
Habitat discrimination of big-scale sand smelt *Atherina boyeri* Risso, 1810 (Atheriniformes: Atherinidae) in eastern Algeria using somatic morphology and otolith shape

Boudinar Sofiane¹, Chaoui Lamy¹, Mahe Kelig², Cachera Marie², Kara Hichem^{1,*}

¹ Marine Bioresources Laboratory, Annaba University Badji Mokhtar, Algeria

² IFREMER, Sclerochronology Centre, Fisheries Laboratory, Boulogne-sur-Mer, France

* Corresponding author : Hichem Kara, Tel: +213 770312458 ; Fax: +213 38868510 ;
email: kara_hichem@yahoo.com

Abstract :

Somatic morphology and otolith shape were used to discriminate four samples of *Atherina boyeri* from three different habitats: Mellah lagoon (n = 269), Annaba Gulf (144 punctuated and 194 unpunctuated individuals) and Ziama inlet (n = 147) in eastern Algeria. For each individual, somatic morphology was described with 13 metrics and eight meristic measurements, while the otolith contour shape of 452 individuals from the three habitats was analysed using Fourier analysis. Then, two discriminant analyses, one using the 13 metric measurements and the other using Fourier descriptors, were used in order to discriminate populations of *A. boyeri*. The results of the discriminant analyses based on the two methods were similar, and showed that this species could be discriminated into three distinct groups: (1) marine punctuated, (2) lagoon and marine unpunctuated and (3) estuarine. These results are consolidated by the comparison of the Mayr, Linsley and Usinger coefficient of difference for the meristic parameters according to the location origin, where the difference reached a racial or even sub-specific level for some characters, depending on which pairs of populations were compared.

Keywords : Sand smelt, otoliths, morphology, Mediterranean, Algeria

1. Introduction

Big-scale sand smelt *Atherina boyeri* (Risso, 1810) is an extremely euryhaline teleost fish, which inhabits coastal and estuarine waters, including coastal lagoons and more rarely inland waters, over a wide range of salinities, from freshwater to hypersaline conditions (110‰ maximum recorded) (Gon & Ben-Tuvia 1983; Henderson & Bamber 1987). This species is common in the Mediterranean and adjacent seas, and is also found in the north-east Atlantic, from the Azores to the north-west coast of Scotland (Kiener & Spillmann 1969; Quignard & Pras 1986). It is composed of local semi-isolated populations, which may be different according to their reproductive biology and life traits (Henderson & Bamber 1987).

Kiener & Spillman (1969) re-examined the description of species within the European Atherinidae, condensing two genera and 20 species into a single genus with three species: *Atherina hepsetus* (Linnaeus, 1758), *Atherina presbyter* (Cuvier, 1829) exclusively marine and *Atherina boyeri* (Risso, 1810) which lives in marine, brackish and freshwater (Milana et al. 2008).

However, several studies based on morphometrical data revealed differences in body shape among populations of *Atherina boyeri* living in the sea compared to those living in lagoons (Kartas & Trabelsi 1990; Trabelsi et al. 2002a, 2004). This conclusion was supported by a biochemical approach by Focant et al. (1992, 1993, 1999) and by a genetic approach by Cammarata et al. (1996), using electrophoresis analysis of a muscle protein and allozymic variation of 20 loci, respectively. More recent investigations also based on allozyme analysis (Mauro et al. 2007) and molecular data tended to considerate *Atherina boyeri* as a taxonomic complex, divided by some authors into two forms: one lagoon type and one marine non-punctuated type (Klossa-Kilia et al. 2002, 2007; Kraitsek et al. 2008). Other authors (Trabelsi et al. 2002a, 2002b, 2004; Astolfi et al. 2005; Milana et al. 2008; Francisco et al. 2008, 2011) recognised three forms: one lagoon type and two marine types (punctuated and non-punctuated on the flanks). The punctuated marine type differs from the other groups by its colouring, morphological and biochemical characteristics and the presence of dark spots along the lateral line (Trabelsi et al. 2000, 2002a). Moreover, these authors found that the difference in cytochrome-*b* sequences amongst the three forms was comparable to or greater than those seen between congeneric species of Teleostei. Consequently, the authors mentioned above proposed to elevate the three forms to the rank of species. This “three-form hypothesis” was supported by subsequent molecular investigations conducted with other mitochondrial markers (Milana et al. 2008; Francisco et al. 2008, 2011). This differentiation could be done with euryhaline characteristic of this species. Indeed the environmental conditions, as is well known, may have a strong impact on individual phenotype. A species experiencing a wide diversity of environmental conditions may thus hold a wide diversity of phenotypes through phenotypic plasticity (Congiu et al. 2002). Our aim was to test the three-form hypothesis using somatic morphology (metric and meristic parameters) and otolith shape analyses on *A. boyeri* from different habitats (lagoon, sea and inlet).

2. Materials and methods

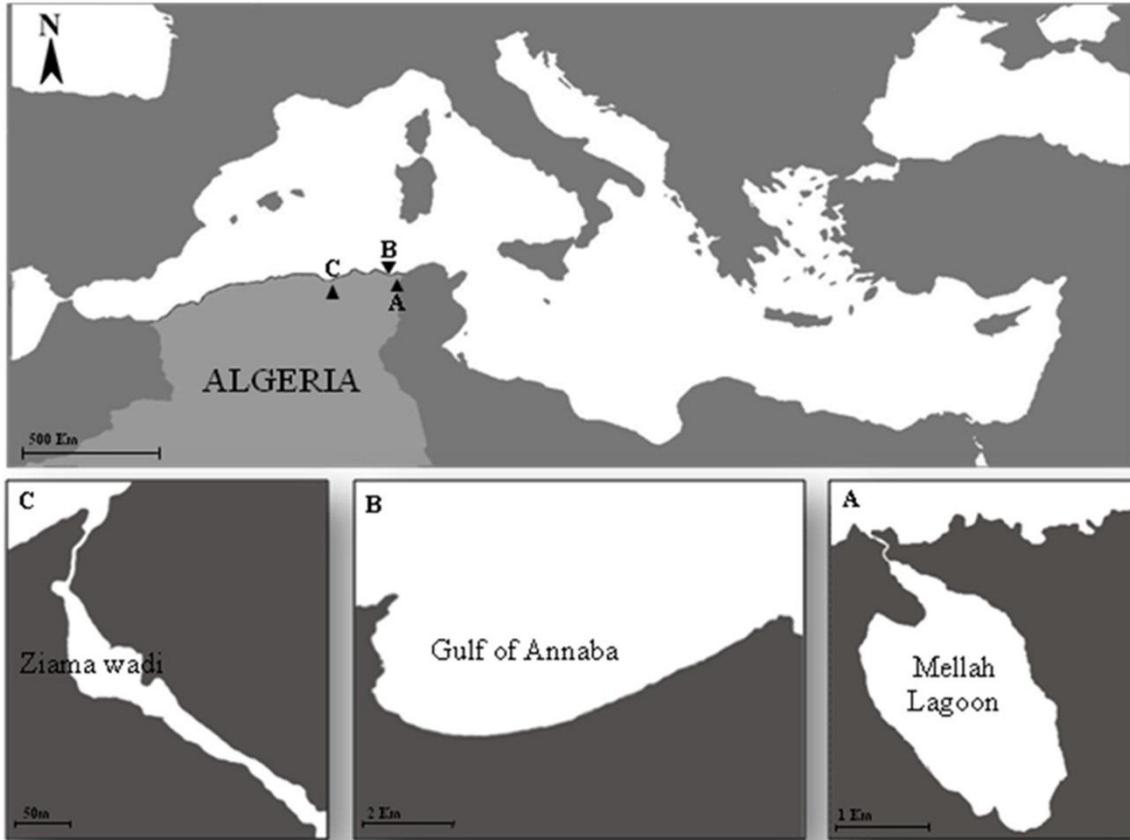
2.1. Study habitats

Fish were caught from three kinds of habitats in eastern Algeria (Figure 1): (1) Mellah lagoon, a water body of 865 ha and 3.5 m depth ($25.4 \leq S\text{‰} \leq 34.8$; $10^{\circ}\text{C} \leq T^{\circ} \leq 30^{\circ}\text{C}$)

(Chaoui et al. 2006), (2) Gulf of Annaba ($35.1 \leq S\text{‰} \leq 37.9$; $14.8^{\circ}\text{C} \leq T^{\circ} \leq 28.8^{\circ}\text{C}$) (Frehi et al. 2007), (3) Ziama inlet with salinity not exceeding 10‰.

The geographical distances between these different area are: Ziama inlet/Gulf of Annaba = 210 Km, Ziama inlet/Mellah lagoon = 250 km and Gulf of Annaba/Mellah lagoon = 40 Km.

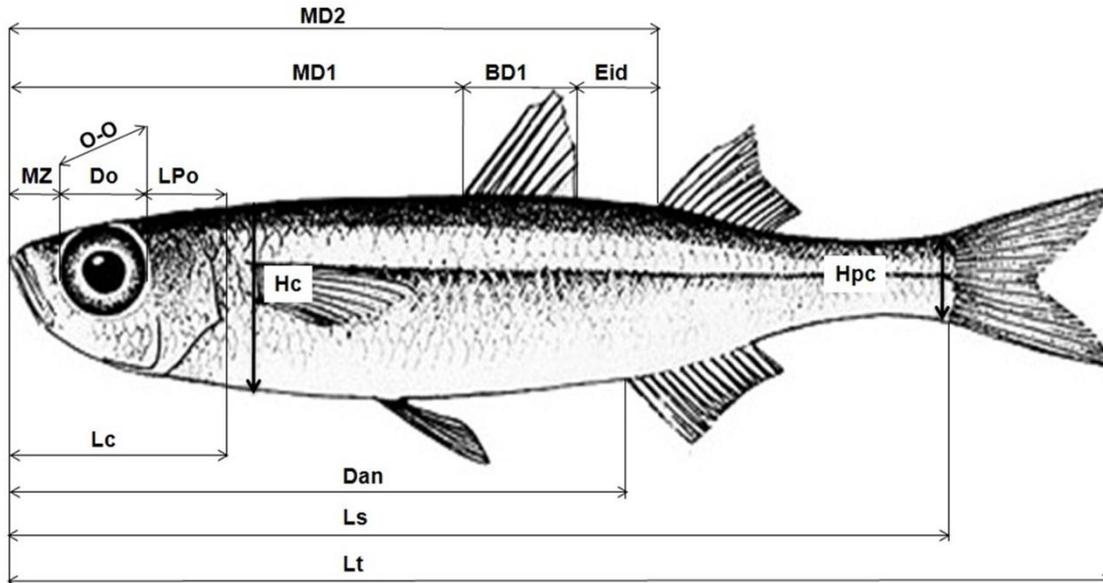
Figure 1. Sampling locations of *Atherina boyeri* in different habitats of eastern Algeria: A: Mellah lagoon ; B: Annaba Gul f ; C: Ziama inle t.



2.2. Somatic morphology

A total of 754 specimens were sampled using beach seine between March 2010 and March 2011: 269 from Mellah lagoon ($2.0 \leq L_t \leq 8.3$ cm), 194 unpunctuated ($4.6 \leq L_t \leq 13.1$ cm) and 144 punctuated ($5.4 \leq L_t \leq 10.7$ cm) from Annaba's Gulf and 147 from Ziama inlet ($2.9 \leq L_t \leq 10.2$ cm). Fishes were transferred in dry ice to the laboratory and stored at -4°C . Thirteen body measurements were measured using vernier calipers to the nearest 0.01 mm (Figure 2) on each individual and eight meristic characters were counted (number of fin rays D1, D2, A, P, number of scales on lateral line (Sc), number of vertebrae (Ve), number of upper (TGsup) and lower (TGinf) gill rakers of the first left branchial arch (TG = TGinf + TGsup).

Figure 2. Measurements taken on specimens examined of *Atherina boyeri*. Lt: total length; Ls: standard length; MZ: muzzle length; DO: orbital diameter; LPO: post-orbital length; Lc: cephalic length; MD1: distance from the end of the muzzle to the beginning of the first dorsal fin; MD2: distance from the end of the muzzle to the beginning of the second dorsal fin; Dan: distance from the end of the muzzle to the beginning of the anal fin; Eid: inter-dorsal I space; O-O: inter-orbital space; Hc: height of the body; HPC: height of caudal peduncle; BD1 : length of the first dorsal fin insertion.



To ensure unbiased comparisons between groups, the size-effect was statistically removed by using residuals from a Principal Component Analysis and each of the four populations was randomly split into two equal parts to verify the intra-specific effect. Also, a primary test was applied to test the influence of sex where a Factorial Discriminant Analysis analysis (FDA) considering sex in each sample was applied. The Factorial Discriminant Analysis, which helps to better discriminate samples of the same or of different species (Semple et al. 1991) was performed using the SPSS software version 13 (SPSS 2004). Using ward's method, a hierarchical clustering was performed with the FactoMineR package with R® software (R Development Core Team 2014). The classification success of the discriminant analysis was assessed using jackknifed cross-validation.

Moreover, in order to detect differences in the meristic characters following the sampling location of specimens, the means of each character's mean were compared using Student's "t" test at probability level $\alpha = 0.001$. When a difference was assessed between two populations, the Mayr, Linsley, and Usinger coefficient of difference (Mayr 1969) was calculated to determine their respective taxonomic level:

$$C.D. = \frac{\bar{X}_1 - \bar{X}_2}{S_1 + S_2}$$

Where X_1 and X_2 : the mean of the meristic characters for the first and second populations respectively, S_1 and S_2 are the standard deviation of the meristic characters for the first and second population.

It is generally accepted (Géry 1962) that when 75% of individuals in a population ("1") differ in one or more characters from 75% of individuals from population "2" ($CD > 0.67$), we are dealing with racial differences between populations "1" and

“2”. When 75% of individuals in the population “1” differ by 97% from population “2” (CD > 1.28), these two populations differ sub-specifically.

2.3. Otoliths shape

A total of 226 fish (69 lagoonal, 51 marine unpunctuated, 40 marine punctuated, 66 from inlet) were analysed. The sagittal otoliths were taken as pairs, washed in clean water, air-dried and stored in plastic tubes. Both right and left otoliths were examined under a stereomicroscope fitted with a numerical camera (Lumenera's INFINITY lite) linked to a computer monitor. Otoliths were orientated in a consistent manner, with the sulcus side up. Numerical pictures were then acquired with the TNPC software (Mahé et al. 2011). For each numerical image, the software Shape 1.2 (Iwata & Ukai 2002) calculated the Fourier coefficients in order to make them invariants to the otolith size, orientation and position, regarding the beginning of the outline, which is arbitrarily defined (Crampton 1995). Also, the Fourier Power (FP) spectrum was calculated to determine the sufficient number of harmonics accounting for the best reconstruction of the otolith outline (Pothin et al. 2006). A subsample of 40 otoliths (10 samples × 4 areas) was randomly created for this purpose. The first 13 harmonics reached 99.99% of the mean cumulated power. The Fourier analysis indicated that the otolith shape of *Atherina boyeri* could be summarised by these 13 harmonics.

Again using SPSS version 13.0 statistical software (SPSS 2004), a second FDA was performed with Fourier descriptors to compare otolith shape variations among the sampling habitats and its classification success was again assessed using jackknifed cross-validation.

A primary test was applied to test the influence of sex, doing an FDA analysis considering sex in each sample. Using ward's method, a hierarchical clustering was performed with the FactoMineR package with R[®] software (R Development Core Team 2014).

3. Results

3.1. Somatic morphology analysis

Populations could be morphologically discriminated into three distinct groups based on the first two functions of the FDA (94.38% of the variance, Wilks's lambda = 0.194, $P < 0.001$). Marine punctuated and Ziama inlet specimens were separated into two groups, while lagoon and unpunctuated marine specimens were gathered in the third group (Figure 3). The classification of samples based on these characters correctly classified 71.9% of samples. The marine punctuated specimens were the most highly ranked (92.36%), followed by those sampled from Ziama inlet (85.03%), then lagoon's specimens (68.40%) and finally marine unpunctuated (51.50%). The test for the intra-specific or sex effect showed that there is no influence of these two parameters.

A comparison of meristic characters (Table I) indicated a significant difference according to the habitat types. This difference concerned most of the 13 characters, with the exception of the number of spines on the second dorsal, the pectoral fin and the number of upper gill rakers when comparing marine punctuated specimens and lagoon specimens; and the number of spines on the first dorsal and the anal fin when comparing with Ziama inlet specimens. Also, the number of anal fin rays was different when comparing lagoon specimens and marine unpunctuated ones.

Figure 3. Discriminant analysis using metric parameters on somatic morphology of *Atherina boyeri* in eastern Algeria (southern Mediterranean Sea). Mellah lagoon (LM: o) ; Ziama inlet (Z: Δ) ; marine punctuated (MP: \diamond) ; marine unpunctuated (MNP: \square) ; group centroid (\blacksquare) ; 95% probability ellipses are indicated for each group.

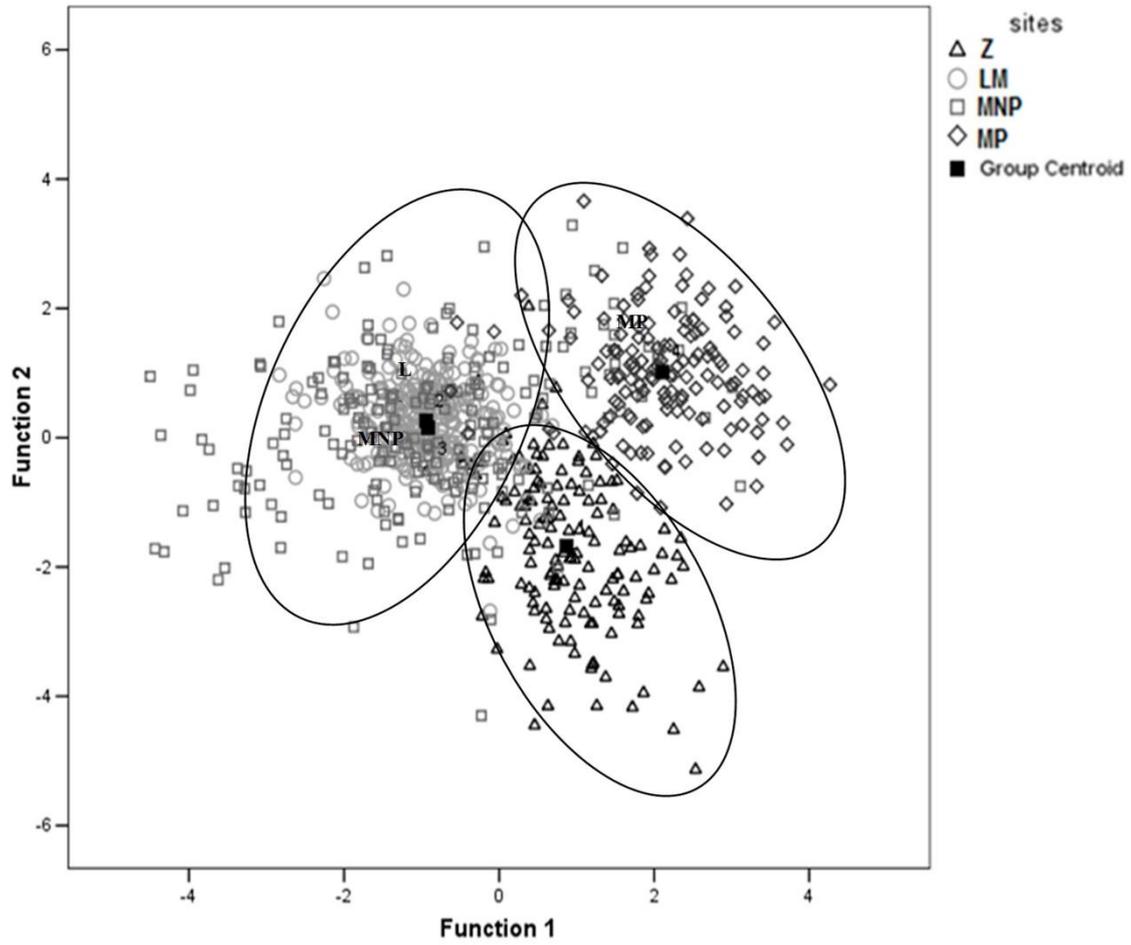


Table 1. Values of „t“ Student’s test, and the coefficient of difference „CD“ calculated from the body shape of *Atherina boyeri* between the three populations in the eastern Algeria (southern Mediterenaen Sea).

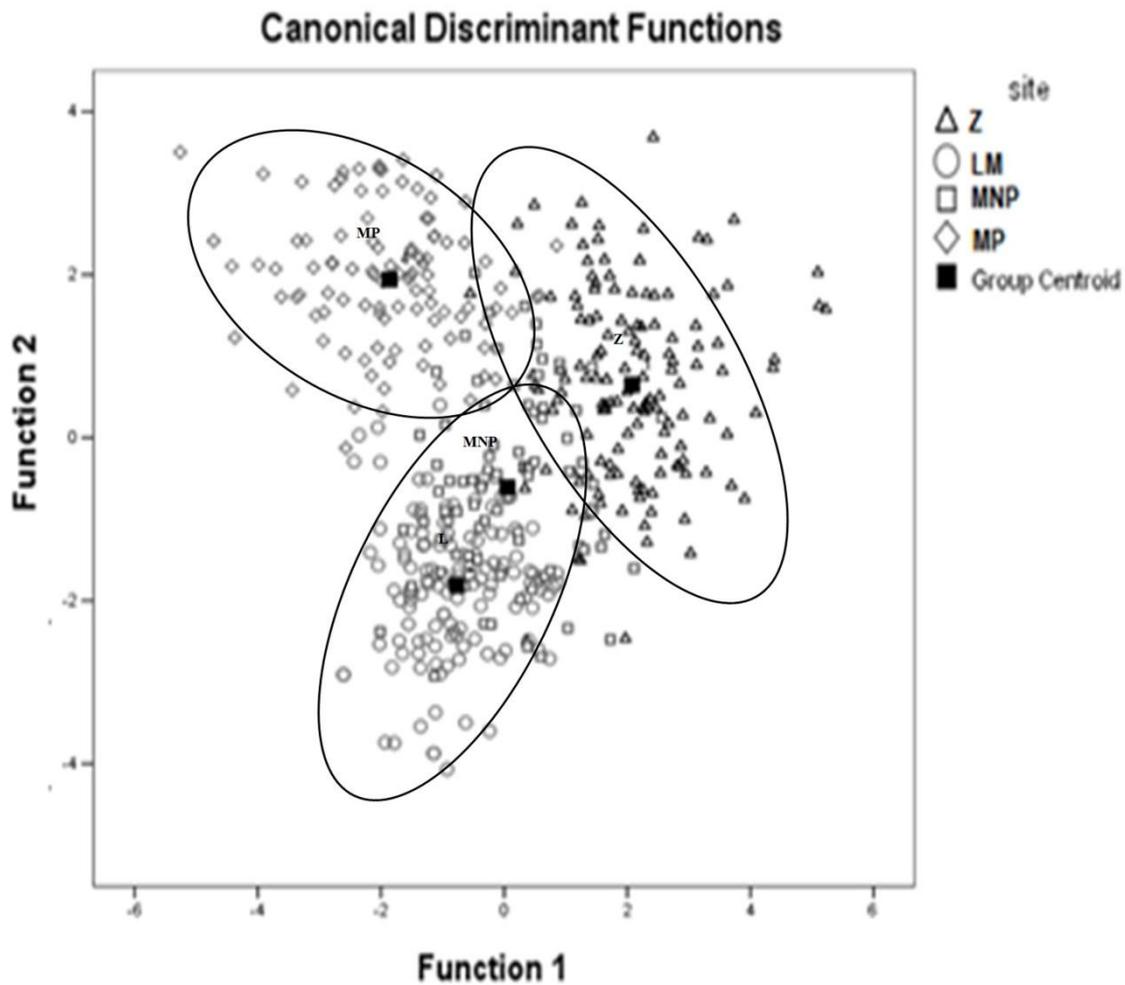
Character s	LM - Z		LM – MP		LM – MNP		Z – MP		Z - MNP		MNP – MP	
	t	C.D.	t	C.D.	t	C.D.	T	C.D.	t	C.D.	t	C.D.
D1	3.07	0.15	2.05	0.10	9.14	0.44	0.74	0.04	11.06	0.60	9.95	0.54
D2	8.39	0.44	0.75	0.03	3.26	0.15	8.05	0.47	4.80	0.26	3.47	0.18
P	10.46	0.53	1.21	0.06	15.40	0.73	10.21	0.59	4.48	0.24	14.56	0.79
Sc	15.75	0.87	4.13	0.21	15.33	0.89	12.52	0.76*	6.94	0.39	13.83	0.83
A	4.41	0.22	3.40	0.17	0.40	0.01	0.52	0.03	3.18	0.17	2.48	0.13
TGsup	3.75	0.19	0.39	0.02	9.15	0.45	2.80	0.16	11.38	0.62	8.25	0.44
TGinf	5.38	0.27	19.20	1.04	24.11	1.26	23.38	1.41*	27.31	1.54*	8.44	0.46
Ve	21.31	1.13	2.88	0.15	17.92	1.04	15.72	0.92*	8.30	0.47	16.14	0.94
TG total	6.13	0.32	15.67	0.86	22.37	1.18	19.82	1.19*	25.53	1.44*	9.27	0.5

LM: Mellah Lagoon, MP: Punctuated marine specimens, MNP: Unpunctuated marine specimens, Z: Ziama inlet specimens, D1, D2, A, P : number of fin rays, Sc: number of scales on lateral line, Ve: number of vertebrae, TGsup: number of upper gill rakers of the first left branchial arch, TGinf: number of lower gill rakers of the first left branchial arch, TG total = TGinf + TGsup : number of total gill rakers of the first left branchial arch, * Racial differences, ** Subspecific differences.

3.2. Otolith shape analysis

The first two discriminant functions of the FDA performed with Fourier descriptors accounted for 87.40% of the variance. Individuals could be discriminated into three distinct groups based on the first two functions (Wilks’s lambda = 0.062; P < 0.001). The first group was composed principally by individuals from Ziama inlet, the second one was mainly composed by marine punctuated individuals and the third group by unpunctuated marine and lagoon specimens (Figure 4). Overall area-classification success was 84.30%, ranging from 68.75% for unpunctuated marine specimens to 91.18% for marine punctuated specimens (86.36% for lagoon specimens and 86.23% for those sampled from Ziama inlet). The primary tests for the intra-specific variation or sex influence showed the same results pattern as for the total population.

Figure 4. Discriminant analysis using Fourier's descriptors on otolith shape of *Atherina boyeri* in eastern Algeria (southern Mediterranean Sea). Mellah lagoon (LM: o) ; Ziamia inlet (Z: Δ) ; marine punctuated (MP: ◇) ; marine unpunctuated (MNP: □) ; group centroid (■) ; 95% probability ellipses are indicated for each group.



4. Discussion

In this study, somatic morphometry and otolith shape analyses of atherinids from eastern Algeria allowed us to clearly discriminate three major groups and to distinguish four groups (lagoon, marine punctuated, marine unpunctuated and inlet specimens).

First, the analysis of body shape by several measurements separated marine punctuated and Ziamia inlet specimens into two distinct morphological groups while lagoon and unpunctuated marine specimens were gathered in a third group. These results were in accordance with those obtained by the analysis of otolith shape which also identified three groups (marine punctuated specimens, and Ziamia inlet specimens constituted two distinct groups; and lagoon with unpunctuated marine specimens composed the third group). Using otolith shape analysis in this work was a reliable choice since it allowed to distinguish between *Atherina boyeri* populations from different habitats and, to our best knowledge, it is the first time that this technique has been used to discriminate fish population from different habitats. Otoliths are used in a wide range of studies, such as species identification (Aguirre & Lombarte 1999; Parmentier et al. 2001) and may vary within species according to fish size or habitat (Begg et al. 2001; Stransky & MacLellan 2005; Hüsey 2008). Several studies have also

shown that otolith shape analysis allowed the discrimination of local fish stocks according to ecological factors (Smith 1992; Friedland & Reddin 1994; Bolles & Begg 2000; Cardinale et al. 2004). In our case study, environmental influences on the observed differences are also likely to play a major role, in terms of the semi-enclosed lagoon compared to the marine water masses. Water temperature and salinity are key factors influencing both physiological properties of fish and biomineralisation processes of the otoliths. Otolith growth is closely coupled to fish physiology, and metabolism can thus influence morphological differences among otoliths (Bang & Grønkvær 2005; Stransky et al. 2008).

Also, meristics parameters were significantly different between the lagoon, inlet, marine punctuated and marine unpunctuated specimens, and, thus, like previous results of Trabelsi et al. (2002a, 2004), who used metrics parameters, we were able to separate between lagoon and unpunctuated marine populations. The difference between these last two populations reached a racial level for the number of vertebrae, the number of scales on the lateral line, the number of spines on the pectoral fin and the number of upper and total gill rakers. This racial level of differentiation was also observed for the number of vertebrae, the number of scales on the lateral line and the number of spines on the pectoral when comparing unpunctuated and punctuated marine fish. Some characters allowed us to differentiate Mellah lagoon and Ziama inlet samples (number of vertebrae, number of scales on lateral line), Mellah lagoon and punctuated marine fish (number of gill rakers), Ziama inlet and marine punctuated fish (number of vertebrae, number of scales on lateral line, number of gill rakers). In some cases (unpunctuated marine - Ziama inlet and punctuated marine - Ziama inlet), these differences even reached sub-specific level considering the number of gill rakers.

Atherina boyeri as a single taxon has been questioned and rather a complex of two or three forms has been proposed. First, previous morphometric data revealed differences among populations living in the sea compared to those living in lagoons (Kartas & Trabelsi 1990; Focant et al. 1992; Trabelsi et al. 2002a, 2004). Such differences were also present between neighbouring marine populations (Trabelsi et al. 1994). Genetic variations in the cytochrome-*b* gene support the idea that *Atherina boyeri* is a taxonomic complex and suggest the presence of only two species, a marine and a lagoon one (in Greek waters, Klossa-Kilia et al. 2002, 2007; Kraitsek et al. 2008). Second, others studies also using cytochrome-*b* were concluded by the description of three species, *A. boyeri* (marine non-punctuated), *A. punctata* (marine punctuated) and *A. lagunae* (lagoon) (Trabelsi et al. 2002a, 2002b; Milana et al. 2008), corresponding respectively to the three forms described by Francisco et al. (2008): marine non-punctuated, marine punctuated and *Atherina boyeri* from lagoons. These differences were significant enough to admit that each of them reaches the specific level, supporting the idea that they should be elevated to the rank of different species and confirming the need for a systematic revision of the genus (Trabelsi et al. 2002a, 2002b; Astolfi et al. 2005; Milana et al. 2008; Francisco et al. 2008, 2011).

In our case of study, our results demonstrated at least three different phenotypes in *A. boyeri* from different habitats. It was demonstrated that the genotype of this species is capable of flexible phenotype responses and can quickly adapt the features of its morphology in environments going from freshwater to polyhaline brackish coastal and ocean waters (Henderson & Bamber 1987). The same difference is observed in other species such as, for example, *Sparus aurata* (Chaoui et al. 2001), *Dicentrarchus labrax* (Kara & Frehi 1997; Bahri-Sfar & Ben Hassine 2009) and *Lithognathus mormyrus* (Hammami et al. 2011) considering lagoon and sea habitats, or even between lagoon habitats only as in *Lithognathus mormyrus* (Hammami et al. 2013). So, considering the three-form hypothesis, we can conclude that, indeed, three phenotypes of *A. boyeri* are distinguishable. These phenotypes may have a genetic determinism linked to the ecological connectivity among the life stages (Lemaire et al. 2000; Chaoui et al. 2012).

This conclusion needs to be supported by genetic studies, particularly concerning the population from Ziana inlet, which clearly exhibits differences at all levels (somatic morphometry and otolith shape) from the other locations. It also stresses the need for a systematic revision of the *Atherina* genus.

Acknowledgements

This work is funded in part by the PHC-Maghreb research program (Partenariat Hubert Curien No. 12MDU880M). The authors thank also the Algerian Ministry for Higher education and scientific research (General directorate for scientific research and technology development, GDRSDT) which financially supported this study, within the framework of the National Funds of Research (NFR).

References

- Aguirre H, Lombarte A. 1999. Ecomorphological comparisons of sagittae in *Mullus barbatus* and *M. surmuletus*. *Journal of Fish Biology* 55:105–114.
- Astolfi L, Dupanloup I, Rossi R, Bisol P, Faure E, Congiu L. 2005. Mitochondrial variability of sand smelt *Atherina boyeri* populations from north Mediterranean coastal lagoons. *Marine Ecology Progress Series* 297:233–243.
- Bahri-Sfar L, Ben Hassine OK. 2009. Clinal variations of discriminative meristic characters of sea bass, *Dicentrarchus labrax* (Moronidae, Perciformes) populations on Tunisian coasts. *Cybiu* 33:211–218.
- Bang A, Grønkjær P. 2005. Otolith size-at-hatch reveals embryonic oxygen consumption in the zebra fish *Danio rerio*. *Marine Biology* 147:1419–1423.
- Begg GA, Overholtz WJ, Munroe NJ. 2001. The use of internal otolith morphometrics for identification of haddock (*Melanogrammus aeglefinus*) stocks on Georges Bank. *Fishery Bulletin* 99:1–14.
- Bolles K, Begg G. 2000. Distinction between silver hake (*Merluccius bilinearis*) stocks in US waters of the northwest Atlantic based on whole otolith morphometrics. *Fishery Bulletin* 98:451–462.
- Cammarata M, Mauro A, Mazzola A, Scilipoti D, Arculeo M, Parrinello N. 1996. A biochemical genetic study of isoenzyme polymorphism within and between two populations of *Atherina boyeri* Risso. *Genetika* 32:1220–1224.
- Cardinale M, Doering-Arjes P, Kastowsky M, Mosegaard H. 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 61:158–167.
- Chaoui L, Gagnaire PA, Guinand B, Quignard JP, Tsigenopoulos C, Kara MH. 2012. Microsatellite length variation in candidate genes correlates with habitat in the gilthead sea bream *Sparus aurata*. *Molecular Ecology* 21:5497–5511.
- Chaoui L, Kara MH, Faure É, Quignard JP. 2006. L'ichtyofaune de la lagune du Mellah (Algérie Nord-Est): diversité, production et analyse des captures commerciales. *Cybiu* 30:123–132.
- Chaoui L, Quignard JP, Kara MH. 2001. Différenciation morphologique de deux populations marine et lagunaire de daurade *Sparus aurata* (Linné, 1785). Rapport de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée 36:371.
- Congiu L, Rossi R, Colombo G. 2002. Population analysis of the sand smelt *Atherina boyeri* (Teleostei Atherinidae), from Italian coastal lagoons by random amplified polymorphic DNA. *Marine Ecology Progress Series* 229:279–289.
- Crampton JS. 1995. Elliptic Fourier shape analysis of fossil bivalves, practical considerations. *Lethaia* 28:179–186.

- Focant B, Rosecchi E, Crivelli AJ. 1999. Attempt at biochemical characterization of sand smelt *Atherina boyeri* Risso, 1810 (Pisces, Atherinidae) populations from the Camargue (Rhône delta, France). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 122:261–267.
- Focant B, Trabelsi M, Kartas F, Quignard JP. 1992. Caractérisation biochimique de trois populations d'*Atherina boyeri* des milieux lagunaires méditerranéens. Rapport de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée 33:94.
- Focant B, Trabelsi M, Vandewalle P, Kartas F, Quignard JP. 1993. Apport de l'analyse biochimique à la caractérisation de populations d'*Atherina boyeri* des milieux lagunaires méditerranéens. Actes du colloque Okeanos, «Le système littoral méditerranéen», Montpellier. pp. 145–148.
- Francisco SM, Congiu L, Stefanni S, Castilho R, Brito A, Ivanova PP, Levy A, Cabral H, Kiliass G, Doadrio I, Almada VC. 2008. Phylogenetic relationships of the North-eastern Atlantic and Mediterranean forms of *Atherina* (Pisces, Atherinidae). *Molecular Phylogenetics and Evolution* 48:782–788.
- Francisco SM, Congiu L, von der Heyden S, Almada VC. 2011. Multilocus phylogenetic analysis of the genus *Atherina* (Pisces: Atherinidae). *Molecular Phylogenetics and Evolution* 61:71–78.
- Frehi H, Couté A, Mascarell G, Perrette-Gallet C, Ayada M, Kara MH. 2007. Dinoflagellés toxiques et/ou responsables de blooms dans la baie d'Annaba (Algérie). *Comptes Rendus Biologies* 330:615–628.
- Friedland K, Reddin D. 1994. Use of otolith morphology in stock discriminations of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 51:91–98.
- Géry J. 1962. Le problème de la sous-espèce et de sa définition statistique. *Revue Suisse de Zoologie* 69:521–541.
- Gon O, Ben-Tuvia A. 1983. The biology of Boyer's sand smelt, *Atherina boyeri* Risso in the Bardawil Lagoon on the Mediterranean coast of Sinai. *Journal of Fish Biology* 22:537–547.
- Hammami I, Bahri-Sfar L, Ben Hassine OK. 2011. Morphological variations of striped seabream, *Lithognathus mormyrus*, populations along the Tunisian coast. *Journal of the Marine Biological Association of the United Kingdom* 91:1261–1271.
- Hammami I, Bahri-Sfar L, Kaouache M, Grenouillet G, Lek S, Kara MH, Ben Hassine OK. 2013. Morphological characterization of striped seabream (*Lithognathus mormyrus*, Sparidae) in some Mediterranean lagoons. *Cybium* 37:127–139.
- Henderson P, Bamber R. 1987. On the reproductive biology of the sand smelt *Atherina boyeri* Risso (Pisces: Atherinidae) and its evolutionary potential. *Biological Journal of the Linnean Society* 32:395–415.
- Hüssy K. 2008. Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects. *Journal of Experimental Marine Biology and Ecology* 364:35–41.
- Iwata H, Ukai Y. 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity* 93:384–385.
- Kara MH, Frehi H. 1997. Etude biométrique du loup *Dicentrarchus labrax* du Golfe d'Annaba. Différenciation d'une population lagunaire voisine. *Journal de Recherche Océanographique* 22:45–50.
- Kartas F, Trabelsi M. 1990. Sur le polymorphisme de l'athérine, *Atherina boyeri* Risso, 1810 (Atherinidae) des eaux littorales tunisiennes. *Cybium* 14:295–311.
- Kiener A, Spillmann CJ. 1969. Contribution à l'étude systématique et écologique des athérines des côtes françaises. *Mémoires du Muséum national d'Histoire naturelle - Série A: Zoologie* 60:33–74.
- Klossa-Kilia E, Papisotiropoulos V, Tryfonopoulos G, Alahiotis S, Kiliass G. 2007. Phylogenetic relationships of *Atherina hepsetus* and *Atherina boyeri* (Pisces: Atherinidae) populations from Greece, based on mtDNA sequences. *Biological Journal of the Linnean Society* 92:151–161.

- Klossa-Kilia E, Prassa M, Papatropoulos V, Alahiotis S, Kiliass G. 2002. Mitochondrial DNA diversity in *Atherina boyeri* populations as determined by RFLP analysis of three mtDNA segments. *Heredity* 89:363–370.
- Kraitsek S, Klossa-Kilia E, Papatropoulos V, Alahiotis SN, Kiliass G. 2008. Genetic divergence among marine and lagoon *Atherina boyeri* populations in Greece using mtDNA analysis. *Biochemical genetics* 46:781–798.
- Lemaire C, Allegrucci G, Naciri M, Bahri-Sfar L, Kara MH, Bonhomme F. 2000. Do discrepancies between microsatellite and allozyme variation reveal differential selection between sea and lagoon in the sea bass (*Dicentrarchus labrax*)? *Molecular ecology* 9:457–467.
- Mahé K, Fave S, Couteau J. 2011. TNPC : User guide. Version 5. Boulogne-sur-mer: Ifremer Institute. Computer Program.
- Mauro A, Arculeo M, Mazzola A, Parrinello N. 2007. Are there any distinct genetic sub-populations of sand smelt, *Atherina boyeri* (*Teleostei: Atherinidae*) along Italian coasts? Evidence from allozyme analysis. *Folia Zoologica* 56:194–200.
- Mayr E. 1969. Principles of Systematic Zoology. New York: Me Graw-Hill Book Company. 428 pp.
- Milana V, Sola L, Congiu L, Rossi A. 2008. Mitochondrial DNA in *Atherina* (*Teleostei, Atheriniformes*): differential distribution of an intergenic spacer in lagoon and marine forms of *Atherina boyeri*. *Journal of Fish Biology* 73:1216–1227.
- Parmentier E, Vandewalle P, Lagardère F. 2001. Morpho-anatomy of the otic region in carapid fishes: eco-morphological study of their otoliths. *Journal of Fish Biology* 58:1046–1061.
- Pothin K, Gonzalez-Salas C, Chabanet P, Lecomte-Finiger R. 2006. Distinction between *Mulloidichthys flavolineatus* juveniles from Reunion Island and Mauritius Island (south-west Indian Ocean) based on otolith morphometrics. *Journal of Fish Biology* 69:38–53.
- Quignard JP, Pras A. 1986. Labridae. In: Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E, editors. Fishes of the North-Eastern Atlantic and the Mediterranean. Paris: UNESCO. pp. 919–942.
- R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: <http://www.R-project.org/>. Accessed Apr 2014 22.
- Semple JC, Chmielewski JG, Leeder C. 1991. A multivariate morphometric study and revision of *Aster* subg. *Doellingeria* sect. *Triplopappus* (*Compositae: Astereae*): the *Aster umbellatus* complex. *Canadian Journal of Botany* 69:256–276.
- Smith MK. 1992. Regional differences in otolith morphology of the deep slope red snapper *Etelis carbunculus*. *Canadian Journal of Fisheries and Aquatic Sciences* 49:795–804.
- SPSS. 2004. SPSS for Windows, Version 13.0. Chicago: SPSS Inc. Computer Program.
- Stransky C, Baumann H, Fevolden SE, Harbitz HH, Nedreås KH, Salberg AB, Skartein TH. 2008. Separation of Norwegian coastal cod and Northeast Arctic cod by outer otolith shape analysis. *Fisheries Research* 90:26–35.
- Stransky C, MacLellan SE. 2005. Species separation and zoogeography of redfish and rockfish (genus *Sebastes*) by otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 62:2265–2276.
- Trabelsi M, Faure E, Quignard JP, Boussaid M, Focant B, Maamouri F. 2002a. *Atherina punctata* and *Atherina laguna* (Pisces, *Atherinidae*) new species found in the Mediterranean sea, Article 1: morphological investigations of three Atherinid species. *Comptes Rendus de l'Académie des Sciences Paris Life Science* 325:967–975.
- Trabelsi M, Gilles A, Fleury C, Quignard JP, Maamouri F, Faure E. 2002b. *Atherina punctata* and *Atherina lagunae* (Pisces, *Atherinidae*), new species found in the Mediterranean Sea. Article 2: molecular investigations of three Atherinid species. *Comptes Rendus de l'Académie des Sciences Paris Life Science* 325:1119–1128.

- Trabelsi M, Maamouri F, Quignard JP, Boussaïd M, Faure E. 2004. Biometric and molecular investigations evidenced allopatric speciation in Western Mediterranean lagoons within the *Atherina lagunae* species (*Teleostei*, *Atherinidae*). *Estuarine, Coastal and Shelf Science* 61:713–723.
- Trabelsi M, Quignard JP, Kartas F. 1994. *Atherina boyeri*: première mention en Méditerranée de deux populations marines sympatriques. *Cybium* 18:457–459.
- Trabelsi M, Quignard JP, Tomasini JA, Boussaid M, Focant B, Maamouri F. 2000. Discriminative value of the meristic characteristics of the punctuated sand smelts marine populations. *Obelia* 26:75–88.