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Why can't young fish eat plants? Neither digestive enzymes nor gut development preclude herbivory in the young of a stomachless marine herbivorous fish

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ABSTRACT

Most young fishes lack the ability to function as herbivores, which has been attributed to two aspects of the digestive system: elevated nitrogen demand and a critical gut capacity. We compared the digestive morphology and biochemistry of two size classes of the marine herbivore *Hyporhamphus regularis ardelio*, pre-ontogenetic trophic shift (pre-OTS, <100 mm) and post-ontogenetic trophic shift (post-OTS, >100 mm), to determine what limits the onset of herbivory and how their digestive processes fit with current models of digestion. Two gut-somatic indices comparing gut length to body length (relative gut length) and body mass (Zihler's Index) demonstrated a significant decrease (RGL 0.59 → 0.49, $P<0.01$; ZI 3.24 → 2.44, $P<0.01$) in gut length relative to body size. There was little difference in enzyme activity between the two classes, with juveniles showing similar levels of carbohydrase and lipase and less protease compared with adults, indicating that juveniles did not preferentially target nitrogen and were as capable of digesting an herbivorous diet. These findings suggest that herbivory in this fish is not limited by the function of the post-oesophageal digestive tract, but rather the ability of the pharyngeal mill to mechanically process plants. Our findings offer partial support for the current model of stomachless digestion, indicating that further refinement may be necessary.

1. Introduction

Ontogenetic trophic shifts are changes in diet that occur during an animal's life history, and are common amongst vertebrates that are herbivores as adults (White, 1985). Such shifts present an opportunity to investigate the developmental and physiological requirements of herbivory, as they represent the point at which herbivory becomes a feasible trophic specialisation. Marine fishes offer a challenging case study for understanding the potential limiting factors of herbivory, as substantially less is known about herbivorous fishes than about terrestrial herbivores (Choat and Clements, 1998; Clements et al., 2009) and comparative studies of digestive physiology on either side of a carnivore-to-herbivore trophic shift have been limited (Moran and Clements, 2002; Elliott and Bellwood, 2003; Drewe et al., 2004; German et al., 2004).

The apparent inability of juvenile fishes to thrive on an herbivorous diet has been attributed to their need to meet an elevated nitrogen (N) demand and the lack of some critical development of the alimentary system and accessory organs. In order to maintain a rapid growth rate, juveniles may have elevated N requirements, forcing them to consume a protein-rich, animal diet until this high N demand subsides (White, 1985). Alternatively, alimentary systems of juveniles may lack the ability to adequately process plant matter, as trophic shifts tend to coincide with gut lengthening (Montgomery, 1977; Stoner and Livingston, 1984; Kramer and Bryant, 1995a; Drewe et al., 2004; German and Horn, 2006). Herbivores tend to have longer, more complex guts than carnivores (Al-Hussaini, 1947; Kramer and Bryant, 1995b; Elliott and Bellwood, 2003), which may enhance digestive efficiency through increased mucosal surface area (Frierson and Foltz, 1992; Horn et al., 2006; German, 2009a; German et al., 2010) or by achieving a necessary gut volume and processing capability to assimilate sufficient energy to meet metabolic needs (Benavides et al., 1994).

The halfbeaks (Hemiramphidae) are a predominantly herbivorous family of fishes (Randall, 1967; Carr and Adams, 1973; Robertson and Klumpp, 1983; Carseldine and Tibbetts, 2005) with ontogenetic trophic shifts from carnivory to herbivory that have been well characterised at species-specific points in development (Robertson

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Abbreviations: AMH, adaptive modulation hypothesis; CV, coefficient of variation; K_m, Michaelis–Menten constant; N, Dietary nitrogen; OTS, ontogenetic trophic shift; PFR, plug flow reactor; post-OTS, pre-ontogenetic trophic shift = <100 mm SL; pre-OTS, post-ontogenetic trophic shift = >100 mm SL, RGL, relative gut length $\frac{\textit{gut length}}{\textit{standard length}}$; SL, standard length, measured from the tip of the upper jaw to the caudal flexure; TSGA, total standardised gut activity; U, enzymatic activity units (1 μmol product liberated per minute); ZI, Zihler's Index $\frac{\textit{gut length}}{10 \times \sqrt[3]{\textit{body mass}}}$.

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Fig. 1. Pre-OTS (65 mm SL) and post-OTS (187 mm SL) *Hyporhamphus regularis ardelio*, below and above respectively, with excised intestines. Intestines are positioned approximately according to where they lie in the visceral cavity. Gut region divisions for digestive enzyme analyses are marked with arrowheads. These sections are referred throughout as posterior, mid and distal segments.

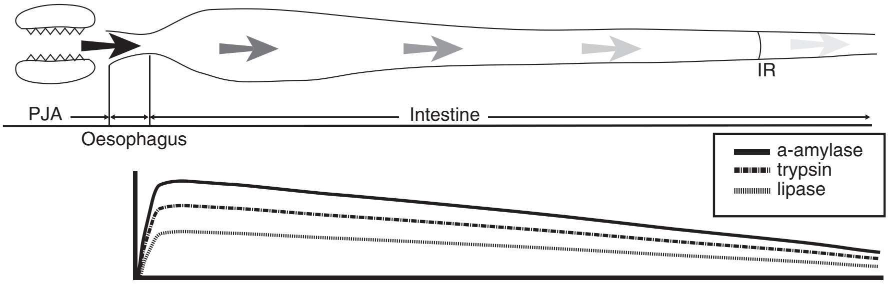

Fig. 2. Schematic diagram of digestive function predicted by Plug Flow Reactor theory. Ingesta enter the intestine directly from the oesophagus, after mechanical disruption by the pharyngeal jaw apparatus (PJA), if present, or oral jaws. Arrow shading corresponds to nutrient concentration and reaction gradient (i.e., digestive enzyme activities), with darker arrows indicating greater levels of both. Ingesta are immediately exposed to pancreatic digestive enzymes, which persist throughout the entirety of the intestine. The PFR is characterised by a high throughput rate and relatively steep gradients of nutrient concentrations and digestive enzyme activities.

and Klumpp, 1983; Tibbetts and Carseldine, 2005). However, little is known about changes in digestive tract morphology that might be associated with the hemiramphid trophic shift. Juveniles have seldom been studied and the bulk of our knowledge about their digestion stems from work on the morphology of adult halfbeaks, which have a digestive tract comprising a short, wide diameter intestine that extends as a straight tube from oesophagus to anus, lacking convolutions, a stomach, and gastric diverticulae (Fig. 1; Klumpp and Nichols, 1983; Robertson and Klumpp, 1983; Tibbetts, 1991; Tibbetts and Carseldine, 2003). Herbivory in these fishes appears to be dependent upon a well developed pharyngeal mill, which mechanically processes food (Tibbetts and Carseldine, 2003). In what appears to be the only ontogenetic trophic study of halfbeaks, the development of the pharyngeal mill, particularly the number, size and coverage area of pharyngeal teeth, has been found to correlate with the onset of herbivory and this development may play a critical role in facilitating the onset of herbivory by conferring the ability to adequately rupture plant cell walls (Tibbetts et al., 2007).

To date, the only model of digestive tract function that fits stomachless fishes is the theoretical plug-flow reactor (PFR) model, proposed by Penry and Jumars (1987) and applied to herbivorous fishes by Horn and Messer (1992). In a PFR gut (Fig. 2), a robust pharynx, if present, mechanically processes food (*sensu* Horn and Messer, 1992) and a relatively short intestine serves as the sole site of chemical reaction. This model is characterised by high intake and throughput rate, which allows the fish to access sufficient nutrients from their relatively poor quality diet. Based solely upon observations of alimentary tract morphology including the milling function of the pharyngeal jaws (Tibbetts, 1991; Tibbetts and Carseldine, 2003) and measurements of a gut passage rate of approximately 4 hours (Klumpp and Nichols, 1983), halfbeaks appear to fit the PFR model, as described by Horn and Messer (1992).

Table 1
Hypothesized patterns of GI tract characteristics in *Hyporhamphus regularis ardelio*.

Characteristic	Function	Hypothesized pattern based on PFR model[a]	Prediction based on AMH[b]
Gut length	Gut capacity	N/A	Larger in post-OTS fish
Enzyme activities			
Amylase	Hydrolyzes starch	Decreasing activity in distal intestine	Elevated in post-OTS fish
Trypsin	Hydrolyzes protein	Decreasing activity in distal intestine	Elevated in pre-OTS fish
Lipase	Hydrolyzes lipids	Decreasing activity in distal intestine	Elevated in pre-OTS fish

[a] Pattern of enzymatic activity based on a plug flow reactor (PFR) model, as described by Horn and Messer (1992).

[b] According to the adaptive modulation hypothesis (AMH; Karasov and Martinez del Rio, 2007) an animal's diet leads to the *a priori* expectation of elevated digestive enzyme activities against those compounds in high concentration in the animal's diet. The carnivorous diet of pre-OTS fish contains approximately 50% protein, 4% carbohydrate, and 9% lipid (Dall et al., 1991), whereas the herbivorous diet of post-OTS fish contains approximately 9% protein (Birch, 1975), 40% carbohydrate (Montgomery and Targett, 1992), and 4% lipid (Nichols et al., 1982). Thus, we expected amylase activity to be elevated in post-OTS fish, whereas trypsin and lipase activities were expected to be elevated in pre-OTS fish.