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# Multi-element otolith fingerprints unravel sea–lagoon lifetime migrations of gilthead sea bream *Sparus aurata*

Lény Mercier<sup>1</sup>, David Mouillot<sup>1</sup>, Olivier Bruguier<sup>2</sup>, Laurent Vigliola<sup>3</sup>,  
Audrey M. Darnaude<sup>1,\*</sup>

<sup>1</sup>ECOSYM, UMR 5119, Université Montpellier 2, Place Eugène Bataillon 34095, Montpellier Cedex 5, France

<sup>2</sup>Géosciences Montpellier, UMR 5243, Université Montpellier 2, Place Eugène Bataillon 34095, Montpellier Cedex 5, France

<sup>3</sup>IRD, UR 227 CoReUs, Centre IRD de Nouméa, BP A5, 98848 Nouméa, New Caledonia

**ABSTRACT:** Precise knowledge of lifetime migrations is vital in exploited fish species, since all essential habitats must be protected to maintain sustainable stock levels. The present study used multi-element otolith fingerprints of the gilthead sea bream *Sparus aurata* (L.) to discriminate its main juvenile and adult habitats in the Languedoc-Roussillon region (Gulf of Lions, northwest Mediterranean) and infer the lifetime migrations of 12 individuals from the area (11 from the present day and 1 from the Roman era). This allowed for the first time the identification of key habitats for the successful completion of the species' life cycle in the Gulf of Lions, and the connectivity between them. Our results revealed that lagoon use by *S. aurata* is probably ancient (>2500 yr) and confirmed its current commonness. Yet, although most observed migration patterns were in accordance with the migratory behavior previously described for the species, strong inter-individual variations and new patterns in habitat use were detected. At the juvenile stage, a preference for shallow lagoons with low salinities was evidenced. Nevertheless, the first year of life can also be successfully completed in marine conditions. At the adult stage, lagoon use was shown to occur until at least age 4 yr, with periods of lagoon residency of up to 11 mo in a year, often including winter months. Because overwintering in the lagoons was previously thought to be impossible for *S. aurata* due to low temperatures, this finding has important implications for future stock management, especially since the species breeds in winter.

**KEY WORDS:** Otolith microchemistry · Trace elements · Random forest · Key habitats · Gulf of Lions

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## INTRODUCTION

Conservation of marine fish resources requires reliable understanding of their lifetime migration patterns, because identification of natal sources, juvenile nursery grounds, and migration routes are key elements of population ecology and sustainable fisheries management (Forrester & Swearer 2002). Potential habitats for fishes are highly diverse, especially in the coastal zone, and are of primary interest for

resource sustainability (Lotze et al. 2006). As anthropogenic pressure on the littoral zone intensifies, coastal ecosystems are increasingly threatened by pollution, eutrophication, and habitat degradation (Nixon 1995, Kemp et al. 2005), resulting in habitat loss, population fragmentation, and erosion of biodiversity (Levin et al. 2001). In this context, understanding and measuring connectivity among coastal habitats used by young and adult fish is essential to define marine protected areas (Mumby et al. 2011),

\*Corresponding author. Email:  
audrey.darnaude@univ-montp2.fr

and quotas for recreational and commercial fisheries (Gillanders et al. 2003). This knowledge remains limited in marine fish (Metcalf et al. 2002), even for some of the most studied and heavily fished species, like the gilthead sea bream *Sparus aurata* (L. 1758).

This highly prized sparid is fished from the Eastern Atlantic to the Black Sea, but principally in the Mediterranean, where it is one of the main targets for coastal fisheries (Bauchot & Hureau 1986). In the Gulf of Lions (northwest Mediterranean), it is known to perform ontogenetic and trophic migrations between coastal lagoons and the sea (Lasserre 1976), but the importance of each habitat for the successful completion of its life cycle remains unclear. Exploitation rates for the species are high (Farrugio et al. 1994), and reliable knowledge of natal origin, population structure, and habitat connectivity is urgently needed to preserve stocks. In the area, *Sparus aurata* spawns in winter, probably at sea, with a peak in January–February (Audouin 1962). Larvae are pelagic for about 3 mo prior to metamorphosis and settlement (Houde 1989). Each year, large numbers of post-larvae enter coastal lagoons (Audouin 1962), where they feed over the summer before migrating back offshore in autumn, when water temperature in the lagoons drops below that of the open sea (Lasserre 1976).

The use of coastal lagoons, at least by juvenile stages, has long been thought to be essential for maintenance of *Sparus aurata* stocks in the Gulf of Lions (Arias 1976). However, how widespread this behavior is, at the population level, and its importance for the maintenance of local exploited stocks, remain unclear. Lagoons are only recent formations in the Gulf of Lions (~5600 yr BC; Sabatier et al. 2010), and are not found in other parts of the species' geographic range. Furthermore, *S. aurata* juveniles have been captured at sea in some parts of the Gulf of Lions (Mosconi & Chauvet 1990, Francour 1997). Thus, the use of coastal lagoons by the species in the area may be facultative. Moreover, the last studies of lagoon use by *S. aurata* in the area were carried out in the 1970s (Lasserre 1976). Since then, intensification of climatic and anthropogenic pressures in the coastal zone (Vitousek et al. 1997, Boesch et al. 2001) have resulted in a significant increase in eutrophication and anoxic crises in the lagoons (Aliaume et al. 2007, IFREMER 2008), and a corresponding loss in biodiversity (Mouillot et al. 2005b). These alterations of lagoon environmental conditions have reduced their quality as habitats for juvenile fish (Quignard et al. 1984, Labourg et al. 1985, Brusle & Cambrony 1992), and might have affected *S. aurata* migratory behavior in the area. In this context, it is urgent to

investigate current coastal habitat use and sea-lagoon migrations of *S. aurata* in the area to ensure the conservation of its local populations. Because migrations and critical habitats can differ according to life stage (Pineda et al. 2007), this necessitates accurate knowledge of fish lifetime movements.

In the last 2 decades, otolith (fish ear-stones) microchemistry has emerged as a powerful tool to explore fish migration patterns. Otoliths grow continuously throughout the lifetime of teleost fishes, with no reabsorption of material incorporated into their structure (Panfili et al. 2002). Their chemical composition reflects that of the surrounding water at the time of deposition (Campana & Tzeng 2000). They are, therefore, natural tags whose structure and chemistry provide a record of the environmental history of individuals throughout their lives (Campana 1999), and can reveal movements of present and ancient fish between different water bodies (e.g. Carpenter et al. 2003, Walther & Thorrold 2006, Clarke et al. 2009, Tanner et al. 2011). Nevertheless, most studies in this field (e.g. Secor et al. 2001, Jessop et al. 2008) have been based on lifetime fluctuations in otolith strontium (Sr) concentrations, which are a proxy for environmental salinity (Campana 1999). However, in order to confidently discriminate between coastal habitats with similar salinities, multi-elemental otolith fingerprints are needed (Forrester & Swearer 2002, Mercier et al. 2011b). Furthermore, in the coastal zone, temporal variations in otolith elemental composition at a given location can be greater than variation among sites, so the collection of multi-annual signatures for each site is necessary to obtain temporally stable signatures that can be exploited to reconstruct lifetime habitat use by coastal fishes (Elsdon et al. 2008).

In the present study, we used otolith microchemistry to evaluate the significance of lagoons in the life cycle of *Sparus aurata* in the Gulf of Lions. We developed pluri-annual and multi-elemental otolith fingerprints from the 4 main coastal habitats used by *S. aurata* in the Gulf of Lions (3 contrasted lagoons and the sea) to infer lifetime sea-lagoon migrations in the species from the signatures in the otoliths of 11 present-day adults, plus an archeological otolith dated from the Roman era (~475 yr BC). This information is needed for better management of lagoon habitats in the Gulf of Lions. It might also be vital for the long-term conservation of *S. aurata* in the Mediterranean since, with the forecasted climate change and erosion of sandy coasts, many of its lagoons may disappear and become embayments in the future (Paskoff 2004).

## MATERIALS AND METHODS

### Study area

In the Gulf of Lions, the coastline of the Languedoc-Roussillon region presents a dense network of 21 coastal lagoons, with various sizes (1 to 75 km<sup>2</sup>) and distances from one another (0.1 to 40 km) (Fig. 1). Among these lagoons, 17 are regularly colonized by *Sparus aurata* juveniles during their first year of life, but only 2 (Salses-Leucate and Thau lagoons) are consistently visited by fish older than 1 yr (Lasserre 1976). All these lagoons are highly variable environments, with strong intra-annual variations in water temperature, salinity, and oxygen levels depending of the wind, air temperature, and precipitation on their catchment area (Mouillot et al. 2007). They show similar annual ranges in water temperatures (between 2 to 5 and 26 to 30°C, with a common mean ~17°C) and all have higher annual productivity levels and, from May to October, 1 to 5°C higher water temperatures than the sea (Table 1). Yet they differ in the chemical composition of their water masses because of different continental and marine influences, anthropogenic loads, and catchment areas (Mouillot et al. 2005a).

With regard to salinity and continental versus marine influence, the 17 lagoons can be grouped into 3 categories (Table 1). The first category includes the Salses-Leucate, Lapalme, and Thau lagoons, which are all under strong marine influence. Due to the high level of water renewal through their direct opening to the sea (IFREMER 2002, 2008), these lagoons exhibit salinities similar to that of the nearby coastal area (37.5), and even higher (40 to 41) in the summer. In contrast, the Canet, Mauguio, and Petite Camargue lagoons are under strong continental influence. They all show very variable salinities, yet these are always lower (<34) than that of the coastal zone. The lagoons from the Bages-Sigean and the Palavas networks exhibit intermediate salinity conditions, especially those in the Palavas lagoon network (Fig. 1), where a marked east-west salinity gradient is observed (IFREMER 2008, 2009).

Pollution by metals in the Languedoc-Roussillon lagoons is generally low (IFREMER 2009). Notable contaminations (by nearby small industry or harbor activities) are rare and localized, and never affect the entire lagoon area (IFREMER 2008). Inter-lagoon variations in water elemental composition in the region therefore are mainly controlled by differences in lagoon freshwater sources. Because the Rhône

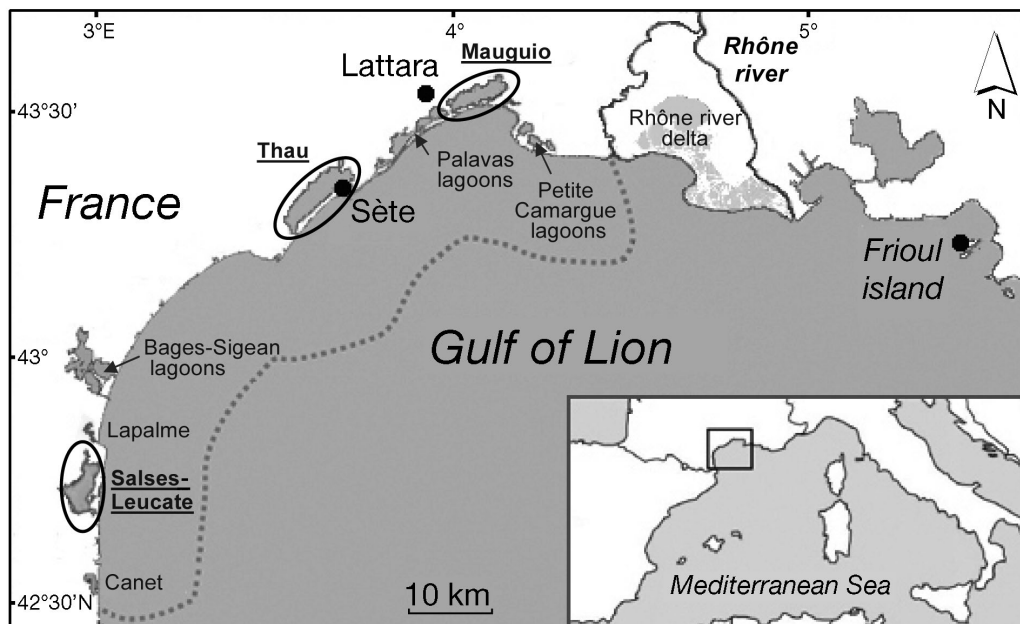


Fig. 1. The study area in the Gulf of Lions (northwest Mediterranean) showing all the coastal lagoons of >2 km<sup>2</sup> area likely to be colonized by *Sparus aurata*. The 3 lagoons (Mauguio, Thau, and Salses-Leucate) retained for the sampling of *S. aurata* juveniles and adults in the lagoons are circled in black. For the marine habitat, all the adults analyzed in the present work came from the fish auction market of Sète, and were caught within the coastal fishing district of the Languedoc-Roussillon region (dotted line). Aquaculture juveniles for detailed intra-annual variations of marine chemical signatures were collected from the nearest offshore fish farm, located on Frioul Island, outside the influence of the Rhône River plume. The fossil otolith analyzed came from the archeological site of Lattara

Table 1. *Sparus aurata*. Characteristics of the 17 lagoons frequented by *S. aurata* in the Languedoc-Roussillon region (Gulf of Lions, northwest Mediterranean), derived from Bec et al. (2011) and IFREMER (2002, 2008, 2009, 2010). For the Bages-Sigean and the Palavas lagoon networks, only the biggest and most contrasted lagoons are presented, with the others (total number indicated in parentheses) showing intermediate conditions. The 3 lagoons sampled are indicated in **bold**. Available hydrological data on the adjacent coastal marine zone (final row) is provided for comparison. chl *a*: chlorophyll *a*; RS: Rhône to Sète; na: not available

Lagoon	Area (km <sup>2</sup> )	Mean depth (m)	Opening(s) to the sea	Watershed (km <sup>2</sup> )	Main freshwater source(s)	Annual median salinity (min–max)	Temperature range (°C)	Annual chl <i>a</i> (µg l <sup>-1</sup> )
Canet	6.0	0.35	1 (direct)	260	Local rivers (Pyrenees)	22.0 (4.9–32.6)	2–27	19.5
<b>Salses-Leucate</b>	<b>54.0</b>	<b>2.0</b>	<b>2 (direct + channel)</b>	<b>162</b>	<b>Karst (Pyrenees)</b>	<b>37.0 (33.3–40.3)</b>	<b>3–29</b>	<b>1.5</b>
Lapalme	6.0	0.5	1 (direct)	65	Karst (Pyrenees)	37.0 (30.2–41.2)	4–31	1.6
Bages-Sigean lagoon network (3)								
Bages-Sigean	37.0	1.3	1 (channel)	443	Canal	34.7 (28.2–40.7)	1–28	2.7
Ayrolle	13.2	0.5	1 (direct)	–	Canal (through lagoon)	35.3 (20.6–38.1)	3–27	1.1
<b>Thau</b>	<b>75.0</b>	<b>4.0</b>	<b>2 (direct)</b>	<b>&gt;285</b>	<b>Local rivers, RS canal (direct)</b>	<b>38.8 (36.4–40.2)</b>	<b>2–29</b>	<b>1.6</b>
Palavas lagoon network (7):								
East (Ingril)	6.8	0.6	1 (direct)	>250	RS canal (direct)	35.8 (23.4–40.0)	1–29	4.7
Middle (Prevost)	3.8	0.75	1 (direct)	>300	RS canal (direct), local rivers	31.5 (14.7–40.8)	3–28	15.0
West (Méjean)	5.5	0.75	1 (channel)	>455	RS canal (direct), local rivers	20.8 (12.1–32.5)	5–25	117.2
<b>Mauguio</b>	<b>32</b>	<b>0.8</b>	<b>1 (channel)</b>	<b>&gt;410</b>	<b>RS canal (direct), local rivers</b>	<b>22.1 (1.0–33.9)</b>	<b>4–30</b>	<b>56.1</b>
Petite Camargue lagoons:								
Ponant	2.0	2.7	1 (direct)	na	Local rivers, RS canal (indirect)	18.7 (0.5–30.4)	8–32	11.4
Médard	1.4	0.6	1 (channel)	na	RS canal (indirect)	20.0 (15.6–23.4)	3–26	8.6
Sea surface (0–1 m)	–	–	–	–	Rhône River	37.5 (36.8–38.7)	7–26	0.4

River accounts for ~80% of the overall riverine input to the Gulf of Lions (Durrieu de Madron et al. 2000) and is the main continental source of particulate and dissolved matter in the local coastal zone (Ollivier et al. 2011), a contrast exists between the lagoons connected to the Rhône River through the Rhône to Sète canal (i.e. Thau, Mauguio, and the lagoons of the Palavas and the Petite Camargue networks), and those outside its influence (i.e. Canet, Salses-Leucate, Lapalme, and the lagoons of the Bages-Sigean network) (Table 1). The latter are mainly under the influence of local rivers and water resurgences (IFREMER 2002). Therefore the water signature within the Canet and the Salses-Leucate lagoons, and to a lesser extent, within the Bages-Sigean lagoon network, reflects the distinct chemical composition (Roussiez et al. 2005) of the water streaming from the Pyrenees that ends in their catchment areas (IFREMER 2002).

## Sampling

The building of a robust statistical classifier for reliable inference of *Sparus aurata* lifetime migrations using otolith fingerprints necessitates a training dataset encompassing the intra- and inter-annual variability in otolith chemical signatures of the main water masses frequented by the fish at each life stage. Adequate estimation of temporal variation of the elemental signatures within each area in particular is crucial, because a sample taken in one particular time period in a certain area might not be representative for samples taken in the same area at a different time (Gillanders 2005). To this end, juvenile and adult *S. aurata* were collected during at least 2 successive years, both within a vast area at sea and in the Salses-Leucate, Thau, and Mauguio lagoons (Fig. 1). These 3 lagoons are the largest in the study area (Table 1). They are abundantly colonized by

juvenile or adult *S. aurata* (Lasserre 1976, Quignard et al. 1984) and, because of their differences in marine influence, main freshwater source, depth, and productivity, are good representatives of the contrasts in lagoon environmental conditions existing within the Languedoc-Roussillon region (Table 1).

Seasonal variations in the signature for the marine habitat were assessed mainly from detailed analysis of the otoliths of 3 aquaculture fish (total length [TL]: 30.4 to 32.4 cm) collected at a local offshore farm (Provence Aquaculture, Frioul Island, 43° 16' N, 5° 18' E; Fig. 1) in December 2007. For these fish, only the otolith signatures corresponding to the last year of life (i.e. after their arrival at the offshore farm) were included in the training dataset. These data were completed by chemical signatures from the edge of the otoliths from 8 wild adults (TL: 26.3 to 39.0 cm), caught in a vast area at sea (Fig. 1) at different dates in 2008, and by those obtained from the core (i.e. the period of early larval life at sea) of 21 juveniles (TL: 16.7 to 20.1 cm) caught in the Mauquo lagoon in September–October 2006, 2007, and 2008. To obtain a comprehensive reference signature for *Sparus aurata* lagoon habitats, detailed temporal variations in otolith composition were measured for 25 juveniles (TL: 11.8 to 20.1 cm) collected in September–October 2006, 2007, or 2008 (i.e. at the end of their annual period of lagoon residency) in the 3 lagoon sites. Emphasis was laid on the Mauquo and Thau lagoons, which are the 2 major putative nursery sites for *S. aurata* in the area (Lasserre 1976). In addition, chemical signatures were measured on the edge of the otoliths of 6 mature females (TL: 33.5 to 38.5 cm) collected between 2006 and 2008 in the only 2 lagoons consistently visited by adults (Lasserre 1976), i.e. Salses-Leucate and Thau. The resulting training dataset for habitat discrimination (Table 2) therefore consisted of 554 seasonal multi-elemental signatures derived from the chronological analysis of 41 otoliths. Because they included detailed seasonal data from all life stages (larvae, juveniles, and adults) and their main habitats (3 contrasted lagoons and a vast area at sea) for at least 2 different years, these chemical signatures covered most of the intra- and inter-individual variation in otolith fingerprints to be expected for *S. aurata* in the Gulf of Lions.

Using the statistical classifier derived from these fingerprints, lifetime migrations of *Sparus aurata* in the Gulf of Lions were investigated from the signatures of 12 otoliths (test dataset; Table 2): 11 from present-day adults (TL: 26.3 to 39.0 cm), collected at sea ( $n = 8$ ) or in the Thau and Salses-Leucate lagoons

( $n = 3$ ) between September 2007 and April 2008, plus a fossil otolith dated from ~475 yr BC (G. Piquès pers. comm.). The latter otolith originated from the archaeological site of Lattara (43° 34' N, 3° 54' E; Fig. 1), an ancient Roman implantation located near the Palavas lagoon network (Chabal et al. 2008). Shape analysis allowed unquestionable species identification for this otolith (Lombarte et al. 2006) and, although it was slightly more opaque than the present-day otoliths, no trace of alteration or burning was observed on its surface. Therefore, it was processed similarly to the other otoliths.

### Otolith preparation and analysis

All the equipment used for otolith extraction and handling was decontaminated with 10% ultra-pure nitric acid, rinsed with ultra-pure water, and left to dry in a Class 100 laminar flow hood, in order to avoid otolith contamination during manipulation (Fowler et al. 1995, Campana et al. 2000). For all fish, sagittal otoliths were extracted using plastic forceps, washed in ultra-pure water, and brushed with a plastic toothbrush to remove all adhering organic tissues. They were then sonicated for 5 min in ultra-pure water and allowed to dry under a Class 100 laminar flow hood before being photographed and weighed. Because otolith photographing and weighing took place under normal laboratory conditions, this step was followed by otolith decontamination in ultra-pure water using ultrasonics. Otoliths were then dried under the same laminar flow hood and stored in 1.5 ml plastic Eppendorf tubes until further processing.

Only right sagittae were prepared for chemical analyses. They were embedded separately in epoxy resin (Araldite 2020) and cut in a transverse plane to expose the core. Individual otolith sections were then polished using diamond-coated polishing paper (ESCIL) and glued on glass slides using a drop of epoxy resin. A last sonication was carried out for surface decontamination before storage in dust-free conditions until analysis.

Otolith signatures were assessed using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS), which allows accurate measurements of element concentrations to the level of parts per billion directly on solid samples (Panfili et al. 2002). All analyses were conducted at the University of Montpellier 2 (UMR 5243 Geosciences), using a 193 nm Excimer Laser System (CompEx 102, Lambda-Physik) coupled to an Element XR sector field

Table 2. *Sparus aurata*. Datasets of multi-annual juvenile (J) and adult (A) otolith fingerprints used for discrimination of coastal habitats in the Gulf of Lions. For each fish of the training dataset, the number of spots wrongly reassigned when using the combination of elements allowing best habitat discrimination gives an estimate of classification error with random forest. TL: total length. Code for each fish includes sampling site (for lettering see Fig. 1 legend)

Fish code (stage)	Capture date (dd/mm/yyyy)	TL (mm)	No. of spots	No. of spots wrongly reassigned
<b>Training dataset</b>				
T01 (A)	09/09/2007	385	1	0
T02 (A)	19/09/2006	347	1	0
T03 (J)	23/10/2008	190	17	0
T04 (J)	23/10/2008	170	17	0
T05 (J)	23/10/2008	191	18	1
M01 (J)	06/10/2008	198	21	0
M02 (J)	06/10/2008	172	19	2
M03 (J)	06/10/2008	189	20	1
M04 (J)	06/10/2008	160	18	1
M05 (J)	06/10/2008	192	19	0
M06 (J)	06/10/2008	118	16	6
M07 (J)	06/10/2008	145	18	3
M08 (J)	06/10/2008	144	17	8
M09 (J)	03/10/2008	192	21	3
M010 (J)	03/10/2008	201	22	3
M011 (J)	03/10/2008	185	24	1
M012 (J)	03/10/2008	201	24	3
M013 (J)	01/10/2008	198	19	0
M014 (J)	01/10/2008	165	17	7
M015 (J)	09/10/2007	172	24	0
M016 (J)	09/10/2007	159	21	1
M017 (J)	09/10/2007	139	18	2
M018 (J)	09/10/2007	175	23	2
M019 (J)	09/10/2007	184	23	2
M020 (J)	29/10/2006	195	23	1
M021 (J)	29/10/2006	200	23	2
SL01 (A)	10/10/2007	356	1	0
SL02 (A)	10/10/2007	338	1	0
SL03 (A)	13/10/2008	335	1	0
SL04 (J)	13/10/2006	146	20	1
S01 (A)	10/04/2008	366	1	0
S02 (A)	06/02/2008	287	1	0
S03 (A)	06/02/2008	290	1	0
S04 (A)	10/04/2008	376	1	0
S05 (A)	06/02/2008	263	1	0
S06 (A)	06/02/2008	273	1	0
S07 (A)	10/04/2008	390	1	0
S08 (A)	10/04/2008	388	1	0
A01 (A)	13/12/2007	308	13	0
A02 (A)	13/12/2007	324	14	1
A03 (A)	13/12/2007	304	12	0
<b>Test dataset</b>				
T01 (A)	09/09/2007	385	1	0
SL01 (A)	10/10/2007	356	1	0
SL02 (A)	10/10/2007	338	1	0
S01 (A)	10/04/2008	366	1	0
S02 (A)	06/02/2008	287	1	0
S03 (A)	06/02/2008	290	1	0
S04 (A)	10/04/2008	376	1	0
S05 (A)	06/02/2008	263	1	0
S06 (A)	06/02/2008	273	1	0
S07 (A)	10/04/2008	390	1	0
S08 (A)	10/04/2008	388	1	0
L01	475 yr BC	–	–	–

ICPMS (ThermoFisher). To assess temporal variations in otolith fingerprints during fish life, the laser was operated in point-by-point mode on each otolith transverse section, following the dorsal axis of the otolith, from the core to the edge. In order to minimize the differences in temporal resolution across the otolith, laser beam diameter was set at 51  $\mu\text{m}$  for the otolith part corresponding to the first year of life and at 26  $\mu\text{m}$  for that corresponding to older years. According to known ontogenetic variations in otolith growth rate for *Sparus aurata* in the study area (Mercier et al. 2011a), the signature for each individual laser spot thereby corresponded to a period of 1 to 2 wk during juvenile life, and of 1 to 3 mo in adult life, depending on the age and the period of the year. For all otoliths, the laser was operated with a repetition rate of 7 Hz and a constant fluence on target of 15 J  $\text{cm}^{-2}$ . Helium was used as the ablation gas, to enhance sensitivity and reduce particle condensation on the surface (Eggins et al. 1998). An argon gas flow was then admixed to the laser-generated aerosol, prior to introduction into the ICPMS for elemental analysis. The instrument was operated in low mass resolution and calcium (Ca) was used as an internal standard using a stoichiometric value of 56 % CaO. A standard reference glass material (NIST 612) was used to calibrate analyses and to control for instrumental drift. Apart from Ca, 14 elements were measured:  $^7\text{Li}$ ,  $^{11}\text{B}$ ,  $^{24}\text{Mg}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{65}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{88}\text{Sr}$ ,  $^{89}\text{Y}$ ,  $^{138}\text{Ba}$ ,  $^{139}\text{La}$ ,  $^{140}\text{Ce}$ ,  $^{141}\text{Pr}$ , and  $^{208}\text{Pb}$ . Calculations of drift and limits of detection (LOD) were made off-line using the software Glitter. Elements for which 25 % of the measures were below LOD were removed from further analysis. Remaining elements were:  $^{11}\text{B}$ ,  $^{24}\text{Mg}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{65}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{88}\text{Sr}$ ,  $^{89}\text{Y}$ ,  $^{138}\text{Ba}$ , and  $^{208}\text{Pb}$ . Data were expressed as ratios to Ca, and element concentrations below LOD were set to zero.

### Data analysis

Habitat discrimination based on otolith fingerprints from LA-ICPMS measurements was achieved using a random forest (RF) algorithm (Breiman 2001), recently shown to be highly accurate for *Sparus aurata* habitat differentiation in the study area (Mercier et al. 2011b). Although the method is still new to ecologists, it has been widely used in bioinformatics (e.g. Cutler & Stevens 2006), and has proved to be superior to most methods commonly used for classification (Cutler et al. 2007). As its name suggests, RF combines many classification trees (5000 in our case, i.e. a forest) to produce more accurate classifications. It

proceeds in 2 steps. First, a RF classifier is built and tested using a training dataset of known origin (here: habitat). Then, the RF classifier is used to predict origin in the tested dataset.

Classification trees follow mathematical rules to recursively split the dataset into binary groups. Group splitting at each node is done by randomly searching a subset of predictors (here: chemical elements) that maximizes homogeneity within each of the 2 groups defined by each node. Each new group is then split again in a recursive manner, and the recursive process stops when further splitting results in no additional gain in homogeneity. Following this procedure, 5000 trees were built, each from a different random subset of the training dataset using bootstrap re-sampling with replacement of individuals. The individuals not used for building a specific tree (i.e. the 'out of bag' individuals) were then used to measure the prediction ability of that tree. Thus, 2 levels of randomization occur in the building of the RF classifier through (1) the initial selection of individuals (here: otolith spots) for the building of each tree, and (2) the selection of predictor variables (here: chemical elements) used for group splitting at each node. The RF classifier is used to predict origin in the tested dataset by running each of the individuals (here: otolith spots) down the 5000 trees of the classifier (i.e. the forest) and using 'majority rules' to reach a consensus in origin prediction between all trees to generate a final prediction for each individual.

In the present work, the RF classifier was trained with the signatures in B, Mg, Cr, Mn, Cu, Zn, Sr, Y, Ba, and Pb from 554 laser spots (training dataset), all with positions on the otoliths corresponding to periods of certain residency at sea or in 1 of the 3 lagoon habitats investigated (Table 2). For aquaculture fish, only the spots corresponding to the second year of life were used to assess the variability in otolith signature for the marine habitat, since these fish arrived in the farm at the end of their first year of life. For wild adults, only the last spot near the otolith edge was used, since it was the sole spot to have unquestionably recorded the signature of the habitat where the fish was caught (i.e. one of the 3 lagoons or the sea). Lagoon entrance is thought to occur around metamorphosis (Lasserre 1974) that leaves a check mark on the otolith at a distance of 300 to 400  $\mu\text{m}$  from the core (Mercier 2010). For the 25 juveniles collected in the lagoons, only the spots at  $>500$   $\mu\text{m}$  from the core were therefore assigned to the lagoon where the fish was captured. Because *Sparus aurata* adults are rarely observed in shallow brackish lagoons (Quignard et al. 1983), spawning was assumed to have occurred at sea for the

21 juveniles caught in the Mauguio lagoon and the very central spots on their otoliths, corresponding to early larval life (i.e. at  $<250$   $\mu\text{m}$  from the core), were assigned to the marine habitat.

Among the chemical elements measurable in the otoliths, some can be less informative than others, and bring more noise than signal in habitat classification (Mercier et al. 2011b). In order to identify the optimal RF classifier for reconstruction of fish movement from otolith signatures, i.e. the element combination allowing the best accuracy in discrimination to be reached between all habitats tested, the following cross-validation procedure (Mercier et al. 2011b) was applied to all possible combinations of the 10 elements measured (B, Mg, Cr, Mn, Cu, Zn, Sr, Y, Ba, and Pb). To avoid circular reasoning when the same data are used to both build and test a classifier, 75% of the spots from the training dataset were randomly selected and only their signatures were used to build the RF classifiers. The signatures from the remaining spots (25%) were then used to test the prediction ability (overall accuracy) of all classifiers, by measuring the proportion of spots correctly assigned to their known origin. Overall accuracies in site discrimination were then compared between all classifiers and only that allowing reaching the maximum accuracy was retained for further analyses.

Before using this optimal RF classifier for lifetime movement reconstruction in the adults from the test dataset, its reliability for each of the 4 habitats studied was assessed, by calculating true skill statistics (TSS) (Allouche et al. 2006). Compared to accuracy, which is the proportion of correct site prediction, TSS also accounts for true negative prediction, i.e. fish for which absence from a given site was correctly predicted by the model (Allouche et al. 2006). It has a range of 0 to 1, with 1 representing 100% correct prediction of presence or absence in a given habitat. Classification error using the optimal RF classifier was also assessed, using a leave-one-fish-out validation procedure in which all the spots from a given fish in the training dataset are removed once, and the spots from all the remaining fish are used to predict successive habitats for this fish. Finally, the contribution of each chemical element to the optimal classifier was assessed by calculating the decrease in global Gini index (GI) before and after the element's removal from the classifier (Breiman 2001). GI calculation over all the trees of the RF provides a global measure of the 'goodness' of a subset of explanatory variables (here: otolith's elemental concentrations) for classification (Breiman 2001). GI ranges from 0 (when all elements equally contribute to the total dis-



crimination) to 100 (when a single element contributes, alone, to 100% of the total discrimination). Therefore, the more an element contributes to the discrimination, the higher the decrease in GI when it is removed from the RF classifier.

Once validated, the optimal RF classifier was used to predict present and past lifetime movements in *Sparus aurata* from the chemical signatures of 545 spots analyzed along the dorsal radius of the 11 present-day adult otoliths, and the fossil one (Table 2). Calendar date for each spot was estimated following the patterns in otolith growth previously described for juvenile and adult *S. aurata* in the Gulf of Lions (Mercier et al. 2011a).

All statistical analyses were carried out using R software (R Development Core Team, 2008 version). More specifically, the packages FactoMineR, class, gtools, klaR, MASS, mda, and randomForest were used. The R scripts created for element selection (Mercier et al. 2011b) are available at [www.ecosym.univ-montp2.fr](http://www.ecosym.univ-montp2.fr).

## RESULTS

### Habitat discrimination accuracy

Optimal discrimination between the 4 habitats (i.e. the 3 lagoons and the sea) was obtained with only 6 out of the 10 chemical elements tested (Mg, Cr, Mn, Cu, Sr, and Ba), with the other elements bringing more noise than signal to the discrimination. Discrimination accuracy with this optimal combination was 92%, with high levels of correct re-assignment (84 to 96%) and high TSS (>0.81) for all habitats (Table 3). Habitat misclassification was shown to be rare for the aquaculture fish and the adults, and uncommon for most lagoon-caught juveniles (Table 2). Nevertheless, 3 individuals from the Mauguio lagoon (M4, M17, and M19) showed notable error rates, with 37 to 47% of misclassified spot per individual. These spots, located right after the metamorphosis checkmark on the otolith, were all assigned to the marine habitat.

Among the 6 chemical elements retained in the optimal RF classifier, none permitted, alone, full discrimination among the 4 potential habitats or, at least, between marine and lagoon environments (Fig. 2). Contributions to habitat discrimination varied greatly between elements. Losses in global GI were 80, 77, 40, 39, 36, and 30 after the removal of Sr, Ba, Cr, Mg, Mn, and Cu, respectively, indicating that Sr and Ba were the 2 main contributors to the RF classifier. Inter-habitat differences in otolith concentrations

Table 3. *Sparus aurata*. Reliability of the optimal random forest classifier obtained for habitat identification in the Gulf of Lions. For each of the 3 lagoon habitats tested (Salses-Leucate, Thau, and Mauguio) and the marine habitat (Sea), overall prediction accuracy and true skill statistics (TSS) indicate the reliability of the classifier in predicting fish presence or absence

Habitat	Accuracy (%)	TSS
Salses-Leucate	88	0.87
Thau	84	0.83
Mauguio	96	0.85
Sea	87	0.81

were apparent for these 2 elements and suggested that their sole combination could allow full discrimination between the 4 habitats (Fig. 2). Hence, otolith Sr:Ca ratios (mean  $\pm$  SD) were significantly higher in the Salses-Leucate lagoon ( $6.6 \pm 0.5 \text{ mg g}^{-1}$ ), and lower in the Mauguio lagoon ( $3.8 \pm 0.6 \text{ mg g}^{-1}$ ), than in both the marine habitat ( $4.7 \pm 1.0 \text{ mg g}^{-1}$ ) and Thau lagoon ( $4.8 \pm 0.7 \text{ mg g}^{-1}$ ), while otolith Ba:Ca ratios in Thau ( $11.9 \pm 6.8 \text{ } \mu\text{g g}^{-1}$ ) and Mauguio ( $11.6 \pm 7.2 \text{ } \mu\text{g g}^{-1}$ ) were significantly higher than in Salses-Leucate ( $7.7 \pm 0.6 \text{ } \mu\text{g g}^{-1}$ ) and the sea ( $6.9 \pm 4.5 \text{ } \mu\text{g g}^{-1}$ ). Despite these inter-habitat differences, however, overall discrimination accuracy with these 2 elements was only 65%, mainly because intra-site variability in their concentrations resulted in overlapping values between habitats (Fig. 2).

Habitat assignments based on multi-elemental signatures further confirmed the limitations of the use of Sr and Ba alone for habitat identification, by revealing non-negligible discrepancies between expected and measured Sr:Ca and Ba:Ca ratios for several fish (Fig. 3). Assignment of some adults to the marine habitat occurred despite very low levels of Sr:Ca (e.g. in the first 2 mo of life for S04 and S08) or very high levels of Ba:Ca (e.g. in the first 2 mo of life for S01, S06, and SL01). Conversely, some major Sr peaks and low levels of Ba:CA were associated with localization in the Thau and Mauguio lagoons (e.g. the beginning of the second year for S01).

### Present-day lifetime migrations

Visual observation of otolith sections for the 11 fish used to assess lifetime migrations in *Sparus aurata* indicated individual ages between 2 (n = 4, TL: 26.3 to 29.0 cm) and 4 yr (n = 2, TL: 37.6 to 38.5 cm). The width of the opaque central zone varied from

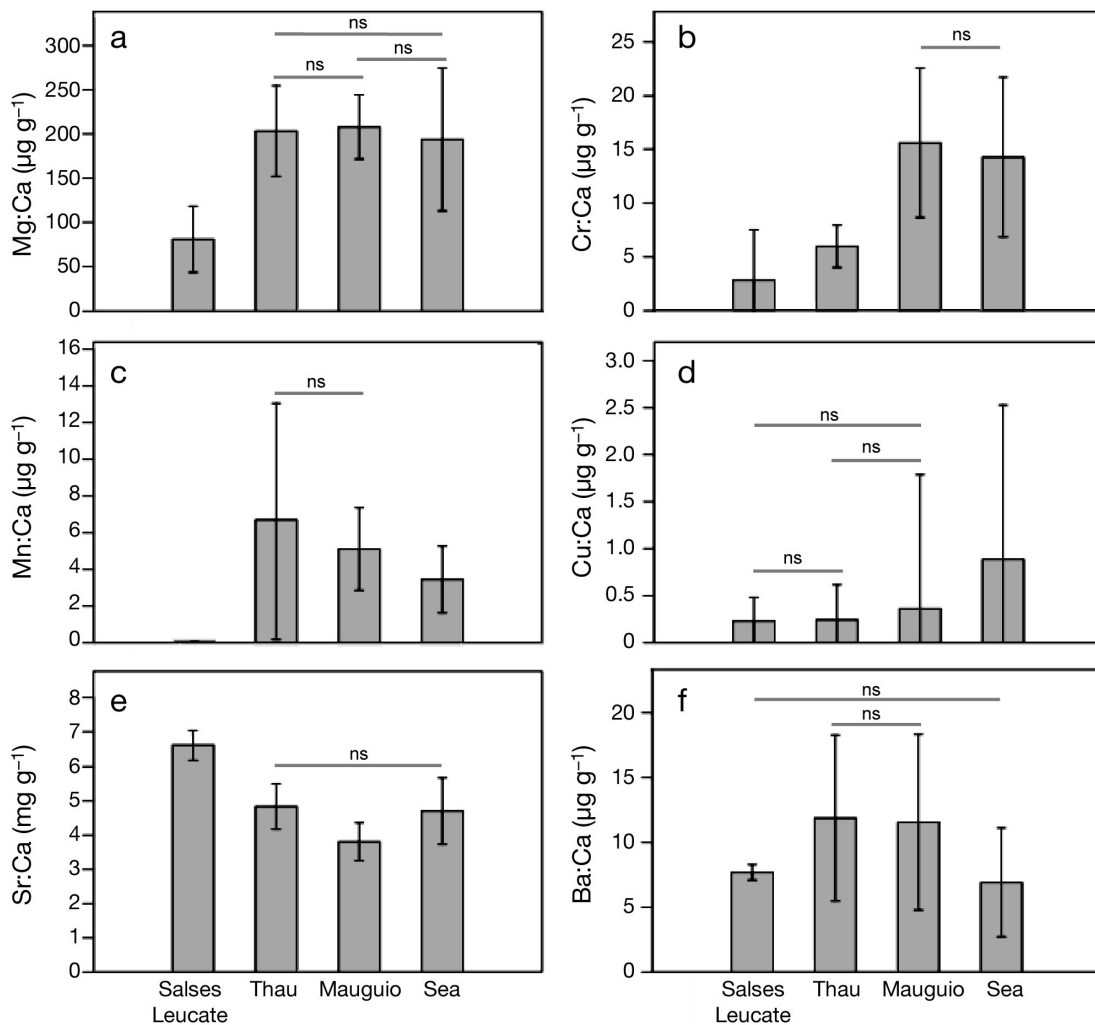


Fig. 2. *Sparus aurata* otolith mean values ( $\pm$  SD) of (a) Mg:Ca, (b) Cr:Ca, (c) Mn:Ca, (d) Cu:Ca, (e) Sr:Ca, and (f) Ba:Ca ratios observed in the 3 lagoons tested (Mauguio, Thau, and Salses-Leucate) and in the marine habitat (Sea). Horizontal bars indicate non-significant (ns) differences in otolith signature between habitats (Mann-Whitney test,  $p \geq 0.05$ )

320 to 440  $\mu\text{m}$  in these fish (Fig. 3), suggesting non-negligible inter-individual differences in birth date and larval growth rate. Variations in multi-elemental fingerprints across otolith sections indicated important inter-individual differences in habitat use over fish life, although the most central zone of the otoliths (distance  $< 300 \mu\text{m}$  from the core) was always assigned to the marine habitat. Habitat assignment with multi-element signatures suggested that all individuals entered the lagoons at least once in their life, but the timing and duration of lagoon use varied greatly among fish.

A lagoon chemistry signal was detected within the otolith growth band corresponding to the first year of life in all fish except for 2 individuals captured in the Salses-Leucate lagoon (SL01 and SL02; Fig. 3). When

present at this life stage, first assignment to a lagoon habitat was detected at distances between 220 and 560  $\mu\text{m}$  from the otolith core (Fig. 4a), before the metamorphosis checkmark in certain fish (T01, S01, S03, and S06; Fig. 3). Given the large inter-individual variation in otolith growth during the first year (Fig. 4b), this indicated a likely arrival in the lagoons between March and May. Subsequent habitat assignments suggested that all the fish studied had returned, or remained (for SL01 and SL02), at sea for their first winter of life (Fig. 3). The last identification of lagoon signatures in the first year of life occurred at distances between 740 and 1690  $\mu\text{m}$  from the otolith core, suggesting a departure from the lagoons between June (S06 and S08) and November (S01 and S02), with a peak in September (T01, S03, S04, S05,

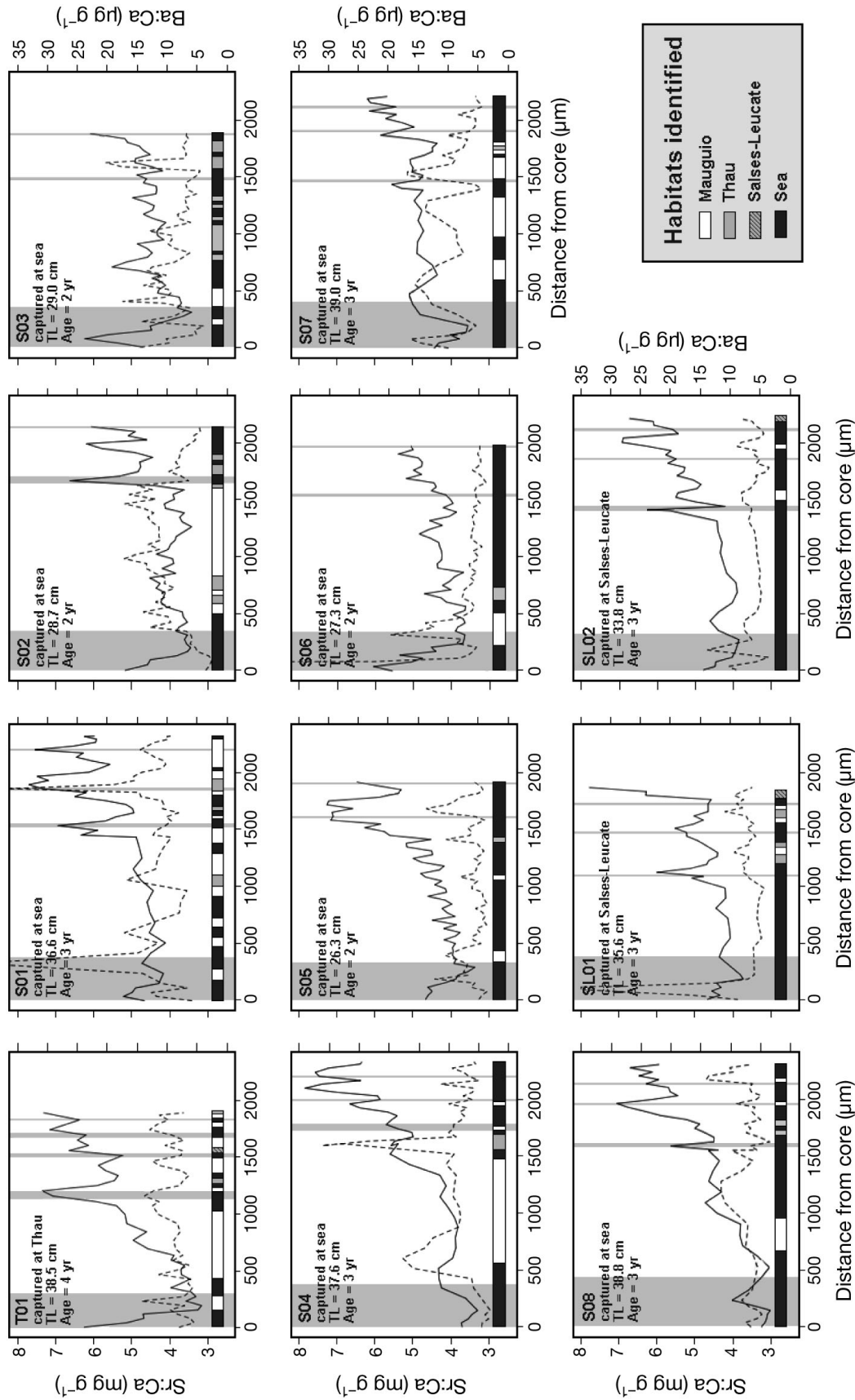


Fig. 3. *Sparus aurata*. Sr:Ca (solid line) and Ba:Ca (dotted line) ratios measured across the otoliths of the present-day adults tested, and corresponding habitat assignment using the multi-element random forest classifier (horizontal bar). For each fish, identification code, place of capture, total length (TL), and age are indicated on the graph. Vertical grey bars represent the position, along the sampled transect, of opaque zones (larval central zone and successive annuli). Because annuli are deposited in the winter irrespective of age in local *S. aurata*, the distance between them corresponds to 1 yr of fish life

and S07) (Fig. 4). In the juveniles, the period for presumed lagoon use therefore lasted from 1 (S08) to 7 (S02) mo depending on the individual, with an average of 4 mo. No assignment to Salses-Leucate was found, with most of the spots (50%) being assigned to Mauguio and only a few (10%) to Thau. Transitional assignments of otolith signatures to the marine habitat during this period suggested that certain fish had experienced marine conditions for several weeks or months, but these were not necessarily followed by a change in lagoon habitat assignment (Fig. 4).

Otolith signatures for adult life were assigned at least once to a lagoon habitat in most of the fish tested (Fig. 3), including those which apparently had stayed at sea as juveniles (SL01 and SL02). Only individuals S05 and S06, both captured at sea, were assigned to the marine habitat at this stage. Putative lagoon use occurred at least up to the age of 4 yr, with most assignments to Mauguio and Thau, irrespective of age. It apparently lasted from 1 (in SL02) to 11 mo (in S01) per year, with an average of 5 mo. A Salses-Leucate signal was only identified once in T01 (during the third year of life, and in SL01 and SL02 (right before their capture, at age 4 yr). Changes in

chemical composition suggested that most adults returned to sea during winter (Fig. 3), except individual S08 (for which the second winter of life was associated with Mauguio) and S01 (for which the second and third winters of life were associated with Thau and Mauguio, respectively).

### Fossil otolith

Internal structure in the fossil otolith was similar to that from present-day *Sparus aurata*. The width of the central opaque zone was 500  $\mu\text{m}$ , and 1 opaque ring was visible at 1470  $\mu\text{m}$  from the core and 610  $\mu\text{m}$  from the edge. Assuming that otolith growth patterns had not changed radically over time in the study area, this suggested a probable death by capture in the second half of the second year of life (i.e. at age 1 yr) for this ancient fish.

The chemical composition of this fossil otolith differed slightly from that of present-day ones, with up to 1.6 times higher Ba:Ca and Mn:Ca ratios (Fig. 5a). However, this was true for only a few of the spots along the otolith radius, and the ranges for the ratios

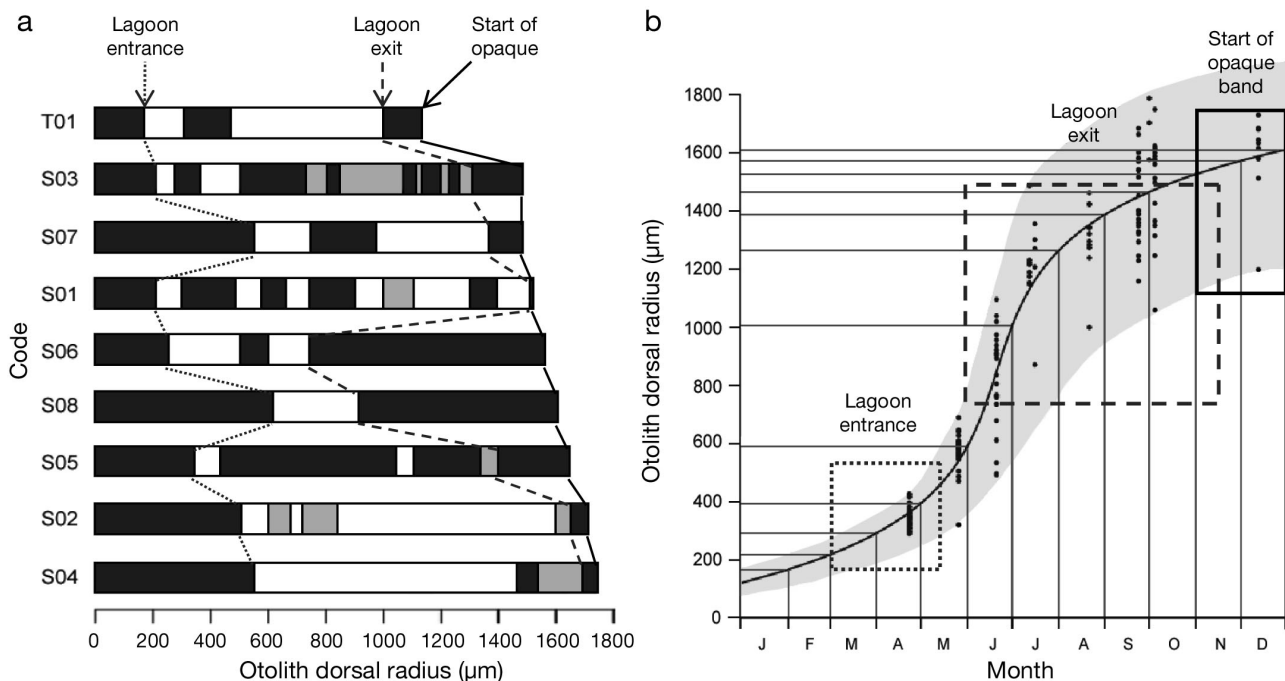


Fig. 4. *Sparus aurata*. Variability in lagoon use during the first year of life: (a) habitat assignment within the otolith band corresponding to the first year of life for the 8 fish entering the lagoons as G0 = group 0 = juveniles of age < 1 yr, (b) calendar for otolith growth in G0 juveniles from the Gulf of Lions (adapted from Mercier et al. 2011a). For shades in (a), see the key in Fig. 3 for the 4 habitats tested (Salses-Leucate, Thau, Mauguio, and Sea). Dotted lines indicate the likely start and end of lagoon residency for each fish. In (b), black dots for each month and the solid line represent otolith dorsal radius lengths observed for G0 juveniles in the study area, and their fit with a Tanaka growth model allowing optimal estimation of average otolith growth during the first year of life (Mercier et al. 2011a). Grey area shows the evolution of otolith radius range throughout the year. The timings indicated on the graph for lagoon entrance, lagoon exit, and start of opaque band deposit are derived from (a)

Mg:Ca, Cr:Ca, Cu:Ca, and Sr:Ca were very similar to those observed in present-day otoliths. As a result, application of the contemporary RF classifier to infer possible movements for this ancient fish produced migration patterns (Fig. 5b) that were consistent with those of modern *Sparus aurata* (Fig. 3). Indeed, only the spots from the very central opaque zone and around the first (and only) opaque ring of this otolith were assigned to the marine habitat, with the rest of the spots being assigned to either Mauguio or Thau lagoons.

## DISCUSSION

### Methodological limitations and usefulness of multi-elemental signatures

Although the chemical signatures obtained from the otoliths of various species indicate that multi-elemental fingerprints are needed to confidently discriminate among coastal fish habitats (Leakey et al. 2009, Mercier et al. 2011b), reconstruction of lifetime migrations from otolith signatures in euryhaline fish has, so far, largely been based on concentrations of only 1 or 2 elements (e.g. Bath et al. 2000, Volk et al. 2000, Elsdon & Gillanders 2005, 2006, Hamer et al. 2006, Jessop et al. 2006, Walther &

Thorrold 2006, Morais et al. 2011). In general, the Sr:Ca signature was used alone as a proxy for salinity (Babaluk et al. 1997, Zlokovitz et al. 2003, Lamson et al. 2006), with Ba:Ca being added when intra-site salinity variations confounded the Sr:Ca signal (Bath et al. 2000, Elsdon & Gillanders 2005, Walther & Thorrold 2006). The present study clearly shows that the use of these 2 signatures alone is not adequate for accurate reconstruction of fish movements in the coastal zone. That is, although Sr and Ba were the main contributors to the optimal RF classifier, neither of them permitted, alone, to discriminate among the 4 habitats accurately or, even, simply between the marine habitat and the lagoons. Similarly, although spatial differences in mean otolith Sr:Ca and Ba:Ca suggested that their combination should allow full discrimination among the 4 habitats, site discrimination by these 2 elements was incomplete. It might even have led to major errors in habitat assignment. This underscores the need for adequate estimation of temporal variations of elemental signatures when building habitat classifiers to track fish by their otolith fingerprints (Gillanders 2005), especially in coastal areas like the Gulf of Lions where water temperature, salinity, and trace-metal bioavailability are particularly variable (Accornero et al. 2008, IFREMER 2008).

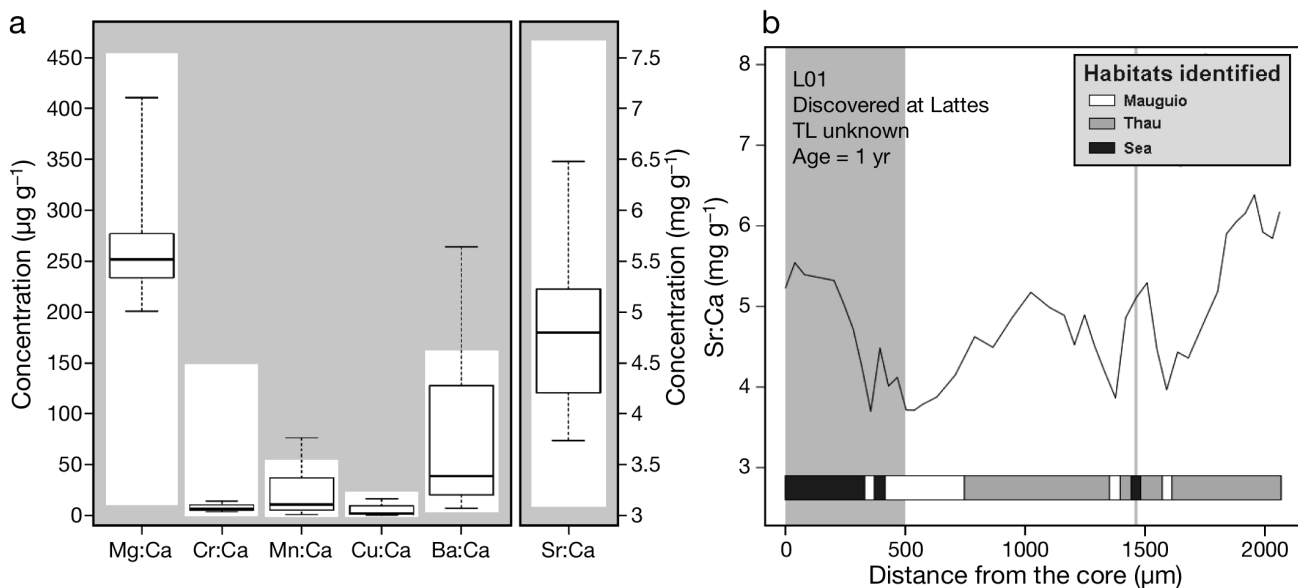


Fig. 5. *Sparus aurata*. For the fossil otolith: (a) chemical signatures measured along the dorsal radius and (b) habitat assignment predicted from the multi-element random forest classifier developed from present-day fish. In (a), the box for each ratio contains the middle 50% of the data, with the thick horizontal line indicating the median; box whiskers (dotted lines) indicate the minimum and maximum values measured; for comparison, the range of values observed in the present-day otoliths is indicated as a vertical white bar for each element; note the separate scale for the Sr:Ca ratio. In (b), vertical grey bars indicate positions for opaque zones on the otolith radius; solid line shows the Sr:Ca ratios measured along the radius; horizontal bar gives the corresponding habitat assignment when using all 6 elements. TL: total length

In our case, adding Mg, Cr, Mn, and Cu to Sr and Ba in the habitat classifier greatly increased discrimination accuracy (from 65% to 92%), due to inter-habitat differences in otolith concentrations for these elements (Fig. 2). The value of Mg, Mn, and Cu for identification of fish habitat by otolith fingerprints has already been acknowledged (e.g. Swan et al. 2006, Clarke et al. 2009, Tanner et al. 2011), although the exact roles played by physiological effects, bioavailability, and water temperature in defining their final otolith concentrations remain unclear (Tanner et al. 2011). In our case, the spatial differences in otolith concentrations observed largely reflect differences in physicochemical conditions and element bioavailability between the 4 habitats, under the combined influence of Rhône River inputs to the marine coastal zone and the Thau and Mauguio lagoons (Accornero et al. 2008, Ollivier et al. 2011), minor localized pollutions by Cu and Cr in the Salses-Leucate lagoon (IFREMER 2009), and differences in water temperature and salinity between the lagoons and the sea (Cacho et al. 1999, IFREMER 2008). Nonetheless, inter-habitat differences in fish physiology might also have contributed to the differences in otolith signatures obtained, because the proportion of juvenile and adult signatures in the training dataset differed between habitats, and inter-habitat differences in juvenile *Sparus aurata* growth have been reported in the Gulf of Lions (Audouin 1962, Mosconi & Chauvet 1990).

The high overall accuracy in habitat discrimination (92%) and the >84% re-assignment rates by habitat obtained with the optimal RF classifier reveal its reliability for inference of *Sparus aurata* lifetime migrations between the coastal zone and the 3 lagoons investigated. This efficiency is partly explained by the differences in elements' bioavailability that exist among these habitats, but is also due in part to the ability of RF to extract signals from very complex data (Prasad et al. 2006). The accuracy of the technique for discriminating among *S. aurata* lagoon fingerprints in the Gulf of Lions has already been demonstrated (Mercier et al. 2011b). Therefore, the main potential methodological limitation in the present study was the otolith signatures dataset used to train the classifier. Although the latter included otolith signatures for multiple months, years, and life stages for all habitats, the chemical signatures gathered did not necessarily encompass all the actual variation within each habitat. Because of the limited number of individuals investigated in certain lagoons (especially Salses-Leucate and Thau), our classifier may have indicated inter-site migrations (assignment to different habitats)

when, in fact, intra-site variations in otolith signatures were involved. Inter-annual differences in otolith fingerprints, which may be as significant within some coastal systems as between systems (Tanner et al. 2011), may have amplified this phenomenon if the signatures for the years not sampled differed from those included in the present work.

Another potential limitation of our classifier was the differences in age among the fish from each lagoon, because several elements can vary in otoliths as a function of life stage (Chittaro et al. 2006). Variation in otolith signature depends on the number of individuals, sites, and seasons analyzed in each habitat. Therefore, the narrower range in otolith signatures obtained for the Salses-Leucate lagoon (Fig. 2) might result from a poorer coverage of the prevailing spatio-temporal and individual variation in that lagoon. Such a bias in the RF classifier could partly explain why otolith signatures for Salses-Leucate were rarely identified in the adult otoliths, contrary to those of Mauguio (for which the number of fish, seasons, and sites analyzed was much higher). However, the high discrimination accuracy and TSS indicate reliable habitat identification in the dataset, even for Salses-Leucate. Errors in habitat assignment were rare and suggested that only some assignments to the marine habitat may be spurious. These were observed only in 3 juveniles, all captured in Mauguio, near the mouth of the lagoon, where salinity may exceed 35 for several weeks at a time (IFREMER 2008) and the organic matter in the water and the sediment is mainly of marine origin (Vizzini et al. 2005). If spot assignments to the marine habitat can correspond to residency near lagoon entrances, they must be considered with caution when reconstructing lifetime migrations. However, the rarity of this error in the training dataset indicates that residing near lagoon mouths is not common in *Sparus aurata*, so it should not greatly confound inferences of migration patterns.

The choice of only 3 sampling sites among the many coastal lagoons colonized by *Sparus aurata* in the area might have generated inconsistencies in our habitat predictions by forcing the classifier to assign otolith signatures from all habitats to 1 of the 4 tested. Because the otolith signature for the marine habitat was robust and the 3 lagoons sampled are those most heavily used by juvenile and adult *S. aurata* in the Gulf of Lions (Lasserre 1976), this should not have a profound effect on our conclusions. Yet, in this eventuality, according to similarities in environmental characteristics among lagoons in the Gulf of Lions (Table 1), false assignment to Salses-Leucate would more likely correspond to a residency in the La

Palme or the Bages-Sigean lagoons, false assignment to Thau to a presence in the western part of the Palavas lagoon network, and false assignment to Mauguio to a residency in the eastern part of the Palavas network or in one of the Petite Camargue lagoons.

### Migratory life history in *Sparus aurata*

The movements inferred from otolith chemistry of the 11 adults revealed wide inter-individual variation in lifetime migration and habitat use. Although a larger sample size is needed to validate information on key habitats for the species in the Gulf of Lions, this result demonstrates that a diversity of migratory strategies are used to complete the species' life cycle in the area.

Inferred migration patterns were consistent with previous knowledge about the reproductive habits and larval ecology of *Sparus aurata* in the area. Spawning of *S. aurata* in the area occurs at sea from November to February, probably off the delta of the Rhône River (Lasserre 1976), and a massive entrance of juveniles is observed in all lagoons around April (Audouin 1962). This generally matches our results, indicating a marine habitat at birth and a first lagoon entrance between March and April. Nonetheless, the high inter-individual variability in the opaque central zone width indicates differences in growth rate and duration of the larval stage. The unexpected assignment of some otolith spots prior to the metamorphosis check to Mauguio in certain fish even suggests an early entrance, as larvae, in lagoons with approximately iso-osmotic waters (i.e. with salinities around 15), such as Mauguio (IFREMER 2008). Because of the limited osmoregulatory ability of *S. aurata* during its first months of life (Bodinier et al. 2010), this might allow the larvae to allocate more energy to growth (Klaoudatos & Conides 1996), by lowering energetic costs for osmoregulation (Bodinier et al. 2009). Rapid growth at this stage increases chances to avoid predation. It might contribute to the bigger sizes-at-age observed for *S. aurata* juveniles in several brackish lagoons of the area (Audouin 1962, Mosconi & Chauvet 1990).

Habitat assignment for the first year of life in the adults tested confirmed the conclusions from previous investigations of local *Sparus aurata* stocks (Arias 1976) and suggested that lagoon colonization by *S. aurata* juveniles still prevails in the area. Yet, 2 individuals apparently remained at sea as juveniles. Since these were the only captured in the Salses-Leucate lagoon, i.e. in a part of the Gulf of Lions where

there are fewer lagoons along the coast (Fig. 1), *S. aurata* larvae may settle at sea only when they fail to find a suitable lagoon habitat. This would explain why *S. aurata* juveniles have been rarely reported at sea in the Gulf of Lions (Mosconi & Chauvet 1990, Francour 1997). However, given the methodological limits of the classifier in discriminating between the sea and some marine-influenced parts of Mauguio, it is also possible that these 2 individuals resided near the entrance of this lagoon. The same bias in the classifier might explain assignment to the sea for the first summer of life in the some other individuals. Therefore, most of the fish tested probably entered Mauguio (or a very similar lagoon) during their first spring of life and remained there until the next autumn, which would be in accordance with the known life cycle of the species in the area (Audouin 1962, Arias 1976), and in other parts of the Mediterranean (Mariani 2006).

Migratory and sedentary behaviors have both been reported for *Sparus aurata* juveniles in their coastal habitats (Sánchez-Lamadrid 2002, Abecasis & Erzini 2008). In our case, assignment during the first year of life suggested that most individuals changed habitat during this period. However, this may partly be an artefact linked to the limitations in the classifier. For example, since the only way to migrate between Mauguio and Thau for *S. aurata* is by the sea (Fig. 1), alternate assignments to Thau and Mauguio without transitional assignment to the marine habitat are unlikely to correspond to an actual migration between the 2 lagoons. It is more plausible that it reflects life in lagoons with environmental conditions intermediate between those of Mauguio and Thau, as observed in the Palavas lagoon network (IFREMER 2008). Therefore, most of the fish tested probably remained within the same lagoon during their first summer of life, the apparent change in habitat found by the classifier resulting from the combined effects of high environmental variability in certain lagoons (IFREMER 2008), and *S. aurata* juveniles' mobility within their nursery grounds. With this regard, our results suggest inter-individual disparities in lagoon habitat use. Hence, while most of the fish tested had remained principally in brackish waters (>75% spot assignment to Mauguio) during their juvenile lagoon life (Fig. 4), others had avoided them (>75% spot assignment to the marine habitat or Thau). These differences in habitat use might reflect individual diversity in osmoregulatory ability, as observed in the European sea bass *Dicentrarchus labrax* (Nebel et al. 2005). However, *S. aurata* may also occupy areas with different salinities from one

lagoon to the other if the benefit from other environmental factors (prey availability, predator density, etc.) compensates the cost for salinity adaptation (Vasconcelos et al. 2010).

The adult migratory behaviors inferred from otolith signatures are in accordance with the great potential for migration of mature *Sparus aurata* (Lasserre 1976, Sánchez-Lamadrid 2002). The observed strategies of habitat use were, however, more diverse than expected from previous studies. Hence, although the captures of adult *S. aurata* in the Gulf of Lions suggested that lagoon entrance was less common for adults than for juveniles (Crespi 2002), most of the fish tested in the present study had spent a considerable amount of time in the lagoons as adults. Only 2 individuals apparently remained exclusively at sea as adults, although, because they both had visited lagoons as juveniles and were killed in their third year, it cannot be concluded that they would not have returned to a lagoon later in life. Lagoon use occurred in the other individuals irrespective of age and season. Contrary to what could be expected from studies in other areas (Abecasis & Erzini 2008), none of the 3 lagoons was the only one that was colonized throughout the fishes' lives, suggesting no lagoon-site fidelity in the Gulf of Lions.

In accordance with previous knowledge on the species' life cycle in the area (Lasserre 1976), most of the fish had spent the winter months at sea, irrespective of age. Because of its poor tolerance of low temperatures, *Sparus aurata* suffers from a 'winter syndrome', with a threshold temperature at 12°C, that causes a cessation of feeding, stress, and lethargy (Gallardo et al. 2003). This syndrome is consistent with *S. aurata* overwintering in the sea (Audouin 1962, Ibarz et al. 2007), as water temperature in the Mediterranean never falls under this critical value, unlike in the lagoons (IFREMER 2008, 2009). Because initial sexual maturity occurs at the end of the second year (Bruslé-Sicard & Fourcault 1997), and the species is known to spawn from November to February in the Gulf of Lions (Lasserre 1976), the prevalence of marine overwintering from year 2 agrees with an offshore location for *S. aurata* spawning in the area (Lasserre 1976). Nevertheless, some winter spots were assigned to lagoons, with potentially important consequences in terms of population dynamics. At present, only the deepest parts of certain lagoons, like Thau or Salses-Leucate, have water temperatures above 10°C in the winter (IFREMER 2008, 2009). However, climate change and water temperature increase in the Mediterranean may disrupt this pattern in the future. Mediterranean Sea tempera-

ture is expected to rise by 3.1°C on average over the next century (Ben Rais Lasram et al. 2010) and some ecological major changes have already been observed (Sabates et al. 2006, Diaz-Almela et al. 2007, Ben Rais Lasram & Mouillot 2009). Because warmer winters may allow increasing numbers of *S. aurata* to overwinter in lagoons, potentially completing their entire life cycle in these habitats, these forecasted environmental changes could have marked impacts on population dynamics, gene flow, and stock structure in the area.

### Lagoons as key habitats

Because the present-day adults investigated had survived the high mortality phase associated with larval and juvenile life (Cushing 1982) and reached the adult stage where they could contribute to the next generation through spawning, their successive habitat choices are those that currently allow successful completion of the *Sparus aurata* life cycle in the area.

Previous investigations of lagoon use by *Sparus aurata* in the Gulf of Lions were carried out some 35 yr ago (Audouin 1962, Lasserre 1974, 1976). Although those studies indicated a major role of lagoons in the successful completion of the species' life cycle, lagoon abiotic and biotic conditions in the area have changed considerably over the intervening years (IFREMER 2009), due to local increases in both climatic and anthropogenic pressures on the littoral zone (Mouillot et al. 2005b). The resulting environmental degradation in certain lagoons, notably Manguio (IFREMER 2009), has caused many authors to question the suitability of lagoons as habitats for juvenile fishes (Quignard et al. 1984, Labourg et al. 1985, Bruslé & Cambrony 1992). Yet, the pronounced lagoon use by all *S. aurata* life stages (including larvae), with unexpectedly similar annual durations of lagoon residency between the juveniles and adults, clearly indicates that lagoons are still key habitats for the maintenance of the species in the area. Nonetheless, although this result needs to be confirmed on a larger sample size, lagoon preferences apparently differ from one stage to the other.

The assignment of most of the fish to Manguio in their first year suggests that shallow and brackish lagoons currently are the main nurseries for *Sparus aurata* in the Gulf of Lions. This is not surprising since the low salinity and the shallowness of these habitats reduce predation risk by the exclusion of many marine predators (Lasserre & Labourg 1974). Moreover, optimal temperatures and salinities for the



growth of *S. aurata* post-larvae are between 20 and 28°C (Requena et al. 1997) and around 28 (Klaoudatos & Conides 1996, Conides et al. 1997), respectively. Because these values are more frequently observed in shallow brackish lagoons like Mauguio (IFREMER 2008, 2009), the juveniles that enter these habitats may optimize their growth during both spring and summer, thereby increasing their chances for survival during the first winter at sea. This had already been hypothesized in the 1970s, when *S. aurata* juveniles were shown to reach bigger sizes in these environments than in the Thau lagoon (Lasserre & Labourg 1974, Lasserre 1976), identified as a major nursery at the time (Lasserre 1976). This differential mortality in the first winter of life could partly explain the observed absence of juveniles from Thau in the adult sample tested here, which is quite surprising since environment quality in Thau is currently among the best in the Gulf of Lions (IFREMER 2008, 2009). Yet, infrequent assignment to Thau at the juvenile stage may also result from the intense fishing effort directed toward *S. aurata* juveniles at the mouth of this lagoon each autumn (Crespi 2002). If this result is validated for a larger number of *S. aurata* individuals, this factor will have to be considered for future management of the local *S. aurata* stock.

At older ages, the fish were assigned to all 3 lagoons, for periods of >3 mo. This is surprising because the capture of adults in Mauguio is rare (Quignard et al. 1983) and it was thought that *Sparus aurata* did not use shallow brackish lagoons after year 1 (Lasserre 1976). Although the preference of adults for deep and marine-influenced lagoons (Audouin 1962, Lasserre 1976) is confirmed by the high number assignments to Thau, assignments to Salses-Leucate were rare during adult life, despite the abundant captures of large *S. aurata* in this lagoon (Crespi 2002). This discrepancy might result from the under-representation of otolith signatures from that lagoon in the training dataset, or precisely from this high capture rate, if the vast majority of adults are harvested. This last hypothesis could explain why no adult residency for periods >3 mo is observed in Salses-Leucate, even for specimens captured in the lagoon. Finally, the low number of assignments of adults to Salses-Leucate might be due to the distance of this lagoon, and all similar ones, to putative *S. aurata* spawning sites in the area (off the Rhône River delta; Lasserre 1976).

Because, in the Gulf of Lions, shallow brackish lagoons seem to be major nursery sites for *Sparus aurata*, but deeper and more saline lagoons like Thau are regularly colonized by adults, even in the winter,

conservation of essential habitats for the species imply the protection of different types of lagoons, and also, the maintenance of a diversity of habitats within each lagoon. These results have implications for the Water Framework Directive (WFD; European Union Directive 2000), which requires all lagoons to achieve a common 'reference state' for water quality in the coming decades. In this respect, urgent attention should be devoted to the Mauguio lagoon, which apparently plays a key nursery role for *S. aurata*, despite its rather poor water quality (IFREMER 2008).

### Historical lagoon use

To date, there have been few attempts to decipher historical fish migration patterns with otoliths from archeological sites (e.g. Carpenter et al. 2003, Zazzo et al. 2006). One of the main limitations is the risk of chemical alteration prior to excavation (Andrus & Crowe 2002). Hence, although diagenetic changes in otolith structure and composition have been shown to be weak, with no chemical element preferentially altered (Dufour et al. 2000), cooking or burning can modify otolith elemental composition (Andrus & Crowe 2002). In our case, visual observation showed no clear alteration of the surface or the internal structure of the fossil otolith, so it is unlikely that it was burnt. However, although the ranges for Sr:Ca, Mg:Ca, Cr:Ca, and Cu:Ca ratios in this otolith were comparable to those of present-day otoliths, high Ba:Ca and Mn:Ca values indicated a potential chemical alteration, probably through cooking since it was found on a Roman-era archeological site. While all elements can be affected by cooking, changes in otolith concentrations depend on the element and the cooking method (Andrus & Crowe 2002). The values for the 6 elements used in habitat classification were compatible with alterations caused by oven roasting (Andrus & Crowe 2002). Despite this, the migratory behavior of this ancient fish was surprisingly similar to present-day *Sparus aurata*.

Another important limit to the inference of lifetime history for ancient otoliths is that otolith growth patterns for a given species might change over time, with the evolution of its physiology and biology, under the forcing of environmental changes. The migratory information obtained for this ancient fish must therefore be considered with caution. Current Mediterranean conditions began around 1300 yr BP in the Gulf of Lions (Leveau et al. 1991), and local climate has not changed much since the Roman era.

Thus, conditions for *Sparus aurata* growth and breeding at that time were quite similar to the present, which is confirmed by the strong resemblance in growth patterns in the fossil and modern otoliths. However, coastal habitats in the Gulf of Lions and their riverine and anthropogenic loads have considerably changed since the Roman era (Chabal et al. 2008, Raynal et al. 2009), with likely modifications in water composition for each environmental type. The establishment of the first lagoon sand barrier near the Lattara archeological site, at 1 km seaward from the present position, has been estimated at around 7500 yr BP, with the actual barrier being dated at 1800 yr BP (Raynal et al. 2009). During the Roman era, the Lattara harbor was located in the Stagnum Latera, a single vast lagoon comprising the current Palavas network and Mauguio (Chabal et al. 2008, Sabatier et al. 2010). Although less isolated from the sea, this vast lagoon was a sheltered brackish environment, connected to the sea by a large permanent channel, and receiving major riverine inputs from the Lez and the Mosson rivers (Raynal et al. 2009). The last major changes in sea surface temperature (SST) and salinity in the Gulf of Lions date from the early to middle Holocene (<4000 yr BP), with SSTs higher and surface salinities lower, by only 2°C and 3, respectively, than the upper Holocene ones (Melki et al. 2009). Therefore, temperature and salinity at sea and in the Stagnum Latera were probably quite similar to those of its modern counterparts. This would explain the similar Sr:Ca profiles in the fossil and modern otoliths. Moreover, since the watershed of the Stagnum Latera corresponded to the current ones for Thau, Mauguio, and the Palavas lagoon network (Chabal et al. 2008), residency in the ancient lagoon should result in assignment to both Thau and Mauguio.

From the growth patterns and habitat assignments observed for the fossil otolith, it can therefore be concluded that the ancient *Sparus aurata* individual it belonged to was fished in the Stagnum Latera at the end of its second year of life. Considering the environmental conditions prevailing in the coastal zone of the Gulf of Lions in the Roman era and their resemblances with those from present-day habitats, the fish probably hatched at sea, entered the Stagnum Latera just before metamorphosis, and spent most of the rest of its life there, returning to sea to overwinter, presumably to avoid the cold water temperatures in the lagoon. Before drawing any further conclusions on the history of *S. aurata* ecology, more archeological otoliths must be found, and elemental alterations estimated. Their analysis may provide important insights into the species' adaptability to environmental

changes. Nonetheless, the data derived from this otolith indicate that lagoon use in the Gulf of Lions probably dates from at least 475 yr BC in *S. aurata*. This raises the issue of climate change: with the erosion of sandy coasts, some lagoons may become embayments in the future (Paskoff 2004), thereby potentially losing their suitability as nurseries for *S. aurata*. The local populations of the species and their dynamics may then be altered.

## CONCLUSIONS

The use of RF allows the extracting of signals from multi-element otolith fingerprints from different years and life stages and building a robust classifier for identification of fish coastal habitats, irrespective of fish age and capture date. Inference of current and historical *Sparus aurata* migrations in the Gulf of Lions with this methodology provided valuable information on the migratory strategies that allow successful stock recruitment in that species. Our results confirm the known migratory behavior of the gilthead sea bream in the area, with colonization of lagoons during the favorable part of the year and a winter migration toward the sea. This pattern in lagoon use is has been established for >2500 yr, which stresses the importance of lagoon habitats for the maintenance of *S. aurata* in the area. However, migration patterns were more diverse than previously described, with heterogeneity in lagoon use at all life stages. Identification of critical lagoon habitats at each stage indicates that shallow lagoons with low salinities and high summer temperatures are of primary importance at the larval and juvenile stages, when most of the mortality occurs. This reveals the need for better management and protection of these fragile coastal habitats, which are facing unprecedented levels of human pressure and global change (Aliaume et al. 2007). Given their importance in the *S. aurata* life cycle, their management is urgently required for the preservation of this marine resource, which is among the most socio-economically valuable in the Mediterranean.

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