

Morphological differentiation of the otoliths of graylings from the Barguzin River and Lake Baikal

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ABSTRACT. A comparative analysis of distinctive morphological features of sagittae in the *Thymallus* species from Lake Baikal and the Barguzin River has been done. The otolith shape was described using values of radii and the Fourier descriptors. It is demonstrated that the otoliths of *Thymallus* species have a similar structure. The otolith outline analysis allowed to differentiate the samplings according to the morphotypes that characterize them. No significant differences in length, width, perimeter and area of the otoliths in the *Thymallus* species have been identified. However some differences in the shape of otoliths were described in *T. baicalolenensis*; they had a less developed pararostrum and more salient ventral edge than the otoliths of *T. baicalensis* from the lakes Balan-Tamur and Baikal. The otolith shape analysis can be widely applied for basic research of microevolutionary processes, delimitation of natural populations and preservation of genetic diversity as well as for defining the structure of the commercial stock.

Keywords: *Thymallus*, otolith, shape analysis, catchment area of Lake Baikal

1. Introduction

The otoliths of the teleosts are of great utility for basic studies of fish biology and ecology as well as for fish monitoring. In particular, the sagittae are used to investigate the fish age and growth, population structure and trophic ecology (Ballagh et al., 2006; Rooker et al., 2008). The species specificity of size and morphology of the otoliths finds a use in the fish species identification (Zischke et al., 2016), including the analysis of gastrointestinal content of predatory fish, piscivorous birds and mammals (Furlani et al., 2007). The sizes and morphology of the otoliths underlie identification guides and keys for full-grown fish (Skalkin, 1963; Skazkina, 1965; Yukhov, 1971; Svetochева et al., 2002) and for young fish (Khrustaleva and Pavlov, 2000). Peculiarities of the variability of otolith size and shape allow to use them for differentiation between closely related species and identification of intraspecific taxa (Campana and Casselman, 1993; Johnson, 1996; Bolles and Begg, 2000; Begg et al., 2001; DeVriesa et al., 2002; Bergenius et al., 2006; Tyagun et al., 2013; Pavlov, 2016).

According to previous studies, the ichthyofauna of the upper Barguzin (Baikal's tributary) catchment area is represented by two species of the genus *Thymallus*: *Thymallus baicalolenensis* Matveev, Samusenok, Pronin & Tel'pukhovskiy, 2005 (Matveev

et al., 2005; Prosekin, 2007) and *Thymallus baicalensis* Dybowski, 1874 that significantly differs in a number of traits from the *T. baicalensis*, inhabiting Lake Baikal and being its geographical form (Prosekin, 2007). The *T. baicalolenensis* mostly prefers mountainous rivers, while the *T. baicalensis* inhabits mountainous lakes of the Amut relict glacial basin located more than 250 km away the Barguzin estuary.

The objective of the study was to define possibilities of otolith differentiation and to identify the most informative traits of their morphology in the *Thymallus* species from various habitats.

2. Material and methods of research

The sagittae from the collection of the Laboratory of Ichthyology of Limnological Institute, Siberian Branch of the Russian Academy of Sciences: *T. baicalolenensis* (25), *T. baicalensis* from the lakes Balan-Tamur (43) and Baikal sampled during the cruise of 2006 (Fig. 1) were used as study material. The fish was first measured for its standard length (*SL*), then the otoliths were extracted for age determination and further analysis. Each otolith was photographed from the exterior side with regard to its anatomic position. The further measurements were done by means of digital imaging. The otolith outline was projected from the digital image onto the image

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plane using Image-Pro Plus software. The outlines of deviant and defective otoliths were excluded from the analysis. Then the area, perimeter, length and width of the projected outline were calculated. The digitized outline was normalized against its position (straight for the right otolith, in inversed manner for the left one), geometrical center superposed on the origin point (maximum in diameter in line with the coordinate axis) and area (the outline was recalculated against the projected area unit).

The polar coordinates were applied for the outline shape analysis. 60 radii (R_i) through equal (6°) angles were obtained from origin point for each otolith. The Excel and Statistika 6.0 software was used to analyze the data. The values of radii (R_i) and the Fourier descriptors were taken as test objects (Castonguay et al., 1991).

3. Results

The structural plan of otoliths in all form of graylings is similar (Fig. 2). The otoliths of *Thymallus* species are flat, without curve, the rostrum is long, rounded or tapered. The antirostrum is round. The postrostrum and parastrostrum are usually divided. The channel (*sulcus acusticus*) is deep. The exterior surface is irregular. The relief corresponds to its optical structure. According to our data, *T. baicalolenensis* (SL 24-32 mm) was 4-6 years old, *T. baicalensis* from Lake Balan-Tamur (SL 32-40 mm) was 5-12 years old and the fishes from Lake Baikal (SL 15-36 mm) were 3-8 years old. When the otoliths become bigger they show a general trend to change their shape: the outline length to width ratio increases and the *excisura minor* and *excisura major* also grow in size (Fig. 2). Although these trends in larger specimens from Lake Balan-Tamur were not pronounced well.

No significant differences in the length, width, perimeter and the outline area of the otoliths, but distinctive features of their shape were found in the fishes from the study sites. Incremental linear discriminant analysis was used to identify the most informative combination of measurements (R_i) and the Fourier descriptors (A_j). Insertion of variables (R_i) and (A_j) stopped at the steps 23 and 18 respectively given F for insertion equal to 1 (Fig. 3). However in the first case two inserted variables R_{51} and R_{27} allow a 90% classification of the otolith samples among the given groups, the results of the classification plateau at the step 4 after insertion of R_{42} having reached 93% of the level of correct classifications. In the second case, the results plateau at the step 10 after insertion of A_{12} reaching 90% of the level of correct classifications. The further insertion of variables may be considered as less informative in both cases because an ulterior growth of classification percentage is due to the otolith that have a low probability to belong to the a priori given groups.

According to the calculations, square of the Mahalanobis distance (R_i at the step 4 and A_j at the step 10) show a higher level of differences between the otoliths samplings of *T. baicalensis* from Lake Balan-Tamur and *T. baicalolenensis* (Table). The distances from



Fig. 1. Sites of the *Thymallus* sampling: 1 – Barguzin River and Lake Balan-Tamur; 2 – Lake Baikal, Svyatoy Nos Peninsula

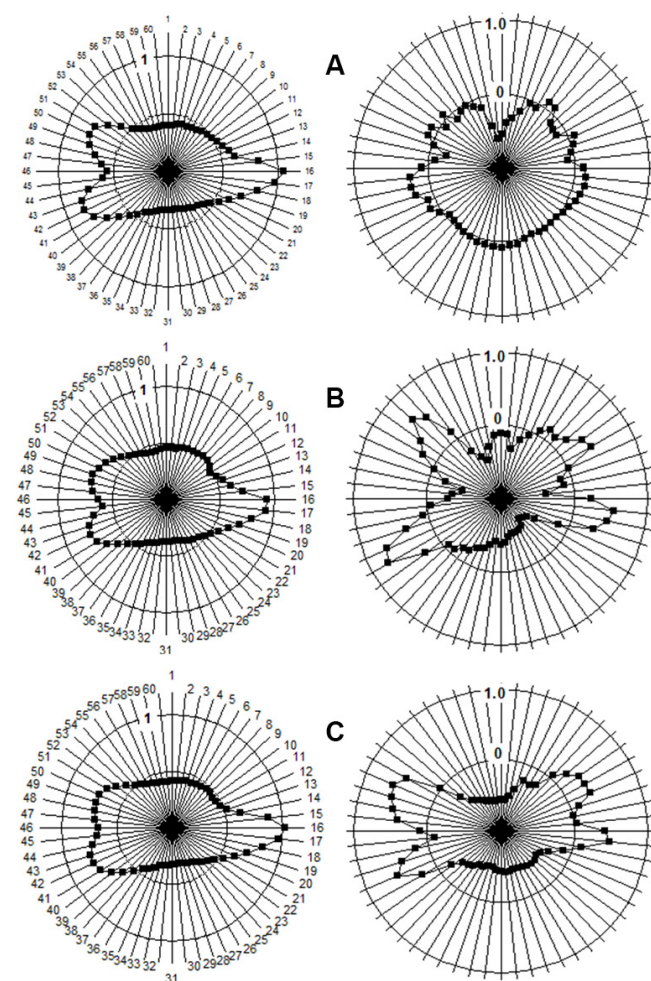


Fig. 2. Diagrams of average values of otolith radii of *T. baicalensis* from Lake Balan-Tamur (A), *T. baicalolenensis* (B), and *T. baicalensis* from Lake Baikal (C) (left panel) and correlation coefficient values against the otolith area (right panel)

the sampling of *T. baicalensis* from Lake Baikal to two other samplings are similar. Thus, the use of the values of radii R_i and the values of the Fourier descriptors A_j in the discriminant analysis yielded the similar results concerning the accuracy of otolith classification and intersample distances.

In coordinates of canonical variables obtained both from R_i and A_j values, the otolith samplings form relatively separate clusters (Fig. 4). As the radii and the descriptors yield the similar results only those based on R_i are given here. Both variables describe a significant proportion of differences: 59 and 41% for the first and the second one, respectively. The value of the first variable depends mostly on R_{51} , while the second one on R_{27} .

The otoliths of *T. baicalolenensis* thus differ in a less developed pararostrum and more convex ventral edge, *T. baicalensis* from Lake Balan-Tamur has otoliths with the least developed pararostrum, while the otoliths of *T. baicalensis* from Lake Baikal have a concave ventral edge. The clusters of other samplings included the otoliths whose morphotype sharply deviated from their group. The shape of these otoliths may be considered deviant for the respective samplings. The sharp deviation from the typical shape, including crystal structure disturbances is of common occurrence in *T. baicalensis* from Lake Baikal (Anoshko et al., 2007). The deviant otoliths were also found in graylings from the Barguzin River and Lake Balan-Tamur. They were thinner, more transparent and lacked a coherent optical structure. It is known that calcium carbonate in such otoliths deposits mostly in the form of vaterite (Gauldie, 1993). However 90% of otoliths have a morphotype characteristic of *Thymallus* species and intraspecific taxa, thus giving a possibility to use them for species identification, e.g. in digestive tracts of predatory fish and piscivorous birds and mammals from the Barguzin catchment area.

4. Discussion and conclusions

The adaptive value of otolith morphological characteristics is unclear (Popper et al., 2005). According to theoretical models there might be differences in the perception of sounds of different frequencies depending on the otolith size (Lychakov and Rebane, 1992; 2000). The intraspecific (and intrapopulation) discrepancies in the otolith morphology may be induced by various reasons or form during the growth as a result of implementation of genetic information under the influence of the environment. A number of papers reports a significant correspondence of the otolith shape to genetic variations on the population level (Jonsdottir et al., 2006; Abuanza et al., 2008; Libungan et al., 2016; Afanasyev et al., 2017). Therefore one may suppose the changes of the otolith shape within specified boundaries could be sporadic. The microevolutionary processes take place on a short-term horizon under the influence both of global and local climatic changes. The otolith morphology as the most morphometric traits is likely a result of expression of many genes.

Table. Square of the Mahalanobis distance between the samplings: *T. baicalensis* from Lake Balan-Tamur (1), *T. baicalolenensis* (2) and *T. baicalensis* from Lake Baikal (3). R_i and A_j are the upper and lower parts of the matrix, respectively

	1	2	3
1		18	11
2	19		12
3	12	14	

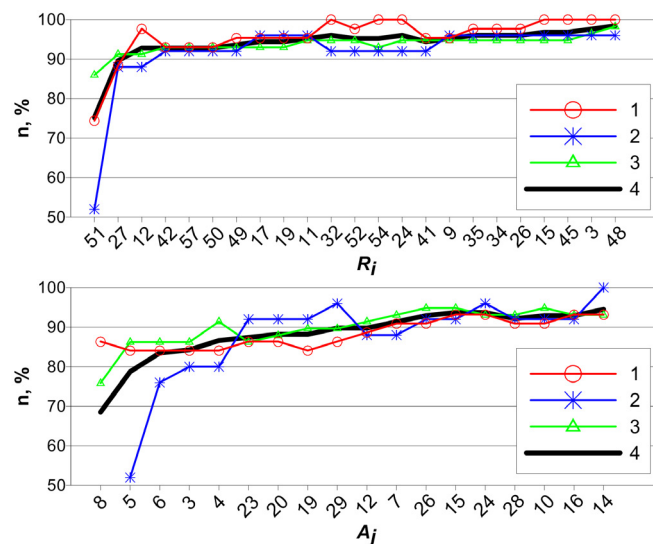


Fig. 3. Results of otolith classification: (1) *T. baicalensis* from Lake Bala-Tamur, (2) *T. baicalolenensis*, (3) *T. baicalensis* from Lake Baikal, (4) all samplings along the stepwise insertion of radii R_i (above) and the Fourier descriptors A_j (below) during the linear discriminant analysis

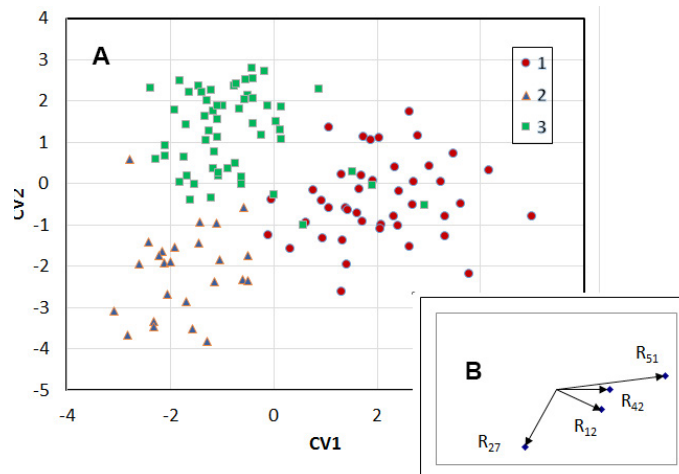


Fig. 4. (A) Differentiation of graylings in coordinates of canonical variables: 1 – *T. baicalensis* from Lake Balan-Tamur, 2 – *T. baicalolenensis*, and 3 – *T. baicalensis* from Lake Baikal. (B) Vectors of factor loadings

Opposed to the morphometric characteristics of fishes, the features of otolith structure depend less on the environment, e.g. short-term changes of the growing period, seasonal action of physical and chemical factors or reproduction cycles (Cadrin and Friedland, 2005). The phenotypical variations of the otoliths do not allow to make conclusions about intraspecific groups of the fishes, however they can evidence that those have been divided for a long time (Campana et al., 1995).

Previously L. Ding and coauthors (Ding et al., 2019) found that the variability of the otoliths of *Schizothorax nukiangensis* Tsao, 1964 could be related to the river dynamics driven by its fall. They showed the otoliths of *S. nukiangensis* sampled in different habitats had a significantly different morphology, in particular they reported an increase of their length. According to Prosekin and Prosekina (Prosekin and Prosekina, 2009) *T. baicalolenensis* and *T. baicalensis* in the Barguzin catchment area inhabit different habitats. *T. baicalensis* gain weight mostly in lakes or in the parts of the river where the stream velocity is less than 1.5 m/s without rapids and shoots. In contrast, *T. baicalolenensis* mainly prefer the river reaches where the stream velocity exceeds 1.5 m/s. In the rare cases when these fishes coinhabit they show a low level of trophic competition as they have different feeding strategies (Prosekin and Prosekina, 2009). However, no significant differences in length and width, perimeter and area of the otoliths between the *Thymallus* species were found during our study. Only the shape of the otoliths had distinctive features: the otoliths of *T. baicalolenensis* had a less developed pararostrum and more convex ventral edge, than those of *T. baicalensis* inhabiting the lakes Balan-Tamur and Baikal.

The studies showed that the otoliths of the *Thymallus* species have a similar structural plan. The analysis of the otolith outline allowed to differentiate the samplings studied pursuant to their typical morphotypes. This analysis has a number of advantages compared to the classical morphological and molecular genetic methods: it is cost effective and allows to quickly obtain primary data for the real time monitoring of population ecology. The otolith shape comparison method could be extensively used for basic research of microevolutionary processes, delimitation of natural populations for the sake of genetic diversity as well as for defining the structure of the commercial stock (Afanasyev et al., 2017).

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