BEAVERS OF THE FISH WORLD: CAN WOOD-EATING CATFISHES ACTUALLY DIGEST WOOD? A NUTRITIONAL PHYSIOLOGY APPROACH

By

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To my parents, John C. and Gillian R. German, for their unending support for whatever it is I choose to do in life.

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BEAVERS OF THE FISH WORLD: CAN WOOD-EATING CATFISHES ACTUALLY DIGEST WOOD? A NUTRITIONAL PHYSIOLOGY APPROACH

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Because wood is composed almost entirely of structural polysaccharides (primarily cellulose) few animals can digest it, let alone thrive on it. Most animals that do subsist on wood require endosymbiotic microorganisms in their digestive tracts to aid in the digestion of structural polysaccharides. Several species of catfishes (family Loricariidae) from the Amazonian basin (South America) were recently described as being "wood-eating", or "xylivorous". However, beyond some cursory analyses of gut contents and digestive enzyme activities, little is known about these animals and whether they can digest wood. In this dissertation I explored the structure and function of the digestive tract in four species of loricariid catfishes: three xylivorous species (*Panaque cf. nigrolineatus* "Marañon", *P. nocturnus*, and *Hypostomus pyrineusi*) and one detritivorous species (*Pterygoplichthys disjunctivus*) that represents the common feeding mode of the family. Thus, I was able to examine whether the xylivorous species had specializations of the digestive tract affording them the ability to digest wood in comparison to the non-xylivorous, detritivorous species.

I measured several aspects of the fishes' gut morphology, including intestinal folding patterns, microvilli surface area, pH and redox potentials, and microbial diversity in different regions of the gut. I also measured the activity levels of 14 digestive enzymes in the guts of the

fish, and determined the sources of these enzymes: endogenous (produced by the fish) *vs.* exogenous (produced by microbes). These data were then compared to concentrations of microbial fermentative end products, called short chain fatty acids (SCFAs), and soluble carbohydrate profiles in the intestinal fluids of the fish to determine where microbes might be most active, and where nutrients were being hydrolyzed and absorbed. And finally, I measured the ability of captive fish to digest wood, to grow on it, and how quickly the fish pass wood through their digestive tracts.

The results of these analyses suggested that, unlike termites, the alleged xylivorous catfishes of the Amazon cannot digest the fibrous components of wood in their digestive tracts. I found no evidence that they harbored endosymbionts in their guts capable of digesting the structural polysaccharides of wood. And, the laboratory feeding trials showed that the fish could not assimilate significant amounts of fiber or energy from wood, resulting in the fish losing weight on a wood diet. The fishes' entire feeding strategy, ranging from intake, to gut passage rates, digestive enzyme activities, intestinal morphology, soluble carbohydrate profiles, and levels of SCFAs throughout the gut suggest that the fish eat as much as they can, pass it through the digestive tract quickly, and assimilate the soluble, non-fibrous components available to them. Unlike many other wood-eating animals (e.g., termites, beavers), these fishes consume decaying wood in aquatic systems; decaying wood is in the process of being degraded by microbes, which produce soluble degradation products (e.g., β-glucosides) that the fish can actually digest and assimilate. Thus, rather than harboring endosymbiotic microorganisms to digest wood fiber within their guts, the fish rely on microbial decomposition occurring in the environment. In this vein, the wood-eating catfishes are actually detritivores like so many other loricariid catfishes.

CHAPTER 1
INTRODUCTION: WHO EATS WOOD AND HOW DO THEY DO IT?

With 10^{12} metric tons produced annually, cellulose is the most abundant organic molecule in the biosphere (Wilson and Irwin 1999; Karasov and Martínez del Rio 2007). Cellulose is the major structural polysaccharide in the cell walls of most photosynthetic organisms and in the sheaths of tunicates, and is found in nearly all habitats. Despite its ubiquitous distribution and overall abundance, relatively few animals can digest it in their alimentary tracts. The β-1,4-linkage between adjacent glucose molecules makes cellulose particularly resistant to hydrolysis; thus, it is considered "refractory" or "recalcitrant" to digestion. In fact, digestion of cellulose requires a specialized set of enzymes, collectively called cellulases, to be degraded. Although many microorganisms (encompassing the phylogenetically disparate groups of bacteria, fungi, and protists) possess genes for these cellulase enzymes, relatively few animals have these genes (Watanabe and Tokuda 2001; Lo et al. 2003). Thus, many herbivorous animals do not digest cellulose in plants they consume, and those that do mostly require the aid of symbiotic microorganisms to extract energy from cellulose (Karasov and Martínez del Rio 2007).

Of all the photosynthetic organisms on the planet, woody plants produce more cellulose on a proportional basis than any group of herbaceous plants or algae; 90% of a tree's biomass is made of cell wall components, primarily cellulose (Karasov and Martínez del Rio 2007). Given the recalcitrance of cellulose to digestion, wood is, therefore, a difficult resource for an animal to rely on to meet its daily energetic needs. Thus, few animals target wood as their primary food source and are considered "xylivorous". The reliance on wood as a food resource is limited to a few families of insects, one family of molluscs, two lineages of mammals, and two genera of fishes. Among insects, silverfish, cockroaches, woodroaches, lower termites, higher termites, beetles, and wood wasps are known to be able to digest wood and subsist on it (Prins and

Kreulen 1991). Shipworms (actually a family of bivalves) represent the only molluscan family to be xylivorous (Xu and Distel 2004), and beavers and porcupines, both of which are large rodents, are the only mammals that are known to be able to extract sufficient amounts of energy from wood (Vispo and Hume 1995; Felicetti et al. 2000). Recently, several new species of Amazonian catfishes (genus *Panaque*, family Loricariidae) were described as xylivorous, with wood composing the only "macroscopic material" in the fishes intestines (Schaefer and Stewart 1993).

The mechanisms of digestion in most xylivorous animals have been described, at least on a basic level; most use symbiotic microorganisms to digest cellulose (Prins and Kreulen 1991; Vispo and Hume 1995; Felicetti et al. 2000; Xu and Distel 2004). However, beyond the measurement of cellulase activity in the guts of *Panaque maccus* (Nelson et al. 1999), little is known about the digestive tracts of the alleged wood-eating catfishes. Vertebrate animals do not possess cellulase genes (Lo et al. 2003) and are, therefore, absolutely reliant upon microorganisms to aid in the digestion of cellulose (Stevens and Hume 1998). For example, beavers and porcupines possess microorganisms in their intestines that ferment cellulose and other refractory polysaccharides (Vispo and Hume 1995), producing byproducts called short chain fatty acids (SCFAs), which the animals then absorb and use as an energy source (Bergman 1990; Stevens and Hume 1998). Thus, the expectation would be that xylivorous catfishes function similarly to beavers and porcupines with a reliance on endosymbiotic fermentation to digest and aassimilate wood.

The family to which the wood-eating catfishes belong, the Loricariidae, is diverse, with over 680 described species in 80 genera, and is Neotropical in its distribution (Armbruster 2004). Most species appear to be herbivorous or detritivorous (Nelson et al. 1999; Delariva and

Agostinho 2001; Pouilly et al. 2003), although xylivory evolved twice in the family, as species in the genus *Hypostomus* (Armbruster 2003) and *Panaque* (Schaefer and Stewart 1993) are considered xylivorous. However, given that many loricariid catfishes consume low-quality food rich in cellulose (i.e., detritus), not too dissimilar from decaying wood, how different are the wood-eating catfishes from other detritivorous loricariid catfishes? Do wood-eating catfishes possess specializations similar to beavers and porcupines allowing them to consume and digest wood, or are these wood-eating fishes simply detritivores that specialize on a form of detritus (i.e., wood) that is ubiquitous in the forested Amazonian basin?

The focus of this dissertation was to investigate the structure and function of the digestive tracts of wood-eating catfishes to determine whether they can actually digest the cellulose in wood, or whether they are detritivores, like so many other loricariid catfishes, and rely more on souble components of their food. In chapter two I describe a detailed suite of analyses of the gut structure and function of three wood-eating species, representing both xylivorous genera within the Loricariidae and a generalized detritivore that represents the most common feeding mode of the family. Specifically, I examined the structure of the fishes' digestive tracts by looking at the gross gut morphology, the folding patterns of the intestine with histological staining, and the surface of the cells lining the gut with transmission electron microscopy (TEM). To determine the conditions of the gut milieu I measured the pH and redox conditions of the fishes' intestines to determine if any region of the gut was hospitable for microbes to reside in and ferment cellulose. Accordingly, I investigated whether the fish used endosymbiotic microorganisms to digest cellulose by measuring concentrations of SCFAs along the fishes' digestive tracts. I also used the TEM micrographs to search for conglomerations of microbes in different regions of the gut. To determine what compounds the fishes were capable of digesting I measured the activity

levels of 14 digestive enzymes, including cellulases, and examined where food was being digested and absorbed in their guts. And, I performed detailed feeding trials to determine whether xylivorous and detritivorous catfish could digest wood and grow on a wood diet. Thus, this study was designed to investigate, on multiple levels, the capabilities of the wood-eating catfishes to harbor endosymbionts in their guts and digest wood.

In chapter three I explored the temporal dynamics of stable isotopic incorporation in *Pterygoplichthys disjunctivus* to determine whether non-invasively sampled tissues – plasma solutes, red blood cells, and fin tissue – could be used to isotopically track the diet of wild-caught fishes. Furthermore, I took advantage of naturally occurring stable isotopic ratios of different plant types to discern whether this detritivorous fish could assimilate carbon from wood cellulose. I then took the lessons learned from laboratory experiments and applied them to data gathered in the field.

The application of this research lies in the potential for biofuel production. With the current interest in cellulosic-ethanol, and the isolation of a fungus from the guts of a wood-eating beetle capable of producing ethanol from wood (Nigam 2001), there is great potential for the discovery of new and novel microorganisms from the guts of the xylivorous and detritivorous catfishes. However, until we understand more about the structure and function of the guts of these animals, this remains a potential rather than a resource. Thus, the point of this dissertation is to reveal whether xylivorous and detritivorous catfishes rely on endogenous or exogenous digestive mechanisms to gain nutrition from their food.

CHAPTER 2
BEAVERS OF THE FISH WORLD: CAN WOOD-EATING CATFISHES ACTUALLY DIGEST WOOD?

Introduction

The consumption of wood for food is rare among animals. Unlike the "greener" portions of plants, woody tissues are made of cells that are dead at functional maturity, and hence, are lacking the nutritional cell contents on which many herbivorous animals thrive. Because wood is comprised almost entirely of structural polysaccharides (e.g., lignocellulose), it is considered nutrient poor (Karasov and Martínez del Rio 2007). Thus, many wood-eating, or xylivorous, animals (e.g., lower termites, beavers) require the aid of symbiotic microorganisms in their alimentary tracts to digest cellulose and make the energy in this compound available to the host (Prins and Kreulen 1991; Vispo and Hume 1995). Indeed, a common theme among xylivorous animals is that they possess an expanded hindgut or caecum in which microbes reside and produce cellulolytic enzymes to aid in the digestion of woody material (Prins and Kreulen 1991; Vispo and Hume 1995; Mo et al. 2004). Because the conditions in this expanded hindgut are typically anaerobic, microbial endosymbionts operate under fermentative pathways, reducing glucose (and other monomers) to by-products called short chain fatty acids (SCFAs; e.g., acetate), which are then absorbed by the host animal and used to generate ATP (Bergman 1990; Karasov and Martínez del Rio 2007).

In 1993, Schaefer and Stewart described several new species as part of a lineage of Neotropical catfishes, genus *Panaque*, family Loricariidae, that appeared to be xylivorous. The enlarged teeth these animals use to scrape wood from the surface of fallen trees in the river, and the presence of wood as the "only macroscopic material" in the fishes' digestive tracts intrigued the authors (Schaefer and Stewart 1993). What's more, xylivory evolved twice in loricariid catfishes, as a clade in the genus *Hypostomus* (formerly *Cochliodon*; Armbruster 2003) is

recognized as wood-eating in addition to the *Panaque* (Figure 2-1). Both xylivorous clades are derived within the larger phylogeny of the family. Although there is some knowledge of the phylogenetic history of loricariid catfishes (Armbruster 2004), including the wood-eating lineages (Schaefer and Stewart 1993, Armbruster 2003), little is known of the digestive physiology in these fishes, and whether they can digest cellulose from wood.

The catfish family to which these alleged xylivores belong, the Loricariidae, is incredibly diverse, with 680 described species in 80 genera, and is entirely Neotropical in its distribution (Armbruster 2004). Although some authors have commented that all loricariids are herbivorous or detritivorous (Nelson et al. 1999), the diets of relatively few species are known (e.g., Delariva and Agostinho 2001; Pouilly et al. 2003), and appear to include animal, plant, and detrital material from the benthos. It is clear, however, that these fishes have undergone evolutionary rearrangements of jaw structure, allowing for diversity in feeding modes and trophic specialization (Schaefer and Lauder 1986). Furthermore, loricariids have extremely long, coiled intestines (Delariva and Agostinho 2001), which suggests they have high levels of intake of low-quality food (Sibly and Calow 1986; Horn and Messer 1992; Karasov and Martínez del Rio 2007), such as detritus (Araujo-Lima et al. 1986).

In the only investigation of digestive physiology in xylivorous catfishes, and in loricariids in general, Nelson et al. (1999) examined digestive enzyme activities and cultured microbes from the digestive tracts of *Panaque maccus*, and an undescribed species of *Pterygoplichthys* (formerly *Liposarcus*; Armbruster 2004), both of which they obtained via the aquarium trade. Nelson and colleagues were able to isolate microbes with cellulolytic capabilities from the guts of the two species, and they were able to measure cellulase activities in the fishes' guts. However, the finding of cellulolytic microbes in the guts of the fish does not mean that those

microorganisms are endosymbionts digesting wood. For example, grass carp, which eat aquatic macrophytes rich in cellulose, have cellulase activities in their guts (Das and Tripathy 1991) and an active microbial population (Trust et al. 1979; Lesel et al. 1986), yet poorly digest the cellulose component of their plant diet (Van Dyke and Sutton 1977). This is likely due to rapid gut transit and low levels of microbial fermentation in the grass carp guts (Stevens and Hume 1998). What is clearly needed is an understanding of the gut structure and function of the wood-eating catfishes, including a traditional fiber digestibility investigation, to determine whether the xylivorous catfishes can digest cellulose from wood and subsist on it. Moreover, such an investigation should be conducted in a theoretical context to better understand exactly how the guts of these fishes function. That is, do their guts act more like those of a terrestrial hindgut fermenter (Penry and Jumars 1987; Breznak and Brune 1994; Vispo and Hume 1995; Felicetti et al. 2000), with some mechanism for slowing the flow of digesta and allowing microbes to ferment structural polysaccharides (Clements and Raubenheimer 2006; Karasov and Martínez del Rio 2007), or are their guts more similar to those of other detritivorous fishes (Horn and Messer 1992; Crossman et al. 2005; German 2008), with rapid gut transit and little digestion of structural polysaccharides?

These divergent digestive strategies not only feature differences in digesta transit rate and gut morphology, but also produce completely different profiles of digestive enzyme activities and SCFA concentrations along the gut (Horn and Messer 1992; Jumars 2000; German 2008). For example, an animal with hindgut fermentation would be expected to show retention of small particles in the hindgut fermentative region (Parra 1978; Vispo and Hume 1995), to have high concentrations of SCFAs in that region (Vispo and Hume 1995; Mountfort et al. 2002; Pryor and Bjorndal 2005), and to have high activities of microbially produced digestive enzymes in the

hindgut (e.g., carrageenase, Skea et al. 2005; cellulase, Potts and Hewitt 1973; Nakashima et al. 2002; Mo et al. 2004). Thus, if xylivorous catfishes are reliant upon an endosymbiotic community to digest wood in their digestive tracts, they should display these patterns.

I examined the gut structure and function of xylivorous and detrivorous loricariid catfishes to determine what traits of the digestive tract, if any, these animals have for digesting a diet rich in refractory polysaccharides. I traveled to the Río Marañon in northern Perú, where xylivorous catfishes are most diverse and abundant (Schaefer and Stewart 1993), and collected animals directly from their natural habitat. In all, I collected two species from the genus *Panaque* (*P. nocturnus* Schaeffer and Stewart 1993, and an undescribed species I am tentatively calling *P. cf. nigrolineatus* "Marañon"), representing the two clades of this genus, and one species of *Hypostomus* (*H. pyrineusi* Ribeiro 1920) representing the other clade of xylivorous catfishes (Figure 2-1). All of these taxa are sympatric in the Río Marañon. Additionally, I made use of an introduced population of a detritivorous loricariid, *Pterygoplichthys disjunctivus* Weber 1991, which has been living in Florida for nearly two decades (Nico 2005). This study, therefore, included both clades of xylivorous catfishes, and a less-derived detritivore from the same family (Figure 2-1). Thus, I was able to examine gut structure and function in these fishes in dietary and phylogenetic contexts.

This study had five main components. I first examined the fishes' gut structure, including the morphology of the digestive tract, to look for the presence of any kinks, valves, or caeca that might serve as a refuge for microbial endosymbionts (Pryor and Bjorndal 2005; Pryor et al. 2006). I also measured the length of the gut (German and Horn 2006), examined the gut ultrstructure with histological and electron microscopical techniques, and qualitatively examined the surface area of the intestine. Second, I measured the pH and redox conditions along the

digestive tract to determine whether any portion of the gut would be hospitable for microbes to operate under anaerobic conditions. Third, I measured the biochemical activity levels of 14 digestive enzymes acting in the gut lumen or along the brush border of the intestine that reflect the ability of the fish to hydrolyze substrates commonly encountered in wood or detritus (Table 2-1). Following the methodology of Skea et al. (2005), I measured enzyme activities relative to location along the gut and determined whether the sources of these enzyme activities were endogenous (host-produced) or exogenous (produced by microorganisms). This was done by collecting three fractions from the gut sections: gut wall tissue (endogenous), gut fluid (enzymes secreted either by the fish or microorganisms) and microbial extract (exogenous). If the fishes were relying on endosymbionts to digest cellulose, I would expect cellulase activities to be highest where the microbes are most densely populated in the gut. Fourth, luminal carbohydrate profiles and SCFA concentrations were measured along the digestive tract to determine where nutrients were being hydrolyzed and absorbed, and where microbes might be most concentrated. Transmission electron micrographs were also used to examine microbial diversity in different regions of the gut. And fifth, two types of feeding trials were performed to determine whether the fish could actually digest wood fiber, how quickly wood passes through their guts, and whether there is selective retention of particles (large or small) anywhere along the digestive tract. Overall, this study was designed to examine, on multiple levels, the capabilities of these fishes to harbor endosymbionts and digest wood. Given the dearth of information available on loricariid digestion, I adopted a null hypothesis for all queries, and expected to find little evidence of microbial digestion and elaborations of the fish digestive tract for harboring endosymbionts.

Materials and Methods

Fish Collection

Twenty five adult individuals each of *Panaque cf. nigrolineatus* "Marañon" and *P. nocturnus*, and seven adult individuals of *Hypostomus pyrineusi* were captured by seine and a backpack electroshocker from the upper Río Marañon in northern Peru (4º58.957' S, 77º85.283' W) in August 2006. Thirty four individuals of *Pterygoplichthys disjunctivus* were captured by hand while snorkeling from the Wekiva Springs complex in north central Florida (28º41.321' N, 81 º23.464' W) in March 2006. Upon capture, fishes were placed in coolers of aerated river water and held until euthanized (less than two hours). Fishes were euthanized in buffered water containing MS-222 (1 g l^{-1}), measured [standard length (SL) ± 1 mm], and dissected on a chilled (~4^{o}C) cutting board. Guts were removed by cutting at the esophagus and at the anus and processed in a manner appropriate for specific analyses (see below). All handling of fish from capture to euthanasia was conducted under approved protocol D995 of the Institutional Animal Care and Use Committee of the University of Florida.

Gut Morphology and Length

Guts from 11 *P. cf. n.* "Marañon", 13 *P. nocturnus*, and 13 *P. disjunctivus* were removed from the fish, uncoiled, and measured. These guts were then immediately frozen and saved for gut content analyses. Gut content analyses were performed following the techniques described by German et al. (2008). However, to save space, the results of this part of the study will not be shown. Briefly, the gut contents of the species of *Panaque* contained wood (55%), amorphous detritus (40%), and diatoms (5%), whereas *Pt. disjunctivus* guts contained amorphous detritus (45%), algae (30%; green filamentous and blue-green algae), diatoms (15%), unidentified insect parts (5%), and sediment (5%).

The measured guts were used to calculate three digestive somatic indices commonly used in studies of fish feeding ecology (German and Horn 2006): relative gut length (RGL = gut length/standard length); gut length as a function of snout-vent length (GL/SVL = gut length/snout-vent length), where snout vent length is the measurement on the ventral surface from the tip of head to the anus; and Zihler's index [Zihler 1982; ZI = gut length/(10 x body mass$^{1/3}$)], which relates gut length to body mass. These indices allow for the comparison of gut length among fishes with different diets while controlling for differences in body size (Kramer and Bryant 1995; German and Horn 2006).

Gut pH and Redox Measurements

Upon dissection, the complete digestive tracts of four individuals each of *P. cf. n.* "Marañon", *P. nocturnus*, and *Pt. disjunctivus* were placed on a sterilized, stainless-steel dissection tray at ambient temperature (22-25ºC) and gently uncoiled without tearing or stretching. The pH and redox conditions of the digestive tracts were measured following Clements et al. (1994) with calibrated pH and redox microelectrodes (models PHR-146S and ORP-146, respectively; Lazar Laboratories Inc., Los Angeles, CA, USA) connected to a portable pH-redox meter (model 601A, Jenco Inc., San Diego, CA, USA). Incisions large enough to allow penetration of the microelectrode tip (~0.25 mm) into the gut fluid were made in the stomach and intestinal wall and the pH and redox conditions were measured immediately after each incision was made. Overall, pH and redox conditions were measured in five sections of the stomach and 10 sections each of the proximal, mid, and distal intestine of each individual fish. The mean pH and redox conditions were then determined for each region of the digestive tract in an individual fish, and mean values determined for each gut region for each species.

Histological and TEM Analyses

Upon removal from the body, the digestive tracts of two individuals of each species 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, and the length of the intestine (IL ± 0.05 mm) was measured with calipers. Six 1-mm sections were excised from each of the proximal, mid, and distal intestine and placed in their own individual vials containing fresh Trump's fixative, and kept cool (~4-10ºC) for transport back to the University of Florida. Three of the sections were designated for analysis with transmission electron microscopy (TEM), whereas the other three were designated for use in histological analyses and light microscopy.

Upon arrival at the laboratory (approximately three weeks), tissues were removed from the fixative and rinsed in 0.1 M phosphate buffered saline (PBS), pH 7.5, for 3 x 20 min, and a final rinse overnight at 4ºC. Following rinsing in PBS, the tissues designated for histological analyses were rinsed for 40 min in running deionized water and dehydrated in a graded ethanol series. The samples were then impregnated in two changes of Citrisolve for 20 min each, and infiltrated in four changes of paraffin (TissuePrep 2, Fisher Scientific, Fair Lawn, NJ, USA) for 30, 45, 60, and 60 min in a vacuum oven at 60ºC. The tissues were then embedded in paraffin at 57ºC and the blocks stored at room temperature before use. Intestinal tissues were serially sectioned at 7 μm, stained in a modified Masson's trichrome (Presnell and Schreibman 1997), and photographed at 40X, 100X, and 400X 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 species) were used to qualitatively examine the gut structure of the fish.

Following the rinsing in PBS, those tissues designated for TEM were then postfixed in 1% osmium tetroxide for 12 h at 4ºC, and rinsed in running deionized water for 40 min. The tissues were then dehydrated in a graded ethanol series, followed by a graded acetone series at room temp (22ºC), and embedded in Spurr's resin (Ted Pella Inc., Redding, CA, USA). Resin blocks were cut into 1-mm thick sections using a Reichert-Jung Ultracut-E microtome (Jena, Germany). The sections were stained with 1% toluidine blue, and examined under a bright-field light microscope (Olympus BX60) to find sections with appropriate intestinal folds (Horn et al. 2006). Ultrathin sections (70 nm) were then cut with a diamond blade from the same central part of each selected mucosal fold, mounted on honeycomb copper grids (Pelco 8GC 180 or 270, Ted Pella) and stained with 1% uranyl acetate and 2% lead citrate. Cross sections of 5–10 enterocytes with undistorted (i.e., cylindrical) microvilli were photographed using a transmission electron microscope (H-7000, Hitachi, Japan). Images (n = 15 per intestinal region per individual fish; 90 images per species) were used to qualitatively assess how the surface area of the intestine changes from the proximal to the distal ends.

Tissue Preparation for Digestive Enzyme Analyses

For fishes designated for digestive enzyme analyses, guts were dissected out, placed on a sterilized, chilled (~4ºC) 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 10,000 x g for 5 min (Skea et al. 2005) in an Eppendorf 5415R desktop centrifuge powered by a 12V car battery via a power inverter. Following centrifugation, the supernatants (heretofore called "intestinal fluid") were gently pipetted into a separate sterile centrifuge vials, and the pelleted gut contents and intestinal fluid were frozen 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 entire liver and hepatopancreas were also excised from each animal. The gut wall sections, livers, and hepatopancreas were placed in sterile centrifuge vials and frozen in liquid nitrogen. All of the samples were then transported on dry ice back to the University of Florida where they were stored at –80°C until analyzed.

The intestinal fluids and pelleted gut contents were homogenized on ice following Skea et al. (2005). Intestinal fluids were defrosted, diluted 5-10 volumes in 0.05 M Tris-HCl, pH 7.5, and gently homogenized using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) with a 7-mm generator at a setting of 1100 rpm for 30 s. The intestinal fluid samples were then stored at –80ºC in small aliquots (100-200 μL) until use. To ensure the rupture of microbial cells and the complete release of enzymes from the gut contents, the pelleted gut contents were defrosted, diluted 3-5 volumes in 0.05 M Tris-HCl, pH 7.5, sonicated at 5 W output for 3 x 20 s, with 40-s intervals between pulses, and homogenized with the Polytron homogenizer at 3000 rpm for 3 x 30 s. The homogenized pelleted gut contents were then centrifuged at 12,000 x *g* for 10 min at 4ºC, and the resulting supernatant designated "microbial extract".

Gut wall, liver, and hepatopancreas samples were homogenized according to German et al. (2004). Gut wall sections were defrosted, diluted in 5-100 volumes of 0.3 M mannitol in 0.001 M Hepes/NaOH (Martínez del Rio et al. 1995; Levey et al. 1999), pH 7.0, homogenized with the Polytron homogenizer at 3000 rpm for 3 x 30 s, and centrifuged at 9,400 x *g* for 2 min at 4ºC. The liver and hepatopancreas samples were treated in the same manner, with the exception that they were diluted 3-10 volumes with 0.05 M Tris-HCl, pH 7.5. Following centrifugation, the

supernatants from the pelleted gut contents (microbial extract), the gut wall sections, liver, and hepatopancreas samples were collected and stored in small aliquots (100-200 μl) at –80°C until just before use in spectrophotometric assays of activities of digestive enzymes. The protein content of the homogenates was measured using bicinchoninic acid (Smith et al. 1985) as detailed by German (2008).

Assays of Digestive Enzyme Activity

All assays were carried out at 25°C, consistent with measured temperatures (24-26°C) of the Río Marañon, in triplicate using the BioRad Benchmark Plus microplate spectrophotomer and Falcon flat-bottom 96-well microplates (Fisher Scientific). All pH values listed for buffers were measured at room temperature (22°C), and all reagents were purchased from Sigma-Aldrich Chemical (St. Louis). All reactions were run at saturating substrate concentrations as determined for each enzyme with gut tissues from the four species. Each enzyme activity (Table 2-1) 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 et al. 2008).

Polysaccharidase activities (i.e., activities against starch, laminarin, cellulose, mannan, and xylan) were measured in the intestinal fluid, pelleted gut contents, liver, and hepatopancreas according to the Somogyi-Nelson method (Nelson 1944; Somogyi 1952). Polysaccharide substrate was dissolved [starch (2%), laminarin (0.5%), carboxymethyl cellulose (0.5%), or mannan (0.5%)] or suspended (xylan, 0.5%) in 0.8 M sodium citrate buffer, pH 7.5. In a microcentrifuge vial, 50 μl of polysaccharide solution was combined with 50 μl of a mixture of sodium citrate buffer and intestinal fluid, tissue, or microbial extract homogenate. Homogenate

volumes ranged from 1-30 µl depending on the enzyme concentration in the homogenates. The incubation period varied with substrate – the assays were carried out for 10 min for starch, two hours for laminarin, each in a water bath, and 24 hours for each of carboxymethyl cellulose, mannan, and xylan, under constant shaking on a rotary shaker in an incubator. The incubation was stopped by adding 20 µl of 1 M NaOH and 200 µl of Somogyi-Nelson reagent A. Somogyi-Nelson reagent B was added after the assay solution was boiled for 10 min (see German et al. 2004 for reagent recipes). The resulting solution was diluted in water and centrifuged at 6,000 x *g* for 5 min. The reducing sugar content of the solution was then determined spectrophotometrically at 650 nm, and polysaccharidase activity was determined from a standard curve constructed with the respective monomer (i.e., glucose for starch, laminarin, and carboxymethyl cellulose; mannose for mannan; and xylose for xylan). Enzyme activities are expressed in U (1 µmol reducing sugar liberated per minute) per gram wet weight of fluid, tissue, or content.

Maltase activity was measured in gut wall tissues and pelleted gut contents following Dahlqvist (1968) as described by German (2008). In a microcentrifuge tube, 10 µL of 56 mM maltose dissolved in 100 mM maleate buffer, pH 7.0, was combined with 10 µL of regional gut wall or pellet homogenate. After 10 min, the reaction was stopped by the addition of 300 µL of assay reagent (Sigma GAGO20) dissolved in 1 M tris-HCl, pH 7.0. The reaction mixture was incubated for 30 min at 37 ^{o}C, and was stopped by the addition of 300 µL of 12 N H_2SO_4. The amount of glucose in the solution was then determined spectrophotometrically at 540 nm. The maltase activity was determined from a glucose standard curve and expressed in U (1 µmol glucose liberated per minute) per gram wet weight of gut tissue or pelleted contents. The

Michaelis-Menten constant (K_m) for maltase was determined for gut wall and pelleted gut content samples with substrate concentrations ranging from 0.56 mM to 112 mM.

Tris is known to be an inhibitor of maltase activity (Dahlquist 1968), but in higher concentrations (e.g., 1 M; Levey et al. 1999) than those used in our homogenate buffer (0.05 M). Nevertheless, to confirm that the different buffers used for the gut wall (Hepes-Mannitol) and microbial extract (Tris-HCl) homogenates did not directly affect the K_m or activity for maltase, the gut walls and pelleted gut contents of the proximal intestine of five additional *P. disjunctivus* were homogenized in the opposite buffers – gut walls in Tris-HCl and pelleted gut contents in Hepes-Mannitol. For maltase, the different buffers did not produce different K_m (Tris-HCl: 7.72 ± 1.91 mM; Hepes-Mannitol: 7.97 ± 0.99 mM; t=0.10, p=0.92, d.f.=10) or activity (Tris-HCl: 20.74 ± 4.76 U · g tissue^{-1}; Hepes-Mannitol: 12.62 ± 1.66 U · g tissue^{-1}; t=1.38, p=0.20, d.f.=10) values in the microbial extract, or K_m (Tris-HCl: 4.98 ± 0.72 mM; Hepes-Mannitol: 3.87 ± 0.58 mM; t=1.20, p=0.26, d.f.=10) or activity (Tris-HCl: 2.05 ± 0.41 U · g tissue^{-1}; Hepes-Mannitol: 2.44 ± 0.37 U · g tissue^{-1}; t=0.70, p=0.50, d.f.=10) values in the gut wall homogenates. The low-concentration Tris-HCl was observed to have little effect on maltase activity in two previous investigations (German et al. 2004, German 2008) in which the gut tissues were homogenized in 0.05 M Tris-HCl buffer. The different buffers also did not affect the K_m and activity levels of the other disaccharidases measured in this study (see below), and thus, I can be confident that any differences in K_m and enzyme activity among the gut wall and pelleted gut content homogenates are not due to the different buffers used in their homogenization.

The activities of the disaccharidases β-glucosidase, β-mannosidase, β-xylosidase, and N-acetyl-β-D-glucosaminidase (NAG) were measured in gut wall tissues and pelleted gut contents using p-nitrophenol conjugated substrates (Nelson et al. 1999; Xie et al. 2007) dissolved in 0.1M

sodium citrate, pH 7.0. In a microplate well, 90 μL of 11.1 mM substrate (1.33 mM for NAG) was combined with 10 μL of gut wall or pelleted gut content homogenate and the reaction was read kinetically at 405 nm for 15 min. The disaccharidase activities were determined from a p-nitrophenol standard curve and expressed in U (1 μmol p-nitrophenol liberated per minute) per gram wet weight of gut tissue or pelleted contents. The K_m was determined for gut wall and pelleted gut content samples for β-glucosidase and NAG. The substrate concentrations ranged from 0.002 mM to 10 mM for β-glucosidase and 0.04 to 1.2 mM for NAG

Chitinase activities were measured following German et al. (2008), but no activity was detected in the four species used in this study. In all assays, the background levels of N-acetyl-glucosamine detected in the blanks (>1 mM) matched what was measurable in the assay mixtures, making activity determinations impossible. However, the measurable N-acetyl-glucosamine in the gut in addition to measurable NAG activities makes it likely that the fish can utilize chitin as a nutrient source.

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) as described by Gawlicka et al. (2000). The substrate, 2 mM Nα-benzoyl-L-arginine-p-nitroanilide hydrochloride (BAPNA), was dissolved in 100 mM tris-HCl buffer (pH 7.5) by heating to 95^{o}C (Preiser et al. 1975; German et al. 2004). In a microplate, 95 μl of BAPNA was combined with 5 μl of homogenate, and the increase in absorbance was read continuously at 410 nm for 15 min. Trypsin was also assayed in the liver and hepatopancreas, but tissues homogenates from these organs were first incubated with enterokinase for 15 min to activate trypsinogen prior to combining the homogenates with substrate (German et al. 2004). Trypsin activity was determined with a p-

nitroaniline standard curve, and expressed in U (1 μmol p-nitroaniline liberated per minute) per gram wet weight of tissue, gut fluid, or pelleted gut contents.

Aminopeptidase activity was measured in gut wall tissues and pelleted gut contents according to Roncari and Zuber (1969) as described by German et al. (2004). In a microplate, 90 μL of 2.04 mM L-alanine-p-nitroanilide HCl dissolved in 200 mM sodium phosphate buffer (pH 7.5) was combined with 10 μL of homogenate. The increase in absorbance was read continuously at 410 nm for 15 min and activity determined with a p-nitroaniline standard curve. Aminopeptidase activity was expressed in U (1 μmol p-nitroaniline liberated per minute) per gram wet weight of gut tissue or pelleted gut contents.

Lipase (nonspecific 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). In a microplate, 86 μl of 5.2 mM sodium cholate dissolved in 250 mM tris-HCl (pH 7.5) was combined with 6 μl of homogenate and 2.5 μl of 10 mM 2-methoxyethanol and incubated at room temperature for 15 min to allow for lipase activation by bile salts. The substrate p-nitrophenyl myristate (5.5 μl of 20 mM p-nitrophenyl myristate dissolved in 100% ethanol) was then added and the increase in absorbance was read continuously at 405 nm for 15 min. Lipase activity was determined with a p-nitrophenol standard curve, and expressed in U (1 μmol p-nitrophenol liberated per minute) per gram wet weight of gut tissue.

The activity of each enzyme was regressed against the protein content of the homogenates to confirm that there were no significant correlations between the two variables. Because no significant correlations were observed, the data are not reported as U per mg protein.

Gut Fluid Preparation, Gastrointestinal Fermentation, and Luminal Carbohydrate Profiles

Measurements of symbiotic fermentation activity were based on the methods of Pryor and Bjomdal (2005). Fermentation activity was 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. As homogenates were prepared from the intestinal fluid samples (see above under "Tissue preparation for digestive enzyme analyses"), 30 μL of undiluted intestinal fluid was pipetted into a sterile centrifuge vial equipped with a 0.22 μm cellulose acetate filter (Costar Spin-X gamma sterilized centrifuge tube filters, Coming, NY) and centrifuged under refrigeration at 13,000 x *g* for 15 min to remove particles from the fluid (including bacterial cells). The filtrates were collected and frozen until they were analyzed for SCFA and nutrient concentrations.

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) and German et al. (2008). Glucose concentrations were analyzed in 2 μL of gut fluid using the same glucose content assay described for the maltase assay above. The only departure being that there was no pre-incubation with maltose; the gut fluid was immediately combined with the assay reagent and incubated at 37 ^{o}C for 30 min, the reaction stopped with 12N H_2SO_4, and the resulting mixture read in a spectrophotometer at 540 nm against a glucose standard curve.

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).

Fiber Digestibility

To evaluate whether *Pt. disjunctivus* and a wood-eating loricariid catfish, *Panaque nigrolineatus*, could digest wood fiber, I performed a traditional fiber digestibility feeding trial using a total collection method (Galetto and Bellwood 1994). Five individuals of *Pt. disjunctivus* (mean ± S.D.; 221.88 ± 9.83 mm SL; 214.44 ± 23.09 g BM) were collected from Wekiva Springs, FL, in September 2007 and brought back to the University of Florida. Seven individuals of *P. nigrolineatus* (176.43 ± 20.73 mm SL; 227.76 ± 78.71 g BM) were obtained from an aquarium wholesaler (5-D Tropical, Tampa, FL) as the fish arrived from Venezuela, where they were captured from their native habitat. Individuals of both species were individually assigned to 75.6-L aquaria equipped with a 2.5 cm plastic mesh at the bottom, which allowed feces and uneaten food ("orts") to fall through and be undisturbed by the fish. The intake tube of the mechanical filter was covered with 250-μm mesh screen to prevent uneaten orts and feces from being sucked into the filter. Every two days the fish were given a new piece (~100 g, wet weight) of water-logged, degraded water oak (*Quercus nigra*) wood on which to graze. The fish were allowed to acclimate to these conditions for at least one month prior to the beginning of the experiment.

Once the experiments were started, they were executed in two-week intervals. Two two-week feeding trials (i.e., a total of four weeks) were performed for *P. nigrolineatus*, and three two-week trials were performed for *Pt. disjunctivus* to obtain enough fecal material for all of the analyses. Each afternoon (~15:00) the fish were gently removed from their tanks into an individual bucket containing aquarium water. The plastic mesh was removed from the bottom of the tank, and the feces, which were completely distinguishable from the orts, were siphoned off with a 25 mL bulb pipette into a weigh boat. Ort debris was siphoned off with a piece of vinyl

tubing onto a 250-μm mesh screen, from which it was scraped with a razorblade into weigh boats for each tank. The feces and debris were dried at 60ºC for 24 hours, weighed, and stored in sealed glass vials. On a daily basis the wood pieces provided to each fish were blotted with a paper towel, weighed (wet mass), and a small subsample was scraped off with a razorblade. The wood was then weighed again before being returned to the tank. Daily diet samples, as well as daily feces and ort samples for an individual fish, were combined to obtain a composite sample for each of the diet, feces, and orts across the feeding trial (Bouchard and Bjorndal 2006).

Wood and ort samples were ground to pass through a 1-mm screen in a coffee grinder, and fecal samples were ground with a mortar and pestle. Fecal, ort, and diet samples were analyzed for dry matter, organic matter, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin+cutin, and %N (Bouchard and Bjorndal 2006). Ort analysis allowed us to examine whether the fishes fed selectively on "more nutritious" portions of the wood.

Dry matter and ash (mineral) content were determined by drying subsamples overnight at 105ºC and then combusting them at 550ºC for 4 h. The difference between these two measures represents the organic matter component of the sample. NDF and ADF were determined by sequentially refluxing samples with neutral detergent and acid detergent solutions (Goering and Van Soest 1970) in an Ankom^{200} Fiber Analyzer according to the guidelines supplied with the equipment (Ankom Technology 1998; Ankom Technology 1999). NDF represents the cell-wall component of the wood (cellulose, hemicellulose, and lignin), and ADF represents the ligno-cellulose component. The actual lignin+cutin portion was determined by refluxing the samples in 72% sulfuric acid for 3 hours at room temp (24ºC; Moran and Bjorndal 2007). The N content of the samples was determined using a Carlo Erba elemental analyzer.

Daily consumption rates, on a dry matter basis, were calculated from a regression of wet weight vs. dry weight of water oak wood pieces minus the mass of orts. Thus, we were able to calculate digestibility coefficients based on total intake and fecal output. Digestibility was determined using the equation (intake-feces)/intake, where intake is total grams of dry matter or organic matter consumed during the trial and feces is grams of dry matter or organic matter in the feces produced during the trial.

Transit Time of Wood in the Digestive Tract

One of the most important elements in studies of digestion is how long food is held in the digestive tract, as this provides key information into the strategy an animal takes to digest a meal (Karasov and Martínez del Rio 2007). At the conclusion of the fiber digestibility experiment, *P. nigrolineatus* and *Pt. disjunctivus* were fed water oak wood that had been stained red with carmine dye (Fris and Horn 1993). The wood pieces were submerged in a 2% carmine dye solution for at least one month. They were then pre-steeped under agitation (3x10 min) in aquarium water to remove loose dye particles and ensure that only fully stained wood was offered to the fish. Wood pieces (~100 g) were placed in tanks as described above under "Fiber digestibility" only the tanks were without the plastic mesh at the bottom. Because *P. nigrolineatus* and *Pt. disjunctivus* are nocturnal, the fish were given the stained wood an hour before the lights turned off in the aquarium laboratory, and the fish usually took 0.5-1 *h* to adjust to the darkness and start feeding (DPG, pers. obs.). Four hours after the commencement of feeding, two individuals were taken and euthanized in MS-222 as described above. After this initial four hour feeding period, the stained wood was replaced with non-stained wood in the tanks of the remaining fish to allow for the tracking of the stained wood (i.e., the pulse) through the gut. Two additional fish were taken and euthanized at six and eight hours, and the remaining

fish at 18 hours post feeding. At each sampling interval, the presence or absence of red-stained feces in the aquaria was noted.

At each sampling interval the fish were dissected on a sterile cutting board, and the gut was removed, uncoiled, measured, and photographed. The red dye on the wood was visible in the gut contents of *P. nigrolineatus*, but not in *Pt. disjunctivus*, mainly because the latter species produces an inordinate amount of bile that is nearly black in color. Thus, I was unable to address particle retention in *Pt. disjunctivus* and only have the approximate amount of time taken for red feces to appear in the aquaria for this species. In *P. nigrolineatus*, the intestine was divided into three sections of equal length and the proximal-most location of the red stained wood in the gut was noted at each sampling interval. The intestinal contents were squeezed from each intestinal section with forceps and the blunt side of a razor blade into sterile centrifuge vials and frozen at -80ºC until analyzed (~one month). When analyzed, the samples were defrosted, and to determine whether specific particle sizes of the digesta were being selectively retained along the intestine, the contents were wet sieved (Vispo and Hume 1995) using mesh sizes ranging from 0.25 to 1.5 mm. Following the sieving, the contents were dried at 60ºC for 24 hours, and the samples of the various particle size classes were weighed. The masses of all of the particle size classes were then added together, and the proportion of each particle size class was determined. This design also allowed me to ascertain whether particles (stained or non-stained) of any size were held longer in one region of the digestive tract than another. This experiment was repeated one month later with an additional seven *P. nigrolineatus* obtained from the same aquarium wholesaler.

Statistical Analyses

Prior to all significance tests, a Levene's test for equal variance was performed and residual versus fits plots were examined to ensure the appropriateness of the data for parametric

analyses. All tests were run using SPSS (version 11) and Minitab (version 12) statistical software packages. The various digestive-somatic indices were compared among species with ANCOVA, using body mass as a covariate, followed by a Tukey's HSD with a family error rate of P = 0.05. The activities of amylase, laminarinase, cellulase, and xylanase were compared between the intestinal fluid and microbial extract fractions of each gut region in each species with *t*-test, using a Bonferroni correction. Interspecific and intraspecific (i.e., among gut regions in a single species) comparisons of total enzymatic activities (intestinal fluid + microbial extract) and total SCFA concentrations were made with ANOVA followed by a Tukey's HSD with a family error rate of P = 0.05. The activities of maltase, β-glucosidase, N-acetyl-β-D-glucosaminidase, β-mannosidase, and aminopeptidase were compared between the gut wall and microbial extract fractions of each gut region in each species with *t*-test, using a Bonferroni correction. Similarly, the K_m values of maltase, β-glucosidase, and N-acetyl-β-D-glucosaminidase from the proximal intestine of the fish were compared between the gut wall and microbial extract fractions of each species with *t*-test.

Results

Gut Length, pH and Redox

The digestive tracts of *P. cf. n.* "Marañon", *P. nocturnus*, *Pterygoplichthys disjunctivus,* and *Hypostomus pyrineusi* were very similar in morphology. All four species have extremely long, thin-walled intestines that exceed 10x the body length of the animal (Table 2-2; Figure 2-2), but *Pt. disjunctivus* possesses a significantly longer gut than either of the *Panaque* species (Table 2-2). I was only able to measure the gut length of two individuals of *H. pyrinuesi*, and thus, they were not included in the statistical analyses. But, the digestive somatic indices in this species (RGL = 11.85; GL/SVL = 20.13; ZI = 40.03) were more similar to the species of

Panaque than to *Pt. disjunctivus*. The pH of the digestive tracts of *P. cf. n.* "Marañon", *P. nocturnus*, and *Pt. disjunctivus* were all neutral, whereas the redox conditions of the stomach were positive (*Pt. disjunctivus*) or less negative (*P. cf. n.* "Marañon"and *P. nocturnus*), and the redox conditions of the intestines of all three species were extremely negative (Table 2-3). Thus, the guts of the three species were aerobic or slightly anaerobic in the stomach region, and extremely anaerobic along the intestine.

Histology and TEM Analyses

The digestive tracts of the four loricariid catfish species were very similar on the histological and transmission electron micrograph levels (Figure 2-3). All four species showed the same pattern of decreasing height of intestinal folds, and decreasing microvilli surface area moving distally along the intestine. Furthermore, no conglomerations of microbes were observed anywhere along the intestines of the four species (Figure 2-3).

Digestive Enzyme Activities

No differences were observed in amylase, laminarinase, or cellulase activities between the intestinal fluid and the microbial extracts of any species (Table 2-4). However, xylanase activity was significantly greater in the microbial extracts of the proximal and mid intestine of *P. nocturnus* than in the intestinal fluids of these regions. Total amylase activity was significantly greater in the proximal intestine than in the distal intestine of all four species (Table 2-5, Figure 2-4). *Panaque nocturnus* possessed significantly lower amylase activity in its proximal intestine than *P. cf. n.* "Marañon" and *H. pyrineusi*, but not significantly lower than *Pt. disjunctivus*. In the mid intestine, *P. nocturnus* possessed lower amylase than *Pt. disjunctivus* and *H. pyrineusi*, but not lower than the mid intestine of *P. cf. n.* "Marañon" (Table 2-5, Figure 2-4). No differences were detected among the species for amylase activity in the distal intestine.

Laminarinase activity was significantly higher in the proximal intestine of all four species than in their mid or distal intestines (Table 2-5, Figure 2-4). No laminarinase activity was detected in the distal intestines of *P. nocturnus* and *H. pyrineusi*. *Pterygoplichthys disjunctivus* possessed significantly higher laminarinase activity in its proximal intestine than in this gut region of *P. cf. n.* "Marañon" and *H. pyrinuesi*, but not *P. nocturnus* (Table 2-5, Figure 2-4). In turn, *P. nocturnus* and *Pt. disjunctivus* possessed greater laminarinase activity in their mid intestines than *P. cf. n.* "Marañon" and *H. pyrineusi*.

Pterygoplichthys disjunctivus and *H. pyrineusi* exhibited significantly higher cellulase activity in their proximal intestines than in their mid or distal intestines (*H. pyrineusi* lacked detectable cellulase activity in its distal intestine), whereas the two species of *Panaque* showed no difference in cellulase activity along the gut (Table 2-5, Figure 2-4). Individuals of *Pt. disjunctivus* possessed significantly greater cellulase activity in their proximal intestines than individuals of *P. nocturnus*, but not greater than *P. cf. n.* "Marañon" or *H. pyrineusi*. And, *Pt. disjunctivus* exhibited higher cellulase activities in its mid intestine than *H. pyrineusi*, but not higher than the species of *Panaque* (Table 2-5, Figure 2-4).

Individuals of *P. cf. n.* "Marañon", *Pt. disjunctivus*, and *H. pyrineusi* possessed significantly greater xylanase activity in their proximal intestines than in their mid or distal intestines (like cellulase, *H. pyrineusi* lacked detectable xylanase activity in its distal intestine). *Panaque nocturnus*, on the other hand, showed a slight increase in xylanase activitiy moving distally along the intestine, albeit not a significant increase (Table 2-5, Figure 2-4). No significant differences were observed in the activity levels of xylanase among the species for any gut region. No mannanase activity was detected in any gut region of any species.

The maltase activity in the microbial extract was significantly higher than the activity of this enzyme in the gut wall of the proximal intestines of all four species (Figures 2-5 and 2-6). No significant differences were observed in the mid intestine. The maltase activity in the gut walls of the distal intestines of the wood-eating taxa was higher than the maltase activity of the microbial extract, whereas the opposite was true for the detritivorous *Pt. disjunctivus* (Figures 2-5 and 2-6). All four species showed decreasing maltase activities in the microbial extract moving distally along the intestine, whereas all four taxa showed slight increases in gut wall maltase activity in the mid intestine in comparison to the proximal intestine (Figures 2-5 and 2-6). *Hypostomus pyrineusi* possessed significantly greater maltase activity in its gut wall fractions (PI: ANOVA $F_{3,22} = 21.84$, $P < 0.001$; MI: ANOVA $F_{3,22} = 55.34$, $P < 0.001$; DI: ANOVA $F_{3,22} = 11.20$, $P < 0.001$, Tukey's $P < 0.011$ for all) than the other species, which did not differ from one another. Similarly, significant differences were detected among the microbial extract maltase activities of the proximal intestines of the species (ANOVA $F_{3,26} = 32.55$, $P < 0.001$; Tukey's $P < 0.011$); *H. pyrineusi* possessed significantly greater maltase activity in its proximal intestine microbial extract than all of the other species, and *Pt. disjunctivus* exhibited greater maltase activity than the species of *Panaque*, which did not differ from one another. A similar pattern was found for the mid intestine microbial extract (ANOVA $F_{3,26} = 20.30$, $P < 0.001$; Tukey's $P < 0.011$), with *H. pyrineusi* and *Pt. disjunctivus* possessing significantly greater maltase than the two species of *Panaque*. There were no differences among the species in the microbial extract maltase activities of the distal intestine.

The β-glucosidase activities in the microbial extracts of the proximal intestines of *P. cf. n.* "Marañon", *P. nocturnus*, and *Pt. disjunctivus* were all significantly higher than the activities of this enzyme in the gut wall fractions, however the opposite was true for *H. pyrineusi* (Figures

2-5 and 2-6). Only *Pt. disjunctivus* showed significant differences in β-glucosidase activity in their mid and distal intestines, with the gut wall activity being significantly higher in the mid intestine, and the activity in the microbial extract being higher in the distal intestine. All four species showed decreasing β-glucosidase activity in the microbial extracts of their distal intestines (Figures 2-5 and 2-6). However, there were several different patterns for gut wall β-glucosidase activity: *P. nocturnus* and *H. pyrineusi* showed decreasing activity in their distal intestine, *P. cf. n.* "Marañon" showed increasing activity towards their distal intestine, and *Pt. disjunctivus* showed a spike in activity in the mid intestine, followed by a decrease in the distal intestine. Like maltase, *H. pyrineusi* possessed significantly greater β-glucosidase in the gut wall fraction of their proximal intestines than in the other species (ANOVA $F_{3,22} = 49.68$, $P < 0.001$; Tukey's $P < 0.011$), which did not differ from one another. *Pterygoplichthys disjunctivus* and *H. pyrineusi* exhibited significantly greater β-glucosidase activity in the gut wall fractions of their mid intestines than *P. nocturnus*, but not *P. cf. n.* "Marañon", which in turn was not different from *P. nocturnus*. Individuals of *P. cf. n.* "Marañon" possessed significantly greater β-glucosidase activity in the gut wall of their distal intestine than in the distal intestine gut wall of *Pt. disjunctivus* ($t = 2.62$, $P = 0.026$, $df = 10$); *P. nocturnus* and *H. pyrineusi* lacked β-glucosidase activity in the gut wall fractions of their distal intestines. No significant differences were detected in the β-glucosidase activities of the proximal and mid intestine microbial extracts of any species. However, *P. cf. n* "Marañon" and *Pt. disjunctivus* exhibited significantly greater β-glucosidase activity in the microbial extracts of their distal intestines than in *P. nocturnus* (ANOVA $F_{3,26} = 4.35$, $P = 0.014$; Tukey's $P < 0.011$), but not greater than in *H. pyrineusi*, which did not differ from *P. nocturnus*.

Panaque nocturnus exhibited significantly greater N-acetyl-β-D-glucosaminidase (NAG) activity in the gut wall of its proximal intestine than in the microbial extract, whereas none of the other species showed differences in NAG activity between these two fractions in their proximal intestines (Figures 2-5 and 2-6). The wood-eating taxa all exhibited significantly higher NAG activity in the gut walls of their mid intestines than in the microbial extracts from this gut region, whereas *Pt. disjunctivus* showed no differences between the two fractions. However, *P. nocturnus* and *Pt. disjunctivus* had significantly greater NAG activity in the gut walls of their distal intestine gut walls than in their microbial extracts, whereas the other species showed no differences between the two fractions (Figures 2-5 and 2-6). *Panaque cf. n.* "Marañon", *P. nocturnus*, and *Pt. disjunctivus* showed increases in their gut wall NAG activities moving distally along the intestine, whereas *H. pyrineusi* showed a decrease. The NAG activities of the microbial extracts were variable, and didn't follow one pattern (increase or decrease) along the guts of any of the four species (Figures 2-5 and 2-6). *Pterygoplichthys disjunctivus* exhibited significantly greater NAG activity in its proximal intestine gut wall than in *P. nocturnus* (ANOVA $F_{3,22} = 4.97$, $P = 0.010$; Tukey's $P < 0.011$), but not greater than the other species, which did not differ from one another. The mid intestine gut wall NAG activities were not different among the species, but *Pt. disjunctivus* displayed significantly greater distal intestine gut wall NAG than the other species (ANOVA $F_{3,22} = 15.39$, $P < 0.001$; Tukey's $P < 0.011$), which did not vary from one another. No differences were detected among the species for NAG activity in the proximal intestine microbial extracts, but *Pt. disjunctivus* had significantly higher NAG activity in its mid intestine microbial extract than in the other species (ANOVA $F_{3,26} = 26.37$, $P < 0.001$; Tukey's $P < 0.011$), which did not differ from one another. *Pterygoplichthys disjunctivus* showed significantly greater distal intestine microbial extract NAG activity than *P.*

cf. n. "Marañon" and *P. nocturnus* (ANOVA $F_{3,26} = 5.25$, $P = 0.007$; Tukey's $P < 0.011$), but not greater than *H. pyrineusi*.

The maltase Michaelis-Menten constants (K_m) from the wall of the proximal intestines of the fish were generally lower, although not significantly so, than the K_m values of the microbial extracts from the proximal intestines (Table 2-6). However, the K_m values of β-glucosidase were all significantly lower in the fish gut walls than in the microbial extracts, and the same is generally true for NAG, except for in *P. nocturnus* (Table 2-6). *Pterygoplichthys disjunctivus* had a significantly higher K_m for maltase in the gut wall fraction than any of the xylivorous species (ANOVA $F_{3,22}$: 9.61, $P < 0.001$; Tukey's $P < 0.011$), which did not differ from one another. No differences in the maltase K_m values of the microbial extracts were detected among the species (ANOVA $F_{3,26}$: 1.80, $P = 0.176$; Tukey's $P < 0.011$). The two species of *Panaque* possessed significantly lower K_m values for β-glucosidase in their gut walls than in *Pt. disjunctivus* or *H. pyrineusi* (ANOVA $F_{3,22}$: 10.28, $P < 0.001$; Tukey's $P < 0.011$), which did not differ from one another. And, *P. cf. n.* "Marañon" possessed significantly lower β-glucosidase K_m in its microbial extract than *Pt. disjunctivus* and *H. pyrineusi* (ANOVA $F_{3,26}$: 7.28, $P = 0.001$; Tukey's $P < 0.011$), but not *P. nocturnus*, and the microbial extract β-glucosidase K_m of the three remaining species were not statistically different from each other. *Panaque cf. n.* "Marañon" displayed significantly lower NAG K_m in its gut wall than any other species (ANOVA $F_{3,22}$: 6.60, $P = 0.003$; Tukey's $P < 0.011$), which were not different from one another. Both species of *Panaque* exhibited significantly lower K_m values for their microbial extract NAG than that of *Pt. disjunctivus* (ANOVA $F_{3,26}$: 4.27, $P = 0.015$), but not of *H. pyrineusi*.

All four species generally possessed significantly greater β-mannosidase and activities in their gut walls than in the microbial extracts (Table 2-7). The activity of β-mannosidase

increased in activity moving distally along the intestine of *P. cf. n.* "Marañon", decreased in activity moving distally along the intestines of *P. nocturnus* and *Pt. disjunctivus*, and spiked in the mid intestine of *H. pyrineusi*. *Panaque nocturnus* possessed significantly greater β-mannosidase activity in the gut wall of its proximal intestine than in the other species (ANOVA $F_{3,22}$: 24.72, P <0.001; Tukey's P <0.011), which didn't differ from one another. *Panaque nocturnus* also showed greater β-mannosidase activity in the gut wall of their mid intestine than *P. cf. n.* "Marañon" and *Pt. disjunctivus* (ANOVA $F_{3,22}$: 8.36, P = 0.001; Tukey's P <0.011), and *H. pyrineusi* exhibited greater activity than *Pt. disjunctivus*, which did not differ from *P. cf. n.*"Marañon". However, *P. cf. n.* "Marañon" displayed significantly higher β-mannosidase activity in the gut wall of its distal intestine than in *Pt. disjunctivus* and *H. pyrineusi*, but not *P. nocturnus* (ANOVA $F_{3,22}$: 4.97, P = 0.010; Tukey's P <0.011). The β-mannosidase activity in the gut wall of the distal intestine of the other taxa did not differ from one another.

All four species generally possessed significantly greater aminopeptidase activities in their gut walls than in the microbial extracts (Table 2-7). Aminopeptidase activities increased in activity moving distally along the intestine in the wood-eating taxa, and spiked in the mid intestine of *Pt. disjunctivus* (Table 2-7). No significant differences were observed in the gut wall aminopeptidase activities of the proximal and mid intestine among the species. However, the three wood-eating species possessed significantly higher aminopeptidase activities in the gut walls of their distal intestines than in this region of *Pt. disjunctivus* (ANOVA $F_{3,22}$: 5.17, P = 0.009; Tukey's P <0.011).

β-xylosidase activity (not shown) was low and only observed in the microbial extracts of the four taxa. The activity of this enzyme, like xylanase, generally decreased in activity moving distally along the intestine.

Trypsin activities significantly decreased moving distally along the intestines of all four species (Figure 2-7). *Panaque nocturnus* possessed significantly greater trypsin activity in their proximal intestine than all of the other species, whereas the two remaining wood-eating species (*P. cf. n.* "Marañon" and *H. pyrineusi*) possessed similar and significantly greater trypsin activity in their proximal intestines than in *Pt. disjunctivus* (Figure 2-7). No differences were detected among the species in their mid or distal intestines. Lipase activities (not shown) followed similar patterns to trypsin and decreased moving distally along the intestines of the fish. The trypsin:lipase activity ratios for all fish exceeded 400:1.

Enzymatic activities of the hepatopancreas and liver (not shown) varied by enzyme. No cellulase or xylanase activities were detected in the hepatpancreas or liver of any species, whereas amylase, laminarinase, trypsin, and lipase were all detected in the hepatopancreas of the fish. Only amylase and lipase were detectable in the liver.

Gastrointestinal Fermentation and Luminal Carbohydrate Profiles

Overall, the concentrations of SCFAs were low in the digestive tracts of all four species of catfish (Table 2-8). Only *H. pyrineusi* showed any significant change in SCFA concentration along the gut, with significantly higher SCFA concentrations in the mid intestine than in the proximal intestine. The trends of SCFA concentrations varied among species, with *Pt. disjunctivus* showing an increasing concentration of SCFAs along the gut, and *P. cf. n.* "Marañon" showing a decrease, albeit no significant change (Table 2-8). The TLC plates (not shown) revealed that all four species had soluble oligo-, di- and monosaccharides in the proximal intestine, and that these concentrations decreased until there were no soluble sugars remaining in the distal intestine. Similarly, measurable glucose was observed in the fluid of the proximal intestine of *P. cf. n.* "Marañon" (2.70 ± 0.29 mM) and *P. nocturnus* (2.86 ± 0.38 mM), but these concentrations disappeared in the mid and distal intestine. Only *H. pyrineusi* showed measurable

glucose in all regions of the intestine and these concentrations decreased, significantly so (ANOVA: $F_{2,14} = 84.75$, $P < 0.001$), from the proximal (4.98 ± 0.43 mM) to the mid (0.93 ± 0.09 mM) to the distal (0.73 ± 0.03 mM) intestine. No glucose was detected in the fluid of any gut region of *P. disjunctivus*.

Fiber Digestibility and Gut Transit

The wood I offered to the fish in the laboratory was almost entirely organic matter (97%), and moderately rich in lingocellulose (~60%; Table 2-9). Because I observed no feeding selectivity on specific types of wood in the wild in Perú, degraded wood of any riparian tree seemed appropriate for this part of the study. Neither *P. nigrolineatus* nor *Pt. disjunctivus* readily assimilated large proportions of the dry or organic matter, or of the fiber types of the water oak (Table 2-10). However, because the organic matter digestibilities are "apparent digestibilities" (because the fish contribute organic waste, like sloughed intestinal cells, to the feces) and the fiber digestibilities represent "true digestibilities" (because the fish contribute no fiber to the feces) it is difficult to match the calculations for the digestibilities for the two fractions to each other. Nevertheless, the digestibilties for NDF and ADF each contributed approximately the same amount of overall organic matter digestion (Table 2-10). For example, 22% digestibility of NDF that composes 86% of the total organic matter equals a total digestibility of 20%. Similarly, 24% digestibility of ADF that composes 61% of the total equals a total digestibility of 15%. Thus, most of the fiber digestibility can be accounted for via ADF and lignin digestion. Furthermore, the lignin:cellulose ratio (Abril and Bucher 2002) of the feces (0.90 ± 0.16) was not significantly greater than the lignin:cellulose ratio of the wood (0.81 ± 0.08; $t = 0.49$, $P = 0.63$, d.f. = 20), suggesting that the fish were unable to assimilate significant amounts of cellulose from the wood diet.

The digestibility data are further confounded by the presence of proportionally more ash in the feces than in the wood (12% ash in the feces *vs.* 3% ash in the wood, overall). On a daily basis two to five times more ash was found in the feces than was consumed with the wood in the first place. This addition of "inorganic" material to the feces is mysterious and may have artificially inflated the levels of fiber digestibility observed in this study.

The fish consumed 2-5% of their body mass (on a wet weight basis) in wood per day, but were not thriving on it, as *P. nigrolineatus* lost 1.8 ± 0.15 % of their body mass over the course of the experiment, and *Pt. disjunctivus* lost 8.4 ± 0.81 % of their body mass. This stands in contrast to a 41% mass gain by *Pt. disjunctivus* on an algal diet in the laboratory (DPG, unpubl. data). Furthermore, *P. nigrolineatus* and *Pt. disjunctivus* excreted more nitrogen in their feces than they consumed in the wood (Table 2-10), and this excretion was significantly greater for *Pt. disjunctivus* than for *P. nigrolineatus*.

Wood traversed the digestive tracts of the two species extremely quickly, with red stained wood appearing in feces less than four hours after its consumption. Furthermore, there appeared to be no retention of the stained wood along the gut of *P. nigrolineatus* (Figure 2-8). The proportion of particles (stained or non-stained) <250 μm in diameter in each region of the intestine at each time interval were as follows (mean ± SEM; n=3-4): 4 hours – PI: 47.59 ± 1.66, MI: 25.44 ± 1.50, DI 22.53 ± 2.77; 6 hours – PI: 44.09 ± 3.61, MI: 22.33 ± 2.33, DI 17.26 ± 2.66; 8 hours – PI: 45.21 ± 7.74, MI: 28.60 ± 4.28, DI 23.31 ± 3.63; 18 hours – DI: 19.05 ± 1.08 (digesta was only present in the DI at the 18 hour interval). Thus, no selective retention of small particles was observed in this study. Additionally, the overall analysis of particle size in the guts of *P. nigrolineatus* revealed that small, more digestible particles were not retained anywhere along the gut, with particles >350 μm making up more of the total moving distally along the

intestine (Table 2-11). However, the results could be interpreted to show selective retention of larger particles (>350 μm in diameter) in the mid and distal intestine.

Discussion

The data gathered in this study overwhelming support the null hypothesis that the "xylivorous" loricariid catfishes are actually detritivores and do not digest the fibrous components of wood in their alimentary tracts. Each of the analyses provided evidence that the fish do not exhibit specialized gut anatomy for harboring endosymbionts: no kinks, valves, or caeca are present anywhere along their long, narrow intestines; the microvilli surface area decreases moving distally along the intestine, indicating that most absorption takes place in the proximal and mid intestine, which was corroborated by the luminal carbohydrate profiles; no conglomerations of microbes were observed in the TEM micrographs; the fish clearly have enzymatic activities geared for the assimilation of soluble components of their diet; they lack significant amounts of gastrointestinal fermentation anywhere within their digestive tracts; they pass wood through the gut too quickly (< four hours) for microbial digestion of cellulose; and the fish do not retain small particles anywhere along their digestive tract. Furthermore, the catfish were unable to digest wood and thrive on it in the laboratory. Each of these components would be expected to be the opposite in an animal that digests wood via an endosymbiotic community of microbes living in their guts. However, loricariid catfishes certainly have interesting digestive tracts (the longest among all fishes measured to date; Horn 1989; Kramer and Bryant 1995) and subsist on detritus in the wild, which they do appear suited to digest (Bowen et al. 1995; German 2008; German et al. 2008).

There has been some debate over the last 15 years as to whether xylivorous catfishes can digest wood. Schaefer and Stewart (1993) suggested that species in the genus *Panaque* "could

be capable of extracting energy from wood" and this assertion has been assumed to be true ever since, especially on the internet and among aquarium fish enthusiasts. However, the one study published to date examining digestion in species of *Panaque* and *Pterygoplichthys* provided only inferential evidence of cellulose digestion (Nelson et al. 1999). The data gathered in the current study systematically refute that wood-eating species in the genera *Panaque* or *Hypostomus*, nor the detritivorous *Pt. disjunctivus*, have the capability to digest and subsist on wood.

Two of the key factors contributing to this inability are the lack of specialized gut anatomy and rapid gut transit. The loricariid catfishes examined in this study clearly have long, narrow, anatomically unspecialized intestines, and no conglomerations of microbes were observed in the fishes' guts. However, unlike terrestrial herbivores and xylivores, a specialized gut anatomy is not a prerequisite for fishes to harbor an endosymbiont community in their guts. Many herbivorous fishes with active endosymbiotic communities and high levels of SCFA production and assimilation (Mountfort et al. 2002) have anatomically unspecialized digestive tracts (e.g., *Odax pullus* and *O. cyanomelas*; Clements and Choat 1995; Clements and Raubenheimer 2006). *Odax pullus* and *O. cyanomelas* do, however, possess voluminous guts, and *O. pullus* has relatively long retention of digesta in the alimentary tract (12-20 hours), mainly because of low gut contractility (Clements and Rees 1998). This low contractility is due to the very thin musculature surrounding the intestine of this species (Clements and Rees 1998), and also may result in significant amounts of axial mixing of digesta in the gut, an important component of microbially-mediated digestion (Horn 1989; Horn and Messer 1992; Karasov and Martínez del Rio 2007). Xylivorous termites have extremely long digesta transit considering their size (~24 hours; Breznak and Brune 1994), porcupines consuming wood have mean retention times of food in the gut exceeding 34 hours (Felicetti et al. 2000), and beavers selectively retain small particles

and fluid in their hindgut caeca (Vispo and Hume 1995). Both long retention time of digesta in the gut, and selective retention of small particles allow resident microbes to remain in the gut and digest cellulose in these taxa. The species of *Panaque*, *Pterygoplichthys*, and *Hypostomus* investigated in this study have relatively thick musculature surrounding their long intestines (Figure 2-3; especially when compared to *O. pullus*; Clements and Rees 1998), have rapid gut transit, and no selective retention of small particles, suggesting more of a unidirectional, "plug-flow" movement of digesta with little axial mixing along the intestine (Penry and Jumars 1987; Horn and Messer 1992; Jumars 2000). Furthermore, the peristaltic contractions of the intestine of *P. nocturnus* are strong and continue after the death of the animal (view video at: http://www.zoology.ufl.edu/dgerman/images/Peristalsis1.AVI). Thus, the combination of long, narrow intestines, rapid gut transit, and strong contractility do not support an active microbial community in the catfishes' digestive tracts. The decreasing intestinal surface area observed in the catfishes is also consistent with other detritivorous fishes (Frierson and Foltz 1992), which typically rely more on endogenous digestive mechanisms than on microbial endosymbionts (Bowen 1984; Smith et al. 1996; Smoot and Findlay 2000; Crossman et al. 2005; German 2008).

Small particles from wood may not always be "higher quality" than larger particles. For example, in ruminant mammals, small, more indigestible particles escape the rumen following digestion, whereas larger, more digestible particles are retained in the rumen (Van Soest 1994). Hence, the reduction of the proportion of small particles in the mid and distal intestine of the fish in this study could indicate a rapid movement of small indigestible particles (perhaps rich in lignin) through their guts, and retention of larger particles in the mid and distal intestine. However, the predominance of small particles in the proximal intestine, which seemingly disappear in the mid and distal intestine, is confounded by endogenous inputs of bile and

digestive enzymes in the proximal intestine in comparison to other gut regions. Endogenous enzyme activities, like those of amylase, laminarinase, and trypsin are all higher in the proximal intestine (Figures 2-4 and 2-7), and these digestive secretions, along with bile, are all part of the "small" particle fraction (<250 μm diameter) of the proximal intestine. The disappearance of this fraction in the mid intestine may simply reflect the reabsorption of bile and endogenous digestive enzymes, which all decrease in activity moving towards the distal intestine. Additionally, the disappearance of smaller particles from the proximal intestine could indicate digestion and assimilation of nutrients from smaller wood degradation products from the degraded wood.

The low wood fiber assimilation efficiencies in the catfishes are highly indicative that they cannot subsist on a wood only diet. Other xylivorous animals that have an active microbial community in their guts are capable of digesting the fibrous cell wall fraction of wood (Breznak and Brune 1994; Felicetti et al. 2000; Karasov and Martínez del Rio 2007). For example, porcupines assimilate about 70% of NDF from wood with similar biochemical composition to that offered to the fish in this study (Felicetti et al. 2000). *Panaque nigrolineatus* and *Pt. disjunctivus* did clearly assimilate some cellulose from wood [given the small increase in the lignin:cellulose ratio (Abril and Bucher 2002) in the feces compared to the wood], and potentially some lignin, although the latter may be an artifact of the detergent fiber analysis system (Jung 1997), which was designed for grasses (Goering and Van Soest 1970), not wood. The small change in the fecal lignin:cellulose ratio in the fish pales in comparison to the change in this ratio observed in termite feces (at least a 50% increase in the ratio in feces relative to wood; Karasov and Martínez del Rio 2007). Furthermore, the low DM and OM digestibilities, and net loss of N combined with a loss of weight while eating wood further show that *P.*

nigrolineatus and *Pt. disjunctivus* cannot thrive on a wood diet. Individuals of *Pt. disjunctivus* likely did worse from weight and fecal nitrogen loss perspectives than individuals of *P. nigrolineatus* beause the former lack the spoon-shaped teeth (Nelson et al. 1999) necessary to gouge wood in significant quantities (i.e., they had lower daily intake rates of wood). This is also supported by the observation that it took six weeks for *Pt. disjunctivus* to produce amounts of feces that *P. nigrolineatus* produced in four weeks.

To my knowledge, no studies have evaluated the digestibility of a diet in an animal that lost weight over the course of the experiment, leaving me with no basis for comparison to the current study. Although nitrogen loss in feces has been observed for rats on a high-fiber, low-protein diet (Jørgensen et al. 2003), there are no observations of mineral (ash) loss in feces. However, there are three possible ways for more ash to be recovered from fishes' feces than from the food they consumed. First, the fish actually deposited minerals into the feces, thereby increasing the ash content. Second, a significant proportion of the fecal organic matter was consumed by microbes over the 12 hours the feces sat in the aquaria. And third, I grossly underestimated the intake of wood by fish in this study. In terms of the first scenario, it is possible that the fish excreted minerals into their feces. Gonzales and Brown (2007) observed that Nile tilapia (*Oreochromis niloticus*) lost body mineral content on low-quality foods that insufficiently met the fishes' daily energetic needs. Perhaps the same pattern was occurring in this study. The loricariid catfishes are characterized as having dermal plates composed of calcium phosphate (bone) on their skin (Armbruster 2004). Just as turtles are known to mobilize the calcium carbonate from their shells to buffer lactate accumulation during periods of anoxia (Jackson 2002; Reese et al. 2004), perhaps the fish in this study, which were in negative energy balance, mobilized the phosphate from their boney plates for ATP production, and excreted the

excess calcium in their feces. The second scenario listed above is not likely, as the amount of ash in the feces is two to five times greater than that of the wood the fish consumed, and a microbial population could not consume that much organic matter in just 12 hours. For example, Galetto and Bellwood (1994) observed little change in fecal organic matter after fish feces sat in an aquarium for 24 hours at 25°C. The third scenario is also not likely because the fish would have produced more "orts" on a daily basis if they had consumed more wood; the loricariids produce a large proportion of debris as they graze on wood (i.e., they are messy eaters), and if intake was higher, then there would have been more debris in the aquaria on a daily basis than was observed. Thus, scenario one above needs to be further investigated to determine whether the fish added inorganic material to their feces. This can be done by determining the concentration of calcium in the feces of the fish relative to the wood they consumed. Furthermore, the mass and calcium content of the dermal plates, as well as circulating blood calcium concentrations can be compared in fed and starved fish to assess whether more calcium was being mobilized in fish in negative energy balance.

The pH levels and redox potentials in the catfish intestines indicated the possibility of supporting a population of anaerobic microbes, but only in the intestine. Many loricariids breathe air and have modified stomachs that qualify as air breathing organs (ABOs; Graham and Baird 1982; Armbruster 1998), which explains why the redox potentials of the stomachs of the fish in this study were positive (*Pt. disjunctivus*) or only slightly negative (*P. cf. n.* "Marañon" and *P. nocturnus*). However, the loricariid stomach is not involved in digestion. For example, the stomach of *Pt. disjunctivus* is usually filled with air, is alkaline, and ingesta are not held in the stomach for any length of time; even individuals of this species killed minutes after consuming food had already passed the ingesta into the proximal intestine, bypassing the

stomach via a small groove at its base (DPG, pers. obs.). Redox potentials measured 1-mm beyond the pyloric sphincter in this study were already -600 mV, indicating that even the most proximal region of the intestine is not oxygenated by the fish's breathing activity. Similar to some detritivorous termites (Kappler and Brune 2002), detritivorous fishes potentially consume large proportions of humic acids, which, in addition to other components of the intestinal fluid (e.g., bile salts, and ingested metals; Kappler and Brune 2002) can increase the reductive potential, thus producing negative redox conditions. Either way, the redox potentials measured in wild-caught fish in this study suggest that the intestinal environment is highly reductive. The digestion of lignin, which composes roughly 18-35% of woody material (Petterson 1984), requires oxidative (positive redox potential) conditions (Zimmer and Brune 2005). Seeing that the stomach is the only oxidative site along the catfish gut, and food is not digested there, I do not see how it is possible for these fishes, via microbial endosymbionts, to digest lignin. That is, unless, similar to termites (Ebert and Brune 1997) and terrestrial isopods (Zimmer and Brune 2005), the catfish possess a radial oxygen gradient in the intestinal lumen that I was unable to detect. However, termites and isopods are extremely small, terrestrial organisms with high surface area:volume ratios in comparison to the loricariid catfishes, so I do not think this is likely.

The low concentrations of SCFAs observed in the fishes' intestines further challenge the hypothesis that wood-eating catfishes use an endosymbiotic community to ferment recalcitrant polysaccharides. Choat and Clements (1998) suggested that fish with less than 20 mM total SCFAs in the "peak" fermentative region of the intestine had low fermentation potential. The highest "peak" concentrations observed in this study were in the mid intestine of *H. pyrineusi* (3.20 ± 0.79 mM), and were far below 20 mM. Furthermore, *H. pyrineusi* was the only species

to show any significant difference in SCFA concentrations along its gut. The low SCFA concentrations coupled with the lack of any localization of SCFAs in one gut region are consistent with other herbivorous and detritivorous fishes that do not rely on gastrointestinal fermentation to digest refractory polysaccharides (Smith et al. 1996; Crossman et al. 2005; German 2008; German et al. 2008). Additionally, the loricariid catfishes are consuming detrital material that is already being degraded by microbes in the environment (Sinsabaugh et al. 1991b; Sinsabaugh et al. 1992; Tank et al. 1998; Hendel and Marxsen 2000). Some of this detritus may already be in the process of fermentative digestion when consumed by the fish (i.e., the food is prefermented), and thus, SCFAs themselves are consumed with the detritus producing the low and unchanging SCFA concentrations along the gut (German 2008).

Perhaps the most informative biochemical data gathered in this study suggesting a lack of microbially-mediated digestion in the catfishes comes from the patterns of digestive enzyme activities along the fishes' intestines. A common pattern in lower termites, which digest cellulose in their hindgut via an endosymbiotic microbial community, is increasing cellulase activities in the hindgut region (Nakashima et al. 2002; Mo et al. 2004). Similarly, marine herbivorous fishes with active hindgut microbial populations have increasing exogenously produced enzyme activities (e.g., carrageenase) in the microbial extracts of their hindguts (Skea et al. 2005). However, none of the catfish species showed increasing cellulase activity in the distal intestine, and instead, showed no pattern (no increase or decrease; *P. cf. n.* "Marañon" and *P. nocturnus*) or decreasing activity (*Pt. disjunctivus* and *H. pyrineusi*) towards the distal intestine. Moreover, the cellulase activities in the catfish guts were five orders of magnitude lower than amylase activities, and one to two orders lower than laminarinase activities. Thus, the

fish clearly digest soluble polysaccharides, like starch and laminarin, more rapidly than structural polysaccharides, especially given the rapid transit time of food through the gut.

Decaying wood in an aquatic environment will likely have more nutritious dietary items collecting on the surface of the wood, and in spaces among fibers, than the wood it self. The epilithic algal complex (EAC), which is a loose assemblage of bacteria, cyanobacteria, filamentous green algae, diatoms, and detritus that grows on hard substrates in aquatic systems (Hoagland et al. 1982; van Dam et al. 2002; Klock et al. 2007; German et al. 2008) contains soluble polysaccharides in the algae (Painter 1983) and in exopolymeric substances produced by microbes (Wotton 2004; Klock et al. 2007). These soluble polysaccharides are likely an important energy source, not only to grazing species like *Pt. disjunctivus*, but also to the "xylivorous" species digging into the decaying wood. Other EAC consuming fishes (e.g., species in the genus *Campostoma*) have very similar patterns and magnitudes of amylase and laminarinase activities to the catfishes (German 2008; German et al. 2008), suggesting that they are targeting similar suites of nutrients from their foods.

The xylanase activities in *P. nocturnus* were the only luminal enzyme activities to be different between the intestinal fluid and the microbial extract, and the activities of this enzyme slightly increased in activity, albeit not significantly so, towards the distal intestine of this species. Xylan is a component of hemicellulose (Petterson 1984; Breznak and Brune 1994), but mammals (and probably vertebrates in general) are not known to possess an endogenous xylanase, nor to be able to metabolize the monomer of xylan, xylose, without the aid of intestinal microorganisms (Johnson et al. 2006a; Johnson et al. 2006b). Additionally, all of the catfish species lacked a β-xylosidase in their gut walls and had low activities of this enzyme in their intestinal contents that decreased moving distally along the digestive tract.

Given the low and variable cellulase and xylanase activities observed in the catfish, and the lack of any consistent pattern of activity along the guts of the fish, it is likely that these enzymes are ingested (and produced by microbes ingested) with detritus rather than produced by a resident endosymbiotic community, *per se*. This is especially true in *Pt. disjunctivus* and *H. pyrineusi*, which showed decreasing cellulase and xylanase activities moving distally along their guts. Furthermore, cellulase and xylanase activities were not higher in the xylivorous catfish species. For example, detritivorous *Pt. disjunctivus* possessed the highest cellulase activity in its proximal intestine, and xylivorous *P. nocturnus* the lowest.

The cellulase activities measured in this study are three orders of magnitude lower than those reported for *Panaque maccus* and *Pterygoplichthys* sp. by Nelson et al. (1999). However, there are several methodological differences between this study and that performed by Nelson and colleagues. First, they used assay conditions designed for ruminant mammals (pH 5, 40°C), which differ wildly from any conditions occurring in the fishes' guts. I designed my assay conditions to reflect the fishes gut pH (pH = 7.5; Table 2-3) and ambient temperatures of their environment (25°C). Second, Nelson et al. (1999) did not specify in which region of the gut they measured the enzyme activities. Third, when performing a general reducing sugar assay for polysaccharidase activity that includes intestinal contents (as was done by Nelson and colleagues, and in this study), it is essential to perform the appropriate blanks to account not only for background reducing sugars in the gut, but also for additional substrate that may be a source of other reducing sugars released during the assay (Skea et al. 2005; German et al. 2008). Not doing so will result in an over-estimation of activity levels, and Nelson and colleagues did not perform this type of blank with their assays (J.A. Nelson, pers. comm.). And fourth, the activities were likely calculated differently between the two studies. All of these reasons (and

more) have led several authors (Peres et al. 1998; Logothetis et al. 2001; Chan et al. 2004; German et al. 2004; Horn et al. 2006) to caution against making comparisons of digestive enzyme activities among different studies. Thus, I will not do so here, referring only to similarities in patterns of digestive enzyme activities along the intestines of different animals.

The most striking digestive enzyme activity data suggesting that the catfishes digest mainly soluble components from their detrital diet comes from the disaccharidase activities. The Michaelis-Menten constants (K_m) for β-glucosidase in the gut walls of the fishes were an order of magnitude lower than those of the microbial extracts (Table 2-6). Although the β-glucosidase activities were higher in the microbial extracts than in the gut walls of the proximal intestines of *P. cf. n.* "Marañon", *P. nocturnus*, and *Pt. disjunctivus*, this may be outweighed by the more efficient (lower K_m) gut wall β-glucosidase of the fish. *Hypostomus pyrineusi* had the double effect of lower K_m and higher activity of β-glucosidase in its proximal intestine gut wall. These results are important because microbes degrading the cellulose of wood in the river excrete enzymes extracellularly (Sinsabaugh et al. 1992; Hendel and Marxsen 2000) and depend on di- and monosaccharides [such as cellobiose (a β-glucoside) and glucose, respectively] to diffuse back to them that they can then further digest and assimilate (Allison and Jastrow 2006). The fishes are consuming wood detritus that is in this process of degradation, and thus, there are likely many soluble components, like cellobiose, on the decaying wood (Sinsabaugh et al. 1992; Hendel and Marxsen 2000). Because the fishes' β-glucosidases are more efficient than those produced by the microbes degrading the wood, the fish quickly digest and assimilate the cellobiose in their detrital diet. Additionally, because microbes in the environment secrete digestive enzymes extracellularly, the enzymes themselves are also likely on the detritus (Sinsabaugh et al. 1992; Hendel and Marxsen 2000), as occurs in soils (Allison 2006; Allison

and Jastrow 2006), and are thus digested within the guts of the fish. This may explain why the microbial extract enzyme activities, almost without exception (Tables 2-4 and 2-7; Figures 2-5 and 2-6), tend to decrease moving distally along the intestines of the fish. This is especially true for β-glucosidase and stands in stark contrast with patterns in lower termites, as β-glucosidase activities increase in the hindguts of these taxa (McEwen et al. 1980). However, decreasing β-glucosidase activity moving distally along the gut has been observed in other detritivorous fishes (Smoot and Findlay 2000).

The more efficient and higher N-acetyl-β-D-glucosaminidase (NAG) activity in the fish gut walls may indicate that chitin, and its degradation products (i.e., chitobiose), are important energy and nitrogen sources to the fish. Fungi, which make cell walls of chitin, are some of the most active microorganisms in wood degradation and digestion (Swift et al. 1979; Breznak and Brune 1994; Hendel and Marxsen 2000), and are likely consumed by the fish with wood detritus. I attempted to measure chitinase activity in the guts of the catfishes, but there was so much background N-acetyl-glucosamine – which is the monomer of chitin and the endpoint of chitin digestion – in the fishes' guts (>1 mM), that the determination of chitinase activity was impossible using colorimetric methods. This stands in contrast to other fishes that consume chitinous arthropods, in which chitinase activity was readily measured (Gutowska et al. 2004; German et al. 2008). N-acetyl-glucosamine is a usable energy source for vertebrate animals (Gutowska et al. 2004), and the presence of such large amounts of this compound in the intestines of the fish suggests that chitin digestion is proceeding rapidly, and thus, fungi may be an important dietary item of the fishes. Indeed, microbes in general may be an important nutrient source to the catfishes, which would make lysozyme an important enzyme for nutrient acquisition in these animals (Krogdahl et al. 2005; Karasov and Martínez del Rio 2007).

Lysozyme is important not only in bacterial cell wall degradation, but also for the degradation of chitin (Marsh et al. 2001; Krogdahl et al. 2005) in fungal cell walls. Thus, future studies of digestion in loricariid catfishes should take lysozyme activity into account, and should explore non-colorimetric methods for the determination of chitinase activity (e.g., release of ^{14}C; Marsh et al. 2001).

Some differences in disaccharidase activities were observed among the catfish species. *Hypostomus pyrineusi* possessed higher microbial extract and gut wall maltase activities than the other species. Because I did not collect enough individuals of this species on which to conduct a thorough analysis of gut contents, it is difficult speculate why they have higher maltase activities than the other species. However, cursory observations of their gut contents did reveal the presence of a large proportion of wood. *Pterygoplichthys disjunctivus* increases its maltase activity when consuming a diet rich in soluble polysaccharides in the laboratory (DPG, unpubl.), which might suggest that *H. pyrineusi* consumes wood with more EAC, and thus, more soluble polysaccharides on it than the other wood-eating species. The presence of measurable glucose in the intestinal fluids in all gut regions in this species, and glucose concentrations in the fluid of the proximal intestine that were double those of the other xylivores further suggest that they consume more soluble polysaccharides than the other taxa. This is offered with the caveat that all four species had abundant soluble oligo- to disaccharides in the fluid of their proximal intestines (as determined with TLC plates) that disappeared by the distal intestine.

Most of the catfish species showed significantly higher β-mannosidase activities in their gut walls than in the microbial extracts in all regions of the intestine. I am not sure what these activities mean for the fish, as I am aware of only one study that examined β-mannosidase activity in other fish taxa: Nelson et al. (1999) found detectable activity of this enzyme in *P.*

maccus and *Pterygoplichthys* sp. Most analyses of mannan, the products of mannan degradation, and the enzymes involved in mannan digestion, have been aimed at bacteria and fungi (Valaskova and Baldrian 2006; Moreira and Filho 2008). Mannan, and the monomer, mannose, are major components of hemicelluloses in wood (Petterson 1984; Moreira and Filho 2008). Thus, an animal digesting wood, or woody detritus, should possess the enzymes to digest this compound. None of the catfish species possessed any activity against the polysaccharide mannan. However, the detectable activity of β-mannosidase in the gut walls of the fish suggest that, like β-glucosidase and cellobiose, the fish may be able to digest the soluble component of mannan degradation, namely, β-mannosides. This may provide yet another example of how the fish are geared for assimilating the more soluble components of their detrital diet.

Many animals, including herbivorous and detritivorous fishes, feed to meet protein requirements (Bowen et al. 1995; Raubenheimer and Simpson 1998; Raubenheimer et al. 2005). The increasing aminopeptidase activities in the distal intestines of the catfishes likely reflect increased efforts by the fish to absorb whatever protein is available in their detrital diet (Fraisse et al. 1981; Harpaz and Uni 1999; German 2008), especially given the decreasing microvilli surface area of the distal intestine. Furthermore, the trypsin activities in the loricariid catfishes are the highest I have measured in a number of fish taxa using identical methodology (German et al. 2004; Horn et al. 2006; German 2008; German et al. 2008). And, as one final piece of information supporting detritivory in these fishes, the extremely high trypsin:lipase ratios (exceeding 400:1) are consistent with other detritivorous animals in marine (Mayer et al. 1997) and freshwater (Smoot and Findlay 2000) habitats.

In conclusion, loricariid catfishes in the genera *Panaque* and *Hypostomus* appear to be little more than detritivores that specialize on a rather ubiquitous form of coarse detritus in their

environment, namely, wood. The digestive tracts of these fishes, and of a closely related non-wood-eating detritivore, *Pt. disjunctivus*, are clearly geared for the consumption of large amounts of low-quality food, and rapid transit of this food through the gut. Enzyme activities in their alimentary tracts hydrolyze soluble components of detritus more efficiently than structural polysaccharides, and the majority of this hydrolysis takes place in the proximal and mid intestine. These patterns match well with the higher microvilli surface area in these regions of the gut. Additionally, even though the guts of these animals are highly reductive and hospitable for anaerobic microbes, the low SCFA concentrations throughout the fishes' intestines show that they are not relying on microbial symbionts to digest structural polysaccharides via fermentative pathways. Further investigations in these fishes should emphasize their ability to digest bacteria and fungi, as these organisms may be likely food sources for the fish. Nonetheless, loricariid catfishes are impressive organisms that are highly abundant in the Amazonian basin, and the wood-eating species likely contribute heavily to nutrient cycling in these habitats by reducing the particle size of wood from coarse debris to particles on the scale of 1-mm in diameter.

Table 2-1. Digestive enzymes assayed in this study of gut structure and function in wood-eating loricariid catfishes.

Enzyme	Location[1]	Substrate	Substrate source	Fractions assayed[2]
Amylase	Lum., cont.	Starch	Algae, detritus	Fluid, contents, HP, Liver
Laminarinase	Lum., cont.	Laminarin	Diatoms	Fluid, contents, HP, Liver
Cellulase	Lum., cont.	Cellulose	Wood, algae, detritus	Fluid, contents, HP, Liver
Xylanase	Lum., cont.	Xylan	Wood, detritus	Fluid, contents, HP, Liver
Mannanase	Lum., cont.	Mannan	Wood, detritus	Fluid, contents, HP, Liver
Chitinase	Lum., cont.	Chitin	Fungi, insects, detritus	Fluid, contents, HP, Liver
Trypsin	Lum., cont.	Protein	Algae, detritus, animals	Fluid, contents, HP, Liver
Lipase	Lum., cont.	Lipid	Algae, detritus, animals	Fluid, contents, HP, Liver
Maltase	BB, cont.	Maltose	Algae, detritus	Contents, gut wall
β-glucosidase	BB, cont.	β-glucosides	Algae, wood, detritus	Contents, gut wall
β-xylosidase	BB, cont.	β-xylosides	Wood, detritus	Contents, gut wall
β-mannosidase	BB, cont.	β-mannosides	Wood, detritus	Contents, gut wall
N-acetyl-β-D-glucosaminidase	BB, cont.	N-acetyl-β-D-glucoaminides	Fungi, insects, detritus	Contents, gut wall
Aminopeptidase	BB, cont.	Dipeptides	Algae, detritus, animals	Contents, gut wall

Notes. [1] Indicates where the enzyme is active. Lum = lumen of the intestine; cont. = contents (ingesta) of the intestine; BB = brushborder of the intestine. [2] The portions of gut content or intestinal tissue in which I assayed the activity of the enzyme.

Table 2-2. Interspecific comparisons of body mass (BM), relative gut length (RGL), gut length as a function of snout-vent length (GL/SVL), and Zihler's index (ZI) in three species of loricariid catfishes.

Species (sample size)	Diet	SL	BM	RGL	GL/SVL	ZI
P. cf. nigrolineatus "Marañon" (11)	W	87.65 ± 9.99	29.59 ± 8.83[a]	11.56 ± 0.50[a]	18.97 ± 0.96[a]	36.71 ± 1.58[a]
P. nocturnus (17)	W	102.24 ± 3.62	32.69 ± 3.79[a]	11.47 ± 0.41[a]	18.44 ± 0.62[a]	37.53 ± 1.28[a]
Pt. disjunctivus (17)	D	203.94 ± 8.23	196.83 ± 23.01[b]	17.24 ± 0.55[b]	27.19 ± 0.92[b]	61.93 ± 1.90[b]
Species			$F_{2,44} = 28.03$	$F_{2,44} = 55.58$	$F_{2,44} = 12.58$	$F_{2,44} = 28.01$
			$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Body Mass			--	$F_{1,41} = 1.42$	$F_{1,41} = 0.72$	$F_{1,41} = 1.21$
			--	$P = 0.240$	$P = 0.401$	$P = 0.279$

Note: Values are mean (± SEM). Abbreviations for diet are as follows: W = wood; D = detritus. Interspecific comparisons of BM were made with ANOVA followed by a Tukey's HSD with a family error rate of P=0.05. Interspecific comparisons of gut dimension parameters were analyzed with ANCOVA (using body mass as a covariate) and Tukey's HSD with a family error rate of P=0.05. Values for a parameter that share a superscript letter are not significantly different.

Table 2-3. pH and redox conditions in four regions of the gut of *Panaque nocturnus*, *P. cf. nigrolineatus* "Marañon", and *Pterygoplichthys disjunctivus*.

Species	Stomach	Proximal Intestine	Mid Intestine	Distal Intestine
		pH		
P. cf. n. "Marañon"	7.42 ± 0.04	7.51 ± 0.11	7.48 ± 0.03	7.50 ± 0.04
P. nocturnus	8.31 ± 0.17	8.29 ± 0.14	8.28 ± 0.14	8.28 ± 0.14
Pt. disjunctivus	7.12 ± 0.15	7.48 ± 0.15	7.28 ± 0.09	7.47 ± 0.12
		Redox (mV)		
P. cf. n. "Marañon"	-59.38 ± 6.33	-605.47 ± 2.87	-611.52 ± 5.54	-616.74 ± 6.51
P. nocturnus	-45.72 ± 5.81	-605.81 ± 8.91	-616.73 ± 9.11	-608.96 ± 8.91
Pt. disjunctivus	105.90 ± 67.80	-595.40 ± 4.16	-596.23 ± 4.04	-599.63 ± 6.39

Note: Values are mean (± SEM).

Table 2-4. Amylase, laminarinase, cellulase, and xylanase activities ($U \cdot g^{-1}$) in the intestinal fluid and microbial extracts of *Panaque cf. nigrolineatus* "Marañon" (*Pm*), *P. nocturnus* (*Pn*), *Pterygoplichthys disjunctivus* (*Ptd*), and *Hypostomus pyrineusi* (*Hp*).

	Amylase			Laminarinase		
Species (n)	Proximal intestine	Mid intestine	Distal intestine	Proximal intestine	Mid intestine	Distal intestine
Pm (6)						
Intestinal Fluid	884.68 ± 114.63	196.94 ± 74.76	214.03 ± 123.06	0.052 ± 0.015	0.015 ± 0.006	0.013 ± 0.006
Microbial Extract	859.09 ± 70.77	451.96 ± 129.06	78.20 ± 26.02	0.125 ± 0.035	0.022 ± 0.009	0.008 ± 0.002
t	0.21	1.71	1.08	1.96	0.66	1.16
P	0.84	0.12	0.31	0.08	0.53	0.27
Pn (6)						
Intestinal Fluid	323.38 ± 40.14	146.59 ± 30.84	88.46 ± 27.17	0.262 ± 0.040	0.093 ± 0.037	n.d.
Microbial Extract	308.31 ± 41.29	142.86 ± 29.53	36.53 ± 8.80	0.222 ± 0.036	0.137 ± 0.027	n.d.
t	0.26	0.09	2.18	0.73	0.96	N/A
P	0.80	0.93	0.06	0.48	0.38	
Ptd (10)						
Intestinal Fluid	581.64 ± 148.54	468.74 ± 68.70	90.59 ± 15.79	0.276 ± 0.050	0.123 ± 0.018	0.018 ± 0.006
Microbial Extract	615.83 ± 150.35	473.46 ± 54.66	90.78 ± 18.80	0.340 ± 0.091	0.128 ± 0.019	0.023 ± 0.010
t	0.16	0.05	0.01	0.62	0.19	0.44
P	0.87	0.96	0.99	0.55	0.85	0.67
Hp (5)						
Intestinal Fluid	804.53 ±120.12	522.44 ± 122.85	105.41 ± 33.79	0.107 ± 0.049	0.006 ± 0.002	n.d.
Microbial Extract	1012.67 ±184.21	533.74 ± 98.99	85.54 ± 18.85	0.083 ± 0.047	0.005 ± 0.001	n.d.
t	0.95	0.07	0.51	0.34	0.68	N/A
P	0.37	0.95	0.62	0.74	0.51	

Table 2-4 (continued)

	Cellulase			Xylanase		
Species (n)	Proximal intestine	Mid intestine	Distal intestine	Proximal intestine	Mid intestine	Distal intestine
Pm (6)						
Intestinal Fluid	0.0034 ± 0.0020	0.0013 ± 0.0003	0.0042 ± 0.0029	0.0051 ± 0.0007	0.0010 ± 0.0002	n.d.
Microbial Extract	0.0034 ± 0.0015	0.0040 ± 0.0014	0.0016 ± 0.0005	0.0071 ± 0.0023	0.0031 ± 0.0014	0.0008 ± 0.0001
t	0.04	1.86	0.89	0.81	1.43	N/A
P	0.97	0.12	0.40	0.44	0.18	
Pn (6)						
Intestinal Fluid	0.0013 ± 0.0003	0.0028 ± 0.0001	0.0022 ± 0.0016	0.0001 ± 0.0001	0.0005 ± 0.0002	0.0024 ± 0.0013
Microbial Extract	0.0010 ± 0.0001	0.0012 ± 0.0002	0.0006 ± 0.0002	0.0004 ± 0.0001	0.0015 ± 0.0003	0.0007 ± 0.0003
t	0.85	1.56	1.22	3.33	3.32	1.61
P	0.41	0.15	0.25	<0.01	<0.01	0.14
Ptd (10)						
Intestinal Fluid	0.0055 ± 0.0010	0.0041 ± 0.0009	0.0002 ± 0.0001	0.0064 ± 0.0025	0.0062 ± 0.0025	n.d.
Microbial Extract	0.0091 ± 0.0023	0.0039 ± 0.0011	0.0005 ± 0.0001	0.0088 ± 0.0035	0.0052 ± 0.0018	0.0005 ± 0.0001
t	1.45	0.07	2.13	0.54	0.34	N/A
P	0.17	0.94	0.05	0.60	0.74	
Hp (5)						
Intestinal Fluid	0.0043 ± 0.0012	0.0007 ± 0.0004	n.d.	0.0037 ± 0.0007	n.d.	n.d.
Microbial Extract	0.0048 ± 0.0009	0.0003 ± 0.0001	n.d.	0.0024 ± 0.0014	0.0003 ± 0.0001	n.d.
t	0.31	0.98	N/A	0.81	N/A	N/A
P	0.76	0.36		0.44		

Note: Values are mean (± SEM). Comparisons were made of the activities of each enzyme between the intestinal fluid and microbial extract of each gut region in each species with *t*-test. Following a Bonferroni correction for each enzyme and species, differences are considered significant at P=0.017.

Table 2-5. Summary of ANOVA and *t*-test* statistics for interspecific comparisons of digestive enzyme activities for each of the proximal intestine (PI), mid intestine (MI), and distal intestine (DI), and intraspecific comparisons of digestive enzyme activities among gut regions.

Enzyme	Interspecific comparisons by gut region			Intraspecific comparisons (among gut regions) by species			
	PI	MI	DI	*P. cf. n.* "Marañon"	*P. nocturnus*	*Pt. disjunctivus*	*H. pyrineusi*
Amylase	$F_{3,26} = 4.87$	$F_{3,26} = 7.12$	$F_{3,26} = 0.93$	$F_{2,17} = 28.30$	$F_{2,17} = 23.34$	$F_{2,29} = 8.56$	$F_{2,14} = 51.68$
	$P = 0.009$	$P = 0.001$	$P = 0.444$	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$
Laminarinase	$F_{3,26} = 3.75$	$F_{3,26} = 12.61$	$t = 0.93$*	$F_{2,17} = 17.66$	$t = 2.68$*	$F_{2,29} = 13.02$	$t = 1.96$*
	$P = 0.025$	$P < 0.001$	$P = 0.368$	$P < 0.001$	$P = 0.023$	$P < 0.001$	$P = 0.086$
Cellulase	$F_{3,26} = 4.37$	$F_{3,26} = 4.08$	$F_{2,21} = 3.06$	$F_{2,17} = 0.20$	$F_{2,17} = 0.74$	$F_{2,29} = 11.11$	$t = 3.86$*
	$P = 0.015$	$P = 0.019$	$P = 0.072$	$P = 0.818$	$P = 0.492$	$P < 0.001$	$P = 0.018$
Xylanase	$F_{3,26} = 0.44$	$F_{3,26} = 0.92$	$F_{2,21} = 1.43$	$F_{2,17} = 6.56$	$F_{2,17} = 0.81$	$F_{2,29} = 3.04$	$t = 3.18$*
	$P = 0.726$	$P = 0.445$	$P = 0.265$	$P = 0.009$	$P = 0.463$	$P = 0.065$	$P = 0.013$
Trypsin	$F_{3,26} = 42.40$	$F_{3,26} = 2.45$	$F_{3,26} = 1.17$	$F_{2,17} = 23.59$	$F_{2,17} = 208.28$	$F_{2,29} = 9.03$	$F_{2,14} = 36.21$
	$P < 0.001$	$P = 0.090$	$P = 0.343$	$P = 0.009$	$P = 0.463$	$P = 0.001$	$P < 0.001$

Note: * If only two values were compared, *t*-test was used instead of ANOVA. For example, *P. nocturnus* and *H. pyrineusi* lacked laminarinase activity in their distal intestines, and thus, for the DI, laminarinase activities were only compared among *P. cf. n.* "Marañon" and *Pt. disjunctivus* with *t*-test. Similarly, intraspecific comparisons of laminarinase activities in *P. nocturnus* and *H. pyrineusi* were only made among the PI and MI with *t*-test. Sample sizes: *P. cf. nigrolineatus* "Marañon" n=6; *P. nocturnus* n=6; *Pt. disjunctivus* n=10; *H. pyrineusi* n=5.

Table 2-6. Michaelis-Menten constants (K_m) of disaccharidases in the gut walls and microbial extracts of the proximal intestines of *Panaque cf. nigrolineatus* "Marañon" (*Pm*), *P. nocturnus* (*Pn*), *Pterygoplichthys disjunctivus* (*Ptd*), and *Hypostomus pyrineusi* (*Hp*).

	Maltase			β-glucosidase			N-acetyl-β-D-glucosaminidase		
Species	Gut Wall	Microbial Extract		Gut Wall	Microbial Extract		Gut Wall	Microbial Extract	
Pm	1.84 ± 0.24	2.66 ± 0.39	*t* = 1.83 P = 0.097	0.041 ± 0.005	0.708 ± 0.042	*t* = 15.94 P < 0.001	0.075 ± 0.004	0.317 ± 0.063	*t* = 3.86 P = 0.003
Pn	2.85 ± 0.60	4.33 ± 0.31	*t* = 2.20 P = 0.053	0.026 ± 0.004	0.976 ± 0.069	*t* = 13.67 P < 0.001	0.146 ± 0.018	0.187 ± 0.015	*t* = 1.76 P = 0.108
Ptd	4.35 ± 0.25	5.47 ± 1.53	*t* = 0.56 P = 0.587	0.121 ± 0.018	1.175 ± 0.113	*t* = 7.12 P < 0.001	0.172 ± 0.012	0.979 ± 0.237	*t* = 3.40 P = 0.008
Hp	2.07 ± 0.19	2.09 ± 0.13	*t* = 0.10 P = 0.923	0.103 ± 0.025	1.391 ± 0.101	*t* = 12.36 P < 0.001	0.141 ± 0.027	0.470 ± 0.095	*t* = 3.34 P = 0.010

Note: values are mean (± SEM), and concentrations are in mM. Gut wall and microbial extract constants were compared with *t*-test for each species and enzyme, and after a Bonferroni correction, are considered significantly different at P = 0.013. Samples sizes were *Pm*: n=6; *Pn*: n=6; *Ptd*: n=6 (gut wall), n=10 (microbial extract); *Hp*: n=5.

Table 2-7. β-mannosidase and aminopeptidase activities ($U \cdot g^{-1}$) in the gut wall and microbial extracts of *Panaque cf. nigrolineatus* "Marañon" (*Pm*), *P. nocturnus* (*Pn*), *Pterygoplichthys disjunctivus* (*Ptd*), and *Hypostomus pyrineusi* (*Hp*).

	β-mannosidase			Aminopeptidase		
Species (n)	Proximal intestine	Mid intestine	Distal intestine	Proximal intestine	Mid intestine	Distal intestine
Pm (6)						
Gut Wall	0.418 ± 0.073	0.399 ± 0.129	1.133 ± 0.403	0.223 ± 0.027	0.421 ± 0.123	1.309 ± 0.361
Microbial Extract	0.078 ± 0.015	0.115 ± 0.045	0.141 ± 0.056	0.045 ± 0.015	0.069 ± 0.011	0.208 ± 0.053
t	4.57	2.08	2.44	5.75	2.86	3.02
P	0.006	0.065	0.035	<0.001	0.017	0.013
Pn (6)						
Gut Wall	1.816 ± 0.209	1.453 ± 0.294	0.455 ± 0.105	0.319 ± 0.057	0.923 ± 0.194	1.294 ± 0.236
Microbial Extract	0.041 ± 0.011	0.102 ± 0.019	0.024 ± 0.006	0.038 ± 0.003	0.078 ± 0.006	0.210 ± 0.025
t	8.48	4.57	4.10	4.93	4.53	4.56
P	<0.001	0.006	0.009	0.001	0.001	0.001
Ptd						
Gut Wall (6)	0.229 ± 0.101	0.240 ± 0.056	0.059 ± 0.019	0.358 ± 0.029	0.712 ± 0.089	0.217 ± 0.052
Microbial Extract (10)	0.162 ± 0.027	0.043 ± 0.008	n.d.	0.254 ± 0.045	0.262 ± 0.030	0.237 ± 0.053
t	0.79	3.49	N/A	1.64	5.78	0.25
P	0.441	0.018		0.123	<0.001	0.805
Hp (5)						
Gut Wall	0.630 ± 0.168	0.931 ± 0.213	0.157 ± 0.058	0.364 ± 0.059	0.973 ± 0.148	1.066 ± 0.318
Microbial Extract	0.134 ± 0.024	0.071 ± 0.021	0.236 ± 0.126	0.111 ± 0.0010	0.134 ± 0.029	0.173 ± 0.052
t	2.92	4.01	0.57	4.21	5.56	2.77
P	0.019	0.004	0.586	0.003	0.001	0.024

Note: Values are mean (± SEM). Comparisons were made between the activities of each enzyme from the gut wall and microbial extract of each gut region in each species with *t*-test. Following a Bonferroni correction for each enzyme and species, differences are considered significant at P=0.017.

Table 2-8. Total short chain fatty acid concentrations (mM) in three gut regions of *Hypostomus pyrineusi*, *Pterygoplichthys disjunctivus*, *Panaque cf. nigrolineatus* "Marañon", and *Panaque nocturnus*.

Gut Region	*P. cf. n.* "Marañon"	*P. nocturnus*	*Pt. disjunctivus*	*H. pyrineusi*	
Proximal	2.95 ± 0.65	1.50 ± 0.23	2.44 ± 0.41	1.00 ± 0.16[a]	
Middle	2.85 ± 1.40	1.94 ± 0.39	2.40 ± 0.44	3.20 ± 0.79[b]	
Distal	2.10 ± 0.33	1.65 ± 0.32	3.50 ± 0.68	2.01 ± 0.40[ab]	
	$F_{2,17} = 0.26$	$F_{2,17} = 0.48$	$F_{2,17} = 1.28$	$F_{2,14} = 4.55$	
	P = 0.77	P = 0.63	P = 0.31	P = 0.03	
Total	7.90 ± 1.95	5.10 ± 0.54	8.44 ± 1.17	6.21 ± 1.00	$F_{3,22} = 1.45$
					P = 0.26

Note. Values are mean (± SEM). Comparisons of SCFA concentrations among gut regions within a species, and for total SCFA concentration between species, were made with ANOVA, with differences considered significant at P = 0.05. If significant differences were detected with ANOVA, this was followed by a Tukey's HSD multiple comparison test with a family error rate of P = 0.05. Those values sharing a superscript letter are not significantly different. Samples sizes were as follows: *P. cf. n.* "Marañon", n=6; *P. nocturnus*, n=6; *Pt. disjunctivus*, n=10; *H. pyrineusi*, n=5. Acetate:Propionate:Butyrate ratios for total SCFAs were as follows: *P. cf. n.* "Marañon" = 62:23:15; *P. nocturnus* = 44:31:25; *Pt. disjunctivus* = 70:16:14; *H. pyrineusi* = 52:28:20.

Table 2-9. Nutritional composition of water oak (*Quercus nigra*) wood consumed by *Panaque nigrolineatus* and *Pterygoplichthys disjunctivus* in laboratory feeding trials.

Component	Percent of total
Dry Matter (DM)	92.16 ± 0.20
Organic Matter (OM)	97.19 ± 0.19
Nitrogen	0.13 ± 0.01
NDF (total fiber)	86.87 ± 3.88
ADF (lingocellulose)	61.16 ± 2.96
Acid detergent lignin	26.65 ± 1.57

Note: Values are mean (± SEM). n = 11.

Table 2-10. Digestibilities (%) of various fractions of wood consumed by *Panaque nigrolineatus* and *Pterygoplichthys disjunctivus* in laboratory feeding trials.

	Panaque nigrolineatus	*Pterygoplichthys disjunctivus*
Dry Matter (DM)	3.35 ± 0.39[b]	1.72 ± 0.41[a]
Organic Matter (OM)	11.37 ± 0.89	12.76 ± 1.52
Nitrogen	-114.93 ± 22.90[b]	-397.78 ± 86.50[a]
NDF (total fiber)	22.95 ± 2.40	31.54 ± 3.56
ADF (lignocellulose)	23.69 ± 2.55	32.08 ± 3.04
Acid detergent lignin	36.87 ± 2.62	32.92 ± 3.51

Note: Values are mean (± SEM). DM, OM, and nitrogen digestibilities are "apparent digestibilities" because of endogenous inputs from the fish, whereas digestibilities for NDF, ADF, and lignin are "true digestibilities" because the fish do not excrete any substances that are considered fibrous. Values were compared between the species for each digestibility coefficient with *t*-test (d.f. = 9), and following a Bonferroni correction, values are considered significantly different at P = 0.008. Values for a particular digestibility coefficient with different superscript letters are significantly different. *P. nigrolineatus*, n = 7; *Pt. disjunctivus*, n = 4.

Table 2-11. Particle sizes of intestinal contents presented as the percent of total contents for each of the proximal, mid, and distal intestine of *Panaque nigrolineatus*.

Region of Intestine	<250 μm	250-350 μm	351-700 μm	701-1000 μm	1001-1500 μm	≥1501 μm
Proximal	44.59 ± 4.97	10.73 ± 0.69	18.10 ± 1.76	9.26 ± 1.19	8.76 ± 1.58	8.56 ± 1.47
Mid	25.42 ± 3.37	13.47 ± 1.75	24.20 ± 2.69	10.17 ± 2.63	13.23 ± 1.50	13.51 ± 1.93
Distal	23.82 ± 2.64	8.56 ± 0.93	30.06 ± 3.51	10.21 ± 1.04	13.09 ± 2.23	14.25 ± 3.90

Note: Values are mean ± SEM. N=12 for proximal and mid intestine, N=14 for distal intestine.

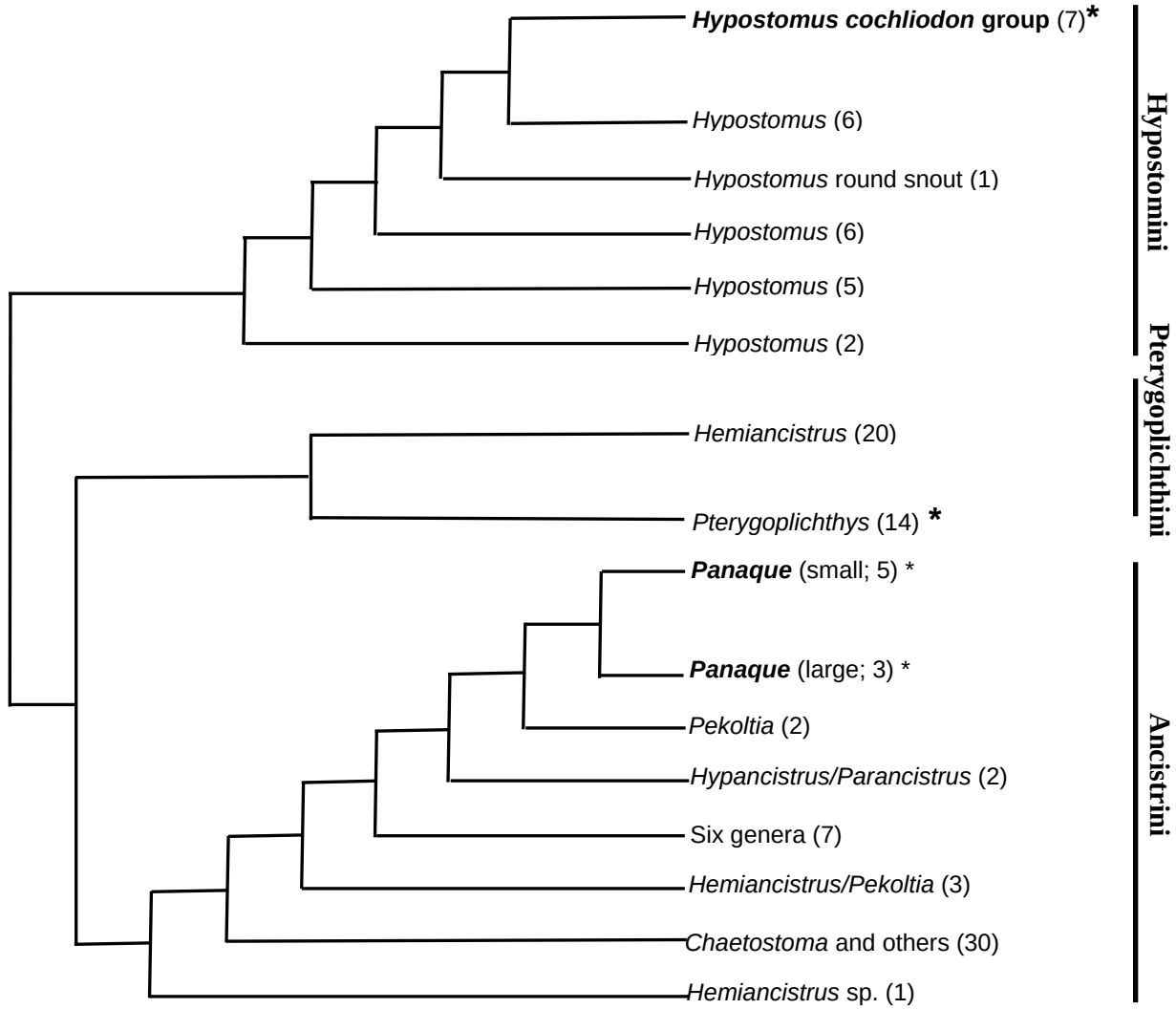

Figure 2-1. Partial phylogenetic hypothesis for three tribes in the catfish family Loricariidae. Phylogeny based on parsimony and summarized from Armbruster (2004). Genera in **bold** include wood-eating species, and the asterisks (*) indicate genera from which species were investigated in this study. Numbers in parentheses indicate approximate number of taxa not shown.

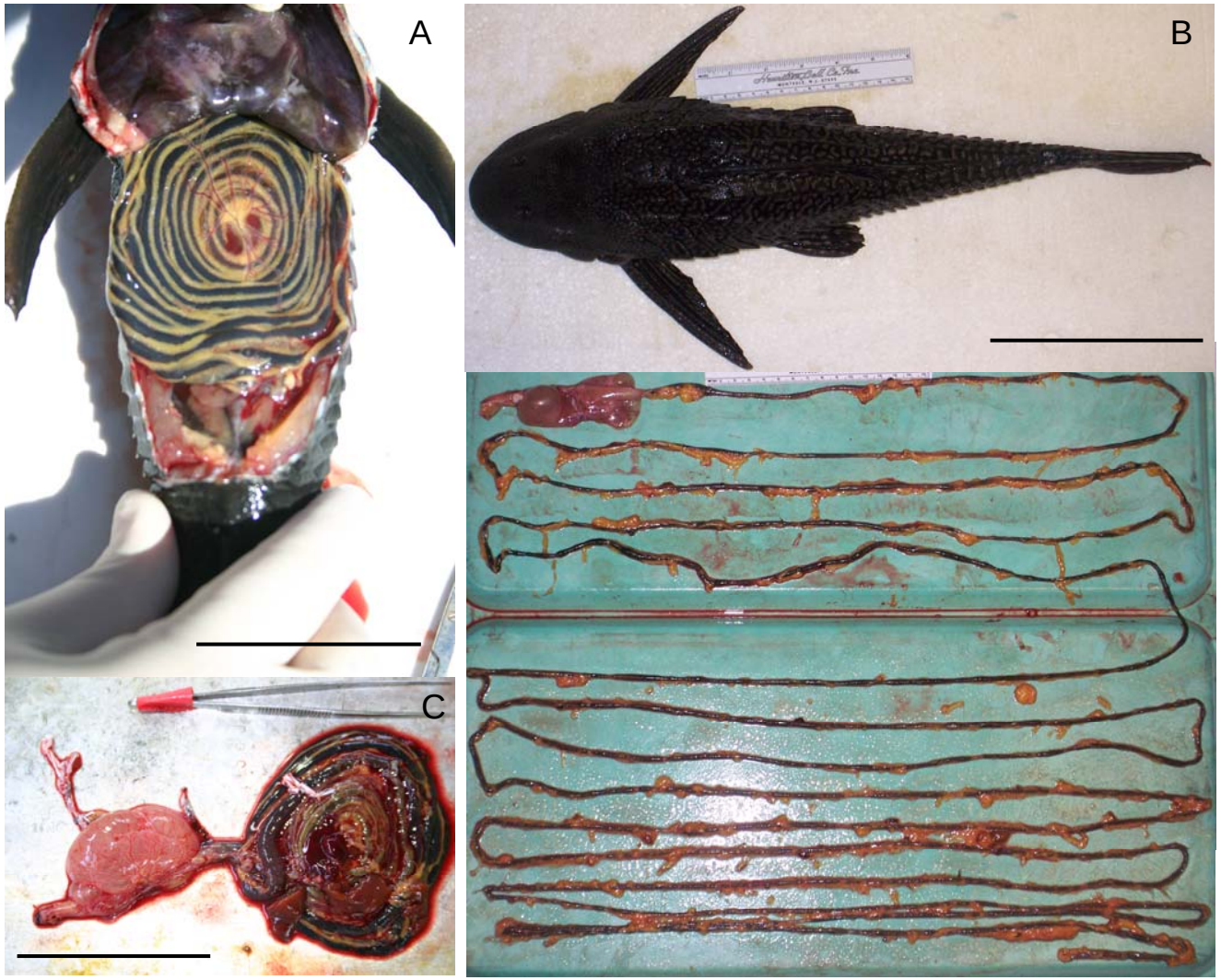

Figure 2-2. Photographs of the digestive tract of *Pterygoplichthys disjunctivus*: coiled within the body cavity (A; scale bar = 75 mm), uncoiled beneath the body of the fish (B; scale bar = 150 mm), and freshly removed from the body, coiled (C; scale bar = 75 mm).

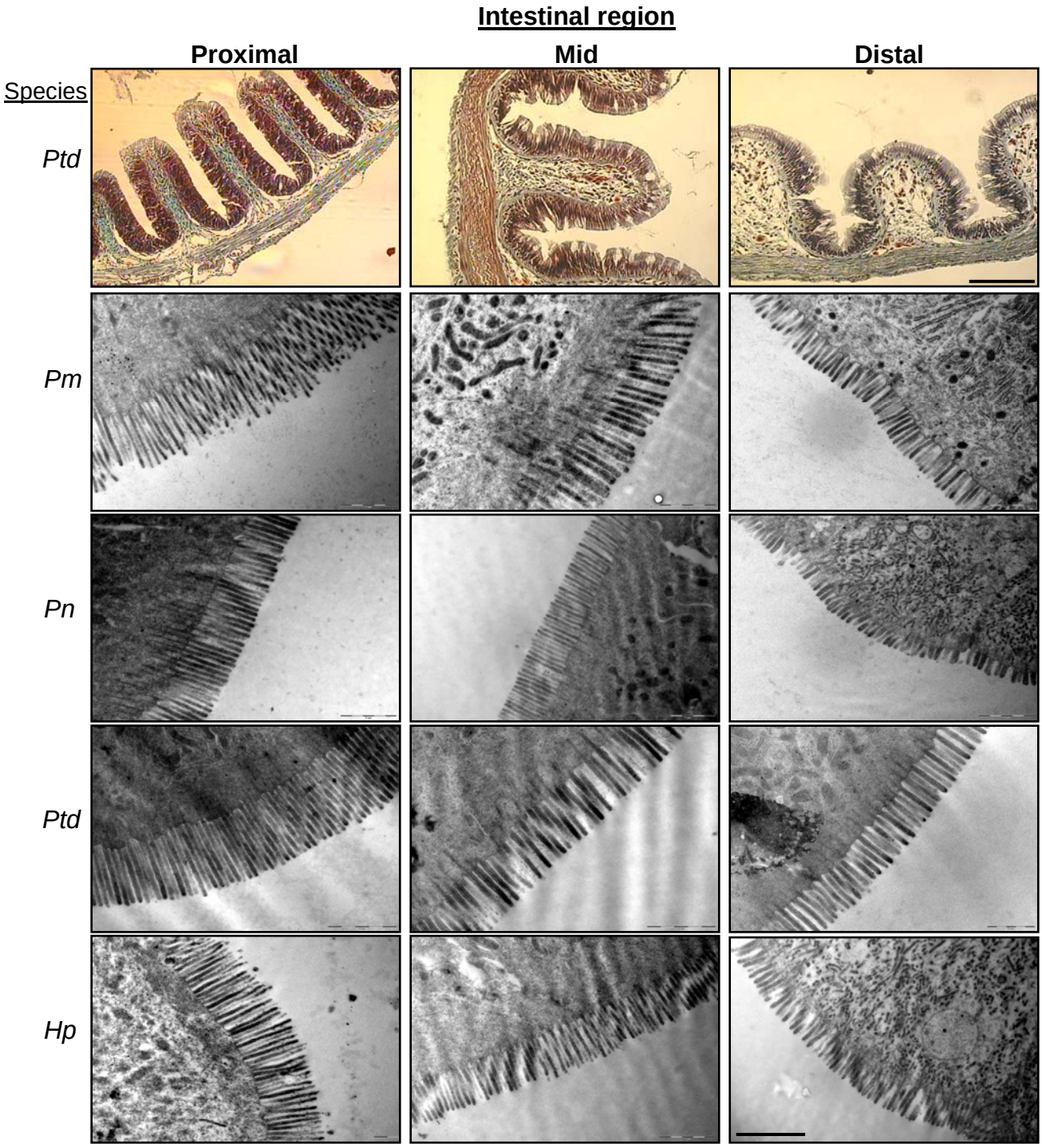

Figure 2-3. Histological images and transmission electron microscope (TEM) micrographs of the proximal, mid, and distal intestine of *Pterygoplichthys disjunctivus* (*Ptd*), *Panaque cf. nigrolineatus* "Marañon" (*Pm*), *P. nocturnus* (*Pn*), and *Hypostomus pyrineusi* (*Hp*). Scale bar for histology = 65 μm, scale bar for TEM = 1 μm.

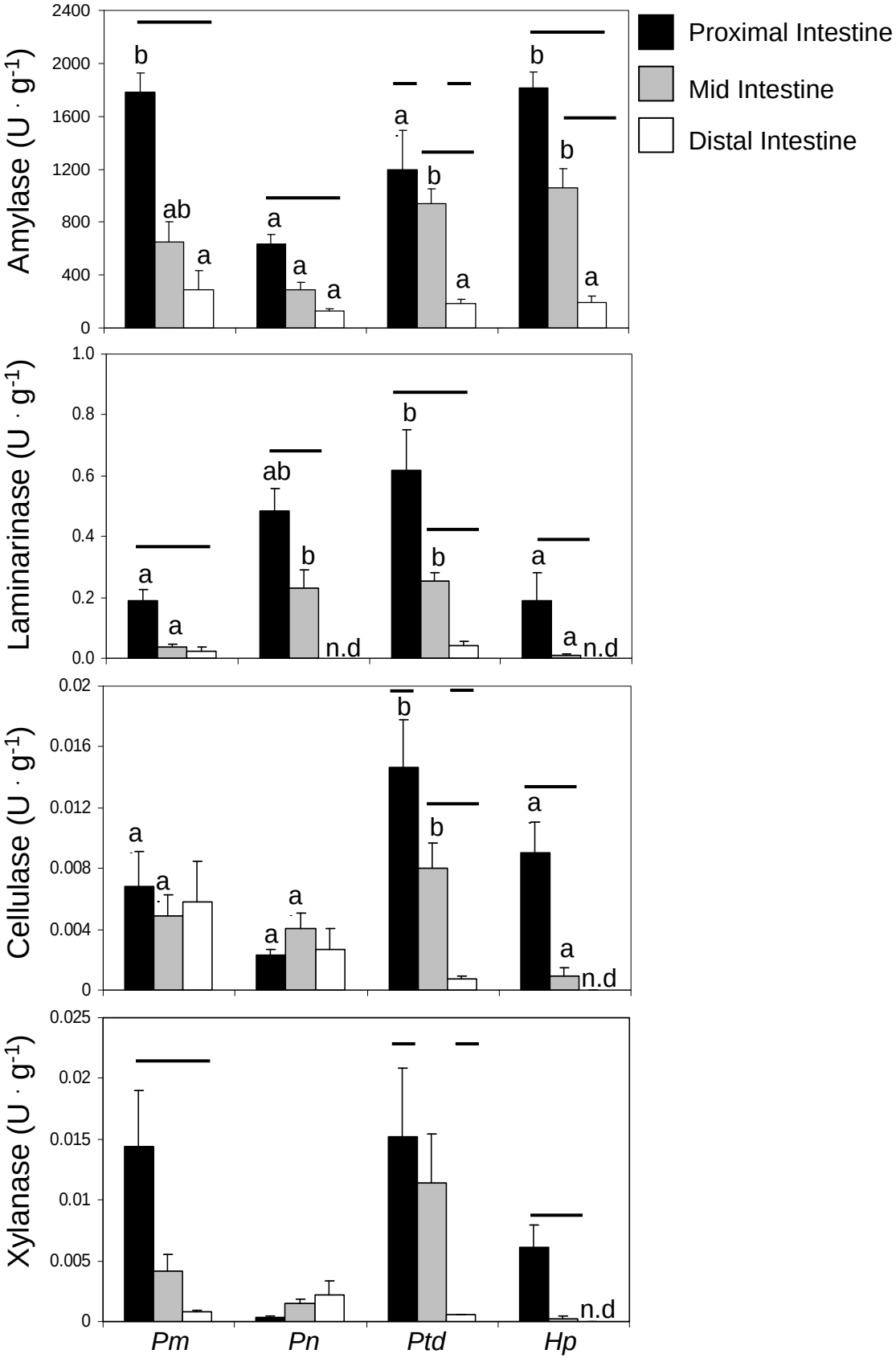
Proximal Intestine
Mid Intestine
Distal Intestine
Amylase (U · g-1)
Laminarinase (U · g-1)
Cellulase (U · g-1)
Xylanase (U · g-1)
2400
2000
1600
1200
800
400
0
1.0
0.8
0.6
0.4
0.2
0.0
0.02
0.016
0.012
0.008
0.004
0.025
0.02
0.015
0.01
0.005
n.d
Pm
Pn
Ptd
Hp

Figure 2-4. Total activities (intestinal fluid + microbial extract) of amylase, laminarinase, cellulase, and xylanase in three regions of the intestine of *Panaque cf. nigrolineatus* "Marañon" (*Pm*), *P. nocturnus* (*Pn*), *Pterygoplichthys disjunctivus* (*Ptd*), and *Hypostomus pyrineusi* (*Hp*). Values are means and error bars represent SEM. Interspecific comparisons of each enzyme activity in each gut region were made with ANOVA followed by a Tukey's HSD with a family error rate of $P = 0.05$. Bars of a specific color and for a specific enzyme sharing a letter are not significantly different. Intraspecific 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 specific elevation passing over two or more bars indicate a significant difference in enzyme activity ($P < 0.01$) among those gut regions.

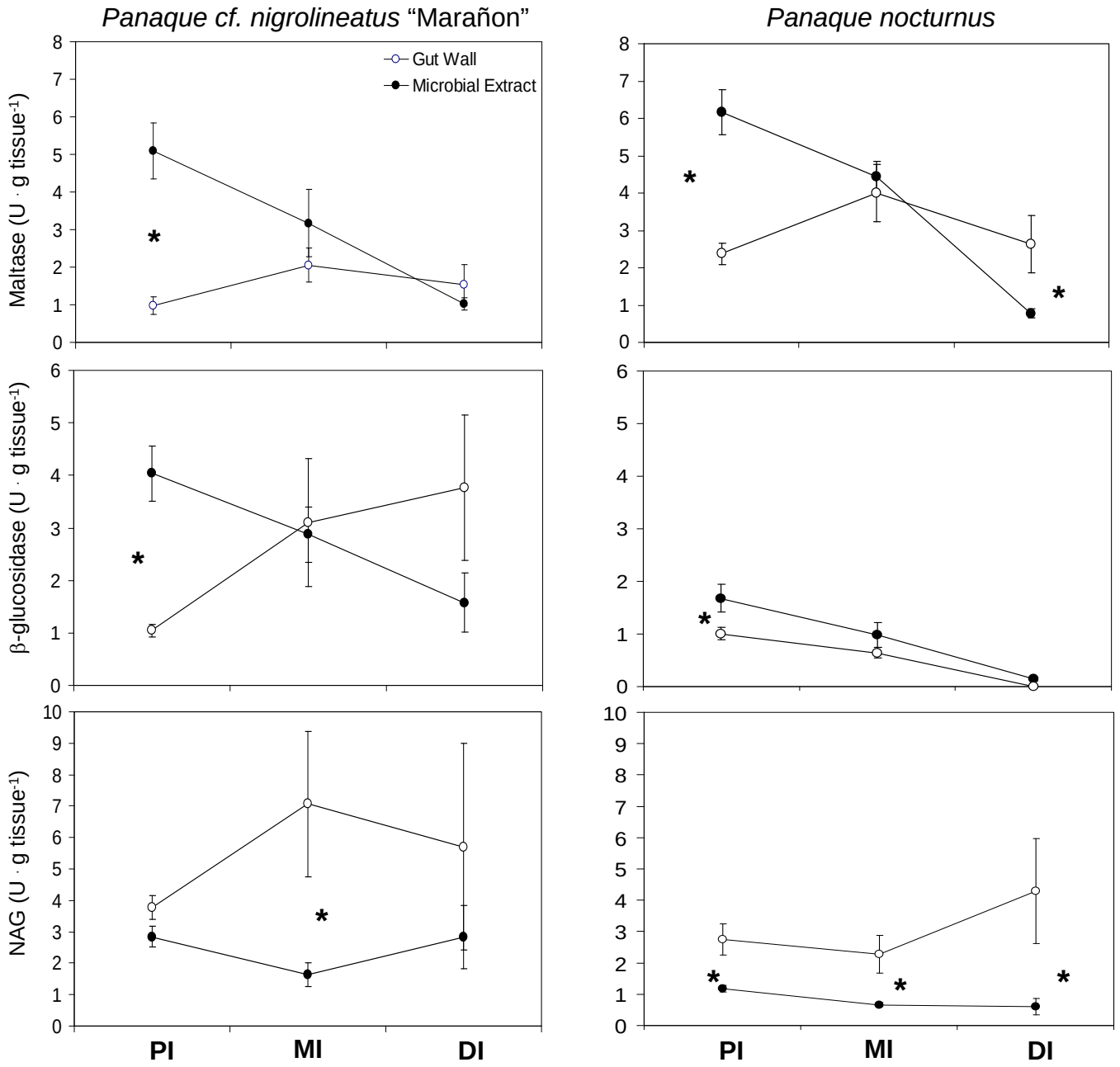
Panaque cf. nigrolineatus "Marañon"
Panaque nocturnus
Gut Wall
Microbial Extract
Maltase (U · g tissue-1)
β-glucosidase (U · g tissue-1)
NAG (U · g tissue-1)
PI
MI
DI

Figure 2-5. Maltase, β-glucosidase, and N-acetyl-β-D-glucosaminidase (NAG) activities in the gut walls and microbial extracts of the proximal intestine (PI), mid intestine (MI), and distal intestine (DI) of *Panaque cf. nigrolineatus* "Marañon" (left column) and *P. nocturnus* (right column). Comparisons were made of the activities of each enzyme between the gut walls and microbial extracts of each gut region with *t*-test. Following a Bonferroni correction for each enzyme and species, differences are considered significant at P=0.013 [indicated with an asterisk (*)].

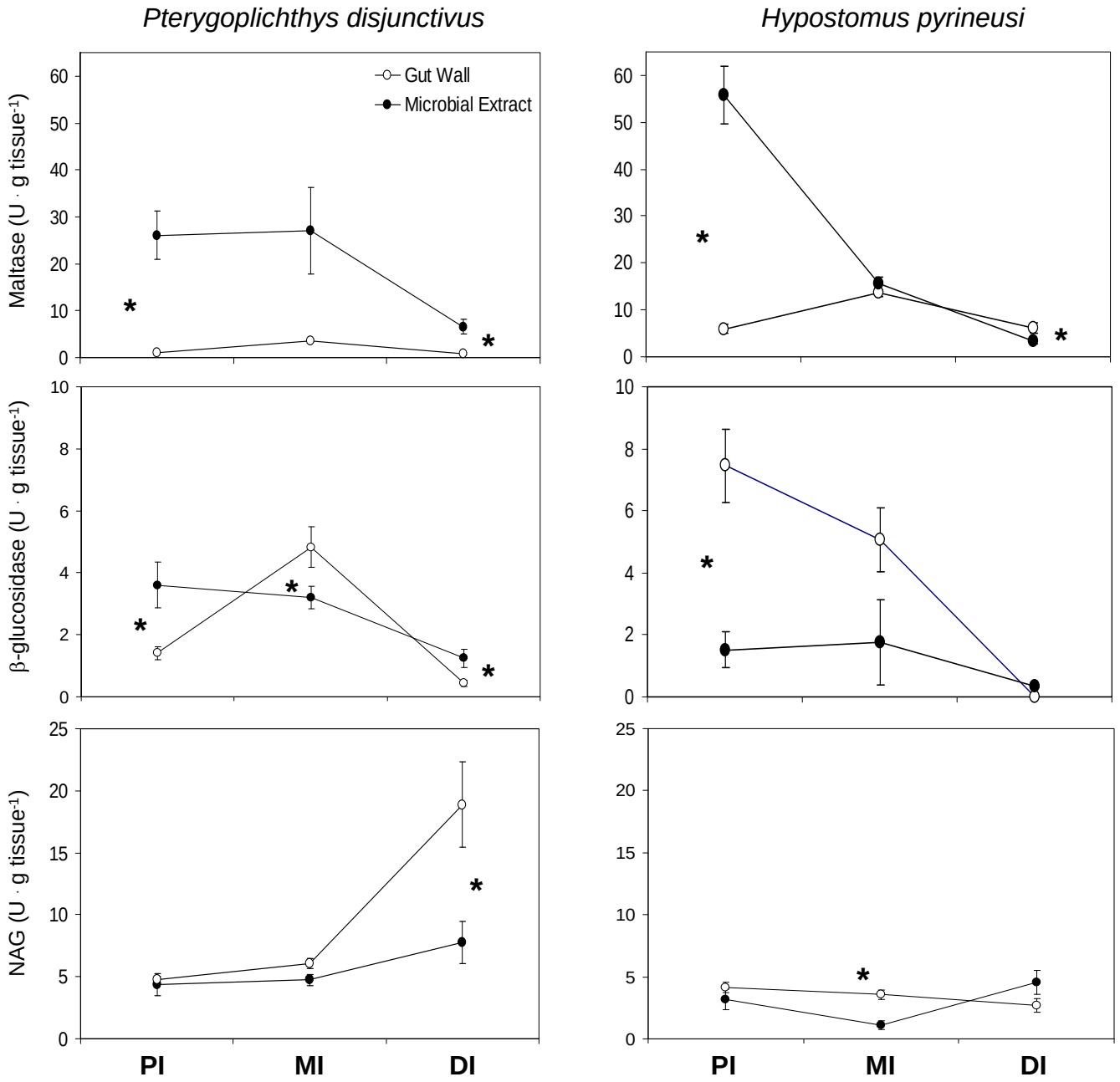
Pterygoplichthys disjunctivus
Hypostomus pyrineusi
Gut Wall
Microbial Extract
Maltase (U · g tissue-1)
β-glucosidase (U · g tissue-1)
NAG (U · g tissue-1)
PI
MI
DI

Figure 2-6. Maltase, β-glucosidase, and N-acetyl-β-D-glucosaminidase activities in the gut walls and microbial extracts of the proximal intestine (PI), mid intestine (MI), and distal intestine (DI) of *Pterygoplichthys disjunctivus* (left column) and *Hypostomus pyrineusi* (right column). Comparisons were made of the activities of each enzyme between the gut walls and microbial extracts of each gut region in each species with *t*-test. Following a Bonferroni correction for each enzyme and species, differences are considered significant at P=0.013 [indicated with an asterisk (*)].

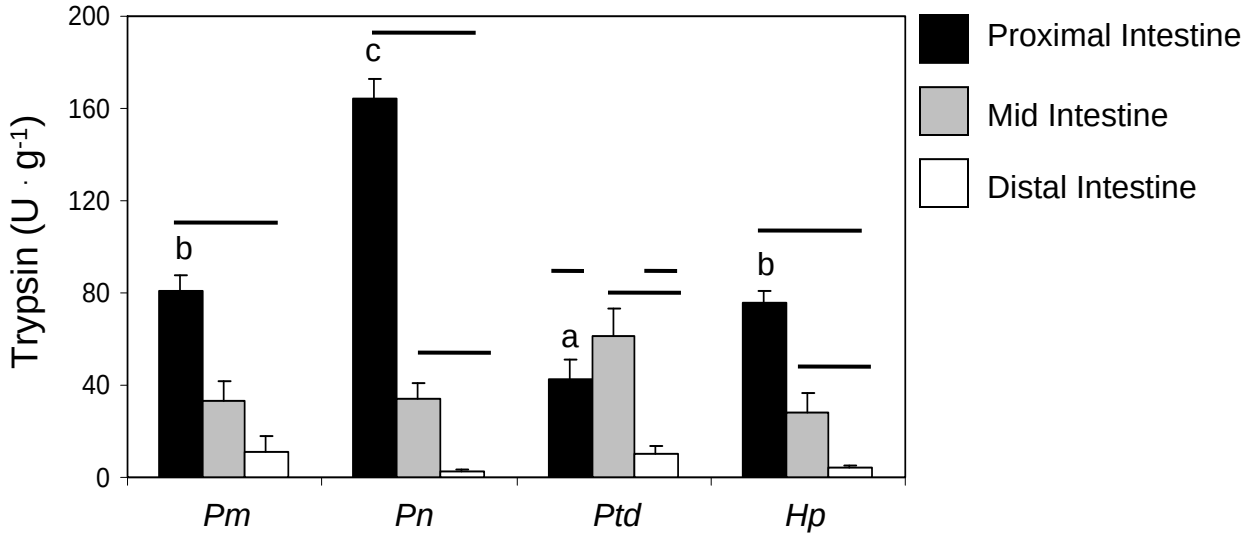

Figure 2-7. Total activities (intestinal fluid + microbial extract) of trypsin in three regions of the intestine of *Panaque cf. nigrolineatus* "Marañon" (*Pm*), *P. nocturnus* (*Pn*), *Pterygoplichthys disjunctivus* (*Ptd*), and *Hypostomus pyrineusi* (*Hp*). Values are means and error bars represent SEM. Comparative data as in Figure 2-4.

Figure 2-8. Position of stained wood in the intestine of *Panaque nigrolineatus* at different time intervals following its consumption. Fish were allowed to graze on the wood for four hours (pulse), at which time the stained wood was taken away from the fish and replaced with non-stained wood so the fish could continue to feed. Four individual fish were sacrificed and the position of stained wood in the gut observed at four, six, eight, and 18 hours following commencement of feeding on the stained wood. No stained wood remained in the intestine at 18 hours post feeding. Stained wood was already present in feces at the four-hour interval, indicating that transit time is less than four hours.

CHAPTER 3
CAN WOOD-EATING CATFISHES ASSIMILATE NUTRIENTS AND ENERGY FROM WOOD? INSIGHTS FROM STABLE ISOTOPES IN THE LABORATORY AND IN THE FIELD

Introduction

Plants and animals are made primarily of carbon, nitrogen, hydrogen, and oxygen. Each of these elements exists in different forms that vary in mass because of different numbers of neutrons in their nuclei (Fry 2007). These different forms of the atoms with different masses are called isotopes, and are denoted by their mass (e.g., ^{13}C has an atomic mass of 13, whereas ^{12}C had an atomic mass of 12). In the atmosphere, the proportional amounts of heavy and light isotopes of carbon, nitrogen, and oxygen are relatively constant. However, plants and animals use these isotopes in different ways resulting in the organisms having varying ratios of heavy and light isotopes relative to one another. For example, many monocot plants, like grasses, use the C_4 photosynthetic pathway, which results in tissues with more of the heavy isotope of carbon (i.e., ^{13}C) than dicot plants, which use the C_3 photosynthetic pathway (Fry 2007). From an isotopic perspective, an animal truly "is what it eats" because the atoms they use to synthesize new tissues come directly from their diet. Thus, the ratios of heavy and light isotopes of different elements in an animal's diet (e.g., $^{13}C/^{12}C$ and $^{14}N/^{15}N$) can be used to trace what foods these animals actually digest, assimilate, and use to make new tissue.

When attempting to discern an animal's dietary habits using stable isotopes it is critical to recognize that the animal's isotopic signature may be similar to that of their diet (DeNiro and Epstein 1978; DeNiro and Epstein 1981), plus or minus some difference, commonly referred to as an isotopic discrimination factor ($\Delta X_{tissue\text{-}diet}$; Reich et al. 2008). The isotopic discrimination factor between an animal's tissue and their diet is typically caused by three mechanisms: first, isotopic memory, which is the observation that the isotopic signature of an animal's tissues do

not immediately match that of the new diet following a dietary switch, and instead follow some temporal dynamics of isotopic incorporation (Fry and Arnold 1982; Phillips and Eldridge 2006); second, metabolic fractionation, which is the isotopic difference between reactants and products in biochemical reactions (Fry 2007); and third, isotopic routing, which is the shuttling of nutrients into pools by nutrient class (e.g., absorbed amino acids enter the amino acid pool, absorbed fatty acids enter the fatty acid pool, etc.; Martínez del Rio and Wolf 2005), as opposed to homogenous mixing of atoms. Each of these factors can produce differences in the isotopic signatures among the nutrient fractions of a single tissue (e.g., lipids tend to be depleted in $\delta^{13}C$; Fry 2007), and produce isotopic differences among tissues in a single animal, especially when compared to that animal's diet. Thus, when using stable isotopes to analyze the dietary history of an animal in the wild, expected discrimination factors for targeted tissue types should be determined in the laboratory for that animal beforehand (Gannes et al. 1997; Reich et al. 2008).

Of course, the advantage of using stable isotopic analyses is that they provide an important dietary reference point integrated over some time period, unlike gut content analyses, which provide only a snapshot view of diet (Fry 2007). However, the length of time it takes for a tissue to "turn over" and equilibrate with the isotopic signature of food is a necessary piece of information in stable isotopic investigations. The turnover time varies with tissue type (Tieszen et al. 1983; Hobson and Clark 1992; Martínez del Rio and Wolf 2005), with dietary quality (Gaye-Siessegger et al. 2003; Gaye-Siessegger et al. 2004), and with growth rate (Hesslein et al. 1993; MacAvoy et al. 2001; Sakano et al. 2005; Trueman et al. 2005; Reich et al. 2008). Therefore, without knowledge of expected discrimination factors and turnover times, it is difficult to discern the dietary history of an animal with stable isotopes and to know the time frame at which you are operating (Parker et al. 2008; Reich et al. 2008). That is, does a tissue

take days, weeks, months, or years to turn over and match the isotopic composition of a diet, and just how large is the discrimination when turnover is reached?

Although the number of studies of fish ecology using stable isotopic analyses has expanded in recent years, few have attempted to accurately determine the turnover times and discrimination factors of tissues in the laboratory (Hesslein et al. 1993; MacAvoy et al. 2001; Jardine et al. 2004; Sakano et al. 2005; Guelinckx et al. 2007) before gathering tissues and dietary items from the field (e.g., Ho et al. 2007). The results of several studies with fish suggest that discrimination factors commonly used in the literature to reflect trophic shifts (e.g., 1‰ for $\delta^{13}C$, 3.4‰ for $\delta^{15}N$; Fry and Sherr 1984; Focken 2004; Fry 2007) are not correct for many species, or dietary items (Hesslein et al. 1993; Jardine et al. 2004; Trueman et al. 2005; Barnes et al. 2007; Guelinckx et al. 2007; Mill et al. 2007; Gamboa-Delgado et al. 2008). Therefore, it is clear that the variability in turnover times and discrimination factors among species obligates laboratory investigations in each species under study, or at least in closely related taxa that are morphologically and physiologically similar to the target species (MacAvoy et al. 2001).

Laboratory isotopic turnover experiments provide valuable information, not only for the better understanding of field isotopic signatures, but also for observing the assimilation of specific nutrients, especially if a component of a diet is indigestible (Gamboa-Delgado et al. 2008). For example, wood is made primarily of cellulose and hemicellulose, but is also composed of 18-35% lignin (Petterson 1984), which is absolutely indigestible by vertebrates, or by endosymbiotic microorganisms inhabiting their digestive tracts (Van Soest 1994; Stevens and Hume 1995; Karasov and Martínez del Rio 2007). Furthermore, different fiber types of wood (e.g., cellulose vs. lignin) have different isotopic signatures, with cellulose being ~2‰ enriched in $\delta^{13}C$ compared to bulk wood (Gaudinski et al. 2005; Bowling et al. 2008), and lignin being

~4‰ depleted (Bowling et al. 2008). Thus, one would expect an animal consuming wood to have discrimination factors skewed towards the digestible, cellulosic portions of their diet rather than the bulk wood. However, few studies have attempted to trace the assimilation of wood carbon using stable isotopes in animals (e.g. Tayasu et al. 1997; Nonogaki et al. 2007), and I know of none that have examined whether a wood-eating animal can specifically assimilate the isotopes from the cellulosic portion of their diet, rather than the overall isotopic signature of the bulk wood (including lignin).

In this study I combined laboratory and field stable isotopic investigations to discern whether wood-eating catfishes (family Loricariidae) can assimilate wood and thrive on a woody diet. I performed turnover studies with captive individuals of the detritivorous loricariid, *Pterygoplichthys disjunctivus*, and used several tissues that could be sampled non-invasively (red blood cells, plasma, and fin tissue) over time. I determined turnover rates and discrimination factors for $\delta^{13}C$ and $\delta^{15}N$ for these tissues, and used this information to guide stable isotopic analyses of this and related species gathered in the field. Although *Pt. disjunctivus* is not a true wood-eating catfish, they were appropriate laboratory surrogates because they are closely related to the wood-eating catfishes (i.e., in the genera *Panaque* and *Hypostomus*; Armbruster 2004), and because they have similar gut morphology, digestive physiology (Chapter 2), metabolic rates (Nelson 2002), and low-quality diet to their wood-eating brethren. Furthermore, the wood-eating catfishes are native to rugged regions of the Amazonian basin and are difficult to obtain in sufficient numbers for use in the laboratory (Schaefer and Stewart 1993; DPG, pers. obs.), even via the aquarium trade. Although native to South America, *Pt. disjunctivus* has been introduced to waterways in Florida, where it has been established for nearly two decades (Nico 2005).

Thus, I had access to an abundant local population of this species within transport distance of the laboratory.

I performed two stable isotopic turnover experiments in this investigation. In trial one, I established turnover rates and discrimination factors for the different tissues (red blood cells, plasma, and fin tissue) of *Pt. disjunctivus* on an algal diet. In trial two I determined whether this species was capable of assimilating the fibrous portions of wood – I fed the fish an artificial wood-detritus diet with fibrous ($\delta^{13}C$ = -26.36‰; $\delta^{15}N$ = 2.13‰) and soluble components ($\delta^{13}C$ = -11.82‰; $\delta^{15}N$ = 3.39‰) of different isotopic signatures to observe whether the fish were assimilating the wood fiber, the soluble components, or a mixture of the two. I also observed whether the fish were growing on a wood-dominated diet. Wood-detritus is not completely made of fibrous material, as degraded wood is typically composed of 20% soluble components (Chapter 2), most likely representing microbes, digestive enzymes of microbial origin, and soluble wood-degradation products (Sinsabaugh et al. 1991b; Sinsabaugh et al. 1992; Tank et al. 1998; Hendel and Marxsen 2000). Thus, I attempted to mimic the biochemical composition of natural wood-detritus with my artificial version for trial two. I also extracted the cellulosic components of wood used in the laboratory, and from that grazed upon in the field to determine whether fishes were specifically assimilating the carbon isotopes of cellulose and hemicellulose as opposed to other elements of bulk wood, such as lignin.

Although Nonogaki et al. (2007) suggested that lorcariid catfishes can assimilate wood carbon (based on bulk wood $\delta^{13}C$), I provided physiological evidence in Chapter 2 of this dissertation showing that wood-eating loricariid catfishes, and *Pt. disjunctivus*, poorly digest the fibrous components of wood in their intestines. These fishes clearly digest the more soluble components of their diet. Thus, I predicted that *Pt. disjunctivus* could not assimilate the carbon

isotopes of wood fiber in the laboratory. However, because many of the soluble components of natural wood-detritus (e.g., β-glucosides) do come from cellulose degradation (Sinsabaugh et al. 1992; Tank et al. 1998; Hendel and Marxsen 2000), I predicted that wild-caught wood-eating catfishes would have carbon isotopic signatures reflecting the cellulosic component of their wood diet, and not that of bulk wood or lignin. Furthermore, I hypothesized that the nitrogen signatures of wild-caught wood-eating catfishes would be much greater (i.e., $\delta^{15}N > 2‰$; Tayasu et al. 1997) than those of wood-eating termites, which are reliant upon endosymbiotic nitrogen fixation to meet their nitrogen requirements (Slaytor and Chappell 1994). Symbiotic nitrogen-fixing bacteria fix atmospheric nitrogen into usable compounds for the host organism (Karasov and Martínez del Rio 2007). However, from a stable isotopic perspective, this results in low $\delta^{15}N$ values for the host (near zero) because the standard used to determine $\delta^{15}N$ is atmospheric nitrogen. Instead, I predicted the catfishes would have enriched $\delta^{15}N$ signatures reflecting additional nitrogen sources in their food; specifically, from detritus, microbes (fungi in particular) that are common in degrading wood in aquatic systems (Sinsabaugh et al. 1991a; Hendel and Marxsen 2000), and potentially from aquatic insects.

Methods

Fish Collection and Maintenance in Laboratory

Twenty-two adult *Pterygoplichthys disjunctivus* were captured from the Wekiva Springs complex in north central Florida (28º41.321' N, 81º23.464' W) in May 2005. The fish were similar in length [mean ± SD; 128.77 ± 2.12 mm standard length (SL)] and mass (51.29 ± 2.19 g). 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 12L:12D light cycle (Chapter 2). The thermostat in the aquarium laboratory was set at 25ºC for the duration of the experiment and the temperature of each tank was monitored daily to confirm that the temperature did not vary by more than 1ºC. The tanks were scrubbed, debris and feces siphoned out, and 95% of the water changed every five to seven days to limit algal and microbial growth in the tanks as possible confounding food sources.

Tissues Used for Stable Isotope Analysis

I analyzed the isotopic composition of red blood cells, plasma solutes, and pelvic and caudal fin tissue. I chose these tissue types because I could sample them in a non-invasive manner. Indeed, one of the goals of this study was to investigate isotopic turnover and discrimination in blood and skin tissue (i.e., fin clips) for use in stable isotopic studies of fishes, as has been done for other ectothermic (Kelly et al. 2006; Reich et al. 2008) and endothermic (Bearhop et al. 2002) vertebrates. Approximately 150 μL of blood was drawn with a 23-gauge needle from the haemal arch just posterior to the anal fin of the fish, transferred to unheparinized capillary vials, and immediately centrifuged at 13,000 x *g* for five min to separate the red blood cells, white blood cells, and plasma. Following the separation, the red blood cells (RBCs) and plasma were placed in separate, sterile centrifuge vials, and the white blood cells were discarded. Fin clips (approximately 0.5 g wet weight) were taken with sterile scissors by cutting membranous tissue and soft rays (no thicker, hardened rays) from left or right pelvic fin, or the dorsal or ventral caudal fin, depending on the individual fish. Because the specific fin taken was also used to identify individual fish in their respective aquaria, the same fin was sampled at each sampling interval from each individual fish. The fin tissue completely regenerated within the 30 days between sampling intervals (a common observation in fishes; Wills et al. 2008), and thus, the isotopic turnover examined for the fin tissue was not for steady-state fin tissue, but rather, for

regenerated fin tissue. The isotopic turnover for this regenerated tissue may differ from non-regenerated tissue, and therefore, may not reflect fin tissue isotopic turnover in nature.

Stable Isotope Trial 1: Initial Turnover

Upon arrival in the laboratory, tissues were taken from four individual fish to provide an initial stable isotopic reading. Thereafter, fish were fed a commercial algal diet (Wardley® Premium Algae Discs, Hartz-Mountain Corporation, Secaucus, NJ) for 203 days (Table 3-1). These algae discs contained primarily the green alga *Spirulina* as an ingredient, but also contained a variety of grains, legumes, and plant proteins, in addition to vitamins and minerals. Fish were offered seven discs per night, which they consumed, equating to approximately 6% of their body mass, on a wet mass basis, per day. For the first 100 days of the feeding trial, tissues were sampled from three to four fish (from different tanks) every five days, whereas six to seven individuals were sampled every 10-11 days for the final 103 days of the experiment. I designed my sampling regime so that individual fish were sampled every 30 days, giving them ample time to recover from the blood draws and fin tissue biopsies. Three additional fish were captured from the wild, and blood and fin clips taken for isotopic analyses on days 100 and 210 to evaluate how the isotopic signature of the fish changed in nature over this same time period. The blood and finclip samples from days 25 to 55 of the experiment were lost due to "non-demonic intrusion" (Hurlbert 1984), which, in this case, was a careless research assistant. However, these missing samples did not necessarily affect the over all analyses because the rates of isotopic incorporation could still be determined (see below).

Stable Isotope Trial 2: Wood Assimilation and Negative Control

After 203 days on the algae diet, the fish were given a refractory period of 40 days, during which they were fed the algae discs but not handled. After this intermission, fish were divided into two groups: one group (n=6) remained on the algae diet (positive controls), and one group

(n=16) was switched to a wood diet (experimental group). An additional six fish were captured from the wild in February 2006 to act as negative controls and were not fed for the duration of the experiment (see below).

The goal of this experiment was to examine whether *P. disjunctivus* could assimilate carbon and nitrogen from the structural components of wood (cellulose, hemicellulose, lignin), or if they simply digested and assimilated soluble degradation products found on wood as it is decomposed. Thus, I designed a diet that featured wood (and its inherent fiber types) of one isotopic signature, and soluble components of another isotopic signature (Table 3-1). The wood was that of decomposed riparian water oak (*Quercus nigra*), collected from the Sampson River, FL (29º51.37' N, 82º13.16' W), and the soluble components were composed of corn gluten meal, corn meal, vitamins, and minerals (Table 3-1). The wood was chopped into smaller pieces with a hatchet, and ground to particle sizes of 0.25–1 mm in a coffee grinder (Krups GX 4100). The wood was then autoclaved to ensure it was sterile, and dried at 60ºC for 24 hours. The corn gluten meal, corn meal, lysine, vitamins, and minerals (gifts from Hartz-Mountain Corporation, Secaucus, NJ, and some of the same ingredients in the algal diet described above), intended specifically for use in fish food, were then added to the dried wood, followed by xanthan gum (6%; Now Foods, Bloomingdale, IN) and a small amount of water (20 mL/100 g dry mass; Table 3-1). The xantham gum and water, combined, acted as binding agents to keep the ingredients bound when submerged in water. The mixture was then homogenized vigorously by hand with a stirring rod, and pressed into 4x2 mm (0.45 g) circular pellets in a hand press (Parr Instruments, Moline, IL). Because dried wood tends to float and the fish primarily feed on the benthos, the pellets were then adhered to a piece of PVC pipe (n=10 pellets per PVC piece) 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 (~0.05g) of each pellet was inedible by the fish. The fish were offered, and consumed, 10 pellets per night, equating to approximately 8% of their body mass, on a wet mass basis, per day. Given that *Panaque nigrolineatus* (a true wood-eating loricariid catfish) was observed to consume 2-5% of the their body mass in wood per day in the laboratory (Chapter 2), I considered 8% of the fishes' body mass per evening in food to be *ad libitum*. The fish were fed this diet for 155 days, and, as with the initial turnover experiment, tissues were taken every five days for the first 50 days of the experiment, and every 10 days for the remaining 105 days. Fish remaining on the algae diet (positive controls) were fed as described above for trial 1.

Because previous observations suggested that *P. disjunctivus* did not gain weight on a wood-only diet in the laboratory (Chapter 2), I included a group of fish that acted as negative controls. Six individuals were deprived of food for the duration of the wood-feeding experiment. These fish were also weighed and tissues taken from individual fish every 30 days. One individual died during the course of the food-deprivation, so I had a final sample size of five for the negative controls.

At the end of trial 2 (155 days) the fish were euthanized in buffered water containing MS-222 (1 g l^{-1}), measured [standard length (SL) ± 1 mm], and weighed [body mass (BM) ± 0.5 g]. Epaxial white muscle samples were taken from each fish for endpoint stable isotopic measurements. The livers of all of the fish were weighed, the hepato-somatic index [HSI = liver mass (g)/body mass (g)] determined, and a subsample of the liver tissue was dried at 60°C and analyzed for %C and %N in a Carlo-Erba elemental analyzer. The HSI and %C:%N ratio were used to assess the health of the fish on the different diets (or deprived of food). The HSI is a

body condition index (lower HSI = lower body condition, poor health; Lloret and Planes 2003) and the %C:%N ratio indicated whether the fish were mobilizing nitrogen from their livers (higher %C:%N ratio = less nitrogen; Karasov and Martínez del Rio 2007), which would indicate that the fish were in negative nitrogen balance. 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.

Stable Isotopic Profiles of Wild-Caught Fish and Resources

Wood, biofilm, algae, seston, abundant invertebrate taxa and fish were collected from the field sites for stable isotopic measurements (Tables 3-2 and 3-3). I was not exhaustive in my sample collection, but I attempted to characterize a small portion of the foodwebs in two locations: first, in the upper Río Marañon in northern Peru (4º58.957' S, 77º85.283' W; Chapter 2), and second, in Wekiva Springs, FL (see above under "Fish collection and maintenance in the laboratory"). Wood, algae, and invertebrates were rinsed with deionized water and frozen in liquid nitrogen in the field. Biofilm was collected by gently brushing the surface debris from wood and collecting it in a ziplock bag. The bag was sealed and the contents of the bag were mixed by shaking the bag. I then pipetted 1 mL of the slurry into 1.5-mL centrifuge vials and centrifuged them at 10,000 x *g* for five min in an Eppendorf 5415R desktop centrifuge powered by a 12V car battery via a power inverter. The supernatant was discarded and the pelleted debris was frozen in liquid nitrogen. Seston was collected by filtering river water through a 0.25 μm glass fiber filter. Blood and fin clip samples were taken from fish as described above under "Tissues used for stable isotope analysis". I also collected epaxial white muscle (Peru) or fin clip (Florida) samples from several fish species that were too small from which to draw an adequate blood sample (Tables 3-2 and 3-3). All fish tissues were frozen in liquid nitrogen in the

field. All samples were shipped back to the University of Florida on dry ice, dried at 60°C, and stored in sealed containers at room temperature until analyzed.

Because wood is composed of compounds indigestible by the fish (e.g., lignin), it was necessary to extract the more "digestible" portions (i.e., cellulose, hemicellulose) of the wood for stable isotopic analyses. Holo-cellulose (Leavitt and Danzer 1993; Gaudinski et al. 2005), which comprises the cellulosic and hemicellulosic compounds of wood, was extracted following a slightly modified Jayme-Wise method (Leavitt and Danzer 1993), essentially as described by Gaudinski et al. (2005). Wood from Perú and Florida was dried at 60°C, ground in a coffee grinder to pass through a 1-mm screen, and 200-500 μg samples of the ground wood were placed in polyester solvent bags (Ankom Technology, Macedon, New York). The samples were then extracted in 2:1 Toluene:Ethanol in a Dionex Accelerated Solvent Extractor (ASE®), followed by a second extraction in 100% ethanol. The samples were then boiled for 4 hours in deionized water to remove soluble components, and allowed to dry in a drying chamber at room temperature. The bags (containing the samples) were then soaked in 1-L of an aqueous bleach solution containing 10 g of sodium chlorite and 6 mL of glacial acetic acid at 70°C for five days. The bleach/acetic acid solution was changed every 12 hours. Following the bleaching, the samples were thoroughly rinsed in running deionized water for 3 hours, and dried at 60°C. The remaining white fibrous material was holo-cellulose as determined by Gaudinski et al. (2005).

Sample Preparation for Mass Spectrometry

Blood (red blood cells and plasma solutes), fin clip, and muscle samples from fish, and, wood, holo-cellulose, algae, invertebrate, biofilm, and seston (on a glass-fiber filter) samples from the environment, were dried to a constant weight for 24-48 hours at 60ºC (Reich et al. 2008). Lipids were extracted from all animal and plant material (except the red blood cells and

plasma solutes, which were too small) with petroleum ether in a Dionex Accelerated Solvent Extractor (Dodds et al. 2004; Reich et al. 2008). Approximately 500 μg of animal, plant, or detrital tissue were loaded into pre-cleaned tin capsules, combusted in a COSTECH ECS 4010 elemental analyzer interfaced via a Finnigan-MAT ConFlow III device (Finnigan MAT, Breman, Germany) to a Finnigan-MAT DeltaPlus XL (Breman, Germany) isotope ratio mass spectrometer in the light stable isotope lab at the University of Florida, Gainesville, FL, USA. Stable isotope abundances are expressed in delta (δ), defined as parts per thousand (‰) relative to the standard as follows:

$$\delta = [(R_{sample}/R_{standard}) - 1] (1000) \quad (1)$$

where R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and standard, respectively. $R_{standard}$ for ^{13}C was Vienna Pee Dee Belemnite (VPDB) limestone formation international standard. $R_{standard}$ for ^{15}N was atmospheric N_2. IAEA CH-6 ($\delta^{13}C$ = -10.4) and IAEA N1 Ammonium Sulfate ($\delta^{15}N$ = +0.4), calibrated monthly to VPDP and atmospheric N2, respectively, were inserted in all runs at regular intervals to calibrate the system and assess drift over time. The analytical accuracy of our measurements, measured as the SD of replicates of standards, was 0.14 for $\delta^{13}C$ and 0.11 for $\delta^{15}N$ (N = 120).

Statistical Analyses

I estimated growth rates (g in days^{-1}) of *Pt. disjunctivus* using an exponential model ($y = ae^{bt}$; Reich et al. 2008). The fractional rate of isotopic incorporation, λ, was estimated (in days^{-1}) with a non-linear fitting procedure using the equation

$$\delta X(t) = \delta X(\infty) + [\delta X(0) - \delta X(\infty)]e^{-\lambda t}, \quad (2)$$

where $\delta X(t)$ is the isotopic composition at time *t*, $\delta X(\infty)$ is the asymptotic, equilibrium isotopic composition, $\delta X(0)$ is the initial isotopic composition, and λ is the fractional rate of isotope incorporation in a tissue (Martínez del Rio and Wolf 2005; Reich et al. 2008). $\delta X(\infty)$ and $\delta X(0)$ were estimated using the same non-linear procedure. λ can be defined as the sum of tissue net growth (k_{gt}) and tissue catabolic turnover (k_{dt}); thus, $\lambda = k_{gt} + k_{dt}$. (Hesslein et al. 1993; Reich et al. 2008). If a tissue is growing exponentially, then I can measure the growth and determine the contribution of growth and tissue catabolic turnover to λ (Reich et al. 2008). Following Reich et al. (2008), I assumed that the fractional growth rate of a tissue was equal to the fractional rate of growth of the whole animal (k_g). I then compared λ to k_g using *t*-tests, to determine whether the contribution of tissue catabolic turnover to isotopic incorporation was significant. A large difference between λ and k_g indicates a large contribution of tissue catabolic turnover to isotopic incorporation as opposed to new tissue accretion. Because I used adult animals in our experiments, I anticipated a large difference between λ and k_g, and thus, a large contribution of catabolic turnover, unlike growing juvenile animals (Trueman et al. 2005; Gamboa-Delgado et al. 2008; Reich et al. 2008).

Isotopic discrimination factors ($\Delta X_{tissue\text{-}diet}$) were calculated as $\delta X(\infty)_{tissue} - \delta X_{diet}$. Turnover times (average residence times) of C and N molecules in trial one were calculated as $1/\lambda$ (Reich et al. 2008). In trial two, I used the following concentration-dependent linear mixing model incorporating digestibility estimates to predict the final isotopic signatures of the fish if they were assimilating the diet:

$$p_{xi} = \frac{e_i p_i x_i}{\sum_{j=1}^{n} e_{x_j} p_j x_j} \qquad (3)$$

where p_{xi} is the expected isotopic signature assimilated of isotope X, e_i is the digestibility coefficient for a dietary ingredient (e.g., wood), p_i is the proportion of the diet composed of that ingredient, x_i is the concentration (%) of the atom in question (e.g., C) in the ingredient, and the denominator is the summed totals of all of the ingredients in the diet (Martínez del Rio and Wolf 2005). Digestibility estimates for wood and cellulose were garnered (for *Pt. disjunctivus* specifically) from Chapter 2 and digestibilities for corn gluten meal (Guimarães et al. 2008) and corn meal (Krogdahl et al. 2005) were taken from the literature. Xanthan gum was considered an indigestible non-starch polysaccharide (Leenhouwers et al. 2006), and the vitamins and minerals were considered 100% absorbable. Because 20% of the bulk wood mass is made of soluble degradation components (Chapter 2), this was also added into the mixing model. However, the isotopic signature and actual digestibility of this fraction is unknown. Thus, I assumed that most of these degradation products are of cellulosic origin (Sinsabaugh et al. 1992), and would, therefore, have an isotopic signature identical to that of cellulose ($\delta^{13}C$ = -25.39‰). *Pterygoplichthys disjunctivus* has digestive enzyme activities in its gut indicative of efficient digestion of soluble wood degradation products (i.e., high α-glucosidase, β-glucosidase, and β-mannosidase activities), so I assumed digestibilies of 90% for this soluble component. With these ingredients and digestibilties, the artificial wood-detritus diet had the following predicted digestible isotopic signature: $\delta^{13}C$ = -21.79‰, and $\delta^{15}N$ = 3.25‰.

Hepato-somatic indices and liver %C:%N ratios were compared among the experimental (wood-detritus diet), control (algae diet), and food-deprived groups with ANOVA, followed by a Tukey's HSD with a family error rate of P = 0.05. The same ANOVA procedure was also used to compare field isotopic signatures in *Pt. disjunctivus* among days 0, 100, and 210 of the experiment. Prior to all *t*-tests and ANOVA, a Levene's test for equal variance was performed to

ensure the appropriateness of the data for parametric analyses. All modeling and tests were run with SPSS statistical software (version 12).

Results

Trial One: Initial Turnover

The exponential growth rate for algae-fed fish was (mean ± SD) 0.0017 ± 0.0006 day^{-1}, and the fish gained 40.86 ± 0.17% of their body mass during the experiment. The exponential model ($y = ae^{bt}$) described the growth rates of the fish reasonably well (r^2 = 0.82-0.99). Equation 2 described the changes in $\delta^{13}C$ and $\delta^{15}N$ through time adequately well in all tissues (r^2 ranged from 0.73 to 0.92; Figure 3-1). All tissues showed isotopic incorporation rates that were significantly greater than those expected by growth alone (0.0017 day^{-1}), indicating a significant contribution of tissue catabolic turnover to isotopic incorporation (Tables 3-4 and 3-5). Tissue catabolic turnover contributed between 72 and 97% of carbon isotopic incorporation, and between 95 and 99% of nitrogen incorporation. $\Delta^{13}C_{tissue\text{-}diet}$ and $\Delta^{15}N_{tissue\text{-}diet}$ varied by tissue, with $\Delta^{13}C_{tissue\text{-}diet}$ ranging from -0.13 to 1.75, and $\Delta^{15}N_{tissue\text{-}diet}$ ranging from 4.08 to 5.17 (Tables 3-4 and 3-5). The turnover times of C varied by tissue, with fin clips showing the shortest residence time of 20 days, and red blood cells the longest at 167 days (Table 3-4). Similarly, nitrogen turnover times varied by tissue, as red blood cells showed residence times of approximately 4.5 days, and fin clips exhibited residence times of 33.2 days (Table 3-5). Wild-caught fishes captured on days 100 and 210 of the experiment had $\delta^{13}C$ signatures that varied from the first day of the experiment (significantly so for plasma solutes and fin tissue), whereas $\delta^{15}N$ was less variable over time (Table 3-6). Nonetheless, the $\delta^{13}C$ of wild fish became more depleted after 100 days, not more enriched as I observed in the algae-fed fish in the laboratory (Table 3-6).

Trial Two: Wood Assimilation and Negative Control

All of the fish lost weight on the artificial wood-detritus diet, with the mean loss being (mean ± SD) 7.87 ± 2.74% of their body mass over 155 days, whereas the control fish on the algae diet gained 31.23 ± 8.10% of their body mass over the same time period. The food-deprived fish lost 3.67 ± 1.49% of their body mass over 150 days. The fish on the artificial wood-detritus diet lost significantly more weight than those that were food-deprived ($t = 10.61$, $P < 0.001$, d.f. = 9), likely because the former were more active on a daily basis than the latter (DPG, pers. obs.). Equation 2 adequately described the changes in $\delta^{13}C$ over time for plasma solutes, but less so for red blood cells (Table 3-7; Figure 3-2). A linear procedure was necessary to describe the incorporation of $\delta^{13}C$ for the fin clip samples. Equation 2 appropriately described the incorporation of $\delta^{15}N$ over time in plasma solutes and fin clips, but not for red blood cells, which required a linear procedure (Table 3-8; Figure 3-2). It is clear that the $\delta^{13}C$ of all tissues became enriched over the course of the experiment, moving toward the predicted signature, but not perfectly so, especially for the fin tissue (Table 3-7). The $\delta^{13}C$ values of control fish (i.e., those consuming the algal diet) were unchanging over the course of the experiment (Figure 3-2). The isotopic incorporation for $\delta^{13}C$ in plasma solutes in trial one was similar to that observed in trial two for the fish consuming the artificial wood-detritus (0.026 and 0.023 day^{-1}, respectively; Tables 3-4 and 3-7, respectively). Comparable results were observed for the fin clips, as the rate of $\delta^{13}C$ incorporation in this tissue was similar between trial one and trial two (0.031 and 0.021 day^{-1}, respectively; Tables 3-4 and 3-7 respectively).

Different patterns of plasma $\delta^{13}C$ incorporation were observed between the fishes consuming the artificial wood-detritus and those that were deprived of food (Figure 3-3). Whereas the $\delta^{13}C$ incorporation in plasma solutes was described by equation 2 for the fish

consuming the artificial wood-detritus (Table 3-7), a polynomial distribution described the pattern of incorporation in the food-deprived fish (Figure 3-3). These differences are likely the result of the assimilation of the soluble components from the artificial wood-detritus diet. The patterns of red blood cell $\delta^{13}C$ incorporation were similar between the fish consuming the artificial wood-detritus, those that were food-deprived, and the control fish consuming the algae diet, as all three were relatively unchanging over the course of the experiment and not well-described by equation 2 or linear procedures (Figures 3-2 and 3-4).

The patterns of plasma $\delta^{15}N$ incorporation were similar between the fish consuming the artificial wood-detritus and those that were deprived of food (Figure 3-3) – both were described by equation 2 (Table 3-8), and the equation for the food-deprived fish was $11.26 - 2.28e^{-0.005(time)}$ ($r^2 = 0.98$). Remarkably, the fractional rate of nitrogen incorporation (0.005 day^{-1}) was identical in the two groups. However, the overall change in plasma $\delta^{15}N$ from the beginning to the end of the experiment was greater for the fish consuming the artificial wood-detritus (mean ± SD; 1.83 ± 0.66‰) than for the food-deprived fish (1.24 ± 0.23‰; $t = 1.74$, P = 0.10, D.F. = 17). Nevertheless, the loss of weight and the pattern of plasma $\delta^{15}N$ incorporation in the fish consuming the artificial wood-detritus indicate that these fish were at least partially starving and in negative nitrogen balance. This is further corroborated by the hepato-somatic indices of the fish, which were significantly lower in the food-deprived fish and in those consuming the artificial wood-detritus than in those consuming algae (Figure 3-5). Protein reserves were definitely mobilized in the food-deprived fish, as the %C:%N ratio in their liver was significantly greater than in the algae-fed fish (Figure 3-5). However, the %C:%N ratios in the livers of the fish consuming the artificial wood-detritus were indistinguishable from either group, suggesting that these fish were assimilating at least some nitrogen from the corn gluten meal and lysine

(Table 3-1) in their diet. Nevertheless, the rate of $\delta^{15}N$ incorporation in the plasma solutes of the wood-fed and food deprived fish was identical ($\lambda = 0.005\ d^{-1}$; Table 3-8), and this stands in starck contrast to the rate of $\delta^{15}N$ incorporation in plasma solutes from trial 1 on the algae diet ($\lambda = 0.213\ d^{-1}$; Table 3-5). Thus, from an isotopic perspective, the fish consuming the artificial wood-detritus appeared to be in negative nitrogen balance.

The muscle tissue of the fish consuming the artificial wood-detritus had the following final isotopic signatures (mean ± SEM): $\delta^{13}C = -23.19 \pm 0.24$, $\delta^{15}N = 8.40 \pm 0.21$, $\Delta^{13}C_{tissue\text{-}diet}$ = 1.40‰, $\Delta^{15}N_{tissue\text{-}diet}$ = 5.02‰. The fish consuming the algae diet had the following final isotopic signatures in their muscle tissue: $\delta^{13}C = -22.03 \pm 0.21$, $\delta^{15}N = 7.39 \pm 0.16$, $\Delta^{13}C_{tissue\text{-}diet}$ = -0.01‰, $\Delta^{15}N_{tissue\text{-}diet}$ = 5.13‰.

Wild-Caught Fish and Resources

The wood-eating catfishes from the upper Río Marañon, Perú, had $\delta^{13}C$ signatures consistent with the assimilation of cellulosic carbon, and were isotopically different from the other loricariid species in the genera *Lamontichthys* and *Spatuloricaria* (Figure 3-6). The $\delta^{13}C$ of plasma solutes, red blood cells, and muscle tissue of *Panaque cf. nigrolineatus* "Marañon", *P. nocturnus*, and *Hypostomus pyrineusi* were all consistent with that of cellulose. Furthermore, assuming a $\Delta^{13}C_{tissue\text{-}diet}$ of 1.75 for fin tissue, the $\delta^{13}C$ of the fins is also consistent with a diet of cellulose carbon (~26.5‰; Figure 3-6). The wood-eating catfish are clearly enriched in $\delta^{13}C$ compared to biofilm and bulk wood, and are depleted compared to seston (Figure 3-6). All three wood-eating catfish species had stable isotopic signatures that varied from one another, especially for $\delta^{15}N$. The two species of *Panaque* had similar $\delta^{13}C$ signatures, but *P. cf. n.* "Marañon" had enriched $\delta^{15}N$ signatures in comparison to *P. nocturnus* and *H. pyrineusi*, which in turn were different from each another (Figure 3-6). When including other animal taxa in the

carbon-nitrogen dual-isotope plot, all of the wood-eating catfishes (including *P. albomaculatus* and *P. gnomus* in addition to those listed above) cluster together and have $\delta^{15}N$ signatures that are likely too enriched to reflect solely the assimilation of wood or of nitrogen fixation occurring in their digestive tracts (assuming $\Delta^{15}N_{tissue\text{-}diet}$ 4-5‰).

Pterygoplichthys disjunctivus is not likely utilizing cellulosic carbon as a food source in Floridian spring habitats (Figure 3-7). This fish has $\delta^{13}C$ signatures that are more depleted than periphyton or coarse benthic organic matter, although, by assuming a $\Delta^{15}N_{tissue\text{-}diet}$ of 4-5‰, these fish may be getting some nitrogen from periphyton sources. Because I was not exhaustive in my collection of potential resources it is difficult to discern the exact carbon sources for *Pt. disjunctivus*, but we can be certain it is not from wood or wood cellulose. When including other animal taxa in the carbon-nitrogen dual-isotope plot, it is clear that *Pt. disjunctivus* is feeding at a lower trophic level than all of the common fish species observed in the spring habitat (Figure 3-7). However, the Wekiva Springs complex in Orlando, Florida is an urban site and is clearly enriched in $\delta^{15}N$ in comparison to the Peruvian riverine habitat (Figures 3-6 and 3-7).

Discussion

To my knowledge, this study is the first to report on the isotopic incorporation and discrimination factors for plasma solutes, red blood cells, and fin tissue in a fish species. In comparison to mammals and birds, fishes (and ectotherms in general; Reich et al. 2008) are less well-studied in this regard. Most of my predictions were supported by the results of this study. First, *Pt. disjunctivus* clearly cannot assimilate carbon from the fibrous portions of wood, and probably doesn't use wood as a resource in the wild. Second, wild-caught wood-eating catfishes from Perú had $\delta^{13}C$ signatures consistent with the assimilation of cellulosic carbon. And third, the $\delta^{15}N$ signatures of these same wild-caught wood-eating catfishes were too enriched to reflect

solely the assimilation of wood, or of nitrogen fixed by endosymbionts in their digestive tracts. Overall, the data presented here support my larger hypothesis that wood-eating catfishes in the family Loricariidae are not capable of digesting wood in their digestive tracts, and instead rely on soluble wood-degradation products produced by microbial digestion of wood in the environment (Chapter 2).

This study is one of very few to determine the effects of growth versus catabolic tissue turnover to isotopic incorporation in fishes. Hesslein et al. (1993) observed very little contribution of catabolic tissue turnover to isotopic incorporation (~0.002 d^{-1}) in liver and muscle of whitefish (*Coregonus nasus*), and Trueman et al. (2005) observed isotopic incorporation rates due to catabolic tissue turnover of approximately 0.005 d^{-1} in muscle and liver of growing Atlantic salmon (*Salmo salar*). However, the *C. nasus* (0.05 d^{-1}) and the *S. salar* (0.02 d^{-1}) in those studies were growing at faster rates than *Pt. disjunctivus* in this study (0.0017 d^{-1}). Obviously, growth will play a more significant role in isotopic incorporation in more rapidly growing animals, especially in juveniles (Gamboa-Delgado et al. 2008; Reich et al. 2008). The *Pt. disjunctivus* used in this study were all adults, as ripe ovaries and testes were observed upon dissection of many of the wild fish, and some fish at the end of the experiment, and this may explain why they did not grow as rapidly as animals in previous studies that focused on juveniles.

The rates of isotopic incorporation varied by tissue in *Pt. disjunctivus*, with plasma solutes and fin tissue turning over more quickly than red blood cells for $\delta^{13}C$, and plasma solutes and red blood cells turning over more quickly than fin tissue for $\delta^{15}N$. The half-lives for $\delta^{13}C$ in these tissues (calculated as $\ln(2)/\lambda$) ranged from 14 to 116 days, and from 3 to 22 days for $\delta^{15}N$. Differences in turnover rates among tissues are not uncommon in fishes and other animals

though. For example, MacAvoy et al. (2001) observed large half-life values of approximately 170 days for $\delta^{13}C$ and $\delta^{15}N$ in channel catfish (*Ictalurus punctatus*) muscle and whole blood, whereas Sakano (2005) observed half-lives ranging from 24 to 65 days for $\delta^{15}N$ in sockeye salmon (*Oncorhynchus nerka*) muscle. McIntyre and Flecker (2006) estimated half-lives of 17 and 12 days for $\delta^{15}N$ in muscle and fin, respectively, in the loricariid catfish *Ancistrus triradiatus*. Sand goby (*Pomatoschistus minutus*) muscle and liver exhibited half-lives of 25 and 9 days, respectively, for $\delta^{13}C$, and 28 and 3 days, respectively, for $\delta^{15}N$ (Guelinckx et al. 2007). Thus, the turnover rates observed in *Pt. disjunctivus* fall within the range reported for other fish tissues.

Given the slow turnover of 167 days for $\delta^{13}C$ in red blood cells, it is difficult to imagine how the $\delta^{15}N$ turnover rate of less than five days was possible for this tissue in *Pt. disjunctivus*. The few previous investigations of whole-blood (RBC + plasma) $\delta^{15}N$ turnover in fish (>250 days, MacAvoy et al. 2001; 25 days, McIntyre and Flecker 2006) varied widely, but were still much slower than five days. Furthermore, my observation of a turnover rate of 167 days for $\delta^{13}C$ in red blood cells is consistent with slow blood isotopic turnover in channel catfish (MacAvoy et al. 2001). Thus, something appears to be wrong with the $\delta^{15}N$ turnover observed for red blood cells in this study. Several possible methodological issues can explain this rapid turnover, and all of them involve an erroneously high starting point for red blood cell $\delta^{15}N$. First, the isotope ratio mass spectrometer can occasionally produce incorrect $\delta^{15}N$ values, and if unnoticed, may have resulted in inflated starting $\delta^{15}N$ values. Second, there may have been incomplete separation of plasma and red blood cells during the centrifugation process, resulting in plasma proteins contaminating the red blood cell samples; this may have produced unusually high starting $\delta^{15}N$ values and rapid turnover. And third, there is some unknown contaminant in

the red blood cell samples skewing the results. The separation of the red blood cell and plasma fractions of the blood was very clear, so I do not think that option two is likely. Therefore, option one or three appear to be the probable possibilities. Both of these can be addressed by simply running the red blood cell samples through the isotope ratio mass spectrometer a second time to observe whether they are erroneously high. A change of just 1‰ in the starting $\delta^{15}N$ of the red blood cells can result in a reduction of the turnover rate from five days to 25 days. Thus, the implications are large and should be addressed. However, they will not be addressed for this dissertation. If the $\delta^{15}N$ turnover rate is found to be correctly rapid for red blood cells in *Pt. disjunctivus*, the mechanism for this turnover, and the complete independence from $\delta^{13}C$ turnover need to be investigated.

The $\delta^{13}C$ of the fin tissue turned over significantly more quickly than the plasma solutes (20 days *vs.* 36 days; $t = 3.71$, $P = 0.021$, d.f. = 4), but the opposite was true for $\delta^{15}N$ (32 days *vs.* five days; $t = 3.46$, $P = 0.026$, d.f. = 4). I consistently sampled the same fin tissue from each individual fish every 30 days, and thus, the fin tissue used in this study was regenerated tissue as opposed to undamaged, steady-state fin tissue (Wills et al. 2008). The regeneration process – which involves several different cell types, cell migration, and substantial new tissue synthesis (Wills et al. 2008) – may have caused the differences in isotopic incorporation among the fin tissue and the plasma solutes. In the only other study of fish fin isotopic turnover, McIntyre and Flecker (2006) sampled undamaged fin tissue from different individual *Ancistrus triradiatus* for each of their sampling intervals. Interestingly, these authors observed $\delta^{15}N$ turnover rates of about 18 days for undamaged fin tissue, a rate that is nearly half of what I observed. This suggests one of two scenarios: either the catfish studied by McIntyre and Flecker (2006) were growing at a faster rate than *Pt. disjunctivus* in this study, resulting in a larger contribution of

growth to isotopic turnover; or the fin regeneration process in this study slowed the isotopic incorporation of nitrogen from the fish's diet into the newly generated fin tissue. McIntyre and Flecker (2006) did not measure fin carbon turnover, and, to my knowledge, no studies have measured carbon isotopic turnover in fish fin tissue. Thus, I am left with no explanation for how the $\delta^{13}C$ turnover over rate is faster in fin (regenerated or non-regenerated) than in plasma solutes. Nevertheless, the observation that regenerated fin $\delta^{15}N$ turned over more slowly than non-regenerated fin, suggests that my findings are more conservative than those of McIntyre and Flecker (2006), and thus, may still apply to turnover rates of fin tissue in wild fish. Additionally, differences in turnover rates of $\delta^{13}C$ and $\delta^{15}N$ in a single tissue are not uncommon in fishes. For example, Guelinckx et al. (2007) observed $\delta^{15}N$ turnover rates that were 3X faster than $\delta^{13}C$ turnover rates in sandgoby liver. Thus, the observation that plasma solutes had faster $\delta^{15}N$ than $\delta^{13}C$ turnover is not unknown, especially in rapidly cycling tissues like plasma and liver.

Differences in turnover rates among tissues are not consistent and can disappear in rapidly growing ectotherms. For example, Reich et al. (2008) observed half-lives ranging from 27-35 days in five different tissues of rapidly growing loggerhead turtle hatchlings, illustrating that turnover rate can become somewhat homogenous if the animal is growing sufficiently fast. Obviously, this can change the interpretation of field isotopic data if the animal is growing quickly and argues that isotopic data gathered in the laboratory should not only target the species of interest, but also should attempt to mimic growth conditions experienced by the animals in nature. Because I did not measure growth of the catfish in the wild, it is difficult to discern whether *Pt. disjunctivus* were growing at similar rates in captivity and in nature.

Growth rate varies as an allometric function of body mass (West et al. 2001), and thus, the rate of isotopic incorporation in an animal's tissues should also vary with body size. This

hypothesis was supported in house sparrows (*Passer domesticus*), which exhibited rates of isotopic incorporation that varied as an allometric function of body mass$^{-0.25}$ (Carleton and Martínez del Rio 2005). In ectothermic vertebrates, which are "indeterminate growers", it is, therefore, essential to study animals in the laboratory that are of a similar mass to those studied in the field. I was able to do so, as I only studied adult animals, and the average mass of *Pt. disjunctivus* used in the laboratory (51.29 g) was similar to the wild-caught fish in Orlando (61.93 g) and Perú (31.55 g). Another caveat in the consideration of growth is that some fish tissues only reflect the isotopic signature acquired during periods of growth. For example, Perga and Gerdeaux (2005) illustrated that the $\delta^{13}C$ and $\delta^{15}N$ of whitefish muscle were relatively unchanging during the non-growing season, and reflected the isotopic signature of food consumed during the growing season. However, liver $\delta^{13}C$ and $\delta^{15}N$ changed with diet across season regardless of growth. The same could be observed in *Pt. disjunctivus* in this study by comparing the patterns of $\delta^{13}C$ incorporation between red blood cells and plasma solutes. In trial two, when the fish were not growing, the $\delta^{13}C$ of red blood cells changed very little, whereas the plasma solute $\delta^{13}C$ changed considerably. Thus, when attempting to use stable isotopes to discern the diets of fishes that grow seasonally, it may help to use multiple tissues, some that turnover slowly, reflecting the longer-term signal, and some that turnover quickly, to address more recent dietary changes (Reich et al. 2008). In this regard, blood makes the perfect tissue to sample non-invasively, as it provides two "tissues" that turn over at different rates – plasma solutes, which are primarily synthesized in the liver (Turner and Hulme 1970; Adkins et al. 2002) and turnover quickly, and red blood cells, which turnover slowly. Fin tissue may also provide an additional tissue that turns over at a different rate than plasma solutes.

Correctly observing seasonal changes in diet is relevant to loricariid catfishes, as they experience wet and dry seasons each year in the Amazonian basin, which translates into drastic changes in water level, habitat, and food availability (Fink and Fink 1979). Nonogaki et al. (2007) measured the $\delta^{13}C$ in otoliths of grazing and wood-eating catfishes from the Brazilian Amazon. Because otoliths are laid down in annual growth increments, the isotopic signature of each otolith annulus can be used to monitor changes in $\delta^{13}C$ that occur over time. Indeed, Nonogaki and colleagues illustrated that the wood-eating *Panaque nigrolineatus* had $\delta^{13}C$ signatures that were relatively unchanging over time, whereas a grazing loricariid with a more variable diet, *Hypostomus regani*, showed temporal changes in otolith annulus $\delta^{13}C$. In agreement with this, all of the wild-caught wood-eating catfishes in this study (*P. cf. nigrolineatus* "Marañon", *P. nocturnus*, and *H. pyrineusi*) had red blood cell and plasma solute isotopic signatures that were similar to one another for each species, showing similarity in short-term and long-term dietary history. This may not be surprising, however, as wood-detritus is available in flooded forests during the wet season and in main river channels during the dry season.

The $\Delta^{13}C_{tissue\text{-}diet}$ values observed in trial one of this study varied by tissue, but were relatively consistent with discrimination factors of -1.5 to 3.4‰ reported for different tissues in the literature (Reich et al. 2008, and references therein). Plasma solutes and red blood cells had $\Delta^{13}C_{tissue\text{-}diet}$ values indistinguishable from the diet, whereas fin tissue was 1.75‰ enriched over lipid-extracted diet. The $\Delta^{15}N_{tissue\text{-}diet}$ values observed in *Pt. disjunctivus* tissues (Table 3-5) were higher than the widely used 3.4‰ for this isotope (Fry and Sherr 1984; Focken 2004; Fry 2007), but not inconsistent with $\Delta^{15}N_{tissue\text{-}diet}$ in herbivorous animals (Robbins et al. 2005). The major contributing factors to $\Delta^{15}N_{tissue\text{-}diet}$ are growth rate and dietary protein quality (Karasov and

Martínez del Rio 2007), with the latter defined as biological value, or how well the amino acid profile of the dietary protein meets the needs of the animal (Robbins et al. 2005). Herbivorous mammals generally consume protein that is considered low-quality, and hence have larger $\Delta^{15}N_{tissue\text{-}diet}$ than carnivores (Robbins et al. 2005). Mill et al. (2007) extended this argument for fishes showing that herbivores with high-intake of a low-protein diet have larger $\Delta^{15}N_{tissue\text{-}diet}$ (> 4‰) because of the low quality of the protein in the food. Undoubtedly, there were differences in the amino acid profile of the algal diet fed to *Pt. disjunctivus* in this study and the amino acid requirements of the fish, as the $\Delta^{15}N_{tissue\text{-}diet}$ varied from 4.08‰ in fin tissue to 5.13‰ in muscle. I do not know the quality of the protein available to the fish in the wild, but am confident assuming it is equal to or lower in quality than the protein in the algal diet offered to the fish in the lab. Therefore, I feel safe assuming the $\Delta^{15}N_{tissue\text{-}diet}$ values observed in the lab translate into the field.

In this regard, it appears possible to predict the trophic standing of the wild-caught wood-eating catfishes based on their isotopic signatures. According to Figure 3-6, all of these species consume carbon sources consistent with the isotopic signature of cellulose. However, the $\delta^{15}N$ signatures indicate that they are clearly enriched >6‰ over the $\delta^{15}N$ of biofilm or bulk wood, suggesting one of two things: first, the protein quality in the wild is so low that the $\Delta^{15}N_{tissue\text{-}diet}$ is larger than that observed in the lab; or second, they are getting their nitrogen from some intermediate source between the wood and the fish, such as wood-degrading microbes or insects. Boström et al. (2008) and Kohzu et al. (1999) showed that wood-degrading fungi can have $\delta^{15}N$ values ranging from -2 to 4‰, depending on the wood being digested. If the fungi degrading the wood in the upper Río Marañon have $\delta^{15}N$ values ranging from 2 to 4‰, and I assume the observed $\Delta^{15}N_{tissue\text{-}diet}$ of 4 to 5‰ for the fish, then the predicted $\delta^{15}N$ for the wood-eating

catfishes would be 6-9‰, exactly the range in which the fish fall in the wild. However, additional sampling to observe the $\delta^{15}N$ of the wood degrading fungi would be necessary to confirm or refute this assertion. Furthermore, species of *Spatuloricaria* are known to consume insects (de Melo et al. 2004), and the wood-eating catfishes have simlar $\delta^{15}N$ signatures to a sympatric species of *Spatuloricaria* collected from the Río Marañon (Figure 3-6). This suggests that the alleged wood-eating catfishes may supplement their diet with protein from animal sources.

True wood-eating termites that rely upon nitrogen-fixing bacteria in their digestive tracts (Slaytor and Chappell 1994) tend to have more depleted $\delta^{15}N$ values because of the depleted $\delta^{15}N$ signature of fixed nitrogen (Tayasu et al. 1997). Not only do they have lower $\delta^{15}N$ signatures (2-4‰), true wood-eating termites also have $\Delta^{15}N_{tissue\text{-}diet}$ values near zero over that of wood, whereas detritivorous termites have larger $\Delta^{15}N_{tissue\text{-}diet}$ values (>3‰), and $\delta^{15}N$ values near 8‰ (Tayasu et al. 1997), consistent with the wood-eating catfishes in this study. Thus, it does not appear likely that wood-eating catfishes rely upon nitrogen fixation in their digestive tracts, despite the presence of species of *Spirochetes* in their guts, as has been suggested (J.A. Nelson, pers. comm.). Furthermore, I did not observe any conglomerations of microbes anywhere along the digestive tracts of the wood-eating catfishes or of *Pt. disjunctivus* (Chapter 2), suggesting that any microbes isolated from their guts (Nelson et al. 1999) are ingested with their detrital diet as opposed to being endosymbiotic.

Wood-degrading fungi in streams are approximately 2‰ enriched in $\delta^{13}C$ in comparison to bulk wood (Kohzu et al. 1999; Boström et al. 2008), consistent with cellulose digestion. Therefore, digestion of fungi may provide an additional avenue for the wood-eating catfishes to have $\delta^{13}C$ signatures on par with that of wood-cellulose. This is further supported by the wood-

eating catfishes having elevated N-acetyl-β-D-glucosaminidase activities in their digestive tracts, and >1 mM N-acetyl-glucosamine in their intestinal fluid, both of which suggest digestion of fungal cell walls (Chapter 2; German et al. 2008).

Pterygoplichthys disjunctivus is clearly feeding at a lower trophic level than other fishes in Florida spring habitats (Figure 3-7). Gut content analyses of this species suggest they consume 45% green algae and diatoms and 45% detritus (Chapter 2). Green algae (Evans-White et al. 2001) and diatoms (Nichols and Garling 2000) can individually be more depleted in $\delta^{13}C$ (< -30‰) than the signature observed for periphyton (-29.26‰) in this study, which may explain the depleted $\delta^{13}C$ (-30.3‰) observed in *Pt. disjunctivus*. Furthermore, I do not know the isotopic signature of fine benthic organic matter (detritus) in the spring habitats. However, what is clear is that despite all of the similarities between *Pt. disjunctivus* and the wood-eating catfishes in terms of gut morphology and digestive physiology (Chapter 2), the former does not rely upon wood-carbon in the wild, at least in Florida. The enlarged spoon-shaped teeth of the wood-eating catfishes allow them to gouge into wood much more efficiently than the villiform teeth of *Pt. disjunctivus* (Nelson et al. 1999).

To my knowledge, this study is only the second to use a diet with ingredients of varying isotopic signatures to monitor assimilation of specific nutrients in a fish (Gamboa-Delgado et al. 2008). Gamboa-Delgado et al. (2008) determined the contribution of a readily assimable food item to growth in Senegalese Sole (*Solea senegalensis*). These authors designed a diet that featured one portion composed of *Artemia* of one isotopic signature, and another portion composed of an "inert ingredient" of a different isotopic signature. They then traced growth and changes in $\delta^{13}C$ over time, ultimately showing that the inert ingredient contributed very little to the growth of the fish. I attempted something very similar, but with results that were more

ambiguous. *Pterygoplichthys disjunctivus* lost weight on the artificial wood-detritus diet and was clearly in negative nitrogen balance. But, the $\delta^{13}C$ signatures of the fish suggest that they were assimilating at least some carbon from the food; the fish on the artificial wood-detritus diet clearly had different patterns of carbon isotopic incorporation than those that were deprived of food, and rates of carbon isotopic incorporation on par with the fish from the initial turnover experiment with the algal diet. This portion of the study suggested that *Pt. disjunctivus* could not assimilate the refractory polysaccharides from wood in any meaningful quantities, a result that is not surprising. In chapter 2, I showed that neither *Pt. disjunctivus* nor *Panaque nigrolineatus* (a true wood-eating catfish) could assimilate significant amounts of organic matter (1.72% and 3.35%, respectively) or cellulose (10% and 9%, respectively) from a strictly wood diet in the laboratory, and both species lost weight when consuming wood. This inability to digest cellulose in their digestive tracts comes from rapid gut transit and low cellulase activities, the latter of which appear to be ingested with their detrital diet rather than produced by endosymbionts in their guts. However, the fish are quite efficient at digesting soluble components of detritus (starch-like polysaccharides, α-glucosides), and disaccharides from wood degradation (β-glucosides, β-mannosides; Chapter 2), and thus, this supports my claim that they assimilated some soluble components from the wood in the artificial wood-detritus. The similarity between the predicted digestible $\delta^{13}C$ of the artificial wood-detritus and the $\delta^{13}C$ observed in the fish tissues further supports this contention (Table 3-7).

The fish consuming the artificial wood-detritus were definitely in negative nitrogen balance despite the presence of corn gluten meal, which is 70% protein and known to be a highly-digestible protein source for detritivorous fishes (e.g., Nile Tilapia, *Oreochromis niloticus*; Guimarães et al. 2008), in their food. Furthermore, we supplemented the artificial

wood-detritus with L-lysine to account for a deficiency of this amino acid in corn gluten meal (Guimarães et al. 2008). However, there are at least two, non-mutually exclusive reasons why the fish eating the artificial wood-detritus were in negative nitrogen balance. First, the diet was so dilute that I did not offer them enough food on a daily basis to meet their energetic or protein requirements (Raubenheimer and Simpson 1998). And second, the xanthan gum I used as a binding agent compromised the digestibility of the artificial wood-detritus via an increase in digesta viscosity (Leenhouwers et al. 2006; Lentle and Janssen 2008). Although I offered the fish 8% of their body mass per day of the artificial wood-detritus, exceeding what the fish consume when they naturally regulate intake on a wood diet in the laboratory (2-5% per day; Chapter 2), the food may have been lower in quality than I intended. Non-starch polysaccharides, such as guar gum and xanthan gum, are commonly used as binding agents in fish feeds. And, at 6% of the total mass of the diet, the chosen concentration of xanthan gum was the minimum at which the artificial wood-detritus pellets remained bound in water. However, this concentration of xanthan gum is also high enough to increase digesta viscosity and compromise the digestibility of energy and protein for fishes (Leenhouwers et al. 2006).

For example, Leenhouwers and colleagues showed that clariid catfishes consuming diets containing 8% guar gum had decreased energy and protein digestibility in comparison to fish eating a guar gum-free diet, and as a result, the fish consuming the 8% guar gum food increased intake (up to 3% of their body mass per day) to meet their nitrogen requirements. However, the diet consumed by those fishes was extremely high in protein (~50%), and thus, the fish were still able to meet their energetic and protein demands by increasing intake. Because the artificial wood-detritus offered to the fish in this experiment started off low in protein (7.75%, indicative of detritus; Bowen et al. 1995), any compromise of protein digestibility may have resulted in the

fish simply not being able to eat enough to meet their protein requirements. Detritivorous fishes are known to target detrital aggregates that are highest in protein (relatively speaking), or to supplement their low-quality diet with animal material (Bowen et al. 1995). Indeed, the natural diet of *Pt. disjunctivus* includes about 5% insects (Chapter 2). Thus, even though I attempted to provide adequate intake to the fish on the artificial wood-detritus, it simply may have not been enough for them to meet their nitrogen requirements given the increased viscosity of the food. However, it is difficult to imagine a different way of feeding a benthic fish the artificial wood-detritus short of force-feeding them, which has its own shortcomings (e.g., daily anesthesia, not allowing them to regulate intake, physical damage to the esophagus, etc.).

In conclusion, this study is one of the first to integrate laboratory and field isotopic investigations in fishes, and to show that blood and fin tissue can be used as non-invasively sampled tissues for stable isotopic analyses (Kelly et al. 2006; McIntyre and Flecker 2006). The results clearly show the importance of estimating turnover times and discrimination factors in the laboratory before gathering data in the field. If I assumed the commonly used discrimination factors of 1‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ I might draw very different conclusions from the field isotopic data. The isolation of the more "digestible" cellulosic portion of wood provided insight into the carbon sources for the wild-caught wood-eating catfishes that would have been missed by assuming a bulk wood signal for $\delta^{13}C$, as was done by Nonogaki et al. (2007). By using the proper positive and negative control groups in the laboratory feeding trial I was able to show that *Pt. disjunctivus* consuming the artificial wood-detritus simply weren't starving, but were definitely in negative nitrogen balance. The observation that plasma solute $\delta^{15}N$ values increased in the food-deprived fish argues for further evaluation of the effects of food deprivation on $\delta^{15}N$ signatures in ectothermic animals, which is not always consistent with

observations in mammals and birds (McCue and Pollock 2008). Overall, this study, in addition to my digestive investigations (Chapter 2), suggests that the wood-eating catfishes are not true "xylivores" like lower termites and beavers, and instead should be called detritivores, which specialize on degraded wood.

Within tropical freshwater habitats, wood is important to fish communities, as it provides structure, and a hard surface from which to feed (Wright and Flecker 2004). In this regard, wood in tropical freshwater systems is like the coral reef in a tropical marine system. Parrotfish are reef eroders in tropical marine systems, literally taking bites from the reef in search of detritus (Crossman et al. 2005). Thus, in the Amazon, the wood-eating catfishes are the "reef eroders", and can literally be considered the "Parrotfish of the Amazon".

Table 3-1. Overall isotopic signatures of the pelleted algae and artificial wood-detritus diets fed to *Ptergoplichthys disjunctivus*. Proportions of ingredients composing the artificial wood-detritus and the isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of each are also shown.

Diet component	g/100 g	$\delta^{13}C$	$\delta^{15}N$
Algae diet			
Total isotopic signature	-	-22.97 ± 0.02‰	2.43 ± 0.25‰
Lipid extracted signature	-	-22.02 ± 0.19‰	2.26 ± 0.16‰
Wood diet			
Bulk Wood[a]	80.0	-26.36 ± 0.25‰	2.13 ± 0.20‰
Wood cellulose[b]	(33.6)	-25.39 ± 0.12‰	N/A
Soluble wood components[c]	(16.0)	?	?
Corn gluten meal[d]	9.0	-12.98 ± 0.11‰	3.26 ± 0.25‰
Xanthan gum[e]	6.0	-9.64 ± 0.05‰	1.46 ± 0.01‰
Corn meal[d]	2.1	-10.65 ± 0.06‰	4.34 ± 0.31‰
L-Lysine[d]	1.0	-14.63 ± 0.00‰	0.01 ± 0.00‰
Vitamin premix[d]	1.0	-27.59 ± 1.12‰	2.63 ± 0.31‰
Trace mineral mix[d]	0.5	N/A	N/A
Water stable vitamin C[d]	0.4	-18.90 ± 0.01‰	N/A
Total isotopic signature	-	-23.42 ± 0.18‰	2.30 ± 0.20‰

Values are mean (± SEM).
[a] bulk-wood from decomposed water oak (*Quercus nigra*)
[b] cellulose isolated from wood following Gaudinski et al. (2005); proportion determined on a dry matter basis, and mass presented represents 42% (Chapter 2) of 80g of bulk wood; does not constitute an additional ingredient.
[c] 20% of water oak wood is composed of soluble degradation products (Chapter 2), the stable isotopic signatures of this fraction is unknown; does not constitute an additional ingredient
[d] gifts from Hartz-Mountain Corp. and intended specifically for use in fish food
[e] indigestible non-starch polysaccharide added as a binding agent to keep pellets bound in water

Table 3-2. Resources and animals collected from the Río Marañon, Perú, and their respective stable isotopic signatures.

Taxa	Diet	N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Plant/detrital material				
Grazed wood	-	4	-28.76 ± 0.39	1.31 ± 0.08
Cellulose (extracted from wood)	-	4	-26.90 ± 0.63	N/A
Epixylic biofilm	-	4	-29.25 ± 0.05	1.19 ± 0.06
Seston	-	4	-21.83 ± 0.36	3.13 ± 0.53
Crustacea*				
Shrimp	Detritus?	3	-25.08 ± 0.18	9.01 ± 0.26
Crabs	Detritus?	3	-27.14 ± 0.68	4.47 ± 0.30
Catfishes*				
Lamontichthys filamentosus	Algae/Biofilm?	6	-29.09 ± 0.38	8.20 ± 0.17
Spatuloricaria sp.	Insects/Seeds[2]	3	-26.32 ± 0.06	8.72 ± 0.09
Panaque nocturnus	Wood/detritus[3]	6	-26.95 ± 0.09	7.84 ± 0.15
P. cf. nigrolineatus "Marañon"[1]	Wood/detritus[3]	6	-26.15 ± 0.36	8.10 ± 0.20
P. albomaculatus	Wood[4]	6	-26.89 ± 0.22	7.55 ± 0.16
P. gnomus	Wood[4]	6	-27.22 ± 0.52	7.89 ± 0.39
Hypostomus pyrineusi	Wood/detritus[3]	6	-26.79 ± 0.21	7.01 ± 0.16

* Isotopes measured in lipid extracted muscle tissue of crustaceans and catfishes.
[1] This is an undescribed species of *Panaque* belonging to the *P. nigrolineatus* clade.
[2] de Melo et al. (2004)
[3] Chapter 2
[4] Schaefer and Stewart (1993)

Table 3-3. Taxa collected from Wekiva Springs, Florida and their stable isotopic signatures.

Taxa	Diet	N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Plant/detrital material				
Duckweed	-	4	-30.45 ± 0.22	18.91 ± 0.21
Lily pads	-	4	-26.51 ± 0.10	13.24 ± 0.13
Periphyton	-	4	-29.26 ± 0.25	7.81 ± 0.31
Coarse Benthic Organic Matter	-	4	-29.23 ± 0.43	3.43 ± 0.29
Wood	-	4	-25.24 ± 0.11	2.01 ± 0.20
Cellulose (extracted from wood)	-	4	-24.09 ± 0.23	N/A
Mollusca				
Snail*	Grazer?	3	-28.83 ± 0.15	20.70 ± 0.05
Crustacea				
Crayfish*	Shredder	3	-29.46 ± 0.21	19.58 ± 0.35
Fishes*				
Lepisosteus platyrhinchus (Florida Gar)	Fish	3	-29.22 ± 0.17	15.00 ± 0.31
Micropterus salmoides (Bass)	Fish	3	-27.27 ± 0.82	20.31 ± 0.40
Lepomis punctatus (Spotted sunfish)	Invertebrates	3	-26.72 ± 0.48	18.56 ± 1.75
L. macrochirus (Bluegill sunfish)	Invertebrates	3	-25.96 ± 0.92	16.46 ± 1.77
Gambusia holbrooki (Mosquito fish)	Invertebrates	3	-25.07 ± 0.29	24.24 ± 0.38
Lucania goodei (Bluefin killifish)	Invertebrates	3	-30.36 ± 1.51	22.13 ± 1.02
Heterandria formosa (Least killifish)	Invertebrates	3	-29.02 ± 0.48	21.51 ± 0.33
Pterygoplichthys disjunctivus	Algae/Detritus	9	-28.62 ± 0.65	11.51 ± 0.42

* Isotopes measured in lipid extracted muscle tissue of molluscs, crustaceans, and small fishes (*G. holbrooki*, *Lu. goodei*, *H. Formosa*), and lipid extracted fin clips from larger fishes.

Table 3-4. The isotopic incorporation of carbon from an algal diet (trial one) into tissues of *Pterygoplichthys disjunctivus* using the equation: $\delta^{13}C(t) = \delta^{13}C(\infty) + [\delta^{13}C(0) - \delta^{13}C(\infty)]e^{-\lambda t}$.

Tissue	Equation	λ vs. k_{gt} *t*-test	$\Delta^{13}C_{tissue\text{-}diet}$	Average residence time (days)
Plasma solutes	$-22.57 - 6.99e^{-0.026(time)}$	13.54**	0.12 ± 0.08	38.46 ± 9.62
Red blood cells	$-20.74 - 8.05e^{-0.006(time)}$	4.15*	- 0.13 ± 0.30	166.67 ± 10.56
Fin clips	$-20.57 - 5.66e^{-0.050(time)}$	9.82**	1.75 ± 0.15**	20.00 ± 2.80

Note: * and ** indicate significant differences ($P < 0.05$, and $P < 0.001$, respectively) between the fractional rate of isotopic incorporation (λ) and the growth rate ($k_{gt} = 0.0017$ day^{-1}) for all tissue types. $\Delta^{13}C$ is the mean (± SE) diet-tissue discrimination factor, and ** indicates significant difference from 0 with 1-sample t-test ($P < 0.001$); no difference from 0 was detected for $\Delta^{13}C_{tissue\text{-}diet}$ for plasma solutes or red blood cells. Because the fin clip tissue was lipid extracted prior to analyses, the $\Delta^{13}C_{tissue\text{-}diet}$ for fin clips were calculated against lipid extracted diet, whereas the $\Delta^{13}C_{tissue\text{-}diet}$ for plasma solutes and red blood cells were calculated against non-lipid extracted diet. Average residence (± SE) time was estimated as $1/\lambda$.

Table 3-5. The isotopic incorporation of nitrogen from an algal diet (trial one) into tissues of *Pterygoplichthys disjunctivus* using the equation: $\delta^{15}N(t) = \delta^{15}N(\infty) + [\delta^{15}N(0) - \delta^{15}N(\infty)]e^{-\lambda t}$.

Tissue	Equation	λ vs. k_{gt} *t*-test	$\Delta^{15}N_{tissue\text{-}diet}$	Average residence time (days)
Plasma solutes	$6.97 + 3.57e^{-0.213(time)}$	33.58**	4.39 ± 0.05*	4.69 ± 0.24
Red blood cells	$8.42 + 5.83e^{-0.216(time)}$	31.66**	5.17 ± 0.13*	4.63 ± 1.94
Fin clips	$6.51 + 4.48e^{-0.031(time)}$	7.20**	4.08 ± 0.14*	32.26 ± 6.24

** indicates significant difference ($P < 0.001$) between the fractional rate of isotopic incorporation (λ) and the growth rate (k_{gt}) for all tissue types. $\Delta^{15}N_{tissue\text{-}diet}$ is the mean (± SE) diet-tissue discrimination factor, and * indicates significant difference from 0 with 1-sample t-test ($P < 0.001$). Average residence time (± SE) was estimated as $1/\lambda$.

Table 3-6. Isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of three tissues of wild-caught *Pterygoplichthys disjunctivus* collected from Wekiva Springs, Florida during the course of the laboratory feeding experiment.

Day	$\delta^{13}C$ Plasma	$\delta^{13}C$ RBC	$\delta^{13}C$ Fin	$\delta^{15}N$ Plasma	$\delta^{15}N$ RBC	$\delta^{15}N$ Fin
1	-29.21 ± 0.42^{a}	-29.26 ± 0.44	-25.85 ± 0.82^{a}	12.82 ± 0.14	13.12 ± 0.45	11.09 ± 0.26
100	-31.78 ± 0.79^{b}	-31.40 ± 0.81	-29.68 ± 0.40^{b}	11.14 ± 1.18	11.54 ± 1.49	12.22 ± 0.54
210	-31.55 ± 0.51^{ab}	-31.26 ± 0.44	-29.60 ± 1.14^{b}	12.82 ± 0.14	13.12 ± 0.46	10.73 ± 1.17
	$F_{2,8} = 5.76$	$F_{2,8} = 4.16$	$F_{2,10} = 8.41$	$F_{2,8} = 1.96$	$F_{2,8} = 0.96$	$F_{2,10} = 1.33$
	$P = 0.040$	$P = 0.074$	$P = 0.011$	$P = 0.221$	$P = 0.436$	$P = 0.317$

Values are mean (± SEM). Isotopic values were compared across days for each tissue with ANOVA followed by a Tukey's HSD with a family error rate of $P = 0.05$. Values for a particular isotope and tissue that share a superscript letter are not significantly different. RBC = red blood cells.

Table 3-7. The isotopic incorporation of carbon from an artificial wood-detritus diet (trial two) into tissues of *Pterygoplichthys disjunctivus*.

Tissue	Equation[A]	r^2	$\delta^{13}C_{tissue}$ (final) – $\delta^{13}C_{diet}$ (final)$_{predicted}$[B]
Plasma solutes	$-21.16 - 1.83e^{-0.023(time)}$	0.71	0.41
Red blood cells	$-20.17 - 2.52e^{-0.002(time)}$	0.48	-0.24
Fin clips	0.014(time) -20.72	0.87	2.18

[A] The non-linear procedure using equation $\delta^{13}C(t) = \delta^{13}C(\infty) + [\delta^{13}C(0) - \delta^{13}C(\infty)]e^{-\lambda t}$ described the isotopic incorporations for plasma and red blood cells reasonably well, but a linear procedure was necessary for the fin clip samples.

[B] This value represents the difference between the final $\delta^{13}C$ observed for the tissues at the end of the experiment minus that predicted by a linear, concentration-dependent mixing model (Martínez del Rio and Wolf 2005) for the artificial wood-detritus ingredients, plus the expected $\Delta^{13}C_{tissue\text{-}diet}$ (i.e., the $\Delta^{13}C_{tissue\text{-}diet}$ observed in trial one) for each tissue; $\delta^{13}C$ (final)$_{predicted}$ = -21.79‰.

Table 3-8. The isotopic incorporation of nitrogen from an artificial wood-detritus diet (trial two) into tissues of *Pterygoplichthys disjunctivus*.

Tissue	Equation[A]	r^2	$\delta^{15}N_{tissue}$ (final) – $\delta^{15}N_{diet}$ (final)$_{predicted}$[B]
Plasma solutes	$10.12 - 3.03e^{-0.005(time)}$	0.83	0.79
Red blood cells	$0.011(time) + 7.21$	0.82	-0.64
Fin clips	$8.57 – 2.15e^{-0.021(time)}$	0.94	1.29

[A] The non-linear procedure using equation $\delta^{15}N(t) = \delta^{15}N(\infty) + [\delta^{15}N(0) - \delta^{15}N(\infty)]e^{-\lambda t}$ described the isotopic incorporations for plasma and fin clips reasonably well, but a linear procedure was necessary for the red blood cell samples.

[B] This value represents the difference between the final $\delta^{15}N$ observed for the tissues at the end of the experiment minus that predicted by a linear, concentration-dependent mixing model (Martínez del Rio and Wolf 2005) for the artificial wood-detritus ingredients, plus the expected $\Delta^{15}N_{tissue\text{-}diet}$ (i.e., the $\Delta^{15}N_{tissue\text{-}diet}$ observed in trial one) for each tissue; $\delta^{15}N$ (final)$_{predicted}$ = 3.25‰.

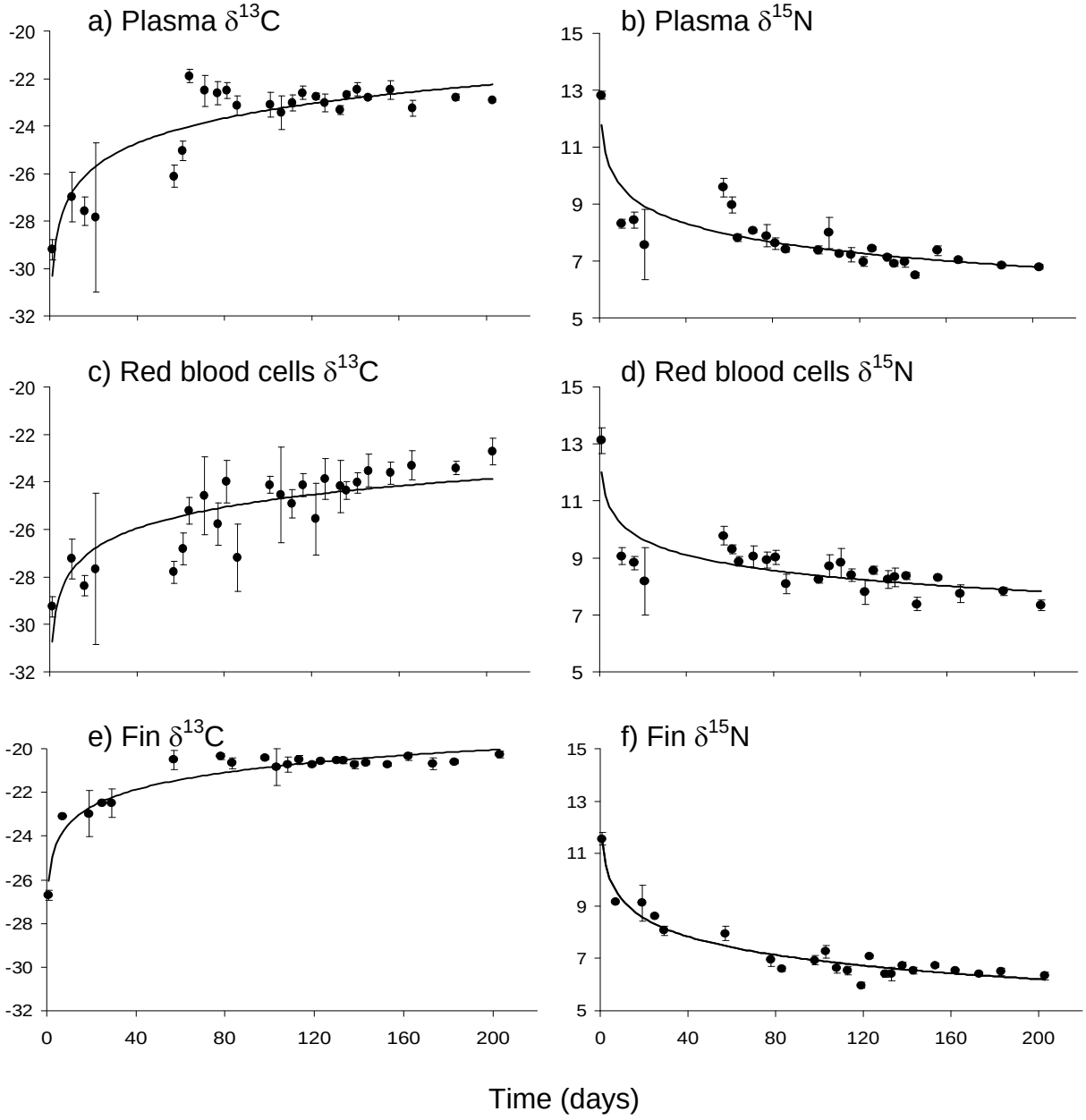

Figure 3-1. Changes in $\delta^{13}C$ and $\delta^{15}N$ in *Pterygoplichthys disjunctivus* 0-203 days after a diet switch. Values are mean (± SEM). Curves were fit by a nonlinear routine with the equation $\delta X(t) = \delta X(\infty) + [\delta X(0) - \delta X(\infty)]e^{-\lambda t}$ (see "Statistical Analyses").

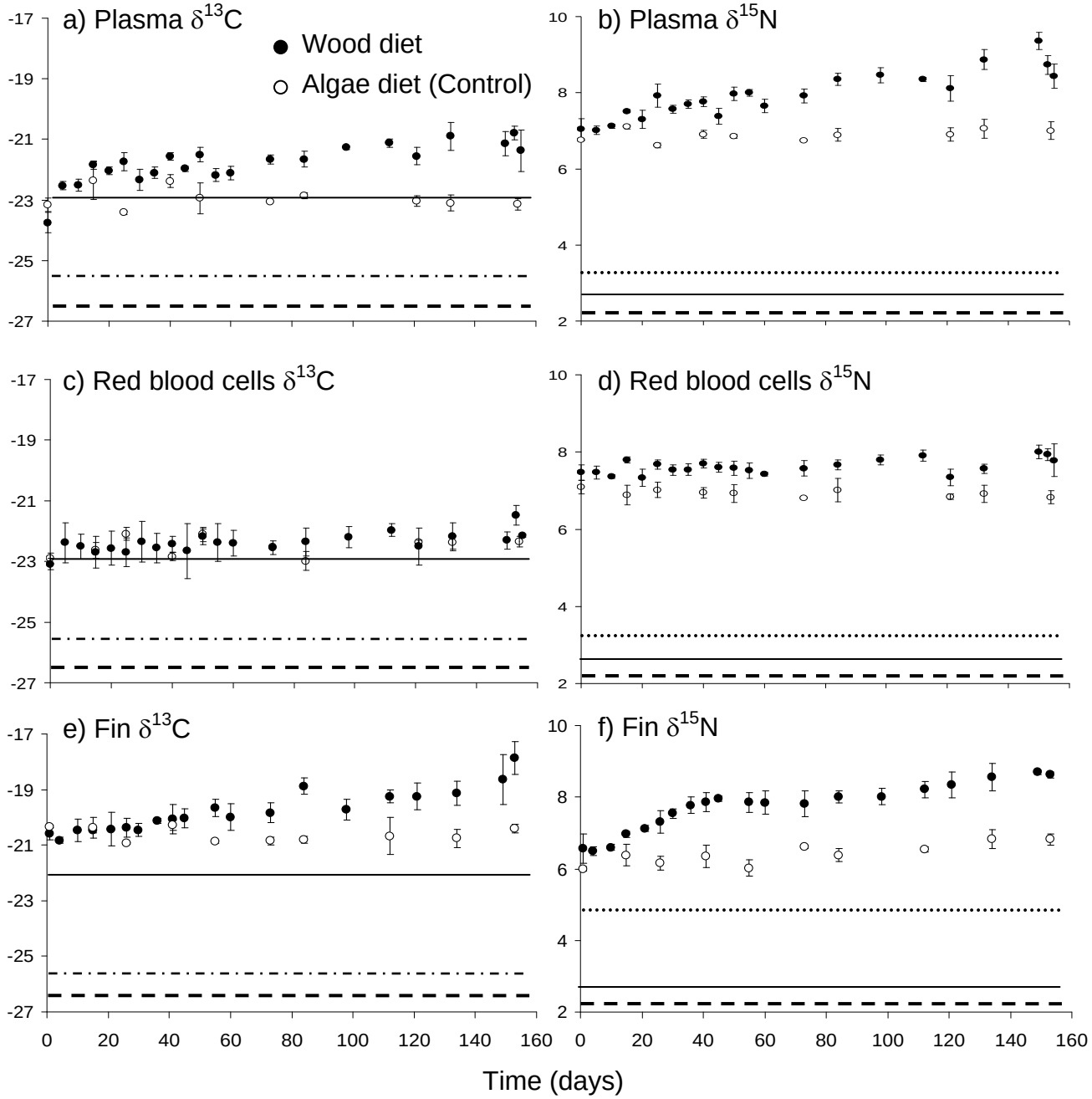

Figure 3-2. Changes in $\delta^{13}C$ and $\delta^{15}N$ in *Pterygoplichthys disjunctivus* across 155 days while consuming an artificial wood-detritus diet or an algal diet (positive control). Values are mean (± SEM). The solid lines represent the $\delta^{13}C$ and $\delta^{15}N$ signatures of the algal diet, whereas the dashed lines represent the $\delta^{13}C$ and $\delta^{15}N$ signatures of the bulk wood in the artificial wood-detritus diet. In the carbon plots, the dash-dot line represents the $\delta^{13}C$ of cellulose isolated from the wood. In the nitrogen plots, the dotted line represents the $\delta^{15}N$ signature of the corn products representing the soluble component of the artificial wood-detritus diet. The $\delta^{13}C$ of the corn products (-11.82‰) is off the scale of the carbon plots. Equations for all relationships presented in Tables 7 and 8.

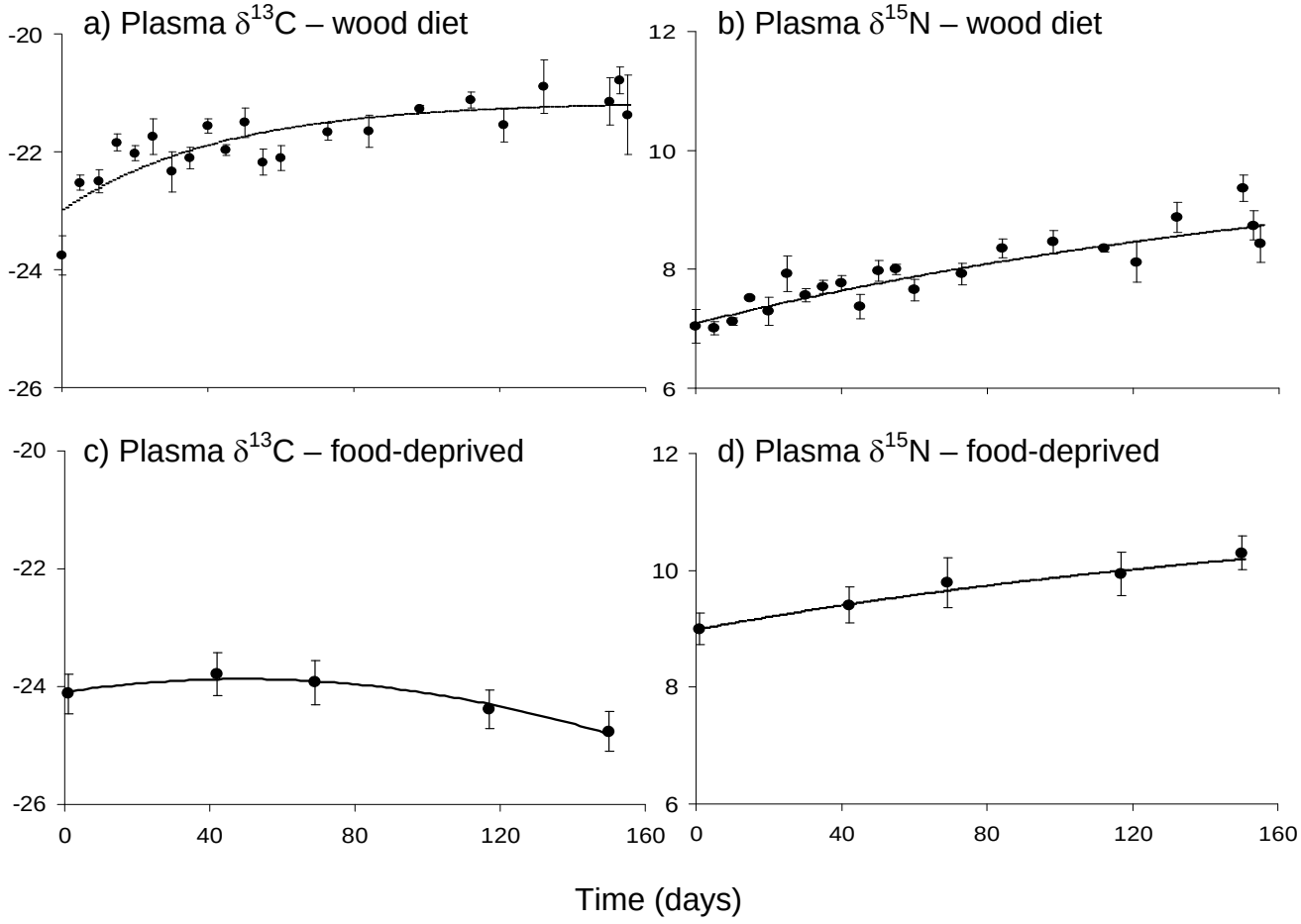

Figure 3-3. Changes in $\delta^{13}C$ and $\delta^{15}N$ in plasma solutes of *Pterygoplichthys disjunctivus* across 155 days while consuming an artificial wood-detritus diet or deprived of food (negative control). Values are mean (± SEM). Curves for the fish consuming the artificial wood-detritus, and the $\delta^{15}N$ of the food-deprived fish were fit with the equation $\delta X(t) = \delta X(\infty) + [\delta X(0) - \delta X(\infty)]e^{-\lambda t}$, whereas the $\delta^{13}C$ of the food-deprived fish was best fit by a polynomial distribution ($y = 0.00009x^2 + 0.009x - 24.09$; $r^2 = 0.97$).

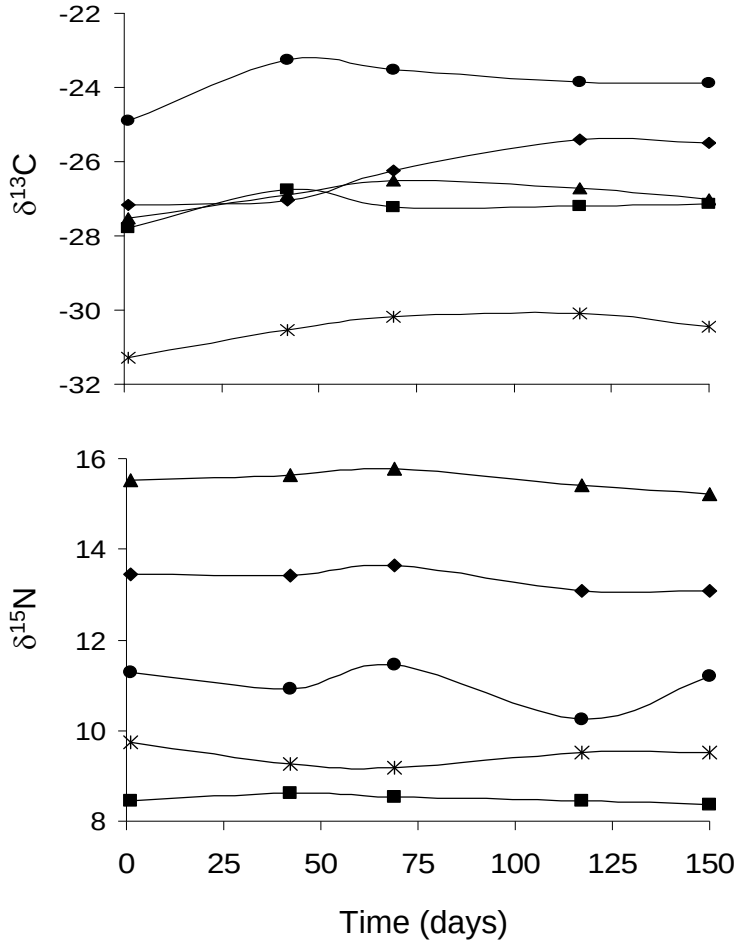

Figure 3-4. Changes in $\delta^{13}C$ and $\delta^{15}N$ in red blood cells of *Pterygoplichthys disjunctivus* that were deprived of food across 155 days. Symbols represent measurements in an individual fish, and lines are provided to trace how individual fish changed over the course of the experiment.

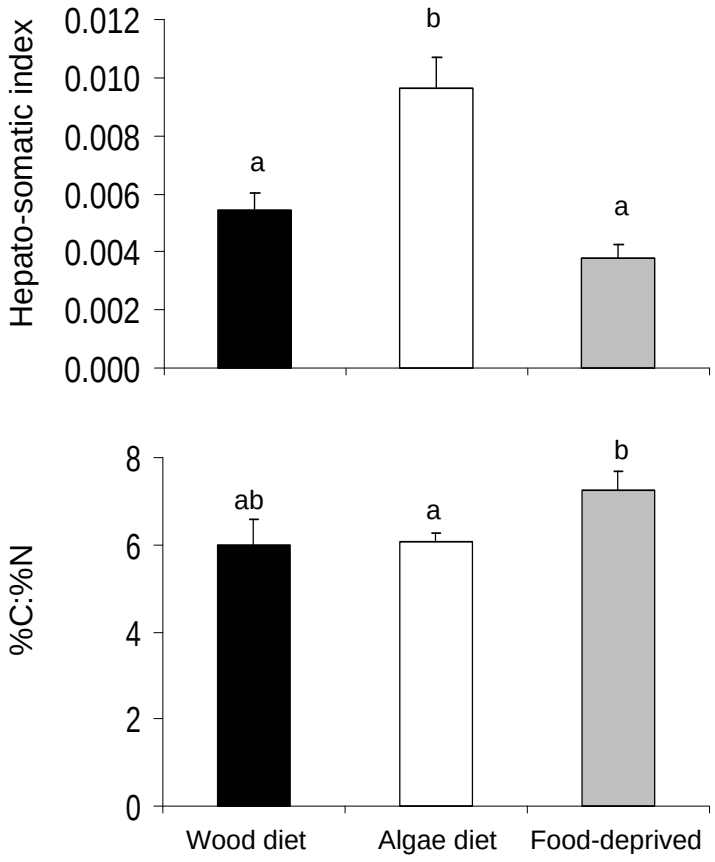

Figure 3-5. Hepato-somatic indices and %C:%N ratios in the livers of *Pterygoplichthys disjunctivus* fed an artificial wood-detritus diet, an algal diet, or those that were deprived of food. Values are mean (± SEM). Values compared among groups for an index with ANOVA followed by Tukey's HSD with a family error rate of P = 0.05. Bars that share a letter are not significantly different.

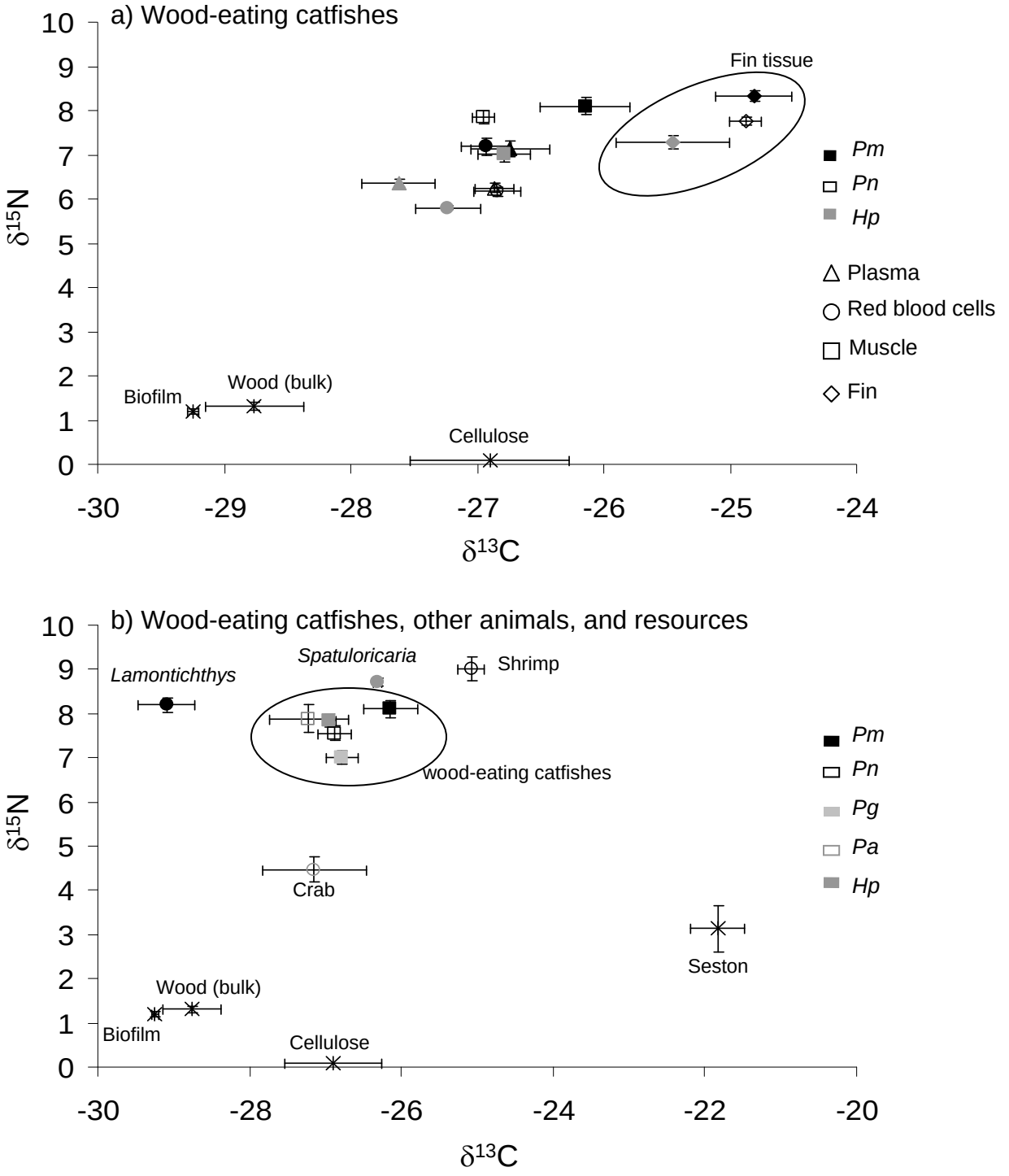

Figure 3-6. Carbon and nitrogen dual-isotope plots of animals and resources collected in the upper Río Marañon, Perú – a) shows the different tissues of only wood-eating catfishes and potential resources, b) shows wood-eating catfishes in addition to other animals. Only lipid-extracted muscle tissue was used to analyze the isotopic signatures of the animals in plot b. *Pm = Panaque cf. nigrolineatus* "Marañon", *Pn = P. nocturnus*, *Pg = P. gnomus*, *Pa = P. albomaculatus*, *Hp = Hypostomus pyrineusi*.

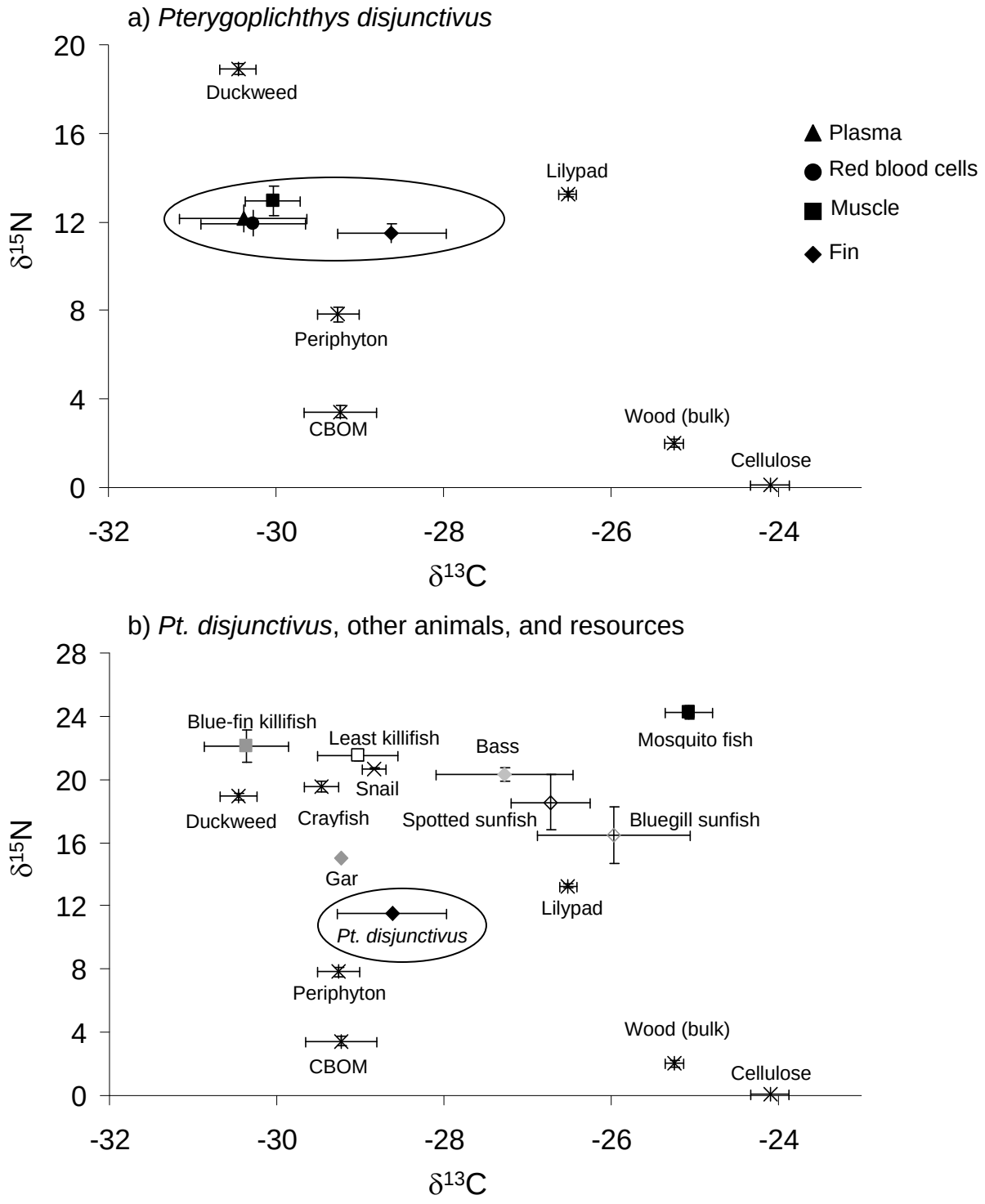

Figure 3-7. Carbon and nitrogen dual-isotope plots of animals and resources collected Wekiva Springs, FL, USA – a) shows the different tissues of only *Pterygoplichthys disjunctivus* and potential resources, b) shows *Pt. disjunctivus* in addition to other animals. Only lipid-extracted fin tissue (large fish) and muscle tissue (invertebrates and small fish, see Table 3) was used to analyze the isotopic signatures of the animals in plot b. CBOM = coarse benthic organic matter.

CHAPTER 4
CONCLUSIONS

The purpose of this dissertation was to explore the capabilities of xylivorous and detritivorous catfishes to digest and assimilate wood, and to determine the extent these animals rely on endosymbiotic microorganisms in this process. The data presented in chapter two unequivocally support the null hypothesis that these fishes do not and cannot digest wood in their digestive tracts in contrast to some of the better known xylivores (e.g., termites, beavers). The following key points all suggest that the alleged xylivorous catfishes are actually just detritivores and digest primarily soluble components of their diet rather than cellulose and other cell wall polysaccharides:

- They have extremely long, narrow digestive tracts (11-18X their body lengths) with no kinks, valves, or caeca to slow the flow of digesta through the gut.
- The intestinal folding patterns and microvilli surface area decrease moving distally along the intestine suggesting that most absorption occurs in the proximal and mid intestine. This is corroborated by soluble carbohydrates being detectable exclusively in the proximal and mid intestines of the fish and disappearing in the distal intestine, indicating that most sugars are actually absorbed in the proximal and mid intestine.
- The pH conditions in the intestine were alkaline, and the redox potentials were clearly negative, signifying that the fishes' intestines are anaerobic. However, the absence of any conglomerations of microbes in the TEM images indicates that the fish do not harbor endosymbionts in their guts.
- The extremely low and unchanging SCFA concentrations along the fishes' intestines suggest that, despite the anaerobic conditions, fermentation of cellulose is not occurring at a rapid pace, and is not likely a mechanism for cellulose digestion in these fishes.
- The low and variable cellulase and xylanase activities, and the fact that these activities did not increase towards the fishes' distal intestines signify that these enzymes are ingested with the food (i.e., decaying wood) rather than produced by endosymbionts. In fact, almost without exception, enzyme activities in the microbial extracts decreased moving distally along the intestine, supporting this supposition.
- Activity levels of enzymes that digest soluble polysaccharides (i.e., amylase, laminarinase) were one to five orders of magnitude greater than the cellulase and xylanase activities, indicating that the fishes preferentially digest soluble polysaccharides rather than refractory ones.

- The soluble components of wood degradation (i.e., β-glucosides, β-mannosides) were efficiently digested and assimilated by the fish. This is especially true for β-glucosidase, as the K_m values of this enzyme in the gut walls of the fish were an order of magnitude lower than the K_m values in the microbial extracts.

- Chitinous compounds may be important energy and nitrogen sources to the fish as significant amounts (>1 mM) of N-acetyl-glucosamine (the monomer of chitin) were detected in the intestinal fluids of the fish. Furthermore, the fishes' N-acetyl-β-D-glucosaminidase (NAG) activities were elevated compared to the microbial extracts.

- The elevated protease activities (trypsin and aminopeptidase), and the heavily skewed trypsin:lipase ratios (400:1) in the fishes alimentary tracts are consistent with other detritivorous animals rather than xylivorous ones.

- The fish passed wood through the gut too quickly (< 4 hours) to allow symbionts to digest cellulose, and there was no retention of small particles along the gut.

- Consequently, the digestibilities of cell wall compounds (NDF, ADF, lignin) were low in the loricariid catfishes, and the fish lost weight on a wood diet, showing that they cannot digest and thrive on a wood diet.

In chapter three, I attempted to discern, using stable isotopes, whether the wood-eating and detritivorous catfishes could assimilate carbon from wood and to what extent they could subsist on a wood diet. The results from this part of the study also clearly suggest that loricariid catfishes primarily digest the soluble components of their diet. The key findings of this chapter were:

- From an isotopic ($\delta^{13}C$ and $\delta^{15}N$) standpoint, that the non-invasively sampled tissues of plasma solutes, red blood cells, and fin tissue turnover and match the isotopic signature of the diet on sufficiently different time scales to be used to track the diets of fishes in the wild.

- Laboratory investigations of wood assimilation using stable isotopes indicate that the detritivorous fish, *Pterygoplichthys disjunctivus*, is incapable of assimilating structural polysaccharides from wood, and that they can only assimilate the more soluble components of wood detritus.

- Wild-caught wood-eating catfishes from Perú do show that they are assimilating carbon ($\delta^{13}C$) with a baseline consistent with that of cellulose, but that their nitrogen ($\delta^{15}N$) signature is too enriched to reflect a digestive strategy consistent with true wood digestion via endosymbionts (like termites and beavers).

- Wild-caught *Pt. disjunctivus* from Orlando, FL, are feeding at a lower trophic level than other native Floridian fishes, and appear to be using detritus, periphyton, and diatoms as a resource as opposed to wood like the true wood-eating catfishes.

The bottom line of this dissertation is that each and every finding in this study says the same thing: the structure and function of the digestive tract of the wood-eating catfishes is not different from detritivorous ones and neither the wood-eating nor the detritivorous species are capable of digesting wood in any significant amount. However, these fish do consume food, detritus, which is in the process of being degraded by microbes in the environment. The fishes take advantage of this degradation by siphoning off soluble components of environmental microbial wood degradation (e.g., β-glucosides, β-mannosides), and probably by digesting environmental microbes themselves. Swift et al. (1979) called this kind of feeding pattern the "external rumen", making reference to the specialized forestomach of a cow. However, rather than harboring microbial endosymbionts in a specialized region of the gut and reaping the benefits internally, the fish allow the microbial action to occur outside of their bodies. Then, they consume the detritus, and take what they are equipped to, the soluble components, and excrete the rest in their feces. Thus, these fishes should not be referred to as "xylivorous", but rather as "wood-eating detritivores".

The way in which these fishes feed is likely very important ecologically in the context of nutrient cycling. Wood-eating detritivorous catfishes take a coarse form of detritus (woody debris) and reduce it to particles generally less than 1-mm in diameter. Furthermore, they add nitrogen to the excreta. Thus, by increasing the surface area of the wood particles and by adding nitrogen, they are creating a perfect milieu, in their feces, for further microbial degradation of wood in the environment. Given that these fishes inhabit rivers and streams in tropical rainforests, the amount of wood that falls in the waterways is enormous. Without the grazing

activities of these fishes on coarse woody debris, the waterways of tropical South America might be choked with natural logjams. Furthermore, with the increase in deforestation in many rainforest habitats, more and more wood is finding its way into Amazonian waterways. At the same time, local human populations in some of these regions (e.g., northwestern Perú) are becoming more reliant on fishes as a source of protein because they have nearly hunted all mammalian and bird taxa to extinction (DPG, pers. obs.). Many of the wood-eating detritivorous fishes have lower fecundity than some of the other fishes (DPG, pers. obs.), and are, therefore, susceptible to overfishing. So, coupled with pollution, the increasing removal of fish from Amazonian waterways, including the wood-eating detritivorous species, may spell serious trouble in terms of wood degradation and nutrient cycling in these habitats. The people are reliant upon the rivers for water, food, and for transportation. There simply are too few roads in the outlying areas of the Amazonian rainforest on which to travel by car. Thus, if continued, the uncontrolled removal of these fishes from the rivers may lead to transportation issues, and unforeseen ecosystem-wide consequences.

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BIOGRAPHICAL SKETCH

Donovan Parks German was born in Orange, CA, in 1975. He received a Bachelor of Arts degree in Marine Science from the University of San Diego where he was a student athlete playing football and lacrosse. During his undergraduate career, Donovan participated in numerous research projects, including one in Bermuda, and another that resulted in a publication (Sturz, A., D.P. German and D. Putnam (1998). Salton Sea Geothermal Area Mud Pots. In *Geology and Geothermal Resources of the Imperial and Mexicali Valleys*, L. Lindsey and W. Hample (eds). San Diego Association of Geologists publication 98-1, Pg. 109-128). He then attended California State University Fullerton, where he received a Master of Science degree. For his thesis research he studied the digestive physiology of herbivorous and carnivorous fishes under the stewardship of Dr. Michael H. Horn. While at CSU Fullerton he was heavily involved in community outreach through the world-peace Buddhist organization, SGI-USA, for which he was awarded the President's Associates Outstanding Graduate Student Award. He also received the Best Thesis award in the Department of Biological Science at CSU Fullerton. He then began his doctoral training at the University of Florida under the tutelage of Dr. David H. Evans, to whom he is gratefully endebted. While at UF, Donovan was part of the NSF SPICE program, which aimed at increasing scientific literacy and general interest in science in under-resourced middle schools in east Gainesville. He also taught Functional Vertebrate Anatomy Lab, and the graduate course Integrative Principles of Zoology at the University. In January 2009, he began a postdoctoral research position in the Department of Ecology and Evolutionary Biology at University of California Irvine under Dr. Steven D. Allison.