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Research paper

Fish otolith asymmetry: Morphometry and modeling

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Abstract

Mathematical modeling suggests that relatively large values of otolith mass asymmetry in fishes can alter acoustic functionality and may be responsible for abnormal fish behavior when subjected to weightlessness during parabolic or space flight [D.V. Lychakov, Y.T. Rebane, Otolith mass asymmetry in 18 species of fish and pigeon, J. Grav. Physiol. 11 (3) (2004) 17–34; D.V. Lychakov, Y.T. Rebane, Fish otolith mass asymmetry: morphometry and influence on acoustic functionality, Hear. Res. 201 (2005) 55–69]. The results of morphometric studies of otolith mass asymmetry suppose that the absolute value and the sign of the otolith mass asymmetry can change many times during the growth of individual fish within the range ±20% [D.V. Lychakov, Y.T. Rebane, Otolith mass asymmetry in 18 species of fish and pigeon, J. Grav. Physiol. 11 (3) (2004) 17–34; D.V. Lychakov, Y.T. Rebane, Fish otolith mass asymmetry: morphometry and influence on acoustic functionality, Hear. Res. 201 (2005) 55–69]. This implies that the adverse effects of otolith asymmetry on acoustic and vestibular functionality could change during the lifetime of an individual fish. The aims of the present article were to examine the nature of otolith mass asymmetry fluctuation and to quantify otolith mass asymmetry in a large number of teleost fishes to verify our previous measurements.

A dimensionless measure of otolith mass asymmetry, χ, was calculated as the difference between the masses of the right and left paired otoliths divided by average otolith mass. Saccular otolith mass asymmetry was studied in 59 Mediterranean teleost species (395 otolith pairs), 14 Black Sea teleost species (42 otolith pairs), red drum (196 otolith pairs) and guppy (30 otolith pairs). Utricular otolith mass asymmetry was studied in carp (103 otolith pairs) and goldfish (45 otolith pairs). In accordance with our previous results the value of χ did not depend on fish size (length or mass), systematic or ecological position of the fish, or otolith growth rate. In the great majority of the fishes studied, the saccular otolith χ was small $|\chi| < 0.05$ (or <5%). Mathematical modeling indicates that values of χ vary among individual fish, but that the value is probably stable during a fish's lifetime.

Keywords: Fish; Sacculus; Utriculus; Otolith asymmetry; Mathematical modeling

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Abbreviations: χ, dimensionless otolith mass asymmetry; m_R and m_L, otolith masses of the right and left paired otoliths; m, arithmetic mean mass of right and left paired otoliths; Δ, otolith mass difference; L, length of the fish; a, coefficient characterizing the growth rate of the otolith; b, growth otolith constant for the given species; χ_{rv}, random variable of the dimensionless otolith mass asymmetry; α, constant related to the systematic difference in growth rates of the right and left otoliths which is independent of the otolith mass; $\beta(m)$, random function of m which is related to fluctuations in the otolith growth rates; m' and m'', integration variables; $\langle \dots \rangle$, symbol of statistical averaging; $\delta(m' - m'')$, Dirac's delta function; A, constant related to the intensity of the stationary white-noise type fluctuations

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1. Introduction

Otolith mass asymmetry may be responsible at least partly for both space illusionary sensations and space motion sickness in human subjects and abnormal behavior when fishes are subjected to weightlessness during parabolic or space flight (De Jong et al., 1996; Egorov and Samarin, 1970; Hilbig et al., 2002; Hoffman et al., 1977; Lychakov and Rebane, 2004; Rahman and Anken, 2002; Takabayashi and Ohmura-Iwasaki, 2003; Von Baumgarten et al., 1982). Mathematical modeling shows that acoustic functionality (sensitivity, temporal processing, sound localization) of a fish can, in principle, be altered by otolith mass asymmetry due to incompatibility and incongruity of the right and left otolith movements (Lychakov and Rebane, 2005). These facts suggest that otolith mass asymmetry can have adverse effects on vestibular and auditory functions, but the precise quantitative morphological and physiological bases of otolith asymmetry remain unclear.

Teleost fishes are a well-suited biological model for assessing the physiological role of otolith mass asymmetry because the compact otoliths can easily be quantitatively assessed. Before starting direct acoustic and space experiments on fishes, which are very sophisticated and technically and logistically complicated, it is important to quantify the natural patterns of otolith mass asymmetry. In previous studies we compared the right and left otolith masses in two species of the Black Sea rays, 15 species of Black Sea teleosts, guppy, carp, goldfish, and pigeon (Lychakov, 1992; Lychakov et al., 1988; Lychakov and Rebane, 2004, 2005; Takabayashi and Ohmura-Iwasaki, 2003). In the overwhelming majority of animals studied, otolith mass asymmetry was within the range of $-0.2 < \chi < +0.2$ or <20%. No relationship was found between the magnitude of otolith mass asymmetry and length or mass of the fish. We attributed these results to otolith mass fluctuation (Lychakov and Rebane, 2004, 2005). We speculated that otolith growth rate varies slightly during the lifetime of an individual fish. Thus, the sign of otolith mass asymmetry would be able to change many times during growth in an individual fish. The aim of the present article is to verify these results and speculations.

2. Materials and methods

2.1. Otolith morphometry

Data used in our analysis were collected from many species and locations by several previous researchers (Table 1). Experimental procedures for extracting and preparing otoliths for study were published previously and are briefly described here. Standard lengths were measured from the tip of the snout to the base of the tail prior to removal of otoliths. In some instances preanal length (three of six *Hymenocephalus italicus*), fork length (*Scomber colias*) and total length (carp, goldfish) or mass (guppy) of fishes were measured. After dissection of the auditory capsules, saccular (utricular in carp and goldfish) otoliths were removed from each side, rinsed in distilled water or fish Ringer's solution, air-dried at room temperature or 60 °C for several days, then weighed on a Sartorius balance to an accuracy 1 μg, on a VLR-200 balance to an accuracy 0.05 mg, on a OHAUS analytical plus balance to an accuracy 0.0001 g. In some instances control tests were performed to assess the purity of the specimens. For this purpose a portion of the dried otoliths was critical-point dried using CO_2, attached to metal stubs with silver conducting paint, and carbon- and gold-coated in a vacuum evaporator. The specimens were then examined with a scanning electron microscope JSM-35. No underlying epithelium or foreign organic materials were found adhering to the otoliths (Fig. 1) (see also electron micrographs in Fermin et al., 1998; Lychakov and Rebane, 2004, 2005). All these contaminations were removed during cleaning. Experiments were carried out in conformity with the guiding principles in the care and use of animals.

2.2. Otolith mass asymmetry calculation and modeling

Otolith mass asymmetry was calculated as otolith mass difference, Δ, ($= m_R - m_L$) divided by the arithmetic mean of the right and left otoliths (m):

$$\chi = (m_R - m_L)/m \tag{1}$$

where χ is the dimensionless otolith mass asymmetry, m_R and m_L are the otolith masses of the dried right and left paired otoliths, m is the arithmetic mean mass of right and left paired otoliths.

Theoretically χ can change from -2 to $+2$, 0 corresponds to absence of mass asymmetry ($m_R = m_L$), -2 or $+2$ corresponds maximal asymmetry (absence one otolith). Correspondingly, χ has positive values if the right otolith mass is larger than left paired otolith mass and negative values for the reverse condition.

We also examined the relation between a species' χ and its otolith growth rate. The species' otolith mass asymmetry, $|\chi|_{\text{species}}$, is calculated as the average of individual values of $|\chi|$ for each species. To evaluate otolith growth rate we described the relationship between otolith mass and fish length, $m = a \times L + b$, where L is the length of the fish, "a" is the coefficient characterizing the growth rate of the otolith, and "b" is a constant for the given species. For these calculations we used 61 species, each including three or more specimens.

Data were processed on IBM PC computer using Microsoft Excel spreadsheet software.

3. Results

3.1. Morphometric measurements

3.1.1. Otolith mass asymmetry

3.1.1.1. Marine fishes. In marine fishes, 97.5% values of χ were between -0.2 and $+0.2$ (Fig. 2). In 83% of fishes stud-

Table 1
Species used in this study[a]

Order	Family	Genus	Species	Ecological type[b]	Number
Marine fish from Mediterranean sea					
Anguilliformes	Congridae	*Conger*	*conger*	L	4
Anguilliformes	Nettastomidae	*Nettastoma*	*melanurum*	B	3
Albuliformes	Notacanthidae	*Notacanthus*	*bonapartei*	B	2
Clupeiformes	Clupeidae	*Sardina*	*pilchardus*	P	3
Clupeiformes	Engraulidae	*Engraulis*	*encrasicolus*	P	2
Osmeriformes	Alepocephalidae	*Alepocephalus*	*rostratus*	B	8
Aulopiformes	Ipnopidae	*Bathypterois*	*mediterraneus*	B	7
Myctophiformes	Myctophidae	*Notoscopelus*	*elongatus*	P	5
Ophidiiformes	Bythitidae	*Cataetyx*	*alleni*	B	4
Gadiformes	Moridae	*Lepidion*	*lepidion*	B	7
Gadiformes	Moridae	*Mora*	*moro*	B	7
Gadiformes	Gadidae	*Gadiculus*	*argenteus*	P	5
Gadiformes	Gadidae	*Trisopterus*	*minutus*	B	7
Gadiformes	Phycidae	*Phycis*	*blennoides*	B	4
Gadiformes	Phycidae	*Phycis*	*phycis*	L	11
Gadiformes	Merlucciidae	*Merluccius*	*merluccius*	B	27
Gadiformes	Macrouridae	*Hymenocephalus*	*italicus*	B	6
Mugiliformes	Mugilidae	*Liza*	*aurata*	L	10
Beryciformes	Trachichthydae	*Hoplostethus*	*mediterraneus*	B	6
Zeiformes	Caproidae	*Capros*	*aper*	P	3
Scorpaeniformes	Dactylopteridae	*Dactylopterus*	*volitans*	L	4
Scorpaeniformes	Scorpaenidae	*Scorpaena*	*notata*	L	6
Scorpaeniformes	Triglidae	*Chelidonichthys*	*obscurus*	L	4
Scorpaeniformes	Triglidae	*Chelidonichthys*	*lucernus*	L	4
Scorpaeniformes	Triglidae	*Chelidonichthys*	*gurnardus*	L	4
Perciformes	Moronidae	*Dicentrarchus*	*labrax*	L	7
Perciformes	Serranidae	*Serranus*	*hepatus*	B	5
Perciformes	Epigonidae	*Epigonus*	*telescopus*	B	4
Perciformes	Carangidae	*Trachurus*	*trachurus*	P	2
Perciformes	Haemulidae	*Pomadasys*	*incisus*	L	12
Perciformes	Sparidae	*Boops*	*boops*	P	9
Perciformes	Sparidae	*Diplodus*	*annularis*	L	11
Perciformes	Sparidae	*Diplodus*	*sargus*	L	6
Perciformes	Sparidae	*Diplodus*	*vulgaris*	L	9
Perciformes	Sparidae	*Lithognathus*	*mormyrus*	L	8
Perciformes	Sparidae	*Pagrus*	*pagrus*	L	10
Perciformes	Sparidae	*Pagellus*	*acarne*	L	11
Perciformes	Sparidae	*Pagellus*	*erythrinus*	L	18
Perciformes	Centracanthidae	*Spicara*	*maena*	P	5
Perciformes	Sciaenidae	*Sciaena*	*umbra*	L	12
Perciformes	Sciaenidae	*Umbrina*	*canariensis*	L	10
Perciformes	Sciaenidae	*Umbrina*	*cirrosa*	L	10
Perciformes	Mullidae	*Mullus*	*barbatus*	L	3
Perciformes	Mullidae	*Mullus*	*surmuletus*	L	4
Perciformes	Cepolidae	*Cepola*	*macrophthalma*	B	7
Perciformes	Pomacentridae	*Chromis*	*chromis*	L	3
Perciformes	Labridae	*Labrus*	*merula*	L	7
Perciformes	Labridae	*Symphodus*	*tinca*	L	4
Perciformes	Labridae	*Coris*	*julis*	L	2
Perciformes	Labridae	*Xyrichthys*	*novacula*	L	6
Perciformes	Ammodytidae	*Gymnammodytes*	*semiesquamatus*	L	3
Perciformes	Uranoscopidae	*Uranoscopus*	*scaber*	L	9
Perciformes	Blenniidae	*Blennius*	*ocellaris*	B	4
Perciformes	Gobiidae	*Gobius*	*niger*	B	3
Perciformes	Gobiidae	*Lesuerigobius*	*suerii*	B	6
Perciformes	Sphyraenidae	*Sphyraena*	*sphyraena*	P	4
Perciformes	Trichiuridae	*Lepidopus*	*caudatus*	B	5
Perciformes	Scombridae	*Scomber*	*colias*	P	14
Perciformes	Scombridae	*Scomber*	*scombrus*	P	9
Marine fish from Black sea					
Clupeiformes	Clupeidae	*Sprattus*	*sprattus*	P	2
Clupeiformes	Clupeidae	*Alosa*	*pontica*	P	4
Clupeiformes	Engraulidae	*Engraulis*	*encrasicolus*	P	1

(*continued on next page*)

Table 1 (*continued*)

Order	Family	Genus	Species	Ecological type[b]	Number
Atheriniformes	Atherinidae	*Atherina*	*mochon pontica*	P	5
Gadiformes	Gadidae	*Gaidropsarus*	*mediterraneus*	B	2
Gadiformes	Gadidae	*Merlangus*	*merlangus euxinus*	B	3
Ophidiiformes	Ophidiidae	*Ophidion*	*rochei*	B	1
Perciformes	Carangidae	*Trachurus*	*mediterraneus ponticus*	P	3
Perciformes	Sparidae	*Diplodus*	*annularis*	L	2
Perciformes	Centracanthidae	*Spicara*	*smaris*	P	4
Perciformes	Sciaenidae	*Sciaena*	*umbra*	L	3
Perciformes	Mullidae	*Mullus*	*barbatus ponticus*	B	8
Perciformes	Uranoscopidae	*Uranoscopus*	*scaber*	B	2
Scorpaeniformes	Scorpaenidae	*Scorpaena*	*porcus*	B	2
Marine fish from the Gulf of Mexico and the Atlantic Coast					
Perciformes	Sciaenidae	*Sciaenops*	*ocellatus*	L	196
Freshwater fish					
Cyprinodontiformes	Cyprinodontidae	*Poecilia*	*reticulata*		27
Cypriniformes	Cyprinidae	*Carassius*	*auratus*		45
Cypriniformes	Cyprinidae	*Cyprinus*	*carpio*		103

[a] The data were obtained by Antoni Lombarte (59 Mediterranean teleost species, $n = 395$), by Dmitry Lychakov (14 Black Sea teleost species, $n = 42$, and guppy, $n = 30$), by Gerald R. Hoff under the supervision of Lee A. Fuiman (red drum, $n = 196$), by Akira Takabayashi and Terue Ohmura-Iwasaki (carp, $n = 103$, goldfish, $n = 45$) (Hoff and Fuiman, 1993; Lombarte and Morales-Nin, 1995; Lombarte et al., 2006; Lychakov, 1992; Lychakov and Rebane, 2000; Takabayashi and Ohmura-Iwasaki, 2003).

[b] B, bottom; L, littoral; and P, pelagic fish.

ied $|\chi| < 5\%$. The mean value of χ for all marine fishes studied was -0.00467 ± 0.00625, $n = 608$ (mean ± SE). The mean value of $|\chi|$ was 0.04298 ± 0.00601, $n = 608$. According to the regression analysis there was no relationship between fish length and $|\chi|$ ($P = 0.075$, $R^2 = 0.005$) There were no apparent differences in χ between benthic/littoral fishes and pelagic fishes (Fig. 2).

For all species, there was an obvious linear correlation between the saccular otolith mass and fish length. For example, for 28 species, each including seven or more specimens, the mean R^2 had a high value 0.86 ± 0.022 (mean ± SE). The regression analysis showed that there was no relationship ($P = 0.70$, $R^2 = 0.003$, $n = 61$ species) between otolith growth rate "a" and $|\chi|_{\text{species}}$ (Fig. 3). For the littoral and bottom fishes (50 species), $P = 0.65$, $R^2 = 0.004$. For the pelagic fishes (11 species), $P = 0.98$, $R^2 < 0.0001$.

Thus, in the great majority (83%) of marine fishes studied irrespective of fish length and systematic and ecological position, saccular otolith mass asymmetry was low ($|\chi| < 0.05$). The value of the saccular otolith mass asymmetry did not depend on otolith growth rate.

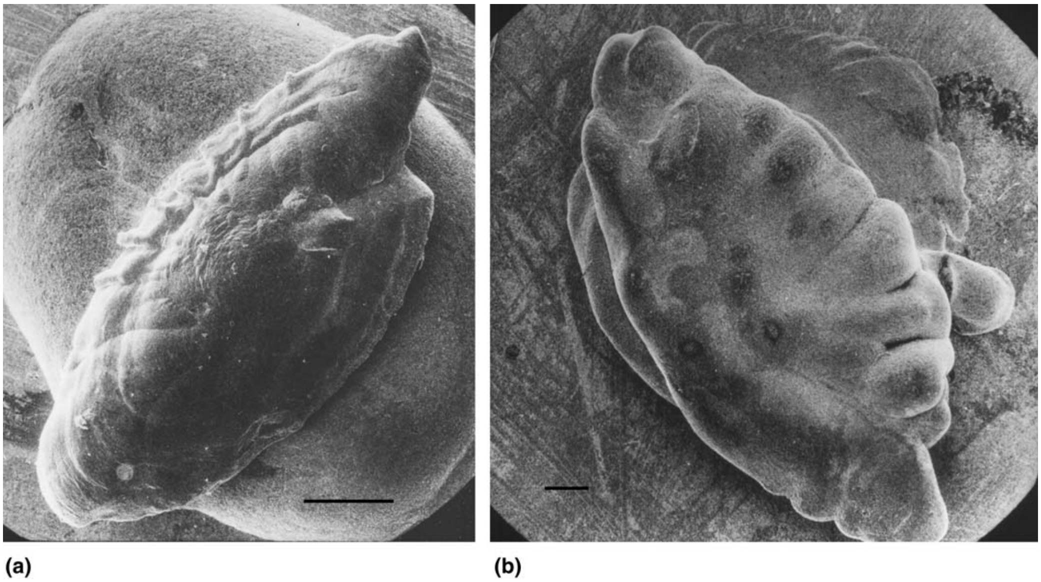

Fig. 1. Saccular otoliths. Scanning electron microscopy (from Lychakov, 2002). Endolymphatic surface view of saccular otoliths of Black Sea fishes (a) *Engraulis encrasicholus*, (b) *Uranoscopus scaber*. Bar 500 μm.

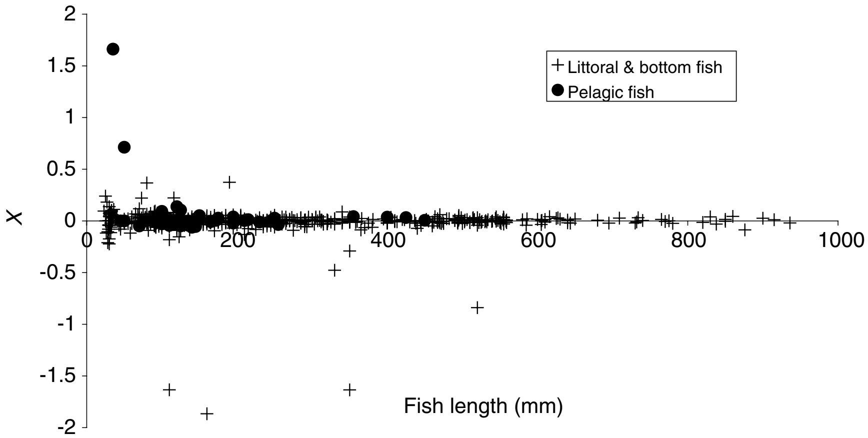

Fig. 2. Saccular otolith mass asymmetry χ in marine fishes as a function of fish length.

3.1.1.2. Individual fish species. Similar results were obtained for otolith asymmetry of individual fish species: *Merluccius merluccius*, *Pagellus erythrinus*, *Sciaenops ocellatus*, *Poecilia reticulata*, *Carassius auratus*, and *Cyprinus carpio* (Fig. 4). For the saccular otolith of *M. merluccius*, $\chi = -0.04533 \pm 0.06278$ (mean ± SE, $n = 27$) and $|\chi| = 0.09326 \pm 0.06071$ ($n = 27$). There was no relationship between the fish length and $|\chi|$ ($P = 0.95$, $R^2 < 0.001$). For the saccular otoliths of *P. erythrinus*, $\chi = 0.00345 \pm 0.00854$ ($n = 18$) and $|\chi| = 0.02374 \pm 0.00637$ ($n = 18$). There was no relationship between fish length and $|\chi|$ ($P = 0.43$, $R^2 = 0.039$). For saccular otoliths of *S. ocellatus*, $\chi = 0.00345 \pm 0.00854$ ($n = 196$) and $|\chi| = 0.03161 \pm 0.00295$ ($n = 196$). The regression of $|\chi|$ on fish length was significant ($P < 0.01$) but R^2 was low (=0.120). For the saccular otolith of guppy, $\chi = 0.02739 \pm 0.01382$ ($n = 27$) and $|\chi| = 0.05797 \pm 0.00951$ ($n = 27$). There was no significant relationship between guppy body mass and $|\chi|$ ($P = 0.53$, $R^2 = 0.016$).

For the utricular otoliths of goldfish *C. auratus*, $\chi = 0.013216 \pm 0.00772$ ($n = 45$) and $|\chi| = 0.03209 \pm 0.00634$ ($n = 45$). There was no significant relationship between goldfish length and $|\chi|$ ($P = 0.78$, $R^2 = 0.002$). For the utriculus of carp, $\chi = 0.00195 \pm 0.00508$ ($n = 103$) and $|\chi| = 0.03615 \pm 0.00361$ ($n = 103$). There was no relationship between carp length and otolith mass asymmetry $|\chi|$ ($P = 0.32$, $R^2 = 0.010$).

3.1.2. Otolith mass difference

3.1.2.1. Marine fishes. The relation between otolith mass difference, Δ, and fish length was more complex than the relation between otolith mass asymmetry, χ, and fish length (compare Figs. 2 and 5). It follows from Fig. 5 that, generally, saccular otolith mass difference increases with fish length. This was more pronounced in littoral and bottom fishes than in pelagic fishes (Fig. 5).

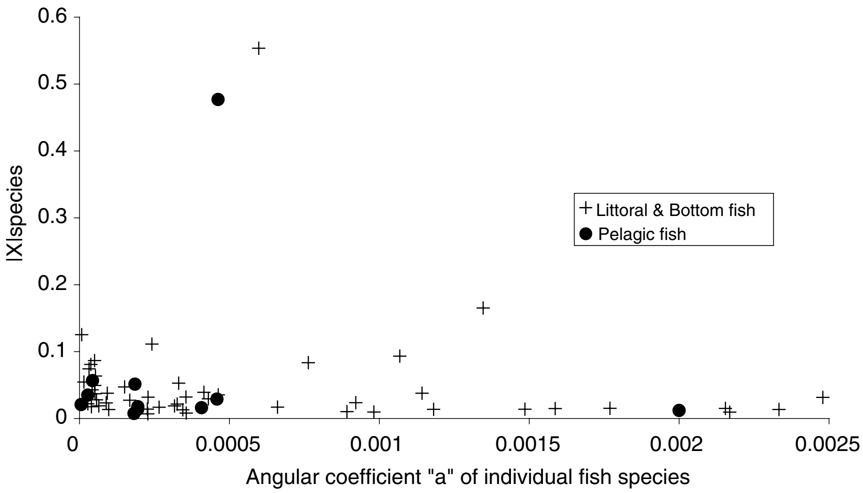

Fig. 3. Species saccular otolith mass asymmetry, $|\chi|_{\text{species}}$, as a function of species otolith growth rate, expressed as angular coefficient “a”. To evaluate the otolith growth rate, we described the relationship between otolith mass and fish length, $m = a \times L + b$, where L is the length of the fish, “a” is the coefficient characterizing the growth rate of the otolith, and “b” is a constant for the given species.

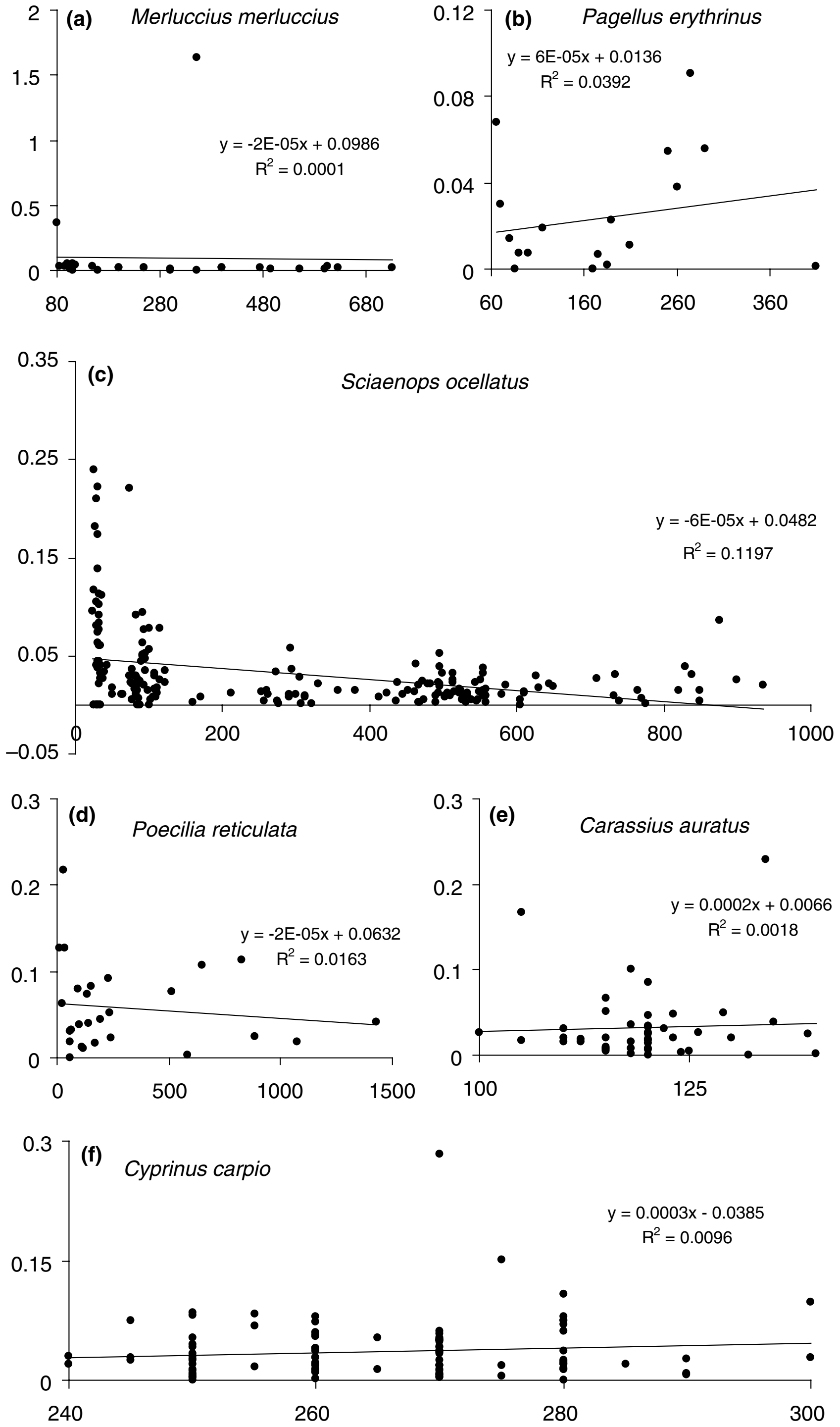

Fig. 4. Saccular (a–d) and utricular (e, f) otolith mass asymmetry $|\chi|$ vs. standard length (mm) (a–c), body mass (mg) (d) or total length (mm) (e, f).

3.1.2.2. Individual fish species. There was no relationship between fish length and saccular otolith mass difference, $|\Delta|$, in *M. merluccius* ($n = 27$, standard length = 80–730 mm; $P = 0.59$, $R^2 = 0.012$). There was also no relationship between fish length and $|\Delta|$ in *P. erythrinus* ($n = 18$, standard length = 65–345 mm; $P = 0.09$, $R^2 = 0.172$). There was, however, an obvious relationship between fish length and $|\Delta|$ in *S. ocellatus* ($n = 196$, standard length = 23–936 mm; $P < 0.001$, $R^2 = 0.772$). That relationship appeared to be curvilinear (Fig. 6). There was also a significant relationship between body mass and $|\Delta|$ in *P. reticulata* ($n = 27$, body mass = 11.3–1427.5 mg; $P = 0.001$, $R^2 = 0.354$).

There was no relationship between the fish length and utricular $|\Delta|$ in *C. auratus* ($n = 45$, total length = 100–140 mm; $P = 0.51$, $R^2 = 0.01$). There was also no relationship

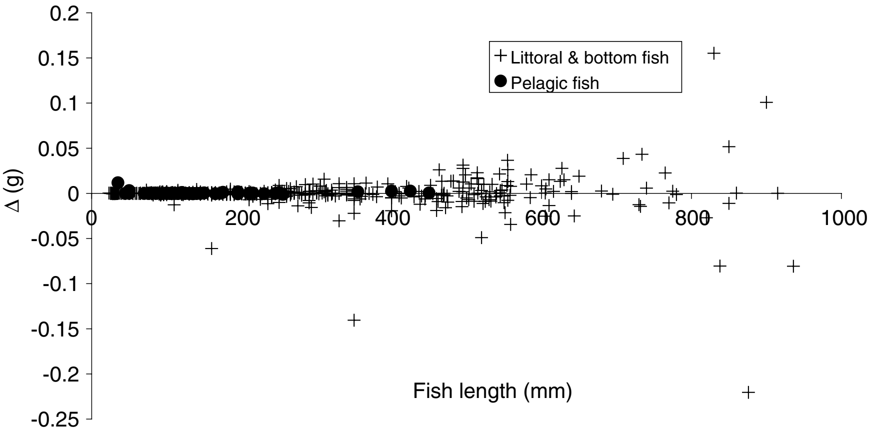

Fig. 5. Saccular otolith mass difference Δ in marine fish as a function of fish length.

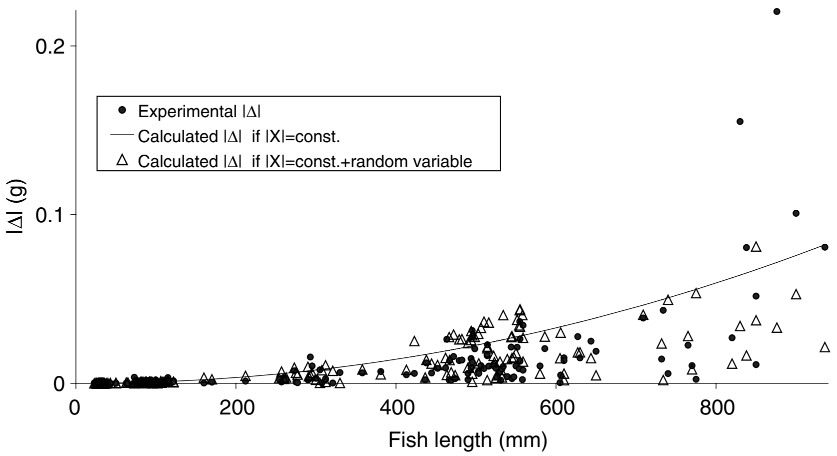

Fig. 6. Experimental and calculated values of otolith mass difference in *Sciaenops ocellatus* as a function of fish length.

between fish length and utricular $|\Delta|$ in *C. carpio* ($n = 103$, total length = 240–300 mm; $P = 0.11$, $R^2 = 0.025$).

3.2. Modeling of otolith mass difference

3.2.1. Empirical modeling

To specify and model the relationship between otolith mass difference and fish length, one species, *S. ocellatus*, was selected because we had more measurements for this species than any others (Table 1, Fig. 6). Data show that otolith mass, m, and fish length, L, are strongly related by a power function, $m = 2 \times 10^{-06} \times L^{2.0576}$ ($R^2 = 0.993$) for *S. ocellatus* (Fig. 7). From Eq. (1), the absolute value of the otolith mass difference was given by $|\Delta| = m \times |\chi|$. Hence

$$| \Delta | = 2 \times 10^{-06} \times L^{2.0576} \times | \chi | \tag{2}$$

The average value of $|\chi|$ in *S. ocellatus* was 0.03161 ± 0.00295 ($n = 196$). If $|\chi|$ were stable during fish growth and equal to the average value, then the relationship between $|\Delta|$ and fish length would follow the curve in Fig. 6. But this does not agree with the observed data (compare filled circles and curve in Fig. 6).

The values of $|\chi|$ of 96.9% of *S. ocellatus* vary within the range from 0 to 0.05. Enter the random variable in Eq. (2)

$$| \Delta|_{\mathrm{rv}} = 2 \times 10^{-06} \times L^{2.0576} \times (| \chi | + \chi_{\mathrm{rv}}) \tag{3}$$

where $|\chi| = 0.03161$, and χ_{rv} is a random variable that varies within the range from -0.03161 to 0.01839. The values of $|\Delta|_{\mathrm{rv}}$ calculated from Eq. (6) (Fig. 6, empty triangles) lay in the same area as the experimental values (Fig. 6, filled circles). Using a random number generator, we entered different sets of random variables in Eq. (6) and the results were the same.

The question arises as to whether this random variable was due to fluctuations in otolith asymmetry in each individual fish during lifetime, or due to inherent small difference in otolith asymmetries between different fish. In the second case, each individual fish has a distinctive invariable otolith mass asymmetry during its lifetime.

3.2.2. Theoretical considerations

To answer these questions let us suppose that the Δ for each fish can arise from two contributions. First, a systematic difference in the growth rates of the right and left otoliths can exist. We believe that the systematic difference in

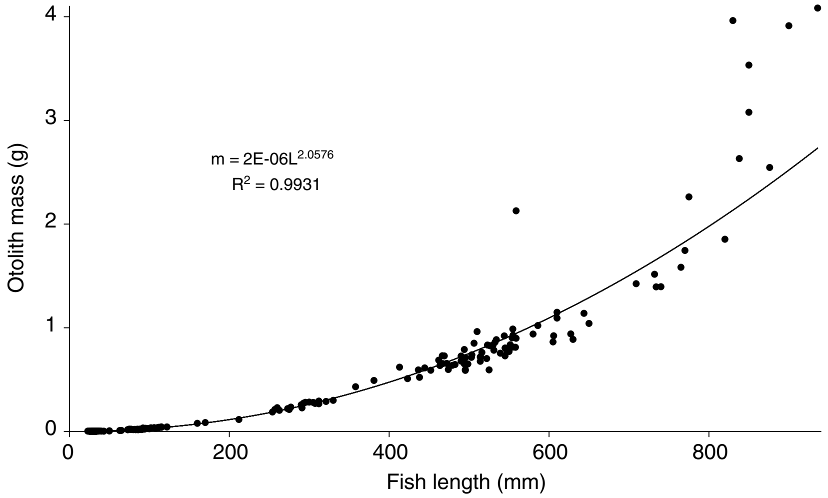

Fig. 7. Saccular otolith mass in *Sciaenops ocellatus* as a function of fish length.

growth rates does not change in the process of the otolith growth and results from the inherent anatomical asymmetry (for example due to the asymmetry of blood supply for the right and left otolith organs). The systematic difference can vary among individuals. Second, fluctuations in the growth rates of the right and left otoliths during life lead to a random difference in growth rates. These two contributions can be described by the following equation, which relates Δ to m.

$$\frac{d\Delta}{dm} = \alpha + \beta(m) \tag{4}$$

where α is a constant related to the systematic difference in growth rates of the right and left otoliths which is independent of the otolith mass, and $\beta(m)$ is a random function of m which is related to fluctuations in the otolith growth rates. The average value of $\beta(m)$ in individual fish during life is equal to zero because random fluctuations can be positive or negative and have different values.

The solution for Eq. (4) for an individual fish is

$$\Delta = \alpha m + \int_0^m \beta(m')dm' \tag{5}$$

where Δ is the mass difference between right and left otoliths of an individual fish for an average otolith mass m, m' is an integration variable, when integrated m' changes from zero when the otoliths are absent to m when the average otolith mass achieves the value m. αm is a systematic component of otolith mass difference, $\int_0^m \beta(m')dm'$ is a random component of otolith mass difference.

The average mass difference for a group of fish of any one species which have the same average otolith mass, m, is given by

$$\langle\Delta\rangle = \langle\alpha\rangle m + \int_0^m \langle\beta(m')\rangle dm' \tag{6}$$

where $\langle\Delta\rangle$ is the average mass difference, $\langle\cdots\rangle$ is a symbol for statistical averaging. The average value $\langle\alpha\rangle$ can be less than, or greater than, or equal to zero because the constant α can have different positive or negative values in different individuals. However, it is reasonable to believe that $|\alpha|$ varies little within a species and that $\langle|\alpha|\rangle$ may vary among species. The average value of the random component $\langle\beta(m)$ will be equal to zero if there is a large number of experimental fish. Hence, from Eq. (6)

$$\langle\alpha\rangle = \frac{\langle\Delta\rangle}{m} \tag{7}$$

Comparing Eqs. (1) and (7), Eq. (7) can be rewritten as

$$\langle\chi\rangle = \frac{\langle\Delta\rangle}{m} \tag{8}$$

where $\langle\chi\rangle$ is the average otolith mass asymmetry for fish that have the average otolith mass, m. Thus, average otolith mass asymmetry is independent of $\beta(m)$ and is equal to the average systematic difference in growth rates of the right and left otoliths.

To investigate contribution of the otolith growth fluctuations to the otolith mass difference we need to study the squared value of the otolith mass difference because the mass difference can be negative or positive. From Eq. (5) we get

$$\langle\Delta^2\rangle = \langle\alpha^2\rangle m^2 + 2m\int_0^m \langle\alpha\beta(m')\rangle dm' + \int_0^m \int_0^m \langle\beta(m')\beta(m'')\rangle dm'dm'' \tag{9}$$

where $\langle\Delta^2\rangle$ is the square of the average otolith mass difference for a group of fish of the same species, which have the same average otolith mass, m; m' and m'' are integration variables.

To calculate the right side of Eq. (9) we need to know the correlators $\langle\alpha\beta(m')\rangle$ and $\langle\beta(m')\beta(m'')\rangle$. The correlator $\langle\alpha\beta(m')\rangle$ is equal to zero because $\langle\beta(m')\rangle$ is equal to zero for a reasonably large group of fish. Thus,

$$< \alpha\beta(m') >= 0 \tag{10}$$

There are no experimental data about the nature of the fluctuations in otolith growth, and as a first approximation we can assume that the fluctuations are absolutely random and have a white-noise type correlator

$$\langle \beta(m')\beta(m'')\rangle = A\delta(m'-m'') \tag{11}$$

where A is a constant related to the intensity of the stationary white-noise type fluctuations, $\delta(m'-m'')$ is Dirac's delta function, which is "infinitely peaked" at $m'=m''$, and zero otherwise.

Substitution of Eqs. (10) and (11) into Eq. (9) gives

$$\langle \Delta^2\rangle = \langle \alpha^2\rangle m^2 + \int_0^m \int_0^m A\delta(m'-m'')\mathrm{d}m'\mathrm{d}m'' \tag{12}$$

Since total area under the delta function is unity, its integration in Eq. (12) gives

$$\langle \Delta^2\rangle = \langle \alpha^2\rangle m^2 + Am \tag{13}$$

From Eqs. (8) and (13) it follows that

$$\langle \chi^2\rangle m = Bm + A \tag{14}$$

where $B = \langle \alpha^2\rangle$. From Eq. (14) it follows that the quantity $\langle \chi^2\rangle m$ should be a linear function of otolith mass.

To compare Eq. (14) with experimental data we need to know average values of the squared otolith mass asymmetry at an otolith mass of m. To do this we ordered experimental data for *S. ocellatus* containing 196 otolith pairs according to their mass increase and then divided them into six sequential sets containing of 32 or 33 otolith pairs. For each set we calculated average mass, m, the average of the squares of individual otolith mass asymmetries $\langle \chi^2\rangle$ and its product $\langle \chi^2\rangle m$. The results are shown in Fig. 8. There was a strong linear relationship between parameters: $\langle \chi^2\rangle m = 0.00055 \times m - 0.00001$ ($P = 0.002$, $R^2 = 0.928$). The value for B was statistically significant ($P = 0.002$), but the value for A was not ($P = 0.855$). This means that the intensity of the white-noise type fluctuations had a low significance for the average mass difference. Hence, it can be proposed that the variability of the otolith mass difference, $|\Delta|$, (Fig. 6) is due to an inherent and stable small difference in otolith asymmetries between individual fish.

4. Discussion

It is important to recognize that we only worked with symmetrical species of teleost fishes, because in flatfishes there may be a significant structural asymmetry (Helling et al., 2005). The nature of this asymmetry will be discussed in a future paper.

Previous mathematical modeling has shown that acoustic and vestibular functionality of a fish ear can be reduced due to otolith mass asymmetry (Lychakov and Rebane, 2004, 2005). However, in the great majority of fishes studied saccular otolith mass asymmetry is very low ($|\chi| < 0.05$ or <5%), irrespective of fish length and systematic position. The low level of otolith asymmetry is typical for utricular and lagenar otolith organs also in symmetric teleost fishes. Takabayashi and Ohmura-Iwasaki (2003) have shown that the mean difference between utricular otoliths in goldfish and carp is 3.035% and 3.491%, respectively. The mean lagenar mass asymmetry is 6.25% in Black Sea fishes and 5.6% in goldfish (Lychakov and Rebane, 2004; Takabayashi and Ohmura-Iwasaki, 2003). On the other hand, only fishes that contain the largest otoliths and $|\chi| > 0.2$ could, in principle, have difficulties with sound processing due to incompatibility and incongruity of the right and left otolith movements (Lychakov and Rebane, 2005). Thus, for most fishes otolith mass asymmetry is well below critical values, so that most fishes avoid functional impairment as a result of otolith mass asymmetry.

The degree of the saccular otolith mass asymmetry does not depend on fish size, otolith growth rate, or systematic or ecological position of the fish (Figs. 2–4). This agrees with our previous results (Lychakov and Rebane, 2004, 2005). However, the relationship between otolith mass

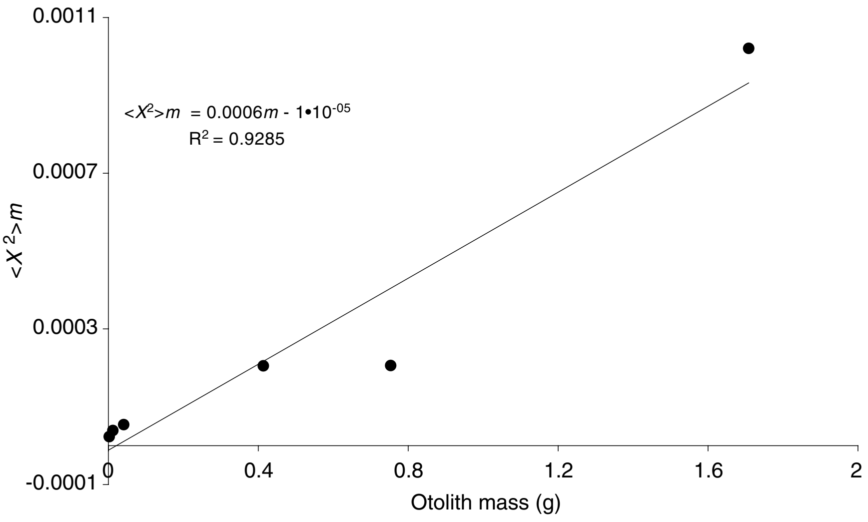

Fig. 8. The dependence of the parameter $\langle \chi^2\rangle m$ on the otolith mass of *Sciaenops ocellatus*.

difference, Δ, and fish length (mass) or mean otolith mass, m, is more complex (Fig. 5).

In previous studies we found that in some cases there is no relationship between fish length (or mass) and otolith mass difference, Δ (Lychakov and Rebane, 2004, 2005). This is especially true for small samples and when the specimens do not differ markedly in size (see data for *M. merluccius*, *P. erythrinus*, *C. auratus*, *C. carpio*). Otherwise there is another pattern. For marine fishes as a group, and for *S. ocellatus* and *P. reticulata* individually, the value of the saccular otolith mass difference, $|\Delta|$, depends on fish length (Fig. 6). This is in accordance with Eq. (1) and the experimental finding that mean otolith mass asymmetry does not change with fish age. However, the experimental data do not exactly fit the calculated curve for the relationship between fish length and otolith mass difference (Fig. 6). To clarify the reasons for this variability we modeled the difference in growth rates between the right and left otoliths and entered a random function $\beta(m)$ which is related to fluctuations in otolith growth rates during a fish's life (Eq. (4)). The comparison of theoretical conclusions (Eq. (14)) with experimental data shows that the intensity of the white-noise type fluctuations in individual otolith growth is statistically negligible. This means that the basic cause of the otolith mass difference variability is probably an inherent trait of individuals. Different individuals from the very outset of growth of the paired otoliths have different values of the otolith mass asymmetry, which are stable throughout life. This conclusion needs to be confirmed by direct observations or experiments.

Our conclusion of inherent and stable otolith mass asymmetry in a fish is very encouraging for verification of the role of otolith mass asymmetry in the context of the otolith asymmetry hypothesis (Egorov and Samarin, 1970; Von Baumgarten et al., 1982). According to this hypothesis, an imbalance between signals from the right and left otolith organs due to otolith mass asymmetry is well compensated on Earth, but in space, the central compensation will no longer be appropriate. These new uncompensated discharge differences may induce sensations of rotation, inclination, space motion sickness, etc. If the values of individual otolith mass asymmetry are stable throughout life, there will be no fundamental problems with carrying out long experiments on fishes. However, gaining a complete understanding of the causes of otolith mass difference variability requires extended investigations on a large sample of fishes of different species and different sizes.

5. Conclusions

This paper is our third work devoted to the problem of the fish otolith mass asymmetry. Within the framework of this problem we tried to elucidate three questions. First, can otolith mass asymmetry contribute to abnormal space motion and space motion sickness (Lychakov and Rebane, 2004)? Second, can otolith mass asymmetry have noticeable adverse effects on acoustic functionality (sensitivity, temporal processing, sound localization) (Lychakov and Rebane, 2005)? Third, do individual fish have a distinctive, invariant otolith mass asymmetry during its lifetime (this paper)? To provide the answers we used our morphometric data together with mathematical modeling of otolith displacement responses to an instant force and the acoustic stimuli and modeled the difference in growth rates between the right and left otoliths (Lychakov and Rebane, 2000, 2004, 2005, this paper). Results of the mathematical modeling say "yes" to all three questions. The following remarks clarify some important topics for understanding these conclusions.

We derived expressions for the critical level of the otolith mass ratio that should be exceeded to avoid abnormal space motion (Lychakov and Rebane, 2004). However, otolith mass asymmetry in that first paper was expressed as the ratio of small and large otolith mass. In the subsequent papers we used the dimensionless coefficient χ. The application of χ offers some advances for calculations (Lychakov and Rebane, 2005) and it is possible to convert the mass ratio used in our first paper to χ.

Our calculations show that, theoretically, otolith mass asymmetry can have adverse effects on auditory functions in fish that contain the largest otoliths and greatest otolith asymmetry $|\chi| > 0.2$ or >20%. With a smaller otolith mass and $|\chi| < 0.2$ or <20% the inconsistency between signals from the right and left ears due to incongruity of the right and left otolith displacements will be negligible (Lychakov and Rebane, 2005). Fortunately, in the overwhelming majority of animals studied otolith mass asymmetry is within the range of $-0.2 < \chi < +0.2$ or <20% (Lychakov and Rebane, 2004, 2005). Moreover, when we processed a large number of fish species (77 in this study vs. 17 teleost species in the previous studies), we confirm that nearly 85% of fishes had $|\chi| < 0.05$.

Thus, it is apparent that the overwhelming majority of fishes do not experience acoustic impairment due to otolith mass asymmetry. But what will happen if $|\chi| > 0.2$? We found that there are some very extreme values of $|\chi| \approx 1.63–1.87$ in what are apparently normal fish (Fig. 2). These are nearly unilateral fish. However, these specimens managed to survive this asymmetry long enough to become samples in our study. Obviously, such large asymmetry could be accommodated by the rest of the ear (at the macular level?) and the central nervous system. "This conclusion suggests that there must be mechanisms of brain plasticity that allow the fish to carry out one of the most difficult tasks – calibration of a very sensitive linear accelerometer, even when the mechanism that determines the function of that accelerometer has these deficits". It is very tempting to assess the extent to which functional accommodation proceeds and to find the morphological bases of brain plasticity.

On the other hand, the overwhelming majority of fishes do not exhibit such extremes. They obey the law of parsimony, keeping their otolith asymmetry at the very low level

$<5\%$ and relieving the central nervous system of having to do redundant coordination. Moreover, according to our modeling, the value of χ is probably stable during a fish's lifetime. The mechanisms by which a fish maintain right–left otolith symmetry at a stable and low level are unknown. Some authors suggest that the weight of the otolith mass on the sensory epithelium is the direct regulating factor controlling the growth of the otolith via negative-feedback loop between the brain and the inner ear (Rahman and Anken, 2002). But other evidence refutes this hypothesis, and otolith weight seems not to be involved in the regulation of its growth (Lychakov, 2002).

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