Strombus 19(1-2): 15-22, Jan-Dez. 2012
www.conchasbrasil.org.br/strombus/

ISSN 1415-2827 (print edition)
Strombus
ISSN 1983-2214 (online edition)

The use of outer lip in age estimation of *Megalobulimus paranaguensis* (Gastropoda, Pulmonata)

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Abstract

The aim of this study was to estimate the age of a population of *Megalobulimus paranaguensis* (Pilsbry & Ihering, 1900), in an urban park in the Santos / SP on the Atlantic costal forest of southeastern Brazil. During the period of 2000 to 2010, the outer lip of snails marked and recaptured was measured, to estimate its growth rate. Non-linear models were used to determinate the relationship between the lip and age. With these values a von Bertalanffy function were made, and sections of the lip were made to see its internal structure. The following equations were developed: Age = $0.6633\ L^{1.8095}$ and Error = $0.6144L^{0.7528}$. The older individuals observed with lip of 6.5 mm had the age of 19.6 years old. The maximum size calculated for the population was estimated in 8.59 mm, size which age was 32.5 years, with a low growth rate. With the sexual maturity time up to three years, it's possible that this species may reach 35.5 years old, possible one of highest age for neotropical gastropods. In the sections were found deposition layers relatively homogeneous with mean thickness of 0.076 mm, reunited in bundles with reduction of layers number with the increase of age. The results indicate that shells of *Megalobulimus* can be used for environmental monitoring.

Keywords: Megalobulimidae, age determination, longevity, non-linear models, land snail, costal Atlantic rain forest, environmental monitoring.

Resumo

O objetivo deste trabalho foi estimar a idade de uma população de *Megalobulimus paranaguensis* (Pilsbry & Ihering, 1900), em um parque urbano em Santos / SP na Mata Atlântica costeira do sudeste do Brasil. Durante o período de 2000 até 2010, lábio externo da concha de caracóis marcados e recapturados foi medido, para se estimar a taxa de crescimento do lábio. Modelos não-lineares foram utilizados para se determinar a relação entre tamanho do lábio e idade. Com estes valores uma função de von Bertalanffy foi feita, e secções do lábio foram feitas para analisar sua estrutura interna. As seguintes equações foram desenvolvidas: Idade = $0.6633\ L^{1.8095}$ e Erro = $0.6144L^{0.7528}$. Os indivíduos mais velhos com um lábio de 6.5 mm tinham idade de 19.6 anos. O tamanho máximo calculado para a população foi estimada em 8.59 mm, tamanho em que a idade seria de 32.5 anos, com uma baixa constante de crescimento. Com o tempo maturidade sexual de até três anos, é possível que esta espécie possa chegar aos 35.5 anos, possivelmente uma das maiores idades para gastrópodes neotropicais. Nas secções do lábio foram encontradas camadas de deposição mineral relativamente homogêneas com espessura média de 0.076 mm, que eram reunidos em feixes com redução do número de camadas com o aumento da idade. Os

resultados indicam que conchas de *Megalobulimus* podem ser usadas para monitoramento ambiental.

Palavras-chave: Megalobulimidae, determinação da idade, longevidade, modelos não lineares, caracol terrestre, Mata Atlântica costeira, Monitoramento ambiental.

Introduction

Megalobulimus paranaguensis (Pilsbry & Ihering, 1900), popularly known as Arua-do-mato, is a neotropical giant land snail of family Megalobulimidae, with mean shell length of 98 mm. It occurs in coast Atlantic forest of southeastern Brazil, between the states of Paraná and southern Sao Paulo (Morretes, 1954; Simone, 2006). Individuals of this species were identified in locations in the São Vicente Island, a local in the coastal region more northern than its original location. Little is known about this species, specially about its biology and ecology. In general, the species of *Megalobulimus* Miller, 1878, have rare and low density populations, with low reproductive potential. These facts difficult studies about its population, except for long-term studies (Simone, 1999). Many species of *Megalobulimus* are endangered, because of the degradation of its natural environment and overkill to be confused with the exotic *Achatina fulica* Bowdich, 1822 (Colley & Fischer, 2009). There are data that suggests some extinction risk for some species of *Megalobulimus* (Leme, 1989; Leme & Indrusiak, 1990; Mansur & Leme, 1996), fact that were suggested by Bequaert (1948), because of the large destruction of the forests in some regions of Brazil. The efforts to the conservation of the genus have increased in the last years, and the genus was one of the first land snails that were being considered flagship and umbrella species of Atlantic rain forest (Santos, 2011).

In Megalobulimidae, the sexual maturity coincides with the formation of the external lip of the shell, and the process of growth stills continues along the life of the individual, and may be an indicative of the relative longevity of the individual (Bequaert, 1948). The objective of this study was to use the external lip to develop a model to estimate the age of *Megalobulimus paranaguensis*, and to analyze biological parameters derived from that.

Material and Methods

The study was conducted in Parque Zoobotânico Orquidário Municipal de Santos (PZOMS); UTM: 23K 362672.57 E; 7348904.53 N; an urban park in Santos / SP, on the island of São Vicente in the coastal Atlantic forest of southeastern Brazil. During the years of 2000 to 2010, collections of mark-recapture were made, in which we mark each individual of *Megalobulimus paranaguensis* found, with the code shown in Fig 1, and we measure the thickness of the base of the external lip of the shell with a caliper, with of 0.01 mm. The same measurement was made when we recapture an animal, to estimate the lip growth rate (LGR). The collections were made from 7:00 AM to 8:30 AM, from Monday to Friday. Eight shells of individuals found dead during the study were sectioned to analysis of the layers and calcification of the lip.

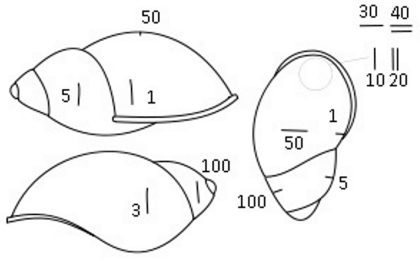

Figure 1

To estimate the relationship between age and growth of the lip we use non-linear models. Logarithmic regression between the lip growth rate (LGR) and the geometric mean (GM) of the data were made, with the fit did by the ordinary least squares method (Keough & Quinn, 2002). With these data, we determinate the time to the lip grow 1 cm for each 1 cm class of size, and we fit an equation size-age. For the age error, the mean difference between the true time interval of the measures and the estimated time interval difference were estimated for each size

class. Both relationships were fitted to an allometric relationship, $y = ax^b$. The models were fitted using program BioEstat5.0 (Ayres et al., 2007).

With the age data, we use the von Bertalanffy model, to see the relationship between age and growth. The curve is descripted by the following equation: $L = L_{\infty}(1 - e^{(-K(t - t0))})$, where L is length of the lip in an certain age; L_{∞} is asymptotic maximum size for the lip; K is a growth constant; t_0 is the hypothetical time when the size of the lip is 0 and t is time in years. The model were fitted in R version 2.14.0 (R Development Core Team, 2011) environment, using library FSA version 0.2-7 (Ogle, 2011).

Results

154 individuals of *Megalobulimus paranaguensis* were marked, which between 69 individuals were captured at least once a time. The interval between the recaptures varies between 7 and 120 months. The relationship between LGR and GM has the following equation: LGR = 1.0025 -0,5 ln (GM) (r^2 = 0.5594; Fig 2).

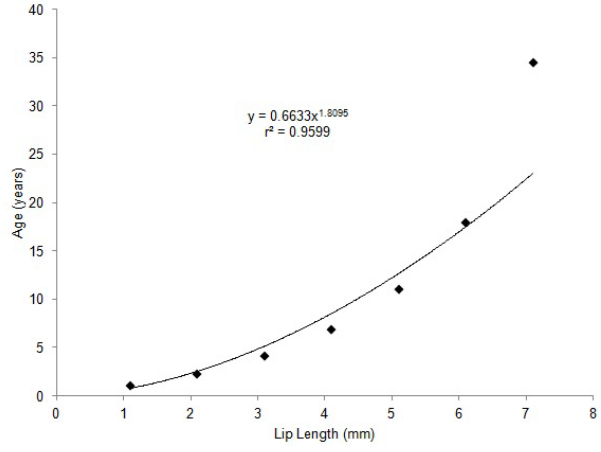

Figure 2

The equations for age estimation and age error derived are: $Age = 0.6633L^{1.8053}$ (r^2 = 0.9599; Fig 3) and $Error = 0.6468L^{0.74525}$ (r^2 = 0.9088; Fig 4). The two models present high fit with the variables. With the age equations, the maximum age found for an individual of *M. paranaguensis* was 19,6 years (Error age: 16.2 – 21.2 years), for two individuals with lip length of 6.5 mm, and minimum age found was 0.18 years old (Error age: 0 – 0.56 years), for 5 individuals with lip length of 0.5 mm.

The resulting equation resulted from the von Bertalanffy model is: $L = 8.59(1 - e^{(-0.088(t - 0,14))})$ (Fig 5). The maximum asymptotic size for the lip of *M. paranaguensis*, suggests that the maximum longevity probably reached for this specie is about 32.5 years old (Error age: 22.7 – 28.3 years old).

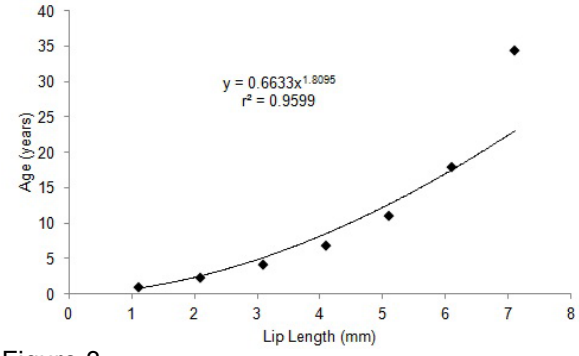

Figure 3

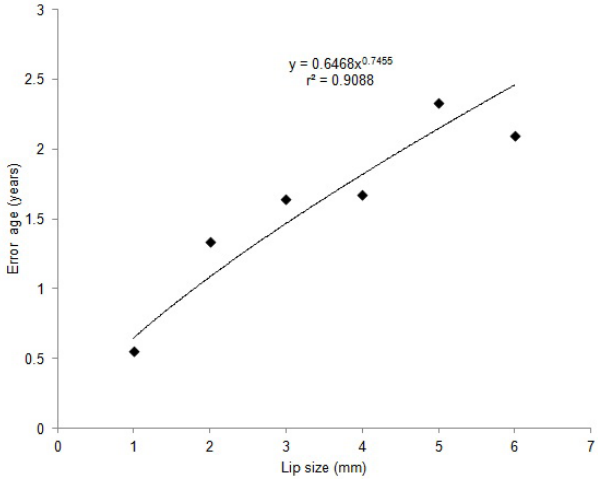

Figure 4

The layer thickness varied between 0.069 to 0.1 mm. with mean of 0.076 mm (Table I). The bundles have 2 to 19 layers. And generally the first two (most outer to the lip) have a greater number of layers, with a progressive reduction in inner direction (Fig 6). The relationship between the calculated age and the number of new layers deposited in the lip is shown in Table III. In the most of the years, 30% of the population has a lip between 3.5 to 4.5 mm, and the less frequency occurs with the young adults, com with between 0.5 to 1.5 mm, with only 8.75% (Table III)

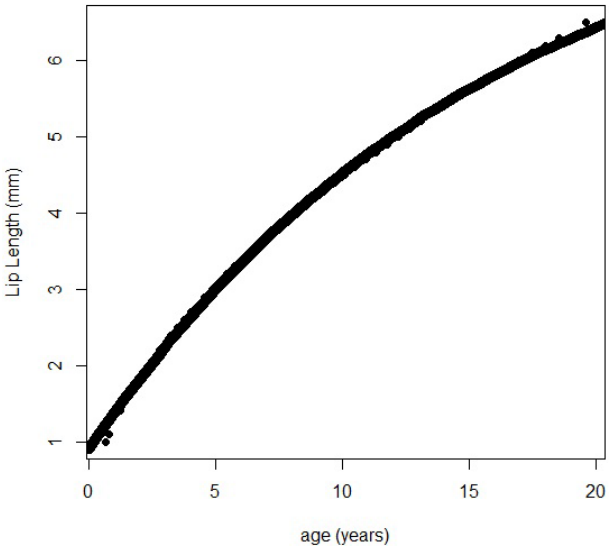

Figure 5

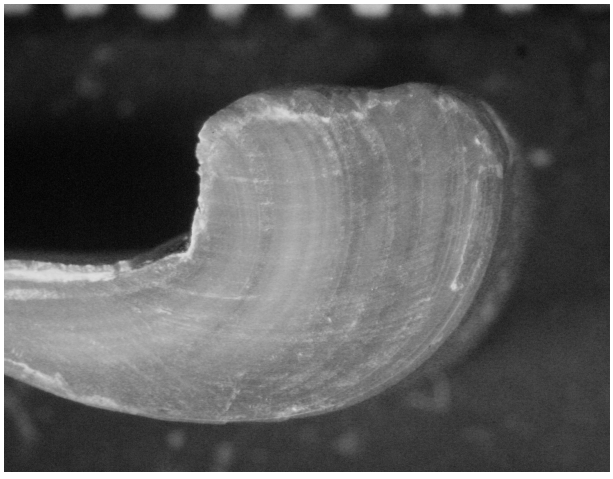

Figure 6

Discussion

Some gastropods thickens their lips when they reach their morphological maturity like *Arianta arbustorum* (Linnaeus, 1758) (Rabound, 1986), the Hawaiian achatinellinae (Hadfield, 1986), the genus *Cerion* Bolten, 1798 (Woodruff, 1978), the genus *Oncomelania* Gredler, 1881 (Van der Schalie & Davis, 1965) and the genus *Megalobulimus*. The main difficulty consists in using this thickness to determinate the growth rate or how many layers are deposited in a determinated time interval.

The asymptotic size of the lip (8.59 mm) with the age estimated for this size (32.5 years) suggests that *Megalobulimus* has a long life cycle, one of the largest life cycle for the neotropical land snails. The time of morphological maturity is a little controversial in the literature. Jacinavicius *et al.* (2004) found that *Megalobulimus ovatus* (Muller, 1774), reaches his maturity in 11 to 13 months; Drouet (1859) found 2.66 years (32 months) for individuals of complex *Megalobulimus oblongus* (Muller, 1774); and Romero (2004), found 1 to 3 years to *Megalobulimus mogianensis* Simone & Leme, 1998, reaches its sexual maturity. It's also possible that the time for maturity to be variable from specie to specie, and may be affected by the environment of the animals, but if *M. paranaguensis* has the same pattern of *M. mogianensis*, its longevity may be 35.5 years old. According to the review of Hellen (1990), short life spam is correlated with the following characters: lack of external shell or shell semitransparent and dwelling in a microenvironment that is exposed with high temperatures and with solar radiations and very small size (in gastropods). This fact explain the long life cycle in *M. paranaguensis*, that has an hard and opaque shell, dwelling in litter

ID (Identification)	Thickness (mm)	Total Layers	Mean Thickness (mm)	Age estimated (years)
A	4.2	61	0.069	8.90
B	1.4	19	0.074	1.21
C	3.5	42	0.082	6.40
22	3.3	48	0.069	5.75
33	6.3	94	0.067	18.54
45	6.3	88	0.072	18.54
79	5.3	53	0.100	13.56
98	4.6	56	0.082	10.49
		Mean Total Thickness	0.076	

Table 1

Interval (Years)	Initial thickness (mm)	Final thickness (mm)	Annual interval (mm)	Number of layers in the interval
0-1	0.00	1.62	1.62	20
1-2	1.62	2.25	0.63	8.3
2-3	2.25	2.74	0.49	6.5
3-4	2.74	3.13	0.39	5.0
4-5	3.13	3.49	0.36	4.6
5-6	3.49	3.80	0.31	4.1
6-7	3.80	4.09	0.29	3.8
7-8	4.09	4.36	0.27	3.5
8-9	4.36	4.61	0.25	3.3
9-10	4.61	4.85	0.24	3.1
10-11	4.85	5.07	0.22	2.9
11-12	5.07	5.29	0.22	2.8
12-13	5.29	5.49	0.20	2.7
13-14	5.49	5.69	0.20	2.6
14-15	5.69	5.88	0.19	2.5
15-16	5.88	6.07	0.19	2.4
16-17	6.07	6.24	0.17	2.3
17-18	6.24	6.41	0.17	2.3
18-19	6.41	6.58	0.17	2.2
19-20	6.58	6.75	0.17	2.1

Tabela 2

Year	Lip Thickness Interval					
	0.5 - 1.5	1.5 - 2.5	2.5 - 3.5	3.5 - 4.5	4.5 - 5.5	5.5 - 6.5
2000	2	10	6	3	**12**	3
2001	0	0	0	**2**	0	**2**
2002	0	0	0	0	0	0
2003	0	0	2	**3**	2	**3**
2004	0	0	1	**3**	**3**	2
2005	1	0	3	**9**	4	6
2006	3	1	7	**13**	7	8
2007	8	11	12	**13**	4	1
2008	4	4	**12**	11	2	0
2009	0	2	7	**8**	2	0
2010	3	4	3	**7**	1	0
Total	21	32	53	72	37	25
%	8.75	13.33	22	**30**	15.4	10.4

Tabela 3

environment, which is not exposed to high solar radiation and temperature and has a big size.

For *Arianta arbustorum*, from the Swiss Alps, in a study using mark-recapture analysis, was determinated that the age of the juveniles can be estimated by the annual marks of interruption in the shells, and in adults by the annual of a new layer to the lip (Rabound, 1986). The author established a life span of 10 years for this species.

In a similar way, D'Orbigny (1837) and Albers (1850) estimated the age of individuals of the complex *Megalobulimus oblongus* in, 10 and 14 years old, respectively, counting the number of layers deposited in the lip and considering that each layer were made after annual dormancy, and both disregarded the time of duration of juvenile

phase. Bequaert (1948), in your review of the family Strophocheillidae, notes that so far there was no real evidence, based in observation or experiment, that each layer corresponds to an year in these species. Recently, using the same arithmetic progression, a shell of *M. intertextus* (Pilsbry, 1895), with 84 mm of length and 27 mm in most thick part of the lip, with 85 layers, had the age estimated in 44 to 88 years, considering respectively two and one layer per year plus 3 years for the juvenile phase (Groh, 2005).

But this arithmetic progression doesn't speak the fact that in some populations of *Megalobulimus* studied there is a proportion of snails with lip thickened, coming to be regarded as a diagnostic character of some species, like *M. haemastomus* (Scopoli, 1786) (Bequaert, 1948) and *M. formicacorsii*, (Barattini & Ledón, 1949). This same distribution was also observed in Parque Zoobotanico Orquidario Municipal de Santos, where the higher frequency occurs in individuals with lip size between 3.5 to 4.5 mm (30%), and lower frequency occurs in the young adults, with lip size between 0.5 to 1.5 mm, in the majority of the years.

The analysis of sections of the lips revealed deposition layers with mean thickness of 0.076 mm. This thickness is less than 0.609 mm found in *M.* cf. *elongatus* (Bequaert, 1948) and 0.318 mm of *M. intertextus* (Groh, 2005). This author suggests that the differences are caused both for individual characteristics of the species, and the calcium concentration of the soil.

The layers are grouped in bundles with variable number. Each layer corresponds to the growth between two dormancies, which corresponds to a year. The layers of the two first bundles has more layers little individual layers, suggesting a rapid process of deposition in this period. As the studied population inhabits a little urban park with perturbations in your microclimate by the edge effect and by the constant management of vegetation and soil, there is individual variations in the growth of the lip and in the formation of new layers and bundles, with some individuals present stop of growth for some years and no dormancy in determinate years. However, the use of the equation obtained for the growth of the lip in each interval of year can provide a standard model with the mean number of new layers deposited in each bundle.

The growth constant (0.08) is low when compared with another neotropical gastropods: Gomes *et al.* (2004) found growth constant of 0.35 for *Simpulopsis ovata* (Sowerby, 1822); Rumi *et al.* (2007) found growth constants varying between 1.30 to 1.51 for *Drepanotrema* spp. and Núñez (2010.), found growth constant of 1.85 for *Stenophysa marmorata*, Guilding, 1828; and 0.85 for *Physa acuta*, Draparnaund, 1805. Probably this is related with its longer life cycle when compared with other neotropical gastropods.

The use of non-linear models and their fit with the data collected in the mark-recapture analysis demonstrate that the lip growth rate in *Megalobulimus paranaguensis* decreases in an exponential way with the age.

This model can explain the frequencies of the lips in *M. paranaguensis*, and another Megalobulimidae, where classes with higher thickness accumulate cohorts of several years, and consequently higher number of individuals.

In a world where the environmental changes are testing the limits of tolerance of the ecosystems, a species with long life and intimately connected with the local microclimate, like *Megalobulimus paranaguensis*, and probably the other species of *Megalobulimus* can be very useful in recording environmental stresses in undisturbed areas. The class frequency of age obtained by biometry, and the lip fragments of the shell, may provide a temporal historic of the responses of the snail to its environment.

Acknowledgments

To Timoteo T. Watanabe, for the English corrections and improvement of vocabulary.

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Received: February 20, 2012. Accepted: july 22, 2012