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The river flood pulse, benthic biofilm, and the nutrition of *Prochilodus lineatus*

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Abstract *Prochilodus lineatus* (Valenciennes, 1837) is one of 270 fish species in the Rio Paraná system yet it comprises > 50% of the fish biomass and is the only fish species in the system known to feed entirely on flocculent benthic biofilm. We studied the feeding behavior and diet of *P. lineatus*, and the composition of benthic biofilm in river channels, the moving littoral, and in isolated floodplain lakes at different stages of the Rio Paraná hydrological cycle. *Prochilodus lineatus* selectively ingests hydrolysis-labile-organic-matter rich in amino acids while selectively rejecting mineral matter and refractory organic matter. Assessed in terms of g AA assimilated · kJ^{-1} energy assimilated, the quality of food ingested by *P. lineatus* ranges from a maintenance level of 5 to 12 mg AA · kJ^{-1}, a level expected to produce near maximum growth. The level of amino acids in benthic biofilm is highest in shallow waters during the advancing flood (the moving littoral), and *P. lineatus* have been observed to feed in littoral areas so shallow that their dorsal fins and backs are exposed. Feeding intensity increases with river height as the moving littoral expands. Feeding site selection, selective ingestion, and conditional feeding intensity each increase amino acid assimilation and together constitute a trophic strategy that allows *P. lineatus* to dominate the fish community of the Rio Paraná.

Keywords Biofilm · Ilyophagus · EPS · Diet selection · River flood pulse

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Introduction

Biofilm is ubiquitous and abundant in freshwater, estuarine, and marine environments. It is a structured aggregation of microorganisms, the extracellular polymeric secretions (EPS) they produce, ad- and absorbed dissolved organic matter (DOM), and often a variety of organic and mineral debris particles entrapped in the aggregate. Of these components, the microorganisms typically comprise < 10% of the organic mass with the EPS making up most of the rest (Flemming et al. 2016b). Biofilm varies in the degree of its attachment to solid surfaces. It occurs firmly attached to stones, submerged wood, and plants in flowing or turbulent waters; it is less firmly attached to surface sediments; and it exists as a loosely attached or unattached flocculent layer lying on sediments and other surfaces and is also present as suspended particles.

Biofilm is an important food resource for a host of invertebrates (Hobbie and Lee 1980; Simon et al. 2003), but the vertebrates believed to depend on it for food comprise a relatively small set of shorebirds (Jiménez et al. 2015; Lourenço et al. 2017), larval amphibians (Ocock et al. 2018), and fishes (Bowen et al. 2006). Although few in species, the fishes that derive their nutrition from benthic biofilm (also known as ilyophagus or mud-eating fishes) are often inordinately successful in establishing large populations that dominate the system fish biomass. For example, *Prochilodus lineatus* is one of 270 fish species described from the Rio Paraná system but makes up > 50% of the fish biomass (Bonetto and Castello 1985; Bonetto 1986). A goal of this study was to determine how *P. lineatus* is so successful utilizing benthic biofilm as its food resource.

A great deal of productive research in the last three decades has focused on biofilm formation, structure, internal dynamics, and interaction with the dissolved milieu (Flemming et al. 2016a; Lawrence et al. 2016; Decho and Gutierrez 2017). Less has been learned about environmental conditions that influence the abundance and nutritional quality of biofilm for consumers and attributes that consumers may have that enable them to use this food resource that is apparently not useful to most vertebrate species. In the work reported here, several characteristics of flocculent benthic biofilm and its use as a food resource by *P. lineatus* in the Rio Paraná floodplain in the vicinity of Corrientes, Argentina, were studied at various stages of the hydrological cycle to evaluate two broad hypotheses. First, that the food quality (capacity to support growth) of biofilm differs by habitat and river height. Second, that *P. lineatus* locates and selectively ingests biofilm of superior food quality and avoids or rejects lower quality biofilm.

Methods

Study sites

This study was conducted in the Rio Paraná basin in the vicinity of Corrientes, Argentina, immediately downriver from the confluence of the Rio Paraná and the Rio Paraguay (Fig. 1). Based on access and the knowledge of local field personnel with experience in collection of *P. lineatus*, three sampling sites were chosen and three habitats were distinguished within sites. The habitats were river channels, discrete floodplain lakes, and river littoral zones (sensu Junk et al. (1989)). In the sense used here, river littoral zones are flooded areas of the flood plain that cover large areas at high water levels but are much reduced in area at low water levels yet maintain connections to river channels such that fish may move between the two habitats. Floodplain lakes are isolated, in some cases for years, and become connected to other waters of the floodplain only during floods. The extent of the littoral depends on the combined effects of river height and local rainfall. River height was recorded daily by the Argentine Directorate of Waterways relative to a datum set at the city of Corrientes (Fig. 2). The areal extent of floodplain lakes is substantially affected by local rainfall. *P. lineatus* is found primarily in the littoral zone, in tributary channels, and in lakes but uses the main channel of the Rio Paraná to move among floodplain habitats and for seasonal spawning migrations during which it does not feed (Bayley 1973; Junk et al. 1989; Agostinho et al. 1993; Sverlij et al. 1993; Pesoa and Schulz 2010).

The Riachuelo study site is a tributary of the Rio Paraná with a central channel and an extensive littoral zone at high water levels but without discrete floodplain lakes in the study reach (Fig. 1). The Madrejon del Puente and Colonia Tacuari sites are discrete floodplain lakes at median water levels but become connected to the Rio Paraná at high water levels and thus function as part of the littoral zone under those conditions. In addition to these three sites, the main channel of the Rio Paraná was successfully sampled on two occasions. Field collections were made on 36 dates during five sampling periods ranging from 7 to 26 days in duration (Table 1).

Field collections

During period 1, sampling was limited to the river channel and the littoral at the Riachuelo site (Fig. 1). During period 2, the Rio Paraná was high enough that all three sites were classified as littoral (Fig. 2). For periods 2 through 5, initial efforts to collect *P. lineatus* included all sites and habitats but subsequent sampling was concentrated where *P. lineatus* was found to be present.

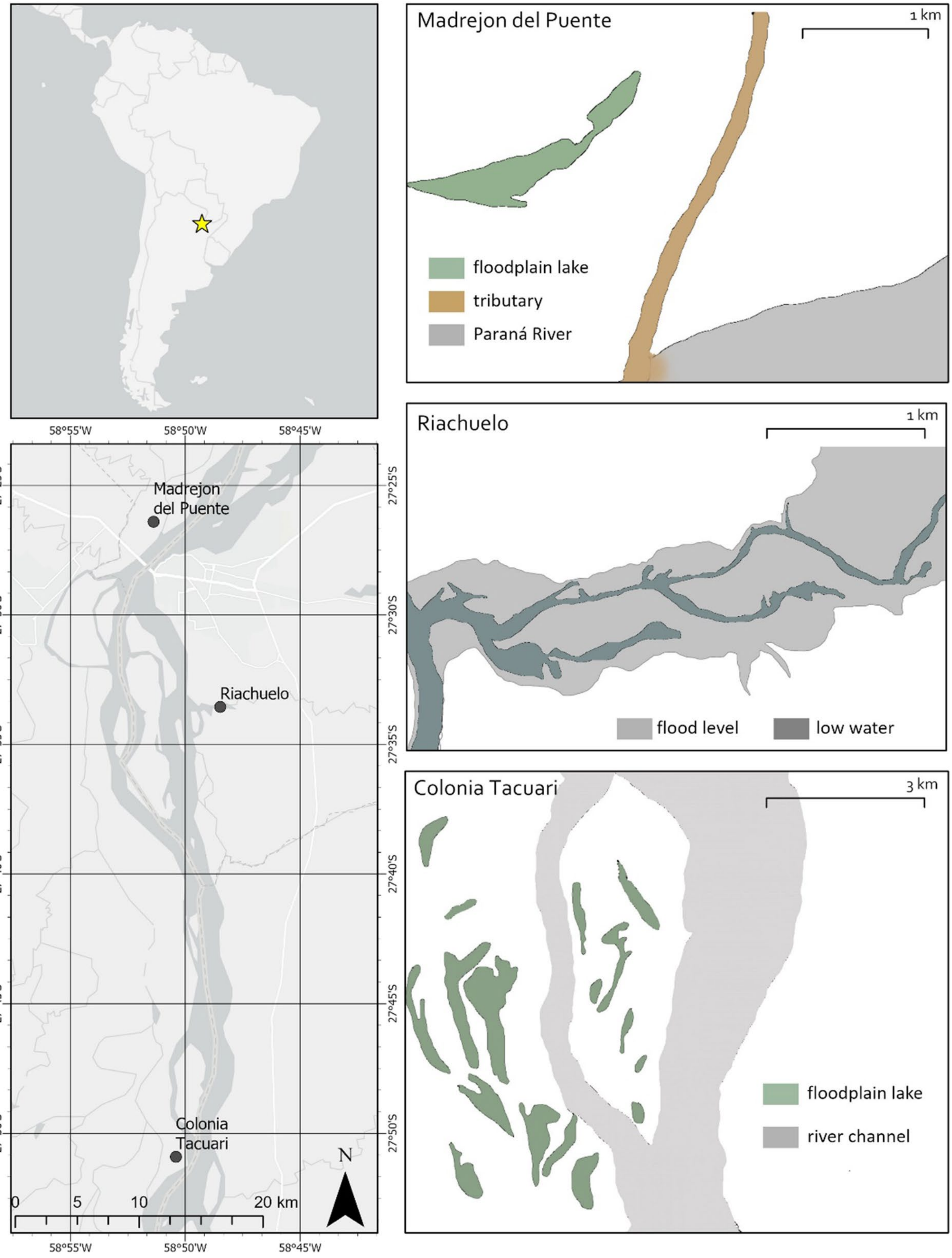

Fig. 1 Map of the Rio Paraná in the vicinity of Corrientes, Argentina, and the three sites used in this study

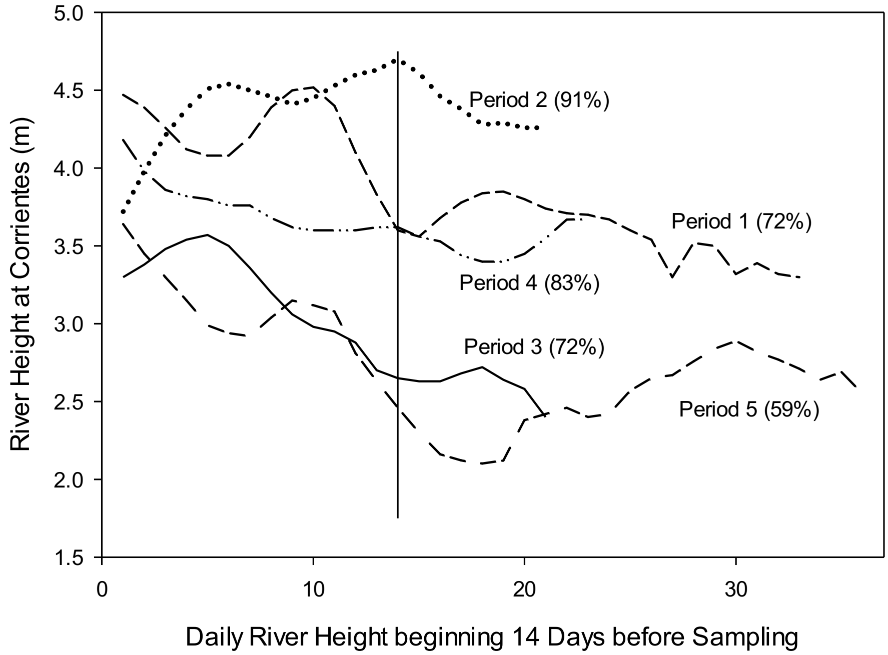

Fig. 2 River height 14 days before and during sampling periods as recorded daily at the city of Corrientes by the Argentine Directorate of Waterways. The vertical line marks the beginning of each sampling period. Values in parentheses are percentages of *P. lineatus* examined from all sites and habitats that had food in the cardiac stomach

Table 1 Numbers of *P. lineatus* collected by habitat and sampling period. Percentages are of numbers across habitats. During sampling period 1, sampling was limited to the Riachuelo site which did not include lakes in the study reach. During flood conditions of sampling period 2, all sites were part of the moving littoral

Sampling period	Moving littoral	River channels	Floodplain lakes	Total
1 (Aug. 6–25, 1987)	37 (30%)	87 (70%)		124
2 (Nov. 25–Dec. 2, 1987)	46 (100%)			46
3 (Nov. 15–23, 1988)	49 (54%)	23 (26%)	18 (20%)	90
4 (Jun. 3–12, 1989)	7 (20%)	24 (69%)	4 (14%)	35
5 (Nov. 21–Dec. 17, 1991)	68 (63%)	15 (14%)	24 (22%)	107
Total	202 (50%)	149 (37%)	51 (13%)	402

Fish were captured using a trammel net with an inside panel mesh of 8 cm stretched measure. Trammel nets are preferred to gill nets or seines in environments where submerged logs may snag the net and they result in minimal delay between capture and examination of fish. The net was set across a point at which a littoral area or river channel narrowed, or perpendicular to shore in floodplain lakes. A boat with an outboard motor swept back and forth across the partially enclosed area to drive fish into the net and then the net was recovered and the fish removed. Additionally, in the Paraná and Riachuelo river channels, the net was laid across the flow and allowed to drift for approximately 15 min. Most collections were made during morning or midafternoon to allow time for subsequent sample processing in the laboratory, but two samples were collected after dark: one at 2 and one at 4 h after sunset. All *P. lineatus* captured were examined except for one large catch of 21 from which 12 were examined and the others returned alive to the water. Immediately following capture each fish was euthanized by cervical dislocation, standard length and weight were measured, and the digestive tract was removed, placed in numbered plastic bag, and stored on ice pending processing in the laboratory.

Benthic biofilm was collected between 5 and 120 cm depth at intervals along transects perpendicular to shore using a 5.7-cm inside diameter coring device made from a transparent acrylic tube. The layer of flocculent benthic biofilm, typically about 5 mm thick, was siphoned from the undisturbed core surface into a plastic bottle using a 3-mm inside diameter glass tube and stored on ice.

Laboratory processing of samples

The digestive tract of *P. lineatus* is comprised of five distinct sections. In order, they are a short esophagus, a cardiac stomach that functions as a holding organ, a muscular pyloric stomach that grinds the food to a small particle size, a pyloric chamber that receives ground food and the common bile duct, and a lengthy intestine (Angelescu and Gneri 1949). The contents of the cardiac stomach were taken to represent the diet with the caveats discussed below.

Immediately upon return from the field, the contents of the cardiac stomach and samples of benthic biofilm were transferred to individual aluminum pans and dried to constant weight at 105 °C. Dried samples were pulverized in a mortar and pestle and stored in sealed glass vials pending chemical analyses. For 80 samples, a small subsample was preserved with formalin and stored in glass vials for microscopy.

Chemical analyses

Samples of cardiac stomach contents and benthic biofilm were analyzed for components found in previous research to be useful indicators of food quality for ilyophagus fishes (Bowen et al. 2006; Bowen and Yap 2018). Ash-free dry mass (AFDM) is a measure of total organic matter and was determined as mass loss on ignition at 550 °C. AFDM was divided into hydrolysis-resistant and hydrolysis labile fractions. Hydrolysis-resistant organic matter (HROM) is a measure of indigestible organic matter including cellulose and chitin and was determined as described by Buddington (1980) with the exception that toluene was substituted for benzene in washing the digested residue. Hydrolysis labile organic matter (HLOM) is the organic fraction removed in this procedure and is material that fish may be able to digest. Total amino acids (AA) were determined by the ninhydrin reaction following alkaline hydrolysis (Makkar et al. 1988) (see supplemental information). This measure is preferred to total nitrogen, much of which may be immobilized in refractory compounds and thus unavailable to the consumer, or total protein which often significantly underestimates the presence of nutritionally valuable amino acids (Bowen 1980). Energy density of the contents of the pyloric chamber was determined for five fish using a Phillipson microbomb calorimeter standardized against benzoic acid (Bowen 1996). In contrast to contents of the cardiac and pyloric stomachs, contents of the pyloric chamber had $\geq$0.22 g AFDM · g^{-1} DM and thus burned completely in the calorimeter to yield accurate measurements (Getachew et al. 2000).

Microscopy

Both fresh and preserved samples were stained with alcian blue, examined under a light microscope at 160 × and 400 × magnification, and descriptions were recorded. Alcian blue stains the acidic mucopolysaccharide portion of extracellular polymeric secretions (EPS) produced by microorganisms which are a major component of organic flocs (Alldredge et al. 1993; Passow and Alldredge 1995; Bar-Zeev et al. 2012).

The proportion of cardiac stomach AFDM that was algae was determined for six arbitrarily selected fish by quantitative microscopy. Algal cells, predominately diatoms, were counted and measured using a drop transect method (Edmondson 1974) and cell volumes were calculated as the volume of a similarly shaped geometric solid (Ahlgren and Bowen 1992). Dry mass of algae was then estimated with the conversion factor of 1 mm^3 fresh algae = 0.1 mg dry mass. This conversion varies among taxa but is accurate enough for present purposes (Kaushik and Hynes 1968) . Coefficients of variation for two sets of three replicates were 15 and 16%.

Selective ingestion experiments

Selective ingestion was assessed in aquarium experiments. Approximately 40L of conditioned water from a large tank holding a variety of fish species was added to a 60-L aquarium and sand and benthic biofilm from the Riachuelo littoral were added and allowed to settle overnight. A sample of the benthic biofilm was collected from the aquarium and then five small *P. lineatus* (9.5 to 10.0 cm SL) were added at 1000 h. After 24 h, the fish were euthanized and the contents of their cardiac stomachs were pooled and dried at 105 °C pending chemical analyses. The experiment was repeated once.

Most statistical analyses were conducted using SigmaPlot 13 (Systat Software, Inc., San Jose, CA, USA). Parametric methods were used unless the data failed a test for normality or homogeneity of variance in which case nonparametric alternatives were used as indicated. Confidence intervals for medians were calculated with the bootstrapping procedure in SPSS 26 (IBM, Armonk, NY, USA).

Results

Field collections of fish

During the five sampling periods, river height ranged from 2.1 to 4.7 m (Fig. 2). Under high-water conditions of sampling period 2, all three sites were connected to the Rio Paraná main channel and thus classified as littoral habitats. During other sampling periods, the Colonia Tacuari and Madrejon del Puente sites were classified as floodplain lakes. The Riachuelo site was classified as all littoral habitat during sampling period 2 when its river channel could not be distinguished from the extensive littoral, but during the other sampling periods this site contained both river channel and littoral habitats. Compared to sampling period 2, littoral areas were substantially reduced in area and depth during other sampling periods. At the lowest water levels (sampling periods 3 and 5), much of the aquatic vegetation that had been in littoral areas and lakes was stranded on land leaving exposed areas of unvegetated sand and mud.

Forty-one of 84 net hauls captured one or more *P. lineatus* for a total of 402 individuals collected. There were twenty successful hauls in littoral areas, seven in floodplain lakes, and fourteen in river channels. The habitats in which *P. lineatus* were found varied among sampling periods (Table 1) Catch-per-effort as a proxy for fish density was not calculated because capture efficiency is likely to have differed with water depth and habitat. Standard lengths averaged 36.1 cm (range 27.0–47.0 cm) and live weight averaged 1.257 kg (range 0.475–2.470 kg).

Evidence of feeding activity

Of the 393 specimens examined, food was found in the cardiac stomachs of 260 (66%). The percentage with food did not differ across sites (ANOVA $p = 0.780$) but habitats were significantly different with the percentage in river channels (48.6%) less than in littoral areas (76.5%) and floodplain lakes (76.2%) (ANOVA $p = 0.025$). During sampling period 1 when sampling was limited to the Riachuelo site, 27.6% of the fish from the channel had been feeding compared to 73.0% from the adjacent littoral zone. For this group of samples, there were no significant differences in AFDM or AA values for cardiac stomach contents for fish from the two habitats (ANOVA $p = 0.677$, 0.835 respectively).

Feeding intensity did not show a clear diel periodicity. Although *P. lineatus* were collected between 0800 and 2230 h, the percentage of each catch with food in the cardiac stomach bore no apparent relationship to the hour of day. Feeding intensity did vary with river height. For the set of samples collected from the littoral and floodplain lakes, the average percentage with food in the cardiac stomach per net haul for a sampling period generally increased with the increase in mean river height for that sampling period (ANOVA of regression statistics $p = 0.049$, adjusted $R^2 = 0.698$) (Fig. 3).

Makeup of cardiac stomach contents

Examined by the unaided eye, the cardiac stomach (seg. 1) contained clusters of aggregated particles, sand, and plant fibers and fragments. Seen under the microscope, sand grains and translucent amorphous material made up most of the volume. The amorphous material stained thoroughly with alcian blue indicating the presence of acidic mucopolysaccharides that are abundant in biofilms (Passow and Alldredge 1995). Vascular plant debris distinguished by conspicuous remnants of cell walls was often present but was not a large fraction of either stomach contents or benthic biofilm.

The chemical composition of cardiac stomachs contents differed by habitat and by sampling period (Table 2). Fish from the littoral and from lakes contained material that was higher in g AFDM · g^{-1} DM and g AA · g^{-1} AFDM, and lower in g HROM · g^{-1} AFDM than fish from either river channel habitat.

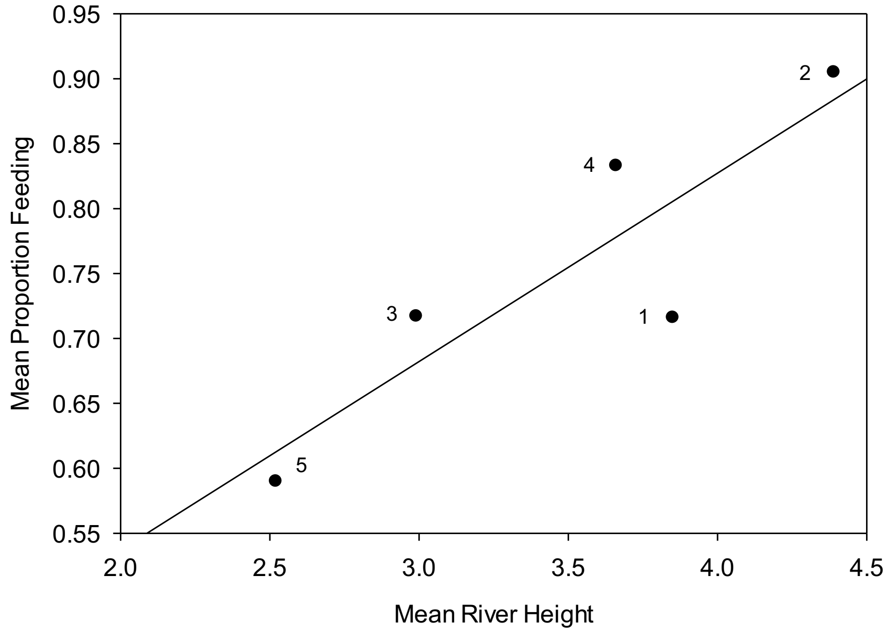

Fig. 3 The relationship between river height during the sampling period and the proportion of *P. lineatus* actively feeding across five sampling periods in Rio Paraná littoral and floodplain lakes in the vicinity of Corrientes, Argentina. ANOVA of regression statistics $p = 0.049$, adjusted $R^2 = 0.698$

Fish from littoral areas had the highest values for g AA · g^{-1} HLOM and fish from floodplain lakes had the highest values for g HLOM · g^{-1} DW. The greatest difference was found for fish from the Paraná channel: these had substantially lower values for each of the beneficial variables and substantially higher values for the non-nutritive variable (g HROM · g^{-1} AFDM). Compared across sampling periods, the greatest effect was for the high-water conditions of sampling period 2 when medians for g AFDM · g^{-1} DM, g AA · g^{-1} AFDM, g AA · g^{-1} HLOM, and g HLOM · g^{-1} DW were highest and HROM · g^{-1} AFDM was the lowest.

Energy density of cardiac stomach contents from five *P. lineatus* collected from the Colonia Tacuari and Riachuelo littoral sites during sampling period 2 averaged 18.9 kJ · g^{-1} AFDM ($n = 5$, range = 15.9 to 22.3).

Chemical composition of benthic biofilm

The chemical composition of benthic biofilm differed by both habitat and sampling period (Table 3). In general, benthic biofilm from littoral areas was highest in g AA · g^{-1} AFDM whereas that from floodplain lakes was highest in g AFDM · g^{-1} DM and g HLOM · g^{-1} DM. Material from the river channels was lowest in g HLOM · g^{-1} DM. Compared across sampling periods, the most prominent results are the high values for g AA · g^{-1} AFDM and g AA · g-1 HLOM for the flood conditions during sampling period 2.

The relationship of benthic biofilm chemical composition to water depth was examined for each site with significant relationships found only for g AA · g^{-1} AFDM and g AA · g^{-1} HLOM at the Riachuelo site (Fig. 4). During periods 1 and 3, g AA · g^{-1} HLOM decreased significantly with increasing depth (linear regression $p < 0.05$). The relationship for period 4 was nearly significant ($p = 0.073$). During period 5, benthic biofilm g AA · g HLOM^{-1} in the Riachuelo backwater did not show a relationship to depth but was related to a sudden > 1 m increase in water level due in part to the rising height of the Rio Paraná (Fig. 2) but more significantly to heavy rainfall in the Riachuelo watershed on November 24 and 25. During this period, benthic biofilm was collected at 5, 30, and 60 cm depths along two transects at the same location on each of 7 dates. The rise in water level was accompanied by a highly significant increase in g AA · g^{-1} HLOM in benthic biofilm between November 26 and December 12, 1991 (linear regression $p = 0.006$, adj$R^2 = 0.773$) (Fig. 5).

Table 2 Cardiac stomach contents compared across habitats and sampling periods in terms of five measures of food quality. Medians (95% confidence intervals) sharing superscripts are not significantly different at $p > 0.05$

	g AFDM · g^{-1} DM	g AA · g^{-1} AFDM	g HROM · g^{-1} AFDM	g AA · g^{-1} HLOM	g HLOM · g^{-1} DM
Habitat					
Moving littoral	0.164[a] (0.150–0.178)	0.101[d] (0.082–0.111)	0.340[e] (0.314–0.408)	0.174[g] (0.151–0.189)	0.101[h] (0.087–0.113)
Floodplain lakes	0.170[a] (0.158–0.226)	0.071[c,d] (0.060–0.102)	0.358[e] (0.348–0.380)	0.111[f] (0.095–0.160)	0.114[h] (0.104–0.150)
Riachuelo channel	0.138[a] (0.110–0.159)	0.053[b,c] (0.049–0.064)	0.492 (0.473–0.526)	0.122[f] (0.099–0.134)	0.069 (0.054–0.078)
Paraná channel	0.056 (0.053–0.069)	0.035[b] (0.016–0.056)	0.667 (0.624–0.738)	0.119[f,g] (0.066–0.152)	0.019 (0.015–0.024)
Sampling period					
1	0.098[i,k] (0.083–0.168)	0.076[m] (0.065–0.090)	0.427[n] (0.347–0.408)	0.132[p] (0.108–0.161)	0.055[q,r] (0.034–0.103)
2	0.190 (0.176–0.203)	0.141 (0.129–0.155)	0.285 (0.267–0.308)	0.205 (0.184–0.213)	0.138 (0.115–0.148)
3	0.092[i] (0.059–0.150)	0.059[l,m] (0.049–0.071)	0.577[o] (0.509–0.652)	0.136[p] (0.104–0.161)	0.040[q] (0.021–0.056)
4	0.139[i] (0.115–0.164)	0.055[l] (0.049–0.061)	0.518[o] (0.482–0.579)	0.122[p] (0.096–0.149)	0.068[q] (0.055–0.078)
5	0.160[k] (0.152–0.164)	0.067[l,m] (0.055–0.078)	0.361[n] (0.344–0.403)	0.115[p] (0.087–0.138)	0.101[r] (0.086–0.112)

Selective ingestion

Aquarium experiments and field data gave qualitatively similar but quantitatively different results (Table 4). Both approaches found that the fish selectively ingested AFDM and a fraction of AFDM that is relatively rich in AA, and selectively rejected Ash and HROM. In quantitative terms, the aquarium experiments showed a higher degree of selection than did the field data with respect to AFDM, HROM, AA, and HLOM. The selection factors that are most similar for the two approaches are those for g HROM · g^{-1} AFDM and g AA · g^{-1} HLOM.

Discussion

Components of the diet

Organic matter in the diet of *P. lineatus* and benthic biofilm examined in this study was predominately an aggregation of amorphous, translucent, non-living particulate organic matter and associated microorganisms termed biofilm sensu Flemming et al. (2016b). It is now well established that 90% or more of this organic material is derived from extracellular polymeric secretions (EPS) of microorganisms, transparent exopolymer particles (TEP) formed abiotically from dissolved organic matter (DOM), and DOM that may be incorporated through microbial absorption or abiotically through adsorption to the biofilm matrix (Cisternas-Novoa et al. 2015; Hongyue Dang 2016; Tansel 2018). Microorganisms typically comprise less than 5% of biofilm organic matter in natural environments (Bowen 1987; Tansel 2018) although they may represent a larger fraction in intensive aquaculture and sewage processing facilities (2–20%, Samocha (2019)). Bacteria make up the smallest fraction of microbial mass, typically < 1% of the AFDM (Geesey et al. 1978; Sutton and Bowen 1994). The fraction of cardiac stomach AFDM that was algae in the present study (mean = 4.73%) is higher than that found in an earlier study of *P. lineatus* at the Riachuelo site (1.1%) (Bowen et al. 1984) but is consistent with the range of values cited above.

Selective ingestion

The very high degree of correlation between stomach contents and benthic biofilm in field data at regular

Table 3 Benthic biofilm compared across habitats and sampling periods in terms of five measures of food quality. Medians (95% confidence intervals) sharing superscripts are not significantly different at $p > 0.05$

	g AFDM · g^{-1} DM	g AA · g^{-1} AFDM	g HROM · g^{-1} AFDM	g AA · g^{-1} HLOM	g HLOM · g^{-1} DM
Habitat					
Moving littoral	0.094 (0.086–0.101)	0.057^{c} (0.051–0.062)	0.540^{d} (0.479–0.568)	0.120^{e} (0.099–0.134)	0.042^{f} (0.037–0.048)
Floodplain lakes	0.179^{a} (0.151–0.202)	0.042^{b,c} (0.033–0.054)	0.434 (0.398–0.456)	0.081^{e} (0.061–0.101)	0.111 (0.090–0.118)
Riachuelo channel	0.131^{a} (0.104–0.167)	0.045^{b,c} (0.030–0.052)	0.554^{d} (0.542–0.612)	0.102^{e} (0.046–0.125)	0.057^{f} (0.045–0.073)
Paraná channel	0.028 (0.027–0.032)	0.036^{b} (0.030–0.038)	0.843 (0.741–0.928)	0.229 (0.137–0.514)	0.005 (0.002–0.008)
Sampling period					
1	0.112^{g} (0.097–0.118)	0.057^{j} (0.050–0.060)	0.385^{k} (0.372–0.459)	0.096^{m} (0.085–0.110)	0.068^{p} (0.054–0.073)
2	0.119^{g,h} (0.098–0.162)	0.086 (0.082–0.099)	0.381^{k} (0.343–0.412)	0.143^{o} (0.125–0.173)	0.076^{p} (0.060–0.102)
3	0.079 (0.075–0.085)	0.054^{j} (0.042–0.061)	0.568^{l} (0.518–0.595)	0.126^{n,o} (0.111–0.145)	0.035 (0.030–0.039)
4	0.175^{g,h} (0.131–0.220)	0.041^{i,j} (0.029–0.047)	0.547^{l} (0.510–0.570)	0.100^{m,n} (0.054–0.119)	0.076^{p} (0.054–0.106)
5	0.112^{g,h} (0.098–0.157)	0.032^{i} (0.028–0.040)	0.512^{k,l} (0.400–0.563)	0.060^{m} (0.046–0.074)	0.056^{p} (0.043–0.076)

time intervals during sampling period 5 provides assurance that the fish were feeding on the benthic biofilm that was sampled (Fig. 5) (Pearson correlation coefficient = 0.944, $p = 0.001$). Both the aquarium experiments and field data comparisons show that *P. lineatus* selectively ingests fractions of benthic biofilm of greater nutritional value (HLOM and AA) and selectively rejects fractions of little or no direct nutritional value (Ash, HROM) (Table 4). Similar selective ingestion for AFDM and AA or total nitrogen and against mineral matter has been demonstrated for other fishes feeding on biofilm, specifically for *Mugil cephalus* (Odum 1968), *Catostomus commersoni* (Ahlgren 1996), *Ichthyomyzon fossor* (Yap and Bowen 2003), and *Dorosoma cepedianum* (Mundahl and Wissing 1988).

The higher degree of selection found in aquarium experiments may be an artifact of the experimental procedure. Movement by the fish in the aquarium partially suspended and may have fractionated the biofilm and thus facilitated selection to a degree that does not normally occur in an undisturbed, natural environment (Odum 1968; Heidman et al. 2012).

Quantitative comparisons of cardiac stomach contents with benthic biofilm are complicated not only by selective ingestion but also by food processing in the cardiac stomach. As food passes through the digestive tract, some Ash including sand and some HROM including plant fibers are retained in both the cardiac and pyloric stomachs (the first two digestive tract segments) while other components are passed on to the pyloric chamber (the third segment). Median values for Ash and HROM are much higher in both stomachs than in the pyloric chamber (Bowen 2021). This means that the benthic biofilm ingested was lower in Ash and HROM than the contents of the cardiac stomach and thus the degree of selection will be underestimated for each of the variables in Table 4 that include Ash or HROM. This limitation does not apply to the variable g AA · g^{-1} HLOM. It does not contain either Ash or HROM and, unlike variables including Ash or HROM, medians for samples from the cardiac stomach, the pyloric stomach, and the pyloric chamber are not significantly different (ANOVA on ranks $p = 0.709$). Thus, the conclusions that can be drawn are that selective ingestion by *P. lineatus* (1) increased g HLOM · g^{-1} DM in the diet by at least a factor of two, (2) increased the g AA · g^{-1} DM in the diet by at least a factor of four, and (3) the fish selectively ingested a fraction of the HLOM with higher AA values, nearly doubling the g AA · g^{-1} HLOM.

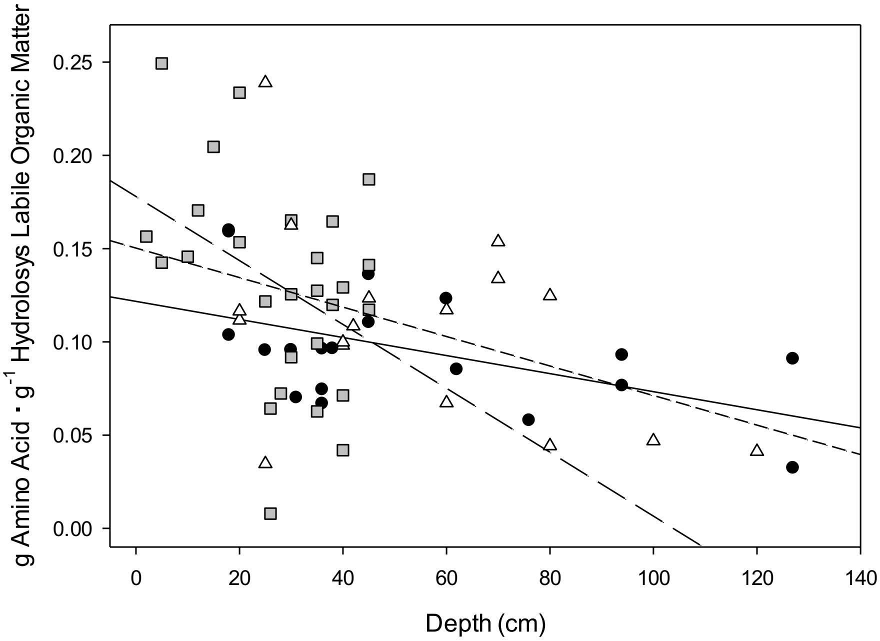

Fig. 4 The relationship between g AA · g^{-1} HLOM in benthic biofilm and water depth at the Riachuelo site during sampling periods 1, 3, and 4. Circles and solid line for sampling period 1 (linear regression $p = 0.022$, adj$R^2 = 0.229$), squares and long dashed line for sampling period 3 ($p = 0.040$, adj$R^2 = 0.124$), and triangles and short dashed line for sampling period 4 ($p = 0.073$, adj$R^2 = 0.145$)

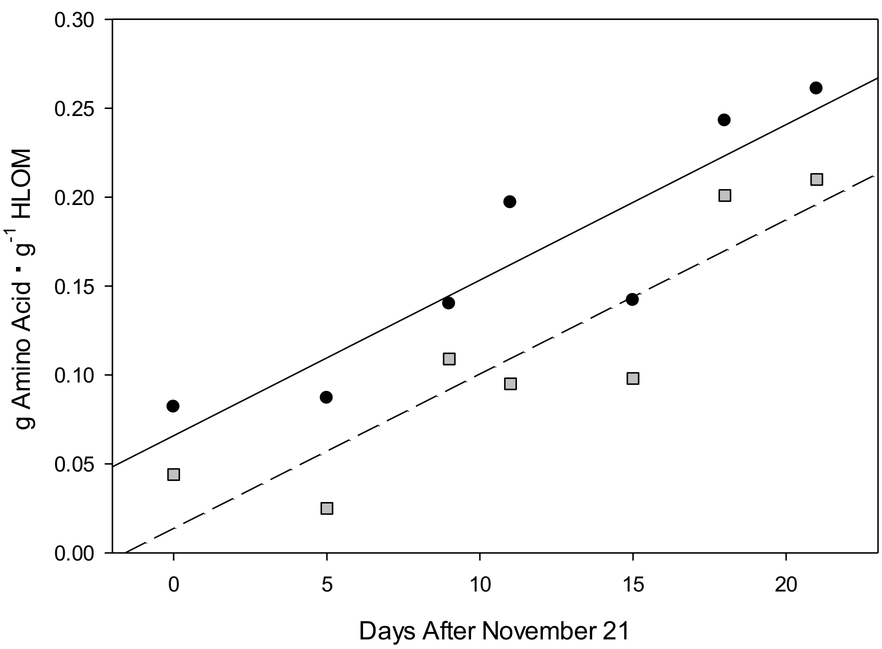

Fig. 5 Change in food quality (g AA · g^{-1} HLOM) following a > 1 m rise in water level in the Riachuelo littoral following heavy rains on November 24 and 25, 1991 (sampling period 5). Solid circles = means for fish cardiac stomachs, gray squares = means for benthic biofilm. Values for fish stomachs and benthic biofilm are correlated (Pearson correlation coefficient = 0.944, $p = 0.001$). Linear regression lines illustrate trends ($p \leq 0.005$)

Dimensions of food quality

A fundamental concept in animal nutrition is that consumers must obtain two macronutrients from their diets: energy and protein (Harper 1976). The food quality of a given diet can be described in terms of the extent to which it provides these two macronutrients. Energy intake is regulated by hunger and depends on how much food is eaten and how much energy the food yields. There is no nutrient-specific hunger for protein

Table 4 Selective ingestion by *P. lineatus*. Data for aquarium experiments are for samples pooled for all fish in the experiment. Field data are means for five dates (43 fish and 42 benthic biofilm samples) from the Riachuelo littoral collected during sampling period 5

	g AFDM · g^{-1} DM	g HROM · g^{-1} AFDM	g AA · g^{-1} DM	g HLOM · g^{-1} DM	g AA · g^{-1} HLOM
Experiment 1					
Cardiac stomachs	0.189	0.571	0.0208	0.081	0.256
Benthic biofilm	0.075	0.648	0.0044	0.026	0.168
Selection factor	2.52	0.88	4.70	3.071	1.53
Experiment 2					
Cardiac stomachs	0.178	0.438		0.100	
Benthic biofilm	0.066	0.761	0.059	0.016	
Selection factor	2.7	0.58		6.342	
Mean selection factor	2.61	0.73	4.70	4.71	1.53
Field data					
Cardiac stomachs	0.183	0.448	0.016	0.100	0.165
Benthic biofilm	0.116	0.575	0.006	0.052	0.107
Mean selection factor	1.60	0.79	3.88	2.03	1.91

or amino acids, so the intake of protein depends on the ratio of assimilable protein to assimilable energy. That is to say, the quantity of protein an animal assimilates depends on the quantity "that comes along with" the assimilable energy in the diet the animal selects. Within a specific range, the amount of assimilable protein per assimilable energy in the diet determines animal growth rate and growth rate is a major factor in ecological fitness. For a more detailed review of these concepts and their application to ilyophagus fishes, see Bowen (1984) and Bowen et al. (1995).

Protein must be digested before it can be assimilated as mono- and dipeptides, so the measure used in the present study, total amino acids, is nutritionally equivalent to dietary protein. Consumers' needs for specific essential amino acids are typically met for wild animals by their natural diets so the protein dimension of food quality is determined by the total amount of amino acid assimilated (Bowen 1980; Bowen et al. 1984; Ekasari et al. 2014).

In the present study, the energy quality of the food ingested was assessed as kJ assimilated · g^{-1} DM and calculated as:

$$Q_E = (gHLOM \cdot g^{-1}DM)_{CS} \cdot AE_{HLOM} \cdot ED$$

where (g HLOM · g^{-1} DM)$_{CS}$ is the g HLOM · g^{-1} DM in the cardiac stomach, AE_{HLOM} is the assimilation efficiency for HLOM (0.667) (Bowen 2021), and ED is the energy density of the diet (18.9 kJ · g^{-1} HLOM). Since the rate of food ingestion is unknown, this value serves as an index by which the relative energy quality of different cardiac stomach samples can be compared. The amino acid quality of the food, mg AA assimilated · kJ^{-1} assimilated, was calculated as:

$$Q_{AA} = (mgAA \cdot g^{-1}DM)_{CS} \cdot AE_{AA} \cdot QE^{-1}$$

where (mg AA · g^{-1} DM)$_{CS}$ is the mg AA · g^{-1} DM in the cardiac stomach and AE_{AA} is the assimilation efficiency for amino acids (0.735) (Bowen 2021).

Amino acid assimilation as the growth-limiting food quality variable

The ranges of values for amino acid and energy dimensions of food quality in the diet are compared by habitat and sampling period in Fig. 6. Blé and Arfi (2009), working with another ilyophagus fish, *Citharinus citharus*, in a shallow lake in the River Niger floodplain, Mali, found similar values for both dimensions (2.9 kJ assimilated · g^{-1} DM, 10.4 g AA assimilated · kJ^{-1} assimilated).

Fish require about 4 mg assimilated AA · kJ^{-1} assimilated energy for maintenance (Bowen 1984) and median values for each habitat and sampling period exceed that requirement. Under aquaculture conditions, most fish achieve maximum growth rate at > 20 mg assimilated AA · kJ^{-1} assimilated but growth rates approaching 90% of the maximum can

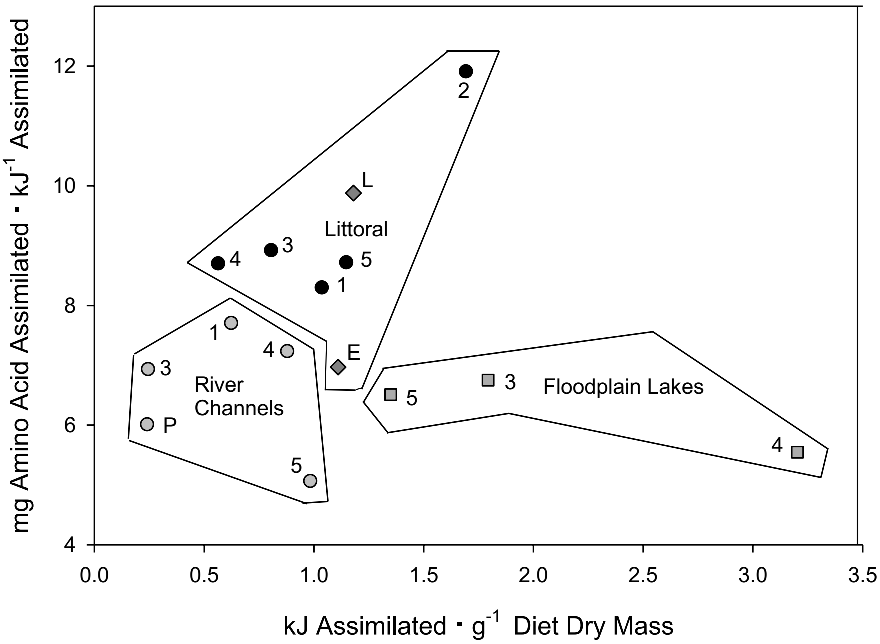

Fig. 6 The diet of *P. lineatus* in terms of two dimensions of food quality. Points are medians for the contents of cardiac stomachs (seg. 1). Samples were collected in three habitats over five sampling periods as denoted by numerals. Symbols labeled E and L are early (Nov. 26 to Nov. 30) and late (Dec. 2 to Dec. 12) in sampling period 5. The symbol labeled P is for fish collected during sampling period 3 along the margin of the main course of the Rio Paraná. The other river channel samples are from the Riachuelo site

be achieved in the range of 11 to 15 mg assimilated AA · kJ^{-1} assimilated (Bowen 1981, 1984; Bowen et al. 1995). Within the range of values found in the present study, we would expect Q_{AA} to be growth limiting, i.e., growth to be proportional to mg AA assimilated · kJ^{-1} assimilated with rapid growth at the higher levels found in this study.

The energy dimension of food quality, Q_E, is not likely to be growth limiting. Fish collected during each sampling period contained large mesenteric fat bodies implying energy assimilation well in excess of needs for metabolism and growth. Additionally, because the proportion of the population that is feeding at any one time ranged from to 59 to 91%, the fish would have the capacity to increase ingestion if their energy needs were not being met. Thus, the available evidence indicates that the AA dimension of food quality is the critical factor determining growth.

Food quality by habitat and water level

Both habitat and sampling period had significant effects on Q_{AA} and Q_E (two-way ANOVA on ranks, all $p \leq 0.007$). During each sampling period, the median Q_{AA} was highest in the littoral. When sampling periods are compared, Q_{AA} was highest during period 2 under flood conditions when all sites were classified as littoral and during rising water conditions during the latter half of period 5. When lake habitat could be distinguished, Q_E was consistently highest in floodplain lakes.

Habitat characteristics affect both formation and retention of benthic biofilm. Floodplain lakes are relatively stable environments where benthic biofilm would be expected to accumulate although wave action may suspend biofilm in very shallow water and cause it to settle in deeper areas. In the moving littoral, biofilm in shallow water is periodically desiccated and fundamentally altered as waters recede (Keitel et al. 2016). In river channels, periods of high flow would be expected to sweep away flocculent material. Values for g AFDM · g^{-1} DM in lake benthic biofilm were 1.4 to 6.3 times higher than in littoral areas and river channels (Table 3). HROM in lake samples is a significantly larger fraction of the AFDM and this is consistent with greater age of the biofilm as microbial metabolism breaks down labile components leaving refractory components to accumulate. Samples of benthic biofilm and fish stomachs from floodplain lakes in sampling period 4 have much higher values for g AFDM · g^{-1} DM and HLOM · g^{-1} DM than other habitat/sampling period combinations and thus those fish had the highest values for Q_E (Fig. 6). These samples were collected in June during the cool season when some aquatic macrophyte and riparian vegetation was senescent and may have released organic matter that contributed to the production of benthic biofilm.

The amino acid dimension of food quality is also related to habitat characteristics. Compared across habitats, in each sampling period, Q_{AA} was highest for fish collected in littoral areas. The littoral is the shallowest of the three habitats and g AA · g^{-1} HLOM values were found to be inversely proportional to depth at some littoral sites. Compared across sampling periods, flood conditions during sampling period 2 greatly increased the area of shallow water at all three study sites and the highest values for g AA · g^{-1} HLOM and g HLOM · g^{-1} DM in both benthic biofilm and *P. lineatus* cardiac stomachs were found under these conditions. The relationship between depth and the amino acid dimension of food quality is complicated by the continuously changing water depth at any specific littoral location. During sampling period 5, not depth *per se* but rising water level was coincident with significant increases in g AA · g^{-1} HLOM in benthic biofilm and g AA assimilated · kJ^{-1} assimilated by fish.

Sites where biofilm is most recently formed appear to provide food of the highest food quality. In earlier research on feeding by ilyophagus fishes, it was inferred that the amino acid dimension of food quality is a function of biofilm age (Bowen 1979a, b; Yap and Bowen 2003). Details of biofilm formation suggest why this may be expected. Early in its formation, biofilm appears to be relatively high in amino acids. Orvain et al. (2014) found that on tidal flats during the summer, freshly produced EPS contained 0.10 g protein · g^{-1} AFDM and as discussed above, EPS makes up > 90% of biofilm AFDM. In the present study, an even higher value (0.144 g protein · g^{-1} AFDM) was found for benthic biofilm in newly flooded littoral areas during the conditions of sampling period 2. Immediately following formation, biofilm may absorb and adsorb additional dissolved amino acids (Decho 2000), but later, both amino acids and HLOM are mineralized by microbes relative to refractory components of the biofilm. Domozych and Domozych (2008) found that protein in biofilm allowed to develop on Plexiglas sheets in a temperate wetland for 90 days first increased to a maximum of 6.5% of dry mass (approximately 9.6% of AFDM) after 30 days but declined thereafter. Dauda et al. (2018) found that newly formed biofilm in aquaculture tanks lost 30% of its total nitrogen in 3 weeks. Diagenetic processes have been shown to convert at least part of the amino acid fraction to humified or otherwise immobilized nitrogen of no nutritional value (Odum et al. 1979; Rice 1982; Decho and Gutierrez 2017). Thus, the available data indicate that newly formed biofilm may initially increase in amino acids but as it ages some of the amino acid is mineralized and another fraction becomes unavailable thereby reducing its value to consumers.

Shallow littoral waters have characteristics that make them well suited for biofilm formation. They have the greatest light intensity and highest temperatures to drive photosynthesis of benthic algae. The proportion of photosynthate lost from and/or released as EPS by photosynthesizing microorganisms increases with both temperature and light intensity so these conditions favor increased biofilm formation (Claquin et al. 2008; Pisani et al. 2011; Decho and Gutierrez 2017). During the advancing flood, benthic biofilm in the shallowest waters will be the most recently formed. Older biofilm in shallow waters will be transported down slope by wave action and wind-driven currents until it settles in deeper water with a lower probability of resuspension (Bowen 1979a, b). In consequence, we would expect biofilm in the shallowest water to be relatively recently formed.

On a longer timescale, receding waters expose littoral sediments which are then dry for days, weeks, or months. The effect of desiccation on benthic biofilm has not been studied directly, but it is reasonable to expect that at a minimum the biofilm would be substantially altered. In contrast, rising waters support new biofilm formation in shallow backwaters. Rewetted sediments and plant debris release phosphorus and nitrogen that stimulate algal growth and dissolved organic carbon that directly stimulates the production of biofilm (Schönbrunner et al. 2012; Keitel et al. 2016; Lu et al. 2017; Tansel 2018). Later in sampling period 5, littoral benthic biofilm and fish samples were collected from sites that had been dry earlier in the sampling period (Fig. 5). Nutrients and dissolved organic matter released from re-wetted sediments could account for the increasing amino acid levels in both benthic biofilm and fish diets as the waters rose. This scenario fits with the available information but the processes of benthic biofilm formation in shallow freshwaters and the effect of age on biofilm nutritional value have not yet been studied in a systematic and comprehensive way that would provide confirmation of the interconnections of these process as inferred here.

Whatever the reason, shallow littoral waters contain benthic biofilm of superior nutritional value and *P. lineatus* takes advantage of it. Artisanal fishermen in the Paraná basin consistently say that *P. lineatus* feed in very shallow water, occasionally in water so shallow that their dorsal fins and backs are exposed. They are the first fish to enter newly flooded backwaters and the last to leave as waters recede. As the river level rises, the extent of littoral areas increases as does the percentage of *P. lineatus* feeding. Higher in the Paraná drainage in the Pantanal do Miranda-Aquidauana, Brazil, Lescano de Almeida et al. (1993) found just half of *P. lineatus* had food in their cardiac stomachs during low water levels compared to 100% when the lowlands were flooded. A similar preference for benthic biofilm in shallow water was found for juvenile *Sarotherodon mossambicus* that make daily migrations to the shallowest waters of sand terraces along the shores of Lake Sibaya, S. Africa to feed on amino acid rich benthic biofilm (Bowen 1979b).

The success of P. lineatus in exploitation of flocculent benthic biofilm

The findings of this study support both hypotheses set out in the "Introduction." The food quality (capacity to support growth) of biofilm does differ by habitat and flood condition and *P. lineatus* does locate and selectively ingest biofilm of superior food quality while avoiding and/or rejecting lower quality biofilm. Thus, there appear to be three elements of its feeding strategy that allow *P.* lineatus to support such a large population by exploiting a food resource unused by most other fishes. The first is behavioral. They feed preferentially in areas where and at times when the biofilm is of high nutritional value. The second may involve both behavior and buccal morphology as they selectively ingest higher quality biofilm within their immediate environment. Third is the behavioral response of increased feeding intensity as waters rise and food quality increases. Each element increases both the amino acid dimension and the energy dimension of food quality, but in each case the greatest benefits are to the amino acid dimension. Together, these constitute an integrated trophic strategy that is highly effective in supporting a large population biomass.

The flood pulse concept illustrated

The flood pulse concept can be distilled to two principal elements (Junk et al. 1989). First, that river/floodplain community production and structure results not from materials imported from upstream but rather from primary production stimulated by the release of nutrients from floodplain soils and vegetation as the flood pulse advances. Second, that in systems in which the flood pulse is regular and of significant duration, consumers will adapt to exploit this productivity. The feeding ecology of *P. lineatus* described in this report illustrates both of these.

The advancing flood across the Paraná floodplain stimulates production of new biofilm on previously dry benthic substratum. The nutritional quality of benthic biofilm in the study area is adequate to support the rapid growth of *P. lineatus* only during the flood pulse and in waters close to the aquatic-terrestrial transition zone. In its digestive morphology (Bowen 2021) and behavior, *P. lineatus* is highly adapted to the use of benthic biofilm as its food resource. Its dependence on the flood pulse is such that *P. lineatus* year class strength in the Rio Uruguay (González-Bergonzoni et al. 2019) and year class strength and condition factors (mass per length) in the Rio Paraná (Gomes and Agostinho 1997) have been found to be directly proportional to the extent of annual flooding. Its ability to utilize benthic biofilm as its food resource allows *P. lineatus* to make up more than half the fish biomass in the Rio Paraná and to significantly contribute to the structure of the river/floodplain community as the primary prey of most piscivores. Other prochilodontids with essentially identical digestive tract morphology appear to play similar roles in the other major neotropical river/floodplain systems (Taylor et al. 2006): *P. mariae* in the Orinoco River (Saldana and Venables 1983; Lasso et al. 2016) and *P. nigricans* in the Amazon River (Yossa and Araujo-Lima 1998; Silva and Stewart 2017; Bayley et al. 2018).

In addition to ilyophagus fishes, benthic biofilm is an important food source for larval amphibians (Ocock et al. 2018), at least a few shorebirds (Jiménez et al. 2015; Lourenço et al. 2017), and freshwater and marine benthic invertebrates (Hobbie and Lee 1980; Simon et al. 2003). In the past, biologists trained to identify plants and animals have produced extensive tables to describe stomach contents in taxonomic terms but tended to dismiss the non-living components of benthic biofilm as incidental. It has become increasingly apparent that in many cases the plant and animal constituents of benthic biofilm are incidental and the non-living component is the source of nutrition. More specific knowledge of where and how animals feed on benthic biofilm is likely to clarify the results of work intended to identify ecosystem-scale energy flow and material cycling using stable isotopes (Dodds et al. 2014; Marchese et al. 2014; Arantes et al. 2019). Even more fundamentally, the processes that produce benthic biofilm in a wide range of environments and determine its nutritional value for diverse consumers will be interesting and potentially important topics for future research.

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Data availability Data on which this manuscript is based are archived in the Emory University open-access archive Dataverse. Reviewers may access them at https://dataverse.unc.edu/privateurl.xhtml?token=b28d5fa9-0ba2-4946-8506-e57935ebe2e1

Code availability Does not apply.

Declarations

Ethics approval No approval was required or sought when this research was conducted. All aspects of the research were conducted in compliance with Argentinian law 14346 Article 3 concerning ethical research using vertebrate animals.

Conflict of interest The authors declare no competing interests.

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