



Natural growth, otolith shape and diet analyses of *Odontesthes nigricans* Richardson (Atherinopsidae) from southern Patagonia[☆]



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ABSTRACT

Age and growth, otolith shape and diet of *Odontesthes nigricans* were analysed in order to provide an insight into the life history of the species and furthermore, to assess their possible use as a tool for discriminating silverside populations from the South Atlantic Ocean (Punta María) and Beagle Channel waters (Varela Bay). The age and growth analysis was performed by counting daily increments and annual marks in *sagittae* otoliths. Length-at-age data of individuals <65 mm standard length (SL) were fitted to the Laird–Gompertz model ($SL_t = 6.22 \exp^{2.45 [1 - \exp(-0.02 t)]}$), which provided an excellent description of the pattern of daily growth for *O. nigricans* juveniles from Varela Bay. The spawning period was also assessed through back-calculation of hatching dates and it extended from November to February. The count of annual marks in larger individuals identified 7 year classes (0+ to 6+) in Varela Bay and 6 year classes (0+ to 5+) in Punta María. The von Bertalanffy growth model explained more than 95% of the growth patterns observed in *O. nigricans* from Varela Bay ($SL_t = 245.49 [1 - \exp^{-0.24(t + 0.46)}]$) and Punta María ($SL_t = 345.09 [1 - \exp^{-0.15(t + 0.31)}]$). Particularly, k and SL_∞ varied significantly between sampling sites; reaching Punta María a larger SL_∞ value with a lower k . Otolith shape variation was also explored using elliptical Fourier analysis and it showed significant differences between Varela Bay and Punta María populations. Furthermore, gut content analysis characterized *O. nigricans* as an invertebrate predator, being benthic organisms the most important components of its diet, which also showed significant site dependence. The use of all these analyses contributed to a holistic approach which maximized the likelihood of correctly identifying both *O. nigricans* populations in the southernmost limit of the species distribution.

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1. Introduction

The silverside *Odontesthes nigricans* (Richardson, 1848) is widely distributed in the South Atlantic Ocean from Orense (39°S, Buenos Aires Province) to Cabo de Hornos (56°S) (Dyer, 2000). In Tierra del Fuego, the southernmost limit of its distribution, this native species can be found in the coastal waters of both the Atlantic Ocean and

the Beagle Channel; and is of considerable economic importance to artisanal and sport fishing. This species, together with *Odontesthes smitti* (Lahille 1929), represents the second most fished species after *Eleginops maclovinus* (Cuvier, 1830), with an average of 2.2 ton landed annually in the last years in Tierra del Fuego. Despite the species importance for small-scale fisheries and its wide distribution range, knowledge of its main biological features is scarce. Moreover, the available information comes from central populations of *O. nigricans*. The morphology of different osteological structures and otoliths were analysed in previous reports (Piacentino, 1990, 1999; Piacentino and Torno, 1987; Tombari et al., 2010) in order to define the taxonomic status of the species. The age and growth of a population from Golfo Nuevo (Chubut province,

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Argentina) were described by López (2009) who characterized *O. nigricans* as a fast-growing species, considering that it reached more than 50% of the L_{∞} in the first year of life. Carballo et al. (2011) also reported on the parasite fauna of *O. nigricans* from the same area of north Patagonia (Golfo Nuevo and Golfo San Jorge) and suggested that silversides are at an intermediate level in the trophic webs of the region. Currently, there is increasing interest in studying peripheral populations within a species' range as they are more likely to be imperilled than central populations. They tend to occur in less suitable environments and are often isolated from more central and continuous populations. Thus, peripheral populations are often smaller and more prone to extirpation due to stochastic or catastrophic events. Available evidence suggests that these populations are often genetically and morphologically divergent from central populations. Distinct traits found in peripheral populations may be crucial to the species, allowing adaptation in the face of environmental change. Therefore, peripheral populations will often have high value for conservation (Lesica and Allendorf, 1995). Only a few morphological (Morriconi et al., 2009) and ecophysiological studies (Fernández et al., 2009; Lattuca et al., 2009, 2013) have been carried out on *O. nigricans* from Tierra del Fuego, the southernmost limit of its distribution, however nothing has been done in order to explore geographic variation in phenotypic traits that often suggest the influence of environmental factors and local habitats (O'Reilly and Horn, 2004).

Age and growth have been the life history parameters most frequently used to distinguish fish populations because they are phenotypic expressions of the interaction between genotypic and environmental influences (Begg, 2005). Very different growth patterns can be exhibited by the same species in different environments or by the same population in different years (Ricker, 1975; Wootton, 1990). The approach of comparing life history parameters as a possible tool for discriminating populations has been successfully applied to other species (Tracey et al., 2006; Moore et al., 2012; Sequeira et al., 2012). Many other techniques, such as geometric morphometric, are considered appropriate for studying population structure (Ihssen et al., 1981; Begg and Waldman, 1999). Geometric morphometric is a robust tool for analyzing both physiological and morphological form, and has been used to discriminate between unique fish populations (Adams et al., 2004). Otoliths are regarded as an ideal subject for morphometric analysis due to their species specificity and limited extent of individual variability in growth, relative to variability in somatic growth (Campana and Casselman, 1993). In the case of otoliths, elliptical Fourier analysis represents a precise method for describing and characterizing outlines, efficiently capturing outline information in a quantifiable manner (Kuhl and Giardina, 1982). Trophic studies are also fundamental components for understanding the fish biology. Information from fish gut content analysis constitutes an important tool that can be used to relate fish and the physical environment in which they inhabit, when fish gut contents are determined (Baker et al., 2013).

In this context, the main goal of this paper was to investigate the contribution of growth parameters, otolith morphology and diet in determining the structure of *Odontesthes nigricans* populations. Furthermore, this study represents a more comprehensive approach about life history of *O. nigricans* from a geographical area where information on this issue is scarce.

2. Materials and methods

2.1. Fish collection and processing

Field works were carried out during 2010–2011 in two different coastal areas of Tierra del Fuego, Varela Bay (54° 52' S, 67° 16' W; Fig. 1) and Punta María (54° 01' S, 67° 20' W; Fig. 1).

Samplings were performed using a seine net in Varela Bay and gill and seine nets in Punta María. Particularly, seine netting (25 m long, 1.5 m high, and 3 mm stretched mesh size) covered, in two to three operations, ca. 100 m of shore line from the shallower littoral zone up to 1.5 m depth. Gill nets (30, 40 and 60 stretched mesh distance, each 10 m long and 2 m high) were placed perpendicular to the shore line in the intertidal zone and operated for ca. 6 h during the night.

Fish were killed by overdose in MS-222 solution and fixed and preserved in 96° alcohol. Standard length (*SL*) of each individual were measured with a digital calliper (± 0.1 mm) and total body mass (*BM*) and gonad mass (*GM*) was recorded to the nearest 0.01 g.

2.2. Fish condition

For each sampling site, fish weight–length relationships of silversides (Varela Bay $N = 100$, 49–250 mm *SL*; Punta María $N = 44$, 60–210 mm *SL*) collected during spring 2010 – summer 2011 were estimated by fitting the model $BM = a SL^b$ (Ricker, 1975). The parameters and their confidence intervals were estimated by linear regression of log transformed data. In addition, the condition of individual fish was calculated as $K = [BM [SL^b]^{-1}] 100$, where b is the slope of the linear regression of log transformed weight–length data.

2.3. Daily growth

Daily growth analyses were conducted using 0+ juveniles ($N = 57$, 30–69.8 mm *SL*) from Varela Bay during summer 2011. *Sagittae* otoliths were removed under a stereomicroscope, placed on a glass slide and included in a drop of transparent thermoplastic glue (Pro-texx)[®]. The embedded otoliths were polished using 12, 9 and 3 μ m lapping film paper to enhance the visibility (Secor et al., 1991). The number and width of each daily increment and otolith radius (*OR*) (Panella, 1980) were recorded under transmitted light, using an Axioscope Zeiss microscope at 200–400 \times magnification with an associated Sony CCD digital camera and an image analysis system (Kontron). All measurements were taken along the longest axis of the *sagittae* otoliths.

Increments were counted in both otoliths of the pair and, when the counts agreed in at least 90% between left and right otolith, the information provided from either of them was considered as the juvenile age. If the difference between both otoliths was greater than 10% neither of them was considered. When only one otolith from the pair was available due to the loss of the other during the extraction procedure, such information was used.

The daily pattern of increments in otoliths in *Odontesthes nigricans* was identified according to the criteria established by Barkman (1978) and Barkman and Bengston (1987) in *Menidia menidia* (Linnaeus, 1766); Peñailillo and Araya (1996) in *Basilichthys australis* Eigenmann, 1928 and *Austromenidia regia* (*Odontesthes regia* (Humboldt, 1821)); Gleason and Bengston (1997) in *Menidia beryllina* (Cope, 1867) and Brown and Fuentes (2005) in *Odontesthes bonariensis* (Valenciennes, 1835).

The length-at-age data of *Odontesthes nigricans* juveniles were fitted to the Laird–Gompertz model (Zweifel and Lasker, 1976) according to $SL_t = SL_0 \exp^G [1 - \exp(-at)]$, where t is the number of increments enumerated in the otoliths (age in days), SL_t standard length at increment t (mm), SL_0 is the size at hatching ($t = 0$) or first increment deposition (mm), G is A_0/α , A_0 is the specific growth rate at $t = 0$ (hatching) (dimensionless), and α represents the rate of exponential decay (dimensionless). The coefficients were fitted using non-linear least square methods. The asymptotic standard length SL_{∞} (mm) in the juvenile size range was then estimated as $SL_{\infty} = SL_0 \exp^G$.

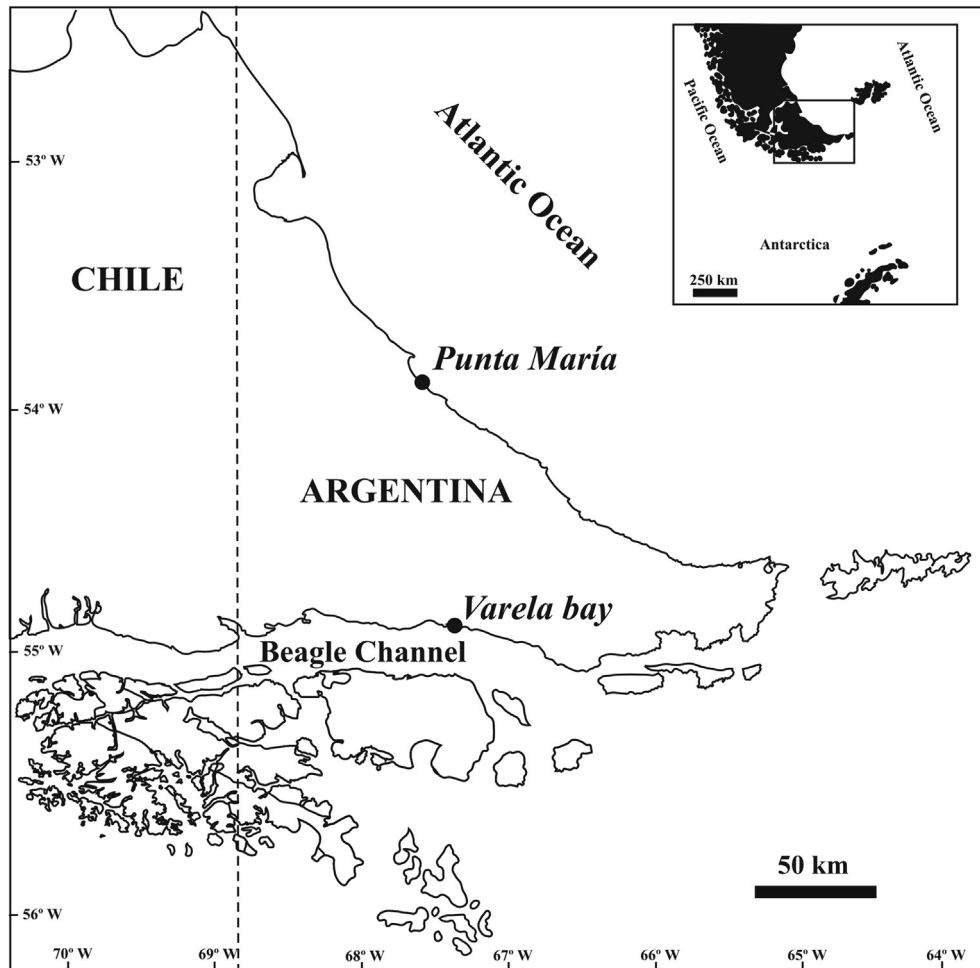


Fig. 1. Study area and location of sampling sites.

After verifying linearity between SL and OR of *Odontesthes nigricans* juveniles, SL at previous ages was estimated for each fish according to the biological intercept method (Campana, 1990; Campana and Jones, 1992): $SL_i = SL_c + (OR_i - OR_c) (SL_c - SL_o) (OR_c - OR_o)^{-1}$, where SL_c and OR_c are the standard length (mm) and the otolith radius (μm) measured at capture; SL_o and OR_o are the standard length (mm) and the otolith radius (μm) estimated at first increment deposition (6.2 mm); SL_i is the back-calculated standard length (mm) corresponding to OR_i ; and OR_i is the otolith radius (μm) at previous ages (1, 2, 3 ... days).

Additionally, the instantaneous growth rate was obtained using the back-calculated SL in individual fish. IGR was obtained as follows: $IGR_i = (SL_{i+1} - SL_i) / t_i$, where IGR_i and SL_i are the instantaneous growth rate at i -th day and fish SL at the age of i -th day-old, respectively.

2.4. Spawning and hatching dates estimates

Hatching dates were determined by subtracting the number of daily increments from the dates of capture of the specimens. The established hatching dates were then contrasted with the analysis of the reproductive cycle, based on the monthly changes in the gonadosomatic index GSI (%) which was calculated as $GSI = GM / BM^{-1} \cdot 100$, where GM is the gonad mass and BM is the total body mass, for each fish (females: $N = 180$, 120–247 mm SL ; males

$N = 178$, 140–229 mm SL) collected during winter 2010 – autumn 2011.

2.5. Annual growth

For annual growth analysis, silversides (Varela Bay $N = 100$, 49–250 mm SL ; Punta María $N = 44$, 60–210 mm SL) were collected during spring 2010 – summer 2011. The *sagittae* otoliths were removed, cleaned mechanically and observed directly in a drop of water. The number, width and radius of each annual mark and otolith radius (OR) were recorded under a stereomicroscope ($10\times$) and an image analysis system (Otolis 32). All measurements were taken along the longest axis of the *sagittae* otoliths.

Whenever the two readings of the same otolith resulted in different age estimates, a third attempt was made and only accepted when it coincided with one of the earliest readings. If not, the data were excluded from the analysis (González Castro et al., 2009). Monthly proportions of opaque and hyaline bands along the edge of the otoliths were examined in order to validate the annual periodicity of mark formation (Campana, 2001). Each specimen was assigned to a year class taking into account the number of marks counted, date of capture, birth date and edge type.

The index of average percent error (IAPE, Beamish and Fournier, 1981) and the coefficient of variation (CV, Chang, 1982, Campana et al., 1995; Campana, 2001) were used to determine the precision level of age interpretations. Additionally, a simple regression

analysis was performed to test the level of agreement in the number of annual rings determined between both readers.

The annual growth curve of *Odontesthes nigricans* from each sampling site was fitted by the von Bertalanffy growth function: $SL_t = SL_\infty [1 - \exp^{-k(t-t_0)}]$, where SL_t is the standard length (mm) at age t , SL_∞ represents the asymptotic standard length (mm), k is the Brody growth coefficient (years^{-1}), and t_0 is the initial condition parameter when the hypothetical length is zero (years) (Wootton, 1990). The estimates of the parameters of the equation and the comparison of curves between both sampling sites were performed using the maximum likelihood method, considering a normal distribution and unequal variances of the residuals per age class (Kimura, 1980; Cerrato, 1990; Aubone and Wöhler, 2000), in an Excel spreadsheet provided by Aubone (2001).

2.6. Otoliths shape analysis

For otoliths shape analysis, silversides from Varela Bay ($N = 33$, 112–250 mm SL) and Punta María ($N = 30$, 85–205 mm SL) were collected during spring 2010 – summer 2011. The right otolith of each individual was viewed under a stereomicroscope ($20\times$); with his external face upward (*sulcus* down) and the rostrum pointing to the right. The binary silhouette of the otolith shape was captured with a digital camera. If the right otolith was unavailable, the left one was used and its image was horizontally flipped using standard images analysis techniques, to ensure that the rostrum was oriented to the right of screen. The program SHAPE (Iwata and Ukai, 2002) was used to extract the contours of the otolith outline in preparation for elliptical Fourier analysis. An elliptical Fourier function fits a closed curve to an ordered set of data points in a two-dimensional plane (Kuhl and Giardina, 1982). It uses an orthogonal decomposition of a curve into a sum of harmonically related ellipses. These ellipses can be combined to reconstruct an arbitrary approximation of the closed curve (Tracey et al., 2006). For each individual, 7 harmonics were generated. Each harmonic was composed of 4 coefficients resulting in 28 coefficients per individual. Each otolith was normalised by the program for size and orientation, which caused the degeneration of the first three coefficients to fixed values: $a_1 = 1$, $b_1 = c_1 = 0$. Therefore, each individual was represented by 25 coefficients for the shape analysis. A principal component analysis (PCA) was performed using the PrinComp module of the SHAPE program. A permutational multivariate analysis of variance (PERMANOVA; Anderson, 2004), was used to assess differences in otolith shape between sampling sites. However, PERMANOVA does not accommodate unbalanced sample designs; therefore, six individuals from the Varela Bay sample were randomly removed to balance the sample sizes. A total of 9999 unrestricted random permutations of the raw data were used (Anderson, 2001). A linear discriminant analysis (DA) was also performed, using the sampling site as classification criterion, and a subsequent cross-classification analysis to assess the correct assignment of individuals to each sampling site. To establish the bias of the classification analysis a Cohen's kappa test was performed, which estimates the improvement over chance of the percent corrected classification rates (Titus et al., 1984). Statistical analyses were conducted using InfoStat v. 2011e (Di Rienzo et al., 2011).

2.7. Diet analysis

The gut contents of *Odontesthes nigricans* from Varela Bay ($N = 56$, 112–250 mm SL) and from Punta María ($N = 48$, 160–205 mm SL), collected during spring 2010 – summer 2011, were analysed. This analysis was restricted to prey items found in the anterior part of the gut to increase the likelihood that the prey

had been eaten recently, and therefore in the habitat where the fish were caught and also to minimise bias arising from differential passage rates of preys. Fish with empty contents were recorded but not considered for the analyses. Prey categories were identified to the lowest possible taxonomic level and counted within each prey category under a stereomicroscope (Wallace, 1981). The volume of each prey category per gut was measured by water displacement in a graduated cylinder. The importance of the different prey categories to the diet was identified using the compound Index of Relative Importance (*IRI*) (Pinkas et al., 1971), as modified by Hacunda (1981):

$IRI_i = (\%N_i + \%V_i) \%F_i$, where $\%F$ is the frequency of occurrence, $\%N$ is the percentage by number and $\%V$ is the percentage by volume of the contribution of each prey category (i). This index has then been expressed as $\%IRI_i = (IRI_i / \sum IRI_i) \times 100$.

Also for each fish, the relative contributions of each prey category i to the total volume of prey items in the gut, were determined as the percentage by volume ($\%v$): $\%v_i = 100 v_i/v_t$, where v_i is the total volume of items of the prey category i in each stomach, and v_t is the total volume of items of all prey categories in the stomach.

The relative contributions of each prey category to the total volume of prey items in the gut were then used in a PCA to analyse diet variability of the species and in a DA to test differences among sampling sites. A subsequent cross-classification analysis was performed to assess the correct assignment of individuals to each sampling site. To establish the bias of the classification analysis a Cohen's kappa test was performed, which estimates the improvement over chance of the percent corrected classification rates (Titus et al., 1984). Statistical analyses were conducted using InfoStat v. 2011e (Di Rienzo et al., 2011).

3. Results

3.1. Fish condition

Weight–length relationships of *Odontesthes nigricans* (Varela Bay: $\log BM = -2.4 + 3.26 \log SL$, $R^2 = 0.99$, $P < 0.001$; Punta María: $\log BM = -2.59 + 3.52 \log SL$, $R^2 = 0.99$, $P < 0.001$) were greater than 3 (Varela Bay: $3.23 < 95\%$ confidence interval < 3.29 ; Punta María: $3.42 < 95\%$ confidence interval < 3.62), indicating a positive allometry. These relationships differed significantly between sampling sites (ANCOVA, $P < 0.001$). Moreover, the condition of fish slightly increased with size (Varela Bay: $K = 0.341 + 0.00352 SL$, $R^2 = 0.16$, $P < 0.05$; Punta María: $K = 0.222 + 0.00195 SL$, $R^2 = 0.16$, $P < 0.05$). However, individuals from Varela Bay showed a better condition than those from Punta María (ANCOVA, $P < 0.001$).

3.2. Daily growth

The mean distance in the otolith from the core to deposition of the first increment (hatch check) was $14.06 \mu\text{m}$ ($\pm\text{SD} = 2.20$). From 50 to 135 increments were counted and measured under transmitted light microscopy.

Assuming that the linear relation between SL and OR seen in the population sample ($SL = 12.59 + 0.07 OR$, $R^2 = 0.94$, $P < 0.001$) also holds true for individual fish within the sample, previous growth trajectories (i.e. previous size at age) of individual fish were back-calculated. In Fig. 2a mean back-calculated SL at previous ages are shown. The IGR of fish (Fig. 2b) were also obtained using the back-calculated SL in individual fish. IGR values increased with increasing age from 0.25 mm day^{-1} at hatching ($6.2 \text{ mm } SL$) to a maximum rate of 0.72 mm day^{-1} at 38.63 mm (day 69) and then decreased with increasing size/age to a rate of 0.27 mm day^{-1} for a $63.08 \text{ mm}/131 \text{ day}$ old larva.

Length at increment data for *Odontesthes nigricans* juveniles were used to fit the Laird–Gompertz model (Fig. 3) and the estimated growth parameters, 95% confidence intervals and coefficients of variation are shown in Table 1.

3.3. Hatching and spawning dates' estimates

Hatching dates varied from November 5 to February 17 (Fig. 4). In order to support this back-calculation, these dates were compared with the monthly evolution of the GSI of *Odontesthes nigricans* captured at Varela Bay.

The GSI values ranged from 1.18% to 22.12% for females and from 0.07% to 10.38% for males. The differences of GSI during the months of observation (Fig. 4) were significant in both sexes (Kruskal–Wallis, $P < 0.001$). In females, GSI values for November differed statistically from December, February and March values (Dunn's Multiple Comparisons test; $P < 0.05$). In males, GSI values for October and November differed from those for December, January and March and values for August differed from those for December and January (Dunn's Multiple Comparisons test; $P < 0.05$). An increase of the GSI of both females and males was observed from August to November, indicating the appearance of pre-spawning individuals. In November, The highest GSI values (22.12% for females and 10.38% for males) were observed in November, which corresponded to the first observation of spawning individuals. A decrease in the GSI from November to January revealed the occurrence of the spawning season, which coincided with the hatching dates previously estimated through back-calculation.

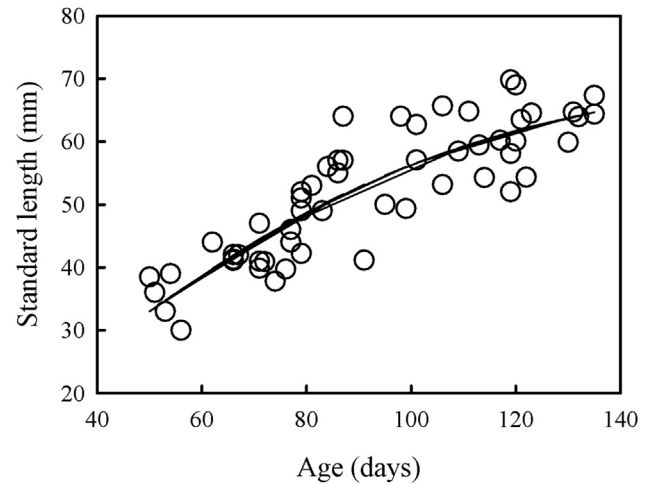


Fig. 3. Length-age data of juveniles of *O. nigricans* (O) from Varela Bay fitted to the Laird–Gompertz model.

Table 1

Estimates (\pm SE), 95% confidence interval (CI) and coefficients of variation (CV) of the Laird–Gompertz parameters for *O. nigricans* juveniles from Varela Bay.

	Estimates	95% CI	CV
SL_0 (mm)	6.22 \pm 5.55	–4.85–17.26	0.89
G (dimensionless)	2.45 \pm 0.81	0.85–4.06	0.33
α (dimensionless)	0.02 \pm 0.01	0.01–0.04	0.32

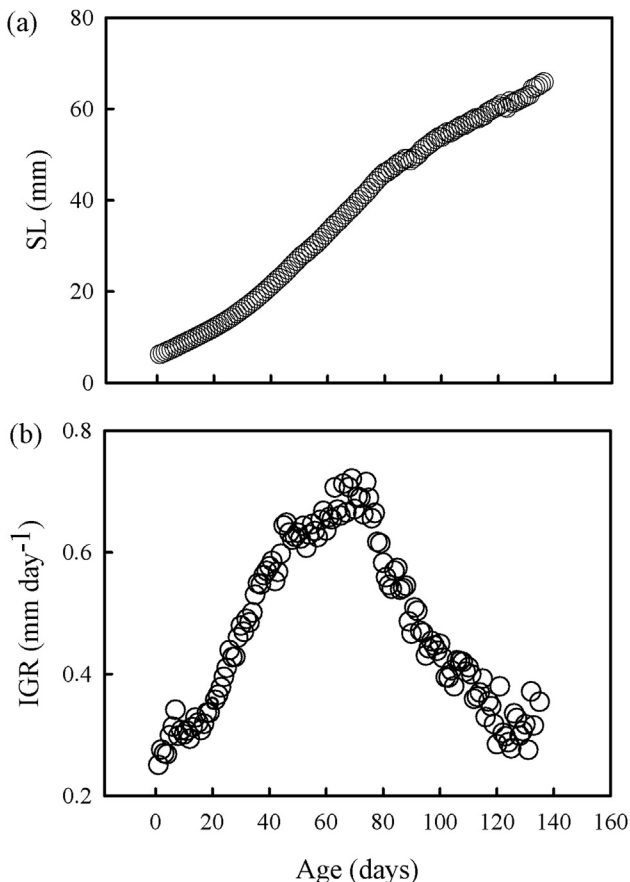


Fig. 2. Mean back-calculated SL (mm) and mean IGR (mm day^{-1}) for juveniles of *O. nigricans* from Varela Bay.

3.4. Annual growth

The analysis of annual changes in the frequency of occurrence of the fast (opaque) and slow (hyaline) growth bands from the edge of the otoliths of *Odontesthes nigricans* showed that opaque bands were more frequent during the warm season (December–March), while hyaline bands predominated during the cold period (April–October). Thus, opaque and hyaline bands are formed annually in the otoliths (Fig. 5).

The simple regression analyses (Varela Bay: $R^2 = 0.81$, $P < 0.001$; Punta María: $R^2 = 0.94$, $P < 0.001$) showed an agreement between the two readers. The values from both the IAPE (Varela Bay: 1.75%; Punta María: 1.92%) and the CV (Varela Bay: 2.48%; Punta María: 2.72%) indicated a good level of precision for readings, suggesting

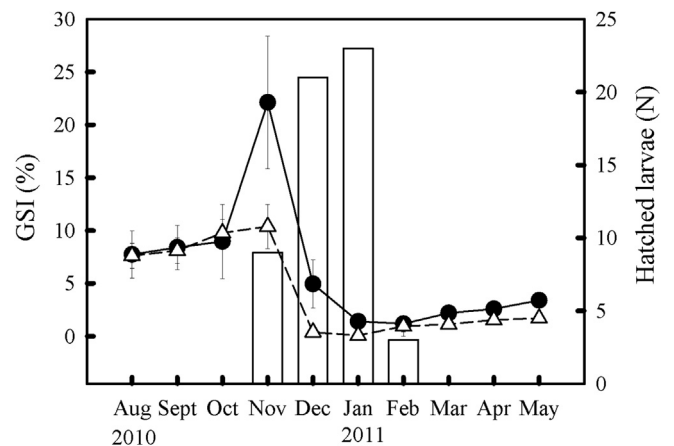


Fig. 4. Gonadosomatic Index (GSI, %) of *O. nigricans* females (●) and males (Δ) and hatching period of *O. nigricans* larvae in Varela Bay.

that the otoliths of *Odontesthes nigricans* are appropriate structures for age estimation.

The maximum age of fish determined was 6+ years for Varela Bay and 5+ years for Punta María, respectively. In Varela Bay, the 1+ and 2+ year classes were dominant in the catches (27% each) and the 5+ and 6+ year classes were the least represented (3% and 4%, respectively). In Punta María, the 4+ year class was dominant (25.6%), the 1+ and 3+ year classes were equally frequent (20.9%), and the 5+ year class was the least represented (4.6%). Sample sizes did not allow growth comparison between sexes within each sampling site. Length-at-age data of both sexes were then pooled and used to fit the von Bertalanffy growth model for *Odontesthes nigricans* from each site (Fig. 6). The estimated growth parameters indicated that *O. nigricans* had a differential growth between both sampling sites. The comparison of the von Bertalanffy growth curves showed significant differences between sites (χ^2 , $P < 0.001$). Fishes from Varela Bay showed significantly higher SL_{∞} (χ^2 , $P < 0.001$) and k (χ^2 , $P < 0.001$) values than those from Punta María (Table 2). The t_0 value did not differ significantly between sampling sites (χ^2 , $P > 0.1$).

3.5. Otoliths shape analysis

A PCA, using the Fourier coefficients as variables, was performed in order to examine potential otolith shape differences between silversides from Varela Bay and Punta María. Although the PCA plot did not reveal a clear separation, some shape differences were observed, otoliths of fishes from Varela Bay had a more curved and rounded dorsal area and a shorter rostrum as compared to those fishes from Punta María. The otolith shape of fishes from Varela Bay was mainly associated to PC1 (46.23% of total variance explained) shape gradient, whereas otolith shape of fishes from Punta María varied mainly associated to PC2 (21.21% of total variance explained) (Fig. 7). When these otolith shape characteristics were analysed using PERMANOVA, significant differences were detected ($P < 0.05$) between sampling sites. Assignment of individuals to the fishing grounds, through the DA, was correctly classified in a 91.67% for Varela Bay and in a 93.33% for Punta María through the elliptical Fourier analysis. The overall percent of agreement of cross-classification was 92.42% against the 50.28% of agreement obtained by chance (Kappa = 0.85 ± 0.06). These results showed that individuals were well separated in morphospace. Sample sizes did

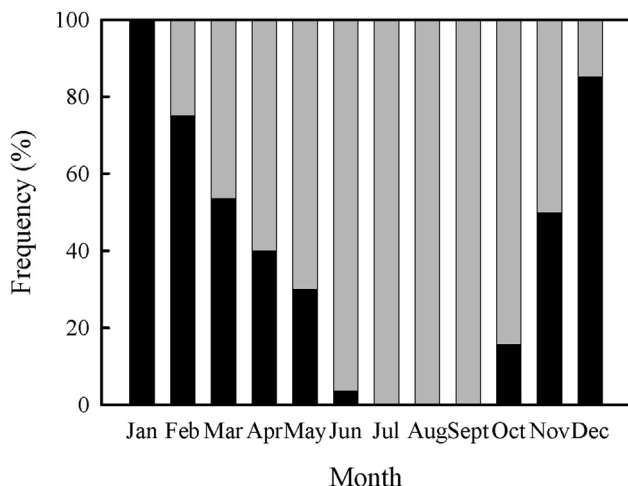


Fig. 5. Annual frequency variation of opaque (black) and hyaline (grey) bands of the edge of *O. nigricans*' otoliths.

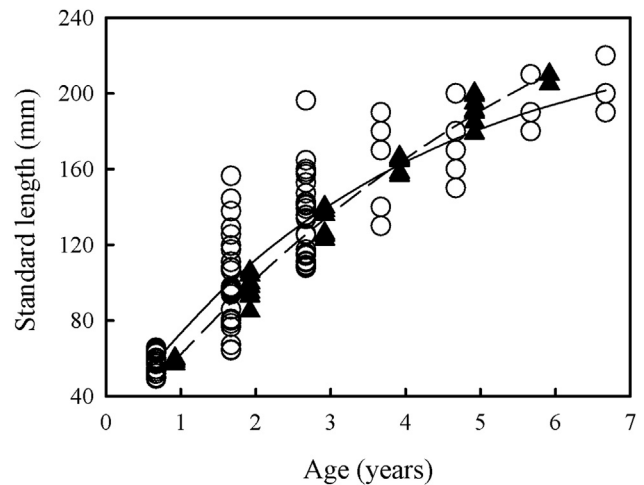


Fig. 6. Length-age data of *O. nigricans* (Varela Bay: O; Punta María: ▲) fitted to the von Bertalanffy growth model (Varela Bay: solid line; Punta María: dashed line).

not allow comparing otolith shape between sexes within each sampling site.

3.6. Diet analysis

The contribution of prey categories, in terms of %IRI, found in all gut contents of *Odontesthes nigricans* from both sampling sites are shown in Table 3. Silversides from Varela Bay had a more diverse diet as compared to those from Punta María; with 7 and 3 prey categories in their guts, respectively.

A PCA, using the relative contributions of each prey category to the total volume of prey items in the gut of each individual, was performed to examine potential diet differences among silversides (Fig. 8). Among them, variables showed significant site dependence (DA, $P < 0.001$); with a zero classification error rate for Varela Bay and a 20% classification error rate for Punta María. The overall percent of agreement of cross-classification was 90.91% against the 52.17% of agreement obtained by chance (Kappa = 0.81 ± 0.06).

4. Discussion

In the present study, we examined the age, growth, monthly evolution of GSI, otolith shape and diet of *Odontesthes nigricans* from the southernmost limit of its distribution. These biological aspects were analysed in order to provide an insight into the life history of the species and furthermore, to assess their possible use as a tool for discriminating silverside populations from the South Atlantic Ocean (Punta María) and Beagle Channel waters (Varela Bay). Punta María is an exposed coast, with a soft bottom and smooth sloping, combined with an intermediate energy coastline and mean tidal amplitude of 4.92 m (www.hidro.gov.ar). On the other hand, Varela Bay is an enclosed coastal embayment with a soft bottom and a smoother sloping and mean tidal amplitude of 1.22 m (www.hidro.gov.ar) that receives the input of freshwater from a small stream. Due to these characteristics, sea temperatures and salinity are expected to be more constant in Punta María as compared to Varela Bay. However, no noticeable differences in physicochemical parameters of seawater were observed between both places at the time of capture. Information on primary productivity is not available for any of the sampling sites.

The maximum lengths of *Odontesthes nigricans* attained in Varela Bay and Punta María (289 and 240 mm TL, respectively) were greater than maximum length previously reported by López

Table 2

Estimates (\pm SE), 95% confidence interval (CI) and coefficients of variation (CV) of the von Bertalanffy parameters for *O. nigricans* from Varela Bay and Punta María.

	Varela Bay			Punta María		
	Estimates	95% CI	CV	Estimates	95% CI	CV
SL_{∞} (mm)	245.49 \pm 7.53	224.58–266.41	3.07	345.09 \pm 41.21	213.94–476.23	11.94
K (years ⁻¹)	0.24 \pm 0.01	0.20–0.29	6.28	0.15 \pm 0.03	0.06–0.24	19.11
t_0 (years)	-0.46 \pm 0.03	-0.56 to -0.36	-7.84	-0.31 \pm 0.08	-0.57 to -0.05	-25.90

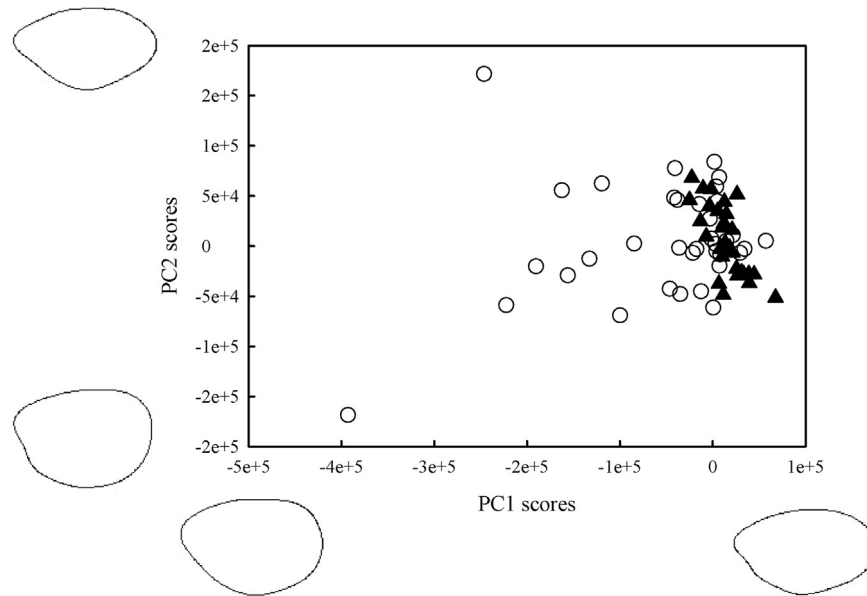


Fig. 7. Analysis of the overall *O. nigricans*' otolith shape variation along the first two canonical axes, and the diagrams of the reconstructed extreme configurations (Varela Bay: O; Punta María: \blacktriangle).

(2009) in Golfo Nuevo, northern Patagonia (236 mm TL); indicating that the species could attain greater sizes at higher latitudes. Additionally, the analysis of $\log BM - \log SL$ and $K - SL$ relationships revealed a positive allometric growth pattern for any of the sampling sites; however individuals from Varela Bay resulted in a better condition than those from Punta María.

Age and growth analyses were performed by counting daily increments and annual marks in *sagittae* otoliths. The otolith has been considered the best structure for age estimates of teleost fishes (Campana and Thorrold, 2001) because they have priority in the utilization of calcium (Carlander, 1987) and they continue grow with the fish ages (Beamish and McFarlane, 1987; Casselman, 1990).

Table 3

Contribution of food items to the diet of *O. nigricans* from Varela Bay and Punta María. Percentage by number (%N), percentage by volume (%V), frequency of occurrence (%O) and percentage by index of relative importance (%IRI). N: number of fish with gut contents present (and without into brackets).

Prey category	Varela Bay				Punta María			
	%N	%V	%O	%IRI	%N	%V	%O	%IRI
Veneridae	13.42	53.71	48	31.01	0.00	0.00	0.00	0.00
Amphipoda	29.55	37.55	78	50.36	45.95	10.63	42.11	28.07
Gasteropoda	0.61	0.10	8	0.06	0.00	0.00	0.00	0.00
Harpacticoida	56.36	7.95	30	18.57	0.00	0.00	0.00	0.00
Mytilidae	0.01	0.07	2	0.01	0.00	0.00	0.00	0.00
Diptera	0.04	0.53	2	0.01	0.00	0.00	0.00	0.00
Isopoda	0.01	0.09	2	0.01	0.00	0.00	0.00	0.00
Euphausiacea	0.00	0.00	0.00	0.00	44.59	29.23	57.89	50.36
Polichaeta	0.00	0.00	0.00	0.00	9.46	60.14	26.32	21.58

In growth studies it is ideal to have a broad range of fish sizes, by incorporating initial larvae. A crucial point is the determination of larval mean size at first daily increment deposition in otoliths (SL_0). As a general trend, it has been observed that first deposition begins when exogenous feeding occurs (Brothers et al., 1976). Due to the absence of those initial larvae in this work, such SL_0 value was obtained as parameter from the Laird–Gompertz model. The obtained value (6.22 mm) was quite similar to the value reported by

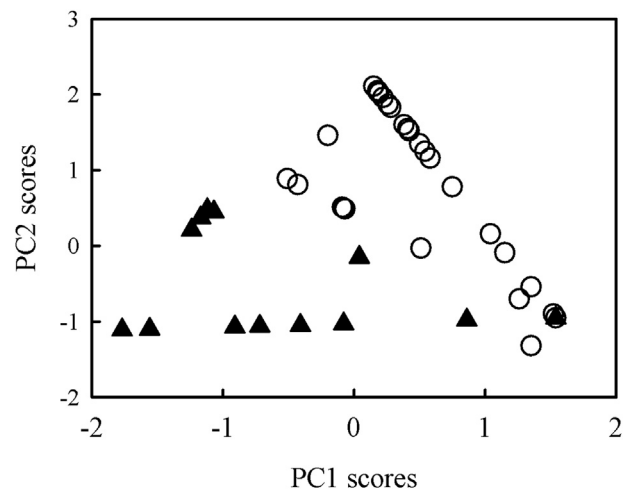


Fig. 8. Ordination results of *O. nigricans*' diet from Varela Bay (O) and Punta María (\blacktriangle) as principal components plot.

Brown and Fuentes (2005) for *Odontesthes bonariensis* larvae (6.7 mm). Freitas et al. (2009) determined size after hatching around 7.2 mm for larvae of *Odontesthes argentinensis* (Valenciennes, 1835). The Laird–Gompertz model has been successfully used to describe larval and juvenile growth of marine fishes (Dulčić and Kraljevic, 1996; Plaza et al., 2008; Brown and Sánchez, 2010). The Laird–Gompertz model explained properly the growth in length for *Odontesthes nigricans* juveniles because an asymptotic trend was detected for larger specimens. According to the model, SL_{∞} was 72.4 mm, resulting lower than 85.4 mm established by Powles and Sandeman (2008) for juveniles of *Labidesthes sicculus* (Cope, 1865), a north American silverside. Daily growth rates of *O. nigricans* varied in a broad range, from 0.25 to 0.72 mm day⁻¹, however these values are within the range established for *Menidia menidia* (Lankford et al., 2001; Munch and Conover, 2004) and for *L. sicculus* (Cope, 1865) (Powles and Sandeman, 2008).

Back-calculation of hatching dates by counting daily rings in otoliths has been a useful method to determine the spawning period of *Odontesthes nigricans* from Varela Bay which was determined in this study for the first time and it extended from November to February (peak in January). Additional evidence for this spawning temporal distribution has also been revealed by the monthly variation of the *GSI* of *O. nigricans* from the same locality, showing a decrease from November to March. Therefore, these results support the assessment of the hatching dates through back-calculation.

The annual frequency of marks formation was validated through the edge analysis (Beamish and McFarlane, 1983; Campana, 2001) in otolith of *Odontesthes nigricans* from Punta María. It was assumed that *O. nigricans* from Varela Bay has the same periodicity of band formation. These results agreed with previous growth studies in *O. nigricans* from lower latitudes (López, 2009) and in other atherinopsids where an annual periodicity in mark formation was also identified (Sverlij and Mestre Arceredillo, 1991; Becker et al., 2003; Llompert et al., 2013). The count of annual marks allowed identifying 7 year classes (0+ to 6+) in Varela Bay and 6 year classes (0+ to 5+) in Punta María, extending the previously estimated life span of the species in lower latitudes, which was from 4 to 6 years (López, 2009). Nevertheless, these age ranges fell within the values registered for other atherinopsid species from South America (Sverlij and Mestre Arceredillo, 1991; Becker et al., 2003; Llompert et al., 2013). The von Bertalanffy growth model explained more than 95% of the growth pattern observed in *O. nigricans*. Particularly, *k* and SL_{∞} varied significantly between sampling sites; reaching Punta María population a larger SL_{∞} value at a lowest rate. Such trade-offs between growth rates and asymptotic length is often found (Dulčić et al., 2011). Probably they are influenced by different environmental and ecological factors, such as temperature, mortality and food availability (Methot and Kramer, 1979; Campana and Jones, 1992; Oxenford et al., 1994) in both sites. The comparison of growth parameters obtained in this study with those reported by Sverlij and Mestre Arceredillo (1991), Becker et al. (2003) and Llompert et al. (2013) showed that the *O. nigricans* populations of Tierra del Fuego approached to the asymptotic length at lower speed. It is worth mentioning that a similar rate was found for *O. nigricans* in Golfo Nuevo, Argentine Sea (López, 2009).

Tombari et al. (2010) described the morphology of *sagittae* otolith of *Odontesthes nigricans* as oblong with a smooth and slightly convex inner face, cup-shaped *ostium*, conspicuous *cisure* and *rostrum*, small and shallow depression in the dorsal area, and wide and straight *cauda*. The posterior end is rounded and the outer face is flat or slightly convex in contrast to the other studied species, whose posterior end has a concave outer face and a conspicuous depression in the dorsal area. The *sagittae* otoliths are characterized by high morphological variability that has proven to be a useful tool

for discriminating between local fish stocks and populations for a range of species (Bolles and Begg, 2000; Stransky, 2005; Mérigot et al., 2007). In the present study, the morphometric analysis suggested significant differences in otolith shape between Varela Bay and Punta María populations. Based on these results, this study supports the potential benefits of otolith shape analysis as a population discrimination tool.

In the present work, feeding habits of the species were described for first time. *Odontesthes nigricans* can be characterized as an invertebrate predator; being benthic organisms the most important components of the diet. However, organisms from the pelagic zone were also present. Species of the family Atherinopsidae have generally been considered as planktivorous (Ringuelet, 1942), nevertheless silversides show an anatomic versatility that allows them to widen their trophic spectrum when zooplankton is scarce (Ringuelet et al., 1980). According to this, many authors reported different diets for the *Odontesthes* genus species (Ringuelet et al., 1980; Ferriz, 1987; García, 1994; Lattuca et al., 2008; Lameiro Rodrigues and Azevedo Bemvenuti, 2011). The analysis of the %IRI showed differences in prey consumption by *O. nigricans* from Varela Bay and Punta María; which are probably due to differences in food availability. Additional studies would lead to a better comprehension of the effect of such different diets on the growth of the species.

Results of the present work contribute to the knowledge of life history of *Odontesthes nigricans* from an area where information for this species is scarce. Moreover, ecological factors seemed important in promoting intraspecific differences on growth parameters, otolith morphology and diet of the individuals. The observed discreteness between Varela Bay and Punta María populations can also be supported by the strong genetic structure revealed by a preliminary phylogenetic assessment of mitochondrial DNA sequences between the former locality and other localities from the Atlantic coast of Tierra del Fuego, including Punta María (Fernández, 2011). Therefore, the use of these several analyses contribute to a holistic approach which maximizes the likelihood of correctly identifying both *O. nigricans* populations in the southernmost limit of the species' distribution. Further analyses must be conducted in order to elucidate the existence of any other population in the Atlantic coast of Tierra del Fuego. Present results also suggest that the development of an optimal strategy for the efficient management of the species must necessarily take into account the differences observed on the characteristics of the life-history of *O. nigricans*.

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References

- Adams, D.C., Rohlf, F.J., Slice, D., 2004. Geometric morphometrics: ten years of progress following the "revolution". *Ital. J. Zool.* 71, 5–16.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46.
- Anderson, M.J., 2004. PERMANOVA 2 Factor: a Fortran Computer Program for Permutational Multivariate Analysis of Variance (For Any Two-factor ANOVA Design) Using Permutation Tests. Department of Statistics, University of Auckland, New Zealand. WWW Page. <http://www.stat.auckland.ac.nz/~mja/>.
- Aubone, A., 2001. Planilla de cálculo para la estimación de los parámetros de la curva de von Bertalanffy por el método de máxima verosimilitud, errores con distribución normal; prueba de comparación de coeficientes. Laboratory of Biomathematics, INIDEP.

- Aubone, A., Wöhler, O.C., 2000. Aplicación del método de máxima verosimilitud a la estimación de parámetros y comparación de curvas de crecimiento de von Bertalanffy. INIDEP. Technical Report 37.
- Baker, R., Buckland, A., Sheaves, M., 2013. Fish gut content analysis: robust measures of diet composition. *Fish Fish.* <http://dx.doi.org/10.1111/faf.12026>.
- Barkman, R.C., 1978. The use of otolith growth rings to age young Atlantic silversides, *Menidia menidia*. *Trans. Am. Fish. Soc.* 107, 790–792.
- Barkman, R.C., Bengston, D.A., 1987. The record of daily growth in otoliths of silversides *Menidia menidia*, from field and laboratory. *J. Fish Biol.* 31, 683–695.
- Beamish, R.J., Fournier, D.A., 1981. A method for comparing the precision of a set of age determinations. *Can. J. Fish. Aquat. Sci.* 38, 982–983.
- Beamish, R.J., McFarlane, G.A., 1983. The forgotten requirement for age validation in fisheries biology. *Trans. Am. Fish. Soc.* 112, 735–743.
- Beamish, R.J., McFarlane, G.A., 1987. Current trends in age determination methodology. In: Summerfelt, R.C., Hall, G.E. (Eds.), *Age and Growth of Fish*. Iowa State University Press, Ames, pp. 15–42.
- Becker, F.G., Bruschi Jr., W., Peret, A.C., 2003. Age and growth of three *Odontesthes* species from Southern Brazil (Atherinopsidae), with reference to phylogenetic constraints in their life-history. *Braz. J. Biol.* 63, 567–578.
- Begg, G.A., 2005. Life history parameters. In: Cadrin, S., Kerr, L.A., Mariani, S. (Eds.), *Stock Identification Methods: Applications in Fishery Science*. Elsevier, Burlington, pp. 119–150.
- Begg, G.A., Waldman, J.R., 1999. A holistic approach to fish stock identification. *Fish. Res.* 43, 35–44.
- Bolles, K.L., Begg, G.A., 2000. Distinctions between silver hake (*Merluccius bilinearis*) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometric. *Fish. Bull.* 98, 451–462.
- Brothers, E.B., Mathews, C.P., Lasker, R., 1976. Daily growth increments in otoliths from larvae and adult fishes. *Fish. Bull. U.S.* 74, 1–8.
- Brown, D.R., Fuentes, C.M., 2005. Validation of daily increment deposition in otoliths of the pejerrey (*Odontesthes bonariensis*) larvae. *Rev. Investig. Desarro. Pesq.* 17, 35–42.
- Brown, D.R., Sánchez, R.P., 2010. Larval and juvenile growth of two Patagonian small pelagic fishes: *Engraulis anchoita* and *Sprattus fuegensis*. *Rev. Investig. Desarro. Pesq.* 20, 35–50.
- Campana, S.E., 1990. How reliable are growth back-calculations based on otoliths? *Can. J. Fish. Aquat. Sci.* 47, 2219–2227.
- Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.* 59, 197–242.
- Campana, S.E., Casselman, J.M., 1993. Stock discrimination using otolith shape analysis. *Can. J. Fish. Aquat. Sci.* 50, 1062–1083.
- Campana, S.E., Jones, C.M., 1992. Analysis of otolith microstructure data. In: Stevenson, D.K., Campana, S.E. (Eds.), *Otolith Microstructure Examination and Analysis*, Canadian Special Publication of Fisheries and Aquatic Science 117, pp. 59–71.
- Campana, S.E., Annand, M.C., McMilland, J.I., 1995. Graphical and statistical methods of determining the consistency of age determinations. *Trans. Am. Fish. Soc.* 124, 131–138.
- Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.* 58, 30–38.
- Carballo, M.C., Navone, G.T., Cremonese, F., 2011. Parasites of the silversides *Odontesthes smitti* and *Odontesthes nigricans* (Pisces: Atherinopsidae) from Argentinean Patagonia. *Comp. Parasitol.* 78, 95–103.
- Carlander, K.D., 1987. A history of scale age and growth studies of North American freshwater fishes. In: Summerfelt, R.C., Hall, G.E. (Eds.), *The Age and Growth of Fish*. Iowa State University Press, Ames, pp. 3–14.
- Casselman, J.M., 1990. Growth and relative size of calcified structures of fish. *Trans. Am. Fish. Soc.* 119, 673–688.
- Cerrato, R.M., 1990. Interpretable statistical test for growth comparisons using parameters in the von Bertalanffy equation. *Can. J. Fish. Aquat. Sci.* 47, 1416–1426.
- Chang, W.Y.B., 1982. A statistical method for evaluating the reproducibility of age determination. *Can. J. Fish. Aquat. Sci.* 39, 1208–1210.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2011. InfoStat Version 2011. InfoStat group, FCA, Universidad Nacional de Córdoba, Argentina. WWW Page. <http://www.infostat.com.ar>.
- Dyer, B.S., 2000. Revisión sistemática de los pejerreyes de Chile (Teleostei, Atheriniformes). *Estud. Ocean.* 19, 99–127.
- Dulčić, J., Kraljević, M., 1996. Larval growth of anchovy, *Engraulis encrasicolus* (fishes) in the eastern Adriatic Sea. *Vie Milieu* 46, 73–78.
- Dulčić, J., Pallaoro, A., Matic-Skokov, S., Dragičević, B., Tutman, P., Grgičević, R., Stagličić, N., Bukvić, V., Pavličević, J., Glamuzina, B., Kraljević, M., 2011. Age, growth and mortality of common two-banded seabream, *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), in the eastern Adriatic Sea (Croatian coast). *J. Appl. Ichthyol.* 27, 1254–1258.
- Fernández, D.A., Lattuca, M.E., Boy, C.C., Pérez, A.F., Cevallos, S.G., Vanella, F.A., Morriconi, E.R., Malanga, G.F., Aureliano, D.R., Rimbau, S., Calvo, J., 2009. Total energy content and energy densities of the main tissues of Sub-Antarctic fishes from the Beagle Channel. *Fish Physiol. Biochem.* 35, 181–188.
- Fernández, D.A., 2011. Informe etapa III Proyecto PFI: Análisis de la estructura poblacional y de las características reproductivas del róbalo y los pejerreyes de la Provincia de Tierra del Fuego con implicancias para el manejo de las pesquerías artesanales. Consejo Federal de Ciencia y Tecnología, p. 8.
- Ferriz, R.A., 1987. Alimentación del pejerrey patagónico, “*Patagonina hatcheri*” (Eigemann, 1909) en el embalse Ramos Mexía, Neuquén, Argentina. *Rev. Mus. Argent. Cienc. Nat.* 6, 29–38.
- Freitas, L.S., Rodrigues, R.V., Okamoto, M.H., Louzada, L.R., Sampaio, L.A., 2009. Effects of photoperiod on survival and growth of the marine pejerrey *Odontesthes argentinensis* larvae. *Aquac. Res.* 40, 252–255.
- García, M.L., 1994. Feeding habits of *Odontesthes smitti* (Atherinidae, Fishes) in the south Atlantic. *Vie Milieu* 44, 151–156.
- Gleason, T.R., Bengston, D.A., 1997. Size selective mortality of inland silversides: evidence from otolith microstructure. *Trans. Am. Fish. Soc.* 125, 860–873.
- González Castro, M., Abachian, V.A., Perrotta, R.G., 2009. Age and growth of the striped mullet, *Mugil platanus* (Actinopterygii, Mugilidae), in a southwestern Atlantic coastal lagoon (37°32'S–57°19'W): a proposal for a life history model. *J. Appl. Ichthyol.* 25, 61–66.
- Hacunda, J.S., 1981. Trophic relationships among demersal fishes in a coastal area of the gulf of Main. *Fish. Bull. U.S.* 79, 775–788.
- Ihssen, P.E., Booke, H.E., Casselman, J.M., McGlade, J.M., Payne, N.R., Utter, F.M., 1981. Stock identification: materials and methods. *Can. J. Fish. Aquat. Sci.* 38, 1838–1855.
- Iwata, H., Ukai, Y., 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Hered.* 93, 384–385.
- Kimura, D.K., 1980. Likelihood methods for the von Bertalanffy growth curve. *Fish. Bull.* 77, 765–776.
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. *Comput. Graph. Image Process.* 18, 236–258.
- Lameiro Rodrigues, F., Azevedo Bemvenuti, M., 2011. Diet and feeding strategy of the silverside *Odontesthes bonariensis* (Valenciennes 1835) (Atheriniformes: Atherinopsidae) from the Mirim Lagoon, Rio Grande do Sul, Brazil. *Pan-Am. J. Aquat. Sci.* 6, 161–169.
- Lankford, T.E., Billerbeck, J.M., Conover, D.O., 2001. Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. *Evolution* 55, 1863–1872.
- Lattuca, M.E., Battini, M., Macchi, P.J., 2008. Trophic interaction among fishes in a northern Patagonian oligotrophic lake. *J. Fish Biol.* 72, 1306–1320.
- Lattuca, M.E., Malanga, G.F., Aguilar Hurtado, C., Pérez, A.F., Calvo, J., Puntarulo, S., 2009. Main features of the oxidative metabolism in gills and liver of *Odontesthes nigricans* Richardson (Pisces, Atherinopsidae). *Comp. Biochem. Physiol. Part (B)* 154, 406–411.
- Lattuca, M.E., Pérez, A.F., Giarratano, E., Malanga, G.F., 2013. Baseline levels of biomarkers of oxidative damage in *Odontesthes nigricans* (Pisces, Atherinopsidae) from two coastal areas of the Beagle Channel, Argentina. *Rev. Chil. Hist. Nat.* 86, 453–464.
- Lesica, P., Allendorf, F.W., 1995. When are peripheral populations valuable for conservation? *Conserv. Biol.* 9, 753–760.
- Llompard, F.M., Colautti, D.C., Maiztegui, T., Cruz-Jiménez, A.M., Baigún, C.R.M., 2013. Biological traits and growth patterns of pejerrey *Odontesthes bonariensis*. *J. Fish Biol.* 82, 458–474.
- López, M., 2009. Estudios preliminares sobre la edad y el crecimiento del pejerrey lagunero *Odontesthes nigricans* (Atherinopsidae) en Golfo Nuevo, Argentina. Licenciatura thesis. Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Argentina. unpublished.
- Mérigot, B., Letourneur, Y., Lecomte-Finiger, R., 2007. Characterization of local populations of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. *Mar. Biol.* 151, 997–1008.
- Methot, R.D., Kramer, D., 1979. Growth of the northern anchovy, *Engraulis mordax*, larvae in the sea. *Fish. Bull. U.S.* 77, 413–423.
- Moore, B.R., Simpfendorfer, C.A., Newman, S.J., Stapley, J.M., Allsop, Q., Sellin, M.J., Welch, D.J., 2012. Spatial variation in life history reveals insight into connectivity and geographic population structure of a tropical estuarine teleost: king threadfin, *Polydactylus macrochir*. *Fish. Res.* 125/126, 214–224.
- Morriconi, E.R., Lattuca, M.E., Boy, C.C., Calvo, J., 2009. Specific testicular morphology in the atherinomorph *Odontesthes nigricans* (Richardson, 1848): a comparison with the sympatric species *O. smitti* (Lahille, 1929). *J. Appl. Ichthyol.* 25, 485–487.
- Munch, S.B., Conover, D.O., 2004. Nonlinear growth cost in *Menidia menidia*: theory and empirical evidence. *Evolution* 58, 661–664.
- O'Reilly, K.M., Horn, M.H., 2004. Phenotypic variation among populations of *Atherinops affinis* (Atherinopsidae) with insights from a geometric morphometric analysis. *J. Fish Biol.* 64, 1117–1135.
- Oxenford, H.A., Hunte, W., Deane, R., Campana, S.E., 1994. Otolith age validation and growth-rate variation in flyingfish (*Hirundichthys affinis*) from the eastern Caribbean. *Mar. Biol.* 118, 585–592.
- Panella, G., 1980. Growth patterns in fish sagittae. In: Rhoads, D.C., Lutz, R.A. (Eds.), *Skeletal Growth of Aquatic Organisms: Biological Records of Environmental Change*. Plenum Press, New York, pp. 519–560.
- Peñailillo, J., Araya, M., 1996. Momento de formación y periodicidad de los micro-incrementos de crecimiento en otolitos de larvas de pejerrey (*Austroromenia regia*) mantenidas en laboratorio. *Investig. Mar. Valpo.* 24, 32–38.
- Piacentino, G.L., 1990. Descripción del esqueleto axial, caudal e inserciones de las aletas dorsales y anal de *Austroromenia nigricans* Richardson, A. *smitti* Lahille y *Austrotherina incisa* Jenyns (Pisces, Atherinidae). *Iheringia Série Zool.* 70, 67–80.

- Piacentino, G.L., 1999. Osteología craneana de *Odontesthes nigricans* (Richardson 1845) y *Odontesthes smitti* (Lahille 1929) de la Ría de Puerto Deseado (Santa Cruz, Argentina) (Teleostei, Atherinopsidae). Bol. Lab. Hidrobiol. 12, 23–47.
- Piacentino, G.L., Torno, A.E., 1987. Osteological observations of the vertebral column in some species of South America atherinids (Osteichthyes, Atheriniformes). Stud. Neotrop. Fauna Environ. 22, 93–98.
- Pinkas, L., Oliphant, M.S., Iverson, Z.L., 1971. Food habits of albacore bluefin tuna and bonito in California water. Dep. Fish Game's Fish Bull. 152, 1–105.
- Plaza, G., Ishida, M., Aoyama, D., 2008. Temporal patterns of growth in larval cohorts of the Japanese sardine *Sardinops melanostictus* in a coastal nursery area. J. Fish Biol. 73, 1284–1300.
- Powles, P.M., Sandeman, I.M., 2008. Growth, summer cohort output, and observations on the reproduction of brook silverside *Labidesthes sicculus* (Cope) in the Kawartha Lakes, Ontario. Environ. Biol. Fishes 82, 421–431.
- Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191, 1–382.
- Ringuelet, R.A., 1942. Ecología alimenticia del pejerrey (*Odontesthes bonariensis*) con notas limnológicas sobre la laguna de Chascomús. Rev. Mus. La Plata Zool. 2, 427–461.
- Ringuelet, R.A., Iriart, N.L., Escalante, A.H., 1980. Alimentación del pejerrey (*Basilichthys bonariensis bonariensis* Atherinidae) en la Laguna de Chascomús (Buenos Aires, Argentina). Relaciones ecológicas de complementación y eficiencia trófica del plancton. Limnobiós 1, 447–460.
- Secor, D.H., Dean, J.M., Laban, E.H., 1991. Manual for Otolith Removal and Preparation for Microstructure Examination. Baruch Institute Technical Report 91.
- Sequeira, V., Neves, A., Barros Paiva, R., Pereira de Lima, J., Vieire, A.R., Serrano Gordo, L., 2012. Life history parameters as possible discriminators of bluemouth *Helicolenus dactylopterus* (Delaroche, 1809) populations in Portuguese waters. Fish. Res. 125/126, 69–76.
- Stransky, C., 2005. Geographic variation of golden redbfish (*Sebastes marinus*) and deepsea redbfish (*S. mentella*) in the North Atlantic based on otolith shape analysis. ICES J. Mar. Sci. 62, 1691–1698.
- Sverlij, S.B., Mestre Arceredillo, J.P., 1991. Growth of the argentine silverside, *Odontesthes bonariensis*, Pisces, Atheriniforme in La Florida Reservoir (San Luis, Argentina). Rev. d'Hydrobiol. Trop. 24, 183–196.
- Titus, U., Mosher, J.A., Williams, B.K., 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. Am. Midl. Nat. 111, 1–7.
- Tombari, A.D., Gosztanyi, A., Echeverría, D.D., Volpedo, A.V., 2010. Morfología de los otolitos y las vértebras de especies de atherínidos marinos (Atheriniformes, Atherinopsidae) que coexisten en el Océano Atlántico sudoccidental. Cienc. Mar. 36, 213–223.
- Tracey, S.R., Lyle, J.M., Duhamel, G., 2006. Application of elliptical Fourier analysis of otolith form as a tool for stock identification. Fish. Res. 77, 138–147.
- Wallace Jr., R.K., 1981. An assessment of diet-overlap indexes. Trans. Am. Fish. Soc. 110, 72–76.
- Wootton, R.J., 1990. Ecology of Teleost Fishes. Chapman & Hall, London, p. 392.
- Zweifel, J.R., Lasker, R., 1976. Prehatch and posthatch growth of fishes. A general model. Fish. Bull. U.S. 74, 609–621.