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Otolith microchemistry in *Sicydium punctatum*: indices of environmental condition changes after recruitment

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Abstract – The amphidromous life cycle and morphological characteristics of Sicydiinae species allow them to colonise tropical freshwater habitats from the river mouth to the upper reaches of the river basin. Otolith microchemistry of 62 *Sicydium punctatum* from the Pérou River, Guadeloupe (French West Indies) was investigated with a femtosecond laser ablation-inductively coupled plasma mass spectrometer (fs-LA-ICP-MS) in order to reconstruct individual life history and follow the possible movements of *Sicydium punctatum* after the recruitment. Sr:Ca fingerprint confirmed the amphidromous cycle of this species. Variations of Ba:Ca in some individuals indicated changes in environmental conditions after the recruitment in the river. Even if results did not allow concluding to the specific origin of these variations, the hydrological regime and the biofilm composition may have a non negligible influence on the Ba availability. Results supported the use of multi-elemental signatures in otoliths and highlight the need for a large geographical and temporal sampling of Ba and Sr in freshwater systems for a better understanding of amphidromous fish species.

Key words: Tropical freshwater goby / Otolith microchemistry / Barium / Strontium / *Sicydium punctatum* / Gobiidae / West Indies

1 Introduction

In tropical island rivers (Indo-Pacific and Caribbean), Gobiidae and Eleotridae are the most represented fish families. In Gobiidae, Sicydiinae exhibit an amphidromous life cycle (McDowall 1988; Keith 2003) adapted to fragmented habitats and oligotrophic freshwater environments, which are subject to extreme climatic and hydrological seasonal variations. Adults spawn in freshwater habitats. After hatching, the free embryos drift downstream towards the sea where the larval development occurs. This marine phase of larval growth lasts three to six months (Bell 1994; Lord et al. 2010) and is a key to species dispersal among insular regions (Keith et al. 2008). In fish species, after larval growth, transparent pelagic post-larvae, feeding on plankton, recruit back to rivers where they undergo an extensive metamorphosis. Individuals become pigmented, benthic and herbivorous, feeding on algae and diatoms by scraping them off the hard substrate (Keith et al. 2008). Although more than 100 Sicydiinae species exhibit this life pattern (McDowall 1988; Ryan 1991), little is known about the details and modalities of this cycle. It is essential to understand each step of this cycle for conservation issues as recruiting post-larvae support fisheries, which are declining (Manacop 1953; Bell 1999). *Sicydium punctatum* (Perugia, 1896), distributed in Caribbean rivers is a key species of Caribbean island freshwater ecosystems. Its post-larvae dominate goby fry fisheries in this area like in Dominica where the species accounts for 95% or more of the catch (Bell 1994).

Tropical rivers support growth, spawning and downstream migration of goby free embryos. After hatching, the survival of larvae depends on the distance from the nest to the sea. Drift is obligate since larvae cannot live long in freshwater (Bell and Brown 1995; Iida et al. 2010). Valade et al. (2009) showed that for *Sicyopterus lagocephalus* from Reunion Island, the maximum survival period in freshwater is four days. Bell and Brown (1995) observed developmental stages of *Sicydium punctatum* larvae found within 0.2 km from the sea in Dominica rivers and found that most larvae reaching

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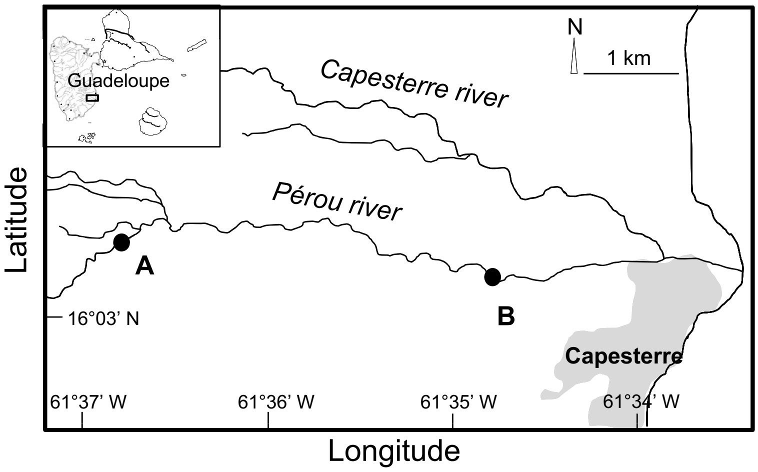

Fig. 1. Sampling sites on Guadeloupe Island (West Indies).

the sea were under one day old. Despite a lower survival of drifting larvae from upstream nest, *Sicydium punctatum* adults live in rivers from the coastal zone to altitudes over 300 m and up to 14 km inland (Bell and Brown 1995). The reasons why Sicydiinae as *S. punctatum* migrate so far upstream in the river remain unclear. Bell (2009) suggested that agonistic behaviour could explain the distribution and the presence of adults far upstream with the possibility for some individuals to migrate downstream after having reached a sufficient length to compete with the large downstream gobies. Recently, the analysis of otolith microchemistry of three *Sicyopterus* species from New Caledonia and Vanuatu rivers showed different behaviour patterns during the freshwater phase especially back and forth patterns between freshwater and estuarine sites tending to support various reproductive or diet behaviour hypotheses (Lord 2009). At present, no other information is available about the habitat use during the adult stage of *Sicydium punctatum*. Such information is therefore of major concern as insular growth habitats can be highly anthropogenized and may affect *S. punctatum* population.

Otoliths are well known calcified structures, metabolically inert and continuously growing throughout life, consisting of successive discrete layers of aragonite crystalline microstructure corresponding to daily growth increments deposited on a protein matrix (Campana 1999). Microstructures and macrostructures are used to accurately estimate the fish's age and to analyse and date specific events such as recruitment, metamorphosis or reproduction (Lecomte-Finiger 1999). In otoliths from amphidromous species, metamorphosis is materialised by a metamorphosis check mark, formed as the fish recruits to the rivers (Shen and Tzeng 2002; Keith et al. 2008). This mark is important to date life history events of the fish. The increments are also able to trap minor and trace elements coming from the surrounding water and food within the matrix during the calcification process. In combination with age data, otolith elemental composition has been used to reconstruct migratory environmental history of fish (Elfman et al. 2000; Arai et al. 2007).

Among elements trapped in the otolith, strontium (Sr), particularly the Sr:Ca ratio, is the most common marker used to describe migratory environmental history of diadromous and catadromous fish (Limburg et al. 2001; Milton et al. 2008). The Sr content in the otolith of teleost fish is positively correlated to ambient salinity and above all Sr concentration of the surrounding environment (Tzeng 1996; Tsunagawa et al. 2009; Tabouret et al. 2010). Elevated Sr:Ca ratios in fish otoliths are widely accepted as proof of occupation of marine habitats (Shen et al. 1998; Howland et al. 2001) or of a marine larval stage (Closs et al. 2003) whereas low Sr:Ca values are attributed to occupation of freshwater habitats. Recent findings (Elsdon and Gillanders 2005; Hamer et al. 2006) have also underlined the potential of the Ba:Ca ratio to track environmental histories of fish, due to the ability of the element to be incorporated into otoliths primarily in relation to ambient concentrations (Bath et al. 2000; Elsdon and Gillanders 2004; De Vries et al. 2005).

In this study, we tried (i) to confirm the amphidromous life cycle and (ii) to determine if there are differences of Sr and Ba otolith composition within two *S. punctatum* groups from a Guadeloupe River and if both elements can be relevant markers of the environmental conditions of the species after the recruitment. This investigation was based on the analysis of otolith microchemistry, especially Sr:Ca and Ba:Ca ratios, using femtosecond laser ablation coupled with inductively plasma mass spectrometry (fs-LA-ICP-MS). This is the first step to a better understanding of the freshwater life history and migratory behaviour of *S. punctatum*. Understanding the distribution and the habitat use of *S. punctatum* is essential for the species conservation and management.

2 Materials and methods

2.1 Sampling

Fish were sampled at two stations (site A (upstream): 16° 03′ 32 N – 61° 36′ 43 W; site B (downstream): 16° 03′ 14 N –61° 34′ 45 W) on the Pérou River located in Basse-Terre in Guadeloupe (French West Indies, Caribbean sea) (Fig. 1). Three sampling surveys were made in 2009: the first one during the dry season in April, the second one at the

Table 1. Total length (mm) of *Sicydium punctatum* caught in April, May and July on site A and site B of the Pérou River (Guadeloupe).

		Sampling month			
Site		April	May	July	Total
A	N	12	10	10	32
	Mean	47.3	40.0	39.3	
	Std. Dev.	13.0	11.4	12.4	
	Min	31	29	24	
	Max	71	65	58	
B	N	8	14	8	30
	Mean	40.3	43.5	35.9	
	Std. Dev.	11.9	11.9	8.4	
	Min	27	25	23	
	Max	57	58	45	
Total					62

end of the dry season in May and the last one in July at the beginning of the rainy season. Individuals were collected using a DEKA 3 000 electrofishing system (Gerätebau, Mårsberg, Germany) in flowing sections and pools (<1 m depth) along a 50 m transect approximately. 136 individuals were collected at the upstream site and 109 at the downstream site. Samples covered the entire size range observed among the catches (20 to 72 mm). From 8 to 14 individuals per survey and site ($N_{\text{total}} = 62$) were selected for the otolith microchemistry analysis (Table 1).

2.2 Otolith preparation and microchemistry analysis by femtosecond laser ablation–ICP-MS

Sagittal otoliths were extracted from the fish under a binocular magnifier (Olympus VMZ), rinsed with distilled water in order to eliminate organic residues and dried. Left otoliths were individually embedded in Araldite epoxy resin (Araldite 2020, Escil, France) and ground on a sagittal section in order to expose the core with 1 200-grain carbide silicon abrasive disc then a finer 2 400-grain disc (Escil Chassieu, France). The sections were polished with diamond paste of decreasing grain diameter (3 to 0.1 µm) and thoroughly rinsed between each step and at the end with milliQ water. Preparations were finally dried and kept in dry clean tubes until microchemistry analysis.

Otolith samples were analyzed with an IR 1 030 nm femtosecond laser (Alfamet-Novalase, France) coupled to an Elan DRC II (Perkin Elmer) mass spectrometer. This analytical method allows the quantitative determination of trace and minor elements within the otolith at a high spatial resolution (Coutant and Chen 1993; Arai and Hirata 2006). A linear raster scan ablation 20 µm width was made along the longest radius of the otolith. The laser beam was applied with a 500 Hz frequency at a 5 µm s^{-1} speed. The transect runs from otolith edge to edge, passing through the core and the metamorphosis check mark (Fig. 2). Ablation conditions are those described by Tabouret (2009) for the analysis of eel otoliths and Lord (2009) for the analysis of otoliths from *Sicyopterus* species. The entire transect was selected in order to determine chronological variation of ^{86}Sr and ^{138}Ba.

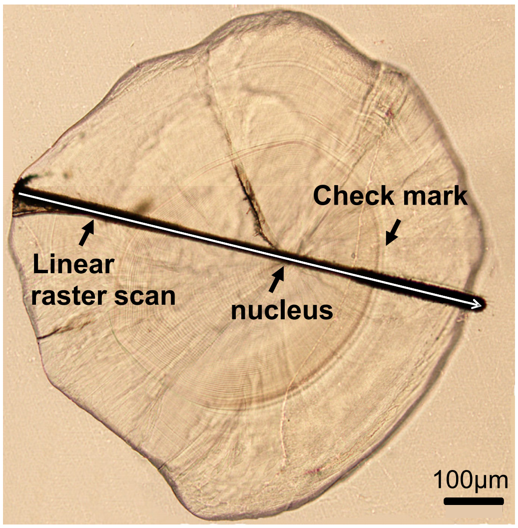

Fig. 2. Sagittal section of the left otolith from *Sicydium punctatum* (total length: 48 mm showing the linear raster scan made by the femtosecond laser ablation-ICP-MS from one edge to the other edge of the otolith and passing through the nucleus and the metamorphosis check mark.

Analytical accuracy was measured with the fish certified otolith reference material No. 22 (National Institute for Environmental Studies, Japan; Yoshinaga et al. 2000). ^{43}Ca was used as an internal standard for each ablation to check for variation in ablation yield. Sr and Ba were standardised to calcium (i.e. Sr:Ca and Ba:Ca) based on the stoichiometry of calcium carbonate (386 000 µg Ca g^{-1} otolith), as these elements can substitute for calcium in the otolith matrix (Campana 1999). Element:Ca ratios are expressed in µg g^{-1}. Quantification of ^{86}Sr and ^{138}Ba concentration was allowed using a standard curve based on the analysis of calcite pellets (Barats et al. 2007). The average detection limit based on three standard deviations (SD) of the blank gas was 767 ng g^{-1} for ^{86}Sr and 32 ng g^{-1} for ^{138}Ba. In the following section, the whole profiles of both elemental ratios are exposed. The ablation from one edge of the otolith to the other passing through the nucleus explains the profile symmetry and allows detection of potential artefacts. After the elemental analysis, the check mark was identified on each otolith using an Olympus BX40 light microscope and placed on the elemental profile.

2.3 Data analyses

In order to test for the amphidromous hypothesis, the average elemental ratios during the larval phase (LP) were compared to the average elemental ratios recorded after the check mark (PR for post-recruitment). Differences were tested using parametric tests (two-sample *t*-test) after checking the data normality using a Shapiro Wilks test. As high Sr value is known as a good marker of the marine environment, the possible recovery of the data from both phases was tested with the comparison of the minimum Sr:Ca value during LP and the maximum Sr value during PR for each individual using a pair-sample *t*-test. The maximum Ba values during the LP were also compared to the minimum values during PR.

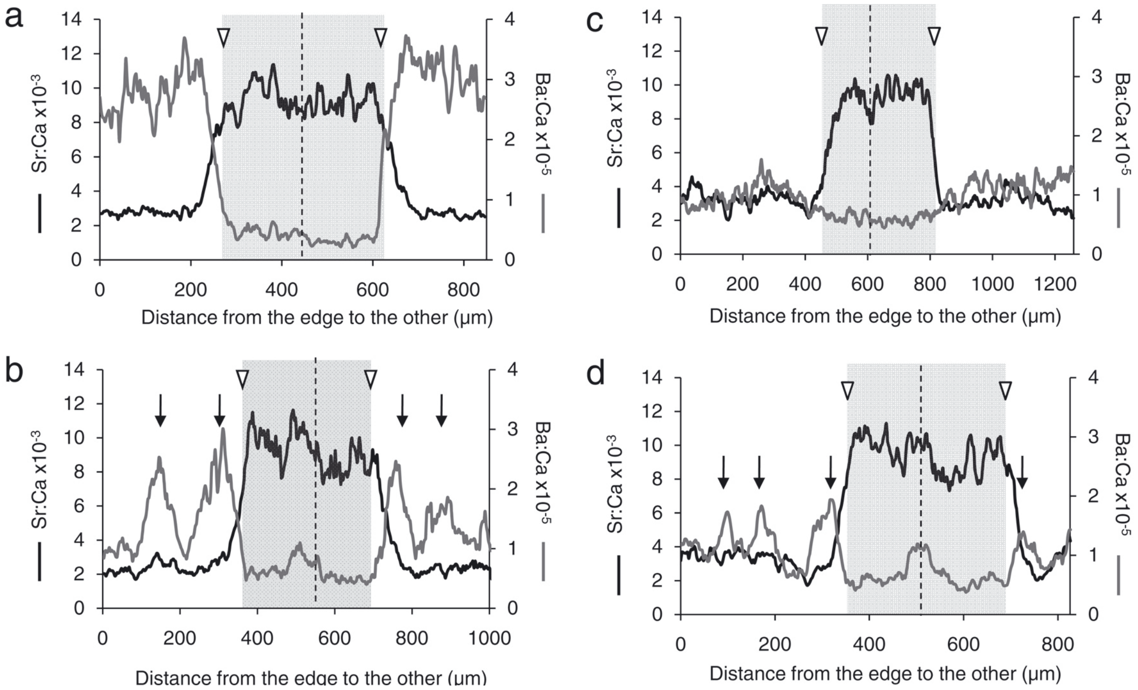

Fig. 3. Profiles of Sr:Ca and Ba:Ca ratios provided by a continuous raster scan with fs-LA-ICP-MS on otoliths of two *Sicydium punctatum* (total length, (a) 48 m, (b) 54 mm) from the site B of the Pérou River (Guadeloupe) and of 2 individuals (c) 60 mm, (d) 44 mm) from site A. Dotted line: nucleus; grey zone: marine larval phase; white arrows: recruitment; white zone: adult phase; black arrows: Ba:Ca increase.

The elemental difference between both sampling sites was also investigated with the comparison of the mean elemental ratios calculated during the PR. When individuals showed significant Ba variation, the average concentration was calculated outside the Ba peaks (i.e. the Ba:Ca ratio baseline) and during the Ba peaks. During the Ba peaks, mean Ba:Ca and Sr:Ca ratios were calculated as the mean of the 5 points before and the 5 points after the centre of Ba peak. Care was taken for variations occurring at the end of the larval phase not to induce bias due to the larval or ontogenic signature. Such a method of calculation was chosen as each Ba peak was defined by more than 15 points. This method also takes into account the peak shape (wide or narrow) and the intra-individual variability. Moreover, without the possibility to exactly determine the fish age after the recruitment (daily increments not clearly distinguished) it was not possible at this time to use a statistical test based on all the profile and a temporal scale. The Ba and Sr baseline signals were calculated as the mean of all the values outside the Ba peaks during the post-recruitment phase. The elemental baselines of both sites were compared using a two-sample *t*-test. The difference between elemental ratios during peak and the baseline was investigated using a pair-sample *t*-test. All results are expressed within a 95% confidence interval. All statistical analyses were performed using Origin Pro8 software (OriginLab, USA).

The occurrence of the Ba variations was investigated to determine the regularity and the origin of the variations. The relation between the Ba peak occurrence, the individual length and the sampling site was tested using the Pearson correlation coefficient. Deposition rate of otolith increments after the metamorphosis check mark in *Sicydium punctatum* is not validated as in other species of Sicydiinae where one increment corresponds to one day (Lord et al. 2010). This prevents the exact dating of elemental events. In order to investigate the synchronicity of Ba:Ca variation events, profiles of 3 individuals of the same size class (54 mm, 54 mm and 56 mm), caught at the same date and site (site B) were compared. This comparison aims at determining the main factor influencing the Ba:Ca composition of the otolith during the adult phase.

3 Results

3.1 Microchemistry analysis: inter-site comparison

For all individuals ($N = 62$) of *Sicydium punctatum* from both sites of the Pérou River, the Sr:Ca ratio shows the same pattern and value range. Within the central part of the otolith, from the nucleus to the metamorphosis check mark, Sr:Ca ratios exhibit high values (mean value: $9.39 \pm 0.50 \times 10^{-3}$) (Fig. 3). In the same otolith part, low values of Ba:Ca are observed (mean value: $0.55 \pm 0.16 \times 10^{-5}$). After the check mark, Sr and Ba ratio patterns change drastically. On both sampling sites, Sr:Ca ratios show a significant decrease down to values comprised between 2 and 4×10^{-3} that remain constant or with low variations until the otolith edge (Fig. 3). Based on the mean ratios within each part (larval phase; post-recruitment phase), Sr:Ca ratios during the larval phase were significantly different from the values recorded during the life after the metamorphosis check mark ($N = 62$; $p < 0.001$) (Fig. 4). This

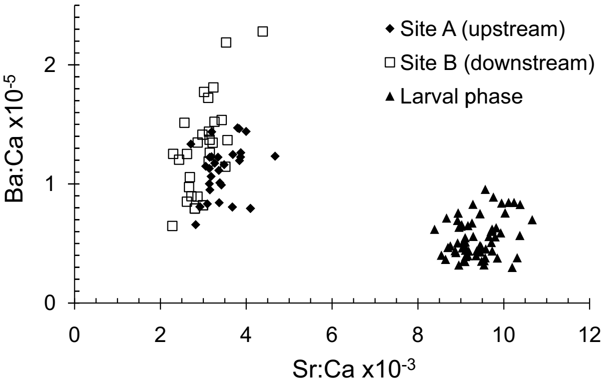

Fig. 4. Mean Sr:Ca and Ba:Ca concentrations in otolith of *Sicydium punctatum* during the larval phase and after the recruitment for individuals from site A (N = 32) and site B (N = 30) sites of the Pérou River (Guadeloupe).

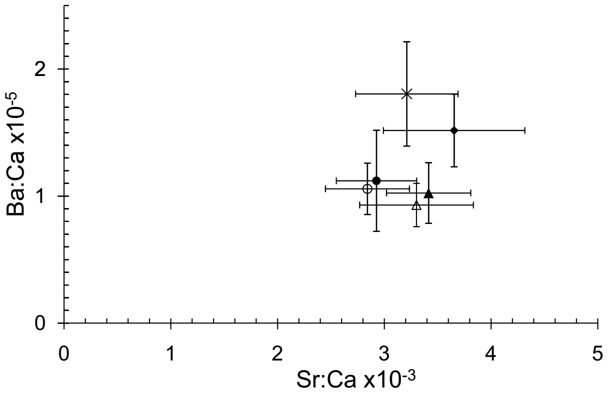

Fig. 5. Mean Sr:Ca and Ba:Ca concentrations in otolith of *Sicydium punctatum* from the Pérou River after the recruitment (◆) in varying individuals from site A during Ba peaks (N = 20), (Δ) in varying individuals from site A outside the Ba peaks (N = 20), (▲) in non varying individuals from site A (N = 12), (X) in varying individuals from site B during Ba peaks (N = 13), (○) in varying individuals from site B outside the Ba peaks (N = 13), (●) in non varying individuals from site B (N = 17). Standard deviation is given by the black bars.

difference is also confirmed by the comparison of the minimum Sr values recorded during LP and the maximum Sr values during PR showing no overlap of the ratio ranges of both phases ($N = 62$, $p < 0.001$). However, the minimum Ba ratios during PR are not significantly different from the maximum Ba ratios during LP ($N = 62$, $p = 0.069$).

Ba:Ca ratio profiles after the check mark are more complex than those of Sr:Ca. For all individuals whatever the site, Ba:Ca ratios increase after the check mark (Fig. 3) but with a high degree of variability. Two main types of profiles are distinguished. The first type gathers profiles showing no or low Ba:Ca variations from the check mark to the edge (Figs. 3a, 3c). 57% (N = 17) of *S. punctatum* from the downstream site and 37% (N = 12) of the individuals from the upstream site exhibit this pattern. Individuals showing this pattern are called "non varying" individuals. These individuals can be subdivided into two other groups, the first with high constant Ba ratios and the second with low Ba ratios. As only one individual from site B (Fig. 3a) shows high constant Ba ratios, high and low Ba groups were considered as one group for the following investigation.

The second type of profile shows multiple marked variations of Ba:Ca ratio along the profile from the check mark to the edge of the otolith (Figs. 3b, 3d). The amplitude of these variations can be several order of magnitude higher than the Ba:Ca ratio baseline. 43% of the individuals caught downstream (N = 13) showed this type of profile as did 63% (N = 20) of fish caught upstream. These individuals are called "varying" individuals.

For all the individuals with Ba:Ca variations, Sr:Ca values observed during the Ba peaks are significantly different from the Sr:Ca values observed outside these peaks ($p < 0.001$; $N = 33$; Fig. 5). The mean Sr:Ca value ($3.44 \pm 0.61 \times 10^{-3}$) during the Ba peak represent an increase of 10% of the Sr:Ca mean value outside the Ba peak ($3.12 \pm 0.54 \times 10^{-3}$). In addition, Sr:Ca otolith background was not different between varying and non varying fish from both sites (upstream: $N = 32$, $p = 0.517$; downstream: $N = 30$, $p = 0.548$). Nevertheless, the baseline ratios were significantly different between sites A and B, site B showing slightly lower values (Fig. 5; $N = 62$; $p < 0.001$). The same trend was observed during Ba peaks (Fig. 5; $N = 33$; $p = 0.0388$).The Ba:Ca mean value during the peaks ($1.59 \pm 0.28 \times 10^{-5}$; $N = 33$) represents a 62% increase of the baseline value ($0.98 \pm 0.19 \times 10^{-5}$; $N = 33$). Mean Ba:Ca values outside the Ba peaks in varying fish were also not significantly different from the mean Ba:Ca background in the non varying individuals from each site (site A: $N = 32$, $p = 0.288$; site B: $N = 30$, $p = 0.393$). Ba:Ca backgrounds on both sites were also very similar (Fig. 5; $N = 62$; $p = 0.046$). The same pattern was observed for the Ba:Ca peak values. Varying individuals from site B showed slightly higher values than the fish from site A (Fig. 5; $N = 33$; $p = 0.043$).

3.2 Investigation of Ba:Ca variation synchronicity

Considering the length of the individuals caught in the Pérou River, no relation was found between this parameter and the peak occurrence ($N = 33$; $r = 0.076$) neither between the occurrence and the sampling site (occurrence per individual in site $A = 2.1 \pm 0.9$ variations; site $B = 2.2 \pm 1.0$ variations).

The three individuals of the same size class from site B show two major Ba:Ca increases between the metamorphosis check mark and the edge of the otolith (Fig. 6). These two ratio increases are simultaneous between both individuals (SL: 54 mm, Figs. 6a, 6b). The first increase is located 250 μm from the nucleus and the second increase 420 μm from the nucleus. These variations are also observed on the third individual (Fig. 6c) even if synchronicity is not as good as the previous one. However, synchronicity of Ba:Ca variations is not observed for all individuals of a given size class. Sr:Ca and Ba:Ca profiles from two *S. punctatum* are shown from site B (Fig. 7). Despite a same length, 38 mm, Ba:Ca profile from the two individuals show two different patterns, one with no high increase of ratio values (Fig. 7a) and one with two increases from 1 to 2.5×10^{-5} (Fig. 7b).

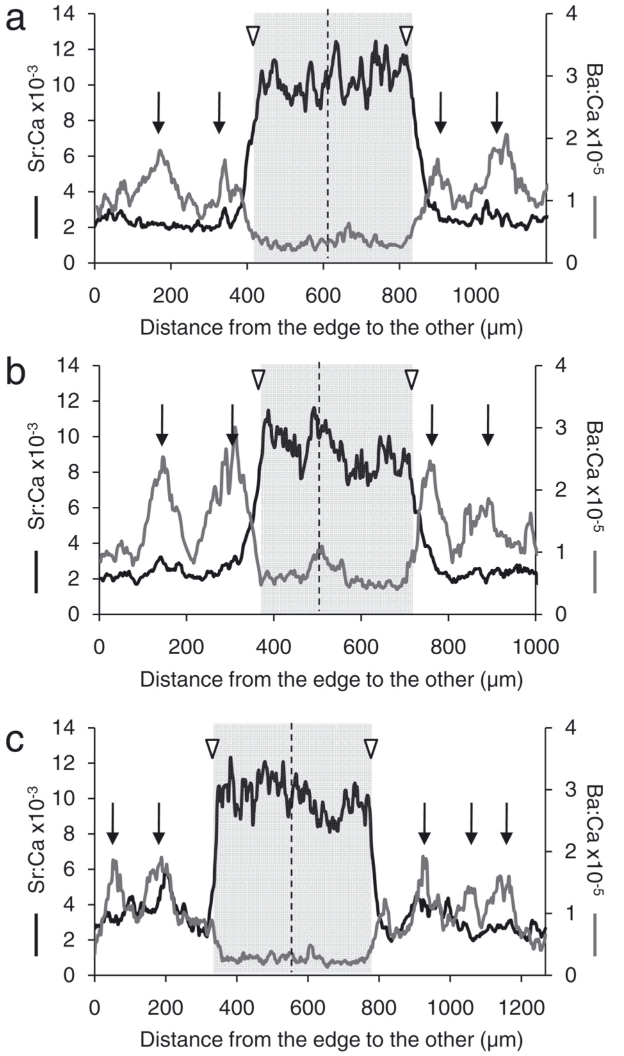

Fig. 6. Profiles of Sr:Ca and Ba:Ca ratios provided by a continuous raster scan with fs-LA-ICP-MS on otoliths of 3 *Sicydium punctatum* of same size class (total length, (a) 54 mm, (b) 54 mm, (c) 56 mm) and caught during the same sampling survey on site B of the Pérou River (Guadeloupe). Dotted line: nucleus; grey zone: marine larval phase; white arrows: recruitment; white zone: adult phase; black arrows: Ba:Ca increase.

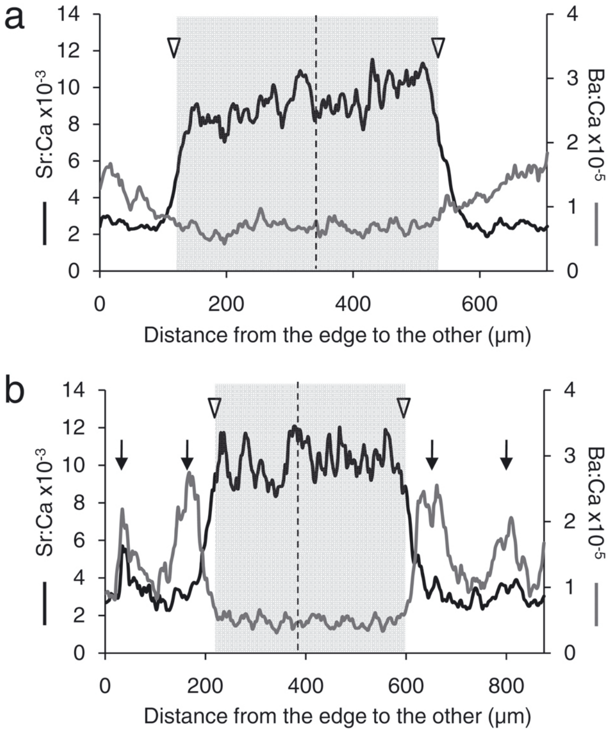

Fig. 7. Profiles of Sr:Ca and Ba:Ca ratios provided by a continuous raster scan with fs-LA-ICP-MS on otoliths of 2 *Sicydium punctatum* with a same length (a) and (b) 38 mm) and caught during the same sampling survey on site B of the Pérou River (Guadeloupe). Dotted line: nucleus; grey zone: marine larval phase; white arrows: recruitment; white zone: adult phase; black arrows: Ba:Ca increase.

4 Discussion

4.1 Amphidromy

Microchemistry analyses of *S. punctatum* otoliths revealed high Sr:Ca ratios and low Ba:Ca values from the nucleus to the check mark. These values are significantly different from the elemental signals observed after the metamorphosis check mark. High Sr:Ca in fish otoliths are attributed to marine habitats and illustrate the marine larval phase of other amphidromous goby species (Radtke and Kinzie 1996; Shen et al. 1998; Tsunagawa et al. 2009). In *S. punctatum*, the drastic decrease of Sr:Ca values in otolith is concomitant with the check mark. After this event, mean Sr:Ca ratios (Sr:Ca = 3.23 ± 0.48 × 10^{-3}; N = 62) are close to those described in otoliths of fish inhabiting freshwater habitats in temperate areas as in volcanic island rivers (Tsunagawa et al. 2009; Tabouret et al. 2010). Furthermore, these values are in agreement with the low Sr:Ca ratios met in waters draining volcanic island arcs such as Guadeloupe and Martinique (4.0 ± 1.7 × 10^{-3} calculated using Sr and Ca data from Rad et al. 2007). This contrasts with the high Sr:Ca ratio observed in the ocean (∼19 × 10^{-3}).

Finally, the Sr:Ca behaviour and values observed during the larval phase and the recruitment of *S. punctatum* is a good marker of the amphidromous life cycle of this species.

4.2 Ba variations: changes of the environmental conditions or inter-riverine movements?

S. punctatum, as other Sicydiinae and goby species, is known to be territorial (Fitzsimmons and Nishimoto 1990; Marquet et al. 2003). However, recent observations based on microchemistry of otolith from *Sicyopterus lagocephalus* (Lord 2009) and *Rhinogobius* sp. (Tsunagawa et al. 2009) suggest that some individuals have the ability to move from freshwater habitats to brackish or marine habitats and to have flexible migratory patterns. Based on the low Sr:Ca ratio values observed in our study between the check mark and the otolith edge, downstream migration to marine habitats does not occur after the recruitment to both sites in the Pérou River. Among the *S. punctatum* population studied here, for 57% of the individuals from site B and 37% of the individuals from site A, Ba:Ca ratios in otolith of *S. punctatum* are stable from the check mark to the otolith edge and tend to support the settlement of individuals in a given freshwater habitat. This agrees with other studies and indicates that at recruitment, all individuals undergo an upstream migration and appear to settle as they reach hard substrates in freshwater habitats to altitudes over 300 m and up to 14 km inland (Bell and Brown 1995).

However, for 43% of the individuals from site B and 63% of the group from site A, Ba:Ca profiles show significant variations with values 2 to 3 fold higher than the baseline values observed between the check mark and the edge. Ba:Ca variations were previously found in otoliths from *Sicyopterus* species during the freshwater growth phase (Lord 2009), as well as in otoliths of some other diadromous fish species (eel, alosa, salmon, trout) of temperate climates (Wells et al. 2003; Tabouret et al. 2010). Temporal variability of Ba in otolith has been reported with a time scale from seasonal to interannual (Thorrold et al. 1998; Gillanders 2002; Feyrer et al. 2007). The regularity of Ba:Ca peaks observed in *S. punctatum* from a same size class suggests a seasonal variability of the Ba:Ca otolith composition. In our study, three processes may explain these variations: (i) the influence of physiological processes on Ba uptake, (ii) potential movements between different habitats having significantly different Ba:Ca signature, (iii) environmental changes of the habitat conditions over short distances and according to time.

Influence of physiological processes

The elemental composition of otolith is known to be influenced by physiological processes like metamorphosis, growth rate, reproductive status and stressful events. Temperature and growth rate are known to be at least as influential as ambient concentration in modifying otolith elemental composition (Campana 1999). However, according Bath et al. (2000) Sr and Ba incorporation in fish otoliths are independent from growth rates of individual fish. In the Pérou River, the average temperature varied between 23 and 26 °C from June 2009 to December 2010 (P. Cattan, pers. comm.). No effect on the Sr and Ba incorporation in otolith was observed for this range of temperature (Bath et al. 2000; Elsdon and Gillanders 2002) and the monthly temperature variations in the Pérou river were too low (below 2 °C; P. Cattan, pers. comm.) to explain the high range of Ba variations in otolith. The reproductive status might explain the Ba peak. However, the absence of a correlation between length, as an age indicator, and the occurrence of variations tends to eliminate a link with the reproduction physiological processes. Finally, the tropical rivers are subjected to extreme climatic and hydrological seasonal variations that may induce a stress to the fish population and may interact with a sudden change of the environmental conditions affecting the fish metabolism as well as the Ba availability.

Migratory hypotheses

In his study of *S. punctatum*, Bell (2009) suggested the presence of non dominant individuals able to settle in low densities at upstream habitats. These individuals could move downstream after having reached sufficient size to compete during agonistic relationships. Density dependant migration was also suggested for *Sicyopterus lagocephalus* as migrant individuals were found in rivers with high densities of Sicydiinae and crustaceans (Lord 2009). Such movements have previously been suggested for other species like Anguillidae (Daverat and Tomas 2006; Tabouret 2009) known to undertake movements between supposed growth habitats in the upstream part of the river and downstream habitats. Due to the short time for larvae to reach the sea (3 days), the migration and the settlement of Sicydiinae in downstream habitats would reduce the larval migration time needed for larvae to reach the estuary and would ensure a greater survival (Bell 2009; Valade et al. 2009).

The recording of such migration in the otolith implies a significant chemical difference between habitats used by individuals. We do not have the elemental composition of the Pérou River water. Since Sr:Ca and Ba:Ca in otoliths are known to mostly reflect ambient water composition (Bath et al. 2000; Elsdon and Gillanders 2004; Tabouret et al. 2010), some assumptions can be made based on Sr:Ca and Ba:Ca ratios in otoliths.

Non-varying individuals were fish that did not move between two environments with different Sr:Ca and Ba:Ca composition. In varying individuals, the slight but simultaneous increase of Sr:Ca ratios during the Ba:Ca peaks may record migration to the upper estuary zone where the salinity is still very low (Sr:Ca staying low) while the Ba could rapidly desorb from suspended sediments when they encounter the high ionic strength of estuarine saline water (Coffey et al. 1997; Moore and Shaw 2008). Variations over time in Ba:Ca ratio in coral cores from reefs have been found to result from the discharge and/or sediment load in rivers (Sinclair and McCulloch 2004). Mobile Ba in sediments could build up in the river catchment during the dry periods and mobilized in high concentration pulses during flood events upon contact with seawater (Coffey et al. 1997). If the migration assumption towards the upstream estuary is true, the Ba:Ca peaks in *S. punctatum* would preferentially record migrations during periods of high-flow. Finally, high flow events in tropical rivers are able to displace a major part of the upstream fauna and could be at the origin of a passive displacement of the individuals to the Ba remobilisation zone.

Changes in Barium biogeochemistry at the habitat scale

Since Ba:Ca in otolith is supposed to mostly reflect ambient water composition, the variability observed in otoliths of *S. punctatum* from a given site likely resulted from processes altering ambient composition. Heterogeneity in Ba:Ca ratios over short distances and time within the river catchment may be a plausible explanation (Wells et al. 2003; Walther and Thorrold 2006; Gillanders 2002). River Ba:Ca data are very scarce especially for areas draining volcanic substrates and no data is available for Guadeloupe. Ba:Ca ratios in volcanic context show very wide variations $3{-}3600 \times 10^{-6}$, subsurface waters (groundwater reflecting magmatic contribution) having significantly higher Ba:Ca ratios than surface waters (Louvat and Allègre 1997, 1998; Dessert et al. 2009). For The Lesser Antilles, several small aquifers are generally in contact with the rivers (Cabidoche et al. 1994). During low water discharges, streams and rivers must be largely affected by the contribution of underground water (Rad et al. 2007), whereas during the rainy season surface runoff has a greater contribution.

Alternatively, the influence of diet in the Sr and Ba incorporation is also widely discussed (Buckel et al. 2004; Marhon et al. 2009). Lefrançois et al. (2011) showed that a mean of 25 to 31% of *S. punctatum* diet is provided by the epilithic biofilm growing on wet stones on the river bed in Caribbean rivers. The colonisation of biofilm on the substrate takes 2−3 weeks. During this period, bacterial populations and pennate diatoms first colonise the stones of the river bed. Strong hydrological pressure and sediment abrasions are thought to maintain the epilithic biofilm at a non-mature state of colonisation (Monti and Lefrançois 2010). If these disturbances affect the major part of the river system, there are some isolated habitats (pools) without permanent hydrological turbulence. Some authors described Ba adsorption to freshwater surface coatings like biofilms (Dong et al. 2003) especially in diatom rich biofilms (Fisher et al. 1991; Bonny and Jones 2007). The omnipresence of diatoms in pool biofilms can therefore lead to the bioaccumulation of Ba. Moreover, the degradation of organic aggregates seems to favour the barytine formation (Jeandel et al. 2000). Since high organic litter degradation takes place in pools and considering the high trophic plasticity of the fish from tropical rivers (Jepsen and Winemiller 2002), peaks of Ba:Ca found in otoliths of some *S. punctatum* individuals could be attributed to shifts in the microhabitat and the grazing of a biofilm rich in diatoms and particulate matter. During the wet season, high flood events could induce the erosion of the biofilm, especially diatom communities, even in these protected habitats, resulting in a decrease of Ba:Ca concentration in otoliths. In these conditions, low Ba values in otoliths could be attributed to the grazing of a non-mature biofilm as observed in the rest of the river. The time necessary to recover stable conditions needed for the biofilm and the diatom colonisation appears as a good explanation for the time observed between each peaks of Ba:Ca in otoliths. Inversely to the upstream estuary migration hypothesis, the habitat scale hypothesis including both water and diet route for the incorporation of high Ba:Ca ratios, would record environmental changes during periods of low-flow.

5 Conclusion

This first study of *S. punctatum* otolith microchemistry supports the amphidromous cycle of the species and highlights the settlement of individuals in freshwater habitats after the recruitment. Despite the absence of environmental Ba data, Ba variations in otolith illustrate a change of environmental conditions during the freshwater adult phase. At this step of the investigation, it is too early to favour one hypothesis or another for the origin of Ba variations. However, these preliminary results on *S. punctatum* otolith microchemistry support the use of the combination of both elemental ratios, Sr:Ca and Ba:Ca, as tracers of variations in environmental conditions during the adult phase of amphidromous fish. The further validation of these tracers is of major concern for the species management and conservation.

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