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First evidence of otolith abnormalities in *Belone belone* from the Sea of Marmara: An integrative approach to morphological deformities with light and scanning electron microscopy

by

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Abstract

This study is the first of its kind, providing detailed evidence of otolith abnormalities in *Belone belone* in the Darıca coast of the Sea of Marmara (Türkiye), using an integrative approach that combines light microscopy and scanning electron microscopy (SEM). Structural anomalies in otoliths were detected across both sexes, and 4% of *B. belone* individuals analyzed were found to suffer from abnormal otoliths. In contrast, distinct differences were detected between normal and abnormal otoliths. Although there were some differences between morphometric parameters, a statistically significant difference was determined only in terms of otolith perimeter. SEM and light microscopy revealed that morphological differences, especially on the lateral otolith surfaces, were key to distinguishing normal and abnormal otoliths. Normal otoliths exhibited typical anatomical features such as aragonite crystal structure, elliptic-lanceolate shape, short-wide rostrum, and generally indistinct antirostrum. In contrast, abnormal otoliths exhibited a high degree of shape variability, with irregular surface protrusions, pits, and disorders in the crystal structure resulting from calcium carbonate polymorphism. The wide range of morphological anomalies observed in *B. belone* across sizes and sexes suggests a potential link to environmental stressors, such as mucilage and pollution, as well as genetically driven developmental disorders.

Key words: abnormal otolith, crystal polymorphism, mucilage pollution, microscopic analysis, environmental stress

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1. Introduction

Three sides of Türkiye are surrounded by four seas, namely the Black Sea, the Sea of Marmara, the Aegean Sea, and the Mediterranean Sea. Although fish biodiversity in these seas varies over the years, its abundance is quite high (Bilecenoğlu et al., 2014; Froese & Pauly, 2025). In fish biodiversity, needlefish are quite popular for their economic value in Turkish fisheries, and they have become one of the important species. Three needlefish species, *Tylosurus acus*, *Belone belone*, and *Belone svetovidovi*, are found in Turkish coasts (Bilecenoğlu et al., 2014). *T. acus* and *B. svetovidovi* are only found along the Mediterranean and Aegean coasts of Türkiye. *B. belone* is distributed along all Turkish coastal waters, indicating its adaptability to diverse marine environments of Türkiye (Bilecenoğlu et al., 2014). *B. belone* (Linnaeus, 1761) is a pelagic, oceanodromous needlefish found in marine and brackish waters of the Atlantic Ocean, Mediterranean, Black, Baltic, Caribbean Seas, and the Sea of Marmara (Froese & Pauly, 2025). Some biological aspects of *B. belone*, such as age, mortality, growth, sexual maturity, and spawning time, have been reported from the Adriatic Sea (Zorica & Čikeš Keč, 2013; Zorica et al., 2011). However, there are limited studies on *B. belone*, which is distributed in the Sea of Marmara (Türkiye).

The inner ear structure of fish consists of three semicircular canals, primarily responsible for detecting angular acceleration, and three otolith organs—asteriscus, lapillus, and sagitta—associated with both auditory and spatial perception (Campana, 2004). The well-defined growth rings of sagittal otoliths have enabled them to be widely used in age estimation of many fish species (Campana, 1999). Moreover, the correctly defined morphology of sagittal otoliths can show geographical variability within the same species, making them valuable tools for stock differentiation (Bostancı & Yedier, 2018). In fact, sagittal otoliths display species-specific characteristics, enabling their application in taxonomic classification and species discrimination (Bostancı et al., 2024; Tuset et al., 2008). Otoliths often display a series of incremental patterns that appear at regular intervals, ranging from daily to annual. The formation of these patterns can be influenced by both internal and external factors that include environmental conditions and genetic influences. The otoliths, composed of calcium carbonate within a protein matrix, exist in three crystal forms: aragonite, calcite, and vaterite. As a result, fish may develop abnormal otoliths with structures and characteristics that deviate from the normal patterns. Otolith abnormalities have been reported in scientific literature, with evidence suggesting that these deformations are influenced by the specific crystalline polymorph of calcium carbonate present, namely calcite, aragonite, and vaterite (Gauldie, 1993). These crystal polymorphs exhibit distinct crystallographic structures: calcite is trigonal, aragonite is orthorhombic, and vaterite is hexagonal. Normal sagittal otoliths primarily consist of aragonite, whereas anomalous otoliths exhibit overgrowth of translucent vaterite and/or calcite formations (Béarez et al., 2005; Gauldie, 1993; Tomás & Geffen, 2003; Yedier et al., 2023). However, in some cases, they may contain a mixture of aragonite and vaterite or consist entirely of vaterite. Otoliths with a vateritic structure are significantly larger and less dense than their aragonitic counterparts, reflecting the distinct physical properties resulting from the different crystal structures.

In many fish species, skeletal crystals can undergo dynamic remodeling, but once formed, otolith crystals tend to remain largely unchanged except under extreme physiological conditions (Mugiya & Uchimura, 1989). The abnormality can be observed in aquaculture species and in wild fish species that live in highly dynamic oceanographic systems. The mechanisms causing otolith abnormalities are not fully understood, and several hypotheses have been proposed to explain their occurrence. One hypothesis suggests that these deformities may be caused by genetic anomalies, while another hypothesis suggests that deformities may be due to environmental stressors (Béarez et al., 2005; Tomás & Geffen, 2003). Abnormal otoliths are observed in various marine and freshwater fish species over time, though they are relatively uncommon. *Odontesthes bonariensis* from Chasicó Lake (Avigliano et al., 2012), cultured and wild *Hypomesus transpacificus* from the US (Lewis et al., 2022), *Sarpa salpa* (Yedier et al., 2024), and *Saurida lessepsianus* from the Aegean Sea (Reis et al., 2025) are some of these studies carried out in different aquatic habitats.

The presence of different polymorphic crystal structures in otoliths poses methodological challenges in data acquisition and interpretation. In addition, these crystalline structures are either unrecognized or misinterpreted, potentially compromising the reliability of otolith-based studies. Although otolith research covers many different scientific fields (Bostancı et al., 2024; Campana, 2004; Tuset et al., 2008), detailed studies on deformed otoliths are still insufficient. Furthermore, the external morphology of normal and abnormal otoliths is not well characterized in several species, including the *B. belone*. This study provides the first detailed morphological assessment of abnormal sagittal otoliths in *B. belone* from the Darıca coastline in the Sea of Marmara, Türkiye.

2. Materials and methods

Sea of Marmara is 70 km in width and 240 km in length and has an area of 11 500 km^2 (Uzer & Karakulak, 2022). The Sea of Marmara, located between two different seas, the Black Sea and the Mediterranean Sea, is a unique environment for the habitat where fish species shelter, feed, and breed, and has a very rich dynamic structure in terms of pelagic and demersal fish species population (Özsoy et al., 2016). Darıca district, located on the borders of the İzmit Bay in the northeast of the Sea of Marmara, is the third largest district of Kocaeli province with a population of approximately 227 892 (Fig. 1) (TSI, 2023). Darıca district has a 16 km coastline and has a rising marine tourism potential. There are also some local fishing activities, such as spearfishing, line fishing, and net fishing.

B. belone (garfish) samples were collected using fishing nets from the Darıca coast between latitudes 40°43′N to 40°46′N and longitudes 29°19′ E to 29°26′ E in the Sea of Marmara (Fig. 1). After sampling, fish samples were immediately placed in refrigerated containers and transported to the laboratory in order to minimize the deterioration of their body integrity. Fish samples were identified, and then their weight (W) (± 0.1 g) and total length (TL) (± 0.1 cm) were also recorded. The sex of *B. belone* samples was determined through microscopic examination of the gonads.

The methodology recommended by Campana (2004) was used with some additions for the removal and preparation of the otoliths for microscopic examination. In this regard, sagittal otoliths were removed in pairs (right and left) from the inner ears of *B. belone* individuals. Right and left sagittal

Figure 1

Sampling area from the Darıca coast in the Sea of Marmara (Türkiye).

otoliths of the *B. belone* were weighed (the nearest 0.0001 g). Otolith width is the longest vertical dimension between the ventral and dorsal ends of the otolith, while otolith length is the longest horizontal dimension between the rostrum and the posterior end of the otolith passing through the center of the otolith (Fig. 2). Otolith length, otolith width, otolith area and otolith perimeter were measured with Digimizer Image Analysis Software program (Ver. 6.4.4) (MedCalc Software Ltd, Belgium) after photographed under a stereo microscope using a DFC 290 Leica digital camera (Leica Microsystems Ltd, Germany). The detailed morphological structures of sagittal otoliths were analyzed using light microscopy and scanning electron microscopy (SEM), with a focus on identifying normal otoliths and abnormal otoliths that show differences resulting from irregular protrusions, crystalline and external shape changes on their surfaces (Tuset et al., 2008; Yedier, 2022).

A parametric t-test was used for comparisons of abnormal and normal otolith morphometric measurements of *B. belone*. SPSS Statistics (Ver. 26.0) (IBM Corp, USA) was used for descriptive statistical values of otolith morphometric measurements and statistical comparisons in the study.

3. Results

In this study, a total of 100 garfish (*B. belone*) were examined from the Darıca coast in the Sea of Marmara. The TL and body weight of *B. belone* samples ranged from 24.6 cm to 44.5 cm and from 15.4 g to 117.2 g, respectively. There is no statistical difference between the otoliths of both *B. belone* male and female individuals ($p > 0.05$) and also between the left and right otoliths ($p > 0.05$). Based on the evaluation of the procedure used to confirm abnormal otoliths of *B. belone* individuals sampled from the Darıca coast in the Sea of Marmara, it was confirmed that *B. belone* individuals had abnormal otoliths. Abnormalities were detected in the otoliths of four individuals of *B. belone* sampled from the Darıca coast, with different TLs ranging from 25.9 cm to 44.2 cm. It was calculated that 4% of individuals of *B. belone* from the Darıca coast in the Sea of Marmara have abnormal otoliths. Among the four samples with abnormal otoliths, two were female and the other two were male. Morphometric measurements of these individuals and their otolith morphometric data, such as width, length, perimeter, weight, and area values of normal and abnormal otoliths, are presented in Table 1. In all

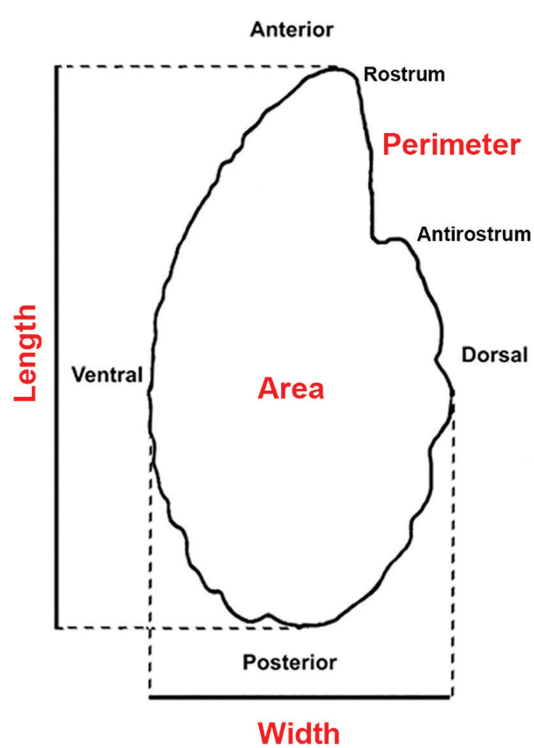

Figure 2

Otolith morphometric and morphological characteristics of *Belone belone*.

otolith measurements evaluated within the scope of the study, it was determined that there was a statistical difference only in the otolith perimeter values between the abnormal and normal otoliths of *B. belone* individuals sampled from the Darıca coast (Table 1).

Light microscopy and SEM showed certain differences between normal and abnormal otolith morphology of *B. belone* from the Darıca coast (Sea of Marmara, Türkiye). Normal left and right otoliths of *B. belone* individuals have aragonite crystalline structure (Fig. 3). The mesial surfaces of these otoliths are convex, the lateral surfaces are concave, and their shapes are elliptic-lanceolate (Fig. 3). The anterior region of normal otoliths is angled with a sinuate margin, while the posterior region is round with entire margin (Fig. 3). The antirostrum of these otoliths is commonly absent or short (Fig. 3). The rostrum of the otoliths is short and broad (Fig. 3). The ventral parts of the left and right normal otoliths are almost straight or less regular with entire-sinuate margins, while

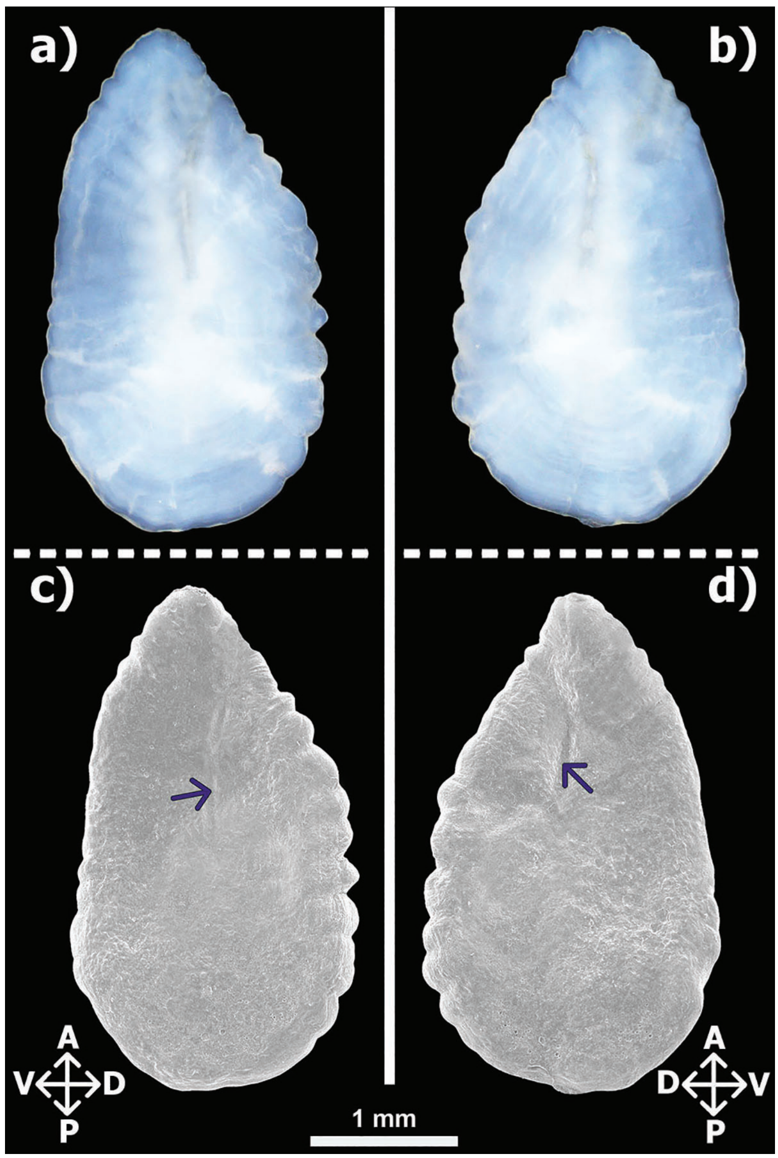

Figure 3

Light microscope images of the lateral surfaces of the normal left **(A)** and right **(B)** otoliths and SEM images of the lateral surfaces of the normal left **(C)** and right **(D)** otoliths of the 38.1 cm TL female *Belone belone* of a specimen from the Darıca coast in Sea of Marmara, Türkiye. A: Anterior; P: Posterior; V: Ventral; D: Dorsal. Purple Arrows: canal. SEM, scanning electron microscopy; TL, total length.

Table 1

Basic measurements of *Belone belone* samples collected from the Darıca coast (Sea of Marmara, Türkiye) and comparisons of their abnormal and normal otolith morphometric data

	W (g)	TL (cm)	Sex	Side	Otolith Weight (g)	Otolith Area (mm²)	Otolith Perimeter (mm)	Otolith Length (mm)	Otolith Width (mm)
Abnormal Samples	19.2	25.9	♂	Left	0.0016	3.2019	8.0820	2.7729	1.6094
				Right	0.0019	2.9783	7.2769	2.7036	1.5305
	69.4	39.0	♀	Left	0.0033	5.4039	11.1767	3.8532	2.1196
				Right	0.0037	5.2827	10.8174	3.9054	1.9942
	69.6	39.8	♀	Left	0.0050	6.3525	12.3690	4.1205	2.2615
				Right	0.0052	5.6110	11.3466	3.9115	2.1118
	115.8	44.2	♂	Left	0.0084	7.6115	12.4390	4.2390	2.4191
				Right	0.0079	7.6756	12.5602	4.3766	2.3989

	W (g) range	TL (cm) range	Otolith Weight (g) Mean ± SE (Min–Max)	Otolith Area (mm²) Mean ± SE (Min–Max)	Otolith Perimeter (mm) Mean ± SE (Min–Max)	Otolith Length (mm) Mean ± SE (Min–Max)	Otolith Width (mm) Mean ± SE (Min–Max)
Normal *n*=96	15.4–117.2	24.6–44.5	0.0034 ± 0.0001	4.6496 ± 0.0564	9.0418 ± 0.0615*	3.4426 ± 0.0242	1.8798 ± 0.0132
			(0.0014–0.0088)	(2.5790–8.3203)	(6.6210–12.9776)	(2.4460–4.9353)	(1.3850–2.3784)
Abnormal *n*=4	19.2–115.8	25.9–44.2	0.0046 ± 0.0009	5.5147 ± 0.6214	10.7585 ± 0.7131*	3.7353 ± 0.2267	2.0566 ± 0.1179
			(0.0016–0.0084)	(2.9783–7.6756)	(7.2769–12.5602)	(2.7036–4.3766)	(1.5305–2.4191)

TL, total length; W, weight; SE, standard error.
* indicates statistically different.

the dorsal parts are wavy and slightly irregular with crenate-lobbed margins (Fig. 3).

Due to the high variability of abnormal otoliths of *B. belone* individuals, it is highly difficult to define their shape, morphology, and other parts compared to normal otoliths. Therefore, abnormal otoliths of *B. belone* individuals sampled in the Darıca coast from the Sea of Marmara were evaluated separately. Thus, more detailed data about abnormal otoliths in this species were obtained. For instance, when abnormal otoliths of *B. belone* individuals sampled from the Sea of Marmara were compared with normal otoliths, no significant difference was observed on the mesial surface. However, the main differences were more pronounced, especially on the lateral surface; therefore, the morphological evaluations of otoliths in the study were carried out especially on the lateral surface. In addition, in this study, both left and right otoliths of four *B. belone* individuals having abnormal otoliths were evaluated morphologically separately. These evaluations are as follows, according to the TL order of the relevant *B. belone* individuals.

For instance, the lateral surface of the otolith of a 25.9 cm TL male *B. belone* specimen is rough, and it contains irregular protrusions and depressions of different sizes and shapes (Fig. 4). The protrusions and elevations are irregularly distributed, especially on the lateral surface of the left abnormal otolith (Figs. 4A and 4C). In addition, calcium carbonate polymorphism is quite evident in the left otolith, and this differentiation is clearly revealed by light and SEM analyzes (Fig. 4C). The crystallization difference in this otolith can be clearly observed, especially with the presence of large aragonite crystal structures in the center and vaterite crystals in other parts of the otolith (Figs. 4A and 4C). There is no problem due to crystallization in the right otolith of this individual, but a large depression is noticeable in the center of the lateral surface (Figs. 4B and 4D). In addition, there is no abnormal structure or condition in the ventral and dorsal parts of the right and left otoliths and in the posterior regions (Fig. 4). Moreover, this individual has a fragment of loss due to abnormality in the anterior part of the left otolith (Figs. 4A and 4C). The otoliths of this individual are less lanceolate in shape (Fig. 4). The center of the lateral surface is convex due to the crystal accumulation in the middle part of the left otolith (Figs. 4A and 4C), while the right otolith is concave due to the depression in its center (Figs. 4B and 4D). The rostrum of the left and right otoliths is short and broad (Fig. 4). Additionally, the antirostrum is absent in both left and right otoliths (Fig. 4). The ventral parts of the left and

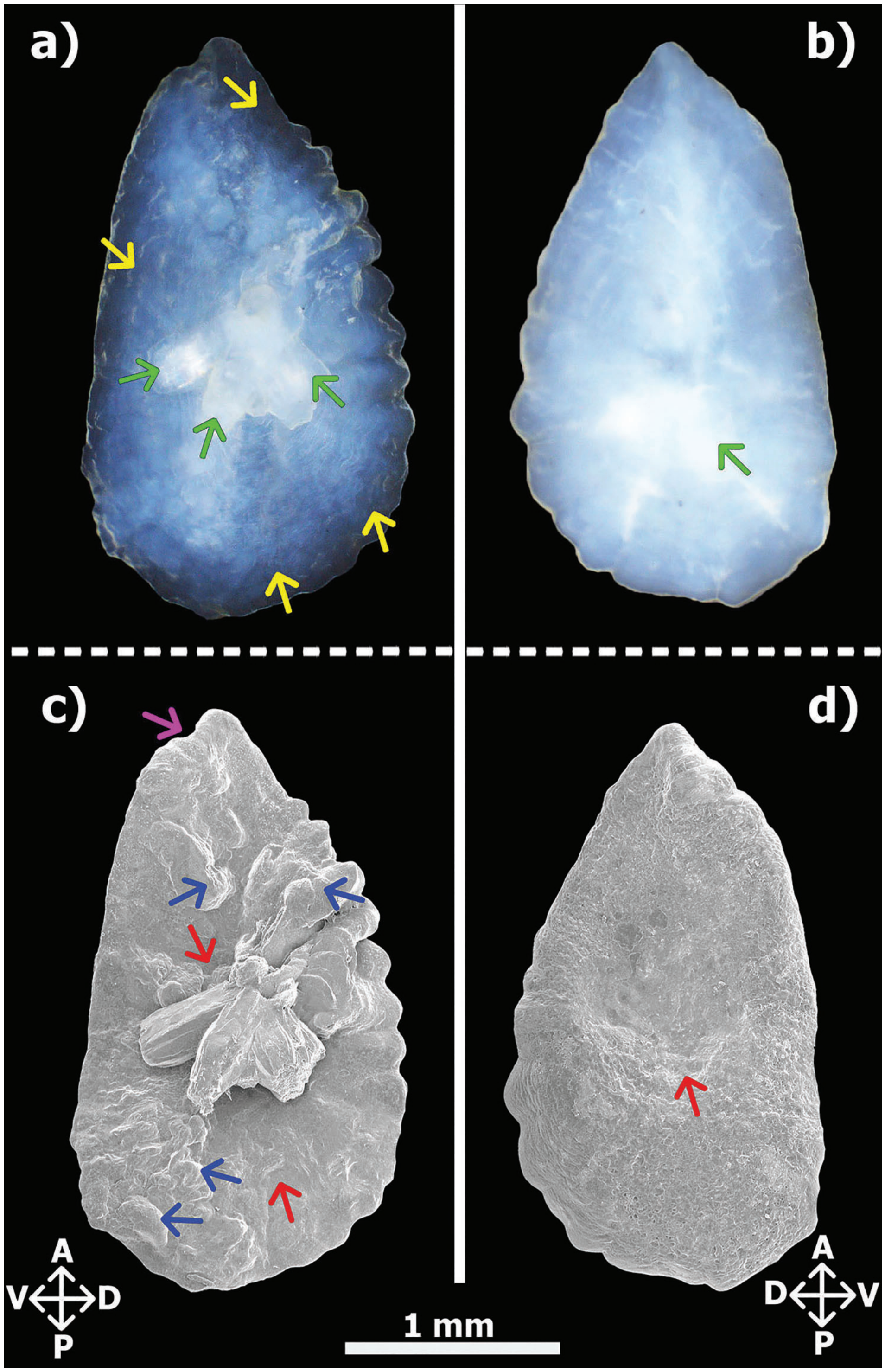

Figure 4

Light microscope images of the lateral surfaces of the left **(A)** and the right **(B)** otoliths and SEM images of the lateral surfaces of the left **(C)** and the right **(D)** otoliths of the 25.9 cm TL male *Belone belone* specimen from the Darıca coast in the Sea of Marmara, Türkiye. Arrows indicate for Yellow: aragonite part, Green vaterite part, Red: depressions, Blue: protrusions, and Pink: fragment losses, D: Dorsal; V: Ventral; P: Posterior; A: Anterior. SEM, scanning electron microscopy; TL, total length.

right otoliths of this individual are less straight with sinuate margins, while the dorsal parts are wavy and irregular with crenate-lobed margins (Fig. 4). The anterior regions of the left and right otoliths are angled with sinuate margins, and the posterior regions of the left and right otoliths are oblique with sinuate margins (Fig. 4).

The lateral surface of the left and right otolith of the female *B. belone* sample with a TL of 39.8 cm is less rough compared to other abnormal otoliths, and some protrusions and pits on its surface are observed (Fig. 5). In particular, a large depression is noticeable in the central part of both otoliths (Fig. 5). This depression is obvious in the SEM analysis (Figs. 5C and 5D). In addition, there are differences in crystal structures originating from calcium carbonate polymorphism in the ventral and dorsal parts and anterior and posterior regions of the right and left otoliths (Fig. 5). This differentiation was revealed in light and SEM analyzes (Fig. 5). The vateritic structure is more obvious especially in the edge parts of these otoliths. However, this difference is less obvious compared to other abnormal samples (Figs. 4 and 7). In addition, there is fragment loss in the ventral and dorsal parts and the anterior and posterior regions of both the left and right otoliths (Fig. 5). The fragment loss is particularly high in the anterior region and the dorsal part of the otoliths (Fig. 5). The shape of the abnormal otoliths is elliptic-lanceolate. Lateral surfaces of the left and right otoliths are concave due to the depression in their center (Fig. 5). Especially, the rostrum of the left otolith is quite short and narrow because large amount of part is lost due to abnormality (Figs. 5A and 5C), while the rostrum of the right otolith is also short and broad (Figs. 5B and 5D). Antirostrum is absent in both left and right otoliths, as in other abnormal otoliths (Fig. 5). The shape of the ventral parts of the abnormal left and right otoliths of this individual is less straight and slightly irregular with sinuate margins, while the dorsal margins are wavy–irregular with crenate-irregular margins (Fig. 5). The anterior regions of the left and right otoliths are angled with lobbed margins, while the posterior region of the left otolith is slightly rounded with crenate margin, but it is oblique with crenate margin for the right otolith (Fig. 5).

The lateral surface of the otolith of the 39.0 cm TL female *B. belone* specimen is rough and contains irregular protrusions and depressions of different sizes and shapes (Fig. 6). The protrusions and elevations are irregularly distributed on the lateral surfaces of both right and left abnormal otoliths (Fig. 6). Additionally, calcium carbonate polymorphism is quite evident in both left and right otoliths (Fig. 6). In particular, there is a large and distinct aragonite crystalline structure in the center of both otoliths, and vateritic structures are present in the other parts of the otoliths (Fig. 6). These differentiations are clearly revealed in light and SEM analyzes. However, no loss of parts due to abnormalities in the right and left otoliths of this individual was observed (Fig. 6). The shapes of the abnormal otoliths are less lanceolate (Fig. 6). The center of the lateral surfaces is convex due to the crystal accumulation in the middle part of the left and right otoliths (Fig. 6). The rostrum of the left and right otoliths is short and broad (Fig. 6). Moreover, the antirostrum is absent for both left and right otoliths (Fig. 6). The shape of the ventral parts of the left and right otoliths is less straight and slightly irregular, with serrate margins, while the dorsal parts are wavy-irregular with lobed margins (Fig. 6). The anterior regions of the left and right otoliths are angled with lobbed margins (Fig. 6), while the posterior region of the left otolith is slightly rounded-oblique sinuate margin (Figs. 6A and 6C), and the posterior part of the right otolith is angled-oblique with sinuate margin (Figs. 6B and 6D).

The lateral surface of the otoliths of the 44.2 cm TL male *B. belone* specimen is rough and contains some irregular protrusions and depressions of different sizes and shapes (Fig. 7). The protrusions and elevations are more pronounced and irregularly distributed on the lateral surface of the otoliths, especially in the anterior parts of the otoliths (Fig. 7). Additionally, there is a structure composed of distinct aragonite crystals in the anterior part of the left otolith (Figs. 7A and 7C). These differentiations were clearly revealed in light and SEM analyses (Fig. 7). Abnormality-related fragment losses in the anterior, dorsal, and ventral parts of the right otolith (Figs. 7B and 7D) and in the anterior region and dorsal part of the left otolith (Figs. 7A and 7C) were observed. Abnormal otoliths are less elliptic–lanceolate shaped (Fig. 7). The right and left otoliths are concave due to the depression in the center of the lateral surfaces (Fig. 7). The rostrum of the right otolith is short and broad (Fig. 7), while the rostrum of the left otolith is also short but broader than the right otolith (Fig. 7). In addition, both left and right otoliths lack antirostrum (Fig. 7). The shape of the ventral parts of the otoliths is less straight and slightly irregular with sinuate margins, while the dorsal parts are wavy and irregular with crenate margins (Fig. 7). The anterior regions of the left and right otoliths are angled with lobbed margins, and the posterior regions of the left and right otoliths are slightly oblique with sinuate margins (Fig. 7).

4. Discussion

This study provides the first and detailed data on the presence of abnormalities in the otoliths of *B. belone*

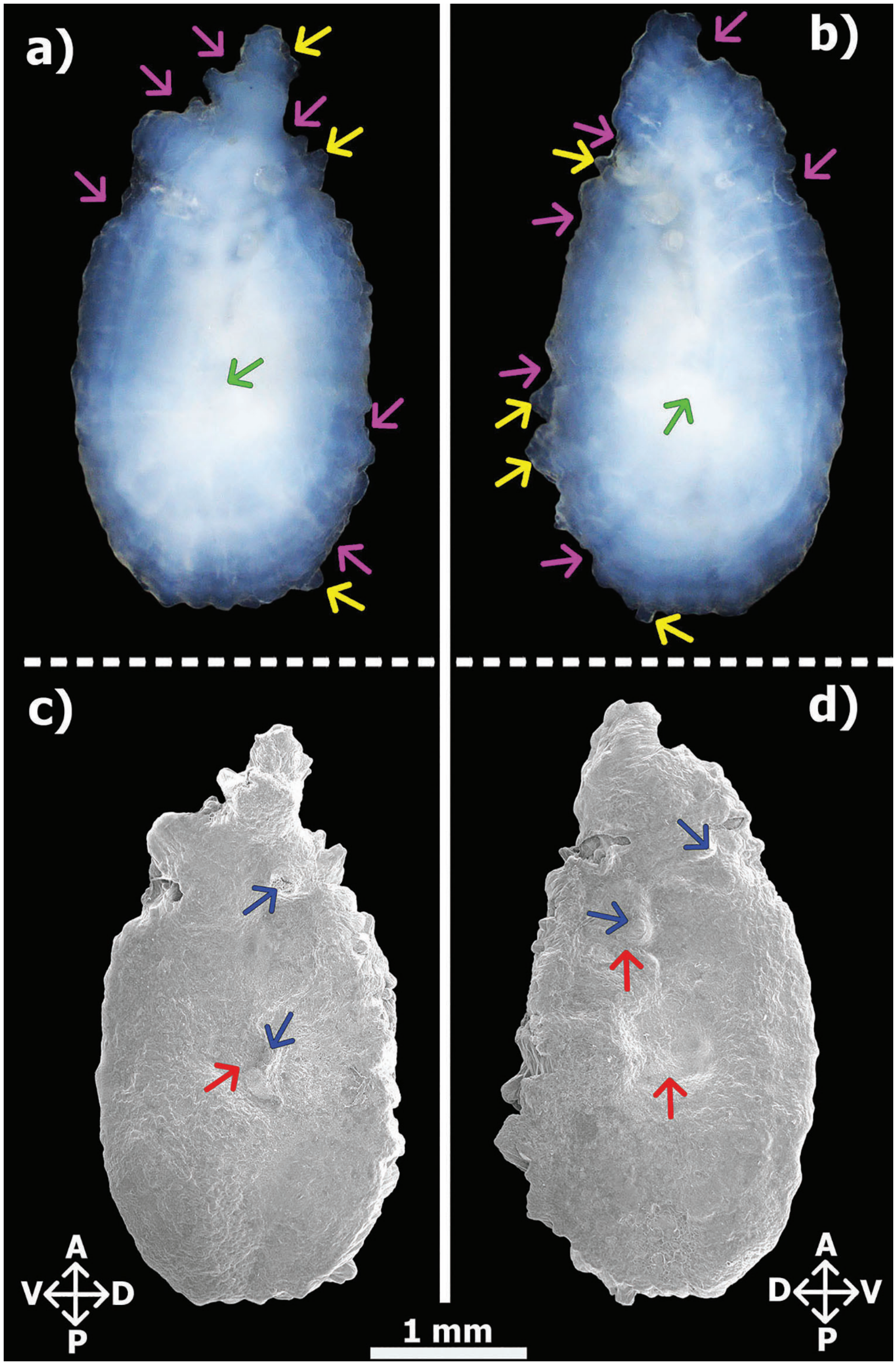

Figure 5

Light microscope images of the lateral surfaces of the left **(A)** and the right **(B)** otoliths and SEM images of the lateral surfaces of the left **(C)** and the right **(D)** otoliths of the 39.8 cm TL female *Belone belone* specimen from the Darıca coast in the Sea of Marmara, Türkiye. Arrows indicate for Yellow: aragonite part, Green vaterite part, Red: depressions, Blue: protrusions, and Pink: fragment losses, D: Dorsal; V: Ventral; P: Posterior; A: Anterior. SEM, scanning electron microscopy; TL, total length.

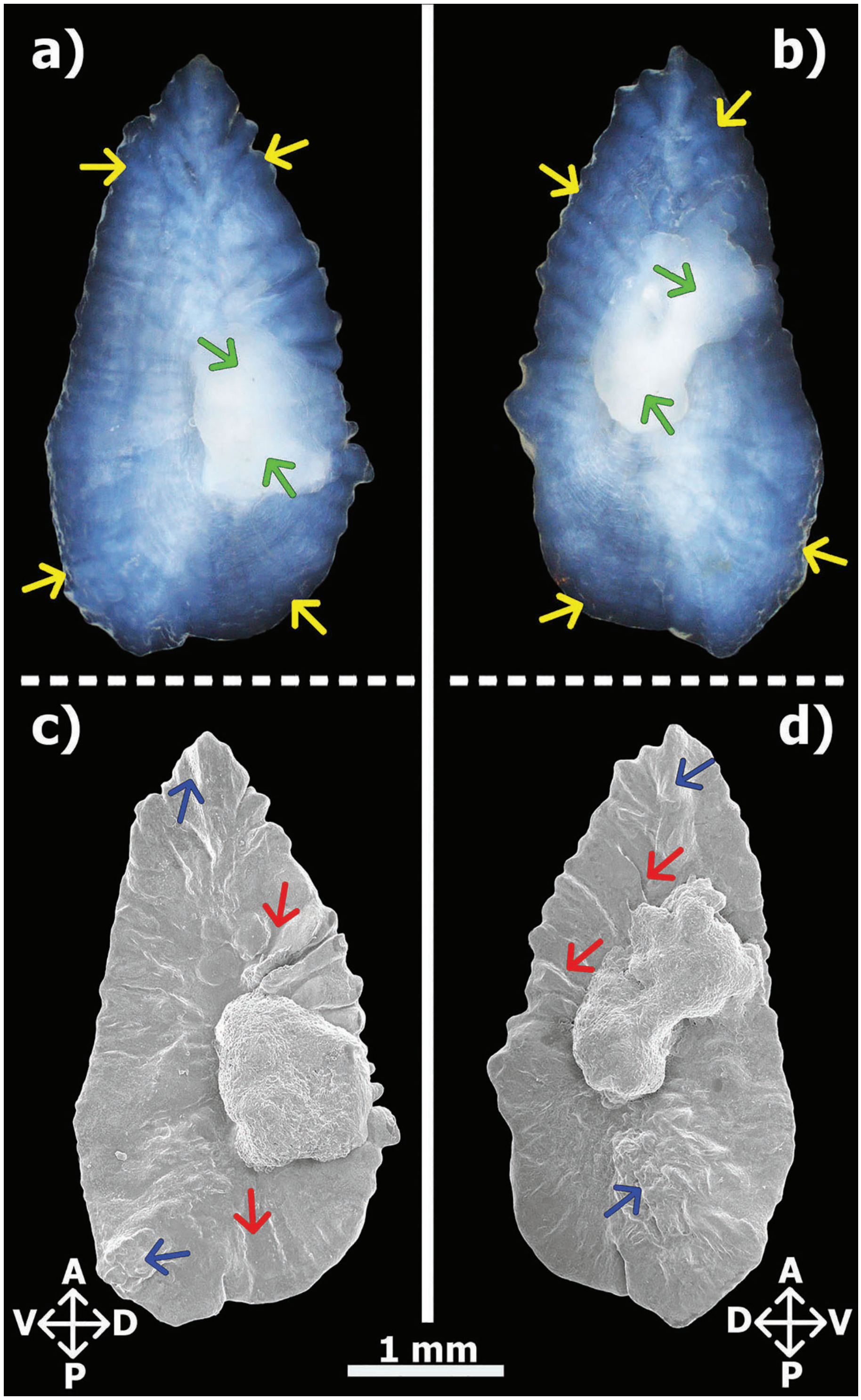

Figure 6

Light microscope images of the lateral surfaces of the left **(A)** and the right **(B)** otoliths and SEM images of the lateral surfaces of the left **(C)** and the right **(D)** otoliths of the 39.0 cm TL female *Belone belone* specimen from the Darıca coast in Sea of Marmara, Türkiye. Arrows indicate for Yellow: aragonite part, Green vaterite part, Red: depressions, Blue: protrusions, D: Dorsal; V: Ventral; P: Posterior; A: Anterior. SEM, scanning electron microscopy; TL, total length.

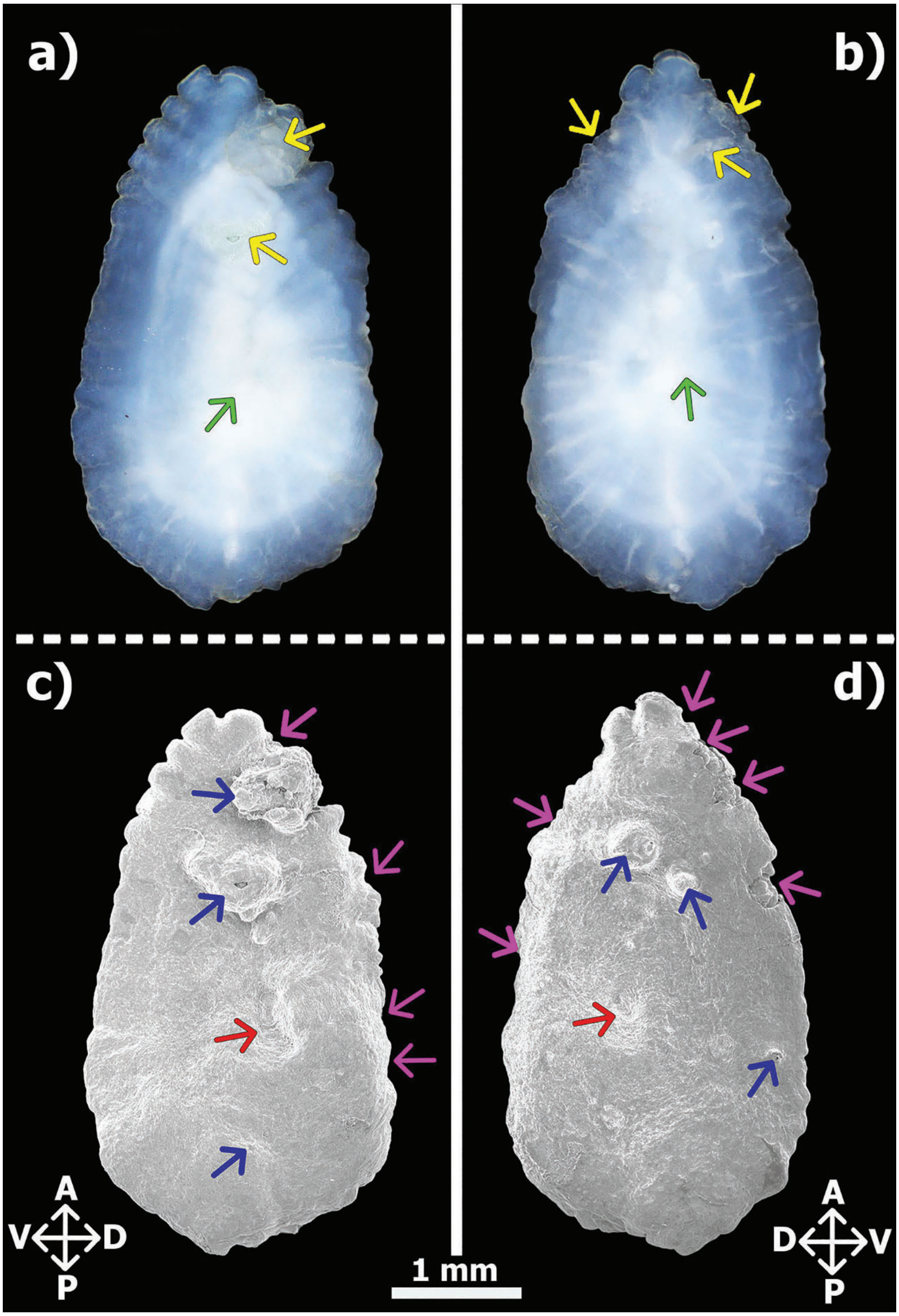

Figure 7

Light microscope images of the lateral surfaces of the left **(A)** and the right **(B)** otoliths and SEM images of the lateral surfaces of the left **(C)** and the right **(D)** otoliths of the 44.2 cm TL male *Belone belone* specimen from the Darıca coast in the Sea of Marmara, Türkiye. Arrows indicate for Yellow: aragonite part, Green vaterite part, Red: depressions, Blue: protrusions, and Pink: fragment losses, D: Dorsal; V: Ventral; P: Posterior; A: Anterior. SEM, scanning electron microscopy; TL, total length.

individuals from the Darıca coast in the Sea of Marmara (Türkiye). In the present study, otoliths of *B. belone* individuals were evaluated by an integrative approach using Light and SEM, and the evaluation revealed that normal and abnormal otoliths of *B. belone* individuals sampled from the Darıca coast of the Sea of Marmara differed morphologically. The abnormalities observed in the otoliths of *B. belone* individuals were the presence of irregular protrusions and pits on the surfaces of the otoliths and crystal and shape differences due to calcium carbonate polymorphism. A similar situation was also reported by Yedier and Bostancı (2019) for the *Lophius budegassa* sampled in the Sea of Marmara, where the *B. belone* samples in the current study were sampled. Yedier and Bostancı (2019) also reported that abnormalities occurred in the left or right otoliths of *L. budegassa* individuals. The current study also revealed that abnormality could occur in both the left and right otoliths of *B. belone* individuals. Similar situations and results of the present study had also been reported in *Sarpa salpa* (Yedier et al., 2024) and *Saurida lessepsianus* (Reis et al., 2025) in the Aegean Sea (Türkiye) and *Trachinus draco* individuals in the Black Sea (Türkiye) (Yedier, 2022). In addition, the difference in TL of *B. belone* individuals with abnormal otoliths sampled from the Darıca coast showed that this abnormality does not only occur in a certain size group but can occur in both small and large individuals of *B. belone*. This situation shows that the abnormality in otoliths of *B. belone* individuals sampled from the Darıca coast of the Sea of Marmara is not related to the TL of the fish. It was reported in the literature that abnormality in otoliths of both freshwater fish, such as *Anguilla anguilla* (Jawad et al., 2024), and marine fish, such as *S. salpa* (Yedier et al., 2024) and *T. draco* (Yedier, 2022), is not related to fish size, as in the current study.

There may be changes in the weight, length, perimeter, width, and area measurements of otoliths due to different conditions such as calcium carbonate polymorphism, accumulation problems, and fragment losses (Tomás & Geffen, 2003; Yedier, 2022; Yedier et al., 2024). For instance, Tomás and Geffen (2003) reported that abnormal otoliths had larger values, especially for perimeter, area, and length, compared to normal otoliths. In the current study, it was determined that the average perimeter, area, length, and width values of abnormal otoliths were larger than those of normal otoliths. Similar results were also reported in Turkish seas in terms of average perimeter, length, area, and width values in otoliths of the *T. draco* (Yedier, 2022) and average perimeter values in otoliths of the *L. budegassa* (Yedier & Bostancı, 2019). Besides, in the current study, there was a statistical difference only in the otolith perimeter value from the measurements of normal and abnormal otoliths of *B. belone* individuals sampled from the Darıca coast of the Sea of Marmara ($P < 0.05$). In addition, Yedier (2022) reported that the otolith perimeter value of abnormal otoliths of the *T. draco* sampled from the Cape Jason in the Black Sea showed a statistical difference between abnormal and normal otoliths, as in the current study. Besides, in the current study, abnormal otoliths were found in both female and male *B. belone* individuals. This situation revealed that the abnormality in the otoliths of this species is not directly related to gender. In the literature, it was reported that abnormalities in otoliths in several fish species such as *S. salpa, Pagellus acarne, Merlangius merlangus, Diplodus puntazzo*, and *Trachurus mediterraneus* sampled from Turkish seas are not related to gender (Yedier & Bostancı, 2020; Yedier et al., 2024). Contrary to the results of the present study, it was reported that abnormalities could be related to gender, as only female individuals of the *A. anguilla* sampled from Lake Bafra (Türkiye) were found to have abnormalities in their otoliths (Jawad et al., 2024).

The formation of abnormal/aberrant otoliths in fish species varies, and it was reported that the rate of individuals with abnormal otoliths in cultured fish is higher than in natural habitats (Lewis et al., 2022; Reimer et al., 2016). For instance, it was reported that the proportion of fish individuals with abnormal otoliths in culture can reach 68% for delta smelt (Lewis et al., 2022), 66%–100% for Atlantic salmon, 14%–60% for herring, 52%–56% for coho salmon, 50% for rainbow trout and 48% for lake trout (Reimer et al., 2016). The presence of abnormalities in otoliths of wild fish species in their natural habitat is much lower than in cultured individuals, with rates of <1% in delta smelt (Lewis et al., 2022), 5%–6% in herring, 1%–12% in coho salmon, 24% in lake trout, and 5% in rainbow trout (Reimer et al., 2016). This ratio varies depending on the species and habitat of wild fish in the natural environment in the Turkish seas. In the current study, this rate was determined to be 4% in *B. belone* individuals sampled from the Darıca coast of the Sea of Marmara. This rate was reported as 3% in the *L. budegassa* sampled from the same sea (Sea of Marmara, Türkiye) (Yedier & Bostancı, 2019). Unlike these, this anomaly was reported as low as 0.16% in *S. lessepsianus* individuals sampled from the Aegean Sea's Muğla coast (Türkiye) (Reis et al., 2025). However, this abnormality was reported as high as 52.42% in *S. salpa* individuals sampled from the North Aegean Sea (Türkiye) (Yedier et al., 2024) and 52.5% in *T. draco* individuals sampled from the Black Sea (Türkiye) (Yedier, 2022). Although the reasons for abnormal formation in otoliths are not fully understood, the fact that this rate varies greatly, especially in fish in wild environments, has a high potential to be affected by both environmental and genetic factors (Jawad et al., 2024; Lewis et al., 2022; Yedier & Bostancı, 2020). When this ratio is detected at a high rate, it is generally more related to the genetic predisposition of the species, while at low and medium levels, it may be closely related to stress, feeding preferences, pollutants, and environmental factors (Yedier et al., 2024). In the literature, it was reported that environmental factors such as temperature changes (Tomás & Geffen, 2003), rapid and irregular growth due to excessive and irregular feeding (Reimer et al., 2016), and stress (Bowen et al., 1999) may cause such abnormalities in fish as in *B. belone*. In aquatic systems, fish, which are often used as indicators, are both directly and indirectly affected by pollution in their habitat (Adams, 2002). Therefore, this abnormal situation in the otoliths of fish was expressed as a potential source of water pollution from the above-mentioned situations, as well as different pollutants in the *T. draco* from the Black Sea (Yedier, 2022), and in four flatfish species from the Aegean, Mediterranean, and Black seas (Yedier et al., 2023).

The Sea of Marmara, which is the sampling area of the current study, is a small sea that is in active exchange with the Black Sea to the north and the Aegean Sea to the south. The Sea of Marmara is exposed to intense inputs of waste pollutants such as ship traffic, oil spills, and ballast water, and domestic and industrial wastewater coming from highly populated areas such as the Sea of Marmara, Istanbul, and the Gulf of Izmit in Kocaeli (Özsoy et al., 2016). Additionally, it was reported that the transport of pollution from the Black Sea to the Sea of Marmara via the Bosphorus upper layer current contributes to this process (Şeker & Öztürk, 2021). The Sea of Marmara is sensitive to pollution as it is a geographically closed inland sea and is heavily polluted, especially in the last 50 years, due to the effects of industrialization, urbanization, and agricultural activities, and this pollution continues (Akoğlu et al., 2024). In addition to these pollutions, various factors such as the temperature increase in the Sea of Marmara due to global warming and ecological imbalance have resulted in the emergence of mucilage crisis/pollution, which significantly affected the Sea of Marmara in 2021 (Albay, 2023; Şeker & Öztürk, 2021). This type of pollution causes serious physiological, behavioral, and developmental deteriorations in fish species in the seas (Jacquin et al., 2020). The mucilage, which is also seen in the Sea of Marmara, has had serious negative effects on fish species living in the Sea of Marmara (Albay, 2023; Şeker & Öztürk, 2021). Garfish, *B. belone*, examined in the present study, is one of the pelagic fish species living in the Sea of Marmara. This species, which generally hunts on the surface and feeds on small fish, can be one of the species with a high potential to be affected by this pollution, as it lives in the upper water layers where mucilage is most concentrated. In *B. belone* individuals, mucilage could affect physiological respiration, egg development, hunting and migration behavior, foraging, and population distribution. Many surface-dwelling fish species, such as *B. belone*, come into direct contact with more mucilage, which can cause mucilage particles to stick to the gills of fish and thus cause respiratory distress in fish (Albay, 2023; Şeker & Öztürk, 2021). Many fish species, such as *B. belone* individuals, are visual hunters; therefore, water turbidity, especially from mucus, can reduce fish's field of vision, making hunting difficult and even preventing them from detecting predators (Utne-Palm, 2002). Additionally, mucilage causes the death of small fish that the garfish feeds on, thus reducing their food resources and affecting the food cycle of the species, which in turn can affect the healthy nutrition of *B. belone* individuals and developmental disorders may occur (Albay, 2023; Şeker & Öztürk, 2021). Besides, garfish may tend to move away from mucilage-covered areas, thus mucilage may change their migration direction and location, forcing them to live in habitats they would not normally prefer (Albay, 2023). In addition, mucilage may cause stress in fish, leading to irregularity and lack of coordination in the swimming and shoaling behavior of *B. belone* individuals (Albay, 2023; Şeker & Öztürk, 2021). All of these may negatively affect the survival, hunting, and prey escape behavior of *B. belone* individuals, as well as other fish (Albay, 2023; Şeker & Öztürk, 2021). It was reported that the oxygen concentration in the Sea of Marmara, especially on the coasts of Istanbul and Kocaeli, has decreased due to mucilage, and many pelagic and benthic fish species have died as a result. Such studies have once again clearly revealed the negative effects of mucilage on fish (Albay, 2023; Karadurmuş & Sarı, 2022; Şeker & Öztürk, 2021). Although mucilage was not reported to have a direct mortality effect on the *B. belone*, it is possible that mucilage may cause serious problems such as stress, reproductive failure, and migration disorders in *B. belone* individuals in the long term, as in many other fish (Albay, 2023; Karadurmuş & Sarı, 2022; Şeker & Öztürk, 2021). Such problems may have induced abnormal otolith formation in *B. belone* individuals. Darıca region in Kocaeli (Türkiye), where *B. belone* individuals were sampled, was also heavily affected by the mucilage crisis experienced in the Sea of Marmara, especially in 2021, and dense mucilage layers were observed on the Darıca coast (Sea of Marmara, Türkiye), especially in the relevant period (Tuzcu Kokal et al., 2022). The pollution and mucilage problem in the Sea of Marmara continues in 2025 and continues to threaten fish species.

Under normal conditions, abnormalities in otoliths can cause different problems with balance, hearing, and lateral line neuromasts of fish (Campana, 2004; Shahjahan et al., 2022), causing fish to be unable to hunt, escape from predators and migrate regularly, and reducing their survival rates (Lewis et al., 2022). However, despite all these negative aspects of their living environment, abnormalities in the otoliths of *B. belone* individuals sampled from the Darıca coast of the Sea of Marmara are at a low rate, contrary to expectations. Additionally, the observation of abnormal otoliths in *B. belone* individuals of different sizes in this region shows that small or large individuals with abnormal otoliths can continue their lives. Behavioral and vestibular disorders resulting from otolith abnormalities may have more widespread and severe effects, especially in smaller-bodied fish (Reimer et al., 2016). Therefore, *B. belone* individuals, which are not small-bodied fish, may have been less affected by this situation. Besides, as in many fish species, *B. belone* individuals may have developed different strategies, adaptations, and/or mechanical methods in order to survive against these negativities (Huey et al., 2012). For instance, like many fish species, *B. belone* can be easily hunted and can hide from predators thanks to color adaptation (Collette, 2016). In addition, *B. belone* individuals can avoid obstacles such as mucilage layers and predators because of their ability to forage on the water surface (Gauzens et al., 2024).

Otolith abnormalities detected in *B. belone* individuals living in marine systems under high anthropogenic pressure, such as the Sea of Marmara, may have occurred as a result of both environmental factors, such as pollution, temperature changes, mucilage, and nutrient imbalances, and genetic factors. Additionally, such morphological disorders in fish species could threaten not only individual development but also the physiological resistance and ecosystem roles of fish populations. Especially in *B. belone* populations exposed to variable environmental conditions in the Sea of Marmara for a long time, abnormal otoliths can be considered as both early warning indicators and biological monitoring parameters in terms of providing information about this habitat. In addition, the results and evaluations of the current study also constitute an important database for further studies on this subject in this region. On the other hand, considering that otolith anomalies can be shaped not only by environmental but also genetic variations, and by regularly conducting otolith morphometry-based biomarker analyzes and genetic structure studies in this region, both long-term protection of *B. belone* and other fish species living in the relevant habitat and regular monitoring of the ecosystem health of the Sea of Marmara with alternative methods can be ensured.

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