

Ecological Plasticity of Apollonia melanostomus (Pisces, Gobiidae) from its Main Habitat Types in Bulgaria

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Abstract. Three native populations of *A. melanostomus* from different Bulgarian habitats were analysed, in view to their morphologic, biochemical-genetic variability and resistance in salinity alterations. Freshwater specimens can survive when fresh water (0‰) is changed rapidly by Black Sea water (16-18‰) and marine vice-versa. In view of the salinity resistance *A. melanostomus* is evaluated as tolerant. Eleven non-enzymatic and 16 enzymatic loci were tested as genetic markers for population identification. Most of them were monomorphic for all populations analysed, with exception of esterases and malate dehydrogenase. These two polymorphic enzyme systems could be used for further analyses of population structure of *A. melanostomus*. The genetic diversity D_{Nei} compared between three populations was low - 0.005-0.016 and the sample from Durankulak occurred to be more genetically divergent. The morphological and biochemical-genetic variability characteristics of the studied populations do not show correlation, but independently vary according to different environmental factors.

Key words: *Neogobius (Apollonia) melanostomus*, genetic markers, morphological variability, invasive species, Black Sea.

Introduction

The round goby *Apollonia melanostomus* Pallas (1814) represents a Ponto-Caspian relict, inhabiting natively Black, Azov and Caspian Seas, as well as rivers, coastal lakes and lagoons connected with these water basins (PINCHUK *et al.*, 2003). In Bulgarian language it is known as “strongyl”. In its native areal it is a significant target species for the artisanal and commercial fishery (MOSKALKOVA, 1996) and together with other gobiids, it represents an important component of sub-littoral fish communities. The round goby is also a well-known inhabitant of the Bulgarian Danube, although in the past it was comparatively rare (MARINOV, 1966).

Although *A. melanostomus* was never attained high population densities in the lower Danube, it is expanded its range to the middle and even upper Danube (ANHELT *et al.*, 1998); STRÁŇAI & ANDREJI, 2004). In the period since 1990, the round goby has extended widely, it has spread through Europe and even North America (PINCHUK *et al.*, 2003; CORKUM *et al.*, 2004; SAPOTA, 2004). The success of the species as an invader has been attributed mainly to its ecological plasticity (HÓRKOVÁ & KOVÁČ, 2014).

A plethora of articles has been published concerning various aspects of the biology and ecology, physiology, behaviour and genetics of the round goby (CORKUM *et al.*, 2004;

CHARLEBOIS *et al.*, 1997). Morphometric comparison of the species from lower and middle Danube was accomplished by POLAČIĆ *et al.*, 2012). Genetic analysis by the aims of enzymatic electrophoresis was performed by DOBROVOLOV *et al.* (1995); WALLIS & BEARDMORE (1984a, b); THACKER & ROJE (2011), etc. Variations in reproductive parameters observed in non-native *A. melanostomus* demonstrate the species' phenotypic plasticity, which appears to be an important attribute that helps potential invaders to establish new populations in unknown environments (HORKOVÁ & KOVÁČ, 2014). The available huge amount of information demonstrates the dynamics of the species' populations, as well as their plasticity, and reminds that a population is a constantly changing system. However, the corresponding prediction for the relationship between heterozygosity and morphological variability among populations and species is unclear, and conflicting hypotheses have been suggested (ZINK *et al.*, 1985).

The objective of this study was to evaluate the ecological plasticity of *A. melanostomus* from selected areas representing the three main habitat types of the species in Bulgaria on the basis of salinity resistance, morphological and biochemical-genetic data: Danube River, Black sea and a coastal brackish lake. These water bodies differ in their basic hydrology and morphology features including water current, depth and substratum, also in salinity. The followed key assumption was that the possible differences in variation reflect the natural response of the round goby to variable environmental features. Their analysis will also reveal more information on the population dynamics of this highly invasive species.

Materials and Methods

The material was collected from different model ecosystem types: Black Sea, a coastal lake, as well as Danube River during the period 2008–2011 (Fig 1). Part of it was fixed in 4% formaldehyde solution for morphological or frozen at -20°C for genetic analyses.

Adaptation. Salinity adaptation was studied in aquariums with 30 marine and 30 brackish specimens from Black Sea and

Durankulak. 1/4 of the initial salinity was lowered daily in a marine aquarium, until it became freshwater. In a second aquarium salinity was decreased to 1/2 and in a third a rapid exchange of Black Sea water (17‰) with freshwater was performed. The exact opposite operations were performed with the freshwater specimens. The water was tempered before every change; salinity was measured and recorded. Behavior and external morphology was examined two times per day for a ten days period after every manipulation.

Morphometrics. Measurements of 23 continuous external morphological characters from fish pictures were accomplished by the aims of digitizing software as described by SIMONVIĆ (1999) with abbreviations presented in Table 1.

Genetics. Proteins and enzymes were separated by horizontal starch gel electrophoresis according to SMITHIES (1955) methods, modified by DOBROVOLOV (1973). Besides it, isoelectric focusing (IEF) on thin polyacrylamide Ampholone gel with 3.5-10.0 pH gradients was applied, as well as IEF on ultra-thin polyacrylamide Servalyte gel plates provided by LKB (Stockholm, Sweden) (Fig 2). The proteins were stained with Commassie Brilliant Blue R-250. Staining of different enzymes was undertaken according to SHAW & PRASAD (1970). Buffer systems described by DOBROVOLOV (1976) and CLAYTON & GEE (1969) were used for the electrophoresis. The nomenclature of mentioned loci and alleles followed the recommendation of SHAKLEE *et al.* (1990). Five enzymatic systems were studied: alcohol dehydrogenase (EC 1.1.1.1. - ADH), esterase (EC 3.1.1.1 - EST), lactate dehydrogenase (EC 1.1.1.27 - LDH (EC 1.1.1.37 - MDH), malic enzyme (EC 1.1.1.40 - MEP), superoxide dismutase (EC 1.15.1.1 - SOD), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8.- G3PDH).

Statistics. Measurements were transformed to ratios, in order to avoid allometric effects, although some basic head lengths do not change according to age SIMONVIĆ *et al.* (2001). Normality tests for the measured morphometric variables were performed according to SHAPIRO & WILK (1965). Principal Component Analysis (PCA) was used in order

to establish which of the measured characters are most flexible. Statistics was computed according to HAMMER *et al.* (2001). The experimental allelic frequencies were tested for deviations with the Hardy-Weinberg equilibrium (HARDY, 1908; WEINBERG, 1908). Intrapopulation genetic diversity H_j was also

calculated. Calculation of genetic similarity and genetic distance indices was performed according to NEI (1972). Phylogenetic analysis was done using PAUP, version 4.0 SWOFFORD (1998), visualized with "TreeView" v1.6.6. The morphometric Euclidean distances were calculated according to CULMAN *et al.* (2009).



Fig. 1. Sampling stations: 1 - Danube, Belene 2 - Durankulak Lake, 3, Black Sea, Sinemorets.

Results

Salinity adaptation

The accomplished in aquariums experiments showed that all examined specimens of *A. melanostomus* from a brackish environment are capable to adapt without visual consequences in behaviour or external morphology (white eyes or other abnormal characteristics) when freshwater (0‰) is changed rapidly by Black Sea water (16-18‰). Marine specimens also survived in freshwater without preliminary adaptation in intermediate salinities.

Morphometric analysis

The investigated specimens from the three selected model ecosystems showed comparatively low rates of variability. The Danube group occurred to be the most variable (Table 1). A Principal Component Analysis based on the measured traits (Fig. 4) configures three not well separated groups, in accordance with their site of origin. Most distinctive traits for the three

independent groups' delimitation are praeanal length, length to anus and praeopercular width respectively in significance.

The calculated Euclidean distances among the three examined groups (Fig. 5) showed, that the specimens from Durankulak Lake are slightly more distinct from the others (Danubian and marine).

Genetic-biochemical analysis

Eleven non-enzyme protein loci were visualized. In Varna Bay and Shkorpilovtsi region (Black Sea) *A. melanostomus* showed completely different position of *PROT-4**, *PROT-5**, *PROT-6** and *PROT-8** loci in comparison with the other two (freshwater and brackish) samples (Fig 2).

Totally 16 enzyme loci were analyzed and polymorphism was found in three of them (Table 2). *EST-3** and *EST-4**- loci (Fig 3), as well as *MDH-2** were polymorphic, with allelic frequencies given in Table 2. The other studied enzyme systems correspond-

ing to 13 loci (LDH, SOD, MEP, ADH and β -GPDH) were invariant.

All the examined Black Sea specimens were monomorphic in regard to the analyzed enzymatic systems. The sample from Durankulak occurred to be more genetically divergent (Fig 6).

The intrapopulation genetic diversity H_j of the examined groups occurred to be relatively low, varying from 0 to 0.041 (Table 2).

Table 1. Estimated allelic frequencies of polymorphic loci of *A. melanostomus* from different Bulgarian localities: a*- slower (anodal), and b*- faster (cathodal) electromorph. H_o = observed heterozygosity, H_e = expected heterozygosity.

Locus	Allele	Danube River Belene N=10	Durankulak Lake N=12	Black Sea N=42
<i>EST-3*</i>	a*	0.7	1	1
	b*	0.3	0	0
	H_o	0.2	0	0
	H_e	0.42	0	0
<i>EST-4*</i>	a*	1	0.6	1
	b*	0	0.4	0
	H_o	0	0.4	0
	H_e	0	0.48	0
<i>MDH-2*</i>	a*	0.917	0.9	1
	b*	0.083	0.1	0
	H_o	0.2	0.2	0
	H_e	0.152	0.18	0
Percentage of polymorphic loci	P	0.125	0.125	0
Intragroup genetic diversity H_j		0.036	0.041	0

Discussion

In view of the salinity resistance test and taking in mind the established thermal tolerance of the species round goby could be assumed as *superinvasive* CROSS & RAWDING (2009).

The specimens from Black Sea (Bulgarian Black Sea coast) were the most genetically conservative, as being monomorphic in view to the analysed enzymes. The majority of the analyzed enzyme systems (LDH, SOD, MEP, ADH

and β -GPDH) are monomorphic for all analyzed populations. Only the high variable system of esterases represents a useful tool for further investigation of the populations' structure of the species. The established comparatively low rates of genetic variability of *A. melanostomus* (0 - 0.041) correspond with the results of WALLIS & BEARDMORE (1984a, b), and these of SMITH & FUJIO (1982).

Durankulak Lake was connected periodically with Black Sea till 60's, so *A. melanostomus* could migrate from the Sea to the lake and vice versa - in case of populations' admixture. This isolation was enough for a genetic distance D_{Nei} = 0.011 between the marine and the lacustrine sample - a higher divergence between this and the marine group, when comparing both with the Danubian one (Table 3). From another point of view, two groups from recently separated ecosystems (Durankulak and Black Sea) are also more morphologically divergent, than the other two analyzed combinations among the analyzed groups (Fig 5). The conclusions of BROWN & STEPIEN (2008) concerning higher established variation in mixed populations could explain this fact, as well as other hypotheses (environmental impact), which should be supported by additional evidence. Morphological traits can be changed according to the environmental factors, mainly the concrete trophic ecology of a species (WOOTTON, 1990; ADAMS *et al.*, 2003; MURAKAEVA *et al.*, 2003). The probability of trophic habits' impact on morphology in this case has to be clarified.

Linear correlation between genetic and morphologic distances between the compared pairs of the round goby from different habitats given in Table 3 was rather strong, but not significant ($R=0.73$, $p=0.4$). The obtained results are based on 3 groups only, but still could not confirm Lerner's postulated association between individual heterozygosity and morphological variability if simply extrapolated to higher levels of organization: a negative correlation between allozymic and morphological variance should be observed among populations and higher taxa (LERNER, 1954). Indeed, any

particular measures of genetic and phenotypic variability will not be linearly or even monotonically related to one another (STRAUSS, 1989). Due to the lack of a theore-

tical expectation and many possible confounding factors, the independence of genetic and morphological variances should be by accepted by definition (ZINK *et al.*, 1985).

Table 2. Morphologic variability of three *A. melanostomus* populations from Bulgaria.

N	Description	Belene	Durankulak	Black
		(Danube)	lake	Sea
		N=29	N=32	N=32
		CV	CV	CV
1	Head length	2.21	1.14	2.32
2	Height at the beginning of the first dorsal fin	1.24	0.5	0.89
3	Height at the beginning of the second dorsal fin	1.5	0.53	1.55
4	Height at caudal peduncle	0.47	0.19	0.24
5	Caudal peduncle length	2.13	1.48	1.81
6	Praedorsal length to the first dorsal fin	0.6	0.92	0.37
7	Praedorsal length to the second dorsal fin	1.17	1.19	0.74
8	Praeanal length	2.02	2.23	2.19
9	Praeventral length	3.14	1.09	1.37
10	Jaws length	1.22	0.38	0.82
11	Length from the beginning of the first dorsal fin to anus	1.6	0.66	0.84
12	First dorsal fin length	1.04	0.32	0.75
13	Second dorsal fin length	3.73	2.68	1.63
14	Anal fin length	3.83	2.98	1.95
15	Pectoral fin length	5.92	2.51	3.58
16	Praeocular length	0.74	0.38	0.77
17	Eye diameter	0.41	0.53	0.41
18	Postocular length	1.18	0.55	0.91
19	Head height	1.31	0.51	1.61
20	Jaws width	1.79	0.73	1.97
21	Praeopercular width measured ventrally	2.73	0.89	1.82
22	Ventral fin length	2.06	1.42	0.97
23	Length to anus measured ventrally	6.53	1.99	5.61
24	Interocular length	0.58	0.22	0.64
25	Praeopercular length measured dorsally	0.7	0.98	0.81
26	Opercular length measured dorsally	0.99	1.06	1.29
Average morphologic variability		1.96	1.08	1.46

Table 3. Genetic identity/distances according to Nei and morphological Euclidean distances between three *A. melanostomus* groups from Bulgaria. (BS-Black Sea, Dk-Durankulak Lake, DB-Danube, Belene).

	D-B/Dk	D-B/BS	Dk/BS
Genetic identity I_{Nei}	0.984	0.995	0.989
Genetic distance D_{Nei}	0.016	0.005	0.011
Euclidean morphological distance	1.6013	1.3025	1.72

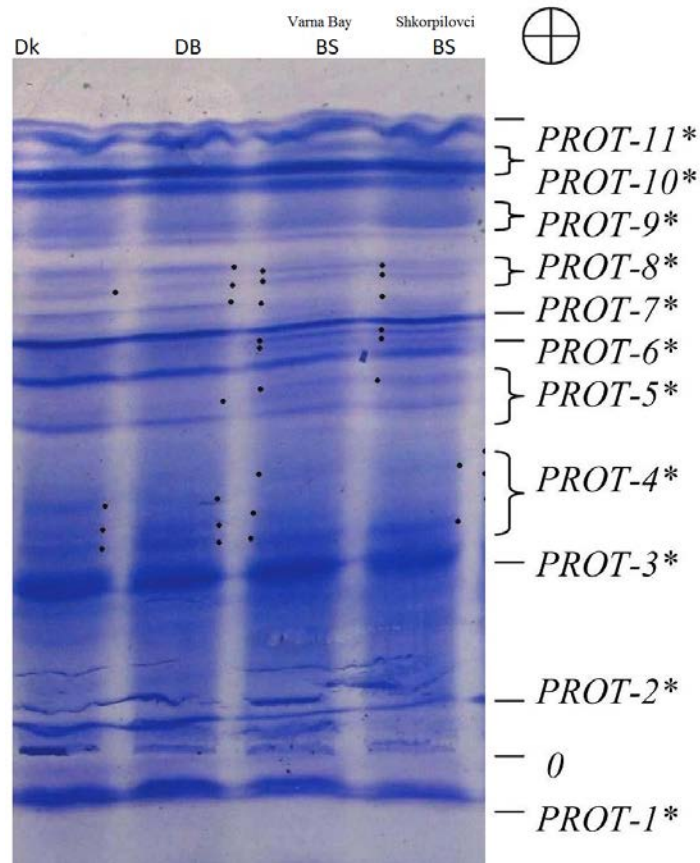


Fig. 2. Isoelectric focusing (IEF) of non-enzyme protein systems on thin polyacrilamide Ampholine gel plate with pH range 3-10 of *A. melanostomus* from different Bulgarian localities: Dk-Durankulak coastal lake, DB-Belene (Danube River), BS-Varna Bay (Black Sea), BS-Shkorpilovci (Black Sea). The differences were marked with black spots, 0 - origin.

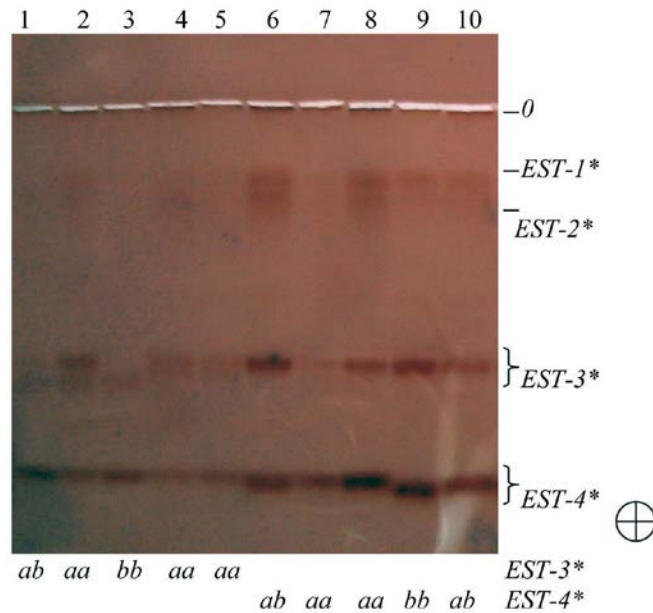


Fig. 3. Electrophoregrams of esterases (EST) on starch gel of *A. melanostomus* from different Bulgarian localities: 1-5-Belene (Danube R.), 6-10-Duranculak lake. EST-3* and EST-4* - polymorphic loci and phenotypes are given under the picture, 0- origin.

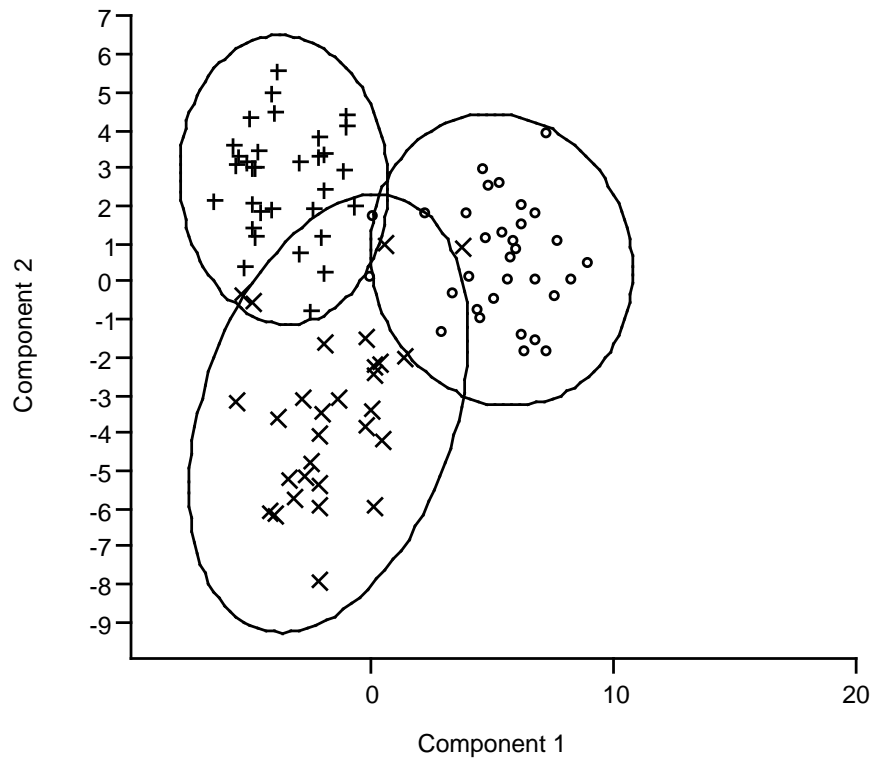


Fig. 4. Comparison of three Bulgarian *A. melanostomus* groups on the basis of 26 morphometric traits. + - Black Sea, o - Durankulak coastal Lake, x - Danube River.

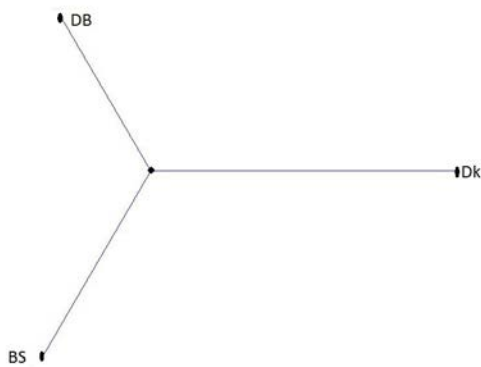


Fig. 5. Euclidean morphologic distances between three *A. melanostomus* groups from Bulgaria on the basis of 26 morphometric traits. (BS-Black Sea, Dk-Durankulak Lake, DB-Danube, Belene).

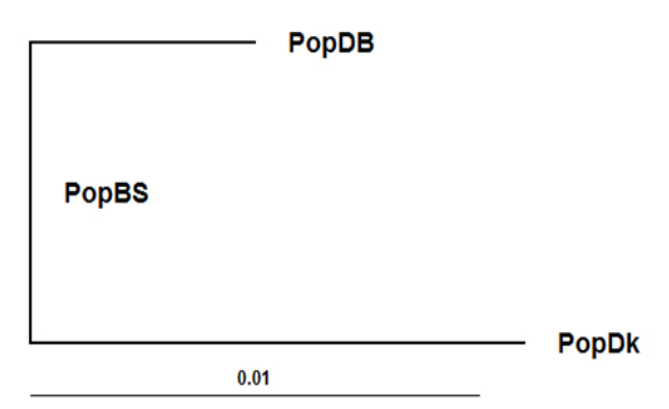


Fig. 6. Genetic distances between three *A. melanostomus* groups (BS-Black Sea, Dk-Durankulak Lake, DB-Danube, Belene), from Bulgaria on the basis of the allelic frequencies of 16 enzymic loci.

Morphologic variability could not be viewed in the “marine-freshwater” axis in this particular case, as well as the correspondence of the environmental salinity with the genetic variability of each group. These data are controversial to SIMONOVIC *et al.* (2001), as only praeanal length is most conservative in the freshwater sample, in comparison with the brackish and marine ones.

On the basis of the obtained results it could be concluded that *A. melanostomus* has a remarkable ability for colonization of new habitats of variant salinities from 0 to 170/00. The morphological and biochemical-genetic variability of the species do not show connection, but independently vary according to different environmental factors (salinity, water velocity, depth, and hydrology). Further comparative research of more localities where the species occurs as well as ecology of mixed populations is essential should be done, in order to receive a better picture of its population structure.

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