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Scrobicularia plana (Mollusca, Bivalvia): impact of chronic pollution on the reproductive cycle of a population living in an estuary under an arid climate

L. Lefrere[1], H. Bergayou[1], A. Moukrim[1]

[1] *Laboratory of Aquatic Systems Marine and Continental Environment, Ibn Zohr University, BP 8106 Dakhla Agadir Morocco*

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l.lefrere@uiz.ac.ma ;
Phone: +212666073159

Abstract

Field investigations, on two populations of *Scrobicularia plana* (*Mollusca*, *Bivalvia*), were carried out over a year (2001-2002) in the estuary of Oued Souss (southwestern Morocco) to determine the effect of chronic pollution (wastewater) on the reproductive cycle of this species under an arid climate. Monthly samples of *S. plana* (length, 25 to 35 mm), were thus collected in two stations of the estuary, the first being contaminated by wastewater while the second was non-polluted. The sex ratio of *S. plana* in the two populations was well-balanced (1:1). In the non-polluted population, two periods of reproduction are determined, the first occurring from January to April and the second in July-August. In contrast, in the community living in the polluted habitat, a single period of reproduction (from January to April), followed by gonadic rest from June to September, was found. The presence of a single reproductive period in the *S. plana* living in the polluted habitat may be explained by the effect of pollution or the cumulative effect of pollution and high temperatures of summer.

1. Introduction

Scrobicularia plana (Da Costa, 1778) is a worldwide species, which lives on the Atlantic coasts, from Norway up to Senegal, and along coasts of the Mediterranean Sea except in the Black Sea [1]. This bivalve is locally abundant in screened zones [2-3]. *Scrobicularia plana* is also an important species in shallow water benthic communities [3-4] and commercially exploited in several European countries [5-6-7].

From the Mediterranean Sea until the north of Europe, the reproductive cycle of *S. plana* showed variations, which were, especially imputed to latitude [8-9-10]. In Morocco, this bivalve's reproduction was studied in several estuaries of the Atlantic coast, such as that of Oued Bou Regreg [11-12-13] or that of Oued Oum Er Rbia ([14-15] under the conditions of a semiarid Mediterranean climate. These authors have mainly studied the periods of settlement for young bivalves and the fluctuations of dry weight of molluscs for a given age group to determine the periods of gonad maturation. However, no follow-up of gametogenesis was performed. Otherwise, a comparative study of the reproductive cycle of this bivalve was conducted in Moroccan lagoon systems under an arid climate, in Oulidia (in the North) and Khnifiss (in the South) lagoons [16].

The first aim of this work was to determine the characteristics of the reproductive cycle of *S. plana* by studying two populations, which live under an arid climate, as those existing in southwestern Morocco. The Moroccan Atlantic coast and, especially, paralic environments (estuaries or lagoons) were subjected to a great anthropic pressure, thus producing pollution due to the discharge of industrial or urban wastewater in the rivers and the Atlantic Ocean, as there were no still efficient systems in Morocco for the treatment of wastewater [17-18]. As the impact of contaminants on the reproduction of marine bivalves, under an arid climate was little known [19-20], one may wonder what changes in the reproductive cycle of *S. plana* occurred when this species was living in an estuary contaminated by wastewater discharges [21]. To answer this second question, a comparative study on the reproductive cycle of two scrobicularidae populations was performed, the first living in a habitat subjected to chronic pollution and the second in a zone located in the same estuary but at 1.5 km from the first population.

2. Material and methods

2.1. Sampling sites

The estuary of Oued Souss is located on the Atlantic coast, in southwestern Morocco, and is subjected to an arid climate. The temperature of air slightly varied throughout the year (14°C in January, 23°C in August) with an annual mean of 18°C. The quantity of water carried out by the river was small enough (in reason of the Aouloz dam, located at 160 km from the river mouth), and rainfall was also limited over time (100 mm per year, especially in winter). The single permanent supply of water was that of wastewater, originating from the neighbouring towns. Conversely, the estuary was swept by an intense marine hydrodynamism, with currents linked to high or low tides. This situation was the cause of a high salinity and of great sandbanks in the estuary, so that the exchanges between fresh and sea water were limited, thus making the departure of pollutants from the estuary more difficult.

The non-polluted station (30° 21' 976" north, 9° 35' 981" west), and the contaminated site (30° 21' 794" north, 9° 35' 313" west) were situated in the estuary (Figure 1). The latter station was located near several discharges of untreated urban wastewater, originating from the towns of Ait Melloul, Inezegane, and Tarast (16,000 m3 per day). The abiotic parameters for both sites were different. The sand from the non-polluted site was silted up and its granulometric median was greater than that recorded in the contaminated site, as the latter, was covered by clayed and silted deposits (150-180 µm instead of 50-70 µm). By contrast, the percentage of lutites (60% instead of 5% in the non-polluted site), the content of sediment in organic matter (5% instead of 1%), and the matters in suspension in water (320-840 mg/l instead of 340-440 mg/l) were greater in the contaminated station. The difference existing between the types of sediment and their texture was the cause of a great dampening rate in the sediment from the non-polluted site.

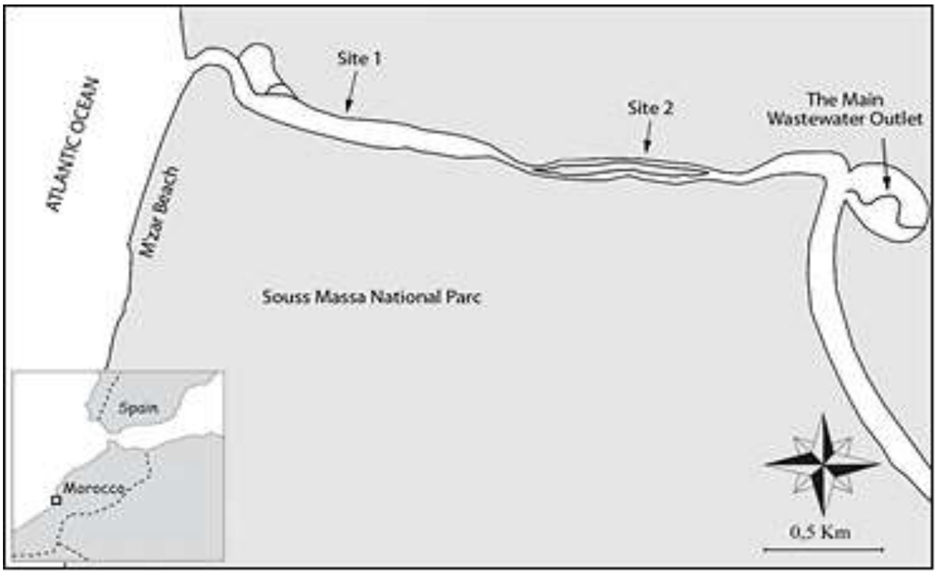


Figure 1. Location of the two sites colonized by Scrobicularia plana in the estuary of Oued Souss in the Bay of Agadir, southwestern Morocco.

2.2. Sampling of Scrobicularia plana

Every month, samples of bivalves (length, 25 to 35 mm) were made, at low tide. The histological examination, the determination of gonadic index, and stereological analysis were performed each month, from January 2001 to March 2002 in the polluted population (30 *S. plana* per sample) to study the impact of pollution on the annual reproductive cycle of these bivalves. However, as gonad smears allowed studying a greater number of *S. plana*, monthly samples, each composed of 100 bivalves, were collected from both stations between August 2001 and August 2002. A similar method was used for the determination of the condition index. However, the latter were only established on a randomly chosen sample of 30 *S. plana* per site and per month.

2.3. Gonad smears and sex ratios

The bivalves were removed from their shells, and their visceral masses were rubbing against histological slides. Microscopic examinations were made to study the reproductive cycle of *S. plana* using the scale of Lucas (1965)

[22] and comprising four stages (from A to D). The sex ratio (number of females in relation with the total number of recognizable-sex bivalves) was performed when this identification was easy. A Khi2 test was used to determine the levels of statistical significance.

2.4. Condition index
It represents the variations of dry weight for a standard bivalve and aims to eliminate the effects of mollusc growth while allowing revealing an accumulation or a loss of organic matter, associated with reproduction. The index selected was that proposed by Lucas & Beninger [23]: IC = [(dry weight of soft masses)/(dry weight of valves) x 1000]. The dry weight was obtained using a dehydration of soft masses (or shell) in a desiccator (60°C, 24 hours).

2.5. Histological study of gonads
The shells of *S. plana* were opened and soft masses were prefixed in the Gendre's fixative for 24 hours. In the laboratory, the shell of each bivalve was removed and small pieces of soft masses were post-fixed in a new solution of Gendre's fixative for 48 hours before being dehydrated through a graded series of ethanol and butanol, and finally embedded in cytoparaffin (56°-58°C). Serial sections, (thickness, 5 µm), were made before being stained with Gabe's trichrome, hemalun-eosin, or Mann-Dominici's method [24]. The maturity of gonads was determined using the scale proposed by Lubet [25] for *Mytilus edulis*.

2.6. Gonadic index
It indicates the state of gonad maturity for each population, and it was evaluated from histological slides. This index [26] was determined by giving a number to each Lubet's gametogenic stage: stage zero (number 1), stages I and II (2), stage IIIA (3), stages IIIB and IIIC (2), and stage IIID (1). For each sample of mussels, the corresponding number multiplies the number of gonads showing a gametogenic stage. The figures obtained were then added and the total number of mussels studied then divided the sum. This gonad index varied from one (all gonads were spent, with completely empty lumina) to three (all gonads were ripe).

2.7. Stereological analysis
The analysis was made on the whole bivalves used for the histological examinations of gonads. For each *S. plana*, three histological slides were randomly chosen through the antero-posterior axis. The different cell categories present in gonadic follicles were counted on five ocular fields (magnification: x 100 for females, and x 400 for males) randomly selected in the visceral mass. In the females, four categories were considered: oogoniae, vitellogenic oocytes, ripe oocytes, and atresic oocytes. In the males, the four categories were the protogoniae and spermatogoniae, the primary and secondary spermatocytes, the spermatids and spermatozoa, and, lastly Sertoli's cells. The mean percentage of each cell category was calculated in relation to the total number of cells counted. For each cell category, mean values and corresponding standard deviations were established.

3. Results and discussion

Out of a number of 1000 bivalves per site, the sex ration of *S. plana* was 52.2% (± 1.8%) in the non-polluted population and 52.7% (± 2.0%) for that which lived in the contaminated habitat. However, the sex ratio was well-balanced at 1:1 (Khi2 = 2.5; $P < 0.05$ in the former population, and khi2 = 2.6; $P < 0.05$ for the latter). In both communities, no hermaphrodite individuals were found.

In both populations studied, the sex ratio of *Scrobicularia plana* was well-balanced so that pollution did not have an influence on the distribution of males and females. Rodriguez-Rua et al. [10] already reported a similar finding in a scrobicularid population, living in the Guadalquivir estuary (Portugal). By contrast, the results of the present study disagreed with the paper described by Roubineau [27]. According to this author, males were slightly greater in numbers to females in the populations living in the estuary of the Loire river (France) but it is important to note that the number of bivalves studied by Roubineau [27] was small (200 only instead of 1000 per population in the present study). For *S. plana* from Moroccan lagoons, and as reported by Lefrere et *al.*, [16], in Oualidia lagoon as in Khnifiss, the sex ratio was averaged 2:1 in favour of females without significant differences. Moreover, both the sex ratio and gametogenesis activity were influenced by other parameters, especially temperature as reported by Zapata-Restrepo et *al.*, [28].

Figure 2 shows several differences in the sexual cycles of both populations. In the non-polluted habitat, two periods of gamete maturation (C-stage), the first ranging from January to April and the other occurring in July-August were noted. In the contaminated habitat, only the first period was observed. From June to September, 75% to 88% of *S. plana* were in sexual rest (A-stage). By contrast, in the non-polluted population, the A-stage was found in 70% of individuals in September-October. However, this fact must correspond to a previtellogenic phase rather than sexual rest, as the percentage of S. plana showing C-stage steadily increased from September.

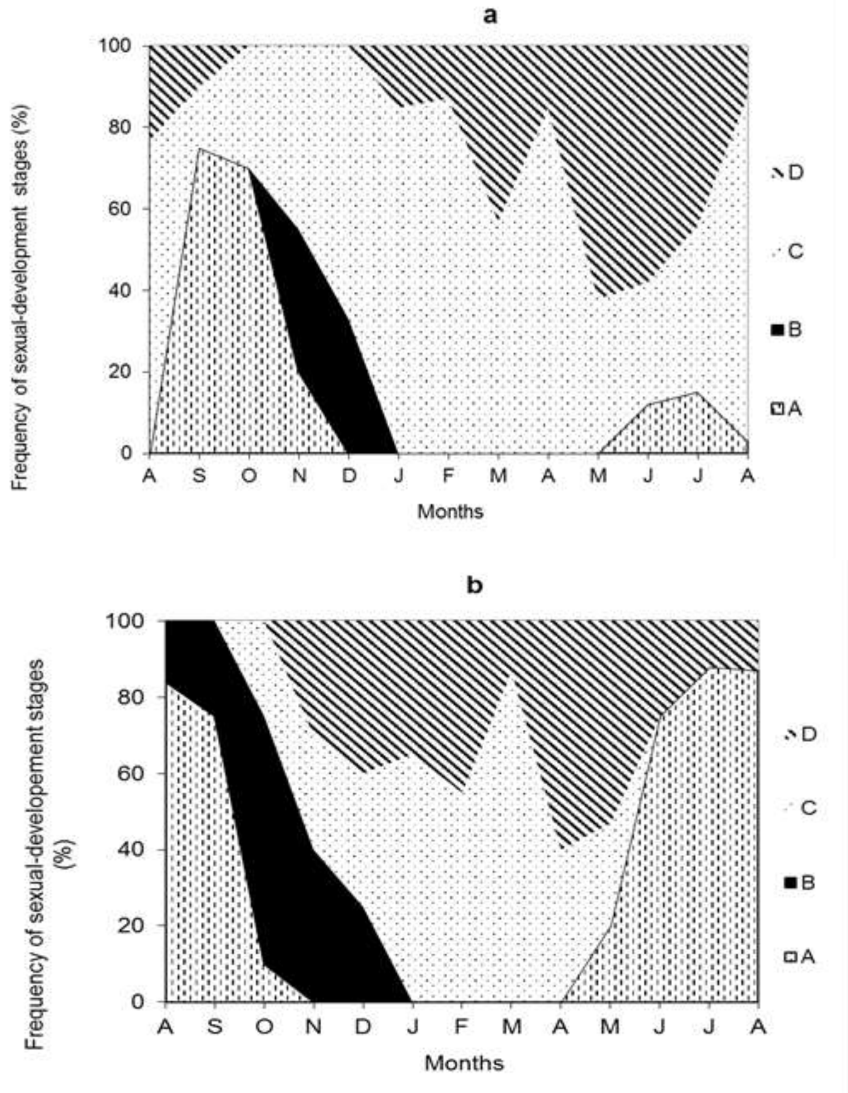


Figure 2. Distribution of sexual-development stages (according to the scale by Lucas, 1965) in both populations of Scrobicularia plana (a: site 1 ; b: site 2) from August 2001 to August 2002. Stages: A (unforeseeable gonad: sexual rest, previtellogenesis, or gamete resorption). B (sex detectable with difficulty to the naked eye). C (identifiable gonad: the foot is salmon-stained in males and pearly white in females). Gonad maturation and spawning occurred during C-stage. D (gonadic reconstitution, with the co-existence of empty follicles and of tubules showing numerous goniae). The cumulated frequencies of the different developmental stages corresponded to the whole bivalves studied (100%).

In the non-polluted population, the condition index (Figure 3) showed a succession of numerical decreases. The most important (in March, May, and September) coincided with the falls noted in the frequencies of C-stage. It is interesting to consider August, as the bivalves recovered some weight in the course of summer before their spawning in September. In the population living in the contaminated site, the index decreased in February and also showed an important fall in April. This last decrease perfectly coincided with the decline in the frequency of C-stage, thus indicating the existence of a spawning period in April. From May to September, the values of this index steadily diminished and this fact corresponds to a steady loss of weight which occurred after spawning in April and became more marked during summer.

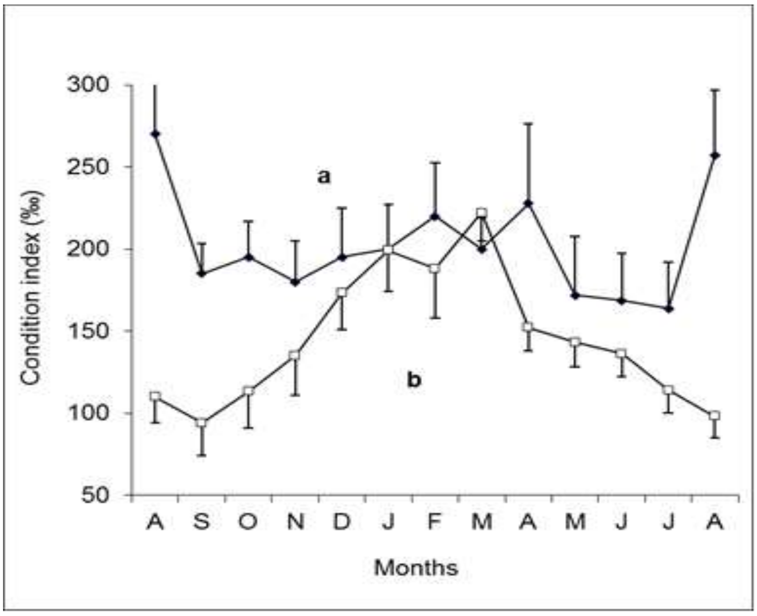
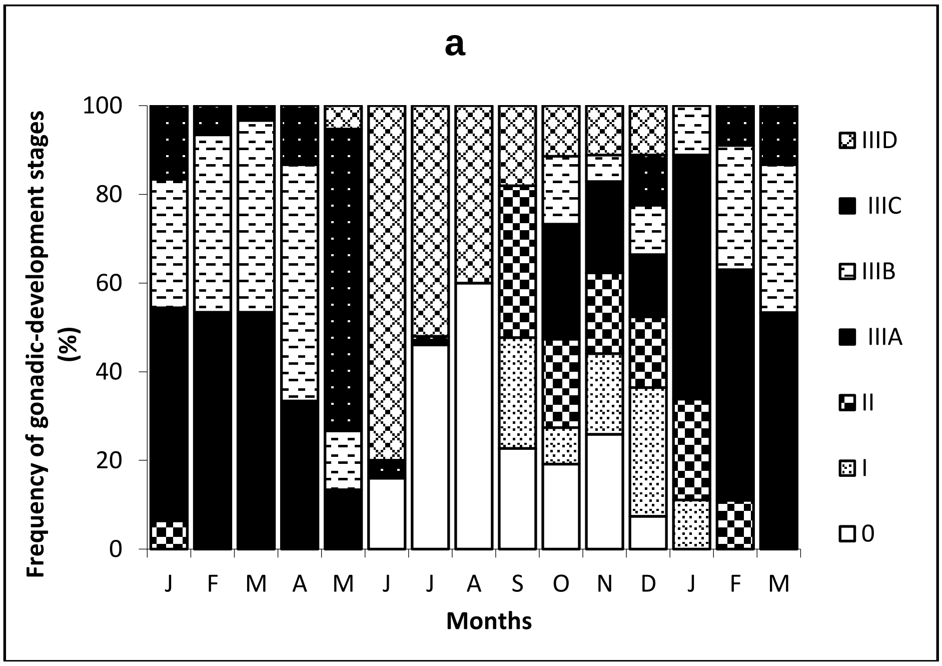


Figure 3. Annual cycle of the condition index in both populations of *Scrobicularia plana* (a: site 1; b: site 2) from August 2001 to August 2002.

A synchronism in the onset and the succession of gonadic-development stages can be observed in males (Figure 4a) and in females (Figure 4b). The start of gametogenesis (I stage) occurred from September and still persisted in January. The population was still heterogeneous, as individuals with 0 stage (sexual rest), IIIB stage (spawning), and IIID (gamete resorption) can be found. The maturation of the gonad (IIIA stage) started in October and its frequency was maximum between January and March (50%). Episodes of partial spawning were noted between October and March. The reconstitution of the gonad (IIIC stage) was dominant in May (> 60%), thus indicating that main spawning occurred between April and May. From June to August, the cumulated frequencies of bivalves with IIID stage and 0 stage were higher than 75%. Moreover, the digestive gland of these last individuals showed areas of epithelial necrosis. The examination of the gonadic index (Figure 5) gives the same conclusions. The lowest values were recorded from June to August (cote 1) and this fact corresponds to sexual rest. By contrast, the highest values ranged from January to May (cote 2.5) and this period corresponds to the maturation of the gonad and spawning.



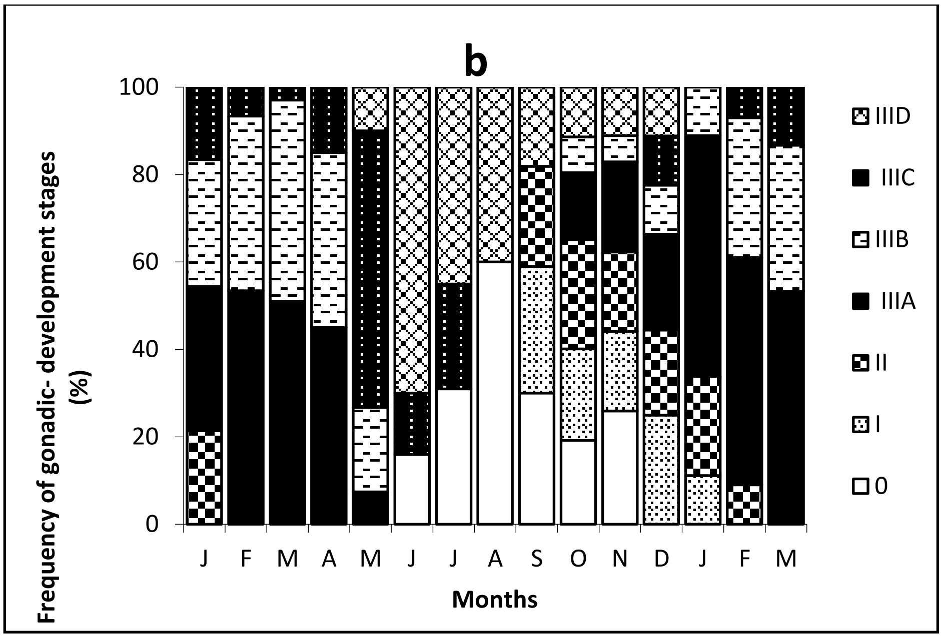


Figure 4. Distribution of gonadic-development stages over 2001-2002 in the males (a) and females (b) of Scrobicularia plana. Stages: 0 (sexual rest), I (early gametogenesis with numerous gonia), II (actively developing gonads but mature gametes were not observed), IIIA (near ripe follicles with mature gametes), IIIB (spawning, follicles distended), IIIC (partially spawn, partially empty lumina), and III D (spent, completely empty lumina). The cumulated frequencies of the different developmental stages corresponded to the whole bivalves studied (100%).

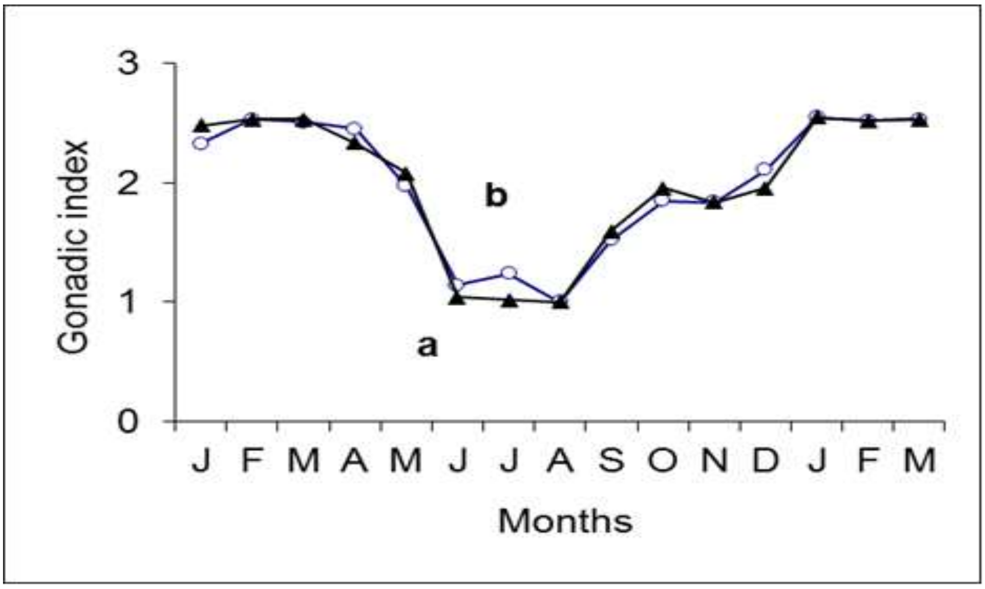


Figure 5. Annual cycle of the gonadic index in the males (a) and females (b) of *Scrobicularia plana* between January 2001 and March 2002.

Figure 6 gives the results of stereological analysis. In females (Figure 6-B), gametogenesis was intense from September to January, as the cumulated frequencies of oogoniae and vitellogenic oocytes were 50% or more during in this period. In males (Figure 6-A), a similar finding was observed with cumulated frequencies of spermatogoniae and spermatocytes higher than 45% between September and February. From January to April, the percentages of mature gametes also increased in the two lines, as the frequencies of spermatids and spermatozoa augmented from 48 to 76% while the rates of ripe oocytes raised from 30 to 68%. During this period, the frequency of Sertoli's cells was also increased. In May, the high percentages of young germinal cells, in males (45%) and in females (52%), indicated a sudden resumption of gametogenesis. By contrast, in June, the female follicles contained more than 85% of degenerated oocytes while the male follicles were contracted, with agglutinated spermatozoa and the presence of a thin layer of spermatogoniae along the inner wall of follicles. In

July and August, the frequencies of degenerated oocytes were more than 80% in females whereas the male follicles were small and flat, with several spermatozoa.

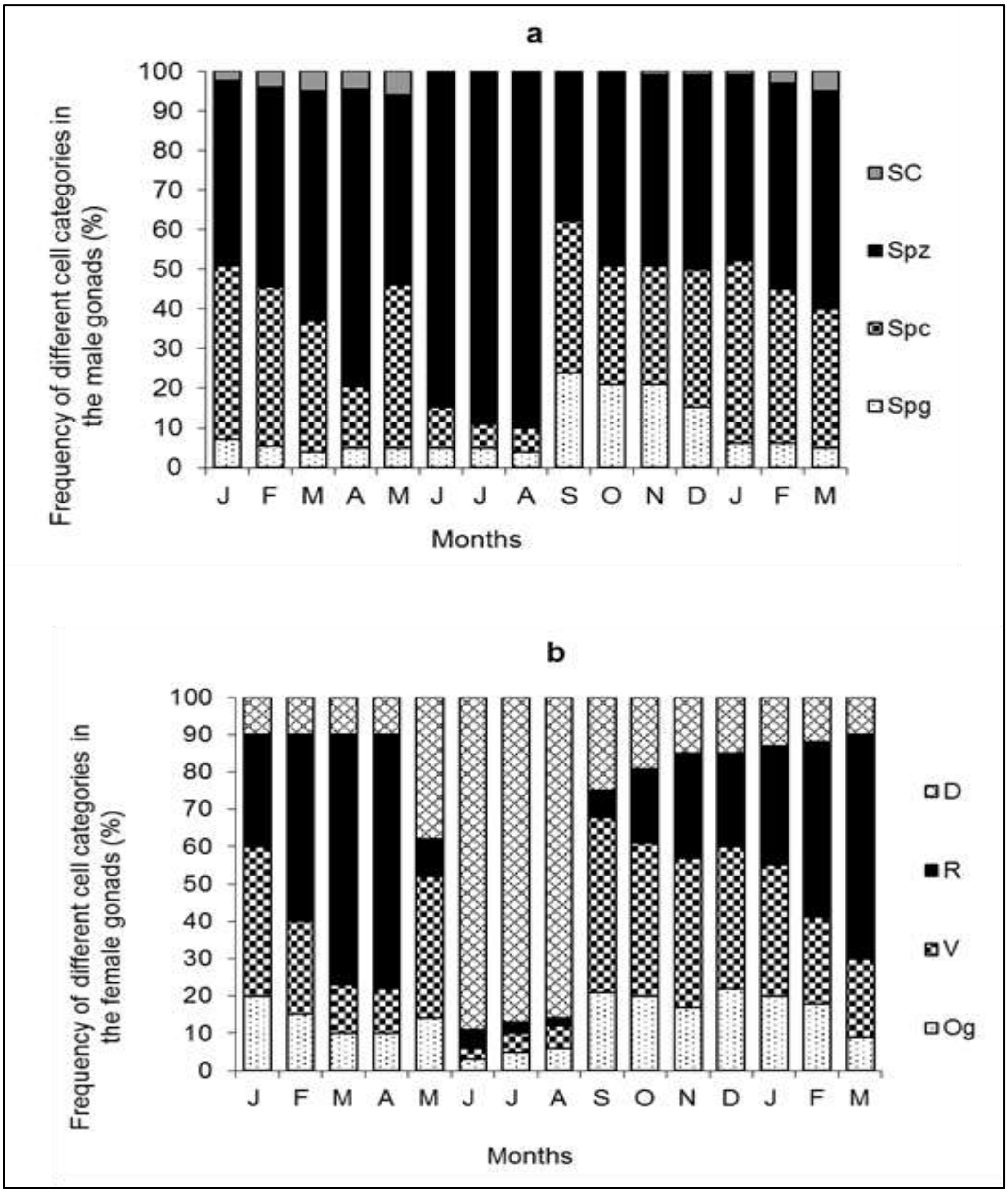


Figure 6. Frequencies of different cell categories in the male (a) and female (b) gonads of *Scrobicularia plana* collected from the contaminated site between January 2001 and March 2002. The cumulated frequencies of the different cell categories corresponded to the whole bivalves studied (100%).

Two periods of reproduction were noted, the first occurring from January to April and the second in July-August in the non-polluted population. The results disagreed with the unimodal and late cycle, described by Elkaim [11] in the populations of *S. plana* living in the estuary of Oued Bou Regreg (Morocco). According to this author, the maturation of gonads was all the more late as the populations were distant from the mouth of the river and the sexual cycle showed a period of rest from October to December. On the other hand, our results agreed with the reports by Kouradi [12] and Cheggour [13] on scrobicularid communities, which were living in the estuary of Oued Bou Regreg, or with the observations by Benabdellaoui [15] on the populations inhabiting the mouth of the Oued Oum Er Rbia. Essink et *al.*, [29], might explain this difference in the reproductive cycles of Moroccan populations of *S. plana* with his paper. According to these authors, the occurrence of spawning period, for the same locality, may show variations from a year to another year in relation to climatic conditions [28-29]. In our opinion, the non-polluted population from the Oued Souss could be placed in the southern group of *S. plana*, known to have a very spread or bimodal reproduction over the year [10-30-31]. The existence of spawning periods staggered over time was explained by an effect of latitude [10-32-33-34] and, in particular, by water temperature [28-30]. When temperature was less than 10°C, it induced early spawning, followed by gonad reconstitution and a second period of spawning. According to Lubet et *al.*,[35], Lubet & Aloui [36], water temperature was not the single acting factor to explain the variations in the reproductive cycle of these bivalves, as other environmental factors may act in synergy in the modulation of this cycle [28].

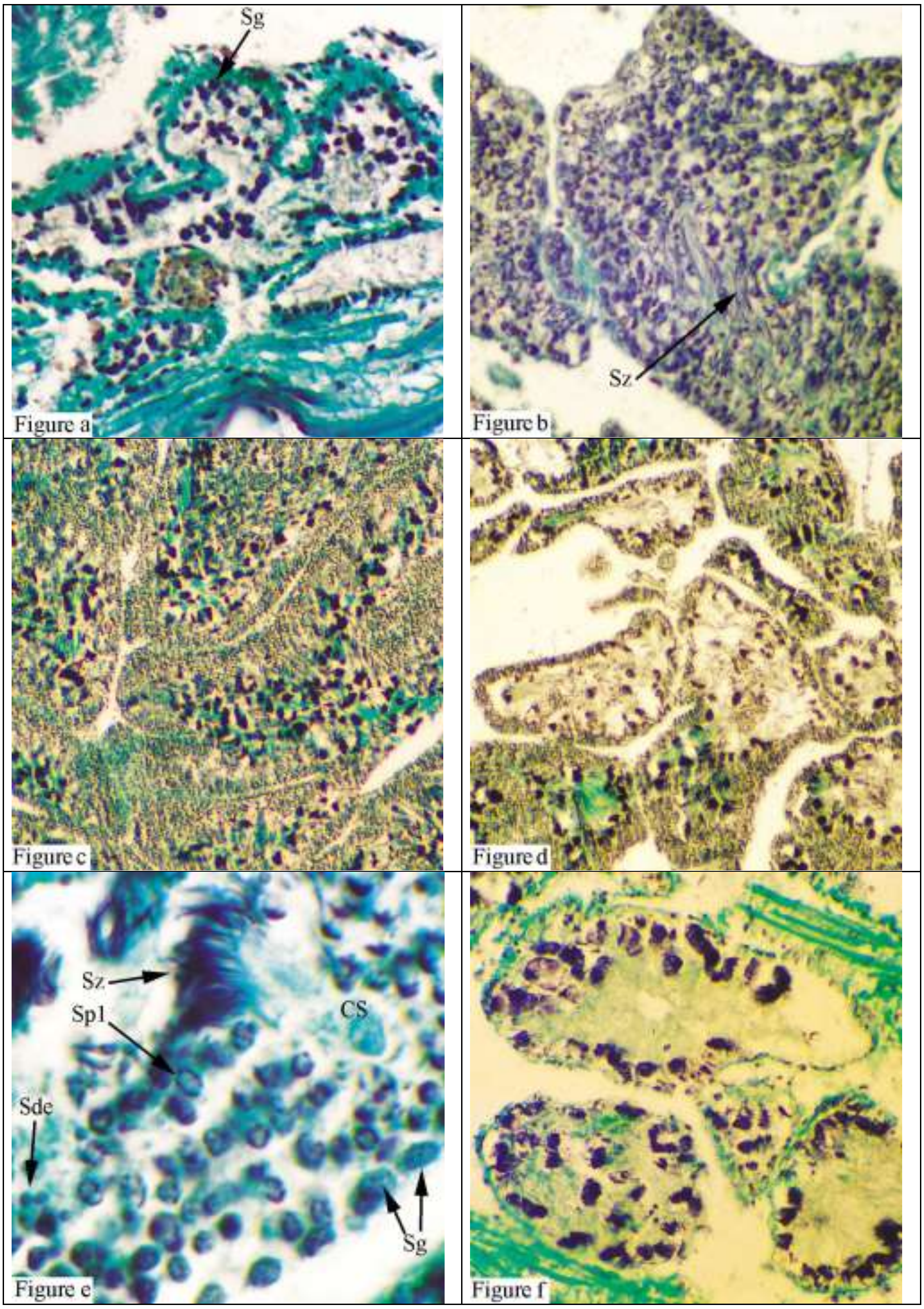


Figure 6-A. Frequencies of different cell categories in male line (figure a, b,c, d, e and f): SC (Sertoli's cells), Spc (primary and secondary spermatocytes), Spg (protogoniae and spermatogoniae), Spz (spermatids and spermatozoa).

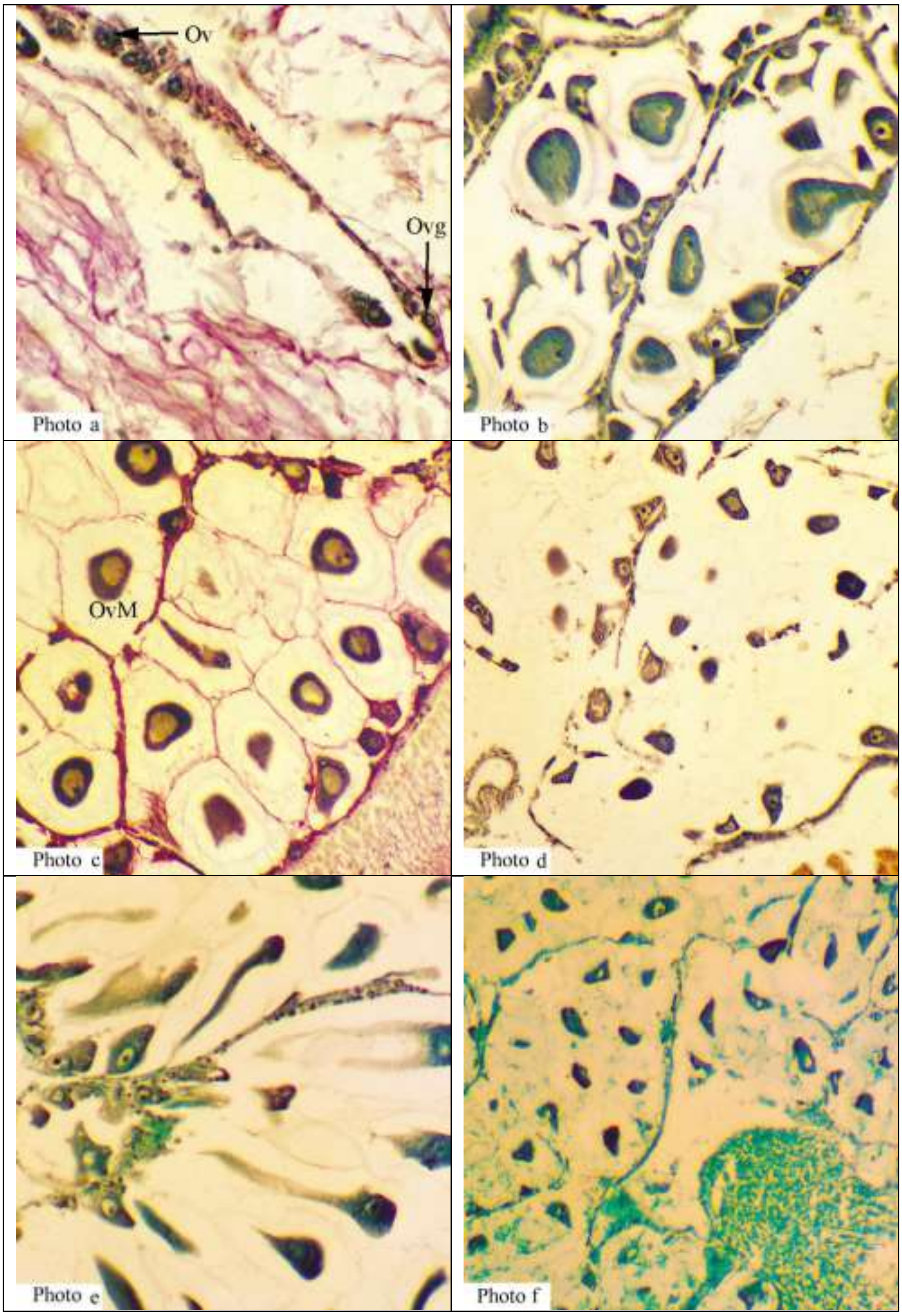


Figure 6-B. Frequencies of different cell categories in female line (photo a, b, c, d, e and f): D (degenerated oocytes), Og (oogoniae), R (mature oocytes), V (vitellogenic oocytes).

Contrary to the non-polluted population, several differences for the *S. plana* were noted, which lived in the contaminated site, as there was a single period of reproduction from September to April, followed by sexual rest between June and September. Moreover, the digestive gland of the bivalves showed areas of epithelial necrosis during summer and this fact indicated that bivalves were situated under conditions of prolonged fast [38]. In our opinion, the results obtained in the population living in the contaminated site might be explained with two probable complementary hypotheses:

- The first supposition is to admit that these bivalves would use up much energy to fight against the effects of pollution, thus causing a deficit in energy elements. Indeed, according to Gabbott & Bayne [39], the carbohydrates of *Mytilus edulis* were used during gametogenesis and served as energy resources for metabolism in autumn and winter before the use of proteins when the fast of mussels persisted. An argument in support of this first hypothesis was the fact that great variations in the gametogenic cycle of bivalves were noted in the seawaters of industrial zones owing to the accumulation of toxic metals within mollusc bodies [40]. The exposure of mytilids to contaminants slowed down gamete development [41-42] and the resorption of gametes found in some bivalves of contaminated zones was often due to environmental conditions [43-44].
- The second hypothesis is to relate the findings noted in the present study to high temperatures of summer which happened from June to September in the estuary of Oued Souss. However, this environmental factor did not act alone, as the non-polluted population showed a second episode of spawning during this period. A mixed effect of pollution and summer temperatures would be more conceivable, as the sediment of both studied sites underwent more easily desiccation at low tide in summer. According to Gäde [45], Meinardus & Gäde [46], glycolysis provided the main part of energy supplies (in ATP equivalents) when anaerobiosis was more than 4 hours (within 4 hours, glycolysis only provided 45% of energy).

The two abovementioned hypotheses proposed for the changes in energy reserves may only be verified by furthers experiments to determine the scrobicularid contents for the three types of metabolites (glycogen, lipids, and proteins), to analyse the synchronism between the reproductive cycle of *S. plana* and that of storage reserves, and to specify the existence of an inter- and intrapopulation variability [47].

Conclusion

The experimental results reported in this paper revealed that the sex ratio of *S. plana* in the two populations was well-balanced (1:1). In the non-polluted population, two periods of reproduction are determined, the first occurring from January to April and the second in July-August. In contrast, in the community living in the polluted habitat, a single period of reproduction (from January to April), followed by gonadic rest from June to September. The presence of a single reproductive period in the *S. plana* living in the polluted habitat may be explained by the effect of pollution or the cumulative effect of pollution and probably to high temperatures of summer.

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