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## Reproductive cycle of *Bolinus brandaris* (Gastropoda: Muricidae) in the Gulf of Gabès (southern Tunisia)

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### Abstract

The reproductive cycle of the purple dye murex (*Bolinus brandaris*) from the Gulf of Gabès was studied through gonad histology and calculation of bio-physiological indices (general condition index - K and gonadosomatic index - GSI). The shell length at first sexual maturity (SL<sub>50</sub>) of *B. brandaris* was also investigated using a macroscopic maturation scale. The population's sex ratio was statistically skewed (M:F = 1:1.5), with a significant dominance of females over males. Monthly variations in the maturation stages of gonads, K and GSI showed that *B. brandaris* has an annual reproductive cycle, a long period of gonadal activity and a slight asynchronicity between the sexes. The spawning season comprised the period between April and July, with a clear spawning peak from May to June. The SL<sub>50</sub> was reached at 56.4 mm in females and 54.6 mm in males. Although at present *B. brandaris* has no commercial value in Tunisia, it is predictable to become a fishery-exploited species in the near future. The present data, particularly the timing and duration of the spawning season and the size of individuals of both sexes at first sexual maturity, provide baseline information to propose the early adoption of fishery management measures, aimed at ensuring sustainable exploitation and long-term preservation of this alternative fishing resource, once commercial exploitation of this species commences.

**Keywords:** Purple dye murex, *Bolinus brandaris*, reproductive cycle, size at first sexual maturity, fisheries management, Gulf of Gabès.

### Introduction

Around the world, there are diverse fishery-exploited gastropod species, which number around 720 (Leiva & Castilla, 2002). Latest global fishery production statistics in respect of miscellaneous marine gastropods (abalones, winkles and conchs) total up to about 131 x 10<sup>3</sup> tons whose approximate value is estimated in US\$ 768 million (FAO, 2011). The purple dye murex *Bolinus brandaris* (Linnaeus, 1758), a commercially valuable muricid species, is found on soft bottoms in the Mediterranean Sea and Atlantic Ocean, from the coast of Portugal to Morocco (Poppe & Goto, 1991). This species has been exploited since ancient times, not only as seafood but also for the purple dye used to decorate garments. Today, it is targeted by small-scale fisheries along the coasts of Portugal (Vasconcelos *et al.*, 2008a), Spain (Martín *et al.*, 1995), Italy (Ramón & Amor, 2001) and along the coastline of Greece (Katsanevakis *et al.*, 2011). In Tunisian waters, *B. brandaris* is found in the Bizerte lagoon and in the Gulf of Tunis (northern Tunisia) (e.g. Abidli *et*

*al.*, 2009, 2011), constitutes an important component of the benthic communities in the Gulf of Gabès (southern Tunisia), and is one of the most common shellfish species caught as by-catch by bottom trawlers. Recent fishing surveys in this area (dealing with stock assessment and population structure of the fishing resources) showed that *B. brandaris* is mainly found in the 10–80 m depth range, with 45 and 60 m depth providing the highest catches per unit effort (CPUE = total biomass / hour) (K. Elhasni, unpublished data).

Many different types of studies have been conducted in regard to the purple dye murex, including the fishery and population structure in the Catalan coast (Martín *et al.*, 1995) and in the Ria Formosa lagoon (Vasconcelos *et al.*, 2008a). As regards *B. brandaris*, the most studied topic is imposex (development of male sexual characters onto females of prosobranch gastropods), both in the Atlantic Ocean, specifically in Portugal (e.g. Vasconcelos *et al.*, 2010; 2011), and in the Mediterranean Sea, especially in Spain (e.g. Solé *et al.*, 1998; Morcillo & Porte, 1999; Ramón & Amor, 2001) but also in Italy (Chiavarini

*et al.*, 2003). In the southern Mediterranean, data on the incidence of imposex and the degree of severity in *B. brandaris* were gathered during monitoring surveys conducted along the Moroccan (Lemghich & Benajiba, 2007) and Tunisian coasts, specifically in the Bizerte lagoon and in the Gulf of Tunis (Abidli *et al.*, 2009; 2011). In contrast, the information available on the reproductive cycle of the purple dye murex is relatively scarce despite the fact that it is commercially exploited both in the Mediterranean and in the Atlantic, especially in Spain (Martín *et al.*, 1995) and in Portugal (Vasconcelos *et al.*, 2008a). Indeed, the availability of data is confined to descriptions of the morphological and ultrastructural aspects of the reproductive system (e.g. Amor *et al.*, 2004; 2007), while two studies relate to the reproductive cycle of *B. brandaris* harvested from the Spanish coasts of Catalonia (Ramón & Amor, 2002) and Andalusia (Tirado *et al.*, 2002).

Although the species is frequently found in the nets of bottom trawlers in the Gulf of Gabès, fishermen sorting the catches retain the commercially valuable target species such as fishes, cephalopods and crustaceans but usually discard the catches of *B. brandaris*. At present this species has no commercial value in Tunisia and is not found in local seafood markets. However, some fishermen retain small amounts of *B. brandaris* for their own consumption, considering its meat to be tastier than that of *Hexaplex trunculus*, another muricid species commonly found in the seafood markets of Tunisia. Therefore, it is predictable that *B. brandaris* might soon become a commercially-exploited species. Moreover, the high value attributed to it in countries of southern Europe (especially in Portugal and Spain, where first sale prices can reach 20 €/kg) (Vasconcelos *et al.*, 2008a) indicates that Tunisia might promote the exportation of the catches *B. brandaris* to foreign countries in the near future. Nevertheless, lack of scientific knowledge persists on the species' biology, especially in this area, with particular reference to its reproductive cycle.

With exploitation of new gastropod species showing an increasing trend in international markets (Averbuj *et al.*, 2010), and given the fishing potential of *B. brandaris* in the Gulf of Gabès, it is of utmost importance to improve the knowledge about this potentially valuable local resource before the development of a commercial fishery. A similar approach, i.e., gathering data on the species biology before or at the early development of new fisheries, was adopted earlier for other gastropod resources all over the world (e.g. Valentinsson *et al.*, 1999; Narvarte, 2006; Averbuj *et al.*, 2010). Similarly, within the plausible scenario of becoming a fishery-exploited species in the Gulf of Gabès in the near future, it is considered prudent to gather baseline information on the biology of *B. brandaris*, which would allow the proposing specific fishery management measures and promoting the long-term sustainable exploitation of the resource. In this context, the present work aimed at describing the reproductive cycle

of *B. brandaris* from the Gulf of Gabès, including aspects such as the sex ratio, gonad maturation and spawning periods, and the size at first sexual maturity.

## Materials and Methods

### Study site and sampling

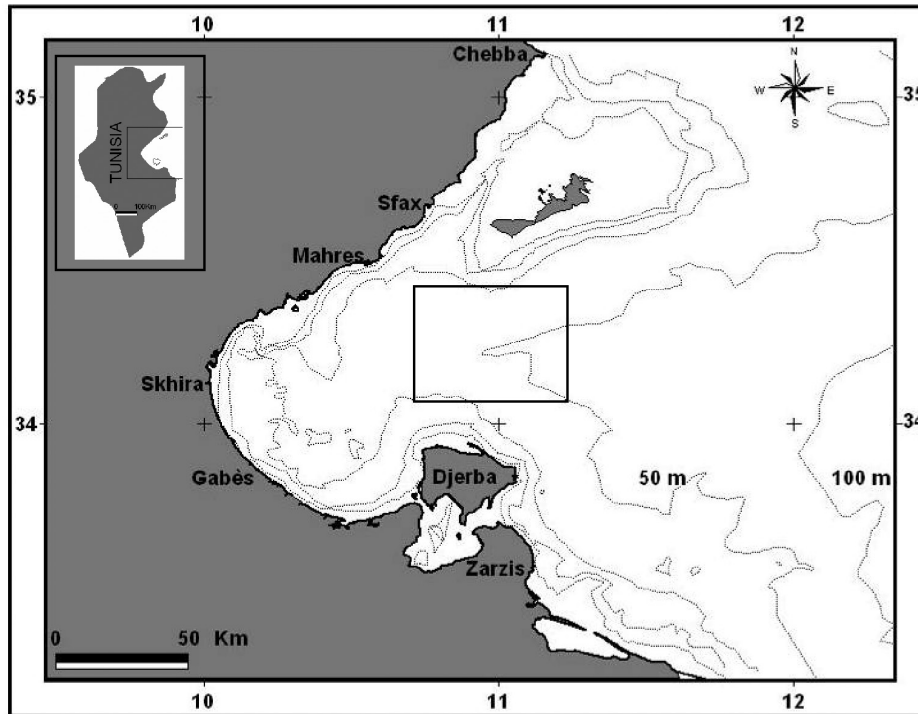
Monthly sampling was carried out from January through December 2007 on board commercial bottom trawlers operating out of the port of Sfax (southern Tunisia). This fishing fleet essentially targets demersal fishes and crustaceans (mainly shrimps), besides the purple dye murex caught as by-catch. 150–180 *B. brandaris* were collected each month (an average of 160 individuals per month), from relatively shallow waters (ranging between 40 and 50 m depth) of the Gulf of Gabès (Fig. 1).

In the laboratory, individuals were measured for shell length (SL) using a digital calliper (precision 0.05 mm) and weighed for total weight (TW) on a digital balance (precision 0.001g). The shell of *B. brandaris* being rather fragile (particularly the siphonal canal), it is highly vulnerable to fishing damage caused by bottom trawling. Therefore, in order to minimise bias in measurement, only those specimens whose shells were intact were included in laboratory analyses. In addition to this, to reduce susceptibility for inaccurate weighment, individuals were first lightly blotted and dried, so as to allow as much water as possible to drain out from the mantle cavity. Since the purple dye murex does not exhibit external sexual dimorphism, individuals were sexed after the sexual organs had been exposed by breaking and removing the shell. Moreover, due to the occurrence of imposex (development of male sexual characters onto females of prosobranch gastropods) in *B. brandaris* collected in the study area (K. Elhasni, unpublished data), females were identified by the presence of a vulva and a capsule gland, and males by the presence of a penis and the absence of a capsule gland.

### Histology of the gonads

For histological purposes, 20 individuals of both sexes were sampled every month. Individuals were de-shelled and the central portion of the complex digestive gland-gonad was dissected. The collected tissues were fixed in Bouin's fluid for 72 hours before being preserved in 70% ethanol. These tissues were dehydrated afterwards in an ethanol series (70–100%), cleared in toluene and embedded in paraffin wax blocks. Gonads were sliced into sections of 4–7 µm thickness using a microtome, before being stained with haematoxylin–eosin. Finally, gonadal sections were mounted between a glass slide and a cover slip, and the assembly was then placed under an optical microscope for observation.

Identifying the various stages of maturation in the histological sections of the gonads was based on a maturation scale (Table 1) adapted from a study with this spe-



**Fig. 1:** Map of the Gulf of Gabès (southern Tunisia) showing the sampling area (rectangle) for *Bolinus brandaris*.

cies by Ramón & Amor (2002), but including two additional maturation stages (pre-active and partially spent), as done successfully in a previous study concerning a sympatric muricid (*Hexaplex trunculus*) by Vasconcelos *et al.* (2008b).

#### *Bio-physiological indices*

In order to allow for the calculation of bio-physiological indices, the tissues of each individual were dried with blotting absorbent paper and thereafter weighed (SPW). In *B. brandaris*, the gonad is intimately linked with the

**Table 1.** Microscopic scale employed to classify the gonad maturation stages of *Bolinus brandaris*.

Maturation stage	Brief description
Stage 0 Immature	Gonads present little detailed internal structure. Very small spermatogonia and oogonia are dispersed in a relatively large and loose connective tissue.
Stage 1 Pre-active	Gonads of both sexes show development of follicle and tubule structures, which are embedded in connective tissue. The first gametogenic stages appear near the walls, but the cytoplasm is still very poor comprising only a few spermatocytes and early growing oocytes, in males and females respectively.
Stage 2 Active	The connective tissue is markedly reduced in size in favour of more developed and closely packed follicles. In males, the spermatogonia grew into spermatids and a few spermatozoa appear in the lumen of the tubules. In females, small and half-grown oocytes (pre-vitellogenic oocytes) appear at the periphery of follicles.
Stage 3 Ripe	The connective tissue almost disappears and the follicles and tubules are highly compressed. In males, tubules are distended and most spermatogonia have finished spermatogenesis and transformed into spermatozoa, particularly in the lumen (although earlier stages still occur near the wall). In females, follicles are filled with mature oocytes packed with yolk globules, especially in the lumen (although a few half-grown oocytes still occur near the wall).
Stage 4 Partially spent	Because spawning is in progress, gonads gradually reduce in size and the connective tissue reappears. The lumen of tubules and follicles becomes emptier and surrounded by some connective tissue. Although gametogenesis decreases near the wall, spermatozoa or ripe oocytes are still present in the lumen.
Stage 5 Spent	Gonads further reduce in size because most gametes were released. Large empty tubules and follicles are scattered in loose matrix of thicker connective tissue. Most tubules and follicles are completely empty, although a few still contain some spermatozoa and ripe oocytes in the lumen.



underlying digestive gland, so it was carefully dissected out under a binocular microscope before being weighed (GW) on the digital balance. The indices were calculated for standard-sized individuals (45–65 mm SL) to minimise bias of increasing somatic weight during ontogeny, besides emphasising the seasonal variations pertaining to the reproductive cycle. The general condition index (K) and the gonadosomatic index (GSI) were calculated using the following equations:  $K = SPW / TW * 100$  and  $GSI = GW / SPW * 100$ , respectively.

#### Size at first sexual maturity

The shell length at first sexual maturity ( $SL_{50}$ ) was estimated by sampling individuals across a broad size range, just before the beginning of the spawning season. Since the sample set of specimens analysed was quite large, the  $SL_{50}$  was based on the macroscopic examination of gonads. An earlier study by Ramón & Amor (2002) found a good match between macroscopic and microscopic maturation stages in *B. brandaris*, thus reaching the conclusion that sufficient information about the reproductive cycle is provided by the macroscopic stages, whenever a detailed histological description of the gonads is unnecessary. Therefore, individuals were classified according to a macroscopic scale based on gonad size and appearance, and also on the development of secondary sexual characters in both sexes (a vesicula seminalis in males, a capsule gland in females) (Table 2). An identical approach was recently adopted with success in studies concerning the sympatric muricid *H. trunculus* (Elhasni *et al.*, 2010; Gharsallah *et al.*, 2010).

For both sexes,  $SL_{50}$  was calculated by plotting the cumulative frequency of individuals with ripe gonads (stage 3) as a function of shell length (grouped into 5 mm SL classes). The FISHPARM software (Pauly, 1984) was employed to fit a logistic model to data, through the following equation:

$$P = 1 / (1 + e^{-r(SL - SL_{50})})$$

where: P = proportion of mature individuals; r = constant; SL = shell length (mm);  $SL_{50}$  = shell length (mm) at which 50% of the individuals are mature.

#### Statistical analysis

The sex ratios (M:F) in monthly samples and size classes (5 mm SL) were compared with parity (1:1) using the chi-square test ( $\chi^2$ -test). Analyses of variance (ANOVA) were employed to compare specimen size (SL) and weight (TW) between sexes and to assess the monthly variations in the bio-physiological indices (K and GSI) around the year. The non-parametric Kruskal-Wallis test was performed whenever the ANOVA assumptions (normality of data and homogeneity of variances) were not fulfilled. Pair-wise multiple comparisons using Tukey or Dunn post hoc tests, respectively, were made each time ANOVA or Kruskal-Wallis tests detected significant differences among groups. Statistical analyses were performed following ZAR (1996), using the software package SigmaStat® (version 3.5) with significance level considered for  $P < 0.05$ .

## Results

#### Size range and sex ratio

A total of 1929 individuals were analysed in order to determine the reproductive cycle of *B. brandaris* harvested from the Gulf of Gabès. Both sexes presented broad size and weight ranges (females: 34.3 – 95.1 mm SL and 1.6 – 39.6 g TW; males: 31.0 – 93.4 mm SL and 1.2 – 34.1 g TW). On average, females ( $61.0 \pm 13.0$  mm SL;  $11.2 \pm 7.4$  g TW) were significantly bigger and heavier (SL:  $H = 22.912$ ,  $P < 0.001$ ; TW:  $H = 27.004$ ,  $P < 0.001$ ) than males ( $58.3 \pm 12.8$  mm SL;  $9.7 \pm 6.6$  g TW).

In the overall samples, females ( $n = 1168$ ) outnumbered males ( $n = 761$ ), revealing an unbalanced sex ratio (M:F) of 1:1.5, significantly different from parity 1:1 ( $\chi^2 = 85.03$ ,  $P < 0.01$ ). As far as the monthly samples are concerned, the sex ratios were balanced from January to March, in September, and from November to December ( $\chi^2$ -test,  $P > 0.05$ ). Females were more abundant than males in the remaining months (between April and August and in October), thus revealing significant ( $\chi^2$ -test,  $P < 0.05$ ) or highly significant ( $\chi^2$ -test,  $P < 0.01$ ) female-biased sex ratios (Fig. 2A).

**Table 2.** Macroscopic scale employed to classify the gonad maturation stages of *Bolinus brandaris*.

Maturation stage	Brief description
Stage 0 Immature	Gonads of both sexes could not be distinguished from the adjacent digestive gland. Males and females present inconspicuous <i>vesicula seminalis</i> and capsule gland, respectively.
Stage 1 Intermediate	Gonads of both sexes are very small and hardly distinguishable from the digestive gland.
Stage 2 Active	Gonads of both sexes are more developed (but still smaller than mature gonads), corresponding to approximately one-third of the area of the digestive gland.
Stage 3 Ripe	Gonads of both sexes are fully developed, inflated and voluminous, corresponding to more than half of the area of the digestive gland. Males have a yellowish testis and a well developed <i>vesicula seminalis</i> , whereas females have a pinkish gonad and a large whitish capsule gland.

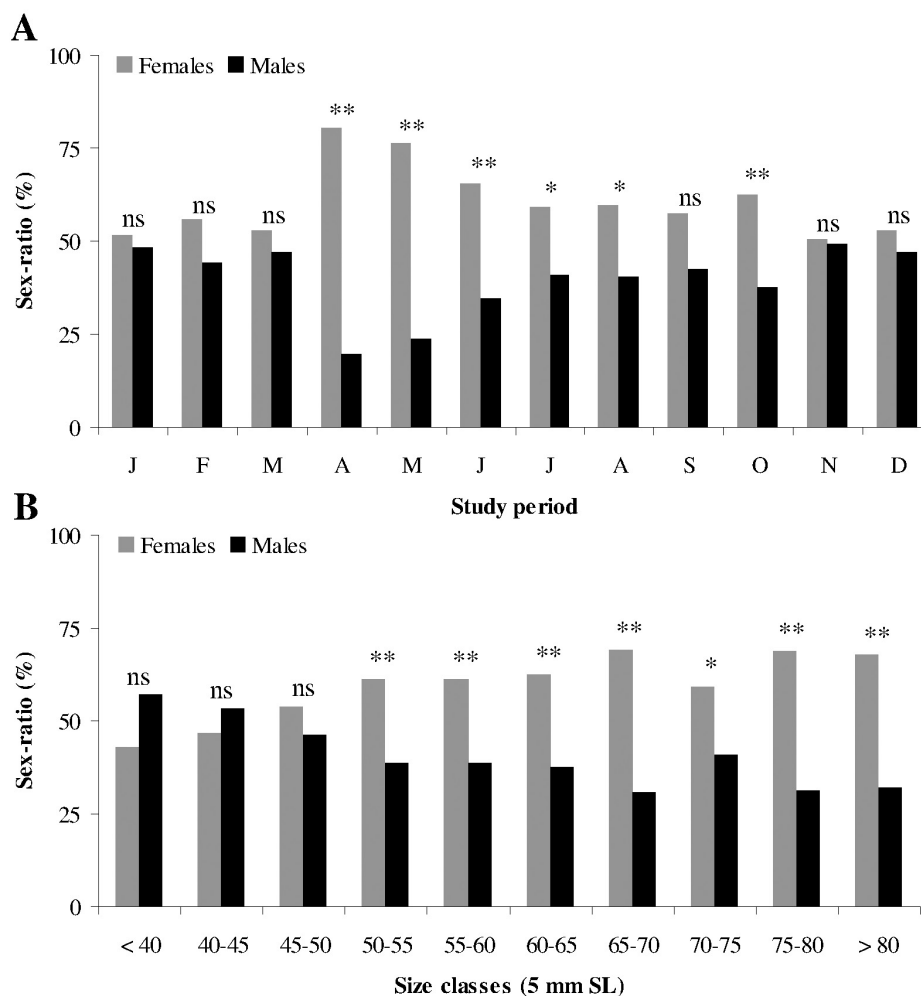
Individuals of both sexes were grouped into size classes (5 mm SL) to assess the variation in the sex ratio (M:F) as a function of size (SL). While the sex ratios were statistically balanced ( $\chi^2$ -test,  $P > 0.05$ ) in the smallest size-classes (i.e.  $< 50$  mm SL), significant ( $\chi^2$ -test,  $P < 0.05$ ) and mostly highly significant female-biased sex ratios ( $\chi^2$ -test,  $P < 0.01$ ) were found to exist in the largest size classes (i.e.  $\geq 50$  mm SL) (Fig. 2B).

#### Histology of the gonads

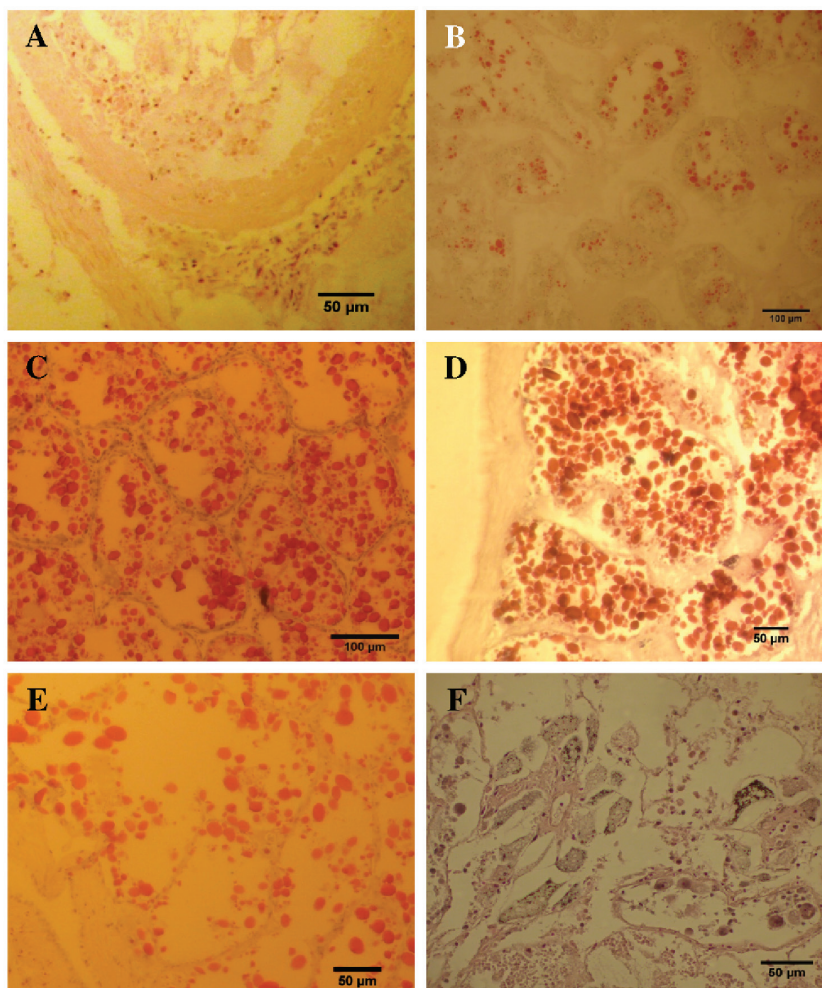
Examination of gonad histological sections at each maturation stage highlighted the most relevant features of gametogenesis in female and male *B. brandaris* (Figs. 3 and 4). Several maturation stages were seen to occur simultaneously in both sexes, although with highly variable relative proportions the year round (Fig. 5). The highest number of different maturation stages registered in a monthly sample was five stages of oogenesis (January and June) (Fig. 5A) and six stages of spermatogenesis (January) (Fig. 5B). In general, the histology of the gonads showed that the reproductive cycle of *B. brandaris*

from the Gulf of Gabès follows an annual cycle, with a long period of gonadal activity and slight asynchrony in gonadal development between sexes (Figs. 5A,B).

In females, active (stage 2) and ripe (stage 3) gonads were observed during most of the year (eight months), although in variable proportions. In contrast, gonads with regression signs, namely partially spent gonads (stage 4) were only observed over a shorter period (four months). Oogenesis began in October-November, as verified by the high frequency of pre-active and active stages, accounting for 77.8 and 75.0% of the gonads, respectively. The highest frequencies of ripe ovaries were observed from December (50.0%) to April (maximum in March, with 100%), followed by a manifest decrease in May (37.5%) and June (22.2%). While partially spent gonads were mostly evident in May (50.0%), the proportion of spent gonads was higher in June (44.4%), July (55.6%) and August (40.0%). Females showed a relatively long spawning season, with a steep decline in the percentage of ripe gonads in May-June, which thus clearly indicated the spawning peak. Most females showed low reproduc-



**Fig. 2:** Variation in the sex ratio of *Bolinus brandaris* (A) during the study period (January – December 2007) and (B) according to specimen size (grouped into 5 mm SL classes). Asterisks denote samples with statistically unbalanced sex ratios ( $\chi^2$ -test): ns, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .



**Fig. 3:** Photomicrographs of *Bolinus brandaris* female gonad maturation stages: (A) immature; (B) pre-active; (C) active; (D) ripe; (E) partially spent; (F) spent.

tive effort from July to September, as revealed by the high percentages of immature, pre-active and spent gonads observed (altogether ranging from 77.8 to 100% during this period) (Fig. 5A).

In males, active (stage 2) and ripe (stage 3) gonads were noted during nine to ten months of the year, respectively. In contrast, immature (stage 0) and pre-active (stage 1) gonadal stages were only found during six months of the study period. The rising percentage of pre-active and active gonads from August to October (both with 66.7%) indicated the beginning of spermatogenesis. Gonad maturation occurred mainly between November and March, when the occurrence of active, ripe and partially spent stages varied between 72.7% and 100%. During this period, highest proportions of ripe males were observed in December (80.0%) and March (75.0%). The steep decrease in the frequency of ripe gonads from March (75.0%) to April (33.3%) marked the peak of gamete release. Subsequently, partially spent and spent gonads predominated in April and May, together accounting for 55.6 and 70.0% of the maturation stages registered in those months. Most males demonstrated

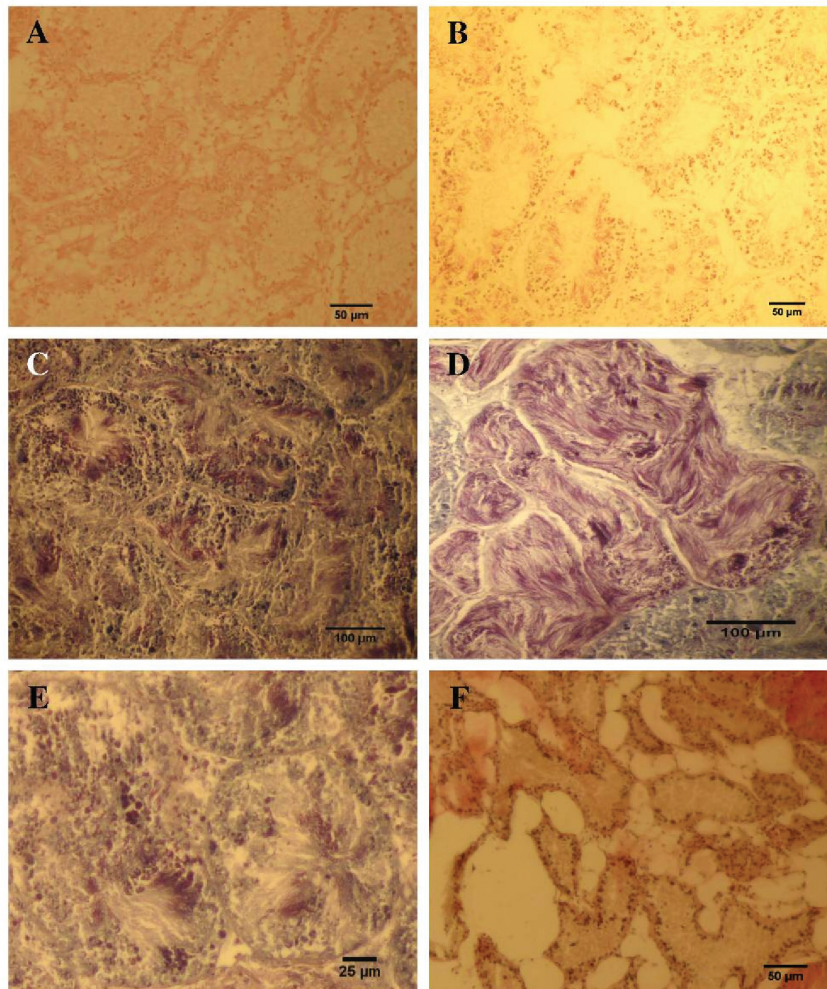
lower reproductive effort in June and July, as revealed by the high occurrences of immature, pre-active and spent gonads (altogether comprising 85.7 to 87.5%, respectively) (Fig. 5B).

#### *Bio-physiological indices*

Monthly variations in the general condition index (K) and gonadosomatic index (GSI) in *B. brandaris* are shown in Figure 6. Both indices presented statistically significant differences between sexes (K:  $H = 6.467$ ,  $P = 0.011$ ; GSI:  $H = 64.792$ ,  $P < 0.001$ ). On average, females showed higher mean annual K ( $36.0 \pm 8.2\%$ ) than males ( $34.9 \pm 8.0\%$ ) (Fig. 6A), whereas mean annual GSI was higher in males ( $6.2 \pm 3.5\%$ ) than in females ( $4.3 \pm 3.2\%$ ) (Fig. 6B).

Throughout the study period, the K index of both females and males showed a similar temporal trend with highly significant monthly variations (females:  $H = 129.958$ ,  $P < 0.001$ ; males:  $H = 76.545$ ,  $P < 0.001$ ). In females, the highest K values were observed in March (39.8%) and April (39.6%), which then decreased during spring, with a steep and significant decline between May and June (Dunn's test:  $Q = 4.576$ ,  $P < 0.05$ ), indicative of





**Fig. 4:** Photomicrographs of *Bolinus brandaris* male gonad maturation stages: (A) immature; (B) pre-active; (C) active; (D) ripe; (E) partially spent; (F) spent.

the spawning peak. The lowest K values were observed between June and October (ranging from 32.2 to 32.6%), coinciding with the period of lower reproductive effort. In males, the highest K values were recorded between December and March (ranging from 37.7 to 37.9%), followed by a steady decline during spring, indicative of male gamete release. The lowest K values were observed between June (31.5%) and September (32.8%), i.e., simultaneous with the period of lower reproductive effort, which was then followed by a steady increase till the year end (Fig. 6A).

The GSI in both sexes also displayed highly significant monthly oscillations throughout the year (females:  $H = 346.065$ ,  $P < 0.001$ ; males:  $H = 208.681$ ,  $P < 0.001$ ) and slight asynchrony between the male and female reproductive cycles. Males matured earlier and released gametes sooner than females. In females, the highest GSI values occurred in March (6.9%) and April (6.8%). This was followed by a steep and significant decline from May (5.6%) to June (1.7%) (Dunn's test:  $Q = 6.825$ ,  $P < 0.05$ ), indicating the peak of spawning. The lowest GSI values

were registered between July and October (between 0.5 and 0.6%), coinciding with the period of lower reproductive effort, which was then followed by a steady and continuous increase in the GSI till the year end. Males registered the highest GSI values from December to March (ranging from 7.8 to 8.3%), defining the period of increased gonad maturation. There was a marked decline from April to May (Dunn's test:  $Q = 3.511$ ,  $P < 0.05$ ), corresponding to the peak in gamete release, further followed by a phase of lower reproductive effort mainly between June and September (with the lowest GSI values ranging from 0.3 to 1.2%) (Fig. 6B).

#### *Size at first sexual maturity*

The shell length at first sexual maturity ( $SL_{50}$ ) of 1151 *B. brandaris* individuals (670 females ranging from 37.4 to 87.3 mm SL, and 481 males between 36.8 to 84.7 mm SL), was investigated during the maturation period of both sexes (Fig. 7). The smallest mature individuals (stage 3, with ripe gonads) were found in the 45-50 mm SL size-class (smallest mature female = 42.2 mm SL;



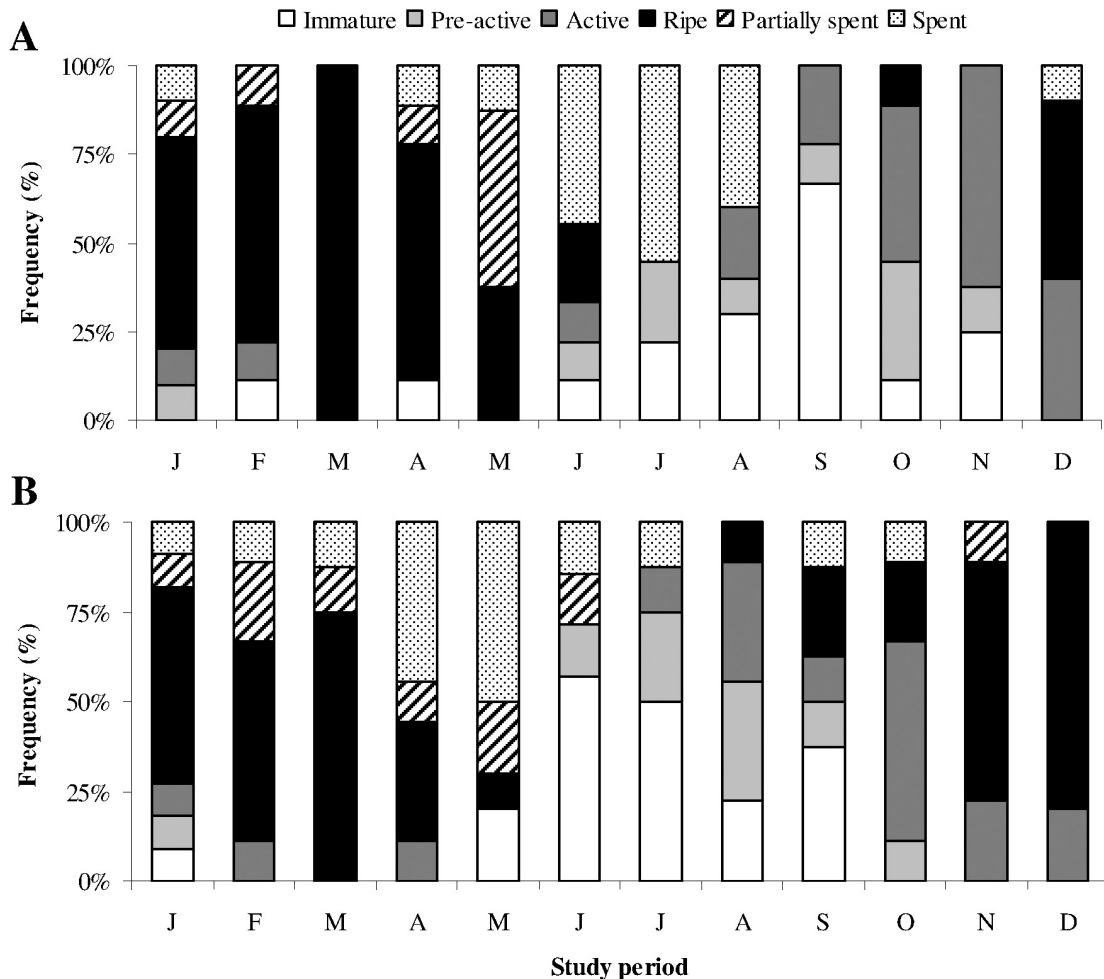


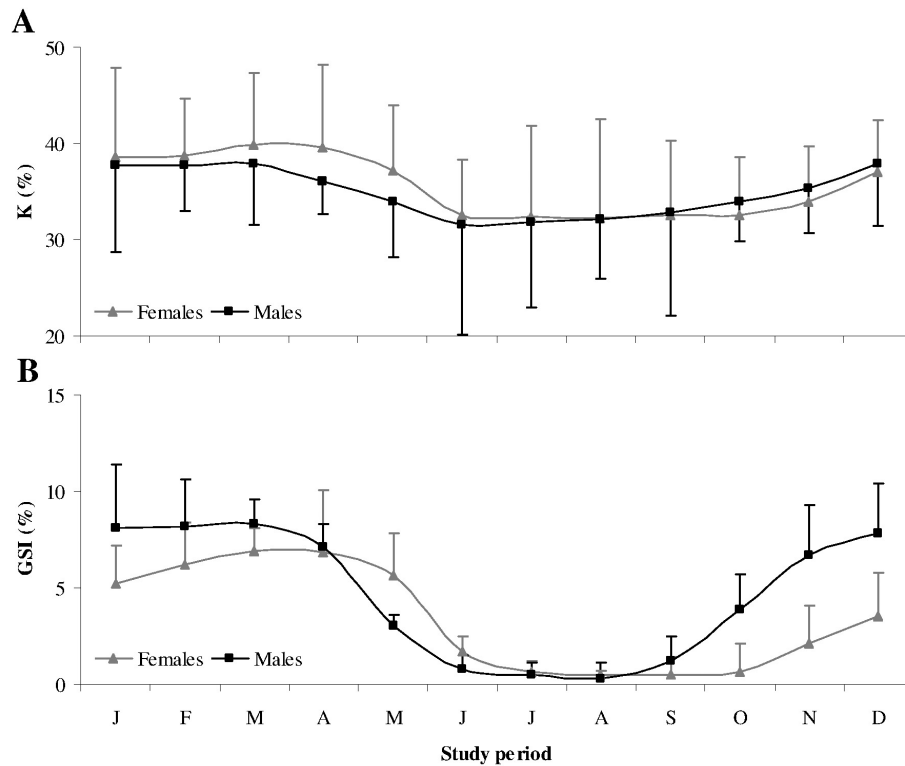
Fig. 5: Monthly frequency of gonad maturation stages in (A) female and (B) male *Bolinus brandaris*.

smallest mature male = 40.3 mm SL). First sexual maturity for females was reached at a size ( $\text{SL}_{50} = 56.4 \pm 0.5$  mm) (Fig. 7A) slightly bigger than that of males ( $\text{SL}_{50} = 54.6 \pm 0.6$  mm) (Fig. 7B). In the 65-70 mm SL size class, > 80% of females and 90% of males were found to be sexually mature. Finally, full sexual maturation (100% mature individuals) was attained in the size-classes 70-75 mm SL for males and 75-80 mm SL for females (Figs. 7A,B).

## Discussion

The sex ratio of the *B. brandaris* population from the Gulf of Gabès was found to be statistically unbalanced, with a clear preponderance of females observed in the overall samples. This finding agrees with other studies conducted on this species along the Catalan coast (north-eastern Spain) (Ramón & Amor, 2001, 2002) and in the Ria Formosa lagoon (southern Portugal) (Vasconcelos *et al.*, 2010), being also commonly observed in another muricid gastropod (the banded murex, *Hexaplex trunculus*) caught along the Tunisian coast (e.g. Elhasni *et al.*, 2010; Gharsallah *et al.*, 2010). In the present study, female-bi-

ased sex ratios occurred mainly between April and August, but with a clear period of highly unbalanced sex ratios from April to June, coinciding with the breeding and spawning season of *B. brandaris* locally. The reproductive behaviour of *B. brandaris*, with females forming crowded aggregations to deposit collective spawns (Martín *et al.*, 1995; Vasconcelos *et al.*, 2008a) is certainly responsible for the female-biased sex ratios found in late spring – early summer. Indeed, if during bottom trawling a breeding aggregation is caught by the trawl path, most likely females will predominate in the catches and generate strongly female-biased sex ratios. A similar predominance of females over males also occurred during the breeding and spawning season of *H. trunculus* in Tunisian waters (Elhasni *et al.*, 2010; Gharsallah *et al.*, 2010). In the present study, while sex ratios were balanced in the smallest size classes (< 50 mm SL), the proportion of females in the samples augmented by increasing specimen size, generating female-biased sex ratios in the largest size-classes (> 50 mm SL). A similar trend of females predominating in larger size classes has also been reported for *H. trunculus* from Portugal (Vasconcelos *et al.*, 2008b) and Tunisia (Elhasni *et al.*, 2010;



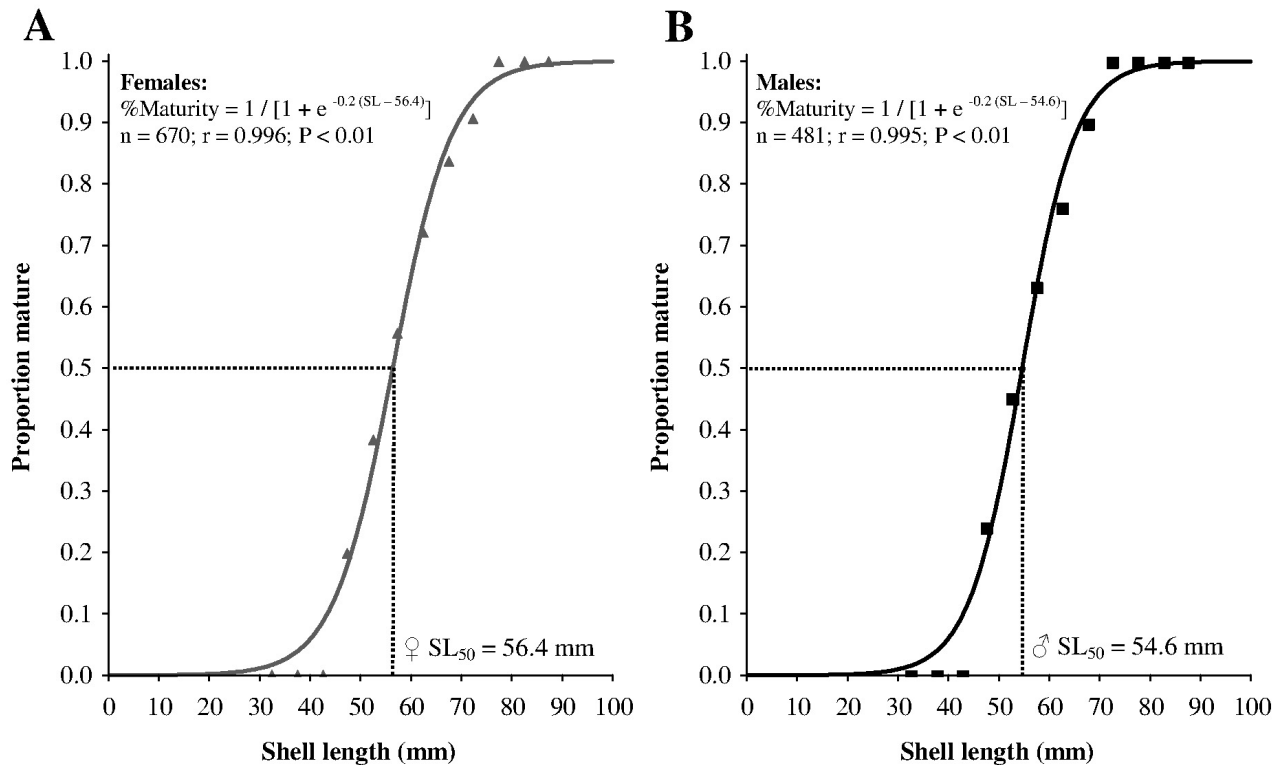
**Fig. 6:** Monthly variation in (A) general condition index (K) and (B) gonadosomatic index (GSI) of *Bolinus brandaris*. Data presented as mean  $\pm$  SD.

Gharsallah *et al.*, 2010). Female-biased sex ratios among larger and older individuals probably reflect differential growth between sexes (particularly after reaching sexual maturity), with females allocating more energy towards growth than reproduction, i.e. with females growing faster and larger as compared to males.

It is unanimously agreed that though costly and time-intensive, histological analysis of the gonads is the most reliable and comprehensive technique for exhaustively describing the gametogenic cycle and assessing gonadal maturation. As mentioned previously, an earlier study had revealed the macroscopic maturation stages could adequately describe the reproductive cycle of *B. brandaris* (Ramón & Amor, 2002). The present study has further corroborated this by examining the bio-physiological indices (K and GSI), the monthly fluctuations of which reflected variations in somatic and gonad weights during the reproductive cycle, clearly highlighting the key-phases of gonad maturation, gamete release and spawning. In both sexes, K and GSI showed analogous and synchronous temporal trends. This indicates that variations in somatic weight were chiefly caused by fluctuations in gonad weight throughout the reproductive cycle. In females, apart from gonad weight, change in the K index is also influenced by the conspicuous fluctuation in capsule gland weight during the reproductive cycle (being practically empty during the period of lower reproductive effort and wholly filled at ripening). Overall, the satisfactory agreement in the monthly variation of gonad

gametogenic stages, K and GSI, established the ability of these bio-physiological indices to follow seasonal trends in the reproductive dynamics of *B. brandaris*, namely for routine analyses that do not require an exhaustive histological description of the gonads.

The principal features of the reproductive cycle of *B. brandaris* include a clearly-defined annual cycle, prolonged gonadal activity and a slight asynchrony between sexes. In accordance with reports relating to some locations along the coast of Spain (Martín *et al.*, 1995; Ramón & Amor, 2002; Tirado *et al.*, 2002), *B. brandaris* from the Gulf of Gabès also displays an extensive period of gametogenic activity. Most mature males were found from November to March, the peak of gamete release having occurred between April and May, whereas most mature females were found from January to April, with the spawning peak occurring between May and June. This slight asynchrony in gonad development, and peaks of gametic emission between sexes (with a gap of approximately two months between males and females), is undoubtedly due to the capacity of females to store viable sperm in the sperm-ingesting gland (*receptaculum seminis*) after copulation and prior to fecundation and spawning. This phenomenon might denote a strategy to increase the number of copulas and thus improve reproductive success, as already reported to occur both in *B. brandaris* (Ramón & Amor, 2002; Tirado *et al.*, 2002) and *H. trunculus* (Lahbib *et al.*, 2004; Vasconcelos *et al.*, 2008b,c; Elhasni *et al.*, 2010). In the Gulf of Gabès, the



**Fig. 7:** Size at first sexual maturity ( $SL_{50}$ ) of (A) female and (B) male *Bolinus brandaris*, showing the proportion of ripe individuals (stage 3) as a function of shell length (grouped into 5 mm SL classes).

spawning season was seen to occur mainly between April and July, with spawning peaking from May to June, as indicated by the microscopic maturation stages and corroborated by the bio-physiological indices (K and GSI).

Information pertaining to other geographical areas also suggests that *B. brandaris* has a long spawning season (Martín *et al.*, 1995). Along the Spanish coast, spawning occurred mainly between May and July (Tirado *et al.*, 2002) with maximum ripening in females being achieved in June and July (Ramón & Amor, 2002). In several locations along the Israel coast, spawn was found between May and July (Barash & Zenziper, 1980). The period with lower reproductive effort in *B. brandaris* from the Gulf of Gabès occurred mainly between June and September in males and between July and October in females. Relatively similar periods of reduced reproductive activity have also been observed in other populations of *B. brandaris*: both sexes in July and August (Tirado *et al.*, 2002), both sexes mainly from August to October (Ramón & Amor, 2002). Therefore, minor variation in the timing of gonad maturation and spawning among geographical areas suggests that environmental cues play a key role in the reproductive cycle of *B. brandaris*. Like many other species of gastropods, the reproductive cycle of *B. brandaris* appears to be controlled by the annual cycle in day length and seawater temperature. Gonad maturation seems to be triggered by the increasing length of the day, and related rising temperatures during late spring and early summer, followed by the period of lower re-

productive effort coinciding with the longest days and warmest summer temperatures. A similar annual pattern was found in the reproductive cycle of *H. trunculus*, both in Gulf of Gabès (Elhasni *et al.*, 2010), in the Bizerte lagoon (Lahbib *et al.*, 2004; Gharsallah *et al.*, 2010), and in the Ria Formosa lagoon (Vasconcelos *et al.*, 2008b,c).

For fisheries management, size at first sexual maturity is a crucial parameter (Appeldoorn, 1988). The estimation of  $SL_{50}$  can be achieved either following histological criteria (e.g. Avila-Poveda & Baqueiro-Cárdenas, 2006; Cledón *et al.*, 2008) or through macroscopic examination of the gonads and development of secondary sexual characters (Berg *et al.*, 1992; Stoner *et al.*, 1992; Elhasni *et al.*, 2010; Gharsallah *et al.*, 2010). An earlier study showed how effective macroscopic examination can be for describing gonadal maturation in *B. brandaris* (Ramón & Amor, 2002), allowing examining and classifying the maturation stage in a much high number of individuals per monthly sample. In the present study, the estimation of the size at sexual maturity revealed that *B. brandaris* males reached maturation at a size slightly smaller than that of females ( $\text{♂}SL_{50} = 54.6 \pm 0.6$  mm;  $\text{♀}SL_{50} = 56.4 \pm 0.5$  mm), which might be due to differential growth between sexes or to a delay in female maturation. Sexual maturity in males at smaller sizes than those observed in females is a common phenomenon in several gastropod species, including *Adelomelon brasiliana* (Cledón *et al.*, 2008), *Buccinanops cochlidium* (Averbuj *et al.*, 2010), *H. trunculus* (Elhasni *et al.*, 2010; Gharsallah *et*



al., 2010) and *Odontocymbiola magellanica* (Bigatti *et al.*, 2008). However, in *B. brandaris* from the Catalan coast, the smallest mature male and female with signs of gametogenic activity measured 29.2 and 26.9 mm SLWS (shell length without siphonal canal), respectively (Ramón & Amor, 2002). These differences confirm that in gastropods, size at first sexual maturity could vary appreciably in populations from different geographical areas (e.g. Martel *et al.*, 1986; Kideys *et al.*, 1993). Additionally, a recent study found temporal variation in the size at first maturity of a marine gastropod (*Zidona dufresnei*) subjected to fishery exploitation (Torroglosa & Giménez, 2010).

As mentioned above, *B. brandaris* is expected to become a fishery-exploited species in the Gulf of Gabès in the near future. Therefore, to ensure sustainable exploitation and long-term preservation of the resource, fishery management measures should be implemented right from the start of its exploitation. As such, planning management measures before the commencement of extensive commercial exploitation provides the possibility of preventing a later collapse of the resource (Averbuj *et al.*, 2010). Viewed in this context, the present data on the reproductive biology of the species, in particular the timing of the spawning season and the size at first sexual maturity, provide information crucial to the proposal of prior and specific management measures. First of all, to protect the spawning stock, *B. brandaris* caught during the spawning season (and particularly during the spawning peak, between May and June) should be discarded live on-board. This also applies to the collective spawns caught in the trawling nets, which should be immediately returned to the fishing grounds to avoid excessive damage to the oothecas, eggs and developing embryos. Finally, because this species lacks external sexual dimorphism, as a precautionary measure a minimum landing size of 60 mm SL should be legally established for the catches of *B. brandaris* in the Gulf of Gabès and commercially under-sized individuals should be discarded live on-board. Although further studies are needed for proposing a comprehensive set of fishery policies, if and when this species is commercially exploited, adoption of these provisional management measures in a timely manner would definitely help to prevent the resource being over-exploited.

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