STUDIES OF AGE AND GROWTH OF THE GASTROPOD TURBO MARMORATUS DETERMINED FROM DAILY RING DENSITY

B. Bourgeois, C. E. Payri and P. Bach

ABSTRACT

This is a study of the age and growth of the gastropod Turbo marmoratus using a sclerochronological method. The shells of an introduced population on Tahiti (French Polynesia) are examined. The confirmation of age, based on a novel marking technique using a lead pencil, reveals a daily rate of deposition within the growth rings. A new method of estimation of the growth parameters of the Von Bertalanffy model from the daily ring density (DRD) is described. The fit to the model allows the estimation of $K = 0.32$ year$^{-1}$ and $D_0 = 30.3$ cm ($D = $ diameter).

INTRODUCTION

The green snail, Turbo marmoratus, was introduced in Tahiti (French Polynesia) waters in 1957. It has thrived in the Polynesian archipelago, constituting a new resource whose stocks are exploited without particular knowledge of its biology. It has a current natural western Indo-Pacific distribution. A renewed interest in nautical products has made it a luxury item, whose price has not ceased to rise over the last decade, while the worldwide stock is decreasing (Yamaguchi 1988a, 1991). Despite the economic value of this species, no growth studies have been undertaken in nature. To our knowledge, only the works of Yamaguchi (1988b) deal with the biology and the ecology of Turbo marmoratus, and are based on observations in pools and aquaria in sub-tropical conditions.

The study of age and growth is of great importance for the biology and ecology of a species. It is the basis for all research in population dynamics and stock management of exploitable species.

The skeletons of invertebrates in general, and molluscs in particular, all show growth rings (Jones 1987; Santarelli 1985; Davenport 1988; Guillaume 1988; Bourget and Brock 1990). These represent abrupt or repeated changes in bimineral accretion (Clark 1974), where the most recently formed layer covers the previous growth increment over a continuous deposition surface.

The growth rings can either be visible directly on the shell and these are the external growth rings, or are visible in cross-sections of the shell and these then represent the internal growth rings. The study of the growth rate of Turbo marmoratus using sclerochronology requires on the one hand the selection of appropriate calcified parts which could be interpreted in terms of age, and on the other the confirmation of the length of formation periodicity of these structures, in other words, confirmation of their deposition rates.

Following age confirmation, it remained to find a relationship between age (or a variable representative of age) and the shell perimeter, which was compatible with the Von Bertalanffy growth model. This relationship is based on the method developed by Bach and Chauvelon (1994) for fish otoliths. With the help of this relationship and the one obtained between the perimeter and diameter of the shell, the values of the parameters $K$ and $D_0$ of the model are estimated.

MATERIALS AND METHODS

Study Site and Marking Methods

This study is incorporated into the investigation of growth by marking and recapture of Turbo marmoratus on the reef at Tautira in Tahiti, between January 1992 and December 1993 (Fig. 1).

During the study, 182 green snails were marked on the shell and operculum using lead pencils (Fig. 1). The recapture of 44 green snails (out of a total of 182 which were marked) for individuals a diameter from 8.8 to 21.5 cm enabled the study of the periodicity of formation of elementary growth structures (Table 1).

During marking operations, several bands of a width from a half to one centimeters are laid on the internal shell surface and on the perimeter of the lip, using a lead pencil as shown in Fig. 2. The marking remains very visible on thin strips, and enables the counting of rings deposited as a function of the time of freedom after marking (At). Counting can hence be done equally well on the mother-of-pearl layer, or the prismatic layer (Bourgeois 1996).

Table 1: Summary of the shell marking operations and results from recaptured individuals

<table>
<thead>
<tr>
<th>Date</th>
<th>Number</th>
<th>During this study</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-01-93</td>
<td>108</td>
<td>26</td>
<td>48</td>
</tr>
<tr>
<td>19-05-93</td>
<td>23</td>
<td>3-02-93</td>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diameter (cm)</th>
<th>At (days)</th>
<th>16-01-93</th>
<th>23-01-93</th>
<th>8-01-93</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.3-15.1</td>
<td>26</td>
<td>7</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>8.8-21.5</td>
<td>8</td>
<td>15</td>
<td>26</td>
<td>32</td>
</tr>
</tbody>
</table>

Fig. 1: Location of study sites. A: French Polynesia, B: Tahiti Island

Fig. 2: Location of study sites. A: French Polynesia, B: Tahiti Island

Table 1: Summary of the shell marking operations and results from recaptured individuals

<table>
<thead>
<tr>
<th>Date</th>
<th>Number</th>
<th>During this study</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-01-93</td>
<td>108</td>
<td>26</td>
<td>48</td>
</tr>
<tr>
<td>19-05-93</td>
<td>23</td>
<td>3-02-93</td>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diameter (cm)</th>
<th>At (days)</th>
<th>16-01-93</th>
<th>23-01-93</th>
<th>8-01-93</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.3-15.1</td>
<td>26</td>
<td>7</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>8.8-21.5</td>
<td>8</td>
<td>15</td>
<td>26</td>
<td>32</td>
</tr>
</tbody>
</table>


Fonds Documentaire ORSTOM
Cote: BX15990 Ex:1
polished on sheets of 9, 3 and 1 mm. The surface to be studied is then glued onto a glass slide using araldite (Durcupan ACM) and then put in an oven for complete polymerization of the resin. The slide is then fixed to an ISOMET diamond saw, and cut to a thickness of about 0.5 mm. The thickness is then reduced to between 25-50 μm as previously described, the result being monitored using a dissecting microscope.

A total of 18 shells of a diameter between 8.8 and 21.5 cm were treated in this manner. The counting of the rings is done both on the prismatic and mother-of-pearl layers (Fig. 3).

In order to improve the determination of the rings in the prismatic layer which correspond to growth in surface area, a new technique was developed. This consisted of putting the sample in an oven at 400°C for six hours prior to gluing with araldite onto the glass slide. This procedure increases the contrast of organic layers, thus making the counting of the rings easier (Fig. 4).

Observation of the growth rings
Using light microscopy: The various observations were done in natural or polarized light using an Olympus BH-2 microscope and a Leica M3z dissecting microscope. Size measurements were done using an ocular micrometer.

Using scanning electron microscopy (SEM): A simple examination of the structure was done using SEM. Fragments were isolated by transverse fractures in different parts of the shell. After cleaning by ultrasonic bath and oven drying (at 60°C for 1 to 4 hours, depending on sample size) the surfaces were metallised using a thin layer of gold-palladium. Observations were made using a Hitachi S 570 microscope.

Study of age by sclerochronology
The study dealt with a batch of 53 individuals of a diameter between 3.4 and 23 cm. All shells had an entire apex, and were subjected to meticulous brushing and washing in hydrochloric acid, removing all epifauna and epiflora. The conispiral perimeter of the shell was measured using a very thin copper wire (0.1 mm) snugly fitted along the spiral, from the lip towards the apex, following the line of suture. On each shell, the number of daily rings was counted under a dissecting microscope along a length of one centimetre in three different places, as shown in Fig. 5 and Fig. 6.

The diameter (d) across the apex and corresponding to half this zone was measured using a calliper rule. The number of rings in each sampled centimetre enables the determination of the daily ring density (DRD) associated to a corresponding value (d) of the shell diameter. A relationship was obtained between the perimeter (p) and the diameter (d) in order to obtain the values of the variables DRD and p onto which are adjusted the growth model.
Age and Growth of *Turbo marmoratus*

**Model based on DRD**

This model is based on the methods developed by Bach and Chevillon (1994) for fish otoliths. The density of daily rings associated with a point on the perimeter \( p \) (corresponding to a diameter \( d \)) is defined by the relationship between the number of rings \( N \) and the width of the zone \( \Delta l = 1 \) cm over which the counting of microstructures was done.

\[
DRD = \frac{\Delta N}{\Delta l}
\]

The function \( DRD(p) \) is expressed in the form:

\[
DRD(p) = \frac{\Delta N}{\Delta l} = DRD = \frac{\Delta N}{\Delta l}
\]

Hence, the age in days of the green snail (i.e., the total number of rings \( N \)) under the hypothesis of daily deposition is obtained by the integration of (2) between \( 0 \) and \( p \)

\[
N = \int_{0}^{p} DRD(p) \, dp
\]

If we assume that the increase in size of the shell is described by the Von Bertalanffy model, the perimeter at a point in time \( t \) is given by:

\[
P_t = P_\infty \left[1 - \exp \left(-\frac{K}{P_\infty - P}\right)\right]
\]

the age associated with the size will be:

\[
t = \frac{N}{\frac{1}{K}} - 1\ln \left[1 - \left(\frac{P}{P_\infty}\right)\right]
\]

knowing that \( DRD(p) = \frac{\Delta N}{\Delta l} \), we deduce the expression for the density of the rings:

\[
DRD(p) = \frac{\Delta N}{\Delta l} = \frac{\Delta l}{\Delta N} = \frac{1}{K} \left(\frac{P}{P_\infty} - 1\right)
\]

Deriving (5):

\[
DRD(p_A) = \frac{1}{K} \left(\frac{P_A}{P_\infty} - P\right)
\]

which is of the form:

\[
DRD(p) = \frac{1}{K} \left(\frac{P_A}{P_\infty} - P\right)
\]

with:

\[
A = K P_\infty \quad \text{and} \quad B = -K
\]

Hence, by adjusting the model to the pair of data \( DRD(p), P \) it is possible to estimate the perimeters \( A \) and \( B \) which enable the calculation of the values \( K \) and \( P_\infty \).

If we assume that \( t(p) = 0 \) when \( p = 0 \), then \( t_0 \) can be determined from (5).

A regression analysis enables the determination of a relationship between the diameter and the perimeter, from which a diameter-age key is obtained.

**RESULTS**

Rate of ring deposition

Examination of sections of the shell under the microscope show that the lead pencil marks are clear and of good contrast both in reflected and transmitted light. It is relatively easy to determine the layers deposited following marking, both within the mother-of-pearl and prismatic layers. Furthermore, a clear improvement in clarity of the rings within the prismatic layer is obtained after oven treatment at 400°C.

For the various shells studied, we show that the number of rings counted corresponds exactly to the number of days of freedom after marking (Table 2).

**Table 2: Number of rings deposited following marking as a function of days of freedom and shell diameter in *Turbo marmoratus***

<table>
<thead>
<tr>
<th>Nb of days of freedom</th>
<th>Diameter (cm)</th>
<th>Nb of rings following marking</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10.3</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>11.9</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>13.1</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>13.1</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>26</td>
<td>16.4</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>16.9</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>18.2</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>18.3</td>
<td>26</td>
</tr>
</tbody>
</table>
Fig. 4: Polished slice of shell after 6 hours in an oven at 400°C. Note the increase of contrast of organics layers. Ir : internal growth ring, er : external growth ring, pl : prismatic layer, ml : mother-of-pearl layer.

Fig. 5: Location of the three counting zone (l1, l2, l3 = lcm) of daily rings corresponding to a diameter across apex (d1, d2, d3) to determine the daily growth density.

Fig. 6: External growth rings on the top of a shell. Arrow shows the mark laid with a lead pencil on perimeter of the lip aperture.

Age and Growth of Turbo marmoratus

Model based on DRD
This model is based on the methods developed by Bach and Chauvelon (1994) for fish otoliths. The density of daily rings associated with a point on the perimeter p (corresponding to a diameter d) is defined by the relationship between the number of rings (AN) and the width of the zone (Δl = 1 cm) over which the counting of microstructures was done.

\[ \text{DRD}(p) = \frac{\text{dN}}{\text{dl}} \]

The function \( \text{DRD}(p) \) is expressed in the form:

\[ \text{DRD}(p) = \frac{N}{d} \]

Hence, the age in days of the green snail (i.e., the total number of rings \( N \)) under the hypothesis of a daily deposition is obtained by the integration of (2) between 0 and \( p \).

\[ N = \int_{0}^{p} \text{DRD}(p) \, dp \]

If we assume that the increase in size of the shell is described by the Von Bertalanffy model, the perimeter at a point in time \( t \) is given by:

\[ P_t = P_0 \left( 1 - \exp^{-K(t-t_0)} \right) \]

The age associated with the size will be:

\[ t = \frac{N}{K} \ln \left( \frac{1}{1 - \frac{P_0}{P}} \right) \]

knowing that \( \text{DRD}(p) = \frac{\text{dN}}{\text{dl}} \), we deduce the expression for the density of the rings:

\[ \text{DRD}(p) = \frac{1}{K \left( P_0 - P \right)} \]

where

\[ \text{DRD}(p) = \frac{1}{K} \frac{A}{B} \]

with:

\[ A = K P_0 \]
\[ B = K \]

Hence by adjusting the model to the pair of data (DRD, P), it is possible to estimate the parameters \( A \) and \( B \) which enable the calculation of the values \( K \) and \( P \).

If we assume that \( t(p) = 0 \) when \( p = 0 \), then \( t_0 \) can be determined from (5).

A regression analysis enables the determination of a relationship between the diameter and the perimeter, from which a diameter-age key is obtained.

RESULTS
Rate of ring deposition
Examination of sections of the shell under the microscope show that the lead pencil marks are clear and of good contrast both in reflected and transmitted light. It is relatively easy to determine the layers deposited following marking, both within the mother-of-pearl and prismatic layers. Furthermore, a clear improvement in clarity of the rings within the prismatic layer is obtained after oven treatment at 400°C.

For the various shells studied, we show that the number of rings counted corresponds exactly to the number of days of freedom after marking (Table 2).

Table 2: Number of rings deposited following marking as a function of days of freedom and shell diameter in Turbo marmoratus

<table>
<thead>
<tr>
<th>Nb of days of freedom</th>
<th>Diameter (cm)</th>
<th>Nb of rings following marking</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10.3</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>11.9</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>13.1</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>15.1</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>10.3</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>11.9</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>13.1</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>15.1</td>
<td>15</td>
</tr>
<tr>
<td>26</td>
<td>10.3</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>11.9</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>13.1</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>15.1</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>16.3</td>
<td>26</td>
</tr>
</tbody>
</table>
Results reveal a daily rate of deposition. The elementary growth rings are daily rings (Fig. 7).

Quantification of mineral deposition on the shell:

In terms of growth, the microscopic examinations following marking gave the following results: The mean thickness of aragonite tablets for shells of a diameter between 13 and 16 cm is 0.8 ± 0.075 μm. The increase in thickness of the mother-of-pearl layer varies in relation to the shell diameter, but also depends on the extent of attack of the shell by boring organisms. For shells between 13 and 16 cm there is a mean daily mother-of-pearl deposition of 35 ± 10 μm. For shells with a diameter above 20 cm, there is a daily deposition of 13 ± 3 μm.

Growth in length (shell perimeter) can vary between 220 and 280 μm per day, for diameters between 13 and 16 cm. For those individuals with a diameter above 20 cm, and which are therefore in shell thickening strategy, the increase in length can vary from 5 to 127 μm per day (Bourgeois 1996).

Relationship between DRD and the perimeter:

A least-square regression analysis, adjusted to the 53 pairs of data (diameter; perimeter) of the sample give the following expressions:

\[ P = 3.6648 D + 6.0804 \quad (r^2 = 0.987) \]  
\[ D = 0.2678 P - 1.437 \quad (r^2 = 0.987) \]

The diameters \( d_1, d_2, d_3 \) are converted to perimeters \( p_1, p_2, p_3 \) using function (11). The infinite diameter can be deduced from function (12) and the growth curve is estimated (Fig. 9).

\[ l = D_m - D - 1.437 = 30.305 \text{ cm} \]

Knowing the values of \( A \) and \( B \), we deduce the values of the parameters \( K \) and \( P_0 \) of the Von Bertalanffy model. We obtain:

\[ K = 0.000886 \text{ day}^{-1} = 0.324 \text{ year}^{-1} \text{ and } P_m = 118.53 \text{ cm} \]

DISCUSSION

Marking Technique

Among the various techniques experimented with during the course of this study about growth of Turbo marmoratus using mark-and-recapture, it was found that the one using marking with a lead pencil was most effective (Bourgeois, 1996). In addition to its simplicity, it enables marking to be done in situ, a continuity of data and near-zero stress to the animal.

Daily Growth Rings

A daily deposition of elementary rings was described for other species of molluscs such as Mercenaria mercenaria and Tridacna squamosa by Pannella and MacClintock (1968), for Tridacna maxima by Henocque (1980) and for Turbo setosus by Sire and Bonnet (1984).

For the green snail, the study of the rate of daily deposition could not exceed 26 days for individuals having a maximum diameter of 17 cm. Beyond about 30 days, the distance between the mark and the edge of the shell precludes the cutting of a thin section on an ordinary microscope slide (76 x 25 mm). As the number of layers increases, so does the number of external events influencing the rate of deposition, and the degree of uncertainty during various countings becomes larger. However, on longitudinal shell sections, a confirmation of the number of days can be obtained from counting the number of rings of surface area increase within the prismatic layer. During the study, the results obtained were satisfactory up to a Dt of 72 days. Beyond this Dt, the counting of the number of growth rings is hampered by...
Age and Growth of Turbo marmoratus


Guillaume M. (1988) La croissance du squelette de Forêts jetée, scléractinaire hermatypique, sur le récif frangeant de la saline, île de la Réunion, Océan Indien. Thèse de doctorat, Université d'Aix-Marseille II


Yamaguchi M (1984a) Sedentary organisms as resources from coral reef : Marine ranching as a means of resource management. Aquabiology 10 : 250-254

Yamaguchi M (1984b) Biology of the green snail (Turbo marmoratus) and its resource management. Workshop on Pacific Inshore Fishery Resources 11

Yamaguchi M (1991) Green snail. Marine Sciences

Acknowledgements

This study could not have been possible without the logistical and financial support of the Établissement de Valorisation des Activités Aquacoles et Maritimes (EVAAM). It has benefited from numerous discussions, criticisms and comments from the whole research team of the Laboratoire d'Ecologie Marine (LEM) of the Université Française du Pacifique and that of CRSTOH. May they all be thanked.

References
