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Wood-eating catfishes of the genus *Panaque*: gut microflora and cellulolytic enzyme activities

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Fresh gut contents of the wood-eating loricariid *Panaque* and a generalized loricariid *Liposarcus* sp. had enzymatic activity directed against both cellulose and hemicellulose. Aerobic cultures made from the guts of *Panaque* exhibited growth on a minimal salts medium containing only crystalline cellulose as a carbon source as well as on a variety of other substrates containing carbon polymers found in wood. Anaerobic cultures made from *Panaque* guts only grew with glucose as a carbon source. Cultures of whole gut contents grown on a yeast extract basal salts medium had significant cellulolytic activity. However, no culture of individual microbes had significant cellulolytic activity, suggesting that any cellulose breakdown which occurs in loricariid guts is by a consortium of micro-organisms. A variety of aerobes, microaerophiles and facultative anaerobes were found in the guts of *Panaque*; several of these bacteria appear to be new species.

Key words: herbivory; Loricariidae; xylophagy; trophic ecology; nutrition.

INTRODUCTION

Among vertebrates, exploitation of wood as an energy resource is rare. Only a few representatives from the mammalia (e.g. *Castor*, *Erethizon*) are known to be specialists for eating woody materials. Furthermore, none of the over 25 000 estimated fish species in the world (Nelson, 1994), which represent more than half of all vertebrate diversity, is recognized to feed specifically on wood. Despite this dearth of wood-eating vertebrates, mechanisms of wood digestion merit attention because of current interest in the degradation of recalcitrant carbon polymers (e.g. cellulose, hemicelluloses and lignins). A major reason for this interest in ways to degrade poorly accessible carbon is the potential for development of more efficient uses of herbaceous biomass (e.g. increasing the assimilation efficiency of livestock through treatment of poorly digestible feeds or degradation of carbon polymers to fuels like ethanol) or more efficient decomposition of waste biomass. Therefore, the discovery of a new wood-eating vertebrate taxon should be of broad scientific interest. The purpose of this paper is to report additional experimental observations on a genus of loricariid catfish that appears to have evolved the ability to exploit wood as a food resource.

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The neotropical catfish family Loricariidae contains at least 70 genera with more than 600 described species (Isbrücker, 1980) and numerous undescribed species. An evolutionary success of this magnitude begs an explanation; however the biology of the loricariids is poorly known. Unlike other more studied radiations in fish (e.g. Cichlidae and Characidae), this radiation of the Loricariidae occurred almost entirely at the trophic level of primary consumer and entirely in the neotropics. The confinement of the Loricariidae to the neotropics suggests that this radiation has occurred since the Cretaceous separation of South America from Africa (*c.* 100 milion years ago; Lundberg, 1993). Novel arrangements of the jaw musculature and new and variable tooth morphology are thought to have contributed to the evolutionary success of the loricariids (Schaefer & Lauder, 1986; Schaefer & Stewart, 1993), but the importance of physiology, behaviour, or symbiotic relationships to this radiation are unknown.

Like most tropical, endemic groups, the trophic ecology of the Loricariidae is virtually unstudied. Besides being mostly herbivorous, loricariids may be specialists for allochthonous carbon (Araujo-Lima *et al.*, 1986; Yossa-Perdomo *et al.*, 1996). In contrast, neotropical, herbivorous characins (Araujo-Lima *et al.*, 1986) and the vast majority of other herbivorous fishes (Opuszynski & Shireman, 1995) derive their energy primarily from autochthonous sources. Two genera of loricariids, *Panaque* (Eigenmann and Eigenmann) and *Cochliodon* (Heckel), possess spoon-shaped teeth that are considered to be specializations for eating a very unique type of allochthonous carbon: wood [Schaefer & Stewart, 1993; Fig. 1(a)–(c)]. Representatives from both these loricariid genera have been collected in the field with gastrointestinal tracts filled entirely with fresh-cut wood shavings [Schaefer & Stewart, 1993; Fig. 1(d)]. One of us (D.J.S.) has examined the gut contents of eight *Panaque* species collected in South America. In all species, the only identifiable, macroscopic food items observed in the foreguts were wood chips. One specimen of an undescribed species from the upper Amazon that attains a large size (>50 cm) had the entirety of its enormous gut length (roughly eight times total length) completely packed with nothing but fresh wood shavings cut across the grain. Native fishermen actually locate this species by listening for the sounds of it scraping submerged wood (D. J. Stewart, pers. obs.). These observations indicate clearly that wood is being ingested actively by *Panaque*; the nutritional significance of this behaviour is, as yet, unknown. The purpose of this paper is to report on the presence of various carbon degradative enzymes found in the gastrointestinal tract of *Panaque* imported to North America and to describe the organisms cultured from these *Panaque* guts.

MATERIALS AND METHODS

ANIMALS

Specimens of *Panaque maccus* (Schaefer & Stewart, 1993) and *Liposarcus* (Günther) sp. (a generalized loricariid) were obtained from aquarium wholesalers. *Panaque maccus* were obtained as they arrived from South America in the hope that they would still contain some of their native gut flora. Animals were maintained in the laboratory in aquaria containing aged tap water on a diet of various temperate woods, summer squash (zucchini *Cucurbita pepo*) and whatever algae grew in their tank. Under these culture

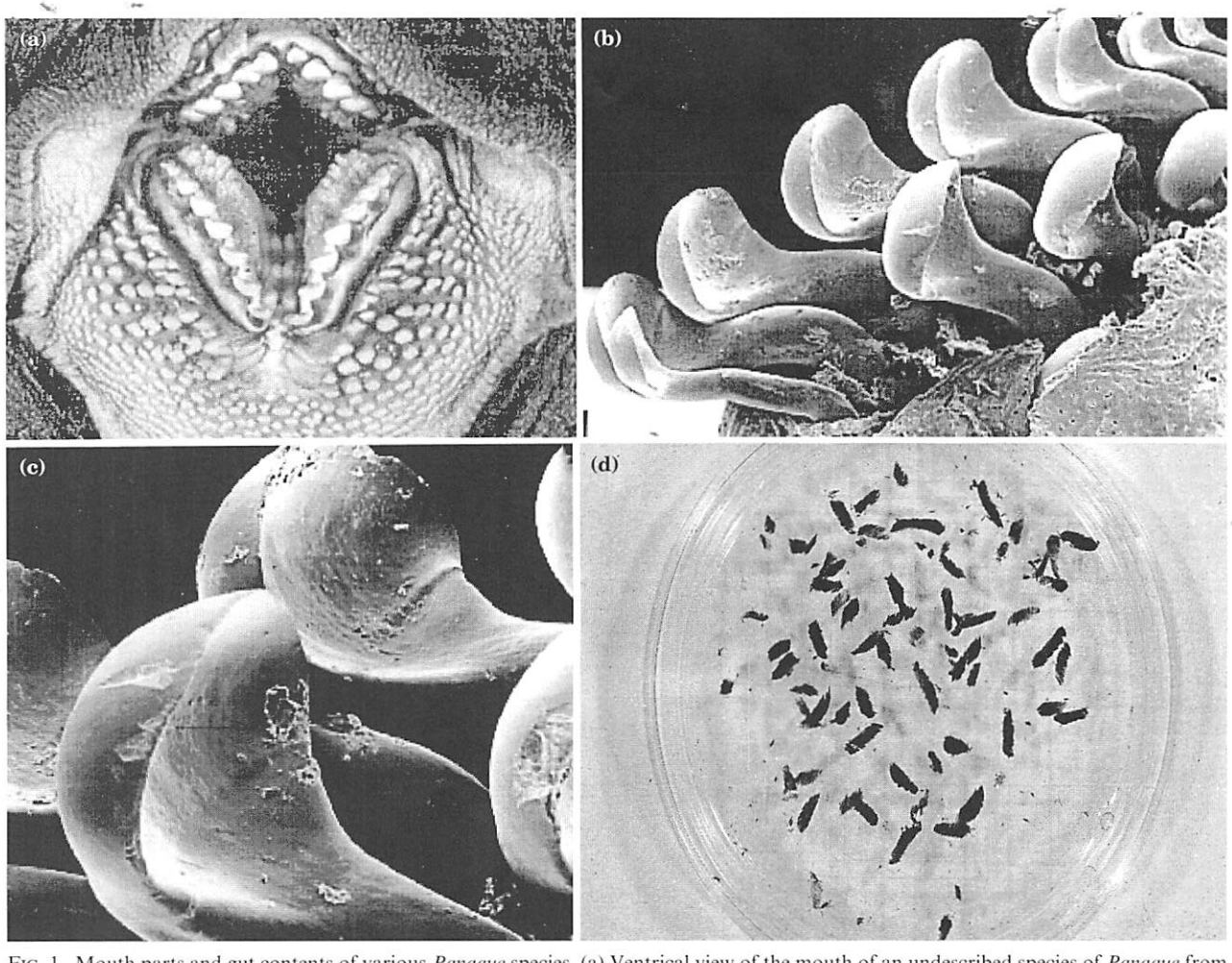

FIG. 1. Mouth parts and gut contents of various *Panaque* species. (a) Ventrical view of the mouth of an undescribed species of *Panaque* from the *nigrolineatus* clade. This particular species can reach standard lengths of 75 cm, although this particular individual measured 34·6 cm. The magnification is 2·55 ×. (b) Scanning electron micrograph (SEM) of the dentary bone and the specialized spoon shaped teeth of an individual *Panaque cochliodon*. The S_L of this animal was 11·5 cm and the magnification was 25 ×. (c) Two of the teeth in (b) depicted with SEM at a magnification of 40 ×. (d) Wood shavings taken from the foregut of the undescribed species shown in (a) captured in the Napo River, Ecuador.

TABLE I. Growth of micro-organisms in broth cultures containing various carbon sources

Culture media	Carbon source	*Panaque maccus*		*Liposarcus* sp.	
		Aerobic	Anaerobic	Aerobic	Anaerobic
Yeast extract–basal salts	Glucose	++	±	++	0
Yeast extract–basal salts	Cellobiose	++	0	+	0
Yeast extract–basal salts	Crystalline cellulose	+	0	±	0
Yeast extract–basal salts	Mannan	++	N	++	N
Yeast extract–basal salts	Xylan	++	N	++	N
Yeast extract–basal salts	No added carbon	0	0	0	0
Minimal salts media	Crystalline cellulose	++	0	N	N

These data are from those culture conditions which were replicated at least three times. The optical density of control media was subtracted from the final score for each culture.

++, Heavy growth; +, medium growth; ±, some growth; 0, control growth level; N, no data.

conditions, *Panaque* would consume both wood and zucchini, whereas *Liposarcus* would only eat zucchini. Food was sterilized by autoclaving (wood) or boiling (vegetables) before being introduced to the tank. *Panaque maccus* undergo an ontogenetic shift in tooth morphology and only acquire the characteristic spoon-shaped teeth (Fig. 1) when they are about 35 mm standard length (L_S) (Schaefer & Stewart, 1993). Therefore, only *P. maccus* that were larger than 35 mm were assayed for enzymatic activity or used for bacterial cultures.

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 or streaked onto agar plates. 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 or 5% hydrogen, 95% carbon dioxide. Initial cultures were made by diluting gut contents 1 : 10 in sterile phosphate-buffered saline (PBS) and inoculating into a yeast extract–basal salts medium with glucose, cellobiose, cellulose, mannan, xylan or carboxymethylcellulose as the sole carbon source (Table I). Anaerobic cultures also included cysteine-HCl (0·1 g l^{-1}) and resazurin (0·001 g l^{-1}) in their media. Control flasks were prepared in an identical manner with the exception that dissecting tools were immersed in the broth but no gut contents were added. Subsequent cultures were also made into a minimal salts medium containing the various carbon sources or into a minimal salts medium containing 0·2% agar and the various carbon sources including filter paper as a cellulose source. Some cultures were also supplemented with a gut extract diluted 1 : 100 and filtered through a 0·2-μM sterile filter. Growth in liquid cultures was assessed by measuring the optical density at 600 nm after subtracting the optical density of a control culture or, when the carbon substrate was too cloudy to permit spectrophotometric evaluation, scored visually.

Cultures made for the sole purpose of identifying organisms were made as follows: gastrointestinal tract contents from *P. maccus* and *Liposarcus* sp. were streaked onto blood agar (Becton-Dickinson) plates. Plates were incubated at 37° C for 48 h as were subsequent incubations. Individual colonies from blood agar plates were transferred into nutrient broth with a sterile inoculating loop using aseptic techniques and incubated. After 48 h, a loopful of bacteria was transferred to trypticase soy agar (TSA) plates and incubated. A quadrant streak was used to obtain individual colonies. Individual colonies were then transferred into trypticase soy broth (TSB) and incubated. This method was repeated three times and a Gram-stain was done each time to ensure the purity of each culture.

IDENTIFICATION OF MICROFLORA

Pure cultures were also identified by Gram-stains for morphological characterization. The following classical biochemical tests were also performed to determine the identity of the pure cultures: (1) sulphur–indole–motility (SIM) test; (2) triple sugar iron (TSI) test; (3) methyl red (MR) test; (4) Voges–Proskauer (VP) test; (5) fluid thioglycollate test; (6) carbohydrate fermentation: dextrose, lactose and sucrose utilization were examined; and (7) Simmon's citrate test. Bacteria were assigned to various taxa according to *Bergey's Manual of Systematic Bacteriology* (1979). The API 20NE test kit (bioMeriuex, Marc-Etoile, France) was also used to determine the identity of pure bacteria cultures. Furthermore, the BIOLOG identification system was used to confirm the identities of the isolates. Bacterial strains used were isolated from the intestines of *Panaque* as described above. Prior to inoculation of the BIOLOG plate, isolates were grown on trypticase soy agar (TSA) at 37° C for 48 h since the BIOLOG system requires metabolically active cells. Cell suspensions were made as described in the manufacturer's manual (Biolog, Inc., Hayward, CA, U.S.A.). To ensure the use of uniform cell density throughout the experiment, an inoculum in the range of 1·2–1·5 colony forming units (cfu) cm^{-3} was used. The BIOLOG multiwell (95 substrate wells) plates were inoculated as described in the user's manual. Multiwell plates were incubated at 37° C for 72 h. Colour changes were read in a Metertech Microplate Reader (Atlas Bioscan, Bognor Regis, U.K.) with a filter cut-off of 600 nm. The substrate wells were read against a substrate blank well. The substrate utilization profile of each isolate was recorded. The data were compared with the currently available BIOLOG database of environmental and medical bacteria. Bacterial suspensions used to inoculate the BIOLOG plates were streaked onto TSA to test their purity.

ENZYME ASSAYS

After dissection of animals, sections of gastrointestinal contents were rinsed first with 70% alcohol and then sterile buffer, split into approximate thirds and weighed. Gut contents were stripped into a Tenbroeck-style tissue grinder then diluted either 10 × or 20 × with 0·1 M citrate buffer (pH 5·0) and homogenized. Gut content density was taken as 1 g cm^{-3} for calculation of dilution volumes and the mass of the extremely thin-walled gastrointestinal tract was ignored. Therefore, reported enzyme activities will be underestimates of the activity that was actually present per unit volume of gut content. Homogenates were filtered through nytex netting or lightly centrifuged to remove coarse particulate matter prior to assaying. Carbon polymer degradative capacity was measured with two broad types of enzyme assay.

(1) Various glycosidase activities, e.g. β-glucosidase (β-D-glucoside glucohydrolase E.C.3.2.1.21) and β-xylosidase (β-D-xylanoxylohydrolase E.C.3.2.1.37), etc., were assayed using a variation of Bahl & Agrawal (1968). The appropriate *p*-nitrophenyl conjugated substrate (Sigma® 0·5 g cm^{-3} in 0·1 M citrate buffer pH 5·0) was incubated with diluted gut contents (volume fraction 0·33) for 2 h at 40° C. Optical density (OD) in excess of a heat-inactivated control at 425 nm was recorded after reacting the liberated *p*-nitrophenol with Na_2CO_3. Concentrations were calculated by referencing the OD to a standard curve prepared with pure *p*-nitrophenol (Sigma®).

(2) β-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 0·1 M pH 5·0 citrate buffer, or a suspension of 20 μm crystalline cellulose (Sigmacell 20) in the same buffer, all to a final sodium azide concentration of 0·001% to prevent bacterial activity during the assay. Hemicellulolytic activity was assayed analogously, except that guar gum mannan was the substrate. Reducing sugar groups were assayed after 24 h of incubation at 40° C, using a modification of the Somogyi method (Nelson, 1944). Optical density at 500 nm, in excess of a control blank which had gut contents added after the 24-h incubation, was measured and recorded on a Beckman DU-6® or Gilford Response® spectrophometer. Concentrations were calculated by reference to a standard curve prepared with glucose.

RESULTS

BACTERIAL ISOLATION AND CULTURING

Quantitative results

Bacterial growth was obtained from gut contents of both *P. maccus* and *Liposarcus* sp. on all carbon substrates under aerobic isolation and incubation conditions (Table I). In contrast, most anaerobic cultures of gut contents produced little to no growth on substrates other than glucose. Also of interest, was the observation that growth occurred in cultures made from *P. maccus* gut contents when crystalline cellulose was the only carbon source available. Micro-organisms from the purported wood-specialist *Panaque* either grew better on cellulose and its constituent dimer cellobiose, or, those organisms that could grow on these substrates were present at an initially higher density than in the more generalized loricariid, *Liposarcus* (Table I). This finding was not tested further because transfection between species was a distinct possibility under the fish culture conditions employed in this part of the study.

Qualitative observations

Although attempts to culture a single organism which produced a true cellulase were unsuccessful (see below), evidence suggests that microflora taken from loricariid guts are capable of degrading and extracting energy from fibrous carbohydrates. Platings of cultures onto minimal salts agar plates containing crystalline cellulose or filter paper strips had significantly more growth than plates containing no added carbon. This growth in excess of agar-only plates was observed both anaerobically and aerobically, however, the filter paper strips were only visibly frayed under aerobic conditions. None of these replates onto minimal salts–cellulose exhibited true cellulase enzyme activity when assayed (cf. Johnson *et al.*, 1982), although some cultures had carboxymethylcellulase activity.

Identification

Since *P. maccus* were obtained upon arrival from South America, and because all food and structure was sterilized before being placed in their tank, it is likely that some of the organisms described here are found in feral *Panaque*. However, since we had no control over the conditions, experienced by the animals in transit, we cannot be certain of this. All of the isolates from *P. maccus* guts were Gram-negative rods (Table II). Both Gram-positive cocci and Gram-negative rods were isolated from the gastrointestinal tract of *Liposarcus* sp. (Table II). Nine pure cultures were isolated from *P. maccus*, whereas *Liposarcus* sp. harboured 10 organisms which we were able to isolate in culture. None of the isolates produced acetoin or H_2S and no isolate utilized indole.

Five and four axenic cultures of bacteria were isolated from the fore- and hindgut of *P. maccus*, respectively (Table II). Isolates from the foregut were either microaerophiles, facultative or obligate anaerobes and were different from isolates cultured from the aquarium itself. Except for one obligate anaerobe, none of these bacteria fermented lactose. All of the isolates utilized citrate, and three were weakly motile. Two distinct types of bacteria were isolated from the hindgut of *P. maccus*. The first type (two strains) were motile facultative

anaerobes that fermented all the carbohydrates tested and utilized citrate. The second type (two strains) were motile aerobes that did not ferment carbohydrates and weakly utilized citrate. Two of the aerobes appeared to be cultureable from both the fish and the tank, but the remainder of the isolates were unique to the fish.

Two Gram-positive cocci and three Gram-negative rods were isolated from the foregut of *Liposarcus* sp. (Table II). Both cocci were motile, but the one which was an obligate anaerobe did not ferment lactose. Of the three rods, the one which was a microaerophile did not ferment dextrose. Three aerobic, one microaerophilic and one facultative anaerobic Gram-negative motile rods were isolated from the hindgut of *Liposarcus* sp. All of these isolates utilized citrate except for one of the aerobes. None of these bacteria fermented the carbohydrates tested. As for *Panaque*, some of the aerobes isolated from *Liposarcus* appeared to be cultureable from both the fish and the tank, but the remainder of the fish isolates were not isolated from the tank. The identities of the isolates from the BIOLOG system confirmed the results from the morphological and physiological studies.

The facultative anaerobes isolated are similar to *Xenorhabdus* sp. which has been isolated from *Neoaplectana* sp., a nematode parasite of neotropical fishes. The other bacterial species appear to be new at this juncture. The only non-bacterial organism found in the gut contents or cultures was a spherical protozoan capable of ameboid motion found in aerobic cultures made from *P. maccus* gut contents. This organism was osmotically fragile and we were unable to isolate it.

ENZYME ASSAYS

Gastrointestinal contents from both loricariid species exhibited hydrolytic activity against the β-glycosidic linkages connecting the various monomers of cellulose and hemicellulose [Fig. 2(b)]. Both species had substantially lower, but still measurable, enzymatic activity in their gut contents against the larger carbon polymers which were tested [Fig. 2(a)]. However, our rough calculations suggest that even this low rate of enzymatic activity would be enough to supply the basal energy requirements of a *Panaque*. For example, an average, resting 10 g *Panaque* consumes about 700 calories (0·7 kcal) of energy each day (oxycaloric coefficient of 5 kcal $l^{-1}O_2$; Nelson, 1999). Estimates from dietary energy extraction suggest that the daily energy required by a free-living *Panaque* is actually close to 4 kcal (J. A. Nelson, unpubl. obs.). Measurements of the V_{max} for various β-glycosidases in *Panaque* [Fig. 1(b)] suggest that these enzymes could supply energy far in excess of these requirements (an activity of 1000 units and gut contents of 0·75 g of substrate would supply about a mole of monosaccharide per day; with a heat of combustion of 686 kcal mol^{-1}, even a modest conversion efficiency of 20% produces at least 30 times the animal's daily energy requirement). Clearly, disaccharidase activities are not limiting the ability of *Panaque* to exploit carbon polymers, even if those disaccharides are connected with β linkages. For β-endoglucanases [Fig. 1(a)], 2 units of activity would produce 1·5 kcal of daily reducing sugar energy in a 10-g animal with 0·75 g of gut contents. If half of this energy was available to the animal, it suggests that the animal could satisfy its entire resting energy budget from cellulose or mannan.

TABLE II. Biochemical characterization of bacteria isolated from fore- and hindgut of loricariid catfish

Isolate no.	Indole util.	H_2S prod.	MR-VP	Oxygen*	Dextrose ferment?	Lactose ferment?	Sucrose ferment?	Motility	Citrate util.
Panaque maccus									
1	—	—	—	Mae	—	—	—	+	++
2	—	—	—	FAn	—	—	—	++	++
3	—	—	—	FAn	—	—	—	+	++
4	—	—	—	FAn	—	—	+	++	++
5	—	—	—	Ana	—	+	+	+	++
6	—	—	—/—	FAn	+	+	+	+	—
7	—	—	—/—	FAn	++	+	++	+++	++
8	—	—	—/—	Ae	—	—	—	+	++
9	—	—	—/—	Ae	—	—	—	+	+
Liposarcus sp.									
1	—	—	—	Mae	—	+	+	+	+++
2	—	—	—	FAn	+	+	+	+++	++
3	—	—	—	FAn	+	—	+	+++	+
4	—	—	—	FAn	+	+	+	+++	+
5	—	—		Ana	+	—	+	+++	+
6	—	—	—	Ae	—	—	—	+	++
7	—	—	—	FAn	—	—	—	+	++
8	—	—	—	Ae	—	—	—	+	++
9	—	—	—	Ae	—	—	—	+	—
10	—	—	—	Mae	—	—	—	+	++

Tabular results from the following classical biochemical tests: (1) sulphur–indole–motility (SIM) test; (2) triple sugar iron (TSI) test; (3) methyl red (MR) test; (4) Vogues–Proskauer (VP) test; (5) fluid thioglycollate test; (6) carbohydrate fermentation: dextrose, lactose and sucrose utilization; and (7) Simmon's citrate test.

*Mae, Microaerophile; FAn, facultative anaerobe; Ana, obligate anaerobe; Ae, aerobe. *Panaque maccus*: isolates 1–5 were from the foregut and isloates 6–9 were from the hindgut. All the isolates were Gram-negative rods. *Liposarcus* sp.: isolates 1–5 were from the foregut and isolates 6–10 were from the hindgut. Except for isolates 4 and 5 which were Gram-positive cocci, all the isolates were Gram-negative rods.

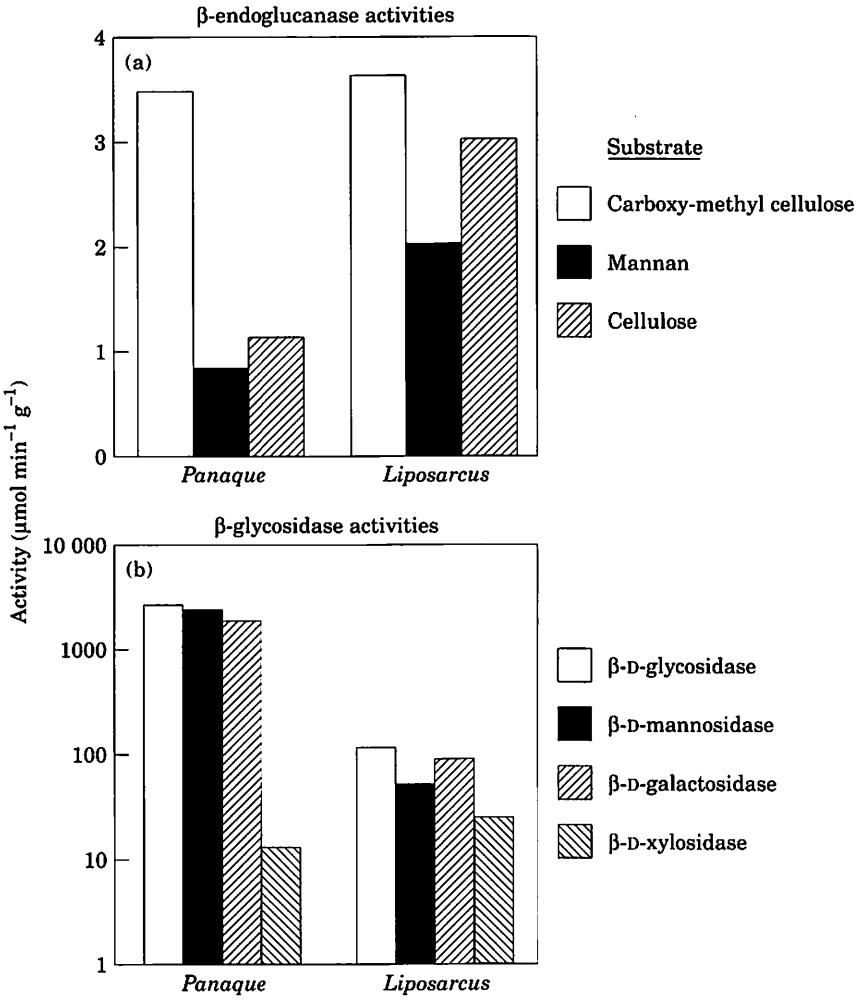

FIG. 2. Activity of various catabolic enzymes in the gastrointestinal tracts of two loricariid catfish genera; representatives of the genus *Panaque* appear to be specialized for wood consumption whereas *Liposarcus* are generalized periphyton and benthos consumers, but will eat wood when no other food is available. Activity is reported in International Units (μmol of reducing sugar equivalents or *p*-nitrophenyl derivative released per minute of incubation with the respective substrate per gram of gut content). Results were standardized to 30° C using a Q_{10} of 2. (a) *β*-endoglucanase activity: substrates were (number of fish assayed in parentheses): crystalline cellulose (Sigmacell 20 average particle size 20 μm) (4); guar gum mannan (gift from T. Highley, USDA Forest Products Laboratory, Madison, WI 53706, U.S.A.) (7) and carboxymethylcellulose (Sigma) (9). Optical densities used to calculate enzymatic activities were in addition to that of a blank containing both substrate and the test solution added after the incubation period. (b) *β*-glycosidase activity: substrates were (number of fish assayed in parentheses): *p*-nitrophenyl *β*-D-xylo-pyranoside (Sigma) (10); *p*-nitrophenyl *β*-D-gluco-pyranoside (Sigma) (12); *p*-nitrophenyl *β*-D-galacto-pyranoside (Sigma) (3); *p*-nitrophenyl *β*-D-manno-pyranoside (Sigma) (3). The number of fish assayed is as indicated, however, there was often within fish replication accomplished by assaying multiple gut segments, in which case the mean of the segments was used. Optical densities used to calculate enzymatic activities were always in addition to that of a blank containing both substrate and the test solution to control for non-enzymatic substate degradation, non-specific absorbance and reducing sugar equivalents already present in the gut contents.

Even if one adopts the more conservative 4 kcal day^{-1} estimate for the animal's daily energy expenditure, the calculations predict that the animal could get up to 18% of its daily energy supply from cellulose or mannan.

Interestingly, despite the observation that microflora from the gut of *P. maccus* showed good growth on media containing cellulose as the only carbon source (Table I), there was lower *in vitro* enzymatic activity against this substrate in gut contents from this species than in the generalized loricariid [Fig. 2(a)]. However, qualitative assays of the supernatants from broth cultures made from both loricariid species did reveal that the organisms which grew in culture had the ability to produce reducing sugars from both cellulose and carboxymethylcellulose. One inference from this result could be that not all of the organisms responsible for the inferred cellulose degradation in *Panaque* guts are expressing their enzymes extracellularly.

DISCUSSION

This work adds to the inferential evidence that some loricariid catfish have evolved the capacity to use wood as a food source. Previous field observations described gastrointestinal tracts from representatives from the genera *Panaque* and *Cochliodon* filled entirely with fresh-cut wood shavings (Schaefer & Stewart, 1993). We now offer observations of wood-eating behaviour and evidence that the enzymatic machinery needed to degrade some of the major carbon polymers found in wood and terrestrial macrophytes is found in loricariids (Fig. 2). The source of these enzymes is, as yet, undetermined. In addition, we report that aerobic micro-organisms isolated from loricariid guts are capable of growth in media containing only poorly digestible carbon polymers as a carbon source (Table I), and that these cultures of micro-organisms are also capable of producing cellulolytic enzymes. Preliminary studies have also shown that some representatives of the genus *Panaque* are capable of extracting energy from a wood diet in algae-free water, and that they are capable of positive somatic growth under such conditions (J. A. Nelson, unpubl. obs.). In contrast, a generalized loricariid, *Hypostomus* (Lacépède) sp. is able to eat and extract energy from wood, but not in sufficient quantities to maintain weight or to grow (J. A. Nelson, unpubl. obs.).

Although never described in fishes, aerobic wood digestion is already well known in aquatic, invertebrate systems (e.g. shipworm Waterbury *et al.*, 1983). Since both generalized loricariids such as *Hypostomus* (Graham & Baird, 1982) and *Panaque* (J. A. Nelson, unpubl. obs.) breath air as well as water, and, since generalized loricariids are the presumed ancestors of *Panaque*, it is interesting to speculate that an aerobic digestive tract may be involved in the evolution of wood eating.

The fact that most of the isolated micro-organisms from both species were aerobes or facultative anaerobes means that they can probably move between fish and environment with relative ease. This suggests a speculative scenario for how relatively generalized algae/periphyton scraping loricariids may have evolved the capacity to digest wood. Fishes scraping periphyton from woody substrates may ingest wood particles and micro-organisms decomposing the

wood incidentally. As the wood decomposition continued in the fish's gut, the fish were able to absorb small carbon molecules such as monosaccharides or short chain fatty acids, generated by microbial action. Animals which were better able to scrape the wood were at an energetic advantage, especially during times of poor periphyton quality. Further specialization on wood may have required development of very specialized teeth and jaw movements capable of cutting large amounts of woody materials (Fig. 1). *Panaque* and *Cochliodon* are most abundant in piedmont areas of the Amazon headwaters (Stewart, pers. obs.) where relatively steep altitudinal gradients change abruptly to lower gradients. Rivers there change course often, undercutting large areas of forest in the process. This meandering produces extensive areas of coarse woody debris in the rivers of these areas. Presumably fishes that acquired the ability to digest wood would be at a competitive advantage in such a habitat, especially during the dry season when many fish species of this region stop feeding entirely because of food scarcity (Fink & Fink, 1979). This process appears to have happened at least twice in evolutionary history since the phylogenetic relationships of loricariids (Schaefer & Stewart, 1993) indicate that the spoon-shaped teeth of *Panaque* and *Cochliodon* are independently derived (convergent). Being able to utilize carbon from woody materials may also be an adaptive advantage in heavily forested areas where algal production may be inhibited by low light levels. Similarly, in large turbid rivers, sunken trees are common, but bottom habitats may be virtually lightless and, thus without significant primary productivity.

Existence of similar enzyme activities in the guts of *P. macus* and *Liposarcus* sp. (Fig. 2) and the similarity of the micro-organisms cultured from the two species (Tables I and II) suggest that if the described microflora are responsible for wood digestion, there are no host specificity limitations. Because we have no knowledge of the fish's handling before arrival in North America, and because we did not employ sterile fish culture, we are unable to speculate about native gut flora from the results reported here, particularly for *Liposarcus* which are often cultured for the aquarium trade. However, the organisms cultured from the guts of these fishes exhibited some interesting properties. Initial cultures from both *Panaque* and *Liposarcus* made into yeast extract–basal salts media exhibited measurable activity against crystalline cellulose and substantial enzymatic activity against both carboxy-methyl cellulose and cellobiose (not shown). Yet, the true cellulase was invariably lost in subsequent subcultures. These results suggest that the degradation of fibrous carbohydrates by these cultures is the net result of a microbial community metabolizing, and not the action of a single microbe. Also relevant, was the observation that cultures of organisms grown with cellulose as the sole carbon source would liquify gelled mannan quickly when replated onto this substrate, whereas cultures grown on cellobiose did not liquify mannan when recultured upon it. This observation suggests that the organisms capable of breaking the more resilient bonds in loricariid diets are probably out-competed by other organisms in an environment where more easily digestible carbon (e.g. cellobiose) is the only substrate.

Although there is presently a surge of interest surrounding marine herbivorous fishes (e.g. Ojeda & Cáceres, 1995; Polunin *et al.*, 1995; Seeto *et al.*, 1996), the biology and ecology of tropical, freshwater herbivorous fishes

remains virtually unstudied. Marine herbivorous fishes have microfloral fermentations occurring in their guts (e.g. Rimmer & Wiebe, 1987; Clements & Choat, 1995; Seeto *et al.*, 1996). Seeto *et al.* (1996) provide evidence that these fermentations proceed at a rate sufficient to supply substantial energy to the host. We know of no comparable work on freshwater herbivorous fishes. Freshwater fishes have been reported to have cellulolytic activity in their GI tracts (e.g. Prejs & Blaszczyk, 1977), however, the significance of this activity and whether it can be completely accounted for by cellulases produced by ingested invertebrates has not been established (Lindsay & Harris, 1980), although there is some evidence for resident cellulolytic organisms in herbivorous fish (Luczkovich & Stellwag, 1993). We know of no known linkage between gut enzymatic activities and a specific symbiont in any freshwater, herbivorous fish. In the present study, we describe enzymatic activity in the guts of loricariid catfishes directed against carbohydrates found in macrophytes and wood. These enzymes are present in sufficient amounts to supply the animal's resting energy supply under saturating substrate conditions. In addition, we found that aerobic community cultures of the microflora were capable of producing similar enzymatic activity *in vitro*. Since the loricariids were fed only on sterilized plant material, these enzyme activities are unlikely to be attributable to coincidental invertebrate ingestion. Although suggestive, these results do not confirm the digestion of the complex polysaccharides found in wood within loricariid guts. Further work to ascertain whether wood digestion actually occurs in loricariid guts and to associate specific enzyme activities to (a) particular organism(s) or community of organisms is justified by our results.

In most aquatic ecosystems, the underwater breakdown of large particulate organic matter like fallen trees is initiated by fungi and bacteria with small particulate matter being ingested by aquatic macro-invertebrates that, in many cases, also derive energy from digestion of the microbes. The presence of wood-eating fishes in an aquatic system, if sufficiently abundant, could radically change the breakdown and cycling of woody carbon into various food chains. Many herbivores have been shown to have very inefficient digestion systems, getting sufficient energy only by passing large quantities of material through the gut. If the same is true of *Panaque* and *Cochliodon* species, they could perhaps significantly influence the carbon recycling rates as well as the rate of accumulation of large particulate organic carbon in neotropical streams. Why fish have filled this ecological niche only in the neotropics and the significance for aquatic ecosystem dynamics should also be the foci of future studies. Unfortunately, the piedmont rain forest habitats favoured by *Panaque* are currently among those tropical ecosystems most threatened by human activity.

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