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Using different hard structures to estimate the age of deep-sea fishes: A case study of the Pacific flatnose, *Antimora microlepis* (Moridae, Gadiformes, Teleostei)

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A R T I C L E I N F O

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A B S T R A C T

Age was estimated for the Pacific flatnose *Antimora microlepis* using the operculum, vertebrae, otoliths, scales and pectoral finrays. Otoliths and vertebrae were the only structures that produced recognizable marks considered to be annuli. Annuli counts from these two structures were similar producing ages ranging from 5 to 35 years. These two hard structures may be useful to estimate the age of other deep-sea fishes.

1. Introduction

Deep-sea fishes are taxonomically diverse and play important roles in marine ecosystems throughout the world. Although the life-histories of many deep-sea species remain poorly understood, some are characterized by long lifespans, low fecundity, and slow growth (Bergstad, 2013), making them vulnerable to overexploitation (Devine et al., 2006). Reliable age data are particularly scarce for many species of deep-sea fishes that occur as bycatch in commercial fisheries.

The two species that comprise the genus *Antimora* (Moridae, Gadiformes) are among the most widespread and abundant deep-sea fishes in the world's oceans (Iwamoto, 1975; Kulka et al., 2003). Both occur commonly as bycatch in deepwater trawl and longline fisheries (Iwamoto, 1975; Kulka et al., 2003; Frey et al., 2017). The Pacific flatnose *A. microlepis* Bean, 1890 inhabits the continental slope and abyssal plain of the North Pacific, while the blue hake *A. rostrata* (Gűnther, 1878) occurs in temperate and cold waters throughout the rest of the world, with the exception of the Arctic Ocean and semiclosed seas of Japan and the Mediterranean (Small, 1981; Cohen et al., 1990). Several studies have examined the age and growth of the blue hake (Magnússon, 2001; Fossen and Bergstad, 2006; Horn and Sutton, 2015; Orlov et al., 2018; Vedishcheva et al., 2019; Korostelev et al., 2020) which is considered a prospective target for commercial fishing (Novikov and Timokhin, 2009; Priede, 2017). Fewer have investigated the age and growth of the Pacific flatnose (Orlov and Abramov, 2001, 2002; Frey et al., 2017) which also might have commercial fishery importance (Davletshina et al., 2019; Orlov et al., 2020). These studies made age estimates by counting rings on broken and burnt otolith (sagitta) using traditional methods (Chilton and Beamish, 1982). However, to the authors' knowledge, no methods for obtaining age estimates using alternate structures have been explored in *Antimora* spp. A number of studies have demonstrated the effectiveness of using several different hard structures to estimate the age of various fishes, including gadiforms, to which *Antimora* spp. belong (Sinis et al., 1999; Kuznetsova et al., 1999; Rovnina, 2006; Buslov, 2009; Ma et al., 2011; Uzunova et al., 2020).

The purpose of this study was to compare various methods for estimating the age of Pacific flatnose speicmens using different hard structures. The relative effectiveness of the methods and structures tested may have implications for obtaining robust age estimates in

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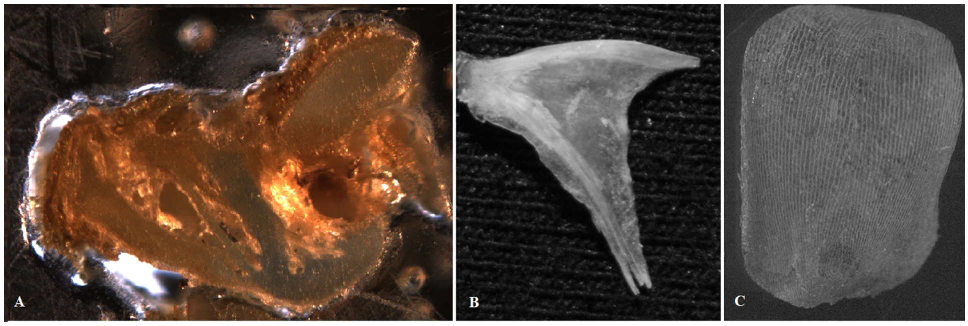

Fig. 1. A typical example of the image of the dorsal finray cross-section (A), operculum (B) and scale (C) of the Pacific flatnose.

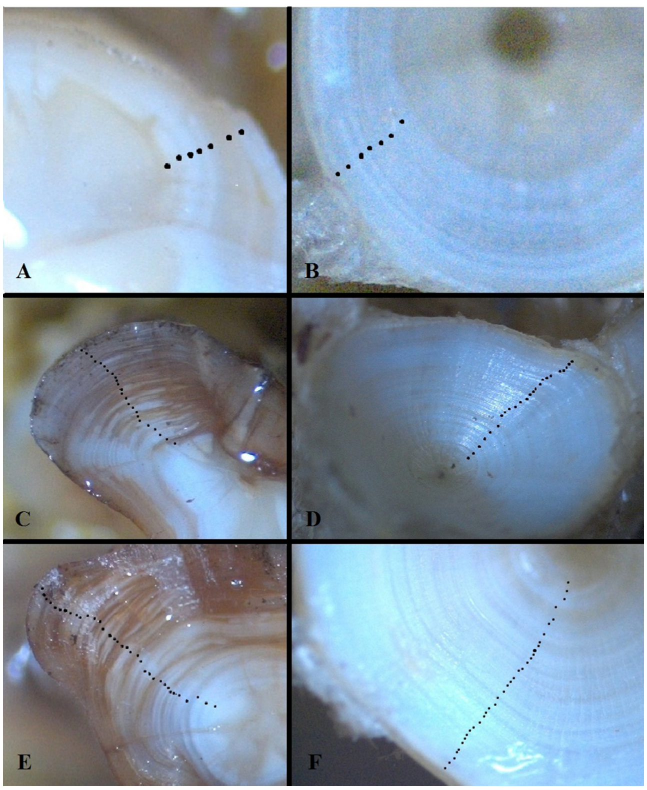

Fig. 2. The surface of the otolith (A, C, E) and the vertebra (B, D, F) of the same individual of the Pacific flatnose: A, B - 7 years with a total length of 19.5 cm; C, D - 24 years with a total length of 39 cm; E, F - 35 years with a total length of 67.4 cm.

other deep-sea fishes.

2. Material and methods

Five hard structures were extracted from 33 frozen individuals of Pacific flatnose caught during bottom trawl surveys in the waters of the West Coast of the United States by the Northwest Fisheries Science Center in 2007, 2010 and 2015 (Keller et al., 2017). These specimens were taken within an area bounded by coordinates 32° - 47°48´N and 117°44´ - 125°42´W, within a depth range 467 – 1256 m. From each individual, gill covers (operculum), trunk vertebrae, otoliths (sagitta), pectoral finrays, and scales were extracted. We used standard break-and-burn methods to make age estimates based on otolith annuli (Chilton and Beamish, 1982). Otoliths were broken in half transversely (through the nucleus) with a lancet and burned over an alcohol flame. If necessary, the exposed surfaces were polished using 0.1–0.9 μm grit abrasive discs (Buehler, USA) coated with aluminium-oxide or silicon-carbide. Burnt surfaces were coated with glycerine and illuminated with reflected light. We used an Olympus SXZ12 microscope (Japan) with a DFPLAPO 1 × PF lens and built-in camera Zeiss Stemi 305 (Germany) to view and photograph the surfaces at 1 × 20–40 magnification.

Age estimation based on vertebrae annuli followed methods that have proven to be effective in other gadiforms (Buslov, 2002, 2009). Four to five vertebrae immediately posterior to the skull were removed from each specimen. Individual vertebrae were separated, cleared of tissue, and then dried. Age estimates were made by counting annuli on one side of the vertebrae under 10-fold magnification.

To prepare finrays cross-sections, the first dorsal finray was cut out with scissors and air-dried in a parchment envelope. We encased the finray in epoxy resin and allowed it to solidify over 24 h. Then, using a microtome SM2000R Leica (Germany), we cut approximately 1 mm cross-sections perpendicular to the ray axis. Sections of each finray were placed on a separate glass slide and viewed under a binocular microscope. Scales were prepared for age reading according to methods that have been applied to a wide range of fish species (Chugunova, 1952, 1959; Pravdin, 1966; Kafanova, 1984). The scales were taken from the lateral surface of the body between the second dorsal fin and the lateral line, attached to a slide, cleaned of mucus with a solution of ammonia, and viewed under a binocular microscope. The bones of the operculum were cut from the head with a scalpel, and the skin, pellicles (membranes), and mucus were removed. Then the bones were dried, degreased with an alcohol solution and viewed under a binocular microscope.

All images of the various structures were photographed using a Zeiss Stemi 305 digital camera (Germany) and were analyzed using Adobe Photoshop CS6, ver. 13.0 × 64 software (Adobe System Incorporated, USA). The surfaces of the otolith and vertebra were photographed in high resolution (minimum 600 dpi). Since the vertebrae is cone-shaped, and it is not always possible to polish otolith sections into a flat surface, multiple images of each structure were taken with different focus settings in order to view all visible growth zones. Images showing the entirety of the growth zone from the nucleus to the edge of the structure were typically used to make final age estimates, though partial views of growth zones in other regions of the structure could also help to distinguish annuli. The annuli were determined as a combination of two adjacent light (translucent) and dark (opaque) bands.

In order to avoid bias, each otolith and vertebrae was examined by three independent readers. The average percent error (APE) index was calculated according to the methods proposed by Beamish and Fournier (1981) for comparison of age determinations by different readers. Age for an individual fish was determined as a mean age based on the three independent readings of otoliths and vertebrae. Between-reader age determinations were based on pairwise comparisons and were considered consistent if APE values were less than 10 % (Arkhipkin et al., 2008). In addition, otoliths and vertebrae were viewed separately, in an arbitrary order, to avoid bias in assessing the age of the same fish using different structures.

A Student's t–test was used to calculate the significance of the

Table 1
The values of APE indexes (%) for annuli counts on otolith and vertebrae surfaces based on the readings of the three independent readers.

Readers/ structure	Reader 1 otolith	Reader 2 otolith	Reader 3 otolith	Reader 1 vertebrae	Reader 2 vertebrae	Reader 3 vertebrae
Reader 1 otolith						
Reader 2 otolith	7.82					
Reader 3 otolith	8.83	9.78				
Reader 1 vertebrae	0.42	4.73	9.23			
Reader 2 vertebrae	4.07	4.09	5.38	3.96		
Reader 3 vertebrae	8.13	8.11	5.36	8.24	8.02	

Table 2
The parameters of von Bertalanffi growth equation of Pacific flatnose *Antimora microlepis* (sample size = 33) and values of approximation confidence for age estimations obtained by otoliths and vertebrae readings.

Structure/ Parameters of von Bertalanffi growth equation	$L\infty$	K	t_0	R^2
Otoliths readings	118.31	0.020	4.7	0.7
Vertebrae readings	127.86	0.018	4.9	0.7

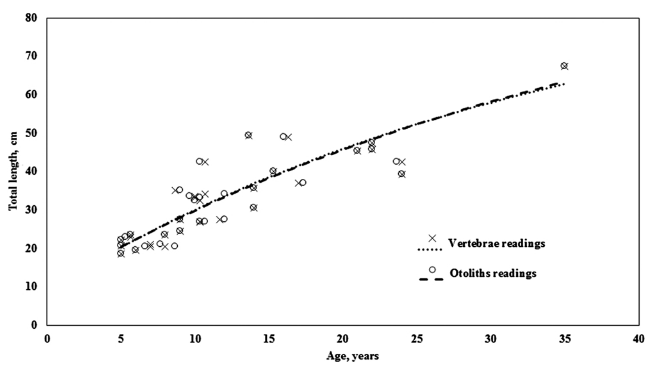

Fig. 3. The relationship between total length of Pacific flatnose and its age estimated by different methods.

differences in age estimates obtained using each method (Zolotov, 2006; Zolotov et al., 2006). Growth was characterized using a von Bertalanffy growth equation calculated using PAST ver. 3.14 software (Hammer et al., 2001), which is well suited for describing the age and growth rates of long-lived fish, including members of the genus *Antimora* (Magnússon, 2001; Fossen and Bergstad, 2006; Horn and Sutton, 2015; Orlov et al., 2018; Vedishcheva et al., 2019; Korostelev et al., 2020). The value of R^2 in relationship between age and total length was determined using the least squares method, reducing the relationship to a linear form (Frey et al., 2017).

3. Results and discussion

Our comparison of various hard structures obtained from Pacific flatnose specimens showed that annuli were not clearly visible on the cross-sections/surfaces of the finrays, scales, or operculum (Fig. 1). The finrays and gill covers appeared slightly calcified, the number of visible rings to be insignificant, and in some individuals they appeared to be absent altogether. On the scales, the sclerites are arranged in rows, but the allocation of annual rings was very difficult to discern. However, annuli were clearly visible on both the broken and burnt otolith surfaces and the cleaned vertebrae (Fig. 2).

The calculations of annuli might be considered as quite repeatable since the APE indexes for readers 1, 2 and 3 were 0.42 %–9.23 % (Table 1). Variations between the age estimates obtained using otoliths and vertebrae for each specimen were insignificant ($p > 0.05$). The von Bertalanffy growth curves produced using each structure were therefore extremely similar (Table 2). High values of the approximation confidence value ($R^2 = 0.7$) indicate that the theoretical curves of this relationship correspond well to the experimental data (Fig. 3).

It is possible that the obtained $L\infty$ values are overestimated due to the very small sample size and the presence in the sample of a single large individual with *TL* 67 cm. Similar overestimations are found in other studies, for example, in Orlov and Abramov (2002), where $L\infty = 125.9$ cm (n = 109) and Fossen and Bergstad (2006), where $L\infty = 2332.0$ cm (n = 68). Frey et al. (2017) observed an $L\infty$ of 61.2 cm (n = 247), but noted that this value was likely low due to length-at-depth and sex ratio data suggesting that the largest and oldest individuals may inhabit depths beyond the 1280 m limit of sampling. A larger, more representative sample would be needed to improve the accuracy of the growth curve for this species.

4. Conclusion

Out of the five types of structures we examined, only otoliths and vertebrae proved suitable for making age estimates of Pacific flatnose specimens. Small differences of one year in the otolith vs vertebrae annuli counts of three individuals were most likely the result of a subjective (human factor), rather than a methodological error. It should be noted that the Pacific flatnose, like many deep-sea fishes (Cailliet et al., 2001), is a long-lived species for which age estimation can be technically difficult due to the density of annuli and other growth checks (Fossen and Bergstad, 2006; Horn and Sutton, 2015). However, the close fit between annuli counts found here using broken otolith and vertebrae surfaces proved encouraging. Based on our results, vertebrae annuli may provide useful supplemental information for making age estimates in deep-sea fish species.

CRediT authorship contribution statement

N.B. Korostelev: Methodology, Formal analysis, Writing - review & editing. **P.H. Frey:** Resources, Data curation, Methodology, Validation, Writing - review & editing. **A.M. Orlov:** Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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