Growth of the purple dye murex, *Bolinus brandaris* (Gastropoda: Muricidae), marked and released in a semi-intensive fish culture earthen pond

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SUMMARY: The present study reports the growth rate of the purple dye murex, *Bolinus brandaris* (Gastropoda: Muricidae), estimated from mark-recapture experiments. A total of 1067 specimens (shell length = 43.4±8.1 mm, range = 14.6−78.4 mm) were marked with Dymo® tape tags and released in a semi-intensive fish culture earthen pond. After a period at liberty ranging from almost two months to around two years, 288 individuals were recaptured (shell length = 67.4±6.2 mm, range = 45.3−88.6 mm), which corresponded to a recapture rate of 27.0%. At recapture, only one specimen had lost the tag (tag loss rate <0.1%) and all remaining tags were intact and legible. Mean monthly growth rates were 0.9±1.0 mm in shell length, 0.4±0.5 mm in shell width and 0.7±0.7 g in total weight. Growth rates showed high inter-individual variability and an evident decreasing trend with specimen size. Comparison of growth rates with similar information available for other muricids confirmed that *B. brandaris* is a relatively slow-growing species. This provides valuable information for both fisheries management and for assessing the potential of *B. brandaris* as a candidate species for molluscan aquaculture.

Keywords: purple dye murex, *Bolinus brandaris*, Gastropoda, Muricidae, mark-recapture, growth rate.

INTRODUCTION

Muricidae is a diverse gastropod family comprising between 1150 (Vokes 1996) and 1300 species distributed worldwide (Houart 2001). The purple dye murex, *Bolinus brandaris* (Linnaeus, 1758), is a common muricid throughout the Mediterranean Sea, whereas its distributional range in the Atlantic Ocean is mainly restricted to Portugal and Morocco (Poppe and Goto 1991, Houart 2001). Nowadays, *B. branda-
ris also occurs in some areas of Galicia (NW Spain), probably as a consequence of accidental introduction of juveniles together with bivalves imported for culture (Bañón et al. 2008). This species generally occurs in the sub-littoral (Dalla Via and Tappeiner 1981), but can also be found at 100-200 m depth (Macedo et al. 1999, Muzzavor and Morenito 1999), inhabiting sandy, sandy-muddy or muddy bottoms (Macedo et al. 1999, Muzzavor and Morenito 1999, Anon. 2001).

Marine molluscs are among the most important invertebrate fisheries in the world and gastropods represent around 2% of the marine molluscs fished worldwide (Leiva and Castilla 2002). Some gastropod species have a high commercial value in international markets and play an important socio-economical role in small-scale fisheries. The purple dye murex is fished for human consumption in France (Bartolome 1985), Italy (Cecalupo et al. 2006), Spain (Martín et al. 1995, Anon. 2001, Mallol et al. 2004), Portugal (Muzzavor and Morenito 1999, Vasconcelos et al. 2008), and occasionally in Turkey (Ramón and Flos 2001). In Portugal, B. brandaris is commercially exploited along the Algarve coast, mainly in the Ria Formosa lagoon, where it is greatly appreciated. There is therefore great demand for it and it has a high commercial value in the seafood market (reaching 20-25 € kg⁻¹ for first sale) (Vasconcelos et al. 2008).

As with other fishing resources, the sustainable management of commercially exploited gastropod species and rational decision-making by fisheries biologists require reliable data on key aspects of the species biology, including information on growth and age. In addition, the culture of some muricids is attracting much attention in several regions (Ramón and Flos 2001). The increasing demand and high commercial value of B. brandaris in the seafood market has generated expectations about its potential as a candidate for molluscan aquaculture, which requires information on the growth features of this species. Previous studies on the biology of B. brandaris have mainly focused on the reproductive cycle, ultrastructural analyses of spermatogenesis and oogenesis (e.g. Ramón and Amor 2002 and references therein), and imposex (e.g. Vasconcelos et al. 2010 and references therein). To our best knowledge, the only information available on the growth of B. brandaris was obtained from spawns collected in the Mediterranean, whose hatchlings were reared in the laboratory and monitored during a few months (Ramón and Flos 2002). Marking methods have long been used in many fields of biology, namely in studies of evolutionary biology, behaviour, conservation biology and population management (Henry and Jarne 2007). However, although they have an external shell on which marks or tags can be attached with little or no adverse effects (Gosselin 1993), mark-recapture studies with gastropods are not very frequent (Henry and Jarne 2007), including for estimating growth (e.g. Appeldoorn 1988, Eversole and Anderson 1988, Kraeuter et al. 1989, Castagna and Kraeutner 1994, Haaker et al. 1998, Vasconcelos et al. 2006, Rogers-Bennett et al. 2007). Diverse techniques are considered suitable for marking gastropods (Hancock and Urquhart 1959, Nielsen 1992, Gosselin 1993, Stewart and Creese 2000, Crowe et al. 2001, Henry and Jarne 2007 and references therein), but probably the most common and reliable method for long-term studies is gluing tags to the shell (Henry and Jarne 2007), a technique that has been successfully employed for several gastropod species (Eversole and Anderson 1988, Kraeuter et al. 1989, Debrot 1990, McShane and Smith 1992, Treble et al. 1993, Castell et al. 1996, Stewart and Creese 2000, Amos and Purcell 2003, Vasconcelos et al. 2006).

The present study reports the growth rate of B. brandaris estimated through mark-recapture experiments in a semi-intensive fish culture earthen pond in the Ria Formosa lagoon (Algarve coast - southern Portugal). The main advantages and disadvantages of the marking technique (plastic tags glued to the shell) are briefly discussed and the growth rate of B. brandaris is compared with that of other muricids (commercially or potentially valuable species, either for fisheries or aquaculture).

**MATERIALS AND METHODS**

### Sampling

The purple dye murexes were gathered during experimental fishing surveys performed monthly during one year (July 2005 - June 2006) in the vicinities of Culatra Island (Ria Formosa lagoon - southern Portugal) (Fig. 1). This area is characterised by narrow shallow channels (2-3 m depth), mainly with muddy bottoms and seagrass coverage (namely Zostera spp.). Specimens were caught using an artisanal fishing gear locally called a “wallet-line”, which is non size-selective and thus catches B. brandaris with a broad size range (for further details see Vasconcelos et al. 2008).

After collection, individuals were transported to the laboratory and maintained in aquaria with aerated seawater. Specimens damaged during fishing and handling procedures (mainly with a broken siphonal canal) were discarded. Intact specimens selected for marking were cleaned with a hard brush to remove colonising algae and/or epibionts (mostly polychaetes) from the shells.

### Marking

A total of 1067 B. brandaris with broad size and weight ranges were marked (Table 1). Specimens were marked with tags of Dymo® plastic tape. Tags of five different colours (black, blue, red, green and yellow) and with two alphanumeric characters were prepared so that a large number of individuals could be tagged. Tags were cut with a size of approximately 6 mm length x 4 mm width, which is a suitable size for marking even the smallest B. brandaris.
the Ria Formosa lagoon and resembles the surrounding earthen pond at the IPIMAR’s Fish Culture Experimental Station (Fig. 1), which receives water directly from the Ria Formosa lagoon and resembles the surrounding environment in terms of sediment type and water quality. To maximize the recapture rate, the earthen pond was surrounded with a plastic net fence (area ≈ 100 m²; maximum depth ≈ 2 m; mesh size ≈ 2 cm). To avoid predation and marked specimens escaping through the fence, the base was buried in the bottom sediment (≈ 20 cm) and the top was above seawater level (≈ 20 cm). At the end of the release operations (June 2006), the cumulative stocking density of marked specimens reached 10.7 ind m⁻² (not accounting for natural mortality that might have occurred in the meantime) (Table 1).

Release and recapture

Marked specimens were released monthly (from July 2005 to June 2006) in a semi-intensive fish culture earthen pond at the IPIMAR’s Fish Culture Experimental Station (Fig. 1), which receives water directly from the Ria Formosa lagoon and resembles the surrounding

Table 1. – Data on the release and successful recapture operations of marked Bolinus brandaris, including number (N), shell length (SL), shell width (SW), total weight (TW), stocking density and recapture method (snorkelling - S and/or “wallet-line” - WL). Data presented as means±SD and range (minimum-maximum).

<table>
<thead>
<tr>
<th>Date</th>
<th>N</th>
<th>SL (mm)</th>
<th>SW (mm)</th>
<th>TW (g)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul-05</td>
<td>87</td>
<td>45.4±8.1 (29.7-77.8)</td>
<td>22.8±4.1 (14.4-36.4)</td>
<td>5.5±3.2 (1.4-22.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>Aug</td>
<td>103</td>
<td>46.8±9.7 (26.0-77.9)</td>
<td>23.8±5.5 (12.1-43.5)</td>
<td>6.6±5.2 (0.9-30.8)</td>
<td>1.9</td>
</tr>
<tr>
<td>Sep</td>
<td>75</td>
<td>43.9±8.9 (31.7-70.2)</td>
<td>22.0±4.7 (16.6-37.3)</td>
<td>5.2±4.2 (2.1-20.9)</td>
<td>2.7</td>
</tr>
<tr>
<td>Oct</td>
<td>97</td>
<td>41.2±5.7 (31.2-63.2)</td>
<td>20.4±2.9 (15.1-31.5)</td>
<td>3.8±1.7 (1.5-11.9)</td>
<td>3.6</td>
</tr>
<tr>
<td>Nov</td>
<td>87</td>
<td>51.0±11.1 (30.5-76.6)</td>
<td>25.7±5.6 (15.4-38.2)</td>
<td>7.5±4.6 (1.7-19.7)</td>
<td>4.5</td>
</tr>
<tr>
<td>Dec</td>
<td>91</td>
<td>45.9±7.0 (36.0-74.2)</td>
<td>22.7±3.3 (17.2-33.9)</td>
<td>5.3±2.9 (2.4-17.3)</td>
<td>5.4</td>
</tr>
<tr>
<td>Jan-06</td>
<td>78</td>
<td>43.7±4.7 (35.9-60.6)</td>
<td>22.0±2.4 (17.8-30.5)</td>
<td>4.5±1.5 (2.4-10.3)</td>
<td>6.2</td>
</tr>
<tr>
<td>Feb</td>
<td>94</td>
<td>40.1±9.4 (14.6-78.4)</td>
<td>20.1±6.6 (6.6-38.1)</td>
<td>4.0±3.6 (0.2-22.7)</td>
<td>7.1</td>
</tr>
<tr>
<td>Mar</td>
<td>101</td>
<td>41.7±4.7 (26.1-49.3)</td>
<td>20.7±2.6 (11.6-24.6)</td>
<td>3.9±1.1 (0.7-6.0)</td>
<td>8.1</td>
</tr>
<tr>
<td>Apr</td>
<td>97</td>
<td>40.5±4.2 (28.5-48.9)</td>
<td>20.6±2.1 (14.0-25.1)</td>
<td>3.8±1.0 (1.3-6.5)</td>
<td>9.1</td>
</tr>
<tr>
<td>May</td>
<td>69</td>
<td>41.6±4.0 (27.6-48.5)</td>
<td>21.4±2.3 (12.4-27.7)</td>
<td>4.3±1.1 (0.9-6.5)</td>
<td>9.8</td>
</tr>
<tr>
<td>Jun</td>
<td>88</td>
<td>38.9±6.1 (23.2-49.3)</td>
<td>19.7±3.3 (11.0-25.4)</td>
<td>3.5±1.5 (0.7-6.6)</td>
<td>10.7</td>
</tr>
<tr>
<td>Total</td>
<td>1067</td>
<td>43.4±8.1 (14.6-78.4)</td>
<td>21.8±4.2 (6.6-43.5)</td>
<td>4.8±3.2 (0.2-30.8)</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Prior to marking, shells were thoroughly dried with absorbent paper to facilitate the adherence of the glue. Since B. brandaris burrows shallowly into soft substrata with the siphonal canal pointing towards the sediment surface (P. Vasconcelos, pers. observ.), tags were adhered to the shell surface with cyanoacrylate glue (to minimise losing the tags) and covered with epoxy glue (to reduce abrasion and avoid fouling). Small amounts of both glues were applied to the shell, i.e. just enough glue to adhere and cover the tag completely. Tags were invariably adhered to the penultimate growth band of the last whorl of the shell (termed “tag position 2”) (Fig. 2b), which helps to prevent spilling glue onto the soft body. After drying for 30 to 60 minutes, marked individuals were rinsed in seawater to remove glue residues and kept overnight in aquaria. This tagging technique has already been employed successfully in previous mark-recapture experiments with Hexaplex trunculus, a sympatric muricid in the Ria Formosa lagoon (for further details see Vasconcelos et al. 2006).
The purple dye murex is a generalist carnivore, which feeds mainly on bivalves, gastropods and barnacles. It supplements its diet through scavenging and cannibalism (Anon. 2001, Ramón and Flos 2001). Therefore, marked specimens were regularly provided with live bivalves (cockles, *Cerastoderma edule*, and mussels, *Mytilus galloprovincialis*) that are among their principal prey in the Ria Formosa lagoon. In addition, the natural colonisation and maturation of the earthen pond, which had been filled months before the beginning of the mark-recapture experiments, provided a diverse food supply for *B. brandaris* (ascidians, barnacles, bivalves, other gastropods, etc.), which was confirmed periodically with snorkelling. Potential predators of *B. brandaris* (e.g. cephalopods, fish and limicoline birds) or competitors for space and food (e.g. the muricids *H. trunculus* and *Stramonita haemastoma*) were not detected inside the fenced area of the earthen pond.

Recaptures were performed mainly by hand collection during snorkelling, but whenever the underwater visibility did not allow marked specimens to be detected, *B. brandaris* were caught using the “wallet-line”. In general, snorkelling was used more often in the warmest months and the “wallet-line” was mainly used in the coldest months. In order to allow for multiple recaptures of the same individual, all recaptured specimens were returned to the earthen pond after sampling. During the study period, seawater temperature and dissolved oxygen in the earthen pond were monitored daily with a multiparameter sonde. The condition of the fence was periodically inspected during snorkelling to detect damage that could allow specimens to escape, and at the same time, shells of dead specimens were collected and inspected for any signs of predation attempts.

**Growth rates**

Both at marking and recapture, specimens were measured for shell length (SL, from the apex to the tip of the siphonal canal) and shell width (SW, width of the last whorl or body whorl) with a digital calliper (precision of 0.01 mm) (Fig. 2A), and weighed for total...
weight (TW) with a top-loading digital balance (precision of 0.01 g). Before weighing, individuals were inspected for shell damage and epibiotic colonisation, and were slightly blotted dry to drain water from the mantle cavity. The position of the tag on the shell surface (determined as the number of growth bands between the tag and the shell aperture) was registered to ascertain the shell deposition during the period at liberty (tag position at recapture vs tag position at marking, i.e. tag position n+2 – tag position 2) (Figs. 2B-D).

Monthly growth rates in SL, SW, TW and number of deposited growth bands were calculated with the following equation:

\[ GR = \frac{(S_r - S_m)}{(t_r - t_m)} \times 30 \]

where \( GR \) is the monthly growth rate (unit / month), \( S_r \) is the size at recapture (SL, SW, TW or tag position), \( S_m \) is the size at marking (SL, SW, TW or tag position), \( t_r \) is the date of recapture, and \( t_m \) is the date of marking.

In the case of multiple recaptures (marked specimens caught more than once), the size increment (SL, SW, TW or tag position) was derived from the period at liberty between consecutive recaptures (i.e. the growth between the previous and the following recapture).

**Statistical analysis**

Analyses of variance (ANOVA) were employed to compare the mean size (SL) of marked and recaptured *B. brandaris* and to compare monthly mean growth rates between size classes (10 mm SL classes). Whenever ANOVA assumptions (normality of the data and homogeneity of variances) were not met, a non-parametric ANOVA on ranks (Kruskal-Wallis test) was performed. Each time ANOVA or Kruskal-Wallis tests detected significant differences among groups, pairwise multiple comparisons were made using Tukey or Dunn post hoc tests (Zar 1999).

The relationships between specimen size at marking (grouped in 10 mm SL classes) and the monthly mean growth rates (in SL, SW, TW and shell deposition) were investigated through regression analysis (least squares method). The linear function \( Y=a+bX \) was fitted to raw data and the degree of association between variables was assessed through the correlation coefficient \( r \). Statistical analyses were performed using the software package SigmaStat\textsuperscript{\textregistered} (version 3.5) and statistical significance was considered for \( p<0.05 \).

**RESULTS**

**Environmental parameters**

Monitoring of environmental parameters in the earthen pond revealed a mean seawater temperature of 20.3±4.7ºC during the whole study period, ranging between 12.9±1.1ºC in January 2006 and 26.7±1.2ºC in July 2005 (Fig. 3A). The lowest and highest temperatures were recorded in January 2007 (9.6ºC) and in June 2006 (29.3ºC) respectively. Mean dissolved oxygen (7.4±1.4 mg l\(^{-1}\)) was within acceptable levels, ranging from 5.7±1.5 mg l\(^{-1}\) in August 2005 to 8.3±1.1 mg l\(^{-1}\) in February 2006 (Fig. 3B). During summer, high seawater temperatures and the consequent low levels of dissolved oxygen meant that floating aerators needed to be used frequently in the earthen pond.

**Recapture rate**

From a total of 24 monthly recapture operations only 14 were successful, either through snorkelling (7), using the “wallet-line” (5) or with both recapture methods (2) (Table 1). After a mean period at liberty of 314±291 days, ranging from almost two months (53 days) to around two years (729 days), a total of 288 marked individuals were recaptured (67.4±6.2 mm SL, range = 45.3-88.6 mm SL) (Table 1). The overall recapture rate was 27.0%, with 113 marked specimens recaptured once (39.2%), 81 caught twice (28.1%), 78 recaptured three times (27.1%), 14 caught four times (4.9%) and only 2 recaptured five times (0.7%).

Highly significant differences (K-W: \( H=770.327, p<0.001 \)) were detected between the mean size of all the specimens released and the mean size at marking of recaptured specimens. Indeed, the size of the individuals released (43.4±8.1 mm SL) was significantly smaller (Dunn test: \( Q=16.724, p<0.05 \)) than the size at marking of the recaptured individuals (58.4±12.4 mm SL) (Fig. 4A). Moreover, the size-frequency distribution of the marked and recaptured specimens confirmed that most of the smaller marked specimens were not recaptured (especially individuals below 40 mm SL, which represented less than 10% of the total recaptures) (Fig. 4B).
Mortality and tag loss rates

The shells of 16 dead *B. brandaris* (44.2±7.0 mm SL, range = 31.2-63.3 mm SL) were collected by snorkelling during the entire study period, corresponding to an overall mortality rate of 1.5%. These shells were intact and without any signs of predation, namely drilling attempts caused by cannibalism among conspecifics.

Among all specimens recaptured (after a maximum period at liberty of around two years), only one had lost the tag (tag loss rate <0.1%). The vast majority of the tags were intact and with the printed characters easily readable without magnification, but in a few cases the cover of epoxy became cloudy (particularly after long periods of immersion), so that it was necessary to scrape the surface of the glue or even remove the epoxy cover from the tag to improve legibility.

Growth rates

The SL, SW and TW of marked-recaptured *B. brandaris* showed a pronounced increase between marking and recapture (Table 1). Highly significant differences (K-W: *H*=770.327, *p*<0.001) were detected in the mean size of recaptured specimens during the period at liberty. This reflects significant growth (Dunn test: *Q*=6.753, *p*<0.05), from 58.4±12.4 mm SL (range = 26.0-77.9 mm SL) at release to 67.4±6.2 mm SL (range = 45.3-88.6 mm SL) at recapture (Fig. 4A), accompanied by progression in the size-frequency distribution towards greater shell lengths (Fig. 4B).

Mean growth rates of *B. brandaris* obtained through mark-recapture were 0.9±1.0 mm SL / month (range: 0.0-6.3 mm SL), 0.4±0.5 mm SW / month (range: 0.0-2.7 mm SW) and 0.7±0.7 g TW / month (range: 0.8-4.5 g TW). On average, individuals deposited 2.3±3.2 shell bands along the body whorl per year (range: 0.0-16.4 growth bands). Monthly growth rates showed high inter-individual variability, independently of the parameter considered for estimating growth (SL, SW, TW or number of deposited shell bands). Furthermore, several marked specimens did not grow between marking and recapture, and some individuals even lost weight during the period at liberty.

Due to these highly variable individual growth rates, specimens were grouped into size classes (10 mm SL) in order to establish eventual relationships between the original size (SL at marking) and mean growth in SL, SW, TW and shell deposition (Fig. 5). Highly significant decreasing trends were detected for growth in SL (*r*=0.973, *p*<0.005), SW (*r*=0.976, *p*<0.004) and shell deposition (*r*=0.972, *p*<0.005) (Figs. 5A,B,D), but not for growth in TW (*r*=0.270, *p*>0.05) (Fig. 5C). The Kruskal-Wallis test detected highly significant differences in growth rates between size classes for all variables considered: SL (*H*=47.919, *p*<0.001), SW (*H*=184.102, *p*<0.001), TW (*H*=25.696, *p*<0.001) and shell deposition (*H*=148.294, *p*<0.001). Pairwise multiple comparison revealed several significant differences in mean growth rates between size classes (Dunn test, *p*<0.05), which occurred mostly between smaller and larger size classes (especially below and above 50-60 mm SL). A decreasing trend in growth rates between size classes (growth slowdown during ontogeny) was also evident for growth in SL, SW and shell deposition (Figs. 5A,B,D), but not for growth in TW (Fig. 5C).

**DISCUSSION**

**Advantages and disadvantages of the marking technique**

A recent review that included a comparison of techniques for marking gastropods (Henry and Jarne...
2007) concluded that glued marks are ideal and the most common technique for individual marking. The present study confirmed that Dymo® tape tags adhered with cyanoacrylate glue and covered with epoxy glue are an appropriate and reliable method for marking *Bolinus brandaris*. This marking technique is inexpensive, but requires some time to prepare the tags (manually engrave the characters and cut the margins of the tags). The tags were suitable for marking fairly small *B. brandaris* (around 15 mm SL), and could be used to mark even smaller specimens. The marks were simple to apply.
and the glue bonded strongly and quickly between tags and shells. The epoxy cover took a little longer to harden, but marked specimens were ready to return to the aquaria within 30 minutes to one hour. Although inconspicuous, tags exhibited excellent visibility and the epoxy cover protected the tags from fouling. In some specimens the visibility of the tags was hampered because they became partially or totally covered by shell deposition during growth, either enclosed in the suture of the shell spire (Fig. 2D) or covered by the development of the inner lip (labrum columnar) of the shell aperture (Figs. 2E,F).

Tags were resistant, durable, and maintained a good legibility (the engraved characters were easily readable two years after marking). Most marked specimens were readily identified at recapture and only a few tags had become difficult to read (whenever the epoxy cover became opaque after long periods of immersion). The purple dye murex is fairly resistant to handling and quickly withdraws into the shell during aerial exposure to retain seawater inside the mantle cavity and avoid desiccation. Therefore, marking did not cause immediate post-tagging mortality and apparently had no harmful effects on *B. brandaris* behaviour (marked specimens resumed crawling within a few minutes after returning to the aquaria). Moreover, due to the negligible size and weight of the tags, which certainly did not impair movement or energy expenditure, this marking technique presumably had no effects on the main life-history traits of *B. brandaris* (survival, burrowing ability, growth and condition).

**Recapture rate**

The present recapture rate (27.0%) can be considered high compared to values generally reported in the literature. However, considering that marked *B. brandaris* were released in a confined environment virtually without potential predators, the recapture rate was unexpectedly low, especially for smaller specimens. Underwater censuses, particularly in conditions of low-visibility, might affect the detection of marked specimens and thus lower the recapture rate (Bell *et al.* 2005). In the present case, the recapture rate during snorkelling might have been affected by the characteristics of the bottom in the earthen pond (muddy sediments that reduced underwater visibility), by the burrowing behaviour of *B. brandaris*, and by the use of inconspicuous tags. Indeed, individuals were frequently found buried shallowly and in some cases were only detected by the siphonal canal pointing out of the surface of the sediment.

Besides affecting the recapture rate, these difficulties probably also biased the size of recaptures, because smaller individuals are less conspicuous, especially with low underwater visibility. In fact, a usual source of variation in capture probability is size: the smaller the individual, the lower the capture probability (Catchpole *et al.* 2001, Henry and Jarne 2007). To overcome this problem and mitigate size bias, supplementary recaptures were made with the “wallet-line”, but the smallest specimens were still almost absent from the catches. Another explanation for this scarcity of small specimens could be competition for food between smaller and bigger individuals, or even mortality by cannibalism among conspecifics, with bigger individuals attacking smaller ones. However, although cannibalism has been observed in *B. brandaris* after long periods of starvation in captivity (P. Vasconcelos, pers. observ.), the few shells of dead specimens found did not show drilling attempts.

**Mortality and tag loss rates**

The mortality rate registered during the study period was fairly low (1.5%) and the 16 shells of dead *B. brandaris* belonged both to small and big specimens, presumably due to natural mortality. However, the total mortality rate is certainly underestimated because the sedimentation in the earthen pond soon covered the shells, making it difficult to collect dead specimens during snorkelling. Henry and Jarne (2007) compared five techniques for marking gastropods and concluded that glued plastic marks were the most resistant in terms of tag loss rate. Our results corroborate this conclusion, since the tag loss rate was quite low (<0.1%, i.e. <0.001) compared to the tag loss rate of 0.01 to 0.1 that is usually expected for most marking techniques in gastropods (Henry and Jarne 2007). For instance, in mark-recapture studies employing glued plastic or metal tags, the following monthly tag loss rates were reported: no tag loss in *Busycon carica* (Kraeuter *et al.* 1989), 0.01 in *Austrovenus stuchburyi* (Stewart and Creese 2000), 0.01 in *Physa acuta* (Henry and Jarne 2007), 0.02 in *Haliotis rubra* (McShane and Smith 1992) and 0.05 in *Cittarium pica* (Debrot 1990).

The present tag loss rate corresponds to a maximum period at liberty of around two years and is likely to increase in the long-term. Tag loss rate may change over time (Henry and Jarne 2007), like with *Cellana tramoserica* marked with glued plastic tags, whose tag loss rate increased from 0.001 after 74 days to 0.1 after 279 days at liberty (Treble *et al.* 1993). This means that whenever the tag loss rate is measured during a short period and increases over time, extrapolation to longer periods leads to under-estimation of the tag loss rate (Treble *et al.* 1993). Studies with gastropods marked with glued plastic tags reported maximum tag retention of ≈2 years in *H. rubra* (Worthington *et al.* 1995), ≈7 years in *Busycon carica* (Kraeuter *et al.* 1989) and ≈11 years in *H. rubra* (McShane and Smith 1992).

**Growth rate**

A typical muricid grows 1-2 mm in shell length per month (Spight *et al.* 1974). Accordingly, the present monthly growth rates (0.9±1.0 mm SL, 0.4±0.5 mm SW and 0.7±0.7 g TW) indicate that *B. brandaris* is
a relatively slow-growing muricid. However, growth rates could have been influenced by high stocking density of marked specimens (reaching 10.7 ind m$^{-2}$), as it was impossible to ascertain if the benthic communities in the earthen pond (i.e., food availability) remained stable throughout the study period. To the authors’ best knowledge, there is no specific information on the density of B. brandaris populations in the wild, although very high densities are reported to occur in the reproductive season, during breeding aggregations and collective spawning (Martin et al. 1995, Mallol et al. 2004, Vasconcelos et al. 2008). Moreover, growth rates might be slightly underestimated, since recaptures were dominated by adults (presumably in the phase of slower growth), a bias that can only be attenuated by correcting for size dependency in capture probability (Catchpole et al. 2001, Henry and Jarne 2007). For this reason, these growth estimates should be interpreted as mean growth rates for the size range of the recaptures, and any extrapolation to smaller sizes (e.g., juveniles) would be problematic and inaccurate. Finally, size-biased data towards larger sizes make using graphical methods (Gulland-Holt plot) for estimating the von Bertalanffy growth parameters ($K$ and $L_{\infty}$) inappropriate and imprecise.

It is well established that muricids exhibit highly variable growth patterns and the growth rates obtained in this study are a confirmation of this phenomenon. In B. brandaris, variable growth occurs since early developmental stages and is further accentuated during ontogeny. Firstly, this species has intra-capular development (Barash and Zenziper 1980, D’Asaro 1991) and larvae/embryos feed on nurse-eggs inside the oothecas (egg-capsules) (Ramón and Flos 2001), a development mode that contributes to highly variable sizes at hatching. Moreover, in the present study several specimens simply did not grow (either in SL, SW, TW or shell deposition) and some lost weight between marking and recapture (even during long periods at liberty). Similar trends were reported for a symetrically and phylogenetically closely related muricid (H. trunculus) (Leitão et al. 2009), namely hatchlings with highly variable sizes (Vasconcelos et al. 2004) and great inter-individual variability in growth, both of juveniles and adults (Vasconcelos et al. 2004, 2006). In B. brandaris, individual variability in monthly growth rates was more evident in the total weight gain. This might be due to bias in weighing live gastropods (the difficulty of ensuring the complete removal of water from inside the shells and standardising weighing). Furthermore, somatic weight (and consequently total weight) exhibits seasonal variation throughout the reproductive cycle (Vasconcelos et al. 2009). In addition, a few recaptured specimens showed shell thickening (authors, unpublished data) that might have resulted from imposex, which affects B. brandaris in the study area (Vasconcelos et al. 2010). Some studies detected morphological variations in the gastropod shell resulting from organotin pollution (TBT and TPT) and subsequent imposex (e.g., Son and Hughes 2000, Plejdup et al. 2006, Bigatti and Carranza 2007, Márquez et al. 2011), including shell thickening and increased shell weight (e.g., Cob et al. 2008, Lahbib et al. 2009).

Despite the inter-individual variability in growth of B. brandaris, a declining trend in monthly growth rates during ontogeny was evident, with growth rates decreasing with increasing specimen size. Negative correlations were detected between specimen size at marking and growth rates in SL, SW and shell deposition. In contrast, the relationship between specimen size at marking and the growth rate in TW was not significant, probably due to the reasons mentioned above. The growth of most gastropods decelerates with increasing size / age (Hughes 1986, Fujiinaga 1987, Fuse 1999), and in some cases a significant proportion of adults simply does not grow for long periods (including individuals considerably smaller than the maximum size reached by the species) (Laxton 1970, Fotheringham 1971, Spight et al. 1974, Tallmark 1980, Appeldoorn 1988, Kraeuter et al. 1989). In addition, growth can be interrupted during the breeding activity (Moran et al. 1984). In many gastropods, including several muricids, the growth rate decelerates after reaching the size at sexual maturation and becoming reproductively active (Laxton 1970, Fotheringham 1971, Spight et al. 1974, Tallmark 1980, Fujiinaga 1987, Appeldoorn 1988, Ishida 2004). This makes growth rates highly variable, even among specimens of similar size, because growth stops earlier in some individuals than in others (Laxton 1970). In the plots of growth increment against size at marking, the existence of a significant fraction of individuals that had reached the size at which growth ceases led to a “tail” of large specimens that did not grow anymore, which biased the estimation of growth rates (Moran et al. 1984). Like several other gastropod species, B. brandaris showed episodic growth, i.e. intermittent and indefinite periods of no growth (or even negative growth in TW) interspersed with periods of rapid growth. This growth pattern leads to highly variable and irregular growth rates (Laxton 1970, Kraeuter et al. 1989, Fuse 1999) and creates “stepped” growth curves (Laxton 1970). In B. brandaris kept in aquaria, the deposition of an entire growth band (easily identified by its lighter colouration and fragility) frequently occurs overnight, followed by a long period without any evidence of shell deposition and growth (P. Vasconcelos, pers. observ.).

The comparison of the monthly growth rate of B. brandaris with similar information available for other muricids (commercially or potentially valuable species, either for fisheries or aquaculture) is compiled in Table 2. Comparison of growth rates is complex and should be interpreted carefully because studies have different objectives and experimental designs (field, aquaculture or laboratory). Caution should also be taken whenever growth rates obtained from individuals with different ages and size ranges (early ontogenic stages, juveniles or adults), and with different feeding regimes and diets are compared. The present growth rate ($0.9 \pm 1.0$ mm

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SL/month) was estimated mainly with adults and thus is lower than the only data available until now for *B. brandaris* (4.3 mm SL/month), obtained following the growth of hatchlings and juveniles in the laboratory over seven months (Ramón and Flos 2001). Likewise, this growth rate of *B. brandaris* is quite similar to that of adult *H. trunculus* (1.0±1.0 mm SL/month), estimated through mark-recapture in the Ria Formosa lagoon (Vasconcelos et al. 2006), but lower than that obtained using hatchlings and juveniles born and kept in captivity (Vasconcelos et al. 2004, Lahbib et al. 2008, 2010) (Table 2). Moreover, the growth rate of *B. brandaris* is lower than that reported for most other muricids. However, most of those species attain bigger sizes than *B. brandaris*, and studies were performed mostly in laboratory or aquaculture facilities using early life-stages (post-metamorphic hatchlings) and small juveniles; therefore, growth rates are presumably overestimated considering the overall size ranges in natural populations.

### Implications for fisheries and aquaculture

The present information has implications for both fisheries management and for assessing the potential of *B. brandaris* as a candidate for molluscan aquaculture. The rising economic value of this species in the seafood market has increased the pressure on this fishing resource, with the consequent risk of overfishing (Martín et al. 1995, Vasconcelos et al. 2008). These facts prompted researchers to perform pilot studies aimed at assessing the technical and economical feasibility of rearing *B. brandaris* (Ramón and Flos 2001, Vela and Moreno 2004, Ramón et al. 2005). Mark-recapture data revealed that this is a relatively slow-growing muricid, whose growth decreases during ontogeny, especially after reaching the adult stage. Therefore, and since the “wallet-line” is not a size-selective fishing gear (Vasconcelos et al. 2008), fishermen must adhere to the minimum landing size legally stipulated for *B. brandaris* (65 mm SL) to ensure long-term sustainable exploitation of the resource. In terms of aquaculture, if the hatchling and juvenile growth rate during the first year of life (4.3 mm SL/month) (Ramón and Flos 2001), reaching 41 mm SL in one year (Ramón et al. 2005), is coupled with the present adult growth rate (0.9 mm SL/month), it appears realistic that *B. brandaris* might reach a marketable size within two years. Overall, this corroborates the opinion that *B. brandaris* might constitute a potentially valuable species for aquaculture (Ramón and Flos 2001) and encourages further research into poorly-known aspects of its biology (e.g. spawning, early development and growth), which are crucial for confirming the feasibility and profitability of rearing this muricid on a commercial scale.

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**Table 2.** Comparison of monthly growth rates in shell length (SL) of commercially or potentially valuable muricid species, either for fisheries and/or aquaculture.

<table>
<thead>
<tr>
<th>Species</th>
<th>Origin</th>
<th>Size range (mm SL)</th>
<th>Growth rate (mm SL/month)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bolinus brandaris</em></td>
<td>Ria Formosa (Portugal)</td>
<td>26.0-77.9</td>
<td>0.9±1.0</td>
<td>Present study</td>
</tr>
<tr>
<td><em>Bolinus brandaris</em></td>
<td>Catalunya (Spain) I</td>
<td>&gt;5 juveniles</td>
<td>4.3</td>
<td>Ramón and Flos (2001)</td>
</tr>
<tr>
<td><em>Chicoreus virgineus</em></td>
<td>Cuddalore (India) L</td>
<td>70-85</td>
<td>3.0-5.0</td>
<td>Ramesh et al. (1992)</td>
</tr>
<tr>
<td><em>Chorusa gigantea</em></td>
<td>Chile L</td>
<td>12.7-15.5</td>
<td>2.0-2.5</td>
<td>Gutiérrez and Gallardo (1999)</td>
</tr>
<tr>
<td><em>Concholepas concholepas</em></td>
<td>Las Cruces (Chile) F</td>
<td>11.3±4.6</td>
<td>3.7</td>
<td>Guisado and Castilla (1983)</td>
</tr>
<tr>
<td><em>Concholepas concholepas</em></td>
<td>Chile L</td>
<td>juveniles</td>
<td>4.7</td>
<td>Lara and Montes (1988)</td>
</tr>
<tr>
<td><em>Concholepas concholepas</em></td>
<td>Chile L</td>
<td>5-20 (juveniles)</td>
<td>2.3</td>
<td>Méndez and Cancino (1992)</td>
</tr>
<tr>
<td><em>Concholepas concholepas</em></td>
<td>Las Cruces (Chile) F</td>
<td></td>
<td>3.7-3.9</td>
<td>Marínquez et al. (2008)</td>
</tr>
<tr>
<td><em>Dicathais orbita</em></td>
<td>Australia F</td>
<td>&lt;30 (juveniles)</td>
<td>2.5</td>
<td>Woodcock and Benkendorff (2008)</td>
</tr>
<tr>
<td><em>Hexaplex trunculus</em></td>
<td>Ria Formosa (Portugal) L</td>
<td>20.7-58.4</td>
<td>1.0±1.0</td>
<td>Vasconcelos et al. (2006)</td>
</tr>
<tr>
<td><em>Hexaplex trunculus</em></td>
<td>Bizerte lagoon (Tunisia) L</td>
<td>hatchlings</td>
<td>3.6</td>
<td>Lahbib et al. (2008)</td>
</tr>
<tr>
<td><em>Hexaplex trunculus</em></td>
<td>Bizerte lagoon (Tunisia) L</td>
<td>juveniles</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hatchlings</td>
<td>3.6</td>
<td>Lahbib et al. (2010)</td>
</tr>
</tbody>
</table>

* A, data from aquaculture facilities; F, data from field measurements; L, data from laboratory experiments.


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