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Assessment of the size distribution of the big-scale sand smelt (*Atherina boyeri* Risso, 1810) in the diet of the Caspian seal (*Pusa caspica* Gmellin, 1788) using fish otoliths from fecal samples

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Abstract
Big-scale sand smelt (*Atherina boyeri*) is an euryhaline pelagic schooling fish found throughout the Caspian Sea. A specialized fishery does not target this species, and its body length can reach up to 13 cm. *A. boyeri* serves as prey for many omnivorous and predatory fish and is one of the primary food sources for the Caspian seal (*Pusa caspica*). The objective of this study was to determine the size composition of *A. boyeri* in the diet of *P. caspica*, a declining species of the Caspian Sea. A coprological method was used, involving the collection of seal feces during spring and autumn haul-outs in the Northern and Middle Caspian, followed by examination of fish otoliths. Before this, otoliths from fish were analyzed to assess morphological diversity and growth patterns in relation to *Atherina boyeri*. Equations were developed to estimate fish length from otolith length and width. Otoliths from feces were classified into three wear categories: lightly, moderately, and heavily abraded. A comparison of otolith sizes from fish and feces allowed the calculation of wear coefficients based on otolith length for each group, which were incorporated into the reconstruction formula. The reconstructed fish length distribution showed that at the Northern Caspian haul-outs, individuals measuring 60–70 mm predominate, while at the Middle Caspian haul-out, individuals measuring 80–90 mm are most common. Overall, this is the first study to confirm that *P. caspica* feeds on both juvenile and mature *A. boyeri*, with lengths ranging from 23.1 to 117.1 mm.

Keywords Coprological method, Sagitta, Shape indices, Fish length, Otolith abrasion, Reconstruction formulas, Haul-out site, Pinnipeds

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Introduction

The Caspian seal (*Pusa caspica* Gmellin, 1788) is the only marine mammal in the Caspian Sea, an endemic species listed as Endangered on the IUCN Red List [1] and holding a similar status at the national levels of Caspian states [2–5]. The species' range covers the entire Caspian Sea basin. *P. caspica* is an obligate ichthyophage. The condition and availability of its food resources largely determine the animals' nutritional health and disease resistance, influencing the species' overall reproductive capacity. In recent decades, a decline in fish stocks—the primary prey of *P. caspica*—has been observed [6–12]. Therefore, it is essential to conduct studies to evaluate the presence of various fish species in the diet of *P. caspica*.

According to retrospective data, fish play the most significant role in the diet of *P. caspica* [13–19], accounting for 98–99% of the stomach content [14, 18], with species of the families *Gobidae* and *Clupeidae* being the dominant ones [13, 18]. The diet al.so includes mollusks and crustaceans (gammarids, shrimps, mysids, crayfish, and crabs).

Big-scale sand smelt (*Atherina boyeri* Risso, 1810) is among these prey items. The frequency of *A. boyeri* in their stomachs was 10.8% during the autumn-winter period and 1.8% during the winter, respectively [13], and reached 50% in April [17]. These data pertain to periods when the seals were in the Northern Caspian. In the Middle and Southern Caspian, *A. boyeri* played a secondary role during the seals' haul-out periods [20]. Later data indicated that *A. boyeri*, along with sprat and gobies, could be considered one of the main fish prey items for the seals [18].

It is clear that the data above, gathered by analyzing the stomach contents of killed animals, are quite scattered in terms of time and sampling locations, requiring more detailed studies to understand the importance of this species in the diet of marine mammals at this stage. However, the high conservation status of *P. caspica* suggests not only a ban on its commercial hunting but also prohibits killing them for scientific research. Therefore, the only feasible approach is to use non-lethal methods to study the seal's diet and assess the role of individual species within it.

The coprological method, which relies on the analysis of fish otoliths, offers excellent opportunities to study the diet of ichthyophagous pinnipeds [21–23], particularly in species under strict protection due to declining populations. Research conducted from 2015 to 2023 on otoliths found in feces collected at island haul-outs showed that *A. boyeri* ranks second in the *P. caspica* diet, after gobies [24, 25]. However, this information alone is not enough to assess the role of this species in meeting the seals' nutritional requirements. An essential first step is to determine the lengths of fish consumed by seals, which can be estimated by back-calculating from otoliths recovered from feces. In this context, studying the relationship between the growth of *A. boyeri* otoliths and the growth of the fish is crucial. To more accurately estimate the lengths of the fish consumed by the seals, it is also necessary to understand the deformations otoliths undergo during passage through the seal's gastrointestinal tract.

Given the above, the present study aimed to determine the size structure of *A. boyeri* eaten by seals based on regression analysis of the relationship between otolith growth and fish growth, considering changes in otoliths during passage through the seal's gastrointestinal tract.

Materials and methods

To describe the morphological diversity and size variation of fish otoliths, a bioanalysis was performed on 61 big-scale sand smelt (*Atherina boyeri*) specimens. Of these, 54 were captured using a 1.9 m × 0.8 m bottom trawl with 10 mm mesh in various parts of the Northern Caspian at depths of 2–4 m by staff from Kazakhstan Applied Ecology Agency LLP in autumn 2017 and 2019, and by Tengizchevroil LLP in autumn 2017. The specimens were then transferred to the Institute of Hydrobiology and Ecology. An additional seven *A. boyeri* were collected by the authors during expeditions in spring 2018 and autumn 2020 using a square-mesh fyke net (2.0 m length, 1.0 m height, 10 mm mesh body, 5 mm cod end) in the coastal zone of the Middle Caspian (Kendirli Bay) at depths up to 1 m. Figure 1 shows a schematic map of the fish sampling points. All fish were euthanized using an overdose of the pharmaceutical "Eugenol" [26] and then fixed in a 10% formalin solution. Species identification was confirmed with the Caspian Sea Fishes and Invertebrates Identification Guide [27].

Fish were measured using a caliper with an accuracy of 0.1 mm. In this study, we used standard length (SL – from the tip of the snout to the end of the scaled portion) [28], and sex and gonadal maturity stage were determined according to Pravdin (1966) [29].

This study focused on the largest otolith of *A. boyeri*—the sagitta [21, 30–33].

Otoliths were extracted from the fish under laboratory conditions. The extracted left and right otoliths were placed in test tubes containing 95% ethanol [21, 33] and then cleaned of any remaining tissue in a sodium hypochlorite solution [34]. Overall, the study used 48 left and 50 right otoliths.

For the dietary analysis of the Caspian seal (*Pusa caspica*) at the haul-outs in the Northern and Middle Caspian, fecal samples were collected between 2015 and 2021 (Fig. 1). Collection was performed manually while wearing nitrile examination gloves. Each sample was placed in an individual zip-top bag and labeled with the date, location of collection, the sample's sequential

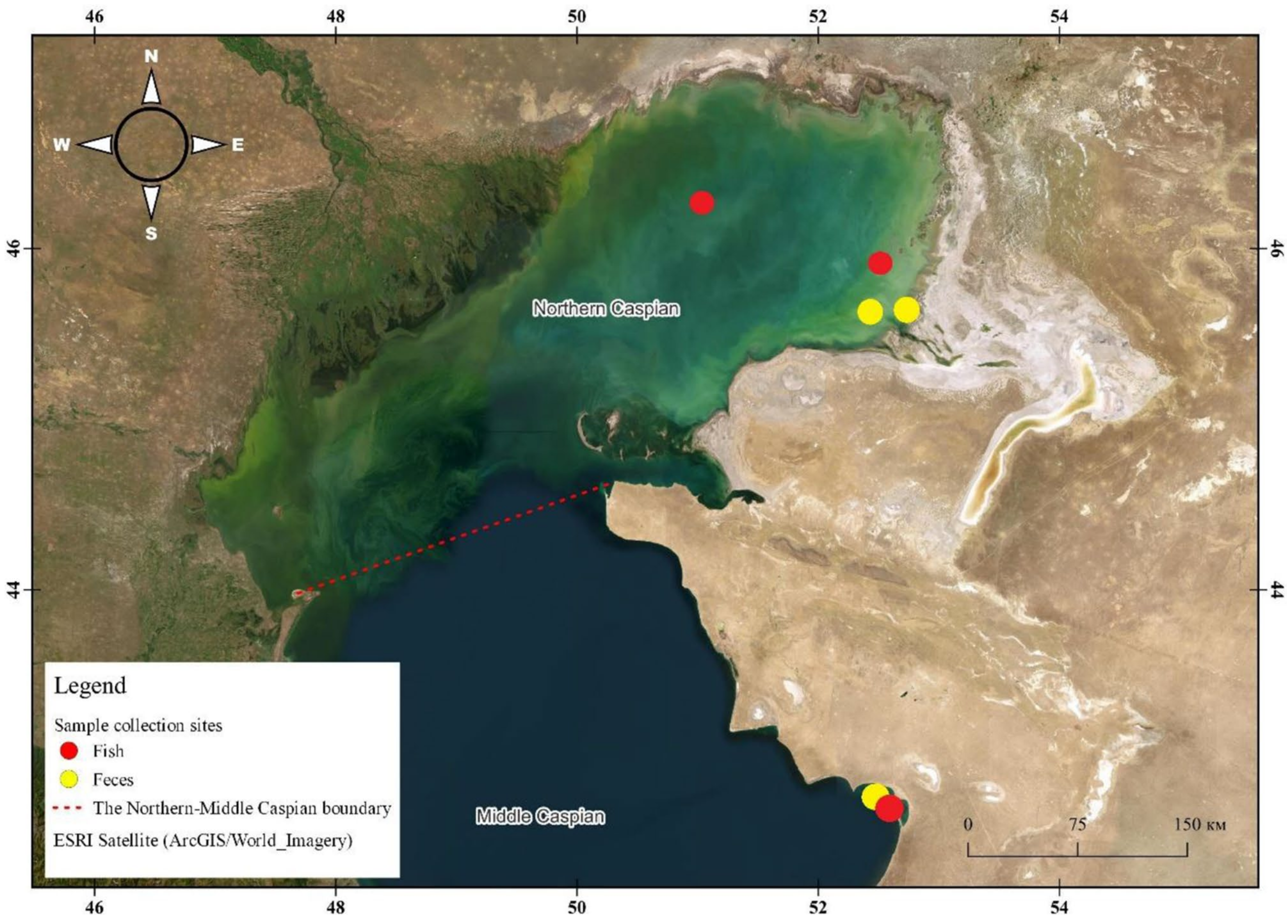

Fig. 1 Sampling sites for big-scale sand smelt (*Atherina boyeri*) capture and Caspian seal (*Pusa caspica*) fecal sample collection

number, and a description (intact or fragmented). When multiple fecal fragments were found nearby, they were combined into a single sample and placed in a shared zip-top bag labeled accordingly. Fecal samples were photographed in situ at the collection sites. A total of 69 samples were collected at the Caspian seal haul-outs in Kendirli Bay (Middle Caspian), and 84 samples from the New Durnev Islands (near Komsomolets Bay, Northern Caspian). To facilitate otolith separation, each sample was soaked in water for several hours to a few days, with a small amount of detergent added in some cases. After soaking, the fecal material was thoroughly rinsed under running water through a 0.355 mm mesh sieve. Rinsing involved gently agitating the material on the sieve with a soft brush until all organic matter was removed. The remaining solid residues were transferred to a smooth surface and air-dried at room temperature. The dried residues were examined and sorted using a binocular microscope. *A. boyeri* otoliths were identified based on their morphological characteristics, as described in otolith atlases [35, 36]. From these samples, 1,093 otoliths of *A. boyeri* were identified and selected for analysis.

Thus, two sample sets were created:

1. Otoliths are directly extracted from fish.
2. Otoliths taken from Caspian seal feces.

Otolith measurements followed the protocols of previous studies, using a Motic trinocular microscope [23, 37–39]. Otoliths were placed on the microscope stage with their inner surface exposed, and the rostrum to the right to display diagnostic features (ostium, cauda, sulcus). They were then photographed with a digital camera (Fig. 2).

To highlight key diagnostic features, otoliths extracted from feces were coated with graphite [36, 40].

The perimeter and area of the otoliths were calculated using the biological image analysis software ImageJ. Otolith shape indices were calculated using the relevant formulas [39, 41–44] (Table 1).

Thorough statistical analyses of the collected data were performed following the outlined methodologies [45, 46]. All calculations were done using the MS Excel analysis toolpak. Pearson's correlation was used, including calculating the error of the correlation coefficient with the formula:

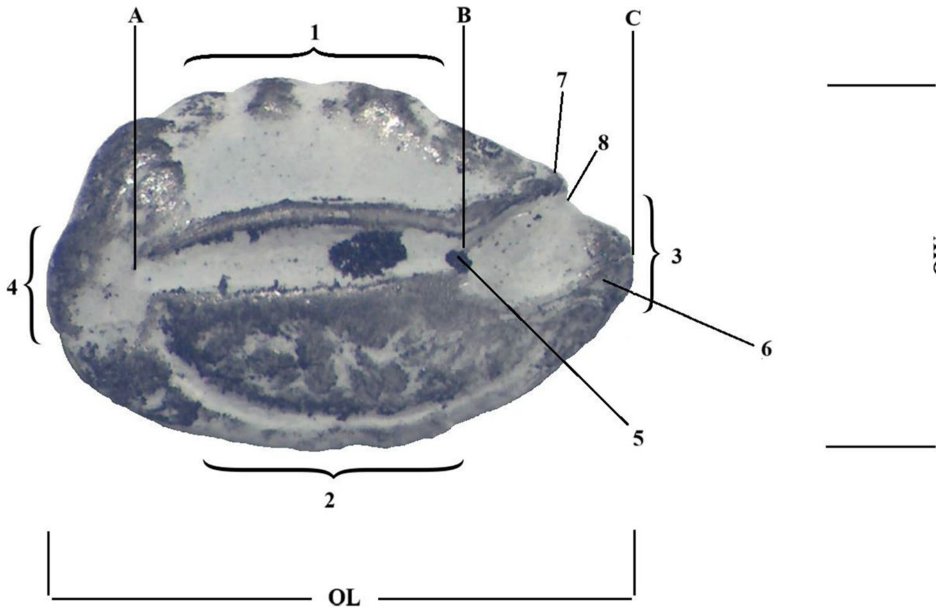

Fig. 2 Internal surface of the big-scale sand smelt (*Atherina boyeri*) otolith: 1 – dorsal part; 2 – ventral part; 3 – anterior part; 4 – posterior part; 5 – neck; 6 – rostrum; 7 – antirostrum; 8 – excisura; OL – otolith length; OW – otolith width; AC – sulcus; AB – cauda; BC – ostium

Table 1 Fish otolith shape indices [39]

Index Name	Symbol	Formula
Aspect ratio	OL/OW	OL/OW
Circularity	P/A	p^2/A
Form Factor	FF	$4\pi A/p^2$
Roundness	RO	$4A/\pi OL^2$
Rectangularity	RE	A/(OL*OW)
Ellipticity	EL	(OL-OW)/(OL + OW)

Note: OL – otolith length; OW – otolith width; P – perimeter; A – area

$$m_r = \sqrt{\frac{1-r^2}{n-2}}$$

Where **r** is the sample correlation coefficient and **n** is the number of paired observations. The significance of the difference between the two correlation coefficients was also assessed. The significance of the difference between the two correlation coefficients was evaluated using the test statistic:

$$t_{dr} = \frac{d}{m_d} \geq t_{crit}$$

Where $\mathbf{t_{dr}}$ is the test statistic for the difference between correlation coefficients, **d** is the difference between the two sample correlation coefficients, $\mathbf{m_d}$ is the standard error of that difference, calculated as $\mathbf{m_d = \sqrt{(m_{r1}^2 + m_{r2}^2)}}$ with $\mathbf{m_{r1}}$ and $\mathbf{m_{r2}}$ being the standard errors of the individual correlation coefficients, $\mathbf{t_{crit}}$ is the critical value of the Student's t-distribution for $\mathbf{v = n_1 + n_2}$ – 4 degrees of freedom at the chosen significance level.

The significance of the difference between mean values was assessed using Student's t-test (t_d). The standard deviation was calculated by the formula:

$$\sigma = \sqrt{\frac{C}{n-1}}$$

Where **σ** is the standard deviation, **C** is the variance, and $n-1$ is the number of degrees of freedom.

The coefficient of variation (**CV**) was calculated as:

$$CV = \frac{100\sigma}{M}$$

Where **σ** is the standard deviation, and **M** is the arithmetic mean.

To create size classes, the following algorithms were applied [45]. The number of classes was calculated using the formula, with the result rounded to the nearest integer:

$$R = 1 + 3{,}3\log_{10} n$$

Where **R** is the number of classes, and **n** is the sample size.

The class interval width was calculated as:

$$k = \frac{p}{R}$$

Where **k** is the class interval width, **p** is the range (max-min), and **R** is the number of classes. The resulting **k** was rounded to the nearest whole millimeter for fish length.

The start of the first class was calculated as:

$$W_{\alpha} = W - \frac{1}{2}k$$

The end of the first class was calculated as:

$$W_{\omega} = W - \frac{1}{2}k - \delta$$

Where $\boldsymbol{W_{\alpha}}$ and $\boldsymbol{W_{\omega}}$ are the lower and upper limits of the first class, $\boldsymbol{W}$ is the minimum observed value, $\boldsymbol{k}$ – class width, $\boldsymbol{\delta}$ is the measurement precision.

In subsequent graphs, only the upper boundary of the class is shown.

For otoliths from feces, specimens from each size class were randomly selected using Excel's random selection function. To evaluate differences in wear, three abrasion levels were established: lightly, moderately, and heavily abraded (Table 2).

Figure 3 illustrates the three categories of otolith wear through photographs. Each category is represented by two images—one depicting the condition of a small otolith and the other a larger one.

To analyze the relationship between fish length and otolith dimensions, scatter plots were created in Microsoft Excel. Otolith length and width were plotted on the X-axis, while the standard lengths of control specimens of *A. boyeri* were plotted on the Y-axis. A linear trendline was added to each plot, and the equation of the regression line was displayed. The coefficient of determination ($\mathbf{r^2}$) was automatically provided in the "Regression Statistics" section of Excel's "Data Analysis" regression tool.

Table 2 Determination of the degree of wear of big-scale sand smelt (*Atherina boyeri*) otoliths from Caspian seal (*Pusa caspica*) feces

№	Characteristic	Light	Moderate	Heavy
1	Rostrum	Not chipped or broken	Shows signs of wear but retains its characteristic shape	Broken or worn, making precise measurement impossible
2	Sulcus	Clearly defined, ostium and cauda are distinctly visible	Still visible as a uniform channel, ostium, and cauda are indistinguishable	Difficult to discern or worn smooth
3	Irregularities on the ventral and dorsal parts of the otolith	Clearly expressed	Weakly expressed	Absent
4	Determination of sides (left/right)	Possible	Possible	Not possible

To assess the normality of the length and width distributions of otoliths from feces, QQ plots were constructed in Microsoft Excel, following the method described by Shahbaba (2012) [46]. Empirical sample quantiles, arranged in ascending order, were plotted against the corresponding theoretical quantiles of the normal distribution on a scatter diagram. The closer the points are to the 45° reference line—indicating perfect normality—the more the sample distribution resembles a normal distribution.

Results

Otolith measurements

The fish sample, carefully selected for otolith extraction, ranged from 47 to 102 mm, with an average length of 81.5 mm. The length distribution shows that many individuals fall within the size ranges of 69.5–78.4 mm, 78.5–87.4 mm, and 87.5–96.4 mm, with one distinct group representing the smallest fish at 42.5–51.4 mm (see Fig. 4).

The coefficient of variation (CV) in otolith length, width, and perimeter is low, ranging from 12 to 15.5%. In contrast, the otolith area exhibits greater variability, with CVs ranging from 23.6 to 26.1%. A comparative analysis of the main parameters of the left and right otoliths of the big-scale sand smelt (*Atherina boyeri*) revealed no significant differences in mean values.

The high correlation coefficients of both left and right otoliths with fish length, along with the lack of significant differences between these coefficients, suggest an interrelated growth of fish and their otoliths (Table 3). Based on these findings, subsequent analyses used right otoliths due to their slightly higher number of measurements (50 versus 48 for left otoliths).

A comparison of fish length and the OL/SL and OW/SL ratios between females and males (juveniles were not compared) was also conducted. The analysis revealed no sexual dimorphism in these traits (Table 4), allowing subsequent otolith analyses to disregard the fish's sex.

Otolith shape characteristics

The otoliths of *A. boyeri* generally conform to the species description [36, 47]. Overall, *A. boyeri* otoliths are elliptical and spindle-shaped, tapering to a point at the anterior end. The sulcus is elongated and well-defined, running along the middle of the otolith. Both the ostium and cauda are distinguishable and separated by a pronounced neck, which renders the sulcus hetero-ciliated (see Fig. 2). Two forms can be distinguished: otoliths with a notch in the ostium area (Fig. 5a) and otoliths without a notch (Fig. 5b). In otoliths with a notch, a pointed rostrum is evident, while the antirostrum is poorly developed; in

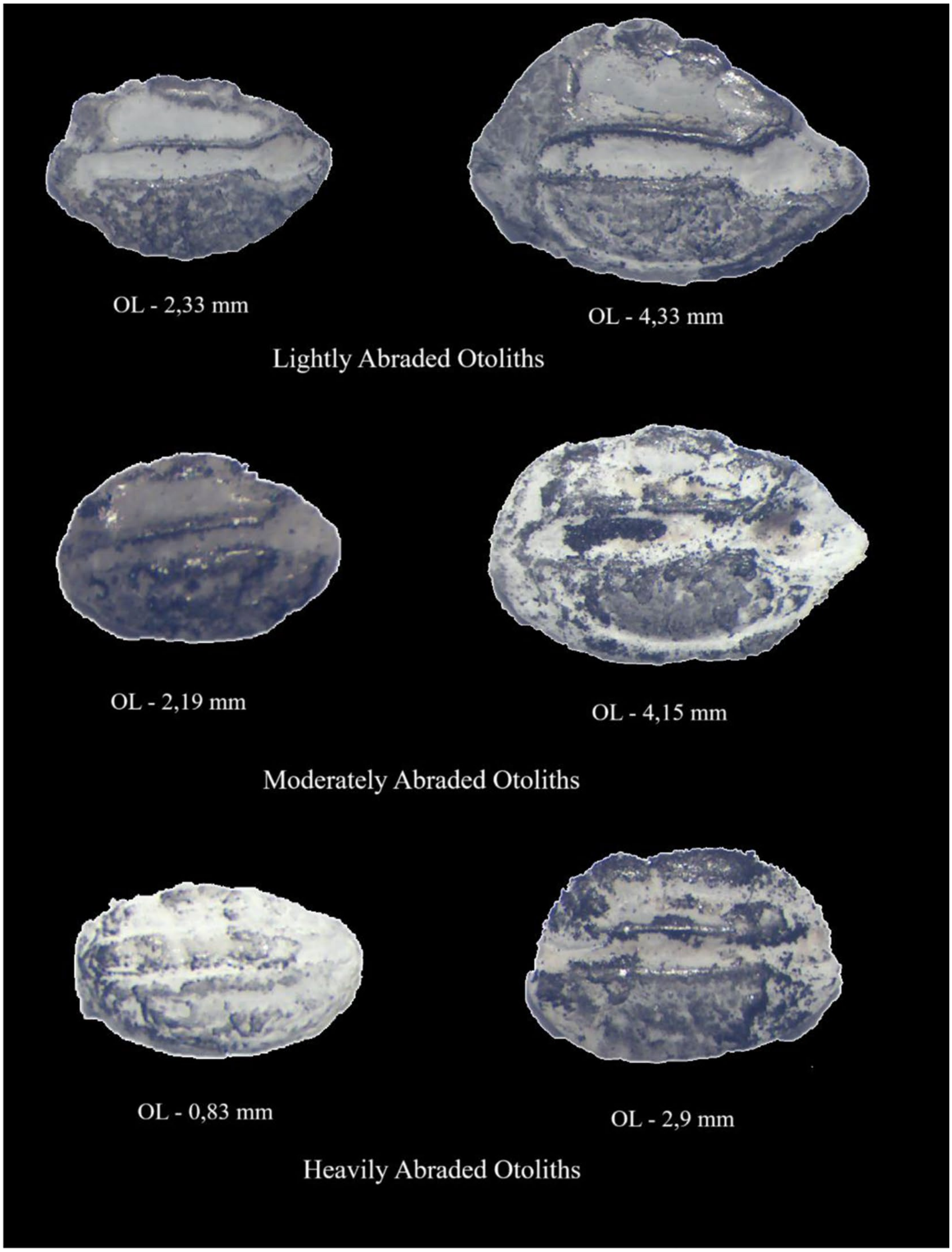

Fig. 3 Examples of the degree of wear of otoliths from feces (OL – otolith length)

otoliths without a notch, the rostrum is faintly expressed, and the antirostrum is absent.

In the fish otolith sample, the number of otoliths with a notch exceeds twice the number of otoliths without a notch. When grouped based on the presence or absence of a notch, fish lengths showed no statistically significant differences in their mean values. Likewise, otolith lengths themselves, depending on whether a notch is present or not, did not display significant differences in mean values (Table 5).

Otolith shape indices

Table 6 presents the calculated shape indices of *A. boyeri* otoliths. Notably, the indices' coefficients of variation (CV) range from 3.24 to 11.91. These low CV values

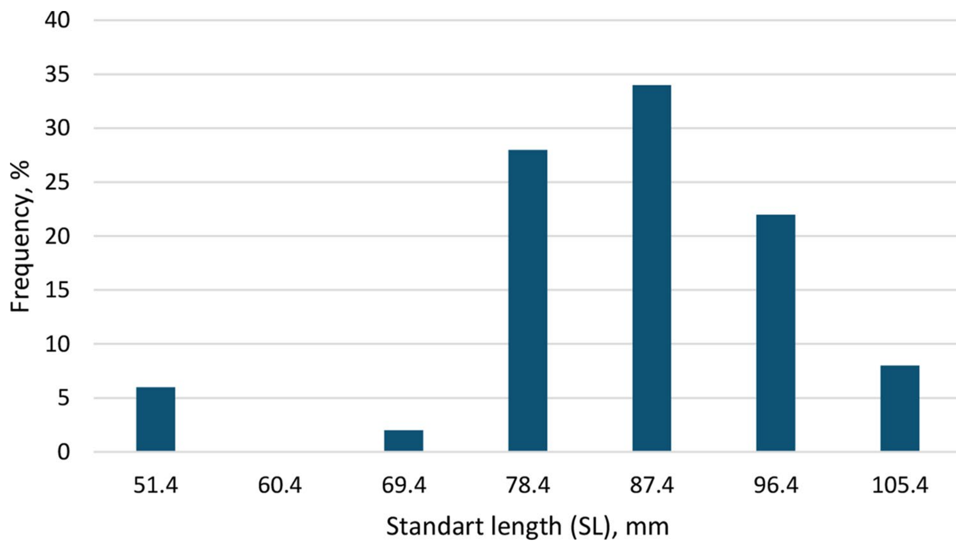

Fig. 4 Size structure of the big-scale sand smelt (*Atherina boyeri*) sample

Table 3 Comparative characteristics of left and right otoliths from fish

Parameter	OL. mm		OW. mm		*P*. mm		A. mm^2	
	Left	Right	Left	Right	Left	Right	Left	Right
min	1.9	2.0	1.3	1.3	5.8	5.6	1.9	1.8
max	4.6	4.6	2.6	2.7	12.4	12.6	8.8	8.9
mean ± SE	3.6 ± 0.07	3.6 ± 0.08	2.2 ± 0.04	2.2 ± 0.04	9.8 ± 0.18	9.9 ± 0.21	5.8 ± 0.20	5.7 ± 0.21
n	48	50	48	50	48	50	48	50
CV (%)	14.2	15.5	12	13.8	12.9	14.7	23.6	26.1
t_d	$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
r with SL	0.92 ± 0.06	0.93 ± 0.05	0.91 ± 0.06	0.92 ± 0.06	0.93 ± 0.05	0.92 ± 0.06	0.92 ± 0.06	0.93 ± 0.05
t_{dr}	$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	

Note: SE – standard error, n – sample size, OL – otolith length, OW – otolith width, P – perimeter, A – area, CV – coefficient of variation, r – correlation, t_d – the Student's t-test statistic for the significance of the difference between means, t_{dr} - the Student's t-test statistic for the significance of the difference between two correlation coefficients

Table 4 Comparative characteristics of otoliths from males (m) and females (f)

Parameter	SL. mm		OL/SL		OW/SL	
	m	f	m	f	m	f
min	71.0	68.0	0.04	0.04	0.02	0.02
max	102.0	101.0	0.05	0.05	0.03	0.03
mean ± SE	82.9 ± 1.8	84.7 ± 2.1	0.04 ± 0.0004	0.04 ± 0.0006	0.03 ±0.0003	0.03 ± 0.0004
n	27	20	27	20	27	20
CV	10.9	10.7	4.7	6.2	5.0	6.9
t_d	$p > 0.05$		$p > 0.05$		$p > 0.05$	

Note: SE – standard error, n – sample size, SL – standard length, OL – otolith length, OW – otolith width, CV – coefficient of variation, t_d – Student's t statistic for the significance of the difference between means

suggest that the examined otoliths exhibit only minor variability.

The fish length distribution graph (Fig. 4) showed that the sample included small fish (42.5–51.4 mm; 3 specimens) and large fish (60.5–105.4 mm; 47 specimens). A visual comparison of the otolith shapes from these groups reveals that both small and large fish have roughly similar forms (Fig. 6). Moreover, a comparison of the distribution graphs for the degree of elongation, roundness, and ellipticity—the indices with the highest coefficients of variation—indicates that the values of these indices for small fish are very similar and fall within the range observed for large fish (Fig. 7). Therefore, it is reasonable to treat the small and large fish as a single homogeneous sample for subsequent analyses.

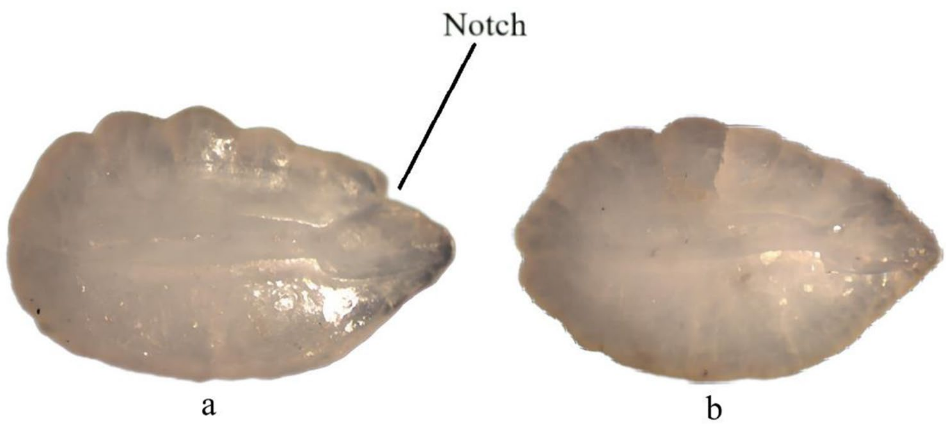

Fig. 5 Otolith samples: **a** – otoliths with a notch, **b** – otoliths without a notch

Table 5 Comparative analysis of big-scale sand smelt (*Atherina boyeri*) standard length and their otolith length, classified by the presence or absence of a Notch

Parameter	SL. mm		OL. mm	
	With Notch	Without Notch	With Notch	Without Notch
min	47.0	70.2	2.0	3.1
max	101.0	102.0	4.6	4.4
mean ± SE	81.4 ± 2.2	81.8 ± 2.7	3.6 ± 0.1	3.6 ± 0.1
n	36	14	36	14
CV (%)	16.2	11.9	17	11.7
t_d	$p > 0.05$		$p > 0.05$	

Note: SE – standard error, n – sample size, SL – standard length, OL – otolith length, CV – coefficient of variation, t_d - Student's t statistic for the significance of the difference between means

Formulas for reconstructing fish length from otolith length or width extracted from the fish

The correlation between otolith length and width was calculated to assess the feasibility of reconstructing fish length from the otolith's dimensions, yielding a high correlation coefficient ($r = 0.95$). This strong relationship indicates that fish length can be reliably estimated using either the otolith length or width. Accordingly, growth equations were derived from the data from otoliths extracted from fish. One equation relates fish length to otolith length ($y = 20.568x + 8.2204$) with coefficient of determination (r^2 – 0.87), and the other relates fish length to otolith width ($y = 36.359x + 0.749$) with r^2 – 0.84, where

Table 6 Otolith shape indices of big-scale sand smelt (*Atherina boyeri*)

Parameter	Aspect ratio	Circularity	Form Factor	Roundness	Rectangularity	Ellipticity
min	1.40	16.11	0.61	0.49	0.66	0.17
max	1.78	20.70	0.78	0.67	0.79	0.28
mean ± SE	1.60 ± 0.01	17.50 ± 0.15	0.72 ± 0.006	0.57 ± 0.004	0.71 ± 0.003	0.23 ± 0.003
n	50	50	50	50	50	50
CV (%)	5.65	3.37	3.29	6.51	3.24	11.91

Note: SE – standard error, n – sample size, CV – coefficient of variation

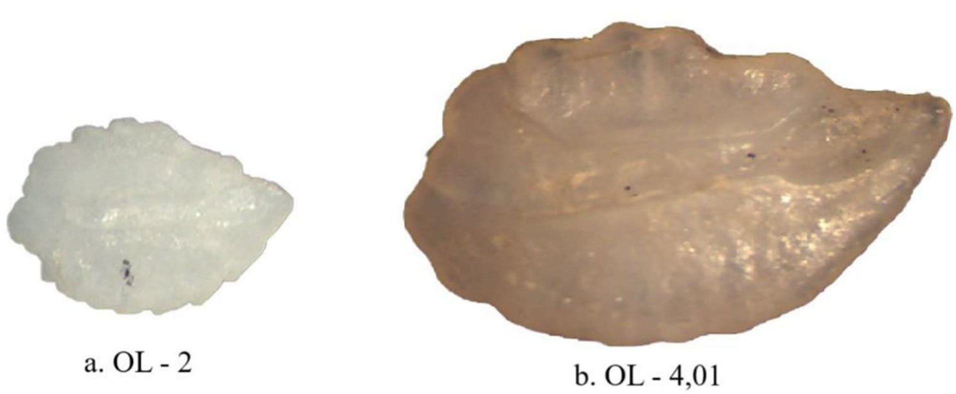

Fig. 6 Comparison of big-scale sand smelt (*Atherina boyeri*) otolith shape: **a** – otolith of a small fish (SL – 47 mm); **b** – otolith of a large fish (SL – 93.1 mm)

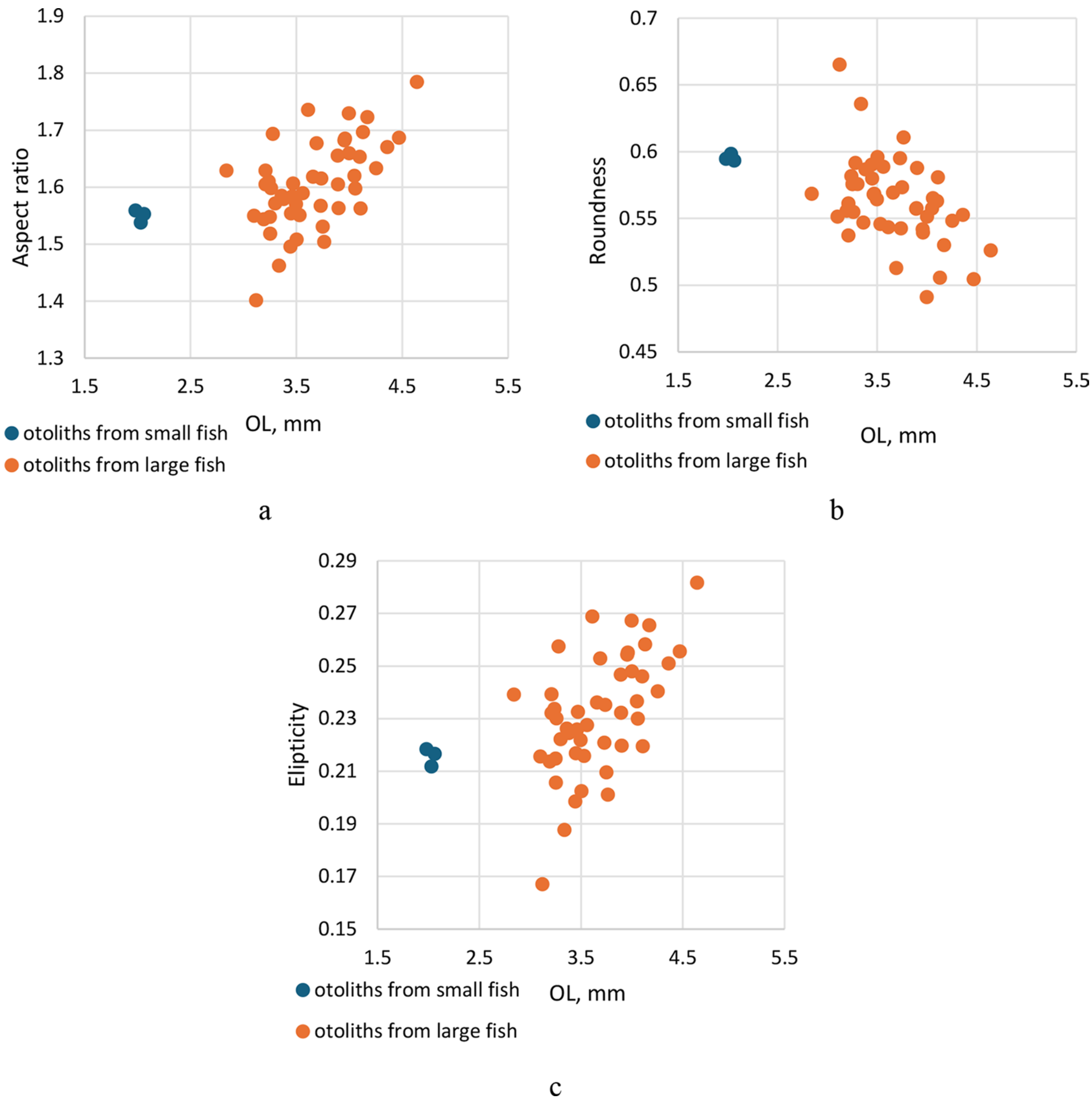

Fig. 7 Comparison of big-scale sand smelt (*Atherina boyeri*) otolith shape indices in small and large Fish: **a** – Aspect ratio; **b** – Roundness; **c** – Ellipticity

y represents fish length and x represents the otolith length or width (Fig. 8).

Reconstructed fish lengths are very similar to the actual fish lengths, and there are no significant differences in the mean values ($p > 0.05$) (Table 7).

The differences between actual and reconstructed fish lengths were calculated for each fish using two formulas. The smallest difference based on otolith length is 0.03%, while the largest difference based on otolith width reaches 16.3% (Table 8). The distribution graph of the differences shows that the calculations for otolith length and otolith width produced similar results, with about 50% of the data falling within a difference range of 1.19–6.32% (Fig. 9).

The results indicate that the formulas can reconstruct fish length from the otolith length or width extracted from feces, with an average error of 4% for otolith length and 4.3% for otolith width.

Otolith sample from feces

Analysis of the distribution of otolith length and width from a sample of 1,093 otoliths extracted from feces reveals that the highest length values occur in two classes: 3.32 mm and 3.73 mm. Similarly, the maximum otolith width values are 2.15 mm and 2.38 mm (Fig. 10).

The normality test of otolith length and width distributions showed that most graph points lie close to the diagonal line, indicating that the overall sample distribution is nearly normal (Fig. 11). Only slight deviations from the line are observed in the distribution's tails (at the lower and upper ends), suggesting minor departures from normality at the extreme minimum and maximum values.

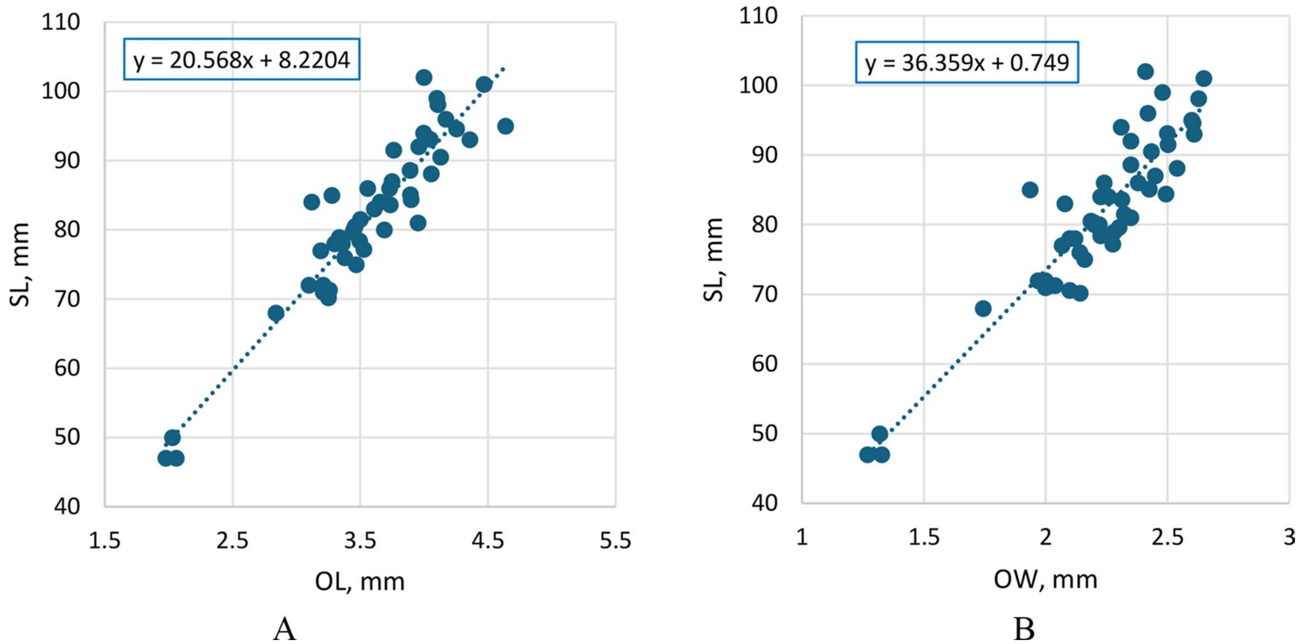

Fig. 8 Relationship between Fish Length and: **A** – otolith length; **B** – otolith width

Table 7 Comparison of actual fish length with reconstructed fish length by formulas

Parameter	Actual FL, mm	Reconstructed FL, mm	
		by OL	by OW
min	47.0	48.9	46.9
max	102.0	103.7	97.1
mean ± SE	81.5 ± 1.7	81.5 ± 1.6	81.5 ± 1.6
n	50	50	50
CV (%)	15	14	13.8

Note: SE – standard error, n – sample size, CV – coefficient of variation

Table 8 Calculation of differences between actual fish length and reconstructed fish length using otolith length and otolith width formulas

Parameter	OL		OW	
	Difference, mm	Difference, %	Difference, mm	Difference, %
min	0.02	0.03	0.08	0.16
max	11.6	13.8	13.9	16.3
mean ± SE	3.3 ± 0.4	4.0 ± 0.5	3.6 ± 0.5	4.3 ± 0.5
n	50	50	50	50

Note: SE – standard error, n – sample size

Considering the large sample of otoliths from feces, a subsample was assembled to study the variability of their shape indices. Since otolith width exhibits lower variability (Table 3), the subsample was selected based on the corresponding otolith widths from 0.43 mm to 2.97 mm. To ensure that the width-based subsample also covered the full range of otolith lengths, otolith lengths were compared within width classes. From the numerous classes, 10 otoliths per class were selected (using Excel's RAND() function); if a class contained 10 or fewer specimens, all otoliths were included. Notably, the four largest otoliths (with lengths ranging from 4.58 mm) were initially excluded from this selection and were subsequently added. Consequently, a subsample of 103 otoliths from feces was compiled, representing the entire linear variability of otoliths in terms of width and length (Table 9).

Determination of the degree of wear of Big-scale sand smelt otoliths from feces

The comparison of otolith length and width from fecal samples, based on the degree of wear, is presented in Table 10. Significant differences in otolith length and width are observed depending on the degree of wear ($p < 0.001$). The smallest otoliths are heavily abraded, while the largest ones are lightly abraded.

Comparison of the shape indices among the three groups (Table 11) revealed that otoliths with a high degree of wear significantly differ from the other two groups in all indices ($p < 0.05$). Otoliths with moderate and light wear differ only in roundness and rectangularity ($p < 0.05$), while no differences were observed for the remaining indices ($p > 0.05$).

A comparison of the shape indices of the three fecal otolith groups with those from fish revealed that all shape indices of otoliths from feces are lower than the corresponding indices of otoliths from fish. Otoliths with heavy wear exhibited significant differences in mean values across all indices ($p < 0.05$). For otoliths with moderate wear, significant differences ($p < 0.05$) were found in the aspect ratio, roundness, rectangularity, and ellipticity.

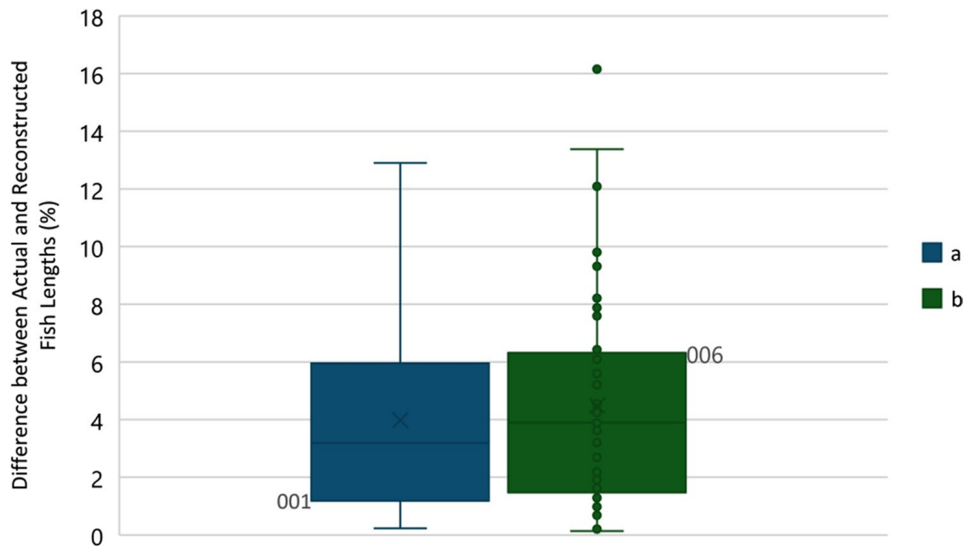

Fig. 9 Distribution graph of the differences between reconstructed Fish Lengths and actual Fish Lengths (%): **a** – differences using the otolith length formula; **b** – differences using the otolith width formula

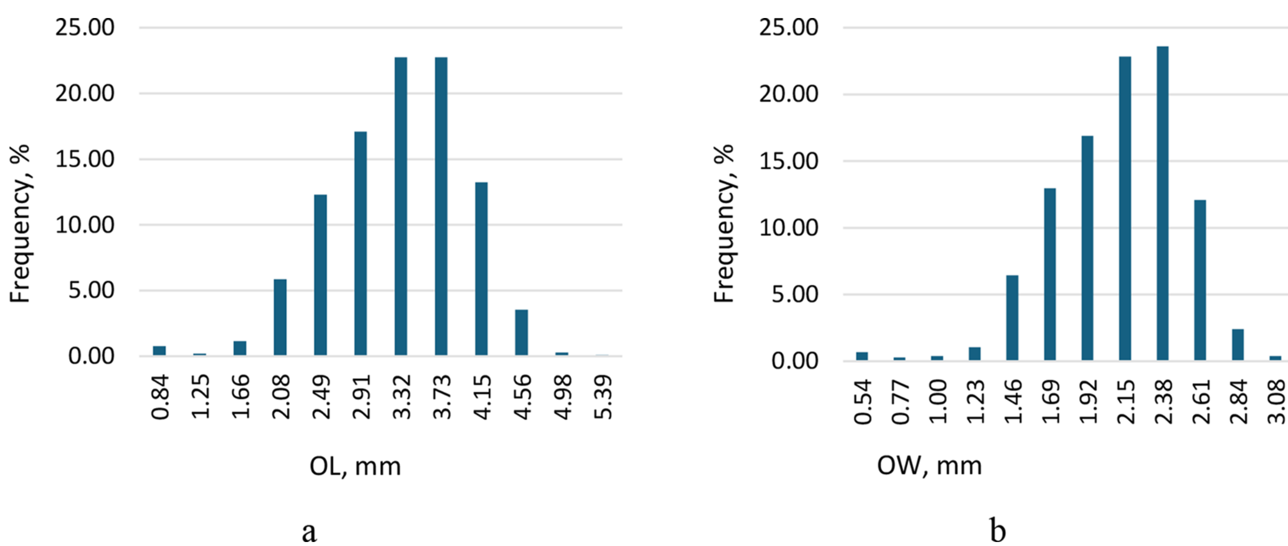

Fig. 10 Distribution of Linear Dimensions of Big-scale Sand Smelt (*Atherina boyeri*) Otoliths from Feces: **a** – by length; **b** – by width

In contrast, the lightly abraded otoliths did not differ significantly from those of fish ($p > 0.05$).

Since the aspect ratio represents the ratio of otolith length to its width, it is possible to calculate a wear coefficient for reconstructing otolith length from fecal samples using this index. Data (see Table 11) indicate that elongation decreases with increasing wear: heavily abraded otoliths exhibit the lowest aspect ratio, while lightly abraded ones show the highest. This suggests a reduction in otolith length relative to its width, supporting the development of corresponding correction factors.

Three coefficients were obtained by sequentially dividing the mean aspect ratio of otoliths from fish by the corresponding index values of the three fecal otolith groups. For lightly abraded otoliths (1.6/1.57), the coefficient is 1.019; for moderately abraded otoliths (1.6/1.54), it is 1.039; and for heavily abraded otoliths (1.6/1.46), it is 1.096. These coefficients can adjust the reconstructed fish lengths using otolith length measurements from feces.

Fish length reconstruction based on the otolith length formula

The distributions of otolith length and width from the fecal sample, categorized by degree of wear, are shown in Figs. 12 and 13. When ordered by the degree of wear, the distribution curves for otolith length and width reveal an overlapping pattern: the right side of the curve for heavily abraded otoliths intersects with the left side of the curve for lightly abraded otoliths. A small portion of the heavily abraded otoliths, reaching their maximum values,

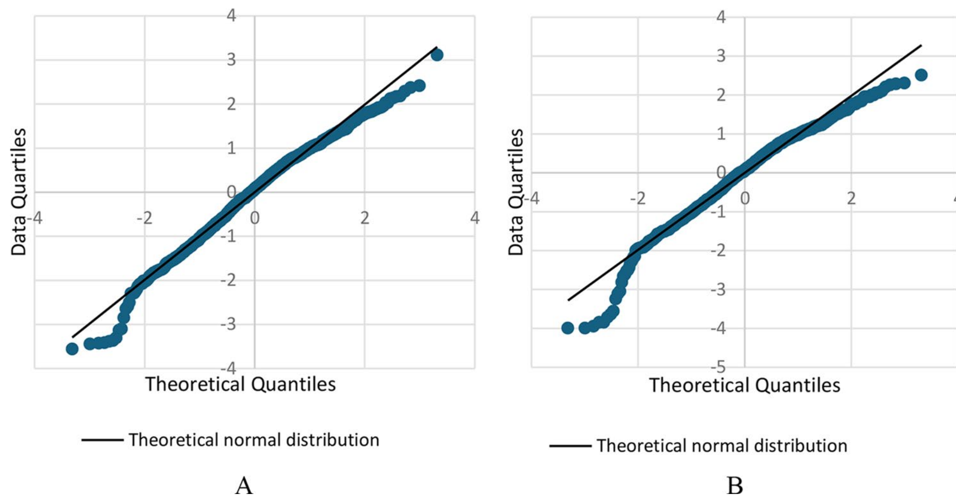

Fig. 11 Normality Test Plots: **A** – Otolith Length; **B** – Otolith Width

Table 9 Subsampling of Big-scale sand smelt (*Atherina boyeri*) otoliths from feces based on otolith width

№	Distribution Classes of All Otoliths by Length (mm) (From–To)	Otolith Width Classes (mm) (From–To)	Corresponding Otolith Length Ranges for Width Classes (mm) (From–To)	Number of Selected Otoliths (*n*)	Total Number of Oto-liths (*n*)
1	0.43–0.84	0.31–0.54	0.64–0.83	6	6
2	0.85–1.25	0.55–0.77	0.79–1.14	4	4
3	1.26–1.66	0.78-1.00	1.28–1.52	5	5
4	1.67–2.08	1.01–1.23	1.52–2.24	10	13
5	2.09–2.49	1.24–1.46	1.67–2.5	10	73
6	2.50–2.91	1.47–1.69	1.88-3	10	151
7	2.92–3.32	1.70–1.92	2.24–3.54	10	200
8	3.33–3.73	1.93–2.15	2.50–4.15	10	245
9	3.74–4.15	2.16–2.38	2.99–4.36	10	242
10	4.16–4.56	2.39–2.61	3.38–4.39	10	125
11	4.57–4.98	2.62–2.84	3.91–4.72	14	25
12	4.99–5.39	2.85–3.08	4.13–5.19	4	4
	Total			103	1093

Table 10 Comparison of otolith length and otolith width from feces by degree of wear

Parameter	OL, mm			OW, mm		
	Lightly	Moderately	Heavily	Lightly	Moderately	Heavily
min	1.8	1.0	0.6	1.1	0.6	0.4
max	5.2	4.2	3.0	3.0	2.6	1.9
mean ± SE	3.7 ± 0.1	2.7 ± 0.2	1.7 ± 0.01	2.3 ± 0.07	1.8 ± 0.1	1.2 ± 0.07
n	41	22	40	41	22	40
CV%	20.0	30.2	36.6	18.8	27.9	36.9

Note: SE – standard error, n – sample size, OL – otolith length, OW – otolith width, CV – coefficient of variation

coincides with the modal values of the lightly abraded group. In contrast, the minimum values of the lightly abraded otoliths extend into the left part of the heavily abraded curve.

Furthermore, the moderately abraded otoliths occupy an intermediate position—their mode falls between the modes of the heavily and lightly abraded groups, with the left tail of the moderately abraded group overlapping the right tail of the heavily abraded group, and the right tail of the moderately abraded group overlapping the left tail of the lightly abraded group. This pattern is also visible in the data shown in Table 12. Notably, more than half of the otoliths (55%– 601 out of 1093) displayed light wear.

The fish length was reconstructed using the formula **y = 20.568*x*k + 8.2204**, where **y** is the fish length (mm), **x** is the otolith length (mm), and **k** is the wear coefficient.

Table 11 Otolith shape indices from feces (Grouped by degree of Wear) compared with otolith shape indices from fish

Parameter	Aspect ratio	Circularity	Form Factor	Roundness	Rectangularity	Ellipticity
Lightly abraded						
min	1.42	15.78	0.58	0.45	0.58	0.17
ax	1.8	21.59	0.80	0.64	0.73	0.29
mean ± SE	1.57 ± 0.012	17.34 ± 0.2	0.73 ± 0.008	0.57 ± 0.005	0.70 ± 0.004	0.22 ± 0.004
n	41	41	41	41	41	41
Moderately abraded						
min	1.34	15.11	0.53	0.53	0.68	0.14
max	1.72	23.59	0.83	0.73	0.81	0.26
mean ± SE	1.54 ± 0.021	17.2 ± 0.4	0.74 ± 0.016	0.61 ± 0.012	0.74 ± 0.008	0.21 ± 0.007
n	22	22	22	22	22	22
Heavily abraded						
min	1.25	14.36	0.61	0.55	0.65	0.11
max	1.82	20.64	0.88	0.81	0.95	0.29
mean ± SE	1.46 ± 0.021	16.69 ± 0.3	0.76 ± 0.011	0.67 ± 0.01	0.76 ± 0.009	0.22 ± 0.004
n	40	40	40	40	40	40

Note: SE – standard error, n – sample size

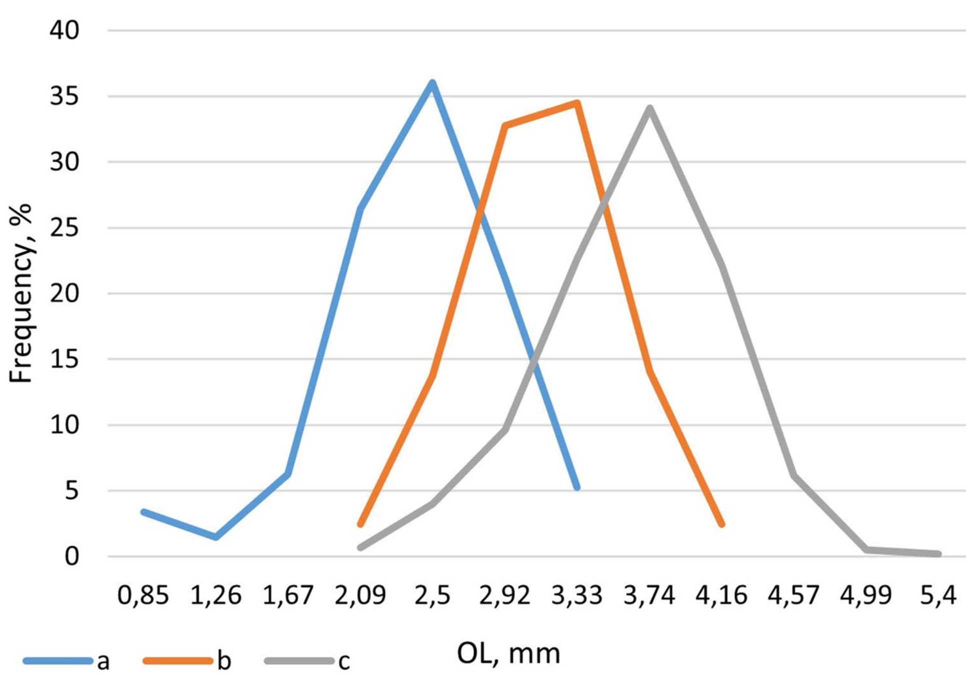

Fig. 12 Distribution of Otolith Lengths from Feces: **a** – Heavily abraded; **b** – Moderately abraded; **c** – Lightly abraded

The coefficient **k** has values of 1.096, 1.039, and 1.019 for heavily, moderately, and lightly abraded otoliths, respectively. An example showing how these coefficients affect the reconstructed fish length is given in Table 13, where one otolith of the same size from each wear category was selected. The comparison of the calculated fish lengths showed differences ranging from 1.9 to 6.5%.

Reconstructed fish lengths based on otoliths from feces are presented in Table 14. A comparison of the mean values of reconstructed fish lengths across the three groups revealed statistically significant differences ($p < 0.0001$).

A comparison of the reconstructed fish lengths from otoliths obtained from fecal samples at the Kendirli and Durnev sites indicates differences in the sizes of big-scale sand smelt (*Atherina boyeri*) consumed at these sites ($p < 0.05$). Analysis of the distribution of reconstructed fish lengths reveals that at the Durnev site, individuals measuring 60–70 mm predominate, whereas at the Kendirli site, individuals measuring 80–90 mm prevail (Fig. 14).

Overall, the analysis of the distribution of reconstructed fish lengths shows that the Caspian seal's diet is predominantly composed of *A. boyeri* measuring 70 to

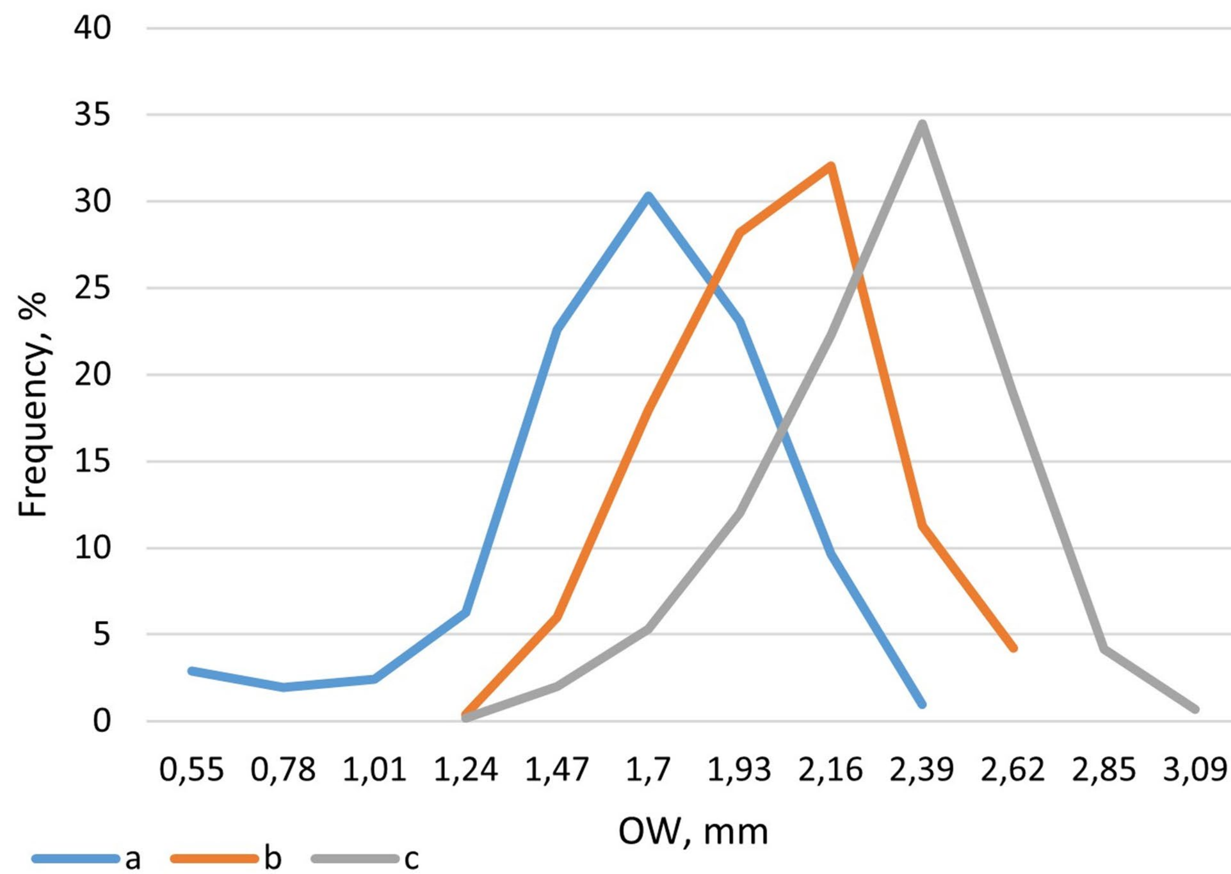

Fig. 13 Distribution of Otolith Widths from Feces: **a** – Heavily abraded; **b** – Moderately abraded; **c** – Lightly abraded

Table 12 Linear parameters of otoliths from feces. a – Heavily abraded, b – Moderately abraded, c – Lightly abraded

Parameter	OL, mm			OW, mm		
	a	b	c	a	b	c
min	0.66	1.84	1.84	0.43	1.24	1.1
max	3.25	3.96	5.19	2.25	2.61	2.97
mean ± SE	2.2 ± 0.03	2.91 ± 0.02	3.45 ± 0.02	1.54 ± 0.02	1.90 ± 0.02	2.18 ± 0.01
n	208	284	601	208	284	601
CV %	22.6	14.4	14.6	22.3	14.1	14

Note: SE – standard error, n – sample size, OL – otolith length, OW – otolith width, CV – coefficient of variation

Table 13 Examples of the influence of the wear coefficient on reconstructed fish length (SL): a – from heavily abraded otoliths; b – from moderately abraded otoliths; c – from lightly abraded otoliths

Parameter	a	b	c
OL, mm	2.6	2.6	2.6
Reconstructed SL, mm	66.8	63.9	62.7
Difference Calculation	a-b	b-c	a-c
Difference, mm	2.9	1.2	4.1
Difference Relative to OL, %	4.3	1.9	6.5

Note: OL – otolith length, SL – standard length

Table 14 Reconstructed fish lengths from otoliths from feces (a – heavily abraded, b – moderately abraded, c – lightly abraded) and results of pairwise comparisons

Parameter	SL, mm		
	a	b	c
min	23.1	47.5	46.8
max	81.5	92.8	117.1
mean ± SE	57.9 ± 0.8	70.5 ± 0.5	80.6 ± 0.4
n	208	284	601
CV %	19	12.8	13
t_d	$p < 0.0001$		

Note: SE – standard error, n – sample size, SL – standard length, CV – coefficient of variation, t_d - significance level for the difference between means according to Student's t-test

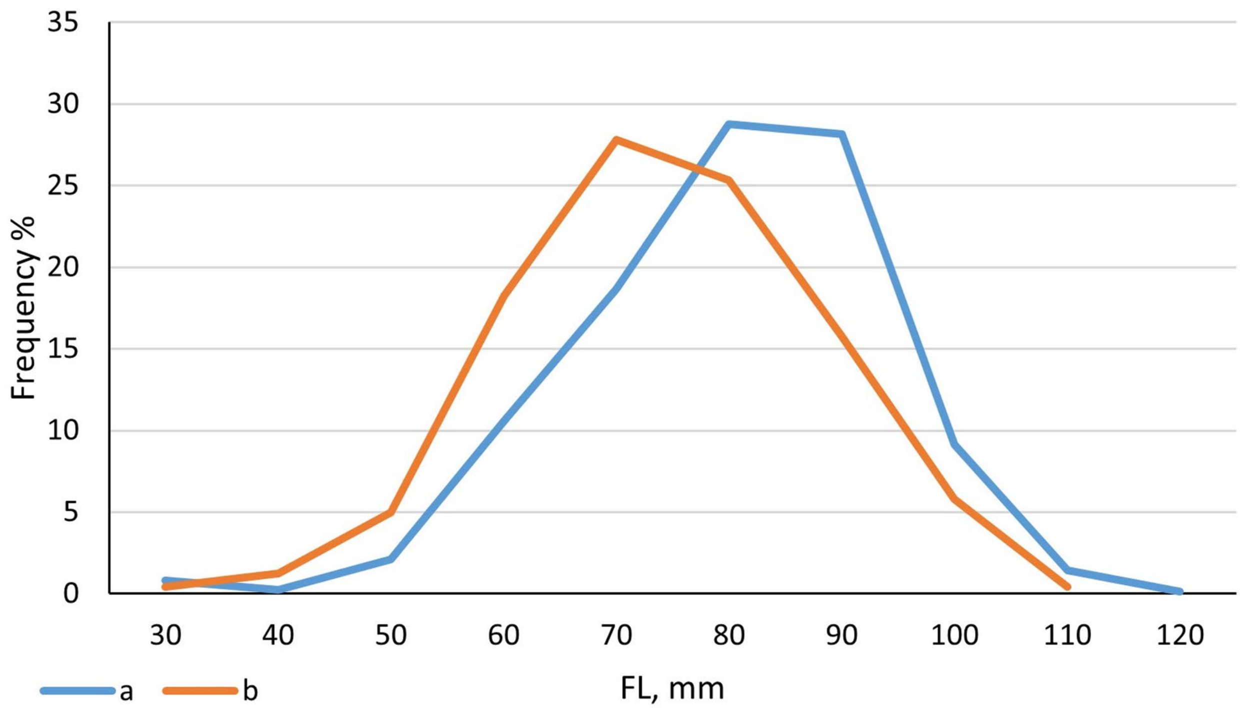

Fig. 14 Sizes of big-scale sand smelt (*Atherina boyeri*) (SL) consumed by the Caspian seal (*Pusa caspica*) at different haul-out sites: **a** – Kendirli, **b** – Durnev

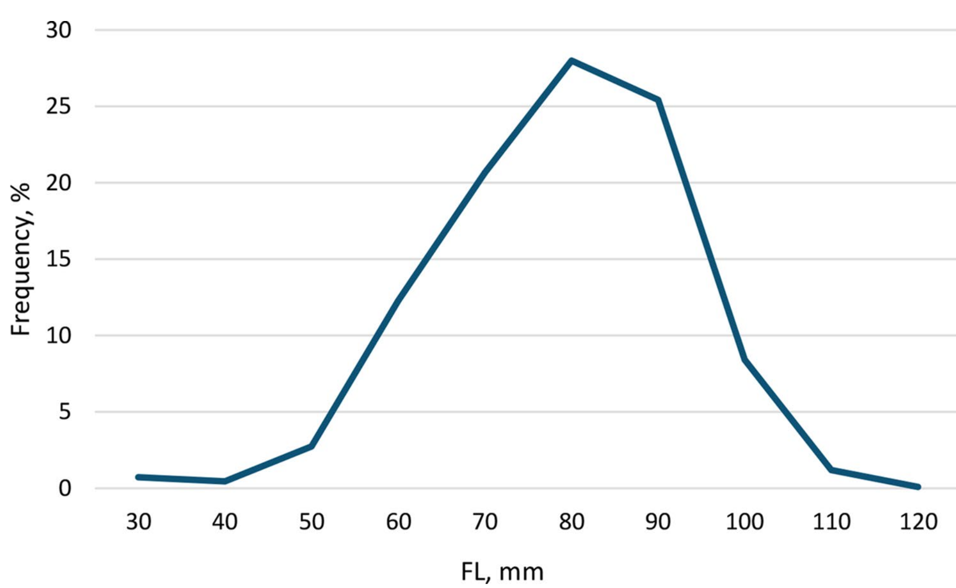

Fig. 15 Distribution of Reconstructed Fish Lengths

90 mm, with individuals ranging from 23.1 to 117.1 mm in length (Fig. 15).

Discussion

Big-scale sand smelt (*Atherina boyeri*) is a euryhaline pelagic schooling fish widespread throughout the Caspian Sea. Specialized fisheries do not target *A. boyeri*, but they typically catch it as bycatch during sprat fishing. The fish can reach a total length of up to 13 cm, have a maximum age of 5 years, and attain sexual maturity at 1 year. Due to its batch spawning behavior, the size range spans a broad age spectrum [48]. *A. boyeri* serves as prey for many omnivorous and predatory fish, as well as for the Caspian seal (*Pusa caspica*) [9].

Our investigation into the diet of *the* endangered Caspian seal (P. caspica) required a unique approach. To avoid harming the seals, we employed a non-lethal research method known as the coprological method, which involved collecting seal feces and identifying fish otoliths within them. As otoliths are species-specific, we conducted individual studies for each fish species [21, 49]. While there is currently no identification key for the otoliths of Caspian Sea fish, we have previously developed a guide, including an atlas, that describes the otoliths of several fish species serving as prey for *P. caspica* [35].

In this study, the researchers investigated otoliths extracted from fish and isolated from feces to clarify the size structure of *A. boyeri* in the diet of *P. caspica*.

The studies demonstrated a strong relationship between otolith growth and fish growth, which is generally characteristic of [50, 51]. There were no significant differences in mean linear parameters between the left and right otoliths, and the correlation coefficients between fish length and both the left and right otoliths did not show significant differences. Comparison of fish length and the ratios of otolith length and width to fish length between females and males revealed no sexual dimorphism. This allowed for the subsequent use of only right otoliths in further analyses, as they were more numerous in the collection, and eliminated the need to separate otoliths by fish sex.

Like other fish species [36], *A. boyeri* otoliths occur in two forms—those with a notch and those without—with the notched otoliths prevailing by more than a twofold margin. However, no significant differences in the mean values were found when comparing fish lengths between the two groups based on the presence or absence of a notch in the otoliths. Likewise, comparisons of both the lengths and the shape indices of otoliths with and without a notch did not reveal statistically significant differences in mean values. Therefore, future studies on otolith growth may proceed without dividing the sample based on the presence or absence of a notch.

Unfortunately, the small number of otoliths from small fish does not allow for a comprehensive comparison of the indices as a function of otolith size (or, more precisely, otolith length growth). However, the low coefficients of variation for the shape indices, and the fact that the ranges of the indices for aspect ratio, roundness, and ellipticity in small otoliths fall within the limits observed for larger otoliths, suggest that the sample can be treated as a single homogeneous group without subdivision based on otolith size.

Therefore, two formulas were developed to reconstruct fish length—one based on otolith length and the other on otolith width. A comparison of the reconstructed fish lengths from these two formulas with the actual fish lengths revealed no significant differences between the two estimates. The differences between the reconstructed and actual fish lengths were minor, with an error of 4% for lengths derived from otolith length and 4.3% for those derived from otolith width. Since the error associated with otolith length is slightly lower, the formula based on otolith length was used in subsequent analyses.

The analysis of the distribution of otolith lengths and widths from feces showed an approximately normal distribution, which enabled the selection of a subsample of 103 otoliths for a more detailed examination of the changes in otolith morphology during passage through the seals' gastrointestinal tract (GIT).

Otoliths from feces were classified into three degrees of wear during their passage through the GIT, following the approach used in studies on *Theragra chalcogramma* and *Pleurogrammus monopterygius* [52]: heavily, moderately, and lightly abraded. Comparison of the mean length and width of *A. boyeri* otoliths revealed significant differences in these parameters among the wear groups. We found that smaller otoliths undergo more intensive abrasion, whereas originally larger otoliths exhibit only light abrasion. Fish with otoliths classified as moderately abraded represent individuals that occupy a medium-length range in the seal's diet. Our earlier findings [53] also showed similar differences in wear degree relative to otolith length for other fish.

Different wear coefficients for otolith length were determined for the three wear groups and incorporated into the fish length reconstruction formulas for a more precise reconstruction of fish length from otoliths obtained from feces. In conclusion, the studies based on the analysis of seal feces collected during their haul-out period at island sites broaden our understanding of the diet of this endangered species. Specifically, the size composition of *A. boyeri*—one of the main prey items of *P. caspica*—was described [24, 25]. We established that the seal, when at the Kendirli haul-out in the Middle Caspian, consumes larger *A. boyeri* (modal range 80–90 mm), whereas in the Northern Caspian, its diet mainly consists of individuals in the 60–70 mm range. Overall, our findings show that *P. caspica* feeds on both juvenile and sexually mature *A. boyeri*, with lengths ranging from 23.1 mm to 117.1 mm.

Conclusions

1. No significant differences were observed between left and right otoliths of Big-scale sand smelt (*Atherina boyeri*), allowing the study to use only right otoliths.
2. The length of *Atherina boyeri* and its relationships with otolith length and width showed no sexual dimorphism, allowing otoliths to be analyzed without separation by fish sex.

3. Although we can divide otoliths based on the presence or absence of a notch, our analysis revealed no significant differences in fish length or otolith length between these groups, suggesting that this characteristic can be disregarded when reconstructing fish length from fecal otoliths.
4. Small and large otoliths from Caspian seal (*Pusa caspica*) feces did not exhibit significant differences in shape indices, which permitted the use of a single reconstruction formula without subdividing the otoliths by size.
5. We derived two formulas for reconstructing fish length: one based on otolith length and the other on otolith width. Testing revealed no significant differences between fish lengths reconstructed by these formulas and the actual fish lengths. However, because the error associated with otolith length is slightly lower, the formula based on otolith length was used in this study.
6. We categorized otoliths from feces into three groups based on the degree of wear. For each group, we calculated a specific wear coefficient for otolith length and incorporated it into the formula for reconstructing fish length.
7. In the diet of *Pusa caspica*, the size composition of *Atherina boyeri* varies by haul-out region: in the Middle Caspian, individuals of 80–90 mm predominate, whereas in the Northern Caspian, individuals in the 60–70 mm range prevail. Overall, the size range of *Atherina boyeri* in the *Pusa caspica* diet is 23.1–117.1 mm.

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Author contributions
All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Mirgaliy Baimukanov, Akzhan Iskakov, and Anuar Shagilbayev. The first draft of the manuscript was written by Akzhan Iskakov, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. M.T. Baimukanov: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration. Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing A.A. Iskakov: Conceptualization, Data curation, Formal Analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editingA.U. Shagilbayev: Formal Analysis, Methodology, Validation, Visualization, Writing – review & editing.

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Data availability
The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The studies on Big-scale sand smelt (*Atherina boyeri*) in this article were conducted on specimens fixed according to generally accepted methods, while the research on Caspian seals (*Pusa caspica*) was conducted using a non-invasive procedure. All studies were approved by the local ethics committee of the "Institute of Hydrobiology and Ecology." As a result, Conclusion No. 1, dated October 4, 2024, was issued, stating that the research is being conducted in accordance with the laws and regulations of the Republic of Kazakhstan, which prohibit animal cruelty.

Informed consent
Informed consent was obtained from the Kazakh Agency of Applied Ecology LLP employees and Tengizchevroil LLP for using Caspian atherina in this study.

Competing interests
The authors declare no competing interests.

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