

**COMPARISON OF METHODS FOR STUDYING THE AGE OF GIANT GRENADEIER
CORYPHAEENOIDES PECTORALIS (MACROURIDAE) FROM THE SEA OF
OKHOTSK BY SCALES AND OTOLITHS**

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Due to the features of their life cycle, deep-sea fishes are more vulnerable to overfishing than many other species. The intensification of fishery for giant grenadier *Coryphaenoides pectoralis* in the Russian Far Eastern seas requires special attention to the rational management of stocks of this species, including through a correct understanding of the size and age structure of its populations. The current and historical estimates of the giant grenadier lifespan vary from 8 to 58 years or more. An analysis was carried out based on the prepared preparations for age determination, which has shown that the age determined by the scales is significantly underestimated compared to that obtained from otolith thin sections. The maximum age of females in the sample, determined by scales, reached 13 years and that determined by otoliths was 23 years; the maximum age of males was 10 and 21 years, respectively. The methodology for determining the age by otolith thin sections does not exclude the underestimation of the first and marginal annual rings of otoliths. The question of interpreting the annual marks on otoliths has not yet been fully resolved. Further research is required to expand the understanding of the growth of the grenadier. For fishery purposes, it is proposed to estimate the age by otoliths.

Keywords: *Coryphaenoides pectoralis*, age, otoliths, scales, Sea of Okhotsk.

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The small-eyed macrurus *Coryphaenoides pectoralis* (Gilbert, 1892) is a broad-boreal bathybenthal species found at depths of 140-3500 m, with maximum concentrations in the range of 500-1200 m (Orlov et al., 2007). It lives in the northern Pacific Ocean from the Pacific coast of the

central part of Honshu Island and the northern regions of the California Peninsula along the Commander-Aleutian Island ridge north to the Navarin underwater Canyon. It is widespread in the Sea of Okhotsk. It is most abundant off the coast of Kamchatka Peninsula and in the Pacific waters off the northern Kuril Islands (Tokranov et al., 2005).

The purpose of this work is to study the seasonal variability of nutrient and DOC concentrations in 2023 in the two most significant rivers of Eastern Kamchatka. A distinctive feature of 2023 was the strongest eruption of the Shiveluch volcano in several decades, which occurred on April 11 (reported by A.Yu. Ozerov and numerous media reports). 0.85–4.75 (optimum 1.5–4.0)°C(Tuponogov, 1991; Orlov, Tokranov, 2008). The species is an important commercial species in the Far Eastern fishery basin, especially in the Sea of Okhotsk, where it lives deeper and its reserves are more significant than in the western Bering Sea and in the Pacific waters near the Kuril Ridge (Tuponogov, 1991, 1997; Tuponogov, Novikov, 2016; Shuntov, 2022). This species is characterized by sexual dimorphism, which is expressed in a significant difference in average and maximum size indicators - females are larger than males (Tuponogov, 2003; Orlov, Tokranov, 2008).

One of the important biological characteristics of the popeye grenadier is its age structure. In general, errors in age determination, apart from giving an incorrect representation of the age structure, can lead to improper commercial exploitation of the species. The interpretation of age assessments for long-lived and deep-sea fish causes particular concern (Savvatimsky, 1994; Bergstad , 1995; Allain, Lorance, 2000). Obviously, this is due to the weakly expressed seasonality at great depths. In the Russian Far East, until now, the method of determining the age of grenadiers by scales has been used. Kulikova (1957) reports a maximum age of eight years for a popeye grenadier specimen with an absolute length (*TL*) of 96 cm. Novikov (1970) and Pautov (1975) reported twice the age at similar lengths (from 15 to 17 years at *TL* 93 cm and 17 years at *TL* 115

cm). Tuponogov (1997) estimated the age of a grenadier *TL* 150 cm at 23 years and reported that the age can reach values over 40 years (Tuponogov, 2003). Soldat (2007) for the roundnose grenadier *C. rupestris* proposed determining age by rows of spinelets on its scales. The maximum age of females in his study was 20 years, and males - 18. Several authors have shown that age estimates based on scales are underestimated compared to age determination using otoliths (Chilton, Beamish, 1982; Wilson, 1982; Treble, Dwyer 2008; Badaev et al., 2023). Orlov et al. (2007), when studying whole otoliths clarified in glycerin under transmitted light against a black background under a microscope, determined the maximum age of a popeye grenadier *TL* 135 cm from waters near the northern Kuril Islands and Southeast Kamchatka at 12 years.

Age estimates based on analysis of thin sections of grenadier (Macrouridae) otoliths indicate that grenadiers are long-lived fish that grow slowly and mature at a late age. Based on otolith increment counts, Bergstad (1990) and Kelly et al. (1997) estimated the maximum age of the roundnose grenadier at 50-72 years. The maximum age of the Pacific grenadier *C. acrolepis* can reach 56-73 years (Matsui et al., 1990; Andrews et al., 1999). For ecologically similar representatives of the Moridae family – small-scaled *Antimora microlepis* and blue *A. rostrata* antimoras – the maximum age of females is estimated at 46 and 47 years, males – at 36 and 44 years, respectively (Korostelev et al., 2020; Korostelev, Orlov, 2020). Burton (1999) and Rodgveller et al. (2010), using thin sections of otoliths and radiometric isotopes with the ratio of ²¹⁰Pb : ²²⁶Ra, indicated the maximum age for the popeye grenadier from the northeastern Pacific Ocean at 58 and 56 years, respectively.

One of the reasons why the TINRO Fish Age and Growth Research Laboratory began studying the accuracy of age determination methods for popeye grenadier using different age-registering structures (ARS) was the intensification of fishing of the studied species in the Far Eastern seas of Russia (Kulik et al., 2023).

The purpose of this work is to compare the results of age determination of popeye grenadier using scales and otolith sections and to identify problems with adequate age assessment using the selected methods.

MATERIAL AND METHODS

The material was collected during monitoring of demersal fish resources in the northern part of the Sea of Okhotsk in 2021 on the longliner "Triumph" (fishing collective farm "Vostok-1"), which carried out commercial fishing of small-eyed grenadier in an area with average coordinates 55°00' N, 148°34' E. The scientific observer's work included mass measurements and biological analyses of the studied species, assessment of catch size and quality for further study of grenadier distribution patterns (collector A.I. Alferov). It is known that during fishing operations, grenadiers often lose the end of their tail, and fish with signs of past tail loss are also found in catches (Matsui et al., 1990; Orlov et al., 2018). In this work, we investigated the age-determining structures (ADS) of fish with intact tails selected from commercial catches. However, during further work with the bulk material, fish with signs of tail loss were encountered, so in addition to absolute length, the length from the tip of the snout to the anal opening was measured with an accuracy of 0.1 cm. The pre-anal distance (from the tip of the snout to the beginning of the anal fin) is less accurately related to absolute length than the more conservative length to the anal opening. It has been previously shown that the distance from the anus to the beginning of the anal fin in fish can vary significantly depending on sex (Voskoboynikova, Balanov, 2019) and individually (Kudersky, 1958).

The methodology for collecting, storing, and processing ADS was adopted based on accumulated global experience with our own modification (Chugunova, 1959; Buslov, 2009;

Hutchinson, Anderl, 2012; Matta, Kimura, 2012; Alferov et al., 2024). Fish of *TL* 69 – 95 cm were studied. To determine age, the largest otoliths (sagittae) were extracted from the head of each individual, and 20 – 30 scales were collected from under the dorsal fin closer to the lateral line. Then a preparation was made with 8 – 10 scales, of which four – five later proved suitable for age determination.

The scales of the small-eyed grenadier are relatively large, of irregular rounded shape (Fig. 1a). Sagittae are somewhat convex in the distal direction and, conversely, concave in the proximal direction . A characteristic feature is the comb-like ventral edge with teeth of varying sizes in different individuals (Fig. 1b) .

For otolith grinding, sandpaper with grit sizes ranging from P180 to P2000 was used. To obtain clear sections with the maximum number of annual marks, the otolith was first ground on the distal side, then on the proximal side. The patterns of ring structures were so clear that immersion liquid was not required to enhance them. As the pattern emerged, the otolith could be broken across closer to the nucleus, and grinding could continue on the larger part, thereby reducing the risk of destroying the thin plate. A similar methodology for otolith sections is used to determine the age of giant grenadier at the Alaska Fisheries Science Center (Hutchinson, Anderl, 2012). When grinding from only one side until a clear pattern is obtained, a portion of the annual marks is ground away. Of the 50 otoliths taken for manual processing, two had to be rejected due to their fragility during the final stages of processing. In total, age was determined for 39 females and 11 males of giant grenadier.

Viewing of cross sections and obtaining their images was carried out using a Micromed MC-4-ZOOM LED trinocular microscope ("Micromed", China) with a Fujifilm X-A2 mirrorless digital camera ("Fujifilm", Japan), using Barlow 2x magnification attachments ("SvBony", China).

For reliability, age assessment of fish was performed by three experienced operators independently of each other.

Comparisons of results between operators and between age determinations using scales and otoliths were performed using the McNemar-Bowker test of symmetry (Ogle, 2021). The test analyzes a $k \times k$ contingency table, where k is the number of age groups. Table rows (i) represent age categories accepted as the standard. Columns (j) represent methods or operators being compared. The table contains frequencies n_{ij} , reflecting the number of individuals in an age category corresponding to the standard for each age category of the compared sample. The null hypothesis is $H_0: n_{ij} = n_{ji} \forall i, j$. The McNemar-Bowker test (T_{MB}) was calculated using the formula:

$$T_{MB} = \sum_{i < j} \frac{(n_{ij} - n_{ji})^2}{n_{ij} + n_{ji}}$$

The criterion follows a χ^2 distribution with $(k-1)/2$ degrees of freedom. At small p values, a conclusion is made about the presence of systematic bias. When comparing the bias in age determination, the results of operator 1 were taken as the standard.

To determine the degree of dispersion of results between the standard and the sample, the coefficient of variation (CV) was used (Campana et al., 1995):

$$CV_j = 100 \times \sqrt{\sum_{i=1}^R \frac{(X_{ij} - \bar{X}_j)^2}{R-1}},$$

where X_{ij} is the age of the i th determination, j th individual obtained in the X_j is the average age of the j th individual, R is the number of age determinations for each fish.

For calculations and plotting, the R scripting language was used with the packages ggplot2 (visualization), data.table (primary data processing) (Barrett T et al. , 2024), FSA (calculation and testing of biases) (Wickham, 2016; Ogle, 2021).

RESULTS

The length (*TL*) of the studied female roughhead grenadier varied within 69.0-95.0 (on average 77.8 ± 0.88) cm, males – 72.0-81.0 (77.1 ± 0.79) cm. The body weight of females was 850.0-3215.0 (1674.6 ± 78.39) g, males – 1130.0-1930.0 (1568.2 ± 82.41) g. The age determined from scales for females was 5-13 years, from otoliths – 9-23 years, for males 7-10 and 13-21 years respectively.

Comparison of age determination results between operators showed a systematic bias in operator 2's data for both structures (table), so his estimates were excluded from further analysis. The data from operators 1 and 3 were combined. It should be noted that for these two operators, the dispersion (*CV*) of age determination results from otoliths slightly exceeded the recommended 10%.

Analysis of bias between operators (McNemar-Bowker test) determined by different recording structures for the age of individuals of grenadier *Coryphaenoides pectoralis*

Sex	Operators	Number of fish, spec.	<i>T</i> _{MB}	<i>p</i>	<i>CV</i> , %
Scales					
Males	1 / 2	11	4.44	1.96×10^{-2}	18.21
Females	1 / 2	39	26.47	2.68×10^{-7}	18.02
Males	1 / 3	11	2.78	9.60×10^{-2}	9.29
Females	1 / 3	39	0.17	7.10×10^{-1}	9.13
Otoliths					
Males	1 / 2	11	3.60	5.80×10^{-2}	17.53
Females	1 / 2	37	23.05	1.57×10^{-6}	19.27
Males	1 / 3	11	2.00	1.57×10^{-1}	10.05
Females	1 / 3	37	1.00	8.57×10^{-1}	13.22

Note. *T*_{MB} – McNemar's criterion, *p* – significance level, *CV* – coefficient of variation.

After combining the data obtained by operators 1 and 3, when comparing (McNemar-Bowker test) the results of age determinations from scales with results from otoliths, the difference was ~40%: for males – $\chi^2 = 22.00$, $p = 2.73 \times 10^{-6}$, $CV = 39.63$ ($n = 22$) ; for females – $\chi^2 = 71.05$, $p = 3.48 \times 10^{-17}$, $CV = 40.31$ ($n = 74$). It is well noticeable (Fig. 2, 3) that when using preparations with scales, the age of the grenadier was significantly underestimated compared to the age determined from otolith sections. Systematic underestimation of age was observed even in pre-recruits and young mature individuals (Fig. 4). For the studied males, this difference reached up to 12 years, for females – up to 15 years.

DISCUSSION

It should be noted that determining the number of years lived by the studied grenadiers using otoliths is not entirely straightforward. We believe that at least some of the age marks identified in our work can be conditionally accepted as annual. The edge of the otolith may appear as a wide growth zone. In their study, Rodgveller et al. , 2010) showed that depending on the processing technology, a significant number of annual rings at the edge of the otolith of the grenadier may be underestimated. The identification of the first annual rings (closest to the nucleus) also raises questions. It is believed that in grenadier, as in most other fish, the largest linear growth occurs in the first years of life (Kulikova, 1957; Novikov, 1970, 1974; Tuponogov, 1991). However, when studying the age of *Macrourus berglax* from the Northwest Atlantic, doubts were expressed that only a clear ring on the scale is annual (Savvatimsky, 1994). Let us try to clarify some biological features of the species that may affect the formation of growth structure rings. The entire life cycle of the grenadier occurs at great depths, where hydrological conditions are relatively stable. Obviously, for larvae and juveniles of grenadier, living in the bathypelagic zone is, on the one hand, a strategy chosen by the species to avoid bottom predators that abound in the

bathybenthal, and on the other hand, certain problems with food availability. Despite the fact that only a small part of grenadier catches retain non-everted stomachs after being raised from depth, the feeding of grenadier $TL > 50$ cm has been relatively well studied (Chuchukalo, 2006). The trophic level of individuals predictably increases as they grow (Gorbatenko, 2018). While juveniles feed mainly on zooplankton, the adult grenadier's diet consists primarily of fish (Pisces), squid (Myopsida + Oegopsida), and decapod crustaceans (Decapoda) (Tuponogov, 1991; Chuchukalo, 2006). Special bioenergetic studies of the species have not been conducted, but it is known that adult fish need to fill their stomachs only once every 2-3 days. Young fish less than 40 cm in size, judging by the limited available material, must feed more intensively to maintain energy balance and grow while consuming food organisms of a lower trophic level (Chuchukalo, 2006). Most likely, the grenadier does not make long intensive movements in search of food, but mainly waits for prey, as indicated by the technochemical characteristics of its muscle tissue, in which the content of proteins and lipids is approximately four times less than, for example, in Greenland halibut *Reinhardtius hippoglossoides* (Kizevetter, 1971). Many representatives of deep-sea nekton (bathylagids (*Bathylagus*), myctophids (Myctophidae), northern smoothtongue *Leuroglossus schmidti* , some squid and others) make daily feeding migrations from deep waters to subsurface waters. But juvenile grenadier constantly inhabits great depths and is probably dispersed throughout a huge water column. According to hydroacoustic studies, it has been suggested that the spatial and temporal distribution of plankton within the depth range of the species is uneven due to both different water dynamics and migrations of interzonal species, which mostly reach the middle mesopelagic zone (Vinogradov, 1968).

The extreme rarity of catches of juveniles (including larvae) of the roughhead grenadier (Endo et al., 2010; Alfyorov, 2022; Saushkina, 2022; Alferov, 2022) does not yet allow for studies of their age, growth, as well as distribution patterns and other characteristics. While for fish $TL >$

30 cm, there is likely some feeding rhythm associated with vertical migrations of interzonal prey organisms, this is unlikely for small grenadiers. Possibly, their growth during this period is not as significant as in shelf species or species that migrate to upper pelagic layers. For example, for Greenland halibut juveniles to grow to 30 - 40 cm under better food availability conditions on the shelf, several years must pass (Dwyer et al., 2016). Even taking into account that until almost the end of the last century, plankton biomass was estimated without considering the catchability coefficient (a reliable catchability coefficient for deep-sea plankton has not been established to this day), Vinogradov (1968) showed that in the lower mesopelagic zone and deeper, the biomass of food zooplankton is an order of magnitude lower than in the upper layer and continues to decrease with depth. At the same time, predatory deep-sea bottom dwellers and near-bottom layer inhabitants are better provided with food than permanent residents of the deep-sea pelagic zone. Adult roughhead grenadier feeds mainly somewhat detached from the bottom (Golovan et al., 1990; Clausen, 2008).

Considering the above, it appears that the less distinct marks located before the first clear ring on the otoliths of grenadiers, between which the distance is not as large as between subsequent clear rings, may well turn out to be annual. This means that the age of this species may be underestimated at this stage as well.

There are no convincing answers to the question of whether the remaining "annual" rings are a zone of annual growth. In the conditions where the grenadier lives, the most likely stressful event that could affect its feeding enough to form a hyaline growth zone on the OPC is spawning. The bioenergetics of the species related to the spawning periodicity of the popeye grenadier, which has not only a large liver that stores fat, but also quite large gonads, has not been studied. Long-term data on seasonal changes in the proportion of pre-spawning and spawning females, seasonal changes in feeding patterns of individuals and their Fulton's condition factor (maximum values at

the end of feeding period and minimum during spawning), fluctuations in condition factor by months in the North Kuril region and the Sea of Okhotsk indicate that the increase in energy expenditure (during spawning intensification) of the popeye grenadier occurs in the warm season (from spring to autumn) and has two peaks. In an interannual context, these peaks may shift (Tuponogov, 1991, 1997). However, throughout the year between these spawning peaks, individuals with both flowing gonads and those that have recently released sexual products can be found. It is quite possible that in the stable deep-water conditions, spawning for some individuals is not tied to a strict annual cycle and, depending on readiness for reproduction, may shift. These shifts do not necessarily have to be in the direction of longer time between growth zone formations than a calendar year.

Ambiguity in determining fish age by different methods (Burton, 1999; Beamish, McFarlane, 2000; Cailliet et al., 2001; Campana et al., 2011; Casselman et al., 2019; Le Bourg, Le Bourg, 2020), including radioisotope and otolith surface grinding methods, as well as the absence of juveniles in the DBRR samples, leaves the question of accurate age determination of the popeye grenadier open. As a consequence, problems related to identifying the onset of sexual maturity, age structure of the commercial part of the population in different areas, fertile age of individuals, and species fecundity remain incompletely resolved. The biochemical composition and mechanisms of ring structure formation in scales and otoliths differ (Mikheev, Sheina, 2020). This may be the reason why some marks that are clearly visible on the otolith are invisible or indistinct on the scale. This makes age determination by scales more subjective and therefore less suitable for practical purposes.

To make progress on this issue, it is necessary, first, to accumulate information on grenadier growth for all size groups, starting from the larval stage. Spawning of grenadier, as probably of other deep-sea fish whose eggs, larvae, and juveniles develop in the bathypelagic zone, must occur

in places with certain current systems that ensure the transit of larvae to areas where food plankton of the required fraction accumulates. The transition to external feeding is critical, and food for larvae must be available by this period. Probably, in areas of increased concentrations of deep-water plankton, there is a high probability of catching juvenile grenadiers and other species with similar ecology. Secondly, important information can be obtained through bioenergetic research using modern methods. Based on more representative material, including hundreds of popeye grenadier otoliths, studies are planned that include the construction of length-age keys for males and females, as well as determining the age of maturity for the species. Cross-analysis of all available methods for determining fish age and identifying the biological characteristics of the species is currently the most likely path.

The age of other long-lived grenadiers and ecologically similar long-lived species is comparable to our results. Kulik et al. (2023) showed that until recently, when determining the total allowable catch of the popeye grenadier, the target exploitation rate was chosen based on the acceptable natural mortality characteristic of fish with a lifespan of up to 25 years. Already for some fishing zones, the level of exploitation of this species' stocks is at the boundary between safe and dangerous. The level of information support for stock assessment corresponds to the second level (Babayay et al., 2018). One of the main conditions for achieving the first level is the identification of the size-age composition of populations over a period exceeding the lifespan of the popeye grenadier. Thus, the relevance of studying the age of this species has great theoretical and practical significance.

CONCLUSION

Comparison of methods for assessing the age of the popeye grenadier confirmed the results of similar studies conducted by foreign colleagues. The results of our research and analysis of

literature data show that age estimates of the popeye grenadier based on scales are significantly underestimated compared to those based on otoliths. The similarity of estimates of the maximum age of the popeye grenadier based on radioisotopes (literature data) and on otolith sections gives reason to consider the latter more suitable for use in fishery management activities compared to the method of determining age by scales. It is necessary to conduct a comparative analysis of various methods for determining age (sections, thin slices, break-and-burn) and verification of age determinations using a wider size and age range of fish, as well as analysis of the annual cycle of otolith development. Without verifying the accuracy of age estimates for the popeye grenadier, problems related to the accuracy of determining its growth, mortality, stock size estimates, and consequently, rational fishery regulation remain unresolved. Until these studies are conducted, intensification of fishing is possible only through newly explored stocks and more complete knowledge of the species' biology.

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COMPLIANCE WITH ETHICAL STANDARDS

Fish for biological analysis were already dead when collected from commercial catches. No special permission is required for such research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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FIGURE CAPTIONS

Fig. 1. Scale (a) and otolith (b) of giant grenadier *Coryphaenoides pectoralis* .

Fig. 2. Preparations from scale (a) and otolith (b) of a female giant grenadier *Coryphaenoides pectoralis* TL 78 cm: (•) – annual rings; age determined from scale is 11+, from otolith – 20+.

Fig. 3. Relationship between absolute length (*TL*) and age in giant grenadier *Coryphaenoides pectoralis* : a – females, b – males; age: (□) – determined from otolith sections, (●) – from scales.

Fig. 4. Difference (bias) between the age determined from scales (*) of males (a) and females (b) *Coryphaenoides pectoralis* and the age determined from otoliths: (– –) – zero difference between age values determined by different methods, (⊥) – minimum and maximum values.