

# Diadromous life cycle and behavioural plasticity in freshwater and estuarine Kuhliidae species (Teleostei) revealed by otolith microchemistry

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**ABSTRACT:** Otolith microchemistry and microstructure were examined in juveniles of 3 *Kuhlia* species (Teleostei) from fresh and brackish environments in order to examine their migratory histories. All species presented with strontium:calcium (Sr:Ca) and barium:calcium (Ba:Ca) profiles in the inner region of the otoliths that suggested an obligatory marine larval phase. At approximately 0.3 to 0.6 mm from the otolith core, all individuals showed a sharp increase in Ba:Ca ratios that were generally associated with variation in the Sr:Ca ratio, indicating recruitment into rivers. Microchemical profiles in the outer region of the otoliths indicated a freshwater habitat for most *K. rupestris* and *K. sauvagii* and an estuarine habitat for most *K. munda*. Microstructure analyses validated the presence of an otolith check mark deposited during the habitat shift in *K. rupestris* and *K. sauvagii*, but not in *K. munda*. We hypothesise that this difference was due to lower osmotic stress for the fish moving from the sea to estuaries than from the sea to freshwater. This study demonstrated the ability of otolith multi-elemental microchemistry and microstructure to provide important insights on life history traits of species that lack basic biological information, such as those in the genus *Kuhlia*. The information provided in this study is critical for the conservation and management of these species.

**KEY WORDS:** *Kuhlia* spp. · Otolith · Microchemistry · Microstructure · Diadromy · Femtosecond laser ablation inductively coupled plasma mass spectrometry · fs LA-ICP-MS

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## INTRODUCTION

The Kuhliidae family (Teleostei) consists of a single genus, *Kuhlia*, and is distributed in the Indo-Pacific tropical region, from the eastern coast of Africa to the western coast of America and from the Ryukyu Archipelago to Australia (Randall & Randall 2001). Among the 12 species recognised in this genus, 6 are primarily marine, 5 inhabit freshwater and 1 is mostly found in estuaries (Randall & Randall 2001, Loiselle & Stiassny 2007). Marine species typically occur inshore and tend to form schools by day. Nothing is known about their reproduction, but juveniles are

often found in tide pools. Freshwater and estuarine representatives of this family mainly live in clear and fast-flowing streams of tropical oceanic islands. The geographical range of these freshwater species varies from a small archipelago (*K. malo* in French Polynesia and *K. salelea* in the Samoa) to a vast region in the Pacific or Indian Oceans (*K. marginata* and *K. munda* in the west and central Pacific, *K. sauvagii* in the western Indian Ocean) or across the Indian and Pacific Oceans (*K. rupestris* from the western Indian Ocean to the central Pacific) (Randall & Randall 2001, Feutry et al. in press). *K. rupestris* is known as a 'legendary angling species' in northeast-

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ern Australia (Merrick & Schmida 1984), while the other riverine species are a source of food in many countries such as Madagascar or Vanuatu.

Similar distributions are also observed in other aquatic taxa typical of insular systems, such as Anguillidae, Gobiidae and Eleotridae for fish, Atyidae and Palaemonidae for decapod crustaceans and Neritidae for molluscs (McDowall 2004). All these groups are diadromous and share a particular life history trait, an obligatory marine phase in their life cycle. According to Myers' definition (Myers 1949), Anguillidae found in freshwater are catadromous, migrating from freshwater to the ocean to reproduce, whereas the other taxa given as examples above are mainly amphidromous (migrate between fresh and saltwater, but not to reproduce). Commonly among amphidromous species, adults live and reproduce in streams, but after hatching, their larvae are swept to the ocean, where they spend several weeks or months before recruitment into freshwater. Because stream habitats on oceanic islands are relatively scarce and subject to climatic or hydrological variations, these types of life histories are the more successful ones (Maeda et al. 2007). Marine larval dispersion is the only way to insure regular recruitment and connectivity among remote islands (MacArthur & Wilson 1963) and seems to be one of the main life history traits of the freshwater Kuhliidae.

The widespread species *Kuhlia rupestris* has long been thought to be catadromous (Hogan & Nicholson 1987, Lewis & Hogan 1987), but this has never been proved with certainty. Recently, the presence of a marine larval phase was demonstrated for *K. xenura* (referred to as big-eyed type *K. sandvicensis*: Benson & Fitzsimons 2002), *K. marginata* (Oka & Tachihara 2008) and *K. malo* (Feutry et al. 2011) based on otolith microchemistry. The life history traits of the other species are unknown.

Otoliths are paired, calcified, inner ear structures used for balance and hearing in all teleost fish. These earstones are of particular interest for fish biologists for 2 main reasons: (1) they are indicators of fish age as they grow continuously with the formation of annual and daily increments (Pannella 1971), and (2) they are environmental recorders, as many elements are incorporated into the calcified matrix in concentrations reflecting their concentration in the fish's environment (Campana 1999). Otoliths have been used to investigate life histories of fish migrating between freshwater and marine habitats since Secor et al. (1995) demonstrated the capacity of strontium:calcium (Sr:Ca) ratios to trace water salinity. More recently, barium:calcium (Ba:Ca) ratios in otoliths have

also proved to be useful proxies for environmental salinities experienced by fish (Thorrold & Shuttleworth 2000, Elsdon & Gillanders 2005a, Tabouret et al. 2010, Feutry et al. 2011). Moreover, drastic environmental change, such as migration between marine and freshwater biomes, can be seen in an otolith's microstructure, as these types of movements may affect otolith growth and produce informative check marks (Crook et al. 2006, Hsu et al. 2009).

In the present study, we examined Sr:Ca and Ba:Ca ratios in the otoliths of 3 *Kuhlia* species found in freshwater (*K. rupestris* and *K. sauvagii*) or estuaries (*K. munda*) in order to test hypotheses about the nature of their presumably diadromous life cycles. Otolith microstructure was also investigated in the search for physical evidence associated with habitat shifts.

## MATERIALS AND METHODS

### Fish collection

A total of 17 *Kuhlia rupestris* individuals were collected during April 2009 in Mayotte ( $n = 7$ ) and during January 2010 in Vanuatu and New Caledonia ( $n = 10$ ). Ten *K. sauvagii* individuals were captured in 2 different rivers of Madagascar in May 2010. *K. rupestris* and *K. sauvagii* individuals were captured in freshwater by electro-fishing (Portable Dekka 3000 electric device). Nine *K. munda* individuals were caught in brackish water using a seine net in January 2010 on the northeast coast of New Caledonia. All fish were anaesthetised and euthanised, then fixed with 3 successive 90% ethanol baths just after capture. All fish captured in the present study were juveniles; their fork lengths (FL), measured to the nearest millimetre, and ranges for each species are given in Table 1. Otoliths were removed later in the laboratory. This method of preservation is commonly used, as Hedges et al. (2004) did not find any significant effect of ethanol preservation on Sr and Ba concentrations in otoliths. Moreover, Proctor & Thresher (1998) showed that this procedure only slightly affects Ca concentrations in otoliths.

### Otolith preparation, microstructural and microchemical analysis

Otoliths were prepared and analysed exactly as described by Feutry et al. (2011). Sagittae, the largest of the 3 pairs of otoliths, were extracted from fish,

Table 1. *Kuhlia* spp. Number (n), fork length (FL) and tag number of the samples per species and sampling location

Species	Island	River	Coordinates	n	FL (mm)	Tag
<i>K. rupestris</i>	Mayotte	Dapani	12° 58' 2" S, 45° 10' 0" E	7	70–142	Kr1 to Kr7
	Efate (Vanuatu)	Eluk	17° 37' 2" S, 168° 30' 0" E	7	90–140	Kr8 to Kr14
	New Caledonia	Kokengone	20° 50' 5" S, 165° 14' 5" E	3	111–150	Kr15 to Kr17
<i>K. sauvagii</i>	Madagascar	Androka	15° 38' 2" S, 49° 58' 2" E	8	69–90	Ks1 to Ks8
		Ambodiforaha	15° 42' 5" S, 49° 57' 6" E	2	75–82	Ks9 to Ks10
<i>K. munda</i>	New Caledonia	Tibarama	20° 56' 1" S, 165° 22' 4" E	4	94–145	Km1 to Km4
		Wan Pwe On	20° 31' 3" S, 164° 46' 3" E	5	42–95	Km5 to Km9

ultrasonically cleaned with triple distilled water and air-dried before being embedded in epoxy resin. Then they were sectioned along the frontal plane with a diamond saw, and the sections were mounted on glass slides and ground on both sides until the core was exposed on one of them. Once polished, each otolith was observed under an optical microscope, Olympus BX51 (100× magnification), equipped with an Olympus DP20 digital camera, and photographed. After eventual marks on the otolith (i.e. discontinuous increment width) were identified by eye, their position (i.e. their distance to the core along the posterior axis of maximal growth) was measured using an image analysis system (Olympus Soft Imaging Solutions). Before analysis with a femtosecond laser ablation inductively coupled plasma mass spectrometer (fs LA-ICP-MS) (LA: Alfamet, Novalase–Amplitude Systemes; ICP-MS: Elan DRC II, Perkin Elmer), the superficial layer of otoliths was removed using a diamond-polishing disc. The fs LA-ICP-MS operating conditions were those used by Feutry et al. (2011). The otoliths were ablated along the posterior axis of maximal growth, from a few microns before the core to the edge. The average detection limit based on 3 standard deviations of the blank gas was 566 ng g<sup>-1</sup> for <sup>86</sup>Sr and 57 ng g<sup>-1</sup> for <sup>138</sup>Ba.

### Data analysis

A 9-point running mean followed by a 9-point running median were applied to elemental ratios to reduce noise (Sinclair et al. 1998, Elsdon & Gillanders 2005b). We distinguished 3 regions in the otoliths when analysing Sr:Ca and Ba:Ca ratios: (1) the core region (CR), which appeared to be chemically distinct from material deposited after hatching (Brophy et al. 2004), (2) the inner region (IR), corresponding to the early life history of the fish, and (3) the outer region (OR), corresponding to juvenile life. The boundary

between the IR and OR was defined according to the drastic elemental ratio changes observed in all individuals at approximately 300 to 500 µm from the core.

A comparison of light microscope images and fs LA-ICP-MS transects showed that the marks observed on the otolith of *Kuhlia rupestris* and *K. sauvagii* may correspond to the boundary between IR and OR. This correspondence was tested for each species using paired Wilcoxon signed tests; distances from the core to these structural breaks were compared to distances from the core to chemical breaks observed in the otoliths.

## RESULTS

### Sr:Ca and Ba:Ca profiles

We measured peaks of Sr:Ca and Ba:Ca ratios in the CR of 82.3 and 100% of *Kuhlia rupestris* individuals, respectively. The IR for this species (and also for *K. sauvagii* and *K. munda*) was characterised by high Sr:Ca and low Ba:Ca ratios (Fig. 1, Table 2). In *K. rupestris*, the boundary between the IR and OR was located at 0.45 ± 0.07 mm from the core, where Sr:Ca ratios decreased suddenly by approximately 2 to 5 mg g<sup>-1</sup> and Ba:Ca ratios increased by 1 or 2 orders of magnitude (Fig. 1). In the OR, Sr:Ca ratios were low and stable (approximately 1 to 2 mg g<sup>-1</sup>; Fig. 1), except for one fish from Mayotte and one fish from New Caledonia (tags Kr4 and Kr15; Fig. 1), whereas Ba:Ca ratios, although variable, were higher than in the CR and IR (Fig. 1, Table 2).

Analyses of otolith microchemistry in *Kuhlia sauvagii* revealed peaks of Sr:Ca and Ba:Ca ratios in the CR of 60% and 90% of individuals respectively (Fig. 1). The IR presented high Sr:Ca and low Ba:Ca ratios (Fig. 1, Table 2). The boundary between the IR and OR was located at 0.38 ± 0.04 mm from the core, where Ba:Ca ratios increased by 1 or 2 orders of mag-

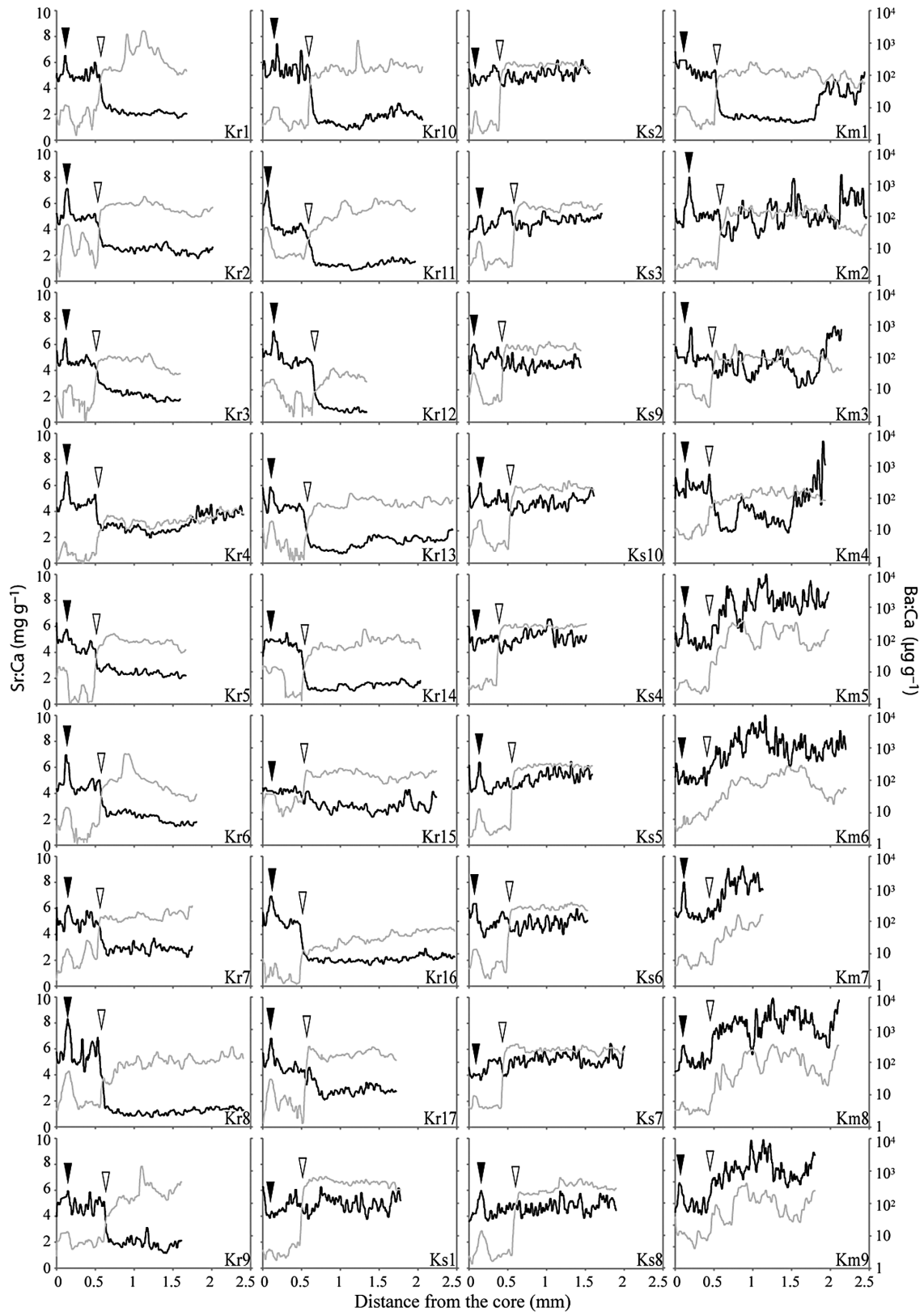


Fig. 1. *Kuhlia* spp. Sr:Ca (black line) and Ba:Ca (grey line) profiles from the core to the otolith edge along the posterior axis of maximal growth in *K. rupestris* (Kr1 to Kr17), *K. sauvagii* (Ks1 to Ks10) and *K. munda* (Km1 to Km9). Ba:Ca ratios have logarithmic scales. (▼) Position of core; (▽) dramatic shift in otolith elemental composition

Table 2. *Kuhlia* spp. Mean Sr:Ca and Ba:Ca ratios ( $\pm$ SD) from the core (CR), inner (IR) and outer regions (OR) per species and per river

Species River	Sr:Ca			Ba:Ca		
	CR	IR	OR	CR	IR	OR
<i>K. rupestris</i>	6.6 $\pm$ 1.2	4.7 $\pm$ 0.4	2.1 $\pm$ 0.7	21.4 $\pm$ 15.7	5.8 $\pm$ 4.1	144.5 $\pm$ 114.7
Dapani	6.6 $\pm$ 0.7	4.7 $\pm$ 0.3	2.5 $\pm$ 0.4	16.8 $\pm$ 15.6	6 $\pm$ 4.3	167.2 $\pm$ 160.1
Eluk	6.8 $\pm$ 1.5	4.8 $\pm$ 0.6	1.4 $\pm$ 0.3	24.1 $\pm$ 15.7	4.4 $\pm$ 1.5	130.5 $\pm$ 79.2
Kokengone	6 $\pm$ 1.6	4.6 $\pm$ 0.4	2.7 $\pm$ 0.5	25.7 $\pm$ 19.6	8.6 $\pm$ 7.5	124.2 $\pm$ 79.4
<i>K. sauvagii</i>	5.4 $\pm$ 1	4.8 $\pm$ 0.2	4.9 $\pm$ 0.3	14.4 $\pm$ 9.4	8.4 $\pm$ 15.6	264.3 $\pm$ 75.6
Androka	5.2 $\pm$ 1	4.7 $\pm$ 0.2	5 $\pm$ 0.2	10.9 $\pm$ 5.5	9.5 $\pm$ 17.5	278.3 $\pm$ 78.8
Ambodiforaha	6.2 $\pm$ 0.2	4.9 $\pm$ 0.1	4.6 $\pm$ 0.2	28.6 $\pm$ 8.6	4 $\pm$ 0.6	208.1 $\pm$ 6
<i>K. munda</i>	6.7 $\pm$ 1.2	5.2 $\pm$ 0.4	6.3 $\pm$ 2.3	8 $\pm$ 4.1	8.7 $\pm$ 11.5	116.2 $\pm$ 24.5
Tibarama	6.3 $\pm$ 1.5	5.2 $\pm$ 0.4	4 $\pm$ 1.2	8.9 $\pm$ 5.4	12.7 $\pm$ 17.5	117.2 $\pm$ 10.7
Wan Pwe On	7 $\pm$ 1	5.2 $\pm$ 0.4	8.1 $\pm$ 0.6	7.3 $\pm$ 3.2	5.5 $\pm$ 2.4	115.3 $\pm$ 33.4

nitude but no changes were observed for Sr:Ca ratios. In the OR, Sr:Ca ratios were stable and remained at values similar to those found in the IR, whereas Ba:Ca ratios were high and variable (Fig. 1, Table 2).

In the CR of 88.8 and 22.2% of *Kuhlia munda* individuals, we measured peaks of Sr:Ca and Ba:Ca respectively. The IR was characterised by high Sr:Ca and low Ba:Ca ratios (Fig. 1, Table 2). An increase of 1 or 2 orders of magnitude of Ba:Ca ratios was associated with a Sr:Ca decrease of approximately 1 to 2 mg g<sup>-1</sup> in samples from Tibarama River (tags Km1 to Km4; Fig. 1), and an increase of approximately 2 to 3 mg g<sup>-1</sup> in samples from Wan Pwe On River (tags Km5 to Km9; Fig. 1) indicated the boundary between the IR and OR in this species. Ba:Ca ratios, although variable, were higher in the OR than in the CR and IR. For all except one *K. munda* individual, Sr:Ca ratios were very variable in this region, fluctuating between values as low as 1 mg g<sup>-1</sup> and values higher than those found in the IR, up to 8 mg g<sup>-1</sup> (Fig. 1). One individual (tag Km1; Fig. 1) presented low and stable Sr:Ca ratios in this region, as found in most of the *K. rupestris*.

### Microstructure

Analysis of otolith microstructure showed a slight mark, corresponding to discontinuous growth increments, at the approximate location of the chemical shift measured with fs LA-ICP-MS in *Kuhlia rupestris* (Fig. 2a) and *K. sauvagii* (Fig. 2b) but not in *K. munda* (Fig. 2c). Paired Wilcoxon signed tests showed no significant difference between the position of these marks and the dramatic chemical events in *K. rupestris* and *K. sauvagii*.

## DISCUSSION

### Sr:Ca and Ba:Ca ratios in otoliths

Drastic changes in the elemental composition of otoliths were observed across all fish analysed in the present study. Sr:Ca and Ba:Ca ratios in otoliths are correlated with ambient Sr:Ca and Ba:Ca ratios (Elsdon & Gillanders 2003), with minimal influence of dietary uptake of elements (Milton & Chenery 2001, Walther & Thorrold 2006) and water temperature and salinity (Bath et al. 2000, Elsdon & Gillanders 2004). Because ambient Sr:Ca and Ba:Ca ratios are often correlated to ambient salinity, Sr:Ca and Ba:Ca concentrations in otoliths are useful proxies to infer past environmental salinities (see Elsdon et al. 2008 for a review).

Sr:Ca ratios in otoliths are generally positively correlated with ambient salinity (Secor et al. 1995, Thorrold & Shuttleworth 2000, Elsdon & Gillanders 2005a), but this correlation is not always verified (Kraus & Secor 2004, Brown & Severin 2009). In contrast, Ba:Ca ratios of fish living in marine water were found to be low compared to those of fish from estuarine or freshwater (Campana 1999). As far as we know, this prediction has never failed. Indeed, bio-availability of Ba is lower in marine than in freshwater (Turner et al. 1981), and although concentrations vary among freshwater locations (Guay & Falkner 1998), ambient Ba:Ca is higher in freshwater than in the sea (McCulloch et al. 2003, Elsdon & Gillanders 2005a). Therefore, we suggest that the abrupt increases in Ba:Ca ratios probably reflect a shift from one habitat to another with a lower salinity, regardless of whether they are associated with variation in Sr:Ca ratios.



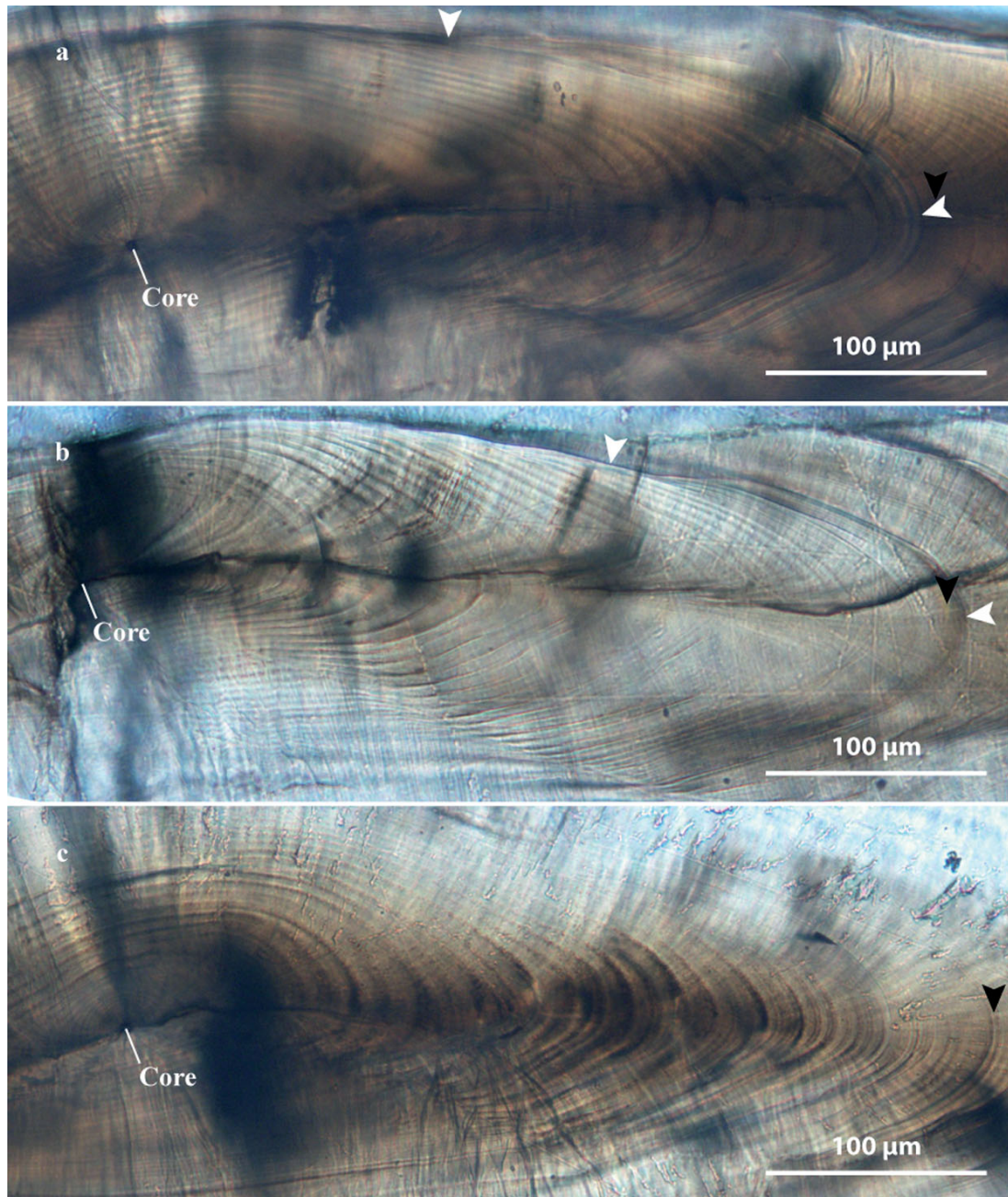


Fig. 2. *Kuhlia* spp. Otoliths of (a) *K. rupestris*, (b) *K. sauvagii* and (c) *K. munda*. (∇) Check marks; (▼) relative position of chemical shifts measured with femtosecond laser ablation inductively coupled plasma mass spectrometry (fs LA-ICP-MS)

Besides environmental variations, physiological state and ontogenetic changes also have the potential to influence the elemental ratios in the otolith (Kalish 1989). Otolith core chemistry is distinct from material deposited after hatching (Brophy et al. 2004). It can reflect the environment in which the mother has lived for Sr (Kalish 1990), or maternal transmission of artificial tags for Ba (Thorrold et al.

2006). In the present study, some individuals in each species showed peaks of Sr:Ca and/or Ba:Ca ratios in the CR and some did not. This inter-specific and inter-individual variability may reflect different environments used by the mothers before spawning, but further investigation of adult behaviour is needed to validate this hypothesis. Arai et al. (1997) and Correia et al. (2003) demonstrated that Sr:Ca ratios in

otoliths decreased during the metamorphosis of eels. The influence of fish metamorphosis on Ba:Ca ratios in otoliths has not yet been investigated. Although we cannot rule out that the major chemical changes observed on the edge of the IR are under ontogenetic influence in *Kuhlia* species, one would expect the variations of element:Ca ratios to be rather similar in all individuals (at least from the same species), which is not the case here.

### Larval life history

High Sr:Ca and low Ba:Ca ratios were measured in the IR of all individuals analysed in the present study, suggesting that the habitat of larvae was relatively chemically homogeneous. These values are in the range of those from fish from a seawater environment; Campana (1999) reported (after standardisation to Ca using the same method we used in the present study) a mean Sr:Ca ratio of  $5.5 \pm 0.3 \text{ mg g}^{-1}$  and a mean Ba:Ca ratio of  $9.5 \pm 1.5 \text{ } \mu\text{g g}^{-1}$  based on 43 and 14 studies, respectively. The results of the present study are also consistent with studies of tropical diadromous species which were known to have a marine phase. In the otolith region of the amphidromous goby *Sicyopterus lagocephalus* corresponding to the larval phase, Lord et al. (2011) found Sr:Ca ratios ranging from 10 to  $12 \text{ mg g}^{-1}$  and Ba:Ca ratios lower than  $10.0 \text{ } \mu\text{g g}^{-1}$ . Working on another goby, Tabouret et al. (2011) found a mean Sr:Ca ratio of  $9.4 \pm 0.5 \text{ mg g}^{-1}$  and a mean Ba:Ca ratio of  $5.5 \pm 1.6 \text{ } \mu\text{g g}^{-1}$  in *Sicydium punctatum*. Similar results found recently in the IR of *Kuhlia malo* (Feutry et al. 2011), *K. marginata* (Oka & Tachihara 2008; Sr:Ca data only) and *K. xenura* (referred to as big-eyed type *K. sandvicensis*; Benson & Fitzsimons 2002; Sr:Ca data only) were attributed to a marine larval phase. *Kuhlia* larvae of the 3 species studied here are assumed to live in the sea before they shift to a less saline habitat, as inferred by the Ba:Ca increases observed in all fish. This habitat corresponding to the OR is characterised by important chemical differences among species and is therefore discussed for each species separately.

### Juvenile life history

#### *Kuhlia rupestris*

Most *Kuhlia rupestris* individuals presented low and stable Sr:Ca ratios that were associated with high and variable Ba:Ca ratios throughout the entire

OR, which is consistent with the freshwater habitat where juveniles and adults of this species are generally observed and where the fish in the present study were caught. Stable Sr:Ca ratios lower than  $3.5 \text{ mg g}^{-1}$  are generally associated with freshwater habitats (Chang et al. 2004, Tabouret et al. 2010, Feutry et al. 2011, Lord et al. 2011, Tabouret et al. 2011). This species is thought to be catadromous and to reproduce at sea or in estuary plumes (Hogan & Nicholson 1987, Lewis & Hogan 1987). We did not observe the chemical signatures of a return to marine environments in the otoliths of *K. rupestris*, but no mature individuals were analysed in the present study. Further research should focus on adults' migratory history to investigate the reproduction location.

#### *Kuhlia sauvagii*

High and unstable Ba:Ca ratios were also reported in this species, but no significant changes in Sr:Ca ratios were observed between the IR and the OR. However, all fish were captured in freshwater at high elevation (>30 m above sea level) and therefore, the elemental composition on the edge of the otolith should be the signature of such habitat. As both Sr:Ca and Ba:Ca ratios remained stable from the chemical break to the edge, we assume that the fish spend their whole life in freshwater after they left the sea. Sr:Ca ratios have long been thought to be lower in fresh than in marine waters (Casselman 1982, Kalish 1989, Radtke 1989, Secor 1992, Secor et al. 1995, Tzeng et al. 1997), but more recently it has been shown that depending on bedrock geology, they can be similar or higher (Kraus & Secor 2004, Brown & Severin 2009). No water samples were analysed in the present study, but we suppose that the Sr:Ca ratios in the rivers we fished in Madagascar were comparable to those of surrounding seawater. No studies on water chemistry or on otolith microchemistry in Madagascar were found in the literature to confirm this hypothesis, and further work on this aspect is needed.

#### *Kuhlia munda*

Fluctuating Sr:Ca ratios with high amplitude, associated with high Ba:Ca ratios in the OR, are consistent with the estuarine environment where the fish were caught. Ambient Sr:Ca ratios in estuaries are often linked to salinity (Kafemann et al. 2000, Howland et al. 2001); therefore, fish movements along the salinity

gradient in this environment would imply Sr:Ca variations in otoliths. Similar associations of Sr:Ca and Ba:Ca ratios were found in the otoliths of some *Kuhlia malo* individuals and were attributed to an estuarine residency (Feutry et al. 2011), and so we conclude the same for *K. munda* here. The Sr:Ca ratios were generally higher in the Wan Pwe On than in the Tibarama river in New Caledonia, and this probably reflects higher ambient Sr:Ca ratios in the Wan Pwe On estuary, which received less freshwater inputs.

#### Flexible habitat use by *Kuhlia* spp.

Two *Kuhlia rupestris* showed chemical signatures in their otoliths similar to those of most *K. munda*, indicating an estuarine habitat, and one *K. munda* seemed to have a freshwater life history after recruitment. These results confirm the high plasticity of the migratory behaviour in the genus *Kuhlia*, as previously reported for the Hawaiian species *K. xenura* (referred to as big-eyed type *K. sandvicensis*: Benson & Fitzsimons 2002, McRae et al. 2011) and the French Polynesian species *K. malo* (Feutry et al. 2011). In the present study, sample sizes for each species were too small to investigate the diversity of migratory behaviours, and we recommend that future research should analyse more individuals.

#### Recruitment mark

A correspondence was found between the mark and the chemical change observed in the otoliths of *Kuhlia rupestris* and *K. sauvagii*. As discussed above, the change in otolith microchemistry is probably due to a habitat shift from marine to freshwater at the end of the marine larval phase. Similar check marks on otoliths associated with recruitment in rivers have been described in other tropical diadromous fish, in particular in freshwater eels (Arai et al. 2001, Réveillac et al. 2008) and amphidromous gobies (Radtke et al. 1988, Yamasaki et al. 2007, Lord et al. 2011, Tabouret et al. 2011). Settlement marks have also been reported in coral reef fish (Wilson & McCormick 1999). In these species, recruitment into the new habitat (i.e. freshwater or coral reefs) is accompanied by metamorphosis, which may be the cause of the check mark on the otolith more than the habitat transition is (Arai et al. 1999, Shen & Tzeng 2002, Keith et al. 2008). According to Campana & Neilson (1985), the formation of marks and discontinuities in otoliths appear during periods of perturbation and stress to

the fish. The presence of check marks in *K. rupestris* and *K. sauvagii*, which live in freshwater at the end of the marine larval phase, but not in *K. munda*, which inhabits estuaries, suggests that the formation of the check mark in this group is more likely to be caused by the stress of the habitat shift and the physiological changes rather than by a metamorphosis. Osmotic stress is less important when migrating from the sea to estuaries than when migrating from marine to freshwater environments, which may explain why check marks are less evident in *K. munda*. Detailed research on the otolith microstructure of *K. munda*, using electronic microscopy, may help to find changes in the otolith structure during recruitment.

#### CONCLUSIONS

In the present study, a marine larval phase was validated for all the *Kuhlia* specimens analysed from the 3 species. This phase ended with recruitment in freshwater (most *K. rupestris* and all *K. sauvagii*) or brackish water (most *K. munda*), where the juveniles spent the rest of their lives. No juveniles or adults of *K. rupestris*, *K. sauvagii* or *K. munda* have ever been observed in inshore marine environments, despite intensive study on tropical islands. We thus consider these species to be diadromous. The presence of a check mark deposited during recruitment in freshwater has been validated in the present study for *K. rupestris* and *K. sauvagii*, whereas no check marks indicating recruitment in estuaries were found in *K. munda*. This information on *Kuhlia* spp. life cycles is of major importance for species conservation and management. It highlights the importance of a sea–estuary–river corridor for both downstream and upstream migrations. Managers of watersheds should ensure that this corridor remains intact. The obligatory marine larval phase revealed here for these species probably promotes the connection of populations from different rivers. The local management of watersheds must be included in regional plans for conservation.

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