Associated fauna and effects of epibiotic barnacles on the relative growth and reproductive indices of *Stramonita haemastoma* (Gastropoda: Muricidae)

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Summary: To better understand the impacts of biofouling on the biological processes of the basibiont, the effects of epibiotic barnacles on the relative growth and reproductive indices of *Stramonita haemastoma* (Linnaeus, 1767) were assessed. A total of 1035 specimens were collected monthly for one year from Bizerta Channel (northern Tunisia). Endobiotic species comprised the lithophagous bivalves *Lithophaga aristata* and *Rocellaria dubia* of different sizes, communicating with the outside through tiny perforations. Intra-shell tunnels and galleries also sheltered annelids and sipunculids. Epibiotic species comprised algae and highly diversified invertebrates represented by crustaceans, polychaetes, molluscs, echinoderms, ascidians, sponges, bryozoans and sipunculids, with barnacles being the most common group. Comparison of growth features between non-fouled and fouled *S. haemastoma* revealed higher growth in non-fouled specimens. Differences in reproductive condition indices were detected in few months, being mostly higher in non-fouled snails, but showed no asynchrony in the spawning period for either fouled or non-fouled gastropods hosts.

Keywords: *Stramonita haemastoma*; biofouling; reproductive indices; relative growth; Bizerta Channel.

Fauna asociada y efecto de los balanos epibiontes al crecimiento relativo e índices reproductivos de *Stramonita haemastoma* (Gasterópoda: Muricidae)

Resumen: Para mejorar la compresión de los impactos del biofouling en los procesos biológicos de los basibiontes, se ha evaluado los efectos de los balanos epibiontes en el crecimiento relativo y en los índices reproductivos de *Stramonita haemastoma* (Linnaeus, 1767). Se recogieron un total de 1032 especímenes mensualmente, durante un año, en el canal de Bizerta (norte de Túnez). Las especies endobióticas estaban compuestas por los bivalvos litófagos *Lithophaga aristata* y *Rocellaria dubia,* de diferentes tamaños, que se comunicaban con el exterior a través de pequeñas perforaciones. Los túneles y galerías del interior de la concha también albergaban anélidos y sipuncúlidos, siendo los balanos el grupo más común. La comparación del crecimiento entre los gasterópodos con y sin fouling mostró un mayor crecimiento en los *S. haemastoma* sin fouling. Las diferencias en los índices reproductivos se detectaron en pocos meses, siendo mayor en los caracoles no invadidos por el fouling, pero ninguno de los gasterópodos hospedadores mostró asincronía en el periodo de desove.

Palabras clave: *Stramonita haemastoma*; biofouling; índices reproductivos; crecimiento relativo; canal de Bizerta.

INTRODUCTION

The mollusc shell is a suitable biotope for the settlement and development of several groups of invertebrates. In molluscs, biofouling has mainly been described in bivalves such as *Pinna bicolor* (Keough 1984), *Chlamys opercularis* (Ward and Thorpe 1991), *Crassostrea gigas* (Duault et al. 2001), *Venus verrucosa* (Trigui El Menif et al. 2005), *Flexopecten felipponei* (Schejter and Bremec 2006), *Lithophaga lithophaga* (Trigui El Menif et al. 2007) and *Pinna nobilis* (Rabaoui et al. 2009). The associated fauna highlighted in these bivalve species belongs to several zoological groups: bryozoans, annelids, serpulid polychaetes, tu-

nicates, sponges, crustaceans, ascidians, cnidarians and echinoderms.

Few studies have as yet dealt with shell fouling and its effects in gastropods. These include the study by Buschbaum and Reise (1999) reporting the effects of barnacle colonization on the shell of *Littorina littorea* and those of Bick (2006) and Vasconcelos et al. (2007) on polychaete fixation in the shells of *Stramonita haemastoma* and *Hexaplex trunculus*, respectively.

Few studies of the biology of *S. haemastoma* have been published, and they deal mainly with reproduction, spawning and intracapsular development (Beliste and Stickle 1978, Lahbib et al. 2011). The most studied aspect in *S. haemastoma* is the imposex phenomenon (Spence et al. 1990, Rilov 1999, Lemghich and Benajiba 2007, Lahbib et al. 2010, El Ayari et al. 2015), the sexual deformity associated with marine pollution by organotin compounds such as tributyltin (TBT) and its derivatives (Terlizzi et al. 2001). Recently, the effect of epibiotic barnacles on imposex in *S. haemastoma* sampled from Tunisian coasts was investigated (El Ayari et al. 2015).

Taking into consideration the well-known effects of biofouling that could possibly affect the biology of the basibiont, the present study aimed to identify species associated with the shell of *S. haemastoma* from Tunisian waters and to investigate whether this fouling could affect the relative growth and reproductive indices of this locally abundant gastropod species.

MATERIALS AND METHODS

Specimens of *S. haemastoma* with a shell length of 20-80 mm were collected monthly (N=80-120) on a rocky bottom from June 2009 to May 2010. Sampling was performed by scuba diving at 11 m depth at a station located in the artificial channel linking the Bizerta lagoon with the Mediterranean Sea (Fig. 1). Seawater temperature and salinity were measured using a multiparameter sounder. In the laboratory, fauna living on the shell external surface was collected by washing the shells using seawater from the same sampling station.

Comparison of relative growth and reproductive indices in *S. haemastoma* was investigated monthly in two groups: 40 to 60 gastropods non-fouled by barnacles (GnFB) and 40 to 60 gastropods fouled by barnacles (GFB). The position and number of barnacles per individual were determined after removing them from the shells of the hosts using a scalpel. Gastropods were then sacrificed by freezing at –20°C; shell length (SL), shell diameter (SD), and penis length (PL) were measured to the nearest 0.1 mm using a digital caliper. After thawing, the shell was broken using a bench vice and the soft part of the organism was carefully removed. Endobiotic fauna was collected after shell breakage, identified following macroscopic observation or under a binocular microscope, and then preserved in 70% alcohol.

In *S. haemastoma,* three gonad maturation stages were detected following macroscopic observations of the gonads in both sexes and of the capsule gland in females, following Ramón and Amor (2002) in the sympatric muricid *Bolinus brandaris*. Stage I (immature) corresponds to undifferentiated gonads from the underlying capsule gland in both sexes and females having an inconspicuous capsule gland; stage II (intermediate) gonads in both sexes are more developed and correspond approximately to one-third of the area of the digestive gland; and stage III (mature) males show a well-developed, light brown testicle corresponding to more than half of the area of the digestive gland, whereas females have a voluminous yellowish ovary and a large yellowish capsule gland.

The flesh wet weight (FwW) was recorded after removing the operculum. The female capsule gland was separated and weighed (CGwW). A cross-section was made on the coiled part of the organism, directly under the stomach, and was photographed to measure the gonad area (GA) and the area of the digestive gland–gonad complex (DGGA) using the software Image J 1.38 x. The shell dry weight (SdW) and the flesh dry weight (FdW) were recorded after drying them at 60°C for 3 days.
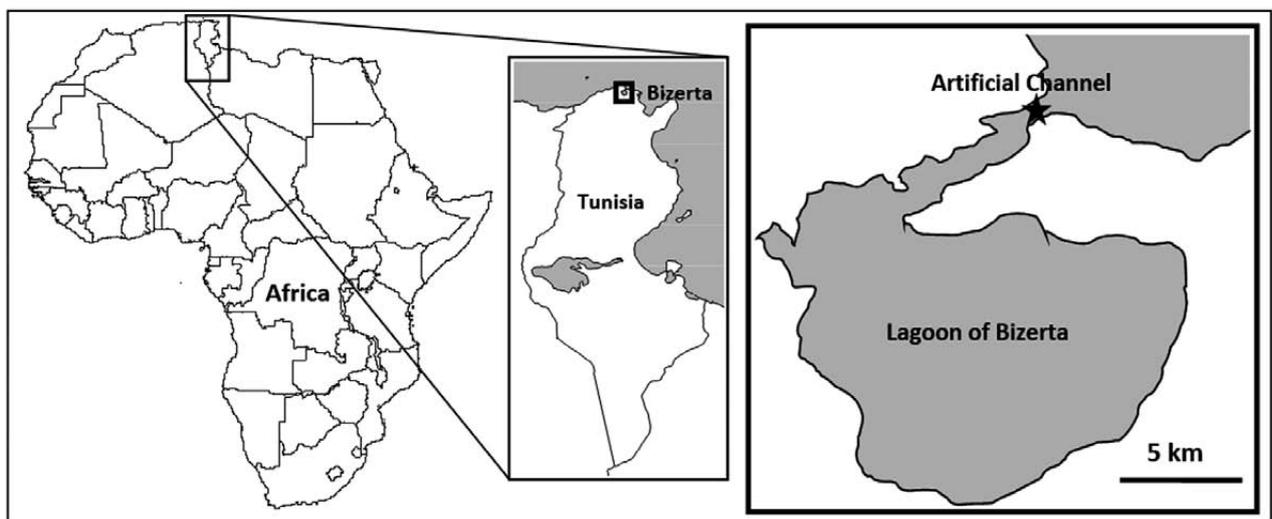

Fig. 1. – Map of Tunisia and sampling site of *Stramonita haemastoma* in the channel of Bizerta.

Table 1. – Monthly seawater temperature and salinity values recorded in Bizerta Channel during the study period (June 2009 - May 2010).

	J	J	A	S	O	N	D	J	F	M	A	M
Temperature (°C)	24.5	25.7	26.7	24.4	20.3	14.0	16.0	15.2	14.0	13.3	17.0	23.9
Salinity	35.8	36.7	37.2	37.9	36.7	27.6	23.1	23.9	28.5	29.3	28.0	31.1

The reproductive condition of *S. haemastoma* was assessed using the following indices, expressed as percentages:

- Gonadosomatic index (GSI) = digestive gland-gonad complex dry weight/SdW (Lucas and Beninger 1985);
- Capsule gland index (CGI) = CGwW/FwW (Giménez and Penchaszadeh 2003);
- Gonad area index (GAI) = GA/DGGA (Poore 1973);
- Penial index (PI) = PL/SL (Vasconcelos et al. 2011).

Relationships between morphometric and ponderal variables (SL, SD, SdW and FdW) were established through regression analysis ($Y=aX^b$). Relative growth between variables was determined by comparing the regression slopes (b) using the Student t-test (Mayrat 1959). A chi-square test was employed to verify a balanced proportion (1M:1F) in the sex ratio of the samples. Comparisons between fouled and non-fouled gastropods were made through analysis of covariance (ANCOVA - model of homogeneity of slopes) using the software STATISTICA 10, with the covariable being always SL. Post-hoc pairwise comparisons were made using the Tukey test. Monthly variations in bio-physiological indices were analysed by one-way ANOVA (Kruskal-Wallis test). In all statistical analysis, significance level was considered for P<0.05.

RESULTS

Seawater temperature and salinity

The average seawater temperature measured in Bizerta Channel during the study period (June 2009 to May 2010) was 19.6°C. The lowest temperature (13.3°C) was recorded in March and the highest (26.7°C) in August (Table 1). The salinity reached a maximum (37.9) in September and a minimum (23.1) in December (Table 1), corresponding to an average value of 31.3.

Population sex ratio

The sex ratio of the studied population showed a higher abundance of males in both fouled and non-fouled gastropods. Unbalanced sex ratios were only statistically significant in the monthly samples of April for GFB (χ^2=4.07, P<0.05) and of December for GnFB (χ^2=4.57, P<0.05).

Associated fauna

Among a total of 1035 individuals of *S. haemastoma* with an average length of 53.4±6.7 mm, an abundant and very diversified associated fauna and flora was identified, composed mainly of crustaceans, polychaetes, molluscs, echinoderms, ascidians, sponges,

Table 2. – Fauna associated with *Stramonita haemastoma* collected from Bizerta Channel (northern Tunisia).

	Epibiotic species	Endobiotic species		Epibiotic species	Endobiotic species
Mollusca	GASTROPODA	BIVALVIA	Errant	SYLLIDAE	
	Diodora graeca	*Lithophaga aristata*	Polychaetes	*Syllis amica*	
	Diodora sp.	*Rocellaria dubia*		*Streptoyllis* sp.	
	Patella sp.			EUNICIDAE	
	Acmaea insessa			*Eunice vitatta*	
	BIVALVIA			*Lysidice ninetta*	
	Anomia ephippium			*Hyalinoecia bilineata*	
	Grassostrea gigas			NEREIDAE	
	Ostrea edulis			*Perinereis cullrifera*	
Crustacea	SESSILIA	Acrothoracica		*Nereis rava*	
	Balanus amphitrite	Unidentified		PHYLLODOCIDAE	
	Balanus perforatus			*Eullalia* sp.	
	AMPHIPODA			CHRYSOPETALIDAE	
	Gammarus aequicauda			*Chrysopetalum debile*	
	Gammarus marinus			SPHAERODARIDAE	
	Gammarus insensibilis			*Sphaerosyllis pirifera*	
	Gammarus pulex		Sedentary	SERPULIDAE	CIRRATULIDAE
	Gammarus chevreuxi		Polychaetes	*Pomatoceros triqueter*	*Dodecaceria concharum*
	Gammarus olivii			*Hydroides uncinata*	*Audonia tentaculata*
	Gammarus locusta			*Hydroides diramphus*	*Heterocirrus* sp.
	Elasmopus rapax			*Serpula concharum*	SPIONIDAE
	DECAPODA			*Serpula vermicularis*	*Pygospio elegens*
	Achaeus cranchi			SABELLIDAE	*Polydora* sp.
	Acanthonyx lunulatus			*Sabella* sp.	LUMBRINERIDAE
	Xantho poressa				*Lumbrinereis* sp.
	ISOPODA		Sipunculoidea		PHASCOLOSOMATIDAE
	Cymodoce sp.				*Phascolosoma stephensoni*
	PYCNOGONIDA				ASPIDOSIPHONIDAE
	Achelia sp.				*Aspidosiphon muelleri*
					Phascolosoma sp.
			Echinodermata	*Amphipholis squamata*	
				Ophiopsila aranea	
				Ophiura sp.	

Fig. 2. – Fauna associated with *S. haemastoma*. A, *Achelia* sp. found in the external shell surface of *S. haemastoma*; B, shell of *S. haemastoma* covered by green algae; C, barnacles entirely covering the gastropod shell; D, oysters randomly distributed on the gastropod shell; E, *Hydroides diramphus* removed from white tube in the shell of *S. haemastoma*; F, shell perforation caused by annelids; G, unidentified cirratulid; H, *Dodecaceria concharum* removed from intra-shell gallery; I, J, K, errant polychaetes (*Aponuphis bilineata* and *Perinereis cullrifera*) removed from tunnels in the shell of *S. haemastoma*; L, *Lithophaga aristata* in gallery on the dorsal side of the shell; M, *L. aristata* in gallery located in the direction "apical axis-siphonal canal"; N, eight-shaped hole showing the penetration of *L. aristata*; O, P, Q, penetration of *L. aristata* in perpendicular direction to the apical axis; R, *L. aristata* reached the visceral mass; S, T, *Rocellaria dubia* removed from breach dug in the last spire of the shell; U, *Phascolosoma* sp. found in gallery in the dorsal surface near the last spire of the host shell; V, *Aspidosiphon muelleri muelleri* removed from a gallery in the dorsal surface; W, *Phascolosoma stephensoni* removed from a gallery located on the edge of the columella; X, unidentified crustacean (*Acrothoracica* sp.) removed from intra-shell cavity with 3 mm.
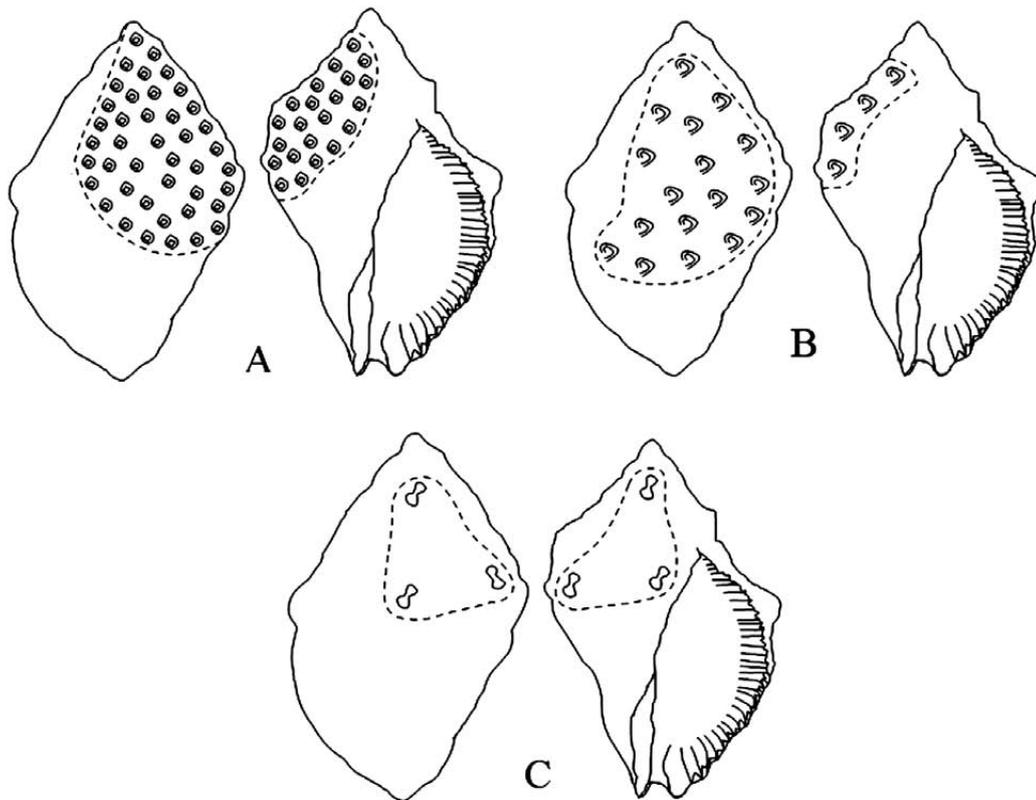

Fig. 3. – Preferential shell colonization zone/location of epibiotic species observed in the shells of heavily fouled *S. haemastoma*; (A) barnacles; (B) polychaete tubes; (C) perforations caused by *Lithophaga aristata*.

Table 3. – Relative growth of non-fouled and fouled *S. haemastoma* (sexes confounded and separate sexes). SL, shell length; SD, shell diameter; SdW, Shell dry weight; FdW, Flesh dry weight; N, number of individuals; M, males; F, females; r, correlation coefficient; t, Student t-test.

Biometric variables	Gastropod non-fouled by barnacles (both sexes) (N=439)		Gastropod fouled by barnacles (both sexes) (N=596)	
SD/SL	$SD=0.58SL^{1.02}$ r=0.95 t=2.48		$SD=0.75SL^{0.95}$ r=0.94 t=4.63	
SdW/SL	$SdW=5E-03SL^{2.63}$ r=0.93 t=22.20		$SdW=5E-05SL^{2.55}$ r=0.76 t=19.81	
FdW/SL	$FdW=9E-06SL^{3.07}$ r=0.88 t=24.04		$FdW=6E-05SL^{2.60}$ r=0.78 t=157.23	
Sexes	M (N=241)	F (N=198)	M (N=331)	F (N=265)
SD/SL	$SD=0.59SL^{1.01}$ r=0.95 t=1.50	$SD=0.56SL^{1.03}$ r=0.95 t=0.19	$SD=0.76SL^{0.95}$ r=0.95 t=3.78	$SD=0.74SL^{0.96}$ r=0.93 t=2.60
SdW/SL	$SdW=6E-03SL^{2.57}$ r=0.94 t=13.45	$SdW=3E-03SL^{2.73}$ r=0.93 t=1.55	$SdW=1E-02SL^{2.60}$ r=0.93 t=31.96	$SdW=5E-03SL^{2.42}$ r=0.91 t=20.02
FdW/SL	$FdW-f=1E-05SL^{2.96}$ r=0.87 t=7.80	$FdW-f=5E-06SL^{3.22}$ r=0.88 t=13.09	$FdW-f=8E-05SL^{2.52}$ r=0.77 t=117.62	$FdW-f=3E-05SL^{2.73}$ r=0.79 t=64.42

bryozoans, sipunculids, and green and brown algae (Table 2).

Epibiotic species associated with S. haemastoma

The associated community removed from the external shell surface of the basibiont is compiled in Table 2, and some photos of the epibiotic species are depicted in Figure 2 (A - E). Among the epibiotic taxa, barnacles were the most represented group (Fig. 2C) and were fixed mainly on the dorsal left face of the apical part of the shell (Fig. 3A). The number of barnacles per host was 23.2±16.5, against 2.3±2.8 for the oysters, which were randomly distributed on the gastropod shell (Fig. 2D). The shell surface also showed the presence of scattered U-shaped tubes that sheltered sedentary polychaetes (Fig. 3B), with Serpulidae and Sabellidae being the most abundant polychaete families (Table 2). Regarding errant polychaetes, the gastropod shell was generally colonized by the families Syllidae, Eunicidae, Nereidae, Phyllodocidae, Chrysopetalidae and Sphaerodaridae (Table 2).

Endobiotic species associated with S. haemastoma

Following shell breakage, a rich endofauna was detected living in breaches, tunnels or galleries dug by annelids, lithophagous bivalves, sipunculids and crustaceans. The last two groups (sipunculids and crustaceans) were less represented than annelids (sedentary and errant polychaetes), which were found in 65.6% of the gastropods, with an average of 3.4±2.7 annelids per host. Polychaetes burrowing the shell of *S. haemastoma* at different positions are shown in Figure 2 (F-H). Tunnels perforated by these polychaetes sheltered errant annelids such as *Aponuphis bilineata* (Fig. 2I-J) and *Perinereis cullrifera* (Fig. 2K). The lithophagous bivalves *Lithophaga aristata* and *Rocellaria dubia* were extracted from breaches having the same size and form as the endobiont. *L. aristata* (4 to 17.5 mm in length) was found in 10.1% of the gastropods examined and occupied galleries situated mainly at the dorsal side of the shell (Fig. 2L), in the direction "apical axis-siphonal canal" (Fig. 2M, N) or in perpendicular direction (Fig. 2O-R). The preferential colonization zone of *L. aristata* in the shell of *S. haemastoma* is schematically illustrated in Figure 3C. In contrast, *R. dubia* (6.6 mm in length) occurred at a very low rate (0.1%) and was removed from breaches dug in the last spire of the host shell (Fig. 2S, T). Five specimens of sipunculids were found in tunnels and galleries belonging to the families Phascolosomatidae (three *Phascolosoma stephensoni* of 10, 13 and 16 mm length and one *Phascolosoma* sp. of 13.5 mm length) (Fig. 2U) and Aspidosiphonidae (*Aspidosiphon muelleri muelleri* of 11 mm long) (Fig. 2V). One specimen of *P. stephensoni* was found in a gallery located on the edge of the columella (Fig. 2W), while all other individuals were removed from tubes dug in the dorsal face nearby the apex of the shell. An unidentified crustacean (*Acrothoracica* sp.) was found in an intra-shell cavity of 3 mm (Fig. 2X).

Relative growth

Stramonita haemastoma fouled by epibiotic barnacles (GFB) displayed negative allometry for the relationships SD/SL, SdW/SL and FdW/SL, indicating that in both sexes SL grows at a faster rate than SD, SdW and FdW. On the other hand, *S. haemastoma* non-fouled by barnacles (GnFB) showed a higher growth of SD and FdW than of SL (Table. 3). The ANCOVA test detected significant differences between GFB and GnFB for the relationships SD/SL and FdW/SL (Table 4).

Table 4. – Results of the ANCOVA performed with data on relative growth of *S. haemastoma*. SL, shell length; SD, shell diameter; SdW, Shell dry weight; FdW, Flesh dry weight: GFB, gastropods fouled by barnacles; GnFB, gastropods non-fouled by barnacles.

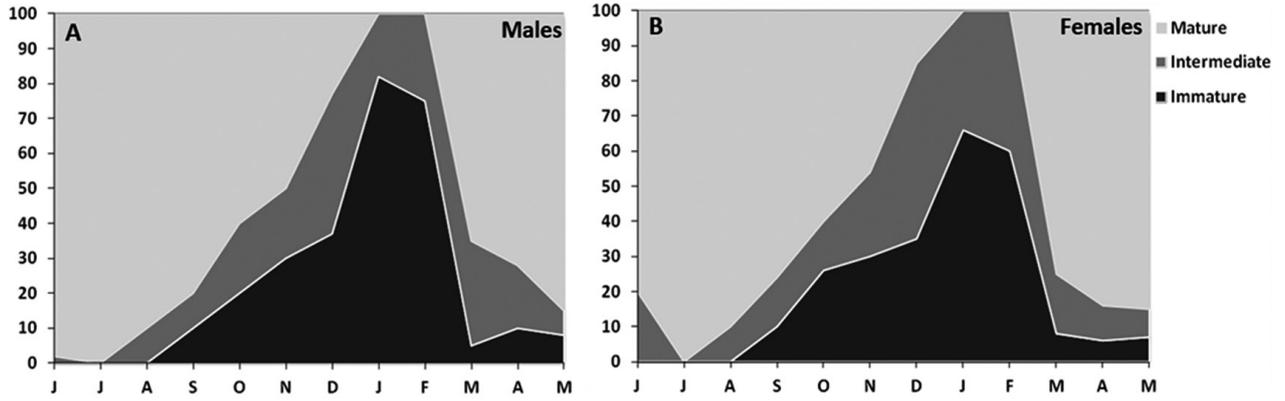| Biometric variables	SD/SL		SdW/SL		FdW/SL	
	F	P	F	P	F	P
Total GnFB vs GFB	1.51	0.21	5.19	0.02	40.65	<0.01
GnFB: Males vs Females	0.36	0.54	4.74	0.03	7.13	<0.01
GFB: Males vs Females	0.24	0.62	2.22	0.13	46.24	<0.01

Fig. 4. – Monthly variation of gonad maturation stages in males (A) and females (B) of *S. haemastoma*, following macroscopic observation of the reproductive organs.
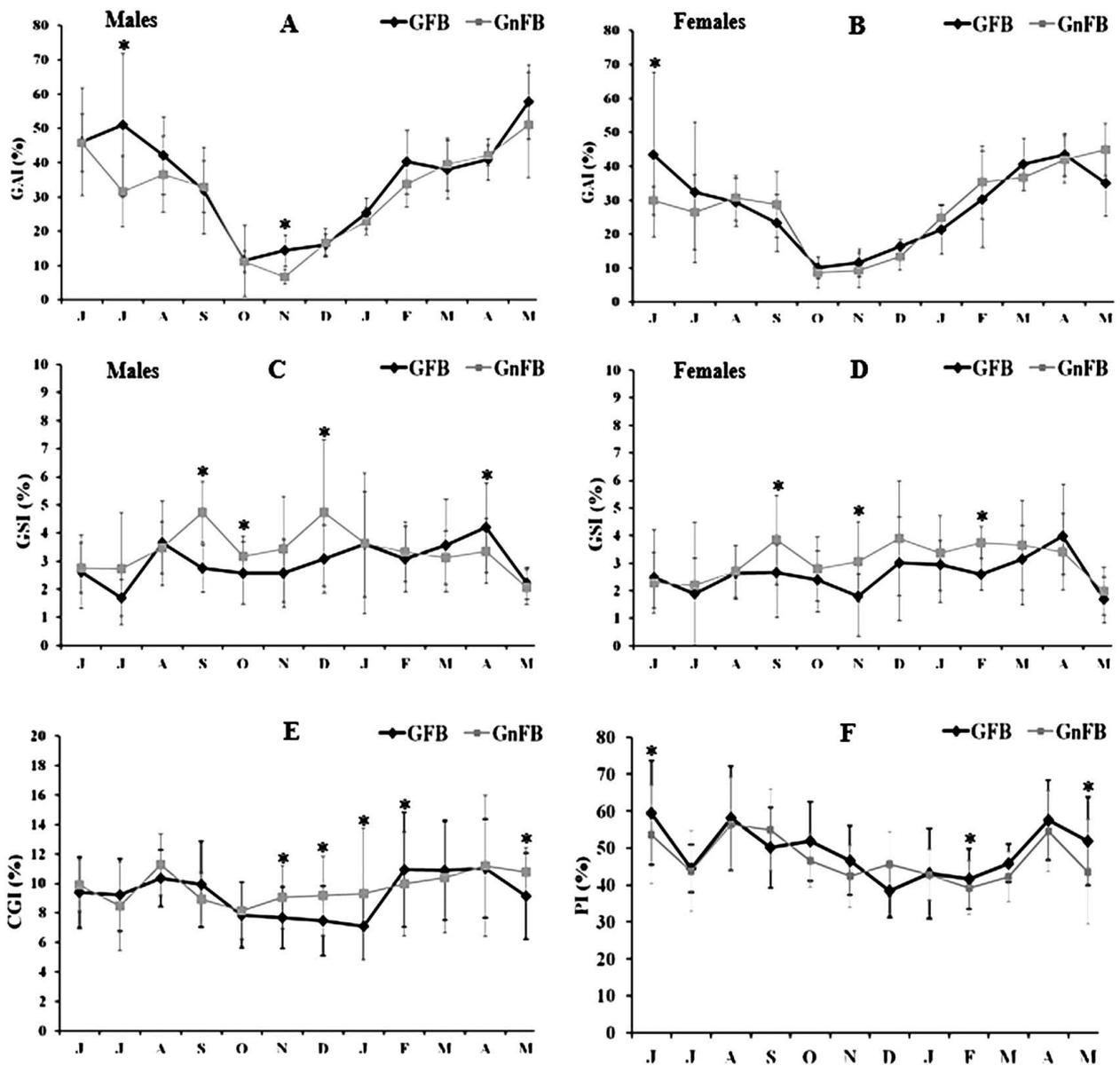

Fig. 5. – Comparison of the monthly variation of the gonad area index (GAI) in males (A) and females (B); gonadosomatic index (GSI) in males (C) and females (D); capsule gland index (CGI) (E) and penial index (PI) (F) in *S. haemastoma* non-fouled (GnFB) and fouled (GFB) by epibiotic barnacles (June 2009 - May 2010). (*) Statistically significant differences GnFB and GFB in each month.

Taking into consideration the sex, both males and females fouled by epibiotic barnacles showed negative allometries in the relationships SD/SL, SdW/SL and FdW/SL, indicating that shell grows faster in length than in diameter, SdW and FdW. This type of allometry was also recorded in non-fouled males for the relationships SdW/SL and FdW/SL. For the relationship SD/SL isometry was recorded in both sexes for the relationship SdW/SL it was recorded in non-fouled females. As for the relationship FdW/SL, positive allometry was detected, indicating higher growth in flesh weight than in shell length (Table. 3). Statistically significant differences between males and females occurred in the relationships FdW/SL for GFB and SdW/SL and FdW/SL for GnFB (Table 4).

Reproductive cycle

Macroscopic observations of the gonads of *S. haemastoma* showed a great similarity in the rhythm of gonad maturation between males and females (Fig. 4A, B), with mature males and females being present throughout the year, except in January and February, when only immature and intermediate individuals were recorded (Fig. 4A, B). Gonadal maturation occurred from May-June to October. The highest percentages of ripe gonads were recorded in July (100%) in both males and females (Fig. 4A, B), with a period of gametogenesis extending from December to April-May (Fig. 4A, B). Once gametes were mature, copulation and capsule deposition occurred about seven days later. All three gonad maturation stages were found almost year-round, except for the absence of stage I (immature) in June, July and August, stage II (intermediate) in July and stage III (mature) in January and February (Fig. 4).

Monthly variations in the reproductive condition indices of *S. haemastoma* during the study period are presented in the Figure 5. All indices showed similar trends in males and females, with slight variations in some months. The GAI showed that gametogenesis occurred from November to April-May. After that, spawning took place between June-July and October. In fouled snails, spawning was significant during this period, while it occurred mainly in September in non-fouled snails. Significant differences between lots were detected during June in males, and during July and November in females, when the highest values of GAI were recorded in GFB (males: H=0.12, P<0.05; females: H=0.16, P<0.05) (Fig. 5A, B).

GSI values displayed more fluctuations than GAI values and were more regular in fouled snails. GnFB displayed three decreasing periods in GSI, with two periods (from September to October and from April to July) probably being associated with spawning, and the third one, from December to February, which was due to another unknown activity. Decreases of the index were displayed in GFB from August to September (in males) and October-November (in females) and from April to July in both sexes (Fig. 5C, D). Significant differences were recorded between lots in September, October, December and April for males and in Novem-ber, February and April for females (males: H=18.23, P<0.05; females: H=30.13, P<0.05).

The monthly variation in the CGI showed that hypertrophy of glands was reached in February and continued until August in both female lots. Atrophied capsule glands were observed from October to January, indicating that spawning occurred between August and October. The values of CGI showed that the capsule gland in GnFB was bigger than that in GFB in August, November, December, January and May (H=7.38, P=0.006) (Fig. 5E).

The PI showed that penis hypertrophy is reached in April, while it becomes atrophied in November-December. Copulation occurred between June and July and between August-September (in GFB) and September-November (in GnFB). The Kruskal-Wallis test (H=4.98, P=0.025) showed significant differences in May and June in favour of GFB and in December in favour of GnFB (Fig. 5F).

DISCUSSION

The present study has shown that the shell of live *S. haemastoma* collected from Bizerta Channel is a suitable biotope for several invertebrate species. The most abundant epibiotic species were barnacles that totally covered the shell of several snails. Sedentary polychaetes and lithophagous bivalves were the most abundant endobiotic species. For polychaetes, a prevalence of cirratulids especially represented by *Dodecaceria concharum* was recorded. In *S. haemastoma* from the Spanish coast, the shell of live individuals sheltered, in addition to *D. concharum*, three other species, namely *Dipolydora armata, Capitella minima* and *Spirobranchus polytrema* (Bick 2006). Seven families of polychaetes found in the present study (Spionidae, Ciratulidae, Syllidae, Nereidae, Sabellidae, Sabellariidae and Serpulidae) were also reported in *H. trunculus* from Portuguese waters by Vasconcelos et al. (2007). These authors showed that the dorsal surface of the shell, mainly in the proximity of the apex, was generally more fouled by epibiotic polychaetes than the ventral surface, a pattern of colonization which is in agreement with the present results and that is probably related to the mode of locomotion of this snail. Indeed, it has been shown that *S. haemastoma* moves by crawling on hard substrate and by burrowing on soft substrate when the rocks become exposed during low tides (Papp and Duarte 2001). In both cases, the continuous abrasion of the ventral surface leads to an erosion of the settled epibionts, with the same finding being also recorded in *H. trunculus* (Vasconcelos et al. 2007).

Fouling is likely to be a function of time, food availability, seawater salinity and temperature (Wahl 1989, Sahu et al. 2013). In fact, it has been shown that salinity influences all physical-chemical variables of seawater and controls the larval dispersal and recruitment patterns of epi- and endobiotic species (Nair 1965), while seawater temperature influences chemical and biological interaction and regulates the growth of benthic organisms (Sahu et al. 2013). Consequently, the shells of smaller gastropods are usually less colonized

by epifauna than those of larger gastropods, which also agrees with our observations. Abundant colonization could have serious and deleterious consequences on the basibiont. Indeed, Thieltges and Buschbaum (2007) and Buschbaum et al. (2007) showed that the presence of the worm *Polydora ciliata* on the shell of *Littorina littorea* facilitates barnacle fixation, leads to a gradual destruction of the shell, reduces the resistance against predators and reduces the fecundity and the growth of the gastropods. According to Lleonart et al. (2003), an infestation of 30% in the gastropod *Haliotis* spp. from the Australian coast by two spionid species (*Boccardia knoxi* and *Polydora hoplura*) leads to the mortality of half of the affected population.

For endofauna, we recorded the presence of *L. aristata* and *R. rubia*, with infestation rates of 10.1% and 0.1%, respectively. Trigui El Menif et al. (2007) isolated those two boring mytilids from the rock hosting *L. lithophaga* collected in the Bay of Bizerta. *Rocellaria dubia* was also found by the same authors in breaches dug in the shell of live *Venus verrucosa*. Tebble (1976) reported the presence of *R. dubia* in various substrates (sand, limestone, sandstone and dead mollusc shells) but not in live specimens. This bivalve, as well as the other lithophagous species, has a pair of pallial glands that secrete a calcium-binding mucoprotein for boring into calcareous organisms (Jaccarini et al. 1968). The continuous growth of the endobiont involves a growth in the volume of the breach causing a progressive perforation of the shell of the basibiont, which in some cases leads to its death. This finding was recorded by Simone and Gonçalves (2006) and by Trigui El Menif et al. (2006), respectively in *Nodipecten nodosus* infested by *L. aristata* and in *V. verrucosa* infested by *Rocellaria dubia*. With regard to our results, the infestation of *S. haemastoma* by *L. aristata* does not seem to act negatively on the gastropod at this level, because the extracted endobiont had a size of 17 mm in length and 6 mm in thickness, which does not exceed either the length or the thickness of the gastropod columella. Negative effects in the basibiont could probably occur with the progressive growth of the endobiont, since *L. aristata* can attain a length of 52 mm (Turner and Boss 1962). According to Simone and Gonçalves (2006), the geographical distribution of the *L. aristata* is probably limited to the Atlantic coasts. Moreover, Ávila et al. (2009) discovered *L. aristata* as a fossil form on the island of Santa Maria (Azores). The presence of this species in Tunisian coasts, in the same habitat as *L. lithophaga* (Trigui El Menif et al. 2006) and in the shell of *S. haemastoma*, lets us suppose that it is an invasive species coming from the Strait of Gibraltar.

A male-biased sex ratio was found in *S. haemastoma* from Bizerta Channel, which agrees with previous studies on this species that concluded that this could be due to either female mortality or imposex in some females (Rilov 1999). Usually, differences in growth between populations collected from different sites are associated with many factors, including environmental conditions, TBT pollution, prey type and parasitism (Crothers 1985). Another factor that seems to affect the growth of gastropods is biofouling, since non-fouled and fouled gastropods were collected at the same sampling site. Indeed, the annual growths in terms of SD and FdW were higher in non-fouled gastropods. In fact, biofouling is time-dependent (Wahl 1989), which means that the growth of *S. haemastoma* is also accompanied by an increase in number and size of epibiotic barnacles. In some cases the weight of the barnacles per gastropod host is equal to its own weight; this most probably has negative effects on locomotion of snails, which must move between different substrates in order to feed, avoid unfavourable environmental conditions and escape predation. Consequently, gastropods fouled by epibiotic barnacles spend more energy during their movements at the expense of their growth than non-fouled gastropods. Indeed, it has been demonstrated that epibiotic barnacles in *L. littorea* decrease the locomotion and consequently the growth of gastropods (Buschbaum and Reise 1999, Thieltges and Buschbaum 2007). Thieltges and Buschbaum (2007) also showed that epibiotic *Crepidula fornicata* reduces the growth and survival of blue mussels (*Mytilus edulis*). Recently, Lacoste et al. (2014) recorded that biofouling reduces the growth of pearl oysters (*Pinctada margaritifera*) in French Polynesia. Our results showed that non-fouled females of *S. haeamstoma* have heavier shells and flesh. In contrast, Lahbib (2004) found that males of *Hexaplex trunculus* have heavier shells and flesh than females, considering this as sexual dimorphism characterizing specimens collected from Menzel Jemil (northern Tunisia).

The study of the reproductive cycle of *S. haemastoma* from the Tunisian coast showed an acceptable agreement between the macroscopic classification of gonad maturation stages and three of the bio-physiological indices used (GAI, CGI and PI). In contrast, the GSI showed significant fluctuations that could not be directly related to the reproductive cycle of *S. haemastoma*. This index is probably influenced by other factors, such as variations in the weight of the digestive gland after food ingestion and also SdW, because it is sometimes difficult to entirely remove all epibionts from the basibiont shell. Therefore, based on this result it would be more suitable to follow the reproductive activity of *S. haemastoma* using macroscopic observations together with GAI, CGI and PI. The simultaneous increase in GAI and decrease in CGI indicate gonad maturation and the beginning of spawning in May, which were probably triggered by an abrupt increase in seawater temperature, from 17°C in April to 23.9°C in May, with a similar finding being recorded in *B. brandaris* from Tunisian waters (Abidli el al. 2012). Vasconcelos et al. (2008) and Elhasni et al. (2010) recommended the use of two indices (GAI and CGI) and considered them simple, practical and efficient for the routine assessment of reproductive activity in a sympatric muricid (*H. trunculus*). Abidli et al. (2012), Vasconcelos et al. (2012) and Elhasni et al. (2013) confirmed these findings in analogous studies with another sympatric muricid (*B. brandaris*). Usually, differences in the reproductive cycle are explained by variation in seawater temperature (Lahbib et al. 2009, Abidli et al. 2012). Another factor that seems to affect the repro-

ductive activity of gastropods is imposex (Lahbib et al. 2009). Results gathered in this study further suggest that the reproductive cycle of *S. haemastoma* is also affected by biofouling. Furthermore, Vasconcelos et al. (2011) established the PI for the study of the reproductive activity of male *H. trunculus*. This index was also practical for the study of the reproductive activity of male *S. haemastoma,* but must be used cautiously at sites highly affected by TBT pollution, such as Bizerta Channel (Lahbib et al. 2011), because it has been shown that TBT contamination might increase male PL (Castro et al. 2007, Abidli et al. 2013).

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