Mediterranean Marine Science

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To cite this article:

Research Article

The gametogenic cycle of the non-native false limpet *Siphonaria pectinata* (Linnaeus, 1758) in the easternmost limit of its distribution range: implications for its future in the Eastern Mediterranean Basin

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Handling Editor: Serge GOFAS

Received: 1 May 2020; Accepted: 8 September 2020; Published online: 7 October 2020

Abstract

The gametogenic cycle of the false-limpet *Siphonaria pectinata* from the Bizerte channel (Northern Tunisia) was studied through histological characterization of the hermaphroditic gonad during a 1-year study period (May 2015 - May 2016). Spawning intensity in the field as well as the gonadic index were calculated monthly. Both female and male gametes were observed simultaneously within acini of adult individuals and continuously throughout the year. Oogenesis started in the mid-autumn (October), with gonads characterized mainly by proliferation of female cells. After that, oocytes progressively increased in number and volume until summer, when the evacuation stage frequency was the highest. From summer to autumn, some acini were empty from their oocyctes while some others had already begun the proliferation stage, suggesting the lack of a resting phase. Spermatogenesis was also a continuous process throughout the year, with spermatozoa being mainly observed in late spring (March). These findings suggested that environmental conditions are suitable for the establishment of *S. pectinata*, first mentioned in 1998 on the Tunisian coast and even suggest that it could continue spreading eastward in the Mediterranean Basin.

Keywords: Siphonariid; reproduction; alien species; invasion success; Tunisia.

Introduction

Siphonariids are grazing herbivore heterobranch gastropods that feed on macroscopic and microscopic algae such as diatoms. *Siphonaria pectinata* (Linnaeus, 1758) in particular, grows on the hard substrata of the upper and midlittoral zones (Ocaña & Fa, 2003). The species has been long considered to be present on both the Eastern and Western Atlantic coasts, in the latter ranging from Florida to the Texas-Mexican border (Voss, 1959). However, recent investigations have proven genetic disconnection between these two Atlantic coasts (Giribet & Kawauchi 2015), and the western Atlantic populations are treated as a different species, *S. naufragum* Stearns, 1872. In the Eastern Atlantic, *S. pectinata* is present from Portugal to Cameroon (Giribet & Kawauchi 2015), but has also established populations in the Western Mediterranean basin (Southern Iberian Peninsula) (Ocaña & Emson, 1999), Central Mediterranean (from Algeria to Northern Tunisia) (Boukhicha *et al.,* 2014) and has reached the Eastern

Mediterranean, settling in Greece and Croatia (Nicolay, 1980; Despalatović *et al.*, 2009). In these last two locations, the species has been accidentally introduced by shipping traffic (Giribet & Kawauchi, 2015). Its presence in Northern Tunisia was first mentioned in 1998 at low densities in Tabarka (western coasts) (Antit *et al.*, 2008), and it reached Kelibia (eastern coasts) in 2012 where this non-indigenous species finds its natural easternmost distribution limit following an intra-Mediterranean climate-driven expansion (Boukhicha *et al.,* 2014).

Siphonaria pectinata, as other Siphonariids, are hermaphrodites with internal fertilization and lay eggs in gelatinous masses well adhered to the substratum (Pal & Hodgson 2005; Slama *et al.,* 2018). The gametogenic cycle of *S. pectinata* remains undescribed. Given that the reproductive behaviour of marine molluscs is related to environmental factors such as temperature, food availability and day length (Hirano & Inaba, 1980), there is a special interest in studying its reproduction in Tunisia: the environmental conditions of their new distribution limit

could potentially be more stressful and have an impact on their reproductive (and invasive) success. Thus, information regarding reproduction is valuable data in order to determine the structure and dynamics of their recently introduced populations. However, the only works addressing *S. pectinata* in Tunisia are reduced to descriptions of the biological and ecological aspects of the species in the area, reports of their geographical expansion range and description of spawning under laboratory conditions (Boukhicha *et al.,* 2014; Slama *et al.,* 2018). Therefore, the present study aimed to histologically characterize the morphology of the hermaphroditic gonad of *S. pectinata* and describe both oogenesis and spermatogenesis. Such data will be very useful in understanding the biology of this newcomer mollusc to the Tunisian coast and assessing its invasive potential.

Methods

The sampling site was a 200-meter long rock-filled mole with Northeast orientation located in Zarzouna disctrict (37°16'06N 9°52'42E), on the mouth of Bizerte channel. Tidal range varied between 0.1 to 0.9 m. Substratum slope was sub horizontal (about 5% inclination) with low wave exposure action (sheltered area) and high anthropogenic pressure (shipping traffic, urban agglomerations). At this location, thirty adults of *S. pectinata* (10-27 mm in shell length) were randomly collected monthly by hand at low tide from May 2015 to May 2016. In all cases, animals were collected 0.4 m high above mean low tide level. To avoid any effects related to the concomitant change in density, the number of individuals fixed on the rocky surfaces was counted before sampling using a 1m²-quadrat. Only quadrats with densities of 20-30 individuals were sampled. After sampling, the coast intervals where animals were collected were marked to avoid re-sampling it again. Environmental variables (i.e. water temperature, salinity, and dissolved oxygen) were monthly monitored. One single measurement was taken for each parameter (one-off values), and these were taken once a month at the same time of the day using a multiparameter sensor (Xylem - WTW 3320). Data on climatic variables such as air temperature, hours of insolation and rainfall were provided by the National Institute of Meteorology of Tunisia. At the same time, fecundity of *S. pectinata* was estimated by counting individuals and egg-ribbons contained in 10 quadrats (1 m² each) placed at 20 m intervals and at the same shore level. Spawning intensity (SI) was calculated by dividing the number of egg masses by the number of individuals as in Ocaña & Emson (1999).

Collected animals were then transferred to the laboratory. This was done without immerging the specimens in water in a cool atmosphere (containers at 9-11°C) and in a maximum time of 10 minutes to minimize stress. Once in the laboratory, individuals were measured (shell length, Fig. 1a) using a digital Vernier caliper (0.01 mm) and soft parts (Fig. 2B) and gonads (Fig. 1c) were immediately removed and weighed with a precision balance to the nearest 0.001g. Gonad index (GI) was calculated using the formula: GI = (wet gonad weight/soft part wet weight) x 100. Thereafter, gonads were fixed in a formaldehyde solution (4%) for 24 h and then dehydrated in increasing concentrations of ethanol until reaching 96%. Tissues were then embedded in paraffin wax, and 5 μm-thick sections were cut using a Leica microtome. These were later stained with hematoxylin and eosin. Sections were examined under light microscope and photographed with a digital camera (Samsung MV800, China).

Five different sections of each gonad were randomly selected and classified independently according to gonad development stage The developmental stage was determined of each acini found in the gonad sections with female and male parts analysed separately. Then the most prevalent stage for each gonad was considered as the stage for a given individual. The frequency of each stages was calculated by dividing the number of individuals under that stage by the total number of gonads analysed monthly (N=30).

Statistical analyses consisted of one-way ANOVAs followed by Tukey pair wise comparison tests to investigate the monthly variation in GI and SI. The values are given as mean ± standard deviation (SD). Analyses were conducted using SigmaStat® 3.5 (Systat Software, CA, USA)
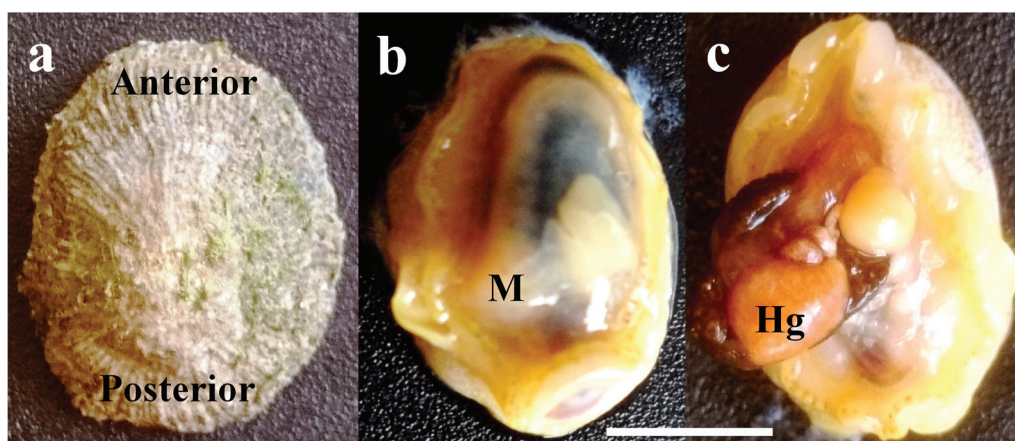

Fig. 1: Dorsal view *of S. pectinata*. (a) with the shell, (b) after removal of the shell, (c) after dissection of the gonad. M: Mantle, Hg: hermaphroditic gonad. Scale bar=1cm.
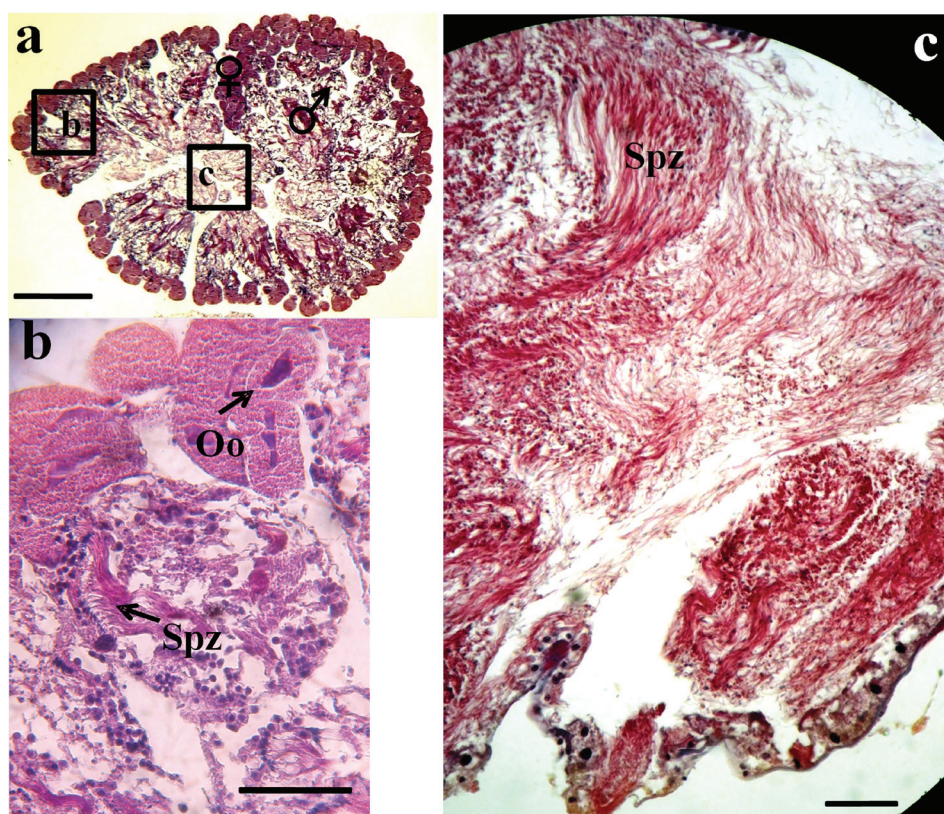
Fig. 2: Histological section of the gonad showing the distribution of female acini at the periphery and male acini at the center (a). (b) Detail of acini showing oocytes and spermatozoa. (c) Detail of the hermaphroditic duct showing spermatozoa. Oo, oocytes; Spz, spermatozoa. Scale bars= 500µm (a), 80µm (b), 3µm (C).

Results

Environmental variables

Seawater temperature showed its minimum values in January (mid winter) (12.2°C) and its maximum in August (late summer) (27.4°C) with an annual average of 18.6±4.4 °C. The highest salinity was recorded in July (mid-summer) (38.0 psu) when the insolation was the highest and rainfall null, while the lowest values were registered in March (beginning of spring) (34.2 psu) when the highest rainfall rate was recorded. The average salinity was 35.9±1.1 psu. Water oxygenation ranged from 5.3 (August) to 8.2 mg l^{-1} (March), with an average of 6.7±0.8 mg l^{-1} (Table 1).

Description of gametogenic stages

Microscopic observations showed the presence of both male and female gametes within the same gonad in all histological sections, confirming the simultaneous hermaphroditism of *S. pectinata* (Fig. 2a). The gonad of *S. pectinata* is generally composed of tubules isolated by a scanty and sparse inter-tubular tissue. Each tubule is composed of a germinal epithelium lining the gonad lumen embedded by a wall of connective tissue. The peripheral part of the gonad is occupied by the female acini while the central part is occupied by the male acini (Fig. 2b, 2c).

Five oogenesis stages were defined for the female part of the hermaphroditic gonad: (1) Proliferation stage:

Table 1. Monthly values of seawater and climatic variables in Bizerte Channel during the study period (May 2015 - May 2016). Data for climatic variables are provided by the National Institute of Meteorology of Tunisia.

	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Sea water variables													
T°C	17.6	19.2	23.0	27.4	24.5	21.2	18.5	14.3	12.2	15.3	14.9	15.4	16.3
O2 (mg/l)	6.8	6.5	6.3	5.3	5.7	5.9	6.2	7.1	7.5	7.4	8.2	7.2	6.7
Salinity (ppt)	36.6	36.2	38.0	37.7	36.1	35.5	35.0	36.0	35.6	35.2	34.2	34.3	35.6
Climatic variables													
Average air T°C	20.5	23.9	27.4	27.7	27.0	21.1	17.0	12.5	12.8	13.4	13.5	17.3	19.7
Insolation (h)	312	345	380	340	266	226	177	157	161	181	228	253	315
Rainfall (mm)	20	1	0	40	36	87	82	20	51	50	106	20	15

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characterized by the presence of oogonia and some previtellogenic oocytes (PVO) bordering the acinus (Fig. 3a). Most acini have rounded oocytes attached to the follicular wall. The size of the oogonia range between 15 and 20 μm. (2) Growth stage: the volume of the gonadal tubules increases toward the reserve tissue. The oocytes become larger as the cytoplasmic volume increases and begin to fill completely the acini. The size of the oocytes varies between 28 and 45 μm. In this stage, the simultaneous presence of three types of oocytes can be observed, which in decreasing order of abundance are: early vitellogenic oocytes (EVO), PVO and late vitellogenic oocytes (LVO) (Fig. 3b). At this stage, some oocytes are still attached to the wall (Fig. 3c). (3) Pre-evacuation stage: characterized by a marked reduction of the connective tissue. There is also an increase in the size of the oocytes (58 - 78 μm) which most have an oblong shape (LVO) (Fig. 3d). (4) Evacuation stage: the LVOs become regular in shape (mature oocytes) and invade the lumen of the gonadal tubules and reach the center of the gonad to join the hermaphroditic duct. Oocytes reach a size ranging from 82.5 to 107.5 μm (Fig. 3e). (5) Post-evacuation stage: reached when most of the acini are empty with the presence of some atretic oocytes, some mature oocytes not yet evacuated, and ovogonia at different developmental stages. Altogether this confirms the lack of a resting phase in *S. pectinata* (Fig. 3f, 3g).

Regarding spermatogenesis, four stages were identified: (1) Early developmental stage, in which most acini are filled with dividing and growing germ cells and spermatogonia. This leads to a slight increase in cell volume and to the differentiation of spermatogonia into spermatocytes (Fig. 4a). The size of the spermatogonia varies between 16 and 18 μm. (2) Differentiation stage: spermatocytes and spermatids are the only cells found in the gonadal sections (Fig. 4b). (3) Ripe (or mature) stage: the acini are mainly filled with mature spermatozoa with the presence of few spermatids (Fig. 4c, 4d). The spermatozoa show a head of variable size according to the gonadal section level (4.5 - 5.5 μm) and a flagellum with an approximate length of 20 to 30 μm. (4) Spent stage: the sperm is evacuated, the acini are partially or completely emptied, some of them still showing residual sperm in their lumen (Fig. 4e, 4f).
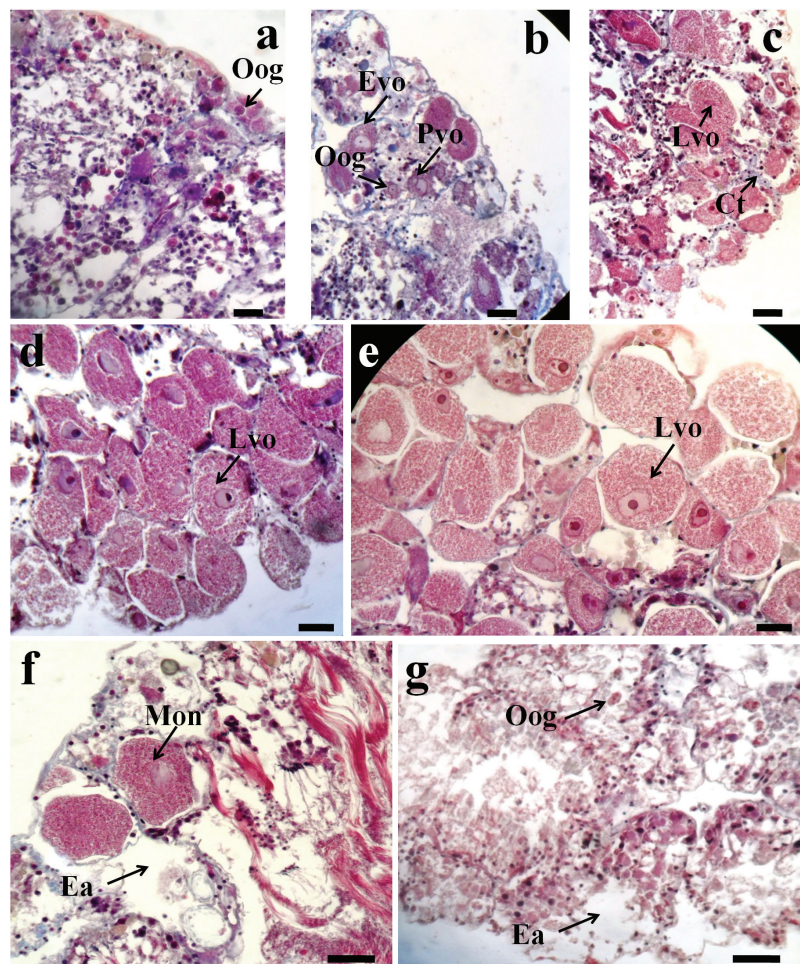

Fig. 3: Female gametogenic stages in *Siphonaria pectinata*. a. Proliferation stage, characterized mainly by the presence of oogonia and previtellogenic oocytes. b-c. Growth stage, characterized by the presence of early vitellogenic and vitellogenic oocytes. d. Pre-evacuation stage, characterized by large vitellogenic oocytes compressed within the acini. e. Evacuation stage, characterized by vitellogenic oocytes detached from the acinar wall and invading the lumen. f-g. Post-evacuation stage, characterized by large empty acini with developing oogonia and non-evacuated oocytes. Scale bar: 50μm. Ct, connective tissue; Ea; empty acinus; Evo, early vitellogenic oocyte; Lvo, late vitellogenicoocyte; Mon, Mature oocyte non-evacuated; Oog, oogonia; Pvo, previtellogenic oocyte.
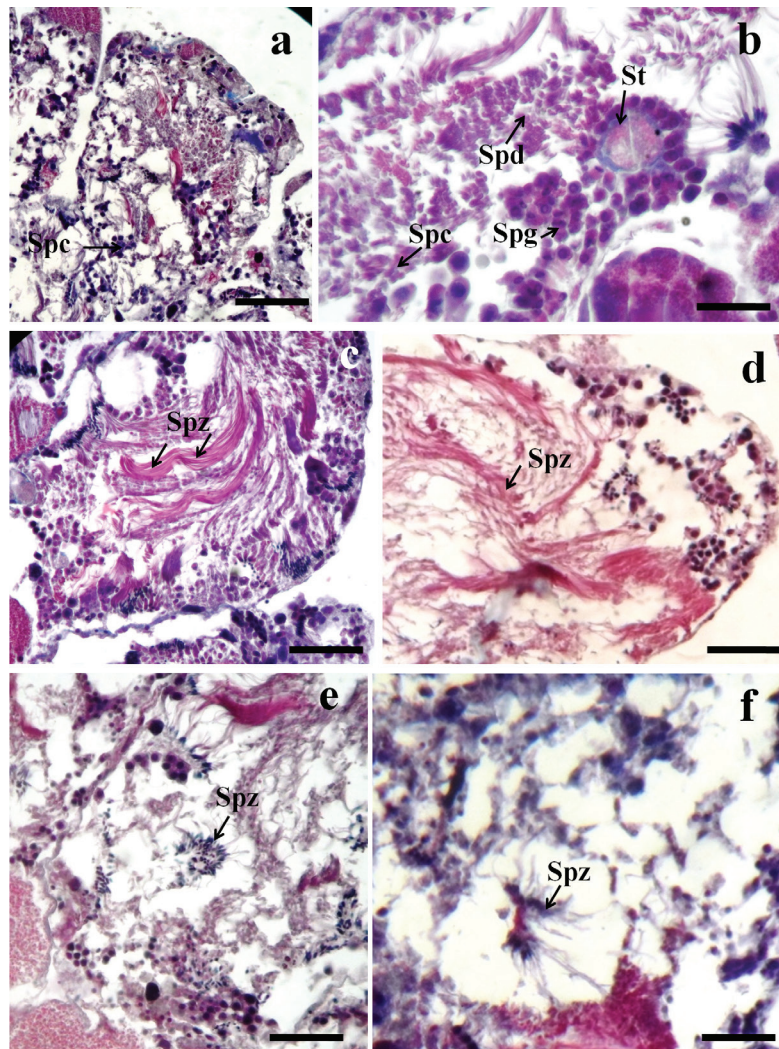
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Fig. 4: Male gametogenic stages in *Siphonaria pectinata*. a. Early development stage, with mainly spermatocytes present. b. Differentiation stage, characterized by numerous spermatogonia and spermatocytes. c. Mature stage, characterized by the presence of spermatozoa with centripetal maturation. d. Advanced mature stage, mainly with spermotozoa. e-f Spent stage, characterized by empty acini. Scale bars=100 μm. Spc, spermatocytes; Ea: empty acini, Spd, spermatids; Spg: spermatogonia; Spz, spermatozoa; St, Sertoli cell.

Gametogenic cycle

The GI of *S. pectinata* increased significantly from November (0.8%) to March, moment when it reached the maximum value of 7.12% (ANOVA Tukey test, $4.7 < q < 41.9$; $P < 0.01$) (Fig. 5). This means that gametogenesis is active and that gonads monthly gain weight. At the histological level, this period was characterised by the dominance of individuals at pre-evacuation stage (63-83.3% of the observed gonads) in the female part, and the dominance of differentiation (60-70% from December to February) and mature (76.7% in March) individuals in the male part (Fig. 6). In the field, no egg ribbons were found neither in December nor January, further confirming that most animals are in gametogenesis phase (Fig. 5). Laid egg ribbons appeared in February while the GI continued increasing, suggestive of an asynchronous reproductive activity among individuals. From March to November, there was a decrease in the GI values mainly from June to July (ANOVA Tukey test, $q=9.9$; $P < 0.001$) and from August to September (ANOVA Tukey test, $q=5.9$; $P < 0.001$),

suggesting that the main spawning events are occurring during these months. These events corroborate the gametogenic stages in the female part showing an increase in the frequency of individuals at the evacuation stage from 16.4% in June to 80% in July. The decrease in GI was accompanied by an increase in the number of egg ribbons laid per individual from May to June and mainly from June to July where the highest value is reached (1.5 egg ribbons per individual) (ANOVA Tukey test, $q=17.33$; $P < 0.001$). Thereafter, egg ribbons continue to be laid in the field but at significantly lower amount (ANOVA Tukey test, $9.38 < q < 14.41$; $P < 0.001$) until November where the number reached 0.03 ribbons per individual (Fig. 5). At the gonad level, this period was characterized by the predominance of the proliferation stage (76.7% of individuals) in October and growth stage in November (53.3%).

In the male gonads, the decrease in GI was marked by high frequencies of individuals at the spent stage from April to July reaching 50-86.7% and in November (76.7%) suggesting that during these months the high
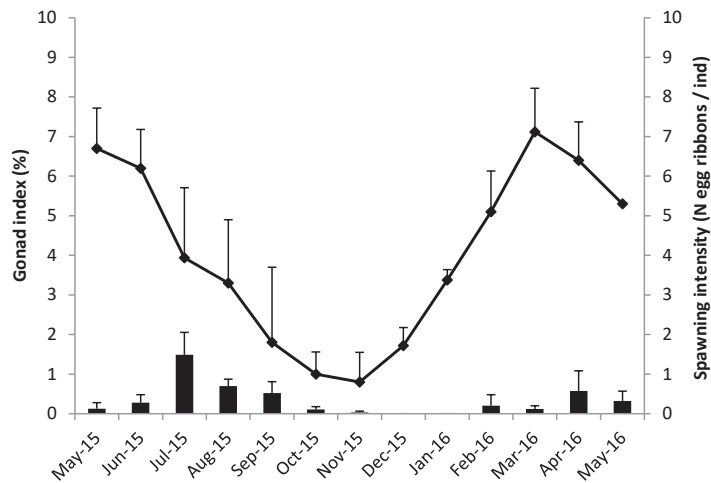
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Fig. 5: Mean monthly gonad index (curve) and spawning intensity (histogram) of *Siphonaria pectinata* during the study period.

a

Proliferation   ■ **Growth**   ■ **Pre-evacuation**
■ **Evacuation**   □ **Post-evacuation**

b

■ **Early developement**   ■ **Differentiation**   ■ **Mature**   □ **Spent**
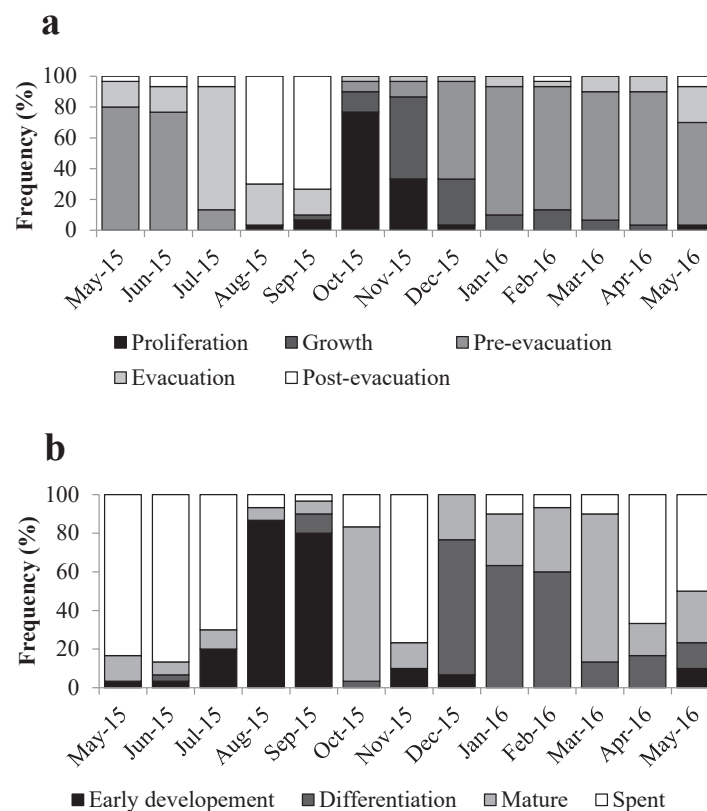
Fig. 6: Monthly frequency of individuals according to prevalent gametogenesis stages in *Siphonaria pectinata* during the study period. a. Female gonad part. b. Male gonad part.

spermatozoa evacuation rate contributed together with the egg-ribbon laying to make gonads lighter in weight.

Discussion

The present work reports the first data on the gametogenic cycle of *S. pectinata* based on histological analysis and calculation of the gonadic index. This study site, Tunisia, is of special relevant given that it represents the easternmost area where the species is naturally present following an intra-Mediterranean climate-driven expan-

sion. The species can be found in the Eastern part of the Mediterranean Sea (i.e. Greece) but there it is believed to have arrived through marine traffic (Giribet & Kawachi, 2015). Our results revealed that *S. pectinata* is a simultaneous hermaphrodite, with female and male gametes being observed at the same time within acini. This finding is consistent with reports from previous studies on other siphonariid species like *S. capensis*, *S. serrata* and *S. lessonii* (Knack de Almeida & Giménez, 2018; Pal & Hodgson, 2005). Zischke (1974) suggested a brief phase of protandry that precedes simultaneous hermaphroditism in *S. naufragum* (identified as *S. pectinata*) collected in

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southern Florida. He reported that egg-ribbons were laid by animals with a minimum size of 7 mm of shell length and that below this size, individuals were males. However, in Gibraltar populations, the presence of mature eggs within gonads was reported in smaller sizes (5.5 mm) (Ocaña & Emson, 1999). These authors considered that this size corresponds to 10 to 15-month-old animals but did not mention the possibility of protandrous hermaphroditism in younger *S. pectinata*. They stated, however, that egg-laying occurring at smaller sizes at Gibraltar may be the result of slower growth rates compared to Florida. A possible explanation for this shift could be the genetic difference recorded between the Eastern and Western Atlantic populations that was evidenced by Puizina *et al.* (2012) and later by Giribet & Kawauchi (2015). The latter indeed proved that the population studied by Zischke (1974) and the populations present in Florida and along the Western Atlantic coast is actually *S. naufragum*. Because we focused on larger specimens in the present work (>10 mm SL), further histological investigation is need to clarify if *S. pectinata* exhibits a brief protandrous hermaphroditism or not.

Our analysis of the monthly variation in the frequency of gametogenic stages indicates that *S. pectinata* has a continuous reproductive cycle and that both oogenesis and spermatogenesis occur throughout the year like in other siphonariids such as *S. capensis*, *S. serrata* and *S. lessonii* (Knack de Almeida & Giménez, 2018; Pal & Hodgson, 2005). Regarding oogenesis, individuals at the proliferation stage were only observed during 6 months (May and from August to December), with the highest frequencies being recorded in October and November (mid-autumn). The growth stage was present during 8 months, from September to April, with a maximum frequency of individuals registered in November and December (late autumn / early winter). Subsequently, individuals at the pre-evacuation stage appeared in October and were present until July, with highest frequencies from December to June (beginning of winter-beginning of summer). The evacuation occurs throughout the year but mainly from May to August (late spring-late summer) with a maximum in July (mid-summer), in agreement with the spawning frequency recorded in the field. No egg masses were observed in December and January, although individuals at the evacuation stage were observed at a 3.3-6.7% frequency, which could be attributed to the environmental stressors. These may include osmotic stress from rain-derived freshwater that may affect spawning or the effect of storms, which are more prevalent during this time of the year and which can cause physical dislodgement (Levings & Garrity, 1986; Iwasaki, 1995). In some siphonariid species, such stressors resulted in an adaptive spawning behaviour consisting in the placement of egg masses in sheltered mirohabitats like depressions, crevices and cracks (Levings & Garrity, 1986).

The post-evacuation stage, characterized by the predominance of empty gonad acini, was present during 8 months, but prevalent in August and September (late summer - beginning of autumn). Although using a different methodology (frequency of individuals in the prevalent gametogenic stages instead of frequency of gametogenic stages), the gametogenic cycle we here describe resembles the one of *S. lessonii* present in Argentina, but with fewer time-shifts (Knack de Almeida & Giménez, 2018). According to these authors, proliferation (5-9 months) predominated in April and May (beginning of austral autumn, growth (6-8 months) in late autumn and beginning of winter and pre-evacuation (9 months) from late autumn, to the beginning of spring. The same work shows the evacuation stage to occur from late winter to mid-summer (9-11 months), and post-evacuation (8 months) from late spring to the beginning of autumn being predominant for 5 months (30-50%) with a maximum in March (late summer) (Knack de Almeida & Giménez, 2018). In *S. pectinata,* individuals at the post-evacuation stage were observed for 8 months but with highest frequencies recorded only in August (70%) and September (73.3%) suggesting that the gonad of *S. pectinata* is more active and returns faster to oogenesis. Indeed, not fully spent gonads were observed in the present study, as there were always regenerated acini. The amount of PVOs found during post-evacuation was higher than that observed during evacuation, further supporting this conclusion. In *S. capensis* and *S. serrata* from the south-eastern coasts of South Africa, despite using a different description of the stages of gametogenesis, the main gametogenetic activity was reported to occur later. Growth was observed especially in early spring, pre-evacuation in summer (peak), evacuation from mid-summer to mid-autumn and post-evacuation from autumn to winter (Pal & Hodgson, 2005).

As regards spermatogenesis, individuals at the early development stage occurred mainly from late summer to the beginning of autumn while individuals at the differentiation stage were mainly present in winter. Mature individuals (evacuation stage) were especially frequent during mid-autumn and mid-spring, and individuals at the post-evacuation stage (spent gonads) from mid-spring to mid-summer. In *S. lessonii*, the early development stage occurs mainly in mid-summer, differentiation in mid spring, evacuation during winter and spring, and post-evacuation by the end of spring and throughout the summer (Knack de Almeida & Giménez, 2018).

The GI values were in agreement with the gametogenic cycle showing a maximum value in March, coinciding with the highest frequencies of limpets at pre-evacuation and maturation stages for oogenesis and spermatogenesis, respectively. The minimum value was reported in November, corresponding to the predominance of the individuals at proliferation and growth stages in female gonad and at the spent stage for male gonad. The GI values were also in concordance with the spawning intensity recorded in the field, thus we suggest the value of using this index for routine and fast monitoring of the reproductive cycle of *S. pectinata* and other siphonariids pending its verification at other locations.

The asynchrony and difference in the extension of the gametogenic stages reported in *S. pectinata* in comparison with other siphonariids could be related to the environmental conditions, mainly sea water temperature,

food availability and day length (Hirano & Inaba 1980; Knack de Almeida & Giménez, 2018). Indeed, it has been shown that reproduction in *S. lessonii* coincides with longer days and warmer temperatures (Knack de Almeida & Giménez, 2018). These authors reported that gonads were in evacuation and post-evacuation stages, with fully developed oocytes, from spring to summer, suggesting that reproduction occurred during the hottest months. Such pattern has also been recorded in the present study, with spawning occurring mainly in mid-summer. However, this point should be verified with a follow-up study outside the Bizerte channel as environmental factors could change within the same location.

However, summer heat may also lead to increased mortality rates, directly as a result of exposure of adults (as reported in populations of *S. japonica* in Hong Kong) (Liu, 1994) or indirectly, due to starvation of individuals because of low algal productivity as shown in *S. diamenensis* on the Australian coasts during the austral summer (Quinn, 1988). Considering that *S. pectinata* is an alien species on the Tunisian coast, such a warm and sunny environment (Table 1) is suspected to be physiologically more stressful in comparison to its original biotope (eastern Atlantic coast). In this case, temperature may affect survival rates and the associated planktotrophic larval development, since larval period will be relatively longer when conditions are suboptimal (lower food availability and higher temperature) (Hoegh-Guldberg & Pearse, 1995; Pechenik *et al.,* 2003). In this sense, larvae with a potentially decreased size and a longer planktotrophic phase will be more vulnerable to predation assuming that they are predated at similar rates compared to their original biotope (Thorson, 1950). The results of the present study support the hypothesis that *S. pectinata* is well adapted to the Tunisian environment as the spawning intensity is higher than in other regions such as Gibraltar, and the spawning period longer (Ocaña & Emson, 1999): these authors reported a spawning period in the field ranging from 4 to 6 months with an intensity of 0.05 to 0.4 egg ribbons per individual against 10 months and 0.03-1.49 egg ribbons recorded in the present study. Furthermore, the fecundity recorded in Tunisian *S. pectinata* was higher than that of Gibraltar population, further suggesting that this alien false limpet is well adapted to the Tunisian environment (Slama *et al.,* 2018). However, as *S. pectinata* fecundity is size dependent (Slama *et al.,* 2018), we should note that the smaller size range sampled for the Gibraltar study may partly be influencing the differences in spawning effort among our results. In that western Mediterranean basin, factors other than temperature (e.g. predation, substrate topography and exposure to wave action) may also be having an impact of the reproductive biology (Ocaña & Emson, 1999), and should be thus considered in inter-site comparisons. Indeed, very significant effects on population dynamics, including spawning intensity, growth rates and mortality were observed in adjacent sites in Gibraltar with distinct topography (Ocaña & Emson, 1999).

Conclusion

S. pectinata is a simultaneous hermaphrodite species, with a continuous gametogenic activity at the Bizerte channel, producing both sperm and eggs throughout the year. Such a reproductive strategy, combined with a free-swimming planktotrophic veliger of 36 days before settlement (Slama *et al.,* 2018), allows this alien gastropod to successfully colonize the Northern Tunisian coastline. Altogether, the results of the present study provide the first histological description of the gametogenic cycle of *S. pectinata* and support the hypothesis that this species has not yet found its easternmost limit of distribution within the Mediterranean Basin.

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