First data on age and sexual maturity of the Tristan klipfish, *Bovichtus diacanthus* (Bovichtidae) from Tristan da Cunha, South Atlantic

MARIO LA MESA¹ and JOSEPH T. EASTMAN²

¹CNR-Consiglio Nazionale delle Ricerche, ISMAR Istituto di Scienze Marine, sede di Ancona, Largo Fiera della Pesca, 60125, Ancona, Italy
²Department of Biomedical Sciences, College of Osteopathic Medicine, Ohio University, Athens, OH 45701-2979, USA

Abstract: The Tristan klipfish, *Bovichtus diacanthus*, an endemic species at Tristan da Cunha Island was successfully aged using whole sagittal otoliths. The annulation pattern was clear, resulting in an alternating combination of opaque and translucent zones that form an annulus. Although sampling limitations did not allow direct validation of annual ring deposition, most otoliths showed a translucent edge, perhaps suggesting that the deposition of translucent zones is a synchronous process which takes place in winter coincident with the spawning season. The reliability of ageing methodology was supported by the good agreement between readings. The maximum age was estimated to be five years in females and four years in males. In order to increase the small number of direct readings, the length at age was back-calculated for each fish by fitting growth curves. The resulting growth of *B. diacanthus* was described by the von Bertalanffy growth model, as summarized by the following parameters: $L_{\infty} = 207.1$ mm TL, $k = 0.49$ per year, and $t_0 = 0.04$ years. The size at which 50% of the population spawns for the first time was c. 147 mm, corresponding to 2.5 years of age.

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Introduction

The Bovichtidae is the phyyletical basal family of the perciform suborder Notothenioidei, based on genetic (Bargelloni et al. 2000, Near et al. 2004, Near & Cheng 2008), cytotenic (Mazzei et al. 2006), morphological (Eastman 2006), and osteological data (Voskoboinikova 2004). Nine of 11 species in the family Bovichtidae are included in the genus *Bovichtus* (Hardy 1988, Gon 1990, Eastman & Eakin 2000). Most are distributed outside the Antarctic Region, except for *Bovichtus elongatus* (recently synonymized with *B. chilensis*, Bravo et al. 1999) which inhabits intertidal waters off the Antarctic Peninsula (Hureau & Tomo 1977). The other species are found off southern Australia, Tasmania and New Zealand (*Bovichtus angustitrons* and *B. variegatus*), southern South America (*Bovichtus chilensis* and *B. argentinus*), and oceanic islands, such as Tristan da Cunha (*Bovichtus diacanthus*), Macquarie (*Bovichtus oculus* and *B. psychrolutes*), Kerguelen, Amsterdam and St Paul (*Bovichtus veneris*). Due to the remoteness of their habitats, bovichtids remains poorly known compared to other notothenioids (Mazzei et al. 2006).

The Tristan klipfish, *Bovichtus diacanthus* (Carmichael, 1819) is a cool temperate species endemic to the Tristan da Cunha and Gough islands (Andrew et al. 1995). It is widespread in rock pools 10–200 cm deep and in subtidal areas down to 20 m, being able to tolerate a wide range of temperatures and salinities (i.e. from 10°C surface waters in winter to 27°C sun-warmed tide pool waters in summer; Andrew et al. 1995). The pronounced seasonality of these environmental factors probably influences their life cycle. The spawning season of the Tristan klipfish takes place from July to at least August. It is a batch spawner, as each female releases eggs in two batches (La Mesa et al. 2010). The early life stages of the Tristan klipfish are pelagic, forming large shoals of silvery larvae. Once attaining 5–6 cm of size, pelagic juveniles move into shallow inshore waters and develop into benthic adults (Andrew et al. 1995). The maximum size of the Tristan klipfish is 15–25 cm TL (Andrew et al. 1995), but no data are currently available on life span and growth rate of this species.

In 2004 the ICEFISH cruise (International Collaborative Expedition to collect and study Fish Indigenous to Sub-Antarctic Habitats) sought to investigate the biology and ecology of non-Antarctic notothenioids, especially members of the families Bovichtidae and Eleginopidae, from the South Atlantic (Eastman 2006). The cruise provided the opportunity to collect samples of the Tristan klipfish, *Bovichtus diacanthus*, at Tristan da Cunha Island. The aims of this paper are to use the pattern of seasonal growth increments (annual) of otoliths to estimate the age of the specimens, as well as to assess the size and age at sexual maturity, coupling ageing data to stage of gonadal development.

Material and methods

Specimens of the Tristan klipfish were taken during the ICEFISH cruise (#4-04) of the RV *Nathaniel B. Palmer* in...
the South Atlantic Ocean. Fish were caught using a dip net in tide pools about 1 m deep at Runaway Beach at Tristan da Cunha (37°06'S, 12°21'W) on 9–10 July 2004. Seawater temperature was 14–15°C.

All fishes were measured for total length (TL, mm) and standard length (SL, mm), weighed (body wet weight, BW, g) and sexed. The stage of gonad development was macroscopically assessed following the five-point scale of Kock & Kellermann (1991). Sagittal otolith pairs were extracted from each fish, cleaned and stored dry in vials. The otoliths were placed in ethanol in a Petri dish with a black background to enhance contrast between translucent and opaque zones and viewed under reflected light using a dissecting microscope at 16–25× magnification. As a result, the nucleus and the opaque zones appeared as light rings and the translucent or hyaline zones as dark rings. The combination of each opaque and subsequent translucent zone was considered to form an annulus, as is the case in other notothenioids (Everson 1980, North 1988). This pattern was also quite clear viewing the whole otolith; nevertheless, otoliths from the older fish were sectioned and the counts obtained by the two methods compared. The count-path was generally from the core toward the rostrum or post-rostrum margin of the otolith, as otoliths grow mostly along the anterior–posterior axis (Fig. 1). The width of each annulus (OL_i) and the maximum otolith length (OL) were measured on this axis, with an accuracy of 0.01 mm. All measurements were performed under a dissecting microscope coupled to a CCD videocamera using an image analysis software (Image-Pro Plus, ver. 4.5.1 Media Cybernetics).

The relationship between fish size (TL) and otolith size (OL) was estimated by applying linear regression analysis. Applying the Fraser-Lee procedure (Carlander 1981, Francis 1990), which is a method for increasing the number of length at age data to be used in fitting growth curves, the length at age (TL_i) was back-calculated for each fish as follows:

$$ TL_i = a + (TL_c - a) OL_c^{-1} OL_i $$

where a is the intercept of the TL-OL regression, TL_c and OL_c are the fish length and otolith size at capture, OL_i is the annulus width. Other more accurate methods, such as the biological intercept (Campana 1990), were not feasible due to the lack of information on the early life stages of this species.

Each otolith was read twice by the same reader without any ancillary data on the fish, in order to estimate the reproducibility of age readings (i.e. ageing precision). Thus, readings were compared using the index of average percent error (APE) (Beamish & Fournier 1981) and the mean coefficient of variation (CV) (Chang 1982). A paired t-test was also applied to estimate the statistical differences between readings.

The von Bertalanffy growth function was fitted to the length at age dataset comprising both direct readings and back-calculated values using the program FISHPARM of the statistical package FSAS, thorough the Marquardt algorithm for non-linear least squares parameter estimation. The von Bertalanffy growth parameters (L_N, k and t_0) were calculated pooling ageing data of males and females, because only a few fish were available. The growth performance index ($\Phi' = 2 \log L_N + \log k$) (Munro & Pauly 1983), was calculated to compare growth of this species with other notothenioids. The length-weight relationship of fish was calculated for each sex and for the whole population applying the exponential function $BW = a TL^{b}$. The equation was linearized applying the log transformation to both terms to determine the regression parameters.

Based on the stage of macroscopic maturity of gonads, the proportion of individuals in stages 3–5 for each centimetre size class was recorded. The length at first spawning (TL_{m50}), representing the length at which 50% of the population spawn for the first time (Kock 1989), was
determined by fitting a logistic equation to the proportion of fish in each size class as follows:

\[ P = \frac{1}{1 + \exp(-r(TL - TL_{m50}))} \]

where \( P \) is the estimated proportion at a given size class, \( r \) is a fitted parameter and \( TL \) is the size for which proportion is estimated. Unfortunately, the few immature specimens available in our sample did not allow estimation of the length at sexual maturity as well (Kock 1989).

Results

Length frequency distribution and sex ratio

Overall, 40 individuals of \( B. \) diacanthus were collected on July 2004, consisting of 17 females from 95 to 186 mm TL and 23 males from 93 to 189 mm TL. Body weight ranged between 9–98 g in females and 9–87 g in males, respectively. Applying the chi-square test for goodness of fit, the sex ratio did not differ significantly from 1:1 (\( \chi^2 = 0.90, \text{df} = 1, P > 0.1 \)). Comparing the length frequency distributions of sexes, no significant difference were found between males and females (Kolmogorov-Smirnov test, \( P > 0.05 \)).

Length-weight relationship

As no difference was found between the allometric coefficients (\( b \)) calculated for males and females (\( F \) test, \( F_{1,36}, P > 0.1 \)), we only report the length-weight relationship calculated for the whole sample (Fig. 2). The allometric coefficient (i.e. the slope of the curve, \( b = 3.32, \text{s.e.} = 0.103 \)) was significantly different from 3.00 (\( t \)-test for allometry, \( P < 0.01 \)), indicating positive allometric growth for the population.

Fig. 3. Proximal side of left sagittal otolith of \( Bovichtus \) diacanthus collected off Tristan da Cunha. Major morphological features are indicated. Scale bar = 1 mm.

Fig. 4. Scatter plot and fitted regression line of fish length (TL) versus maximum otolith size (OL).

Fig. 5. Average (above) and frequency distribution (below) of annulus width calculated on whole otolith of \( Bovichtus \) diacanthus. Bars indicate standard deviation.
Otolith size and morphology

The morphology of the sagittal otolith of *B. diacanthus* is as follows (Fig. 3): roughly ovate shape, prominent rostrum broad proximally and slender and rounded distally, minute rounded antirostrum, high relief of the medial side, ostio-caudal sulcus acusticus, notch in the postero-dorsal margin and the dorsal and ventral grooves (Hecht 1987).

The maximum otolith length (OL) ranged from 2.25 to 3.47 mm. The relationship between OL (mm) and fish length (TL, mm) was described by the following equation (Fig. 4):

\[
TL = -68.43 + 73.93 \text{OL} \quad n = 33 \quad r^2 = 0.80
\]

Annulation pattern

The annulation pattern consisted of a wide opaque nucleus, surrounded by a rather narrow translucent zone forming the first annulus. The second annulus was generally formed by a clear and wide opaque zone and a narrower translucent zone. Subsequent annuli became more regular and were composed of opaque and translucent zones of comparable width, showing an abrupt decrease of thickness and becoming progressively narrower (Fig. 1). The overlap between annulus width measured in the first three years of life was negligible (Fig. 5a & b), indicating a discrete and progressive decrease in growth with age, as well as the regularity of the increment pattern. Unfortunately, we were not able to assess the annual periodicity of annulus deposition due to time limitations of sampling. Nevertheless, the edge of most otoliths was translucent, indicating that translucent zones are presumably formed in winter (i.e. when the fish were collected).

Age and growth

Based on the count of presumptive annuli laid down on sagittal otoliths, we estimated the age in years for each specimen sampled. Only three otoliths (7.5%) were discarded because they were unreadable. Age estimates ranged between 1–4 years for males and 1–5 years for females. The number of annuli counted in fish older than 2 years (i.e. 3–5 years) was the same using whole or sectioned otoliths, supporting the reliability of age estimates. However, the sample consisted mainly of 2–3 year old fish, representing approximately 73% of individuals successfully aged. Comparisons of readings indicated good consistency or reproducibility (APE = 2.65%, CV = 3.75%), with a high percentage agreement (88%). There was no statistically significant difference between readings (t = 1, df = 37, P > 0.05).

Given the relatively few specimens available for each sex, the von Bertalanffy (VB) growth parameters fitted to the age-length dataset were estimated for the whole sample (Fig. 6). The growth parameters and the derived growth

<table>
<thead>
<tr>
<th>Age class</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Mean length</td>
<td>73.4</td>
<td>119.0</td>
<td>149.1</td>
<td>171.0</td>
</tr>
<tr>
<td>Growth rate</td>
<td>73.4</td>
<td>45.6</td>
<td>30.1</td>
<td>21.9</td>
</tr>
</tbody>
</table>

Table I. Mean back-calculated total length (mm) at age and annual growth increment calculated for *Bovichtus diacanthus*.
performance index (Φ’) are reported in Table I. The estimate of L∞ was c. 207 mm, slightly exceeding the maximum fish size collected in the field. The mean length at age and the mean estimated annual growth increment were calculated through the back-calculation (Table II). The species exhibited rapid body growth during the first two years of life; afterwards, the annual growth progressively declined with attainment of sexual maturity (see below) when a fraction of energy reserves is annually shifted to gonad development.

Size and age at first spawning

The proportion of mature individuals (i.e. at macroscopic stage of maturity 3–5) in relation to fish size and the fitted logistic equation are shown in Fig. 7. Data for males and females were pooled together due to the few available individuals. The size at which 50% of population spawn for the first time (TL₅₀) was c. 147 mm (Fig. 7). The mean age at first spawning of B. diacanthus, as derived from the von Bertalanffy growth equation, was about 2.5 years.

Discussion

Although based on relatively few specimens, the present study provides the first data on age and growth rate of B. diacanthus, a temperate species endemic to the Tristan da Cunha and Gough Islands (Andrew et al. 1995). This species belongs to the Bovichtidae, a primitive family distributed in temperate areas north of the Southern Ocean, and representing the phyletically basal lineage of notothenioids. As such, it is an ideal subject for comparison of its life history strategies with those of derived Antarctic and non-Antarctic notothenioids (Eastman 1993).

Generally, the sagittal otoliths of B. diacanthus exhibited clear and unambiguous patterns of alternating opaque and translucent zones. The good consistency or reproducibility between readings indicated the reliability of the ageing procedure. Unfortunately, the restricted sampling time did not allow us to validate the annual periodicity of growth ring deposition using the most common procedures, such as marginal increment analysis. The otolith margin of most specimens was clearly translucent, suggesting that ring deposition should be a fairly synchronous process which takes place in winter (July). Interestingly, the prevalence of translucent zones on the edge of the otolith coincided with the spawning season of B. diacanthus (La Mesa et al. 2010). Therefore, the translucent zones are probably laid down during periods of high reproductive activity coupled with low water temperature, as reported for a sub-Antarctic nototheniid, Patagonotothen ramsayi (Regan), from the Falkland Islands (Brickle et al. 2006a).

Coupling the length at age data derived from both direct readings and back-calculation, we obtained a successful set of samples, especially for the earlier age classes, giving a reliable estimate of the von Bertalanffy growth parameter k. On the other hand, the asymptotic length (L∞) and life span of the species could be slightly underestimated, as the maximum size reported in literature slightly exceeds the largest individuals found in our sample (25 cm TL, Andrew et al. 1995). A reasonable explanation could be that larger fish preferably inhabit deeper waters instead of tide pools, where all individuals used in the present study were caught.

As there are no data on age and growth for other bovichtids, it is worthwhile to compare present results with those obtained for other notothenioids. Examples include the temperate/sub-Antarctic euryhaline Eleginops maclovinus, which belongs to another primitive basal family of notothenioids (Eleginopidae), and the sub-Antarctic Patagonotothen ramsayi (Nototheniidae) from the Falkland Islands (Brickle et al. 2005, 2006a). Taking into account the maximum estimated age and the growth performance (Φ’) for these species, there is a latitudinal trend from temperate to Antarctic waters towards higher life span and slower growth (Table III). In comparison, B. diacanthus is a relatively fast growing and short-lived fish, which attains a smaller size at a faster rate than their sub-Antarctic counterparts. A further comparison can be made with Harpagifer antarcticus (Harpagiferidae), a relatively small Antarctic notothenoid which shares a similar coastal habitat (such as tide pools) off the Antarctic Peninsula. Compared to B. diacanthus (Table III), H. antarcticus is a slow-growing, relatively long-lived fish (Daniels 1983), typical of most Antarctic notothenioids (Kock 1992).

Based on our results, B. diacanthus spawns for the first time at c. 70% of the estimated asymptotic length. This percentage is slightly lower than those calculated for H. antarcticus and P. ramsayi, which are 75% and 78%, respectively (Kock & Kellermann 1991, Brickle et al. 2006b). Similarly, the age at first spawning estimated for B. diacanthus is 50% of the maximum age, which is within the values calculated for H. antarcticus and P. ramsayi (55% and 36%, respectively).

Based on life history strategies, B. diacanthus could be considered a typical r-selected species, attaining a small

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (years)</th>
<th>L∞ (mm)</th>
<th>k (mm)</th>
<th>Φ’</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eleginops maclovinus</td>
<td>11</td>
<td>1244</td>
<td>0.14</td>
<td>5.33</td>
<td>Brickle et al. 2005</td>
</tr>
<tr>
<td>Patagonotothen ramsayi</td>
<td>14</td>
<td>338</td>
<td>0.25</td>
<td>4.45</td>
<td>Brickle et al. 2006a</td>
</tr>
<tr>
<td>Harpagifer antarcticus</td>
<td>9</td>
<td>105</td>
<td>0.25</td>
<td>3.44</td>
<td>Daniels 1983</td>
</tr>
</tbody>
</table>

*Values calculated from published VB growth parameters
size at fast growth rate coupled with short life span and a relatively early sexual maturity. At the same time, its main reproductive traits are unique in combination among notothenioids, such as low reproductive effort, low fecundity, small eggs and batch spawning (La Mesa et al. 2010). Perhaps, as a result of its long separation from other notothenioids, this species has evolved a suite of different and unique characters to cope with pronounced seasonality and the unpredictable tide pools habitat.

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