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Sexual maturity of female golden grey mullet, *Chelon aurata* and leaping mullet, *Chelon saliens* in the Southwest Caspian Sea using otolith

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ABSTRACT

Otoliths were used to investigate the first sexual maturity, an important factor for conservation, in females of two economically and ecologically important mullet species, *Chelon aurata* and *Chelon saliens*. Samples were taken at the Kiashahr port in the Southwestern Caspian Sea during 2020-2021. The chi-square independence test and repeated measures analyses were used to examine the age/length composition and to compare the width of the annual rings of each species. The length range of *C. aurata* (n = 38) and *C. saliens* (n = 48) were 20.5-42 and 18-26.5 cm respectively. The width of the dark ring in the second year of growth in the females of these two species were measured. The results indicated that the female *C. aurata* and *C. saliens* reach sexual maturity at 2nd and 3rd year of age, respectively. These findings provide valuable benchmarks for the timing of first sexual maturity of mullets in the Southwestern Caspian Sea and highlight the significance of age-based otolith analysis in fisheries management.

Keywords: Mullet fish, Otolith, Sexual maturity; Age determination; Opaque and translucent zones.
Article type: Research Article.

INTRODUCTION

Mullets rank as the second most abundant bony fish caught along the Iranian Caspian Sea coast, just after *Rutilus kutum* (Ghaninejad *et al*. 2017). If artificial reproduction and release programs for *R. kutum* were not implemented, mullet would take first place (Fazli *et al*. 2008), exhibiting the significance of these species in bony fish catching (Fazli *et al*. 2008). Fisheries biologists analyze calcium structures to estimate the age, growth, mortality rate, and time of first sexual maturity of fish, and also use these parameters to model life history and fish population dynamics (Ebener *et al*. 2008; Muir *et al*. 2008; Beverton & Holt 2012). The otolith is less vulnerable to mineral degradation compared to other calcium-based structures, making it particularly useful in the study of microchemical compositions and life history (Thorrold & Hare 2002). A pair of translucent and opaque rings compose the annual rings of the otolith. Opaque (calcified) bands are formed through the spring and summer, while less calcified and transparent (protein-rich) bands are formed during fall and winter (Davenport & Stevens 1988; Moulton *et al*. 1992). Genetic, physiological, and environmental factors influence the fish first sexual maturity and life history events (Nikolskii 1969). At the first sexual maturity, it is not possible to determine the exact body size of the fish; thus, the body size at which 50% of the fish enter the stage of sexual maturity is regarded the body size at first sexual maturity (Vazzoler 1996). The abrupt change in the band width of the annual growth rings of fish otolith, and also their distance from the central core of the otolith, are used to investigate the effects of environmental changes and events that occur in fish life history (Wilson & McCormick 1997; Bakhshalizadeh *et al*. 2017). When energy is diverted to reproductive care, physical growth is restricted (Roff 1983; Saborido-Rey & Kjesbu 2012). The reduction in growth of the body's hard structures is displayed as a

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decrease in the distance between the growth rings, which is used as an indicator to identify whether the fish is mature or immature (Trippel & Morgan 1994). Furthermore, significant energy consumption in reproduction causes a decrease in otolith growth and a change in the proportional width between translucent and opaque areas (Irgens *et al*. 2020). This transition zone implies a physiological shift accompanied by a reduction in physical growth (Francis & Horn 1997). Previous studies have used otolith-based research for the study of age, growth, and sexual maturity timing in a diverse range of teleost species (Campana & Thorrold, 2001; Calizo *et al.* 2024). For instance, research on *Gadus morhua* (Irgens *et al.* 2020) and *Micropogonias undulatus* (Hales & Reitz 1992) revealed that a decrease in the otolith increment width indicates the onset of sexual maturity, which coincides with a physiological reallocation of energy from somatic to reproductive growth. Microstructure analysis of otoliths has also been used successfully to assign maturation status in indeterminate growers with overlapping age classes (Hüssy 2010). Even though such techniques are common in the majority of marine and temperate fishes, hardly any studies have explored maturity assessment based on otoliths in the Mugilidae family, mainly in *Chelon aurata* and *Chelon saliens*. This study fulfils an important niche using otolith ring structure analysis to establish the size at first sexual maturity in *C. aurata* and *C. saliens*—two ecologically important and economically relevant mullet species of the Southwest Caspian Sea.

MATERIALS AND METHODS

Sampling was conducted at a depth of 5-7 meters, 800 to 1000 meters from the coastline of Kiashahr Port, Guilan Province, in the Southwestern Caspian Sea during the fishing season. The sampling of the first batch of golden mullet was carried out in the first half of October 2020, while the sampling of the second batch was done at the beginning of April 2021. Small mesh nets of 16 mm were used to collect samples of *Chelon saliens* in Kiashahr Port by the end of May 2021. The sampling times were chosen based on the fact that the gonads of the specified species are ripe at these times, so mature individuals could be easily distinguished. A total of 38 samples were taken for each mullet species. Fish handling was performed under the European and Iranian guidelines on animal welfare (Ref No: 6959/P15; 20.4.2024). Length was measured with a biometric board to the nearest 1 cm, and the weight of the fish was measured using a digital scale to the nearest 1 gram (0.001 lb). Necropsies were carried out to ascertain the sex of the fish, after which female specimens were set aside for otolith removal. The age of the fish was determined by examining the removed otoliths. Otoliths were prepared using the burn and crack technique. Each otolith was heated over a flame until brown, and then broken in half with a scalpel across the short lateral axis, as close to the nucleus as possible. The surface of a transverse section of the prepared otoliths was covered with a drop of emersion oil on the surface and examined under a dissecting microscope. To investigate and identify the age and sexual maturity of the fish, the dissected otolith was placed under a stereo microscope with 10× and 15× magnification. While stereo microscope lighted from above, the age of the fish was determined by counting the light rings (translucent zones). To strengthen the contrast between annual growth rings, pure glycerin was employed (Gebremedhin *et al*. 2019). To assess the first sexual maturity of mullet species, we employed a detailed otolith analysis technique. Initially, the central focus of the otolith was identified. Subsequently, the ventral surface of the otolith was imaged, and the axis was marked to ensure consistent orientation. Using a calibrated stereomicroscope, we measured the distances from the central focus to each annual growth ring along the predetermined axis. This method allowed for precise quantification of growth increments, which are indicative of age and, by extension, sexual maturity. The application of this technique is well-documented in ichthyological studies. For instance, the FAO's guidelines on otolith preparation and measurement emphasize the importance of consistent orientation and precise measurement techniques to accurately determine fish age and growth patterns. Furthermore, studies have demonstrated the effectiveness of stereo microscopy in visualizing and measuring otolith growth rings. For example, research on the forkbeard (*Phycis phycis*) utilized stereo microscopy to photograph otoliths and measure growth rings, confirming the reliability of this method for age determination (Dulčić 2011). In order to determine sexual maturity, the central area of the otolith was identified first. Then, the distances of the annual growth rings from the center along the axis were measured. A calibrated stereo microscope was used to take the measurements on the right annulus. False rings, which were close, compressed, and irregular, made difficult determining the age of fish, particularly those that were over 5 years old. As a result, the first reader (student) read both halves of the same otolith twice at different times. The Paired T-Test compared the age read from each pair of left and right otoliths of the mullets, then all the otoliths on one side of the fish were read by the second expert reader and the percentage of agreement (PA) was calculated.

If the ages read by two readers were close to each other, it was recognized as the age of the fish. For the fish above 5 years old, a one-year difference between two readers was accepted, and the age read by the first person was recorded as the final age. The coefficient of variation (CV) was also used to calculate the accuracy of the age read by two readers (Chang 1982). The coefficient of variation is the ratio of the estimated age's standard deviation to its mean:

$$CV_j = 100 \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(x_{ij} - x_j)^2}{R-1}}}{x_j}$$

where Xij denotes the determined age for month j, Xj is the estimated average age for month j, and R denotes the number of times the age is determined. The average error percentage (APE) between two age reading periods was obtained using the formula of Beamish & Fournier (1981) to check the accuracy of the predicted age of otoliths:

$$APE = \frac{100}{N} \sum_{j=1}^{N} \left[\frac{1}{R} \sum_{i=1}^{R} \frac{|Xij - Xj|}{Xj} \right]$$

Xij is the age established for the jth fish, and Xj is the average age of the jth fish. To compare the ages of fish at two separate times, a paired sample T-test was performed. Finally, the analyses were performed on the otoliths of the right side of the mullet, whose age was read three times by the first person. The average of the three age readings by the first person differed by less than 10%. The cumulative width of the first year of growth was calculated as the sum of the width of the otolith (natal) and the width of the opaque and translucent rings of the first year of growth. "The width of each year's growth ring was added to the cumulative width from the previous years for subsequent years. Since the otoliths were hand-cut and sometimes broke, some samples became unsuitable for measurement due to severe wear, breakage, or extensive burning, and were unavoidably destroyed. Finally, 37 otoliths of *C. saliens* and 35 otoliths of *Chelon aurata* were measured to determine the first sexual maturity of these two species. Annual ring widths, distances from each other, distances from the center, as well as the cumulative width of the annual rings from the center to the end of each ring, were measured along the main axis (Brennan & Cailliet 1989) to the nearest 1 micrometer (Chosen side were done by agreement). The lengths of the portions were measured and documented. By default, in otoliths whose last age ring was not finished, the last year of growth was not measured. Kolmograph-Smirnov (One-Sample K-S) test was used to check the normality of data. After verifying the normality of data, the age and length composition of each species was examined using the chi-square independence test. The width of annual rings was compared using the repeated measures statistical method to calculate the age of first sexual maturity (Chambers 1995). Greenhouse-Geisser F-correction was employed with a probability level of 95% for the violation of symmetry (Winer 1971). The level of significance was determined using the Bonferroni procedure. The paired sample t-test was used to compare averages that showed a significant difference from each other. Furthermore, it was used to assess the ring width before- and after the first sexual maturity. The tests' reliability coefficient was 0.05, and the graphs were generated using the mean standard error. Sections were photographed using KEW software on a computer linked to a micrometer-calibrated stereo microscope. The SPSS, and Excel software was used for analyzing and graph plotting respectively.

RESULTS

The average length, weight, and age of female *Chelon aurata* and *Chelon saliens* were calculated (Table 1). The former fish body length ranged from 20.5 cm to 42 cm, while the latter ranged from 18 cm to 26.5 cm. *C. aurata* was estimated to be between 2 and 8 years old, with an average age of 3.5 years. *C. saliens* ranged from 3 to 6 years in age, averaging 4.1 years. There was no statistically significant difference in age determination between the two readers (golden mullet: $p = 0.44$, $df = 40$, t = 0.781; narrow-snouted mullet: $p = 0.10$, $df = 78$, t = -2; Figs. 1 and 2).

Average error percentages (APE) for *C. aurata* and *C. saliens* were 100% and 95% respectively. In addition, calculated CV for these species were 6.42 and 6.92 respectively. The investigation of the growth rings revealed that the compression of the growth rings (annual rings) along the otolith's edge was considerably stronger, and they were farther apart close to the center (natal). This distance steadily reduces as they move away from the center towards the edges, and also compression increases as they come closer to the edge (Figs. 3, 4, and 5).

Table 1. The length, weight, and age structure of Female's *Chelon aurata* and *Chelon saliens* in the southwestern of the Caspian Sea.

parameter		**Female**	
		Chelon aurata	***Chelon saliens***
Total length (cm)	Average	31.15	23.50
	Minimum	20.5	18
	Maximum	42 26.5	26.5
Weight (gr)	Average	270.72	86.49
	Minimum	158	36.34
	Maximum	582	117
Age (years)	Average	3.5	4.1
	Minimum	2	3
	Maximum	8	6
Total		38	40

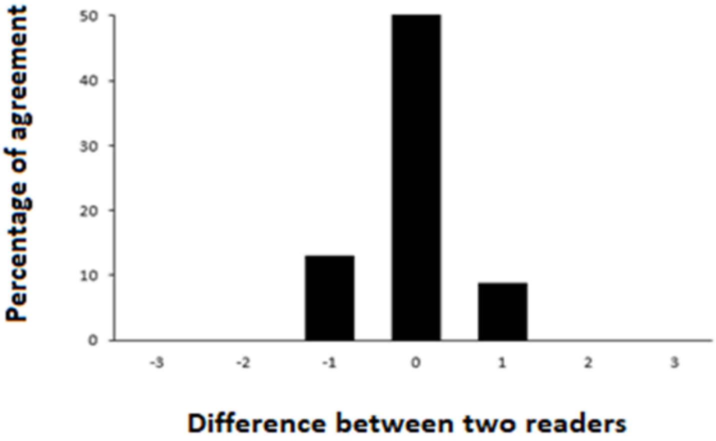

Fig. 1. The percentage agreement between two age readers on the age of *Chelon aurata*.

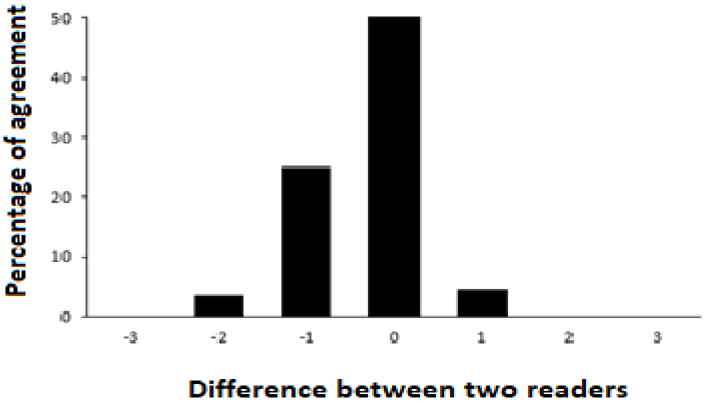

Fig. 2. The percentage agreement between two age readers on the determined age of *Chelon saliens*.

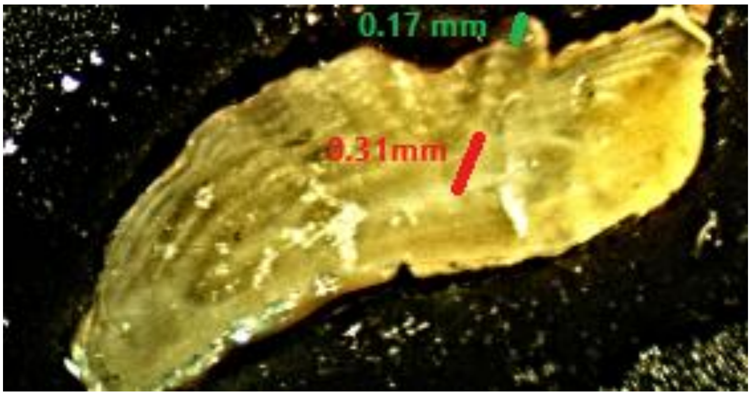

Fig. 3. Otolith of a female Chelon saliens (L = 24.2 cm, W = 102 g, age = 6). The otolith center width is 0.15, the first-year growth ring width is 0.31 (the highest value), and the last-year growth ring width is 0.17 (the lowest value).

As the section edge was approached, the annual rings became more compressed. The cumulative width of the rings, as depicted in Figs. 4 and 5, demonstrated a decline in the otolith diameter growth by elevating fish age. (Figs. 4 and 5).

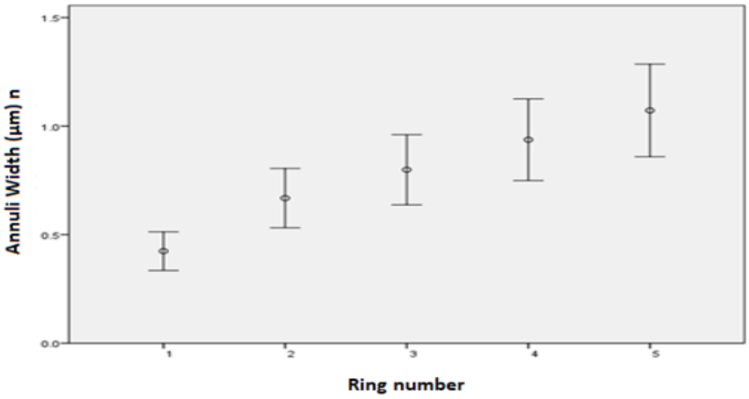

Fig. 4. Cumulative mean width (o) and standard error of mean width (I) of annulus profiles over the previous five rings from the centrum along the main axis of the female otolith section of *Chelon aurata* in the southwestern Caspian Sea.

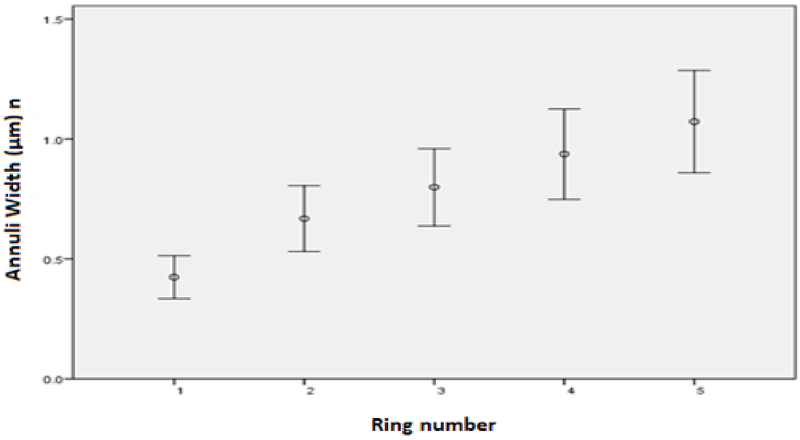

Fig. 5. Cumulative mean width (o) and standard error of mean width (I) of annulus profiles over the previous five rings from the centrum along the main axis of the female otolith section of *Chelon saliens* in the southwestern Caspian Sea.

While the annulus width of female *Chelon aurata* differed significantly in the second year of growth from the width of the annulus before and after it, the width of the annulus of female *C. saliens* differed significantly in the third year of growth (Figs. 6 and 7).

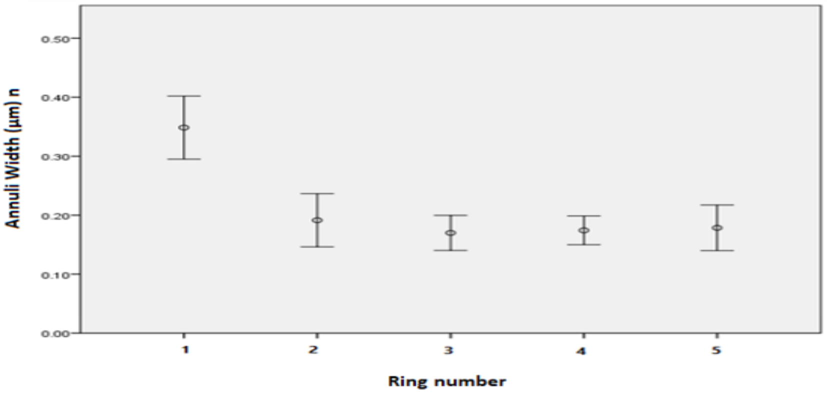

Fig. 6. Mean of annuluses profile (o) and standard error of mean width (I) of Chelon aurata otolith growth.

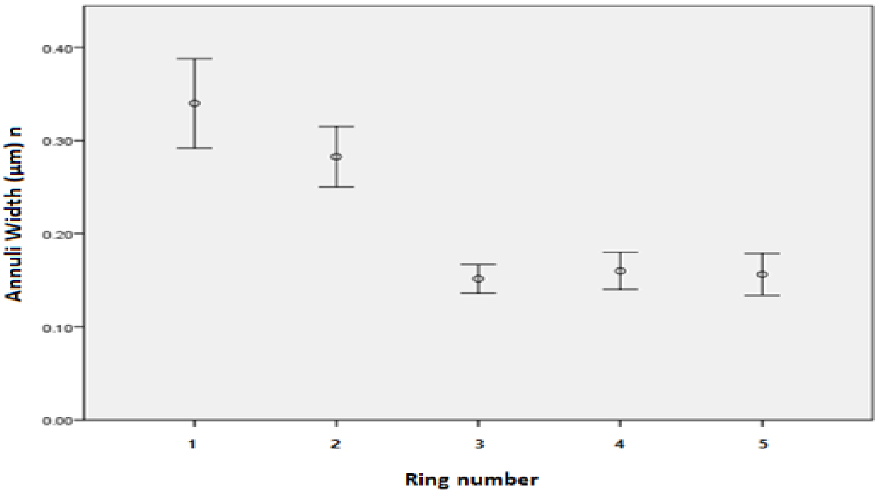

Fig. 7. Mean of annuluses profile (°) and standard error of mean width (I) of *Chelon saliens* otolith growth.

DISCUSSION

Most of the previous researches suggested that otoliths could be a useful tool for estimating fish dynamics and evolution. The average error percentage (APE) and coefficient of variation (CV) were deemed acceptable, as indicated by Campana (2001), which stipulates that APE and CV levels below 5.5% and 7.6%, respectively, are satisfactory. While gonadal examination provides direct insights into the reproductive status of fish, otoliths offer several advantages. Otoliths form annual growth rings, allowing precise age estimation, which is crucial for understanding reproductive cycles and maturity stages (Agostinho 2000). Although otoliths can be analyzed by sacrificing the fish, they retain growth information throughout the fish life, enabling studies of long-term growth patterns and environmental influences (Silva *et al.* 2024). Therefore, combining gonadal examination with otolith analysis enhances the accuracy and depth of reproductive studies. Otolith rings, particularly the first few annual rings, can indicate the age at which fish reach sexual maturity. The number of rings correlates with the age at which fish attain sexual maturity, providing a timeline for reproductive readiness (Silva *et al.* 2024). Changes in otolith growth patterns can reflect shifts in reproductive investment, such as energy allocation to gonad development (Palazzo *et al.* 2022). By comparing otolith growth patterns across different populations or environmental conditions, researchers can infer variations in reproductive strategies and maturity (Palazzo *et al.* 2022). So, otolith analysis provides a chronological framework that, when integrated with gonadal examination, offers a comprehensive understanding of fish reproductive biology. Characteristics such as annual ring patterns, distance between them, and regularity among them were highlighted as valuable features to identify indirectly distinct mullets' first sexual maturity. Besides the environmental factors that impact the formation of annual otolith rings, ontogenetic factors like the quality and availability of food resources have also been suggested to influence the annual ring patterns (Bakhshalizadeh *et al.* 2019, 2021). The results of this study indicated that the width of the translucent and opaque rings in the second year of growth of female *Chelon aurata* and the third year of growth of female *Chelon saliens* had a significant difference with the width transluscent and opaque rings before and after it. Therefore, the first sexual maturity of *C. aurata* occurred at the 2nd year, while that of *C. salien* at the 3rd. Tereshchenko (1950) stated that in the Caspian Sea, male *C. aurata* matures at the 3rd year while female *C. aurata* at 4th year. In previous studies, maturity was measured using A50%, the age at which 50% of individuals are mature, and L50%, the length at which 50% of the fish are adults. The age of first sexual maturity of female *C. aurata* was estimated 4.8 years in Mazandaran Province (Daryanabard *et al*. 2008), while the L50% of female *C. aurata* was 43.26 cm in Guilan Province (Hajivand *et al.* 2014). The reduction in the age of sexual maturity, relative to other studies, serves as a strategy to sustain the population under adverse environmental conditions, where the significantly higher mortality rates of smaller and younger individuals, along with constraints on adult growth, significantly influence the selection for earlier sexual maturity. Since the age of the first sexual reproduction was estimated by inspecting the gonads, determining the maturity of fish during the non-reproductive season is a difficult task (Fontoura *et al.* 2018). Furthermore, the sample may not be typical of the full population, particularly if sampling event is conducted at or around spawning season, and the spatial distribution of spawners and non-spawners may differ significantly (Evans *et al.* 2012). Moreover, the age at first sexual reproduction is typically estimated by examining gonads, determining the maturity of fish outside the reproductive season can be challenging (West 1990; Murua *et al.* 2003). Additionally, the sample may not accurately represent the entire population especially if sampling occurs during or near the spawning season, when the spatial distribution of spawners and non-spawners can differ significantly (Hunter & Macewicz, 1985; Lowerre-Barbieri *et al.* 2011).

Environmental factors may decrease the age of sexual maturity. As fishing mortality increases in the adult and immature populations, evolution moves towards earlier maturity (Browman *et al*. 2000). Moreover, Coulson (2010) posited that, under certain conditions, the substantially higher mortality rates observed in smaller and younger individuals, coupled with constraints on adult growth, may play a critical role in the evolutionary selection for early onset of sexual maturity and prolonged lifespan. While this adaptive strategy is uncommon among fish that have previously attained maturity under favourable biological conditions, it may result in inaccuracies in age determination due to the difficulty in discerning changes in growth ring width, particularly in males (Francis & Horn 1997). Due to the more accessible economic costs, this method requires far fewer samples compared to traditional techniques. Considering the growing importance of animal rights, it is essential to understand the life history conditions of species in reserves to evaluate habitat health and the intensity of environmental pressures. Our study's findings provide a clear estimate of the age at sexual maturity for female mullets, offering both economic and ecological insights. By utilizing otolith profile sections, this crucial data contributes to understanding stock conditions, monitoring the exploitation rates of young fish, and supporting sustainable fishing practices. It also aids in predicting and evaluating fish stock levels. Moreover, incorporating otolith analyses across geographically diverse stocks and environmental gradients in future research will further clarify the interplay of regional and ecological variables affecting reproductive dynamics, thereby supporting robust, ecosystem-based management of these critical mullet resources.

CONCLUSION

Understanding the life history events on otolith profiles is crucial for fisheries management and conservation efforts. It aids in evaluating the adaptive management and harvesting practices in relation to the resilience and health status of the fish and fish populations. Ultimately, adaptive management techniques and conservancy techniques facilitate the establishment of sustainable harvesting. In addition, otolith analysis as an indirect method reveals in more detail the previously documented aspects of a fish's reproductive biology.

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