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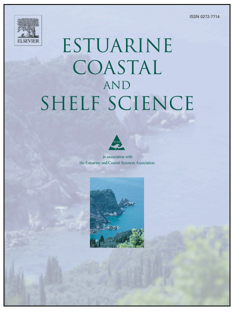

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Environmental migratory patterns and stock identification of *Mugil cephalus* in the Spanish Mediterranean Sea, by means of otolith microchemistry.

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Abstract

The Flathead grey mullet, *Mugil cephalus* is the most globally-distributed Mugilidae species and its migrations and movement patterns have been studied globally but not in-depth in the Mediterranean region. Thus, the present study aimed: (1) to identify migratory patterns throughout the life-history of the *Mugil cephalus* in different Spanish Mediterranean wetlands, and (2) to study the presence of potential fish stocks of the species in the region by means of otolith microchemistry. Specimens (n = 43) were obtained in three wetlands: Parque Natural Delta de l'Ebro (DE), a stratified estuary; Parque Natural de l'Albufera de Valencia (AV), a Mediterranean lake; and Parque Natural Salinas de Santa Pola (SP), a coastal salt marsh. Otolith microchemistry was studied using LA-ICP-MS (chronological variation of Sr:Ca and Ba:Ca ratios). The analysis of lifetime profiles revealed four behavioral patterns: Type I: most frequent use of estuarine environments (estuarine resident); Type II: freshwater behaviour during early life history, moving though estuarine to marine waters at the end of their profile (freshwater migrant); Type III: estuarine water use in early life stages moving then towards sea waters (estuarine migrant); and Type IV: sea/high salinity water habitat use during their entire lifetime (seawater resident). A Canonical Discriminant Analysis, using Sr:Ca and Ba:Ca ratios from core and edge as variables, assigned individuals to the detected patterns with high accuracy (Type I > 95%; Type II and Type III > 83%; and Type IV > 88%). Moreover, two potential fish stocks were identified by the analysis of Sr:Ca otoliths-edge ratios: one in the Valencian Gulf, DE-AV areas presented similar ratios, and the other in the southern location, SP (higher Sr:Ca values). *Mugil cephalus* presented diverse life patterns on the Valencian Community Mediterranean coast. Different strategies could be identified by the used methodology: some particular to an area (Type IV-SP); others shared among areas, changing environments in different stages of their life. The presence of different fish stocks could be influenced by a mesoscale current phenomenon observed in the region associated with the Balearic front.

Keywords

Mugilidae, life-history movements, otolith, microchemical ratios, Mediterranean Sea.

Introduction

The study of migratory history in diadromous fish is very important to understand habitat use and preferences so as to generate proper management regulations for the selected fish areas (Beck et al. 2001; Payne Wynne et al. 2015). Different methods have been used to study movements and habitat selection of fish such as mark-recapture and the analysis of otolith features such as morphometry and chemical composition (Sturrock et al. 2012; Avigliano et al 2014; Clément et al. 2014). The latter methodology has facilitated not only the study of fish movements and migrations but also the stock identification of important commercial species (Gillanders 2005; Tracey et al. 2006; Tabouret et al. 2010; Avigliano et al. 2014, 2015). The use of otolith elemental signatures throughout fish growth serves as a sensitive natural tag and can be used to track the entire life history of fish, reconstructing their environmental migratory patterns and habitat use (Campana et al. 2000; Wang et al. 2010). Elemental deposition in the otolith is influenced by physiological and environmental factors, most particularly by the concentration of elements in the surrounding water (Wang et al. 2010). The chemicals deposited represent a permanent record of the environmental conditions experienced by the fish at a particular time (Campana et al. 2000; Ruttenberg et al. 2005). Sr:Ca and Ba:Ca otolith ratios have been simultaneously used by some authors for stock and migration studies as well as for indicators of habitat (Schuchert et al. 2010; Tabouret et al. 2010; Avigliano et al. 2015). The ratio of these elements varies between freshwater and seawater and is of value to understand the migratory behaviour of diadromous fish (Milton et al. 2008; Campana et al. 2009; Wang et al. 2010).

The Flathead grey mullet *Mugil cephalus* is the most worldwide distributed species of the Mugilidae family, it can be found in all the major oceans, mainly between latitudes 42° N and 42° S (Whitfield et al. 2012). This euryhaline species is thought to spawn offshore and its larvae to migrate from the sea to estuarine or freshwater areas until reaching sexual maturity (Whitfield et al. 2012). It has been studied in great depth because of its important economic value as a commercial species and in aquaculture, for their roe (Chang et al. 2004a). *In situ* as well as laboratory studies have been performed to better understand its biology and ecology (Whitfield et al. 2012).

Migrations and movement patterns of *Mugil cephalus* have been studied in Caribbean (Ibáñez et al. 2012), Pacific (Chang et al. 2004a; Lester et al. 2009; Wang et al. 2010; Górski et al. 2015) and Atlantic waters (Bacheler et al. 2005), using tagging, parasites or otolith analysis techniques. This species has diverse strategies for spawning and nursery area use and different use of habitats (Chang et al. 2004a; Wang et al. 2010; Ibáñez et al. 2012; Górski et al. 2015). Moreover, Ibáñez et al. (2012) proposed that the euryhaline behaviour enables this mugilid to search for the best environment not only for reproduction but also for survival and growth; they found a more diverse use of habitats in the Caribbean than the catadromous behaviour previously expected. The wide range of distribution, the flexible physiology and the position at the base of the aquatic food web, confer to *Mugil cephalus* the adaptability to tolerate a wide range of environmental conditions, thus showing an extensive and differing use of coastal environments (Whitfield 2015). Rearing experiments of this species revealed that their otolith Sr:Ca ratios are positively correlated with the salinity of ambient water (Chang et al. 2004b). For Barium, the relationships are yet not validated for this mullet but other fish have shown a negative correlation between otolith Ba:Ca ratios and salinity (ratios were higher in freshwater environments) (Elsdon and Gillanders 2005).

In the Mediterranean Sea *M. cephalus* is the most abundant Mugilidae and represents an important human food resource by being traditionally cultured in coastal lagoons (Gisbert et al. 1995; Turan 2016). This region has an extensive variety of environments that are used by this mullet. However, studies on displacements, strategies of habitat use and stock identification of *M. cephalus* in the Mediterranean region are scarce. As such, the present study has two main objectives using otolith microchemistry: (1) to identify migratory patterns throughout the life-history of *Mugil cephalus* in different Spanish Mediterranean wetlands with diverse ecological features, and (2) to study the presence of potential fish stocks of the species in the region.

Materials and Methods

Study area

Three wetlands in the Spanish Mediterranean region were selected: Parque Natural Delta de l'Ebro, Parque Natural de l'Albufera de Valencia and Parque Natural Salinas de Santa Pola; all situated in or near the Valencian Community (Figure 1). All wetlands are connected to the Mediterranean Sea either permanently or by manually operated channels. The Parque Natural Delta de l'Ebro (Deltebre) is a stratified estuary that presents a river plume (Romero et al. 2001). This area has a 350 km long floodplain that forms one of the largest deltas of the Mediterranean (Llorente et al. 1991). Its salinity ranges from 1.1 to 33.9 PSU. The Parque Natural de l'Albufera de Valencia (Albufera), the second largest wetland on the Mediterranean coast (Benedito et al. 2011), presents a lake - with low salinities throughout the year (around 1.5 PSU (Confederación Hidrológica Jucar 2016)) - that is connected to the sea by three artificial channels (flood-gates) that regulate water flow throughout the year (Soria et al. 2002). These gates are closed during May-June and November-December for rice fields. Finally, the Parque Natural Salinas de Santa Pola (Santa Pola) is a wetland system formed by canals, ponds and dams highly modified by human activities. It is a high salinity area (37.3 PSU) connected to the Mediterranean Sea (Belda Antolí et al. 2008).

Sample collection

Adult individuals (n = 43) of *Mugil cephalus* were captured between April 2013 and April 2014 with gill nets in artisanal catches of the local communities. Samples were taken during non-reproductive seasons in the estuary portion near the sea in the three selected wetlands, always in months when all areas were open to the sea. Collected individuals were taken to the laboratory, their standard length (SL in mm) was recorded and their *sagittal* otoliths removed. After extraction otoliths were dried and stored for posterior use.

Determination of Otolith microchemistry

Right otoliths of individuals captured in the three studied areas (Deltebre: n = 19; SL = 395 – 519 mm; Albufera: n = 11; SL = 355 – 577 mm; and Santa Pola: n = 13; SL = 359 – 419 mm) were weighed to the nearest 0.0001 mg and then embedded in crystalline epoxy resin (EC 141). Transversal sections just above the core were made using a Buehler Isomet low speed saw. The core was then exposed using sandpaper (4000 down to 500-grit) and its surface smoothed using a 1 m [correct at proof] polishing cloth (©Buehler). Sections were sonicated in Milli-Q water for 5 min and dried under a laminar flow for 24 h prior to the laser ablation ICP-MS analysis. An Agilent 7500ce (Tokyo, Japan) inductively coupled plasma mass spectrometer (Q-ICP-MS), coupled to a 213nm Nd:YAG laser unit (LA) LSX-213 (Cetac Technologies, Omaha, USA) was used in order to analyse the chronological variation of ^{88}Sr, ^{138}Ba and ^{43}Ca in the otoliths. Sectioned samples were placed in the ablation chamber and a transect was ablated from core to edge (crater width: 50 µm) along the longest radius of the otolith. The laser operated at a pulse rate of 20 Hz, a scan speed of 10 µm/s and an energy output of 100% (5.6 mJ max.). LA-ICP-MS coupling was daily optimized using a SRM NIST 612 silicate glass standard (National Institute for Standards and Technology-NIST, Gaithersburg, MD, USA) for high sensitivity and low background intensity. Otolith element composition was obtained and element:Ca ratios were calculated for statistical analysis and the profile construction of each fish.

Statistical Analysis

To identify migratory patterns, chronological Sr:Ca profiles were obtained for each specimen and then classified according to their environmental components. Microchemical thresholds previously described by Chang et al. (2004a) were used to identify environments: Sr:Ca ratios below 3×10^{-3} mmol/mol corresponded to freshwater habitats; ratios between 3 to 7×10^{-3} mmol/mol represented estuarine-brackish waters, and higher than 7×10^{-3} mmol/mol were high-salinity water habitats. Migratory patterns were identified and a Canonical Discriminant Analysis (CDA) was performed to study the correct assignment of individuals to the identified environmental patterns using InfoStat® software. When analysing life-history profiles, all three previously described environments (freshwater, estuarine and marine water) were found to be used by fish at different extents. Hence, the microchemical variables chosen to execute the CDA were Sr:Ca and Ba:Ca ratios from the core and edge of each profile, so as not to obscure any of the migratory patterns observed. Moreover, these microchemical variables were corrected, prior to any analysis, using the common within-group slope (Campana et al. 2000; Galley et al. 2006; Burke et al. 2008) as a fish size effect was detected (ANCOVA analysis: $p < 0.01$). The constants used were: Sr:Ca-core, b = 0.001; Sr:Ca-edge, b = 0.01; Ba/Ca-core, b = 0.00028, and Ba/Ca-edge: 0.0006; thus successfully removing the significant correlation with fish length.

Finally, to analyse the presence of potential fish stocks in the studied region, averages of Sr:Ca and Ba:Ca at otolith edges were calculated for each fish in all sampled sites, given that this region contains the most recent deposited material. Then, element:Ca ratios were compared among areas with a non-parametric Kruskal-wallis test, given that the variables did not meet the assumptions of normality (Shapiro–Wilk, $p < 0.0001$) and homogeneity of variances (Levene, $p < 0.05$).

Results

The analysis of the lifetime profiles revealed four migratory patterns. The most representative of each recognized habitat use are shown in Figure 2, to indicate differentiation. Pattern Type I corresponds to the most frequent use of estuarine environments (estuarine resident) (Figure 2a); Type II corresponds to a freshwater behaviour during early life history, using estuarine waters during juvenile and adult stages and moving at the end of their profile to the sea (freshwater migrant) (Figure 2b); Type III corresponds to estuarine water use in early life stages and later moving towards sea/high salinity waters (estuarine migrant) (Figure 2c); and Type IV corresponds to sea/high salinity water habitat use during their entire lifetime (seawater resident) (Figure 2d).

The CDA correctly assigned the individuals to the recognized patterns with high accuracy (Type I > 95%; Type II and Type III > 83%; and Type IV > 88%) (Table 1). The plot shows a clear separation of types I and II towards positive values of the first canonical axis; and Type IV towards the negative ones (Figure 3). Type III individuals are distributed in between the other recognized groups (Figure 3) along the mentioned canonical axis. The variable that best explained this variation was Sr:Ca-edge (discriminant coefficient: -0.70). Regarding the second canonical axis, there was not as clear a separation as in the first, but Types II and III tended to be distributed towards the positive values (Figure 3). The variables that best explained the second canonical axis variation were Sr:Ca-core and Sr:Ca-edge (discriminant coefficients: 0.87 and -0.74 respectively). Variability explained by the first and second canonical axis was 96.2% (78.2% and 18% respectively).

The most common pattern among individuals was Type I (Table 2). Not all movement patterns were identified in every studied area. Fish captured in Deltebre showed Type I as their most usual behaviour (78%), but also showed types II and III. Albufera individuals showed only Types I and II, with the first as the most common one (64%). Santa Pola fish were the only ones that presented Type IV behaviour, being this the most common one (70%), individuals also showed Type III pattern (Table 2).

When analysing element:Ca ratios, Sr:Ca of otolith edges revealed a similar ratio pattern for Albufera and Deltebre (Figure 4) but a different one for Santa Pola (Kruskal–Wallis $p < 0.0001$) allowing discrimination of the most southern location from the northern ones because of this chemical feature. Ba:Ca ratios of otolith edges did not reveal any significant differences among the analysed areas (Kruskal-Wallis $p = 0.34$) (Figure 4).

Discussion

Our results show that *Mugil cephalus* presents diverse migratory life patterns on the Spanish Mediterranean coast of the Valencian Community. The microchemical analysis of otoliths showed two different diadromous movement profiles and two constant patterns with residency in estuarine or marine waters. Otolith composition is highly influenced by habitat use (Chang and Geffen 2013), reflecting population movements as well as preferred habitats and their connectivity (Morales-Nin et al. 2014). Our findings agree with those reported by Chang et al. (2004a), who strongly suggested that Taiwanese specimens of *M. cephalus* could be divided into two groups, one that presented a freshwater component in their life cycle and another that did not.

Even though *M. cephalus* is considered a marine estuarine-opportunist species (Chang and Iizuka 2012; Potter et al. 2015), Sr:Ca ratios found in otolith cores of some analysed specimens showed concentrations corresponding to freshwater (Type II) or estuarine waters (Types I and III). This has also been reported for Australian individuals of the species that remained near shore during their marine spawning (Smith and Deguara 2002; Fowler et al. 2016), and for Taiwanese ones that showed freshwater or estuarine water residencies (Wang et al. 2010). Our results may indicate that *M. cephalus* has more diverse spawning and nursery ground behaviours than previously expected, or that there could be a rapid migration from sea to lower salinity environments, not allowing the uptake of elements into the otolith to reflect the environment where they were spawned. However, it is also important to consider that the low number of fish studied per area could be masking other patterns that could have been found with a more abundant otolith sample.

The studied Mediterranean region presents wetlands with specific ecological features (different salinity values, connection to the sea, hydrological regime) in which this widely commercialized mullet showed adaptability to the conditions of the areas by having different environmental strategies reflected in its diverse habitat uses. Thus, *Mugil cephalus*, as well as other reported euryhaline species such as *Centropumus parallelus* or *Lysengraulis grossidens* (Mai et al. 2014; Daros et al. 2016), presented high plasticity related to their habitat use and movements, and showed diverse displacement patterns throughout their life history.

When analysing the presence of potential fish stocks, Ba:Ca ratios of the otoliths edge did not reveal differences among the sampled areas. Sr:Ca edge values, on the other hand, showed differences among individuals captured in Santa Pola with specimens from the northern sampled areas. When comparing the latter, no significant differences were found, showing the values of both areas (Deltebre and Albufera) Sr:Ca associated to estuarine environments. As previously mentioned, the incorporation of elements depends on different factors such as temperature and salinity. Sr concentration in otoliths correlates positively with the salinity found in the surrounding water (Kraus and Secor 2004; Sturrock et al. 2012), while Ba values correlate negatively with salinity or conductivity of the water masses used by the fish (Elsdon and Gillanders 2005; Tabouret et al. 2010; Miller 2011). In this research, Sr:Ca ratios, as seen in other studies, were useful to identify fish that used areas with high salinities like the Santa Pola region (Chang et al. 2004b; Chang and Iizuka 2012; Kerr and Campana 2014; Górski et al. 2015). In contrast Ba was not useful in our study, as it did not reflect the environmental features of the areas, agreeing with findings of Fowler et al. (2016), who reported that this microchemical variable had a very low utility in distinguishing estuarine and marine environments used by *Mugil cephalus* on and off the Australian east coast.

The Mediterranean Sea presents a complex circulation. The study region is influenced by the superficial current of the Atlantic Ocean (AW) and the Levantine Intermediate Water current (LIW) which moves water masses from east to west (Millot 1999; Bergamasco and Malanotte-Rizzoli 2010; Skliris 2014). Moreover, the area is particularly influenced by a mesoscale phenomenon: a superficial current from the eastern Ligurian Sea to the Gulf of Valencia (from The Ebro Delta to the La Nao Cape), following the shelf-slope front that merges with the north Balearic front (Estrada 1996; Salat 1996; Pascual et al. 2002). This current dynamic could be influencing stock displacements of *Mugil cephalus*, representing a geographical impediment in the southern end of the Valencian Gulf. This agrees with the present results which found two potential fish stocks: one associated to areas north of La Nao Cape (Deltebre-Albufera) and the other, south of it (Santa Pola) (Figure 1).

This broadens the knowledge of the environmental requirements of this cosmopolitan species in the Mediterranean area and the different use of the studied wetlands during their lifetime. These results are necessary for the proper management of these protected areas and also for this and other species that make vital use of them throughout their life cycle, hence ensuring their correct preservation.

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Figure and Table Titles and Legends

Fig. 1 Location of the three sampled wetlands (Parque Natural Delta de l'Ebro, Parque Natural de l'Alfubera de Valencia and Parque Natural Salinas de Santa Pola) in the Mediterranean region (black stars).

Fig. 2 Otolith life history profiles of Sr/Ca ratios for the 4 identified Type patterns of *Mugil cephalus* in the three sampled areas measured by LA-ICP-MS from core to edge. Letters refer to identified groups: (a) Type I: estuarine resident (specimen from Deltebre, LS = 413 mm); (b) Type II: freshwater migrant (specimen from Albufera, LS = 355 mm); (c) Type III: estuarine migrant (specimen from Santa Pola, LS = 405 mm); and (d) Type IV: seawater resident (specimen from Santa Pola, LS = 418 mm). Critical levels for the limits of the environments concerning water salinity were taken from Chang et al. (2004a).

Fig. 3 Canonical Discriminant Analysis of otolith microchemical variables for the four identified migratory patterns (Type I, estuarine resident; Type II, freshwater migrant; Type III, estuarine migrant; Type IV, seawater resident) of *Mugil cephalus*.

Fig. 4 Otolith edge Sr:Ca (a) and Ba:Ca (b) ratios for the three analysed sampling areas (Deltebre: Parque Natural Delta de l'Ebro; Albufera: Parque Natural de l'Alfubera de Valencia; Santa Pola: Parque Natural Salinas de Santa Pola). Squares indicate median values and bars indicate minimum and maximum ones. Different letters show significant statistical differences ($p < 0.01$).

Table 1 Cross-classification matrix of the Canonical Discriminant Analysis of otolith, core and edge, microchemical variables (Sr:Ca; Ba:Ca) to differentiate migratory patterns: Type I, estuarine resident; Type II, freshwater migrant; Type III, estuarine migrant; Type IV, seawater resident.

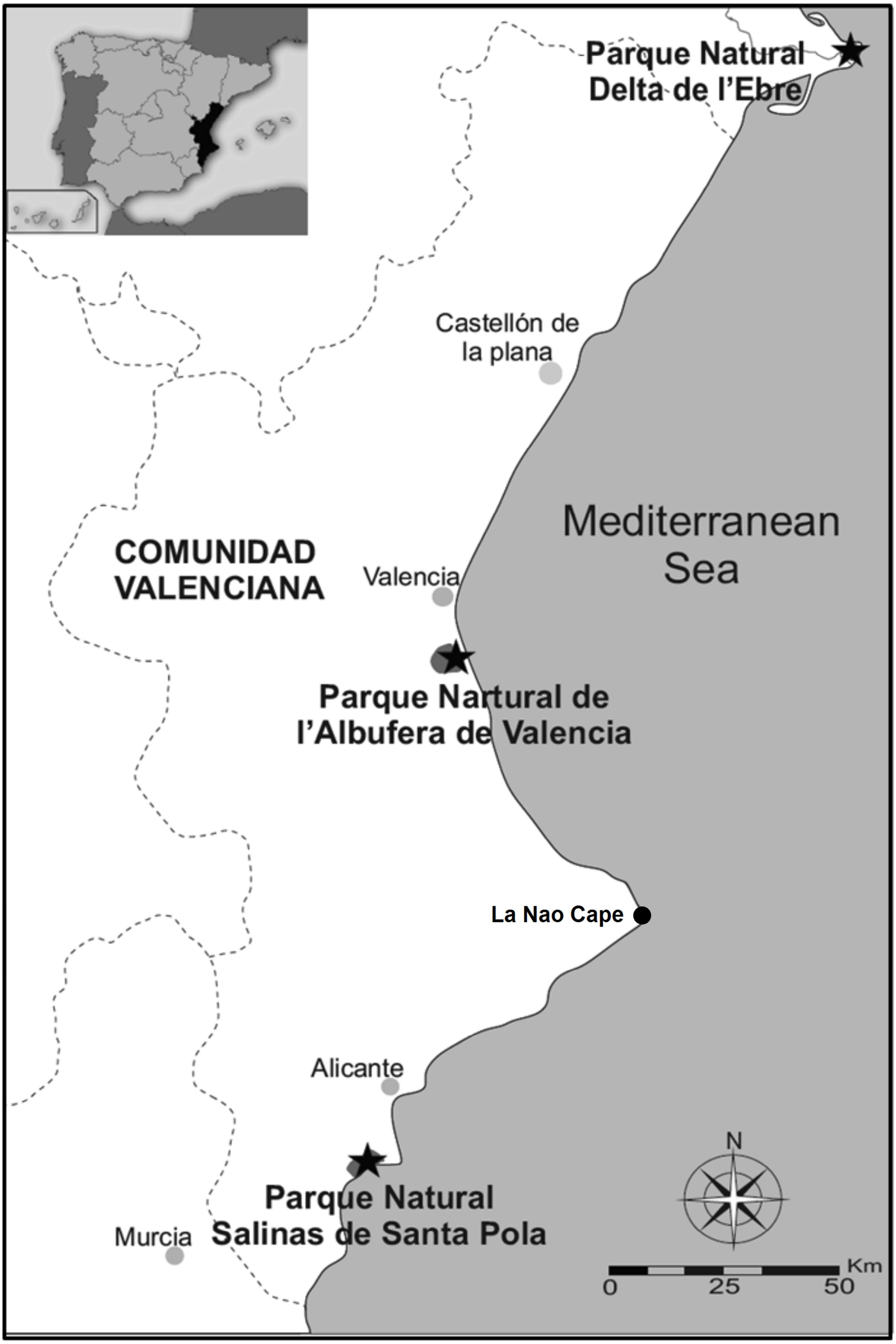
Table 2 Migratory patterns identified (Type I, estuarine resident; Type II, freshwater migrant; Type III, estuarine migrant; Type IV, seawater resident) for individuals of *Mugil cephalus* of the studied areas on the Mediterranean coast.

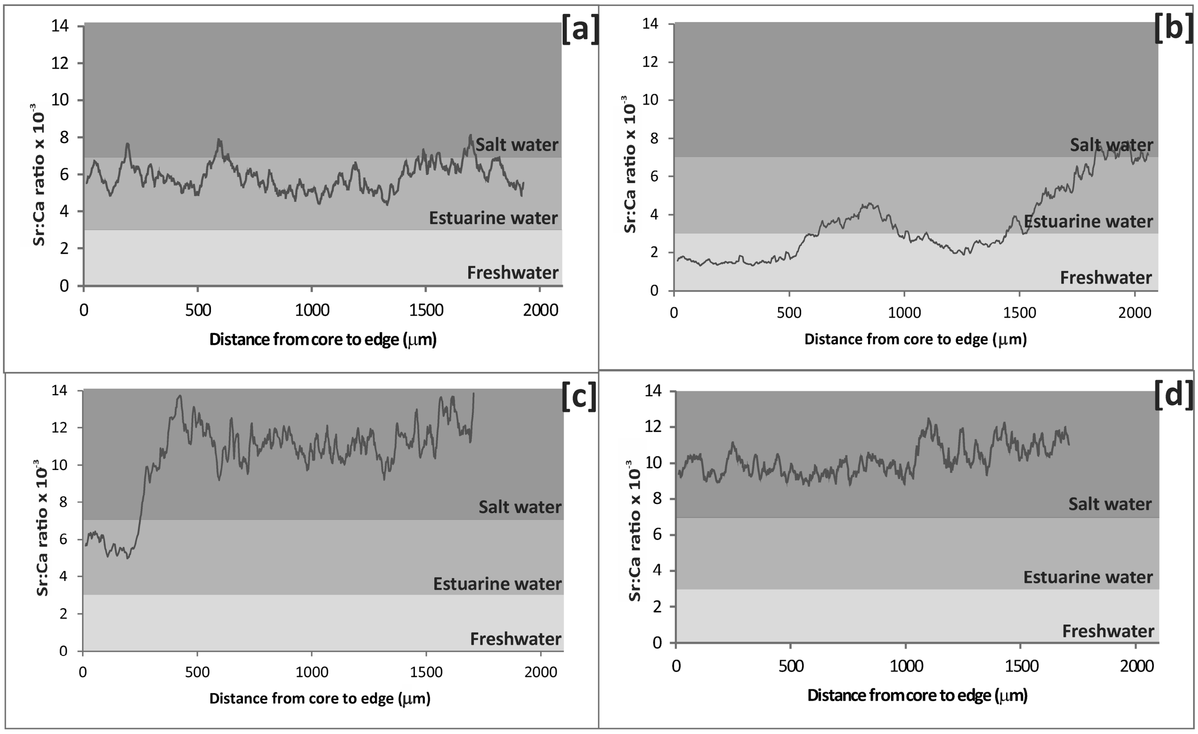
Table 1. Cross-classification matrix of the Canonical Discriminant Analysis of otolith, core and edge, microchemical variables (Sr:Ca; Ba:Ca) to differentiate among migratory patterns: Type I. estuarine resident; Type II. freshwater migrant; Type III. estuarine migrant; Type IV. seawater resident.

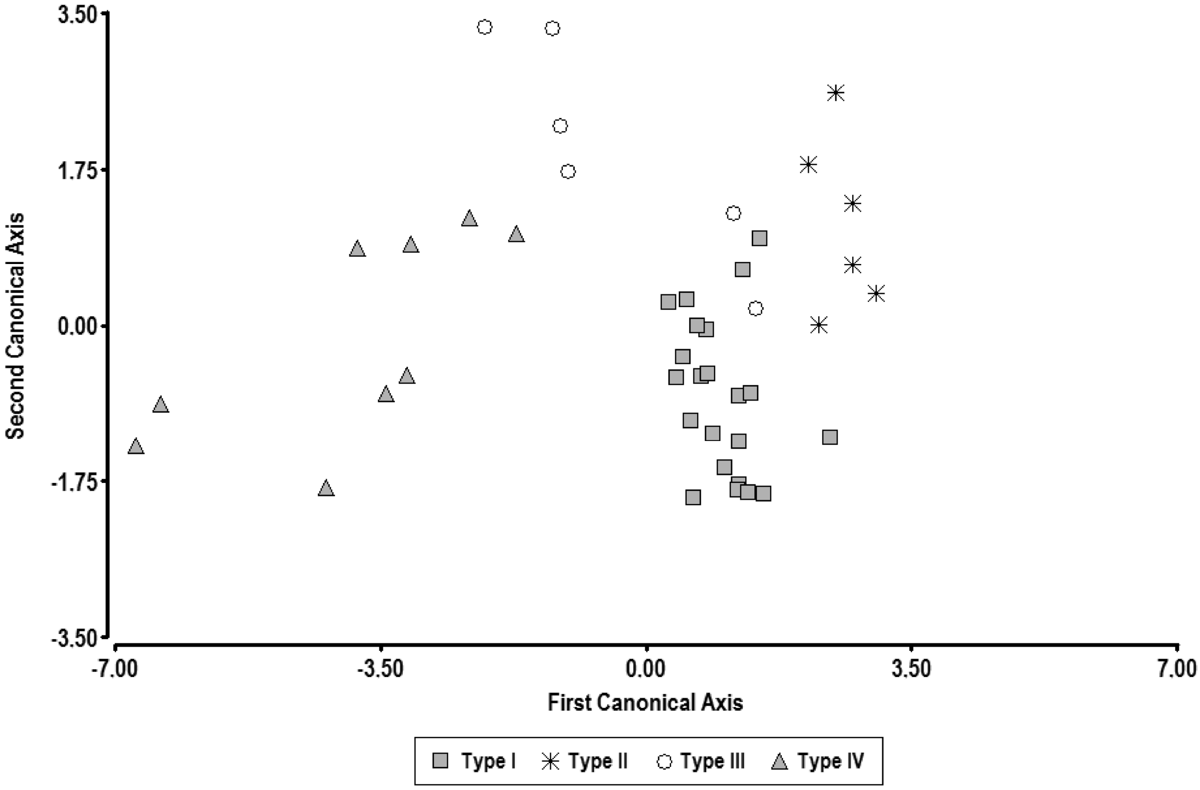
	Type I	Type II	Type III	Type IV	Total sample size of each Type
	Percentages				
Type I	95.45(21)	4.55(1)	0	0	22
Type II	16.7(1)	83.3(5)	0	0	6
Type III	16.7(1)	0	83.3(5)	0	6
Type IV	0	0	11.1(1)	88.9(8)	9

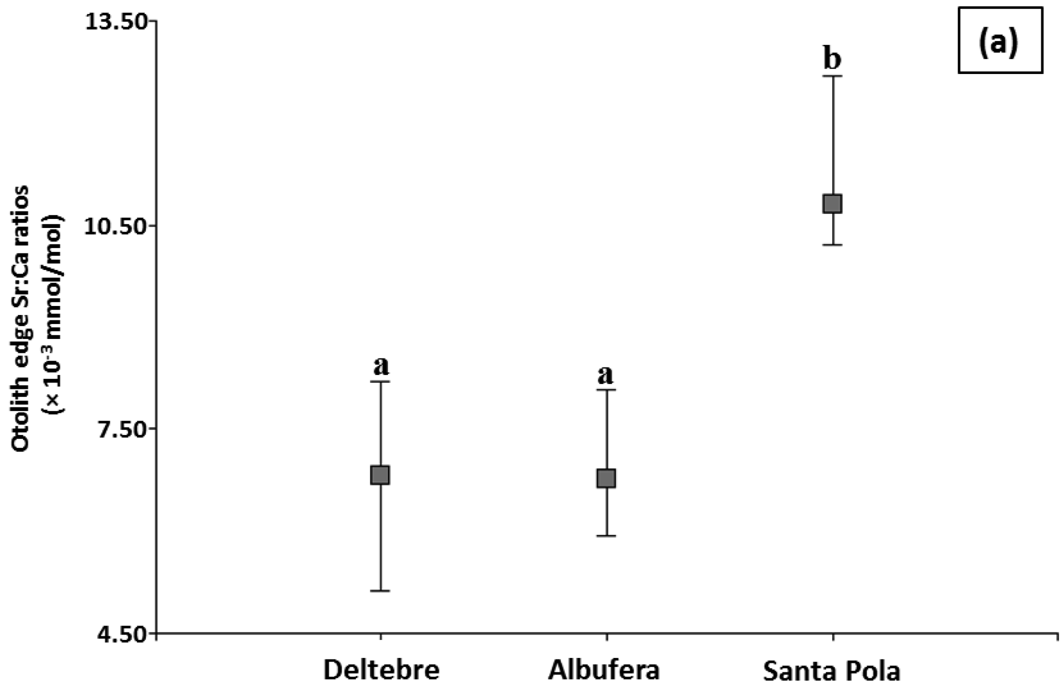
Table 2. Migratory patterns identified (Type I: estuarine resident; Type II: freshwater migrant; Type III: estuarine migrant; Type IV: seawater resident) for individuals of *Mugil cephalus* of the studied areas in Mediterranean coast.

	Type I	Type II	Type III	Type IV	Total individual number
Deltebre	15	2	2	0	19
Albufera	7	4	0	0	11
Santa Pola	0	0	4	9	13

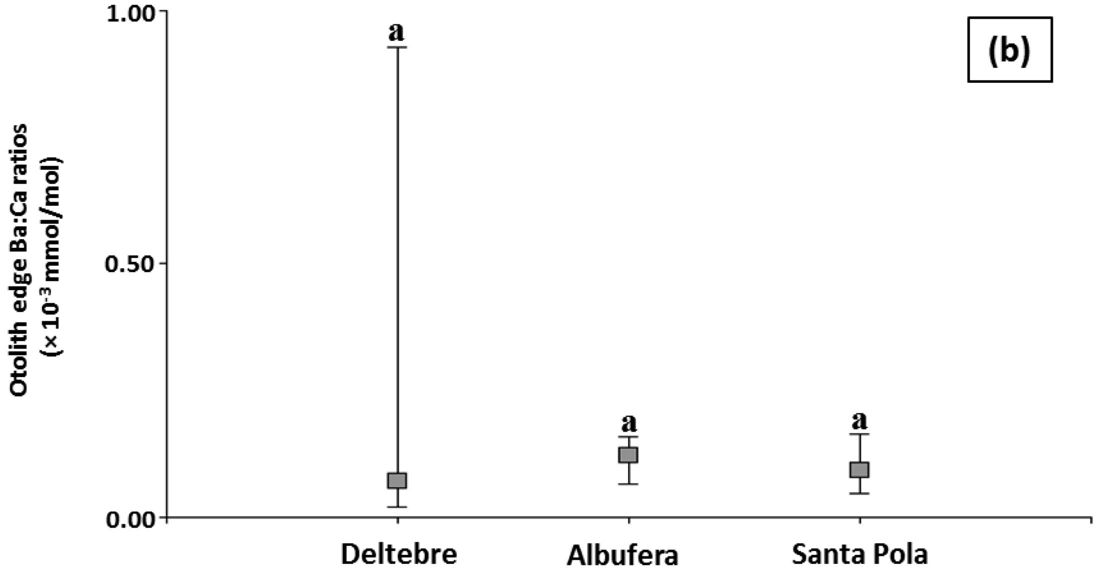

Parque Natural
Delta de l'Ebre
Castellón de
la plana
Mediterranean
Sea
COMUNIDAD
VALENCIANA
Valencia
Parque Nartural de
l'Albufera de Valencia
La Nao Cape
Alicante
N
Parque Natural
Salinas de Santa Pola
Murcia
Km
0
25
50


[a]
[b]
[c]
[d]
Sr:Ca ratio x 10-3
Salt water
Estuarine water
Freshwater
Distance from core to edge (μm)


3.50
1.75
0.00
-1.75
-3.50
Second Canonical Axis
-7.00
-3.50
0.00
3.50
7.00
First Canonical Axis
Type I
Type II
Type III
Type IV



(a)
Otolith edge Sr:Ca ratios (× 10-3 mmol/mol)
13.50
10.50
7.50
4.50
a
a
b
Deltebre
Albufera
Santa Pola



(b)
Otolith edge Ba:Ca ratios (× 10-3 mmol/mol)
1.00
0.50
0.00
a
a
a
Deltebre
Albufera
Santa Pola