Oceanologia, 66 (4)/2024, 66410
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https://doi.org/10.5697/VZSR4828

Decreasing otolith length-to-width ratio with fish length – Atlantic cod (*Gadus morhua*), southern Baltic Sea

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Abstract
The observation concerns otoliths of Atlantic cod from the Słupsk Bank. A total of 100 pairs of otoliths were selected from 100 specimens, the total length of which ranged from 5.2 cm to 62 cm. It was found that the greater the length of the fish, the smaller the ratio of otolith length to width. Whether this is a regular trend that was observed for the first time, or a matter of analyzing otoliths from different populations, remains an unresolved question. No differences were found between males and females in the relationship between fish size and otolith size, or changes in the ratio of otolith length to width.

Keywords
Atlantic cod; Otoliths; Stock; Słupsk Bank

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Received: 5 April 2024; **revised**: 16 October 2024; **accepted**: 25 October 2024

Cod (*Gadus morhua*) belongs to the order Gadiformes (Nelson, 2006), and its size depends on the region of occurrence. In the case of cod from the Baltic, their average size ranges from 30 cm to 60 cm. However, the size of the oldest individuals can reach up to 200 cm (HELCOM, 2013).

Cod is a demersal fish. It occurs in the waters of the North Atlantic and adjacent seas (Cohen et al., 1990). In spring, cod are found in deep waters, while in other seasons they can be found in smaller schools in the pelagic zone (Keats and Steele, 1992).

Two distinct cod stocks exist in the Baltic Sea, i.e., the Western Baltic cod (WBC, ICES subdivisions 22–24) and the Eastern Baltic cod (EBC, SDs 24–32; Schade et al., 2022). Mixing of the stocks is a well-known phenomenon. Adult cod migrate both west and east, even east of the Bornholm Basin (Hüssy, 2011).

Collecting otoliths is an integral part of ichthyological analysis. Otoliths are aragonite bone elements located in the sac-like extensions of the labyrinth in the skull. They are part of the organs responsible for balance and hearing in fish. Because otoliths grow throughout a fish's life, they can provide a wealth of data useful in ecological studies. For example, the growth rate trajectory can be back-calculated (Wilson et al., 2009). However, for this to be possible, otolith growth characteristics must be known, such as the relationship between fish size and otolith size, changes in the otolith length-to-width ratio with fish size, and the possible effects of fish sex on these relationships.

The objective of the study was to determine changes in the length, width, and shape of otoliths with the growth of the fish. An additional aspect that yielded unexpected results was the analysis of changes in the ratio of otolith dimensions (length-to-width ratio) with fish growth. The results most likely represent changes in the dimensions of otoliths belonging to both Western and Eastern cod stocks.

Otoliths used for analysis were collected from Atlantic cod (*G. morhua*) caught using bottom gillnets during expeditions on board r/v *Oceanograf* in the Słupsk Bank region. Fish were collected in 2020 and 2021 in all seasons (January, April, August, November). Only sagittal otoliths were analyzed.

To ensure a representative dataset for analyzing the results, a list of total fish lengths was prepared. We selected 100 pairs of intact otoliths from 100 specimens, in such a way that they represented the widest range of lengths. Otoliths were collected by excision through the upper neurocranium. The total length (TL) of the fish ranged from 5.2 cm to 62 cm. A Canon G9 camera and a corresponding tripod were used to photograph the otoliths. Each otolith was placed with its convex side facing upward in the middle of a black sheet to provide a contrasting background. The camera was positioned parallel to the otolith. Photographs were taken individually for each otolith. To determine whether the otolith was right or left, we checked its anatomical structure, in particular the position of the rostrum (Irgens, 2018). All photographs were imported to AxioVision 4.8 software. The software was used to measure the maximum length and width of otoliths, expressed in pixels. Measurements were taken for all otoliths. Pixel measurements were converted to millimeters using a measuring reticle. In AxioVision 4.8 software, the pixel scale was measured on a 1 mm reticle. The obtained data were transferred to MS Excel 365, where the length and width of otoliths were converted to millimeters.

The variability in the length and width of otoliths with fish length was described. Subsequently, the relationship between otolith length-to-width ratio and fish length was examined, as well as the relationship between otolith length and width, taking the variability between right and left otoliths and between males and females into account. For this purpose, we used a polynomial function. The statistical analyses were conducted using Statistica 13.0.

Corel PHOTO-PAINT X7 software was used for the graphical presentation of otoliths. By adjusting the contrast and grayscale, uniformly black images of otoliths on a white background were obtained. Eleven pairs of otoliths, representing various fish size classes, were included in the presentation of shape changes. The smallest and largest otoliths belonged to fish with lengths of 9.4 cm and 62 cm, respectively. The remaining fish for which the shape analysis was presented differed in length by 5 cm.

The length of the fish ranged from 5.2 cm to 62 cm. The largest number of otoliths was obtained from fish with lengths in the middle of the length range (25–30 cm), while the smallest number from fish with the smallest (5–13 cm) and largest (58–62 cm) lengths. Among the analyzed fish, the sex ratio was equal. The analysis included 50 females and the same number of males.

It was observed that both the length of the otolith and its width increase proportionally to the length of the fish (Figure 1a,b). No differences were observed between males and females. The changes in the ratio of otolith length to its width (Figure 2) show a clear decreasing trend with the total length (TL) of the fish, which, however, does not represent a linear relationship. The larger the length of the fish, the smaller the ratio of otolith length to width. The trend of decreasing ratios indicates that as the fish grows, the width of the otolith increases disproportionately to its length – the otolith becomes wider (Figure 2). The length of right otoliths increases proportionally to the length of left otoliths. The same relationship applies to the otolith width (Figure 3a,b). The obtained results clearly indicate that otoliths belonging to the same pair do not differ from each other.

It can be observed that as the fish grows, the increase in the width of the otolith is more pronounced than its length. In fish with greater total length (TL), both the rostrum and the lobes at the ventral edge of the otolith are more pronounced. For the largest otoliths, the range of lobes is 18 to 21. Intermediate-sized otoliths have lobes ranging from 13 to 16, while the smallest otoliths have approximately 14 lobes (Figure 4).

In previous studies of cod otoliths, similar parameters were considered, such as fish length, otolith length and width, and the correlation between these parameters. The shape of the otolith was also analyzed. The results obtained in the present research on cod confirm what was found in previous studies on other fish species – otolith growth is strongly dependent on fish growth (Sapota and Dąbrowska, 2019). Regarding the appearance of the otolith itself and how its shape changes, it can be observed that in larger individuals, the number of otolith lobes is greater, and they are also more pronounced. Similar observations were made by Hüssy (2008).

Factors affecting the growth of the otolith include genetic variations and, above all, environmental conditions (Campana and Casselman, 1993). A living organism is always exposed to the environment in which it occurs. Therefore, the prevailing environmental conditions will affect the organism. The temperature of the surrounding environment has a direct impact on its metabolism and ontogenesis. The higher the temperature, the faster the growth of the fish, and, consequently, the growth of the otolith (Hüssy, 2008).

The available literature does not mention that temperature changes can affect otolith proportions or the size of the lobes formed (Hüssy, 2008; Mosegaard et al., 1988;

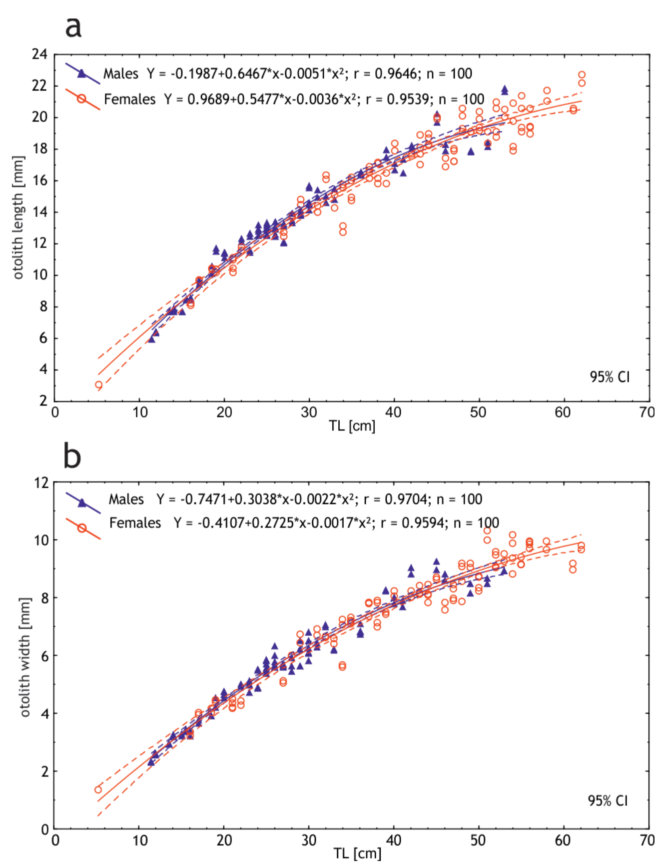

Figure 1. Changes in the length (a) and width (b) of the cod otolith in males (triangles) and females (circles) with the length of the fish.

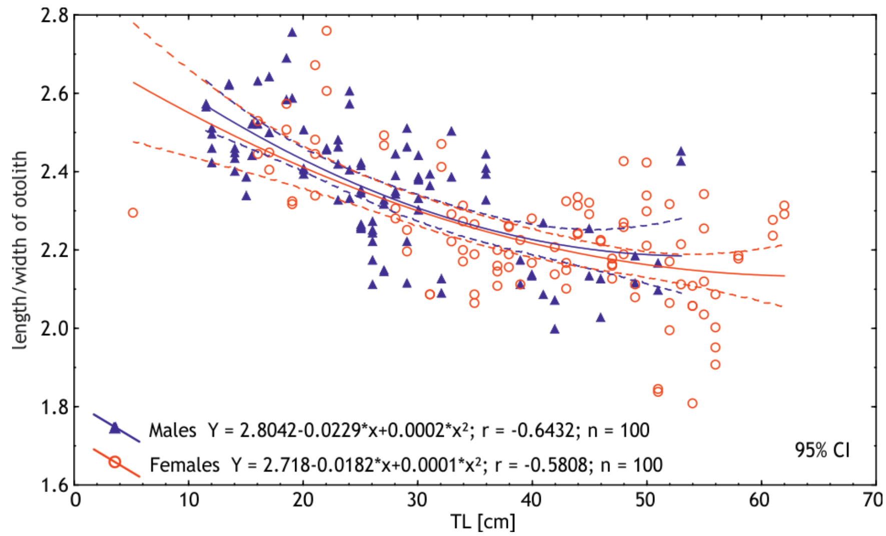

Figure 2. Changes in the ratio of cod otolith length to width in males (triangles) and females (circles) with fish length.

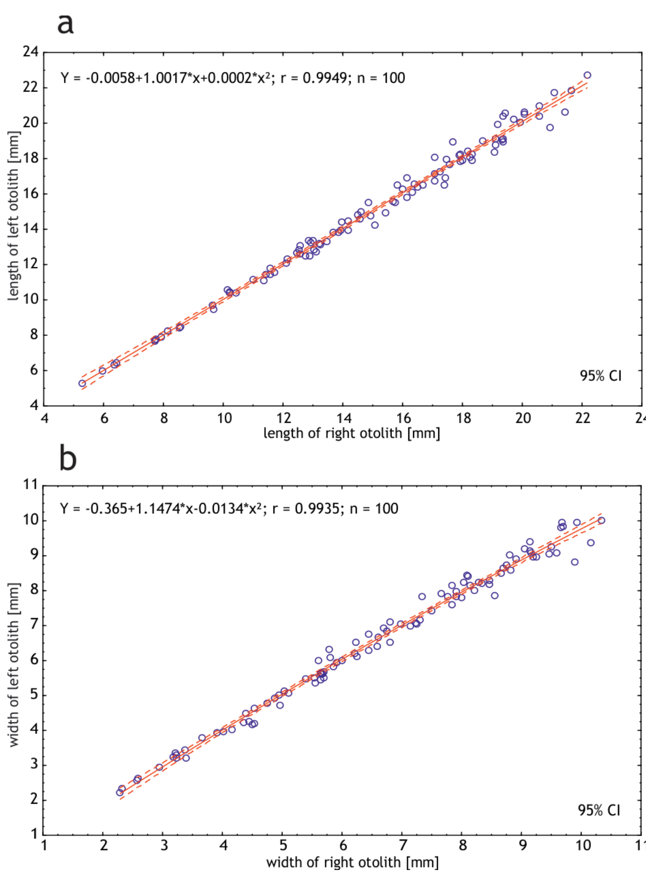

Figure 3. Relationship of the length (a) and width (b) of the right and left otoliths.

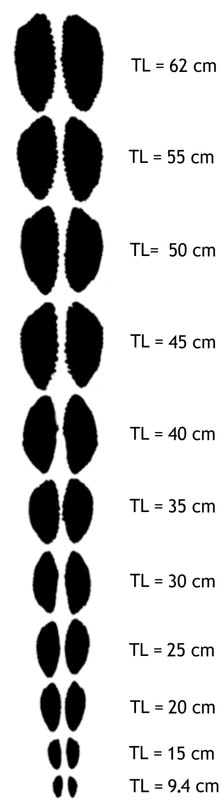

Figure 4. Changes in the otolith shape with the length of the fish.

Folkvord et al., 2004). However, it has been proven that otoliths from fish of the same species and population can show certain differences if the growth of individuals takes place under different environmental conditions, such as different temperatures. On the other hand, otoliths from individuals belonging to different populations, but growing under similar environmental conditions, showed similarities in their structure (Cardinale et al., 2004). The analyzed otoliths were collected from cod living in the same area, so it can be assumed that their growth took place under similar conditions, but it should also be noted that the Słupsk Bank area is a region where individuals from two different populations can meet. As described by Hüssy et al. (2016), otoliths belonging to individuals from the Western Baltic stock are relatively wider than otoliths from individuals from the Eastern Baltic stock.

The Baltic Sea is characterized by lower salinity than, for example, the North Sea. This primarily affects the size to which individuals in the Baltic Sea can grow. Since one of the obvious factors affecting otolith size is the size of the individual, otoliths obtained from Baltic cod will be smaller than those obtained from fish in fully saline waters. In addition, low oxygen zones and an increased number of parasites cause changes in the growth rate and feeding behavior of Baltic cod (Niiranen et al., 2019). Due to a decline in the number of larger cod and a greater abundance of smaller individuals, there is a decrease in predation on larger prey. Changes in the feeding regime of cod occurred during the decline of sprat abundance in the Baltic Sea, which is one of its prey species. This resulted in a decrease in feeding intensity and, consequently, a slowdown in cod growth (Neuenfeldt et al., 2020).

A problem affecting Baltic cod populations is the declining gene pool. This is a result of intensive fishing, which has led to a decline in the number of large individuals in the Baltic Sea (Birgersson et al., 2022). In addition, gene flow from other populations at the entrance to the Baltic Sea is virtually impossible (Andersson et al., 2023). This may be the reason why our results show low variability in the data.

In conclusion, both the length and width of cod otoliths increase proportionally to the growth of the fish. There are no differences in the dimensions of the left and right otoliths, and no differences are observed between otoliths depending on the sex of the fish. However, there is a difference in the shape of the otoliths – in larger fish, the otoliths are wider and have more pronounced lobes. Changes in the proportions of otoliths in cod with its length have not been previously described. Although the analyzed otoliths were from cod occurring in the same area, it should be noted that the fish analyzed probably included specimens from two cod stocks, which could have affected the results.

Funding

The research was financially supported by the Ministry of Science and Higher Education, Republic of Poland (Decision Nos: 11/526929/SPUB/SP/2022 and 531-O402-D031-23). This financial support allowed us to collect material and conduct laboratory analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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