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# INPUTS FROM MICROCHEMISTRY TO THE UNDERSTANDING OF THREE SICYDIINAE SPECIES' LIFE CYCLE

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AMPHIDROMY
MICROCHEMISTRY
SICYDIINAE
LIFE HISTORY

ABSTRACT. – Amphidromous fish species contribute most to the diversity of fish communities in the tropical insular rivers. Hence, their biological life cycle remains poorly known. The otolith elemental composition of three Sicydiinae species *Sicyopus zosterophorum*, *Smilosicyopus chloe*, *Akihito vanuatu* was investigated to describe their biological life cycle for the first time. The otolith analysis using a femtosecond laser ablation – inductively coupled plasma mass spectrometer (fs-LA-ICP-MS) revealed an amphidromous life history for the three species as it suggested a habitat shift from a marine habitat to a freshwater environment. Also, several types of elemental profiles were observed in the adult stage suggesting a diversity of behaviors and/or habitats. Water chemistry analyses would help understanding the origin of the ratios variations in the adult stage and would push forward our general understanding of Sicydiinae life cycle.

## INTRODUCTION

Otoliths are paired calcified structures in the inner ear of the fish. They are made of successive discrete layers of aragonite ($CaCO_3$) crystalline microstructure growth increments that are deposited over a nucleus on a protein matrix (Campana & Neilson 1985, Campana 1999). They are metabolically inert and grow continuously on a regular time period basis during the entire life of the fish (Lecomte-Finiger 1999). Because they are unlikely to undergo any mineral resorption (Mugiya & Uchimura 1989) otoliths are good candidates to date and establish the chronological sequence of events during the fish life as well as estimate the fish environment. The micro- and macrostructure of the otoliths are used to measure the age of fish as well as dating the important events occurring during their life (Lecomte-Finiger 1999). The increments of the otoliths constitute traps for the minor and trace elements that come from the water in the environment (Farrell & Campana 1996, Walther & Thorrold 2006) but as well from the nutrients (Kennedy *et al*. 2000). Amongst the elemental prints, strontium (Sr) was revealed to be a good element to trace the environmental past of the fish in particular migrating species (Secor *et al*. 1995, Gao & Beamish 1999, Tsukamoto & Arai 2001, Limburg *et al*. 2003, Milton *et al*. 2008). Indeed, the concentration of Sr on a precise area of the otolith is positively correlated to the concentration of strontium at the time of the depostition. Studies have shown that a high strontium:calcium (Sr:Ca) ratio is an evidence of a marine environment (Tzeng 1996, Tsunagawa & Arai 2009, Tabouret *et al*. 2010) whereas low values of this ratio may be attributed to species living in a freshwater environment (Shen *et al*. 1998, Howland *et al*. 2001, Closs *et al*. 2003, Thorrold & Shuttleworth 2000). More recently, the barium:calcium (Ba:Ca) ratio was used as a successful indicator of the salinity of the species' habitat (Thorrold & Shuttleworth 2000, Elsdon & Gillanders 2005, Hamer *et al*. 2006). Moreover, Mcculloch *et al*. (2005) and Tabouret *et al*. (2010) have revealed a negative correlation between Sr:Ca and Ba:Ca in the otolith of the barramundi *Lates calcarifer* (Bloch 1970) and the European eel *Anguilla anguilla* (Linnaeus, 1758).

Many fish realize migrations during their life cycle and the most spectacular surely are the one that diadromous fish realise when they migrate alternatively between the ocean and freshwater (Myers 1949). Amongst the diadromous species, anadromous fish such as salmons migrate from the ocean to the rivers to reproduce (McDowall 1988, Dittman & Quinn 1996, McDowall 2001) whereas catadromous fish like eels migrate downstream towards the ocean for mating and reproduction (Schmidt 1923, Tesch 2003). The specialised diadromous life cycle called amphidromy involves two migrations between the sea and freshwater that are not directly for reproduction purposes. The adults reproduce in freshwater and the larvae drift downstream to a pelagic marine environment (McDowall 2007, Closs *et al*. 2013).

The species of the Sicydiinae subfamily (Teleostei: Gobioidei) are amphidromous and they are the biggest contributors to the diversity of fish communities in tropical insular river systems (Keith 2003, Keith & Lord

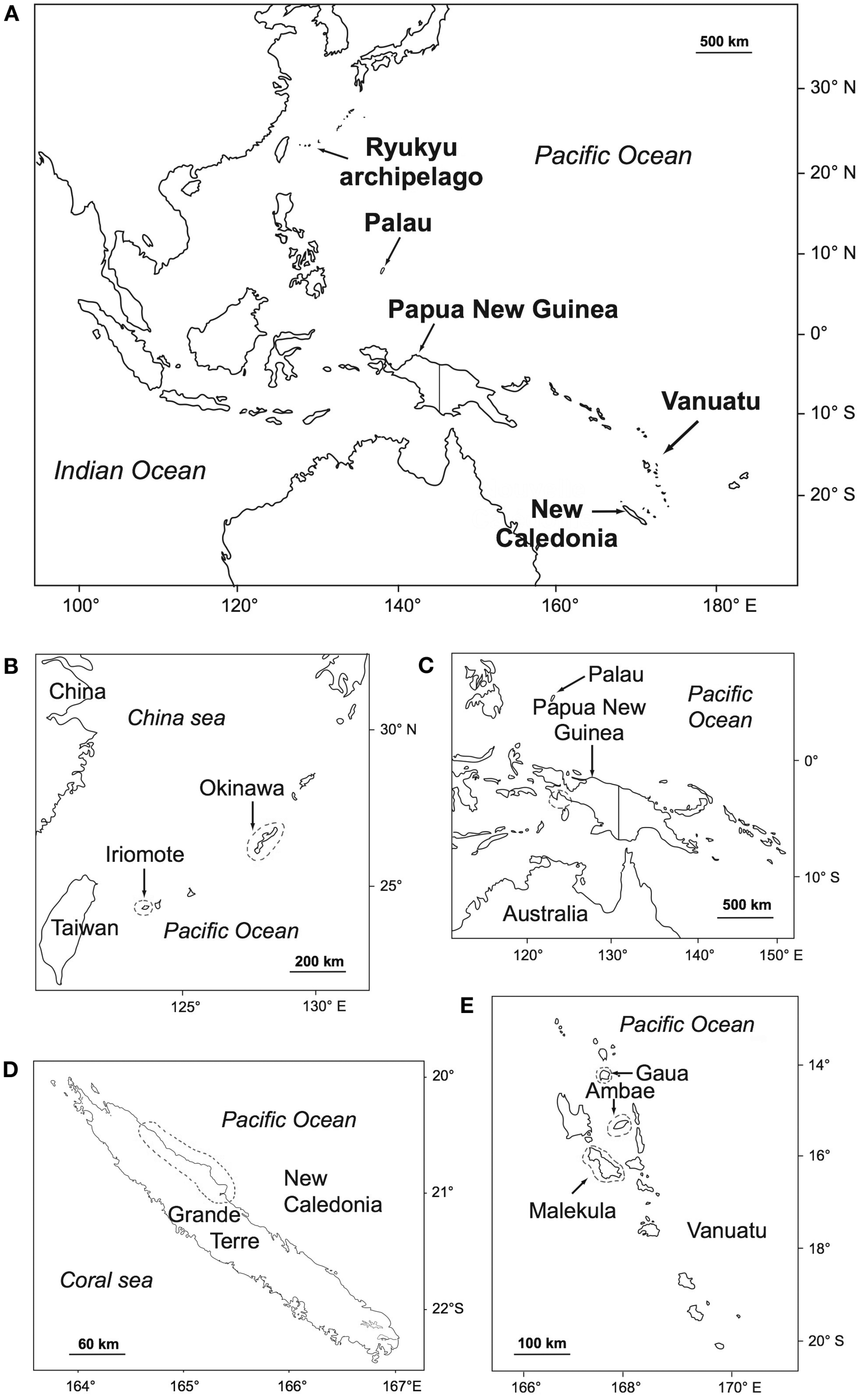

Fig. 1. – Sampling sites in the Pacific Ocean. **A**, Ryukyu archipelago; **B**, Palau; **C**, Papua New Guinea, **D**, New Caledonia; **E**,Vanuatu.

2011). The adults of the Sicydiinae grow, feed and reproduce in the streams (Keith 2003) and hatched larvae drift downstream towards the sea (Luton *et al*. 2005, Maeda & Tachihara 2010) where they spend two to six months (Iida *et al*. 2008, Lord *et al*. 2010, Taillebois *et al*. 2012) for growth. After this pelagic marine larval phase, the post-larvae return to the rivers and undergo a metamorphosis (Taillebois *et al*. 2011) while migrating upstream to settle (Keith 2003). This metamorphosis is materialized by a checkmark made of very close increments on the otolith of the fish (Shen & Tzeng 2002, Keith *et al*. 2008).

At this time, for most of the Sicydiinae, the amphidromy is attributed following visual observations and fisheries. These observations were confirmed for some species by the microanalysis of the otolith composition (Shen *et al*. 1998, Lord *et al*. 2011, Tabouret *et al*. 2011) that brought scientific evidence of migrations and/or movements during the fish life. So far all the Sicydiinae species are known to be amphidromous (Keith & Lord 2010). Several Sicydiinae species may coexist in the same rivers sharing the space and habitat along the river gradient: some species are only found in the lower course of the rivers, or the upper course whereas others may be found all along the river gradient. The genera *Smilosicyopus* Watson, 1999 and *Sicyopus* Gill, 1863 are rather found in the medium and upper courses (Marquet *et al*. 2003, Keith *et al*. 2010, Taillebois 2012).

This study focuses on three species of Sicydiinae that show three different geographic distributions and ranges but present similar types of habitats. They all prefer swift, clear and high gradient streams with rocky and boulder strewn bottom (Watson *et al*. 2001). *Sicyopus zosterophorum* (Bleeker, 1856) is a widely distributed species across the Central West Pacific bioregion, covering a longitudinal distance of more than 15,000 km extending from southern Japan and Palau to southern Papua, Vanuatu, New Caledonia and Fiji (Marquet *et al*. 2003, Keith *et al*. 2010, Watson 1999, Keith *et al*. 2011, Taillebois *et al*. 2013); it lives in the medium and upper courses of the rivers. *Smilosicyopus chloe* (Watson, Keith & Marquet 2001) is endemic to New Caledonia and Vanuatu (Marquet *et al*. 2003, Keith *et al*. 2010) and lives in the same part of the rivers as *S. zosterophorum*. *Akihito vanuatu* Watson, Keith & Marquet 2007 is endemic to Vanuatu and Fiji and is only found in the upper reaches of the rivers above steep and high waterfalls that sometimes may present a strong overhang (Keith *et al*. 2010).

Our aim is two-fold with regards to the biology of Sicydiinae. First, we wish to confirm the amphidromous life cycle of these species (*i.e.* hatch in rivers, marine larval phase and recruitment to the rivers). Second, we would like to take advantage of our microchemistry data to explore, as a preliminary study, the elemental ratio patterns for each species within the adult stage.

## MATERIALS AND METHODS

*Sample collection and sampling sites:* Specimens were collected from different parts of their distribution area (Fig. 1). Four rivers of New Caledonia (Kokengone, Po Vila, Wéwec & Wan Pwé On) were sampled in January and October 2010, all of them being on the northwest coast in the Mont Panié region between Ponérihouen and Pouebo (Fig. 1D). The riv-

Table I. – Sampling sites in the Pacific Ocean for each of the three species *Sicyopus zosterophorum*, *Smilosicyopus chloe* and *Akihito vanuatu*. The number of samples and the nature of the riverbed are given.

| Species | Archipelago-Region | Island | River | Number of samples | River bed |
|---|---|---|---|---|---|
| *Sicyopus zosterophorum* | New Caledonia | Grande Terre | Po Vila | 7 | Volcanic + metamorphic |
| | | | Wewec | 1 | Volcanic + metamorphic |
| | Vanuatu | Malekula | Brenwé | 6 | Raised limestone |
| | Japan-Ryukyu | Okinawa | | 6 | ? |
| | | Iriomote | | 1 | ? |
| | Palau | Palau | Ngmeskang | 4 | Raised limestone |
| | | | Mesekelat | 3 | Raised limestone |
| | Papua New Guinea | Papouasie | Bichain creek | 4 | Raised limestone |
| | Total | | | 32 | |
| *Smilosicyopus chloe* | New Caledonia | Grande Terre | Po Vila | 2 | Volcanic + metamorphic |
| | | | Wewec | 3 | Shale |
| | | | Wan Pwé on | 1 | Volcanic + metamorphic |
| | | | Kokengone | 1 | Volcanic + metamorphic |
| | Vanuatu | Malekula | Brenwé | 5 | Raised limestone |
| | | Gaua | | 1 | Volcanic |
| | Total | | | 13 | |
| *Akihito vanuatu* | Vanuatu | Ambae | Duviara | 5 | Basalt (Alkalin) |

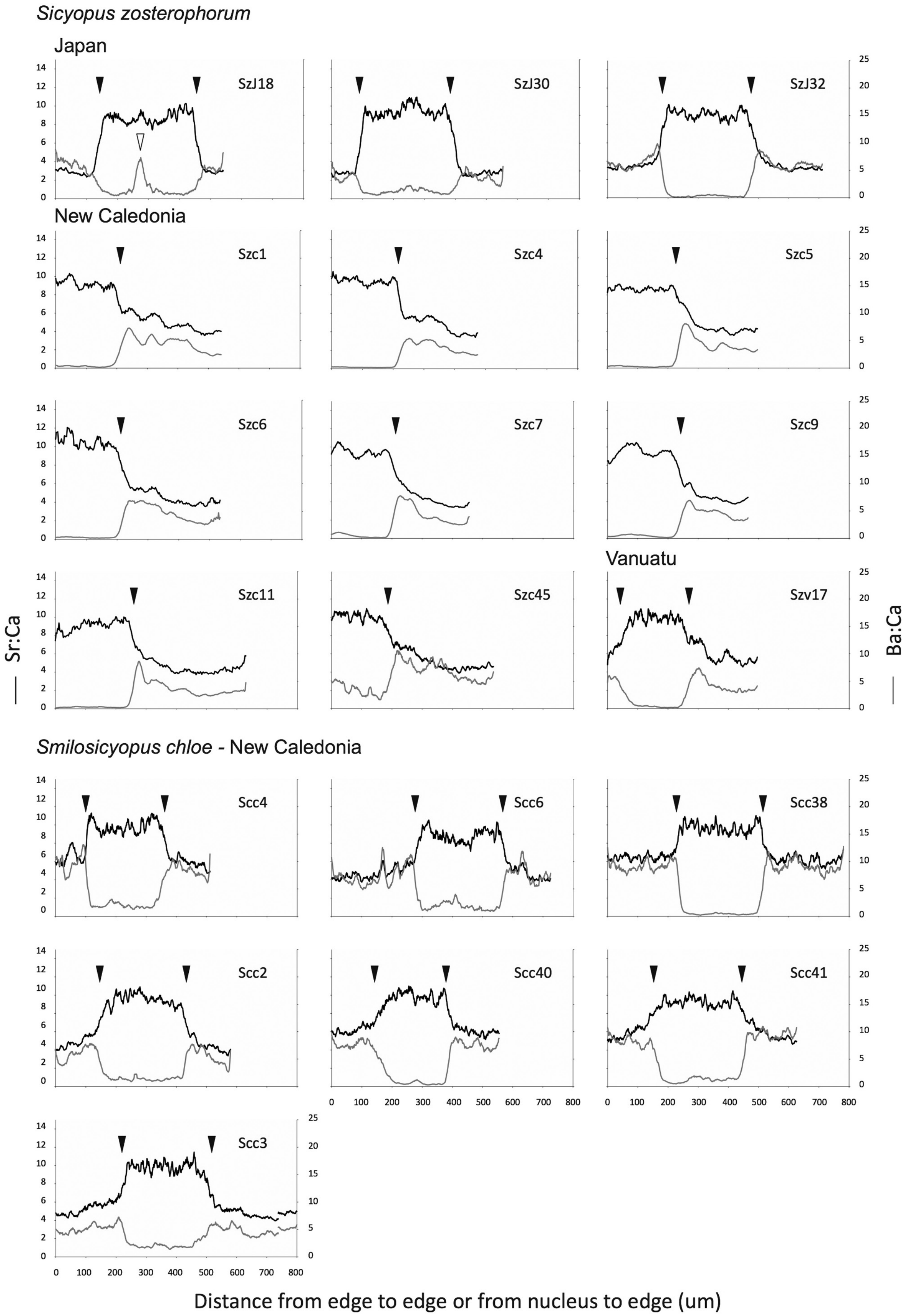

Fig. 2. – Variations of Sr:Ca and Ba:Ca ratios along the otolith transects for 12 specimens of *Sicyopus zosterophorum* from Japan (3 samples), New Caledonia (8 samples), Vanuatu (1 sample) as well as for 7 specimens of *Smilosicyopus chloe* from New Caledonia. The black arrows coincide with the recruitment mark. Ratios are given in × $10^{-3}$ for Sr:Ca and × $10^{-5}$ for Ba:Ca.

ers were sampled at an altitude from 80 to 350 m. The Wéwec River has schist substratum and the three others have schist and basalt beds (Table I). Three islands in Vanuatu were prospected: Malekula and Ambae islands in February 2010 on Brenwé and Duviara Rivers and Gaua island in July 2005 (Fig. 1E). These three islands belong to the volcanic arc of Vanuatu. Malekula is part of the occidental area formed during the upper Oligocene, Ambae and Gaua belong to the central area formed later during the upper Pleistocene. All these three islands are volcanic (basalts) with a tendency to be more alkaline in Gaua and Ambae (Table I). However, some parts of Malekula are made of raised limestone. Japanese rivers in the Ryukyu archipelago were sampled on Okinawa and Iriomote islands (Fig. 1A, B). They both belong to the volcanic arc of the Ryukyus and are made of continental beds. Other areas in the Pacific were sampled such as Palau in Micronesia and Papua New Guinea (Fig. 1c). Palau and the site in Papua New Guinea are made of raised limestone (Table I). All the specimens were collected by electro-fishing (Portable Dekka 3,000 electric device, Dekka Ltd, Germany) or snorkelling and using large hand nets. The fish were killed with an overdose of clove oil (10 %) and stored in 95 % ethanol. Species were morphologically identified based on field guides (Keith *et al*. 2002, Marquet *et al*. 2003, Keith *et al*. 2006, Keith *et al*. 2010).

*Otoliths preparation and microchemistry analyses: Sagitta* otoliths were extracted under an optical magnifier (Olympus VMZ; www.olympus.com) using entomologist tweezers, carefully rinsed, dried and embedded in an epoxy resin (Araldite 2020, Escil, France). They were ground along a transverse section to expose the edge using sandpaper (1,200 and 2,400 grains per inch), and then polished with decreasing abrasive powders (grain diameter 0.5-3.0 µm) and rinsed with MilliQ water. The otolith's sections were dried and stored in clean eppies until further analyses. Thirty-two otoliths of *Sicyopus zosterophorum*, 13 otoliths of *Smilosicyopus chloe* and 5 otoliths of *Akihito vanuatu* were analyzed with an IR 1030 nm femtosecond laser (Alfamet-Novalase, France) coupled to an Elan DRC II (Perkin Elmer) inductively coupled plasma-mass spectrometer (ICP-MS). The ablation followed a linear transect from edge to edge passing through the nucleus or from the nucleus to one edge. The width of the ablation was 20 $\mu$m with a frequency of 500 Hz and a speed of 5 $\mu\text{m.s}^{-1}$. Ablation conditions are those described by Tabouret *et al*. (2011) for the analysis of Gobiidae species. Quantification of $^{86}$Sr and $^{138}$Ba was allowed using three certified reference glasses: NIST614, NIST612 and NIST610 (National Institute of Standards and Technology, USA). Strontium and barium were standardized to calcium (*i.e*. Sr:Ca and Ba:Ca) based on the stoichiometry of calcium carbonate (380 000 $\mu$g Ca $\text{g}^{-1}$ otolith) (Campana 1999), because these two elements can substitute to calcium in the carbonated matrix (Campana 1999). The average detection limits based on three standard deviations (SD) of the blank gas were 4.47 $\mu\text{g.g}^{-1}$ for Sr and 0.27 $\mu\text{g.g}^{-1}$ for Ba.

*Data analyses:* Graphs of Sr: Ca and Ba: Ca ratios as a function of the distance followed by the laser were drawn and both ratios for each otolith are shown on the same graph. To reduce the noise of the elemental ratios we calculated a mobile mean around 9 points (Sinclair *et al*. 1998, Elsdon & Gillanders 2005). Each otolith was photographed before and after laser ablation. A comparison of light microscope images and femtosecond laser ICP-MS transects showed that the checkmark observed in the otoliths corresponds to brutal changes in elemental ratios. Arrows were positioned on the graphs according to the position of the checkmark (Figs 2, 3, 4, 5 and 6). Because our sample size was quite low and uneven, we used non-parametric methods to test if two or more than two groups of samples were identical. A Mann-Whitney U test was performed when two groups were compared (comparing two localities, two life stages). A Kruskal-Wallis one-way analysis of variance was performed when more than two groups were compared (comparing more than two localities, comparing three species).

## RESULTS

### *Sr:Ca ratio along the otoliths' transects*

The ratio Sr:Ca was high ($9.47 \times 10^{-3} \pm 0.76$) in the central region of the otoliths' transects for all the individuals of the three species *Sicyopus zosterophorum*, *Smilosicyopus chloe* and *Akihito vanuatu,* no matter which locality was considered (Table II). The central region of the otolith that goes from the nucleus to the metamorphose check mark corresponds to the larval phase. After this mark until the edge of the otolith the ratio Sr:Ca dramatically drops for all the species (Figs 2, 3, 4, 5 and 6, Table II).

The variability of the Sr:Ca ratio was low during the larval phase and increased in the adult phase. Within the species *S. zosterophorum*, there were significant differences in the Sr:Ca ratio of the adult stage between individuals from different localities (Kruskal-Wallis; p-value $< 0.0001$; Table II(1)). Indeed, specimens from Papua New Guinea, New Caledonia and Vanuatu exhibited Sr:Ca ratio twice higher than specimens from Japan or Palau (Table II). For the species *S. chloe* the Sr:Ca ratio of the adult stage between the two sampled localities were very close even though they were significantly different (Mann-Whitney; p-value $< 0.0001$; Table II(2)). In the same way, in Vanuatu, the three species *A. vanuatu*, *S. zosterophorum* and *S. chloe* presented similar Sr:Ca ratio in the adult phase even though they were significantly different (Kruskal-Wallis; p-value $< 0.0001$; Table II(3)).

### *Ba:Ca ratio along the otoliths' transects*

The ratio Ba:Ca was low ($0.69 \times 10^{-5} \pm 0.65$) in the central region of the otolith (larval phase) for all the individuals of the three species *Sicyopus zosterophorum*, *Smilosicyopus chloe* and *Akihito Svanuatu,* no matter which locality was considered (Table II). After the recruitment mark, until the edge of the otolith, the ratio increased and

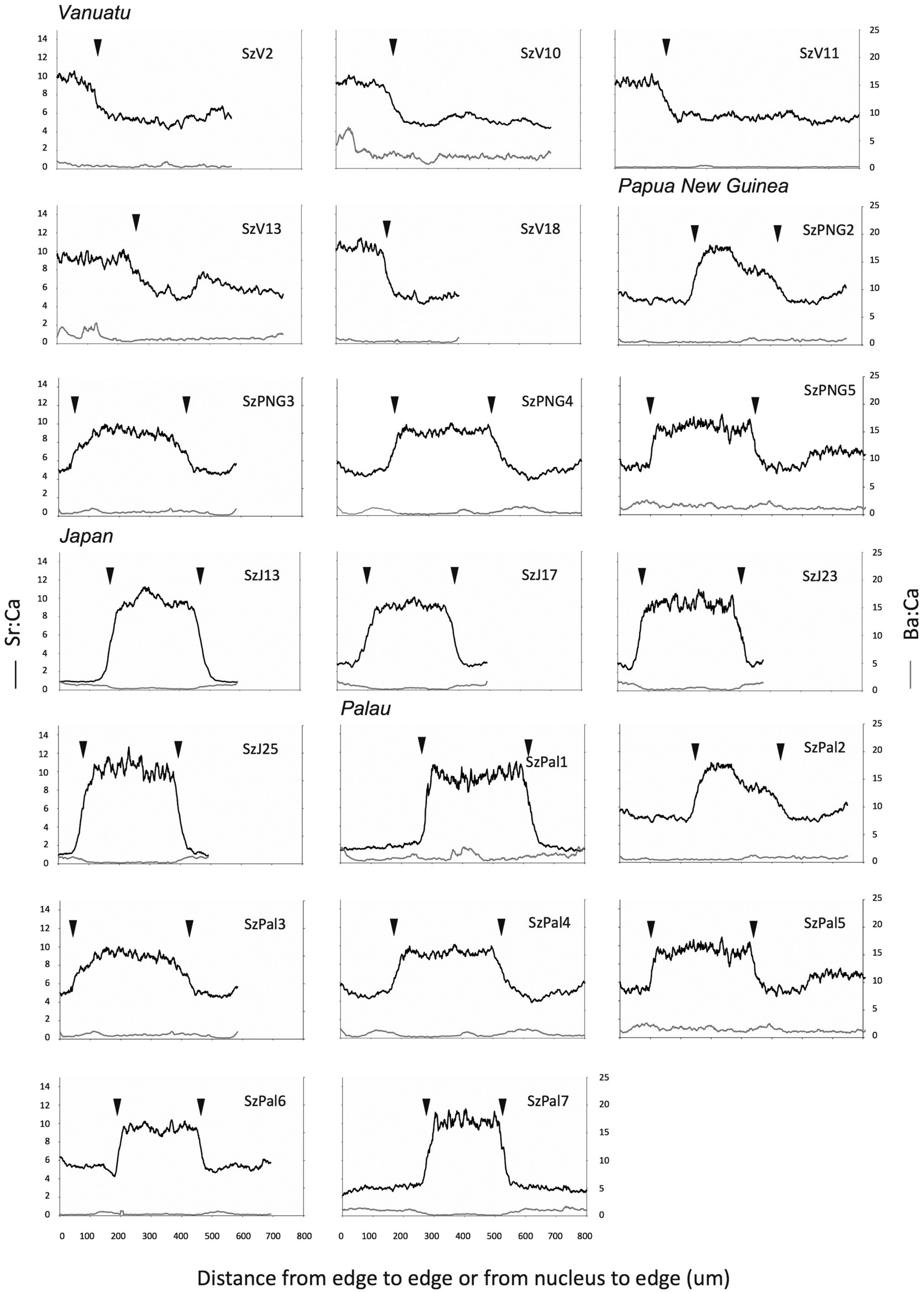

Fig. 3. – Variations of Sr:Ca and Ba:Ca ratios along the otolith transects for 9 specimens of *Sicyopus zosterophorum* from Vanuatu (5 samples), Papua New Guinea (4 samples), Japan (4 samples) and Palau (7 samples). The black arrows coincide with the recruitment mark. Ratios are given in × $10^{-3}$ for Sr:Ca and × $10^{-5}$ for Ba:Ca.

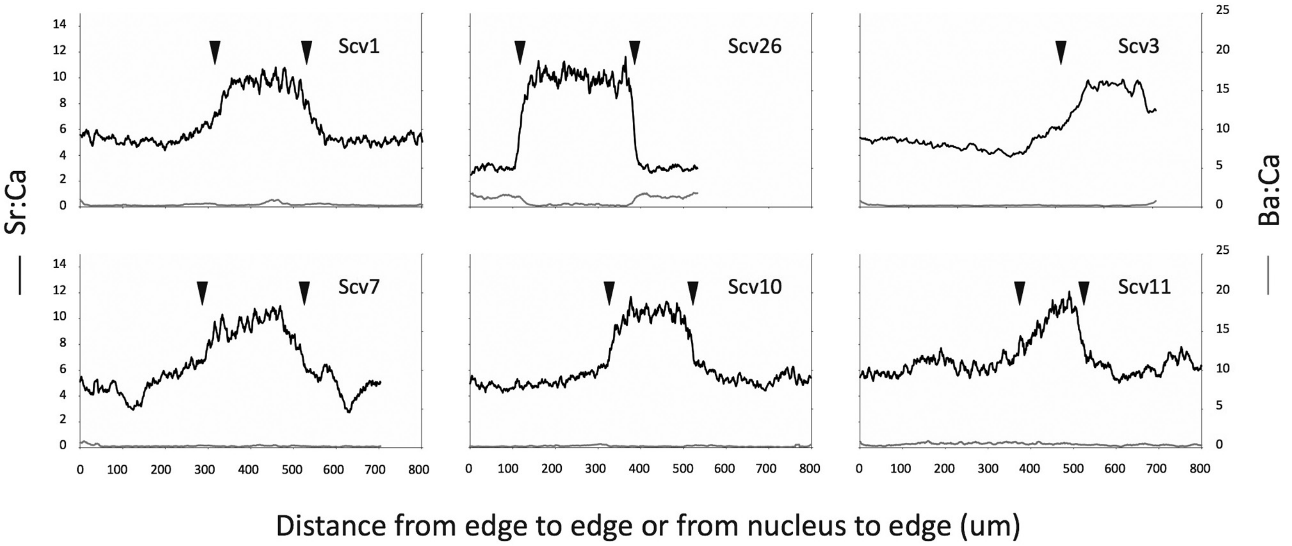

Fig. 4. – Variations of Sr:Ca and Ba:Ca ratios along the otolith transects for 6 specimens of *Smilosicyopus chloe* from Vanuatu. The black arrows coincide with the recruitment mark. Ratios are given in $\times 10^{-3}$ for Sr:Ca and $\times 10^{-5}$ for Ba:Ca.

was very variable depending on the individuals, the locality and the species (Figs 2, 4, 5 and 6; Table II).

For all the individuals collected on Malekula in Vanuatu as well as for some specimens of *S. zosterophorum* from Japan, Palau and Papua New Guinea the Ba:Ca ratio in the otolith was very low ($0.58 \times 10^{-5} \pm 0.46$) all along the transects (Fig. 3). Despite the low values of Ba:Ca during the adult phase, there was a significant difference between the larval and adult stages for specimens of *S. zosterophorum* from Japan and Palau (Mann-Whitney; p-value $< 0.0001$; Table II, Fig. 3) which was not the case for specimens from Vanuatu and Papua New Guinea (Mann-Whitney; p-value $< 0.0001$; Table II, Fig. 3).

Some patterns showed a high and stable Ba:Ca ratio (Fig. 2) in the adult phase, others showed an increasing Ba:Ca ratio (Fig. 5) while other showed peaks of the Ba:Ca ratio (Fig. 6). Those peaks coincided with the recruitment (metamorphose check mark) and in some cases they appeared later after the recruitment of post-larvae once the individual was in the river. Three specimens of *Smilosicyopus chloe* (Scc7, Scc11 and Scc10) that were caught in the same river on the same day showed a difference in the position of the main peak along the otolith transects.

## DISCUSSION

### *Elemental ratios and amphidromy*

### Elemental ratios during the larval phase

High levels of Sr:Ca associated with low levels of Ba:Ca (Sr:Ca = $9.47 \times 10^{-3} \pm 0.76$ and Ba:Ca = $0.69 \times 10^{-5} \pm 0.65$) were observed in the central part of the otolith between the nucleus and the metamorphose check mark for the three species studied *Sicyopus zosterophorum*, *Smilosicyopus chloe* and *Akihito vanuatu*. This region of the otolith corresponds to the larval stage of the fish. The Sr:Ca ratio found in the central region was comparable to the one reported in other Sicydiinae species (Radtke & Kinzie III 1996, Chang *et al*. 2006, Lord *et al*. 2011, Tabouret *et al*. 2011) and also to other amphidromous species such as *Plecoglossus altiveltis* (Kuroki *et al*. 2006), *Awaous stamineus* (Kido & Heacock 1992) and *Rhinogobius* sp. (Chang *et al*. 2008, Tsunagawa & Arai 2009, Tsunagawa & Arai 2011). Such values were also reported in other diadromous species such as *Anguilla anguilla* (Tabouret *et al*. 2010) and *Anguilla japonica* (Arai & Hirata 2006). The Sr:Ca ratio found in the three species studied in the central region of their otolith is in accordance with the observations of Keith *et al*. (2010) who related river recruitment of each species and the catch of post-larvae during their upstream migration. This validates that the larvae evolved in a marine environment, and the studied species are so not anadromous.

### Elemental ratios during the adult phase

For all the species and the localities, the recruitment check mark matched with the drop of Sr:Ca that reached low values ($4.82 \pm 1.56 \times 10^{-3}$) and was associated in some localities for all the three species with a raise of Ba:Ca that reached a mean value of $6.14 \pm 3.03 \times 10^{-5}$.

The low value of the ratio Sr:Ca in the external region of the otolith was similar to what was found in some marine species (mean Sr:Ca ratio for 43 marine species = $5.3 \times 10^{-3}$, Campana 1999) or in estuarine species (Kuhlia species, Feutry *et al*. 2011, Oka & Tachihara 2008). However, input of freshwater with high Sr:Ca is not uncom-

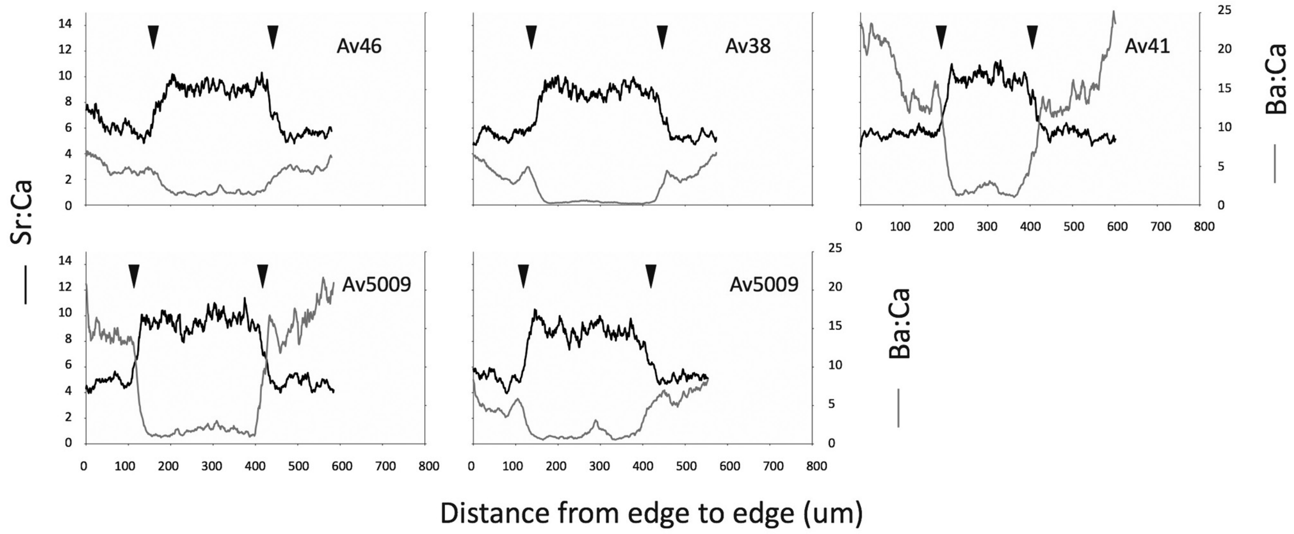

Fig 5. – Variations of Sr:Ca and Ba:Ca ratios along the otolith transects for 5 specimens of *Akihito vanuatu*. The black arrows coincide with the recruitment mark. Ratios are given in × $10^{-3}$ for Sr:Ca and × $10^{-5}$ for Ba:Ca.

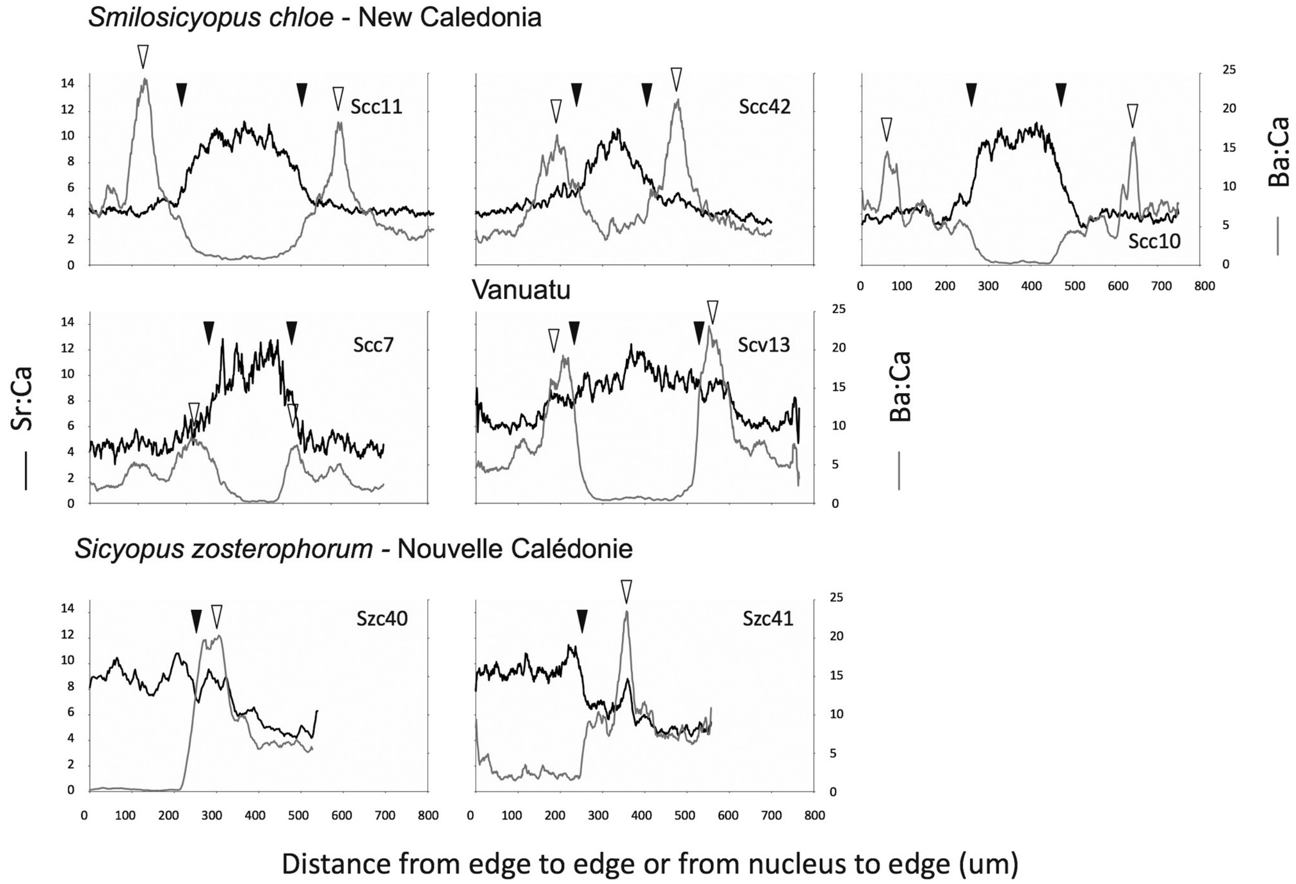

Fig. 6. – Variations of Sr:Ca and Ba:Ca ratios along the otolith transects for 6 specimens of *Smilosicyopus chloe* from New Caledonia (1 sample) and Vanuatu (1 specimen) and for 2 specimens of *Sicyopus zosterophorum* from New Caledonia. The black arrows coincide with the recruitment mark and the white arrow represents the Ba:Ca peak. Ratios are given in × $10^{-3}$ for Sr:Ca and × $10^{-5}$ for Ba:Ca.

mon and has already been observed in other systems (Gillanders 2005). Nevertheless, these ratios were also similar to those observed in the freshwater adult phase of *Stiphodon percnopterygionus* (Gobiidae:Sicydiinae) from the Aha River in Japan (Iida, personal communication). Such high levels probably reflected high concentrations of strontium in the river due to the limestone riverbed or low concentrations of calcium in the case of volcanic

Table II. – Mean values (bold) and standard deviation (italicised) of Sr:Ca (× $10^{-3}$) and Ba:Ca (× $10^{-5}$) ratios for the species *Sicyopus zosterophorum*, *Smilosicyopus chloe* and *Akihito vanuatu* during their larval and adult stages and for the different localities sampled. (1), (2) and (3) show significant differences between localities and species.

| | ***Sicyopus zosterophorum*** | | | | ***Smilosicyopus chloe*** | | | | ***Akihito vanuatu*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Larvae | | Adult | | Larvae | | Adult | | Larvae | | Adult | |
| | Sr:Ca | Ba:Ca | Sr:Ca | Ba:Ca | Sr:Ca | Ba:Ca | Sr:Ca | Ba:Ca | Sr:Ca | Ba:Ca | Sr:Ca | Ba:Ca |
| New Caledonia | **9.536** | **0.991** | **4.833** (1) | **5.028** | **9.604** | **1.148** | **5.266** (2) | **7.185** | | | | |
| | *0.721* | *1.34* | *1.03* | *1.902* | *0.725* | *0.604* | *1.236* | *2.851* | | | | |
| Vanuatu | **9.632** | **0.582** | **5.429** (1,3) | **0.926** | **9.599** | **0.374** | **5.286** (2,3) | **1.462** | **9.298** | **0.791** | **5.546** (3) | **4.744** |
| | *0.665* | *0.394* | *0.572* | *1.42* | *0.961* | *0.253* | *1.093* | *3.499* | *0.643* | *0.574* | *0.796* | *1.320* |
| Japan | **9.369** | **0.329** | **2.705** (1) | **2.527** | | | | | | | | |
| | *0.746* | *0.225* | *1.086* | *2.361* | | | | | | | | |
| Palau | **9.612** | **0.286** | **2.43** (1) | **0.829** | | | | | | | | |
| | *0.788* | *0.151* | *0.927* | *0.297* | | | | | | | | |
| Papua New Guinea | **9.248** | **0.883** | **5.259** (1) | **0.946** | | | | | | | | |
| | *0.64* | *0.499* | *0.75* | *0.522* | | | | | | | | |

riverbed rather than low salinity. This is comforted in our study by an increase of the Ba:Ca ratios in the individuals from New Caledonia and Japan. It is more complex to state about the habitat for the individuals caught in Palau, Vanuatu and Papua as their Ba:Ca ratios in the adult phase were very low and did not significantly differ between the larval and adult phases. Without any data on water composition along the river gradient, the only evidence of the freshwater habitat after the recruitment is the capture of the specimen in strict freshwater which was the case for all of our specimens. As the final Sr:Ca and Ba:Ca ratios did not significantly differ from the recruitment to the capture, the freshwater habitat for adults is the best hypothesis. Marquet *et al.* (2003) for *S. chloe* and *S. zosterophorum*, and Keith *et al.* (2010) for all three species, related freshwater habitats for adults and described the reproduction in river.

## Variations of the elemental ratios and amphidromy

The brutal variations of the Element:Ca ratios might be due to the switch of environment, from high salinities to low salinities. Sicydiinae post-larvae usually undergo a metamorphosis when they recruit to the rivers (Valade *et al.* 2009, Taillebois *et al.* 2011) and the variation in the ratios might be attributed to the morphological and physiological changes occurring at that time. Indeed, in the flat fish *Solea solea* (de Pontual *et al.* 2003) the drop in Sr:Ca is due to internal and physiological factors such as a high metabolic rate associated to the transition from a marine to an estuarine environment. In two species of moray eels *Gymnothorax reticularis* and *Muraenesox cinereus*, which do not change environment during their life cycle, the Sr:Ca ratio drops during the metamorphosis (Ling *et al.* 2005). However, for some individuals of gobie species (*Sicyopterus lagocephalus*, *Rhinogobius giurinus*, *Tridentiger kuroiwae*) no drop in the ratio Sr:Ca was observed despite the presence of a metamorphosis check mark in the otolith (Lord *et al.* 2011, Iida, pers comm). In that case, the absence of variation in the Sr:Ca ratio was linked to an estuarine habitat during the adult stage. In that particular species, the variations in the Sr:Ca ratio was highly influenced by the environment rather than physiological processes as it may be the case for the species *Sicyopus zosterophorum*, *Smilosicyopus chloe* and *Akihito vanuatu*.

According to microchemistry, the studied species have a marine larval phase and a freshwater adult habitat meaning that they're diadromous. We can precise that they are amphidromous and not catadromous as first, all of our individuals were caught in the rivers and no evidence of returning to a high salinity environment (*i.e.* high Sr:Ca ratio) during the adult phase was observed and secondly, Marquet *et al.* (2003) for *S. chloe* and *S. zosterophorum*, and Keith *et al.* (2010) for all three species described the reproduction in river, with a nest dug under rocks where the male attracts the female to lay eggs. We also observed in New Caledonia and Vanuatu nuptial parades taking place in the middle (*S. zosterophorum* and *S. chloe*) and upper (*A. vanuatu*) reaches, and Ellien *et al.* (2014) describes the morphological changes of larvae during the transition from freshwater to sea water. Amphidromy is, as far as we know, the only type of life cycle in Sicydiine gobies (Keith & Lord 2011).

### *Ratios Ba:Ca in the adult stage and link with the environment*

Different types of Ba:Ca ratio pattern were observed during the adult stage, depending on the species, individuals and localities.

Some profiles showed no variation in the Ba:Ca ratio along the otolith transects for *Sicyopus zosterophorum* and *Smilosicyopus chloe* from Malekula Island in Vanuatu, and for some *Sicyopus zosterophorum* from Palau, Japan or Papua New Guinea. At the same time the ratio Sr:Ca was classic with high levels between the nucleus and the metamorphose check mark and low levels between the check mark and the edge (Fig. 3). Low ratios of Ba:Ca in the adult phase, similar to ours, were found in species of *Sicyopterus* in Vanuatu (Lord *et al.* 2011). These low levels of barium found in the adult phase might be linked to the low concentration of barium in the river waters due to the nature and origin of that water. Studies showed that the barium might vary depending on the origin of the waters. In a volcanic context, Ba:Ca was much lower in the surface waters than in the underground waters (Louvat & Allègre 1997, Louvat & Allègre 1998).

All the other profiles showed a much higher Ba:Ca ratio in the adult phase than in the larval phase. However, three different types of patterns in the adult phase were spotted out.

First, individuals of *Sicyopus zosterophorum* from Japan, Vanuatu and New Caledonia and individuals of *Smilosicyopus chloe* from New Caledonia presented high and stable Ba:Ca ratios in the adult phase (Fig. 2). This is in accordance with the fact that these two species were always found in the same rivers and in the same parts of the rivers. When they entered the rivers, the post larvae underwent a metamorphosis and immediately migrated upstream to settle in the adult habitat. Even if only little is known about the adult habitat, they were both found in the middle reaches and altitudes on the same substrates of blocs and pebbles in clear swift and well-oxygenated waters.

The second type of patterns corresponded to profiles where peaks of Ba:Ca were observed during the adult stage either at the time of river recruitment or in between the recruitment and the edge of the otolith. These patterns were found in individuals of *Sicyopus zosterophorum* and *Smilosicyopus chloe* (Fig. 6). Such peaks have already been observed in previous studies in Sicydinae species (Lord *et al.* 2011, Tabouret *et al.* 2011) but as well in diadromous species such as trout and eels under temperate climates (Wells *et al.* 2003, Tabouret *et al.* 2010). In three samples of *S. chloe* collected in the same river, the observed peak is shifted along the three transects (Scc7, Scc11 and Scc10; Fig. 6). These three samples presented some differences in their standard length suggesting they were collected at different ages. They probably recruited in the river at different times. The older specimen had the ratio Ba:Ca peak the most distant from the nucleus compared to the other specimens (Fig. 6). The best guess to explain these peaks at different distances from the recruitment mark according to the age of the fish would be that a geochemical event occurred in the river and affected the three specimens at the same time at a different stage of their growth.

The presence of these different patterns (with peaks and classic) may be explained by either a migrating behaviour and/or variable environmental conditions within the river or a settled behaviour and/or stable environmental conditions. However, the Sr:Ca ratio was always low and stable within the adult phase which favours the hypothesis of variable environmental conditions rather than a migrating behaviour for specimens presenting Ba:Ca peaks.

Finally, the third type of pattern was found in *Akihito vanuatu* individuals that were only collected on Ambae island. The Ba:Ca ratio kept increasing during the adult stage (Fig. 5). *Akihito vanuatu* colonizes the higher reaches of the rivers and can be found at altitudes above 300 m (Watson *et al.* 2007). That pattern of variation may be linked to the biology of the species or the specificity of its habitat.

Our study analysed the elemental Sr:Ca and Ba:Ca ratios along the otoliths transects of three species of Sicydiinae: *Sicyopus zosterophorum*, *Smilosicyopus chloe* and *Akihito vanuatu*. The profiles confirmed their amphidromous life cycle and the concordance between the metamorphosis check mark and the recruitment in rivers. Indeed, we clearly showed that the larvae live in a high salinity environment (the ocean) whereas the adults live and spawn in a freshwater environment (the river). Several types of elemental profiles were observed in the adult stage suggesting a variety of environmental conditions across the species, locations but also during the adult life in the river. Water chemistry analyses would help understanding the origin of the ratios variations in the adult stage and would push forward our general understanding of Sicydiinae life cycle. Indeed, it would be relevant to investigate on the Ba geochemistry in studied insular rivers for a better understanding of the Ba biogeochemical cycle and it would be necessary to use a multimarker approach (such as $^{87}$Sr:$^{86}$Sr ratio) to describe better the fish behaviour.

Finally, we hope that our preliminary study will give helpful data to managers to better understand the life cycle of these rare and threatened species, to help us to manage them and their habitats.

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