Polar Science
February 2016, Volume 10 Issue 2 Pages 167-172
http://dx.doi.org/10.1016/j.polar.2016.02.003
http://archimer.ifremer.fr/doc/00333/44440/

Growth of an Inshore Antarctic fish, *Trematomus newnesi* (Nototheniidae), off Adelie Land

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Abstract :

Dusky rockcod, *Trematomus newnesi*, is a widely distributed neritic circumpolar Antarctic fish species. We conducted a study on age and growth of *T. newnesi* in coastal waters of Adélie Land in East Antarctica. A total of 289 specimens were collected in 2003, 2005, 2006 and 2009. They consisted of 122 females, 132 males and 35 immature specimens. Total length (TL) and total weight (W) of these fish ranged from 13.5 to 25 cm and 19.7–174 g respectively for females and from 12 to 20.9 cm and from 24.1 to 144.1 g for males. The TL/W relationship was described by the following parameters: a = $7.2.10^{-3}$ and b = 3.127, showing no significant difference between sex (ANCOVA, $P < 0.05$). Fish age was estimated by counting annual growth increments on polished transverse sections of sagittal otoliths. Age estimates varied from 3 to 14 years. There was a significant relationship between otolith morphological features (weight and *radius*) and age with no difference between males and females ($p > 0.05$). The estimated values of Von Bertalanffy growth curve $L\infty$ (cm), W_∞ (g) and *k* were 26.6, 200.6 and 0.13 for females and 24.5, 147.0 and 0.15 for males respectively. The indices of growth performance between sexes were not significant. However, potential difference in growth rate between the morphs cannot be neglected.

Keywords : Antarctic fish, Trematomus newnesi, Otoliths, Growth, Age, Adélie land

1. Introduction

Dusky rockcod, *Trematomus newnesi* Boulenger 1902 is a member of the Antarctic/Sub-Antarctic endemic family Nototheniidae (Suborder Notothenioidei) (DeWitt et al., 1990). It is the only known example of phenotypic plasticity in Antarctic notothenioid fish, existing as populations of a typical common morph (benthic morph *versus* semipelagic morph), a large mouth morph and forms which are intermediate between the two morphs. Its large morphological plasticity distinguishes *T. newnesi* from all other notothenioids (Eastman and De Vries, 1997; Eastman and Barrera-Oro, 2010; Barrera-Oro et al., 2012).

The depth range of this species varies from shallow waters to 400 m (Tiedtke and Kock, 1989), but it is more abundant in inshore waters within the 20-50 m depth range, mainly over rocky bottoms or macroalgae beds (Moreno et al., 1982; Barrera-Oro, 2002). Andriashev (1970) and Williams (1988) identified *T. newnesi* as a coastal cryopelagic species which is associated with the underside of the sea-ice. Its diet varies in accordance with the sea ice cover (La Mesa et al., 2000). Gammarideans amphipods and copepods are the main prey of this species (Casaux et al., 1990; Vacchi and La Mesa, 1995; Eastman and De Vries, 1997; La Mesa et al., 2000; Barrera-Oro and Piacentino, 2007; Casaux et al., 2003). *T. newnesi* spawns from March to April in the Ross Sea (Shust, 1987).

T. newnesi is a common species in the Pointe Géologie Archipelago in East Antarctica where large shoals have been observed by Remotely Operated Vehicles (Koubbi, unpublished). It is less common offshore (Causse et al., 2011). Together with *Pagothenia borchgrevinki*, it constitutes the coastal cryopelagic fish community in this area. Larvae are known to be present in this area but are rarely collected (Koubbi et al., 2009). Some isolated individuals were also caught on the upper side of the Adélie Basin, just north of the archipelago (Koubbi et al., 2009).

The *sagittae*, the largest of the three pairs of otoliths, are widely used to describe age and growth in fish (Campana and Thorrold, 2001). Radtke et al. (1989) estimated the age of early life stage of *T. newnesi* of the Antarctic Peninsula population on 390 larvae and juveniles and only on 32 adults. The first year of *T. newnesi* was validated (Radtke et al., 1989). Age was determined by micro-increments with the daily periodicity because the annual growth rings were very difficult to analyse on these very small otoliths. The first year of *T. newnesi* was validated (Radtke et al., 1989). This paper presents the analysis of the growth of adult *T. newnesi* in coastal waters of Adélie Land.

2. Materials and methods

Fish were collected in Pierre Lejay Bay covering an area of 25 km² and around Pointe Géologie Archipelago where the French Scientific station Dumont d'Urville is located (66°40'S and 140°00'E). Fish were caught by lines through holes drilled in the ice during 2003, 2005, 2006 and 2009, in the coastal zone around the French Scientific station Dumont d'Urville mainly from August to November about depths from 20 to 40 m. For each fish, total (TL) and standard (SL) length (measured to the nearest millimetre), sex, macroscopic maturation stage and total weight (W±0.1 g) were recorded. Sagittal otolith pairs were removed from the cranium, cleaned by ultrasonic bath and stored in plastic tubes. The weight of both otoliths from left and right head sides of each specimen was measured from an electronic balance (Wo±0.0001 mg). The weight of both otoliths was used to check of a potential asymmetry between left and right otoliths. Only the right sagittal otolith was used for age estimation. Different techniques (whole otolith, burnt otolith, frontal and transverse sections) were used in order to gain the most precise evaluation of the fish age. The transverse sectioning technique was the most suitable technique for age determination, as it improves the detectability of slow growth areas. The otoliths were embedded in epoxy resin and transverse sections (TS) through the core (or *nucleus*), were made using a precision saw with a blade thickness of 0.3 mm. Finally, the TS were ground and polished on both sides until the core was visible (thickness of 0.21 mm). Under transmitted light, TS were examined using 50x magnification connected to a video camera and an image-analysis system (TNPC software, www.tnpc.fr). Growth rings were counted following the ventral otolith *radius* from the *nucleus* to the otolith edge (Ro±mm). When viewed with transmitted light, alternating translucent and opaque bands were visible. It was assumed that one annular growth ring consisted of one opaque and one translucent band while allowing for the presence of many split increments. About one month after the first reading, all otoliths were drawn at random and read a second time by the same reader. This double reading of otoliths was carried out to measure the precision of ageing data. Precision is defined as the reproducibility of repeated measurements on a given otolith, whether or not measurements are accurate (Chilton and Beamish, 1982). Three common statistical measures were used to determine the amount of variation between the two sets of readings: coefficient of variation (CV), percent agreement (PA) within one year (+/- 1 yr) and absolute percent error (APE). CV and PA were proposed by Kimura and Lions (1991). Beamish and Fournier (1981) suggested use of an average percent error (APE), which is dependent on the average age of the fish species investigated:

$$CV\mathrm{j}(\%) = 100.\frac{\sqrt{\sum_{i=1}^{R}\frac{(X_{ij}-X_j)^2}{R-1}}}{x_j}$$

$$PA = \frac{\sum\left|n_{diff} \leq 1\right|}{n}$$

$$APE\mathrm{j}(\%) = 100.\frac{1}{R}\sum_{i=1}^{R}\frac{|\mathrm{Xij} + \mathrm{Xj}|}{\mathrm{Xj}}$$

Where R is the number of times each fish is aged, Xij the i(th) age determination of the j(th) fish, Xj is the mean age calculated for the j(th) fish, and n_{diff} is the difference in age determination between the first and second readings.

Variations of the relationship between age and morphometric parameters of otolith, according to the sex (S), were investigated with a complete Generalized Linear Model. The model was build taking into consideration that individual age depends on otolith shape parameters (continuous effect) and sex (factor):

$$Age \sim Ro + Wo + S + Ro * S + Wo * S$$

Total Length (TL)/Total Weight (W) regressions were calculated according to Ricker's (1975) formula expressed as:

$$W = a.TL^b$$

Where a and b are the constants of the regression. Growth rate (k), asymptotic length (TL_∞) and weight (W_∞) were estimated, from the Von Bertalanffy (1938) growth equation (t_0=0; Age where length or weight is equal zero, is 0):

$$TL_t = TL_\infty(1 - e^{-k(t)})$$

$$W_t = W_\infty(1 - e^{-k(t)})^b$$

Where TLt and Wt refer to length and weight at age t, respectively translucent. Three length/weight relationships of males, females and both sexes combined were fitted using linear regression after log transformation of the length and weight data. Analysis of covariance (ANCOVA) was used to estimate the differences between the fitted length/weight relationships for males and females.

Growth performance was estimated using the index of growth performance (φ') (Pauly and Munro, 1984):

$$\varphi' = \log k + 2.\log TL_\infty$$

Likelihood Ratio Tests were used to compare the von Bertalanffy growth curves between sexes (Kimura, 1980). Moreover, in order to compare growth between sexes, the index of growth performance also was used, because the two parameters L_∞ and K are inversely correlated (Pauly, 2010).

Statistical analyses were conducted using the open-source statistical package "R" (R Core Team, 2015).

3. Results

Total and standard lengths ranged from 14.2 to19.4 cm (mean 16.5±2.2 cm) and 12.5 to 17.0 cm (mean 14.6±2.0 cm) respectively. The TL-SL relationship (TL=1.114.SL+0.272; N=289) showed a statistically significant correlation of the two parameters (P=0). Of the 289 fish analysed, 132 (45.7%) were male, 122 (42.2 %) were female and 35 (12.1%) were immature. The TL of males ranged from 12.0 to 20.9 cm (mean 18.0 ± 2.0 cm), and their W from 24.1 to 144.1 g (mean 47.0 ± 24.0 g). The TL of females ranged from 13.5 to 25.0 cm (mean 18.0±2.3 cm), and their W from 19.7 to 174 g (mean 62.0 ± 26.0 g). Females attained larger sizes than males (Fig. 1). All immature fishes were identified at size of 12.0 to 14.9 cm, and weights of 21.0 to 36.0 g. TL was correlated significantly with W ($W=7.2.10^{-3}.TL^{3.13}$; $P<2.2.10^{-16}$). The length–weight relationship (Fig. 1) showed a positive allometric growth, regardless of sex. The slopes of the relationships did not differ significantly between sex (*t*-test, P=0.102).

Growth increments counted on transverse otolith sections was represented by an opaque and a translucent zone. However, some split increments were observed in the prepared otoliths. The age bias was evaluated and the results indicated relatively good agreement (agreement=76.2%, CV=3.5% and APE=4.13%). The deviation between two readings was apparent in the older age (after 10 years) (Fig. 2).

In the present study, multiple regression models were used to investigate the potential relationship between age and simple parameters describing otolith morphometry, such as Ro (the ventral otolith *radius* from the *nucleus* to the otolith edge) and Wo (weight of both otoliths) according to the sex. Among the morphometric parameters of otolith, the weight (p =

5.12 10^{-8}) and the otolith radius (p = 1.99 10^{-9}) were significantly correlated with the age of *T. newnesi* with no difference between males and females (p>0.05) (Table 1; Fig. 3).

Length-at-age and the Von Bertalanffy growth parameters for males, females and all fish were shown in Figure 4 and Table 1. Maximum age of our fish sampled was 14 years (Fig. 4). No significant differences in growth parameters was found between sexes (likelihood ratio test: $\chi2$ = 3.21, df = 3, p = 0.11). Accordingly, growth performance indexes calculated for males and females were very similar to each other (Table 2).

4. Discussion

Age determination in teleosts is commonly carried out by counting the sequence of opaque and translucent zone deposited on the sagittal otolith structures within otoliths, such as translucent and opaque growth zones presumed to be laid down annually. In temperate waters, the formation of translucent zones is generally considered to occur during winter, whereas opaque zones are formed during rapid growth periods like spring and summer. These differences in otolith biomineralization are linked to temporal fluctuations of metabolism and water temperature (Høie et al., 2008; Neat et al., 2008). For Antarctic species, interpretation of the seasonal patterns in otoliths is rather complex because of a lower contrast between translucent and opaque zones. This characteristic is possibly due to the low variation of water temperature during the year (Littlepage, 1965; Kock 1992; La Mesa and Vacchi, 2001). Radtke et al. (1989) observed the daily increments by Scanning Electron Microscopy (SEM) but this method was very applied on the adult specimen. Consequently, we developed the other ageing technique to observe the annual growth rings. However, our counts of opaque zones, as growth rings by taking account of split annuli, were carried out on otolith sections after polishing to improve readability and with the results of previous validation study on the first growth *annulus* (Radtke et al.; 1989). Age reading proved both reliable and precise, as indicated by the low APE (Average Percent Error) and CV (coefficient of variation) values and a high agreement between the two readings.

Otolith biomineralization results from multi-causal processes due to the interaction of many internal (physiological) and external (environmental as temperature) factors (Campana and Nielson, 1985; Morales-Nin, 2000; Allemand et al., 2007). In fact, the age could be predicted by the otolith morphological features unlike fish length (Ochwada et al., 2008). During the first life stages of *T. newnesi*, Radtke et al (1989) obtained a multivariate model relating age to the otolith morphometric features (Wo and otolith length). The significant relationship between age and Wo was observed in previous studies on several species during fish life history, such as red snapper (*Lutjanus campechanus* Poey, 1860; Beyer and Szedlmayer, 2010), yellowfin bream (*Acanthopagrus australis* Günther, 1859; Ochwada et al., 2008), sand whiting *(Sillago ciliata* Cuvier, 1829; Ochwada et al., 2008), Chilean jack mackerel (*Trachurus murphyi* Nichols, 1920; Araya et al., 2001), cod *(Gadus morhua* Linnaeus, 1758; Cardinale et al., 2000); european plaice (*Pleuronectes platessa* Linnaeus, 1758; Cardinale et al., 2000), haddock (*Melanogrammus aeglefinus* Linnaeus, 1758; Cardinale and Arrhenius, 2004), Macrouridae species *(Nezumia sclerorhynchus* Valenciennes, 1838 and *Coelorhynchus coelorhynchus* Risso, 1810; Labropoulou and Papaconstantinou, 2000) and gray angelfish (*Pomacanthus arcuatus* Linnaeus, 1758; Steward et al., 2009). Some experimental studies have analyzed the effect of the somatic growth in the otolith size (Reznick et al., 1989; Secor and Dean, 1989) suggesting the importance of Wo. It appears that for two similar sized fish of different ages, the older and slower-growing fish will have a heavier otolith because the deposition of otolith material has continued for a longer time. Inversely, a significant relationship between otolith *radius* and fish age was only observed for a flatfish species (long rough dab, *Hippoglossoides platessoides* Fabricius, 1780; Fossen et al., 2003). In fact, Wo is the only otolith morphometric feature considering the growth of the otolith in a three dimensional space. Inversely, otolith radius takes only the two dimensional space into account. For flatfish species, a significant relationship between otolith *radius* and fish age was explained by the small thickness (Fossen et al., 2003) whereas the rounded otoliths of *T. newnesi* presented potentially the symmetric biomineralization in the three space dimensions resulting from a proportional accretion rate to the axis size through the whole life of an individual. The otolith morphometrics features could be used as age predictors for *T. newnesi* as a faster alternative to the traditional annulus count method, considering similarly males and females since no difference between them was detected and limiting to the individuals of a single population.

Von Bertalanffy parameters were also estimated. Females reached larger asymptotic total length (TL_{∞}) and weight (W_{∞}) than males, although the growth rate (k) of females was lower than the males' one. It was lower because of the larger asymptotic length. The differences between sexes were not significant as for the relationship between otolith morphometric features and age. Therefore, the index of growth performance for males and females are very close, with a mean value of 1.96. This value, between 1 and 2, falls within the range of many Antarctic nototheniids (Kock, 1992; La Mesa and Vacchi, 2001). The asymptotic total length (TL_{∞}), for combined sexes, reached 25.9 cm. This value is higher than the measurement calculated in the Antarctic Peninsula (22.48 cm) (Radtke et al., 1989) where the sampling mainly was on larvae and juveniles in order to study the growth of the early life stages of *T. newnesi*. Conversely, this work mainly examines growth of the adults. The results showed that females were the largest by length and weight. Moreover, *T. newnesi* present morphological variability especially head morphology. For others species, different of head morphology could be reflect food availability and feeding behaviour (Silva, 2003; Janhunen et al., 2009; Mahé et al., 2014). These morphs were characterized by the large mouth morph which could be more benthic than the typical semipelagic morph (Eastman and De Vries, 1997). Consequently, the proportion for each morph in the sampling could influence the observed growth in the same area. Von Bertalanffy parameters of *T. newnesi* are similar to those of other *Trematomus* species (La Mesa et al., 1996; La Mesa and Vacchi, 2001). Growth characteristics of *T. newnesi* could be the result of a combination of environmental factors such as low temperature, extreme seasonality of light and food availability (Kock and Everson, 1998; La Mesa and Vacchi, 2001). High-Antarctic fish growth is slow. Individuals can attain the age greater than 20 years (La Mesa et al., 1996; Kock and Everson, 1998). Extreme environmental conditions in the Antarctic area, such as seawater temperature near its freezing point (-1.86°C, Kock, 1992; La Mesa and Vacchi, 2001), restrict the growth rate of most antarctic ectotherms in spite of their physiological adaptations (Heilmayer et al., 2004; Fraser et al., 2007).

5. Conclusions

To conclude, this study of the otoliths of *Trematomus newnesi* adults in the Eastern part of Antarctica has provided new information on the growth of this fish species. The growth of *T. newnesi* is similar to other *Trematomus* species. There was no significant growth difference between sexes. The otolith morphometrics features could be used as age predictors for *T. newnesi*. This study was restricted to the age and growth of the species *T. newnesi* and therefore the morphs present within the sample obtained in Adélie Land, were not identified. Nevertheless, the ecological implications, e.g. potential difference in growth rate between the morphs cannot be neglected and this should be a high priority for future work (Eastman and Barrera-Oro, 2010).

Acknowledgments

This study is part of the IPEV-ICOTA (Ichtyologie Côtière en Terre Adélie) and ICO²TA (Integrated Coastal Ocean Observations in Terre Adélie) programmes. It is a contribution to the Census of the Antarctic marine environment. We would like to thank all the volunteers who collected samples during the winter and the Dumont d'Urville station team for their assistance. We would especially thank Mandy Gault and Gordon Henderson for their valuable help in correcting this manuscript.

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Table 1: Summary of statistics for of *T. newnesi.* The table give F value and P value of a completed Generalized Linear Model between age and morphometric parameters of otolith according to sex (S).

Factors	F	P
Ro	33.03	$5.12\ 10^{-8}$
Wo	40.98	$1.99\ 10^{-9}$
*Ro * S*	0.13	0.72
*Wo * S*	0.24	0.62

Table 2: Growth parameters and the index of growth performance (φ') for both sexes combined of *Trematomus newnesi* sampled at Adélie Land.

Sex	N	L8 (cm)	W8 (g)	k	t_0	φ'
Female	116	26,62	200,63	0,129	-2,43	1,961
Male	126	24,49	146,99	0,148	-1,262	1,948
Both sexes combined	289	25,92	189,57	0,135	-1,206	1,957

Figure 1: Total Length-Total weight relationship of *Trematomus newnesi* at Adélie land in female (grey line) and in male (black line) fitted to the data (female: □ ; male : ◊ ; immature : ○) (N=298).

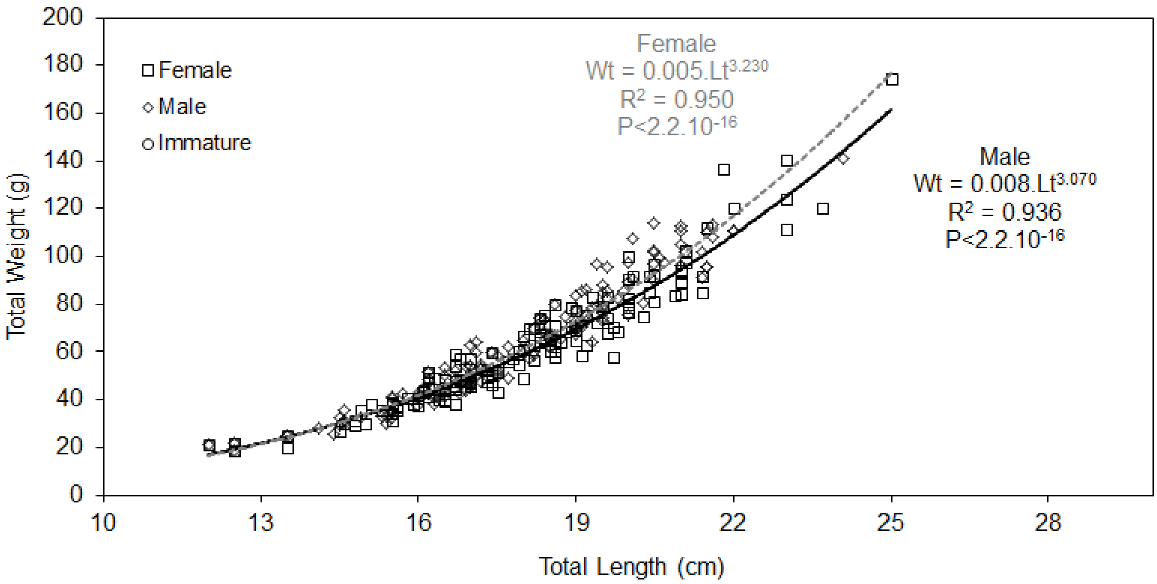


Figure 2: Age bias plots for the reader comparisons based on *Trematomus newnesi* otoliths at Adélie land. Each error bar represents the standard deviation around the mean age assigned by one reader for all fish assigned a given age by the second reader. The 1:1 equivalence (straight line) is also indicated.

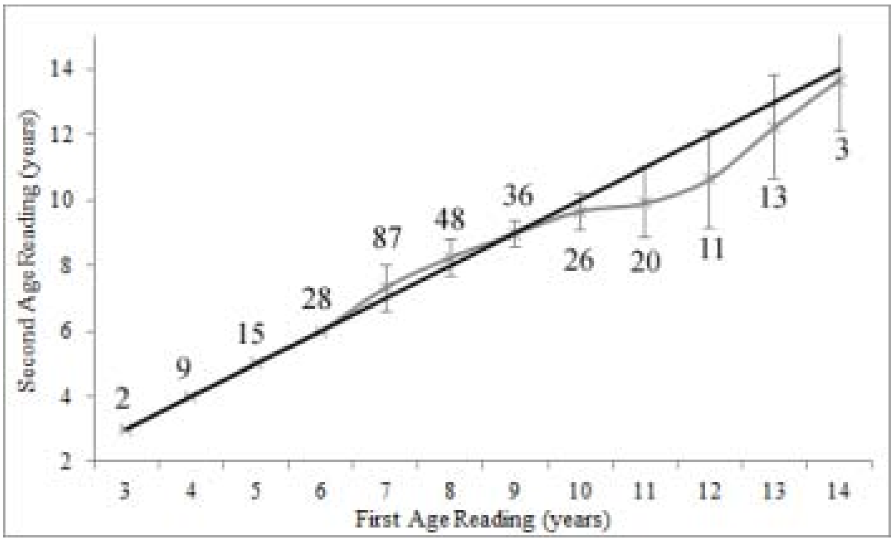
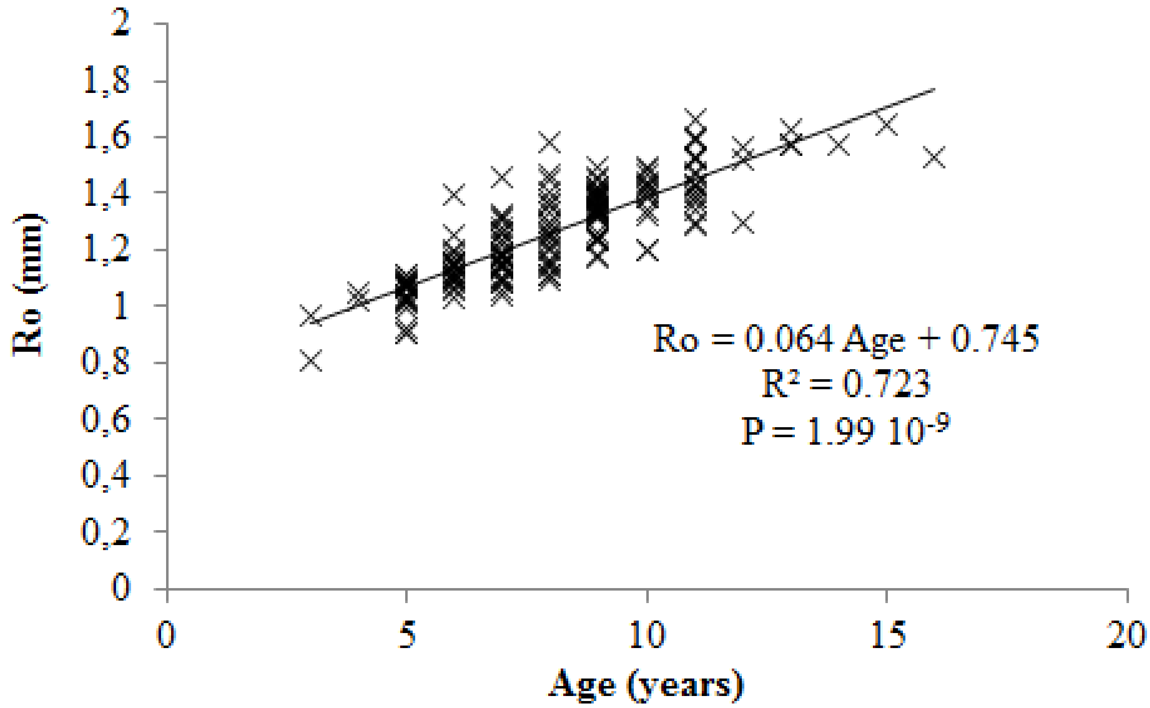


Figure 3: Age- otolith shape parameters (Ro : otolith radius; Wo :otolith weight) relationships of *Trematomus newnesi* at Adélie land fitted to the data (N=289).



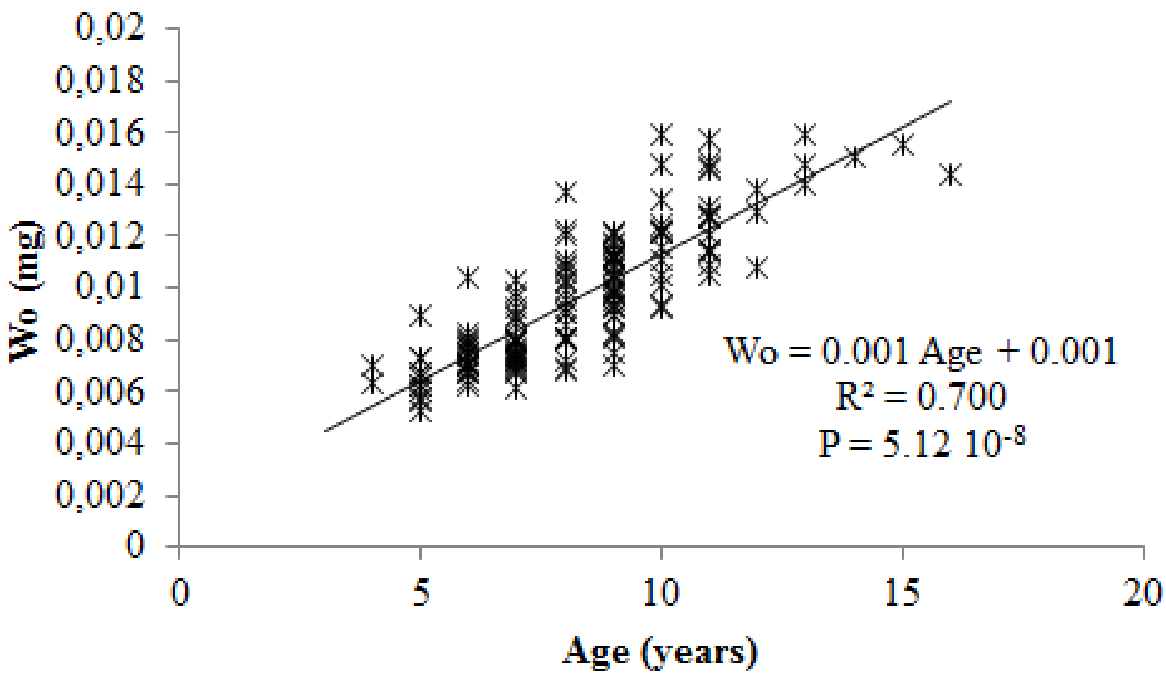


Figure 4: Von Bertalanffy growth curves (female: dotted line; male: black line) and *Trematomus newnesi* data of females (□) and males (◊) at Adélie Land.