Foot Abnormalities in *Venericardia antiquata* and *Venus verrucosa* from the Bizerte Lagoon Complex (Northern Tunisia): Hydrodynamics and Sediment Texture Inductions

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Abstract

The Examination of the soft part of the two bivalve species *Venericardia antiquata* (Linnaeus 1758) and *Venus verrucosa* (Linnaeus 1758), that occur together in northern coast of Tunisia, allowed us to discover for the first time the presence of morphological abnormalities affecting the foot of many individuals (annual rate of 31.6%). The presence of a developed byssus was also detected in some specimens of *V. antiquata*. A classification scale of this malformation, established depending on the degree of this anomaly, showed six initial types that evolve to form two or three feet, at the posterior and/or anterior sides of the animal. In order to determine the causes of this malformation, experiments of transplantation were carried out. Specimens of *V. verrucosa* collected from Zarzouna station were transplanted in Chaâra station which is characterized by low rate of malformations, low hydrodynamics and different sediment type and vice versa. Results revealed that foot malformations degree is highly correlated with both hydrodynamics and substrate type. This may be the main cause of foot malformations. The present study provides data on foot malformations in *V. antiquata* and *V. verrucosa* from the Tunisian coast that could be used as a starting point for future monitoring programs.

Keywords: *Venericardia antiquata; Venus verrucosa; Foot malformation; Transplantation; Zarzouna; Chaara*

Introduction

Many bivalve species play important role in marine ecosystem by filtering the water and serving as habitat and prey for a variety of sea life. In addition, they contribute to the modifications of the substrata [1].

During the past few decades, many bivalve species have been studied to determine their potential as bio-monitoring organisms and they become a popular choice for pollutants, parasites and natural factors (abiotics factors, hydrodynamics…) monitoring for several reasons. They are sedentary, regionally abundant, long lived, have adequate tissue mass for analysis, filter feeder (accumulate pollutants from food, water and also from the ingestion of inorganic particulate materials), hence fulfilling the criteria as good bio-indicators [2]. Consequently, such organisms have been largely used in programs of biological monitoring as mussels and oysters which are widely used as biological indicators in national and international monitoring programs [3].

Structural abnormalities caused by predators or parasites as the formation of mud blisters on the nacre of oysters by the annelid *Polydora* [4], Supernumerary siphons, either functional or nonfunctional, occur on the hard clam, *Mercenaria* and the soft-shell clam, *Mya arenaria* [5]. The causes of these deformities remain undetermined. Similarly bifurcation of the foot of clams has been reported by Atkins [6] and the adductor muscle of oysters by Pauley and Sayce [7]. The presence of parasites, *Cartlia australis* and *Himasthla sp.* in the foot of the cockle *Cerastoderma edule*, reduces the burrowing capacity of this endogenous bivalve due to foot atrophy [8,9]. In some other cases, the causes of tumors and abscesses in the mantle of *Crassostrea gigas* [10,11] and malformations of the labial palps and differentiation of a supernumerary foot in *Mytilus edulis* [6] are still unknown. It has been shown that algal blooms have a direct effect on bivalves, causing inhibition of valve activity and production of lesions in the mantle, gills and digestive gland [12]. Marteiliosis and Bonamiosis, caused by the protozoan *Marteilia refringens* and the bacteria *Bonania ostrea* respectively, induce the digestive gland destruction and gills ulceration in *Ostrea edulis* [13]. Moreover, behavior changes of mollusks have been reported by several authors. The causes of such alterations could be infestation by parasites as in *Mesodesma donacium* affected by the polychaete *Polydora biocippitalis* secreting conchylony and calcite around this spionid for isolation [14].

In Tunisia, studies on mollusk disturbances are scarce. In fact, in Bivalves, the examination of the soft part of the European clam *Ruditapes decussatus* and the shell of the date mussel *Lithophaga lithophaga* collected from the north lake of Tunis and the bay of Bizerte, respectively, revealed a deformation in the European clam which consists on the differentiation of two siphons inhaling and exhaling originating from both usual siphons, in the form of Y so possesses four functional openings instead of two [15]. In the date mussel, Trigui El Menif et al. [15] showed the presence of shell disturbances in external and internal sides of the valve. In Gastropods, Trigui El Menif et al. [16] and Lahbib et al. [17] described differentiation of a male genital tract (imposex phenomenon) in females of the gastropods *Hexaplex trunculus* and *Bolinus brandaris* [18] collected along the Tunisian coast. Furthermore, Lahbib et al. [17] and Abidi et al. [18] reported the presence of malformations and excrescences affecting the penis and the vas deferens in both sexes of *H. trunculus* and *B. brandaris* from some stations of Tunisian coast.

A comparative bio-ecological study of the two bivalve species *Venus verrucosa* and *Venericardia antiquata*, collected from a site...
subjected to intense anthropogenic pressure and high hydrodynamics, was conducted. The examination of the soft part showed a very pronounced malformation of the foot in both species. The present study was designed to describe this malformation, to establish a gradual developmental scale and to determine the possible causes.

Materials and Methods

A total of 1393 specimens of the clam *Venus verrucosa* and 1354 of the cockle *Venericardia antiquata* with a shell length ranging from 10 to 60 mm for the clam and 5 to 40 mm for the cockle were collected monthly from July 2008 to August 2009 by SCUBA at Zarzouna station (Figure 1). This sampling site is located in the first part of the artificial channel of Bizerte which is characterized by important biodiversity [19], high anthropic activity [20] and strong hydrodynamics [21]. This channel (200 m width, 1500 m length and about 12 m depth [23]) and is directly connected to the Mediterranean Sea. The sediment of this site is characterized by fine fraction: 0.063 mm < sediment texture ≤ 0.25 mm (83.32%) and gross fraction: 0.5 mm < sediment texture ≤ 2 mm (16.68%) [22].

In the laboratory, animals were divided into 10 class sizes for the clam [(10-15), (15-20), (20-25), (25-30), (30-35), (35-40), (40-45), (45-50), (50-550 and (55-60)] and 7 class sizes for the cockle [(5-10), (10-15), (15-20), (20-25), (25-30), (30-35) and (35-40)].

In April 2010, additional samples of *V. verrucosa* and *V. antiquata* were investigated at the Zarzouna and Chaâra stations. The Chaâra station is located in the second part of the channel of Bizerte (1000 to 1500 m wide, 5500 m long and about 12 m depth [23]) and is directly connected to the Bizerte lagoon (Figure 1). The number of specimens sampled was 95 and 60 of *V. verrucosa* and 61 and 50 of *V. antiquata*, respectively at Zarzouna and Chaâra stations. Morphometric (Length (L)) and ponderal measurements (Total Wet Weight (TWW)) of each individual of both species were measured. Subsequently, the soft parts of each bivalve were examined macroscopically and microscopically using a stereomicroscope to that in bivalves showing malformations were counted and photographed.

According to the status and the intensity of the malformation affecting the foot, we established a developmental scale with various stages. In July 2011, *V. verrucosa* was transplanted aiming to determine possible causes of this abnormality. Two hundred specimens of the almost affected class (25-30) were collected from Bizerte channel (Zarzouna) and were transplanted in the large station (Chaâra), located at the entrance of the Bizerte lagoon and vice versa.

Fifty specimens were collected from each station, dissected and examined to determine the intensity of malformation. The other 100 specimens were marked and transplanted in the two selected stations (50 individuals per station). The monitoring of the transplanted individuals was performed bimonthly. At the end of the experiment (March 2013), the number of remaining clams was 32 specimens in Chaâra and 21 specimens in Zarzouna.

Statistical analysis

Percentages were compared among samples using the χ² test in the software R and significance level was considered for p<0.05.

Results

Developmental scale of foot abnormality

A classification scale was established to classify the different stages of malformed foot development in the two studied bivalve species (Figure 2).

Stage 0: Normal foot (Figures 3A and 4B).

Stage 1: This stage is expressed by five types. It starts by ventral and central groove in the antero-posterior part of the foot (Stage 1a, Figures 3B and 4B). This groove can present or not a byssus (Figure 3B'). In the second type, the groove is present in the sagittal plane (Stage 1b, Figures 3C and 4C). The stage 1c (Figure 4D) appeared with a transverse groove with circular section perpendicular to the antero-posterior foot. The stage 1d (Figure 4E) showed a bifurcation of the posterior tip of the foot. The stage 1e (Figures 3E and 4F) revealed a bifurcation occurring in the anterior tip of the foot.

Stage 2: This stage is expressed by seven types and characterized by the groove evolution in the anterior direction. In this case, the anterior foot tip grows and becomes hypertrophied (Stage 2a, Figures 3F and 4G). Furthermore, the groove can deep and grows in the anteroposterior direction (Stage 2a, Figure 3G). A central groove appears to grow in depth from the anterior side and accompanied with an early anterior bifurcation oriented from the left or right side of the foot (Stage 2a, Figure 3H). Otherwise from Stage 1a and 1b, the antero-posterior central groove was present simultaneously with an anterior sagittal groove (Stage 2ab, Figure 3I). Besides, the groove developed in depth and width giving two ventrally separated feet and dorsally joined. Depending on the location of the groove, both tips of foot, anterior or posterior, are unequal. The anterior (Figures 4I and 4J) or the posterior (Figure 3J) tip may be larger (Stage 2c). Moreover the posterior bifurcation evolves toward the anterior direction of the foot, thus giving two ventrally and dorsally separated tips. Anterior tip remains undivided (Stage 2d, Figure 4K). In addition, the anterior bifurcation evolves towards the posterior direction of the foot, giving two ventrally and dorsally separated tips. The posterior tip remains undivided (Stage 2e, Figures 3K and 4L-4M).

Stage 3: Appears with three types. The stage 3a is an advanced stage of 2a. The groove evolves more toward the antero-posterior direction reaching both tips of the antero-posterior foot. The stage 3a is an advanced stage of 2a. The bifurcation evolves in the posterior direction.
other stages were observed at variable rates depending on the size of specimen and season. Table 1 showed different types of malformations and their rates according to the season. We noted that in *V. antiquata*, malformation generally increased according to specimen size. The first five class sizes are dominated by specimens with a thin or deep antero-

giving two posterior unequal feet, still joined at the posterior level. The groove continues its evolution in depth and length toward the anterior tip from the side of the large portion of the foot (Figure 3L). The stage 3e showed a complete separation of the two anterior tips at dorsal and ventral sides (Figure 3M).

**Stage 4:** It showed two types. The stage 4a, is characterized by the presence of two equal feet completely separated from the ventral side (Figure 3N). The stage 4a, is characterized by the presence of a small foot completely separated from the two other feet which are separated only at the ventral side (Figures 3O and 4N). The stage 4e showed the presence of two unequal feet completely separated (Figures 3P and 4O).

**Foot abnormality in Venericardia antiquata**

The examination of the cockle soft parts showed the presence of 194 individuals (out of 1354 collected over a year) with foot abnormality (14.33%). Seasonal variations of foot malformation showed highest rates in spring (19.11%) and winter (18.21%) against 11.78% and 9.81% in autumn and summer, respectively. The chi-square test revealed a significant difference between winter/Summer ($\chi^2=9.39$, df=1, $p=0.002$), Winter/autumn ($\chi^2=5.08$, df=1, $p=0.02$), Spring/summer ($\chi^2=11.15$, df=1, $p=0.0008$) and between autumn and spring. Taking into account different class sizes, we found that most affected individuals, on an annual and seasonal scale, belong to the classes (25-30) and (30-35) that represent 81% of the total affected sample. The remaining 19% belong essentially to the class (20-25). It should be noted that in *V. antiquata*, and with exception of type 1d, all
posterior groove (type 1a: 28.79% and 2a 2: 31.41%). Advanced stages generally concerned large specimens. The rate of individuals showing the types 3 and 4 do not exist with smaller class sizes and passed from 22.05% at the class (25-30) to 33.33% at (35-40). Specimens coming from type 1a are the most dominant (47.42%). The remaining affected individuals are in type’s b, c and d (52.58%). It should be noted that among the 194 specimens affected by foot deformity, 61 showed a byssus at the anterior or posterior part of the groove (Figure 3B). This brownish organ, with a length of 1.5-16 mm has the form of a silk tuft combined together by a gelatinous substance.

Foot malformation in *Venus verrucosa*

From 1393 collected specimens during the sampling period, 158 individuals were identified with abnormal foot corresponding to a rate of 11.34%. Seasonal variations of abnormality showed the highest rate in spring (16.42%), while in the other seasons, values were lower and similar (8.86%, 17.1% and 6.33%, respectively). In spring, the highest rate (6.96%) is the class (40-45). Previous different stages and types of abnormality were also observed in *V. verrucosa*. All types of stage 1 were reported in this species, whereas only types 2a1, 2c, 3e, 3a3, 4a2 and 4a3 were revealed at variable rates depending on specimen size and season for the stages 2, 3 and 4 (Table 2 and Figure 4). The most reported types of malformations were 2a and 3a (Table 2).

Spatial variation

In Zarzouna and Chaâra, the rates of affected *V. antiquata* were 67.21% and 18.00% respectively, against 10.53% and 5% for *V. verrucosa*. These results showed a higher rate in the first station for both bivalves. Comparing the two stations using the chi-square test (Table 3), we highlight significant difference was found only for *V. antiquata* ($\chi^2=24.93$, p<0.001). The observed malformation stages, differed according to stations. We noted the presence of a single initial stage 1a that occurs in the same form or under stage 1a’, where a groove

<table>
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<tr>
<th>Class size (mm)</th>
<th>Nt</th>
<th>Na</th>
<th>F%</th>
<th>I%</th>
<th>Stages</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
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<td>14.33</td>
<td>100</td>
<td>45</td>
<td>56</td>
<td>56</td>
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Table 1: Main stages of foot malformation observed in *Venericardia antiquata* in Zarzouna station. (F: frequency, I: intensity of stage, Na: number of specimens affected, Nt: total number of examined specimens).
Table 2: Main stages of foot malformation observed in the *Venus verrucosa* in Zarzouna station. (F: frequency, I: intensity of stage, Na: number of specimens affected, Nt: total number of examined specimens).

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<th>Class size (mm)</th>
<th>Nt</th>
<th>Na</th>
<th>F%</th>
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| Total          | 1393| 158| 11.34| 100  | 26 | 44 | 56 | 32 |

is provided with a byssus for cockle (Figures 3B and 3B). The stage 1a' was dominant in individuals of Chaâra with a single stage 2e for *V. antiquata* (Figure 3K).

At the zarzouna station, in addition to the stages 1a and 1a', we recorded more advanced stages, respectively 2a1, 2a2, 2a3, and 2e (Table 3).

As for *V. verrucosa*, the observed stages in Châara station were 1a, 1b, 1c, 2a, 2b, 2c, and 2e (Figures 4M and 4N and Table 3).

Transplantation

The examination of clam soft part showed that the number of affected individuals and intensity of malformation have not changed in individuals of Zarzouna that were collected and transplanted in Châara (Table 4). In contrast, individuals taken from Chaâra and transplanted into Zarzouna station showed an increase in the rate of affected specimens (7.5%) and the observed stages were 1e and 1d.

Discussion

The present study showed a very noticeable abnormality affecting the foot of the two studied bivalve species *V. antiquata* and *V. verrucosa*. More types of malformations were observed in the same individual in *V. antiquata*. However, in *V. verrucosa*, we registered only one type in the same individual (groove or bifurcation). These malformations come from an initial stage 1 characterized by six different forms. Each form gradually evolves to give different intermediate and final type. The early malformation development begins with a ventral anteroposterior groove (in *V. antiquata*), sagittal from the anterior side or transversal (in *V. antiquata* and *V. verrucosa*) or with an anterior or posterior bifurcation of the foot (in *V. verrucosa*). In *V. antiquata*,
only a posterior bifurcation was registered. Development continues to give advanced stages manifested by the presence of two feet, equal or unequal, welded from posterior or anterior in *V. antiquata*. In *V. verrucosa*, we observed two unequal feet in anterior side. Specimens with three feet, in which one is separated from the two others, are observed in both species. Some specimens with (in *V. antiquata*) or without groove (in *V. verrucosa*), showed an exaggerated outgrowth of the anterior part of the foot. It is noted that among the affected cockle specimens, we found 31.6% of individuals with byssus. This finding is explained by the bivalve lifestyle. Indeed, despite that the two bivalves live closely, the clam is found in abundance and in groups under small rocks while the cockle is found dispersed on the surface slightly covered by sediment. This assumes that the environment of *V. verrucosa* is more sheltered against the strong current of the communication channel. In the endogenous cockle, the strong hydrodynamics of the communication channel (surface currents in order of 0.4 m/s in winter and 0.2 m/s in summer; [21,24]) prevent this bivalve from sinking into the sediment.

When collecting samples, we found a deep currentology much more important than superficial. Under these conditions, it is probable that *V. antiquata* requires an adaptation to these conditions to allow burrowing into the sediment. This adaptation is manifested differently by foot developing in each specimen, related to environmental conditions. Atkins [6] showed the presence of malformations of unknown origin affecting the *Mytilus edulis* foot. This malformation results found differentiation of a small dorsal or ventral foot taking origin from the base of the main foot. In addition, we found in the cockles that the length of siphons is much shorter (Figure 5A) than that registered in clams (Figure 5B). Consequently, burrowing activity is lower with cockles, which explain the high rate and degree of the
malformation compared to clams. Therefore, cockles provided more pressure to run away in the sediment.

In addition, some specimens, shown by their location in the biotope, develop byssus to resisting strong currents. Moreover, most of specimens showing byssus have shelly debris and small stones attached to this byssus. De Montaudouin [25] and Dabouineau and Ponsero [26] noted byssus development in young cockle Cerastoderma edule when sediment presents low water retention. In this case, the developed byssus increased the bivalve bearing surface which can be easily moved by currents to other sites. Some studies showed that parasitism may affect foot growth. In fact, Bartoli [27] showed the presence of metacercariae Gymnophallus fossarum in the foot of Venerupis aurea. According to the same author, this infestation decreased normal foot growth and consequently changed the burrowing behavior of the bivalve. Similarly, high infestation of the cockle foot of Venerupis aurea showed the presence of metacercariae Gymnophallus fossarum bearing surface which can be easily moved by currents to other sites. Thus, animals cannot live a normal endogeneous life. This investigation suggested that malformation is probably related to sediment type, hydrodynamics and borrowing mode of the species. Furthermore, a genetic study is also needed to know, if these abnormalities are of a genetic origin.

Conclusion

The above results showed the presence of foot abnormalities in V. antiquata and V. verrucosa. The foot malformation scale showed that V. antiquata is the most affected by these abnormalities. Transplantation experiments showed low rate of affected specimens. This can be explained by the difference of the sediment structure in the considered sites.

The present study provides data on foot malformations in V. antiquata and V. verrucosa from the Tunisian coast that could be used as a starting point for future monitoring programs.

References