*Marine Biology Research*, 2015
http://dx.doi.org/10.1080/17451000.2015.1031799

**ORIGINAL ARTICLE**

# The use of otolith microstructure in resolving issues of ageing and growth of young *Micropogonias furnieri* from southern Brazil

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**Abstract**
The corvina *Micropogonias furnieri* is a long-lived coastal sciaenid fish that sustains an important demersal fishery from southern Brazil to northern Argentina. The ageing of young corvinas in the Patos Lagoon estuary has been controversial. In former studies, up to six thin opaque bands in their otolith sections were considered annuli, supporting the hypothesis of slow growth and long permanence in the lagoon. However, corvinas caught in coastal waters with the same number of annuli were much larger. This fact raised doubts regarding the periodicity of the band formation in the otoliths of lagoon specimens. To better understand the growth of young corvinas, we examined the microstructure of otoliths. First, we validated the daily periodicity of growth increments using an oxytetracycline marking experiment. Second, 130 wild specimens were aged: 88 from the lagoon (30–285 mm TL, 51–678 days) and 42 from adjacent marine waters (133–275 mm TL, 212–514 days). The thin opaque bands were found to be formed on an average of 159, 269, 341, 418, 477 and 547 days and their number was higher in those corvinas remaining in the lagoon. Specimens of a similar size were older in the lagoon, suggesting slower growth than those that move to the marine environment around the first year of life. These differences may be due to growth constraints on those juveniles that remain in the more variable estuarine environment in the lagoon, or to a larger food supply in the more stable coastal environment.

**Key words**: *Ageing, checks, corvina, daily growth increments, marking, otolith, oxytetracycline*

## Introduction

Ageing is a highly relevant issue in ecological studies of fish populations, not only to estimate growth, mortality and longevity, but also to date events along their life history (Stevenson & Campana 1992). Many marine fishes have complex life histories in which juveniles and adults occur in spatially distinct habitats (Gillanders et al. 2003). For these fishes, the identification of the first annulus is an important component of ageing (Campana 2001). Otoliths (ear bones) are apposition structures which have alternate layers with different proportions of aragonite and an organic matrix – called increments – that may form on a daily or subdaily basis in addition to alternate larger bands with different transparency (more opaque or translucent) that, when formed on an annual basis, are called annuli (Morales-Nin 2000). Events such as changes in the habitat, movements between freshwater and seawater or from the pelagic to the demersal environment are common in the first years of the life of fishes (Spounagle & Cohen 1994; Wilson & McCormick 1999). These kinds of events may result in opaque and translucent bands in otoliths called checks or false annuli. Errors in the identification of the first annuli may be critical for the correct ageing of fishes, even if the periodicity of the former annuli has been validated in older fishes (Campana 2001). The analysis of the microstructure of the sagittal otoliths is a powerful tool to study the growth of young-of-the-year fishes, provided that the periodicity of the growth increment formation can be validated (Campana & Neilson 1985; Campana 1992, 2001). Chemical markers,

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*Published in collaboration with the Institute of Marine Research, Norway*

*(Accepted 10 March 2015)*

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such as oxytetracycline (OTC) or tetracycline, under experimental conditions, have been used to investigate the periodicity of the formation of these growth increments in the microstructure of otoliths (Hernaman et al. 2000; Zerbi et al. 2001; Cermeno et al. 2003). OTC is an antibiotic that chelates the calcium and magnesium present in calcified tissues and appears as an opaque ring on the otolith soon after being injected into the fish (Morales-Nin 1992).

The corvina *Micropogonias furnieri* (Dermarest, 1823) sustains the most important demersal coastal fishery along the southeastern and southern coasts of Brazil as well as along the coasts of Uruguay and northern Argentina (Vasconcellos & Haimovici 2006). It spawns in coastal waters near freshwater runoffs at the Rio de la Plata estuary (Macchi et al. 2003; Jaureguizar et al. 2003) and the narrow mouth of the 300 km long Patos Lagoon (Figure 1) between November and April (Haimovici & Ignácio 2005). In its estuarine region, the salinity of the Patos Lagoon is low when freshwater discharge is strong and increases when strong southwest winds reverse the outflow; thus, the change between low and high salinities occurs with a periodicity of weeks or months (Möller et al. 2001). Changes are faster in the channel along which the most water flows, and slower in the large, shallow, marginal embayments (Figure 1).

*Micropogonias furnieri* spawns in coastal waters and pelagic eggs and larvae are carried passively into the estuarine region of the lagoon (Sinque & Muelbert 1997). Pelagic larvae of 20–30 mm length recruit to the bottom where they feed actively on infauna and epifaunal organisms (Gonçalves et al. 1999), while the juveniles remain in the central and lower Patos Lagoon (Figure 1), where they find adequate conditions for faster growth (Garcia & Vieira 1997; Vieira 2006; Costa et al. 2013) until attaining a total length (TL) of 150–250 mm. After leaving the lagoon, young corvinas are found in the coastal region, mainly in shallow waters; later, they join the adult stock along the shelf (Haimovici et al. 1996). *Micropogonias furnieri* has large otoliths that show alternate opaque and translucent bands when sliced (Figure 2A–H). Cotrina & Lasta (1986) and Schwingel & Castello (1990) studied the annual periodicity of the formation of alternate opaque and translucent bands in the otoliths of specimens recruited to the adult stock. The corvina is a long-lived species that can reach ages over 35 years (Haimovici & Umpierre 1996). However, the interpretation of the alternate opaque and translucent bands in sliced otoliths is not straightforward, as small-sized specimens from the Patos Lagoon may show up to six bands, while larger specimens from coastal waters may have as few as one or two bands (Figure 2E,F). Castello (1986) observed up to five rings in the scales and otoliths of corvinas in the Patos Lagoon and concluded that, in this environment, at the age of two years, males measured on average 181 mm and females 205 mm. These values were much lower than the 311 mm TL reported by Schwingel & Castello (1990) and 323 mm TL reported by Haimovici & Umpierre (1996) for the two-year-old corvinas sampled from marine coastal waters. This contrast raised doubts not only about the annual formation of the rings in the scales and bands in the otoliths of small specimens caught in estuarine waters, but also on the existence of substantially different growth patterns between the lagoon and the marine environment.

The aim of this study was to better understand the growth of juvenile corvinas from the Patos Lagoon estuary and adjacent coastal waters, to estimate the age at which the displacement between these two environments occurs, and to compare their growth using the microstructure of their otoliths. For this, we experimentally validated the daily deposition of increments in the otoliths, assigned the ages at which the thin opaque bands form and identified the checks that hamper ageing. Juvenile ageing may help us understand the connectivity between estuarine nursery habitats and adult marine populations (Mateo et al. 2011) and provide information on the relative value of different habitats by comparing their relative growth (Ross 2003).

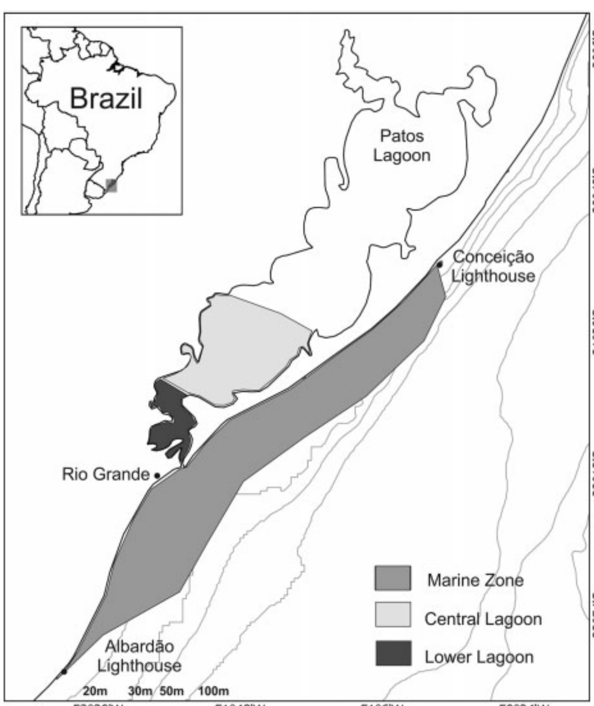


Figure 1. Sampling area for 130 juvenile corvinas, *Micropogonias furnieri*, in southern Brazil.

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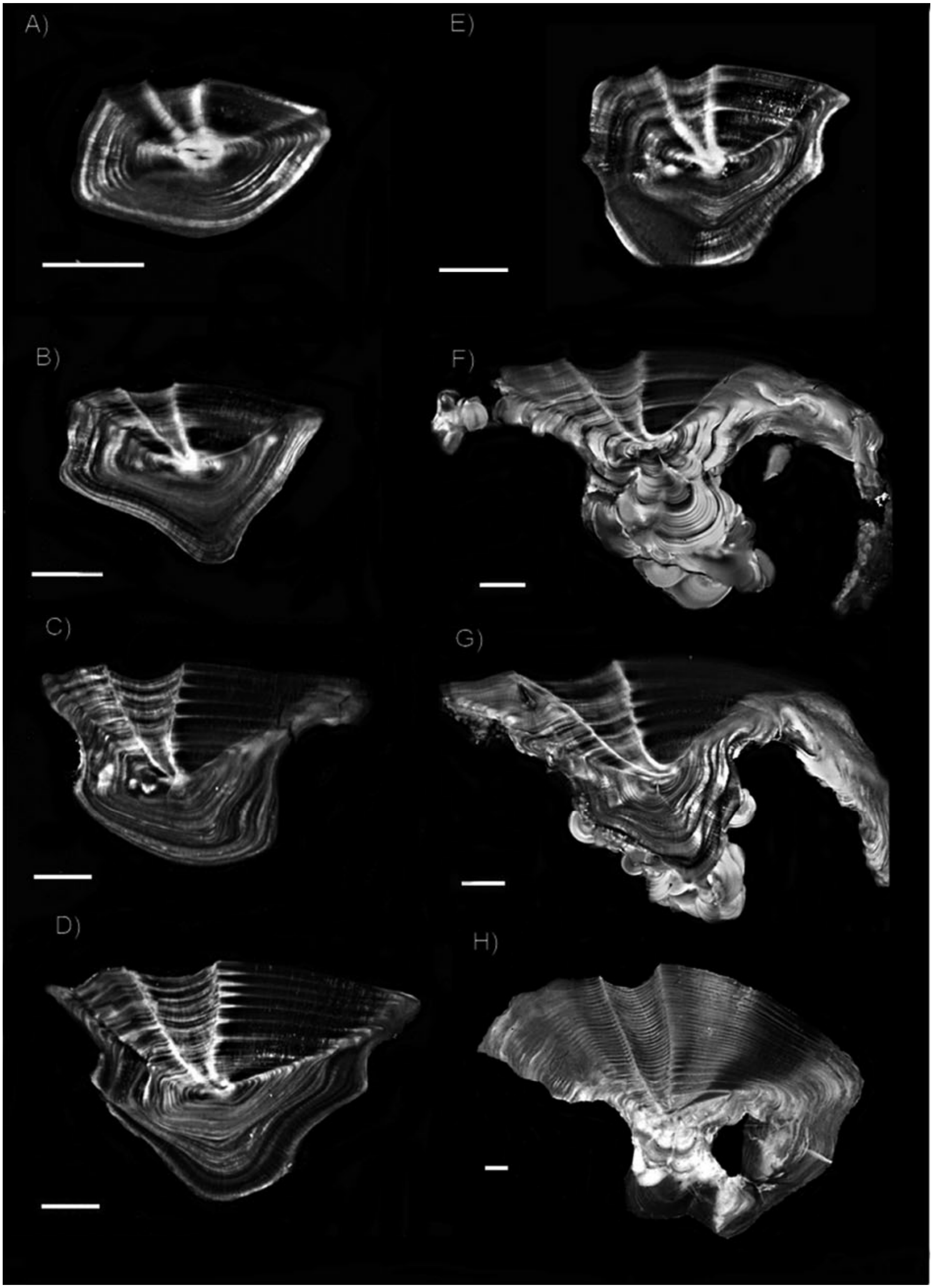


Figure 2. Saggital otolith sections from young specimens from the central and lower lagoon; (A) 85 mm and 145 days, (B) 134 mm and 260 days, (C) 215 mm and 464 days, (D) 220 mm and 678 days, and larger specimens from the marine coastal zone; (E) 149 mm and 384 days, (F) 400 mm and 2 years, (G) 454 mm and 5 years and (H) 471mm total length and 27 years. Scale bar corresponds to 1 mm.

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## Materials and methods

### *Validation of growth increment periodicity*

Validation of the daily formation of the increments was performed by a marking experiment in which 18 juveniles were caught with hook and line or cast nets in the Patos Lagoon estuary in March 2012. All specimens were measured (TL, mm) between the tip of the snout and the caudal fin and their total weight obtained in grams. The juveniles were divided into two tanks: seven specimens of 195–254 mm were placed in one tank and 11 specimens of 112–162 mm were placed in another. Tanks contained 300 l of water with a salinity of 31.9 ± 1.2 and were kept constantly aerated at 23.5 ± 0.9°C, under a photoperiod of 14 hours of light followed by 10 hours of dark in an acclimatized laboratory at 18°C. All specimens were acclimated for 12 days before being anaesthetized and receiving an intraperitoneal injection of 100 mg/kg fish of an aqueous solution of oxytetracycline (OTC). The fish were fed twice a day with the fish *Macrodon atricauda* (Günther, 1880) and the squid *Doryteuthis pleii* (Blainville, 1823). Additionally, the water in the tanks was changed daily. Only 15 specimens survived past the first seven days. Smaller specimens fed actively while the larger ones often rejected food. During the experiment, the mean daily growth of the group of small specimens was observed to be 0.51 mm/day, significantly higher than the growth of the group of large ones, which was 0.075 mm/day (Mann–Whitney, $p = 0.011$). Three specimens died 2, 3 and 17 days after marking, respectively, probably due to the handling stress for the OTC injection. The surviving juveniles were anaesthetized with benzocaine (50 ppm), measured, weighed and sacrificed in lots after 7, 15, 41 and 51 days (Table I).

Sagittal otoliths were cleaned with water and embedded in a transparent polyester resin. Cross-sections passing through the nucleus of 0.25–0.35 mm were obtained with a low-speed precision saw and diamond wafering blades (Isomet Buehler Ltd). The counting of the growth increments after OTC marking was performed using an epifluorescence microscope with ultraviolet light wavelength of 400 nm. The presence of OTC appeared as a fluorescent yellow ring near the edge of the otolith sections (Figure 3A). The dates of hatching of specimens were back-calculated from the date of capture and the age readings.

### *Ageing data*

A total of 130 juvenile corvinas with a total length between 30 and 285 mm were collected for ageing between February 2008 and August 2011 (Table II).

Table I. Total length (mm), death day, number of days after OTC injection and the major count of growth increments on the otoliths of 17 juveniles (*Micropogonias furnieri*) injected with oxytetracycline (OTC) on 12 April 2012 at the Marine Station for Aquaculture, RS, Brazil.

| Total length (mm) | Death day | Days after OTC | Counting |
|---|---|---|---|
| 118 | 14 April 2012 | 2 | 2 |
| 148 | 15 April 2012 | 3 | 3 |
| 237 | 19 April 2012 | 7 | 6 |
| 225 | 19 April 2012 | 7 | 6 |
| 209 | 19 April 2012 | 7 | 6 |
| 114 | 19 April 2012 | 7 | 5 |
| 149 | 19 April 2012 | 7 | 7 |
| 240 | 29 April 2012 | 17 | 17 |
| 250 | 27 April 2012 | 15 | 14 |
| 200 | 27 April 2012 | 15 | 12 |
| 186 | 22 May 2012 | 41 | 37 |
| 196 | 22 May 2012 | 41 | 29 |
| 163 | 22 May 2012 | 41 | 37 |
| 183 | 2 June 2012 | 51 | 48 |
| 152 | 2 June 2012 | 51 | 47 |
| 125 | 2 June 2012 | 51 | 49 |
| 134 | 2 June 2012 | 51 | 49 |

These fish were from three different fishing zones: (1) the central portion of the Patos Lagoon between 70 and 130 km from the estuary mouth, (2) the lower portion (estuarine) of the Patos Lagoon up to 70 km from the estuary mouth subject to salinity changes (Fernandes et al. 2005) and (3) the adjacent marine continental shelf between the Conceição (31° 45′S) and Albardão lighthouses (33° 15′S; Figure 1).

For age determinations, the otolith sections were progressively polished with 1500 to 12,000 grit silicon carbide paper (Micro-Mesh). Subsequently, sections were etched with 0.5% HCl by volume, for up to one minute, and immersed in water for 24 hours before being mounted on histology slides with synthetic resin (Entellan Merck) and covered with cover slips. On the sections, under a stereo microscope at 10× magnification, well-defined thin opaque bands were visible and their outer margins were determined by reducing the magnification of the objective (Figure 2). Using a transmitted light microscope at 400× magnification, daily growth increments were observed (Figure 3B). Readings were taken twice by the same reader; the last was considered as the specimen's age in days and a third reading was made if the first two differed by > 10%. Despite the first daily ring being deposited two or three days after hatching (Albuquerque et al. 2009), no corrections were made to add these rings in the final counting because they were not clearly discerned on the otoliths of the juveniles. Checks associated with the transition between the pelagic

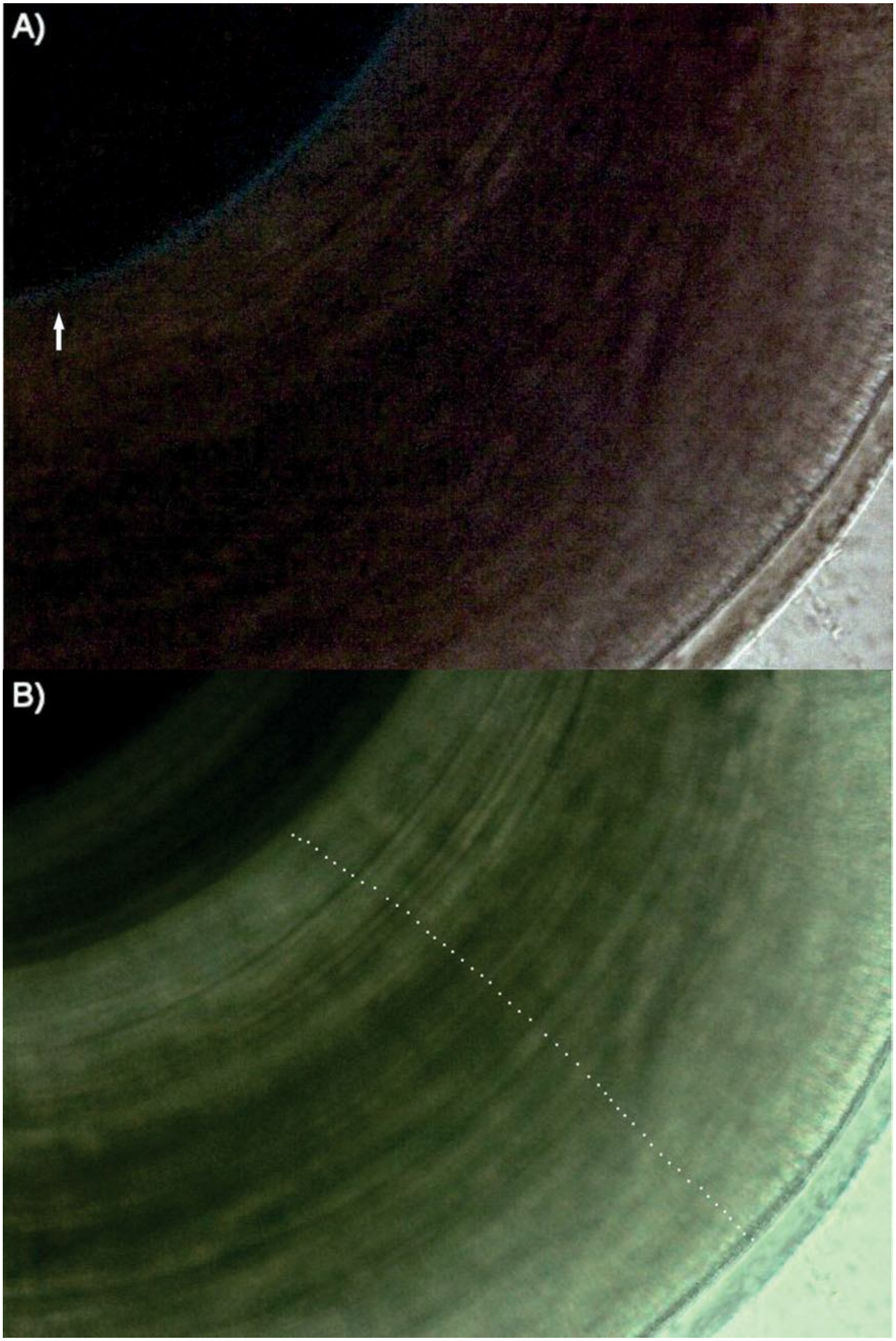


Figure 3. Daily growth increments (white dots) of an otolith of a juvenile marked with oxytetracycline at 162 mm total length and sacrificed 51 days later with a total length of 183 mm; (A) light at wavelength of 400 nm, and (B) normal light. Arrow indicates OTC marking.

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Table II. Total length range (mm), number of specimens ($n$) and number of daily growth increments on otoliths of juvenile *Micropogonias furnieri* from three different environments.

| Habitat | Total length range (mm) | $n$ | Daily growth increment |
|---|---|---|---|
| Lower lagoon | 30–273 | 50 | 59–644 |
| Central lagoon | 35–285 | 38 | 51–678 |
| Continental shelf | 133–275 | 42 | 212–514 |

and demersal habitat observed by Braverman (2011) in small juveniles in the La Plata estuary were not observable in our otolith sections. Daily growth increments were counted between the nucleus and the dorsal edge of the *sulcus acusticus* (Figure 2). The precision of the readings was estimated by calculating the coefficient of variation (CV) between the last two readings (Chang 1982).

To test the hypothesis of differences in the growth of juvenile *Micropogonias furnieri* between environments, we used an analysis of covariance (ANCOVA) in which the number of daily growth increments was the independent variable and the total length was the dependent variable after testing for normality (Kolmogorov–Smirnov one-sample test) and homogeneity of variance (Levene's test; Zar 1984).

At low magnification, distances between the focus and the end of the opaque bands ($B_1$ & $B_i$) and the edge (Bt) along the dorsal edge on the otolith sections were measured to back-calculate the total length of the fishes at their formation ($TL_{Bi}$). Because the relationship between the distances from the focus to the edge of the otolith sections and the total length of the fish was approximately linear through the origin (Figure 4), the relationship TLi = TL × $B_i$/Bt was used to back-calculate the total length at the end of each opaque band.

To analyse the relationship between the position of the thin opaque bands in the otoliths of the juveniles and the annuli in the otoliths of adults, the distances between the focus and the end of the first three annuli along the internal dorsal radius on the otolith sections of 1700 corvinas from 300 to 750 mm of TL were measured (Manuel Haimovici et al., unpublished data). These corvinas were collected between 2007 and 2010 by commercial fishing in coastal waters along southern Brazil for stock assessment studies. The criteria for the identification of the annuli followed Haimovici & Umpierre (1996).

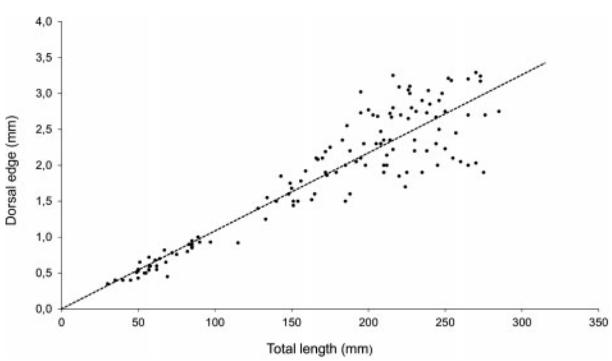


Figure 4. The relationship between the total length of juveniles (mm) and the length of the dorsal edge (mm) in 130 otolith sections.

## Results

### *Validation of daily deposition of growth increments*

Growth increments after OTC marking were observed in all specimens. Increments followed the same width pattern as those formed before marking for the specimens that did not present a disruption in their somatic growth. For those that did not accept food and almost ceased their somatic growth, the increments were narrower than the previous markings. The difference between the number of days after marking and the number of increments was zero for five specimens, up to four for 12 specimens and, exceptionally, 12 for a single specimen (Figure 5; Table I). The correlation between the number of days after OTC marking and of the major increment counts was high ($R$ = 0.992) and the slope of age regression was only slightly lower than unity

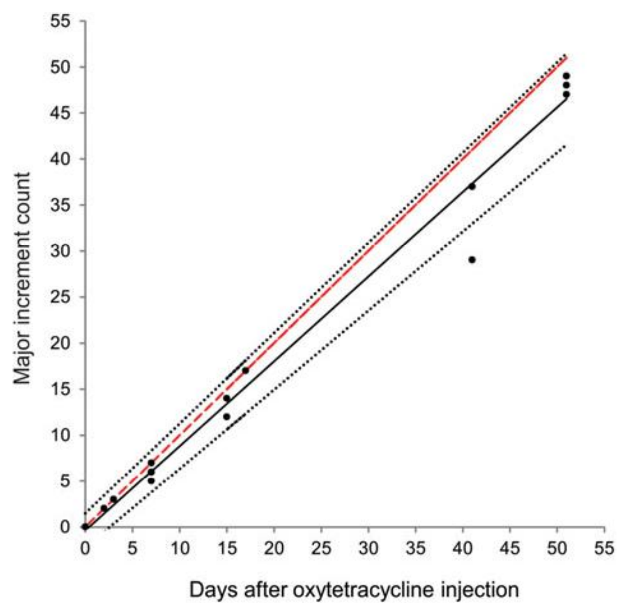


Figure 5. Number of daily growth increments observed using epifluorescent microscopy according to the number of days after OTC injection in 17 juveniles of *Micropogonias furnieri* under experimental conditions. The trend line is shown as the solid black line and the confidence intervals are shown as the dashed black lines. The reference line (slope = 1, intercept = 0) is indicated as the red solid line.

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($b$ = 0.9197; s.e. = 0.028; $p < 0.05$). For these reasons, although the number of counts may underestimate the number of days, the daily formation of the growth increments was considered validated. Growth increment formation was independent of feeding and somatic growth as all specimens, including those that stopped feeding, formed daily increments in their otoliths and this supported the hypothesis of an endogenous rhythm in their daily formation.

### *Ageing and checks*

In the otolith sections, daily growth increments were clearly discriminated along the axis between the nucleus and the dorsal edge of the *sulcus acusticus* (Figure 3B). Their daily number ranged from 51 in a 30 mm juvenile to 678 in a 220 mm female sampled in the central lagoon; the youngest corvina captured in coastal waters was 212 days old and measured 133 mm (Table II). The CV between the last two readings was 4.06%.

The number of thin opaque bands observed in the sectioned otoliths of all sampled corvinas ranged from zero (Figure 2A) to six (Figure 2D). The end of the first band corresponded on average to 159.6 days and decreased with the increasing number of bands from 109.6 days between the first and second bands to 70.5 days between the fifth and sixth bands (Table III). Specimens with one to six bands in their otoliths measured on average 93.9, 137.9, 171.1, 201.5, 232.6 and 255.4 mm, respectively (Figure 6; Table III). Therefore, the hypothesis that the thin opaque bands have a yearly pattern of formation was discarded and the thin opaque bands were considered to be checks.

When only corvinas with thin opaque bands in their otoliths were considered, the proportion of specimens with five and six bands was high in the central Patos Lagoon (61%, $n$ = 13) and lower Patos Lagoon (52%, $n$ = 25) and rare in the marine continental shelf (3%, $n$ = 37). The relationship between the number of bands and the total length as the covariate showed a significantly larger number of bands in the lower and central lagoon than the ones in coastal waters (ANCOVA $F$ = 17.54, $P$ = 7E–0.5).

Table III. Mean number of daily growth increments and back-calculated mean total length at the end of the thin opaque bands on sectioned otoliths of *Micropogonias furnieri* from the Patos Lagoon estuary and adjacent marine coastal waters ($n$, number of specimens; sd, standard deviation).

| | Daily growth increment | | | Back-calculated total length | | |
|---|---|---|---|---|---|---|
| Thin opaque bands | Mean (days) | *sd* | *n* | Average (mm) | *sd* | *n* |
| 1 | 159.6 | 45.4 | 93 | 93.9 | 16.7 | 95 |
| 2 | 269.2 | 49.2 | 87 | 137.9 | 18.1 | 93 |
| 3 | 341.7 | 41.5 | 62 | 171.1 | 18.2 | 65 |
| 4 | 418.6 | 47.5 | 45 | 201.5 | 21.5 | 51 |
| 5 | 477.1 | 49.0 | 25 | 232.6 | 23.1 | 32 |
| 6 | 547.6 | 54.1 | 13 | 255.4 | 13.6 | 19 |

### *Growth*

To compare the growth between juvenile corvinas caught in the Patos Lagoon and those caught in coastal waters, specimens smaller than 100 mm, absent in coastal waters, were randomly distributed between both groups (Figure 7). The difference was investigated by comparing the relationship between

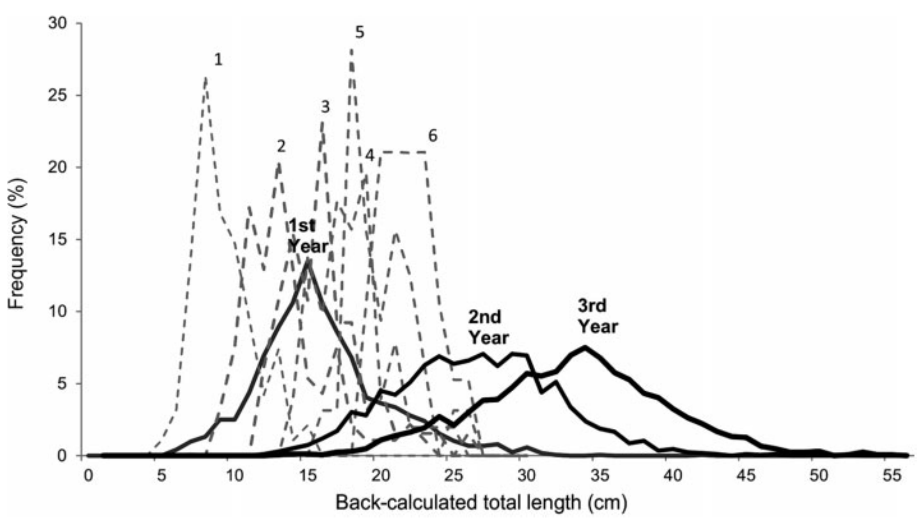


Figure 6. Back-calculated total length at the formation of each of the six thin opaque bands on the otoliths of *Micropogonias furnieri* juveniles from the Patos Lagoon (dotted lines) and back-calculated total length at the formation of the first three annuli of adult fish from the continental shelf (solid lines).

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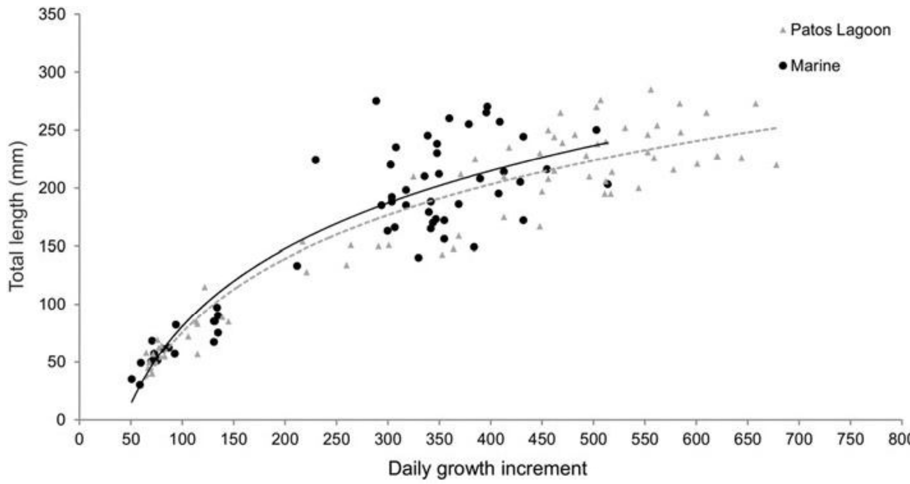


Figure 7. Relationship between the total length of juveniles and the number of daily growth increments in transverse sections of sagittal otoliths ($n$ = 130). Specimens caught in the lagoon are shown as a dotted line and grey triangles; those caught in adjacent coastal waters are shown as a solid line and black circles.

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their total lengths (mm) and their ages in days as covariants, after logarithmic linearization of both variables. The group from marine coastal waters was larger at similar ages than those that remained in the lagoon (ANCOVA $F$ = 24.32, $P$ < 0.0001). When only specimens over 100 mm TL were considered, those caught in the marine continental shelf grew on average 204.7 mm in 356.9 days ($n$ = 41), while those in the Lagoon only grew 213.6 mm in 467.9 days ($n$ = 53). At the age of one (365 days), specimens that remained in the Patos Lagoon estuary measured on average 195 mm while those from coastal waters had an average TL of 206 mm.

## Discussion

### *Validation*

The analysis of the microstructure of otoliths contributed to age determintion and to a better understanding of the corvina life cycle. Validation of daily formation of growth increments in otoliths of young corvinas was demostrated by the high correlation between growth increment counts and days after the OTC marking experiment. The slightly lower growth increment counts than the number of days after marking can be attributed to metabolic stress after OTC injection (Morales-Nin 1992). The consistency of the counting of increments was also supported by the correspondence between 80.3% of the back-calculated birth dates (October to March) with the spawning period of the corvinas in the marine coastal waters of southern Brazil (Vazzoler 1991; Haimovici 1998) and larval recruitment to the Patos Lagoon estuary (Muelbert & Weiss 1991; Sinque & Muelbert 1997).

### *Ageing and checks*

The identification of the first annulus is not an easy task in the corvina because it is obscured by the frequent occurrence of checks in the same size range at the formation of the first two annuli (Figure 2). However, it was observed that checks in specimens from the estuary have well-defined narrow opaque bands (Figure 2B–D), while the first annuli in corvinas larger than 400 mm were wider (Figure 2F,G) and became increasingly narrower in older individuals (Figure 2H). Therefore, in ageing adults the first annuli on the otoliths were identified as the the first large opaque band following a more translucid central zone. There is no simple way to distinguish checks from annuli. In fact, Castello (1986) used scales to age small corvinas from the Patos Lagoon, recording up to five rings; this author observed that they corresponded to opaque bands in the otoliths. He attributed slow growth to environmental stress in the estuary; the hypothesis of slow-growing corvinas that mature at a small total length in the Patos Lagoon was accepted in subsequent years (Vazzoler 1991). However, Cabral (2002) could not replicate Castello's validation or find fully mature small corvinas in the Patos Lagoon estuary, and raised doubts about the annual nature of the rings in the scales. The counting of these checks as annuli in former studies led to both the overestimation of ages and the underestimation of growth, besides attributing an estuarine phase up to four years to corvinas (Albuquerque et al. 2012).

We associated the higher number of thin opaque bands of the otoliths to those fishes that remained longer in the Patos Lagoon. Although an explanation for the nature of the thin opaque bands is beyond the scope of this study, some incidental evidence points

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to associating their formation to the influence of the temperature and/or salinity in the Patos Lagoon estuary. In this system, the distribution of salinity correlates with wind forcing and variations in freshwater input (Garcia 1997); during periods of low discharge, SE and SW winds force seawater into the lagoon, along the channel and beyond its mouth. In contrast, NE winds combined with high fluvial discharge significantly reduce estuarine salinity. Salinity changes are faster and more intense in the channel and deeper waters and slower and less pronounced in the shallow waters. Costa et al. (2013) observed that all size groups of juvenile corvinas occurred in all depth ranges of the estuarine region of the Patos Lagoon, but found a positive canonical correspondence between total length and depth. Small juveniles with a TL of 30–100 mm and up to around 150 days of life had no checks, suggesting they grew in the more stable shallow-water areas. The decreasing mean number of days between thin opaque bands (checks) for corvinas with an increasing number of daily growth increments and total length may be attributed to their greater mobility within the estuary between areas with different temperatures and/or salinities. Furthermore, corvinas larger than 150 mm that migrated to coastal waters, where the salinity and temperature are less variable, had fewer checks than those from the lagoon.

### *Growth and migration from the estuary to the ocean*

The observed larger size at age of the juvenile corvinas from the marine coastal region (Figure 7) may be the result of the slower growth of those remaining in the central and lower lagoon or the faster growth of those that migrated. Environmental stress may slow down juvenile corvina growth in those that remained in the estuary, where large salinity fluctuations of the Patos Lagoon are associated with river discharge changes and wind action (Möller et al. 2001). Moser & Gerry (1989) observed that *Micropogonias undulatus* (Linnaeus, 1766) actively avoids areas of unstable salinity in estuaries and assemble themselves in more stable areas, which implies that there must be an additional metabolic cost of fluctuating salinity for this species. The lagoon is connected to the sea by a narrow channel 800 m wide, and the avoidance of salinity changes by juvenile *M. furnieri* is far more difficult than in estuaries with an enlarged mouth in which a salinity front divides fresh and marine waters, such as the Río de la Plata estuary (Acha et al. 1999, 2004).

Although the metabolic cost in the Patos Lagoon may be higher, it is compensated by the availability of food, as young corvinas prey on few and abundant small epifaunal and infaunal organisms, including the tanaid *Monokalliapseudes schubartii* (Mañé-Garzón, 1949) and polychaetes such as *Nephtys fluviatilis* Monro, 1937 (Figueiredo & Vieira 2005). On the other hand, it is likely that feeding conditions for larger juveniles are more favourable in the coastal waters between 10 and 20 m in depth, which is rich in macrobenthic polychaetes, epifaunal gastropods and decapod crustaceans (Capitoli & Bemvenuti 2004). Whatever the cause of the migration, the observed fact is that juvenile corvinas that move to the coastal marine waters by the end or shortly after their first year of life grow faster than those that remain in the lagoon.

## Conclusions

1. Daily growth increments can be observed in sectioned otoliths of corvinas up to over one year old.
2. Opaque thin bands on the otoliths of young corvinas in the estuary were found to be formed over unequal time periods and should be considered checks.
3. Ontogenetic movements of the corvinas towards the coastal marine region begin by the end or shortly after their first year of life.

## Acknowledgements

We are grateful for the field and laboratory assistance provided by colleagues at the Marine Station for Aquaculture and at the Institute of Oceanography at the Federal University of Rio Grande. Dr Leandro Castello is thanked for his comments on the draft.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was supported by the Brazilian National Scientific and Technological Research Council (CNPq) under the scholarship granted to L.M.C. (Proc. 132469/2012-0) and the fellowship granted to M.H. (Proc. 309282/2011-0).

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