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# Morphometric comparison of native and non-native populations of round goby *Neogobius melanostomus* from the River Danube

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**Abstract.** The external morphology of native (Bulgarian) and non-native (Slovak) populations of *Neogobius melanostomus* was examined using standard distance-based measurements and, in addition, comparison of fin ray number between populations was made. Five of the 29 morphological characters measured differed between populations, both for males and females. The non-native population was characterised by a smaller mouth and eye, shorter pre-dorsal distance and greater inter-orbital distance. Three characters, post-orbital distance, head depth and minimal body depth, differed between populations only in females, though the overall trend was the same in both sexes. A lower head depth value, greater post-orbital distance and minimal body depth was recorded for females from the non-native population. The range in fin ray numbers was similar between native and non-native populations. Populations differed, however, in the frequency of pectoral fin rays. Individuals with 17 pectoral fin rays were more common in the non-native range, their proportion in the native range being low. In contrast, individuals with 19 pectoral fin rays occurred more commonly in the native range. This difference was consistent for both sexes. The differences between populations are discussed within the context of differing environments and the “ship transport” hypothesis.

**Key words:** plasticity, invasive species, morphological shift

## Introduction

The invasive character of the spreading of Ponto-Caspian gobies into upstream reaches of the River Danube has stimulated a number of studies aimed mainly at their distribution (Erős et al. 2005, Jurajda et al. 2005, Wiesner 2005), life history strategies (e.g. Lavinčiková & Kováč 2007) or ecology (e.g. Simonović et al. 2001, Copp et al. 2008). Currently, it is generally believed that their range expansion in recent decades has been, at least to some extent, human-facilitated (Copp et al. 2005) and that ship transport (via ballast water) was the main vector of introduction of distinct goby populations into the middle and upper Danube in the 1990's (Ahnelt et al. 1998, Wiesner 2005, Polačik et al. 2008).

Of the invasive goby species, the round goby *Neogobius melanostomus* (Pallas, 1814) has become

most widespread, causing detrimental effects on local ecosystems, especially on the North American continent (e.g. Corkum et al. 2004). Further, in its non-native range in the upstream reaches of the Danube, *N. melanostomus* now outnumbers other invasive goby species present (Copp et al. 2008), underlining its importance as the species with, potentially, the most profound ecological impact on this river (Jurajda et al. 2005).

Though range expansion has taken place along the same river course, the native (lower Danube) and non-native ranges (middle or upper Danube) of invasive gobies (Ahnelt et al. 1998) differ markedly regarding environmental conditions, and this may play an important role in their biology. For example, in the upstream sections of the river there is a high availability

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of the coarse bottom substrates with interstitial spaces preferred by *N. melanostomus* (e.g. Jurajda et al. 2005, Erős et al. 2005, Moog et al. 2008, Polačik et al. 2008), whereas lower sections are dominated by fine substrata (Moog et al. 2008, Polačik et al. 2008). Both ranges also differ in terms of food resources for *N. melanostomus*, which feed primarily on benthic invertebrates (Pinchuk et al. 2003). In the Danube, the abundance of non-mollusc macrozoobenthos decreases in a downstream direction, while the abundance of molluscs increases in the lower stretch of the river (Literáthy et al. 2002, Polačik et al. 2009).

Suitable environmental conditions upstream of its native range have resulted in thriving non-native populations of *N. melanostomus*. In the upper and middle Danube, the fish have been found to reach higher population densities, larger size, to grow faster and to have better somatic condition, presumably as a consequence of increased feeding on their preferred food, non-mollusc macrozoobenthos (Adámek et al. 2007, Copp et al. 2008, Polačik et al. 2008, Polačik et al. 2009).

In fishes, a change in diet and/or habitat use is often reflected in morphology, a process commonly termed resource polymorphism (e.g. Smith & Skúlason 1996). Within species, major morphological changes due to a combination of phenotypic plasticity and genetic change have been detected in a period as short as two decades. Such a morphological shift was documented in a Lake Victoria (Africa) population of *Haplochromis pyrrhocephalus* (Witte & Witte-Maas, 1987), which had undergone a population bottleneck. In addition to a dietary shift, changes in both internal and external morphology were reported for this cichlid species following increased eutrophication of the lake (Witte et al. 2008).

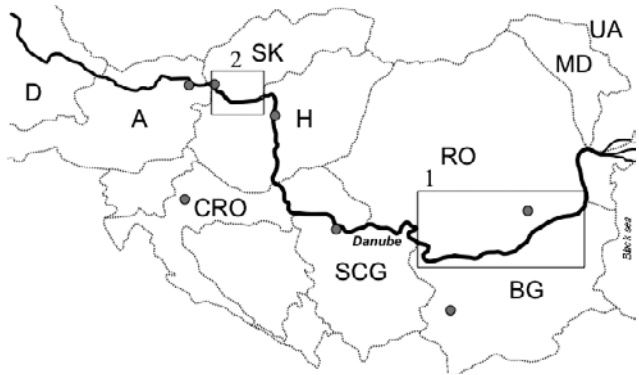
Goby expansion in the Danube offers an analogous example as the duration of their range expansion is similar (Copp et al. 2005) and, presumably as a result of ship transport of a limited number of founder individuals, the non-native populations have undergone a genetic bottleneck (Brown & Stepien 2008).

One of the traits of a successful invader is the ability to produce different phenotypes under different conditions (e.g. Záhorská et al. 2009). In this study, we investigated whether there was any change in external morphology of two distinct *N. melanostomus* populations (native and non-native) following the species' expansion into a new environment. Specifically, we compared the external morphology of *N. melanostomus* from the lower Danube in Bulgaria (native range) with the non-native population from the upper Danube in Slovakia.

## Material and Methods

### Material

In 2004 and 2005, Slovak (r. km 1706-1873) and Bulgarian (r. km 376-836) stretches of the Danube were sampled to obtain specimens of non-native and native populations of *N. melanostomus*, respectively (Fig. 1). The fish were captured using backpack electrofishing gear and through beach seining. Fish were stored in 4 % formaldehyde for at least one month before further processing.



**Fig. 1.** Course of the River Danube with sampling locations indicated. 1 – native range, 2 – non-native range.

During ontogeny in *N. melanostomus*, only a minority of morphometric characters show non-abrupt linear growth (Ľavrinčíková et al. 2005). In order to avoid problems linked to non-isometric growth, which could potentially emerge when comparing fish samples of different size (Kováč & Syriová 2005), only fish between 70-80 mm SL (no break point in measured characters; Ľavrinčíková et al. 2005) were chosen (Table 1). In these fish, 29 morphometric and five meristic characters were measured and counted by the same person following Holčík et al. (1989) (Tables 2 and 3). Morphometric characters were measured to the nearest 0.1 mm using a digital calliper and all measurements for bilateral characters were taken from the left side of the body.

Throughout any given year, the overall shape of a fish's body, and hence values for morphometric characters, may vary due to a change in gonad size (Ibanéz-Aguirre et al. 2006). As *N. melanostomus* from the two ranges differed in mean size (Polačik et al. 2008), only a limited number of fish collected overlapped in size. Thus, to obtain an adequate sample size for statistic analysis, our samples were partly composed of fish collected from both before and after the spawning season (Table 1). Therefore, selected body shape characters prone to the effects of changes in gonad size (i.e. body depth, body

**Table 1.** Mean standard length (SL), mean total weight and number of fish sampled between 2004 and 2005 from the native (Bulgarian) and non-native (Slovak) ranges of *N. melanostomus*. M = males, F = females.

population	sex	mean SL (mm)	SD	mean total weight (g)	SD	Oct 2004	Apr 2005	Jul 2005	Oct 2005	Total n
native	M	73.9	2.9	9.4	1.3	-	14	6	11	31
	F	74.3	2.6	10.2	1.4	-	21	9	-	30
non-native	M	75.2	2.8	10.9	1.3	16	14	-	-	30
	F	75.0	2.8	11.2	1.3	46	-	-	-	46

**Table 2.** Mean ( $\pm$  SD) morphometric characteristic measurements (presented as % of SL) in native and non-native populations of *N. melanostomus*. Sig. = significant difference between the two populations; significance level = 0.001. \*\*\* = significance  $P < 0.001$ . D1 = first dorsal fin, D2 = second dorsal fin, A = anal fin, P = pectoral fin, C = caudal fin.

Characteristic (as % of SL)	Code	males					females				
		native		non-native		Sig.	native		non-native		Sig.
		mean	SD	mean	SD		mean	SD	mean	SD	
head length	lc	29.1	0.8	28.4	0.8		28.5	0.8	28.5	0.8	
head width	lac	22.2	0.9	22.2	0.7		21.7	0.6	21.9	0.7	
head depth	hc	19.8	0.8	19.5	1.1		19.5	0.7	18.8	0.8	***
upper jaw length	lmx	9.0	0.5	8.4	0.4	***	9.0	0.7	8.3	0.4	***
lower jaw length	lmd	10.1	0.5	9.4	0.3	***	9.8	0.6	9.3	0.4	***
pre-orbital distance	pro	10.1	0.6	9.8	0.5		9.9	0.6	9.9	0.6	
post-orbital distance	poo	13.5	0.6	13.8	0.5		12.9	0.7	13.6	0.6	***
eye diameter	oh	7.8	0.6	7.0	0.4	***	7.8	0.5	7.1	0.3	***
inter-orbital distance	io	4.1	0.4	4.6	0.5	***	4.1	0.5	4.7	0.4	***
body width*	laco	17.9	1.3	17.9	1.1		18.8	1.4	18.5	0.9	
body depth*	H	21.6	1.4	21.9	1.0		22.3	1.1	22.4	1.0	
minimum body depth	h	10.4	0.6	10.8	0.4		10.3	0.5	10.8	0.5	***
length of c. peduncle	lpc	20.8	1.1	21.3	0.9		20.8	0.9	20.8	1.2	
caudal peduncle depth	hpc	12.4	0.8	13.0	0.8		12.3	0.5	12.6	0.6	
pre-dorsal distance	pD	35.5	0.9	34.7	0.9	***	35.7	0.7	34.6	0.8	***
pre-ventral distance	pV	31.6	0.9	31.0	1.1		31.8	0.9	31.4	0.9	
pre-anal distance	pA	56.1	0.9	55.7	1.1		57.2	1.2	56.3	1.2	
P-V distance	p-v	15.9	0.9	16.0	0.6		16.7	0.9	16.4	0.7	
V-A distance	v-a	26.3	1.1	26.3	1.2		27.1	1.1	26.8	11.1	
D1 fin base length	lD1	17.6	0.9	17.3	1.0		17.8	1.2	17.3	1.0	
D2 fin base length	lD2	31.4	1.1	31.7	1.9		31.0	0.9	31.2	1.2	
D1 fin depth	hD1	15.1	1.1	15.4	1.1		14.7	1.1	14.9	1.0	
D2 fin depth	hD2	14.7	1.0	14.7	0.9		14.4	0.9	14.3	1.2	
A fin base length	lA	24.7	1.1	24.3	1.3		24.0	1.4	24.4	1.6	
A fin depth	hA	12.1	0.9	12.3	0.9		11.8	1.0	11.5	1.2	
P fin length	lP	23.5	0.8	23.3	1.1		23.4	0.8	23.2	1.1	
V fin length	lV	20.5	1.0	19.9	1.6		20.1	1.1	19.7	1.0	
C fin length	lcaud	20.0	0.7	19.6	1.5		19.2	0.9	18.7	0.9	
urogenital papilla length	upl	4.4	1.0	4.0	1.5		4.0	0.3	3.8	0.5	

\* Excluded from comparison.

width, pre-ventral distance, P-V distance, and V-A distance; Table 2), or reported by other authors to change during reproduction (length of pectoral fin; Pinchuk et al. 2003), were tested for inter-seasonal differences within a population sample (Bonferroni corrected ANCOVA, significance level  $\alpha' = 0.008$ ). The tests indicated that body depth and body width were significantly influenced by season ( $P < 0.001$ ) and, therefore, these two characters were excluded from all subsequent analyses.

In addition to morphometric characters, selected meristic data were also obtained. Fin rays in five fins (D1, D2, P, V, A; see Table 2 for abbreviations) were counted using a binocular microscope. "The number of fin rays" refers to all rays in a fin, regardless of whether they are branched or unbranched.

To compare somatic condition between populations, Fulton's condition coefficient was calculated according to Anderson & Neumann (1996):  $K = (W/L^3) \times 100\,000$  where  $W$  = eviscerated body weight and  $L$  = standard length.

#### Data analysis

No sexual dimorphism has previously been reported regarding the external morphology of examined populations (Simonović et al. 2001, L'avrínčiková et al. 2005). Our sample size, however, allowed us to treat males and females separately in all analyses. The distinction among sexes lends greater credibility to the results, provided that a difference is found in both sexes independently.

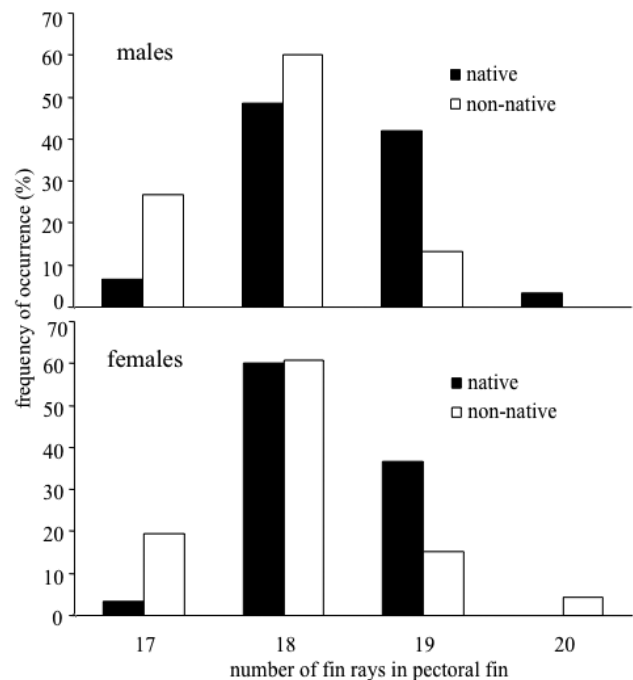
Since the parametric analyses used in this study are sensitive to the influence of outliers, we excluded outliers (caused by deformations in the preserved material) to avoid any possible analysis bias (Almeida et al. 2008). Six data points (three individuals from each population) were subsequently excluded from parametric analyses.

As morphometric characters were linearly related to fish size (SL; Pearson correlations all  $P < 0.05$ ), multivariate analysis of covariance (MANCOVA) was employed to test for differences in morphometric characters between the two population samples. All morphometric data complied with MANCOVA requirements (i.e. normal distribution, linearity, and homogeneity of variance and covariance).

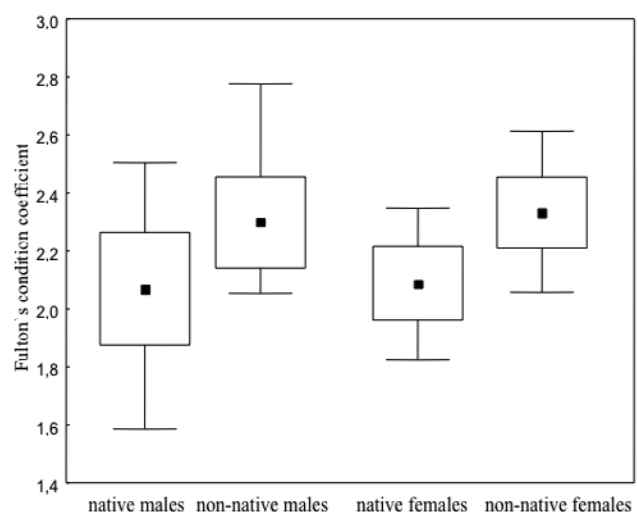
The ANCOVA univariate test was applied after multivariate testing in order to determine those characters accounting for differences between population samples. Fin ray number in adult fish is unlikely to be influenced by fish size. F-tests were employed to test for possible variance in fin ray number between native and non-

native populations. Chi-square tests were then used to reveal differences in the frequencies of fin ray counts between the surveyed populations.

In order to decrease the probability of committing a type I error, the significance level was corrected using the Bonferroni method, thus decreasing the significance level in multiple testings to  $\alpha = 0.00185$  and  $\alpha = 0.01$  for morphometric and meristic characters, respectively.



**Fig. 2.** Frequency of occurrence of number of pectoral fin rays in native and non-native populations of *N. melanostomus*.



**Fig. 3.** Fulton's condition coefficient for native and non-native populations of *N. melanostomus*. The centre point denotes the mean, the box denotes SD, and the whiskers denote the range.



**Table 3.** Fin ray number in native and non-native populations of *N. melanostomus* according to sex. Sig. = significance of Chi-square test, significance level = 0.01. \*\* & \*\*\* = significant differences at  $P < 0.01$  and  $P < 0.001$ , respectively. Fin abbreviations explained in Table 2.

Fin	native males			non-native males			Sig.	native females			non-native females			Sig.
	mean	SD	range	mean	SD	range		mean	SD	range	mean	SD	range	
D1	6.0	0.0	6-6	6.0	0.0	6-6		6.0	0.2	5-6	6.0	0.0	6-6	
D2	14.9	0.6	14-17	14.9	0.6	13-16		14.8	0.5	14-16	14.8	0.5	13-16	
P	18.4	0.7	17-20	17.9	0.6	17-19	***	18.3	0.5	17-19	18.0	0.7	17-20	**
V	12.0	0.0	12-12	12.0	0.2	11-12		12.0	0.0	12-12	11.9	0.4	10-12	
A	12.1	0.6	11-13	12.0	0.6	11-14		11.8	0.6	11-13	11.8	0.6	10-13	

In both sexes and both populations, Fulton's condition coefficient indicated normal distribution and homogeneity of variances between the compared groups and, therefore, parametric t-tests were applied to discover whether the differences were statistically significant.

## Results

In both males and females, external morphology differed significantly between native and non-native populations (both MANCOVA,  $P < 0.001$ ).

Five morphometric characteristics accounted for the between-population differences in external morphology: upper jaw length, lower jaw length, eye diameter, inter-orbital distance and pre-dorsal distance (ANCOVA,  $P < 0.001$ ; Table 2). Significant differences in these morphological characteristics were also found in both sexes. Three characteristics – head depth, post-orbital distance, and minimum body depth – differed significantly only in females. The non-native *N. melanostomus* had higher values for post orbital distance, inter-orbital distance and minimum body depth, and lower values for upper jaw length, lower jaw length, eye diameter, head depth and pre-dorsal distance (Table 2).

The range in fin ray numbers was similar in both populations (Table 3). No significant decrease in variability of fin ray number was found for any of the fins in the non-native population (F test,  $P > 0.05$ ). Native and non-native populations, however, differed significantly in pectoral fin ray frequency (both sexes; Chi-squared test,  $P < 0.001$ ). Individuals with 17 and 19 pectoral fin rays occurred at different frequencies in the surveyed ranges. Individuals with 17 fin rays were more common in the non-native range, whereas their proportion in the native range was low. In contrast, individuals with 19 pectoral fin rays occurred more commonly in the native range. This difference was consistent in both sexes (Fig. 2).

The non-native population exhibited significantly higher Fulton's condition coefficient values in both sexes (t-test,  $P < 0.001$ ; Fig. 3).

## Discussion

Ecological polymorphism is well documented in a number of widespread generalist percid and salmonid fishes (e.g. Smith & Skúlason 1996, Olsson & Eklöv 2005, Bhagat et al. 2006). There is, however, a lack of studies documenting ecological polymorphism in benthic fish species. Goby species have restricted swimming abilities and are, therefore, strictly benthic and incapable of the dramatic lifestyle shifts, such as switching between pelagic and benthic habitats, typical for many other polymorphic fish species (Robinson & Parsons 2002). On the other hand, the invasive nature of *N. melanostomus* necessarily includes considerable adaptation capacity as new ecosystems are being invaded. It is reasonable, therefore, to assume that differing conditions in the upper and lower sections of the same river (see Introduction) would be reflected in changes in *N. melanostomus* morphology.

Numerous differences in external morphology have already been reported for a number of distinct *N. melanostomus* populations (reviewed in Simonović et al. 2001), documenting a high level of phenotypic plasticity in this species. Both the native and non-native *N. melanostomus* populations sampled in our study appear to be morphologically similar to the Prahovo (lower Danube) population examined by Simonović et al. (2001), in that both populations show a significant difference from the Prahovo population in only two out of the 12 characters that could be compared (t-test). Moreover, in both cases the difference was significant in one sex only.

It should be noted that the link between shape and function may be obscure or almost unidentifiable, and finding a direct relationship is often impossible. The following discussion on morphological shifts observed

between native and non-native *N. melanostomus* populations and their possible adaptive function, therefore, should be viewed as a hypothesis needing further examination, rather than an authoritative explanation of the functionality of the shifts.

The two populations differed in the same five morphometric characters in both sexes. The proportionally higher values of upper and lower jaw length (i.e. bigger mouth) in the native population (Table 2) may reflect their disparate diet compared to the upstream fish, similarly as Witte et al. (2008) found an increase in mouth size in *H. pyrrhocephalus* following an increase in the proportion of molluscs in the diet. *N. melanostomus* in the lower Danube feed mainly on molluscs, such as bivalves and gastropods (Simonović et al. 1998, Simonović et al. 2001, Polačik et al. 2009), which are more difficult for the fish to handle than gammarids due to their hard shells (Wainwright & Richard 1995). In the upstream sections, however, gammarids are the principal prey of non-native Danubian populations (Adámek et al. 2007, Copp et al. 2008, Polačik et al. 2009).

Further, we found that the native *N. melanostomus* population had larger eyes and a smaller inter-orbital distance (most likely a consequence of the larger eyes). In comparative studies, a relative increase in eye size is traditionally explained as an adaptation towards a darker environment (e.g. Ibanéz-Aguirre et al. 2006, Witte et al. 2008), provided that size of retinal cones is a function of eye size (Van der Meer & Anker 1984). Aside from true flood events, however, our field observations do not support the view that there is increased water turbidity in the lower Danube; rather substantial sediment retention in upstream sections increases clarity downstream (Teodoru & Wehrli 2005). As an alternative, Pankhurst & Montgomery (1994), in a study on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1972) suggested that growth of the eye is age-dependent rather than affected by somatic growth rate. In other words, up to a certain level of food deprivation, eye growth is maintained at the expense (or in spite) of low somatic growth. Although our comparison of condition indices showed that the native *N. melanostomus* population may have been relatively food deprived, further study is needed, including experiments under controlled conditions to reveal the potential linkage between eye size and food availability in *N. melanostomus*.

In the native population, all unpaired fins were shifted towards the rear (Table 2), although the difference was statistically significant for the first dorsal fin only.

At this time, we are unable to provide a plausible explanation for the observed differences in the fin placement, or for the three significantly differing characters in females only (head depth, minimum body depth, and post-orbital distance).

The range of fin ray counts in both native and non-native populations is well within the values published by previous authors (Gheorghiev 1966, Smirnov 1986, Simonović et al. 1998, Stráňai & Andreji 2004). Native and non-native populations differed significantly, however, in the frequency of occurrence of fish with 17 and 19 pectoral fin rays (Table 3). While specimens with 17 fin rays rarely occurred in the sample from the lower Danube, they were common in the non-native population. The situation was reversed as regards specimens with 19 fin rays. These differences may have arisen via a founder effect (Brown & Stepien 2008) during, for example, the introduction of restricted numbers of individuals in the holds of ships (e.g. Ahnelt et al. 1998, Wiesner 2005, Polačik et al. 2008). If, on the other hand, the character's value represents phenotypic plasticity, its change is likely to be driven by disparate environmental characteristics. Any adaptive function of this meristic trait, however, remains unknown (Kristjansson et al. 2004).

In conclusion, native and non-native Danubian populations of *N. melanostomus* differ in their external morphology; some of these differences being potentially explainable by disparate environments or a founder effect. Future investigations should involve experimental studies under controlled conditions to corroborate the relationship of particular morphological changes with the effects of diet, food availability and/or genetic heritability.

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