STATE OF OUR KNOWLEDGE ON THE DIVERSITY AND FEEDING ECOLOGY OF THE SUCKERMOUTH ARMORED CATFISHES (LORICARIIDAE)

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EXTENDED ABSTRACT - ONLY DO NOT CITE

With currently about 640 species recognized as valid, the Loricariidae (suckermouth armored catfishes) of South America represents the largest catfish family, and perhaps the largest algivorous family of fishes. With such a tremendous assemblage of species, it is clear that loricariid catfishes have a great impact on stream ecosystems; however, it is currently unknown how so many loricariid catfishes can exist and how so many can occur at the same location at the same time. I will discuss what is known about feeding in loricariids and current hypotheses on their evolution and the reasons why they are so diverse.

Loricariid catfishes are recognized by a ventral, suctorial mouth and jaws that are designed for rasping along submerged surfaces (Schaefer, 1987, 1988). Loricariids typically feed on algae and the organic biofilm that covers submerged surfaces (Power, 1984). Loricariids typically possess a very long gut where the food is processed.

To date, there has been no satisfactory hypothesis to explain the diversity of loricariid catfishes. Schaefer and Lauder (1986, 1996) suggest that the diversity of morphology in loricariids is a result of the loss of a biomechanical couple linking the opercle and the lower jaw. Loss of this couple has allowed loricariid to radiate into a wide variety of niches involving algivory/detritivory. The decoupling hypothesis has also been used to explain the large number of cichlids in Africa vs. South America (Liem, 1973). However, the decoupling hypothesis is a circular argument – find a group that is diverse, find a mechanical couple that has been lost, and then use the lost couple to explain the diversity. The decoupling hypothesis does not take into account the ecology of the organisms and fails for many reasons with loricariid catfishes. Phylogenetic analysis suggests that primitive loricariids retain the couple suggested by Schaefer and Lauder (1986, 1996) to be important in the evolution of loricariids, and many loricariid groups have re-evolved a similar couple. Given that decoupling is unlikely to be a major influence on the evolution of loricariids, what can explain the diversity of loricariids?

I have examined the diversity of loricariids from an ecological and biogeographical stand-point. I have attempted to determine the feeding-modes followed by loricariid catfishes. Although most are algivorous/detritivorous, many are insectivorous, granivorous, moluscivorous, or scavengers. In addition, exploitation of the algivorous/detritivorous niche is complex. By examining jaw and tooth morphology, it is clear that not all loricariids are exploiting algae and detritus in the same way.

I hypothesize that a particular substrate can be fed upon by several different groups of loricariids. Long, straight-jawed species feed on filamentous algae. Short, slightly-angled-jawed species feed on tougher material closer to the surface. And, on logs, short, highly-angled-jawed species feed on either wood or material growing in the wood. In addition, observations in the field suggest that loricariids are found on particular substrates such as plants, rocks, sand, or logs in a particular flow regime (riffle, run, pool). By conservatively factoring in the number of permutations of feeding areas available at a particular location, it can be determined that many potential feeding areas can be available at a given location. The value would be decreased somewhat by species that are attracted to a particular habitat regardless of flow or if a particular habitat is not available at a location. A particular niche may be further subdivided by species using the feeding area at different times during the day or season or in slightly different ways. This method will be used to explore the loricariid assemblages at certain well-studied localities.

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HERBIVORY BY FISHES ON CORAL REEFS;

THE CURRENT STATE OF PLAY

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Coral reefs covering 600,000 km ^{2} represent only a small area (0.17%) of the planet's surface but support a disproportionately high component of the world's shallow water fish fauna. The 3,000 species of fishes that occur in the Indo-West-Pacific, a region that includes approximately 73% of the world's coral reefs constitutes 12% of the worlds fish species and 22% of marine fish species (Helfman *et al* 1997). These fishes are dominated by a single order the Perciformes which includes the majority of the marine herbivorous fishes. Coral reefs are biogenic structures that occur in shallow waters, subject to intense tropical light regimes and characterised by complex structural features. Reefs also exhibit high levels of primary productivity (1500 to 5000 g C/m^{2}

/yr). The dominant primary producers are assemblages of algal turfs that occur in complex mixture of green, red and brown algae and subject to rapid turnover with 20-90% of turf algal production being consumed daily (Hatcher 1997). A variety of experimental investigations have identified perciform fishes as important consumers of reef algal turfs (Hatcher & Larkum 1983).

Herbivory through grazing fishes is a defining feature of coral reefs, especially in the Indo-Pacific (Steneck 1988). The high local diversity of this fish fauna, with up to 40 species of vertebrates co-occurring within small areas makes this a unique herbivore assemblage. Analysis of the patterns of carbon flow in reef systems supports the view that herbivorous grazing fishes are a key part of the reef ecosystem processes. Coral reefs constitute oases of high primary productivity in tropical oceans (Hatcher 1997). It is assumed that the low standing crops of nutritious plant assemblages are rapidly consumed, assimilated and incorporated into food webs that underwrite the high levels of secondary production of coral reefs. A closer examination of coral reefs as the exemplar of marine fish herbivory reveals a lack of critical information relating to the process itself. For example, herbivory in terrestrial systems is understood in functional terms, the ability to derive energy from the fibrous portions of plants through fermentation by symbiotic microbes. This approach provides a link between the structure of plant foods, the nature of the resources accessed by herbivores and the internal processes by which materials are processed, assimilated and incorporated into food chains. In marine systems herbivores are defined as animals that remove plant material, a definition that is inadequate for understanding food processing and energy flow in marine ecosystems. The problem becomes more acute when one considers the wide variety of nominally herbivorous species on coral reefs and the varied composition of the algal materials that must serve as substrates for fish digestion (Kloareg & Quatrano 1988).

Compared to terrestrial system we are ignorant of important functional aspects of herbivory on coral reefs. This review will address some of the consequences of this and suggest a bipartite approach to future investigations of herbivory. Firstly we need better information on the nature of the food resources harvested by the different taxa and diversity of food processing modes that occur. Secondly we require information on the digestive physiology of the herbivores themselves. Our approach includes a survey of the taxa of nominally herbivorous fishes, surgeonfishes (Acanthuridae), parrotfishes (Scaridae) and drummers (Kyphosidae). Microbial fermentation is a key digestive process in terrestrial herbivores. We commenced our study with a comparative survey of short chain fatty acids in the alimentary tracts of nominally herbivorous fish species. This survey suggests a wide variety of food-processing modes confirmed by studies of diet and alimentary tract turnover times. An important finding of the survey was the dominance of fishes consuming detrital materials. An analysis of detrital material itself has revealed that detritus has twice the total hydrolysable amino acids of algae. Our conclusions are; i) there is a wide variety of food processing modes amongst many of the nominal herbivores on coral reefs; ii) many herbivores are in fact detritivores; iii) detritus itself has a surprisingly high higher nutritional profile; iv) although we can identify dietary items and feeding modes we lack information on the digestive physiology of the fishes concerned. In order to understand herbivory in marine and terrestrial ecosystems we need to initiate a program of study into the digestive physiology of the major groups of nominal herbivores in marine systems.

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DIGESTION IN MARINE HERBIVOROUS FISHES:

IS SOME FIBRE NEEDED IN THE DIET?

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At present we lack a framework that allows us to understand the relationships between different diets and the diversity of digestive systems seen in marine herbivorous fishes. Such a framework would allow us to interpret diet composition, e.g. secondary metabolites, in the context of nutrition. An appropriate context does exist in the terrestrial literature (e.g. Van Soest 1994), where the fate of plant production is modeled with respect to the digestive processes of herbivores. I will review five inter-related areas (digestive mechanisms, plant composition, the concept of fibre, ingestion and throughput rates, and microbial processes) where I believe our progress is hampered by lack of knowledge and erroneous assumptions.

Digestive mechanisms

An examination of the literature suggests digestion in marine herbivorous fishes is largely understood in the context of the mechanisms for lysis of algal cells first proposed by Lobel in 1981 and developed by Horn (e.g. Horn 1989). These mechanisms are acid lysis in the stomach, trituration in a gizzard-like stomach, trituration in a pharyngeal mill, and microbial fermentation in the hindgut. While these were originally (and sensibly) proposed as mechanisms for cell lysis, they seem to be understood by many workers as digestive mechanisms, i.e. mechanisms that enable fishes to digest algal nutrients. Perhaps as a result, we know very little about the digestive enzymes of these fishes, a problem that has been identified previously (Horn 1989).

Plant composition

Marine algae contain a higher proportion of protein and a smaller proportion of structural components than terrestrial plants (Choat and Clements 1998). On this superficial basis the algal diet of marine herbivorous fishes may seem less refractory than the diet of terrestrial herbivores. However, the diversity of marine algae, and their disparity in terms of constituent polysaccharides, may constrain digestive specialization in herbivorous fishes. This may be especially marked in grazing fishes, which ingest a broad range of dietary substrates. It is widely acknowledged that algal cell wall polysaccharides are unlikely to be hydrolysed by endogenous digestive enzymes, yet even chlorophyte and rhodophyte starches vary in their susceptibility to fish amylases (Zemke White and Clements 1999). We need a better understanding of algal carbohydrates, so the relationships between diet choice (e.g. secondary metabolite deterrence) and digestive systems can be determined.

The concept of fibre

The diets of terrestrial vertebrate herbivores are generally explained in terms of fibre content. Animals with high fibre diets typically rely on gastrointestinal symbioses with microorganisms to extract sufficient energy from their food. We have no concept of fibre for marine herbivorous fishes. In other words, we are unable to predict how different algal components are degraded by endogenous (i.e. host fish) and exogenous (i.e. microbial) enzymes. As a result, we have no framework within which to characterise diets in relation to different digestive systems, i.e. predict diet quality. Given the disparity of algal carbohydrates, it seems likely that much of the material ingested by herbivorous fishes will be refractory to endogenous enzymes, i.e. fibrous.

Ingestion and throughput rates

Fish could respond to fibrous algal diets in at least three ways: (a) by employing high ingestion rates and rapid turnover of gut contents, (b) supplement their algal diet with animal material (or detritus) if they are incapable of properly digesting the plant component, and (c) salvage energy via the use of digestive symbioses with gut microorganisms. We have an extremely poor understanding of throughput rates in fishes, as illustrated by comparison with terrestrial studies. A model for quantifying relationships between diet quality, digestive processes and body weight in ungulates (Illius and Gordon 1992) contains 9 rate variables.

We do not have estimates for any of these variables in marine herbivorous fishes.

Microbial processes

It is becoming increasingly apparent that microorganisms play a role in digestion in some marine herbivorous fishes, as they do in most terrestrial vertebrate herbivores. However, work in this area is in its infancy. To date no published data exist on fermentation rates in these fishes. We have very little idea of the substrate utilisation (for an exception see Seeto et al. 1996) or species composition of these microbial communities. No published information exists on the stoichiometry of fermentation in these systems, i.e. the energetics of fermentation and the loss of short-chain fatty acids (SCFA) to microbial processes. We do not know whether the metabolic physiology of host fishes is geared towards a metabolic diet of SCFA. One current problem seems to be a reliance upon *in vitro* studies of isolated microorganisms, as these studies tell us little about processes taking place in microbial communities *in vivo*.

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HINDGUT FERMENTATION IN THE SILVER DRUMMER,

A MARINE HERBIVOROUS FISH

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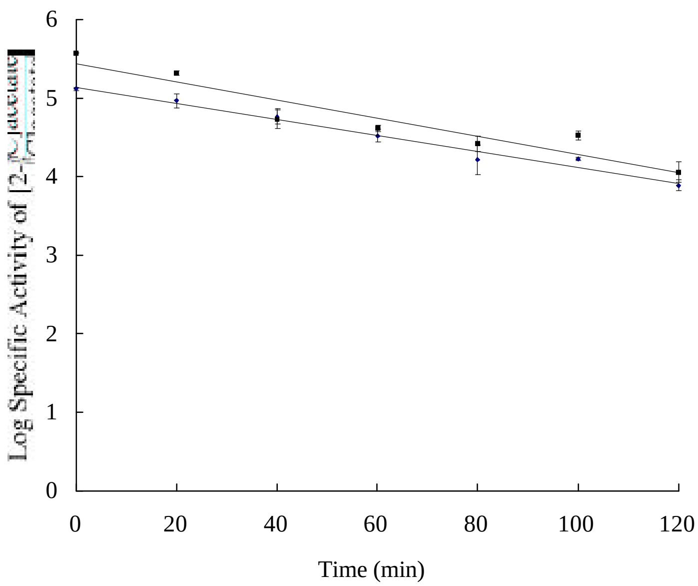
EXTENDED ABSTRACT ONLY – DO NOT CITE

Gastrointestinal fermentation by symbiotic microorganisms facilitates plant digestion in herbivorous reptiles, birds and mammals[1,2]. Gut symbionts ferment cell wall components of the diet to short-chain fatty acids (SCFA), which are then taken up and used for metabolism by the host animal. Some marine herbivorous fishes contain elevated levels of SCFA, especially acetate, in the gut.[3] However, the contribution of fermentation to energy balance in fishes is unknown[4]. We estimated acetate production in herbivorous silver drummer, *Kyphosus sydneyanus* (F. Kyphosidae), collected from coastal reefs off northeastern New Zealand. E*x vivo* preparations of freshly caught silver drummer were maintained with the respiratory and circulatory systems intact. Labelled acetate was injected into ligated hindgut sections, and gut fluid sampled at intervals for periods of up to 3 hours. Acetate production rates were comparable with those found in the hindgut of herbivorous reptiles[5] and mammals[6]. These and other stoichiometric measurements indicate over 98% of microbially-produced acetate is taken up across the gut wall by silver drummer. This work suggests hindgut fermentation can function as an effective digestive strategy in an ectothermic, warm temperate marine fish.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ARE SEDIMENT-INGESTING GIZZARD SHAD

DETRITIVORES, BACTERIVORES OR HERBIVORES?

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Gizzard shad, *Dorosoma cepedianum*, are often important members of temperate freshwater ecosystems and, due to their high biomass, can influence ecosystem nutrient transport (Schaus et al. 1997). These fish “telescope” the pelagic food web by feeding on detritus. Many detritivores ingest sediment, a nutritionally dilute diet, and glean their metabolic requirements from it. Detritivores have anatomical and physiological adaptations to survive on this low energy diet by maximizing energy and nutrient recovery while minimizing their losses (Penry and Jumars 1987). Although gizzard shad show anatomical features indicating that they are adapted to consuming detritus including subterminal mouths, pharyngeal organs and long digestive tracts approximately 3-fold greater than the body length of the fish, adult gizzard shad are known to consume other food items in addition to detritus and are described as omnivores (Bodola 1966; Yako et al. 1996). The life history, ecological importance to aquatic systems, feeding preferences, and anatomical features of the digestive system of the gizzard shad are well studied but knowledge of the food resource of sediment-ingesting gizzard shad is limited. To determine the food resource of sediment-ingesting gizzard shad we characterized their digestive physiology and used solubilization of fatty acids within their digestive tract as tracers for digested foods.

We characterized digestive physiology of sediment-ingesting gizzard shad by determining gut enzyme activities using fluorescently labeled artificial substrate and gut surfactant concentration by drop diameter measurements of serial diluted gut fluid. Sediment-ingesting gizzard shad showed gut juice lipase:protease ratios of approximately 1 and gut surfactant concentrations 10 times critical micelle concentration (Figure 1). The digestive physiology of sediment-ingesting

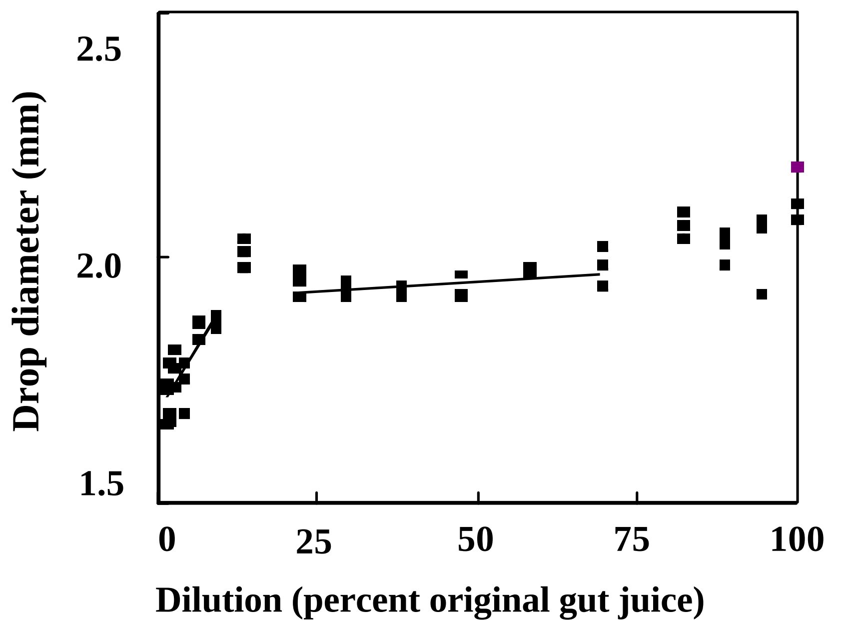

Figure 1. Serial dilution of ceca region gut fluid demonstrating the presence of surfactants at concentrations above critical micelle concentration. The rapid and linear decrease in drop diameter at dilutions < 13% original gut juice results from the loss of micelles in the solution.

invertebrate detritivores are characterized by having gut juice lipase:protease ratios <1 and gut surfactant concentrations 10 times critical micelle concentration (Mayer et al. 1997). In contrast, sediment-dwelling invertebrate predators are characterized by having gut juice lipase:protease ratios >1 and gut surfactant concentrations lower than critical micelle concentration. Gut juice lipase:protease ratios for gizzard shad were not as low as found in invertebrate detritivores. The extended length of the gizzard shad digestive tract may allow a degree of evolutionary flexibility not present in many invertebrate phyla; this is supported by our observation that protease activity was high for the greatest distance in the intestine. These data indicate that gizzard shad digestive physiology is adapted to sediment ingestion, which allows for the efficient utilization of the food resources available in mud meals.

The type and quantity of food digested by gizzard shad was estimated by determining the dissolved fatty acids (both neutral and polar lipid) in four sections (ceca region [CR], fore intestine [FI], mid intestine [MI], hind intestine [HI]) of the shad's digestive system. Dissolved lipid was present in the ceca region at a concentration of approximately 5 mg ml^{-1} gut fluid and decreased to less than 2 mg ml^{-1} gut fluid in the mid and hind intestine. It was assumed that all the fatty acids present in the gut fluid originated from the membranes of living organisms. Based on this assumption, three estimates of calories gained from living biomass present in the sediment were made and compared to a previously measured estimate of basal metabolism (Table 1). All three estimates indicated that there was sufficient digestible living biomass in sediments to support gizzard shad caloric requirements. Fatty acids from both microeukaryotes and bacteria were detected in gizzard shad gut fluid. Community structure analysis of the digested food resources indicates that photosynthetic microeukaryotes and bacteria comprise 55% and 45% of the food resource, respectively.

Table 1. Calories gained from living biomass (vs. 1,004 calories respired day^{-1}*)

Method	Parameters	Calories day^{-1}
Digested	Lipid solubilized in CR Passages through CR day^{-1} Assume equal dissolution Assume dissolution is the rate limiting step	1,268
Absorbed	[$lipid_{CR}$ – $lipid_{MI\text{-}HI}$] Passages through gut day^{-1} Assume equal dissolution	1,077
(Table 1 con't)		
Assimilated	[$ASDM_{GZ}$ – $ASDM_{FECES}$]* Passages through gut day^{-1} Cal_{ASDM}*	1,122

* Data from Pierce (1980).

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DIGESTIVE AMYLOHYDROLITIC PROFILE

OF *Colossoma macropomum, Piaractus mesopotamicus*

AND THEIR HYBRID:

A COMPARATIVE APPROACH

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Carbohydrates constitute one of the three prime components of the fish diet that are used as an energy source to support animal growth and occur as natural constituents or are added in food as ingredients or additives. It has been proposed that an appropriate level of this nutrient in the diet can ensure maximum utilization of the other nutrients (Voragen, 1998; Peragon *et al*., 1999).
Enzymatic digestion of carbohydrates (starch) has been detected in the digestive tract of several species so far studied. This enzymatic hydrolysis of starch is started by the action of amylase activity.
According to Smith (1980) the enzyme activity reflects the herbivorous, omnivorous or carnivorous nature of the species studied. The knowledge of digestive enzymes in fish is important because it can clarify some aspects of their nutrient physiology and help to solve some nutritional problems on fish feeding in aquaculture.

Many authors agree that a study of amylohydrolitic profile in fish can reveal the capacity of different species to use carbohydrates. Therefore, the amylohydrolitic profile of two omnivorous *Colossoma macropomum* (tambaqui) and *Piaractus mesopotamicus* (pacu) and their hybrid tambacu were studied in the digestive tract (stomach, cecus and proximal and distal intestine).
C. macropomum inhabits Amazonas-Orinoco drainage system and is one of the most promising native freshwater fish. *P. mesopotamicus* inhabits the Paraguai-Uruguai rivers, which is extended also into the swamp of Mato Grosso, Brazil. Tambacu is a hybrid resulted from the crossing of *C. macropomum* female and *P. mesopotamicus* male.

Young fish of tambaqui (n=6), pacu (n=6) and tambacu (n=10) were kept in well-aerated tanks of 250 liters at 25 º c under natural photoperiods. Fish were fed pellets with 7 percent of cornstarch, protein contents of 26 percent and total energy of 2700 kcal. The nourishment period was 15 days. Then they were killed by a blow to the head and punched in the spinal cord. The digestive tract was excised, rinsed with cold saline, separated and kept in a freezer at -20°c for the posterior analysis.

Amylohydrolitic assays were performed in 0.2M citrate/phosphate buffer pH 7.0 by using 2.7% of starch solution and 0,5% NaCl as cofactor, as described by Bernfeld (1955). The enzyme reaction was performed at 25ºC lasting 30 minutes. The reaction was killed by addition of barium-zinc solutions' to a final concentration of 5% $ZnSO_4$ and 0.3N $Ba(OH)_2$. Glucose, as reaction product, was determined by Park-Johnson's method (1949).

Dates were analyzed by non-parametric method of wilcoxon and mann-whitney (zar, 1984). The accepted level of confidence was 5%.
The results (Table 1) show that the starch was hydrolyzed in every region of digestive tract of pacu, tambaqui and the hybrid tambacu. However, cecus was significantly more active than the other regions. The presence of amylase activity in the three species indicates that the starch hydrolyzed probably has an important role in the energetic metabolism.

+Table 1. Amylase activity in digestive tract in three species of fish, determined at 25 º C.

Species	Digestive Tract			
	Stomach	Cecus	Proximal Intestine	Distal Intestine
Tambaqui	40.64 ± 0.39	137.1 ± 8.1	74.1 ± 3.3	23.33 ± 0.14
Pacu	16.16 ± 3.5	78.09 ± 3.5	58.92 ± 7.6	45.02 ± 3.1
Tambacu (Hybrid)	37.56 ± 4.9	382.4 ± 19.3	100.8 ± 3.8	42.38 ±5.9

Amylase activity was expressed as µmols of glucose. $Min^{-1}.g^{-1}$. Wet tissue. Values are mean ± se. The comparison among the species showed the amylohydrolitic profile from the tambacu hybrid cecus more efficient than pacu and tambaqui one, suggesting a remarkable expression of the hybrid carbohydrases.

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CELLULOLYTIC ENZYMES PRODUCED BY

AEROBIC FUNGI ISOLATED FROM THE GUT

OF *Panaque maccus*.

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The family Loricariidae is restricted to the Neotropics, yet contains at least 70 genera with more than 600 described species (Isbrücker 1980) and many additional undescribed species. An evolutionary success of this magnitude in such a restricted geographic region demands an explanation, however the biology of the loricariids is virtually. This radiation of the Loricariidae occurred almost entirely at the trophic level of primary consumer and entirely since the Cretaceous separation of South America from Africa, *circa* 80 million years ago. Novel arrangements of the jaw musculature and new and variable tooth morphology are thought to have contributed to the unprecedented evolutionary success of the loricariids (Schaefer and Lauder 1986; Schaefer and Stewart 1993), but the importance of other biological factors to this radiation are unknown. Besides being mostly herbivorous, some loricariids may be specialists for terrestrial plant material that falls into the water (allocthonous carbon). The few loricariids that have been analyzed derive most of their carbon from allocthonous sources (Araujo-Lima *et al.* 1986). One genus of loricariid catfish, *Panaque*, even has representatives that have been shown from field collections and laboratory experiments to be utilizing wood in their diet. Seven species of *Panaque* have been found to consume wood in their native habitats and two species will consume, extract energy from, and grow on a wood-only diet in the laboratory (Schaefer and Stewart 1993; Nelson *et al.* 1999). This is the first and only suggestion of wood-eating in bony fishes (class Osteichthyes), a taxonomic grouping of more than 25,000 species.

The purpose of the present work was to see whether enzymes involved in the break down of complex carbon polymers found in wood are made by several species of fungi recently found in *Panaque* guts.

Methods

Specimens of *Panaque maccus* (Schaefer & Stewart, 1993) were obtained from aquarium wholesalers as they arrived from South America and were either sacrificed immediately or held in untreated water in aquaria well innoculated with the water they were transported in. These steps were taken to maximize the chance that the fungi we were working with were actually native gut flora.

Microbial cultures

Animals were killed by an overdose of the anaesthetic 3-amino benzoic acid ethyl ester (MS-222). Gastrointestinal tracts were isolated under sterile conditions, divided into sections and inoculated into broth cultures. Aerobic isolations took place on a sterile bench top while anaerobic cultures were started in a sterile, anaerobic glove box (Coy Laboratories) under an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen. The methods of Wubah *et al.*, (1991) were used to start fungal cultures. Briefly, an antibiotic cocktail solution (0.1 ml per tube) containing streptomycin sulfate (20 µg/ml), penicillin G (80 µg/ml), chloramphenicol (30 µg/ml) and oxytetracycline (15 µg/ml) were added to each Hungate tube to inhibit bacterial growth. After 48 h, the tubes were checked for fungal growth. When fungal thalli and zoospores were observed, 0.5 ml. of the culture supernatant was inoculated onto a 100 x 15 mm Petri plate and covered with BAM containing antibiotics and 2% agar. Further purification was carried out until axenic cultures were obtained.

Enzyme assays

Broth cultures were dialyzed against 0.1M citrate buffer (pH 5.0) containing 0.01% sodium azide prior to being assayed. Carbon polymer degradative capacity was measured with two broad types of enzyme assay:
1) various glycosidase activities *e.g.* ß-glucosidase (ß-D-glucoside glucohydrolase E.C.3.2.1.21) and ß-xylosidase (ß-D-xylanoxylohydrolase

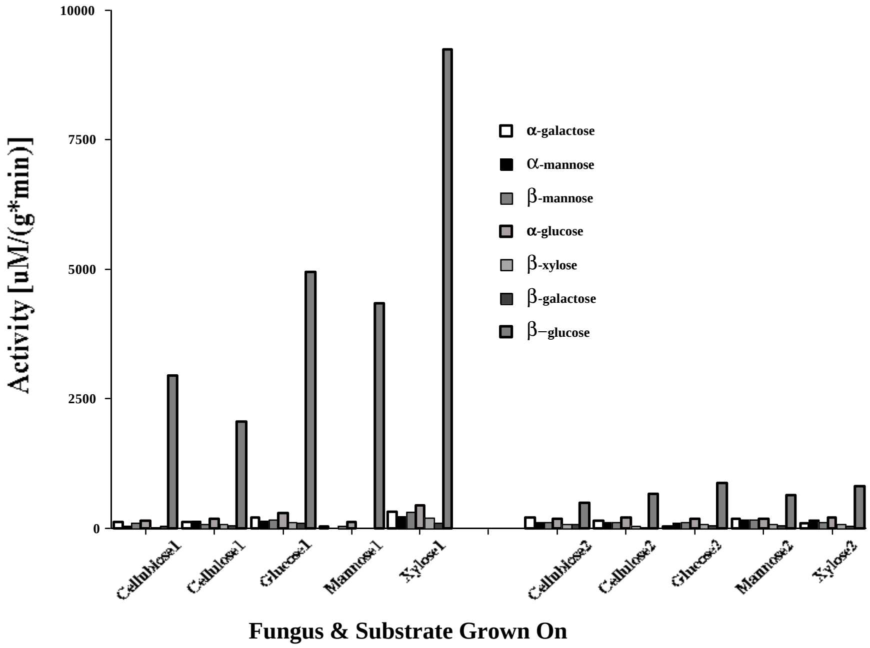
E.C.3.2.1.37) etc. were assayed using a variation Agrawal & Bahl (1968). The appropriate *p*-nitrophenyl conjugated substrate (Sigma® 0.5g•cm^{-3} in 0.1M citrate buffer pH 5.0) was incubated with diluted gut contents (volume fraction 0.33) for 2 hours at 40°C. After terminating the reaction with Na_2CO_3, the liberated *p*-nitrophenol was quantified by recording optical density (OD) in excess of a heat-inactivated control at 425nm. Concentrations were calculated by referencing the OD to a standard curve prepared with pure *p*-nitrophenol (Sigma®)

2) ß-endoglucanase (endo 1,4,-D-glucan 4-glucanohydrolase(E.C.3.2.1.4) activity was assayed by incubating diluted gut contents with either a 1% solution of carboxymethylcellulose in 0.1M pH 5.0 citrate buffer, or a suspension of 20µ crystalline cellulose (Sigmacell 20) in the same buffer, all to a final sodium azide concentration of 0.01% to prevent bacterial activity during the assay. Hemicellulolytic activity was assayed analogously, except that mannan and xylan were the substrates. Reducing sugar groups were assayed after 24 hours of incubation at 40 C, using a modification of the Somogyi method (Nelson, 1944). Optical density at 500nm, in excess of a control blank which had gut contents added after the 24 h incubation, was measured and recorded on a Zeiss™ PM6 spectrophotometer. Concentrations were calculated by reference to a standard curve prepared with glucose. All activities are expressed per gram of soluble protein as determined by the bicinchoninic acid method (Pierce®).

Results and Discussion

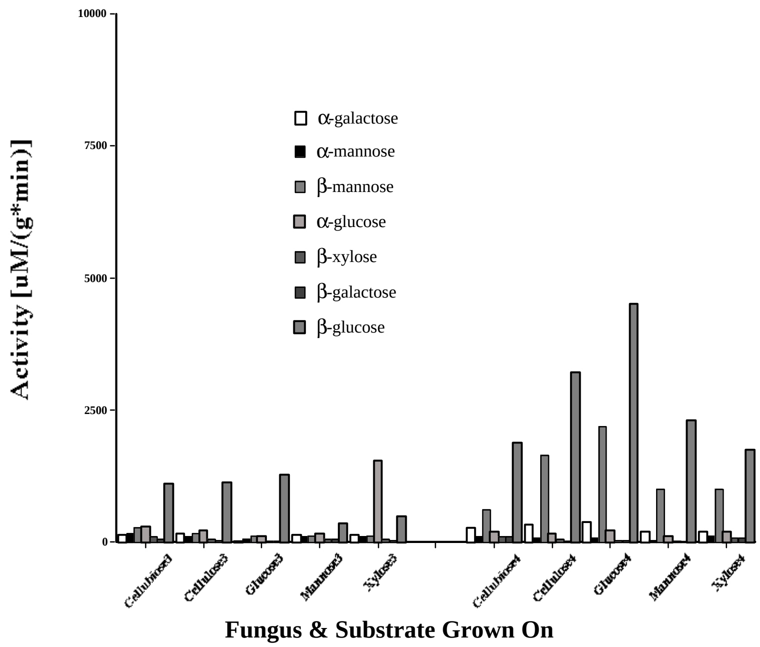
We were able to develop pure cultures of four separate fungi from the gastrointestinal tracts of *Panaque maccus*. Two of the pure fungal cultures (1 & 4) exhibited substantial hydrolytic activity against the ß-glycosidic linkages connecting the monomers of cellulose (ß-glucosidase activity) and, to a lesser extent, the various disaccharide linkages found in hemicellulose (Fig. 1).

Figure 1a: glycosidase activities of unidentified fungi # 1 and 2



The other two pure fungal cultures (2 &3) had some activity against ß-glycosidic linkages and one culture of fungus # 3 grown on xylan had substantial α-glucosidase activity (Fig. 1b). Interestingly, except for this one culture, substantial α-glycosidic activity, like that common to vertebrate intestines, was virtually absent from these fungal cultures (Fig. 1). Extracellular enzyme activity was relatively independent of the media upon which the fungi grew.

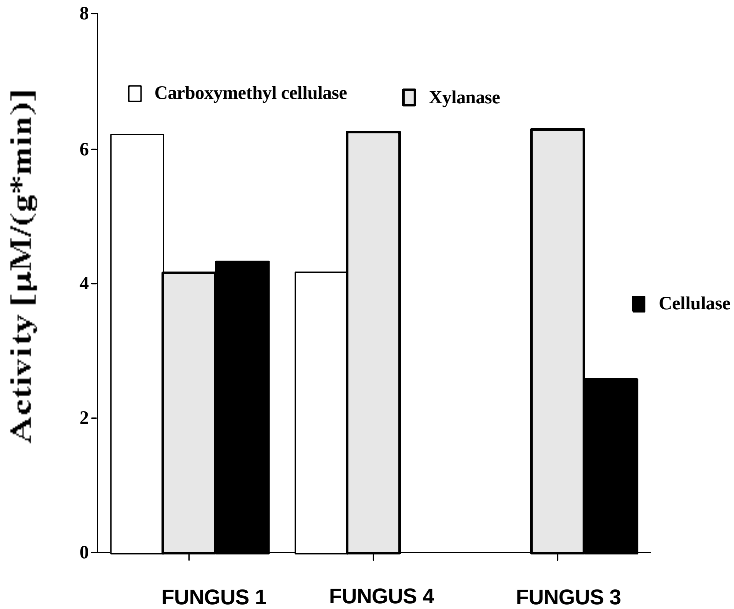
Figure 1b: glycosidase activities of unidentified fungi # 3 and #4



There are several possible explanations for why these fungi only made extracellular enzymes directed against the more difficult to break ß-glycosidic linkages and not the easier α-glycosidic ones. The simplest is that these fungi are descended from wood-decomposition specialists that stopped producing α-glycosidases because of the paucity of α-linkages in the carbon polymers of wood. A second possibility is that because there are bacteria present in *Panaque* guts that produce α-glycosidases (Nelson *et al*. 1999), and because the fish undoubtedly also produce α-glycosidases, the fungi are merely "avoiding" the waste of energy inherent in competing for these resources. A third possibility is that the fungi are quite capable of producing α-glycosidases, but our culture conditions did not induce them to do so. The production of α-glucosidase by fungus #3 when grown on xylan supports this latter interpretation.

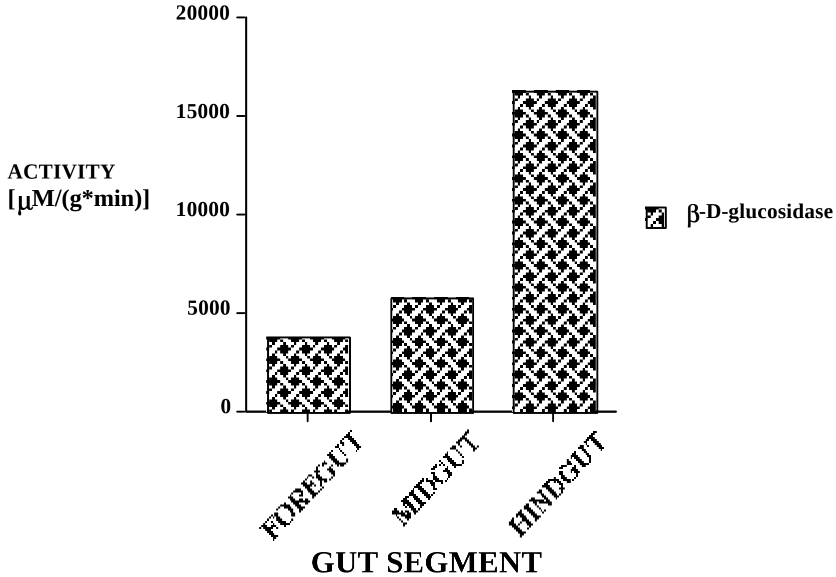
Three of the fungal cultures had substantially lower, but still measurable, enzymatic activity in their gut contents against the larger carbon polymers that were tested (Fig. 2). Only positive results are shown. There was insufficient material to run this assay based upon the substrate that the fungi grew on.

Figure 2: Endoglucanase activities of fungal cultures



There appears to be some localization of these fungi within the gastrointestinal tracts of *Panaqu*e. For instance, for two separate fish, the initial cultures were begun from approximate "one thirds" of the GI tract. The extracts from these initial cultures of both fish showed a trend towards increasing ß-glucosidase activity as one proceeded through the GI tract (*e.g.* Figure 3). Again, one interpretation of these results is that the fungi aren't competing with the animal or the bacteria for the "easy energy" to be obtained in the forgut; instead the fungi are focusing on the more difficult to obtain carbon as it appears in later gut segments. Confirmation of this idea awaits more thorough experimentation.

Figure 3 βD-glucosidase activity from cultures of different gut segments



Cultures of fungi have also been analyzed for biochemical growth characteristics, colony morphology and spore morphology. To date, we have been unable to identify them as any described species. Interestingly, none of the four fungal species were found on the surface of the animal. Light micrographs of the four fungi will be presented.

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ALGAL SECONDARY METABOLITES AND DIGESTION

IN MARINE HERBIVOROUS FISHES

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Much of the work on secondary metabolites has defined organismal responses to compounds through behavioral assays. However, more recent studies have begun to address the physiological and biochemical mechanisms underlying these responses. Given the number and diversity of algal secondary metabolites, and their broad range of pre-ingestive bioactivities, it is not surprising that most of the work examining the interaction of secondary metabolites and digestion has occurred in algal-herbivore systems. The gut environment determines how efficiently an herbivore digests the suite of primary and secondary metabolites present in algae, for it is in the gut that biochemical interactions are mediated by gut histology, chemistry, and microorganisms. Specialization upon given food types requires particular sets of morphological and physiological adaptations for ingestion, digestion, and absorption of nutrients. I will review the status of our knowledge of the effects of secondary metabolites on herbivore digestion in general, and then focus specifically on the well-studied interaction of brown algal phlorotannins and herbivorous fishes.

Because secondary metabolites can be quite reactive, and because they can comprise up to 20% of the dry mass of some species of algae, their effect on digestive processes and of the digestive processes on the metabolites is of particular interest. Herbivorous fishes consume food of lesser quality (more structural material, less protein) and must access the nutrients stored inside algal cell walls. To gain access to these nutrients, herbivorous fishes employ several distinct digestive processes.

The effect of plant secondary metabolites on herbivore digestion and nutrition is dependent not only on the characteristics of the metabolites but also on subtle interactions between the metabolite(s), gut environment, and plant nutritive qualities. In the marine environment, such interactions are complex. This is due, in part, to the diversity of marine plants, the diverse array of secondary metabolites that may be encountered by grazers, and the diversity of herbivore gut environments.

To date, most studies describing the effect of plant secondary metabolites on herbivore digestion in marine systems have focused upon the macromolecular-complexing activity of algal phlorotannins. While these compounds have demonstrated negative effects on marine herbivore food preference and digestion efficiency in some circumstances, it is clear that they can no longer be considered as broad–scope plant defenses. Rather, their anti-digestive activity can be counteracted in marine herbivores possessing guts with certain chemical (pH, redox condition, cation concentration, surfactants etc) and biological (microbes) characteristics. As a result of numerous *in vivo*, *ex vivo*, and *in vitro* studies, a framework linking the bioactivity of dietary phlorotannins with general herbivore gut features has emerged. Additional studies are required to test this framework and to further enhance it by considering other variables such as plant nutritional quality, degree of plant calcification, presence of antioxidants, and the ramifications of multiple defenses. No such framework yet exists for algal derived non-phlorotannin secondary metabolites. Modifications of diet-derived non-phlorotannin secondary metabolites have been identified in some herbivores and this represents a first step toward understanding how herbivore digestive processes effect metabolite changes.

ENERGY EXTRACTION AND FIBER DEGRADATION

OF *PANAQUE MACCUS*

ON THREE DIETS VARYING IN DIGESTIBILITY

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Introduction

Relatively few studies have been performed on the nutritional physiology of Neotropical freshwater fishes. Yet, because the Amazon basin has the richest freshwater fish fauna in the world despite areas of restricted primary productivity due to canopy overhang, dystrophic and/or turbid water, we can predict intense competition for food resources among the fishes there. Intense competition for food often leads to the evolution of novel nutritional strategies. Indeed, members of the loricariid genera *Panaque* (Eigenmann and Eigenmann) and *Cochliodon* (Heckel) have been found to have wood as the only macroscopically observable item in their gastrointestinal tracts (Schaefer and Stewart, 1993). Furthermore, several species of *Panaque* have been found to consume a variety of North American temperate woods in the laboratory, and some individual *Panaque* will exhibit positive growth on a wood-only diet in the lab (Nelson *et al*.1999).

The two genera of loricariids suspected of deriving energy from wood (*Panaque* and *Cochliodon*), both possess spoon-shaped teeth thought to be specializations for gouging wood (Schaefer and Stewart, 1993). Generalized loricariids which feed primarily by scraping algae and aufwuchs from substrate (e.g. *Hypostomus*, *Liposarcus*) possess blade-shaped teeth thought to be specialized for scraping. Whether *Panaque* and *Cochliodon* possess any further specializations for exploiting wood as an energy resource is unknown. The central question we address in this study is whether the ability of one species of *Panaque* to extract carbon from their diet is dependent upon the polymeric form of that carbon in the diet.

Methods

Animals

Specimens of *Panaque maccus* (Schaefer & Stewart, 1993) were obtained from aquarium wholesalers as they arrived from South America and held in untreated water in aquaria well innoculated with the water they were transported in. These steps were taken to maximize the chance that fish retained their native gut flora. Fish were eventually transferred to individual containers that facilitated feces collection. Algal growth was restricted in these individual tanks by covering the entire tank with opaque plastic.

Collection of Feces

The fish were fed one of three diets though to vary in their fiber content (broccoli stems, carrots or wood of the red maple tree) every night. The food was taken out each morning (16h) and feces were collected at that time. The feces from four individuals on each diet were pooled and frozen immediately.

Bomb Calorimetry

Food and feces were dried and ground to pass a 1 mm mesh screen. A weighed portion of the sample was combined with a known amount of carrageenan (Tic Gums Inc.®). Deionized water was then added and pellets were molded and placed back in the drying oven 60 ºC. The pellets were weighed and fuse wire wrapped around them and placed in a Parr® 1341 oxygen bomb calorimeter. The calorimeter was then filled with oxygen to 30 atm and ignited. The temperature rise was recorded by an Instrulab® 4000 temperature detector whose output was recorded by a PowerLab® 4S analog/digital module and Chart® software running on a Power MacIntosh® 7200/120. The gross heat of combustion was calculated from the temperature rise and the energy equivalent of the calorimeter. The latter was determined by igniting benzoic acid standards. The heat of combustion was corrected for nitric acid formation by titrating the bomb washings with 0.0709 M Na_2CO_3.

Fiber analysis

Fiber decomposition was assesed by analyzing diet and feces for various fiber fractions following a micro-modification of the hot detergent procedures of Robertson & van Soest (1981),van Soest (1994) and van Soest *et al*. (1991). These methods allowed us to separate hemicellulose, cellulose and lignin/cutin fractions from soluble cellular components and starch and were quite reproducible. Degradation of the various fiber fractions were estimated from the diet-feces difference. Ash content was used as the indigestible marker.

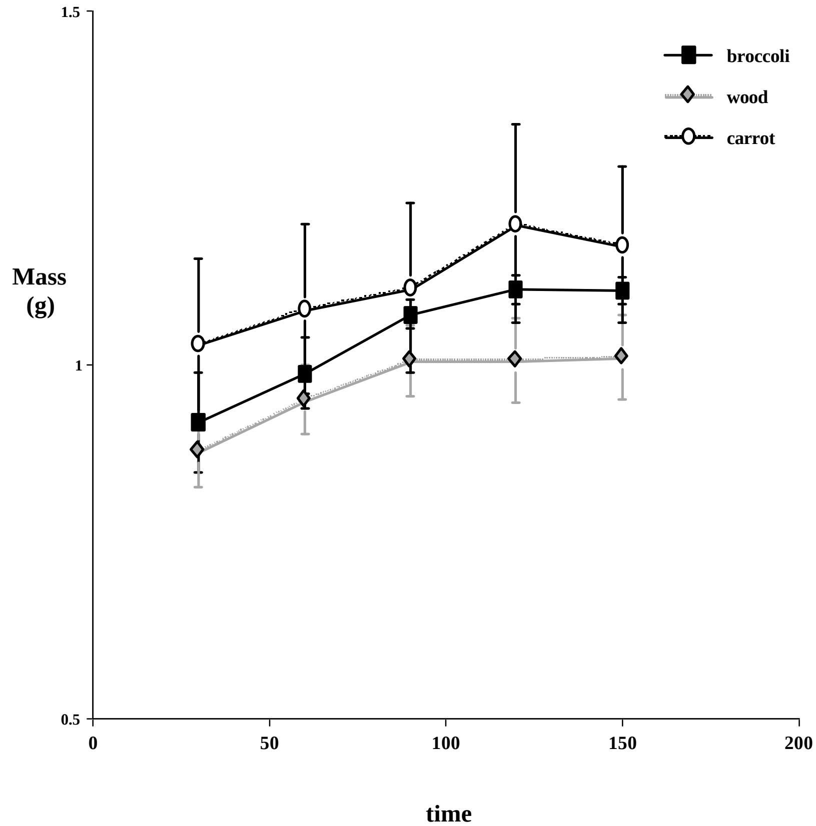
Results and Discussion

Growth

Individual *Panaque maccus* grew in the feces collection tanks despite the exclusion of algae from the water. Growth rate did not appear to depend upon diet for these animals (Figure 1).

This result is in contrast to our results for *Panaque nigrolineatus* and earlier experiments on *Panaque maccus* where growth in animals on a wood-only diet was substantially slower than in animals fed algae or vegetables. One

Figure 1: Growth of fish during feces collection period



explanation for this is that the red maple used in this experiment may be more digestible than the woods used in the earlier experiments. Another rationale explanation for this result is that we were able to culture fungi from the group of *Panaque maccus* that were used for these experiments (see Nelson *et al.* this volume); we were unable to culture fungi from previous shipments of *Panaque maccus.*

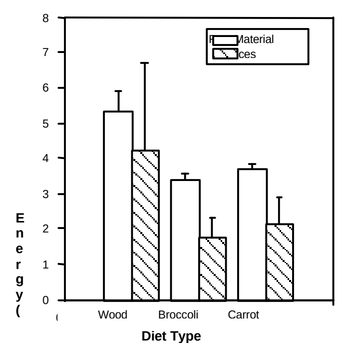
It is likely that these cellulolytic fungi are involved in the fiber decomposition observed *in vivo*.

Energy Extraction

Red maple wood contained more energy per gram than either the carrot or broccoli diets (Figure 2). *Panaque maccus* extracted roughly the same amount of energy from each of the diets.

Figure 2

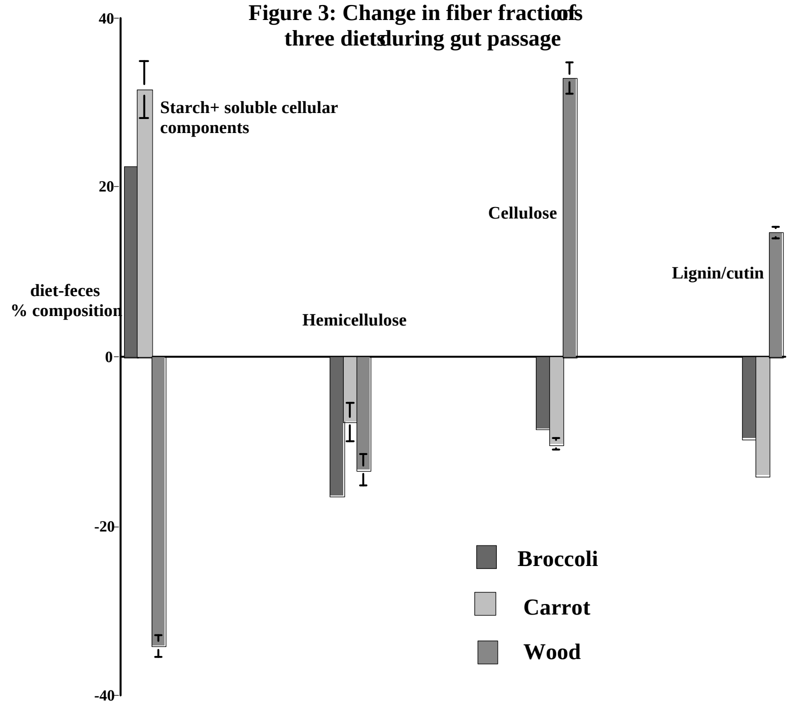
Energy removed from two diets by *Panaque maccus*



Fiber Degradation

Figure 3 presents the results from the hot detergent analysis of fiber fractions in food and feces. The data is presented as the per cent composition of a particular fiber component in the dietary item minus the per cent composition for that fiber fraction in the feces of animals eating only that diet. Thus, a high positive number is indicative of dietary assimilation of that component whereas a large negative number indicates little dietary importance for that particular fiber fraction.

Figure 3: Change in fiber fractions of three diets during gut passage



The primary message from this analysis is that the fiber degrading ability of *Panaque maccus* is very dependent upon what they are eating. Fish on a broccoli or carrot diet digested primarily the soluble cellular components and starch in their diet. There was very little evidence for degradation of the more recalcitrant carbon polymers. Fish on a red maple diet, on the other hand, appeared to digest primarily cellulose and the lignin/cutin fraction. The large negative number for the soluble fraction of the wood diet probably stems from the presence of bacterial and fungal material in the feces.

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