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

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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

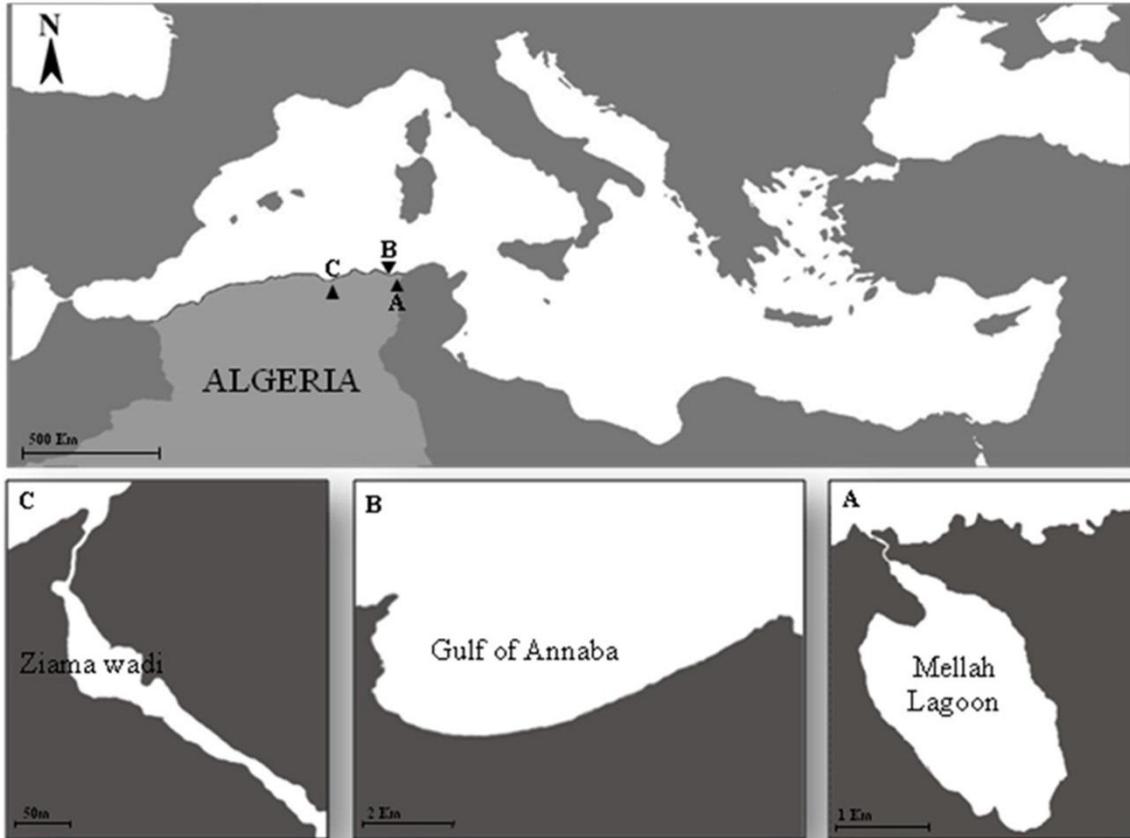
2.1. Study habitats

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.

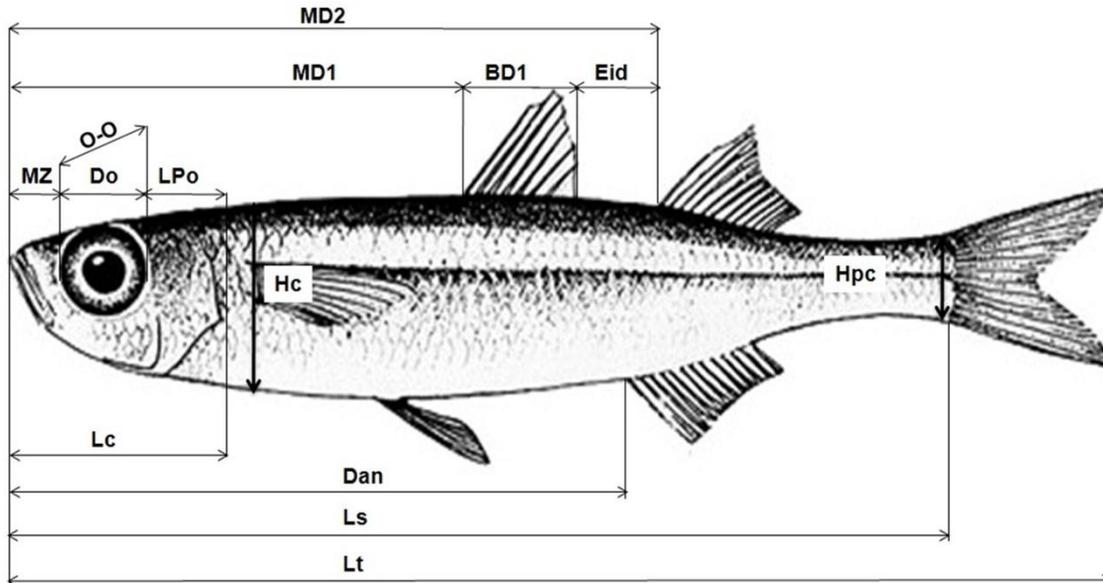
Figure 1. Sampling locations of *Atherina boyeri* in different habitats of eastern Algeria: A: Mellah lagoon ; B: Annaba Gul f ; C: Ziama inle t.



2.2. Somatic morphology

A total of 754 specimens were sampled using beach seine between March 2010 and March 2011: 269 from Mellah lagoon ($2.0 \leq Lt \leq 8.3$ cm), 194 unpunctuated ($4.6 \leq Lt \leq 13.1$ cm) and 144 punctuated ($5.4 \leq Lt \leq 10.7$ cm) from Annaba's Gulf and 147 from Ziama inlet ($2.9 \leq Lt \leq 10.2$ 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.

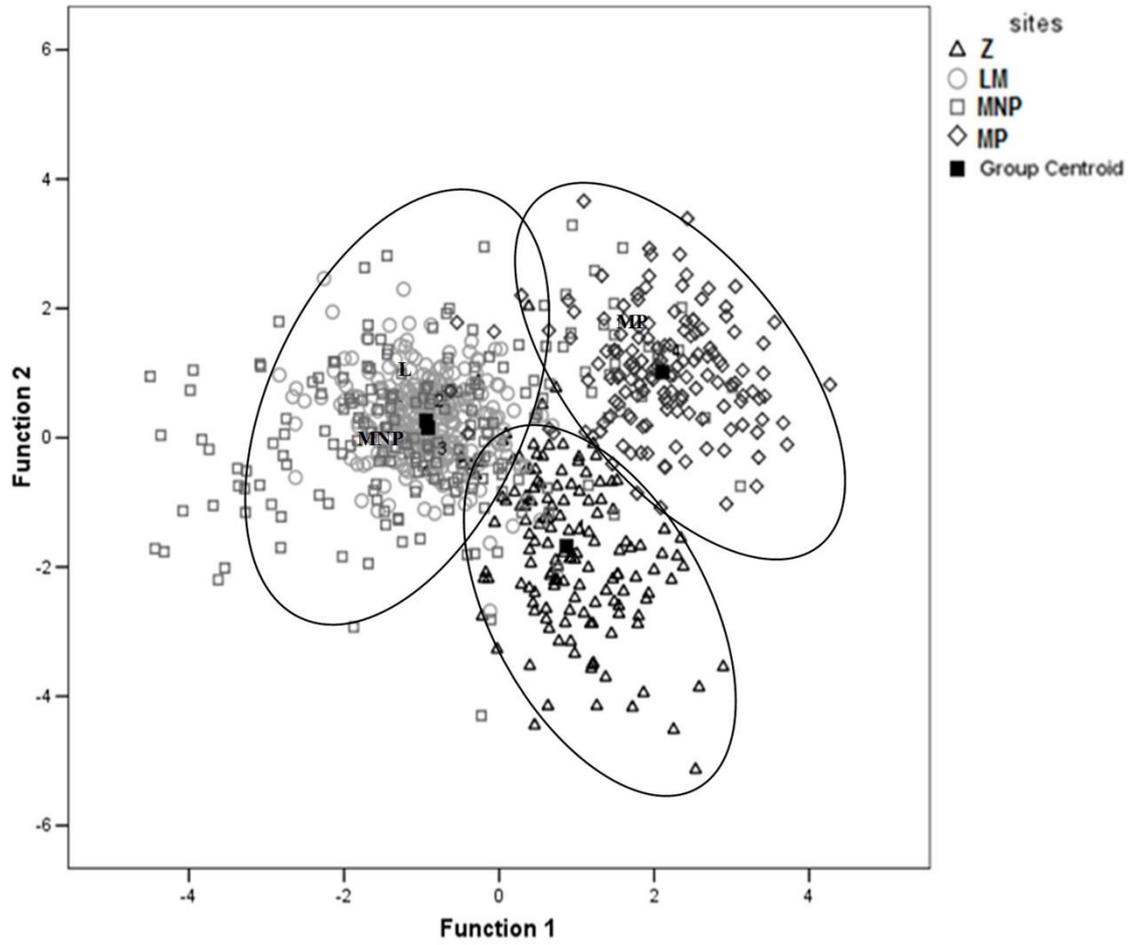


Table 1. Values of „t“ Student’s test, and the coefficient of difference „CD“ calculated from the body shape of *Atherina boyeri* between the three populations in the eastern Algeria (southern Mediterenaen Sea).

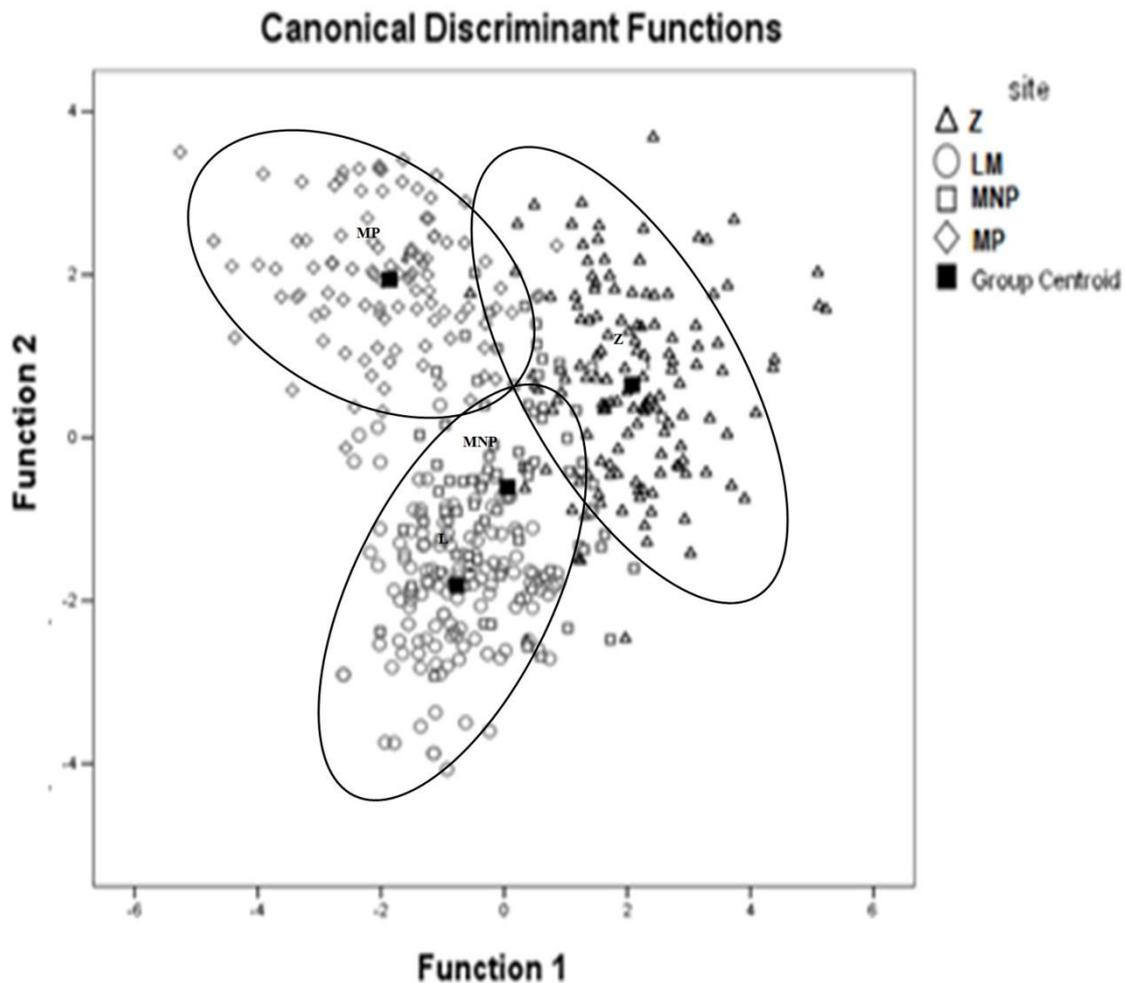
Character s	LM - Z		LM – MP		LM – MNP		Z – MP		Z - MNP		MNP – MP	
	t	C.D.	t	C.D.	t	C.D.	T	C.D.	t	C.D.	t	C.D.
D1	3.07	0.15	2.05	0.10	9.14	0.44	0.74	0.04	11.06	0.60	9.95	0.54
D2	8.39	0.44	0.75	0.03	3.26	0.15	8.05	0.47	4.80	0.26	3.47	0.18
P	10.46	0.53	1.21	0.06	15.40	0.73	10.21	0.59	4.48	0.24	14.56	0.79
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) ; Zياما 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 Zياما 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 Zياما 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, meristic 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 Ziamia 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).

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