Theoretical and Applied Veterinary Medicine

ISSN 2663-1156 (print)
ISSN 2663-1164 (online)
Theoretical and Applied Veterinary Medicine, 7(3), 147–152
doi: 10.32819/2019.71026

Original researches

Fluctuating fish asymmetry in natural and artificial reservoirs of Dnipro region on example of invasion types

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Received: 12 June 2019
Revised: 19 July 2019
Accepted: 08 August 2019

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Cite this article: Horchanok, A. V. (2019). Fluctuating fish asymmetry in natural and artificial reservoirs of Dnipro region on example of invasion types. *Theoretical and Applied Veterinary Medicine*, 7(3), 147–152. doi: 10.32819/2019.71026

Abstract. Researches of fluctuating asymmetry of the bilateral features of fish were conducted in 2018 on the natural reservoirs (the Samara River within Novomoskovsk and Pavlograd districts of Dnipropetrovsk region, the reservoirs of the Dnipro-Oril Nature Reserve). Insignificant deviations from the bilateral symmetry of external features in living organisms may indicate a decrease in the vitality of certain populations. Using of mass, widespread fish species as the test objects is the best way to detect the levels of fluctuating asymmetry. The fluctuating asymmetry of four bilateral features was estimated within 146 units of the common sunfish *Lepomis gibbosus*, the stone moroco of *Pseudorasbora parva* and the round goby *Neogobius melanostomus* in different habitat conditions, the stability of their life circle was evaluated. The number of rays in the interbranchial septum, the number of branching rays in the pectoral and abdominal fins, the number of scales in the lateral line were analysed. The variability of these features on the left and right sides of the fish was evaluated. The lowering of the natural fish populations vitality because of the influence of negative anthropogenic pressure is currently minor. The stability of fish development by the analysis of bilateral features is noted within normal limits of 1 point (*Neogobius melanostomu*s from the reservoirs of the Dnipro-Oril Nature Reserve, *Lepomis gibbosus* and *Pseudorasbora parva* from the middle section of the Samara River) or deviates insignificantly from the statutory indicator in 2 points (*Lepomis gibbosus* from the lower section of the Samara River). Species of *Neogobius melanostomus* from the reservoirs of the Dnipro-Oril Nature Reserve show the lowest quantitative indices of the bilateral features asymmetry (0.17 of asymmetric detections per feature). This may demonstrate the absence of negative anthropogenic impact on ground fish within the particularly guarded water areas. The application of the analysis of the fluctuating fish asymmetry is the simplest and most accessible way of rapid testing of the water environment state, but complex laboratory studies, including molecular and biochemical ones, must be used to obtain a more accurate and reliable estimate.

Keywords: fluctuating asymmetry; *Lepomis gibbosus*; *Pseudorasbora parva*; *Neogobius melanostomus*; reservoirs of Dnipro Region.

Флуктуюча асиметрія риб природних і штучних водойм Придніпров’я на прикладі інвазійних видів

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Анотація. Дослідження флуктуючої асиметрії білатеральних ознак риб проводили у 2018 р. на природних водоймах (ріки Самара у межах Новомосковського та Павлоградського районів Дніпропетровської області, водойми природного заповідника «Дніпровсько-Орільський»). Незначні відхилення від білатеральної симетрії зовнішніх ознак у живих організмів можуть вказувати на зниження життєздатності певних популяцій. Використання масових, широко розповсюджених видів риб, як тест-об’єктів – оптимальний спосіб виявлення ступеня флуктуючої асиметрії. Досліджували флуктуючу асиметрію чотирьох білатеральних ознак у сонячного окуня (*Lepomis gibbosus*), чебачка амурського (*Pseudorasbora parva*) та бичка-кругляка (*Neogobius melanostomus*) в різних умовах існування, оцінили стабільність їх життєвого розвитку. Аналізували кількість променів у міжзябровій перегородці, кількість гіллястих променів у грудних і черевних плавцях, кількість лусок у бічній лінії. Визначали мінливість цих ознак на лівому та правому боці риб. Установлено, що зниження життєздатності природних популяцій риб за впливу негативного антропогенного тиску наразі незначне. Стабільність розвитку риб за аналізом білатеральних ознак відмічається у межах норми – 1 бал (*Neogobius melanostomus* із водойм природного заповідника «Дніпровсько-Орільський», *Lepomis gibbosus* і *Pseudorasbora parva* – із середньої ділянки ріки Самара) або незначно відхиляється від нормативних показників – 2 бали (*Lepomis gibbosus* із нижньої ділянки Самари). Найнижчі кількісні показники асиметрії білатеральних ознак спостерігаються у *Neogobius melanostomu* з водойм природного заповідника «Дніпровсько-Орільський» (0,17 асиметричних виявлень на одну ознаку). Це може свідчити про відсутність негативного техногенного впливу на донних риб у межах акваторій, які перебувають під особливою охороною. Застосування аналізу показників флуктуючої асиметрії риб – найбільш простий і доступний спосіб експрес-аналізу стану водного середовища, але для отримання більш точного та достовірного оцінювання необхідно застосовувати складні лабораторні дослідження, у тому числі молекулярні та біохімічні.

Ключові слова: флуктуюча асиметрія; сонячний окунь (*Lepomis gibbosus*); чебачок амурський (*Pseudorasbora parva*); бичок-кругляк (*Neogobius melanostomus*); водойми Придніпров’я.

Introduction

Fluctuating asymmetry (FA) indicates minor and non-directional deviations of the body's features from strict bilateral symmetry (Zakharov, 1987), which appear and can be taken into account by researchers in the case of the stability distortion of the organism (Zorina & Korosov, 2009). Numerous studies have proved that the level of FA of ichthyofauna is minimal under favourable conditions of natural reservoirs, but under the influence of a stress factor it significantly increases. It should be noted that these demonstrations of destabilization, for example, of meristic (counting) features can be detected even at low levels of anthropogenic disturbances in ecosystems. According to the recommendations of many researchers (Leung & Forbes, 1997; Gelashvili et al., 2004; Korona & Vasiliiev, 2007; Novitskiy, 2007), fluctuating asymmetry can be used as a reliable indicator of even insignificant deviations of environmental parameters from the norm, which do not yet cause irreversible decrease in the vitality of units. That is, the index of fluctuating asymmetry is a kind of indicator which shows stability of an organism development (Zakharov, 1987; Leung et al., 2000; Gileva et al., 2007; Graham et al., 2010; Khrystenko et al., 2016).

Currently, asymmetry indicators are used in population biology to study phenotypic variability, to evaluate the functional significance of properties and their liability to breeding effects, to estimate the state of natural populations, to study micro evolutionary transformations, and also for biomonitoring studies (Valen, 1962; Palmer, 1986; Zakharov, 1987; Leung et al., 2000; Dongen, 2006).

Today, the number of scientific researched dedicated to various issues of the influence of environmental factors on fluctuating asymmetry is constantly increasing, with the scientific community pointing out that the phenomenon of FA requires additional studying (Palmer, 1996).

Compared to other vertebrates, fish are appropriate as test objects for biomonitoring. Important information about the stability of life development can be obtained through the so-termed «morphological approach» when the exterior and interior morphological features of fish are analysed. Using of the morphological approach in ichthyologic and ecological researches as the simplest and at the same time the most informative allows to analyse the unit variability of organisms within the conditions of anthropogenic pressure, to estimate their vitality, and to analyse the state of the natural environment. Invasive fish species, which today continue to cover new habitats, are favourable objects for assessing the status of reservoirs (McCallum et al., 2014; Novitskiy et al., 2019).

Common sunfish (*Lepomis gibbosus*) is a widespread species, both in Ukraine and in Europe broadly, and its habitat continues to increase. Invasive species such as *L. gibbosus* are known to adapt well to a variety of habitats. In order to assess the morphological changes of this invasive species, researches were conducted in the Aegean and Thracian regions. Here, body shape variability in sampling populations of *L. gibbosus* showed significant geographical and environmental differences with and without allometric standardization, it indicates that depending on the morphometric research hypothesis, species may be important implementation of allometric regression and standardization (Mangit et al., 2018; Reis et al., 2018).

Stone moroco (*Pseudorasbora parva*) is an invasive species which range is greatly expanded due to the sudden introduction. The native range of the species includes eastern Asia, from the Amur basin to northern Vietnam, and Europe. Expansion of the stone moroco is promoted by anthropogenic transformation of reservoirs and high flexibility of the species. This species is an insalubrious invader that sometimes forms large populations, has no industrial value, and is capable to cause significant damage to the forage of native fish species (Karabanov et al., 2010; Angradi, 2018).

The next invader species is the round goby (*Neogobius melanostomus*). After its invasion, as a highly invasive species, long-term changes are gradually occurring in populations. The round goby also represents threat to native species, competes with them for fodder resources, habitat, etc. (Prysiazhniuk et al., 2019). The round goby as a eurybiotic species contributes to the migration of pollutants to higher trophic levels (Hubanova et al., 2019) in transformed ecosystems with different levels of pollution (Novitskiy & Gubanova, 2016). Monitoring researches indicate that the number of round goby species decreases in areas with low levels of contamination, while remaining stable in areas with high levels of pollution. The average body size decreases and reproductive capacity increases, which emphasizes the impact of the invasive species on the population and indicates the need to control the species in the contaminated environment (McCallum et al., 2014).

The dynamics of invasive species after their naturalizing is underinvestigated. Understanding this dynamic is important for the management of invasive species, determining their functional value in different types of reservoirs.

The aim of this work is to evaluate the fluctuating fish asymmetry (on the example of the common sunfish *L. gibbosus*, stone moroco *P. parva*, round goby *N. melanostomus*) in the natural reservoirs of the Dnipro region as an index of stability of the morphosis.

Material and methods

Ichthyologic studies and sample collecting were performed in 2018 from natural reservoirs (lower section of the Samara River near village Odynkivka Novomoskovsk district, middle section of the Samara River near village Kocherezhky Pavlograd district).

The analysis included 146 mature fish: 118 units of common sunfish *L. gibbosus*, 10 units of stone moroco *P. parva*, 18 units of round goby *N. melanostomus*. These species have been used as test objects for assessing the stability of fish organism development.

The materials provided for analysis by an employee of the Dnipro-Oril Nature Reserve D. L. Bondariev were analysed in the research (units of the round goby *N. melanostomus*).

The sampling of juvenile fish was carried out in July and August in the riverfront of reservoirs by a small mesh trawl (15.0 m in length, with a mesh of 0.7 cm in the wings and 0.3 cm in the ball). For catching *L. gibbosus* amateur fishing gear (fishing rod with float) also was used. According to the methodology (Lalumière, 2004), the samples were monoject.

Sampling of ichthyologic material was performed according to the method of I. F. Pravdin (1966). The processing of the collected material was carried out in accordance with standard methods of ichthyologic researches (The method of collection..., 1998; Romanenko, 2006; Pryachin & Shkitckiy, 2008). The measurements were performed on fresh material.

Four morphological bilateral features of the fish were analysed: the number of rays in the interbranchial septum (RIM); the number of branching rays in the pectoral (P) and abdominal (V) fins; the number of scales in the lateral line (LL). The variability of these features on the left and right sides of the fish was analysed (Figure). Researches of morphological bilateral features were performed according to the instructions of V. M. Zakharov (1987).

The processing, analysis and generalization of the results were performed using methods of mathematical statistics (Lakin, 1990).

Statistical data processing and the search of multidimensional regression models of the research results were performed with using Excel and *Statistica 10*.

Results

The random sampling of fish was analysed in the research; therefore, a large number of asymmetric detections indicates a decrease in the vitality of natural populations (groups) of fish under the influence of negative anthropogenic pressure. Summaries of the obtained material and the variability of the bilateral features of the

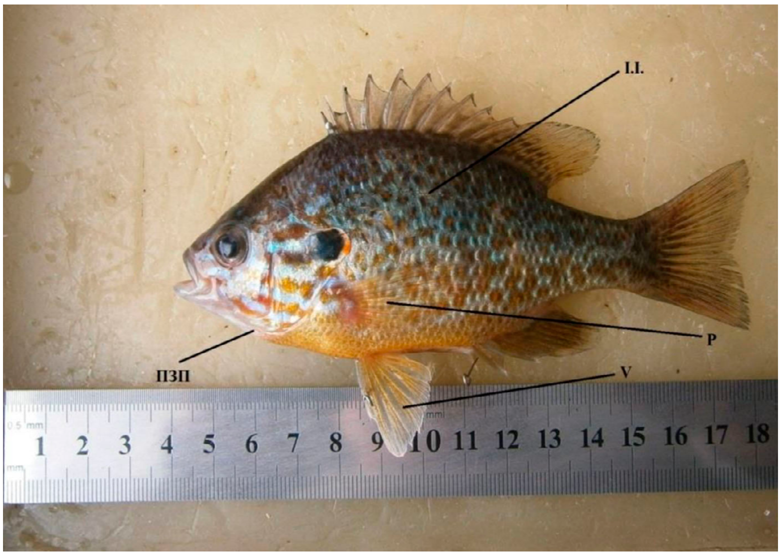

Figure. Bilateral features of the common sunfish *Lepomis gibbosus*:
(RIM) the number of rays in the interbranchial septum; the number of branching rays in the (P) pectoral and (A) abdominal fins; (LL) the number of scales in the lateral line.

three species of studied fish from different reservoirs of the Dnipro region are represented in Tables 1–2.

According to the research method of fluctuating asymmetry the number of asymmetric features (NAF) obtained for each group of fish should be divided by the number of studied units.

20 units of *L. gibbosus* from lower section of the Samara River (village Odynkivka), 74 units of *L. gibbosus* from middle section of the Samara River (village Kocherezhky), 10 units of *P. parva* from middle section of the Samara River (village Andriivka), 18 units of *N. melanostomus* from Dnipro-Oril Nature Reserve were examined.

Table 1. Variability of bilateral features of *Lepomis gibbosus* from different reservoirs of Dnipro region

Features	Side of body	M ± m	Min – max	Ϭ
Samara River (lower section, village Odynkivka, n = 20)				
Number of rays in the interbranchial septum	left	4.20 ± 0.121	3.00 – 5.00	0.52
	right	4.00 ± 0.152	3.00 – 5.00	0.65
Number of rays in paired fins (pectoral respectively)	left	11.35 ± 0.134	10.00 – 12.00	0.59
	right	11.10 ± 0.121	10.00 – 12.00	0.55
Number of rays in paired fins (abdominal respectively)	left	5.95 ± 0.053	5.00 – 6.00	0.22
	right	5.90 ± 0.072	5.00 – 6.00	0.31
Number of scales in the lateral line	left	37.35 ± 0.431	34.00 – 41.00	1.93
	right	37.00 ± 0.514	32.00 – 41.00	2.22
Samara River (middle section, village Kocherezhky, n = 74)				
Number of rays in the interbranchial septum	left	4.04 ± 0.041	3.00 – 5.00	0.31
	right	4.05 ± 0.031	3.00 – 5.00	0.28
Number of rays in paired fins (pectoral respectively)	left	11.66 ± 0.072	10.00 – 13.00	0.58
	right	11.58 ± 0.061	11.00 – 13.00	0.55
Number of rays in paired fins (abdominal respectively)	left	5.96 ± 0.022	5.00 – 6.00	0.20
	right	5.99 ± 0.013	5.00 – 6.00	0.12
Number of scales in the lateral line	left	36.03 ± 0.242	31.00 – 45.00	2.05
	right	36.12 ± 0.241	32.00 – 43.00	2.05

Note: M – average value; m – average inaccuracy; min – max – minimum and maximum value; Ϭ – standard deviation; n – number of units.

Table 2. Variability of bilateral features of stone moroco *Pseudorasbora parva*

Features	Side of body	M ± m	Min – max	σ
Samara River (village Andriivka), n = 10				
Number of rays in the interbranchial septum	left	3.00 ± 0.001	3.00 – 3.00	0.00
	right	3.00 ± 0.004	3.00 – 3.00	0.00
Number of rays in paired fins (pectoral respectively)	left	10.60 ± 0.44	9.00 – 12.00	1.26
	right	10.80 ± 0.389	9.00 – 13.00	1.23
Number of rays in paired fins (abdominal respectively)	left	8.50 ± 0.167	8.00 – 9.00	0.53
	right	8.30 ± 0.201	7.00 – 9.00	0.67
Number of scales in the lateral line	left	33.60 ± 0.721	30.00 – 38.00	2.27
	right	33.70 ± 0.778	30.00 – 39.00	2.45
Dnipro-Oril Nature Reserve, n = 18				
Number of rays in the interbranchial septum	left	4.00 ± 0.112	3.00 – 5.00	0.49
	right	4.06 ± 0.091	3.00 – 5.00	0.42
Number of rays in paired fins (pectoral respectively)	left	19.06 ± 0.221	18.00 – 21.00	0.94
	right	19.11 ± 0.278	17.00 – 22.00	1.18
Number of rays in paired fins (abdominal respectively)	left	4.94 ± 0.056	4.00 – 5.00	0.24
	right	4.94 ± 0.091	4.00 – 6.00	0.42
Number of scales in the lateral line	left	48.94 ± 0.467	44.00 – 53.00	1.98
	right	48.94 ± 0.456	44.00 – 53.00	1.95

Determine how many asymmetric features one unit has on average:

common sunfish (lower section of the Samara River village Odynkivka):	29 : 20 = **1.45**
common sunfish (middle section of the Samara River village Kocherezhky):	82 : 74 = **1.11**
stone moroco (middle section of the Samara River village Andriivka):	11 : 10 = **1.1**
round goby (Dnipro-Oril Nature Reserve):	12 : 18 = **0.67**

For reliable comparison of the analysed samples, the obtained data are divided into the number of investigated morphological features (four), obtaining the frequency of asymmetric detection per feature (ADF):

common sunfish (lower section of the Samara River village Odynkivka):	1.45 : 4 features = **0.36**
common sunfish (middle section of the Samara River village Kocherezhky):	1.11 : 4 features = **0.28**
common sunfish (artificial reservoir village Mykolaivka-1):	1.54 : 4 features = **0.39**
stone moroco (middle section of the Samara River village Andriivka):	1.1 : 4 features = **0.28**
round goby (Dnipro-Oril Nature Reserve):	0.67 : 4 features = **0.17**

V. M. Zakharov (1987) proposed to use a scoring of the fish life development stability as an indicator of the water environment state. When specifying the frequency of asymmetric detection per feature (ADF):

to 0.35 – development stability can be assessed 1 point (norm);
at a value from 0.35 to 0.40 – **2 points (small deviations from the norm);**
from 0.40 to 0.5 – **3 points (serious environmental situation);**
from 0.45 to 0.50 – **4 points (environmental crisis);**
more then 0.50 – **5 points (environmental catastrophe).**

The stability of the fish development from different reservoirs of the Dnipro region according to the analysis of bilateral features is within the norm (1 point) or deviates in a minor way from the statutory indicators (2 points).

Discussion

Bilateral features were counted in units of common sunfish *L. gibbosus* and stone moroco *P. parva*, which are non-indigenous species of ichthyofauna, and now are naturalized in the resevoirs of Ukraine (Novitskiy et al., 2019). In addition, the deviation from the bilateral symmetry in the Ponto-Caspian species of the round goby *N. melanostomus* was investigated.

It is known that adequate assessment of the biological organism status and the natural environment in general can be obtained already in the analysis of samples from 10–20 units (Pitelka, 1950; Zakharov, 1987). We have analysed the morphological bilateral structures of 118 units of *L. gibbosus*, 10 units of *P. parva*, 18 units of *N. melanostomus*. The introduction of morphological features is represented in tables 1–2 (the number of features for each unit is indicated in the rightmost columns, for features the values on the left (l) and right (n) are not equal).

Researches of deviations from the bilateral symmetry were carried out in different ecological groups of fish (common sunfish - predator, stone moroco – euryphagus, round goby – benthophagus), which were caught from reservoirs with different anthropogenic load, from the great (middle and lower sections of the Samara River) to relatively free from anthropogenic pressure (inland reservoirs of the Dnipro-Oril Nature Reserve) (Slynko et al., 2011).

Obtained data about the stability of fish development indicate that the decline in the vitality of natural fish populations under the influence of negative anthropogenic pressure is negligible (Graham et al., 2010). It is necessary to note the low quantitative indices of the bilateral features asymmetry of the round goby from the reservoirs of the Dnipro-Oril Nature Reserve (0.17 detections per one feature). It is known that demersal and bottom fish are particularly vulnerable to contaminations of different origins, so the absence of morphological abnormalities, deviations from the bilateral symmetry of the body in group of round gobies may indicate the absence of negative technogeneous influence on these species within closely guarded waters.

A little increased degree of variability of the bilateral features of common sunfish from the lower section of the Samara River (see figure) can be explained by two reasons: a) the presence of significant negative pressure on the water of river; b) significant flexibility of the non-indigenous species *L. gibbosus*, which is currently undergoing a rapid period of naturalization in the Dnipro region reservoirs (Novitskiy et al., 2014; Hubanova, 2019).

In all conscience, to obtain scientifically reliable information about the state of the water environment due to the analysis of the bilateral morphological features of the fish, it is necessary to conduct certain monitoring researches of different ecological groups of fish. In our opinion, determining the indices of fluctuating fish asymmetry is the first, simplest and most accessible way to express the state of the water environment. To obtain a more accurate and reliable assessment, complex laboratory researches, including molecular and biochemical studies, such as the assessment of species' stress resistance, are the main source of studying reaction of the organism to negative impact (Almeida et al., 2008; Allenbach, 2010).

Conclusion

The decline in the vitality of fish natural populations from different reservoirs of the Dnipro region under the influence of negative anthropogenic pressure is currently negligible. Stability of fish development according to the analysis of bilateral features is noted within the norm – 1 point (round goby from reservoirs of the Dnipro-Oril Nature Reserve, common sunfish and stone moroco from the middle section of the Samara River) or deviates insignificantly from the statutory indicators in 2 points (common sunfish from the lower section of the Samara River).

The lowest quantitative indicators of asymmetry of the bilateral features are observed in the group of round goby *N. melanostomus* from the reservoirs of the Dnipro-Oril Nature Reserve (0.17 detections per one trait). This may indicate that there is no negative anthropogenic impact on bottom fish within closely guarded waters.

The application of the analysis of the fluctuating fish asymmetry is the simplest and most accessible way of rapid analysis of the water environment state, but complex laboratory studies, including molecular and biochemical, must be used to obtain a more accurate and reliable estimate.

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