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Preliminary analysis of jack mackerel otolith microstructure

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The preliminary microstructure analysis of juvenile jack mackerel (*Trachurus murphyi*) collected in the north coast (Arica to Coquimbo) 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 were formed between 23 to 51 days after hatching, and SP formation in sagittal otolith is same to 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 um of the first increment, peaked about of 20 um ranged 50 to 80 days, then became progressively narrow until they reached a value of 2,1 um at 170 days old.

The relationship between length of fish and post-rostrum radium of otolith was described by a linear regression than explained the 90% of variance. This indicated than 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 98% of variability and fitted well the data to fishes smaller than 16 cm FL. The preliminary results, with micro-increments analysis presumably of daily periodicity, show high growth in juvenile Chilean jack mackerel.

INTRODUCTION

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

In the period 1960-1995 the capture of jack mackerel grew in ascending form arriving at a maximum of 4.6 million tons, beginning 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 south central area, by Russian, Cuban, Chinese and Vanuatu fleets, and by Peru and Ecuador fleets in their respective EEZs.

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 ranging from 35º to 40ºS and to 90ºW; a coastal feeding habitat of adults in the central-southern area off Chile (33ºS-40ºS), where the juveniles are recruited, and a nursery habitat north of 30ºS in warm oceanic and coastal waters (Arcos et al., 2001).

Peruvian researchers suggest an isolated stock off the coast of Peru. However, the Jack mackerel scientific work group of SPRFMO considered the existence of a whole stock for the South Pacific and in 2012 they have made the united stock assessment with this stock definition.

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 on a quarterly basis, which 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 results with L asymptotic ranged from 65 to 80 cm fork length (FL), K up to 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 has medium longevity with maximum age observed between 9 and 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 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 of jack mackerel tests carried out have shown that the precision of the regular readers is high and adequate, while the variability between readers is 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 the 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 on the counting of microincrements previously validating the daily periodicity of these in adult fish by Araya et al. (2003). These results indicated that the mean length at 365 days was 17.7 cm fork length with the mean otolith radio of 2.49 mm. Other results were the indirect validation of second and third annulus with length frequency mode and estimated the von Bertalanffy growth parameters: L_∞= 75.17; k=0.165; t0=-0.817.

The prevalence and impact of inaccurate age determinations on the accuracy of population dynamics studies cannot be overstated. There are many instances in which ageing error has contributed to the serious overexploitation of a population species. The problem is often on age underestimation rather than overestimation, resulting in overly optimistic estimates of growth and mortality rate (Campana, 2001).

The objective this study was to explore the otolith microstructure patterns and estimate the daily age of juvenile Chilean jack mackerel and preliminary estimation of length-at-age relationships.

MATERIALS AND METHODS

The juveniles of jack mackerel were captured aboard of scientific vessel off west northern coast of Chile (18º25' to 31º30' S; 70º06' to 71º43' O) in December 2012 and February 2013. A total of 44 pairs sagittae otoliths corresponded to fishes between 3 to 21 cm of fork length (FL) that were prepared (mounted and polished), of these 21 otoliths, with adequate readability, were analyzed. The left otolith was initially photographed under a stereo microscope, 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 µm-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 pictures 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 QImagen Evolution 5.0 camera on light microscopy connected to a computer. After polishing, counting of the primary increments was conducted twice, across the area of the otolith with the best resolution increments by two independent readers at different times; the result of each reader was overage. A comparison between readers was carried out applying 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 assume 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*. The latter authors indicated that the first increment was formed on 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, the microstructure analysis included the counting and measurement of: 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 the relationship between *FL* and age of juvenile was fitted by Laird-Gompertz model. The equation for the model was:

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

were FL is the fork length at age, X is age in days, L_{∞} 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).

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 of the zone of the otolith; SP in ventral or dorsal zone was 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 a new sequence of concentric microincrements born from the SPs (**Fig. 1 c y d**) was observed. The total numbers of SPs in an otolith (except in the rostrum area) ranged from 2 to 8 with mean ± s.d. of 5.3±1.5.

The counting of increments was sometimes difficult at the beginning of SGZ, with diffuse areas between the increments 50 to 80, where the increment width were significantly larger than anterior and posterior area, with mean of 19,9 μm ± 8.1 (**Fig. 2 d**). According to this area, the width of increments gradually decreases and it is possible to observe growth bands formed by thin rings that sometimes are not located at regular distances (Fig. 2 e). Where the periodicity of the microincrements has not been validated, makes it difficult since, two reading criteria could be used: one called Group Band Reading (GBR) i.e., counting the packages of rings; the other is called Individual Mark Reading (IMR) that we used for jack mackerel, involving counting of 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 no significant difference between readers (P= 0.239).

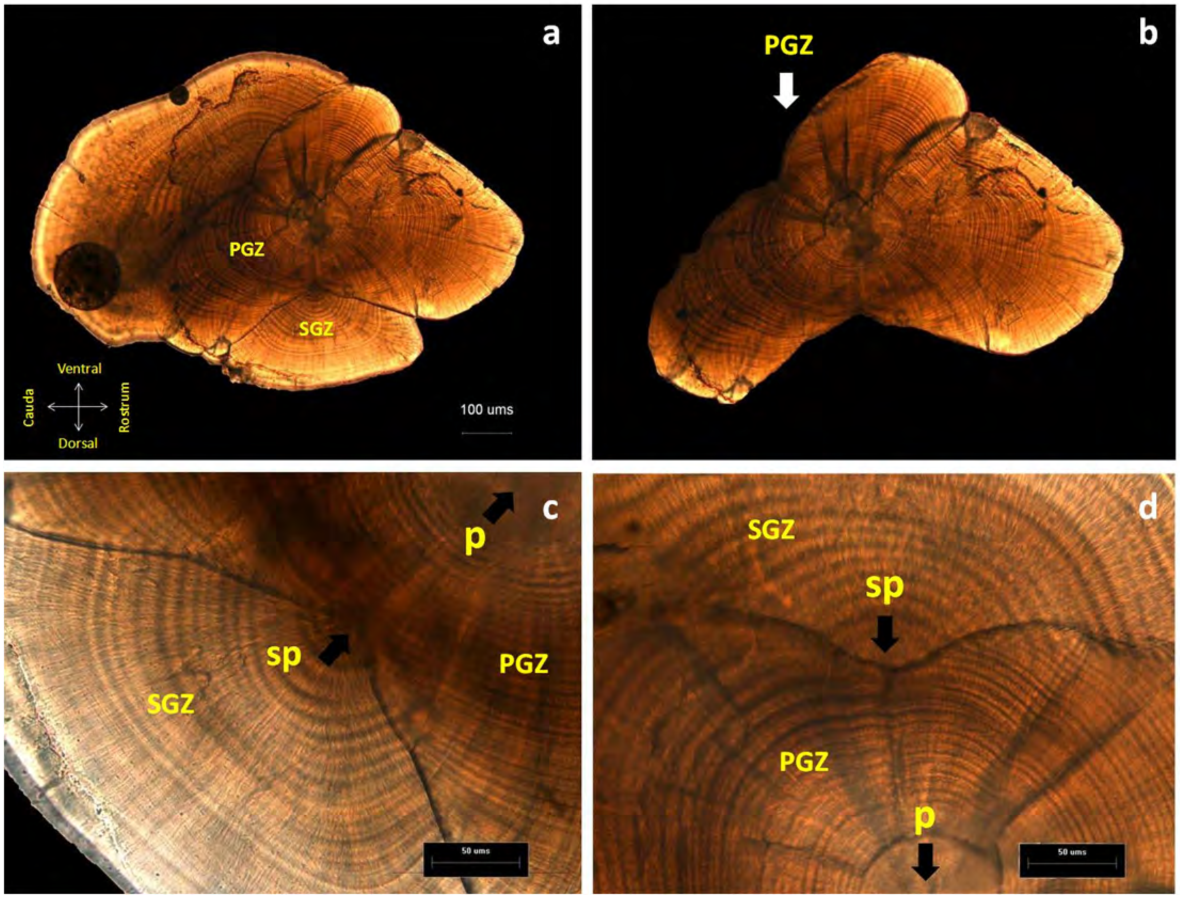

Figure 1. Microestructure of otolith sagitta of *Trachurus Murphyi* of 3,6 cm FL and 62 days, captured in the North of Chile. Pictures **c** and **d** show the primordium (p) and secondary primordium (sp) that divided the otolith in a Primary Growth Zone (PGZ) and a Secondary Growth Zone (SGZ). Picture **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 um of the first increment, peaked about of 20 um ranged 50 to 80 days, then became progressively narrow until they reached a value of 2,1 um 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 the increment width is used like proxy of growth rate, a high growth rate occurred after 40 days old possibly associate to end of larval stage and beginning the juvenile period with a necessary change of diet and habitat. After day 80 when ending the bigger growth period, the rate begins a gradual decline.

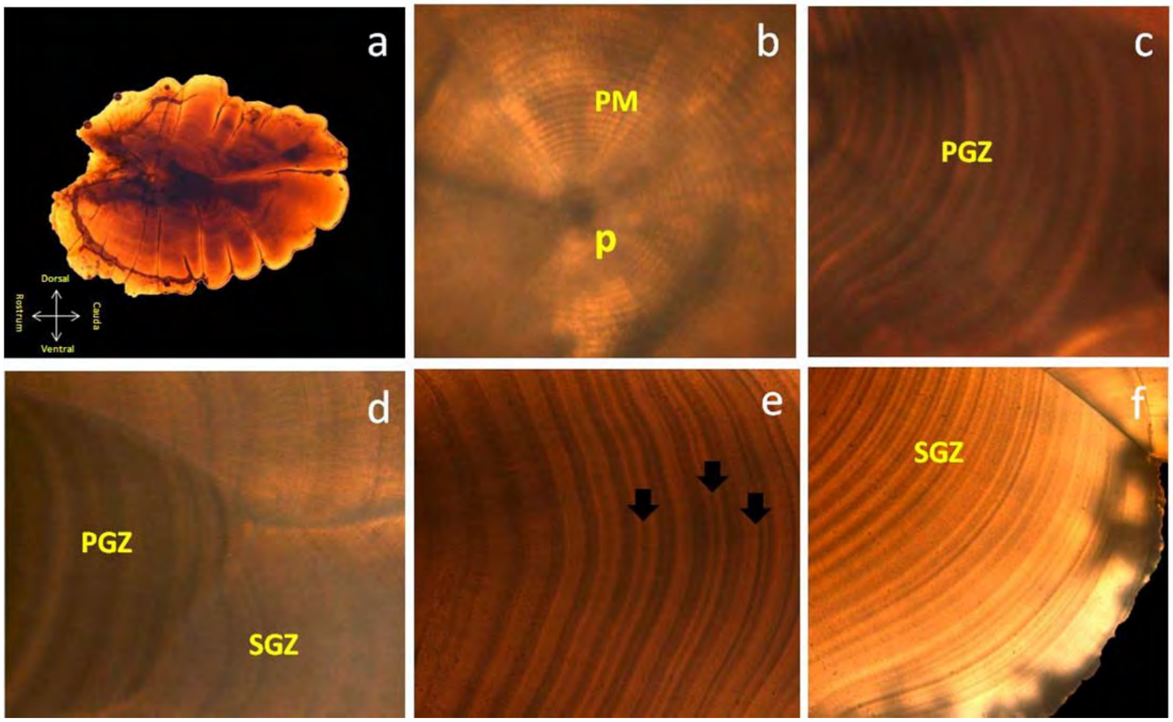

Figure 2. Sagittae otolith microstructure of *T. murphyi* with 10.7 cm FL. The pictures show the whole reading axis toward the post-rostrum. The picture **a)** shows the whole otolith; **b)** shows the initial primordium (p) and primary microincrement (PM); c) the middle area of PGZ; **d)** the end area of PGZ and start of SGZ**; e)** corresponds to 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.

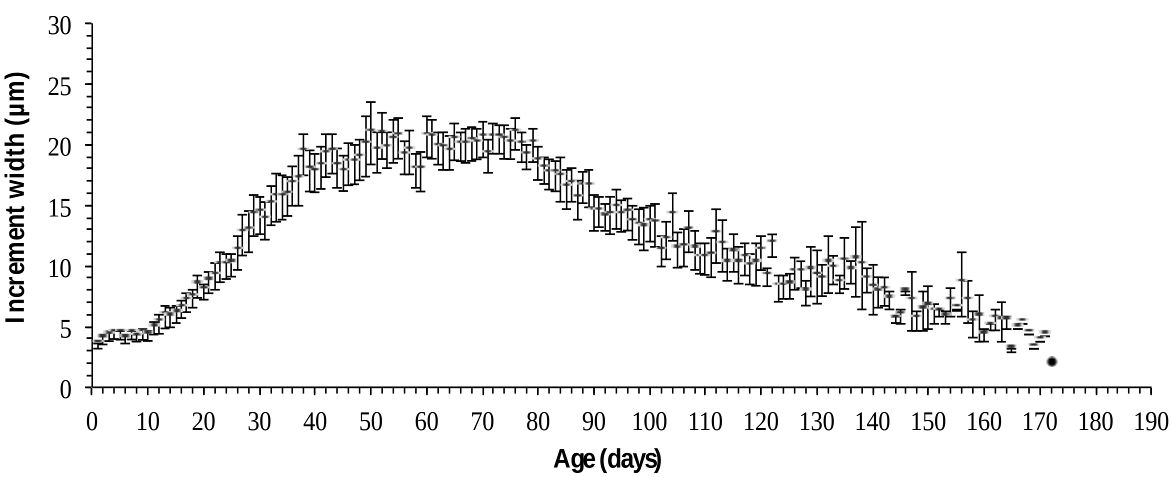

Figure 3. Increment width profile along the post-rostrum radius of sagittae otolith *T. murphyi*. Values are mean with standard error measurement in 21 otoliths of juvenile fish, collected in the north coast (Arica to Coquimbo) off Chile.

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The relationship of FL to R_0 was well described by a linear regression fitted by $FL=0.0077\ R_0-1.1394$ with $r^2=0.90$ to 21 otolith reading (Fig. 4).

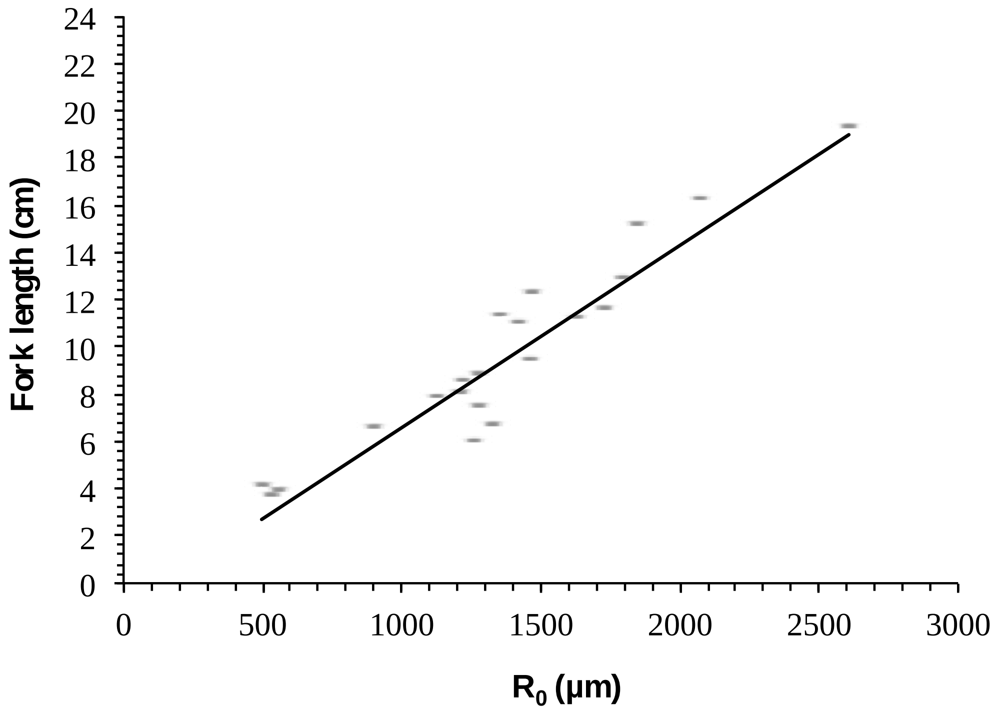

Figure 4. Regression of fork length and otolith radius of juvenile *Trachurus murphyi*, collected in the north coast (Arica to Coquimbo) off Chile.

Length-at-age plots of the fish were significantly explained by the Laird-Gompertz growth equation **(Fig 5**). The parameters value obtained were L_∞=36.4 cm; G=0.007 cm*days^{-1} and X_0=186.4 days, with coefficient of determination of r^2=0.98. The parameters are preliminary since the relationship was fitted with insufficient data for fish over 16 cm FL and the 91% of age estimated did not exceed the 200 days.

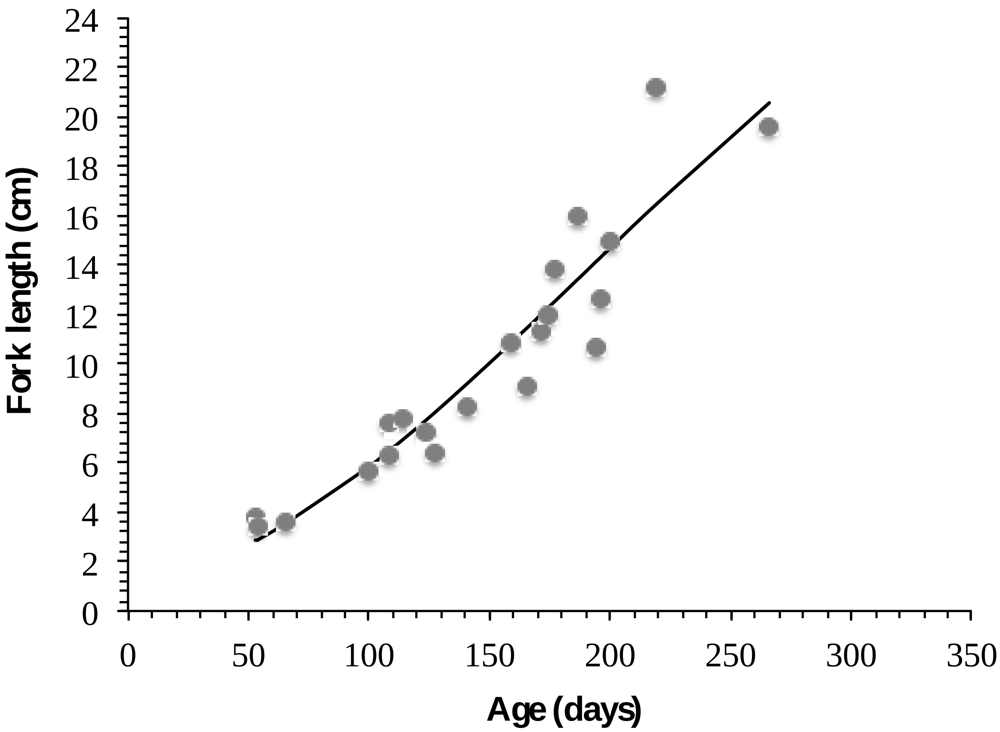

Figure 5. Curve that fitted the length-at-age plots of *Trachurus murphyi* juvenile collected in the north coast (Arica to Coquimbo) off Chile.

DISCUSSION

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 have an initial primordium and variable numbers of secondary primordium than begin to form around the 20 or 25 days after hatching. In Chilean jack mackerel we observed between 2 to 8 SPs, slightly different at 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 general 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 characteristic 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 the causes of SPs form include habitat shift (Campana 1984; Sogard, 1991), ontogenetic dietary shift (Marks & Conover, 1993), and 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), *Trachurus trachurus* (Waldron & Kerstan, 2001). The Peak of the increment is similar to *T. japonicus* with increments over 20 um between 40 to 80 or 100 days depending on 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.

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 that have been validated the microincrements like *T. japonicus* (Xie et al. 2005), allows us to verify 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 radium of otolith was described by linear regression that explained the 90% of variance. This indicated than 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.

Although the curve length-at-age did not take sigmoidal growth form, typical of Laird-Gompertz model, probably due to lack of data for large fish, however, the equation explained the 98% of variability and fit well to data of fishes lower of 16 cm FL. The growth curve in the length interval of 3 to 16 cm was similar to the reported for Goicochea et al. (2013) to jack mackerel captured off the Peruvian coast. However, our data have not allowed to estimate the length at the 365 day old because it is necessary to complete the age estimation for larger fish.

The preliminary results show high growth in juveniles of Chilean jack mackerel with microincrement analysis presumably of daily periodicity. As said previously, the reading criteria used was IMR that consists of counting every clear increment independently from its appearance (Fig. 2 e). The experts in daily increment analysis, based on validation studies and experience, suggest GRB reading criteria, because the IMR criteria may lead to error at counting sub-daily increments, checks or discontinuities like daily increments (Cermeño et al., 2006; Campana & Neilson, 1985; Campana, 1992). However, using GBR criteria, that consists of counting every repetitive cyclic set of growth bands or groups of microincrements as one (usually 2 but occasionally more), in Chilean jack mackerel may cause growth at a higher rate than the estimated in the present study.

To resolve this controversy, the validation in juvenile fish is an essential aspect before any otolith microstructure investigation. This is especially true in Salmonidae and pelagic fishes, where sub-daily increment are more common than in others species (Neilson, 1992), such as 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).

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