DOI: 10.2478/cjf-2023-0018

CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

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FLUCTUATING ASYMMETRY IN MORPHOLOGICAL CHARACTERS OF THE INVASIVE ROUND GOBY *Neogobius melanostomus* POPULATIONS FROM DIFFERENT HABITATS OF THE SAVA RIVER BASIN, CROATIA

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ARTICLE INFO

Received: 7 July 2023
Accepted: 29 August 2023

Keywords:
Bilateral asymmetry
Invasive fish
Environmental stress
Bioindicator

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 and is more abundant in highly polluted areas. The objective of this study was to determine the fluctuating asymmetry of four morphometric characters, as well as otolith width and length. Thirty individuals of *N. melanostomus* were collected and analysed at two sampling sites in the main course of the Sava (Babina Greda, Slavonski Brod) and in a modified habitat (Sava-Odra canal). The squared coefficient of asymmetry variation was used to analyse potential differences between populations. We identified variations in the asymmetry values of the analysed characters, as well as otolith width and length between sampling sites. The highest fluctuating asymmetry was observed in individuals at the Babina Greda sampling site, which could be due to high environmental stress. The results of the study show that the study of fluctuating asymmetry can be used on invasive fish species as a simple approach to identifying habitat quality.

How to Cite

Planchet, V., Špelić, I., Piria, M. (2023): Fluctuating asymmetry in morphological characters of the invasive round goby *Neogobius melanostomus* populations from different habitats of the Sava River basin, Croatia. Croatian Journal of Fisheries, 81, 167-172. DOI: 10.2478/cjf-2023-0018.

INTRODUCTION

Asymmetry represents a possible differential development of a bilateral character between the right and left sides of an organism (Jawad et al., 2020). In previous studies, asymmetry was observed on various vertebrates such as mammals (Markowski 1993), reptiles (Castillo and González-Rivas, 2023) and birds (Lens et al., 2002). Asymmetry analysis can also be applied to fish (Allenbach, 2011), where otolith asymmetry can be studied among other morphological structures (Al-Rasady et al., 2010; Jawad et al., 2020). The deviation from perfect bilateral symmetry (fluctuating asymmetry) can be caused by several factors such as environmental stress or developmental instability (Valentine et al., 1973; Palmer and Strobeck, 1986; Markowski 1993; Livshits and Kobyliansky, 1991). As a result, fluctuating asymmetry can be used to assess the suitability of habitats for the development of thriving populations of various animals, to determine the impact of pollutants on the populations (Jawad et al., 2012) or as a biomonitoring tool (Allenbach, 2011). The asymmetric individuals may have lower probabilities of survival than more symmetric ones, and single-trait asymmetry can serve as an early warning system in conservation (Lens et al., 2002).

The demersal and bottom-dwelling fish are particularly vulnerable to contaminants of different origins (Horchanok, 2019). Therefore, generally abundant gobies have been used previously to identify environmental stress (Jumawan et al., 2016). Round goby *Neogobius melanostomus* is a small benthic fish, highly invasive, pollution-tolerant, more abundant at highly polluted sites (Kornis et al., 2012; Marentette et al., 2010; McCallum et al., 2014; Piria et al., 2016) and an invasive non-native fish in Croatia (Mihinjač et. al., 2019). The fluctuating asymmetry of *Neogobius melanostomus* performed on several meristic characters was low under anthropogenic environmental stress (Horchanok, 2019).

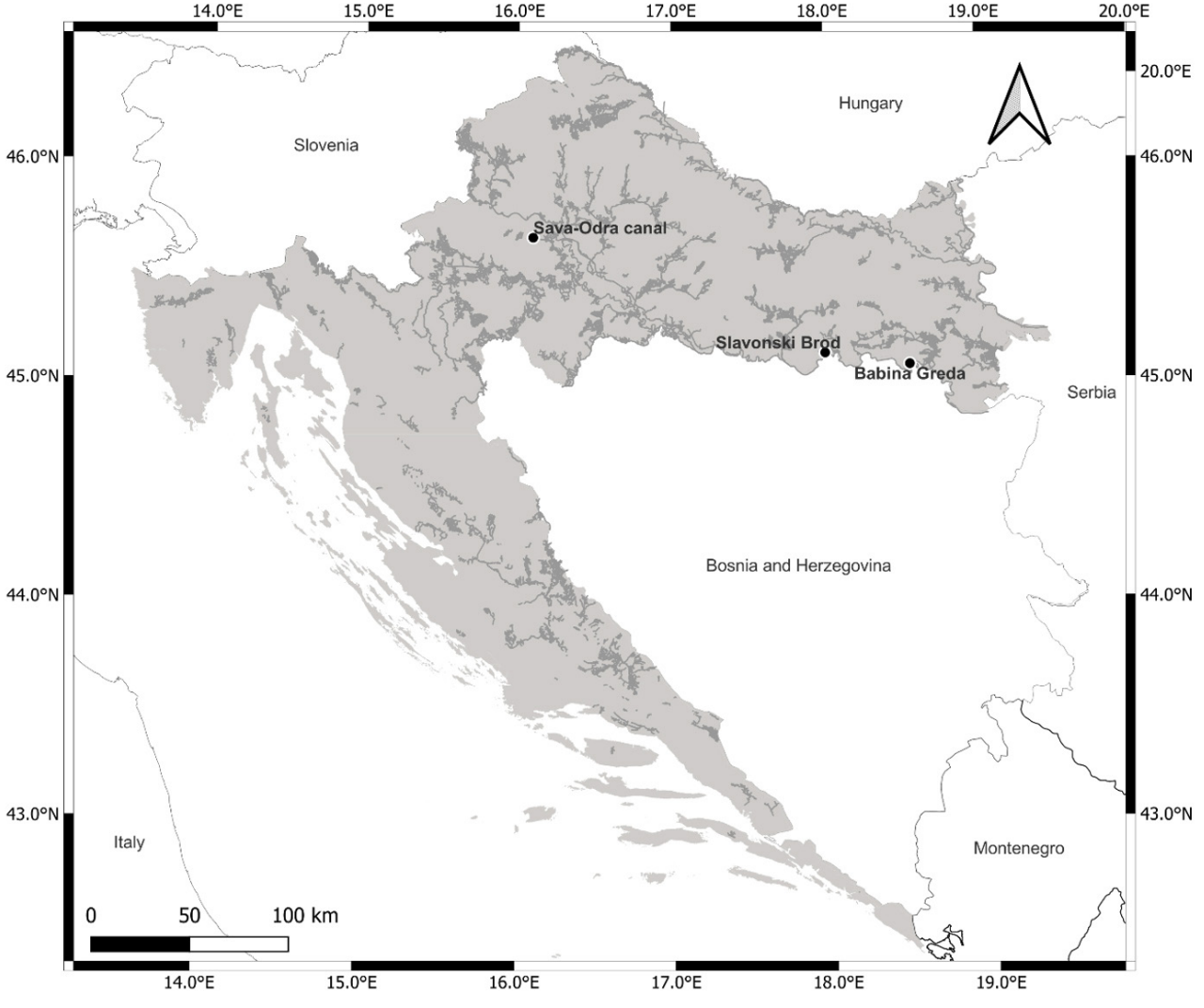

Fig 1. The map of the sampling sites with larger watercourses marked in dark grey

However, a study of signatures of contamination in *N. melanostomus* indicates that there is likely a causal link between contaminant exposure and shifts in the morphological and physiological characteristics of the species (Marentette et al., 2010). Hence, morphometric characters and otolith size may be important indices in the quantification of environmental stress, which were not tested by previous studies on *N. melanostomus* using fluctuating asymmetry analyses.

This work studies fluctuating asymmetry for both morphometric characters (head length, length of the snout, length of the upper jaw and post-orbital length) and otoliths of *N. melanostomus* collected at three different locations. The results of this study may be useful in the identification of environmental stress on aquatic habitat quality.

MATERIALS AND METHODS

The fish were sampled using the electrofishing method in September 2012 on the Sava River near the towns of Slavonski Brod (SBB) and Babina Greda (BGG), and in July 2019 near Zagreb at the Sava-Odra canal (CSO) (Figure 1) (for details see Jakovlić et al., 2015; Planchet et al., 2023). Electrofishing permits were issued by the Ministry of Agriculture. All caught specimens of *N. melanostomus* were removed from the water, taken to the laboratory and stored at -20°C.

In the laboratory, each specimen of *N. melanostomus* was measured for total length (TL) to the nearest 0.1 cm. Four morphometric characters were measured at the left and right sides of each specimen, and used for fluctuating asymmetry analyses: head length, snout length, length of the upper jaw and post-orbital length (Figure 2).

The left and right otoliths were taken from each specimen. 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 4.10 (ToupTek, Zhejiang, China). The measurement of otolith length and width for fluctuating asymmetry analyses (Figure 3) was done by TpsDig software.

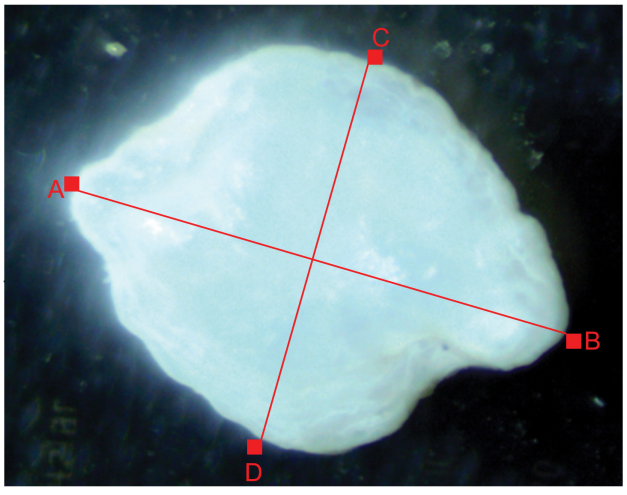

Fig 3. Otolith of round goby *Neogobius melanostomus* and taken measurement (distance between point A and B indicating otolith length, and distance between point C and D indicating otolith width)

The squared coefficient of asymmetry variation (CV^2) was calculated according to Valentine et al. (1973) by the following formula:

$$CV^2 = (Sr - 1 \times 100 / Xr + 1)2$$

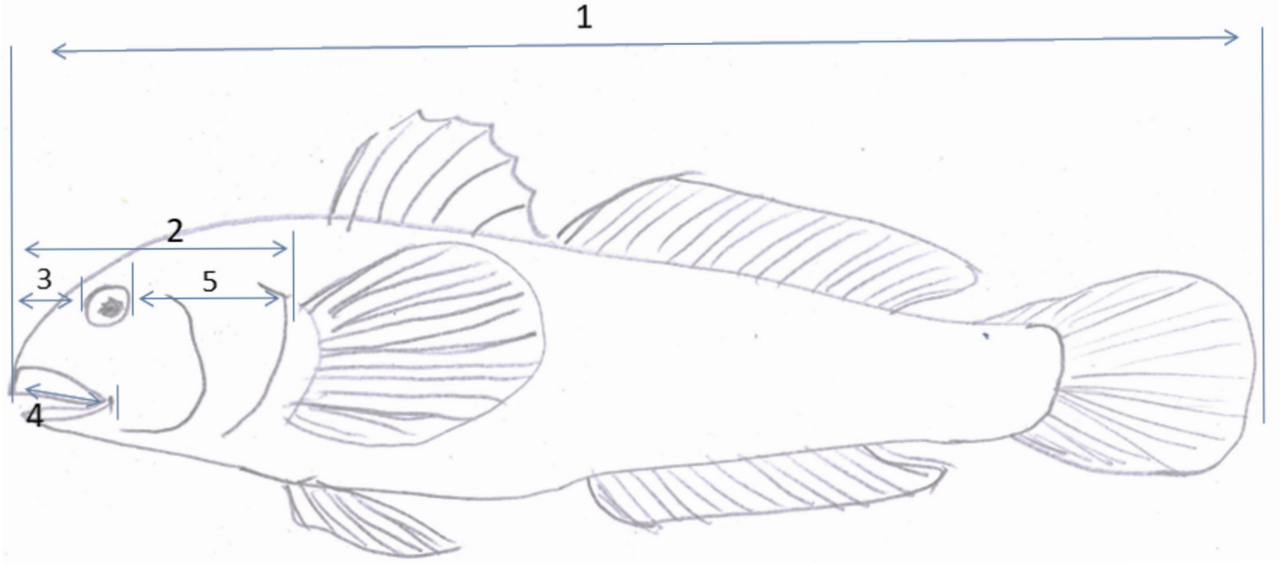

Fig 2. Measurement of body characters of round goby *Neogobius melanostomus*: (1) total length; (2) head length; (3) snout length; (4) length of the upper jaw and (5) post-orbital length

where Sr – 1 is the standard deviation of signed differences and Xr + 1 is the mean of the character. The mean was calculated by adding the absolute scores for both sides and dividing by the sample size.

RESULTS

In total, 11 specimens of *N. melanostomus* from SBB (TL_{min} 4.3 - TL_{max} 7.3), 16 specimens from BBG (TL_{min} 4.2 - TL_{max} 9.1) and 3 specimens from CSO (TL_{min} 6.7 - TL_{max} 8.4) were analysed for fluctuating asymmetry.

The percentage of individuals with asymmetry ranged from 63 to 100%. The results of the CV^2 showed the highest level of asymmetry in BBG in three morphometric characters (head length, snout length and postorbital length) and otolith width. At SBB, the length of the upper jaw and otolith width showed the highest values (Table 1).

DISCUSSION

This study confirms the variation in the asymmetry values of the measured characters between sampling sites of studied fish individuals, which may indicate severe environmental stress. Previous studies already showed a relationship between organism asymmetry and environmental stress (Jawad et al., 2012). In this study, the bilateral asymmetry of *N. melanostomus* was identified at all investigated sites of the Sava River. Indeed, the Sava River is polluted by organic and inorganic compounds from a variety of domestic and industrial activities that may affect the health of humans and wildlife (Källqvist et al., 2008; Malev et al., 2020).

Sediments are a potential source of potentially toxic elements, especially for fish species that feed on bottom-dwelling invertebrates (Zuliani et al., 2019), such as *N. melanostomus* (Piria et al., 2016). The highest concentrations of potentially toxic elements were detected in fish from sites with intensive industrial and agricultural activities (Zuliani et al., 2019). As bottom-dwelling fish that feeds primarily on invertebrates (Piria et al., 2016), *N. melanostomus* may be affected by severe pollutants throughout its life cycle.

The higher toxic potential along the Sava River was identified at specific "hot spots" and in its lower reaches (Källqvist et al., 2008; Ščančar et al., 2015; Marković et al., 2018). The sampling sites in this study were characterised by different environmental factors and conditions, resulting in higher anthropogenic pollution around BGG and further downstream (Milačič et al., 2017). BGG is the site with the abundant population of *N. melanostomus* (Piria et al., 2016) and the high environmental stress (Ščančar et al., 2015; Marković et al., 2018; Zuliani et al., 2019). This could be a reason for the higher values of the bilateral asymmetry of *N. melanostomus* taken from the most downstream point (BGG) in this study. This study also showed higher abundance of *N. melanostomus* at the most downstream sampling site (see Jakovlić et al., 2015). The high abundance of the species in certain sites may indicate the high pollution levels since it is known that populations of this species tend to be large in polluted areas (McCallum et al., 2014). In addition, numerous pharmaceuticals and illicit drugs were found in the Sava River, with the highest concentrations observed for antibiotics, allergy/cold medications and analgesics (Malev et al., 2020), which may also be reflected in the variation in body asymmetry.

Although this study is preliminary due to the small number of samples, the obtained results indicate that the stretch of the Sava River around BGG is more polluted than the SBB and CSS sampling areas. This study also shows that *N. melanostomus* can be used as a bioindicator for water quality analysis due to its fluctuating asymmetry in response to environmental conditions. Once caught, this invasive fish must be removed from the water (Mihinjač et al., 2019) and therefore can be used for rapid identification of habitat quality.

Table 1. Squared coefficient of asymmetry (CV^2) value and % of individuals of *Neogobius melanostomus* with asymmetry (values in bold represent the highest obtained CV^2 for each trait)

Character	Slavonski Brod n = 11		Babina Greda n = 16		Canal Sava Odra n = 3	
	CV^2	% of individuals	CV^2	% of individuals	CV^2	% of individuals
Head length	1.63	100	**2.51**	100	1.6	100
Snout length	3.37	90.91	**5.24**	87.5	4.66	66.67
Length of the upper jaw	**4.99**	63.64	3.68	87.5	4.8	100
Post orbital length	3.67	90.91	**5.26**	93.75	0.29	100
Otolith length	**7.77**	100	6.90	100	3.06	100
Otolith width	4.51	100	**5.12**	100	4.72	100

ACKNOWLEDGEMENTS

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. We thank our colleagues at the University of Zagreb Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology for their help with sampling in 2012 and 2019 on the Sava River and Sava-Odra canal. I am grateful to my internship tutor, Marie Laure Navas, for her constructive advice before and during my internship at University of Zagreb Faculty of Agriculture.

FLUKTUIRAJUĆA ASIMETRIJA MORFOLOŠKIH ZNAČAJKI INVAZIVNIH POPULACIJA GLAVOČA *Neogobius melanostomus* IZ RAZLIČITIH STANIŠTA SLIVA RIJEKE SAVE, HRVATSKA

SAŽETAK

Glavočić okrugljak, *Neogobius melanostomus*, jedan je od tri invazivna ponto-kaspijska glavoča pronađena u slivu rijeke Save u Hrvatskoj. Ima negativan učinak na izvorne ekosustave, a veći broj individua pronalazi se na jače zagađenim mjestima. Cilj ovog istraživanja je utvrditi fluktuirajuću asimetriju morfometrijskih značajki tijela te širine i dužine otolita. Na dva mjesta uzorkovanja u glavnom toku rijeke Save (Babina Greda, Slavonski Brod) i izmijenjenom staništu (kanal Sava-Odra) prikupljeno je i analizirano 30 jedinki *N. melanostomus*. Kvadratni koeficijent varijacije asimetrije korišten je za analizu potencijalnih razlika između populacija. Identificirane su varijacije u vrijednostima asimetrije analiziranih morfometrijskih značajki te širini i dužini otolita između mjesta uzorkovanja. Najveća fluktuirajuća asimetrija uočena je kod jedinki na mjestu uzorkovanja Babina Greda, što može biti posljedica visokog pritiska onečišćenja okoliša. Rezultati studije pokazuju da se metoda fluktuirajuće asimetrije može koristiti na invazivnim vrstama riba kao jednostavan pristup u identifikaciji kvalitete okoliša staništa.

Ključne riječi: bilateralna asimetrija, invazivne ribe, okolišni stres, bioindikator

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