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CJM Validation of Daily Growth Microincrements in Otoliths

Chile

VALIDATION OF DAILY GROWTH MICROINCREMENTS IN OTHOLITS OF JACK MACKEREL

EXTENDED SUMMARY

FIPA Project No. 2017-61

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INTRODUCTION

Jack mackerel (*Trachurus murphy*) has a wide distribution in the south eastern Pacific and it has been one of the most important pelagic resources of commercial activity in Chile, focusing in three fishing areas: northern zone (18 ° 21 '- 24 ° 00' SE), Caldera-Coquimbo zone (24 ° 00'-32 00'S) and central-south zone (32 ° 00 '- 43 ° 30' S). In the last-mentioned area, but outside the Exclusive Economic Zone (EEZ), jack mackerel has also been exploited by a foreign fleet of Russian, Chinese, European, Korean and Vanuatu vessels. In addition, Peru and Ecuador also capture this species within their respective EEZs (Serra 1991, Elizarov *et al.* 1993, Taylor 2002, Canales 2013). Currently, in the stock assessment carried out by the Scientific Committee of the South Pacific Regional Organization for Fisheries Management (SPRFMO), the age at length keys of Chile are used to estimate the age structure of the catches of this country as also from the catches of the international fleet that operates in front of Chile. To include the information on the fisheries off Ecuador and Peru are being used growth functions either from Chile, Russia and recently from Peru.

The estimation of age in commercial species is essential for the application of models where the structure of the stock is analyzed, based on ageing and their respective cohort trends, in which allows to obtain estimates of recruitment, mortality and other characteristics related to the condition of the stock (Summerfelt and Hall 1987, Catalano *et al* . 2010, Francis 2016). Age estimation is also useful to determine longevity and other life history parameters that are important for fisheries management (Canales and Leal 2009, Kousteni and Megalofonou 2015). Therefore, the age estimation must be carried out the most accurately as possible to guarantee levels of reliability in the different output products associated with the stock assessment process.

One aspect associated with age studies is the validation of the first *annulus,* because if there is an error fixing their position, can transfer a constant error (or bias) throughout the age estimate, which can affect biomass estimation in those cases in which stock evaluation models are based on age (Campana 2001, Natanson *et al.* 2002). Although the impact of this difficulty may be less significant in old fishes, it can influence the proportions of juveniles and adults in the population, the maturity ogive at age and/or life history parameters like natural mortality. In the case of jack mackerel, Goicochea *et al.* (2013) based on the analysis of the microstructure in cross sections of the otoliths collected from the coastal zone of Peru, reported an average length of 17.7 cm FL at 365 days and the average caudal radius of the otolith at the formation of the first *annulus* correspond to 2.49 mm. Additionally, Cerna *et al.* (2016) demonstrated that in adult specimens of this species it is possible to observe micro-increments sequences starting from the primordium and towards the periphery of the otolith, using light microscopy. The number of visible micro-increments even exceeded 365 in an uninterrupted sequence, particularly in specimens smaller than 20 cm FL. In larger specimens were observed diffuse areas where the micro-increments could not be distinguished; however, distinctive micro-increments were observed between transition zones. However, the number of fish analyzed in the study was reduced to stablish conclusions with confidence. These authors estimate an average length of 22 cm FL at 365 days, similar to that recorded by Gretchina *et al.* (2017), where an average length of 21.4 cm FL for the first year of life was determined.

Cerna *et al.* (2016) emphasized that the sagittal otolith of this species presents a large number of disturbances that make it difficult to identify true *annuli,* particularly during the first years of life. This characteristic has been considered one of the main sources of uncertainty in the estimation of the annual age of this species, due to the impact that it can generate in the stock assessment (Dioses 2013, Goicochea *et al.* 2013, Díaz 2013, Channels 2013). However, at the microstructural level, the large number of macroscopic disturbances tend to disappear when the otolith is polished, being reduced to discontinuities that do not seem to interrupt the sequence of micro-increments. Furthermore, the transition zones presumably linked to growth stunting and/or growth stoppage are much more distinctive and feasible to quantify. It is important to highlight that at the microstructural level a true *annulus* is characterized by a gradual decrease in thickness and/or interruption of the sequence of micro-increments that precede it, followed by a recovery of the sequence of micro-increments of greater thickness (Victor and Brothers 1982, Wright *et al*., 2020a). Therefore, these observed transition zones appear to be true *annuli* in this species, although it is highly recommended to increase the sample size to strengthen this inference.

In certain cases, achieving levels of precision in the estimation of age is a great challenge, such as *Trachurus* genus, because the annual growth rings in the *sagittae* otoliths are difficult to interpret, particularly in the first years of life.

The FIP 2014-32 study of the National Fond of Fisheries and Aquaculture of Chile, "Jack mackerel reading protocol" (Cerna *et al.* 2016) was carried out to develop an age reading protocol as a guide for determining the age of jack mackerel for states members of the SPRFMO. Along with the reading protocol, progress was also made in the validation of age, establishing the following: 1) high growth during the first year of life, derived from the analysis of primary micro-increments, and in the following two years derived from the monitoring of year classes, and 2) a low growth rate in adult fish (lengths > 40 cm TL), estimated by the radiocarbon pump method. The high growth in the first years of life of jack mackerel suggest new growth parameters and natural mortality that would probably modify some indicators of the stock assessment and the status of this resource. These new findings should be confirmed, since the error in the estimation of age in many cases has contributed to the overexploitation of commercial fish populations (Campana 2001), is not a minor impact on resources such as jack mackerel, whose fishery is shared with other nations of the Southeast Pacific.

Within the framework of the SPRFMO science group, the data used in the evaluation model has been critically examined and one of the pieces of information on which a discrepancy has emerged is the estimation of the age of jack mackerel, mainly between Chile and Peru. This difference gave rise to the realization of an inter-age calibration workshop held in Lima in 2011 and the main recommendation being to continue working on the comparison of readings and identification of standard criteria for estimating age and the elaboration of a common reading protocol to being used by participating countries in the fishery. For the development of the age calibration work, aspects of age validation such as the identification of the first annual ring and the formation of the following annual rings were recommended.

In the working group to estimate the age of jack mackerel at the 4th Meeting of the Scientific Committee of the SPRFMO 2016, it was defined as a priority to continue the research work on the issues of validation of age and the reading protocol, mainly due to the doubts that have arisen between the different laboratories that work on these issues. This information is decisive for the stock assessment process, a good estimate of the growth curve of this resource will provide the basis to obtain less uncertainty in the stock assessment of this resource. For example, one aspect that impacts the precision of age estimation in accelerated growth species is the difficulty of identifying the first *annulus*. Although the impact of this difficulty may be less in old fishes, its influence increases when occurs a juvenilization process of the population, due to the fact that the age structure of the catches moves to younger age groups.

However, these results could not be confirmed due to the lack of a validation process of the periodicity of formation of micro-increments in otoliths of juvenile fish. It is important to highlight that the daily periodicity of micro-increments formation in *sagittae* otoliths has only been validated in adult fish through a chemical marking study, carried out in adults (> 27 cm FL) kept in confined conditions (Araya *et al*. 2003).

It is also important to underline that one of the recommendations from the workshops of the FIP Project 2014-32 "Protocol for reading jack mackerel otoliths" (Cerna et al. 2016) was the need to validate the periodicity of the primary micro-increments, as a way of evaluating the presumably high growth rate, estimated for the first year of life for this species. For this, it is crucial to maintain juveniles of known age in confinement conditions that allow validating the interpretation criteria of daily micro-increments, to later apply them in the estimation of the age of wild juveniles for the validation of the first annulus.

Once the periodicity of formation of the micro-increments and the time of formation of the first *annulus* has been validated, it is also necessary to move forward in the estimation of age and growth of wild juveniles less than one year old, using the already validated interpretation criteria, because the microstructural differences of the otoliths and the early life history information they contain can be an important ecological marker which help to reveal aspects of the population structure of this species. Therefore, this report provides the following results:

1. To validate the periodicity of the primary micro-increments in otoliths of jack mackerel juveniles keeping fish in captivity.
2. To validate first *annulus* from the count of primary micro-increments previously validated.
3. To determine dates of birth and age of recruitment in wild fish less than one year of life.

Validate the periodicity of the primary micro-increments in otoliths of jack mackerel juveniles keeping fish in captivity.

The fish maintenance stage for experimental purposes was carried out in the Aquaculture facilities of the Faculty of Renewable Natural Resources of the Universidad Arturo Prat at Iquique (20 ° 16'14” S; 70 ° 07'52” W).

The water exchange rate was 100% renewal per hour. The fish were received in the Aquaculture facilities (Fig. **1)** and a gradual conditioning protocol was applied that minimized the stressful effects derived from transport.

Fish were led to a condition of artificial food adaptation based on dry pelletized or fresh food alternatively combined with ground pelletized which gave satiety. Simultaneously during maintenance, were recorded water quality parameters such as temperature and oxygen levels.

On April 12, 2019, 44 and 39 specimens were marked between 6.5 and 12 cm FL, immersing them in a solution of 50 $mg \cdot l^{-1}$ red alizarin (Fig. 2) and a second marking procedure was finally performed 46 days after. The count was carried out between the marks left by the alizarin and the manipulation of the specimens. The analyzed specimens formed daily micro-increments and the marks were discernible under magnification.

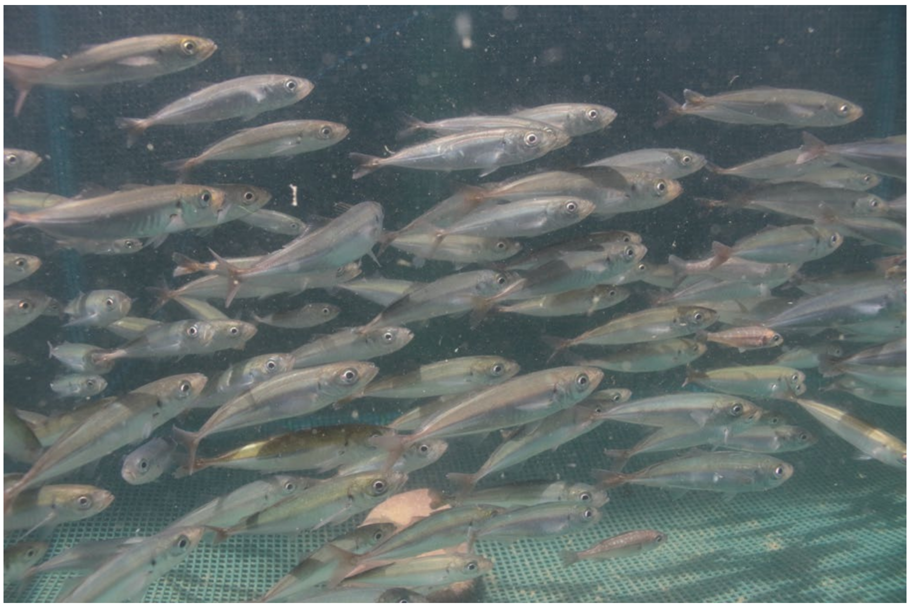

Figure 1. Jack mackerel between 6.5 and 12 cm FL kept in captivity.

Figure 2. Jack mackerel marking process in red alizarin solution.

The otoliths obtained did not need to be observed in a fluorescence microscope, since the fuchsia marks could be observed in a bright-field microscope (Liu *et al.* 2009).

The counting and measurement of micro-increments, between marks, was performed directly from a Zeiss Axio Scope A1 brightfield microscope at 40X magnification. Micro-increments were counted twice by two people (JP and MA) and the average was used for calculation purposes.

In **Figure 3** is observed the fluctuation of the temperature and pH of the two pools where individuals of jack mackerel were kept for 6 hours for marking with alizarin. The temperature fluctuated between 18.2 ° C and 19.2 ° C and pH between 8.2 and 8.5. Later during the period, fishes remained in captivity, the temperature fluctuated between 15.8 and 19.8 ºC and the oxygen level between 4 and 8.9 $mg \cdot l^{-1}$ (**Figure 4**).

Preparation and observation

To observe the otoliths under magnification with reflected light (**Fig. 5)** formed a translucent area when they were handled for marking, as it can be confirmed observing it in brightfield microscope with transmitted light **(Fig. 6)**. This translucent zone presented an average distance, from the nucleus of the otolith to the initial edge of this zone, of 101.7 µm (n = 44; s.d. = 72.5 µm), at the same time the distance from the distal edge of translucent area to the edge of the otolith averaged 650.1 µm (n = 44; s.d. = 112.4. µm). The first alizarin mark appears in fuchsia in the analyzed samples, both at the macro and microstructural level (**Fig. 6, 7 and 8)** and the second mark appears as a thin line of different appearance from the rest of the underlying micro-increments and that follows a largely otolith trajectory. Both markings appear in all the otoliths of the marked specimens (**Fig. 8**) as a discontinuity in the otolith formation pattern which is characterized by a much wider D-zone.

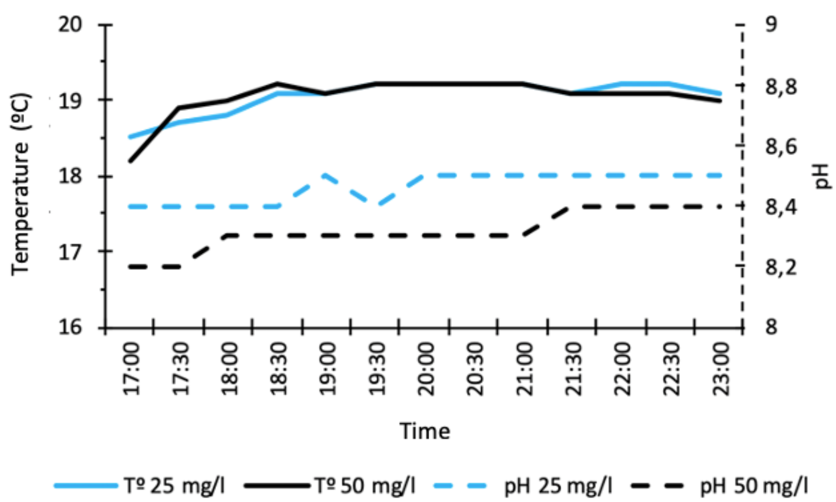

Figure 3. Record of temperature and pH during 6 hours of jack mackerel in a bath of alizarin (February 26, 2019).

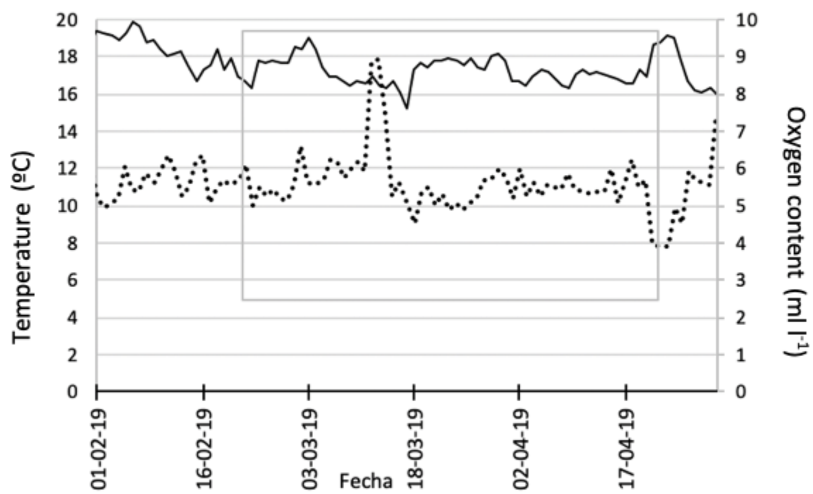

Figure 4. Temperature and oxygen fluctuations in the fish tank. (Square indicates the period of experimentation with jack mackerel).

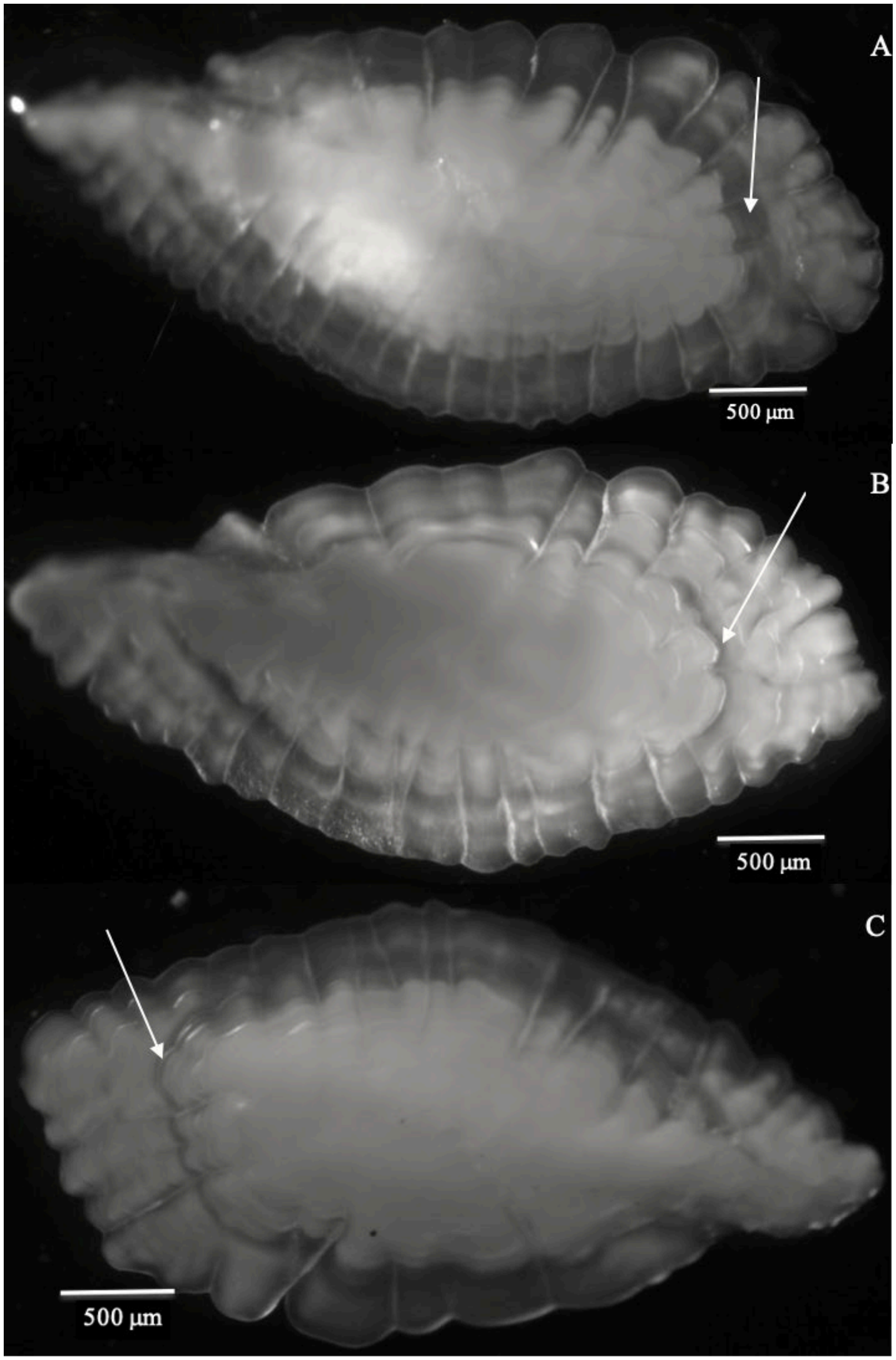

Figure 5. Photographs of jack mackerel from the second experiment under a stereo microscope, the translucent mark left by the first days of arrival in captivity and a concentration of 50 mg / l of alizarin is shown with arrows. (A) 14.2 cm, (B) 12.9 cm, (C) 12.5 cm FL.

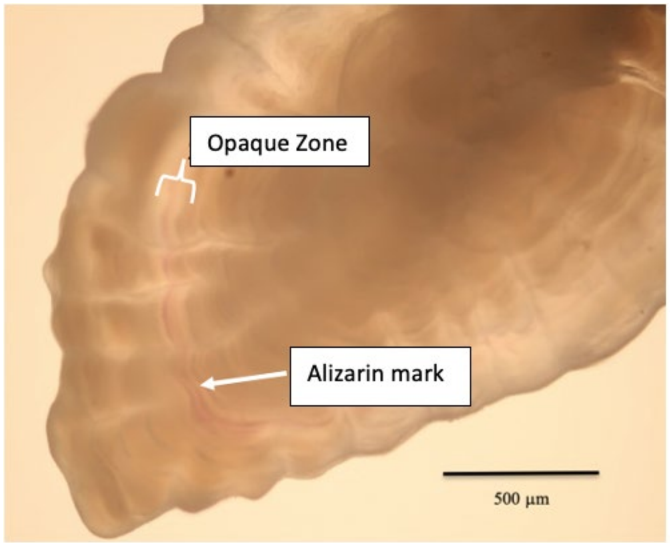

Figure 6. A 12.4 cm FL jack mackerel otolith from the 50 mg l^{-1} alizarin solution is observed with transmitted light, where the first mark left by alizarin is seen. The translucent area observed in **Figure 5.C** appears in this photo opaque.

Figure 7. Photographic sequence of an otolith of a 10.7 cm FL jack mackerel, where can be seen from center to the edge the micro-increments and marks that left the alizarin (40X).

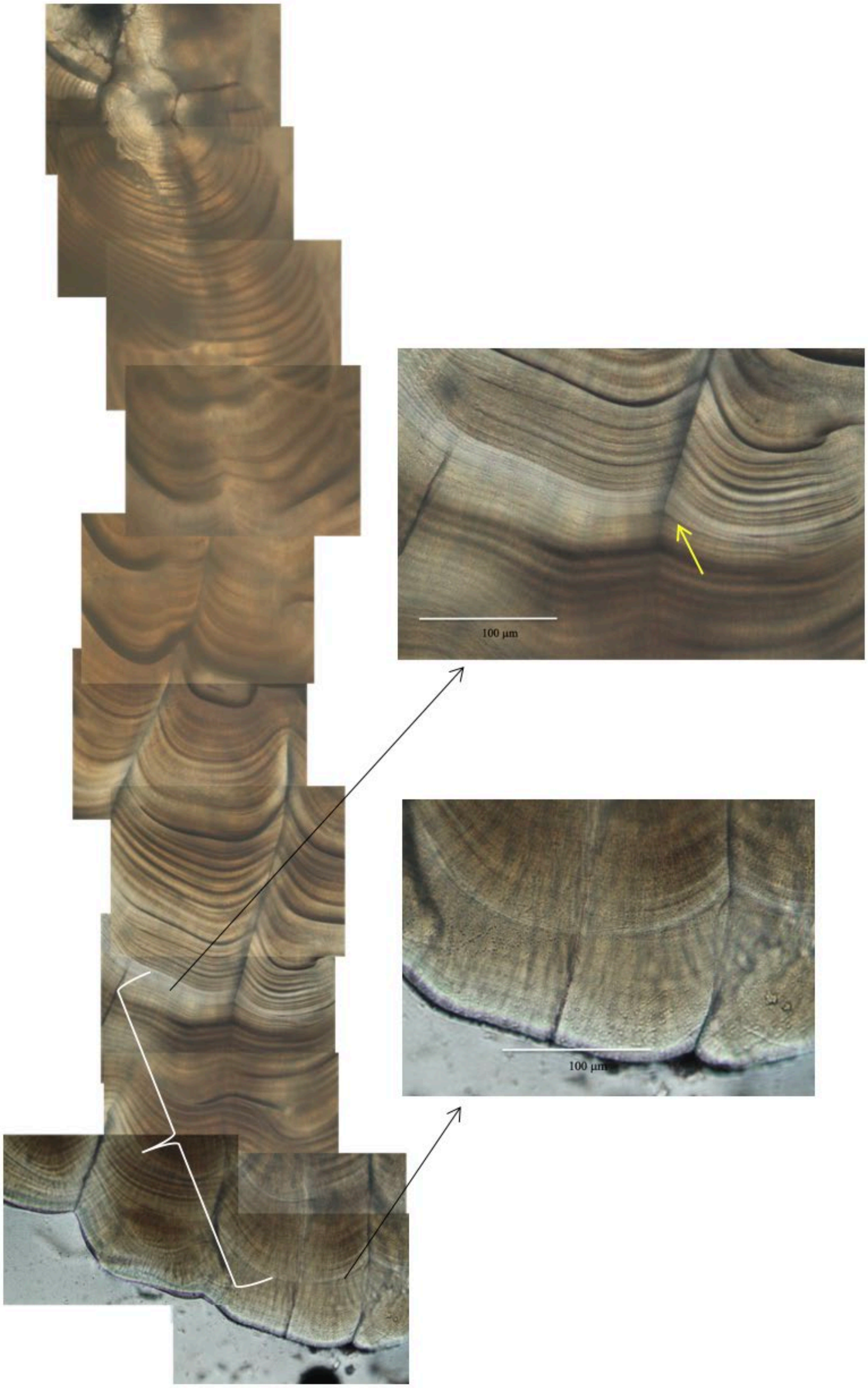

Figure 8. Photographic sequence of an otolith of a 12.6 cm FL jack mackerel, where can be seen from the center to the edge the micro-increments with the marks left by alizarin (40X)

The width of the micro-increments between the two marks fluctuated between 5 and 15 µm (**Fig. 9)**, when the mark appears the micro-increments are thin and later they become wider taking the appearance of the group of bands (**GBR**) for later begin to decrease as you approach the second mark formed.

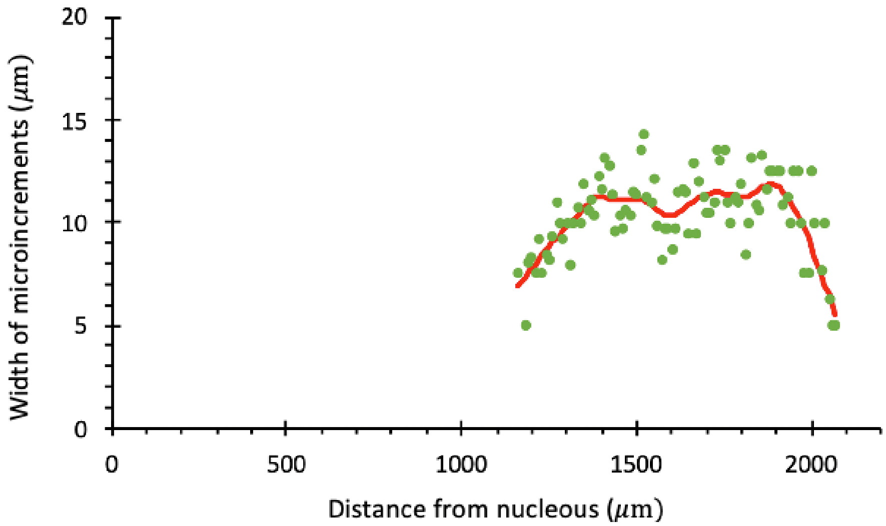

Figure 9. Micro-increments width between marks with respect to the center of the otolith. The red line is a fit with the LOWESS algorithm (Cleveland 1981) to show the trend.

44 preparations were obtained that allowed the counting of micro-increments between marks, the reader J.P could observe in 43 and the average number was 45.8 (s.d. = 0.83) and the reader M.A in 40 preparations with an average of 45.7 (s.d. = 1.1). Both medians are not significantly different from 46 (t-test; $p = 0.1075$ and $p = 0.114$, respectively) which are the days that the jack mackerels remained in captivity between marks.

Table 1 shows the fork lengths and total weight of the specimens to which the micro-increments in the otoliths were counted.

Table 1. Fork length (cm) and total weight (g) registered at the end of the experiment, and average number of micro-increments recorded by reader of jack mackerel otoliths kept in captivity.

		Número promedio microincrementos	
Longitud Horquilla (cm)	Peso Total (g)	Lector J.P.	Lector M.A.
14,4	29,5	45	45,5
15,8	35,9	46,5	45
15,3	49,1	46,5	
12,3	30	47,5	43,5
14,2	31	45	43
15,6	34,9	46,5	45,5
13,7	35	45,5	47,5
14,5	30,5	45,5	46,5
15	36,3	46	46
15	41,5	44,5	45,5
14,5	38,4	46	47
15	43,4	45,5	46
13,3	26	44,5	45,5
14,6	38,8	47	46
12,8	25,5	45	45,5
12,7	25,1	45	44,5
15,5	42,3	45,5	45
14,3	32,2	45	43,5
11,4	15,5	46	46
13,4	29,7	45,5	46,5
12,6	21,3	46	45,5
14,5	36,7	45,5	46
15,4	42,7	47,5	44
15,7	45,2	45	45,5
13,9	31,5	47	47
13,6	30,4	46,5	46
14,1	31,9	46	46
13,4	30,5	45,5	46
14,9	39,3	45,5	
12,9	26,7		46,5
14,9	39,2	45,5	44
14,6	42,9	45,5	47,5
14,9	40,3	45,5	47
15,4	45,6	45,5	46,5
15	40,8	44	43,5
15,1	43,2	46	
14	33,7	46,5	47
14,9	39,4	45,5	46
14,6	37,5	46	45,5
13,9	35,2	46	
16,6	55,3	47	46
13,6	31	47,5	46,5
13,8	30,8	44,5	46
15,2	40,8	46	47

The translucent area of the otoliths, observed under a stereo microscope, may be product of post- capture stress and marking of the specimens, in this sense it has been reported that captivity stress leads to changes in the structure of the otoliths forming discontinuities, which are thinner and more regular marks (Pannella, 1980, Geffen 1992, Panfili and Tomas 2001). Marks of stress were found in *Oreochromis niloticus* (Panfili and Tomas 2001) and also in *Etropus crossotus* (Reichert *et al.* 2000).

The otoliths of juvenile jack mackerel marked with alizarin, at least the first mark could be obtained with sufficient reliability, without the need to observe it with a fluorescence microscope, this is very encouraging for future otolith marking experiments. This has been noted in other species they have been possible to perform more than two alizarina marks in small sizes specimens (Liu *et to the.,* 2009).

The second mark, despite of not marking a fuchsia color as well as the first mark, was visible and allowed to count the number of primary micro-increments between marks of the otolith. The number of micro-increments coincided with the number of days elapsed between the two marking events of fish in captivity. The appearance of the marks could be also associated with the stress process that the specimens were subjected when they were submerged in alizarin. It has been reported that the width and contrast characteristics of the micro-increments can be affected by the growing conditions, not affecting the number of these (Moksness *et al.* 1995, Massou *et al*. 2002). Even when it is deprived of food, micro-increments are formed, these being smaller (Campana and Neilson 1985). Payan *et al.* (2004) also found that in *Oncorhynchus mykiss* individuals that stress produces a wider D-zone.

The average number between the marks confirms that the micro-increments formed in the jack mackerel otoliths of average 10 cm FL are of a daily nature, the results obtained are in agreement with those found by Araya *et al*. (2003) in the same species and with individuals between 28.4 and 37.7 cm FL.

Validate the first *annulus* from the count of previously validated primary micro-increments.

For the development of the validation objective of the first annulus, a comparative analysis was carried out between the macro and microstructure of sagittal otoliths of the jack mackerel *Trachurus murphy*, between 13 and 23 cm FL, collected in Chilean waters during the years 2008 and 2009, which they were complemented with samples of otoliths collected in international waters in 2017. The otoliths were prepared through a double sagittal polishing, avoiding over-polishing the edges, but ensuring sufficient resolution throughout the structure. The readings were performed in duplicate, using images digitized through image analyzers. The macro and microstructural comparison showed the following major findings (ii) the presence of micro-increments daily deposited continuously and sequentially from the nucleous to the edge otolith (**Fig. 10**). ; (ii) age vs. length and age vs. caudal radius curvilinear type relationships for the length range of specimens analyzed (13-23 cm FL); (iii) existence of three characteristic microstructural zones; (iv) existence of CSC; and (v) occurrence of discontinuities.

The micro-structure of the sagittal otoliths of specimens between 18 and 23 cm FL, evidenced the presence of three characteristic micro-structural zones, a first zone that extended between the first and third week of life associated with the larval stage, where a gradual increase in thickness of micro-increments was observed (8.27 ± 6.87; range: 1-23 µm), a second zone of greater growth of the otolith that began with the appearance of secondary growth centers and that extended until approximately the fourth month of life (20.77 ± 7.40; range: 6-38 µm), and a third microstructural zone that was characterized by a noticeable reduction in otolith growth rates (4, 30 ± 1.84; range: 2-11 µm) (**Fig. 2**).

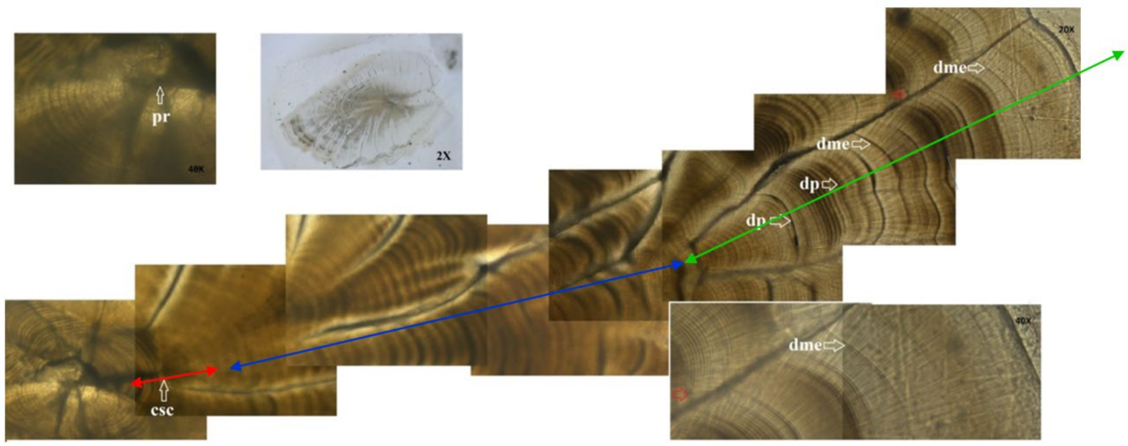

Figure 10. Photographs of the comparison between the macro and microstructure of a jack mackerel specimen, 18 cm FL, collected off the Chilean coast. **CSC**: Secondary growth center; DME: discontinuity of greater extension; PD: partial discontinuity of less extent. The image can be zoomed in to better view the IPs. The red marks indicate the exact correspondence between sections of different magnification. The red, blue and green arrows indicate the extent of Zones A, B and C, respectively.

The relationship age vs FL was significantly described by Laird-Gompertz models, from which length sizes between 21.2 and 21.8 cm were estimated for the first year of life, which were similar to previous studies for *T. murphy,* using the same methodology in Chilean waters. The Laird-Gompertz models adjusted to the age vs FL relationship estimated maximum absolute growth rates at the inflection point between 0.46 and 0.48 mm / day, reached between 70 and 78 days of age for specimens between 13 and 23 cm FL for this species (Fig. **11)**. Similarly, the age vs caudal radius relationship was significantly explained by a logarithmic curvilinear model and its inverse function was significantly adjusted to exponential models, from which were estimated average radius at the first year of life of between 3 and 3.1 mm. Both functions (age-length & age-caudal radius) showed that there was a substantial reduction in growth towards the end of the first year of life, which was independent of the year analyzed.

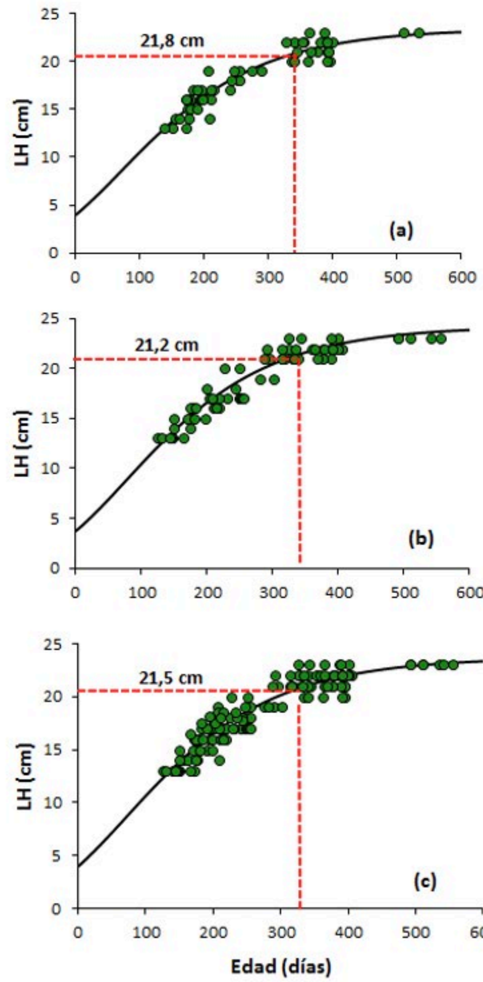

Figura 11. Laird-Gompertz models adjusted to the age vs. fork length relationship for specimens between 13 and 23 cm FL of jack mackerel collected on the Chilean coast. The red segmented lines illustrate the average length estimated by the function for the first year of life, for the year 2008 (**a**), year 2009 (**b**) and when all the information was combined (**c**).

The comparison between the age, the daily age and the annual age registered in the traditional way, in two years of study (2008 and 2009), and when the information was combined, showed that the first translucent zone completed its formation at median ages of 180 days, far below of 365 days required to be considered the first annulus in this species and therefore it is recommended that it be omitted from the age determination process (**Fig. 12**). On the other hand, the second most prominent translucent zone began to form after less opaque growth and completed its formation at approximately 300 days. Consequently, the results showed that the second band completed is very close to the end of its first year of life and the second band it is very far from a new annual cycle.

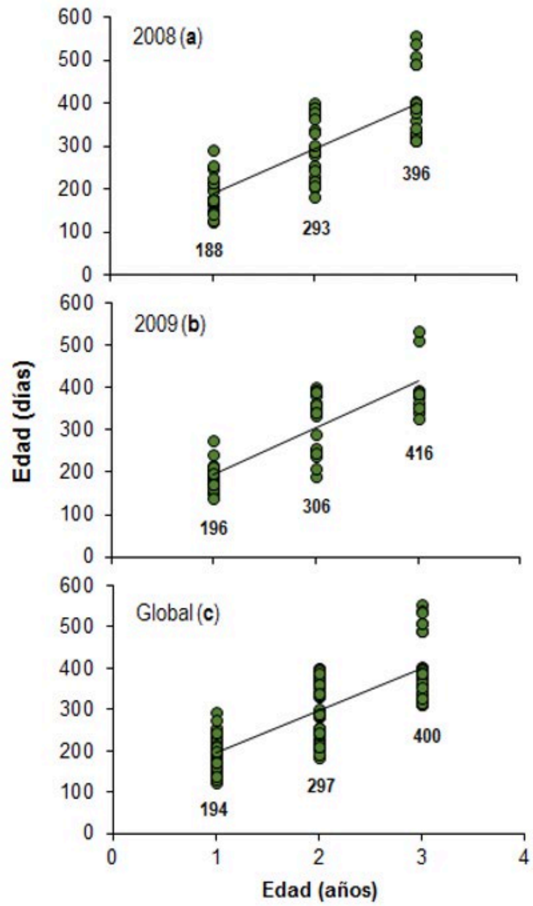

Figure 12. Linear relationships between annual age obtained by traditional reading, and daily age obtained by the microstructure of sagittal otoliths analysis of jack mackerel specimens between 13 and 23 cm FL, for each year and for the combined information. The numbers below the values represent the average number of days predicted by the model to the formation of each macroscopically identified annulus.

The comparative analysis between the macro and microstructure of the sagittal otoliths of this species proved to be an effective tool to validate the time of formation of the first annulus in this species. However, the microstructure also evidenced the existence of disturbances, among which the CSC, discontinuities and phase changes between opaque to translucent deposits are distinguished that make it difficult to properly interpret a daily ring, even if the criteria for its identification have been validated under experimental conditions. Therefore, it is essential to move towards the integration of information between different laboratories to generate interpretation and identification protocols of daily micro-increments for this species and thus elaborate graphic catalogs and reference collections.

Dates of birth and recruitment age in wild fish under one year of life

According to the results obtained in the first specific objective of the present project, the criterion of groups of bands (GBR) was used to read the micro-increments; These bands appeared under the light microscope as rings formed by alternately light and dark sub-micro-ring packages, which together formed a micro-increment (Cermeño *et al* . 2008). From the northern zone of Chile, a total of 126 jack mackerel specimens sampled between the months of September 2017 and May 2018 were analyzed, whose sizes varied between 7.1 and 22.5 cm of FL. From the south-central zone, a total of 55 specimens were analyzed, sampled in 2008, 2009 and 2014, whose lengths varied between 8.5 and 19 cm FL.

Prior to the preparation of the microstructure, only jack mackerel otoliths that were intact were selected and weighed (mg), and the fish's age in days was determined from the otolith weights (Araya *et al*. 2001; Cisterna 2014), the linear relationship ($r^2 = 0.95$) between the variables FL (cm) and otolith weight (g) is presented in **Figure 13**. While in **Figure 14** is presented the linear relationship between the age of the fish in days and the weight of the otolith (mg) ($r^2 = 0.93$)

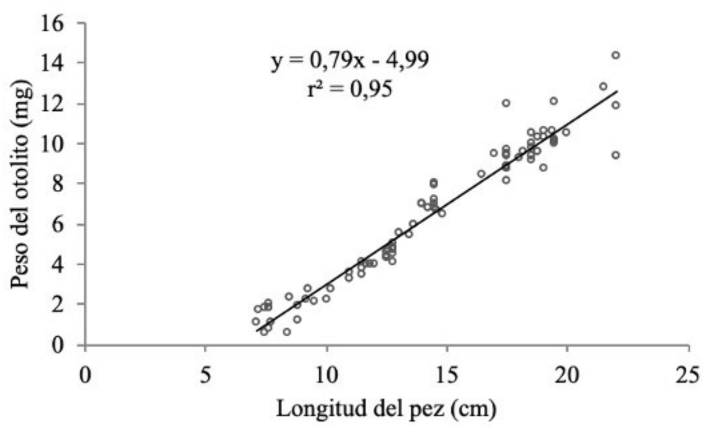

Figure 13. Linear regression between FL (cm) and weight of its otolith (mg), n = 79.

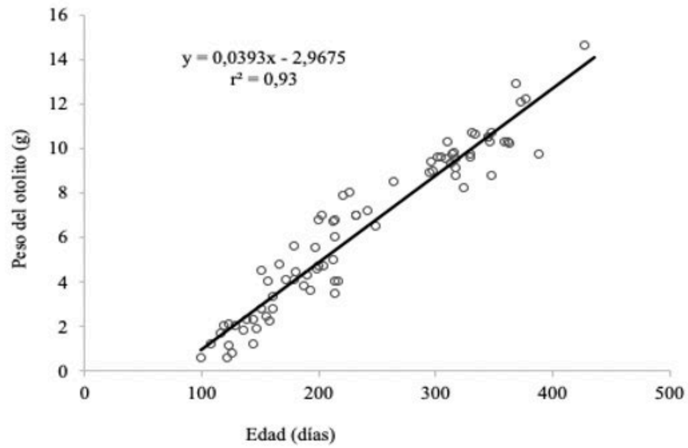

Figure 14. Linear regression between the weight of the otolith (mg) and the age determined in days, n = 79.

From the analysis of the microstructure were determined the dates of birth of the fish analyzed in the northern zone, subtracting the number of daily increases identified in the otolith from the date of capture of the fish. The results indicate that the analyzed individuals were born between the months of August and January, with a higher frequency between October and November, these results agree with the high values of the gonadosomatic index (GSI) registered for the same period in the northern area of Chile and the main reproductive time of this species (**Fig. 15**).

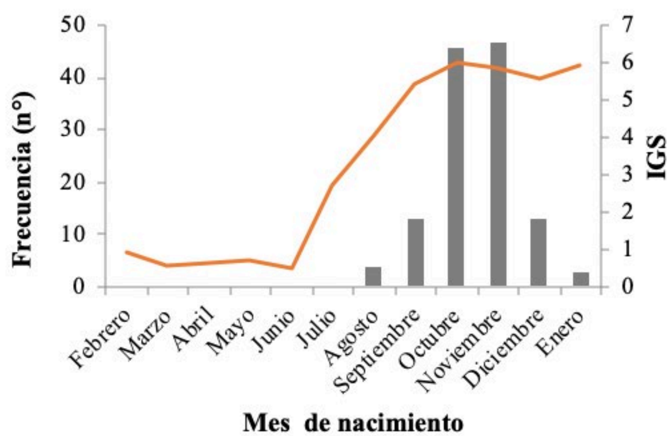

Figure 15. Frequency distribution of individuals respect to their date of birth determined by reading of daily micro-increments contrasted with the GSI values for the same period. The years of birth correspond to 2016, 2017 and 2018.

From the thicknesses of the micro-increments measured and their distance from the center of the otolith, it was possible to obtain the thickness profile of the micro-increments through the radius of the otolith, measured from the center to the post-rostral edge (**Fig. 16**). The model fitted to the observations showed high significance for the three estimated parameters (p <2e-16), the highest rates of growth (increases of 25 µm) was reached at a distance of 800 µm of the center of the otolith.

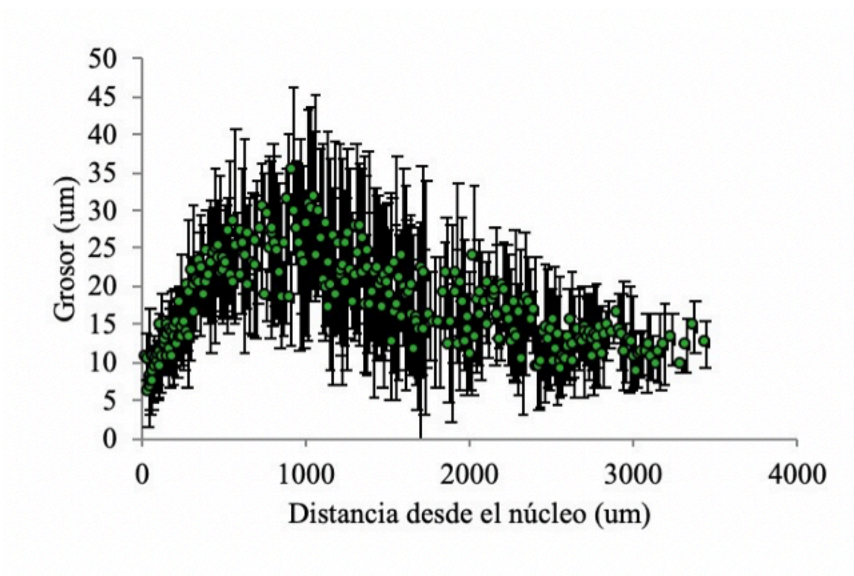

Figure 16. Relationship between the average thickness of the micro-increment (μm) and its distance from the center of the otolith. The bars correspond to the standard deviation.

Conclusions

1. It was possible to produce both marks on the otoliths of 12.4 cm FL jack mackerel, the first one of fuchsia color with a wide D-zone and the second also with a bit thinner D-zone.
2. The average number of micro-increments between marks was 45.8 (0.83) and 45.7 (1.13) according to both readers. Both means are not significantly different from 46 (t-test; p = 0.107 and p = 0.114, respectively), which are the days that the jack mackerels remained in captivity between marks.

3. The microstructure of the *sagittae* otoliths of jack mackerel *Trachurus murphy*, collected in Chilean waters between 13 and 23 cm FL, allowed to record an uninterrupted sequence of daily micro-increments, from the first mark surrounded by the primordium to the edge of these, which allowed a daily age estimate with reliability.
4. The microstructure of the *sagittae* otoliths of *Trachurus murphy* specimens between 18 and 23 cm FL presented three zones, a first zone characterized by a gradual increase in the thickness of the micro-increments between the first and fourth week of life, a second zone of greater otolith growth that began with the appearance of CSC and that lasted until approximately the fourth month of life, and a third microstructural zone that was characterized by a marked reduction in otolith growth rates.
5. The first year of life is reached at mean lengths between 21.2 and 21.8 cm FL.
6. It was also demonstrated through additive general linear models where FL was incorporated as a covariant variable, that the month of collection had a significant impact on the fluctuations of the translucent and opaque bands, demonstrating that there is a factor of seasonality acting in this process.
7. The comparison between the age, the daily age and the annual age registered in the traditional way, in two years of study (2008 and 2009), showed that the first translucent zone completed its formation at average ages of 180 days, well below 365 days required to be considered the first *annulus* in this species.
8. The daily age estimate showed that the second translucent zone was formed at mean ages between 293 and 323 days at an estimated mean radius of 2.9 mm in the caudal direction of the *sagittae* otoliths, values that were close to the theoretical age expected and therefore should be considered as the first *annulus* for this species.
9. The analysis of the dates of birth indicated that the individuals analyzed were born between the months of August and January; with a peak between October and November that corresponded to 73% of the individuals. The dates of birth widely overlap with the period with the highest GSI reported for this species, which registers the highest values between the months of September and December.