AGE AND GROWTH OF WHITEMOUTH CROAKER (*Micropogonias furnieri*) LARVAE AND JUVENILES*

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SUMMARY. With the aim of studying the age and daily growth of whitemouth croaker (*Micropogonias furnieri*) larvae and juveniles, the microstructure of *sagittae* otoliths of 122 individuals 3-25 mm standard or notochord length (SL or NL) size range and 7-92 days old was analyzed. Specimens derived from the turbidity front of the Río de la Plata area, the main spawning zone of the species. Counting and measuring of the increments width along the otolith major radius were performed. The length-at-age data were fitted to the linear model (R^2 = 0.875) and that of Laird-Gompertz (R^2 = 0.881) with L_0 = 2.44 and 2.76 mm, respectively, that represents the average length at hatch. With the Laird-Gompertz model an inflection point at 31 days and 10.23 mm SL was obtained. The instantaneous growth rates as a function of length showed a bell shape with an increment of 0.19-0.28 mm day^{-1} (27 mm SL asymptotic length). The growth rate derived from the linear model was 0.25 mm day^{-1}. A linear relationship between the otolith major radius and length was found. The analysis of increments width as a function of age showed a trend similar to the one described in the Laird-Gompertz model.

Key words: Otoliths, fish larvae, microstructure, growth rings, age, *Micropogonias furnieri*.

EDAD Y CRECIMIENTO DE LARVAS Y JUVENILES DE CORVINA RUBIA (*Micropogonias furnieri*)

RESUMEN. Con el objetivo de estudiar la edad y el crecimiento diario de larvas y juveniles de corvina rubia (*Micropogonias furnieri*) se analizó la microestructura de los otolitos *sagittae* de 122 individuos de un rango de longitud de 3-25 mm de longitud estándar o notocorda (LS o LN) y 7-92 días de edad. Los ejemplares se obtuvieron en el frente de turbidez del área del Río de la Plata, principal zona de desove de la especie. Se realizó el conteo y medición del espesor de los incrementos a lo largo del radio mayor del otolito. Los datos longitud-edad se ajustaron al modelo lineal (R^2 = 0,875) y al de Laird-Gompertz (R^2 = 0,881) con L_0 = 2,44 y 2,76 mm, respectivamente, que representa la longitud promedio al nacimiento. Con el modelo de Laird-Gompertz se obtuvo un punto de inflexión a los 31 días y 10,23 mm LS. Las tasas instantáneas de crecimiento en función de la longitud presentaron forma acampanada con un incremento de 0,19-0,28 mm día^{-1} (longitud asintótica de 27 mm LS). La tasa de crecimiento derivada del modelo lineal fue de 0,25 mm día^{-1}. Se encontró una relación lineal entre el radio mayor del otolito y la longitud. El análisis del espesor de los incrementos en función de la edad mostró una tendencia similar a la descripta en el modelo de Laird-Gompertz.

Palabras clave: Otolitos, larvas de peces, microestructura, anillos de crecimiento, edad, *Micropogonias furnieri*.

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INTRODUCTION

Fish larvae and juveniles age and growth analysis is important to understand population dynamics and its impact on exploitation. According to Jones (1992), the information related to age structure in early life stages can be used to clarify the effects of environmental changes on growth and survival and improve understanding of the factors that affect recruitment success.

Since the 80s fishery biologists have used the otolith daily increment technique to calculate hatching dates and study the effect of the environment on early growth stages and survival (Campana and Neilson, 1985; Jones, 1986, 1992, 2002; Brown *et al.*, 2004). Otolith microstructure can be systematically used to study the daily events that occur in early life history and growth rates variation, information kept unaltered through time (Stevenson and Campana, 1992). In other words, it constitutes an important tool to enhance understanding of the oceanographical and ecological processes of the ecosystem dynamics that may affect fish early life stages (Sponaugle, 2010).

The first study on whitemouth croaker (*Micropogonias furnieri*) larvae daily growth, made by Albuquerque *et al.* (2009), consisted of an experimental validation of otoliths daily increment deposition. The study included fitting of a growth model and determination of daily growth rates in individuals from hatch through 29 days old. As for other sciaenid species, mention is made of accessory nuclei (Na) formation in otoliths of larger larvae. Braverman *et al.* (2015), using an indirect method, also validated larvae otoliths daily deposition pattern in the Río de la Plata system (Argentina).

Ciechomski (1980) found significant weight variations (up to five times) in *M. furnieri* juveniles of similar length. The species larval growth studies should imply a compound analysis of length, weight and age variations. In the present work larval growth in terms of length and age is described.

Whitemouth croaker, the species with the largest biomass in the Río de la Plata system and the Argentine-Uruguayan Common Fishing Zone, is an important target for the coastal fisheries of Argentina and Uruguay. It is a demersal fish of up to 39 years life span in the region, an age structure that shows predominance of certain year classes and recruitment fluctuations that display variability at 3-7 years old (Carozza *et al.*, 2004; Acha *et al.*, 2012).

The bottom salinity front at the innermost part of the Río de la Plata estuary is the main spawning ground where *M. furnieri* pelagic eggs are spawned (Macchi *et al.*, 1996; Acha *et al.*, 1999) and where their larvae are retained (Braverman *et al.*, 2009). *M. furnieri* reproductive success seems to be linked to the dynamics of the estuarine waters (Acha *et al.*, 1999; Acha and Macchi, 2000; Berasategui *et al.*, 2004; Simionato *et al.*, 2008). The species is a multiple spawner with an indeterminate annual fecundity: unyolked oocytes continuously mature are spawned throughout the reproductive season that takes place from November through April. A female spawns, on average, a new batch of eggs every 3-4 days during said period (Macchi *et al.*, 2003). Its planktonic larvae are subject to both physiological and ecological processes that affect recruitment to the nursery grounds (Acha *et al.*, 1999; Braverman *et al.*, 2009).

The objective of this work is to analyze, for the first time, the age and daily growth of *M. furnieri* larvae and early juveniles from the Río de la Plata estuarine system. The results obtained will be useful to develop models to estimate population density since they will contribute length-at-age data to the spatial distribution of individuals (Braverman *et al.*, 2009).

MATERIALS AND METHODS

A vertically stratified plankton sampling was designed to study the ecology of *M. furnieri* early stages during a cruise carried out on board of the RV "Capitán Cánepa" from 10th through 20th March 2006 (late summer) along the turbidity front of the Río de la Plata area (Figure 1). Samples were collected at three depth levels, in day light (8:00 a.m.-6:00 p.m.), at stations separated 10-12 km. Two were taken in the water column at 2.5 ± 1.1 m and 6.2 ± 1.4 m depth with a Motoda sampler equipped with a mechanical opening-closing device and a 200 µm mesh size (Wiebe and Benfield, 2003). To obtain the third, at the deepest sampling level (7.6 ± 0.9 m), an epibenthic sampler furnished with a 500 µm mesh size was used. The volumes of filtered water were taken with flow meters placed in the net mouth.

Whitemouth croaker individuals were identified on board and kept frozen for consevation. After that, an optical dissecting microscope was employed to take the individual length as standard length (SL) or notochord length (NL) on

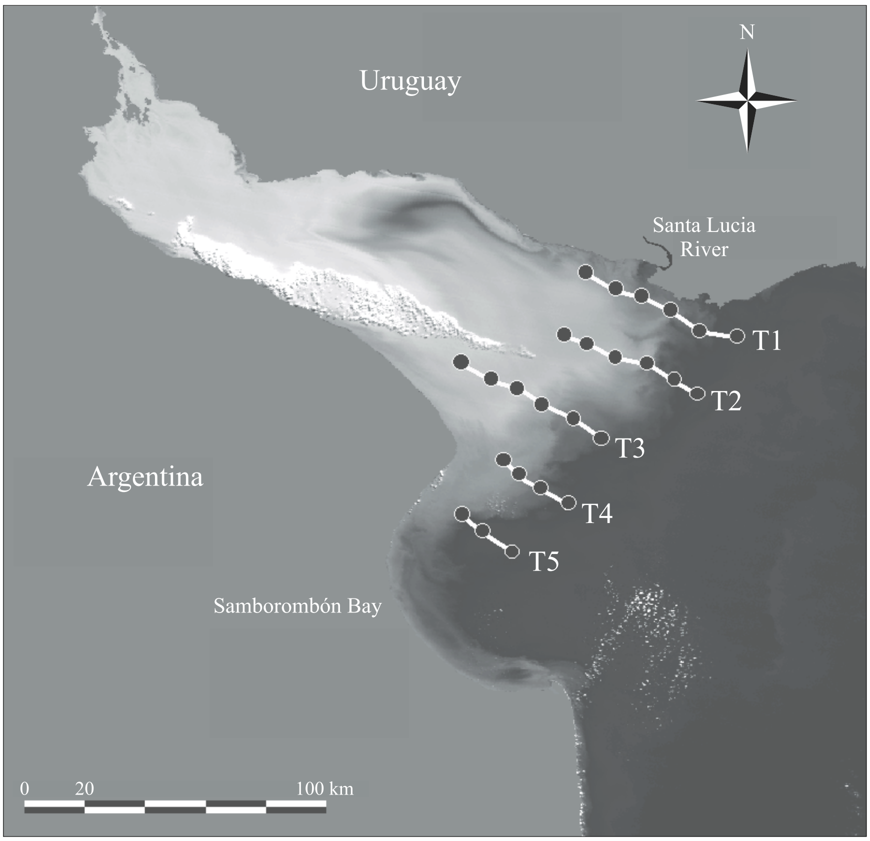

Figure 1. Sampling stations (black dots) along five transects (T1-T5) performed in March 2006 during the "CC-03/2006" coastal survey carried out in the turbidity front of the Río de la Plata area, whitemouth croaker (*Micropogonias furnieri*) spawning zone.

Figura 1. Estaciones de muestreo (puntos negros) a lo largo de cinco transectas (T1-T5) de la Campaña Costera "CC-03/2006" realizada en marzo de 2006 en la zona del frente de turbidez del Río de la Plata, zona de desove de la corvina rubia (Micropogonias furnieri).

pre-flexion larvae. Then, the *sagittae* otoliths were extracted by dissolving the head of each larva in a concentrated sodium hypochlorite solution (NaClO). In juveniles, extraction was performed with dissecting forceps. Later, otoliths were washed with distilled water to dissolve NaClO crystals, dried, mounted in a numbered glass slide with transparent resin (PRO-TEX or nail polish) and fixed in the *sagittal* plane (Secor *et al*., 1992).

To process digital images, the microstructure analysis was performed under a transmitted light microscope (Zeiss Axioscop model) connected to a computer equipped with an image analyzing system consisting of a video camera and a measuring software (Kontron 3.0). When the increments were not easily identified in the same plane, otoliths were polished using 12, 9 and 3 μm porosity lapping film paper.

The increments near the nucleus, quite narrow, were analyzed at 400X magnification. When wider, they were counted and measured at 200X magnification. At the microscope, improved visualization and contrast were obtained with filters of polarized light. The daily deposition pattern was considered according to the criteria established by Braverman *et al.* (2015).

To describe otolith development, images from individuals of different size were taken. The major radius of each otolith (R_m), total number of daily increments (I), increments width (E) throughout the R_m and the major radius of the first increment (R_1) were registered. When the number of increments in both otoliths of the pair coincided in, at least, 90%, age determination was made randomly selecting one of them. If a large difference was found, the otolith with increments easier to identify was considered. In the case of big ones, only those in which the microstructure in the accessory nuclei region was well visualized were taken into account. Those having fuzzy increments along the major radius were counted throughout other radii and E was calculated interpolating measurements to the major radius. Only the otoliths measured along the major radius were used to establish the length-radius relationship. Hatching dates were determined subtracting the number of daily increments from the dates specimens were caught.

The Laird-Gompertz (Zweifel and Lasker, 1976) growth model was fitted to the length-at-age data:

$$LS\ (t) = L_0 * \exp\ (k*(1-\exp\ (-\alpha * t))) \qquad (1)$$

where L_0 is the length at t = 0 (age 0); k, α, are parameters of the model and t is the age in days. Age was determined as the number of increments counted in the otolith + 3 that, according to Albuquerque *et al*. (2009), represents the number of days until deposition of the first increment occurs. The R^2 value was compared to the one obtained with a linear regression model also fitted to data. The first increment deposition was estimated through a linear regression analysis after checking independence of the specimen length. To relate somatic growth to otolith growth, the R_m with respect to length was analyzed. Finally, mean width values against age were plotted.

The instantaneous growth rates (G) for each individual were calculated deriving the Laird–Gompertz model (equation 1) with respect to time (Sánchez *et al*., 1999):

$$G = dLS/dt = \alpha\ LS * \ln((LS/L_0) - k) \qquad (2)$$

The inflection point coordinates (length and age) were calculated following Zweifel and Lasker (1976) expressions (equation 1):

$$LS_i = L_0 * e^{(k-1)} \qquad (3)$$

$$t_i = (1/\alpha) * \ln k \qquad (4)$$

Later, the asymptotic limit (L_∞) –the length at which the individual starts growing more slowly– was determined as:

$$L_\infty = L_0 * e^k \qquad (5)$$

Outliers were identified using the Bonferroni test: t = SSres/gl ≈ τ student, where SSres = sum of squares of standardized residuals and gl = degrees of freedom of the error. All the statistical analyses were made using a significance level $\alpha = 0.05$ (Sokal and Rohlf, 1999).

To relate somatic growth to otolith growth, the mean increments width and the fitted Laird-Gompertz model were represented in the same graph.

RESULTS

Individuals were in a good state of conservation since all of them had the three pairs of otoliths in their inner ears. A total of 122 pairs of otoliths from individuals 3-25 mm SL (Figure 2 A) were in a condition to be analyzed. The ages estimated ranged 7-92 days old. The major radius (R_m) varied from 31 through 806 μm (n = 98). The otolith radius at the first increment deposition (R_1) was 11.5-21.1 μm, with 16.67 μm mean value and a confidence interval ($CI_{95\%}$) = [16.30-17.00]. Whitemouth croaker larvae hatching period extended from December 2005 through March 2006, at the end of which a peak was detected (Figure 2 B).

The Laird-Gompertz (L-G) model was properly fitted to larvae length-age data (Figure 3 A). According to the model, L_0 = 2.76 mm ($CI_{95\%}$ = [2.12-2.79]) constitutes the mean larval length at the first increment deposition. The estimates for the rest of parameters were: k = 2.31 [$CI_{95\%}$ = 1.99-3.52] and α = 0.027 [$CI_{95\%}$ = 0.019-0.035]. The inflection point was observed at t_i = 31 days, SL_i = 10.23 mm and (L_∞) 27.81 mm asymptotic length. The linear model was also properly fitted

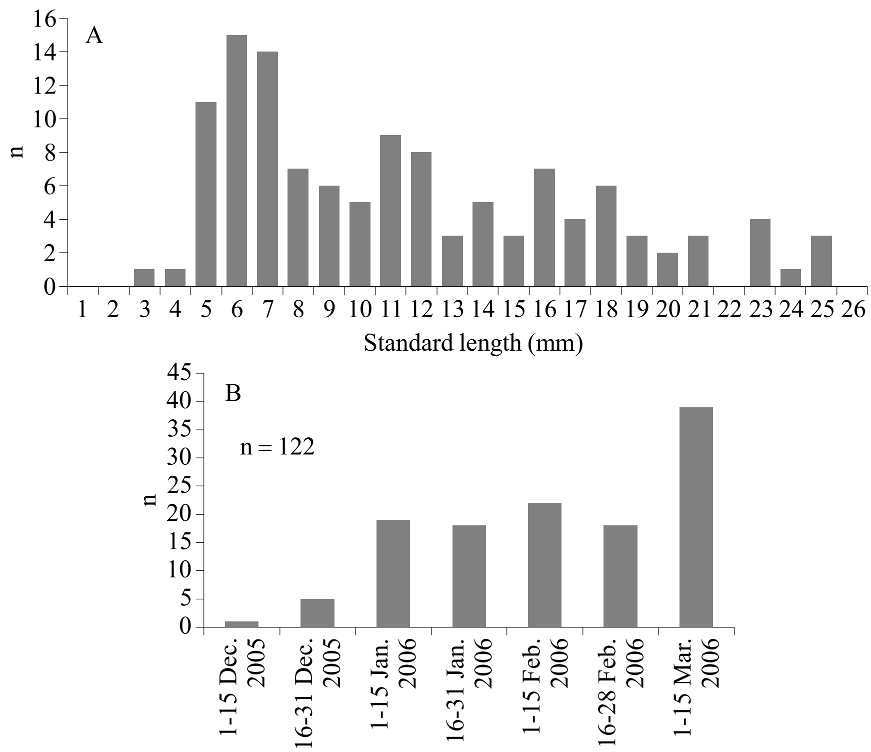

Figure 2. Length distribution (A) and hatching dates estimated (B) of whitemouth croaker larvae and juveniles.
Figura 2. Distribución de longitudes (A) y fechas de nacimiento estimadas (B) de larvas y juveniles de corvina rubia.

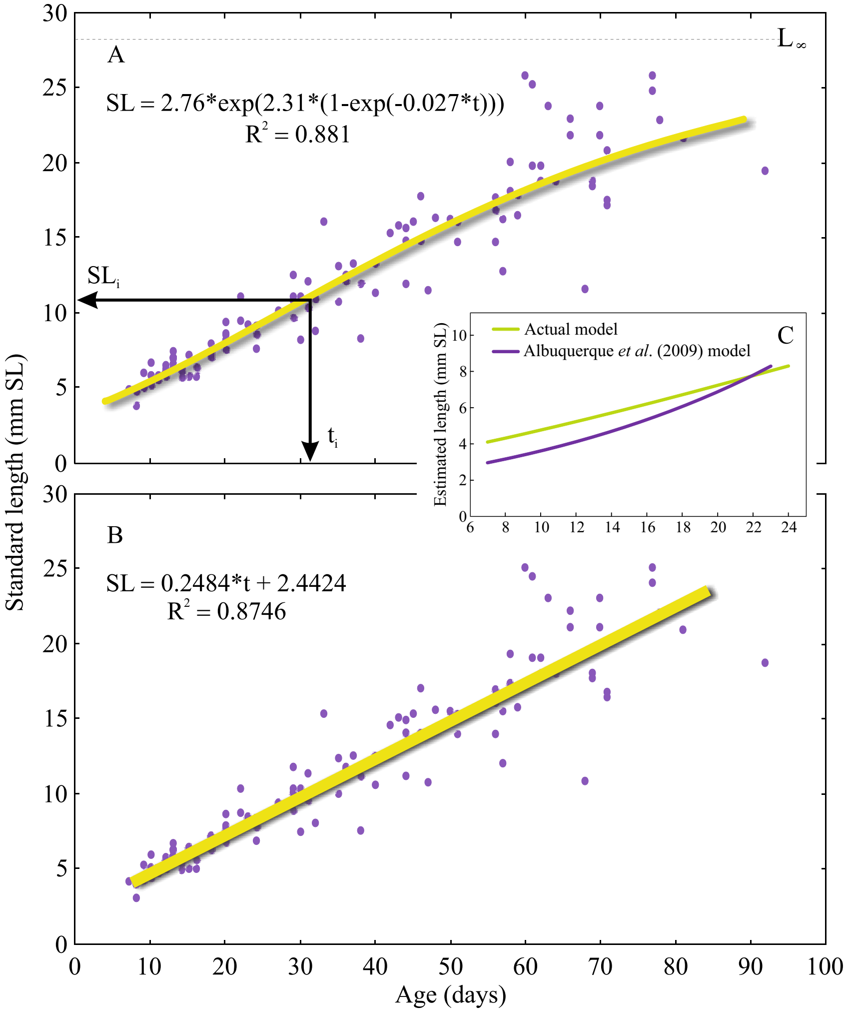

Figure 3. Length-age relationship of whitemouth croaker larvae and juveniles of up to 90 days old in the Río de la Plata system. Laird-Gompertz (A) and linear (B) models fitted to data. Length range: 3-25 mm SL; N: 117. C) Comparison of the Laird-Gompertz model presented in this work with the one fitted by Albuquerque *et al.* (2009) for up to 24 days old larvae in Lagoa dos Patos (Brazil).

Figura 3. Relación largo-edad de larvas y juveniles de corvina rubia de hasta 90 días de edad en el sistema del Río de la Plata. Modelos de Laird-Gompertz (A) y lineal (B) ajustados a los datos. Rango de longitud: 3-25 mm LS; N: 117. C) Comparación del modelo de Laird-Gompertz presentado en el presente trabajo con el ajustado por Albuquerque et al. (2009) para larvas de hasta 24 días de edad en Lagoa dos Patos (Brasil).

to the length-age data (Figure 3 B) with a slope representing the mean growth rate of all individuals (0.25 mm day^{-1}).

The instantaneous growth rates obtained deriving the L-G model showed a bell shape appearance when plotted against age (Figure 4), rose from 0.19 through 0.28 mm d^{-1} (the maximum value representing the inflection point of the model) and then decreased to 0.13 mm d^{-1}. The last value corresponded to the lowest growth rate estimated for the largest individuals (around 24 mm SL) with a size close to the theoretical asymptotic length (L_∞ = 27.8 mm LS) obtained with the L-G model.

The relationship between the otolith major radius and individual length was linear (Figure 5). As regards increments width, an increasing trend was detected when plotted against the 0-37 days interval. Above that age the indefinite trend observed suggests that the older the individual the larger the width variability. In the L-G model somatic and otolith growth showed a similar trend (Figure 6).

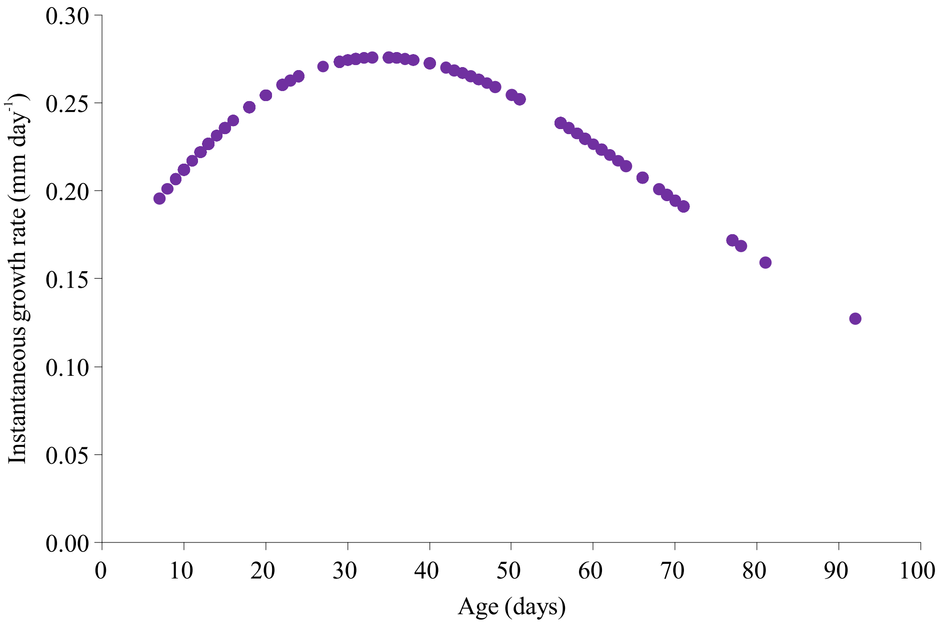

Figure 4. Instantaneous growth rate derived from the Laird-Gompertz model.
Figura 4. Tasa de crecimiento instantánea derivada del modelo de Laird-Gompertz.

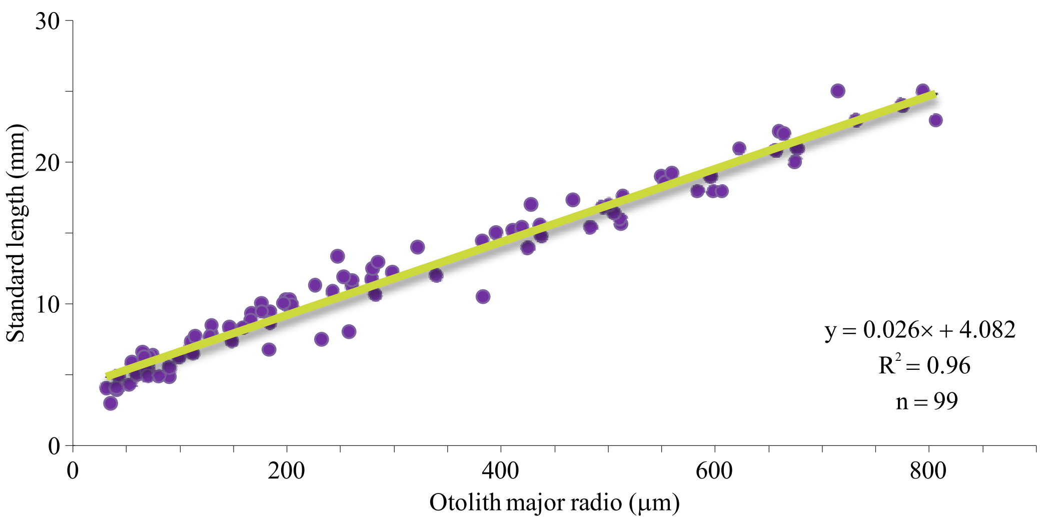

Figure 5. Relationship between the otolith major radius and whitemouth croaker larvae and juveniles length.
Figura 5. Relación entre el radio mayor del otolito y la longitud de larvas y juveniles de corvina rubia.

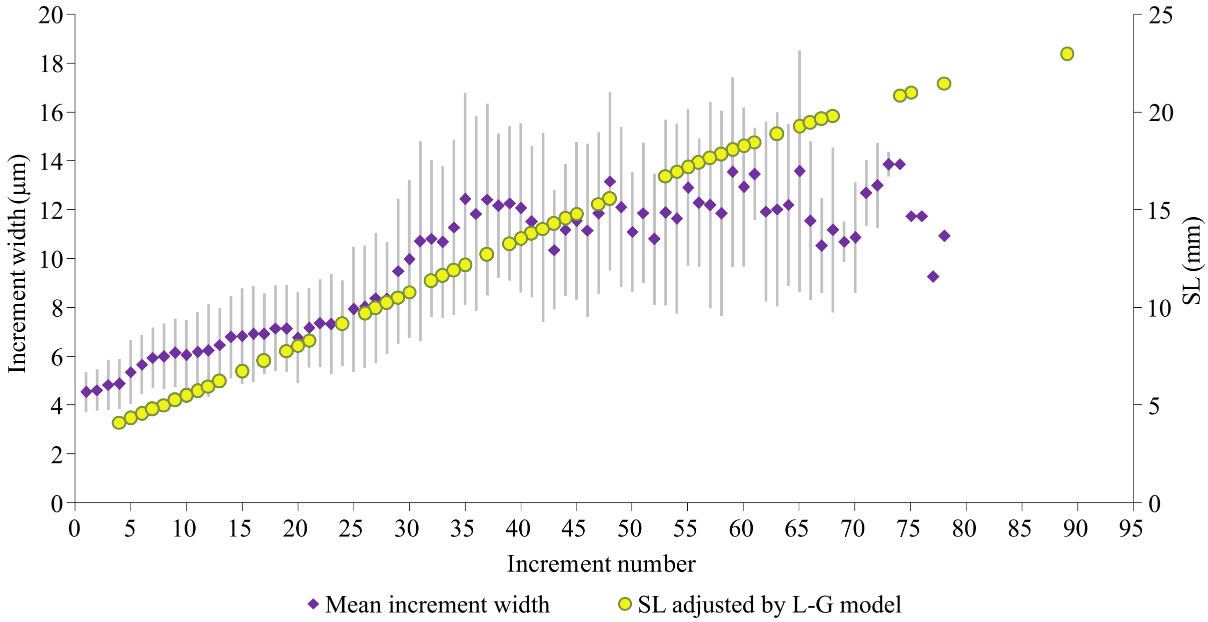

Figure 6. Comparative graph of *Micropogonias furnieri* larvae somatic and otolith growth. The Laird-Gompertz (L-G) model and the mean increments width with respect to age are presented. The vertical bars indicate the standard deviation of the mean width values.

Figura 6. Gráfico comparativo del crecimiento somático y del otolito de las larvas de Micropogonias furnieri. *Se presenta el modelo de Laird-Gompertz (L-G) y el espesor medio de incrementos con respecto a la edad. Las barras verticales indican la desviación estándar de los valores medios del espesor.*

DISCUSSION

It was proved that, depending on the temperature at which they are born and grow, fish cohorts experience growth differences (Houde, 1996). The results presented in this work indicate that individuals derived from cohorts spawned mostly in summer 2006 and that, based on the reproductive biology of adult specimens, birth occurred within the spawning period (Macchi *et al*., 2003). According to historical records, said period (austral summer) corresponds to the highest temperature registered in the Río de la Plata system, *c.a.* 22 °C (Guerrero *et al*., 1997). Thus, taking into account that a high temperature level favors larval growth, it may be considered that the individuals analyzed were grown in good temperature conditions (Houde, 1996; Werner, 2002). Consequently, the daily growth values determined in this study should be placed within a favorable context.

Both fitted models (linear and L-G) properly describe for the first time the larval growth of *M. furnieri* from the Río de la Plata mixohaline zone. Interpretation of the linear model constitutes a simple and useful tool to compare slopes. However, despite the good statistical fit obtained with it, taking into account genetic events, the L-G model should be considered more appropriate to describe larval growth since the main features it comprises allow a more accurate understanding of the changes that occur throughout larval life: 1) the asymptotic limit, 2) the inflection point (Zweifel and Lasker, 1976). The strong linear relationship between otolith radius and larval length implies coupling of somatic and otolith growth, the latter as an indicator of larval growth. From this point of view, the increment width with respect to age (otolith growth) analysis could be considered a more sophisticated tool to evaluate individual growth than the bare fit of a model to length-age data. Such analysis and the L-G model showed a similar trend (Figure 6) that indicates a slow growth phase of the mean increment width followed by a fast rise of values and a variable phase with an indefinite trend with respect to age. The phase of slow growth could be related to the asymptotic larval length that corresponds to the lowest daily growth rate values. Different events such as metamorphosis and settlement in fish life history are often related to the L-G model inflection point where a change from a fast larval phase to a slow juvenile stage growth is shown (Zweifel and Lasker, 1976). According to Braverman (2011), *M. furnieri* metamorphosis occurs at 9-18 mm SL that corresponds to 30-60 days old individuals. In that sense, the inflection point obtained with the L-G model and the change observed in otolith growth (Figure 6) would coincide with the onset of metamorphosis. Such a coincidence was also detected in other marine fish species (La Mesa *et al*., 2009; Günther *et al*., 2012; Brown *et al*., 2014).

The parameters of the fitted L-G model used in this work are similar to the ones obtained for *M. furnieri* larvae under experimental conditions (Figure 3 C) or wild specimens from Lagoa dos Patos, Brazil (Albuquerque, 2003; Albuquerque *et al*., 2009). One important parameter to define is the larval size at age 0 (L_0). L_0 determination represents a crucial point when fitting growth models since an overestimated or underestimated L_0 could artificially increase or reduce daily growth rate values (Ekau, 1998). To avoid such a problem, it is important to have a high number of newly hatched larvae. The L_0 value of 1.85 mm NL registered by Albuquerque *et al.* (2009) is slightly lower than the ones determined in this study: 2.76 and 2.44 mm NL corresponding to the L-G and linear model, respectively. The difference could be attributable to the presence of larger individuals.

From an ecological point of view, the first increment deposition represents another larval feature to focus on. In some species it occurs at hatch (Fey *et al*., 2005); in others it is related to the start of exogenous feeding (Gjøsaeter and Øiestad, 1981; Lough *et al*., 1982). Through experimental observations, Albuquerque *et al.* (2009) demonstrated that *M. furnieri* otoliths appear only 24 hs after larval hatching and that the first increment is deposited 2 days later. According to this, to determine larval age it would be appropriate to add 3 days to increments count.

Few studies are available on age and growth of the *Micropogonias* Genus larvae, probably due to the difficulties encountered in daily increments reading. Table 1 shows growth models fitted to different length and age ranges of larvae of two species of *Micropogonias*. Nixon and Jones (1997) obtained L_0 values similar to the ones registered in this work and higher than estimated by Warlen (1982) and Cowan (1988). Considering the different length-age ranges and the criterion used to represent daily growth (mean vs. range of values), the growth rate obtained in this study fitting the linear model (0.25 mm d^{-1}) results fairly lower than that reported by Albuquerque (2003) for the brazilian ecosystem. Nevertheless, such a rate is contained in the interval observed by the author and within the range of values defined by Warlen (1982) and Nixon and Jones (1997). Cowan (1988), working with a linear model, established a daily growth rate of 0.19 mm d^{-1} for *M. undulatus* 40-80 days old larvae, value similar to the growth rate (0.18 mm d^{-1}) of the larval asymptotic length (27 mm SL) registered in this study for specimens of approximately 70 days old. Recently, Kupchik and Shaw (2016) provided data related to *M. undulatus* larvae otolith reading obtained fitting the L-G and the linear models. The respective results were: 0.12-0.196 mm d^{-1} growth rate and 0.20 mm d^{-1} mean value. To conclude, the daily growth estimates for larvae of the *Micropogonias* Genus mentioned in this work are similar to the ones reported by other authors.

Table 1. Comparison of the results of this work with previous studies about the daily growth of larvae and juvenile of the *Micropogonias* Genus.
Tabla 1. Comparación de los resultados del presente trabajo con estudios previos sobre crecimiento diario de larvas y juveniles del Género Micropogonias.

Species	Local	T (°C)	Length (mm SL)	Age (days-old)	Mean growth rate range (mm d^{-1})	Model	Source
Micropogonias furnieri	Río de la Plata estuary, Argentina	18-23	3-25	7-92	0.24	L(t) = 2.76*exp2.31*(1-exp(-0.027*t))	This work
	Lagoa dos Patos, Brazil	23-25	1.5-17.3	0-29	0.36 0.14-0.8	L(t) = 1.85*exp4.66*(1-exp(-0.018*t))	Albuquerque *et al.* (2009)
Micropogonias undulatus	Middle Atlantic Bight, USA	¿?	5-65	20-142	0.18-0.41	L(t) = 2.66*exp4.66*(1-exp(-0.0081*t))	Nixon and Jones (1997)
	Newport River estuary, USA	¿?		40-80	0.19	L(t) = 0.189*t + 0.634	Cowan (1988)
	North of Gulf of Mexico	¿?	3-14	15-64	0.16-0.27	L(t) = 0.93*exp2.88*(1-exp(-0.043*t))	Warlen (1982)
	Bayou Tartellan, Loussiana, Gulf of Mexico	10.2-17	3.5-15.3	20-70	0.12-0.20	L(t) = 1.5*exp2.61*(1-exp(-0.026*t))	Kupchik and Shaw (2016)

This work constitutes the first study on daily growth of *M. furnieri* larvae from the Río de la Plata estuary performed with specimens derived from an only cruise (synoptic sampling). Further efforts are needed to analyze possible spatio-temporal variations of the species larval growth and to evaluate the impact said variations may have in recruitment success.

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