DOI: 10.2478/cjf-2023-0011
CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

VARIATIONS IN OTOLITH AND SCALE SHAPE OF THE INVASIVE ROUND GOBY *Neogobius melanostomus* POPULATIONS FROM DIFFERENT HABITATS OF THE SAVA RIVER BASIN, CROATIA

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ARTICLE INFO

Received: 20 March 2023
Accepted: 18 May 2023

ABSTRACT

Round goby *Neogobius melanostomus* is one of the three invasive Ponto-Caspian gobies found in the Sava River basin in Croatia. It has a negative effect on the native ecosystems due to its high invasive potential that enables its rapid spread upstream in inland watercourses. The objective of this study was to determine the differences in sagittal otolith and scale shape of the frontal and established populations of *N. melanostomus* in two different habitat types. At two sampling sites in the main course of the Sava (Babina Greda, Slavonski Brod) and in a modified habitat (Sava-Odra canal), 20 individuals of *N. melanostomus* were collected and analysed. The geometric morphometric method was used together with Procrustes analysis of variance, canonical variate analysis and discriminant function analysis to analyse the otolith and scale shape. Significant differences in both otolith and scale shapes were found between settled (Babina Greda) and frontal (Slavonski Brod) populations from the main course of the Sava River. The main differences were the wider anterior part of the scales and a more pronounced open indentation on the otolith of the Babina Greda population. The settled population in the Sava-Odra canal was significantly different in otolith shape from the Babina Greda population but not from the Slavonski Brod population, suggesting that the upstream migration consisted of individuals with specific phenotype and genotype. Otoliths generally expressed greater shape variability than scales and thus may be more useful for differentiating between closely related populations on a finer scale. The results of this study suggest that the shape of otoliths and scales can be used to distinguish between closely related populations of *N. melanostomus* from different habitats but more comprehensive studies are needed due to the small number of samples.

Keywords:
Geometric morphometry
Shape
Frontal and settled populations
River
Canal

How to Cite

Planchet, V., Špelić, I., Piria, M. (2023): Variations in otolith and scale shape of the invasive round goby *Neogobius melanostomus* populations from different habitats of the Sava River basin, Croatia. Croatian Journal of Fisheries, 81, 93-101. DOI: 10.2478/cjf-2023-0011.

INTRODUCTION

In recent decades, several dramatic expansions of invasive species have been found (Roche et al., 2013). These invasions create global environmental change as invasive species can cause environmental impacts on ecosystems (Glamuzina et al., 2021). A major part of continental Croatia belongs to the Sava River basin, comprising a network of tributaries and sub-tributaries (Jakovlić et al., 2015). The Sava River flows through Slovenia, Croatia and Serbia, and joins the Danube in Belgrade after a course of 926 km. Throughout this course, the Sava River has a very wide spectrum of different habitats, hosting 74 native and 15 non-native fish species (Simonović et al., 2015). Among the non-native species in the Sava River basin, three Ponto-Caspian gobies (monkey goby *Neogobius fluviatilis*, round goby *Neogobius melanostomus*, bighead goby *Ponticola kessleri*) native to the Black and Caspian seas, have considerably extended their distribution upstream in the river (Jakovlić et al., 2015). All three species showed very high invasive potential (Radočaj et al., 2021). However, most recently, *N. melanostomus* became the most abundant in the Sava River basin (Piria et al., 2011a, b; Piria et al., 2016; Jakšić et al., 2020).

Neogobius melanostomus is a small benthic fish and is among the most frequently unintentionally introduced fish species outside of its natural habitat (Corkum et al., 2004; Kornis et al., 2012). Its fast expansion has considerable deleterious impacts on the ecosystem (Kornis et al., 2012), which was also noted in the Sava River (Piria et al., 2016).

Otoliths are small calcium carbonate structures located in the inner ear cavity (directly behind the brain) of bony fish. These pearly white stones serve as a balance organ and contribute to hearing (Rodriguez-Mendoza, 2006). The structure of otoliths is three-dimensional and all three otolithic organs respond to sounds and vestibular movements of the head (Popper and Fay, 1993). They do not necessarily grow at the same rate, and their size and shape vary considerably among species, but also among individuals of a species (Morales-Nin, 2000; Campana and Thorrold, 2001). In contrast to otoliths, fish scales (ctenoid and cycloid) are determined by the growth of circular lines that form yearly increments (annuli) (Iversen, 1996), and due to seasonal changes in temperature or food availability, major life history events are recorded as markings on fish scales (Tattam et al., 2017).

The shapes and sizes of otoliths and fish scales are important because of their variability. Since 1990, significant developments have been made in fisheries science due to technological advances in extracting information from these hard structures in fish (Begg et al., 2005). Otolith and scale morphometry are used in numerous studies of fish populations because their analysis can provide information on the life history of fish, such as the age, migration pattern or genetic diversity (Biolé 2019; Gebremedhin et al., 2021). They can also incorporate some environmental constituents, depending on the ambient concentration, and can be used to reconstruct chronological life history patterns (Walther and Limburg, 2012). The shape of otoliths and scales has also been used to differentiate genera, species and different phenotypic stocks of local populations (Ibáñez et al. 2017; Milošević et al., 2021), as their variations are influenced by habitat and environmental conditions (Vergara-Solana et al., 2013).

Studies on otolith and scale shape to distinguish phenotypic stocks have been done in both marine and freshwater environments (Campana and Casselman, 1993; Richards and Esteves, 1997; Khan et al., 2022), using both contour (elliptic Fourier analysis) and landmark-based methods (e.g. geometric morphometric). The contour analysis was used more often, but landmark-based methods have a greater power to detect and quantify shape differences between closely related species (Rufino et al., 2006). The geometric morphometric method has proven to be a powerful tool for distinguishing differences in the shape of otoliths and scales even at the level of local populations and has been used in comparative studies to identify fish stocks (Poulet et al., 2005; Ibáñez et al., 2017). The otoliths and scales in *N. melanostomus* are mainly used for age determination and growth patterns (Sokołowska and Fey, 2011; Gruľa et al., 2012; Florin et al., 2018), and less often for shape variability. Variability in otolith shape between two groups of *N. melanostomus* with alternative reproductive tactics and between different populations in the native area was performed using a contour-based (elliptic Fourier analysis) method (Bose et al., 2018; Zamorov et al., 2021). However, there is no comparative study using the landmark-based geometric morphometric method for otolith and scale shape variability on invasive *N. melanostomus* expanding their populations in new areas.

The main objective of the current study was to identify the sagittal otoliths and scale shape of the established and frontal populations of *N. melanostomus* from the Sava River main course and its channelized tributary. Previous reports on other fish species suggested significant differences between otoliths and scales within a species (Poulet et al., 2005; Ibáñez et al., 2017). Therefore, we expected to find differences in otolith and scale shape between the settled and frontal *N. melanostomus* populations found in semi-natural and artificial habitats.

MATERIALS AND METHODS

Study area

The Sava River belongs to the Black Sea basin and represents a right tributary of the Danube River. It flows through Slovenia, Croatia and along the border with Bosnia and Herzegovina, finally joining the Danube in Serbia. The Croatian part of the Sava River is characterized by fast-flowing water in its upper part near the border with

Slovenia and a slow and wide river section downstream of the town of Sisak (Piria et al., 2019). Due to frequent floods in the past, the Sava River in Croatia is semi-regulated by banks. In addition, to improve flood protection the Sava–Odra–Sava canal was built connecting the Sava River with the Odra River and the Odra Field which has become a natural retention basin.

The Slavonski Brod (SBB) and Babina Greda (BGG) sampling sites are located in the downstream section in the main course of the Sava River, with vegetation along the bank dominated by shrubs and submerged logs. The sampling site at SBB is characterized by a silty-sandy substrate with the occasional appearance of gravel and larger stones. The BBG sampling site is characterized by silt, small and larger gravel and large stones. The Sava-Odra canal (CSO) represents artificial habitat because the canal is covered by concrete, and permanently contains low water level and muddy substrate at the bottom. The population sampled at the BGG sampling site in 2012 represented the recently established population at the early-stage invasion, the population from SBB sampled in 2012 represented the first recorded frontal population that was spreading upstream, and the population from CSO sampled in 2019 represented the settled population at a later stage of invasion. The sampling sites were chosen because, at the time of sampling, the SBB population was the most upstream population found in the Sava River. At the BGG location, the individuals had already been present for at least several generations (Jakovlić et al., 2015). The CSO was sampled seven years later when *N. melanostomus* established self-sustaining populations in the main course of the Sava River and its natural and artificial tributaries.

Fish sampling, otolith and scale sample preparation

The fish were sampled in September 2012 (SBB and BGG) and in July 2019 (CSO) (Figure 1) using the electrofishing method as described by Jakovlić et al. (2015). *Neogobius melanostomus* represents an invasive non-native fish in Croatia, hence all caught specimens were removed from the water, euthanized, taken to the laboratory and stored at -20°C.

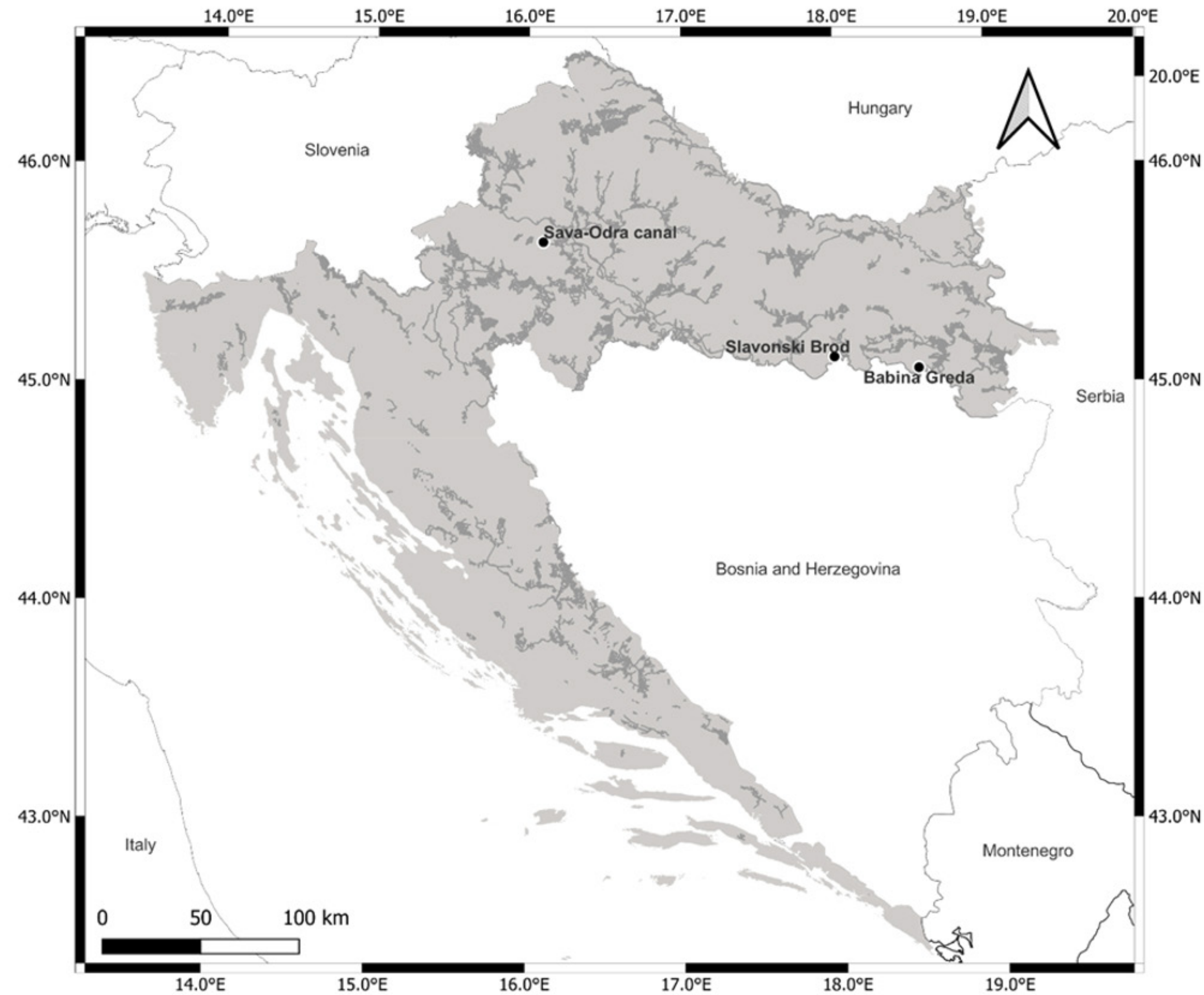


Fig 1. The map of the sampling sites with larger watercourses marked in dark grey

In the laboratory, each specimen of *N. melanostomus* was measured for total length (TL) to the nearest 0.1 cm. The scales from the right side of the body were removed and dried. The left and right otoliths were taken from each specimen but only the right was used for further geometric morphometric analyses. Scales and otoliths were cleaned, labelled and examined under a stereomicroscope (model BTC STM-8, with a maximum magnification of 45x) with a visible scale. Photographs were taken with a digital camera (Toupcam UCMOS05100KPA) mounted on a microscope, using ToupView version 4.10 (ToupTek, Zhejiang, China).

Preparation of shape variables

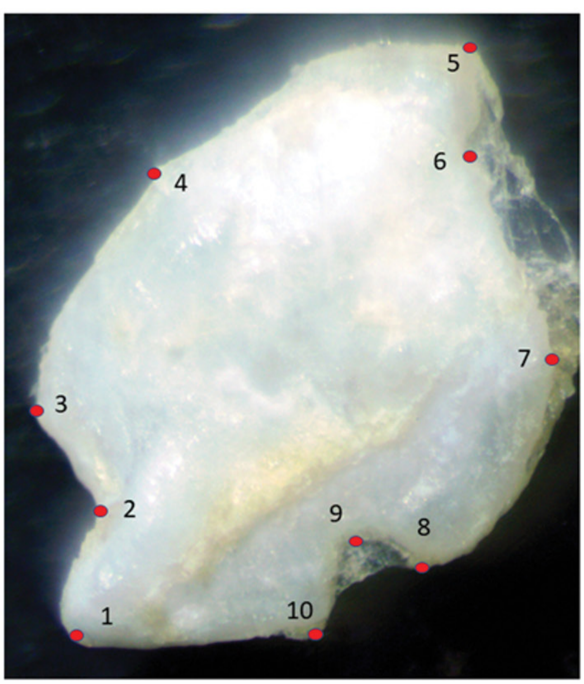
TpsUtil software was used to build the initial .tps file from the captured photographs. In each image, 10 and 7 landmarks of otoliths and scales, respectively, were marked using TpsDig2 version 2.32 to obtain the X and Y coordinates of the landmarks (Rohlf, 2017). The selection of landmarks on scales was based on a combination of points from Ibáñez et al. (2017). The selection of landmarks on otoliths was chosen in places which can appropriately describe the shape (Figure 2).

Non-shape variation and allometric effect removal

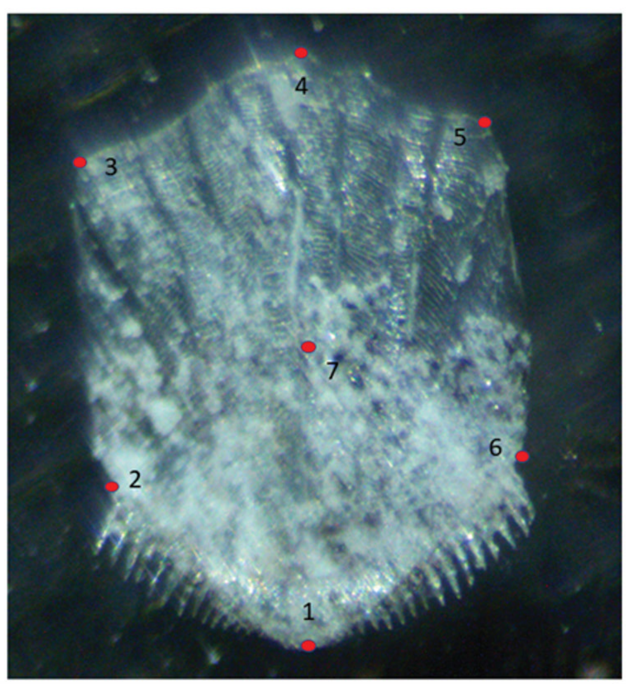
Outliers in data were explored based on squared Mahalanobis distance available in MorphoJ software (Klingenberg, 2011), and specimens that deviated very strongly from the others were excluded prior to further analysis. The removal of non-shape information (such as differences due to rotation, translation and scaling) was performed using generalized Procrustes analysis (GPA) in MorphoJ software (Klingenberg, 2011). The results of this procedure are Procrustes coordinates that represent shape variables, two for each landmark since the photographs are two-dimensional, and the centroid size that represents the size variable, as the value of the square root of the sum of squared distances of each landmark from their respective centroid (Zelditch et al., 2004; Špelić et al., 2021). Permutation tests of Procrustes distances and the T-square statistic were used with Procrustes ANOVA to test the null hypothesis of equal group means by MorphoJ software (Klingenberg, 2011). Procrustes ANOVA was applied to shape data to test the shape differences of specimens among all sites. Allometric shape variation of data was removed in MorphoJ software by regressing shape variables onto centroid size. The residuals resulting from the regression were used to generate a covariance matrix and were used as shape data analyses (Klingenberg, 2011).

Shape analysis and visualization

Variation of shape using shape data residuals first was explored and visualised by principal component analysis (PCA). The possible shape differences between every group of specimens were analysed by canonical variate analysis (CVA) (Špelić et al., 2021). Discriminant function analysis (DFA) was applied to the shape data for cross-validation to assess classification reliability and accuracy (Klingenberg, 2011). The scores of the first two CVA axes were plotted and the shape changes were visualized by wireframe graph using MorphoJ software (Klingenberg, 2011).



A)



B)

Fig 2. Homologous landmarks A. Right otolith; landmarks 8, 9, 10 of otolith represent open indentation; B. Scale taken from the right side of fish; landmarks 3, 4, 5 represent anterior and 1,2,6,7 posterior portion

RESULTS

In total, 3 specimens from CSO, 9 specimens from BBG and 8 specimens from SBB were analysed for otolith and scale shape using geometric morphometry. On average, the smallest specimens were captured at the BBG sampling site, but the greatest size variation was observed at the SBB sampling site (Table 1). One specimen from SBB analysed for otolith shape was excluded from geometric morphometric analyses because it was identified as an outlier.

Table 1. Descriptive statistic of analysed *Neogobius melanostomus* populations (n = number of specimens; SD = standard deviation; CV = coefficient of variation; TL = total length; min = minimal; max = maximal)

Sampling site	n	Mean ± SD	CV %	TL_{min}	TL_{max}
CSO	3	7.66 ± 0.87	7.04	6.7	8.4
BBG	9	6.40 ± 0.45	11.40	5.9	7.3
SBB	8	7.16 ± 1.35	18.86	4.8	9.1

The results of Procrustes ANOVA of Procrustes coordinates on the dataset before allometry removal did not show significant differences in otolith and scale ($P > 0.01$) shape between sampling sites (Table 2).

Table 2. Procrustes ANOVA results on centroid size and shape effect of otoliths and scales by the sampling site classifiers (SS – sum of squares, MS – mean squares, df – degrees of freedom, F – F ratio)

Effect	Scales		Otoliths	
	Centroid size	Shape	Centroid size	Shape
SS	0.473542	0.0204950	227360.417	0.0414626
MS	0.236771	0.0010247	113680.208	0.0012957
Df	2	20	2	32
F	1.72	1.23	0.66	0.74
P (parametric)	0.213	0.239	0.529	0.841

However, statistical analyses of the shape of Procrustes distances using CVA and DFA among groups that were done on residuals produced by the regression after allometry removal, showed significant differences between BGG and SBB sites in both scale and otolith shapes ($P < 0.01$). The discriminant analysis cross-validation testing correctly classified between 62 – 100% BGG and SBB samples of both scales and otoliths (Table 3).

Table 3. Results of Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) statistic tests for compared groups of *Neogobius melanostomus* otoliths and scales with results of cross-validation test in DFA for the compared groups. Numbers in brackets represent the correctly assigned individuals in relation to the total number of compared individuals. BGG – Babina Greda; CSO – Sava-Odra canal; SBB – Slavonski Brod (bold *P* values represent significant differences).

Compared groups	Canonical Variate Analysis (CVA)		Discriminant Function Analysis (DFA)				DFA Cross-validation test
	Procrustes distance among group	Permutation test *P*-value	Procrustes dis-tance	Permutation test *P*-value	T-square	T-square *P*-value (permu-tations)	
BGG-CSO otoliths	0.059	0.9880	0.059	0.9830	11.18	0.795	(37.5%) 3/8 – (100%) 3/3
BGG-SBB otoliths	0.092	0.2250	0.092	**0.0090**	1497438.28	**<.0001**	(50%) 4/8 – (62.5%) 5/8
CSO-SBB otoliths	0.0824	0.8169	0.0824	**0.0031**	5030693.89	**0.0050**	(100%) 3/3 – (62.5%) 5/8
BGG-CSO scales	0.0665	0.3458	0.0664	0.6224	94.06	0.1060	(75%) 6/8 – (33%) 1/3
BGG-SBB scales	0.0655	**0.0500**	0.0655	**0.0338**	242.67	**0.0360**	(87.5%) 7/8 – (85.7 %) 6/7
CSO-SBB scales	0.0500	0.6449	0.0500	0.626	18.44	0.741	(33%) 1/3 – (85.7%) 6/7

The first two canonical variates (CV1 86% and CV2 13%) explained 99% of the total variation in the shape of both otoliths and scales (Figure 3). The shape differences in otoliths and scales were found between populations at BGG and SBB, which confirmed a cross-validated DFA (Figure 3A, B; Table 3). The CSO population was significantly different only in otolith shape from the BGG population by DFA, but not from the SBB population (Table 3).

On the wireframe of graphical visualisation, it is evident that the BGG population possess a wider anterior part of the scales (landmarks 3, 4, 5; Figure 2A; Figure 3A), which shows rectangularity and possesses more pronounced open indentation in the right otolith (landmarks 8, 9, 10; Figure 2B; Figure 3B), which is the main difference in shape compared to the SBB population. The otolith shape of the individuals from the CSO population showed a rounded shape with a less pronounced open indentation on the right side (positive extreme on CV2), but also the posterior part of the scales seems stronger and the anterior part wider, which represents the shape between the BGG and SBB populations (positive extreme of CV1 and CV2) (Figure 3 A, B).

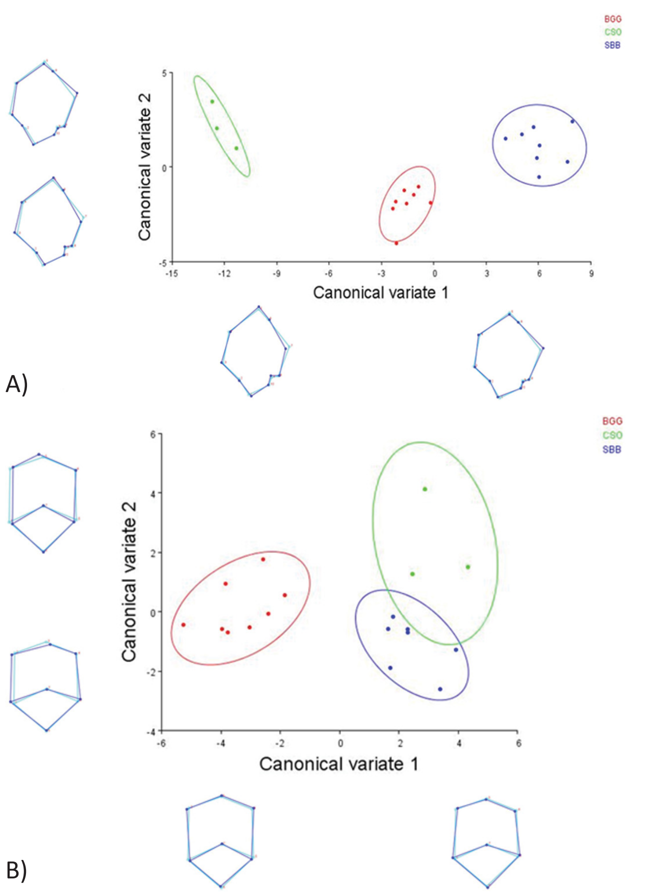


Fig 3. Scatterplot of the first two canonical variate axes of CVA depicting *Neogobius melanostomus* otolith (A) and scale (B) shape variation of collected specimens, equal frequency ellipses. Wireframe graphs with 10 (otolith) and 7 (scale) marked landmarks (see Figure 2) represent shape change along the first and second CV axes, from negative to positive extremes. Light blue outlines represent the average shape and dark blue outlines represent extreme shape changes. BGG – Babina Greda; CSO – Chanal Sava Odra; SBB – Slavonski Brod.

DISCUSSION

This study confirmed that it is possible to discriminate between local invasive populations of *N. melanostomus* by otolith and scale shape variation. The previous analysis of the outer shape of the otolith exhibited great phenotypic variability (Więcaszek et al., 2020) and was confirmed in this study, as this differentiation is not limited only to distant populations which occupy different habitats (Poulet et al., 2005). The scales of *N. melanostomus* exhibited much less variability than the otoliths; however, only a small number of specimens were examined in this study, resulting in limited overall variation of scale shape. This study was performed on specimens found upstream in the Sava River in 2012 at the beginning of the invasion (Piria et al., 2011a; Jakovlić et al., 2015), and their settled populations were sampled in 2019 in an artificial canal connected to the main course of the Sava River. The sampling sites were characterized by different environmental factors and conditions, which can be a reason for the variability of otoliths and scales of the same species (Mille et al., 2016). Besides, great variability in otoliths and likely in scales may also be associated with the size of the fish (Więcaszek et al., 2020), and this is most likely the reason why Procrustes ANOVA showed no significant differences between three populations of *N. melanostomus*. However, allometry removal by regressing shape variation is an important part of the classification reliability and accuracy (Klingenberg, 2011) of closely related populations. This suggests that the allometry of the otoliths and scales of individuals in this research affected the results of Procrustes ANOVA, but this limitation was corrected by regression and further analysis of regression residuals.

Previous research suggested that local adaptation and phenotypic plasticity can lead to environmental-related morphological and genetic variations in freshwater fish, as a response to latitude, thermal and hydrological conditions (Quadroni et al., 2023). However, it was found that the fish scale form was least effective in discriminating populations from nearby areas but better when populations are more geographically dispersed (Ibáñez et al., 2007) or when there is a previously recorded pattern for each location (Ibáñez, 2014). In our study, the frontal population (SBB site) of *N. melanostomus* that was spreading upstream likely consisted of individuals of specific phenotype and genotype due to differences in scale and otolith shape from a nearby settled population (BGG site). This could be a reason that could influence the shape of settled CSO populations taken seven years later. In addition, the CSO populations came from different environmental conditions that permanently contain low water levels and muddy bottom substrate, which could be an additional reason for otolith and scale variation. Additionally, it seems that the CSO population formed the specific shape of otoliths and scales but further studies are needed to confirm this finding due to the small number of specimens analysed. The results of this study confirm the results of previous research on rostrum dace *Leuciscus burdigalensis* from the Viaur River in France, suggesting that differences in the scale shape can be found at the local level (Poulet et al., 2005). Furthermore, research on white mullet *Mugil curema* reveals that scales may better discriminate fish populations than otoliths, and authors describe this method as simple, non-destructive, accessible, fast and inexpensive (Ibáñez et al., 2017). On the contrary, Campana and Casselman (1993) hypothesise that otoliths are an ideal natural marker for fish populations and describe them as an easily determined measure of stock identity for Atlantic cod *Gadus morhua*. Our results indicate that in the case of *N. melanostomus* otoliths may be a better choice, but the disadvantage is that fish must be sacrificed to extract them.

In conclusion, our results suggest that scale and otolith shape morphology can be used for the spatial structure of freshwater fish populations at a fine scale and that the settled and frontal populations of *N. melanostomus* from different habitats can be differentiated by a geometric morphometric approach. However, these findings are preliminary due to the small number of analysed individuals, thus further comprehensive studies are recommended.

RAZLIKE U OBLIKU OTOLITA I LJUSKI INVAZIVNIH POPULACIJA GLAVOČA *Neogobius melanostomus* IZ RAZLIČITIH STANIŠTA SLIJEVA RIJEKE SAVE, HRVATSKA

SAŽETAK

Glavočić okrugljak *Neogobius melanostomus* jedna je od tri invazivne vrste ponto-kaspijskih glavoča prisutnih u hrvatskom dijelu slijeva rijeke Save. Ima negativan učinak na zavičajne ekosustave zahvaljujući visokom invazivnom potencijalu koji omogućuje njegovo brzo širenje uzvodno u kopnenim vodotocima. Cilj istraživanja bio je utvrditi moguće razlike u obliku otolita i ljuski između ustaljenih i frontalnih populacija invazivnog *N. melanostomus* u dva različita staništa. Uzorkovane su dvije lokacije u glavnom toku rijeke Save (Babina Greda, Slavonski Brod) i jedna lokacija u izmijenjenom staništu (kanal Sava Odra) pri čemu je prikupljeno i analizirano 20 jedinki *N. melanostomus*. Za analizu oblika otolita i ljuski primijenjena je geometrijska morfometrija, Prokrustova analiza varijance, kanonička analiza varijacije i diskriminantna funkcijska analiza. Utvrđena je statistički značajna razlika u obliku otolita i ljuski između naseljene (Babina Greda) i frontalne (Slavonski Brod) populacije u glavnom toku rijeke Save. Glavne razlike bile su širi prednji dio ljuski i izraženije otvoreno udubljenje na otolitu populacije naseljene na lokaciji Babina Greda. Naseljena populacija u kanalu Sava-Odra značajno se razlikovala od populacije Babine Grede, ali ne i od populacije Slavonskog Broda, što sugerira na mogućnost da se uzvodna migracija sastoji od jedinki specifičnog fenotipa i genotipa. Otoliti općenito izražavaju veću varijabilnost oblika nego ljuske i stoga mogu biti korisniji za utvrđivanje razlika između bliskih populacija. Rezultati istraživanja sugeriraju da se oblik otolita i ljusaka može primijeniti za razlikovanje bliskih *N. melanostomus* populacija iz različitog staništa, ali zbog malog broja uzoraka se predlaže opsežnija studija.

Ključne riječi: geometrijska morfometrija, oblik, frontalne i ustaljene populacije, rijeka, kanal

ACKNOWLEDGEMENTS

We thank our colleagues of the Department of Fishery, Apiculture, Wildlife Management and Special Zoology for their help with sampling in 2012 and 2019 at the Sava River and Sava-Odra canal. This research was supported by the EIFAAC Project “Management/Threat of Aquatic Invasive Species in Europe”, by the fish monitoring program of the Ministry of Agriculture in Croatia and by the IAS monitoring program of the Ministry of Economy and Sustainable Development, Institute for Environment and Nature.

REFERENCES

Begg, G. A., Campana, S. E., Fowler, A. J., Suthers, I. M. (2005): Otolith research and application: Current directions in innovation and implementation. Marine and Freshwater Research, 56, 477-483. https://doi.org/10.1071/MF05111

Biolé, F. G., Callicó Fortunato, R., Thompson, G. A., Volpedo, A. V. (2019): Application of otolith morphometry for the study of ontogenetic variations of *Odontesthes argentinensis*. Environmental Biology of Fishes, 102 (10), 1301–1310.

Bose, A. P., McCallum, E. S., Raymond, K., Marentette, J. R., Balshine, S. (2018): Growth and otolith morphology vary with alternative reproductive tactics and contaminant exposure in the round goby *Neogobius melanostomus*. Journal of Fish Biology, 93 (4), 674-684

Campana, S. E., Casselman, J. M. (1993): Stock discrimination using otolith shape analysis. Canadian Journal of Fisheries and Aquatic Sciences, 50 (5), 1062–1083. https://doi.org/10.1139/f93-123

Campana, S. E., Thorrold, S. (2001): Otoliths, increments, and elements: Keys to a comprehensive understanding of fish populations?. Canadian Journal of Fisheries and Aquatic Sciences, 58, 30-38. https://doi.org/10.1139/cjfas-58-1-30

Corkum, L. D., Sapota, M. R., Skora, K. E. (2004): The Round Goby, *Neogobius melanostomus*, a Fish Invader on both sides of the Atlantic Ocean. Biological Invasions, 6, 173–181. https://doi.org/10.1023/B:BINV.0000022136.43502.db

Florin, A. B., Hüssy, K., Blass, M., Oesterwind, D., Puntila, R., Ustups, D., Albrecht, C., Heimbrand, Y., Knospina, E., Koszarowski, K. Odelström, A. (2018): How old are you—Evaluation of age reading methods for the invasive round goby (*Neogobius melanostomus*, Pallas 1814). Journal of Applied Ichthyology, 34(3), 653-658. DOI: 10.1111/jai.13596

Gebremedhin, S., Bruneel, S., Getahun, A., Anteneh, W., Goethals, P. (2021): Scientific Methods to Understand Fish Population Dynamics and Support Sustainable Fisheries Management. Water, 13, 574. https://doi.org/10.3390/w13040574

Glamuzina, B., Tutman, P., Glamuzina, L., Vidović, Z., Simonović, P., Vilizzi, L. (2021): Quantifying current and future risks of invasiveness of non-native aquatic species in highly urbanised estuarine ecosystems—A case study of the River Neretva Estuary (Eastern Adriatic Sea: Croatia and Bosnia–Herzegovina). Fisheries Management and Ecology, 28, 138– 146.

Gruľa, D., Balážová, M., Copp, G., Kováč, V. (2012): Age and growth of invasive round goby *Neogobius melanostomus* from middle Danube. Open Life Sciences 7 (3): 448–459. Available at: https://dx.doi.org/10.2478/s11535-012-0024-5.

Ibáñez, A. L. (2014): Fish scale shape variation by year and by geographic location, could scales be useful to trace fish? A case study on the Gulf of Mexico. Fisheries Research, 156, 34-38.

Ibáñez, A. L., Cowx, I. G., O'Higgins, P. (2007): Geometric morphometric analysis of fish scales for identifying species and local populations within the Mugilidae. Canadian Journal of Fisheries and Aquatic Sciences, 64, 1091-1100.

Ibáñez, A. L., Hernández-Fraga, K., Alvarez-Hernández, S. (2017): Discrimination analysis of phenotypic stocks comparing fish otolith and scale shapes. Fisheries Research, 185, 6–13. https://doi.org/10.1016/j.fishres.2016.09.025

Iversen, E. S. (1996): Age and Growth of Resource Species. Living Marine Resources: Their Utilization and Management, 81-101.

Jakovlić, I., Piria, M., Šprem, N., Tomljanović, T., Matulić, D., Treer T. (2015): Distribution, abundance and condition of invasive Ponto-Caspian gobies *Ponticola kessleri* (Gunther, 1861), *Neogobius fluviatilis* (Pallas, 1814), and *Neogobius melanostomus* (Pallas, 1814) in the River Sava basin, Croatia. Journal of Applied Ichthyology, 31 (5), 888–894. http://dx.doi.org/10.1111/jai.12803

Jakšić, G., Jadan, M., Pinder, A. C., Tomljanović, T., Zanella, D., Treer, T., Piria M. (2020): Genotyping of invasive Ponto-Caspian gobies in Croatia. Journal of Central European Agriculture, 21 (4), 733-740. https://doi.org/10.5513/JCEA01/21.4.2989

Khan, U., Ateşşahin, T., Eroğlu, M., Düşükcan, M. (2022): Morphological variations of an invasive cyprinid fish (*Carassius gibelio*) in lentic and lotic environments inferred from the body, otolith, and scale shapes. Acta Zoologica, 1-15. https://doi.org/10.1111/AZO.12431

Klingenberg, C. P. (2011): MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources, 11, 353–357.

Kornis, M. S., Mercado-Silva, N., Vander Zanden, M. J. (2012): Twenty years of invasion: a review of round goby *Neogobius melanostomus* biology, spread and ecological implications. Journal of Fish Biology, 80, 235-285.

Milošević, D., Bigović, M., Mrdak, D., Milašević, I., Piria, M. (2021): Otolith morphology and microchemistry fingerprints of European eel, *Anguilla anguilla* (Linnaeus, 1758) stocks from the Adriatic Basin in Croatia and Montenegro. Science of the Total Environment, 786, 147478 https://doi.org/10.1016/j.scitotenv.2021.147478

Mille, T., Mahé, K., Cachera, M., Villanueva, M. C., de Pontual, H., Ernande, B. (2016): Diet is correlated with otolith shape in marine fish. Marine Ecology Progress Series, 555, 167-184. https://doi.org/10.3354/meps11784

Morales-Nin, B. (2000): Review of the growth regulation processes of otolith daily increment formation. Fisheries Research, 46 (1–3), 53-67. https://doi.org/10.1016/S0165-7836(00)00133-8

Piria, M., Simonović, P., Zanella, D., Ćaleta, M., Šprem, N., Paunović, M., Tomljanović, T., Gavrilović, A., Pecina, M., Špelić, I., Matulić, D., Rezić, A., Aničić, I., Safner, R., Treer, T. (2019): Long-term analysis of fish assemblage structure in the middle section of the Sava River – The impact of pollution, flood protection and dam construction. Science of the Total Environment, 651, 143–153. https://doi.org/10.1016/j.scitotenv.2018.09.149

Piria, M., Jakšić, G., Jakovlić, I., Treer, T. (2016): Dietary habits of invasive Ponto-Caspian gobies in the Croatian part of the Danube River basin and their potential impact on benthic fish communities. Science of the Total Environment, 540 (1), 386–395. http://dx.doi.org/10.1016/j.scitotenv.2015.05.125

Piria, M., Šprem, N., Jakovlić, I., Tomljanović, T., Matulić, D., Treer, T., Aničić, I., Safner, R. (2011a): First record of round goby, *Neogobius melanostomus* (Pallas, 1814) in the Sava River, Croatia. Aquat. Invasions, 6, S153–S157.

Piria, M., Treer, T., Tomljanović, T., Šprem, N., Matulić, D., Aničić, I., Safner, R. (2011b): First record of monkey goby, *Neogobius fluviatilis* (Pallas, 1814) in the barbel zone of the Sava River, Croatia. Journal of Applied Ichthyology, 27, 1383–1384.

Popper, A. N., Fay, R. R. (1993): Sound detection and processing by fish: critical review and major research questions. Brain, Behavior and Evolution 41, 14– 38.

Poulet, N., Reyjol, Y., Collier, H., Let, S. (2005): Does fish scale morphology allow the identification of populations at a local scale? A case study for rostrum dace *Leuciscus leuciscus burdigalensis* in River Viaur (SW France). Aquatic Science, 67, 122–127. https://doi.org/10.1007/s00027-004-0772-z

Quadroni, S., De Santis, V., Carosi, A., Vanetti, I., Zaccara, S., Lorenzoni, M. (2023): Past and present environmental factors differentially influence genetic and morphological traits of Italian barbels (Pisces: *Cyprinidae*). *Water*, 15(2), 325. https://doi.org/10.3390/w15020325

Radočaj, T., Špelić, I., Vilizzi, L., Povž, M., Piria, M. (2021): Identifying threats from introduced and translocated non-native freshwater fishes in neighbouring countries under current and future climatic conditions. Global Ecology and Conservation, 27, e01520 https://doi.org/10.1016/j.gecco.2021.e01520

Richards, A., Esteves, C. (1997): Use of scale morphology for discriminating wild stocks of Atlantic striped bass. Transactions of The American Fisheries Society, 126, 919-925. 10.1577/1548-8659(1997)126<0919:UOSMFD>2.3.CO;2.

Roche, K. F., Janač, M., Jurajda, P. (2013): A review of Gobiid expansion along the Danube-Rhine corridor – geopolitical change as a driver for invasion. Knowledge and Management of Aquatic Ecosystem, 411, 01.

Rohlf F. J. (2017): TPSDig2, version 2.30. Stony Brook NY: Department of Ecology and Evolution, State University of New York. Available at: http://www.sbmorphometrics.org/ (accessed 10.2.2023)

Rodriguez-Mendoza, R. (2006): Otoliths and their applications in fishery science. Croatian Journal of Fisheries, 64, 89-102.

Rufino, M. M., Gaspar, M. B., Pereira, A. M., Vasconcelos, P. (2006): Use of shape to distinguish *Chamelea gallina* and *Chamelea striatula* (Bivalvia: Veneridae): Linear and geometric morphometric methods. J. Morphol., 267, 1433-1440. https://doi.org/10.1002/jmor.10489

Simonović, P., Povž, M., Piria, M., Treer, T., Adrović, A., Škrijelj, R., Nikolić, V., Simić, V. (2015): Ichthyofauna of the River Sava System In: R. Milačič et al. (eds.), The Sava River, The Handbook of Environmental Chemistry 31, Springer-Verlag Berlin Heidelberg, 361-400. http://dx.doi.org/10.1007/978-3-662-44034-6_14

Sokołowska, E., Fey, D. P. (2011): Age and growth of the round goby *Neogobius melanostomus* in the Gulf of Gdańsk several years after invasion. Is the Baltic Sea a new Promised Land?. Journal of Fish Biology 78 (7), 1993–2009. https://doi.org/10.1111/j.1095-8649.2011.02986.x

Špelić, I., Rezić, A., Kanjuh, T., Marić, A., Maguire, I., Simonović, P., Radočaj, T., Piria, M. (2021): Application of the geometric morphometrics approach in the discrimination of morphological traits between brown trout lineages in the Danube Basin of Croatia. Knowledge and Management of Aquatic Ecosystem, 422, 22.

Tattam, I. A., Li, H. W., Giannico, G. R., Ruzycki, J. R. (2017): Seasonal changes in spatial patterns of *Oncorhynchus mykiss* growth require year-round monitoring. Ecology of Freshwater Fish, 26, 434-443. https://doi.org/10.1111/eff.12287

Vergara-Solana, F. J., García-Rodríguez, F. J., De La Cruz-Agüero, J. (2013): Comparing body and otolith shape for stock discrimination of Pacific sardine, *Sardinops sagax* Jenyns, 1842. Journal of Applied Ichthyology, 29, 1241-1246. https://doi.org/10.1111/jai.12300

Walther, B. D., Limburg, K. E. (2012): The use of otolith chemistry to characterise diadromous migrations. Journal of Fish Biology, 81, 796–825.

Więcaszek, B., Nowosielski, A., Dąbrowski, J., Górecka, K., Keszka, S., Strzelczak, A. (2020): Fish size effect on sagittal otolith outer shape variability in round goby *Neogobius melanostomus* (Pallas 1814). Journal of Fish Biology, 97, 1520– 1541. https://doi.org/10.1111/jfb.14521

Zamorov, V., Zamorova, M., Krupko, D., Matvienko, N., Leonchyk, Y., Kvach, Y. (2021): Shape analysis of otoliths of the round goby, *Neogobius melanostomus* (Gobiiformes, Gobiidae), from the Black Sea Basin. Zoodiversity, 55 (4).

Zelditch, M. L., Swiderski, D. L., Sheets, H.D. (2004): Geometric Morphometrics for Biologists: a primer. Amsterdam: Elsevier, 478.