Age validation and growth of yolked larvae of *Patagonotothen tessellata* (Richardson, 1845) (Pisces: Nototheniidae) from the rocky littoral of the Beagle Channel, Argentina*

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SUMMARY: Age and growth estimation of *Patagonotothen tessellata* larvae over the endogenous period was estimated by examining their otolith microstructure and the associated increment deposition rate. Analyzed specimens were incubated from egg-masses collected in Lapataia Bay, Beagle Channel, Argentina. Validation was performed by monitoring reared larvae of known ages. Actual age and increment count were regressed suggesting a daily deposition of one ring in the otolith, which began at hatching time and continued until larvae reached sixteen days old (time of massive mortality). Mean lengths at each age were estimated using Richards´ growth function; their constant (m) attained a value close to zero indicating that the data fitted to the von Bertalanffy model. Absolute growth rate was 0.55 mm*d^{-1}of total length at hatching, dropped near zero on the fifth day after hatching and afterwards, there was no growth. Yolk absorption rate (expressed as a ratio between minor axis/major axis of the vitelline-sac) showed a similar variation as that observed in absolute growth rate during the endogenous period. The total yolk absorption took five days in the unfed larvae group and seven days in the fed larvae group. The rapid absorption of the yolk and the observed growth pattern suggest that *Patagonotothen tessellata* larvae have a short endogenous period, reaching the state of first food uptake five days after hatching.

Key words: Fish larvae, otolith, age determination, daily growth, Notothenioidea, *Patagonotothen tessellata*.

RESUMEN: VALIDACIÓN DE LA EDAD Y CRECIMIENTO EN LARVAS VITELADAS DE *PATAGONOTOTHEN TESSELLATA* (RICHARDSON, 1845) (PISCES: NOTOTHENIIDAE) DEL LITORAL ROCOSO DEL CANAL DEL BEAGLE, ARGENTINA. – Las estimaciones de la edad y del crecimiento en larvas de *Patagonotothen tessellata* durante el periodo de alimentación endógena fueron realizadas analizando la microestructura de los otolitos y la tasa de deposición de incrementos. Los ejemplares analizados provienen de masas de huevos recogidas en Bahía Lapataia, Canal del Beagle, Argentina. La validación se realizó por medio del seguimiento de larvas de edad conocida mantenidas en acuarios. La regresión entre la edad real y los incrementos indicó la deposición diaria de un anillo en el otolito, comenzando al nacer y continuando hasta que las larvas tuvieron dieciséis días de edad (momento en el cual se registró la mortalidad masiva). La talla promedio en cada edad fue estimada usando el modelo de crecimiento de Richards, cuyo constante tuvo un valor cercano a cero, señalando un mejor ajuste al modelo de von Bertalanffy. La tasa absoluta de crecimiento fue de 0,55 mm d^{-1} de longitud total al nacer, descendió a valores cercanos a cero al quinto día, a partir del cual no se registró crecimiento alguno. El rápido consumo del vitelo junto a los patrones de crecimiento observados indican que las larvas de esta especie tienen un corto periodo de alimentación endógena, comenzando el periodo de alimentación exógena después de los cinco días de edad.

Palabras clave: Larvas de peces, otolito, determinación de edad, crecimiento diario, Notothenioidea, *Patagonotothen tessellata*.

INTRODUCTION

Otolith microstructure analysis is a powerful method to determine age structure of the larval fish population and to describe growth pattern (Jones, 1992; Lough *et al.*, 1982; Moksness and Wespestad, 1989; Radtke and Kellermann, 1991), especially in those species which show slow growth rates such as Subantarctic and Antarctic fishes. In addition, otolith microstructure was also employed to describe environmental histories (Radtke *et al.*, 1990).

The formation of daily rings in the otoliths of Antarctic larval fishes was first described by Townsend (1980) but microstructure technique was only employed in a few cases (Hourigan and Radtke, 1989; Radtke *et al.*, 1989; Radtke and Kellermann, 1991; Ruzicka and Radtke, 1995).

This study is the first time that otolith microstructure technique is applied to the analysis of growth patterns and the validation of ring formation in Subantarctic fish larvae belonging to the Nototheniidae family.

Patagonotothen tessellata (Richardson, 1844) shows the typical reproductive behaviour described for intertidal gravel spawners by Balon (1975; 1981) and Rae and Calvo (1995a). The same spawning area is used by this species and by *P. cornucola* (Richardson, 1899), *P. sima* (Richardson, 1844) and *Harpagifer bispinis* (Schneider, 1801) following a temporal sequence and producing one cohort in winter and another in summer (Rae and Calvo, 1995a;

1996). The early stages of each species are observed in the environment at the same time suggesting an overlap in the use of the habitat.

As a result of this reproductive behaviour, the larval winter cohort hatches just before the phytoplankton spring bloom, when the sea is warming up and food availability is high (Rae and Calvo, 1995b). Contrarily, newly hatched larvae from the summer cohort are exposed to a habitat where sea temperature and phytoplankton biomass are decreasing (Hernando, pers. comm.) and lower than during the spring season.

Similar seasonality was observed in the Southern Ocean and was described as a key factor in the life of polar ectotherms (Clarke and North, 1991). In addition, a direct relationship was observed between the distribution of the early life stages and the transition time from endogenous to exogenous periods of nothothenioid larvae and food availability (moment of highest zooplankton biomass) in the Scotia and Weddell seas (Kellermann and Koch, 1984; Permitin, 1973).

To relate environmental factors with growth parameters, accurate estimates of larval growth are essential. This requirement could be fullfilled by the validation of increment deposition rate and the use of microstructure techniques (Geffen, 1992).

The focus of this study is to validate the rhythm of increment deposition and to describe the growth pattern during the endogenous feeding period in *Patagonotothen tessellata* larvae.
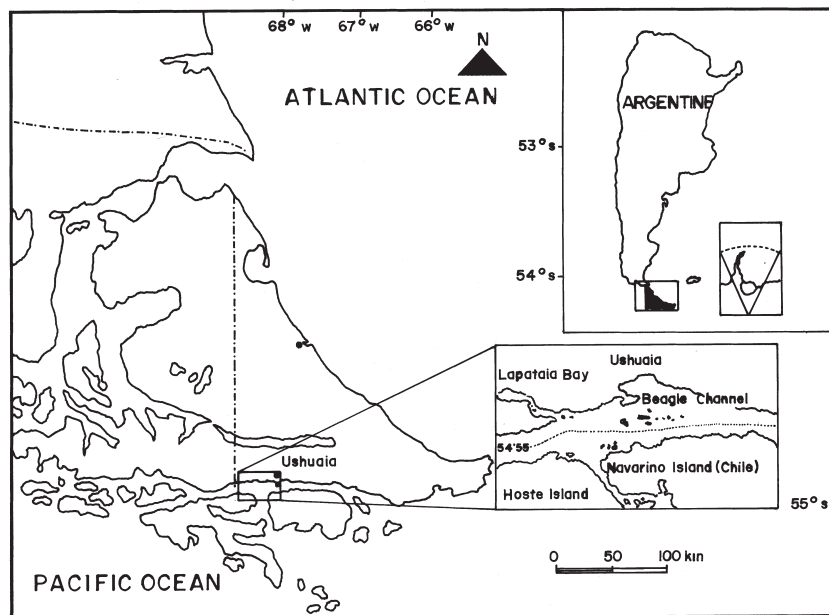

Fig. 1. – Study area in Lapataia Bay, Tierra del Fuego, National Park with sampling sites (enlarged).

MATERIAL AND METHODS

Collection of larvae

Patagonotothen tessellata egg-masses were collected from the natural spawning sites by hand during winter and summer in 1995 (Lapataia Bay, located within Tierra del Fuego National Park; Fig 1). After a nest was detected, each egg-mass was placed into a labelled individual container and transported to the aquarium, in which temperature, salinity and photoperiod were kept close to natural conditions. In those cases where egg-masses and adult males were found together in the same nest both of them were picked up.

Egg-masses were untouched until embryos had developed their eye pigmentation. Thereafter, embryos were placed one by one into a culture cell of 5 ml just prior to hatching and divided into two groups. One of them was unfed during the following sixteen days and the other was fed *ad libitum* with *Artemia* sp. from the third day. The whole volume of seawater in each culture cell was replaced three times a week with new filtered and oxygen saturated seawater.

Yolk-sac larvae were sorted based on their hatching date to get six specimens from each age-class obtaining thus a total of 90 specimens from 15 age classes and their otoliths. Standard length was registered to the nearest mm on the larval left side using a Zeiss dissecting microscope equipped with an ocular micrometer (10x/21).

Otolith preparation

Each pair of Sagittae and Lapilli was dissected using fine needles, transferred to a clean slide using a micropipette; washed in distilled water; dehydrated with ethyl alcohol and mounted using Hyrak mounting media. Each otolith was included on a numbered slide to guarantee random reading. Thereafter, otolith diameters (μm); hatching check radius (μm), and first feeding check (μm) were measured using a Zeiss compound microscope equipped with an ocular micrometer (12x/21).

Reading techniques

A total of 218 readings were performed on all available Lapilli and Sagittae. Otoliths were selected from larvae of one to sixteen days old (time of massive mortality). To obtain age determination,

each otolith was read twice and the corresponding weighted mean was calculated for each otolith type and finally for each specimen.

Estimation of the error was performed calculating percentage average error (Beamish and Fournier, 1981).

Validation of otolith increment deposition rate

The hypothesis that in otoliths, one increment is laid down daily was tested by following a single cohort of larvae kept in an aquarium and by determining if the change in number of increments matched the actual age. Once each larva was aged, the resulting increment counts were regressed to actual age (Wesolowsky, 1976; Zar, 1984).

To describe the growth during the endogenous feeding period, Richards' function was fitted to the data using the maximum likelihood method. The absolute growth was calculated according to the von Bertalanffy model (Campana and Jones, 1992; Kamler, 1992):

$$\text{Standard length} = L_\infty * (1 - \exp^{[-k*(\text{age} - t_0)]})$$

and

$$\text{Absolute growth rate} = k*(L_\infty - Sl_x)$$

where L_∞ is the asymptotic length, k is the von Bertalanffy growth coefficient, t_0 is the predicted age at which fish length is zero (this parameter has no biological sense) and Sl_x is the larval mean standard length (in mm) at age x.

Yolk absorption rate was estimated as a ratio between the major and minor axis of the vitelline-sac, and then it was regressed to the actual age.

RESULTS

Validation of daily growth increments

Daily growth increment was measured by regressing increment count of the larval otoliths to their actual age. During the exploratory data analysis it was found that five readings coming from different specimens exhibited absolute standard residual values greater than two and average percentage error (APE) greater than 30 %. Consequently, they were considered as outliers and removed from the analysis.

The best fit of the data was obtained by a linear model where 97% of the increment count variation
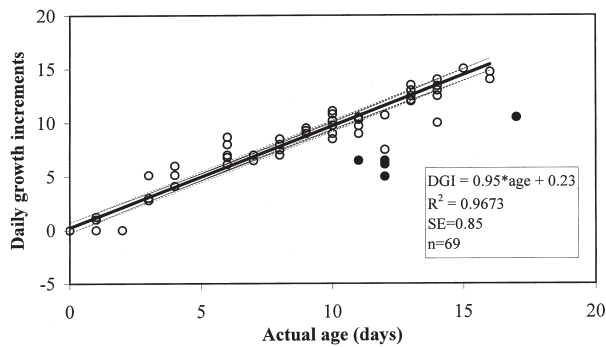
FIG. 2. – Estimated relationship between the number of daily growth increments (DGI) versus actual age (days) during the endogenous phase of larvae of *Patagonotothen tessellata* (broken line: 99 % confidence interval; 1 outliers).

was explained by the model (Fig. 2). The rate of increment deposition was close to 1 increment/day (H_0: b=1; $|t|$=2.42; T 0.01(2), 67=2.65), suggesting a daily deposition rate in larvae of *P. tessellata*. Moreover, the elevation was not different from 0 (p>0.05), denoting that deposition of the rings began just after the hatching time.
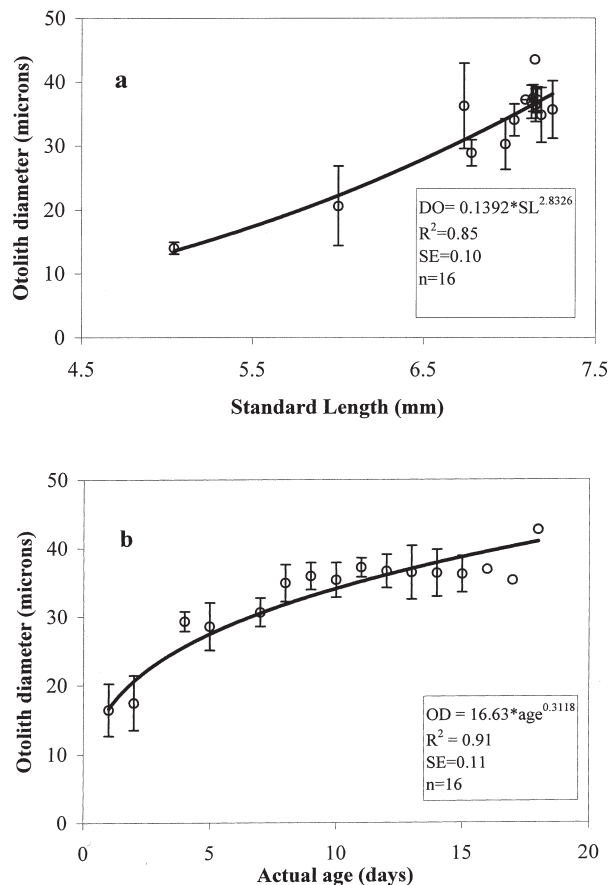
Otolith microstructure

All of the analyzed otoliths showed evidence of deposition of a growth zone during the endogenous feeding period and two marks were noticed. The first one corresponded to the hatch check with a mean radius of 10.8 μm (sd=2.29, n=62), whereas the second check (called first feeding check, FFC)was laid down as a result of the total absorption of the yolk with a mean radius of 15.26 μm (sd=1.67, n=43).

Two other marks were noticed in the otoliths, the "pre-hatching rings", which were found in larvae from 0 to 5 days old and "subdaily increments" that were observed interposed between daily increments. Accuracy and precision of the readings were estimated using average percentage error (APE). Precision estimates showed a range among 0% and 53% with a mean of 8.5% (sd=11.67, n=64), while accuracy estimation exhibited a range from 0% to 66% with a mean of 13.4% (sd=16.56, n=64).

FIG. 3. – Relationships of otolith diameter (OD) with (a) standard length (Sl) and (b) actual age. Bars represent standard error.
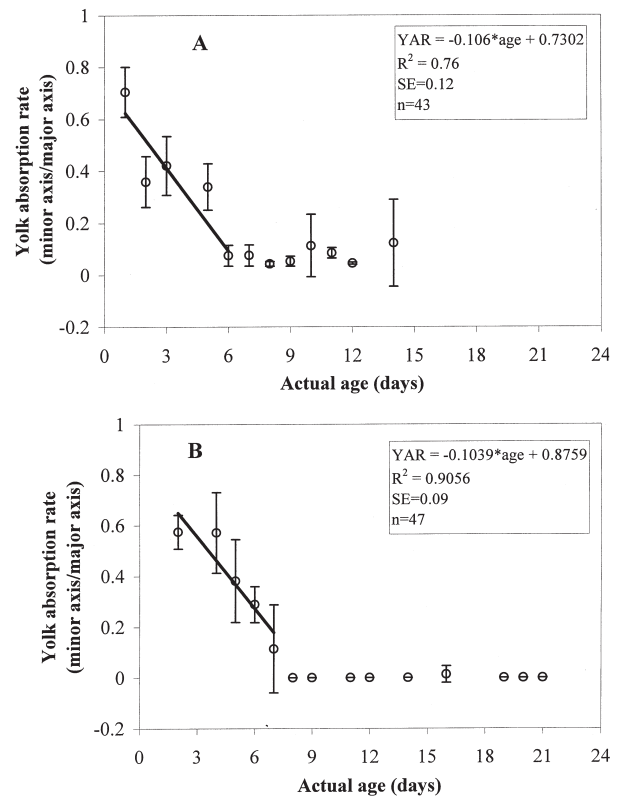

FIG. 4. – Relationship between yolk absorption rate and the actual age of the larvae of *Patagonotothen tessellata*. Yolk ratio is equal to minor axis/major axis of the vitelline-sac (values of x axis were displaced one unit, thus larvae of 0 day old correspond to value 1 in the x axis). (A) unfed larvae, and (B) fed larvae.
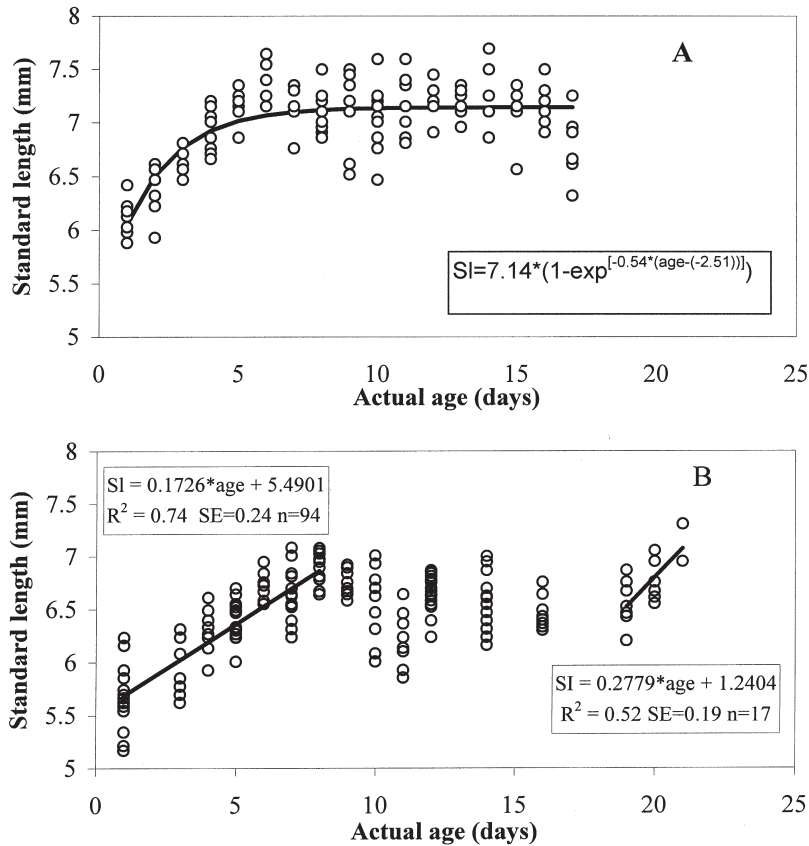
FIG. 5. – Relationship between standard length and actual age of *P tessellata* larvae (A) for unfed larvae and (B) for fed larvae. Solid line represents theoretical values from the model (values of x axis were displaced one unit, thus larvae 0 day old correspond to value 1 in the x axis).

Statistical comparisons were made between the diameter measurement of both Sagittae and between the number of increments observed in each type of otolith belonging to the same specimen. A non-significant difference was observed between the diameter of both Sagittae (Wilcoxon test, P>0.05; n=55), and between increment number of both Sagittae and Lapilli (Wilcoxon test, P>0.05; n=20). However, Sagittae were chosen for ageing because they registered a lower occurrence of subdaily rings and they were bigger than the Lapilli.

The relationship between otolith diameters of Sagittae and standard length was evaluated by fitting a power curve (Fig. 3a). The data showed a good fit and 85% of the diameter variation was explained by changes in total length. The few data observed for <7 mm larvae were a consequence of the fast growth rate noticed in larvae from 1 to 5 days old (see below).

In relation to the otolith diameters and the actual age, power model gave the best fit, explaining 90% of the diameter variation. The maximum value of 40.32 μm was attained at 16 days old.

Larval growth

During the endogenous period, growth in *P. tessellata* yolk-sac larvae showed its maximum value in larvae that were 5 days old. This pattern was also noticed in the yolk absorption, growth trend and absolute growth rate.

Yolk absorption rate was a linear function of the actual age showing similar slopes in unfed and fed larvae. However, the minimum values were attained on the 6th (Fig. 4A) and the 8th day (Fig. 4B), respectively.

In the unfed group, the growth trend was analyzed using Richards´ growth function and their constant (m) value was close to zero, indicating the data fitted to the von Bertalanffy model (Fig. 5A). According to the K coefficient, the endogenous stage asymptotic length of 7.14 mm was reached at the mean age of 5 days. In the fed group, the growth trend showed a similar pattern as that observed for the starved larvae during the first sixteen days (Fig. 5B). Afterwards, larvae began to growth faster.
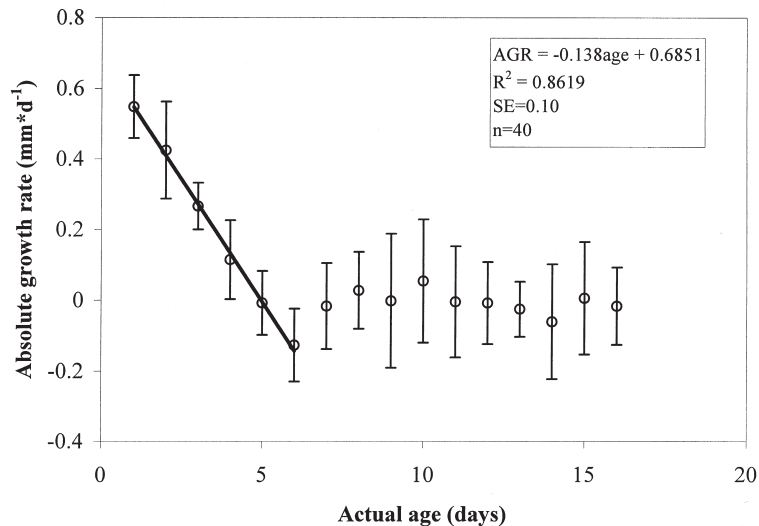
FIG. 6. – Relationship between absolute growth rate (derived from the von Bertalanffy model) and actual age (values of x axis were displaced one unit, thus larvae 0 day old correspond to value 1 in the x axis).

Absolute growth rate was a linear function of the actual age during the endogenous period. The slope indicated a fast decrease reaching its minimum value on the sixth day. Afterwards, AGR remained constant (Fig. 6).

DISCUSSION

To date, few studies have been done using microstructure analysis in Antarctic nototheniids (Hourigan and Radtke, 1989; Radtke et al., 1989; Radtke and Kellermann, 1991; Ruzicka and Radtke, 1995). The present study is the first for Subantarctic nototheniids.

The results of this study show evidence of daily increments in *Patagonotothen tessellata* larvae. From Figure 2, no difference between estimated and actual age of the larvae was observed. The first increment was laid down at hatching time and it was noticed as a check in the microstructure of the otolith. In addition, another check was observed as a consequence of the total absorption of the yolk.

Several authors found the same mark in other species at the end of the endogenous feeding period (Moksness and Wespestad, 1989; Radtke and Kellermann, 1991; Warlen, 1992; White, 1991), but variation as to when the first increment is laid down was observed even in the same species (Maillet and Checkley, 1990; Sepúlveda, 1994; Warlen, 1992). In *P. tessellata* larvae, this second check matched with the total consumption of the yolk, which occurred on the fifth day for unfed larvae (Fig. 4A) and on the

seventh day for the fed group, indicating the beginning of the exogenous feeding period.

Typically, growth in otolith diameter is curvilinear, suggesting that the growth rates are initially high and then decrease as does larval growth during the endogenous period (Lougth et al., 1982; Morales-Nin, 1986; Neilson and Geen, 1986; Sepúlveda, 1994). *Patagonotothen tessellata* otoliths follow this general growth pattern (Fig. 3b) and a marked decrease in the diameter growth rate was noticed to occur after the sixth day, time that coincides with the total yolk absorption time (Figs. 4A and B). Anyway, fish otoliths and their growth patterns record information about developmental processes and allow us to evaluate the past environments of the larvae.

Growth in *P tessellata* larvae coincided with the general pattern observed for the growth of larvae (Kamler, 1992). During the endogenous stage asymptotic length derived from the von Bertalanffy model was attained on the fifth day. Beyond this point, growth was interrupted and even negative growth could be registered. Similar situations were reported by Eldridge et al. (1981) for *Morone saxatilis* Walbaum, 1792 and by Quantz (1985) for *Scophthalmus maximus* Linné, 1758. The multifold increase of body size that characterizes embryonic and larval growth (Kamler, 1992) is noticed in the fed larvae group. Figure 5 reveals that during the endogenous period, growth patterns were similar in both larval groups during the first fifteen days. From the sixteenth day onward, acceleration of growth was registered only in the fed larvae group indicat-

ing the beginning of the next growth stanza, the exogenous feeding period.

During the endogenous period, absolute growth rate derived from the von Bertalanffy model was related to actual age. From Figure 6, two periods could be noticed. The first one is characterized by a linear relationship between the absolute growth rate and the first five days, while the second does not show any trend with values close to zero. Mean absolute growth rate has a value of 0.13 mm*day^{-1}. Similar values were found in pelagic larvae of Antarctic notothenioids (Clarke and North, 1991; Hubold, 1985; North, 1991; White and Burren, 1992).

In summary, the endogenous feeding period in *Patagonotothen tessellata* larvae extends from hatching time until the fifth day, when the yolk reserve is totally consumed. This event was registered in the otolith as a check (called "first feeding check"). *P. tessellata* larvae begin to feed just five days after hatching but the exogenous period is registered in the growth curve only after the sixteenth day (new acceleration of the growth).

These results give us a baseline to investigate starvation effects on the larval growth and to describe the next growth stanza: the exogenous feeding period.

ACKNOWLEDGEMENTS

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