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

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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 $D_\infty = 30.3$ cm (D = diameter).

INTRODUCTION

The green snail, *Turbo marmoratus*, was introduced in Tahiti (French polynesia) waters in 1967. 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 natural products has made it a luxury item, whose price has not ceased to rise over the last decade, while the world-wide stock is decreasing (Yamaguchi 1988a, 1991). Despite the economic value of this species, no growth studies have been undertaken *in natura*. 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 1983; Santarelli 1985; Davanzo 1988; Guillaume 1988; Bourget and Brock 1990). These represent abrupt or repeated changes in biomineral 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 required 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 and 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 L∞ 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 after a period varying from 8 to 328 days of 44 green snails (out of a total of 182 which were marked) for individuals of 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 centimetre 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 (Δt). Counting can hence be done equally well on the mother-of-pearl layer, or the prismatic layer (Bourgeois 1996).

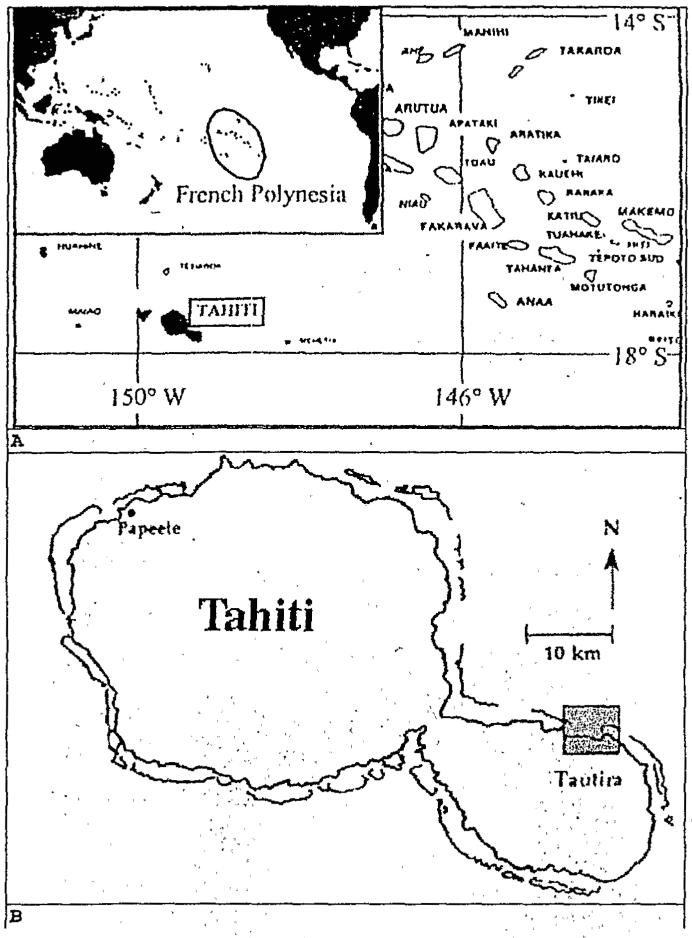


Fig 1 : Location of study sites. A : French Polynesia, B: Tahiti island

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

MARKING

Date	8-01-93	19-05-93	26-10-93	TOTAL
Number	108	26	48	182

RECAPTURE

Date	16-01-93	23-01-93	3-02-93	During this study	TOTAL
Number	7	7	11	19	44
Diameter (cm)	10.3-16.1	10-17	10.4-16.9	8.8-21.5	8.8-21.5
Δt (days)	8	15	26	9-328	8-328

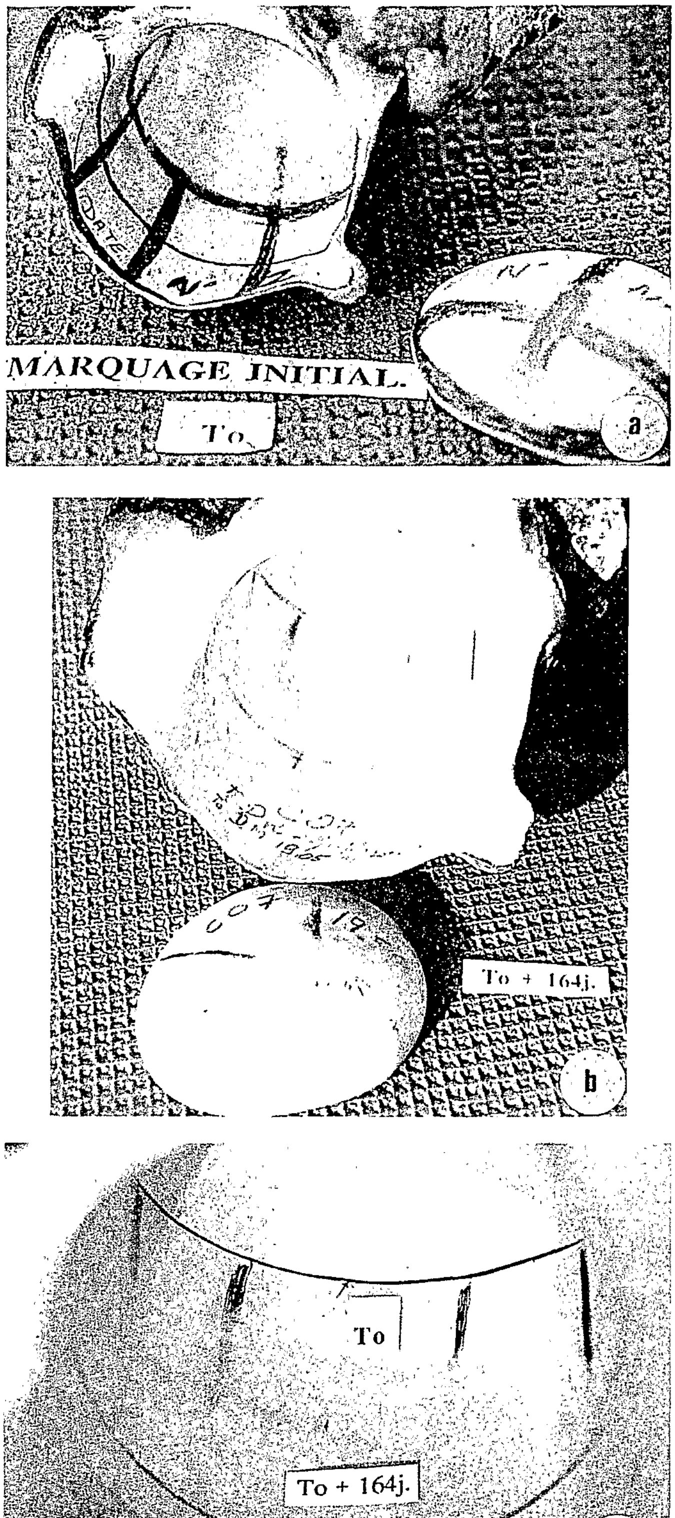


Fig. 2a: Initial marks laid on shell and operculum with a lead pencil.
2b: Shell and operculum after 164 days of freedom.
2c: Enlargement of Fig. 2b. The arrows show the closed position of the operculum.

Confirmation of age

Preparation of thin sections :
For each shell, a section 1 to 2 cm thick is cut on either side of a lead pencil mark, using a fast diamond saw. The slice is embedded in epoxy resin, and thinned-out by hand over sheets of silicon carbide of decreasing grain (180, 240, 400, 600, 800, 1000 and 1200), then polished on sheets of 9, 3 and 1 µm. The surface to be studied is then glued onto a glass slide using araldite (Durcupan ACM) and then put in an oven for complete polymerisation of the resin. The slide is then fixed to an ISOMET diamond saw, and cut to a thickness of about a 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).

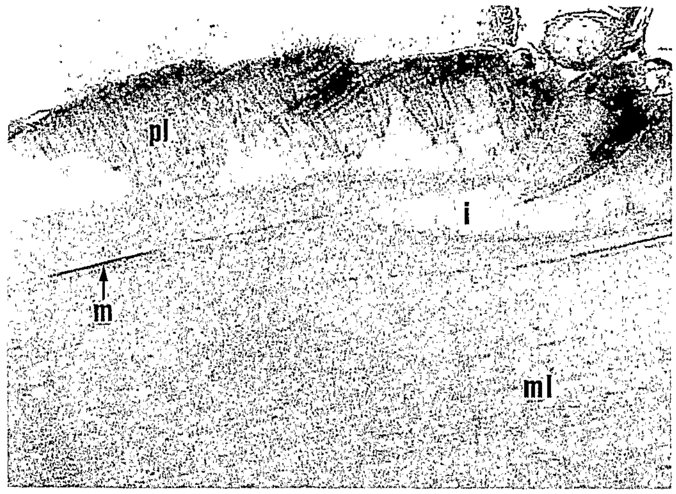


Fig. 3: Slide of thin section. m : mark of a lead pencil, pl : prismatic layer, i : indentation, ml : mother-of-pearl layer.

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

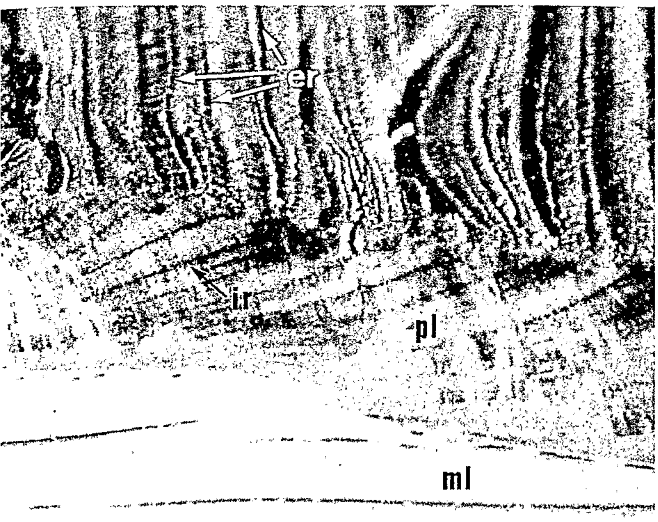
Using light microscopy :
The various observations were done in natural or polarised light using an Olympus BH-2 microscope and a Leica Wild M3z dissecting microscope. Size measurements were done using an ocular micrometer.

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.

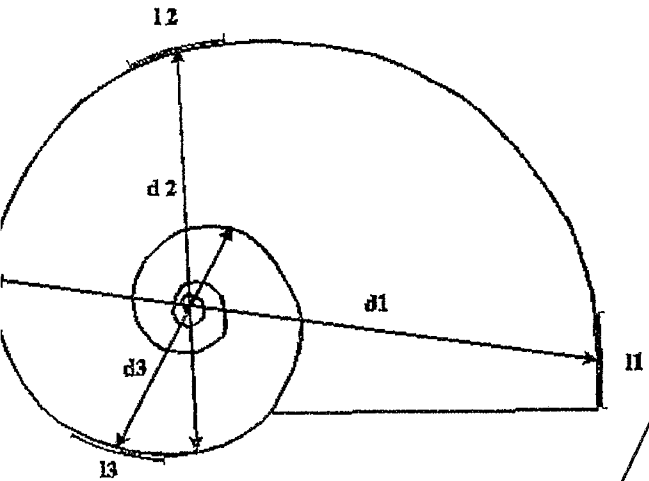
Study of age by sclerochronology
The study dealt with a batch of 53 individuals of a diameter between 3.4 and 23 cm. All shells had an entire apex, and were subjected to meticulous brushing and washing in hydrochloric acid, removing all epifauna and epiflora. The conispiral perimeter of the shell was 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.

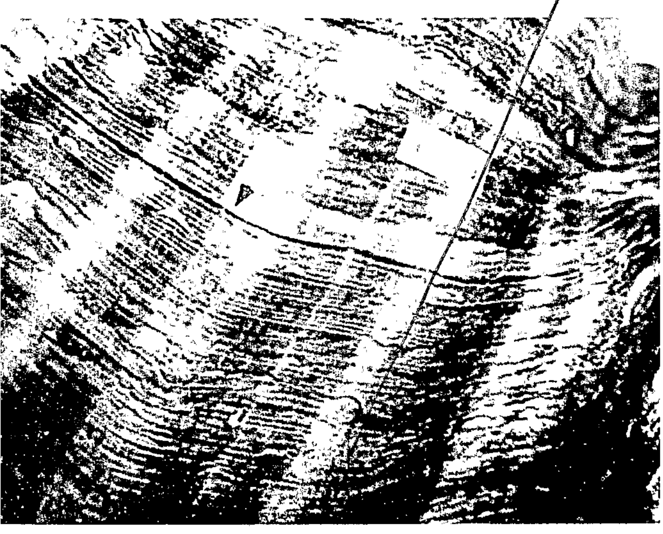
The diameter (d) across the apex and corresponding to half this zone was measured using a calliper rule. The number of rings in each sampled centimeter enables the determination of the daily ring density (DRD) associated to a corresponding value (d) of the shell diameter. A relationship was obtained 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.



.g. 4: Polished slice of shell after 6 hours in an oven : 400°C. Note the increase of contrast of organics ıyers. Ir : internal growth ring, er : external growth .ng, pl : prismatic layer, ml : mother-of-pearl layer.



.g. 5: Location of the three counting zone (l_1, l_2, l_3 = :m)) of daily rings corresponding to a diameter accross ›ex (d_1, d_2, d_3) to determinate the daily growth :nsity.



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

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 (ΔN) and the width of the zone (Δl = 1 cm) over which the counting of microstructures was done.

$$DRD = \Delta N / \Delta l$$

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

$$DRD(p) = dN/dl = DRD = \Delta N / \Delta l \quad (1)$$
$$dN = DRD(p)\, dl \quad (2)$$

Hence, the age in days of the green snail (ie. the total number of rings (N) under the hypothesis of a daily deposition) is obtained by the integration of (2) between O and P

$$N = \int_0^P DRD(p)\, dl \quad (3)$$

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 :

$$P_t = P_\infty\,[1 - \exp^{-K(t - t_0)}] \quad (4)$$

the age associated with the size will be :

$$t_p = N = t_0 - (1/K) - \ln\,[1 - (P/P_\infty)] \quad (5)$$

knowing that DRD (p) = dN / dl, we deduce the expression for the density of the rings :

$$DRD(p) = (1/K)\,[1/(P_\infty - P)] \quad (6)$$

deriving (5) :

$$DRD(p) = 1/K\,(P_\infty - P) \quad (7)$$
$$DRD(p) = 1/(K P_\infty - KP) \quad (8)$$

which is of the form :

$$DRD(p) = = 1/(A + BP) \quad (9)$$
$$1/DRD(p) = A + BP \quad (10)$$

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.

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*

Nb of days of freedom	Diameter (cm)	Nb of rings following marking
8	10.3	8
8	11,9	8
8	13	8
8	16.1	8
15	10	15
15	13.1	15
15	15	15
15	17	15
26	10.4	26
26	12.2	26
26	15.1	26
26	16.9	26

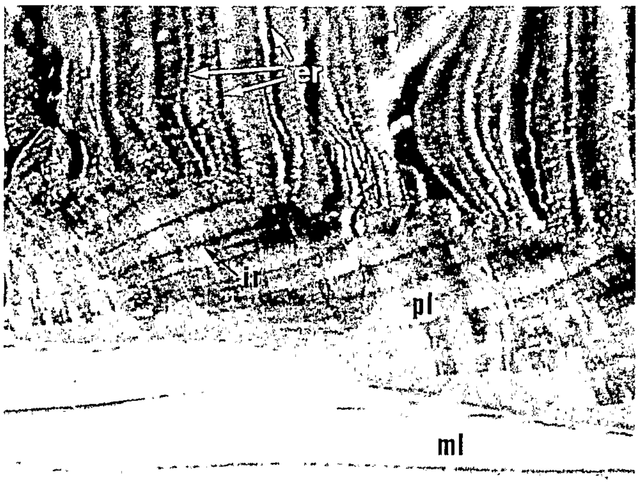
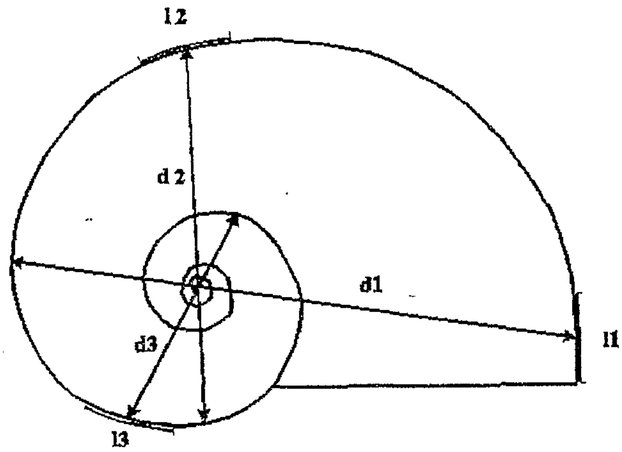


Fig. 4: Polished slice of shell after 6 hours in an oven at 400°C. *Note the increase of contrast of organics* layers. Ir : internal growth ring, er : external growth ring, pl : prismatic layer, ml : mother-of-pearl layer.



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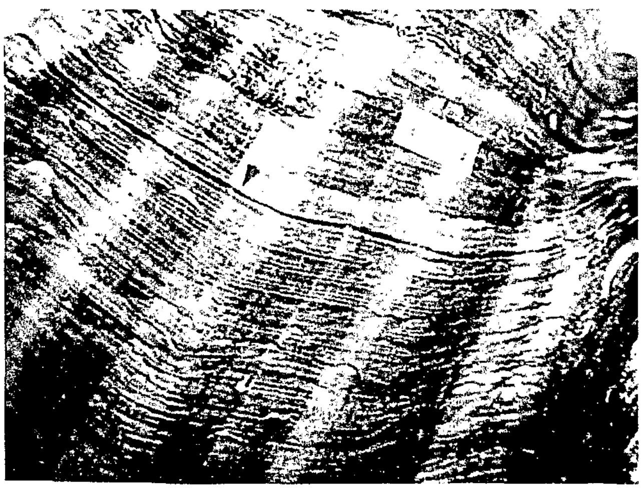

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8	16.1	8
15	10	15
15	13.1	15
15	15	15
15	17	15
26	10.4	26
26	12.2	26
26	15.1	26
26	16.9	26

Results reveal a daily rate of deposition. The elementary growth rings are daily rings (Fig. 7).

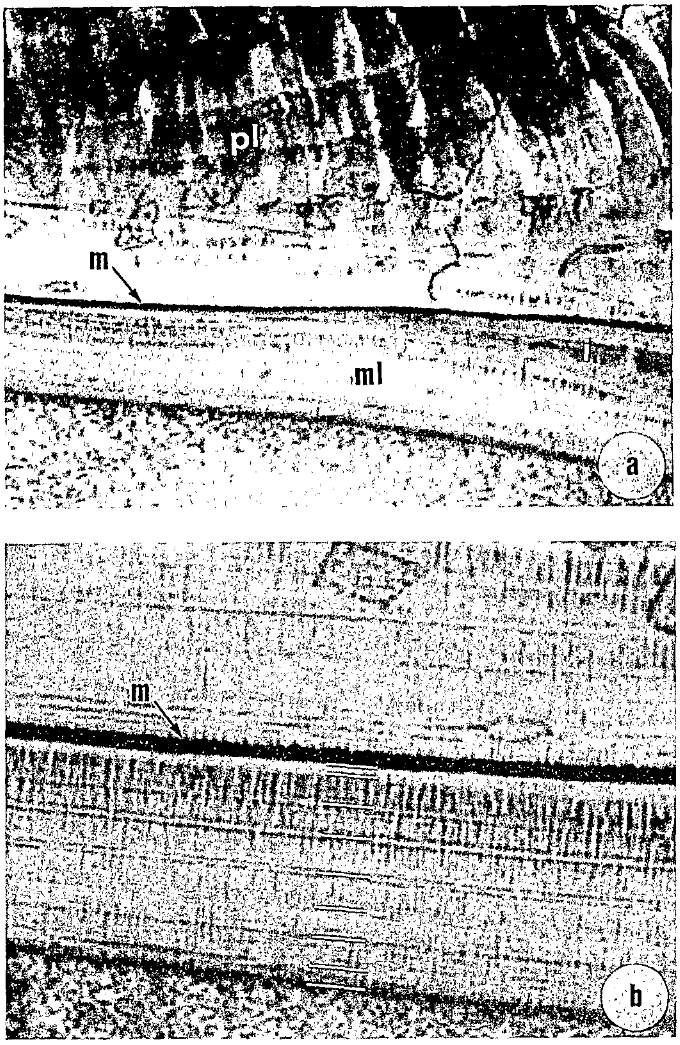

Fig. 7: 7a : Microphotography *showing* the incrementation after a Dt=8 days. 7b : Enlargment of the same slide. The incrementation fit to 8 layers. m : mark of lead pencil, pl : prismatic layer, ml : mother-of-pearl layer, i : indentation, scale bar = 200 µm.

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 ñ 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 ñ 10 µm. For shells with a diameter above 20 cm, there is a daily deposition of 13 ñ 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 :
The non-linear adjustment function of the SAS software was used to estimate the parameters of the model

$$1/DRD(p) = A + B\ P$$

The adjusted model, whose parameter values are :

$A = 0.10502$ with a standard error of 0.00304

$B = -0.000886$ with a standard error of 0.00006

accounts for 57.44% of the variation in 1/DRD(p) ($r = 0.758$) (Fig. 8).

Knowing the values of A and B, we deduce the values of the parameters K and P_i of the Von Bertalanffy model. We *obtain :*

$K = 0.000886\ day^{-1} = 0.324\ year^{-1}$ and $P_\infty = 118.53$ cm

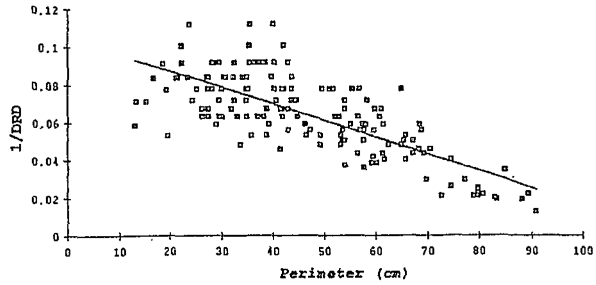

Fig. 8 : Relationship between the variable *1/DRD* and the perimeter

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

$$P = 3.6848\ D + 6.0804 \quad (r^2 = 0.987) \qquad (11)$$
$$D = 0.2678\ P - 1.437 \quad (r^2 = 0.987) \qquad (12)$$

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).

$$L_\infty = D_\infty = 0.2678\ P_\infty - 1.437 = 30.305 \text{ cm.}$$

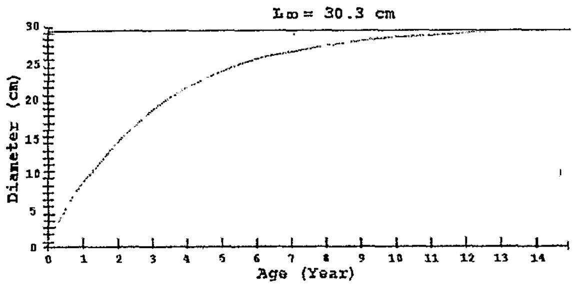

Fig. 9 : Growth curve for *Turbo marmoratus* according to the Von Bertalanffy model, estimated using the DRD method.

DISCUSSION

Marking Technique
Among the various techniques experimented with during the course of this study about growth of *Turbo marmorartus* 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 25mm). As the number of layers increase, 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 the unsolvable problem raised by the conispiral shape of the shells.

Beyond a shell diameter of 18 cm, the thickness of the deposition decreases, organic components dominate and the growth of the perimeter is minimal. The deposition of mother-of-pearl layers via the coat is done in a retrograde fashion, which favours the thickening of this layer. These mother-of-pearl depositions are interspersed with much more frequent, oblique prismatic layers (Bourgeois 1996). The same strategy (except for the oblique deposition of prismatic layers) takes place promptly during repair of the shell edge following mechanical stress. This translates externally as a discrepancy in the succession of growth rings, and internally as an indentation.

Growth Model

The method of estimation by DRD allows estimation of the Von Bertalanffy growth parameters by studying a relatively small sample, as long as it is representative. This method is less suited for the study of seasonal growth variations, but is very powerful when one wishes to obtain the growth parameters without using a mark and recapture method.

The proposed DRD method is subject to several sources of variation. There could exist a local heterogeneity in the width of growth rings counted in the examination zone (intrazonal variation). Furthermore, the zones selected on the shell correspond to different growth schemes owing to seasonal fluctuations in growth rates, which directly affect the density of rings (interzonal variation).

As noted in many molluscs, there is a strong variation in individual growth rates. From a technical aspect, it is very difficult to collect well-standardised data. The problem of distinguishing between daily and sub-daily deposits during counting remains a delicate one. Finally, the degradation factor is proportional to the increase in size of the epibiota in general, and of boring organisms in particular. It acts on the one hand on the reading of the growth rings, and on the other over the reaction of the animal in the deposition of elementary structures.

The DRD method is based on the numerical integration of the growth rate function. It owes its simplicity to the biological and mathematical hypotheses which support it (linearity of the relationship between the diameter and the perimeter of the shell, relationship between DRD and the perimeter, and Von Bertalanffy growth model).The usefulness of its application will only be confirmed after numerous checks and comparisons with other conceivable methods.

In their monograph on the genus *Haliotis*, Shepherd and Hearn (1983) noted that comparison between K and L_∞ is not always possible because of different study techniques. They stress that the data from mark-and-recapture over too short periods of time have a tendency to underestimate K and overestimate L_∞. Furthermore, if the marking data do not cover a wide range of representative sizes for the population under study, we see distortions in the growth parameters. A strong representation of small size-classes tend to overestimate K while underestimating L_∞, and vice versa. For *Turbo marmoratus*, the growth parameters obtained by mark-and-recapture were $K = 0.26\ yr^{-1}$ and $L_\infty = 26.88$ cm (Bourgeois 1996). Hence, one will have to exercise caution in using the results obtained in this study during eventual comparisons with future studies in the growth of *Turbo marmoratus*.

ACKNOWLEDGEMENTS

This study could not have been possible without the logistical and financial support of the Etablissement pour la Valorisation des Activitées 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 Francaise du Pacifique and that of ORSTOM. May they all be thanked.

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