Transitional Waters Bulletin
TWB, Transit. Waters Bull. 6 (2012), n. 1, 20-33
ISSN 1825-229X, DOI 10.1285/i1825229Xv6n1p20
http://siba-ese.unisalento.it

RESEARCH ARTICLE

Associated and digenean fauna of the mussel *Mytilus galloprovincialis* cultured on shellfish tables in the lagoon of Bizerta (Tunisia)

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Abstract

1 - Shellfish tables used for the breeding in bags of the oyster *Crassostrea gigas* and the mussel *Mytilus galloprovincialis* are suitable support for rapid colonization by marine living organisms.

2 - During the period, June 2008 to May 2009, monthly samples of *M. galloprovincialis* were collected from the mytiliculture farm situated in Menzel Jemil (Bizerta Lagoon). Specimens were analyzed to identify their associated and digenean fauna.

3 - Overall samples contain 68 taxa belonging to 12 zoological classes. Crustaceans, Polychaetes and Bivalves dominated largely the associated fauna, with frequencies of 34.7%, 22.9% and 10.6% respectively. The Polychaete *Myrianida pinnigera* was identified for the first time in the Tunisian coast. The remaining groups of associated fauna (Ascidia, Ophiuroids, Gastropods, Actinaria, Gymnolaemata, Demosponges, Calcispongia, Turbellaria, and Echinids,) accounted for only 31.8%. Diversity indices showed a temporal variation of the taxonomic structure of listed groups related to the environmental conditions and the biological cycles.

4 - The examination of ~1400 individual mussels showed the occurrence of a low number of *Proctoeces maculatus* sporocysts (Digenea, Fellodistomodae). Bag-shaped sporocysts containing, according to the maturation stage, germinal balls, daughter sporocysts and or cercariae colonize at the beginning of the infection, the digestive gland then gonads and totality of the mantle.

Keywords: *Mytilus galloprovincialis*, associated fauna, digenean fauna, biodiversity, Bizerta lagoon, Tunisia.

Introduction

The Lagoon of Bizerta, located in the North east of Tunisia has been exploited in conchyliculture since 1964 (Khessiba et al., 2005) and represent the only Tunisian site of growing of the mussel *Mytilus galloprovincialis* and the oyster *Crassostrea gigas*. The growing of these two bivalves is carried out in rope in the northern part of the Lagoon (Companies CULTUMAR and MTRAD) and in tables in the southern part is (Society Tunisia Laguna). This last site contains 15 tables with an average dimension about 20 x 40m. At the same time, this area represents a receptor of several pollutants. Indeed, this site has been exposed to a high anthropogenic pressure coming from its bordering cities and the rather important number of industrial units surrounding its coast (steel complex of El Fouledh, Ciment factory, SOCOMENA in Menzel Bourguiba, oil refinery….). The environment richness in organic matter with the noticeable global warming and the low hydrodynamism in the southeast of the Lagoon have resulted in the confinement conditions. The mussel *M. galloprovincialis* was the most produced mollusc in the Lagoon of Bizerta with a production of 116.5 t/year (93%), 4.6 oyster t/year (4%) and 4.1 t/year of clams (3%) in 1998 (CRDA, 1998). This production has not stopped decreasing for some years. In 2002, mussel production reached only 61.06 tons (CRDA, 2002) and 65 tons in 2004 while in summer 2008, massive mortalities were recorded. The causes of the mortality are still unknown. The reports which were raised consist to the detachment from the rope and the major production part has fallen to the bottom. Therefore, the control of the chemical and biological quality, the parasites of mussels and the environmental factors constitutes the research to be developed by some research center and laboratories in order to determine the causes of this mortality in the Lagoon of Bizerta. Mussels play an important role in the creating of ecosystem engineers. This creation species can modify the biotic and abiotic factors in the local environment which can alter or create habitats that can have positive or negative effects on other species (Bruno and Bertness, 2001; Crooks, 2002; Gutierrez et al., 2003). The mussel beds can persist for many years and are characterized by high mussel densities, large aerial extent and production of organically rich wastes (Commito and Dankers, 2001).

In Tunisia, most studies on *M. galloprovincialis* are related to reproduction and growth (Aloui Bejaoui, 1998) and ecotoxicology (Khessiba et al., 2005; Dellali et al., 2001). Investigations on the associated fauna are scarce and limited to those on *Lithophaga lithophaga* (Trigui El-Menif et al., 2007a), *Venus verrucosa* (Trigui El-Menif et al., 2007b) and *Pinna nobilis* (Rabaoui et al., 2009). The associated fauna of *Mytilus galloprovincialis* was studied by Fazzani et al. (2001) in specimens collected from the Bizerta Lagoon during 1994, 1995 and 1996 only on three seasons. Until now, there is no research dealing with parasitic fauna of this mollusc from the Tunisian coasts.

To remedy to this deficiency and to do a comparative study with the available data, we have done a temporal monitoring of *M. galloprovincialis* in one shellfish table at Menzel Jemil to determine the associated and parasitic fauna.

Materials and methods

From June 2008 to May 2009, a mussel rope of approximately 2 m was monthly raised from the accessible table number 10 of the mytiliculture farm situated in Menzel Jemil. This site, which extends on a surface of 130 km² and an average depth of 7 m, is situated in the northeast of the Lagoon of Bizerta (Fig. 1). On the boat, the rope was shaken several times on a piece of plastic to recover the associated fauna called 'G1'. This group was identified, analyzed and preserved in alcohol 70%. Fifty mussels (G2) were collected monthly at different levels of the same rope so as to list the epibionts of this bivalve.

To study the parasitic fauna, 100 to 150 specimens of mussels were monthly collected, from the table 10 at different levels of the same rope. Each Mollusc was measured for shell length to the nearest 0.1 mm using a vernier calliper and weighted for total weight using a digital balance (precision 0.001g). The parasites harvested after dissection of the mussel were examined and identified under the binocular microscope. Parasites were studied directly in vivo and from

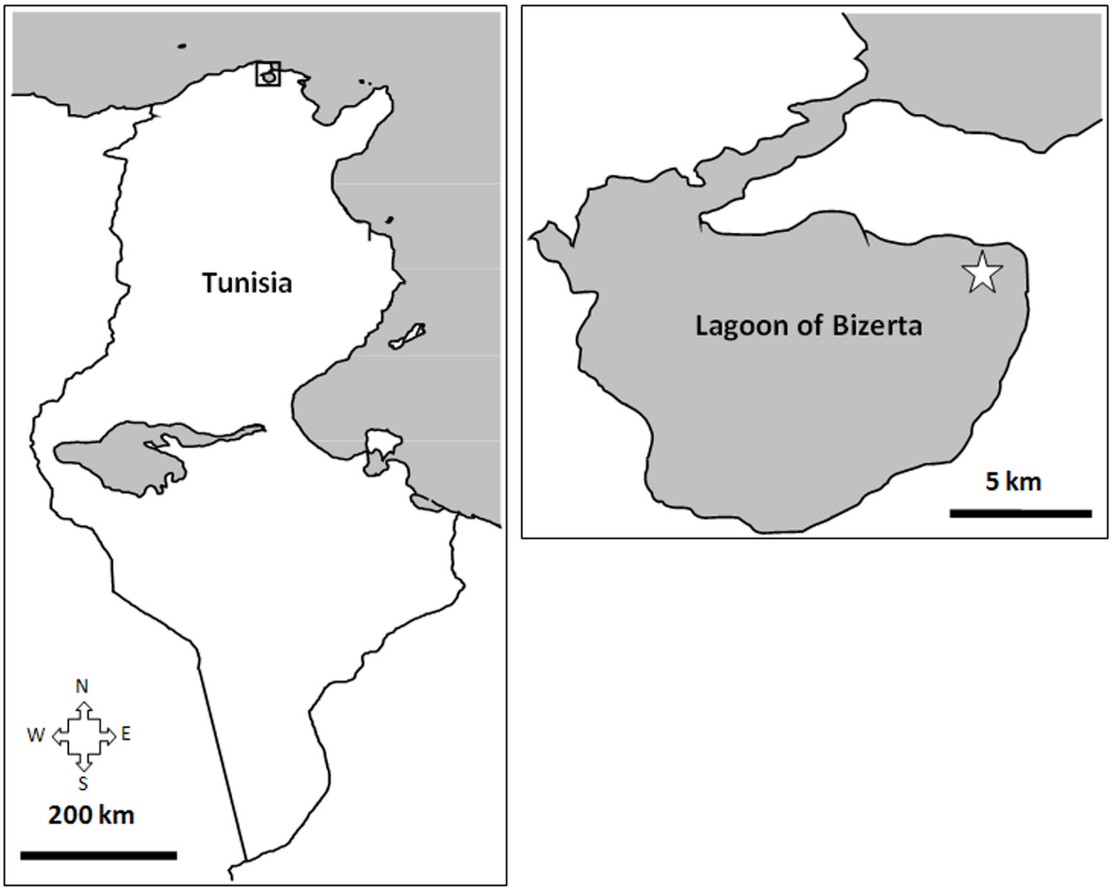

Figure 1. Location of the sampling station (Menzel Jemil Station) in the Lagoon of Bizerta.

individuals fixed in Bouin's fluid, stained by boric carmine, dehydrated then cleared in Gaulteria oil and fixed in Canada balsam.

To localize the site of the parasite occurrence and its effect on the host, we used the histological sections of the visceral mass and the mantle of the mussel fixed in Bouin's fluid, dehydrated and embedded in paraffin. The 5-µm sections were stained with Masson trichrome (Martoja and Martoja-Pierson, 1967).

The nomenclature used in the analyses of the frequencies of larval-stage parasites follows Margolis et al. (1982) and Buch et al. (1997).

To study the associated fauna diversity, we tested the following indices:

(1) Specific richness, (2) The diversity index of Shannon and Weaver (1963) $H' = -\sum (n_i/N \times \log_2 n_i/N)$, n_i = number of the individuals of each species, N = total number of the various species (3) Constancy (C) is the percentage of the relationship between the number of samples which contain this species during the study period and the whole of the collected samples during the year in the same site, (4) the relative abundance ($p_i = n_{i/}N \times 100$) which is defined as the ratio between the individual number of the species i for example (n_i) and the total individual number of all species (N) identified in the sampled site.

The data on the numbers of individuals of epifauna and infauna were log (x+1) transformed prior to analysis in order to satisfy the assumptions of normality. Specific richness and Shannon and Weaver indices were determined using the software Primer 6.

Results

Associated fauna to Mytilus galloprovincialis

Specific richness

Examination of species richness within both groups, during the four seasons of study, showed a low variation. The highest values which are found in the summer, winter and spring, were 23, 28 and 27 species in G1 and 17, 19 and 17 species in G2, respectively. In autumn, a clear decrease was registered with 15 taxa in G1 and 13 taxa in G2 (Fig. 2a). It should be noted that among the 68 taxa, the Polychaeta *Myrianida pinnigera* was identified for the first time in the Tunisian coast, 10 and 5 species for G1 and G2, respectively (Table 1-2) were not identified.

Shannon-Wiever index

The values of Shannon-Weaver index recorded in this study showed that the mussel rope has high diversity during the sampling period. Indeed, the recorded values, within each group, showed a higher diversity in G1 (2.97 bits) than in G2 (2.66 bits). The seasonal values of this index in G1 had a maximum in spring and in winter (3.16 bits) and a minimum in autumn (2.61 bits). Moreover, epibionts showed a maximum diversity in winter (2.79 bits) and a minimum in autumn (2.44 bits) (Fig. 2b).

Constancy

Constancy values of species collected during the study period are summarized in Tables 1 and 2. Considering G1, we noted that 18.6 % of identified species are permanent at least one season of the year (listed in more than 75% of the samples). These permanent species were represented by the Bivalves Lima inflata in summer, winter and in spring, the Gastropod Nudibranch in winter and in spring, the Ophiuroid Amphipholis squamata

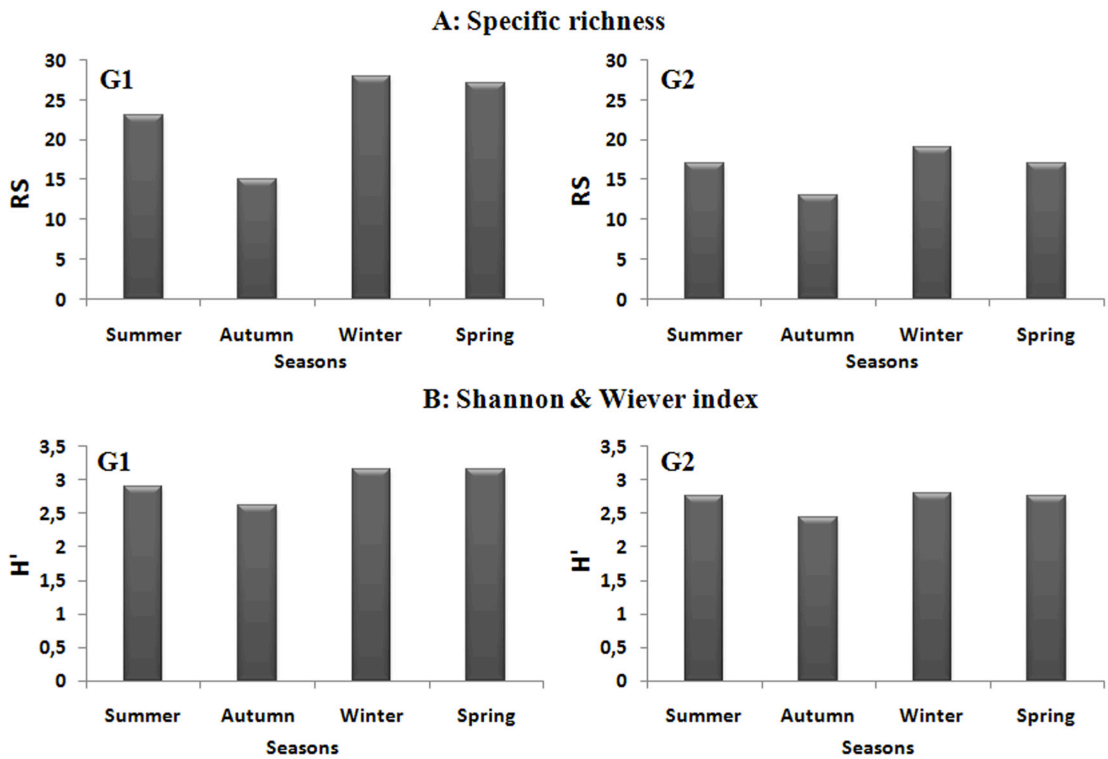

Figure 2.Seasonal variation of motile and sessile species associated with the mussel Mytilus galloprovincialis cultured in Bizerte lagoon (2008-2009), a: Specific richness, b: Shannon and Wiever index.

Table 1 - List of motile species (G1) associated to *Mytilus galloprovincialis* cultured in Bizerta Lagoon, the constancy (C %) and abundances (pi %).

Classes	Taxon	Summer		Autumn		Winter		Spring		Annual average
		C%	pi%	C%	pi%	C%	pi%	C%	pi%	Pi%
Polychaeta	*Perinereis cultrifera*	33.33	0.46	0.00	0.00	33.33	0.94	66.67	0.82	0.55
	Ceartonereis costae	66.67	8.20	0.00	0.00	33.33	1.89	33.33	3.83	3.48
	Platynereis dumerilii	33.33	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	Nereis sp.	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.55	2.41
	Marphysa sanguinea	66.67	2.73	33.33	9.09	0.00	0.00	33.33	0.55	0.82
	Lepidonotus clava	66.67	0.46	0.00	0.00	66.67	0.94	0.00	0.00	0.35
	Legisca extenuata	33.33	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	Harmothoé sp	33.33	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.17
	Audonia tentaculata	66.67	0.91	0.00	0.00	0.00	0.00	33.33	0.55	0.36
	Myrianida pinningera	0.00	0.00	0.00	0.00	33.33	1.57	33.33	2.46	1.01
	Phyllodoce paretti	0.00	0.00	0.00	0.00	0.00	0.00	66.67	2.46	0.61
	Amphitrite ruba	0.00	0.00	0.00	0.00	33.33	0.63	33.33	1.64	0.57
	Sabella pavonina	33.33	1.14	33.33	5.79	0.00	0.00	66.67	14.75	5.42
	Terebella lapidaria	0.00	0.00	0.00	0.00	33.33	0.63	66.67	1.91	0.64
	Total pi%		**15.03**		**14.88**		**6.60**		**29.51**	**16.51**
Bivalvia	*Modiolus barbatus*	33.33	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	Chlamys varia	66.67	12.07	33.33	0.83	33.33	0.94	33.33	4.37	4.55
	Venerpus rhomboides	66.67	3.19	0.00	0.00	33.33	0.31	33.33	0.55	1.01
	Venerpus decussatus	33.33	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	Pinctada radiata	0.00	0.00	0.00	0.00	33.33	0.31	0.00	0.00	0.08
	Lima inflata	100.00	10.25	33.33	6.61	100.00	0.31	100.00	6.83	6.00
	Total pi%		**25.97**		**7.44**		**1.89**		**11.75**	**11.76**
Gastropoda	*Hexaplex trunculus*	66.67	1.59	0.00	0.00	33.33	0.63	33.33	0.27	0.62
	Diodora gracea	0.00	0.00	33.33	1.65	0.00	0.00	33.33	0.27	0.48
	Gibulla commune	33.33	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	Nassarius corniculus	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.82	0.20
	Nudibranche	0.00	0.00	33.33	2.48	100.00	4.72	100.00	3.01	2.55
	Total pi%		**1.82**		**4.13**		**5.35**		**4.37**	**3.92**
Turbellaria	*Proshéceraeus moselyii*	0.00	0.00	0.00	0.00	33.33	0.31	0.00	0.00	0.08
	Planaria sp1.	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.27	0.07
	Planaria sp2.	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.82	0.20
	Planaria sp3.	0.00	0.00	33.33	0.83	33.33	0.63	0.00	0.00	0.36
	Planaria sp 4.	0.00	0.00	0.00	0.00	33.33	0.63	0.00	0.00	0.16
	Yungia aurantica	0.00	0.00	0.00	0.00	33.33	0.63	0.00	0.00	0.16
	Total pi%		**0.00**		**0.83**		**2.20**		**1.09**	**1.03**
Ophiuroides	*Amphipholis squamata*	100.00	16.86	33.33	19.83	100.00	14.15	66.67	6.83	14.42
	Total pi%		**16.86**		**19.83**		**14.15**		**6.83**	**14.42**
Echinids	*Paracentritus lividus*	0.00	0.00	0.00	0.00	33.33	0.31	33.33	0.27	0.15
	Total pi%		**0.00**		**0.00**		**0.31**		**0.27**	**0.15**
Crustacea	*Paracerceis sculpta*	100.00	14.58	33.33	15.70	100.00	16.35	100.00	21.58	17.05
	Cymodoce truncata	100.00	10.25	33.33	14.05	100.00	15.72	100.00	14.48	13.63
	Caprella sp.	0.00	0.00	0.00	0.00	33.33	0.94	0.00	0.00	0.24
	Caprella aequilibra	100.00	4.56	33.33	7.44	66.67	5.97	66.67	1.64	4.90
	Cymadusa hirsuta	33.33	0.23	33.33	1.65	66.67	5.35	33.33	0.82	2.01
	Pilumnus sp.	0.00	0.00	33.33	1.65	33.33	0.31	0.00	0.00	0.49
	Erichtonius difformis	0.00	0.00	0.00	0.00	66.67	2.20	0.00	0.00	0.55
	Corophium sp.	100.00	7.74	33.33	8.26	100.00	12.89	100.00	4.92	8.46
	Carcinus sp.	0.00	0.00	0.00	0.00	33.33	0.31	0.00	0.00	0.08
	Elasmopus rapax	100.00	2.96	33.33	4.13	100.00	3.46	66.67	2.73	3.32
	Total pi%		**40.32**		**52.89**		**63.52**		**46.17**	**50.7**

Table 2 - Abundance (pi %) and constancy (C %) of epibiotic species (G2) on the shell of *Mytilus galloprovincialis* cultured in Bizerta lagoon.

		Summer		Autumn		Winter		Spring		Annual Average Pi %
Classes	**Taxon**	**C%**	**pi%**	**C%**	**pi%**	**C%**	**pi%**	**C%**	**pi%**	
Polychaeta	*Serpula vermicularis*	100.00	53.25	33.33	13.22	100.00	14.34	100.00	25.25	26.5
	Total pi%		**53.25**		**13.22**		**14.34**		**25.25**	**26.5**
Bivalvia	*Anomia ephipium*	0.00	0.00	0.00	0.00	33.33	0.75	0.00	0.00	0.2
	Ostrea edule	100.00	6.80	0.00	0.00	66.67	1.13	100.00	4.46	3.1
	Total pi%		**6.80**		**0.00**		**1.89**		**4.46**	**3.3**
Actinairia	*Actinia equina*	100.00	8.58	33.33	20.66	100.00	20.38	100.00	16.34	16.5
	Total pi%		**8.58**		**20.66**		**20.38**		**16.34**	**16.5**
Crustacea	*Balanus perforatus*	66.67	2.66	0.00	0.00	33.33	1.51	33.33	3.47	1.9
	Balanus amphitrite	66.67	2.07	33.33	5.79	33.33	0.75	0.00	0.00	2.2
	Balanus eburneus	100.00	5.33	33.33	16.53	100.00	11.70	100.00	17.82	12.8
	Total pi%		**10.06**		**22.31**	0.00	**13.96**	0.00	**21.29**	**16.9**
Ascidia	*Ciona intestinalis*	100.00	1.78	33.33	1.65	100.00	4.15	100.00	6.93	3.6
	Diazona sp.	33.33	0.30		0.00	33.33	0.75	33.33	0.50	0.4
	Styela clava	66.67	1.18	33.33	0.83	0.00	0.00	0.00	0.00	0.5
	Botrylus schlosseri	66.67	1.18	33.33	0.83	33.33	1.13	33.33	2.48	1.4
	Botrylus leachi	66.67	1.78	33.33	0.83	0.00	0.00	0.00	0.00	0.7
	Phallusia mammilata	0.00	0.00	0.00	0.00	33.33	0.38	33.33	0.50	0.2
	Phallusia fumigata	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.50	0.1
	Ascidia sp.	33.33	0.59	0.00	0.00	33.33	0.38	0.00	0.00	0.2
	Didemnum coriaceum	33.33	0.89	33.33	9.92	66.67	6.79	100.00	3.47	5.3
	Didemnum albidum	100.00	2.96	33.33	11.57	100.00	14.34	100.00	5.94	8.7
	Didemnum sp.	0.00	0.00	0.00	0.00	33.33	0.38	0.00	0.00	0.1
	Total pi%		**10.65**		**25.62**		**28.30**		**20.30**	**21.2**
Gymnolaemata	*Scrupocellaria scruposa*	66.67	3.55	33.33	1.18	66.67	4.14	33.33	0.89	2.4
	Cellaria sinosa	66.67	1.48	33.33	1.48	66.67	2.07	0.00	0.00	1.3
	Schizoporella errata	100.00	5.62	33.33	3.85	100.00	9.76	100.00	4.44	5.9
	Total pi%		**10.65**		**6.51**		**15.98**		**5.33**	**9.6**
Demosponges	*Dysidea sp.*	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.59	0.1
	Pleraplysilla spinifera	0.00	0.00	0.00	0.00	0.00	0.00	66.67	0.89	0.2
	Hipposponge communis	0.00	0.00	0.00	0.00	33.33	0.75	0.00	0.00	0.2
	Total pi%		**0.00**		**0.00**		**0.75**		**1.48**	**0.6**
Calcisponges	*Sycon sp.*	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.59	0.1
	Total pi%		**0.00**		**0.00**		**0.00**		**0.59**	**0.1**

in summer and winter and the Crustaceans *Paracerceis sculpta*, *Cymodoce truncate* and *Corophium* sp. in summer, winter and in spring, *Caprella aequilibra* in summer and *Elasmopus rapax* in summer and in winter. The number of constant species is 14 (Tables 1). Seasonal values have shown that the Polychaeta *Ceartonereis costae*, *Marphysa sanguinea*, *Lepidonotus clava* and *Audonia tentaculata* appeared as a constant species in summer and *Phyllodoce paretti*, *Sabella pavonina* and *Terebella lapidaria* in spring. The Bivalves *Chlamys varia* and *Venerpus rhomboids* and the Gastropod *Hexaplex trunculus* are constant in summer. The only Ophiuroid *Amphipholis squamata* is constant in spring. The Crustaceans, *Caprella aequilibra*, *Cymadusa hirsute* and *Erichtonius difformis* appeared as a constant species in winter, while *Caprella aequilibra* and *Elasmopus rapax* are constant in spring. Accessory species were represented by 12 species of Polychaeta, 6 species of Bivalves, 4 species of Gastropods, 6 species of Turbellaria, 1 Ophiuroid, 1 Echnid and 9 Species of Crustaceans. The constancy of each species depends on the season (Table 1). The number of accidental species, which present a constancy inferior to 25%, is very big. For example in autumn, we registered the absence of 28 species belonging to all zoological groups with high number of Polychaeta (Table 1). Regarding G2, the percentage of identified permanent species is 32 %. These species are represented by the Polychaeta *Serpula vermicularis* in summer, winter and in spring, the Bivalve *Ostrea edule* in Summer and Spring, the *Actinaria Actinia* equina in summer, winter and in spring, the Crustacean *Balanus eburneus* in Summer, Winter and in Spring, the *Ascidia Ciona* intestinalis and *Didemnum albidum* in Summer, Winter and in Spring and *Didemnum coriaceum* in Spring and the Gymnolaemata *Schizoporella errata* in Summer, Winter and in Spring. Constant species were represented by the Bivalve *Ostrea edule* in Winter,the Crustaceans *Balanus perforates* and *Balanus amphitrite* in the summer, the *Ascidia Styela clava*, *Botrylus schlosseri* and *Botrylus leachi* in Summer and *Didemnum coriaceum* in winter, the Gymnolaemata *Scrupocellaria scruposa* and *Cellaria sinosa* in Summer and in winter and the Demosponge P*leraplysilla spinifera* in Spring. Accessory species were represented by the Polychaeta *Serpula vermicularis* in autumn, the Bivalve *Anomia ephipium* in winter, the Actinaria *Actinia equina* in autumn, all species of Crustacea, Ascidia, Gymnolaemata, two Species of Demosponges *Dysidea* sp. and *Hipposponge communis* in spring and winter, respectively and the Calcisponge *Sycon* sp. in spring. 14 accidental species in G2 were absent in autumn (Table 2). Taking into account seasonal variations, we note that the majority of G1 and G2 species were absent during at least two seasons out of four.

Relative abundance

In both zoological groups, the relative abundance index calculated for each species is wildly variable (Table 1, 2). In G1, values oscillate between 0.06 % for *Platynereis dumerilii*, *Legisca extenuata*, *Modiolus barbatus*, *Venerupis decussatus*, *Gibulla comune* and 17.05 % for *Paracerceis sculpta*. Crustacean species dominate with 50.7%. Polychaeta, Ophiuroids, and bivalves are represented by 16.51%, 14.42% and 11.76%, respectively. Gastropods, Turbellaria and Echinids are scarce with 3.92%, 1.03 % and 0.15%, respectively. The highest abundance was recorded in the spring for *Sabella pavonina* (14.8%). About 42% of the G1 species are not reported in three seasons and the abundance recorded during the fourth season does not exceed 5.30%. Only nine species of G1 were found during these four seasons with an abundance ranging between 2.01% and 20.68% recorded in the two crustaceans *Cymadusa hirsuta* and *Paracereis sculpta*, respectively. Crustaceans are most abundant throughout the study period and the lowest quantitative values were attributed to Turbellaria and Echinids groups which are absent in summer and in summer and autumn, respectively. Serpulids are the most representative in group G2 with 26.5% in total species. The abundance oscillates between 13.2% (autumn) and 53.3% in the summer. *Actinia equin*a was very abundant in autumn (20.7%). After Polychetae, Ascidians and Crustaceans showed a high abundance of 21.2% and 16.9%, respectively. The maximum abundance of both classes was recorded in winter and autumn respectively, while the lowest values were reported in summer. As for the Actinaria and Gymnolaemata, they present an annual average abundance of 16.5% and 9.6% respectively. The highest values are recorded in autumn for the Actinaria (20.7%) and in winter for the Gymnolaemata (16%). The lowest values are recorded in summer and spring, respectively. Bivalves which are represented only by *Anomia ephipium* and *Ostrea edulis* had an annual abundance of 3.3%. Finally, Sponges are the least abundant. Demosponges and Calcisponges have abundances of 0.6% and 0.1% respectively and are signaled in winter and spring respectively.

Digenean fauna

During our research for parasites of the mussel *Mytilus galloprovincialis*, larval stages (sporocyst and cercaria) of *Proctoeces maculatus* (Digenea, Fellodistomatidae) were collected. This mollusc plays the role of first intermediate host in the life cycle of this digenea.

Bag-shaped sporocysts, measuring 1.103 mm (0.445-2.340) in length and 0.481 mm (0.250-0.680) in width were provided with a thin tegument. The white young sporocysts changed into yellow-orange colour when maturity is achieved.

The cercaria (0.269 mm (0.190-0.340mm) in length and 0.132 (0.070-0.175mm) in width) released from sporocyst by a birth pore, was characterized by elongate oval body. The tegument is devoid of spines. Ventral sucker oval to round situated in anterior third of body. Intestinal bifurcation take place in mid-forebody. Caeca extend to middle of post-testicular region or beyond.

The sporocysts colonize, at the beginning

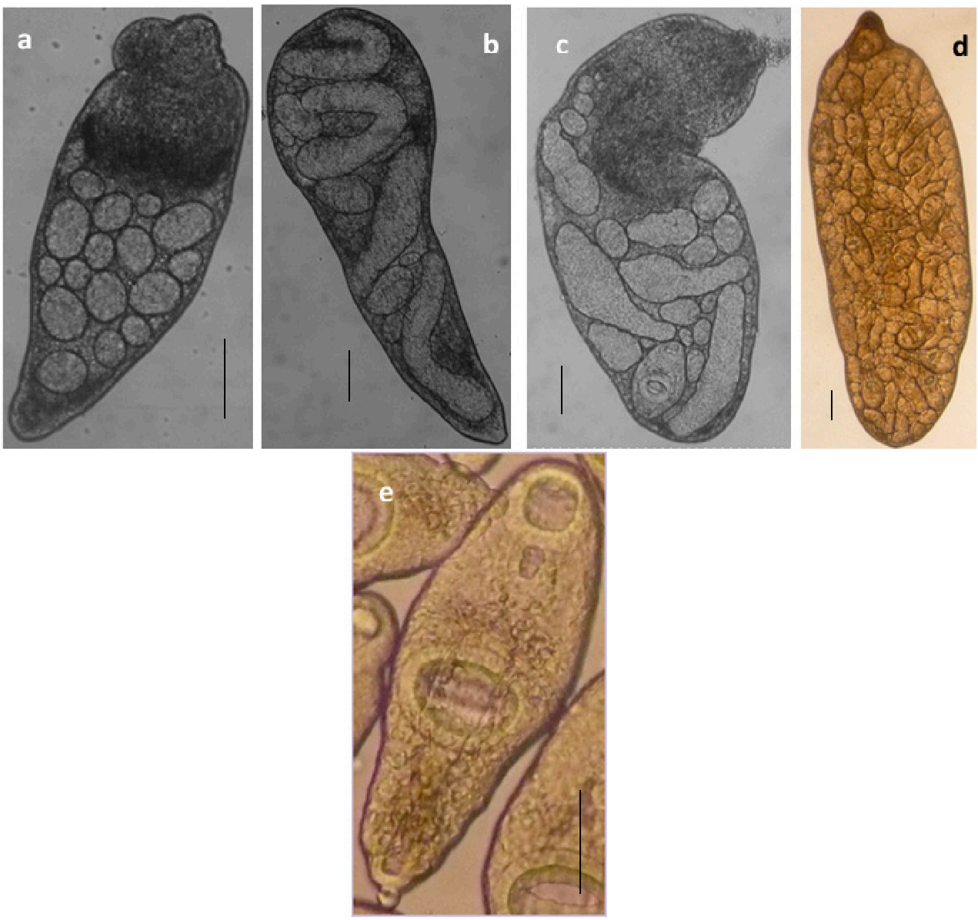

Figure 3. Development stages of sporocysts, (a) sporocyst containing germinal cells, (b) sporosysts containing daughter sporocysts, (c) sporocysts containing daughter sporocysts and cercariae, (d) sporocysts with cercariae, (e) *Cercaria*. (Scale bar: 100µm).

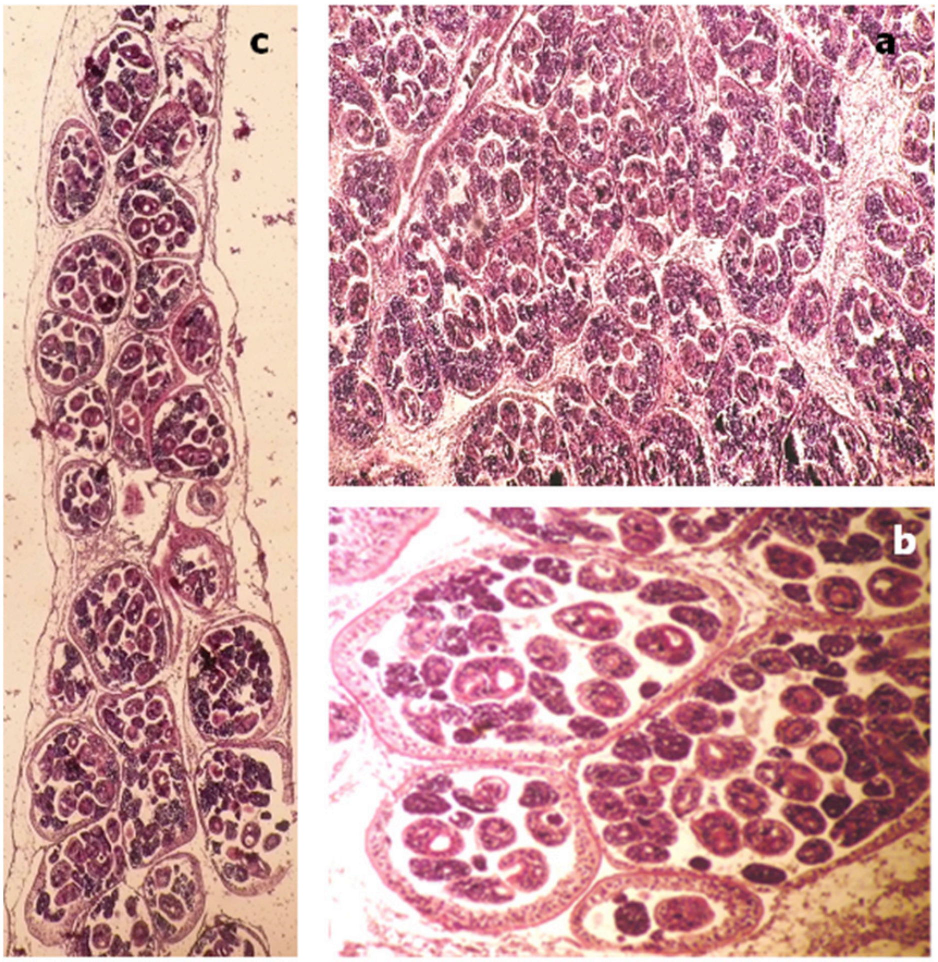

Figure 4. Transverse (a-b) and longitudinal (c) histological sections.

of the infection, the digestive gland. These sporocysts can contain germinal cells (Fig. 3a), daughter sporocysts (Fig. 3b) and or cercariae (Fig. 3c,d). The number of sporocysts exceeds 1000 individuals per parasitized mollusc. Each sporocyst can contain to 80 cercariae (Fig.3e). At the end of the infection and following an intense asexual reproduction of the sporocysts, the gonads and totality of the mantles were colonized by the sporocysts. The transverse and longitudinal histological sections on the level of the mantle confirmed these observations (Fig. 4a,b,c). The intense infection transforms the mantle into a fine transparent membrane emptied of its reserves substances and its active germinal cells. The global prevalence of *P. maculatus* is relatively low and does not exceed 0.35 %. The seasonal evolution of parasitism shows a perennial infestation in the mussels. The highest frequency is noted in autumn (0.52%); this frequency shows a little decrease in winter (0.45%) and becomes minimal in spring and in summer (0.25%).

The distribution of the *P. maculatus* following the size of the host shows that this digenea infects molluscs with size varying from 3.83 to 6.08 cm; this parasite seems prefer the hosts of medium size (size of the examined individuals varying between 2.43 and 7.58).

Discussion

About 68 taxa in Menzel Jemil site were listed belonging to 12 zoological groups. The presence of each taxa depends of the season. The combination of the two groups G1 and G2 showed 40 taxa in summer, 28 taxa in autumn, 47 taxa in winter and 44 taxa in spring. These seasonal substitutions can be explained by the vital cycle of the taxa and the environment factors. The Polychaeta *Myrianida pinnigera* was recorded for the first time in Tunisian coast. Most abundant taxa were the Polychaeta *Serpula vermicularis* (13%), the Crustaceans *Paracerceis sculpta* (9.9%), *Cymodoce truncata* (7.6%) and *Corophium* sp. (4.7%) and the Ophiuroid *Amphipholis squamata* (7.7%). Several studies were interested to the fauna associated with mussels. In the same station of Menzel Jemil farm, Fazzani et al. (2001) reported 26 taxa in which specific richness depends on the season with 20 taxa in summer, 21 in winter and 15 in spring. Most abundant species were *Elasmopus rapax*, *Hydroides elagans* and *Paracerceis sculpta* with frequencies of 27, 23 and 16% of the total number (3136), respectively. The high richness and high abundance in Crustacean (62%) and Polychaeta (35%) species determine the organisation structure of the animal community associated with mussels (Fazzani et al., 2001). These results are in agreement with our data, in fact in 2008, we noticed that Crustacean (34%), Polychaeta (22.9%) and Bivalvia (10.6%) constitute the dominant groups. Working with the same species, Çinar et al. (2008) confirmed the dominance of these groups with 155 species associated to *M. galloprovincialis* collected from the Izmir Bay. These authors showed that Polychaeta are presented by 46%, bivalves and gastropods, 32% and 11.2% Crustaceans. Tsuchiya and Nishihira (1985) studied the fauna associated with *Mytilus edulis* in the North of Japan and counted 69 taxa, belonging to 8 zoological groups. In this fauna, the Polychaeta which represent 46 % of the associated fauna dominate in particular Crustacea (39%). On the other hand Briggs (1982) determined 34 taxa associated with *Mytilus* collected from stations in the North of Ireland. This author underlines the importance of Mollusks and some Crustaceans which dominate in term of specific richness and number. In the Pacific Coast, Suchanek (1980) studied the fauna associated to *Mytilus californianus* and noticed 38% of Crustaceans and 35% of Mollusks. In the south of Chili, Duarte et al. (2006) showed a numerical dominance of Polychaetes, Oligochaetes and Crustaceans associated with *Mytilus chiliensis*. In Bizerta Lagoon, the motile fauna associated with *M. galloprovincialis* represented by 43 taxa against 25 taxa for the sessile fauna. A important decrease of the specific richness was registered in Autumn for both groups. This decrease is probably due to the sampling number, relatively low compared to that performed during the other seasons. This decrease is probably due to the sampling number of species, relatively low compared to that performed during the other seasons either to the raised temperature registered during this period and to the environment confining (Bejaoui et al., 2008) or then to the conjunction of all the environment parameters. Indeed, the high mortality, which was recorded from August to October 2008, reduces the specific richness and the abundance of the associated fauna. This mortality can be also attributed to the proliferation and the development, on the mussel surface, of sedentary organisms which prevent the opening of valves (Fig.5). The diversity within both groups showed a seasonal fluctuation which should be related to the temperature fluctuations and to the life cycle for some species. The epibionts fauna is more important in this site compared to results cited by Fazzani et al. (2001). These authors mentioned only three species:

Not identified Not identified *Schizoporella errata* (different stage)

Figure 5. Epibionts prevent the opening valves of the mussel.

Hydroides elegans, *Ostrea edulis* and *Ciona intestinalis*. In this study, about 19.9% of epibionts are represented by the two Acidians species *Didemnum coriaceum* and *Didemnum albidum* and the Gymnoleamata *Schyzoporella errata*. Their growth on the surface, cover the valves during the worm season and, consequently prevent its bivalvular opening. These epibionts may be the cause of mortality observed in August 2008 in the shellfish station of Menzel Jemil. Moreover, Osman et al. (1989) and Zajac et al. (1989) noted that the presence of Ascidians on the oyster shell decreases the survival and the growth of bivalve. Ascidians use the bivalve shell as a support and also like a food source since Bingham and Walters (1989) found larvae of bivalves in the stomach-contents of many species of Tunicates.

Serpulidae and Balanidae species, with 26.5% and 16.9%, living fixed to the mussel's shell covered an important surface of one or both valves. These epibionts occupy the posterior part of the valve, the open region of the Serpulids tubes extended over the valve. It is probably an adaptation for feeding by these commensally Polychaetes and Barnacles. A similar behaviour was observed by Trigui El Menif et al. (2007a) on *Venus verrucosa* sheltering *Gastrochaena dubia* in the Shelly layer. The endobiont openings are always located in posterior side of *Venus*, exit place of siphons, so *Gastrochaena* takes advantage of the contribution of food. This phenomenon was also observed in *Flexopecten felipponei* colonized by Serpulids, Barnacles and Oysters from Argentina by Schejter and Bremec (2007). Epibionts, are more frequent and abundant in the suspended farming sites; they can weaken the mussels and could contribute to the detachment of the bivalve from the rope, and consequently to its death. Regular cleaning of bags, especially in sensitive environments as the case of Bizerta Lagoon the only Tunisian site sheltering the ongrowing of mussels and oysters is more recommended to minimize the mortality caused by epibionts organisms.

As regard the digenean fauna, the heteroxonic life cycle of *P. maculatus* starts with the infection of *Mytilus galloprovincialis* which is known to be first intermediate host for larval stages, sporocysts and cercariae. Cercariae emerge from the host and invade Annelid Polychaete, *Leptonereis glauca* which act as a second intermediate host for metacercariae. The life cycle is complete when the definitive host, fish is infected by ingesting the second intermediate host (Prévôt, 1965). This cycle can be reduced to 2 invertebrate hosts by removal of the final host fish (Dollfus, 1964; Bray and Gibson, 1980; Pondick, 1983). The ovigerous development of the parasites occurs in the second intermediate host, invertebrates (Annelids Polychaetes, Bivalves, and Gastropods). Stunkard and Uzmann (1959) recorded the presence of all larval stages and adult in the mussel. The cycle of this parasite would be reduced in colder water (Prévôt, 1965; Bray and Gibson, 1980).

P. maculatus is collected, for the first time, in Tunisia with a relatively low frequency (0.35%). This result is in agreement with that signaled by Prévôt (1965) in the mussels coming from the Gulf of Marseille (2.5%). Also Le Breton and Lubet (1992) have noted prevalence about 1.42% for the mussel *M. edulis* cultured in the West coast of Cotentin. The lower rate of infection can be the result of an under estimation since the specimens presenting an intensive proliferation of the parasite end by dying and detaching from the rope. A mortality of at least 33% in mussel's population, resulting from the parasitosis of *P. maculates*, was reported in the farms of west coast of Cotentin (Le Breton and Lubet 1992). Machkevskii and Parukhin (1981) have reported that this parasite is in the origin of massive mortality and the decrease of the speed growth rate of the host from 1.5 to 2 times in the case of intensive infestation with larval stages of *P. maculatus*. The affected growth of molluscs leads to reduce the farms productivity. Concerning gonads with intensive infestation we found, as Stunkard and Uzmann, (1959), Le Breton and Lubet (1992), that they are completely emptied of sexual cells and entirely colonized by sporocysts. This parasite leads to the host castration. Similar results were found by Hopkins (1954) with another species of digenea *Cercaria brachidontis* parasite of *Mytilus recurvus*. The decrease of the speed growth rate, the castration and the mortality of infected mussels led to consider the larvae of *P. maculatus* as a potential high danger for mytiliculture.

The study of the seasonal evolution of *P. maculatus* shows that it is present throughout the year. The perennial infestation can be attributed to the adaptation of the free larval stages (miracidia and cercariae) to the seasonal variations of temperature, salinity and with wide spectrum of second intermediate host (Annelides Polychetes, Bivalves, Gastropods) and final host (Labridea, Sparidea, Blenniidea).

Our results concerning the parasitism evolution according to the size are in agreement with those of Martinez (1972); the mussels infected are of medium size. Le Breton and Lubet (1992) confirm our results concerning the absence of the larvae of *P. maculatus* in the mussel's spats.

Conclusion

The ropes of mussels *M. galloprovincialis* reared on shellfish tables in the station of Menzel Jemil provide a suitable habitat for about 68 taxa distributed on 43 motile species and 25 species fixed on the shell. A trematoda digenea *P. maculatus* was identified as parasite infesting the mussel during the study period. The mortality registered during August essentially can be attributed to the high sea water temperature and to the environment confining, to a physiological weakness of mussels further to an intense infestation by the larvae of *Proctoeces* and also to the development of epibionts species which prevent the opening of valves. More studies on the biology and microbiological analyses are recommended to clarify the various types of associations between the individuals of the fauna community and to reveal the main components responsible of the mortality of mussels in breeding.

Acknowledgements

We thank the manager of the farming mytiliculture station (STL) of Menzel Jemil for providing mussel samples needed to conduct this study.

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