ARE ELEMENTAL AND STRONTIUM ISOTOPIC MICROCHEMISTRY OF OTOLITH AND HISTOMORPHOMETRICAL CHARACTERISTICS OF VERTEBRAL BONE USEFUL TO RESOLVE THE EEL ANGUILLA OBSCURA STATUS IN LALOLALO LAKE IN WALLIS ISLAND?

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ABSTRACT – Otolith Sr/Ca, Ba/Ca and 87Sr/86Sr ratios and vertebral bone histomorphometry were used to investigate the life-history of Anguilla obscura eels from an enclosed lake, Lalolalo (Wallis Island in the Pacific), with no apparent connection with the sea. 87Sr/86Sr isotopic ratio from the core region gives evidence of indisputable marine origin of eels caught in the lake suggesting that underground connections between the lake and the sea exist. Sr/Ca and Ba/Ca ratios recorded after the elver mark are undifferentiated from seawater ones, thus limiting their used as marker of entrance in the lake and movement. However, 87Sr/86Sr isotopic ratio demonstrated that: 1) eels enter in the lake just after their metamorphosis and spend their entire adult life in the lake and 2) the lake chemistry is consistent with 1 % seawater-like contribution and 99 % basalt weathering and/or geothermal spring contribution. The vertebrae do not show a particular demineralization of bone matrix (45-52 %). Reversely, the study of bone compactness shows an important loss of bone that favors the hypothesis of a genital maturation that arises in the ecological state of the lake.

INTRODUCTION

During an hydrobiological survey in Uvea Island (Wallis, 14°18S, 176°10W, Pacific Ocean) in 2004, three eels Anguilla obscura Günther, 1872 were collected in a small closed ecosystem, the lake Lalolalo located in a volcanic crater. Because this lake has no visible connection with the sea, one important question is to know how the population of Anguilla is maintained in this lake. Indeed, there is no way out for the silver eels to leave the lake, at least via surface hydrological systems, since the crater walls are very steep (rising 40 to 50 m above the water lake level). As all known anguillid eel species are thought to breed in the ocean, the eels of the Lalolalo lake need to recruit the lake either by transport or by underground communication. Remaining questions are to know when the eels are entering the lake and if they are also able to leave the lake with these communications or if the lake represents a dead end for the trapped eels.

Otolith microchemistry, through elemental or isotopic ratios, has proven to be a useful tool to describe migratory environmental history of catadromous and anadromous fishes (see review in Elsdon et al. 2008), as well as natal freshwater habitats of anadromous fishes (Kennedy et al. 1997, Ingram & Weber 1999, Hobbs et al. 2005). Sr/Ca and Ba/Ca ratios and the 87Sr/86Sr isotopic ratio are the most commonly used tags because their records in otoliths have been shown to be reliably related to the concentrations of these elements in water bodies where the fishes are living (Bath et al. 2000, Barnett-Johnson et al. 2008, Tabouret et al. 2010) with no significant influence of diet (Marhon et al. 2009). Primarily focusing on Sr/Ca ratios, anguillid eels are all catadromous but while some have a freshwater growth stage, others should instead be seen as ocean or estuarine residency (e.g. Tseng et al. 1997, Tsukamoto et al. 1998, Limburg et al. 2003, Araï et al. 2004, Daverat & Tomas 2006, Jessop et al. 2006, Shiao et al. 2006, Tabouret et al. 2010). However freshwater reproduction has never been reported for eel species.

Relatively distinct breaks were reported in the Sr/Ca ratio of otolith core between freshwater resident and anadromous salmonids (Kalish 1990, Zimmerman & Reeves
Otolith core region prior to the exogenous feeding check have been shown to record a very strong Sr/Ca ratio in eels (Tsukamoto & Arai 2001, Tzeng et al. 2003, Tabouret et al. 2010), stronger than the one only due to maternal investment in the yolk material during their mother’s marine residency for spawning (Tsukamoto & Arai 2001). Otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio has been shown to be positively correlated with $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in rearing waters (Kennedy et al. 2000, 2002, Hobbs et al. 2005, Weber et al. 2005) without any fractionation (Kennedy et al. 2000). Consequently, this isotopic ratio should shift towards modern marine values (0.70918, Miller & Kent 2009) in the case of a marine origin, while significantly lower $^{87}\text{Sr}/^{86}\text{Sr}$ ratio than ocean waters would be expected when eels have joined the lake when isotopic ratio as low as 0.705 can be found for volcanic hydro systems (Barnett-Johnson et al. 2008, Miller & Kent 2009, Walther & Limburg 2012).

During their sexual maturation migratory teleost fish are characterized by important histomorphological transformations of bones (Tchernavin 1938a, b, Meunier & Desse 1978, Persson 1997, Kacem et al. 1998, Yamada et al. 2002, Kacem & Meunier 2009) and scales (Crichton 1935, Van Someren 1937, Kacem et al. 2013). During anadromic migration and sexual maturation for the Atlantic salmon bone demineralization (Kacem et al. 2000) and resorption (Kacem & Meunier 2000, 2003) have been evidenced. During catadromic migration and sexual maturation for the Japanese eel, bone mineralization is stable whereas bone resorption is significant (Yamada et al. 2002). Intense bone resorption was also shown during experimental maturation for the European eel (Fontaine et al. 1964, Lopez et al. 1970, Lopez & Martelly-Bagot 1971, Lopez 1973, Sbaihi et al. 2009), in conger eel (Lopez & Deville-Beignoux 1974) and in Japanese eel (Yamada et al. 2002). So the histomorphological characteristics of Anguilla obscura’s vertebrae may provide arguments to infer the real state of gonadic physiology in the lake specimens, while other morphologic criterions like eye diameter, pectoral fin dimension are missing (D’Ancona 1960, Locomte-Finiger 1990, Acou et al. 2003).

Therefore, the objectives of this study were: 1) to test whether the depositional patterns of trace elements in the otoliths of Anguilla obscura eels could provide an answer on the ecological status of these eels in Lalolalo Lake. In particular, we attempted to determine if otolith Sr/Ca, Ba/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios from the core region and outside the core region could give evidence on marine birth, on when eels have joined the lake and on possible movements between the lake Lalolalo and the ocean via underground connections; 2) to test whether the histomorphometrical characteristics of vertebral bones could provide some information about a possible physiological genital maturation and so favor a reproductive trend either in the lake or in the sea after a catadromic migration. For this second part of the work we compared two eels of Wallis Island with two New-Caledonian specimens of A. obscura, with one adult maturing A. anguilla and with an immature and a maturing conger eels (Conger conger) from Brittany shore (see the study of Sbaihi et al. 2001). We studied the mineralizing rate and the compactness of vertebral bone, that are good proxy information on the maturation state of eels.

MATERIALS AND METHODS

Study area: Wallis (also called Uvea Island, 77.5 km$^2$) is one of the three volcanic islands of the French archipelago of Wallis and Futuna located in the South Pacific Ocean, west of Samoa and north-east of Fiji (Fig. 1). The lavas of the Wallis Islands belong to the alkaline suite of the central Pacific volcanoes and consist primarily of oceanic olivine basalts extruded from 19 separate vents. Though lacking permanent streams due to the extreme permeability of the soils and a weak relief, Uvea has several large crater lakes such as Lalolalo. There is significant seasonal variation with abundant rainfall from approximately October to May. Only little information is however available on Lalolalo lake which is supposed to be 80 meters deep. Within the framework of an extensive survey aimed to improve the knowledge of the flora, fauna and environmental conditions of freshwater ecosystems of Wallis and Futuna Islands, three adult specimens of Anguilla obscura were collected in Lalolalo lake in October 2004. Furthermore, physico-chemical variables were also measured during this survey and are reported in Meisch et al. 2007. The water pH of the lake is relatively alkaline (pH ≈ 9), temperature is around 30 °C, the conductivity is rather high for a freshwater ecosystem (1.646 $\mu$S.cm$^{-1}$), and the water chemical composition reveals low concentration in Ca (7 ppm) and high proportion in Cl, Na, SO$_4$, Mg and K (440, 264, 67, 23 and 7.7 ppm, respectively). Unfortunately Sr and Ba concentrations were not analyzed in the water of the lake.

Biological material: Three specimens of Anguilla obscura (> 600 mm TL) were caught in Lalolalo Lake (Fig. 1). For histomorphometrical comparative studies of vertebrae we had two specimens of A. obscura (835, 525 mm TL) from New Caledonia, one specimen of Anguilla anguilla (590 mm TL) from the Alsacian part of the Rhine River and three specimens of Conger conger (740, 940 and 1,070 mm TL) from the Brittany shore (see the study of Sbaihi et al. 2001).

Otolith preparation and microchemical analyses: Sagittal otolith pairs were extracted from eels, cleaned with H$_2$O$_2$ (ultrix), rinsed with 18.2 M$\Omega$ MQ water and kept dry. They were then embedded in araldate resin (araldate DBF CH Escil, Chassieu, France), ground on a sagittal plane to expose their cores and polished with diamond paper of decreasing grain diameter (9.0, 3.0 and 0.3 $\mu$m) (Escil, Chassieu, France). Then, the right one was etched with EDTA, 5 % during one minute and then, stained with Toluidine Blue for ageing. The left
one was rinsed with 18.2 MΩ MQ water and dried prior to the analysis of $^{86}$Sr, $^{138}$Ba and $^{43}$Ca and $^{87}$Sr/$^{86}$Sr isotopic ratio using an inductively-coupled plasma mass spectrometer (ICP-MS DRC II, Perkin Elmer) and a multicollector inductively-coupled plasma mass spectrometer (Nu-Plasma MC-ICP-MS) coupled to a high-repetition-rate infrared femtosecond laser ablation (fs-LA Alfamet-novalase, France) system according to Tabouret et al. 2010 and Martin et al. 2013 procedures, respectively. Two parallel linear raster scan of 30 µm width were made with a 500 Hz frequency from the primordia to the edge of the otolith at a speed of 5 µm s$^{-1}$ for both elemental and isotopic measurements. Ablated material was carried by a He gas stream (carrier gas flow rate 0.68 L min$^{-1}$) from the laser cell to the ICP-MS where it was mixed with an Ar sample gas and a wet aerosol (2 % HnO$_3$). Prior to ablation a gas blank measurement of 20 s duration was made. $^{43}$Ca was used as an internal standard for each ablation (Whitledge et al. 2007) for the elemental analysis to avoid variation in ablation yield and thus improve the reliability of the elemental abundance at each point of the transect. The average detection limit based on three standard deviations (SD) of the blank gas was 4 µg g$^{-1}$ for $^{86}$Sr and 0.03 µg g$^{-1}$ for $^{138}$Ba. For the Sr isotopic determination the contribution of $^{87}$Rb to $^{87}$Sr intensity was deduced from the $^{85}$Rb signal (Barnett-Johnson et al. 2010). $^{83}$Kr intensity was monitored to control any potential interference of $^{84}$Kr and $^{86}$Kr on $^{43}$Sr and $^{86}$Sr respectively. $^{40}$Sr/$^{86}$Sr invariant ratio was used to correct the $^{87}$Sr/$^{86}$Sr ratio from instrumental mass bias using an exponential law (Walther et al. 2008). Analytical accuracy was achieved through the analyses of the marine fish otolith Certified Reference Material NIES 22 (National Institute for Environmental Studies, Japan Yoshinaga et al. 2000). Element data are reported as element to calcium ratios (i.e. Sr/Ca, Ba/Ca ) based on the stoichiometry of calcium carbonate (389,000 µg Ca g$^{-1}$ otolith, Brown & Severin 2009), as these elements can substitute for calcium in the otolith matrix (Campana 1999). The $^{87}$Sr/$^{86}$Sr ratio obtained in NIES 22 pellets (0.70927 ± 0.00011 2 SD, n = 5) fell within the expected range for nearly constant modern sea water (0.70917) (Allègre et al. 2010).

**Bone preparation and histomorphometrical analyses:** Two or three adjacent vertebrae were sampled either in the abdominal region and/or in the caudal one. They were slightly boiled to remove the flesh from the vertebrae, brushed in running tap water and dried.

**Bone mineralization (BM):** Water and fat were removed from bone with successive graded levels of alcohol, acetone and trichloroethylene baths. The vertebrae were weighed to the nearest mg (dry weight), then incinerated for six hours in a muffle furnace (750 °C). The ashes were weighed to the nearest mg (mineral weight), and bone mineralization (BM) was calculated (%) by the ratio of ash weight to dry weight.

**Bone compactness: analysis of the vertebral bone profiles:** We can assimilate the vertebrae of the Anguillidae to bony cylinders. Then to measure vertebral bone compactness of our samples we have used the “vertebral bone profiles” technique developed for the study of long bone diaphysis compactness in tetrapods (Girondot & Laurin, 2003) suited to vertebrae of Salmonidae (Deschamps et al. 2009). The vertebral bone profiles
allow comparison of vertebral bone areas (B.Ar.) between the various samples and so to decipher eventual significant differences of the bone compactness.

Vertebras were embedded in resin (98 % stratyl, 2 % Luper-ox catalysor) and 125 ± 10 µm-thick, transverse sections using a Leitz 1600 Saw Microtome (Leitz Company, Wetzlar, Germany). One single section through the mid-region of the vertebrae, in which the notochord canal is the narrowest and the bone tissue area the largest was retained for bone area (B.Ar.) analyses. The sections were X-rayed using a CGR Sigma 2060 generator (CGR-GE, Buc, France), adjusted to 8 kV and 6 mA, on a Kodak Industrex film Ready Pack (Eastman Kodak, Rochester, NY, USA) set at 30 cm from the X-ray source. The enlarged X-rays (35x) were digitized using a Olympus Camedia digital camera mounted on an Olympus SZX12 binocular microscope. Vertebral bone profiles were obtained from these images using BONE PROFILER 3.23. Owing to this software, bone area was measured in 50 concentric zones, each representing 2 % along the radius length from the periphery of the notochord canal to the periphery of the vertebra. Transverse section through the mid-region of vertebrae can be divided into four distinct regions (Fig. 2): i) a central ring of compact bone surrounding the notochord that we call “notochord area”; ii) immediately adjacent to this first area, the “transition area” that represents the proximal part of bone trabeculae; iii) a “middle area” representing the region in which bone trabeculae are most often subjected to resorption; iv) and the “peripheral area”, i.e. the distal region of bone trabeculae (but see Deschamps et al. 2009 for more mathematical details). The notochordal canal does not contain mineral tissues, so this central region of the vertebral section was not included in our analysis of vertebral profiles. The diameter of the notochordal canal being variable between sections, we took into consideration the relative distance (d = proportion of the radius of cross-sectioned vertebra) from the periphery of the notochord canal (value: 0) to the periphery of the vertebra (value: 1) to ensure accurate comparison between vertebral sections with various total bone areas (Fig. 2). The main pattern of B.Ar. from the notochordal canal to the vertebral periphery shows a sigmoid signature (Fig. 2). The profiles from eel vertebrae were characterized by eight parameters (P_0, S_0, Min_0, P_3, S_3, P_4, S_4, Min_3) which are specified in Fig. 2. In the present study, it is the curve section corresponding to the “middle area”, that gives us the relevant bone physiological information.

**RESULTS AND DISCUSSION**

Among the three Anguilla obscura eels from Lalolalo lake aged between 7 and 10 years, “life-history transects” passed through the nucleus only for two otoliths (Fig. 3). As core location was not really discernible, adopted trajectory for the latter otolith probably missed it.
According to our hypotheses, one would expect different microchemical patterns along the radius of each otolith. In the case of marine origin, trace element trapped in the otolith core including both their nuclei and otolith accreted before the onset of exogenous feeding would thus reflect sea water composition. Trend in the chemical profile of the otolith from the elver mark to the edge will depend on when eels are entered the lake. Entrance just after the metamorphosis would mean that eels spend their entire life in the freshwater lake, thus otolith elemental signatures would record typical Lalolalo Lake freshwater characteristics during all eel life stages. In the case of a later entry in the lake, then the otolith signature after the elver mark will reflect the marine environment, whereas a more or less important part of the otolith will be related to the lake chemistry.

“Marine” stages : leptocephalus and glass eel

As previously found in all studies regarding eels and using chemical information stored in otoliths (Tzeng et al. 1997, Tsukamoto et al. 1998, Tsukamoto & Arai 2001, Limburg et al. 2003, Arai et al. 2004, Daverat & Tomas 2006, Jessop et al. 2006, Shiao et al. 2006, Tabouret et al. 2010), for the two otoliths where linear raster scan crossed the core region, very high Sr/Ca ratios (Sr/Ca > 11 × 10⁻³, Table I, Fig. 3) and low Ba/Ca ratios (4 × 10⁻⁶ Table I, Fig. 2) were observed in the central region from the primordium to the metamorphosis check mark corresponding to the leptocephalus stage. In addition, in this region of the otolith the strontium isotopic ratio ($^{87}$Sr/$^{86}$Sr) is close to the global marine value in one eel (L2, Fig. 3) but lower in the second one (L3, Table I, Fig.3). This lower $^{87}$Sr/$^{86}$Sr value is coincident with visual passage of raster scan across the zone outside the elver mark, zone characterized by lower $^{87}$Sr/$^{86}$Sr ratio (Table I), thus leading to a lower isotopic ratio than that of seawater. Consequently, these results clearly revealed a marine origin of the eels from Lalolalo Lake. Moreover, despite ontogenic processes that largely affect the incorporation of Sr in the core region of eels (Otake et al. 1997, Tsukamoto & Arai 2001), our $^{87}$Sr/$^{86}$Sr results tend to attest that the high Sr/Ca ratios reflect a marine origin. These results are consistent with the observations of Fontaine et al. (1987) who found a relatively low gonadosomatic index in the eels of this lake suggesting that the eels were not maturing.
their gonads in the lake, excluding that the reproduction may occur in the lake. Furthermore, eel larvae have never been observed in the lake. A marine origin of eels supposes that underground connections may exist between the lake and the Ocean or that eels are artificially stocked or transported (for example by human or piscivorous birds). Underground connections will favor glass eel to colonize the lake when their size is small, but probably preventing their leave as soon as they reach a certain size. Taking into account of the inaccessible edge of the lake (40–50 m steel wall) a human introduction is however unlikely.

**“Continental” stages: elver, yellow and silver eel**

_Anguilla_ spp. should exhibit a clear decrease in Sr/Ca ratio to value lesser than $5 \times 10^{-3}$ and a significant increase in Ba/Ca ratio to value higher than $4 \times 10^{-6}$ when they migrate from the marine environment to freshwater (Otake et al. 1994, Tzeng et al. 1997, Tabouret et al. 2010). Otolith Sr/Ca ratio of _A. obscura_ from the Lalolalo lake decreases just after the metamorphosis and remains stable from the elver mark until the eels were caught, but with ratio values between 4 and $6 \times 10^{-3}$ (Fig. 3A). Moreover, the Ba/Ca ratio exhibits a large peak just after the metamorphosis, then decreasing to values largely lower than $4 \times 10^{-6}$ until the edge of the otolith (Fig. 3B). Both Sr/Ca and Ba/Ca ratios in the otolith part accreted after the elver mark have thus values above $4 \times 10^{-3}$ and lower than $4 \times 10^{-6}$ respectively (Table I), very similar to values found for brackish or marine resident eels (Tsukamoto & Arai 2001, Daverat & Tomas 2006, Tabouret et al. 2010). The occurrence of a relatively strong Ba/Ca peak just after the metamorphosis can be related to ontogenetic effects on otolith chemistry, such as metabolic changes from larval to juvenile that are liable to affect the otolith chemical patterns, irrespective of environmental conditions (De Pontual et al. 2003, Martin et al. 2013). Furthermore, because both ratios were almost constant from the elver mark until the eels were caught, this suggests that the eels spend their whole life in the lake waters. Under the assumption that elemental composition of fish otoliths is directly influenced by properties of the surrounding water, this would suggest that Sr/Ca and Ba/Ca chemical signatures of the Lalolalo lake are not significantly different from that of seawater, limiting thus their use to evaluate when eels are entered in the lake and migration between the lake and the ocean. Sr/Ca freshwater signatures close to or exceeding seawater ones have already been observed in few cases, less than 3% of hydrosystems (Limburg 1995, Wells et al. 2003, Kraus & Secor 2004, Brown & Severin 2009).

$^{87}$Sr/$^{86}$Sr isotopic ratio found from the elver mark to the edge ($=0.707$, Fig. 3C) is definitely different from that of marine water (0.70917, Allègre et al. 2010) suggesting that eels have joined the lake just after the metamorphosis. Furthermore, $^{87}$Sr/$^{86}$Sr isotopic ratio showed slightly

### Table I. Mean and 2σ of Sr/Ca, Ba/Ca and $^{87}$Sr/$^{86}$Sr for two life stages of _Anguilla obscura_ eels from Lalolalo Lake, Wallis Island.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sr/Ca (mg g⁻¹)</th>
<th>2σ</th>
<th>Ba/Ca (µg g⁻¹)</th>
<th>2σ</th>
<th>$^{87}$Sr/$^{86}$Sr</th>
<th>2σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to the exogenous feeding check</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11.5</td>
<td>4.3</td>
<td>4.1</td>
<td>1.4</td>
<td>0.7095</td>
<td>0.0006</td>
</tr>
<tr>
<td>3</td>
<td>12.9</td>
<td>4.1</td>
<td>4.4</td>
<td>1.7</td>
<td>0.7081*</td>
<td>0.00001</td>
</tr>
<tr>
<td>Posterior to the feeding check</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.3</td>
<td>1.4</td>
<td>2.1</td>
<td>2.1</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>1.3</td>
<td>2.0</td>
<td>1.8</td>
<td>0.7074</td>
<td>0.0004</td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>1.2</td>
<td>3.0</td>
<td>2.1</td>
<td>0.7068</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

*linear raster scan partly cross the zone outside the elver mark; n.d.: not determined

### Table II. Mineral rate of vertebral bone in eel and conger specimens.

<table>
<thead>
<tr>
<th>Dry weight (mg)</th>
<th>Ash (mg)</th>
<th>Mineral Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. obscura 1 Wal</td>
<td>521</td>
<td>271</td>
</tr>
<tr>
<td>A. obscura 2 Wal</td>
<td>653</td>
<td>314</td>
</tr>
<tr>
<td>A. obscura 3 Wal</td>
<td>476</td>
<td>216</td>
</tr>
<tr>
<td>A. obscura 4 NC</td>
<td>347</td>
<td>178</td>
</tr>
<tr>
<td>A. obscura 5 NC</td>
<td>107</td>
<td>53</td>
</tr>
<tr>
<td>A. anguilla 6 abd</td>
<td>323</td>
<td>155</td>
</tr>
<tr>
<td>A. anguilla 6 caud</td>
<td>122</td>
<td>61</td>
</tr>
<tr>
<td>C. conger 9</td>
<td>702</td>
<td>351</td>
</tr>
<tr>
<td>C. conger 30</td>
<td>440</td>
<td>218</td>
</tr>
<tr>
<td>C. conger 33</td>
<td>420</td>
<td>211</td>
</tr>
</tbody>
</table>
higher values than expected from weathering reaction with the basaltic rocks of Wallis Island only ($^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratios from 0.7035 to 0.706 for basaltic substrate, Rad et al. 2007). This probably suggests that lake water $^{87}\text{Sr}/^{86}\text{Sr}$ ratio reflects the mixture of chemical weathering of basaltic rocks with oceanic-like sources (rains or seawater intrusion) as already found for river draining volcanic areas (Louvat & Allègre 1997, 1998, Rad et al. 2007). Oceanic-like $^{87}\text{Sr}/^{86}\text{Sr}$ sources to the lake can be linked either to atmospheric or to underground inputs. Using $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio end-members, 0.70917 for oceanic-like water (Allègre et al. 2010) and 0.7035–0.706 (Rad et al. 2007) for weathered basaltic rocks, and mass balance equation (see Allègre et al. 2010), we estimate that about 47±15 % of the strontium present in the lake must be of marine origin to explain the observed lake $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio recorded in eel otoliths. As Sr and Ca are usually conservative during freshwater/saline water mixing (Walther & Limburg 2012) and the Ca concentration of the lake is around 7 mg l$^{-1}$ (Meisch et al. 2007), a marine-like Ca contribution of 3.3 ± 1.0 mg l$^{-1}$ would be also expected. High concentrations of Cl, Na, SO$_4$, Mg and Ca are found in the Lalolalo lake water (440, 264, 67, 23 and 7 mg l$^{-1}$, respectively) compared to other lakes from the Wallis Island (Cl < 10 mg l$^{-1}$, Na < 10 mg l$^{-1}$, SO$_4$ < 30 mg l$^{-1}$, Mg and Ca < 5 mg l$^{-1}$) (Meisch et al. 2007), suggesting that the input via the atmosphere is not a main source of majors ions to the Lalolalo lake. This is reinforced by the generally low concentrations encountered in rains under tropical climate (less than 7 mg l$^{-1}$, 4 mg l$^{-1}$, 2 mg l$^{-1}$, 1 mg l$^{-1}$ and 0.2 mg l$^{-1}$ respectively for Cl, Na, SO$_4$, Mg and Ca, Louvat & Allègre 1997, 1998). Input of seawater via underground connections between the lake and the sea would be thus a more plausible source of

### Table III.

Table III. – Total bone area and parameters (unresorbed and middle area; see Deschamps et al. 2009)* of the model describing bone area profiles of vertebral transverse sections (see Fig. 4) of two congers (Conger conger) and two eels (Anguilla obscura). $Tt-B.A. = \text{Total bone area or bone compactness}; min_0 = \text{maximal bone area value in the periphery area}; min_{3,4} = \% \text{ of bone area lost compared with } min_0; P_0 = \text{relative distance from the border of the chordal area where a 50 }\% \text{ decrease in bone area is observed}; P_3 = \text{relative distance from the border of the chordal area where a 50 }\% \text{ decrease in bone area is observed between } min_0 \text{ and } min_{3,4}; S_0 = \text{reciprocal of the slope at point } P_0; S_3 = \text{reciprocal of the slope at point } P_3.$

<table>
<thead>
<tr>
<th>Specimens</th>
<th>$Tt-B.A.$ (%)</th>
<th>$min_0$</th>
<th>$P_0$</th>
<th>$S_0$</th>
<th>$min_{3,4}$</th>
<th>$P_3$</th>
<th>$S_3$</th>
<th>$P_4$</th>
<th>$S_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. conger 9A</td>
<td>25.0</td>
<td>0.33</td>
<td>0.04</td>
<td>0.02</td>
<td>0.04</td>
<td>0.20</td>
<td>0.04</td>
<td>0.70</td>
<td>-0.04</td>
</tr>
<tr>
<td>C. conger 33B</td>
<td>42.7</td>
<td>0.37</td>
<td>0.06</td>
<td>0.10</td>
<td>0.04</td>
<td>0.30</td>
<td>0.04</td>
<td>0.45</td>
<td>-0.05</td>
</tr>
<tr>
<td>A. obscura 1</td>
<td>28.5</td>
<td>0.42</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.20</td>
<td>0.02</td>
<td>0.60</td>
<td>-0.06</td>
</tr>
<tr>
<td>A. obscura 2</td>
<td>19.8</td>
<td>0.30</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
<td>0.20</td>
<td>0.02</td>
<td>0.85</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

* Parameters of the model for the transition area (min$_{3,4}$, $P_4$, $S_4$) are not presented.

The parameters of the model are described in the legend of Fig. 2.
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strontium and major elements, especially as it is suspected for glass eel colonization into the lake.

Neglecting the atmospheric source and assuming a mean seawater Ca concentration of 412 mg l\(^{-1}\), less than 1% of seawater contribution into the lake via underground connections would be required to explain such a marine Ca contribution. Such a moderate intake of seawater would also significantly contribute to raise the concentrations of Cl, Na, SO\(_4\), and Mg to the levels found in the lake water (Meisch et al. 2007). This would lead to a lake Sr concentration about 130 ± 20 µg l\(^{-1}\) and thus a Sr/Ca ratio (=15-20) of the same order of seawater. Applying the partition coefficient of 0.33 [(Sr/Ca\(_{\text{otolith}}\))/(Sr/Ca\(_{\text{water}}\))\(^{-1}\)] found by Tabouret et al. 2010 for eel living in brackish water, Sr/Ca\(_{\text{otolith}}\) of eel living in the lake Lalolalo would be thus between 4.6 × 10\(^{-3}\), similar to that measured.

Finally, as \(^{87}\)Sr/\(^{86}\)Sr isotopic ratio exhibits no variation across the entire lake life stage of \(A. obscura\), movements between the lake and the sea through underground connections did not occur following entrance of glass eels in the lake. But this is not a proof that these connections may not allow adults to migrate to the sea.

What vertebral bone structure is saying?

The mineralization rate of vertebrae varies between 45.4% to 52.0% in \(Anguilla obscura\) and 49.5% to 50.2% in \(Conger conger\) (Table II). These values are equivalent to the mineralization rate of \(A. anguilla\): 45.6% (Shaihi et al. 2007) or \(Oncorhynchus mykiss\): 57.3% (Cassadvall et al. 1990) and 49.6% to 52.7% (Kacem et al. 2004). In adult Atlantic salmon, \(Salmo salar\), the mineral rate of vertebrae ranges between 49.4% and 52.3% at the beginning of the ascent versus 48.4% to 51.5% after spawning (Kacem et al. 2003). So this histophysiocal characteristic of the bones of the studied eels of Lake Lalolalo is normal. In return, the study of vertebral bone compactness is more instructive. The results obtained with the Bone Profiler software give a bone compactness of 19.8% and 28.5% for the two analyzed specimens of \(A. obscura\), 42.7% and 25.0% respectively for the immature (GSR = 0.07) and maturing (GSR = 0.45) specimens of \(C. conger\) (Table III). Moreover, the vertebral profiles clearly indicate a loss of bone matrix in the two eels like in the mature conger (Fig. 4). We do not know the value of the GRS for the two \(A. obscura\) specimens, but the X-rays of the vertebral sections clearly show important resorption areas in the vertebral frame. The microradiographies of the analyzed sections effectively show that vertebral bones look more spongy in the two \(A. obscura\) and the maturing conger eel than in the immature conger eel (Fig. 5). These observations seem to be in favor of a gonadic activity for these two eels or of a precocious regression of bones linked with the specific water conditions in the Lake (i.e. anoxic and very sulfuric waters below 7 m depth associated with past volcanic activity).

Fig. 5. – X-ray of vertebrae cross sections. A, Immature \(Conger conger\). B, Maturing \(C. conger\). C, \(Anguilla obscura\) N° 2. D, \(A. obscura\) N° 1. (Scale bar = 1 mm).
Indeed, even if the dissected eels were not tested for GRS, gonadic activity in eels can be reversible if silvering eels did not find a way to go out from the lake. If this maturing phenomenon occurs several times in the life of the eels, it could explain the low values of bone compactness observed in the studied eels. In the same way, even if water conditions have not been studied properly, results have shown that below 7 m depth, the water of the lake is anoxic and sulfidic. Such conditions may also have some effects on bone compactness. However, more sampling needs to be done in the lake in order to test these assumptions.

**CONCLUSION**

Otolith $^{87}$Sr/$^{86}$Sr isotopic ratio proves to be useful to solve the *Anguilla obscura* status of the lake Lalolalo (Wallis Island) where otolith Sr/Ca and Ba/Ca are not suitable because of values close to the one found for seaway resident eels. $^{87}$Sr/$^{86}$Sr isotopic ratio measured in the core region demonstrated the marine origin of *A. obscura* as value of 0.709 was found. $^{87}$Sr/$^{86}$Sr isotopic profile from the elver mark to the edge suggested first, eels have entered the lake as glass eels and second, the absence of movements since they joined the lake as $^{87}$Sr/$^{86}$Sr isotopic ratio remains variable after the elver mark. The “Bone Profiler” analysis of bone vertebrae texture indicates that the eels have undertaken their gonadic maturation. Yet new field observations are necessary to know if the adult eels are able to leave lake Lalolalo for their migratory journey to their spawning area, in other words if they can undertake a catadromic migration.

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