostat solution (250 mM Tris buffer, 0.002 mg ml^{-1} horseradish peroxidase, 10 mmol l^{-1} *p*-hydroxybenzoic acid, 0.2 mmol l^{-1} aminoantipyrine, and 0.0334 mg ml^{-1} glucose oxidase) was added and allowed to incubate for an additional 30 min. Absorbance was read at 500 nm and compared to a glucose–glucostat standard curve to calculate activity, which was expressed as U (1 μmol glucose liberated) min^{-1} g^{-1} wet mass of tissue. For both APN and maltase, we calculated total intestinal capacity for activity by summing the product for each segment of mass-specific activity and segment wet mass.

Nutrient uptake assays

We measured rates of intestinal brush border transport of the amino acids L-leucine and L-proline and of the sugar D-glucose using the everted sleeve technique (Karasov and Diamond 1983; Buddington et al. 1987; Secor et al. 1994). Intestinal and cecal segments were everted and divided into 1-cm sleeves. Sleeves were mounted on grooved rods and pre-incubated in aerated fish Ringer's solution at 25 °C for 5 min. Sleeves were subsequently incubated for 4 min in aerated 25 °C fish Ringer's containing both unlabeled (amino acids at 50 mmol $l^{-1}$and D-glucose at 20 mmol l^{-1}) and radiolabeled nutrient (^{3}H-L-leucine, ^{3}H-L-proline or ^{14}C-D-glucose) and an adherent fluid marker labeled with a different radioisotope (^{14}C polyethylene glycol for the amino acids and ^{3}H-L-glucose for D-glucose). In this procedure, we measured total uptake (passive and carrier-mediated) of each amino acid and carrier-mediated uptake of D-glucose as nmole min^{-1} mg^{-1}. We likewise calculated total intestinal uptake capacity for each solute by summing the product for each segment of mass-specific uptake rates and segment wet mass.

Statistical analysis

Intraspecific comparisons of body mass between the two feeding treatments were tested for normality using Shapiro–Wilks W tests and then compared using Welch two-sample t tests with the significance level set at $\alpha = 0.05$ in R v2.10.1 (R Foundation for Statistical Computing, Austria). Intraspecific comparisons of organ masses, nutrient

Table 1 Effect of fasting and feeding on the digestive system in our three study taxa

Variable	*C. idella*			*I. punctatus*			*M. salmoides*		
	Fasted (5)	Fed (6)	*P*	Fasted (6)	Fed (7)	*P*	Fasted (5)	Fed (6)	*P*
Body mass	204 ± 17	225 ± 8	0.265	382 ± 24	357 ± 55	0.701	304 ± 19	295 ± 16	0.772
Liver (wet)	3.43 ± 0.25	4.70 ± 0.37	0.062	4.18 ± 0.38	4.20 ± 0.88	0.572	2.93 ± 0.27	2.85 ± 0.12	0.933
Liver (dry)	0.95 ± 0.10	1.33 ± 0.10	0.062	0.98 ± 0.11	0.83 ± 0.18	0.553	0.79 ± 0.08	0.79 ± 0.03	0.660
Gall bladder (full)	3.38 ± 0.34	1.17 ± 0.30	*0.003*	1.00 ± 0.15	0.33 ± 0.06	*0.010*	0.36 ± 0.04	0.13 ± 0.01	*0.016*
Gall bladder (empty)	0.20 ± 0.02	0.15 ± 0.01	0.054	0.09 ± 0.01	0.07 ± 0.01	*0.034*	0.07 ± 0.01	0.06 ± 0.01	0.268
Gall bladder (dry)	0.03 ± 0.00	0.03 ± 0.01	0.989	0.02 ± 0.00	0.01 ± 0.00	*0.048*	0.02 ± 0.00	0.02 ± 0.00	0.446
Stomach (full)	–	–	–	6.30 ± 1.41	17.7 ± 10.8	*0.043*	4.06 ± 0.83	3.57 ± 0.53	0.423
Stomach (empty)	–	–	–	6.20 ± 0.58	6.80 ± 3.01	*0.037*	3.92 ± 0.51	3.01 ± 0.50	0.267
Stomach (dry)	–	–	–	1.10 ± 0.09	1.02 ± 0.19	0.936	0.83 ± 0.10	0.62 ± 0.09	0.170
Intestine (full)	3.74 ± 0.46	6.27 ± 0.67	*0.044*	4.29 ± 0.39	11.0 ± 2.8	*0.001*	1.44 ± 0.02	1.95 ± 0.14	*0.002*
Intestine (empty)	3.30 ± 0.47	4.16 ± 0.32	0.372	4.14 ± 0.35	4.30 ± 0.56	0.752	1.24 ± 0.08	1.32 ± 0.17	0.577
Ceca mass (empty)	–	–	–	–	–	–	1.55 ± 0.17	1.72 ± 0.29	0.146
Rectum (full)	–	–	–	1.48 ± 0.22	3.34 ± 1.58	*0.001*	–	–	–
Rectum (empty)	–	–	–	1.18 ± 0.27	1.60 ± 0.43	0.196	–	–	–
Intestine (total)	3.30 ± 0.47	4.16 ± 0.32	0.372	5.32 ± 0.40	5.90 ± 0.97	*0.002*	2.79 ± 0.21	3.16 ± 0.34	0.142
Spleen (wet)	0.25 ± 0.02	0.23 ± 0.03	0.301	0.35 ± 0.03	0.21 ± 0.02	*0.002*	0.36 ± 0.04	0.32 ± 0.09	0.720
Spleen (dry)	0.06 ± 0.01	0.07 ± 0.01	0.812	0.08 ± 0.01	0.04 ± 0.01	*0.002*	0.12 ± 0.01	0.10 ± 0.02	0.515
Visceral fat bodies	0.82 ± 0.17	3.31 ± 0.59	0.124	4.27 ± 0.72	3.40 ± 1.91	0.590	3.02 ± 0.61	2.94 ± 0.76	0.823

Intraspecific comparisons of body mass between treatments which were performed using Welch's *t* test. All other statistical analyses were performed using one-way ANCOVA with body mass as the covariate

Significant results are italicized. Sample size for each treatment is noted in parentheses

uptakes, and histology measurements between fasted and fed treatments from each of the three studied taxa were tested for normality using Shapiro–Wilks *W* tests, tested for equality of variances using Levene's test and compared using one-way ANCOVA with body mass as the covariate. Significant results were followed with a Tukey's HSD multiple comparison test with a family error rate set at $P \leq 0.05$. All ANCOVA and post hoc tests were performed using Statistica 9 (StatSoft, Tulsa, OK). Intraspecific comparisons of brush border enzyme assays were performed using *t* tests between treatments using R v2.10.1. We designate the level of significance as $P \leq 0.05$ and report mean values as mean ± -SEM throughout the manuscript.

Results

Body size, gastrointestinal pH, and organ masses

For each taxon, standard length, total length, and body mass did not statistically differ between fasted and fed fish (Table 1). Fed individuals were found to possess food within their stomachs (*I. punctatus* and *M. salmoides*) and chyme within their intestines. Fasting *I. punctatus* and *M. salmoides* possessed, on average, a slightly acidic environment within their stomachs (pH of 5.0 and 5.79, respectively) with individual luminal gastric pH ranging from 3.0 to 6.9 (Fig. 1). Feeding generated significant (*P* values <0.013) decreases in gastric pH for both of these fishes as their stomach increased HCl production (Fig. 1). Digestion also induced significant decreases in luminal pH of the proximal intestine ($P < 0.037$) and rectum ($P < 0.0021$) of *I. punctatus*, but no change in intestinal pH for *M. salmoides* (Fig. 1).

In this study, *C. idella* did not experience postprandial changes in the wet and dry masses of the liver, empty gall bladder, spleen, or of the wet mass of the empty intestine; however, full gall bladder mass did decrease ($P < 0.0026$) by 65 %. Similarly, *I. punctatus* exhibited no postprandial change in either the wet and dry masses of the liver, stomach, and empty gall bladder, or in the wet masses of the empty intestine and empty rectum. *Ictalurus punctatus* did undergo significant (*P* values <0.034) postprandial decreases in full gall bladder mass and wet and dry masses of the spleen. Likewise for *M. salmoides*, feeding resulted in no significant change in the wet and dry masses of the liver, empty stomach, empty gall bladder and spleen, and no change in wet mass of ceca and empty intestine. As observed for the other two species, *M. salmoides* experienced a significant ($P < 0.016$) decrease (64 %) in the mass of the full gall bladder.

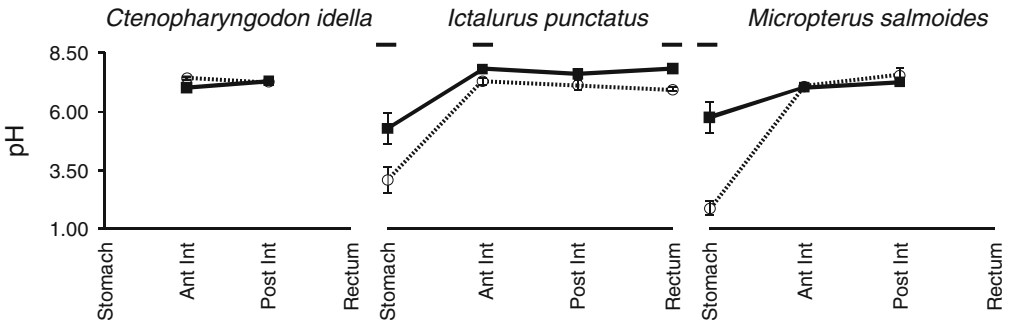

Fig. 1 Gastrointestinal pH measurements of fasted (*closed square* and *solid line*) and fed (*open circles* and *dashed line*) *Ctenopharyngodon idella*, *Ictalurus punctatus*, and *Micropterus salmoides*. A significant difference between fasted and fed treatments is marked with a bar over the alimentary segment. In this and other figures, *error bars* represent SE and are omitted in this figure if the SE is smaller than the symbol. However, for *C. idella* no *error bars* are present because pH measurements were made for only one specimen. Note the rise in gastric pH with fasting for *I. punctatus* and *M. salmoides*

Intestinal morphology

Intestinal length varied significantly among the three species. When analyzed based on absolute length (total body length as a covariate), relative intestinal length, and Zihler index, *C. idella* possessed a significantly longer intestine than *I. punctatus*, which possessed a significantly longer intestine than *M. salmoides* (Fig. 2a–c). However, if the combined length of the pyloric ceca was added to intestinal length for *M. salmoides*, it increased this fish's intestinal length by 254 ± 21 %. This combined intestinal length of *M. salmoides* did not statistically differ from the intestinal length of *C. idella*, and was significantly longer than the intestine of *I. punctatus* (Fig. 2a–c). Several other interspecific differences in intestinal morphology were noted. In fasted fishes, *I. punctatus* had a thicker ($P < 0.001$) mucosa/submucosa layer than that of the other two taxa and *M. salmoides* had microvilli that were significantly thinner than that of the other two taxa. Among fed fishes, *C. idella* and *M. salmoides* had taller enterocytes than *I. punctatus.* Fed *C. idella* also had longer microvilli than the other taxa (Fig. 3a–e).

Feeding induced a significant ($P < 0.0034$) increase (~50 %) in intestinal length for *I. punctatus* (Fig. 2a–c). Neither intestinal length for *C. idella* and *M. salmoides*, nor ceca length for *M. salmoides*, were affected significantly by feeding (Fig. 2a–c). Of the three species, only *I. punctatus* experienced significant (P values <0.033) postprandial changes in the thickness of the mucosa/submucosa and muscularis/serosa layers, with both decreasing after feeding (Fig. 3a, b). The thickness of these layers was nearly identical for fasted and fed *C. idella*. In fed *M. salmoides*, mucosa/submucosa and muscularis/serosa layers (Fig. 3a, b) had on average increased by 40 % and decreased by 50 %, respectively, however, these differences were not statistically significant (mucosa/submucosa $P > 0.162$; muscularis/serosa $P > 0.116$). Enterocyte height (Fig. 3c) significantly (P values <0.041) increased after feeding for both *C. idella* and *M. salmoides*, and significantly (P value <0.001) decreased for *I. punctatus*. Feeding generated significant (P values <0.031) increases in the enterocyte width for both *I. punctatus* and *M. salmoides* (Fig. 3d). With increases in enterocyte height and width, enterocyte volume significantly (P values <0.015) increased after feeding on averaged by 88, 34, and 64 %, respectively, for *C. idella*, *I. punctatus* and *M. salmoides* (Fig. 3e). The pyloric ceca of the *M. salmoides* exhibited no postprandial changes in either tissue thickness or enterocyte dimensions (Fig. 3a–e). We observed only *C. idella* to experience a significant (P values <0.048) postprandial increase in microvillus length (by 70 %; Fig. 4a) and MVSA (by 60 %; Fig. 4c).

Intestinal brush border enzymes

With feeding, *C. idella* experienced significant (P values <0.034) increases in the specific activities of APN and maltase for the proximal intestine, though neither hydrolase was upregulated in the distal half of the intestine (Fig. 5). Feeding also triggered significant (P values <0.004) increases in APN-specific activity for both the proximal and distal intestine and rectum of *I. punctatus* (Fig. 5). Neither the intestine nor the rectum of *I. punctatus* responded to feeding with an increase in maltase activity (Fig. 5). Within 24 h after feeding, *M. salmoides* pyloric ceca and proximal intestine had significantly upregulated specific activity of APN (P values <0.05), and the distal intestine had significantly ($P < 0.05$) upregulated maltase activity (Fig. 5). Total intestinal capacity, calculated by summing the products of intestinal segment mass and specific activity, did not vary significantly between fasted and fed *C. idella* for either APN or maltase (Fig. 6). Both *I.*

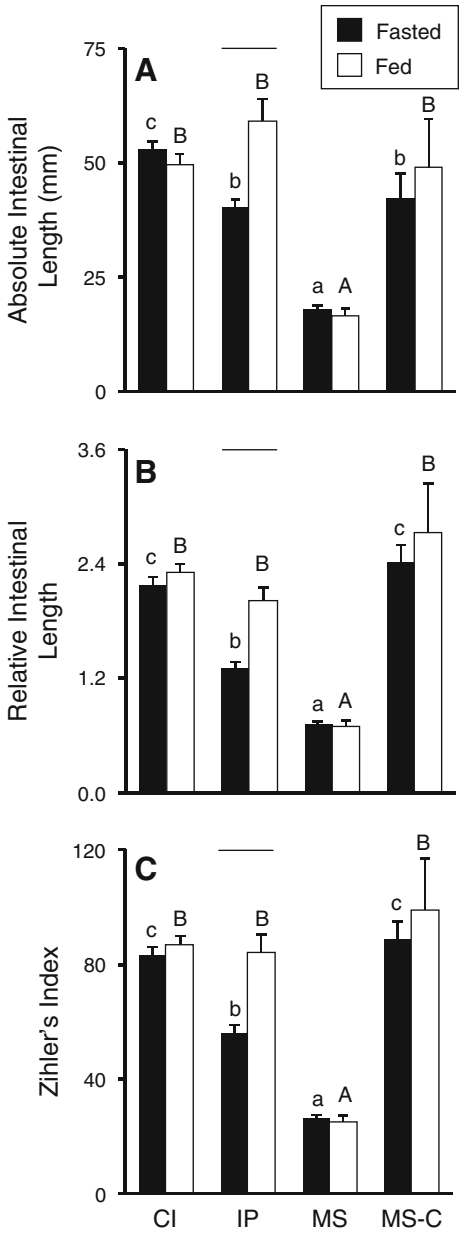

Fig. 2 **a** Absolute intestinal length, **b** relative intestinal length (to body length), and **c** Zihler's index (intestinal length relative to body mass) of fasted and fed *Ctenopharyngodon idella* (CI), *Ictalurus punctatus* (IP), *Micropterus salmoides* (MS), and *M. salmoides* pyloric ceca (MS-C). In this and following figures: (1) *filled bars* depict fasted treatments and *open bars* depict fed treatments; (2) significant intraspecific difference between fasted and fed treatments are denoted by a *horizontal line* above bars; and (3) For each treatment, *differing letters* (*lower case* for fasted and *upper case* for fed) indicate significant differences ($P < 0.05$) between species. Note the significant postfeeding increase in the length of the small intestine of *I. punctatus* and the significant variation in intestinal length among fish species

punctatus and *M. salmoides* experienced significant (P values <0.008) postprandial increases in intestinal APN capacity; however, neither fish upregulated with feeding total intestinal maltase capacity (Fig. 6).

Intestinal nutrient uptake

For *C. idella*, feeding failed to induce any significant change in the uptake of L-leucine, L-proline, or D-glucose for either the proximal and distal halves of the intestine (Fig. 7). In contrast, feeding sparked significant (P values <0.03) increases in the uptake of all three nutrients for the proximal portion of the intestine of *I. punctatus*, as well as for the uptake of L-proline and D-glucose by the distal portion of the intestine and of L-leucine and L-proline by the rectum (Fig. 7). The only significant ($P < 0.02$) change in nutrient uptake for *M. salmoides* was 120 % increase in L-leucine uptake by the proximal intestine (Fig. 7). Neither the distal intestine nor pyloric ceca of *M. salmoides* experienced significant postprandial regulation in the uptake of the three nutrients.

As a reflection of their different feeding habits, these fishes varied in the ratio of amino acid to glucose uptake rates. The herbivorous *C. idella* exhibited L-leucine to D-glucose and L-proline to D-glucose uptake ratios that averaged 4.6 and 6.1, respectively. For the omnivorous *I. punctatus*, the ratios averaged, respectively, 4.3 and 13.0. Ratios were notably greater for the carnivorous *M. salmoides*, 65.9 and 83.3, respectively. These differences in uptake ratios stem largely from a decrease in specific uptake of D-glucose from *C. idella* to *I. punctatus* and again to *M. salmoides*, rather than any notable interspecific differences in amino acid uptake.

From fasted to fed, *C. idella* experienced a significant ($P < 0.0038$) increase (65 %) in total intestinal capacity for L-proline uptake (Fig. 8). Neither the total intestinal capacity of L-leucine nor D-glucose uptake for this fish varied significantly with feeding. In contrast, *I. punctatus* experienced significant (P values <0.011) increases in intestinal uptake capacity with feeding for all three nutrients; most notably was a near ninefold increase in L-leucine uptake capacity (Fig. 8). Feeding failed to generate any significant change for *M. salmoides* in nutrient uptake capacity of their ceca, intestine or combined ceca and intestine (Fig. 8).

Discussion

A priori predictions based on the adaptive regulation of digestion displayed by other ectothermic vertebrates led to the hypothesis that the three species of fish investigated in this study would display an adaptive correlation between the capacity to modulate intestinal form and function with fasting and feeding and dietary habits vis-a-vis the frequency of feeding on their natural diet (Buddington et al. 1987; Secor 2005; Karasov and Martinez del Rio 2007). This hypothesis was supported in part by the results of this

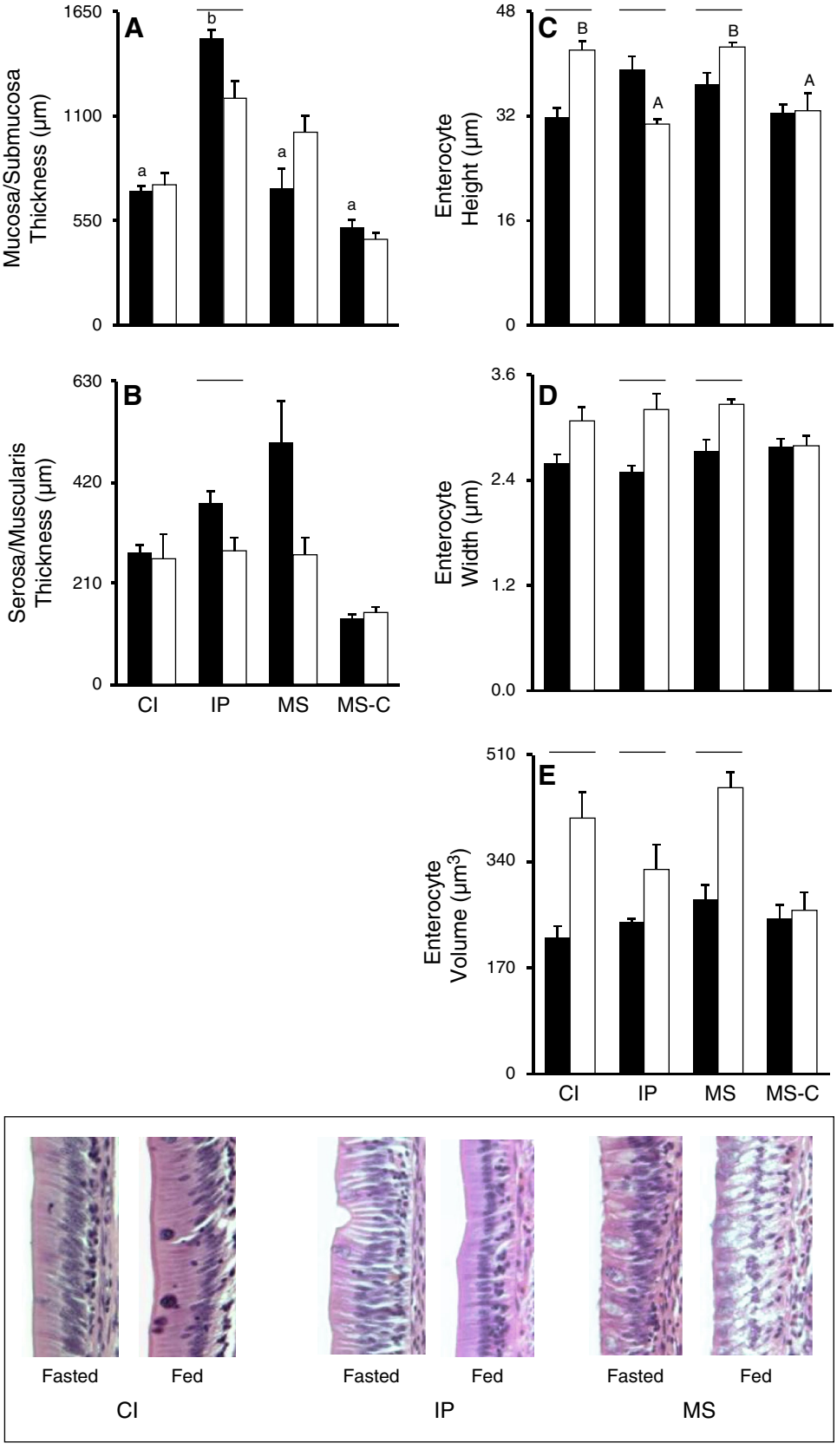

Fig. 3 **a** Mucosa/submucosa thickness, **b** serosa/muscularis thickness, **c** enterocyte height, **d** enterocyte width, **e** enterocyte volume, and micrographs (*below*) of the intestinal epithelium of fasted and fed *Ctenopharyngodon idella* (CI), *Ictalurus punctatus* (IP), *Micropterus salmoides* (MS), and *M. salmoides* pyloric ceca (MS-C). Note that all three species experienced significant postprandial increases in intestinal enterocyte volume

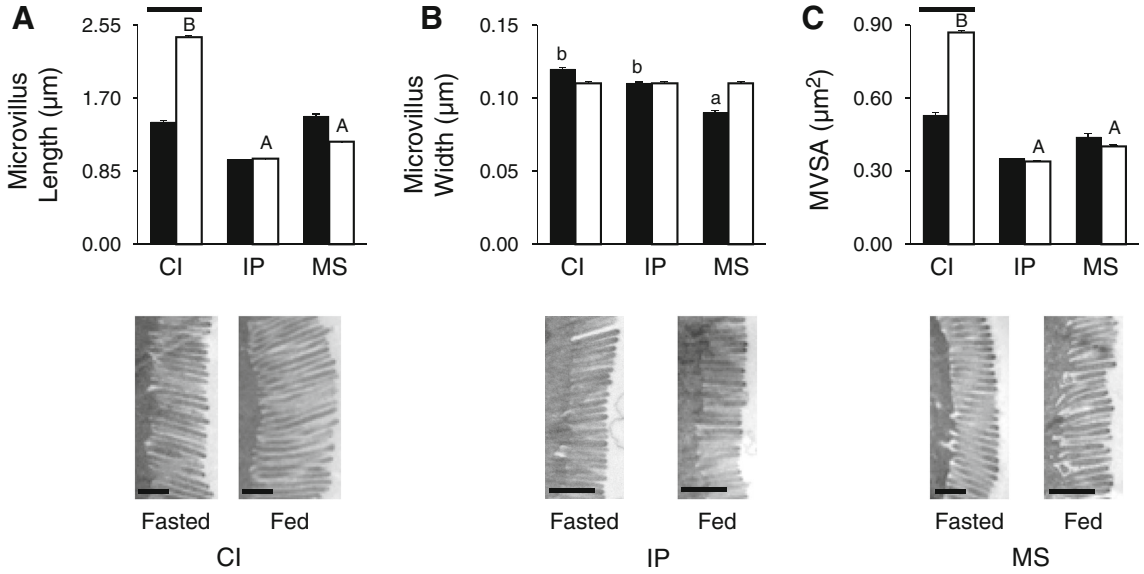

Fig. 4 **a** Intestinal microvillus length, **b** microvillus width, and **c** microvillus surface area (MVSA), and transmission electron micrographs of microvilli of fasted and fed *Ctenopharyngodon idella* (CI), *Ictalurus punctatus* (IP), and *Micropterus salmoides* (MS). The *scale bar* on all micrographs indicates 1 μm. Only *C. idella* experienced a significant postprandial increase in microvillus length and MVSA

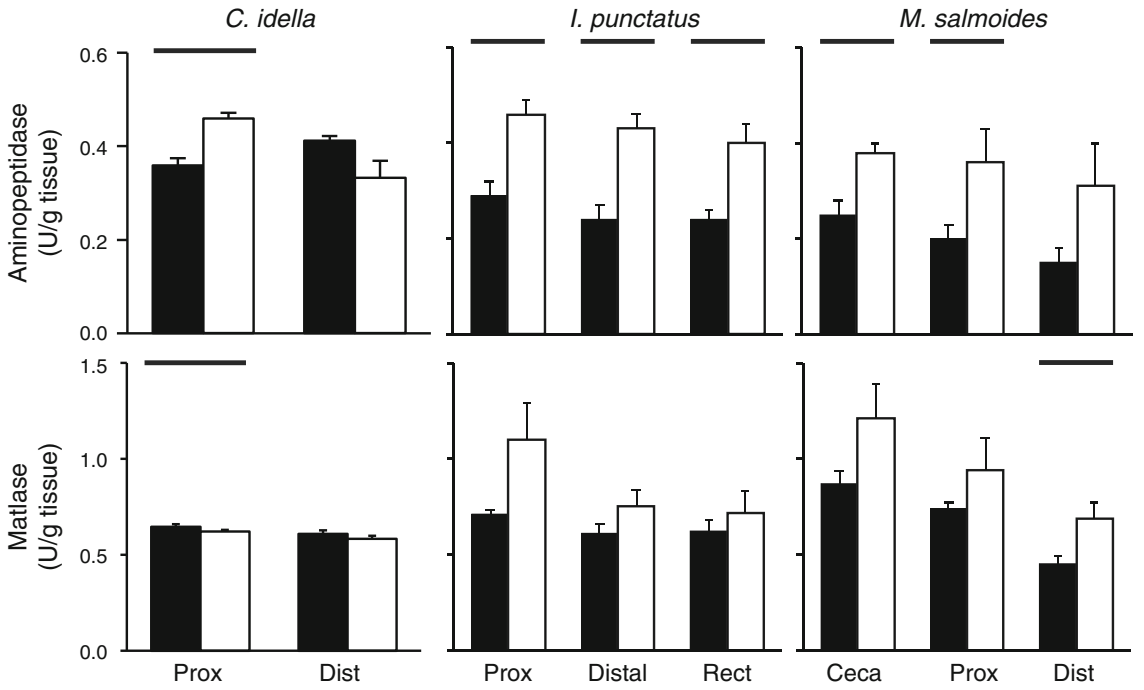

Fig. 5 Intestinal aminopeptidase and maltase activities for the pyloric ceca (Ceca), proximal (Prox) and distal (Dist) small intestine, and rectum (Rect) for fasted and fed *Ctenopharyngodon idella*, *Ictalurus punctatus*, and *Micropterus salmoides*. Enzyme activities are presented as μmol substrate liberated min^{-1} g^{-1} wet mass of tissue. Note that all three species experience significant postprandial increases in aminopeptidase activity for the proximal small intestine

study, as *C. idella*, a frequently feeding herbivore, experienced modest regulation of intestinal structure and function between feeding and fasting. Also supportive is that the magnitude of regulation was greater for the omnivorous *I. punctatus* presumably reflecting their potentially more episodic feeding habits. However, this difference ended

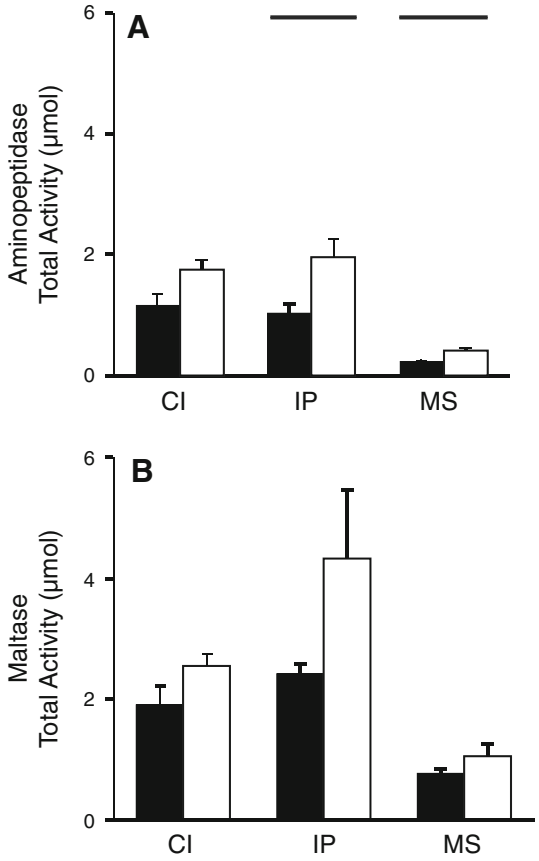

Fig. 6 Summed intestinal (including the cecum or rectum if present) capacities for the activity of aminopeptidase and maltase for fasted and fed *Ctenopharyngodon idella* (CI), *Ictalurus punctatus* (IP), and *Micropterus salmoides* (MS). For only aminopeptidase did *I. punctatus* and *M. salmoides* experience significant postprandial increases

with the carnivorous *M. salmoides*, which was predicted to exhibit the greatest regulatory changes with feeding and fasting. In fact, its response was relatively modest. We shall discuss the functional and morphological responses of these three taxa to a short bout of fasting and refeeding and explore the evidence of integrated responses that underlie adaptive correlations between their feeding habits and digestive response.

Gastrointestinal pH

The active gut exhibits considerable regional variation in luminal pH; the stomach is very acidic (pH = 1–3) whereas esophagus, intestine, and rectum are relatively neutral in pH (pH = 6–8) (Fox and Musacchia 1959; Meldrum et al. 1972; Evans et al. 1988; Secor 2003; Bucking and Wood 2008a; Mekapati et al. 2008). Amongst vertebrates, at least two patterns of gastric acid regulation are apparent: continuous acid production during and between meals, which is characteristic of mammals (Youngberg et al. 1985; Armstrong et al. 1992), and the cessation of acid production following gastric emptying and the subsequent resumption of production upon refeeding, which has been observed for turtles and snakes (Wright et al. 1957; Fox and Musacchia 1959; Secor 2003; Cox and Secor 2010). Both of these patterns have been demonstrated in elasmobranch and teleost fishes (Papastamatious and Lowe 2004, 2005; Bucking and Wood 2008b; Yúfera et al. 2012).

Amongst the fishes investigated in this study, the empty stomachs of fasted *I. punctatus* and *M. salmoides* possessed luminal pHs that were mildly acidic though not functionally digestive. With feeding, gastric pH of both fishes dropped to very acidic levels that are normally recorded for active stomachs of other vertebrates (Fox and Musacchia 1959; Youngberg et al. 1985; Secor 2003; Papastamatious and Lowe 2005). These two fishes apparently downregulate a significant portion of their gastric HCl production upon the completion of digestion. Since we did not observe gastric luminal pH in fasted fish to increase to neutrality, as has been recorded for turtles (Wright et al. 1957; Fox and Musacchia 1959), snakes (Secor 2003), and nurse sharks (Papastamatious and Lowe 2005), we surmise that these fish either maintain a very basal level of acid production between meals, which may be an adaptation to ward off the colonization of bacteria between meals, and/or to facilitate the upregulation of HCl production with feeding (Koelz 1992), or that the fish of our study had not been fasted long enough to cease total HCl production. Recent studies of the transcriptional factors underlying gastric regulation, such as the finding that mRNA expression of pepsinogen and gastric proton pumps are regulated in response to circadian rhythm, rather than in response to feeding (Yúfera et al. 2012), suggest that this line of research may offer a better understanding of acid production.

Intestinal morphology

For many vertebrates, the intestine (particularly the small intestine) experiences a distinct trophic response with fasting and feeding. Often observed following the completion of digestion is some degree of atrophy of the intestinal mucosa which is usually generated by a reduction in enterocyte volume (Dunel-Erb et al. 2001; Lignot et al. 2005). This response may lead to a shortening of the villi and thus a decrease in mucosa/submucosal thickness (Carey and Zafirova 1990; Dunel-Erb et al. 2001; Karasov et al. 2004). Cellular atrophy and shortening of the villi reduces the effective intestinal surface area and hence may lessen performance (Lignot et al. 2005; Ott and Secor 2007a; German et al. 2010a). The onset of these morphological changes occurs soon after the cessation of feeding (Krogdahl and Bakke-McKellep 2005; Gaucher et al. 2012) and, upon refeeding, are reversed just as rapidly, resulting

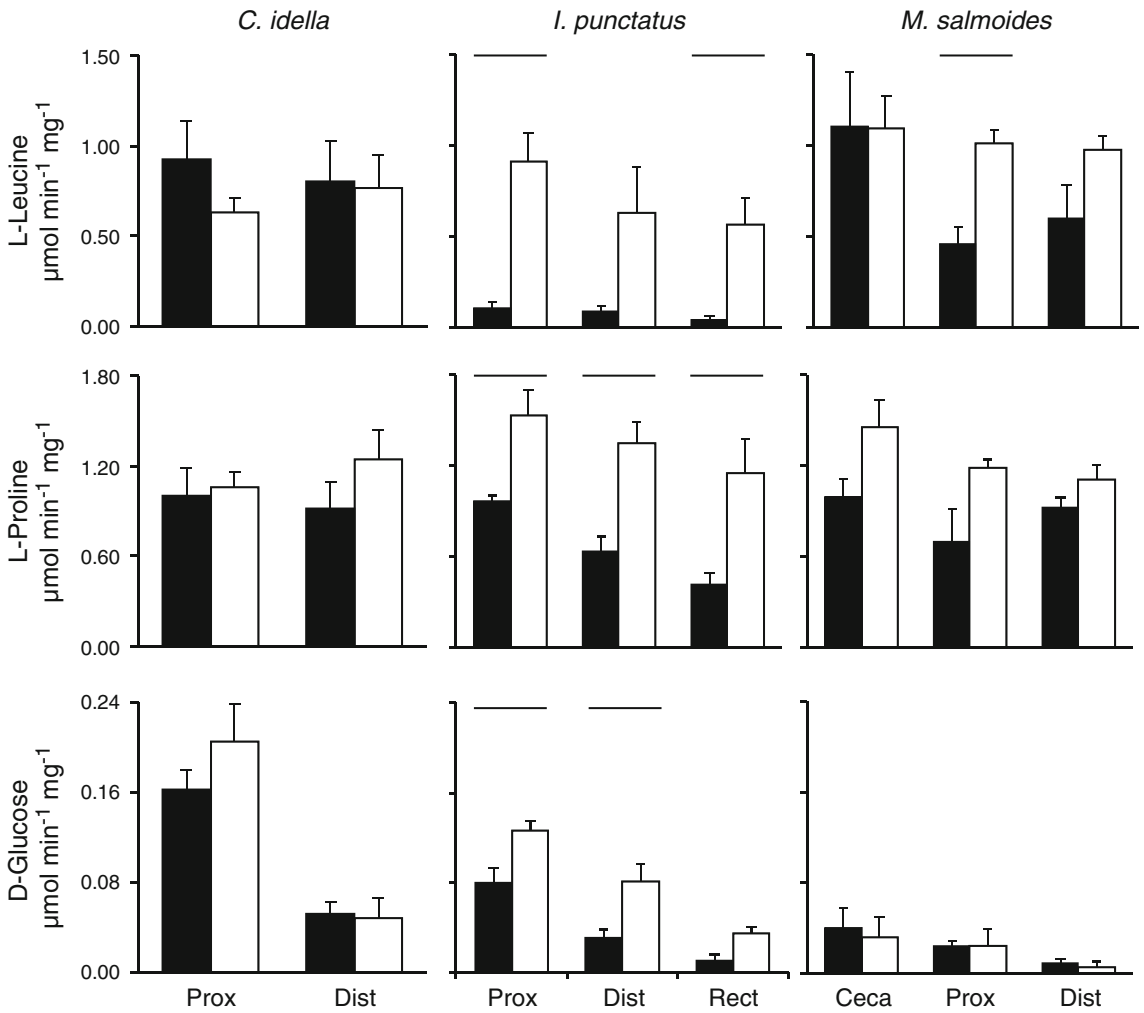

Fig. 7 Uptake rates of L-leucine, L-proline, and D-glucose of the pyloric ceca (Ceca), proximal (Prox) and distal (Dist) small intestine, and rectum (Rect) of fasted and fed *Ctenopharyngodon idella*, *Ictalurus punctatus*, and *Micropterus salmoides*. Only *I. punctatus* experienced significant postprandial increases in intestinal uptake of all three nutrients

in increases of enterocyte volume, lengthening of the villi, and thickening of the mucosae (Carey and Zafirova 1990; Dunel-Erb et al. 2001; Karasov et al. 2004; Abolfathi et al. 2012).

In this study, all three fish species shared an obvious architectural change of the intestinal epithelium, which transitioned from a tightly packed and commonly overlapped pseudostratified arrangement of enterocytes in fasted fish to a more stratified arrangement of the enterocytes after feeding, a shift similar to that reported throughout ectotherms, including snakes (Starck and Beese 2002; Secor 2008), frogs (Cramp and Franklin 2005; Secor 2005), lizards (Christel et al. 2007) and other fishes (Gaucher et al. 2012). This response (pseudostratified to stratified) for each species was largely generated by an increase in enterocyte volume. Interestingly, they did not necessarily share the same dimensional response of hypertrophy; enterocyte volume increased for *C. idella* by increasing enterocyte height, for *I. punctatus* by increasing enterocyte width, and for *M. salmoides* by increasing both enterocyte height and width. The postprandial widening of enterocytes for *I. punctatus* and *M. salmoides* was not substantial enough to increase their mucosa/submucosa thickness. In contrast, the mucosa/submucosa layer decreased in thickness with feeding for *I. punctatus*, due expectedly to the stretching of the intestinal wall from the influx of food.

Intestinal microvilli characteristically do not alter length by more than 25 % between the states of feeding and fasting (Misch 1980; Avella et al. 1992; Dunel-Erb et al. 2001; Secor 2005; Cox and Secor 2010). The exception is the microvilli of infrequently feeding snakes (e.g., pythons and boas), which increase in length by four to sixfold with feeding and subsequently shorten back to original length after the completion of digestion (Lignot et al. 2005; Cox and Secor 2010). In light of this generality, we predicted the lack of any change in microvillus length for *C. idella* and possible alteration of

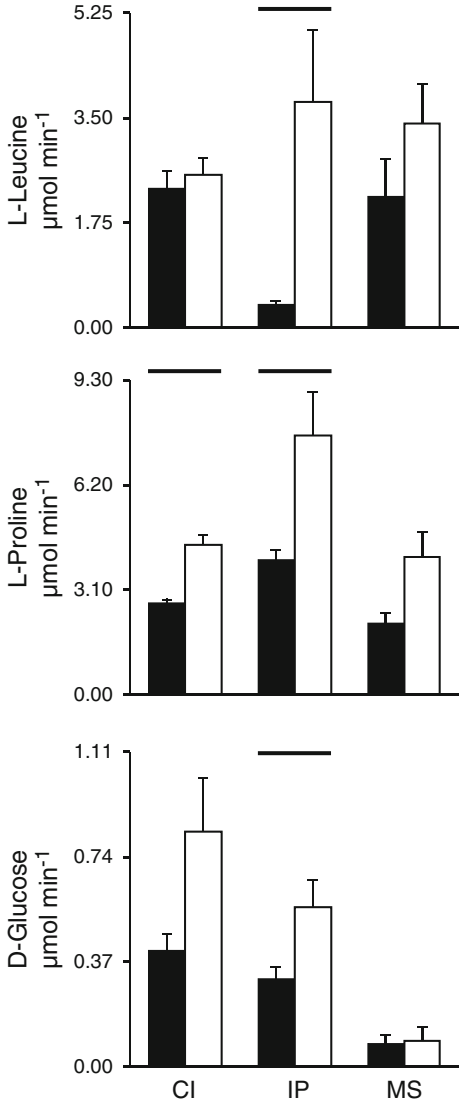

Fig. 8 Summed intestinal (including the cecum or rectum if present) uptake capacities for L-leucine, L-proline, and D-glucose for fasted and fed *Ctenopharyngodon idella* (CI), *Ictalurus punctatus* (IP), and *Micropterus salmoides* (MS). Likewise, *I. punctatus* was the only fish to experience a postprandial increase in intestinal uptake capacity for all three nutrients

the microvilli for *M. salmoides*. Instead, we observed no postprandial change in microvillus length for *I. punctatus* and *M. salmoides*, but a significant postprandial increase in microvillus length, and hence surface area (by 65 %), for *C. idella*. The postprandial increase in microvillus length for *C. idella* (by 70 %) was much less than the increases (by more than 300 %) experienced by infrequently feeding snakes (Cox and Secor 2010). They were similar in scope to the changes in microvillus length experienced by the fasting fishes *Dicentrarchus labrax*, *Silurus meridionalis* and *Pterygoplichthys disjunctivius*, and during estivation for the anurans *Cyclorana alboguttata*, *Ceratophrys ornata*, *Pyxicephalus adspersus,* and *Scaphiopus holbrooki* (Avella et al. 1992; Cramp et al. 2005; German et al. 2010b; Secor and Lignot 2010; Zeng et al. 2012).

Intestinal function

In this study, we focused on two compartments of intestinal performance—brush border hydrolase activity (digestive compartment) and brush border nutrient transporter (uptake compartment)—to assess whether each fish species significantly regulates intestinal function between feeding and fasting bouts. We expected both compartments to be matched in response, either no regulation by both, or both to be regulated. We also predicted *C. idella* to experience no significant postprandial changes in hydrolase or transporters activity, and *M. salmoides* to up and downregulate all hydrolase and transporters activities with each meal. Neither of these predictions was fully met. Whereas *C. idella* did not experience any postprandial changes in intestinal uptake (mass-specific rates or total capacity) for any of the three solutes, they did experience a postprandial increase in intestinal APN activity. *Micropterus salmoides* did experience postprandial increases in APN and maltase activities, but only L-leucine uptake increased. Feeding did generate significant increases in APN activity (but not maltase) and the uptake of all three nutrients for *I. punctatus*. In summary, *C. idella* did exhibit the least comprehensive regulatory responses, whereas *I. punctatus*, rather than *M. salmoides*, experienced the more comprehensive intestinal upregulation with feeding.

Our findings exemplify the spectrum of responses observed for fish intestines with fasting and feeding. After a 3-month fast, the flounder (*Psudopleuronectes americanus*) exhibited no significant change in APN activity or in the uptake of phenylalanine (McLeese and Bergeron 1990). Four and 6 weeks of fasting resulted in a respective decrease in intestinal valine uptake and no change in alanine uptake for the sea bass *Dicentrarchus labrax* (Avella et al. 1992). The Atlantic salmon (*Salmo salar*) experienced significant decreases in the activity of intestinal APN and five intestinal dissacharidases within several days of fasting, a response that was quickly reversed with feeding (Krogdahl et al. 1999; Krogdahl and Bakke-McKellep 2005).

Ratios of amino acid uptake to glucose uptake can serve as a proxy to illustrate the relative importance of each nutrient to a species' uptake performance and nutritive requirements (Buddington et al. 1987; Karasov and Diamond 1988; Secor and Diamond 1995). In this study, amino acid to glucose uptake ratios averaged lowest for the herbivorous *C. idella*, intermediate for the omnivorous *I. punctatus*, and highest for the carnivorous *M. salmoides*. Buddington et al. (1987) observed the same trend with similar fishes; the proline to glucose uptake ratio was

lowest for *C. idella*, intermediate for *I. punctatus*, and highest for the striped bass, *Morone saxatilis*. In that same study, low ratios were also calculated for the herbivorous tilapia (*Tilapia zillii*) and the detritivorous/omnivorous common carp (*Cyprinus carpio*), and higher ratios for the omnivorous surgeon (*Acipenser transmontanus*) and carnivorous trout (*Salmo gairdneri*). The variation in amino acid to glucose ratios in Buddington et al. (1987) and the current study is largely due to a gradient of glucose uptake, decreasing from herbivore to omnivore to carnivore, rather than differences in amino acid uptake.

Integration of intestinal form and function

The completion of digestion, followed by the resumption of feeding, sparks a coordinated set of morphological and functional responses that range from being relatively modest in scope to significantly large (Secor 2005). At one end of the spectrum, fasting is met with no significant changes in gastric acid production, intestinal microvillus length, nutrient transport rates, or brush border enzyme activities. Any loss of intestinal mass with fasting is relatively slight. Consumption of the next meal is answered with minor adjustments in gastrointestinal form and function. Such a scenario is played out for many frequently feeding amphibians and reptiles (Secor 2005; Cox and Secor 2010). At the other end of the fasting/refeeding continuum, as exemplified by infrequently feeding snakes, gastric acid production is turned off, intestinal mass is halved, and intestinal microvillus length, nutrient uptake rates, and enzyme activities are reduced by 70–80 % upon the completion of digestion (Ott and Secor 2007b; Secor 2005, 2008). Feeding is thus accompanied by rapid upregulation of gastric acid production and intestinal function and hypertrophy of the intestinal epithelium (Secor and Diamond 2000; Ott and Secor 2007b; Cox and Secor 2010).

The collective responses of each fish in this study lay part way along this continuum. Each exhibited significant regulation of one or more (but not all) measured features of intestinal morphology and/or function, with *I. punctatus* and *M. salmoides* also regulating gastric HCl production (*C. idella* lacks a stomach). A combination of significant and non-significant postprandial changes in intestinal form and function has been similarly noted for the diamondback water snakes (*Nerodia rhombifer*), blood python (*Python brongersmai*), and Gila monster (*Heloderma suspectum*) (Christel et al. 2007; Ott and Secor 2007a; Cox and Secor 2010).

Underlying the regulation of intestinal function are specific (e.g., changes in protein expression or activity) and/or non-specific (e.g., increase surface area) mechanisms. For infrequently feeding snakes feeding triggers a four to sixfold increase in microvillus surface area, resulting from the lengthening of the microvilli, that matches the magnitude of increases in nutrient uptake rates and hydrolase activities (Secor and Diamond 1995; Lignot et al. 2005; Secor 2008; Cox and Secor 2010). Upon the completion of digestion, microvillus surface area decreases as the microvilli shorten, thereby downregulating their function (Secor 2008). Snakes that do not significantly regulate intestinal function with each meal correspondingly do not modulate microvillus length and thus surface area (Cox and Secor 2010). This mechanistic link between modulating microvillus surface area and the regulation of intestinal function was not strongly evident in this study. The postprandial lengthening of the microvilli of *C. idella* was not matched by a concurrent increase in intestinal function. The postprandial upregulation of nutrient uptake rates and APN activity for *I. punctatus* and *M. salmoides* was not generated by increases in microvillus length. Similarly, moderate changes in microvillus length with different diets did not match diet-induced changes in disaccharidase activities for the catfish *Pterygoplichthys disjunctivus* (German 2009), perhaps because each diet triggered the expression of a different disaccharidase isoform(s) that operated more efficiently via a lower K_m (German 2009). Seemingly, fish employ different specific and non-specific mechanisms to modulate intestinal performance.

Each of the three fishes examined in our study experienced significant regulation of several components of gut morphology and/or function in response to a short-term fast and refeeding. Evident was the regulation of gastric HCl production (*I. punctatus* and *M. salmoides*) and intestinal enterocyte volume (all species), microvillus length (*C. idella*), hydrolase activity (all species) and nutrient uptake (*I. punctatus* and *M. salmoides*). The magnitude of these responses, however, was relatively modest, similar in scope to that observed for frequently feeding amphibians and reptiles (Secor 2005). While we did not observe our predicted distinct step-wise gradient of response based on feeding habits (i.e., no response by *C. idella*, moderate response by *I. punctatus*, and full response by *M. salmoides*), *C. idella* did exhibit the most modest response and glucose uptake capacity did track feeding habits (highest for the herbivorous *C. idella* and lowest for the carnivorous *M. salmoides*). The observed moderate scopes of response and the lack of large magnitudes of regulation as seen in infrequently feeding amphibians and reptiles stem from the fact that fish were fasted for only 7–10 days, which is possibly not long enough to achieve the complete downregulation of their intestine. However, in studies where fish were fasted longer (30–150 days), the magnitude of fasting or feeding responses by the intestine was similarly modest in scope (Avella et al. 1992; Krogdahl et al. 1999; Krogdahl and Bakke-McKellep 2005; German et al. 2010b). A

better explanation may be that these fish feed enough such that the much wider regulation of intestinal performance holds no adaptive advantage (Secor 2005). Such an adaptation, because it can reduce daily maintenance costs, may rather be possessed by fish species that predictably experience much longer episodes of fasting or employ a foraging mode that allows for extended senescence (Fu et al. 2009). Candidate fishes that for this reason widely down and up regulate gastrointestinal performance would include salmon (Kadri et al. 1995) that migrate while not feeding, cod (Gadidae; Schwalme and Chouinard 1999) that fast during the winter when food is scarce, deep sea pelagic fishes (Mauchline and Gordon 1986) that presumably feed infrequently on much larger fish (akin to sit-and-wait foraging snakes), particularly sluggish ambush feeders that live a sedentary lifestyle (Fu et al. 2009) and African lungfish (*Protopterus*; Greenwood 1987) that estivate for 4–8 months during the dry season. Subsequent studies on fasting and feeding responses would also be strengthened by employing some degree of phylogenetic control (e.g. Horn et al. 2006; German et al. 2010a) and expanding species and feeding habits coverage over a broader range of taxa to allow for a more powerful determination of adaptive responses than was possible with the disparate taxa used in this study. Exploring the fasting and postprandial morphology and function of the guts of these fishes may demonstrate that fishes also possess the broad continuum of gastrointestinal responses that are adaptively correlated to fasting durations (Secor 2001; Secor and Diamond 2000; Secor 2005).

Acknowledgments We thank Scott Bessler, Miles Cobia, David Hall, and Brytney Snow for their assistance in the undertaking of this study; Simon Blomberg for statistics consultation; and two anonymous reviewers who offered comments that strengthened the manuscript. This study was funded by fellowships and travel awards from the University of Queensland (to RDD) and the National Science Foundation (IOS 0466139 to SMS).

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