Appraisal of the usefulness of operculum growth marks for ageing *Hexaplex trunculus* (Gastropoda: Muricidae): Comparison between surface striae and adventitious layers

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Abstract
This study estimated the age of *Hexaplex trunculus* (Gastropoda: Muricidae) by analysing operculum growth marks. Surface striae and adventitious layers were counted in opercula of *H. trunculus* from the Ria Formosa Lagoon (southern Portugal) and from the Bizerte Lagoon (northern Tunisia), and in aquacultured individuals used for validation of the ageing technique. Operculum growth features were also compared between *H. trunculus* populations under contrasting environmental conditions (namely seawater temperature). Despite high inter-individual variability, all *H. trunculus* populations displayed a clear increasing trend in the number of striae and layers with increasing specimen size. The operculum marginal growth revealed that opercular deposition is not annual. Instead, a few growth marks are formed in the operculum each year, which was confirmed with reared specimens. In both ageing methods, considerable variation in the number of counts at a determined shell length indicates that operculum growth marks should be interpreted cautiously because they might not correspond accurately with individual age. Because of the subjectivity inherent to either method, counting striae and layers in opercula only provides rough age estimates of *H. trunculus*.

Key words: Adventitious layers, age estimation, Gastropoda, Hexaplex trunculus, Mollusca, operculum, surface striae

Introduction
The understanding of population dynamics, adequate fishery management and sustainable exploitation of gastropod populations requires information on the species biology, namely the estimation of growth, age and longevity. Compared with other molluscs (particularly bivalves), methods of age estimation in gastropods have received less attention (Kraeuter et al. 1989; Chen & Soong 2002) and few studies have addressed the problem of ageing gastropod species (Richardson et al. 2005). This is probably because gastropods show fewer recognizable growth marks (Kideys 1996; Chen & Soong 2002) and also because the geometry of gastropod shells (the whorl-like shape) complicates the use of certain ageing methods, namely conventional shell-sectioning techniques (Richardson et al. 2005). Nevertheless, diverse methods have been employed to estimate the age of gastropods, including analysis of size–frequency distributions, growth checks on the shell surface, acetate peel replicas of sectioned shells, stable isotopes and element ratios in the shell, growth marks in the operculum, growth rings in statoliths, and mark–recapture experiments (see review by Richardson 2001 and references therein).

In general, the gastropod operculum is a disc-shaped structure located on the dorsal surface of the foot and associated with the shell aperture (Fretter &
The operculum serves for covering and blocking the shell aperture, providing shelter for the body when retracted inside the shell (Checa & Jiménez-Jiménez 1998). In addition, the operculum allows organisms to retain inner water and to survive during long aerial exposures, avoiding severe dehydration even under high temperatures (Alyakrinskaya 2004). The gastropod operculum has been studied to ascertain the potential of the visible rings and checks for age estimation. In several species, this has been done by counting the number of surface striae on the opercula, including *Megastraea undosa* (Cupul-Magaña & Torres-Moye 1996; Gluyas-Millán et al. 2000), *Buccinanops globulosum* (Narvarte 2006), *Buccinum isanotikii* (Ilano et al. 2004), *Buccinum undatum* (Santarelli & Gros 1985; Kideys 1996; Valentimson et al. 1999; Shelmerdine et al. 2007), *Coralliophila abbreviata* (Johnston & Miller 2007), *Coralliophila violacea* (Chen & Soong 2002), *Neptunea antiqua* (Power & Keegan 2001), *Neptunea arthritica* (Miranda et al. 2008) and *Nucella heyseana* (Selin 2003). In species where this method was ambiguous and did not produce reliable results, age was estimated by counting the number of adventitious layers (plates added at the underside of the operculum) in sectioned opercula, namely in *Busycon carica* (Kraeuter et al. 1989), *N. antiqua* (Richardson et al. 2005) and *Turbo setosus* (Sire 1984; Sire & Bonnet 1984).

The banded murex, *Hexaplex trunculus* (Linnaeus, 1758) (Figure 1), is distributed throughout the Mediterranean Sea, whereas in the Atlantic Ocean it only occurs from Portugal southward to Morocco and to the Madeira and Canary Archipelagos (Poppe & Goto 1991; Macedo et al. 1999; Houart 2001). This muricid is regularly or occasionally fished/harvested for human consumption in several countries (Poppe & Goto 1991; Houart 2001), where it is caught using diverse fishing gears (pots, basket traps, dredges, scoop nets, trawl nets, gill nets, trammel nets) (Gaillard 1987; Malaquias 2007). This species is commercially valuable in Portugal and Tunisia, targeted by locally important artisanal fisheries mainly in the Ria Formosa Lagoon (southern Portugal) (Muzavor & Morenito 1999; Vasconcelos et al. 2008a), in the Gulf of Gabès (southern Tunisia) (Elhasni et al. 2010) and in the Bizerte Lagoon (northern Tunisia) (Gharsallah et al. 2004, 2010).

In spite of being the subject of an economically important fishery, much remains to be known about population dynamics of *H. trunculus*, particularly age and growth. Besides some studies on the early development and juvenile growth rates (Vasconcelos et al. 2004; Lahbib et al. 2008, 2010), the only age estimation for this species was based on mark-recapture (Vasconcelos et al. 2006a), an accurate but impractical method for routine analyses. Therefore, a simple and reliable ageing method would be desirable. In this context, we assessed the usefulness and accuracy of two alternative methods.

Figure 1. (A) Live specimen of banded murex, *Hexaplex trunculus* (ventral view); (B) soft part of the organism after shell breakage, showing the location of the operculum (dotted ellipse); (C) some examples of damaged and unreadable opercula of *Hexaplex trunculus*: distorted/deformed operculum; operculum missing the nucleus; operculum with eroded surface and broken border (labial edge); operculum with missing and loose layers. f, foot; op, operculum.
based on growth marks in the operculum (surface striae and adventitious layers) of *H. trunculus* from Ria Formosa Lagoon and Bizerte Lagoon. In addition, we compared operculum growth features in these *H. trunculus* populations inhabiting contrasting environments (namely in terms of seawater temperature).

Material and methods

Sampling

Specimens of *Hexaplex trunculus* (Figure 1A) were caught monthly during 2007 by professional fishermen in the Ria Formosa Lagoon (RF, southern Portugal) and the Bizerte Lagoon (BL, northern Tunisia). In the laboratory, individuals were measured for shell length (SL, mm) with a digital calliper (precision of 0.01 mm). Subsequently, shells were broken in a bench vice to remove the soft part of organism (Figure 1B) and the operculum was carefully detached from the foot with a scalpel and forceps. After scraping off the adhering flesh, opercula were washed in running water and air-dried. Distorted, damaged (i.e. broken, eroded, lacking the nucleus or with missing layers) or otherwise unreadable opercula (roughly 5–10%) were discarded (Figure 1C). The operculum length (OL, mm) and width (OW, mm) were also measured (Figure 2A) with the digital calliper.

The operculum of *H. trunculus* is corneous, roughly oval-shaped, concentric, moderately slim and rigiclaudent (aperture-fitting) (Figures 1A and 2B). A detailed description of the operculum, including information on the type, function, morphology, structure and composition, is available as online-only supplementary material. This information is helpful to elucidate the underlying principle of using the operculum for ageing purposes.

Analysis of operculum growth marks

A preliminary observation was made to determine the most suitable surface of the operculum (external or internal) for counting growth marks: using the external surface would eventually allow ageing live specimens, but it is frequently eroded (particularly in the nucleus) or damaged (mainly near the margins); using the internal surface implies killing the specimens, but being attached to the foot it is protected from erosion and damage. This examination revealed that the internal surface is unsuitable for ageing, because the varnish layer underneath the operculum partially covers the inner surface and makes counting growth marks impossible (Figure 2A).

Additionally, in an attempt to enhance the readability of growth marks, some opercula were briefly immersed in methylene blue. This staining technique was not helpful, as surface striae (SS) were not clearer or easier to count (especially near the nucleus, where striae are narrowly deposited close to each other). Illuminating the operculum by using strong backlighting also did not improve the readability of the striae (namely in older and thicker opercula). In contrast, slightly wetting the operculum with tap water often increased the contrast of growth marks and simplified counting striae on the external surface of the operculum (Figure 2B).

The external surface of the operculum of *Hexaplex trunculus* presents a sequential pattern of concentric striae. Despite the existence of many marks (most of which could be seen with the unaided eye), only the well-defined striae were counted, i.e. those that were continuous all around the operculum and that corresponded to a slight depression on the external surface (Figure 2B). Counting SS was made by using a stereomicroscope with transmitted light. Simultaneously, to determine the periodicity of the formation of SS and detect eventual seasonal trends in opercular...
deposition, monthly marginal growth and monthly marginal growth ratio were calculated in 10–15 opercula each month. Marginal growth (MG), i.e. the distance from the most recently deposited stria to the sutural edge of the operculum (Figure 2B), was measured using a stereomicroscope with a calibrated eyepiece. Because specimen size (and consequently operculum length) might influence the marginal growth, marginal growth ratio \[ \text{MGR} = \frac{\text{MG}}{\text{OL}} \times 100 \] was also calculated. To further reduce inter-individual variability in marginal growth between smaller and bigger individuals, MG and MGR were calculated for specimens within a narrow size range (size class of 40–60 mm SL).

Two techniques were essayed for counting the adventitious layers (AL): thin transverse sections of opercula and acetate peel replicas of sectioned opercula following Richardson (2001). Opercula were embedded in polyester resin and cut using a rotating precision saw (BUEHLER® Isomet 1000) through the longest axis, i.e. across the nucleus and along the anterior–posterior axis (Figure 3A). One half-block of resin was used to prepare the thin sections. Slices about 2 mm thick were cut using the precision saw, ground and polished on progressively finer sandpaper using an automated grinder-polisher (BUEHLER® Beta), rinsed with tap water and air-dried (Figure 3B). Then, layers visible in the thin sections were counted using a stereomicroscope (magnifications of ×2.5 and ×4) (Figure 3C).

The other half-block was used to prepare the acetate peels. The cut surfaces were also ground and polished, rinsed with tap water and left to dry. Acetate peels were prepared, removed from the sectioned opercula and mounted between a glass slide and a cover slip. Then, layers visible in the acetate peels were counted using a light microscope (magnifications of ×10 and ×40) (Figure 3D). In both cases, counting was made by two independent observers and a second reading was performed whenever the number of growth marks did not coincide. In some thin sections, air bubbles became trapped in the operculum–resin interface, making the interpretation of growth marks difficult. In contrast, in the acetate peels the recognition of the nucleus was clearer and the identification of layers was easier; therefore, this ageing technique was adopted for counting AL in the opercula of *H. trunculus*.

For validating the deposition rhythm of the growth marks, individuals of three generations of *H. trunculus* born and reared in the IPIMAR Molluscan Aquaculture Experimental Station (Vasconcelos et al. 2004), and therefore with known age (1, 2 and 3 years in early summer), were subjected to the same ageing procedures. To facilitate counting SS in live *H. trunculus*, individuals were anaesthetized with magnesium chloride (MgCl₂) (for details see Vasconcelos et al. 2006b). For counting AL, individuals were sacrificed to remove the operculum.

Figure 3. (A) Location of the cut made along the operculum of *Hexaplex trunculus* (dotted line) to obtain the thin section and prepare the acetate peel replica; (B) thin section of the operculum; (C) counting of adventitious layers (white arrows) in the thin section; (D) counting of adventitious layers (black arrows) in the acetate peel replica; (E) folded margin of a sectioned operculum, containing trapped material within the folds. n, nucleus.
and prepare the acetate peels. The same approach, i.e. comparison between laboratory-reared individuals and age estimates obtained from opercula, was also used for *Buccinum carica* (Kraeuter et al. 1989) and *Buccinum isaotakii* (Ilano et al. 2004). It is recognized that growth in captivity might differ from that in the wild. However, since in the present case the rearing conditions closely resemble the natural environment, it is realistic to presume that deposition of opercular growth marks would be reasonably similar between aquacultured (AQ) and field-collected specimens (RF and BL). This information is highly valuable to determine the number of growth marks (SS and AL) formed in the operculum during the first three years of life. Although the lifespan of this species is around 10 years, at 3 years of age *H. trunculus* already reached 60 mm SL (Vasconcelos et al. 2006a), which comprises a significant fraction of the size range analysed in this study.

Overall, 680 individuals were subjected to the morphometric study of the operculum (340 from Portugal and 340 from Tunisia). For the different ageing approaches, SS were counted in 201 specimens from Portugal and 196 specimens from Tunisia, of which 50 opercula from each population were subjected to counting of AL. In addition, SS were counted in 120 individuals from aquaculture (42 1-year-old, 44 2-years-old and 34 3-years-old), of which AL on 10 opercula from each age group were counted.

**Data treatment and statistical analysis**

To describe relative growth, allometric relationships were established between individual size (SL) and operculum size (OL and OW). The type of growth was assessed through the allometry coefficient, expressed by the exponent $b$ of the allometric relationship ($Y = aX^b$) (Huxley & Tessier 1936). In relationships between linear variables, isometry occurs for $b=1$ (i.e. growth rates of both variables are identical during ontogeny). Therefore, a $t$-test (Sokal & Rohlf 1994) was applied to determine if the regression slopes were included in the isometric range ($b=1$) or allometric ranges (negative allometry: $b<1$; positive allometry: $b > 1$).

For each population, relationships between shell length and number of striae and layers in the operculum were established through regression analysis, by fitting a linear function ($Y=a + bX$) to raw data. The association between variables was assessed by the correlation coefficient ($r$) and the regression slopes were compared between populations using a $t$-test (Zar 1999). Shell length and number of SS and AL were compared between populations using analyses of variance (ANOVA). Whenever ANOVA assumptions (normality of data and homogeneity of variances) failed, the Kruskal–Wallis test (ANOVA on ranks) was performed. Each time significant differences were detected, pairwise multiple comparisons were made through the Tukey test and the Dunn test, respectively (Zar 1999). The number of SS and AL in the opercula of individuals subjected to both ageing techniques was compared using a paired $t$-test. Whenever the normality assumption failed, the Wilcoxon signed-rank test was performed (Zar 1999). Statistical analyses were made using SigmaStat (version 3.5), with significance level considered for $P<0.05$.

Von Bertalanffy growth functions were fitted to the growth marks–length key obtained from the two ageing methods (surface striae and adventitious layers) using the nonlinear model estimation (NLLIN procedure) of the software Statistica (release 6). This iterative curve fitting procedure employs a nonlinear least-squares regression (Gauss–Newton method), providing estimates of the growth coefficient ($K$), asymptotic shell length ($L_\infty$) and theoretical age at shell length zero ($t_0$), through the following equation (von Bertalanffy 1938):

$$L_t = L_\infty \left[1 - e^{-K(t-t_0)}\right]$$

**Results**

The descriptive statistics of *Hexaplex trunculus* analysed in this study are compiled in Table I. Both populations presented broad size ranges (15.7–82.8 and 19.0–77.3 mm SL for Portugal and Tunisia, respectively), without significant differences in specimens mean size between populations ($K$–$W$: $H=0.001$, $P=0.972$). Reared specimens also presented a broad size range, reflecting high individual variability in shell length among individuals within the same age class (1-year-old: 15.7–38.1 mm SL; 2-years-old: 37.5–55.5 mm SL; 3-years-old: 57.8–67.1 mm SL). Although including only relatively young individuals ($\leq 3$ years old), the size range of the reared *H. trunculus* (maximum = 67.1 mm SL) encompasses a considerable fraction of the size range of the specimens sampled from wild populations in Tunisia and Portugal (maximum = 77.3 mm SL in the Bizerte Lagoon; maximum = 82.8 mm SL in the Ria Formosa Lagoon) (Table I).

The allometric relationships established between individual size and operculum size of *H. trunculus* from both populations are presented in Figure 4. All relationships were highly significant ($P<0.001$) and presented high correlation coefficients (ranging from 0.945 to 0.995). Allometry coefficients were invariably higher than 1 ($b=1.048 - 1.133$),
reflecting positive allometries ($t$-test: $P<0.01$). Comparison of regression slopes did not detect significant differences in the allometric relationships between populations ($t$-tests, $P>0.05$) (Figure 4).

Highly significant differences in number of striae (K–W: $H=168.578$, $P<0.001$) and layers (K–W: $H=18.562$, $P<0.001$) were detected in both populations. Opercula from Portugal averaged more SS (Dunn test: $Q=8.034$, $P<0.05$) but fewer AL (Dunn test: $Q=4.260$, $P<0.05$) than opercula from Tunisia. The relationships established between *H. trunculus* size and number of striae and layers in opercula from Portugal, Tunisia and aquaculture are presented in Figure 5 and 6, respectively. Both data sets presented high inter-individual variability and overlap, i.e. specimens with similar shell length displayed a highly variable number of striae and layers in the operculum. For example, in reared *H. trunculus*, 1-year-old individuals had 3–6 SS and 3–5 AL, 2-years-old had 5–11 SS and 4–8 AL, and 3-years-old had 10–12 SS and 6–9 AL. In general, compared to surface striae, counting adventitious layers decreased the inter-individual variability in the number of growth marks formed in the operculum at a determined shell length. In addition, all regressions were highly significant ($P<0.001$) and displayed positive correlation coefficients ($r=0.715–0.908$), reflecting a clear trend of increasing striae and layers in the operculum with increasing specimen size. The comparison of regression slopes between populations detected significant differences ($t$-tests, $P<0.05$), with individuals from Portugal ($b=4.197$ and 4.348, respectively) depositing striae and layers at a higher rhythm than individuals from Tunisia ($b=3.377$ and 3.520, respectively) (Figures 5 and 6).

The comparison of data obtained from opercula of *H. trunculus* subjected to both ageing techniques is illustrated in Figure 7. In all populations, significant differences in the number of striae and layers deposited in each operculum were detected, namely in Portugal (Wilcoxon: $W=1275$, $P<0.001$), Tunisia (Wilcoxon: $W=1041$, $P<0.001$) and aquaculture (Wilcoxon: $W=378$, $P<0.001$): 1 year old (Wilcoxon test: $W=28$, $P<0.05$), 2 years old (paired $t$-test: $t=7.421$, $P<0.001$) and 3 years old (paired $t$-test: $t=9.851$, $P<0.001$). The number of striae (RF = 12.1 ± 2.0; BL = 10.6 ± 2.4; AQ = 8.8 ± 2.4) was almost invariably higher than the number of layers (RF = 5.0 ± 1.9; BL = 7.1 ± 2.8; AQ = 6.0 ± 1.7), indicating that some growth marks visible in the surface of the operculum had no correspondence in its internal structure. Only 7 opercula (4 from BL and 3 from AQ individuals 1-year-old) showed exact correspondence in the number of striae and layers, whereas only two opercula from Tunisia had more layers than striae (Figure 7).

Monthly variation in marginal growth and marginal growth ratio in opercula of *H. trunculus* is illustrated in Figure 8. MG (mm) and MGR (%) presented high monthly variation, further confirming that opercular deposition is not annual; instead, a few striae are formed in the operculum each year. In addition, MG and MGR displayed roughly the same trend in opercula from both populations, although without an evident seasonal variation. Apparently, *H. trunculus* from Portugal deposited more and narrower striae per year than *H. trunculus* from Tunisia, as evidenced by the higher monthly variation and lower values of MG and MGR (Figure 8).

Data from reared individuals (i.e. with known age) confirmed that counting surface striae and adventitious layers in the operculum only allows obtaining rough age estimates of *H. trunculus* (Figure 9). The estimation of the mean age at each stria and layer provided the following information: individuals in the age class 0+ are expected to have less than 3 SS and 3 AL; the age class 1+ has between 3 and 7 SS and between 3 and 5 AL; the age class 2+ has between 7 and 12 SS and between 6 and 9 AL; older individuals (age class 3+ and beyond) are expected to have more than 12 SS and 9 AL.

[Table I. Descriptive statistics (number and size) of *Hexaplex trunculus* used in the morphometric study of the operculum and subjected to different ageing techniques (counting of surface striae and adventitious layers).]

|          | Morphometrics | Surface striae | Adventitious layers | Aquaculture |
|----------|---------------|----------------|--------------------|-------------|
|          |               | 1 year old     | 2 years old        | 3 years old |
| Portugal | $n$           | 340            | 201                | 50          |
|          | mean SL±SD    | 51.4 ± 12.9    | 55.4 ± 9.5         | 51.2 ± 11.4 |
|          | (min–max)     | (15.7–82.8)    | (38.4–82.8)        | (38.4–82.8) |
| Tunisia  | $n$           | 340            | 196                | 50          |
|          | mean SL±SD    | 51.8 ± 12.1    | 50.7 ± 12.7        | 50.6 ± 13.8 |
|          | (min–max)     | (19.0–77.3)    | (19.0–75.5)        | (20.1–71.7) |

n, number; SL, shell length (mm); SD, standard deviation (mm); (min–max), size range (mm)
**Discussion**

**Operculum morphometrics**

The highly significant correlations obtained in the relationships established between specimen size and operculum size of *Hexaplex trunculus* revealed a clear proportionality between these morphometric variables. This results from the immediate addition of a new layer to the operculum as the shell aperture increases during shell growth, i.e. operculum length and width intimately reflect shell length. Similarly, highly significant relationships between SL and OL were observed in *Buccinum isaotakii* (Ilano et al. 2004), *Buccinops globulosum* (Narvarte 2006) and *Neptunea arthritica* (Miranda et al. 2008), between shell height and OL in *Turbo setosus* (Villiers 1981) and *Nucella heyseana* (Selin 2003), between shell basal diameter and OL in *Megastraea undosa* (Cupul-Magaña & Torres-Moye 1996; Gluyas-Millán et al. 2000), and between shell height and operculum diameter in *Turbo setosus* (Sire & Bonnet 1984).

The allometric relationships established between specimen size and operculum size showed that during *H. trunculus* growth the operculum scales allometrically, i.e. changes shape in response to size changes. Indeed, in both *H. trunculus* populations, positive allometries revealed that during ontogeny OL and OW increase at a higher rate than SL, and also that the operculum grows faster in width than in length. Similarly, a positive allometry was observed in the relationship established between SL and OW in *Turbo setosus* (Villiers 1981).

![Figure 4. Allometric relationships established between shell length (SL) and operculum length and width (OL and OW) of *Hexaplex trunculus* from Ria Formosa Lagoon and Bizerte Lagoon.](image-url)
Operculum growth marks

All *Hexaplex trunculus* populations (Portugal, Tunisia and aquaculture) displayed an increasing trend in the number of striae and layers with increasing specimen size. The highly significant correlations obtained in the relationships between SL, SS and AL revealed that the number of opercular growth marks increases during ontogeny and therefore might reflect individual age. Similarly, significant correlations were found between shell length and number of striae in *Buccinum isaotakii* (Ilano et al. 2004) and *Neptunea arthritica* (Miranda et al. 2008), and between shell aperture length and number of striae in *Coralliophila violacea* (Chen & Soong 2002), suggesting that operculum growth marks could be used for ageing those species. In *N. arthritica*, significant correlations between shell length and number of striae led the authors to consider these growth marks as a robust age indicator in this species (Miranda et al. 2008).

In *H. trunculus*, despite the high inter-individual variability and overlap in the number of both opercular growth marks at a determined specimen size, counting adventitious layers in acetate peels helped decreasing the variability. Overall, this variation in the number of striae and layers in the operculum reflects the inter-individual variability in *H. trunculus* growth rates, which occurs since the early life-stages (during embryonic and hatchling development) and extends throughout the juvenile and adult phases (Vasconcelos et al. 2004, 2006a; Lahbib et al. 2008, 2010). Likewise, high variability in the size of individuals with the same number of striae (great overlaps in length-at-age) was also observed in other gastropod species, such as *Buccinum undatum* (Kideys 1996) *B. isaotakii* (Ilano et al. 2004) and *N. arthritica* (Miranda et al. 2008).

Figure 5. Linear regressions established between shell length (SL) and number of surface striae (SS) in opercula of *Hexaplex trunculus* from Ria Formosa Lagoon, Bizerte Lagoon and aquaculture (1, 2 and 3 years old). Horizontal bars represent mean SL at each surface stria.
some growth marks on the operculum surface had no continuity in its internal structure and therefore might represent false rings. In gastropod opercula, concentric lines corresponding to narrow areas of slow growth might occur during short-term unfavourable environmental conditions. In general, seasonal growth marks differ from irregularly formed marks by their greater extension, contrast and definite formation. Nevertheless, false rings might be difficult to discern in both extremities of the operculum, because of problems related both to erosion (mainly in the siphonal edge, near the nucleus) and deposition of striae close to each other (particularly in the sutural edge). Some discrepancies between the number of striae and layers were possibly due to inexact cut of the operculum to

![Figure 6](image1.png)

**Figure 6.** Linear regressions established between shell length (SL) and number of adventitious layers (AL) in opercula of *Hexaplex trunculus* from Ria Formosa Lagoon, Bizerte Lagoon and aquaculture (1, 2 and 3 years old). Horizontal bars represent mean SL at each adventitious layer.

![Figure 7](image2.png)

**Figure 7.** Comparison between the number of surface striae and adventitious layers in opercula of *Hexaplex trunculus* from Ria Formosa Lagoon, Bizerte Lagoon and aquaculture (1, 2 and 3 years old). The $X = Y$ line indicates equal number of surface striae and adventitious layers.
prepare the acetate peels. In fact, the cut is critical (especially in the smallest opercula) and must pass across the operculum at the proper angle relative to the origin. Otherwise, the asymmetrical growth pattern of the operculum might cause miscounting of the earlier growth marks (near the nucleus) (Kraeuter et al. 1989). Moreover, whenever the acetate peel technique does not disclose the nucleus, it is virtually impossible to ascertain how many adventitious layers were lost in sectioning the operculum and/or revelation of the acetate peel replica. Overall, these facts revealed that counting striae might overestimate the age of *H. trunculus*. Similarly, counting striae on the operculum of *Neptunea antiqua* was also a subjective method, because their clarity was equivocal and thus could not be counted with any certainty (Richardson et al. 2005). In *N. antiqua*, counting adventitious layers noticeably underestimated the age of one individual, which was explained by analysing reared individuals. In contrast, other species display an annual periodicity in the formation of striae (age is read as \( n_{SS} \) years): *Megastraëa undosa* (Cupul-Magaña & Torres-Moye 1996; Gluyas-Millañ et al. 2000), *Buccinum isostakii* (Ilano et al. 2004), *Buccinum undatum* (Santarelli & Gros 1985; Valentinsson et al. 1999; Shelmerdine et al. 2007), *Busycon carica* (Kraeuter et al. 1989), *Coralliphila abbreviata* (Johnston & Miller 2007), *Coralliphila violacea* (Chen & Soong 2002), *Neptunea antiqua* (Power & Keegan 2001), *Neptunea arthritica* (Miranda et al. 2008), and *Nucella heyseana* (Selin 2003).

Marginal growth of *H. trunculus* opercula displayed an evident monthly variation, but without a clearly discernible seasonal pattern, except for a marked decrease between March and May followed by a period with higher marginal growth during early summer. The first phase coincides with the spawning period and the second corresponds to the resting period in the reproductive cycle of *H. trunculus* in

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**Figure 8.** Monthly variation in marginal growth (MG) and marginal growth ratio (MGR) in opercula of *Hexaplex trunculus* (size class of 40–60 mm SL) from Ria Formosa Lagoon and Bizerte Lagoon. Horizontal bars represent mean MGR.
both Ria Formosa Lagoon (Vasconcelos et al. 2004, 2008b, c) and Bizerte Lagoon (Lahbib et al. 2004, 2009; Gharsallah et al. 2010). These trends suggest a shift in the energy allocation between reproductive effort and growth (somatic, shell and operculum growth). Similar findings were reported for *N. arthritica*, whose opercula showed a slow increase in marginal growth during the reproductive season, then increased faster in summer and decreased again in winter. This seasonal variation in marginal growth was attributed to the fact that during the period of copulation and egg deposition, individuals are more active and spend more energy for reproduction than for somatic growth (Miranda et al. 2008).

Operculum marginal growth was slightly different between populations, with *H. trunculus* from Portugal presenting higher monthly variation and lower values of MG and MGR than *H. trunculus* from Tunisia. This revealed that *H. trunculus* from Ria Formosa Lagoon deposited more and narrower striae per year than *H. trunculus* from Bizerte Lagoon, eventually reflecting contrasting environmental conditions (namely seawater temperature) in Portuguese and Tunisian lagoons. Indeed, seawater temperature in Ria Formosa Lagoon (range: 12 – 27°C) (Newton & Mudge 2003) is slightly lower than in Bizerte Lagoon (range: 11.5 – 29.5°C) (Béjaoui et al. 2008), particularly during summer. These differences might also be due to genetic differentiation of *H. trunculus* throughout its distributional range, promoted by the considerable distance (over 1500 km) between these coastal lagoons. Moreover, monthly variation in marginal growth might also be due to asynchronous rhythm of opercular deposition within *H. trunculus* populations, influenced by factors as feeding activity, food availability and nutritional value of the food supply. For instance, the deposition of yearly striae in *B. undatum* displays a certain temporal variability (Santarelli & Gros 1985). On the contrary, non-overlapping average operculum length between successive year classes indicates low variation in individual growth of *M. undosa* (Cupul-Magaña & Torres-Moye 1996). Finally, as both populations of *H. trunculus* included specimens of both sexes, monthly variation in marginal growth could have also been influenced by differential growth between sexes and by the presence of individuals at different stages of sexual maturation. For example, *N. arthritica* mature females presented lower marginal growth than immature females, because growth slows down after reaching sexual maturity (Miranda et al. 2008).

### Constraints of age estimation based on operculum growth marks

Both ageing methods (counting surface striae or adventitious layers) have advantages and disadvantages. Counting striae is sometimes a fairly subjective method. In general, interpretation of striae is easier...
in younger specimens, whereas older individuals frequently have eroded or damaged opercula, with smoothed and poorly visible growth marks (e.g. Figure 1C), which makes counting striae increasingly difficult. Counting layers in acetate peel replicas of sectioned opercula is more time-consuming, but a less subjective method. In general, acetate peels reveal a clear pattern of growth lines and easily countable layers, particularly in younger individuals. Nevertheless, sometimes the operculum margin is folded and contains material enclosed between the layers (e.g. Figure 3E), and sectioning often leads to the opening of the operculum edge, altogether making the detection and counting of layers more difficult (Richardson et al. 2005). Besides, some opercula display discontinuities in the layers that result from the repair of damage caused by unsuccessful predation or fishing impacts.

In Hexaplex trunculus, the readability of the striae was somewhat equivocal and could not be counted with a great deal of certainty. The acetate peels displayed a clearer pattern of layers, which could be counted more easily and presented lower inter-individual variability and overlap. However, in both ageing methods, considerable variation in the number of counts at a determined shell length indicates that operculum growth marks should be interpreted cautiously because they might not correspond accurately with individual age. Because of the subjectivity inherent to either method, counting striae and layers in opercula only provides rough age estimates of H. trunculus, inadequate to employ in classic growth models. In addition, despite the effort to sample small individuals, opercula with very few striae and layers were quite scarce. For these reasons, fitting the von Bertalanffy growth function to opercular data invariably produced unrealistic estimates of growth parameters (data not shown). Underestimations of the growth coefficient \( K \) and overestimations of the asymptotic shell length \( L_\infty \) were evident, namely when compared with the length-at-age key obtained from mark-recaptured individuals (Vasconcelos et al. 2006a) and corroborated with reared individuals (mean SL from mark-recapture and aquaculture: 1-year-old = 29.0 and 26.8 mm; 2-years-old = 47.0 and 47.3 mm; 3-years-old = 59.1 and 61.0 mm).

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