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**Analysis of jack mackerel otolith microstructure**

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## Analysis of jack mackerel otolith microstructure

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### ABSTRACT

The microstructure analysis of juvenile jack mackerel (*Trachurus murphyi*) collected in the northern coast (Arica to San Antonio) off Chile was carried out. The otoliths were examined on posterior sagittal plane (postrostrum) by light microscopy.

The general pattern of jack mackerel otolith growth shows the formation of secondary primordium (SP) that divided the sagittal plane of the otolith into a primary and secondary growth zone (PGZ and SGZ). The SP was formed between 23 to 51 days after hatching, and the SP formation in sagittal otolith is the same of the *T. japonicus* pattern, where the PGZ was enclosed by the SGZ in the dorsal, posterior and ventral areas, but not anteriorly (rostrum).

The increment width profile along the post-rostrum radius of sagittae showed the characteristic curve of fish on early stages. The increment width increased gradually from 3,4  $\mu\text{m}$  of the first increment, peaked about of 20  $\mu\text{m}$  ranged 50 to 80 days, then became progressively narrow until they reached a value of 2,1  $\mu\text{m}$  at 170 days old.

The relationship between length of fish and post-rostrum radius of otolith was described by a linear regression than explained the 90% of variance. This indicated that in the posterior axis (post-rostrum) of Chilean jack mackerel's sagittal otolith grew in a consistent direction during juvenile stage and proportional at size of fish.

The length-at-age was fitted with Laird-Gompertz model that explained the 80% of variability and fitted well the data to fishes less than 23 cm FL. The results of primary micro-increments analysis, presumably of daily periodicity, show high growth in juvenile Chilean jack mackerel.

Although the procedures used both in the preparation and the readings of primary micro-increments correspond to the ones used in this discipline, the presence of multiple rings, with a high presence of bi and tripartite structures in the fast growing zone (between 50 and 80 days), certain doubts arise regarding the interpretation which suggests the need to conduct a validation study of the periodicity of primary micro-increments in otolith of juvenile and adult fish.

## INTRODUCTION

Chilean jack mackerel (CJM), *Trachurus murphyi*, is a very important commercial pelagic fish of *Carangidae* family that inhabits the southern Pacific Ocean. Three fishing areas for Chilean jack mackerel can be identified on the coast of Chile: Northern fishery (18°21'S-24°S), Caldera-Coquimbo fishery (24°S-32°S), and southern-central fishery (32°S-43°30'S).

In the 1960-1995 period, the capture of jack mackerel grew in ascending form arriving at a maximum of 4.6 million tons. Later it began to decline until reaching 230,000 t in 2013 (Aranis et al. 2014). Jack mackerel is also exploited by foreign fleets outside the Chilean EEZ, mainly off the southern central area, by Russian, Cuban, Chinese and Vanuatu fleets, and Peru and Ecuador fleets in their EEZ.

The Chilean jack mackerel is widely distributed throughout the southeastern Pacific, ranging from the Galapagos Islands and south of Ecuador to southern Chile. Its current distribution also extends from south-central Chile across the Pacific Ocean, to New Zealand and Tasmanian waters (Evseenko, 1987; Serra, 1991; Elizarov et al., 1993; Taylor, 2002).

The jack mackerel population can be characterized by a spawning area from 35° to 40°S and to 90°W; a coastal feeding habitat of adults in the southern-central- area off Chile (33°S-40°S), where the juveniles are recruited, and a nursery habitat north of 30°S in warm oceanic and coastal waters (Arcos et al., 2001).

Peru's researchers suggest an isolated stock off the Peruvian coast, but the SPRFMO Jack mackerel scientific working group considered the existence of a whole stock for the South Pacific.

The age composition, in the jack mackerel catch, has particularly been a relevant element for understanding the changes in the stock. The Fisheries Research Institute (IFOP)

has been carrying out studies on ageing and growth since late 1970's, which have been used for developing size-age keys and catch-at-age matrix per zone in quarterly basis, that are input data for the stock assessment.

The von Bertalanffy growth parameters of Chilean jack mackerel estimated by different authors through whole otolith analysis show similar result with  $L_{\infty}$  asymptotic ranged 65 to 80 cm in fork length (FL),  $K$  until 0.07 to 0.1 (Kochkin, 1994; Castillo and Arrizaga, 1987; Gili et al., 1996; Gang Li et al., 2011; Cerna and Bocic, 2011). These authors estimated that Chilean Jack Mackerel have medium longevity with maximum age observed between 9 to 17 years old.

The ageing of jack mackerel is supported in indirect age validation as periodicity of growth increment formation, which indicated the hyaline ring formation by year in winter seasons (Serra and Gili, 1995; Castillo and Arrizaga, 1987). Other studies allowed to confirm the ring identification criteria through comparison or precision analysis with senior research that shows high accuracy in most ages (1 to 10 years) and a slight underestimation of age by the IFOP reader from age 11, but in general concluded that the reading jack mackerel tests carried out have shown that the precision of the regular readers is high and adequate, while the variability between readers was considered acceptable and within normal limits of error (Morales-Nin, 1997).

The actual indirect validation of Chilean jack mackerel only considered determining the frequency of formation of a growth increment for sample of fish, with edge analysis method, could be necessary but insufficient to obtain an accurate age determination.

Recently Goicochea et al. (2013) using microstructure analysis of transversal section of otoliths validated the first annual ring, based in the count of microincrements previously validated the daily periodicity of these in adult fish by Araya et al. (2003). This result indicated that the mean length at 365 day was 17.7 cm in fork length by the mean otolith radio of 2.49 mm. Other results included the indirect validation of second and third annulus with length frequency mode and estimated the von Bertalanffy growth parameters:  $L_{\infty}$ =

75,17;  $k=0.165$ ;  $t_0=-0.817$ .

The objective of this study was to analyze the otolith microstructure, estimate the daily age of juvenile Chilean jack mackerel and length-at-age relationships in order to identify the first annulus.

## MATERIAL AND METHODS

The juvenile jack mackerel was captured aboard of scientific vessel off west northern coast of Chile ( $18^{\circ}25'$  to  $31^{\circ}30'$  S;  $70^{\circ}06'$  to  $71^{\circ}43'$  O) in December 2012 and February 2013 and second batch correspond to the central area ( $33^{\circ}00'$ –  $34^{\circ}00'$  S). A total of 64 pairs sagittae otoliths corresponded to fishes between 3 to 25 cm in fork length (FL) that were prepared (mounted, polished and photographed), of these 44 otoliths, with adequate readability, were analyzed. The left otolith was initially photographed under a stereomicroscope, with 20x magnification and reflecting light, to determine the annual age according to reading IFOP's criteria and the total radius (distance from core to edge of post-rostrum) was measured in each otolith. After that, the same otolith was mounted in epoxy resin on slide glass and hand polishing along sagittal plane using 30 and 1  $\mu\text{m}$ -grit sandpaper, according to the Plaza (2005) method.

The microstructure was analyzed using microscopy with 100x and 400x magnifications. The proceeding consisted of three steps: a) take one or two picture at 100x magnification; b) take a sequence of pictures at 400x from primordial to border; c) link the sequence of images for a full reading axis of each otolith. As the otoliths curved to the distal face, all the increments could not be observed in a single sagittal plane. The images were taken using Q-Imagen Evolution 5.0 camera on light microscopy connected to computer. After polishing, count of the primary increments were made twice, across of area of the otolith with best resolution increments by two independent readers in different time, the result of each reader was overage. A comparison between readers was carried out applied ANOVA in order to evaluate the precision on reading. Otolith radii ( $R_0$ ) and increment width were measured through a linear axis for increment counting and

measurement, using Caliper tool of Image-pro plus software. The widths were standardized to the maximum radius to minimize any possible variation from the linear trajectory of measurement.

We assumed that all otoliths's primary increments of juvenile *T. murphyi* have a daily periodicity, based on the experimental validation studies in adult fish of Araya et al. (2003) and work of Xie et al. (2005) that validated the daily increments in recruits and pre-recruits of *Trachurus japonicus*. These last authors indicated that the first increment was formed on the third day after the hatching in *T. japonicus* (Xie et al. 2005).

The otoliths of *T. murphyi* present secondary primordium (SP) that divided the otolith in primary growth zone (PGZ) and secondary growth zone or marginal growth zone (MGZ), similar to observed in *T. japonicus* by Xie et al. (2005). The differences in the physical and biological environments of spawning and nursery areas can cause morphometric variation, in the microstructure analysis included the count and measure as: numbers of SP, age of formation of each SP, formation time of PGZ, MGZ and width increments in each growth zone. The overage ring width for each day with the standard error was obtained as proxy of growth rate.

The relationship between  $FL$  and  $R_0$  was described by a linear regression and relationship between  $FL$  and age of juvenile was fitted by Laird-Gompertz model. The equation for the model was:

$$FL = L_{\infty} \exp[-\exp(-G \{X - X_0\})],$$

where  $FL$  is the fork length at age,  $X$  is age in days,  $L_{\infty}$  is the asymptotic length,  $G$  is the instantaneous rate of growth at age at  $X_0$ ,  $X_0$  is the inflection point of the curve and the age at which absolute growth rate begins to decline. These parameters were calculated by maximum likelihood using non-linear regression function in R software (Ihaka & Gentleman, 1996).

In order to validate the periodicity of the formation of the first macro-ring using

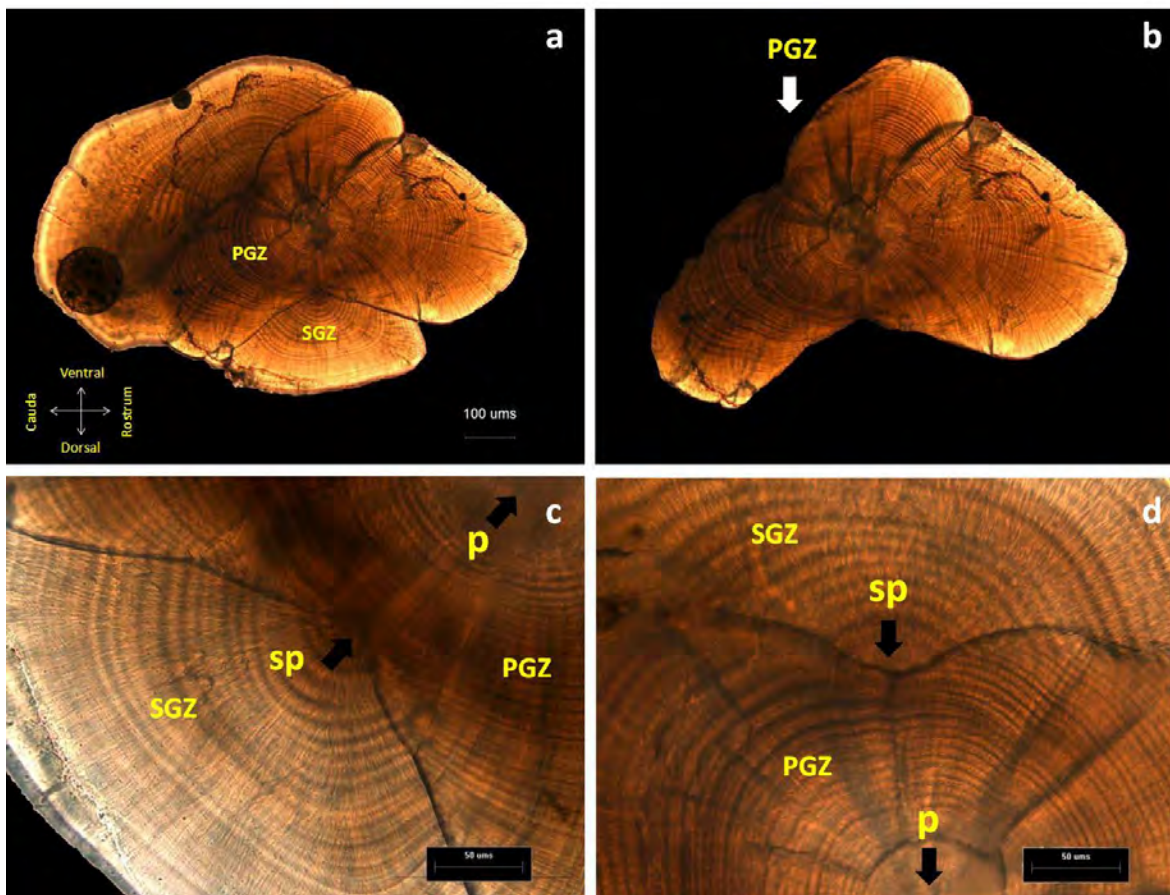
primary micro-increments, otoliths from fish of near one year old currently assigned were prepared. For the correct validation of the formation time of the first macro-ring, a comparative analysis between the reading of primary micro-increments and the prior reading of macro-rings was conducted, that is, age in years according to the current reading criteria.

## RESULTS

The general jack mackerel otolith growth pattern shows the formation of secondary primordium (SP), that divided the sagittal plane of the otolith into a primary and secondary growth zone (PGZ and SGZ) (**Fig. 1**). The time of formation of SP depends on the zone of the otolith, SP in ventral or dorsal zone were formed between 23 and 40 days after hatching, however the SP located in the rostrum-ventral, post-rostrum-ventral and post-rostrum-dorsal could be formed between 37 to 51 days after hatching. The PGZ extended from the first increment until about the 50th increment, in which increment was clear and without sub-daily rings, except to rostrum axis where PGZ extended until the edge (**Fig. 2 b y c**). In the outer margin of this zone began the formation of SGZ where was observed a new sequence of concentric microincrements born from the SPs (**Fig. 1 c y d**). The total numbers of SPs in an otolith (except in the rostrum area) ranged from 2 to 8 with mean  $\pm$  s.d. of  $5.3 \pm 1.5$ .

The counting of increments was sometime difficult at the beginning of SGZ, diffuse area between the increments 50 to 80, where the increment width were significantly larger than anterior and posterior area, with mean of  $19,9 \mu\text{m} \pm 8.1$  (**Fig. 2 d**). After this area the width of increments gradually decreases and it is possible to observe growth bands formed for thin rings that sometimes are not located at a regular distance (**Fig. 2 e**). When the periodicity of the microincrements has not been validated, two reading criteria could be used to solve this difficulty: one called Group Band Reading (GBR) ie. counts the packages of rings, that we used by jack mackerel, or other criteria called Individual Mark Reading

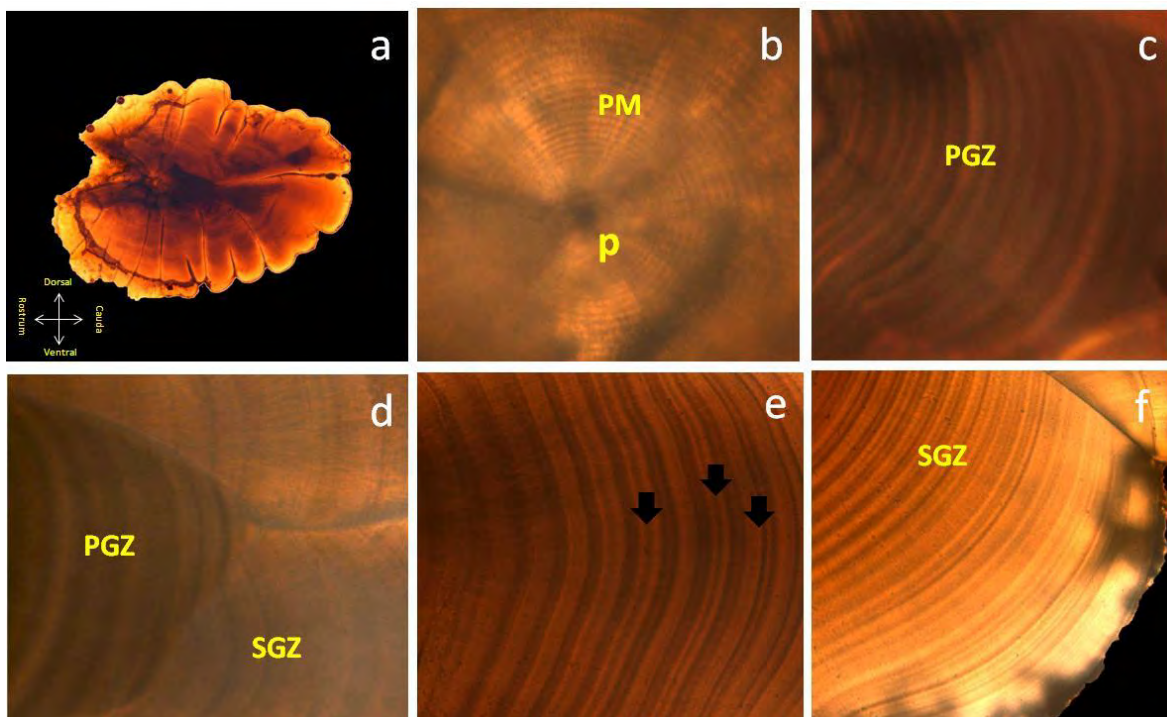
(IMR), that involve counting every clear increment independently from its appearance (Cermeño et al. 2006). For example, the otolith presented in figure 2 has 134 microincrements with the GBR method, or 191 microincrements with IMR reading method. Finally we observed thinner and clear rings near the edge of otoliths (**Fig. 2 f**). This pattern of micro-increment presented a high resolution in otolith by fish < 16 cm FL, into fish larger, the resolution is irregular and it is often necessary to change the reading axis. An ANOVA for the total microincrement counting showed not significantly different between readers ( $P=0.239$ ).



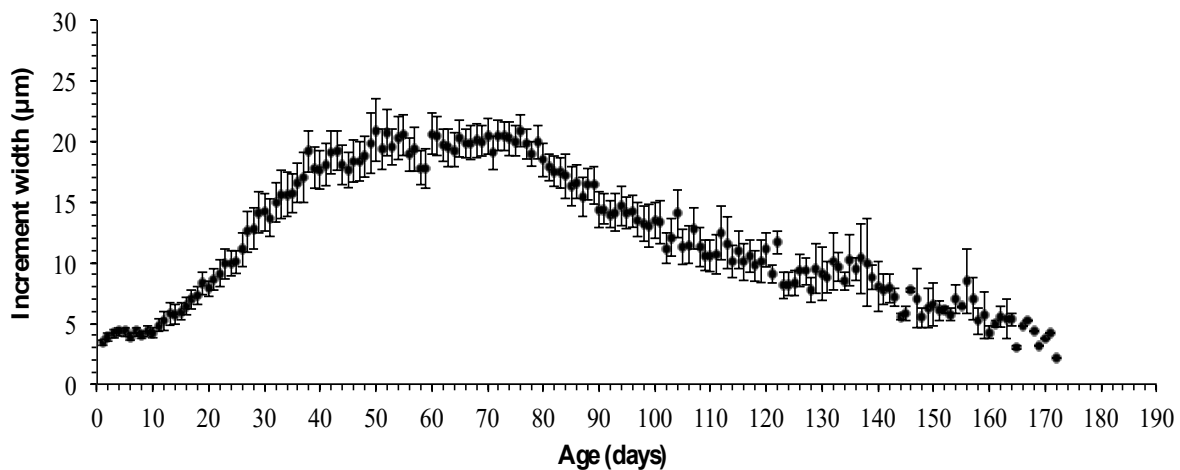
**Figure 1.** Microstructure of otolith sagitta of *Trachurus murphyi* of 3,6 cm FL and 62 days, catch in the North of Chile. The pictures **c** and **d** show the primordium (p) and secondary primordium (sp) that divided the otolith in a Primary Growth Zone (PGZ) and Secondary Growth Zone (SGZ). The picture **b** shows all extension of PGZ, that in the rostrum area reaches the edge.



The increment width profile along the post-rostrum radius of sagittae showed the characteristic curve of fish on early stages. Increment width increased gradually from 3,4  $\mu\text{m}$  of the first increment, peaked about of 20  $\mu\text{m}$  ranged 50 to 80 days, then became progressively narrow until they reached a value of 2,1  $\mu\text{m}$  at 170 days old (**Fig. 3**). The observed variation in otolith microstructure of Chilean jack mackerel juvenile may indicate variations in growth and developmental rates during larval and early juvenile stage. So, if used the increment width as the proxy of growth rate, a high growth rate occurred after 40 days old possibly associated to the end of larval stage and beginning of the juvenile period with a necessary change of diet and habitat. After the day 80 when ending the bigger growth period, the rate begins a gradual decline.

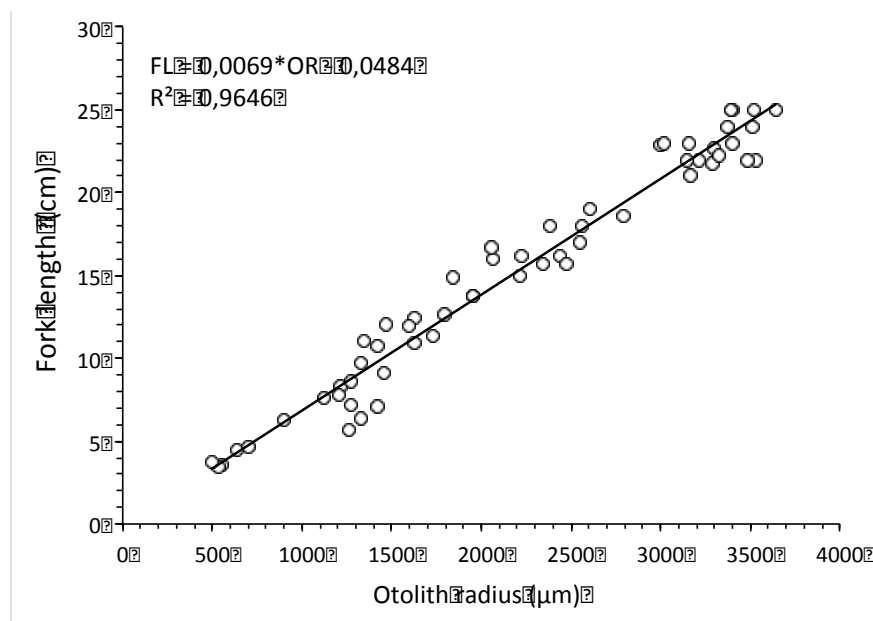


**Figure 2.** Sagittae otolith microstructure of *T. murphyi* with 10.7 cm FL. The pictures show the whole reading axis toward the post-rostrum. The picture **a)** shows the whole otolith; **b)** shows the initial primordium (p) and primary microincrement (PM); **c)** the middle area of PGZ; **d)** the end area of PGZ and start of SGZ; **e)** correspond at the middle area of SGZ where was observed the growth bands formed for two thin rings, the arrow indicated an possible sub-daily ring; **f)** shows the thin rings near to edge of otolith.



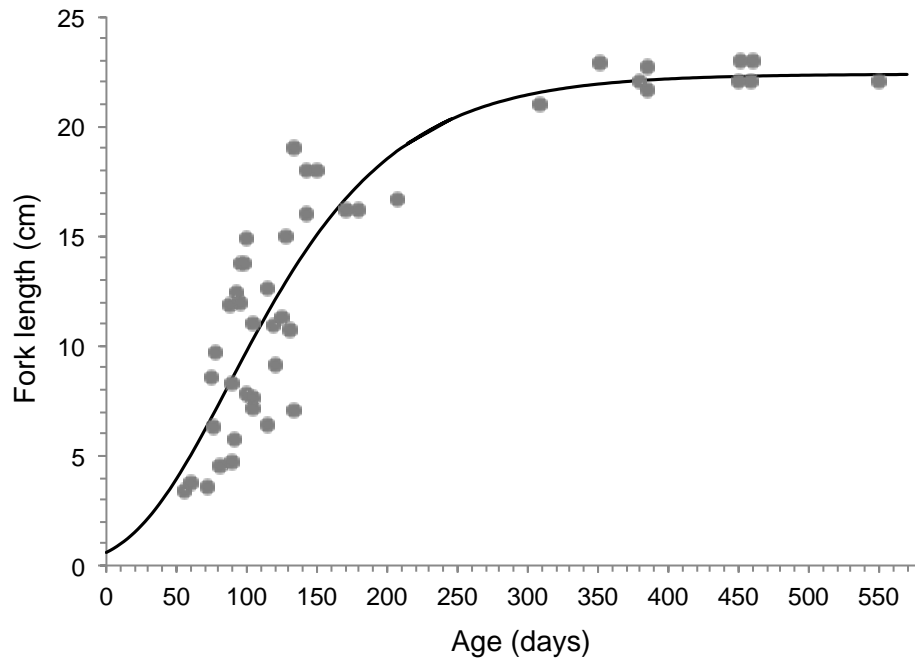
**Figure 3.** Increment width profile along the post-rostrum radius of sagittae otolith *T. murphyi*. Values are mean with standard error measurement in 21 otoliths of juvenile fish, collected in the north coast (Arica to Coquimbo) off Chile.

The relationship of FL to  $R_0$  was well described by a linear regression fitted by  $FL = 0.0069 R_0 - 0.0484$  with  $r^2 = 0.96$  to 57 otolith (Fig. 4).



**Figure 4.** Regression of fork length and otolith radius of *Trachurus murphyi* juvenile, collected in the northern coast (Arica to San Antonio) off Chile.

Length-at-age plots of the fish were significantly explained by the Laird-Gompertz growth equation (**Fig 5**). The parameter value obtained with standard error was  $L_{\infty}=22.4\pm0.97$  cm;  $G=0.015\pm0.003$  cm\*days<sup>-1</sup> and  $X_0=87.2\pm5.6$  days, with coefficient of determination of  $r^2=0.80$ .



**Figure 5.** Curve that fitted the length-at-age plots of *Trachurus murphyi* juvenile collected in the northern coast (Arica to San Antonio) off Chile.

For the macro-microstructure comparison, 51 early juvenile and adult fish with sizes between 3.4 and 23.0 cm FL, around the mean size of maturity (22.7 cm FL: Leal et al., 2013) were used. This allowed the identification of the first annulus using the relationship with the primary microincrements (PM) that we assume have a daily formation. Fish with an annual age, through the conventional otolith reading, of 0 and 1 year, present an age, through PM reading, down than 365 days. Fish with an annual age of 2 years have a mean age with their standard error of  $352\pm79$  days. Fish with annual age of 3 years have an age in days of  $489\pm58$  days, above one year of age (Table 1).

**Table 1.** Results of macrostructure and microstructure reading of 51 early juvenile and adult jack mackerel. The annual estimated age is compared to the range of for lengths, maximum radius of the otolith and the mean of the age in days with its standard error (DE), n corresponds to the number of otoliths read.

Age (year)	Fork length (cm)	Otolith radius ( $\mu\text{m}$ )	Age (days)	
	min - max	min - max	Mean $\pm$ SE	n
0	3.4 - 13.8	469.4 - 1954.9	96 $\pm$ 20	28
1	15.7 - 17.0	2060.0 - 2550.0	185 $\pm$ 34	5
2	18.6 - 23.0	2790.0 - 3290.0	352 $\pm$ 79	5
3	22.0 - 25.0	3150.0 - 3640.0	489 $\pm$ 58	13

## DISCUSSION

### *Characterization of the otolith microstructure*

The analysis of juvenile otolith of *Trachurus murphyi* shows the same general pattern of microstructure observed in *Trachurus japonicus* (Xie et al., 2005; Xie & Watanabe, 2005; Kanaji et al., 2010). In this species the otolith has an initial primordium and variable numbers of secondary primordium that begin to form around the 20 or 25 day after hatching. In Chilean jack mackerel we observed between 2 to 8 SPs, slightly different to Japanese jack mackerel with range 2-15 SPs (Xie et al.2005). These authors show that the numbers of anterior SPs continued to increase with otolith growth.

The generally pattern observed when the microstructure of otoliths involving multiple SPs, after a certain juvenile age the PGZ is totally enclosed by SGZ and after this closed no additional SPs are formed (Sogard et al. 1992; Lee and Kim 2000; Morales-Nin, 2000). However, the distinctive feature of jack mackerel observed in juvenile of *T. japonicus* and *T. murphyi* is that SP formation in sagittal otolith differs from this general pattern, because

the PGZ was enclosed by the SGZ in the dorsal, posterior and ventral areas, but not anteriorly (rostrum).

It has been proposed that SPs form because of habitat shift (Campana 1984; Sogard, 1991), ontogenetic dietary shift (Marks & Conover, 1993) and because of physiological changes (Hare & Cowen, 1994).

The increment width profile along the post-rostrum radius of *T. murphyi* sagittae showed a characteristic curve comparable to *Trachurus japonicus* (Xie & Watanabe, 2005; Kanaji et al., 2010), and *Trachurus trachurus* (Waldron & Kerstan, 2001). The Peak of increment is similar to *T. japonicus* with increments over 20  $\mu\text{m}$  between 40 to 80 or 100 days depending on the hatching date (Xie & Watanabe, 2005). These authors suggest the 40 days correspond to the ending of metamorphosis and the high growth rate of jack mackerel could be associated with trophic shift.

#### *Growth of fish younger than one year old*

It is known that daily periodicity of micro-increments was validated in adult of *T. murphyi* in fishes over 27 cm FL (Araya et al., 2003) but not in juvenile. The similar patterns of width micro-increment of juvenile Chilean jack mackerel with species in that have been validated the microincrements like *T. japonicus* (Xie et al. 2005), allows us to verify, but not to validate, the correct identification of microincrements performed in our study and supports our assumption that these rings could have daily periodicity.

The relationship between length of fish and post-rostrum radius of otolith was described by linear regression that explained the 80% of variance. This indicated that in the posterior axis (post-rostrum) of Chilean jack mackerel's sagittal otolith grew in a consistent direction during juvenile stages. This confirms the correct choice of reading axis in Chilean jack mackerel because according to Xie et al. (2005), otolith measurement along the posterior axis may minimize measurement bias resulting from shifts in otolith growth axes.

The age-size estimation to the date of catch was fitted for 45 fish that presented a resolution from the MP that allowed their reading. Data fitted properly to non-linear Laird-Gompertz model, showing a high growth rate in the first 200 days. This growth rate is higher than the one estimated for jack mackerel from Peru by Goicochea et al. (2013). Although such authors did not include the growth parameters, the same Laird-Gompertz model allows to observe a mean size at 150 days of approximately 9.3 cm total length, compared to the 15 cm in FL, at the same age, estimated in our study.

It is important to keep in mind that, the reading criterion used was GBR, that consists of counting the packages of rings. The experts in daily increment analysis, based on validation studies and experience, suggest GRB reading criteria, because the IMR criteria can lead to error at counting sub-daily increments, checks or discontinuities like daily increments (Cermeño et al., 2006; Campana & Neilson, 1985; Campana, 1992). However, the GBR criteria tend to get like result higher growth rates than the IMR criteria, as in the case of Chilean jack mackerel.

To resolve this controversy, the validation in juvenile fish is an essential aspect before any otolith microstructure investigation. Especially in Salmonidae and pelagic fishes, where sub-daily increment is more common than in others species (Neilson, 1992) for example, king mackerel *Scomberomorus cavalla*, and Spanish mackerel *Scomberomorus maculatus* (De Vries et al., 1990), *Engraulis japonicus* (Tsuji & Aoyama, 1984), *Engraulis encrasicolus* (Cermeño et al. 2006).

#### *Identification of the first annulus*

In the case of jack mackerel, the otolith macro-structure presents a series of growth permanent disturbances or growth stopping that generates wide areas with translucent rings, making it difficult to observe a clear alternation of opaque and translucent macro-rings.

This difficulty is also observed in the microstructure in which translucent macro-rings

do not represent a micro-structural pattern that accounts for a growth stoppage, with very fine or unresolved PM. Contrary, a continuity in the pattern of microincrement was observed with no apparent stoppage, being necessary to identify the position of the correct annulus, relate the number of microincrements to the distance (radius) which annulus are assigned by reader.

The relationship between microincrements and the radius of translucent macro-rings (distance from the core to the edge of the macro-ring) showed that the first macro-ring assigned to a year, in accordance with the annual reading criteria, does not correspond to the annulus, because the number of PM was lower than 200. Although in some otoliths small areas with unresolved PM were observed, adjacent areas show a clear sequence of PM, giving continuity to the microstructure pattern.

This pattern allows us to suggest that the first annulus would correspond to the second translucent macro-ring identified from the conventional ageing method of whole otoliths. Even though the translucent ring in some cases is not completely formed until the day 365, this approach support it in the fact that the year of life and/or annulus's position may vary depending on the birth cohort and spawning area, in a resource with wide distribution.

Although the procedures used both in the preparation and readings of microincrements correspond to the ones used in this discipline, the presence of multiple rings, with doubles and triple sets of microincrements in the fast growing zone (between 50 and 80 days), certain doubts arise regarding the interpretation, that will only be resolved with a validation study of the periodicity of primary micro-increments in otolith of juvenile and adult fish.

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