



## Early developmental aspects and validation of daily growth increments in otoliths of *Micropogonias furnieri* (Pisces, Sciaenidae) larvae reared in laboratory

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**Abstract.** The rate of growth increment deposition in otoliths and the early development of *Micropogonias furnieri* larvae were studied in laboratory under salinity and temperature of 30-35 and 23-25 °C respectively. Hatching occurred between 20 to 22 h after the *in vitro* fecundation. Larvae presented closed mouth, unpigmented eyes and average standard length at hatch about 1.85 mm. Larvae started to feed around the 2<sup>nd</sup> day after hatch, when the eyes became fully pigmented. Laird-Gompertz growth model was applied and average, maximum and minimum instantaneous growth rate were estimated as 0.36, 0.78 and 0.14 mm.d<sup>-1</sup>, respectively. First growth increment in the otoliths was observed at the 3<sup>rd</sup> day after hatch and the increment formation rate was 1.044 per day. Average percent error in increment reading was 13.75, and lower values were observed after age 5 d. Present results support the assumption of daily growth increments in otoliths of *M. furnieri* larvae.

**Keywords:** Daily growth increments, ear stones, whitemouth croaker, fish larvae.

**Resumo.** Aspectos do desenvolvimento inicial e validação da formação de anéis diários em otólitos de larvas de corvina *Micropogonias furnieri* (Teleostei, Sciaenidae) cultivadas em laboratório. O desenvolvimento inicial de larvas de corvina e a taxa de formação de anéis de crescimento em otólitos foram estudados em laboratório. A eclosão das larvas ocorreu aproximadamente 22 horas após a fecundação *in vitro*. As larvas eclodiram com boca fechada, olhos não pigmentados e comprimento padrão em torno de 1,85 mm. A boca abriu cerca de 24 horas após a eclosão e a alimentação iniciou-se quando os olhos tornaram-se pigmentados, no segundo dia após a eclosão. O modelo de crescimento de Laird-Gompertz foi aplicado e as taxas de crescimento média e instantânea máxima e mínima foram respectivamente 0,36, 0,78 and 0,14 mm.d<sup>-1</sup>. O primeiro anel de crescimento nos otólitos surgiu em média no terceiro dia após a eclosão e a taxa de formação de anéis foi de 1,044 anel por dia. O índice de erro médio percentual na contagem de incrementos foi 13,75, com valores menores a partir do quinto dia. Os resultados encontrados permitem validar a taxa diária de formação de anéis de crescimento em larvas de *M. furnieri*.

**Palavras-chave:** anéis de crescimento, otólitos, corvina, larva de peixe.

### Introduction

Whitemouth croaker, *Micropogonias furnieri* (Desmarest, 1823), is one of the most important fish species in the Southern Brazilian

continental shelf. Together with *Cynoscion guatucupa*, supports both artisanal and industrial fisheries of the Argentinean, South Brazilian and Uruguayan coastal regions (Haimovici *et al.* 1989;

Jaureguizar *et al.* 2006). *M. furnieri* is considered an over-fished stock since the 90s (Paiva 1997, Vasconcelos & Haimovici 2006). It is an euryhaline species distributed from Gulf of Mexico, 20° N, to the Gulf of San Matias, 41° S (Chao 1978). In Southern Brazil, spawning occurs during summer (Ibagy & Sinque 1995), mainly in coastal waters of the Patos Lagoon estuary (32° 10.18'S and 52° 7.17'W). Eggs and larvae are passively transported into the estuary, where better environmental conditions favour their development (Muelbert & Weiss 1991). To analyze the effect of these estuarine conditions on larval development it is important to understand larval growth in the field. Besides, the comprehension of changes in growth and mortality of fish may improve the understanding of environmental factors that may affect fish survival and recruitment (Cushing 1988).

One of the most used techniques for studying larval growth in the environment is the analysis of daily growth increments in otoliths (Power *et al.* 2000, Nakaya *et al.* 2008). Studying the growth of fish larvae in field through otoliths requires preliminary knowledge on the rate of increment formation in the otoliths (validation), and on the existing relationship between otolith and larval growth. Validation has been approached by analysing otolith marginal increment (Moku *et al.* 2005), following larval cohorts (Morley *et al.* 2005) and by rearing larvae in laboratory, that represents one of the most used validation techniques (Campana 2001).

In this study we aimed to describe the periodicity of otolith increment formation and to evaluate the first increment deposition on sagittal otoliths of *M. furnieri*. Additionally we intended to describe some poorly known aspects of the early development of that species like size at hatch, growth rate and first feeding. The information presented here will be important to support ongoing studies approaching *M. furnieri* growth in the estuary of Patos Lagoon.

## Materials and Methods

Wild broodstock of *M. furnieri* were captured in the vicinity of Patos Lagoon estuary (South Brazil) during December 2002, and induced to spawn in the laboratory, with gonadotrophin injections (500 UI Kg<sup>-1</sup>). Eggs were artificially fertilized, and before hatching they were transferred to small larviculture tanks (15 L). After hatching started, cultured rotifers and the algae *Tetraselmis tetrathale* were added daily to the tanks to maintain a final concentration of 20 and 2000 ind mL<sup>-1</sup>, respectively. Seventeen days after hatch *Artemia franciscana* nauplii were also offered (2 – 3 ind mL<sup>-1</sup>). Salinity was held between 25 and 30, and temperature between 23 and 25 °C during the whole experiment.

Initially, after hatch 7 to 10 larvae were fixed each day using an anaesthetic solution of benzocain, and placed in alcohol 98%. After 7 days of hatching larvae were fixed at intervals between one and five days up to the 29<sup>th</sup> day. Larval standard length was measured, and sagittal otoliths were removed with surgical needles.

After removal, sagitta otoliths were glued on glass slides with immersion oil. All otoliths were photographed with a digital camera coupled to a light microscope (200, 400, 500 and 1000 x). Otoliths of larvae older than 10 days were mounted in epoxy glue, sanded with fine silicon carbide paper (2000, 8000 and 12000) and polished with car wax. Otoliths were measured on screen using UTHSCSA ImageTool software (University of Texas Health Science Centre at San Antonio, Texas, <http://ddsdx.uthscsa.edu/dig/itdesc.html>) and growth increments were counted by two independent readers on the digital images.

The rate of daily increment deposition was validated by comparing the number of increments counted in the otoliths to the known age of each larva. Otoliths from 82 larvae (from 132 examined) were successful read. The number of otolith increments was plotted against real age using each reading from the individual readers as one data point in order to consider the uncertainty between readers. Precision of counting was evaluated applying the Average Percent Error (APE) (Campana 2001):

$$APE = 100 \frac{1}{R} \sum_{i=j}^R \frac{|x_{ij} - x_j|}{x_j} \quad (1)$$

where  $x_{ij}$  is the  $i^{th}$  age determination of the  $j^{th}$  fish,  $x_j$  is the mean age estimate of the  $j$  fish and  $R$  is the number of times that each fish was aged.

A linear regression model was used to verify increment formation rate, and the slope was compared to 1 using an *f* test (Zar 1984).

The age of formation of the first increment was determined by the frequency of occurrence of known ages with one increment and confirmed by the intercept of the linear regression between real age and growth increment number.

Growth analysis was performed using linear regression and the Laird-Gompertz growth model (Ricker 1979), fitted to the length at age data using a non-linear estimation. This model is described as:

$$S_l = L_0 e^{k(1-e^{-at})} \quad (2)$$

where  $S_t$  is standard length (mm),  $L_0$  is the length at hatch (mm),  $k$  is a dimensionless parameter,  $\alpha$  is the exponential decay rate and  $t$  is the time (days).

To obtain the instantaneous growth rate the first derivative of equation (2) was considered:

$$G' = \alpha \cdot k \cdot L_0 e^{k(1-e^{-\alpha t})} - \alpha t \quad (3)$$

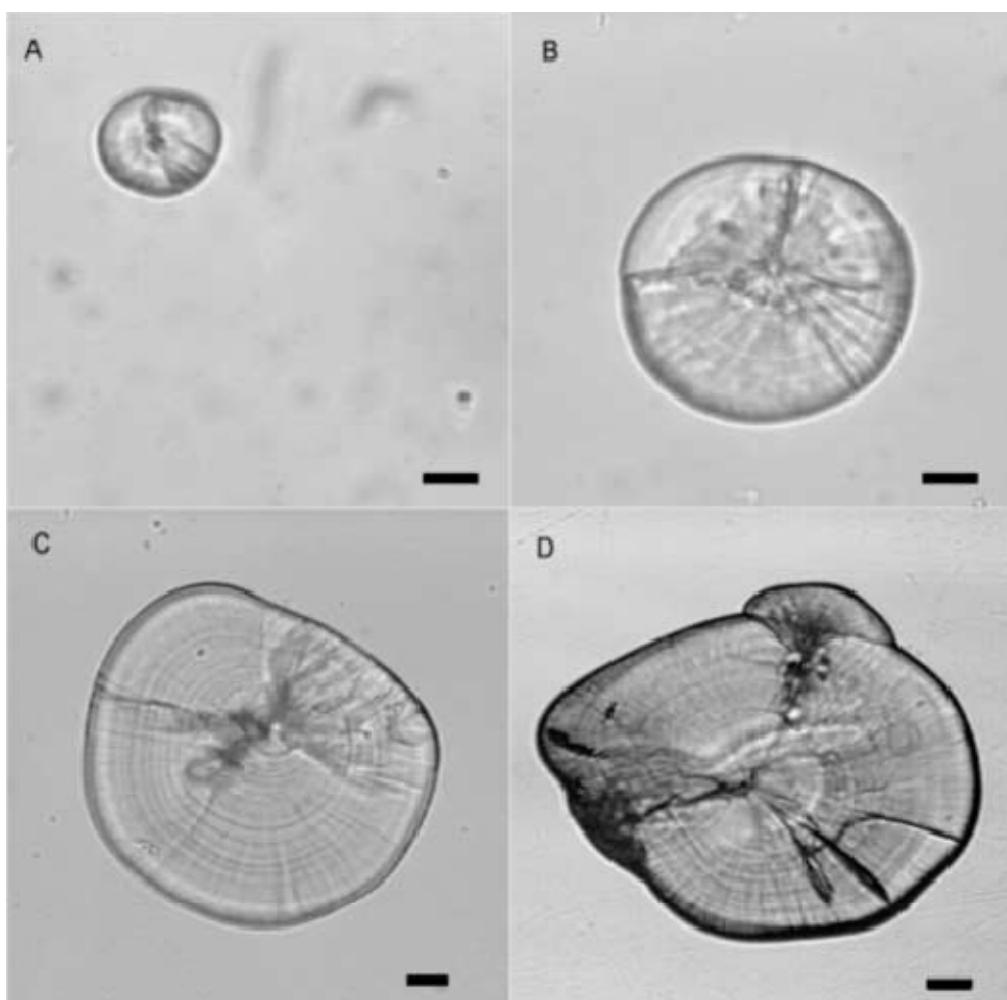
where  $G'$  is the instantaneous growth rate for each day  $t$ .

## Results

Larvae hatched with a large yolk sac, unpigmented eyes and closed mouth. Average initial standard length ( $L_0$ ) *in vivo* was 1.85 mm ( $\pm 1.17$  S.D.). Mouth opened twenty-four hours after hatching, however feeding initiated only 24 hours later (2 day old larvae), when the eyes became fully pigmented. The yolk sac was totally reabsorbed by

the 9<sup>th</sup> day, with larval standard length of about 3.5 mm.

Sagittal otoliths of *M. furnieri* were first observed no earlier than 24 hours after larval hatch, therefore, there was not hatching checks in otoliths of *M. furnieri*. Otoliths extracted from the youngest larvae measured 17  $\mu\text{m}$  in diameter, were flat at the inner surface (Fig. 1A) and showed one single primordium. Initial growth increments were easily observed (Fig. 1B) and the sulcus was not formed before the 19<sup>th</sup> day, when no differences between anterior and posterior edges were noticed (Fig. 1C). After 25 days, the rostrum, which defines the anterior margin of the sagitta (Secor *et al.* 1991) was evident, and the surface of otoliths became rugged and opaque and the first accessory primordium was observed (Fig. 1D).



**Figure 1.** Sagittal otolith photomicrography from *M. furnieri* larvae reared over 24 h (A), 7 days (B), 19 days (C), 25 days (D). Bar (A, B) = 6  $\mu\text{m}$ ; Bar (C) = 28  $\mu\text{m}$ ; Bar (D) = 35  $\mu\text{m}$ . Note the round shape during the initial stages (A and B) and the presence of accessory nuclei (D).

On day 29 the mean standard length was 12.99 mm ( $\pm 3.61$  S.D.), with a minimum of 6.58 and a maximum of 17.43 mm (Table I). Two stages with

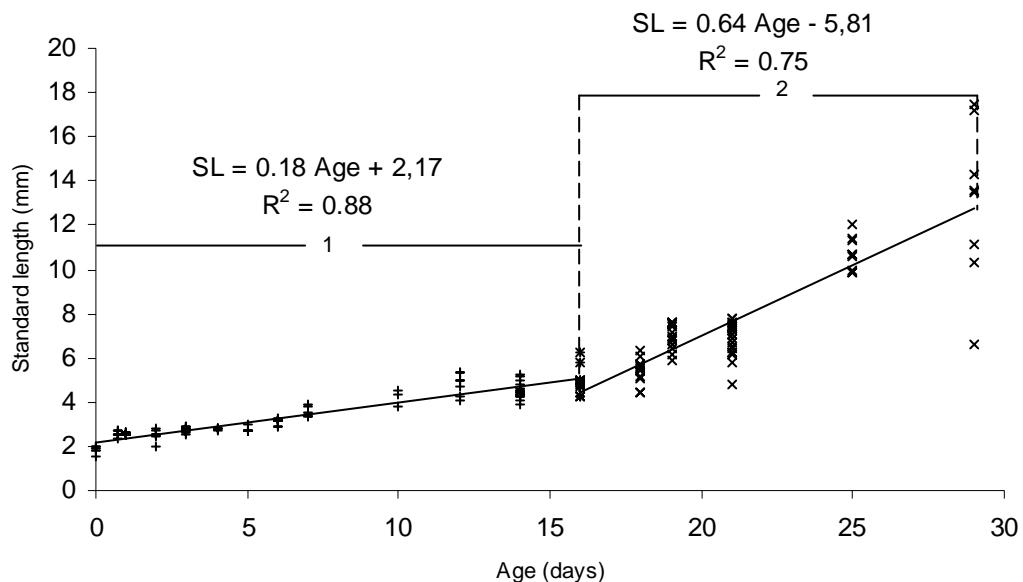
reasonably different growth rates were observed (Fig. 2). Laird-Gompertz growth model parameters were estimated as  $\alpha = 0.018$  and  $k = 4.66$ , while  $L_0$

was fixed at 1.85 mm (Fig.3). Instantaneous growth rate ( $G'$ ) showed increasing values, with minimum, average and maximum of 0.14, 0.36 and 0.78  $\text{mm.d}^{-1}$ , respectively (Fig. 3).

**Table I.** Mean standard length (SL) of larvae of *M. furnieri* reared in laboratory from hatching to the 29<sup>th</sup> day. S.D. = Standard deviation.

Day	SL (mm)	S.D.	Range (mm)	n
0	1.85	0.17	1.98 – 1.50	7
0.75	2.54	0.12	2.67 – 2.34	6
1	2.58	0.05	2.64 – 5.50	8
2	2.51	0.26	2.84 – 2.00	7
3	2.57	0.07	2.87 – 2.55	9
4	2.67	0.10	2.84 – 2.70	4
5	2.69	0.12	2.97 – 2.70	5
7	2.84	0.05	3.86 – 3.33	6
12	4.82	0.50	5.35 – 4.10	7
14	4.51	0.37	5.21 – 3.86	11
16*	4.99	0.6	6.24 – 4.28	12
18*	5.40	0.6	6.29 – 4.41	13
20*	6.43	0.5	7.40 – 5.39	12
21*	6.91	0.75	7.81 – 4.80	8
25*	10.72	0.75	12.00 – 9.86	9
29*	12.99	3.61	17.43 – 6.58	8

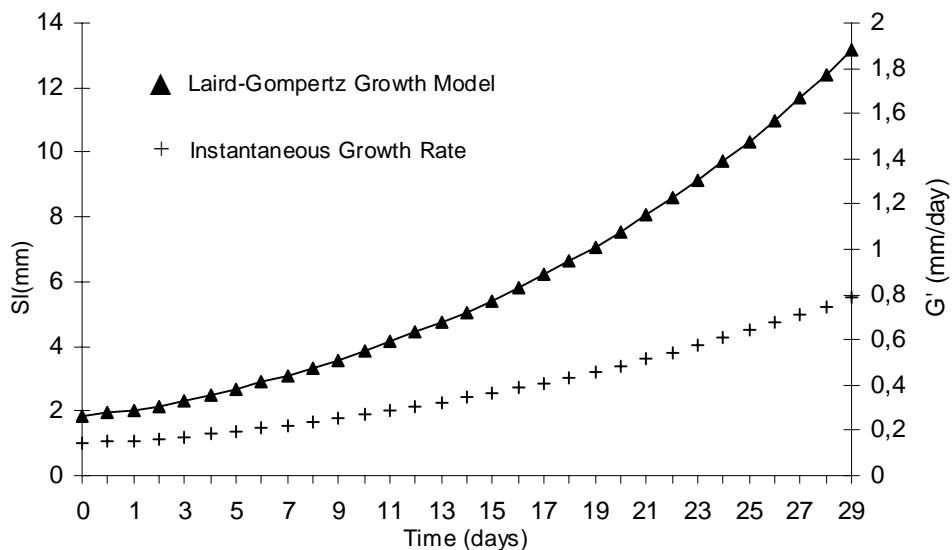
\* Fed with *Artemia salina* and rotifers



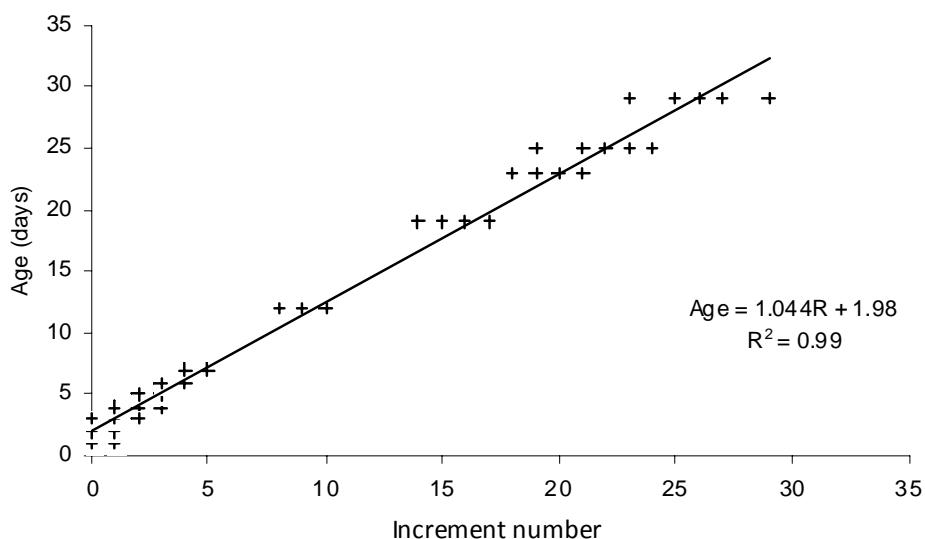
**Figure 2.** Age (days) and standard length (mm) for *M. furnieri* reared in laboratory. (1) Regression line for day 0 to 16; (2) for day 16 to 29.

The relationship between real age and growth increment number showed a linear pattern (Fig. 4), with slope of 1.044 ( $\pm 0.008$  S.E.) being significantly different from one if based on  $P < 0.05$ , or not significantly different when considered at  $P < 0.1$ . Analyzed otoliths showed from 0 to 29 increments that began

to appear at the 1<sup>st</sup> and 2<sup>nd</sup> days, but were most often observed at the 3<sup>rd</sup> day. This result associated with the regression intercept at 1.98 days indicated that, in average, two day old larvae have otolith with zero increments and, therefore, the first increment is deposited three days after hatch.



**Figure 3.** Laird-Gompertz growth model ( $\blacktriangle$ ) and instantaneous growth rate (+) for all reared *M. furnieri* larvae during the complete experiment.



**Figure 4.** Linear regression between known age (days) and increment number (R) for otoliths of *M. furnieri* reared in laboratory.  $n = 164$  (82 readings x 2 readers).

Average APE for all ages of *M. furnieri* larvae was calculated as 13.7 (Table II). The highest APE values were present at initial ages, reaching 37.0 for age 3 days. Absolute age estimates calculated from the linear model agreed better with known ages for young larvae than for older ones. The error observed for estimates at the end of the experiment (29 days) was approximately 1 day (Table II).

## Discussion

Growth of *M. furnieri* larvae in captivity can be separated in two stages. Initial slow growth, when

fed only with rotifera, was followed by faster growth when *A. franciscana* was added to the tanks. This growth feature is reported for fish larvae and related to the onset of external feeding (Zweifel & Lasker, 1976). Additionally, it is already known that larger prey promote better growth conditions than small prey (Hunter 1981). We suggest that the addition of *A. franciscana*, or its combination with rotifers, improved the quality of the diet and consequently larval growth. Accelerated growth rates have also been observed for sciaenidae larvae after 20 days of hatch as a result of settlement (Rooker et al., 1999) which could explain variability on growth rates in

nature. Other experiments should be conducted with the specific objective of examining what promotes that differential growth and the effect of adding larger prey to the diet of larval white-mouth croaker.

Except for the first 7-8 days, otoliths of *M. furnieri* larvae were not easy to read. Albuquerque & Muelbert (2004) used haematoxylin to improve contrast between growth bands in *M. furnieri* larvae collected from an estuary. We did some tests with haematoxylin following these authors but the visualization of growth increments from the larvae held in laboratory did not improve. Nevertheless, approximately 62% of the examined otoliths were successfully read. Most of the otoliths in our study presented the first increment completed three days after hatch. The time of first increment deposition is a species-specific feature (Ekau & Blay 2000) that allows to improve the accuracy in age and growth evaluations when it is correctly estimated

(Campana 2001). The time of first increment deposition can be influenced by environmental conditions like temperature and food availability (Radtke & Fey 1996) and can be influenced or coupled to some important life change, like hatch (Humphrey *et al.* 2005), beginning of active swimming (Laroche *et al.* 1982) and complete absorption of yolk sac (Peñaillo & Araya 1996). Since *M. furnieri* larvae started to feed around the third day after hatch, it is reasonable to suppose that the first growth increment deposition is associated to beginning of feeding, as suggested by Campana & Mosksness (1991). The variability observed on the age of first increment deposition seems to be induced by different time of first feeding. Nevertheless, for practical purposes, two days must be added to the total growth increment number on each otolith analyzed in order to estimate the real age of the larvae.

**Table II.** Average percent error (APE) for age determination of larval *M. furnieri* reared in laboratory from day one to 29. The estimated age was calculated using the linear model (eq. 6) and the 95% confidence intervals (CI) are presented.

Age (days)	Expected increment number	APE	Estimated age (days ± CI)	n
1	0	37.50	-	5
2	0	14.29	-	4
3	1	37.04	3.02 (±0.13)	7
4	2	26.67	4.07 (±0.13)	8
5	3	10.00	5.11 (±0.15)	7
6	4	8.16	6.16 (±0.15)	9
7	5	12.04	7.20 (±0.16)	8
12	10	2.79	12.42 (±0.20)	8
19	17	1.91	19.73 (±0.26)	7
23	21	2.57	23.91 (±0.29)	6
25	23	4.86	26.00 (±0.30)	7
29	27	7.23	30.18 (±0.33)	6
Average =		13.75		

Despite the fact that the age of first increment formation is apparently species specific, our results support the assumption made by Flores-Coto *et al.* (1998) that added two days to the total growth increment number for three sciaenid species, meaning that the time of first increment deposition is assumed to be about the third day after hatch. At a previous study Nixon & Jones (1997) added five days to the number of increments in otoliths of *M. undulatus* based on the conclusions presented for a co-family species (*L. xanthurus*). Considering the results presented here, for a co-gender species, the five days assumed by those authors could promote some underestimation on the estimated growth rates, particularly for the younger fishes.

In reference to age validation, the slope of the regression should be close to one since it is expected that one growth increment is deposited each day. Our results showed a slope of 1.044 (Fig. 4) which was significantly different from 1 when at a P-level of 0.05 or not significantly different at a P-level of 0.1 (*t*-test). This result highlights an important paradox based on the acceptance of a statistical result against a biological meaning. The difference from unity found at our study (0.04) has little biological importance and can only be clearly observed at advanced ages. If larger larvae and a wider age range than that used had been examined in our samples, probably the slope would be closer to unity and this difference would have not occurred.

There is a general acceptance for daily growth increment to occur on otoliths from larvae living in favourable environmental conditions (Campana & Neilson 1985), but it was suggested that starvation could disrupt the daily formation of increments (Method & Kramer 1979). According to Siegfried & Weinstein (1989) short periods of low food availability appear not to affect increment deposition in adults or large juveniles, but may change increment formation in otoliths of larval and early juvenile fish. Additionally, Campana *et al.* (1987) and Jones & Brothers (1987) argued that rough environmental conditions could induce the formation of narrower growth increments hardly resolved at common microscopy. In our experiment temperature, salinity and food were controlled and we do not believe that the experimental environment presented any restriction. Therefore, we conclude that in otoliths of *M. furnieri* larvae, increments deposition rate is one increment per day.

There are only few available studies assessing validation of daily growth increments in otoliths of Sciaenidae larvae. Our results reinforce studies developed for other Sciaenid species, as *Cynoscion nebulosus* (McMichael & Peters 1989), *L. xanthurus* (Siegfried & Weinstein 1989) and *Bairdiella chrysoura* (Hales & Hurley 1991) and strengthen the premise that otoliths of Sciaenid fish present daily growth increments during their entire larval stage. This is the case for most species in nature, when good otolith preparation techniques are used and good environmental conditions for growth are available.

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