Epilithic biofilm as a key factor for small-scale river fisheries on Caribbean islands

E. LEFRANÇOIS & S. COAT
EA 926 DYNECAR, Département de Biologie, Université des Antilles et de la Guyane, Pointe-à-Pitre, Guadeloupe, France

G. LEPOINT
MARE Center, Laboratoire d’Océanologie, Université de Liège, Sart Tilman, Belgium

N. VACHIÉRY
UMR 15 CIRAD-INRA, Domaine Duclos, Prise d’eau, Petit Bourg, Guadeloupe, France

O. GROS
UMR-CNRS 7138, Département de Biologie, Université des Antilles et de la Guyane, Pointe-à-Pitre, Guadeloupe, France

D. MONTI
EA 926 DYNECAR, Département de Biologie, Université des Antilles et de la Guyane, Pointe-à-Pitre, Guadeloupe, France

Abstract  Numerous freshwater species are consumed or exploited through artisanal fisheries in the rivers of the islands of Guadeloupe, French West Indies. Autochthonous production of organic matter is limited in these flowing rivers and is mainly represented by scarce filamentous green algae and an abundant epilithic biofilm growing on wet stones in the river bed. Stable isotope analysis was used to quantify the relative importance of biofilm and other riverine allochthonous and autochthonous food sources in the diet of tropical shrimps (Palaemonidae, Atyidae and Xiphocarididae) and fishes (Gobiidae and Eleotridae) consumed by the local people. The epilithic biofilm was exploited by most species, constituted an important source of autochthonous carbon and was an important source of organic matter production at the base of freshwater food webs in Caribbean rivers. Biofilm percentages in the diet reached 32% for molluscs, 85% for atyid shrimps, 29% for xiphocaridid shrimps, 14% for palaemonid shrimps and 13% for fish. Assessment of biofilm in nine rivers showed that blue and red cyanobacteria were quantitatively dominant with a moderately rich diatom flora. These results address the interactions between river biofilm and Caribbean freshwater fauna where trophic links between consumers and their potential resources have poorly been documented.

KEYWORDS: crustacean, epilithic biofilm, fish, Guadeloupe, rivers, tropical.

Introduction
The aquatic fauna of mixed saline waters of river estuaries is a resource used for food in tropical islands. Transition-zone biotopes where salinity changes according to distance from the ocean, water depth, run-off and season are among the most ecologically productive in the world (Day et al. 1989). According to Rothschild (1996), 70% of the global fish resources spend critical parts of their lives near the shore or near...
river mouths. In tropical islands, people who choose to fish in river mouths have a chance of catching record-size specimens, higher quantities than in the open sea or specimens at particular life stages, especially juvenile fish and shrimp. Fourteen of the 26 native freshwater species common in Guadeloupe, French West Indies, are harvested for human consumption. The most exploited taxa are the Palaemonidae and Atyidae families of crustaceans, which are found in rivers and exhibit benthic and territorial behaviours. Fishing in rivers is usually carried out on foot, without boats or sophisticated fishing gear; and many microhabitats are exploited by the local people using a variety of simple and well-designed implements, such as bamboo baskets. The popular crustaceans of the Atyidae family [Atya innocuous (Herbst) or Atya scabra (Leach)] are a category of special interest because of their lack of claws, their aggregative distribution (Monti & Legendre 2009) and non-aggressive behaviour. Atyid shrimps represent an easy-to-catch source of protein and are locally known as ‘the poor man’s meat’.

In the Caribbean Islands, species found in freshwater biota are practically all diadromous, with amphibromy being the most common strategy (Pringle 1997; Keith 2003). In this type of life cycle, the larva drift towards the sea, and the post-larvae or juveniles re-enter the rivers 2–3 months later (Lord-Daunay 2009). These migrations are made on a regular basis, usually once or twice a year, in massive rhythmic inputs of live organisms from the sea (Monti 2005; Zimmermann 2009). At this time, the post-larvae and juveniles of the fish families Gobiidae and Eleotridae (locally called ‘titiris’ or ‘tritiri’) are also consumed by the Guadeloupean people, as these fishes re-enter fresh waters in large numbers.

Moreover, throughout the Caribbean region, wild populations of Palaemonidae shrimps Macrobrachium spp. are of special interest because they often support commercial or artisanal fisheries (Holthuis 1980; Hunte & Mahon 1983). The largest individuals sometimes fetch a better price than the spiny lobster, Panulirus argus (Latreille). Thus, in Guadeloupe, as in developing countries (FAO 2008), small-scale fisheries based on river crustaceans and fish contribute to nutrition, to sustainable livelihoods or to poverty alleviation.

The trophic webs of these small tropical-island rivers have peculiarities caused by a unique combination of migrant species, tropical climate, steep catchment areas and strong disturbance regimes. Variation in flow regime is considered the most important source of natural disturbance in highly turbulent tropical stream systems (Jackson et al. 2001). Due to turbulent flows (waves and bubbles caused by large rocks), high water velocity, frequent flood events and moderate water depths, phytoplanktonic and zooplanktonic compartments are poorly represented. Such rivers are strongly heterotrophic (Ortiz-Zayas et al. 2005). Autochthonous production is limited and mainly represented by scarce filamentous green algae and an epilithic biofilm growing on stones in the river bed (Coat et al. 2009). In such conditions of limited primary production, animals need to develop optimal strategies to catch and use allochthonous organic matter such as leaves, fruits and drifting particulate matter (DPM; Covich 1988; Henderson 1990; Iwata et al. 2003; Coat 2009). Moreover, species are able to adapt their diet, revealing high trophic plasticity (Winemiller 1991; Jensen & Winemiller 2002). A better knowledge of the dependence of these animals on autochthonous and allochthonous production and an evaluation of the key compartments in energy fluxes are vital for the sustainable management of small-scale river fisheries on Caribbean islands. The resilience of such aquatic ecosystems and their ability to sustain high species richness will be dependent on their capacity to assimilate energy inputs.

The objectives of this study were: (1) to quantify the incorporation of autochthonous organic production in the diets of Caribbean fish and crustacean species, with a particular focus on epilithic biofilm consumption; and (2) to couple these results with qualitative and quantitative data obtained on the composition of the biofilm (bacterial and photosynthetic elements) to be able to offer greater insight into the functioning of the Caribbean freshwater ecosystem.

Methods

Evaluation of food sources in animal diets

Sample collection Aquatic organisms were sampled in an 800-m long stretch of the lower part of the Rivière Grande Anse, Guadeloupe (16°00’N and 61°30’W, Fig. 1) during the rainy season, between September and October 2006. The lower part of the Rivière Grande Anse was chosen as being suitable for the first objective of the study due to its high aquatic biodiversity (Coat 2005). All the elements of the aquatic food web were sampled. Biofilm was scraped from the surface of submerged rocks, both in rapids and in calm biotopes. Filamentous green algae were collected from the river bed, leaf and fruit detritus were sampled from depositional areas and drifting organic matter was collected with a net (35 μm mesh) set for 1 h in the
water column. Fishes and shrimps were captured using backpack electric fishing gear (DEKA 3000; Gerätebau, Marsberg, Germany). Molluscs and crabs were picked by hand, and aquatic insects were collected using a hand-made Surber sampler. Plants and animals were frozen at −30 °C before being identified, enumerated and prepared for isotopic measurements.

Isotopic measurements Muscle tissue of fishes, shrimps and crabs was dissected, and molluscs were analysed whole (shell excluded). Samples were oven dried for 48 h at 50 °C and then ground to a homogenous powder using a pestle and mortar. Measurements of carbon and nitrogen isotopic ratios were carried out with a mass spectrometer (Optima; GV Instruments, Cambridge, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Pisa, Italy) for combustion and automated analysis. Isotopic ratios were presented as δ values (‰) expressed in relation to the vPDB (Vienna PeeDee Belemnite) standard and to atmospheric N₂ for carbon and nitrogen, respectively. Reference materials were IAEA-N1 (δ¹⁵N = 0.4 ± 0.2‰) and IAEA CH-6 (δ¹³C = −10.4 ± 0.2‰). Experimental precision (based on the standard deviation of replicates of an internal standard) was 0.3‰ and 0.4‰ for carbon and nitrogen, respectively. A lipid
normalisation procedure, essential for lipid-rich tissues (C:N > 4), was used to take into account the effects of lipid isotopes (DeNiro & Epstein 1977).

**Data analysis and food source modelling** $\delta^{13}$C vs $\delta^{15}$N values were plotted to determine the trophic linkages between producers and consumers. For each species, the potentially exploited diet items (identified from isotopic signatures and literature) were tested with the Isosource 1.3 software (Phillips & Gregg 2003), a program used to estimate the proportion of the multiple potential food sources in the consumers’ diets when $n$ isotopes are being used and more than $n + 1$ sources are likely to be contributing to a mixture. Isosource uses stable isotope data to calculate feasible ranges of source contributions through the following procedure. First, all possible combinations of source proportions that sum to 100% were calculated in user-specified increments (set at 1%). Second, the predicted isotope values of each animal were computed using linear mixing-model equations that preserve mass balance. Isotope values of computed mixtures are then compared with the observed isotope values, and the range of combinations that match within a user-specified tolerance value (set at 0.4%) was then described.

Trophic fractionation for C (i.e. the shift in stable isotope ratio between a consumer and its diet) was set at 1‰ for all consumers (DeNiro & Epstein 1981; Rau et al. 1983). Trophic fractionation for N is greater and increases with high-protein diets (McCutchan et al. 2003). Fractionation values for N were assumed to be 2‰ for molluscs, 2.5‰ for detritivorous shrimps and algivorous fishes and 3‰ for omnivorous shrimps, crabs and fishes. These presumed feeding modes are based on dietary data from the literature coupled with the interpretation of the $\delta^{13}$C vs $\delta^{15}$N plot. The mass balance tolerance was set at 0.4‰/‰ for each sample and the source increment was set at 1‰. A spreadsheet available from the U.S. Environmental Protection Agency (http://www.epa.gov/wed/pages/models.htm) was used to perform calculations for the Isosource model. Differences between the isotopic signatures of sources and consumers were tested using the Mann–Whitney or Kruskal–Wallis nonparametric tests (XLSTAT-PRO version 7-5-2; AddinSoft, Paris, France).

**Determination of epilithic biofilm components**

**Sample collection** Biofilm was concurrently sampled in the downstream reaches of nine rivers, including Rivière Grande Anse. At each site, five previously cleaned natural stones were placed in riffles to constitute replicates. Riffles were selected to avoid sediment deposition. After 3 weeks of colonisation, the biofilm was collected according to the European standardised method NF T90-354 (AFNOR 2007). Briefly, this procedure included the collection of the biofilm sample from 100 cm² artificial or natural substrate placed in a sunny riffle. Samples were stored in flasks containing 40 mL of river water at 4 °C. Half of each sample was processed by flow cytometry within 3 h of collection. The other half of each sample was fixed in formaldehyde at a final concentration of 3% for species identification.

**Flow cytometry analyses** After filtration through a 40 μm nylon filter (Becton Dickinson, San Jose, CA, USA), samples were analysed on a FACSCalibur flow cytometer equipped with an argon-ion excitation laser (blue light, 488 nm) and the CellQuest 3.01 software (Becton Dickinson). A forward and side scatter gate was set to exclude events characterised by very low forward light intensity caused by the abundance of dispersed particles resulting from the biofilm suspension. One hundred thousand events within this gate were acquired per sample.

Aliquots were taken from each sample and stained with propidium iodide (PI) and Syto 9 (Molecular Probes, Invitrogen, Cergy-Pontoise, France) at a final concentration of 3% for 20 min at room temperature and in the dark. The fluorescence of cells stained with PI and Syto 9 was measured to study cell viability; Syto 9 produces green fluorescence when excited by blue light and PI suppress this fluorescence specifically in dead or damaged cells. It follows that green fluorescence identifies the living part of the cell population.

Measurement of the forward light-scatter signal (correlated with the size of the cells) and of the side light-scatter signal (correlated with the granularity of the cells) allowed the identification of several groups of cells. The size scatter was calibrated using 1 μm fluorescent polystyrene balls. Cytometric analysis of a pure *Nitzschia palea* diatom culture was undertaken to confirm that diatoms result in large size and high granularity events and that they produce a red fluorescence under a 488 nm incident light due to chlorophyll $a$ and carotenoid pigments. The only other auto-fluorescent pigments are phycobilins found in cyanobacteria and cryptomonads (Gregor & Marsálek 2005).

All cyanobacteria species synthesise phycocyanin (non-fluorescent under blue light); but only a few and mostly marine species (‘red cyanobacteria’; Parésys et al. 2005) synthesise phycoerythrin, which emits an
orange fluorescence ($\lambda = 575$ nm) under green light (optimum $\lambda = 532$ nm). Several measurements were, therefore, carried out: (1) the proportion of red and orange auto-fluorescence in the subset characterised by large size and high granularity events; (2) the mean fluorescence intensity of each subset, which depends on the number of pigment molecules per cell; and (3) cell viability (green fluorescence after staining) expressed as the percentage of viable cells in all the events analysed by flow cytometry.

**Species identification** Species were identified according to European standardised methods NF EN-14407 (AFNOR 2004). Diatom identification was based on microscopic observation of the frustule. Samples preserved in formaldehyde solution were first processed with boiling hydrogen peroxide (30%) and, when necessary, with hydrochloric acid to eliminate cell protoplasm. They were then centrifuged and rinsed. After dehydration, a drop of the pellet was included in a very highly refractive medium (Naphrax; Brunel Microscopes Ltd, Chippenham, UK) between the slide and coverslip. Four hundred valves per sample were observed using a polarising microscope at 1000× and determined to species level. Some samples were analysed with a scanning electron microscope to assist taxonomic identification. Formalin-preserved diatoms were rinsed three times in distilled water before dehydration through an ascending series of acetone, critical-point dried in CO2 and sputter-coated with gold before observation under a Hitachi S 2500 scanning electron microscope (Hitachi France, Paris, France) at an accelerating voltage of 20 kV.

**Results**

**Food web structure and contributions of food sources to animal diets**

Algae, biofilm, leaves, fruits and DPM made up the basal resources of the food web. Insect biomass was found to be negligible in the Rivière Grande Anse. The consumer community included molluscs (Neritidae, Thiaridae and Ampullaridae), shrimps (Atyidae, Xiphocarididae and Palaemonidae), crabs (Pseudothelphusidae) and fishes (Gobiidae and Eleotridae). Figure 2 illustrates the food web structure, with nitrogen isotopic ratios ($\delta^{15}$N) indicating the trophic levels of organisms and carbon isotopic ratios ($\delta^{13}$C) estimating the energy links between basal food sources and consumers. The dominant carbon sources of producers (squares in Fig. 2) displayed the lowest $\delta^{15}$N values, indicating their basal positions in the food web. Isotopic signatures of these dominant carbon sources were distinguished, including DPM, biofilm and algae. The proximity between DPM and leaves originating from terrestrial ecosystem suggested that DPM was mainly composed of plant fragments. $\delta^{15}$N signatures of consumers were higher and were found to increase from mollusc to fish species. Crustaceans displayed intermediate nitrogen signatures.

Relative contributions of the food sources calculated using the Isosource model revealed that biofilm was commonly and heavily exploited by the freshwater fauna (Fig. 3). All but the crab species appeared to consume this autochthonous resource. The mean proportion of biofilm in the consumers’ diets varied among groups and ranged from 32% for molluscs to 13% for fishes. Focusing on crustacean species targeted by small-scale fisheries, the mean proportion of biofilm in the diet reached 96% for *Atya innocus* (Herbst), 73% for *Atya scabra* (Leach), 29% for *Xiphocaris elongata* (Guérin-Méneville), 13% for *Macrobachium acanthurus* (Wiegmann), 15% for *Macrobachium crenulatum* Holthuis, 16% for *Macrobachium faustinum* (De Saussure) and 12% for *Macrobachium heterochirus* (Wiegmann).

**Biofilm composition**

The identification and assessment of the relative abundance of biofilm components were performed through flow cytometry. Three subsets (R1, R2 and R3 regions) were distinguished according to size, granularity and fluorescence measurements. Only events of a size between 1 and 100 μm (belonging to
the R1 region) can be defined as eukaryotic cells. These cells are also characterised by a high granularity (Fig. 4A). Events smaller than 1 μm include debris and some prokaryotes, and these were not analysed by fluorescence. Among these events (R1 region), photosynthetic cells exhibited a red fluorescence (R3 region), while some other events displayed an orange fluorescence (R2 region) (Fig. 4B). The orange fluorescence was confirmed by fluorescence microscopy, and the cells were, therefore, considered to be cyanobacteria. The other events of the R1 region were not fluorescent under blue light and included empty frustules and blue cyanobacteria (those containing only phycocyanin).

The biofilm analysed for the nine rivers showed a strong dominance of cyanobacteria among fluorescent cells. Although blue cyanobacteria are usually found in rivers (Parésys et al. 2005), cytometric analysis revealed a high proportion of red cyanobacteria, which varied from 77% of all fluorescent cells in Rivière Moustique Sainte Rose to nearly 100% in Grande Rivière de Vieux-Habitants (Fig. 5).

The biofilm diatom community in the nine sampled river mouths was made up of both cosmopolitan taxa and those that are specific to the region. The results revealed low diversity, with only 58 species, and a strong dominance of a few taxa. Three families of pennate diatoms (Naviculaceae, Nitschiaceae, Monoraphideae) made up 99% of the diatom community, and Araphideae and Epithemiaceae were scarce. Cymbella tropica Krammer, Eolimna verecundaeformis (Manguin), Gomphonema brasiliense ssp. pacificum

Figure 3. Mean contribution of biofilm, algae, plant detritus and animal resources to the diet of freshwater consumers in the Rivière Grande Anse.

Figure 4. (A) Cytometric profile of epilithic biofilm. (B) Fluorescence under blue light of R1 events: (a) chloroplasts of diatoms (Gomphonema sp.); (b) chloroplasts of chlorophyta; (c) protoplasm of cyanobacteria (fluorescence microscopy, ×1000).
Moser, *Navicula incarum* Lange-Bertalot & Rumrich, *Navicula quasidisjuncta* Lange-Bertalot & Rumrich, *Naviculadicta nanogomphonema* Lange-Bertalot & Rumrich were the main taxa found in Guadeloupe rivers.

**Discussion**

**Biofilm as a significant food source**

Food webs in the rivers of Caribbean islands are essentially based on biofilm, filamentous algae and terrestrial or plant detritus (Coat et al. 2009). In these rivers, flow is a major factor in aquatic ecosystem organisation due to the limitation of allochthonous food source residence time (litter, fruits or terrestrial products) and the negatively affected capacity for development of macroalgae under high velocity conditions. The results obtained during this study show that epilithic biofilm is the autochthonous producer most exploited by a wide range of freshwater species, and that it could even constitute the dominant resource for some species, such as atyid shrimps, in running waters. One reason why biofilm production supports an important part of animal biomass in these systems could be that allochthonous carbon is mostly recalcitrant (i.e. it resists bioactivity). On the other hand, carbon from autochthonous primary production, although much less plentiful, is generally more labile (easier to assimilate), contains more energy per unit mass and is typically preferred by metazoans (Thorp & DeLong 2002).

Another reason could be that the biofilm, tightly affixed to the stony substrate, is one of the few perennial resources available. Considering the extreme variability of floods determining allochthonous inputs in Guadeloupean rivers (Chaperon et al. 1985), epilithic biofilm could be considered pivotal to the sustainability of aquatic biodiversity. This greater-than-expected trophic role of biofilms in animal diets is supported by research on aquatic ecosystems that has demonstrated unexpected biofilm grazing behaviours in species other than invertebrates or fish, as observed in shorebirds (Kuwae et al. 2008).

**Scarcity of microalgae**

The biofilm composition (i.e. the proportion of each of its components: heterotrophic bacteria, cyanobacteria, microalgae and extracellular matrix) and its nutritional quality are well known in temperate aquatic systems (Liess & Kahlert 2009) and in some tropical areas (Pringle 1996; Burns & Ryder 2001), but little is known about the biofilm composition in the Caribbean islands. The results obtained on the epilithic biofilm of nine rivers located in Guadeloupe Island show a relative scarcity of diatomic compartments. One consequence is that the use of diatomic richness and diversity as a biological indicator of trophy and pollution of running waters (Prygiel & Coste 1999; Prygiel et al. 2002) may be less powerful in these Islands than in other countries.

Abreu et al. (2007) demonstrated that biofilm is an important food source, especially as a nitrogen
source, for juvenile penaeid shrimp. These authors also showed that juvenile shrimp preferentially consume centric diatoms, and they assume that this selective consumption stimulates biofilm chlorophyll a. This study found that centric diatoms were scarce in the biofilm, and the diatoms were mostly pennates. These results could be because of selective grazing of centric diatoms, but, unfortunately, the impact of animal behaviours on biofilm components and diatom species selection is still undocumented in Caribbean rivers. Another reason for the scarcity of centric diatom could be the influence of the strong hydrological disturbance regimes of the Caribbean rivers. Lopes Thompson et al. (2002) demonstrated that during the colonisation of biofilm on the substrate, which takes 2–3 weeks in these tropical rivers (Monti & Lefrançois 2010), bacterial populations and pennate diatoms first colonise the stones of the river bed. Perpetually exported by hydrological pressures and sediment abrasions, the epilithic biofilm of these rivers could be considered as being maintained in a non-mature state of colonisation. In Caribbean rivers, the enrichment and diversity of diatomic populations included in epilithic biofilm could have consequences for animal feeding and for the sustainability of the aquatic biodiversity. This needs to be evaluated.

Importance of cyanobacteria in biofilm

In the present study, the high proportion of cyanobacteria in the biofilm linked to the low fraction of microalgal components calls for additional studies to improve the identification of the epilithic cyanobacteria populations consumed by aquatic species, including the evaluation of a possible toxicity risk. Cyanobacteria are opportunistic organisms with high adaptive capacities, and some species are likely, under still poorly understood circumstances, to synthesise toxic metabolites called cyanotoxins (Araoz et al. 2007). Usually, among aquatic species, zooplankton is considered as one of the main targets of cyanotoxins Lampert 1981; De Bernardi & Guissani 1990; Ferrão-Filho et al. 2009), with proven ecophysiological consequences on planktonic crustaceans affecting motility, reproduction, filtering rates and growth (DeMott et al.1991; Lurling 2003). In humans, cyanotoxins provoke health disorders depending on the route of exposure, the quantities absorbed and the toxicity of the cyanobacterial strain (Cecchi et al. 2009). Freshwater contamination by cyanobacteria and the toxins they synthesise can limit utilisation of water resources (Chorus & Bartram 1999). In Guadeloupe Island, 84.6% of the tap water comes from water abstraction points located directly in rivers (DIREN 2010). Given the importance of cyanobacteria in river biofilms, the level of cyanotoxins in tropical fresh waters should be of interest.

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BIOFILM AS A KEY FACTOR FOR SMALL-SCALE RIVER FISHERIES


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