



Ultrastructural correlates of acoustic sensitivity in Baikal coregonid fishes

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With 7 figures and 3 tables

Abstract: We investigated the ultrastructure of the sensory saccular and lagenar epithelia of the coregonid fishes: pelagic planktophage Baikal omul (*Coregonus migratorius*), benthophage lacustrine-riverine whitefish, or Baikal pidschian (*C. pidschian*), a typical inhabitant of the shallow water of the lake, and their hybrids of the first generation (F1). We made a few suggestions on the possible adaptive significance of ultrastructural specializations to acoustic sensitivity in different species of coregonid fishes. Unlike the shallow-water pidschian, both the pelagic omul and F1 hybrids possess higher morphological diversity of sensory cells. However, sensory cells of pidschian are characterised by sensory bundles with shorter stereocilia that presumably could perceive higher frequency sound oscillations. The saccular orientations are different from the earlier described ones in other whitefishes. The predominance of any direction in the morphological polarization of the sensory macula of different fish species is expected to be attributed to their ecology.

Keywords: coregonid fishes; Lake Baikal; sensory system in fish; ultrastructure; types of sensory cells; morphological polarization; sacculus; lagena

Introduction

One of the main functional hearing peculiarities in fish is their ability to distinguish the direction of the sound source in 3 dimensions (directivity of hearing or directional hearing) and frequency characteristics of sounds perceived by an auditory system (Platt & Popper 1981; Fay 1988; Lombarte & Fortuco 1992; Lu & Popper 1998; Lovell et al. 2005a; Lovell et al. 2005b; Popper et al. 2005; Popper & Fay 2011; Schulz-Mirbach et al. 2011; Smith et al. 2011; Ladich & Fay 2013). The unique diversity of functional abilities of the auditory apparatus in fish is expressed in morphological specific characteristics (Popper et al. 2005; Popper & Fay 2011; Ladich & Schulz-Mirbach 2016). Despite some

knowledge of inner ear variation, the main question – how the morphological variability is linked to hearing abilities – remains unanswered (Popper et al. 2005; Ladich & Schulz-Mirbach 2016).

Of three otolith organs (sacculus, lagena and utriculus), the sacculus and the lagena are considered to be the major auditory organs in most teleosts, but it is assumed that in a few fish taxa the utriculus is a highly effective transducer for linear acceleration, and besides hearing function, it serves as a gravitation sensor (Popper & Fay 1993; Popper & Fay 2011). Moreover, the largest diversity in ultrastructural characteristics for teleosts occurs on the sacculus (Platt & Popper 1981; Popper & Coombs 1982; Popper & Fay 1993). Therefore, this work was aimed at estimation

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of morphological diversity of ultrastructure of the sensory saccular and the lagenar epithelia in Baikal coregonid fishes and search for receptor apparatus of morphological correlates of functional characteristics of fish hearing.

To study interspecific variability in ultrastructure of the sensory auditory epithelium, we have chosen species that differ significantly in lifestyle, taking into account their high level of adaptation to conditions of the occupied ecological niches as well as their pidschian ♀ × omul ♂ hybrid.

Baikal omul (omul) and Baikal lacustrine-riverine whitefish, or Baikal pidschian (pidschian), are representatives of different phylogenetic branches within the monophyletic group of true whitefishes (*Coregonus* sp.). Branch divergence from each other and from the members of the group happened approximately 1.5 million years ago, being most likely associated with the Pleistocene glaciation events on the territory of the Baikal Rift Zone (Sukhanova et al. 2012). Pelagic planktophage omul (*Coregonus migratorius*) is endemic to the lake. Benthophage lacustrine-riverine whitefish, or pidschian (*Coregonus pidschian*) is a

typical inhabitant of the shallow water area originated allopatrically in a refugia somewhere near Lake Baikal. There are natural hybrids of these species in Lake Baikal (Skryabin 1969), but their ecology is not currently understood.

The adaptation of fish to habitat conditions in different ecological niches may cause the divergence of populations and the further emergence of new species. Comprehensive investigations of coregonid fishes, as well as their representatives in Lake Baikal, performed over several decades, have proven these species to be appropriate models for studying adaptations of fish to environmental conditions and searching for some possible ultrastructural correlates of acoustic sensitivity (Bernatchez et al. 2010; Smirnov & Shumilov 1974; Bychenko et al. 2012; Bychenko et al. 2014; Sukhanova et al. 2012).

The results of some morphological investigations of the auditory organ in fish allowed the supposition that mechanisms involved in the directivity of hearing are associated with the specific structural characteristics of the sensory epithelium – morphological polarization of sensory cells and integration of similar polar-

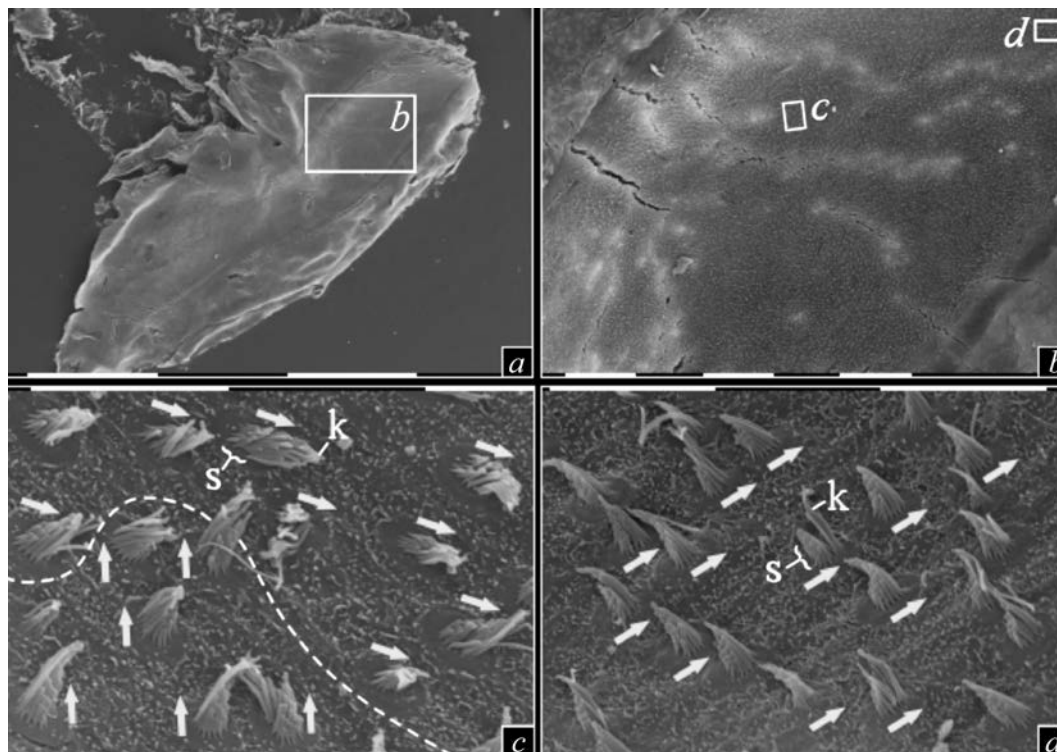


Fig. 1. Sensory elements of the saccular macula in Baikal omul: **a** – general view of the saccular macula (scale bar = 1 mm); **b** – central zone of the macula rostral part (scale bar = 0.1 mm); **c** – macula zone with adjacent areas of multidirectional horizontally and vertically oriented sensory elements (higher magnification; scale bar = 10 μm); **d** – macula area in horizontally oriented sensory elements (higher magnification; scale bar = 10 μm). Scanning electron micrographs (SEM Philips 525 M). The dashed line denotes boundary between different zones of polarization of hair cells; k – kinocilium, s – stereocilia.

ized cells into local groups (Hawkins & Sand 1977; Fay 1997; Lovell et al. 2005a; Lovell et al. 2005b; Popper et al. 2005). Morphological polarization means the specific location (orientation) of sensory hairs (singular kinocilium relative to the bundle of stereocilia) on the top of receptor cells of the saccular epithelium where the vector of polarization is directed from stereocilia to kinocilium (by Platt & Popper 1981; Ricci et al. 2002). Several zones can be identified within the saccular macula, which differ in the polarization vector (Fig. 1). A sensory macula with groups of cells differing in orientation of their tips responds unequally to the otolith shift under the influence of particle motion (Lovell et al. 2005a; Lovell et al. 2005b). The strongest electric response appears in the groups of cells in which morphological polarization coincides with the direction of the otolith shift (Flock 1971; Ricci et al. 2002; Kasumyan 2005).

The experiments showed the regions with different tonal specialization in the auditory maculae of otophyan fish. Intense acoustic stimulation caused different degree of hair cell damages in different parts of the macula (Enger 1981; Smith et al. 2006). According to the data on goldfish (*Carassius auratus*), the acoustic stimuli also caused different electrophysiological responses in hair cells with different length of cilia (Sugihara & Furukawa 1989). In addition, the lengths of stereocilia and kinocilia in the caudal zone of the sacculus were nearly double that of rostral hair cells (Platt & Popper 1984). The results of the combined studies indicated that caudal afferents had relatively lower best frequencies of 120–290 Hz, compared to the afferents innervating the rostral zone of the sacculus, which had best frequencies in the range of 790–1770 Hz (Furukawa & Ishii 1967a; Furukawa & Ishii 1967b; Fay 1978; Fay & Ream 1986; Fay 1988; Saidel et al. 1995; Lanford et al. 2000). The Tetrapoda case study indicated also that hair cells with short cilia were more sensitive to high-frequency acoustic signals than hair cells with long stereocilia (Saunders & Dear

1983). However, the data from tetrapods are not easily extrapolated to the function of the teleost ear since the roles of sacculus and lagenae in the two groups may be significantly different (Popper & Coombs 1982).

Material and methods

Sensory saccular and lagenar epithelia were collected from the omul caught with gill nets of different mesh size during August–October, 2013 in Maloye More, Lake Baikal, from pidschian during its spawning in October, 2014 in the Belaya River (Irkutsk Region), and from hybrids of pidschian ♀ and omul ♂ reared during artificial fertilization under control conditions in the Joint Instrumentation Centre (JIC) “Freshwater Aquarium Complex” at Limnological Institute SB RAS. The detailed characteristics of the material are provided in Table 1.

Histological processing of samples of sensory epithelium was performed according to the standard technique using scanning electron microscopy (Sapozhnikova et al. 2007; Sapozhnikova et al. 2016). The epithelium was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 hours, then washed in the same buffer, fixed in 1% osmium tetroxide and dehydrated in elevated concentrations of ethanol (30%, 50%, 70% and 98%).

Samples of sensory epithelium were dried at critical point on a Balzers CPD 030, coated with gold thickness of 3–6 nm and analysed under a scanning electron microscope Philips 525 M (Holland). The distance between stereocilia was studied by transmission electron microscopy. After fixing, the sensory epithelium was separated from the otolith under a binocular microscope, embedded in epoxy resin, and cut with an ultramicrotome (Leica Ultracut R, Austria) into 70 nm sections, which were examined under a LEO 906 E transmission electron microscope (Germany).

Morphometric analysis yielded 10–50 images of each macula along the length and depth (depending on the length of the epithelium) at an interval of 30 µm. The area of the macula and length of sensory elements (kinocilium and stereocilia) were measured using the licensed programme Image-Pro Plus. The percentage ratio of areas of the macula to different types of cells in all studied whitefishes was calculated from the total area of the sensory macula.

To analyse and classify sensory cells, we used a cluster method, including methods of k-mean and hierarchical clustering with the help of Statistica 8.0. Quantitative parameters of the similarity of hair cells belonging to the same cluster were

Table 1. Characteristics of Baikal coregonid fishes used for studying sensory epithelium.

Species	Sex, mean length of specimens in samples, TL, mm	Age (from otoliths) years	Sampling depth, m	Number of specimens of the same species
Baikal omul	♀, 310 ± 4.45	7+– 10+	200–350	10
	♂, 292 ± 8.23	8+– 9+	200–350	6
Hybrids of Baikal pidschian ♀ and Baikal omul ♂	♀, 160 ± 1.39	2+	–	9
	♂, 139 ± 2.27	2+	–	7
Baikal pidschian	♀, 206 ± 8.47	7+	0–20	6
	♂, 260 ± 6.63	7+	0–20	2

estimated from the following characteristics: maximal and minimal lengths of stereocilia, length of kinocilium, a number of stereocilia in the sensory bundle, and thickness of stereocilia and kinocilium.

Results

The results obtained with scanning electron microscopy showed that macro- and ultra-structures of the sensory epithelium were species-specific in the Baikal coregonid fishes. The saccular and lagenar maculae were located in contact with the medial sulcus or sulcus acusticus of the corresponding otolith.

The sensory saccular macula with hair cells was an elongated oval region on the inner endolymphatic side of the otolith organ with a macula area of $1.63 \pm 0.009 \text{ mm}^2$ for omul, $0.45 \pm 0.006 \text{ mm}^2$ for hybrids of pidschian ♀ and omul ♂, and $1.80 \pm 0.009 \text{ mm}^2$ for pidschian (Table 2). The maximal width of the saccular macula in whitefishes was, as a rule, in its rostral edge $-0.54 \pm 0.004 \text{ mm}$, whereas the macula narrowed to $0.23 \pm 0.003 \text{ mm}$ towards the caudal edge. The lagenar macula was a crescent region on the same side of the otolith organ with a macula area of $0.27 \pm 0.035 \text{ mm}^2$ for omul, $0.08 \pm 0.010 \text{ mm}^2$ for hybrids of pidschian ♀ × omul ♂, and $0.26 \pm 0.010 \text{ mm}^2$ for pidschian (Table 3). The maximal width of the lagena in whitefishes

was in its ventral edge $-0.25 \pm 0.040 \text{ mm}$, whereas the macula narrowed to $0.07 \pm 0.050 \text{ mm}$ towards the dorsal edge.

The sensory macula in the studied fish were represented by 3 elements: two variations of cells – sensory (hair or receptor) and supporting epithelial cells and the otolith membrane, which in fact serves as a contact between the sensory epithelium of the macula with otolith and limits the movement of otolith relative to the sensory epithelium.

The abundance of receptor cells per unit of sensory epithelium surface in all studied fish was relatively higher in the rostral and caudal edges of the saccular and lagenar maculae compared to the central zone. In contrast, in the central zone, receptor elements were located at some distance from each other, something that was clearly expressed in pidschian. The average density of hair cells (n) in the saccular epithelium was $571500 \pm 8975 \text{ cells mm}^{-2}$ in omul (n = 50 squares with an interval of 30 μm), $498700 \pm 3005 \text{ cells mm}^{-2}$ in hybrids of pidschian ♀ × omul ♂ (n = 30 squares with an interval of 30 μm), and $379200 \pm 4854 \text{ cells mm}^{-2}$ in pidschian (n = 30 squares with an interval of 30 μm) (Table 2). The average density in the lagenar epithelium was $487150 \pm 1095 \text{ cells mm}^{-2}$ in omul (n = 10 squares with an interval of 30 μm), $420700 \pm 6850 \text{ cells mm}^{-2}$ in hybrids of pidschian ♀ and omul ♂ (n = 10

Table 2. Morphological characteristics of the saccular macula in Baikal coregonid fishes.

Species	Macula area, mm^2	Ratio of the macula area to the mean length of specimens in samples	Density of hair cells on the macula (cells mm^{-2} , $M \pm m$)	Proportion of the saccular macula occupied by different types of hair cells, %							
				k6s5	k11s4	k5s4	k4s3	k7s2	k6s2	k3s2	k2s2
Baikal omul	1.63 ± 0.009	0.0053	571500 ± 8975	31	13	0	0	32	0	24	0
Hybrids of Baikal pidschian ♀ and Baikal omul ♂	0.45 ± 0.006	0.0030	498700 ± 3005	0	0	21	19	0	36	24	0
Baikal pidschian	1.80 ± 0.009	0.0065	379200 ± 4854	0	0	0	43	0	6	0	51

* The numbers in cell types are mean lengths of kinocilium (k) and of the highest stereocilia (s), μm.

Table 3. Morphological characteristics of lagenar macula in Baikal coregonid fishes.

Species	Macula area, mm^2	Ratio of the macula area to the mean length of specimens in samples	Density of hair cells on the macula (cells mm^{-2} , $M \pm m$)	Proportion of the lagenar macula occupied by different types of hair cells, %			
				k11s9	k5s4	k8s4	k10s2
Baikal omul	0.27 ± 0.035	0.0008	487150 ± 1095	18	17	51	14
Hybrids of Baikal pidschian ♀ and Baikal omul ♂	0.08 ± 0.010	0.0005	420700 ± 6850	13	26	43	18
Baikal pidschian	1.26 ± 0.010	0.0045	287900 ± 9045	11	28	41	20

* The numbers in cell types are mean length of the kinocilium (k) and of the highest stereocilia (s), μm.

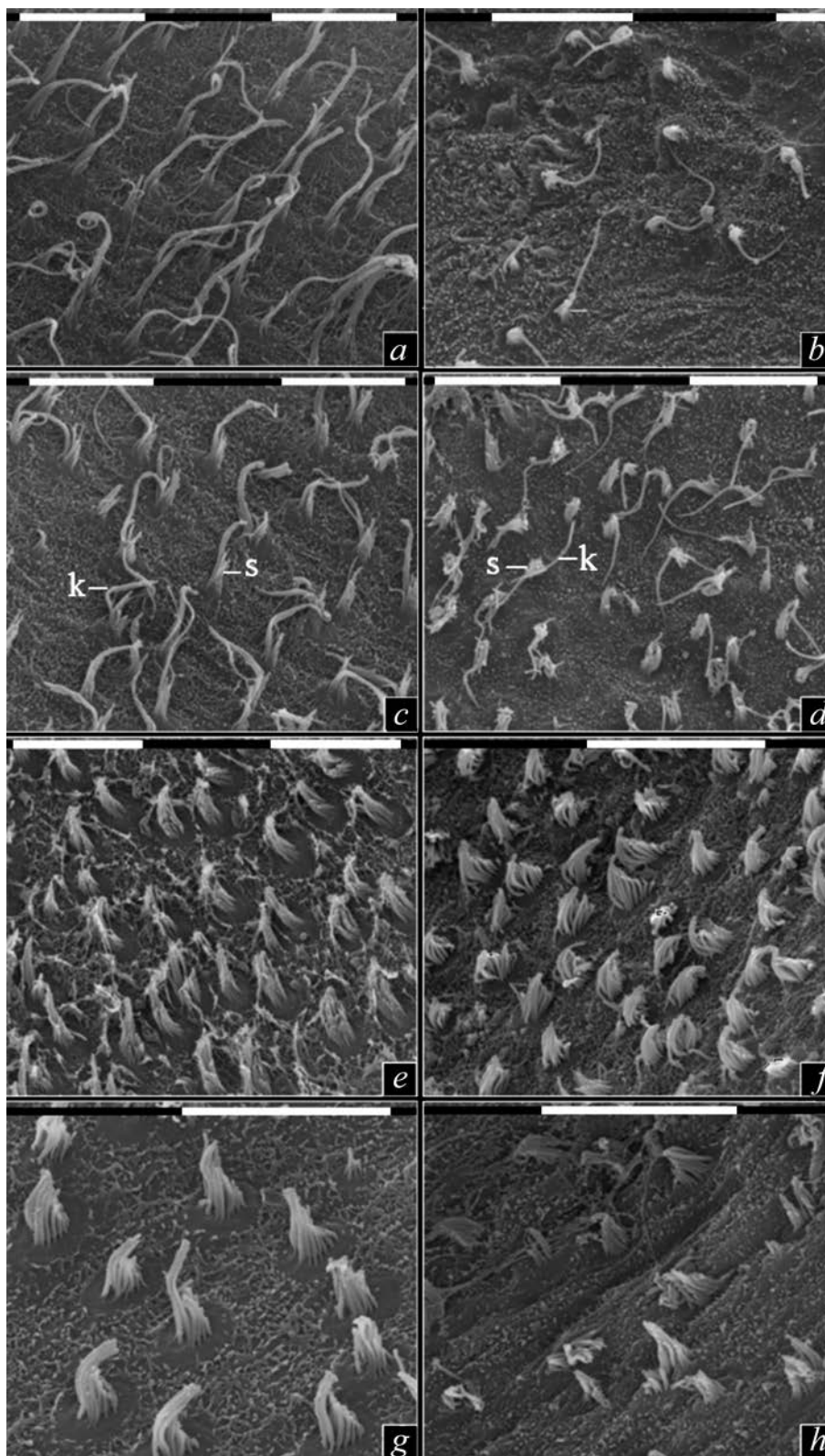


Fig. 2. Eight types of sensory elements of the saccular macula in Baikal coregonid fishes: **a** – k11s4; **b** – k7s2; **c** – k6s5; **d** – k6s2; **e** – k5s4; **f** – k4s3; **g** – k3s2; **h** – k2s2. k – kinocilium, s – stereocilia. Scale bar = 10 μ m. Scanning electron micrographs (SEM Philips 525 M). a–d – most abundant cells at the periphery of the saccular macula; e–h – types found in the saccular macula centre.

squares with an interval of 30 μm), and 287900 ± 9045 cells mm^{-2} in pidschian ($n = 10$ squares with an interval of 30 μm) (Table 3).

There were 10–30 stereocilia located in 3–5 regular rows confronting the kinocilium on the apical surface of each receptor cell of the saccular and lagenar maculae. The distance between stereocilia in a row was up to $0.44 \pm 0.001 \mu\text{m}$. This distance was shorter than that between stereocilia in the neighbouring rows – up to $0.87 \pm 0.001 \mu\text{m}$.

The peripheral marginal part of the macula consisted of sensory cells, which varied from 1.60 ± 0.002 to $3.20 \pm 0.001 \mu\text{m}$ in diameter on the apical surface, being smaller than the diameter of apices of the receptor cells located in the macula centre (from 2.90 ± 0.002 to $4.50 \pm 0.002 \mu\text{m}$). The cilia narrowed towards the cuticular part of the sensory cell. The diameter of gradually narrowing stereocilia towards the cuticle of stereocilia varied from 0.38 ± 0.001 to

$0.17 \pm 0.001 \mu\text{m}$, whereas the diameter of kinocilia was between 0.51 ± 0.002 and $0.35 \pm 0.001 \mu\text{m}$.

The differences between peripheral and central sensory hair cells were predominantly in the sizes of cilia in the bundle. Maximal lengths of stereocilia were found in the row closest to the kinocilium.

Cluster analysis united sensory cells into classes (types) in such a way that maximally similar cells were included in one class. The most significant characteristics for the integration of sensory cells into different clusters were the length of kinocilium (k) and maximal length for stereocilium in one sensory bundle (s) (designation for cilia was used by Platt & Popper 1981). These most variable characteristics were regarded as criteria for identification of types of sensory saccular cells. As a result of the analysis, four types of sensory saccular cells ($k3s2$, $k11s4$, $k6s5$ and $k7s2$) were distinguished in omul, four types of sensory saccular cells ($k3s2$, $k5s4$, $k4s3$ and $k6s2$) in hybrids of

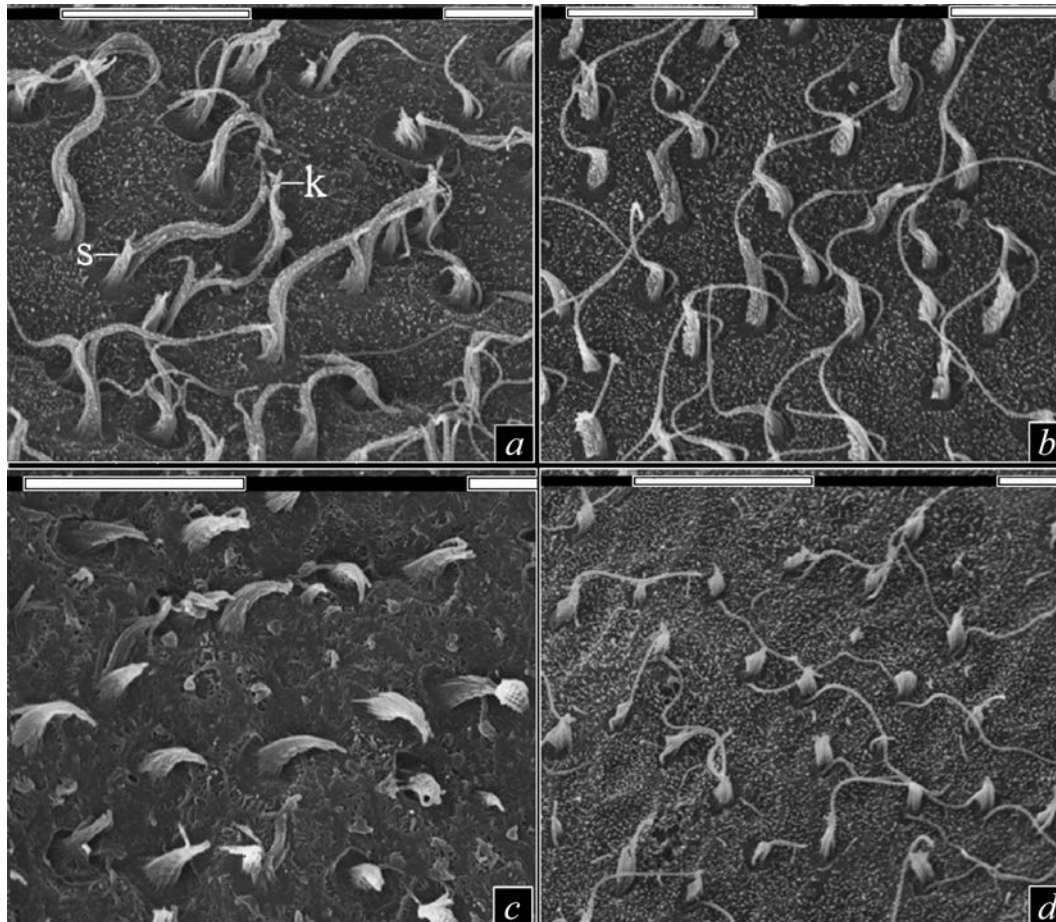


Fig. 3. Four types of sensory elements of the lagenar macula in Baikal coregonid fishes: **a** – $k11s9$; **b** – $k8s4$; **c** – $k5s4$; **d** – $k10s2$. k – kinocilium, s – stereocilia. Scale bar = 10 μm . Scanning electron micrographs (SEM Philips 525 M). **a**–**b** – most abundant cells at the periphery of the lagenar macula; **c**–**d** – types found in the lagenar macula centre.

pidschian ♀ × omul ♂, and three types of sensory saccular cells (k4s3, k2s2 and k6s2) in pidschian (Fig. 2, Table 2). In addition, four types of sensory lagenar cells (k11s9, k5s4, k8s4 and k10s2) were distinguished in all these species (Fig. 3, Table 3). The numbers indicated lengths of kinocilium (k) and maximal stereocilia (s) in micrometres (µm).

Based on the images of the sensory macula of coregonid fishes and using the Image-Pro Plus programme, we compared the areas on saccular and lagenar maculae occupied by sensory elements of different types. Their percentage ratio on the saccular macula for different species of coregonid fishes was as follows: k7s2 (32%), k6s5 (31%), k3s2 (24%), and k11s4 (13%) for omul; k6s2 (36%), k3s2 (24%), k5s4 (21%), and k4s3 (19%) for hybrids of pidschian ♀ and omul ♂; k2s2 (51%), k4s3 (43%), and k6s2 (6%) for pidschian (Table 2). Moreover, the percentage ratio on the lagenar macula was: k11s9 (18%), k5s4 (17%), k8s4 (51%), and k10s2 (14%) for omul; k11s9 (13%), k5s4 (26%), k8s4 (43%), and k10s2 (18%) for hybrids of pidschian

♀ and omul ♂; k11s9 (11%), k5s4 (28%), k8s4 (41%), and k10s2 (20%) for pidschian (Table 3).

Hence, the cluster analysis made it possible to detect a great variety of sensory types of cells on the macula in the studied fish. However, the division of the macula into zones with different types of sensory cells does not always coincide with the division of this macula into the zones with different morphological polarization of sensory cells described for coregonid fishes.

Morphological polarization of the epithelial sensory elements slightly differed from that already described in the lacustrine whitefish *Coregonus clupeaformis* (Popper 1976; Popper 1977) (Fig. 4a).

The dimensions of the entire macula and separate zones with a different orientation of cells were given schematically. Morphological polarization was slightly simplified and described according to the earlier studies (Popper 1977). Each macula was divided into anterior (rostral), posterior (caudal), and central zones, which corresponded to areas of rostral and

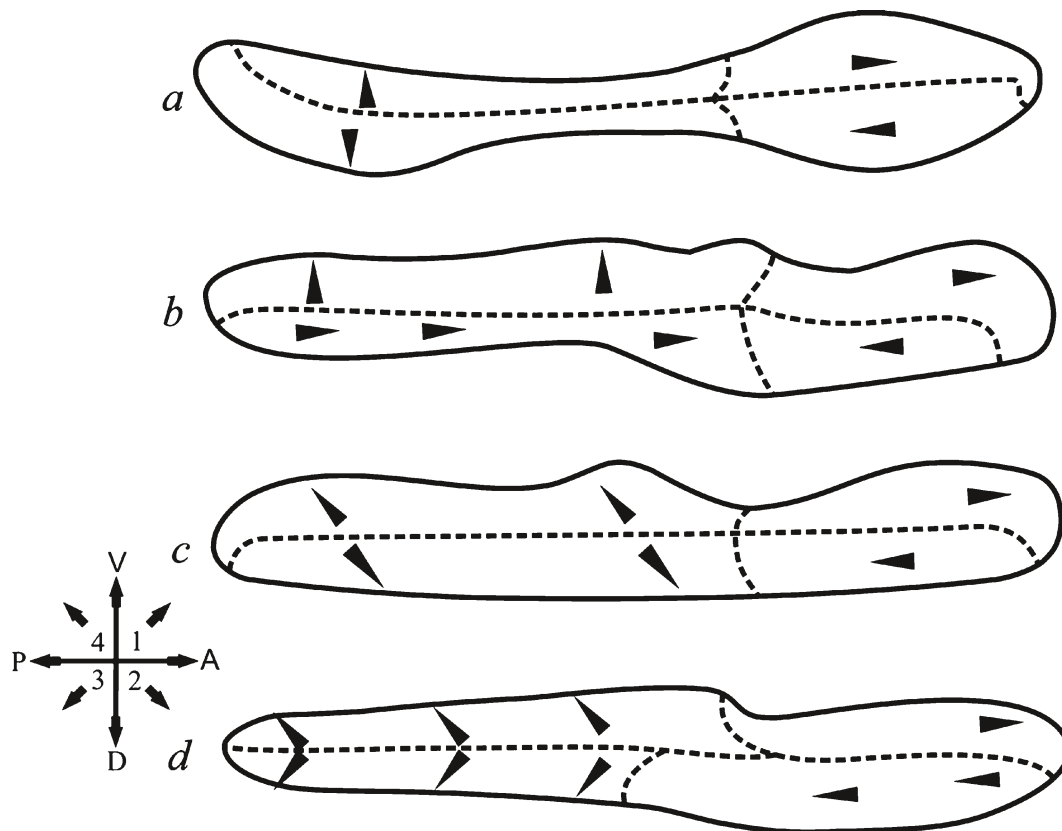


Fig. 4. Morphological polarization scheme of sensory cells of the saccular macula and the integration of similar polarized cells into local groups: **a** – lacustrine whitefish *Coregonus clupeaformis* (by Popper 1976), **b** – Baikal omul, **c** – hybrids of Baikal pidschian ♀ and Baikal omul ♂, **d** – Baikal pidschian. The arrows indicate the orientations of hair cells. Orientation of sensory cells: V – ventral, D – dorsal, A – anterior (rostral), P – posterior (caudal), 1 – rostroventral, 2 – rostrodorsal, 3 – caudodorsal, 4 – caudoventral.

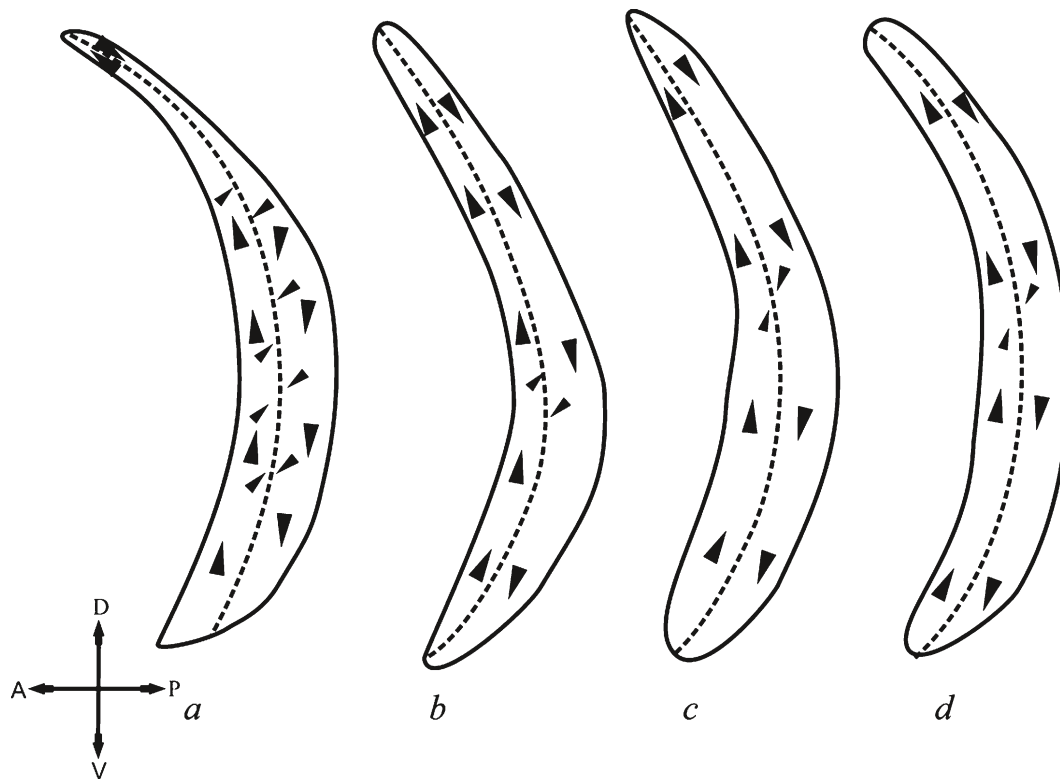


Fig. 5. Morphological polarization scheme of sensory cells of the lagenar macula and integration of similar polarized cells into local groups: **a** – lacustrine whitefish *Coregonus clupeaformis* (by Popper 1976), **b** – Baikal omul, **c** – hybrids of Baikal pidschian ♀ and Baikal omul ♂, **d** – Baikal pidschian. The arrows indicate the orientations of hair cells. Orientation of sensory cells: V – ventral, D – dorsal, A – anterior (rostral), P – posterior (caudal).

caudal fish ends. The horizontal orientation of sensory cells corresponded to the rostrocaudal axis of the macula and fish, whilst the vertical orientation of the sensory cells suggested their focus on the dorsoventral axis of the macula and fish (Fig. 1).

The saccular macula of omul, pidschian, and their hybrids can be divided into four sections with a specific orientation (Fig. 4). The anterior zones of the sacculus had two similar adjacent areas with opposing horizontally oriented sensory cells (Fig. 1c), whereas the posterior zones of the sacculus had two areas with sensory cells oriented at different angles in these species. Moreover, the anterior zones of the sacculus had a small area with sensory cells oriented in the caudorostral direction, the dimensions of which varied in these species.

The posterior zone of omul sacculus had two adjacent areas with opposing horizontally and vertically oriented sensory cells (Fig. 2c, Fig. 4b). In the dorsal side of the sacculus, sensory cells had a strict horizontal orientation and in the ventral side of this macula, they had a strict vertical orientation. The macula of hybrids of pidschian ♀ and omul ♂ in the posterior zone

was also divided into two sections. Sensory cells of caudoventral and rostrordorsal directions were detected from dorsal and ventral sides in this zone (Fig. 4c). The posterior zone of pidschian sacculus was occupied by sensory cells oriented towards the caudodorsal and caudoventral axes, forming two adjacent sections (Fig. 4d).

The lagenar maculae differed insignificantly in various species as compared to the sacculus. The lagenae of these species had two similar adjacent sections with opposing vertically oriented sensory cells (Fig. 5). There were also some areas with horizontally oriented sensory cells in the transition zone.

Discussion

Despite the overall similarity for coregonid fishes of Lake Baikal inhabiting different ecological niches, the area of sensory saccular and lagenar maculae, the location density of sensory cells, their variety, their length and features of the morphological polarization are not equal (Tables 2, 3).

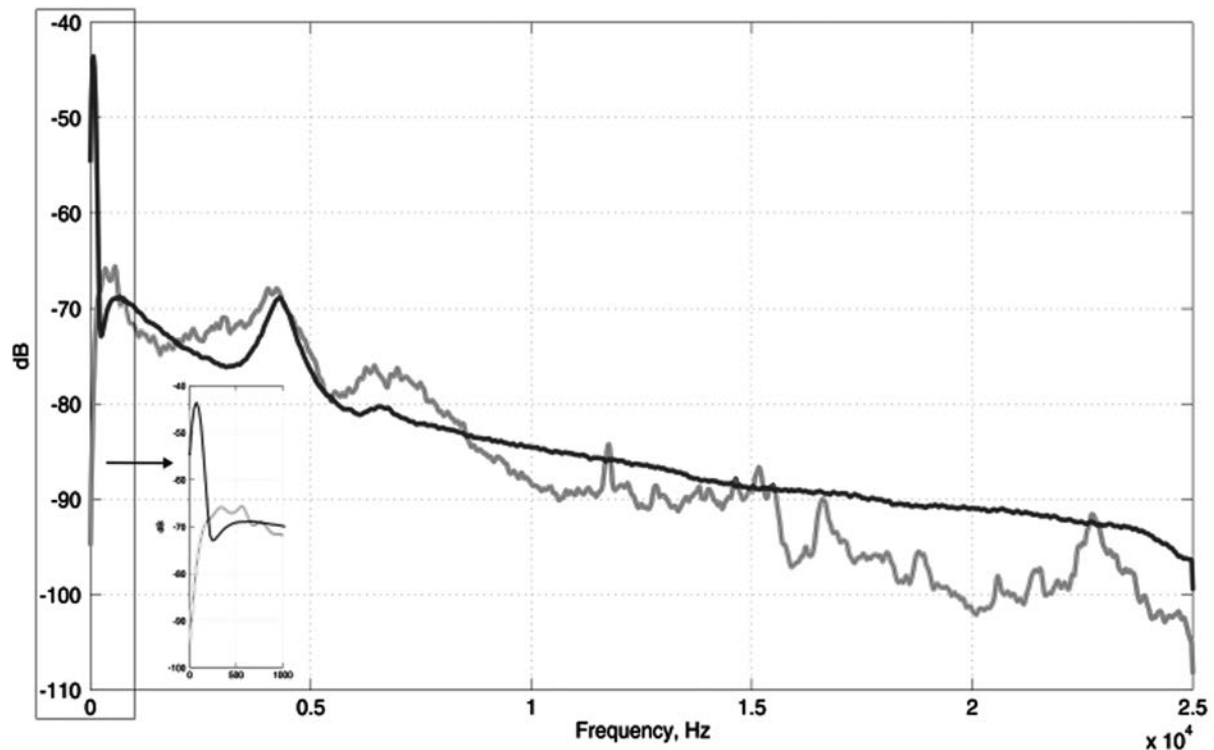


Fig. 6. Distribution of acoustic waves within the spectrum of the open pelagic area (black line, at a depth of 150 m) and the shallow water area (grey line, at a depth of 0–4 m).

The dominance of the vertical orientation of sensory cells was observed in the benthic pidschian and their hybrids in comparative analysis of morphological polarization of the saccular macula. On the contrary, the horizontal orientation prevailed over the vertical one in the pelagic omul (Fig. 4).

These morphological characteristics revealed in artificially grown hybrids are hereditary. However, no experimental evidence has been detected in correlation between functional localization abilities and hair cell orientation patterns so far. Nevertheless, we can assume that morphological characteristics reflect fish capabilities in hearing, apparently, in determining the source of acoustic signals in the water and environment associated with these fish. Omul is an active pelagic migrant of the lake (Smirnov & Shumilov 1974). The more complex polarization of the saccular macula in omul is presumably the most optimal at its lifestyle. Pidschian is a bathypelagic benthophage, feeding at relatively low speeds near the bottom (Skryabin 1969). The sacculus of pidschian perceives acoustic signals from horizontal and vertical planes. Morphological polarization of the sacculus is presumably attributed to the confinement of pidschian to shallow areas of habitation, in particular to river systems. The spatial

isolation of this species likely favours the formation of a highly specialized auditory system. Therefore, the predominance of any direction in the morphological polarization of the sensory macula of different fish species is presumably attributed to their ecology.

It should be noted that there are sensory bundles of various types in all zones of the sensory macula with differing polarization. Peculiarities of morphological polarization of the sensory macula apparently reflect the abilities of the auditory apparatus of fish to determine the direction of acoustic signals in the water towards the source, whereas the availability of any sensory cells on the saccular macula predetermines the functional abilities of the auditory apparatus concerning the frequency coding of the incoming information. As proved in earlier experimental works, long cilia are involved in the perception of lower frequency acoustic signals (Saunders & Dear 1983; Schulz-Mirbach et al. 2011; Smith et al. 2011). However, the significance of this finding for the physiology of different hair cell types is speculative (Popper & Coombs 1982).

The pelagic species, a planktophage omul, is characterised by rather large areas on the macula occupied by sensory bundles with elongated cilia (k11s4, k7s2, k6s5 on the sacculus and k11s9, k8s4 on the lagena).

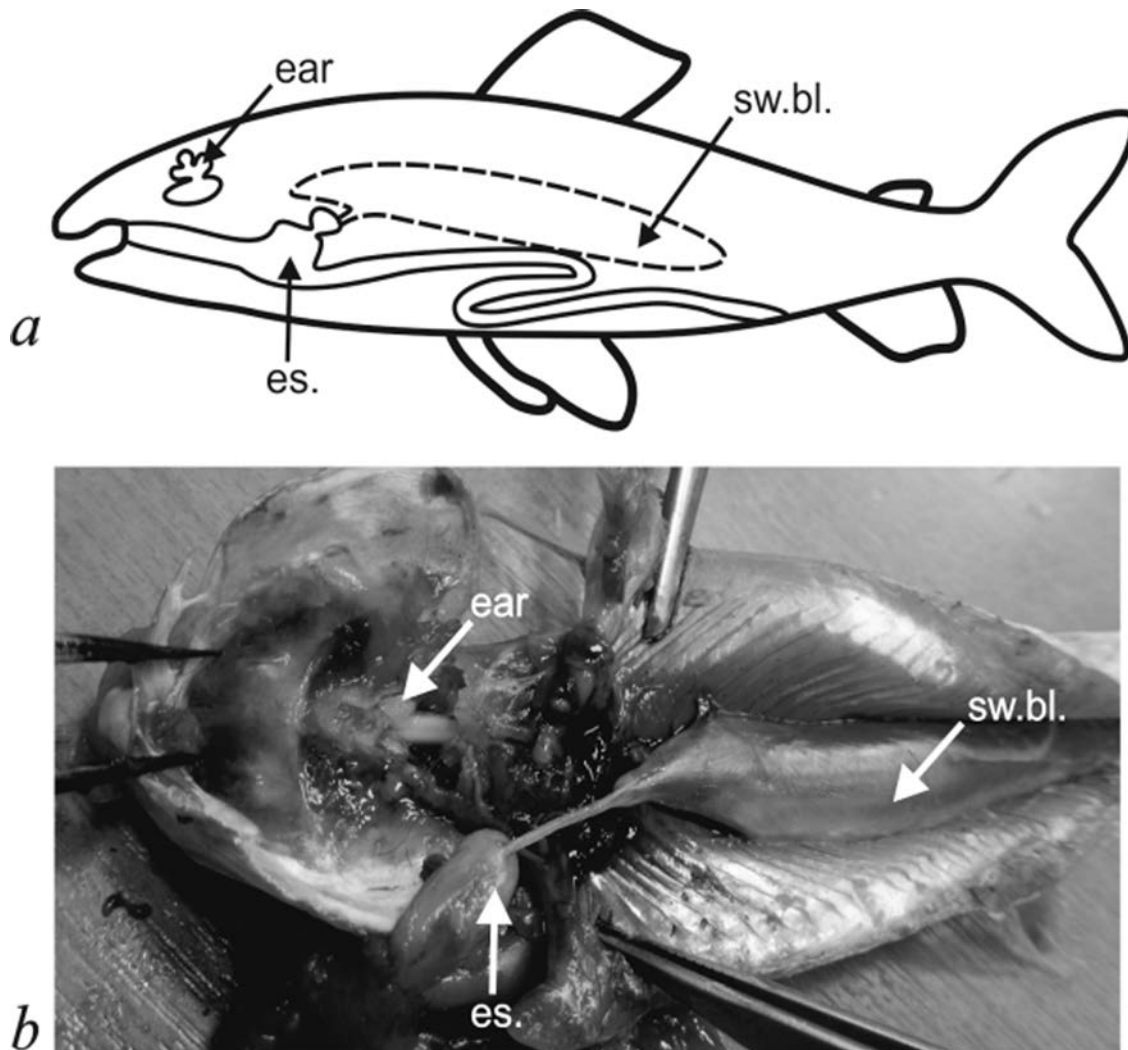


Fig. 7. Relationship between inner ears and accessory hearing structures in coregonid fishes: **a** – scheme of location of swim bladder, inner ears, and esophagus; **b** – dissected Baikal omul. ear – inner ear, es. – esophagus, sw. bl. – swim bladder

This likely favours the most adequate perception of lower frequency acoustic waves by pelagic fish. Low-frequency sounds are better spread at long distances. A general rule works in the distribution of acoustic waves: low-frequency sounds fade weaker than high-frequency ones (Urik 1978; Rogers & Cox 1988). Moreover, there are practically no high-frequency sounds within the spectrum of the open pelagic area of the water body in Lake Baikal, as there are few sources of high-frequency noise. Therefore, the pelagic spectrum is presented mainly by low-frequency sounds (Fig. 6).

Unlike the pelagic inhabitants, a benthophage pidschian, a typical inhabitant of the shallow water area, is characterised by low morphological diversity of types of sensory cells, which are not so densely spaced on the sensory macula (Tables 2, 3). Moreover, sen-

sory bundles with short stereocilia (k4s3, k2s2, k6s2 on the sacculus and k8s4, k5s4 on the lagena) presumably perceiving higher frequency acoustic oscillations are more typical of sensory cells of pidschian. These high-frequency oscillations probably are more easily identified at the background of low-frequency noise in the shallow water area. The acoustic spectrum is enriched with high-frequency sound signals, which are informative for organisms and are better distributed in the shallow zone of freshwater bodies than low-frequency signals (Fig. 6).

One of the important assumptions in assessing hearing sensitivity of fish is considered to the relationship between ears and the swim bladder. Species of fish possessing a connection between inner ears and the swim bladder are known as hearing specialists and can detect sounds up to 10 kHz, or even up to 180 kHz

in case of the American shad (*Alosa* spp.) (Hawkins 1981; Popper & Fay 1999; Mann et al. 2001). Baikal coregonid fishes are physostomous, but non-otophyllous, and their swim bladder has no connection to inner ears through the Weberian apparatus (Fig. 7). Based on works by Coombs & Popper (1979; 1982), it would be predicted that they also lack accessory (ancillary, peripheral) hearing structures because of the standard pattern that is typical of other coregonid species (Fig. 4a). Although it is known that fishes with more complex hair cell patterns may have closer connection between air bubbles (most likely the swim bladder) and inner ears; it is most clearly shown in studies of squirrelfish (Holocentridae) (Coombs & Popper 1979).

It is possible to conclude that coregonid fishes differ in high plasticity in the existing acoustic factors of the environment, and adaptations are formed, first of all, at the ultrastructural level. Due to the increasing accessibility of new methods of the ultra-structural analysis of sensory systems, fish that are studied in their ecological aspect play a more important role and can serve as an adequate model for revealing functional and cause-and-effect relations between a phenotype and natural selection. The data obtained allow us to better understand fundamental evolutionary processes responsible for the formation of behavioural adaptations occurring in unique natural ecosystems.

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