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Digenean larvae parasitizing *Ruditapes decussatus* (Bivalvia) from Tunisian coastsE. DHRIF¹, R. ANTAR¹, S. ABIDLI², L. GARGOURI¹

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Summary

Eleven species of trematode larvae (sporocysts and cercariae of *Bucephalus labracis* Paggi et Orecchia, 1965 and *Cercaria lata* Lespes, 1857 and metacercariae of *Acanthoparyphium* sp. Dietz, 1909, *Caecicola parvulus* Marshall et Gilbert, 1905, *Curtuteria australis* Allison, 1979, *Gymnophallus fossarum* Bartoli, 1965, *Gymnophallus rebecqui* Bartoli, 1983, *Lepocreadium pegorchis* Stossich, 1901, *Parazoogonus* sp. Looss, 1901, *Psilostomum brevicolle* Creplin, 1829 and *Robphildollfusium fractum* Rudolphi, 1819) were found in the clam (*Ruditapes decussatus*) from Tunisian coasts. A part from *B. labracis* and *Cercaria lata*, the other trematode species were recorded for the first time in Tunisia. The examination of the frequency of recorded parasites reveals some monthly variation. This variation is statistically significant by comparing the sampling sites. These results indicate that more frequent parasites (*G. fossarum*, *Cercaria lata*, *Curtuteria australis* and *Acanthoparyphium* sp.) could be considered as biological indicators making it possible to predict the capture localities of the clams.

Keywords: *Ruditapes decussatus*; Digenea; larval stages; Tunisia

Introduction

Ruditapes decussatus (Veneridae) is a widespread bivalve in the world (from southern and western England to the Iberian Peninsula and into the Mediterranean, south to western Morocco and Senegal, west Africa) (Poppe and Goto, 1991) and on the Tunisian coasts (Zammouri-Langar, 1991; Trigui El Menif, 1995; Hamida, 2004; Attia El Hili *et al.*, 2007; Dhraief *et al.*, 2009). It has an important economic value since its commercialization in the domestic markets and exportation to Europe. The abundance and biomass of this benthic animal have changed significantly. In fact, this bivalve is considered a benthic organism living in the sediment-water interface, and is in constant contact with several pathogens. Among these pathogens, Digenean trematodes are the dominant macroparasites (Lauckner, 1983), that represent a real danger for bivalves and are often cited as a limiting factor for their production in several countries (De Montaudouin *et al.*, 2009; Gam *et al.*, 2008; Oie, 2006; Potasman *et al.*, 2002). They have been described in many exploited mollusks as mussels, cockles, oysters and carpetshell clams (Bartoli, 1981; de Montaudouin *et al.*, 2000;

Kim & Powell 2006; Krakau *et al.*, 2006; Thieltges *et al.*, 2006; Thieltges & Reise, 2006, 2007). They generally infect individuals of low immunity and may be the main reason of the phenotypic and genotypic perturbations in the host (Poulin, 1995; Poulin *et al.*, 1998; Poulin, 2013). These disturbances are obviously involved in changing of the behavior of these bivalves (Moore, 2002; Mouritsen & Poulin, 2003; Leung & Poulin, 2007, 2011). *R. decussatus* seems to be exploited by digenean species; indeed, the trematode larval stages can use this mollusk as the first intermediate host but most of them use this bivalve as the second intermediate host (Bartoli, 1965, 1973, 1981; Hanafy *et al.*, 1997; Gargouri Ben Abdallah, 2001; Gargouri Ben Abdallah & Maamouri, 2005; Gargouri Ben Abdallah *et al.*, 2009). In Tunisia, parasitological research on this shellfish has been of little attention and limited to clams from the lagoon of Tunis (Gargouri Ben Abdallah, 2001; Gargouri Ben Abdallah & Maamouri, 2005; Gargouri Ben Abdallah *et al.*, 2009). The aims of this work are to make an inventory of the parasite species of the clam *Ruditapes decussatus* within Tunisian coasts, to evaluate the changes in infection parameters with respect to months and localities and to know if these parasites can be con-

sidered as biological indicators making it possible to predict the capture localities of the clams.

Material and Methods

Sampling sites

Three sampling sites were selected representing different environmental characteristics. The first station (Rades), has sand to sandy-mud bottom sediments, situated in northern Tunisia (Gulf of Tunis) between 36°47'N, 10°17'E and 33°26'N, 07°53'E. Over the year, the water temperature fluctuated between 12.4 °C to 28.4 °C. The salinity ranged from 35.1 psu in winter and 37.0 psu in summer (Zarrad *et al.*, 2003). This station is bordered on the north side by the thermal power plant and the channel of Rades, on the west side by the cities and the east side by the sea.

The other sampling sites are situated in southern Tunisia (Gulf of Gabes) between 35°40'N, 10°40'E and 47°72'N, 38°15'E (port of Sfax), 34°34'N, 10°36'E and 50°80'N, 41°84'E (Gargour). Port of Sfax is limited on the north side by Kettana Oued, east by the Mediterranean, south by Oued Hagouna and the west side of the continent where are the sources of human pollution as areas of landfill, a slaughterhouse and wastewater treatment stations. Station of Gargour is bordered on the north by the port of Sfax, on the east by the sea, on the south by Sidi Farah and west by the village of Gargour. The purification station of shellfish and factories are direct source for pollution for this site. The Gulf of Gabes is characterized by sandy, muddy, sandy-muddy, carbonated and sometimes swampy sediment (Drira, 2009). The mean temperature and salinity are 21.5 °C and 39.83 psu respectively (Dhraief *et al.*, 2009).

Sampling and handling of clams

Between October 2009 and October 2010, a total of 3457 *Ruditapes decussatus* was collected monthly from these three stations: 1734 (84 – 176 per month) specimens from Rades, 1234 (54 – 136 per month) specimens from port of Sfax and 489 (20 – 91 per month) from Gargour. This lamellibranch lives burrowed in sand and silty mud. The clam population was situated 2 to 5 cm above mean low water level and can go deep up to 10 – 20 cm in the soil at very low winter temperatures. In this mollusk, the sexes are separate but the hermaphrodites can be found infrequently. Both sexes show a synchronism in gonadal development (Hamida, 2004). Sexual maturity is reached during the first year. The lifespan is variable and can reach 20 years. The clam, suspension feeder, seems to have a wide feeding spectrum (bacteria, algae, rotifers, detritus...). Its main predators are birds (gulls and oystercatcher...), fish (flounder, sea bream and triggerfish...) and invertebrates (gastropods, crabs and starfish) (Le Treut, 1986; Fischer *et al.*, 1987). The clams were located visually and hand picked. Back to the laboratory, the samples were placed into glass containers with filtered sea water and aerated by a diffuser. This method allows bivalves to get rid of most sand and impurities located between the gills and mantle. Afterwards, the mollusks were observed twice daily, under a stereomicroscope for the presence of naturally emitted cercariae. After two weeks, the clams were measured, weighed and dissected to detect the presence of young sporocysts or metacercariae. The site of infection and the number

of each metacercariae were noted; metacercariae were excysted by coverslip pressure. Different reports on parasites in clams were used for species identification (Bartoli, 1967, 1984; Allison, 1979; Bowers *et al.*, 1996; Skirnisson *et al.*, 2004; Gargouri & Maamouri 2005; Russell-Pinto *et al.*, 2006; Gargouri *et al.*, 2009; Fredensborg *et al.*, 2013).

The parasitological terminology (prevalence, abundance and mean intensity) followed the definitions of Bush *et al.* (1997).

The significance of the variation of the values of the frequency according to the month and localities of capture was calculated based on the χ^2 -distance.

Results and Discussion

The digenean fauna of clams from the three sites was represented by 11 species: sporocysts and cercariae of *Bucephalus labracis* Paggi et Orecchia, 1965 (Bucephalidae Pocket, 1907) and *Cercaria lata* Lespes, 1857 (Faustulidae Pocket, 1926) and 9 metacercariae: 1 Cryptogonimidae Ward, 1917 (*Caecincola parvulus* Marshall et Gilbert, 1905), 2 Echinostomidae Looss, 1902 (*Acanthoparyphium* sp. Dietz, 1909; *Curtuteria australis* Allison, 1979), 1 Gyliauchenidae Fuki, 1929 (*Robphildollfusium fractum* Rudolphi, 1819), 2 Gymnophallidae Odhner, 1905 (*Gymnophallus fossarum* Bartoli, 1965; *Gymnophallus rebecqui* Bartoli, 1983), 1 Lepocreadiidae Odhner, 1905 (*Lepocreadium pegorchis* Stossich, 1901), 1 Psilostomidae Looss, 1900 (*Psilostomum brevicolle* Creplin, 1829) and 1 Zoogonidae Odhner, 1902 (*Parazoogonus* sp. Looss, 1901). Nine digenean species were found in the north of Tunisia (Rades) and 6 species in each site in the south (Gargour and port of Sfax). Four species are common in all investigated localities (*Cercaria lata*, *Acanthoparyphium* sp., *Curtuteria australis*, *Gymnophallus fossarum*). *Bucephalus labracis*, *Caecincola parvulus*, *Lepocreadium pegorchis*, *Robphildollfusium fractum*, *Parazoogonus* sp. were only found in Rades whereas *Gymnophallus rebecqui* and *Psilostomum brevicolle* were harvested in both Gargour and port of Sfax (Table 1). The relatively low numbers of clams inspected in port of Sfax and Gargour could bias the comparison between sites.

The analysis of all parasites present in an individual host of *Ruditapes decussatus*, revealed that most of these hosts harboured 1 (58.80 %) or 2 (31.32 %) and more rarely 3 (7.14 %) or 4 (2.74 %) species of parasites. These relatively poor infracommunities can be explained by the low rate of transmission or by competitive exclusions or insufficiency of resources.

The study of the distribution of digenean species within the host showed that sporocysts of *B. labracis* and *Cercaria lata* occupied the gonad and digestive gland and rarely the gills. Metacercariae of *G. fossarum* and *G. rebecqui* were localized between the mantle and the shell. *Acanthoparyphium* sp. colonized only the foot and *P. brevicolle* was restricted to the digestive gland and rarely to the gonad. *Curtuteria australis* invaded the digestive tract, gonad and the foot. *L. pegorchis*, *Caecincola parvulus*, *R. fractum* and *Parazoogonus* sp. were harvested from the gonad of clams. The segregation of ecological niches for most frequent parasites (case of Gymnophallidae and Echinostomatidae) is probably related to interspecific competition. In fact, by restricting the di-

Table 1. Total prevalence (P%) and mean intensity (MI) of digeneans species in *Rudilapes decussatus* from sampling sites
 1. Int. host: first intermediate host, 2. Int. host: second intermediate host

Digenean species	Families	Intermediate host	Final host	Rades		Port of Sfax		Gargour	
				P (%)	MI	P (%)	MI	P (%)	MI
Species using <i>R. decussatus</i> as 1. Int. host									
Second intermediate host									
<i>Bucephalus labracis</i> Paggi et Orecchia, 1965	Bucephalidae	<i>Atherina Boyeri</i>	Fish	0.11	-	0	-	0	-
<i>Cercaria lata</i> , Lespes, 1857	Faustulidae	?	?	10.2	-	23.82	-	17.79	-
Specieess using <i>R. decussatus</i> as 2. Int. host									
First intermediate host									
<i>Caecincola parvulus</i> Marshall et Gilbert, 1905	Cryptogonimidae	<i>Amnicola lustrica</i>	Fish	0.11	1	0	0	0	0
<i>Acanthoparyphium</i> sp. Dietz, 1909	Echinostomatidae	?	Bird	2.08	16.75	1.05	4.15	22.69	4.17
<i>Curtuteria australis</i> , Allison, 1979	Echinostomatidae	<i>Cominella glandiformis</i>	Bird and fish	53.29	7.28	13.9	6.87	61.14	12.24
<i>Gymnophallus fossarum</i> Bartoli, 1965	Gymnophallidae	<i>Scrobicularia plana</i>	Bird	23.24	7.14	94.73	213	98.36	152.3
<i>Gymnophallus rebecqui</i> Bartoli, 1983	Gymnophallidae	<i>Abra tenuis</i>	Bird	0	0	3.16	7.64	3.47	3.7
<i>Lepocreadium pegorchis</i> Stossich, 1901	Lepocreadiidae	<i>Nassarius corniculum</i> , <i>N. mutabilis</i>	Fish	0.06	1	0	0	0	0
<i>Psilostomum brevicolle</i> Creplin, 1829	Psilostomidae	<i>Hydrobia ulvae</i> , <i>Hydrobia ventrosa</i>	Bird	0	0	1.21	1.13	0.4	1
<i>Robphildollfusium fractum</i> Rudolphi, 1819	Gyliauchenidae	?	Fish	0.06	1	0	0	0	0
<i>Parazoogonus</i> sp. Looss, 1901	Zoogonidae	?	Fish	0.11	1	0	0	0	0

Table 2. The monthly variation in the prevalence (%) of digeneans species in *Rudilapes decussatus* from sampling sites

Sampling Sites	Parasites	O	N	D	J	F	M	A	M	J	J	A	S	O
		2009	2009	2009	2010	2010	2010	2010	2010	2010	2010	2010	2010	2010
Rades	<i>Gymnophallus fossarum</i>	0	5.88	12.42	11.97	61	12.92	22.3	18.33	69.16	14.28	45.45	17.58	21.43
	<i>Curtuteria australis</i>	58.52	78.82	88.88	50	95.59	42.85	24.46	16.66	40.83	22.32	69.42	21.98	39.29
	<i>Cercaria lata</i>	3.4	11.76	1.96	4.22	3.77	4.76	1.44	1.66	0.83	5.35	15.7	63.74	48.81
	<i>Acanthoparyphium</i> sp.	0	0	0	0	0	0	0	0	0	0	23.14	5.49	3.57
Port of Stax	<i>Gymnophallus fossarum</i>	100	88.88	90.9	89.7	79.68	96.73	98.33	95.79	100	100	100	100	100
	<i>Curtuteria australis</i>	0	25.92	30	41.18	14.84	6.52	0.83	10.53	0	2.5	24.36	9.3	4.65
	<i>Cercaria lata</i>	40.54	42.59	20	22.06	32.03	38.04	8.33	3.16	8.42	7.5	6.41	43.02	51.16
	<i>Acanthoparyphium</i> sp.	0	0	1.82	5.15	0	0	2.5	0	0	0	0	1.16	0
Gargour	<i>Gymnophallus rebecqui</i>	0	0	19.09	2.9	4.68	1.08	2.5	4.2	0	0	0	0	0
	<i>Gymnophallus fossarum</i>	-	89.65	100	95.65	98.51	100	100	100	96.15	100	100	100	92
	<i>Curtuteria australis</i>	-	44.82	89.01	43.48	29.85	66.66	96	28.88	42.3	75.67	60	80	92
	<i>Cercaria lata</i>	-	62.07	23.08	26.09	55.22	37.5	0	0	0	0	0	0	0
	<i>Acanthoparyphium</i> sp.	-	0	34.06	23.91	0	0	88	26.66	23.07	8.1	20	70	24
	<i>Gymnophallus rebecqui</i>	-	0	12.09	10.87	1.49	0	0	0	0	0	0	0	0

mension of the site, the parasite limits the competition with other parasite species. For sporocysts of *Cercaria lata* and *B. labracis* that colonize the same microbiotope they have never been found in the same bivalve; it seems that the infections of mollusk by a digenean species prevents the installation of other species and leads consequently to their exclusion. Otherwise sporocysts of *Cercaria lata*, and a few metacercariae of *Curtuteria australis* can occupy the same microbiotope (gonad). It seems that resources are sufficient if the number of metacercariae is lower. In the host's foot, *Curtuteria australis* can elsewhere coexists most often with *Acanthoparyphium* sp., a species belonging to the same subfamily Himasthinae and having ecological similarity. Other authors (Leung and Poulin, 2007, 2011) have found that these two species occupy different microbiotopes in the host's foot; *Acanthoparyphium* sp. colonizes the base of the foot whereas *Curtuteria australis* is concentrated at the tip. They elsewhere believe that the concomitant presence of these two species is due to a change in the immune system of the host. In fact, Leung and Poulin (2007, 2011) thought that *Curtuteria australis* can act on the host genotype by changing some redundant genes considered as suppressor genes of the immune response or genes that tolerate the installation of new specimens of the same species or of other communities such as *Acanthoparyphium* sp. or *G. fossarum*.

Global values of parasitological indices of different digenean species in *R. decussatus* (Table 1) show that *Gymnophallus fossarum* and *Cercaria lata* which are common in three localities, reached their highest level in the south of Tunisia (port of Sfax and Gargour). *Curtuteria australis*, was less frequent in the port of Sfax and showed the highest prevalence in Gargour and at lesser degree in Rades. *Acanthoparyphium* sp. colonized widely the shellfish collected from Gargour and had very low levels of infestation in the other two stations. *G. rebecqui*, harvested only in southern Tunisia, has a low infection rate. Cercaria of *Bucephalus Labracis* and metacercariae of *Caecicola parvulus*, *Lepocreadium pegorchis*, *Robphildollfusium fractum*, *Parazoogonus* sp. and *Psilostomum brevicolle* are rare parasites. The scarcity of these parasites may be related to low frequency of hosts (intermediate or final) or non-specificity of these digeneans to *Ruditapes decussatus*. Apart from *B. labracis*, signaled by Gargouri Ben Abdallah and Maamouri (2005), others species (*Caecicola parvulus*, *L. pegorchis*, *P. brevicolle*, *R. fractum* and *Parazoogonus* sp.) were recorded, for the first time, in *R. decussatus*; this lamellibranch seems to be an accidental host for the last species. Indeed, metacercariae of *L. pegorchis* have been reported in other bivalve species such as *Cerastoderma glauca*, *Paphia aurea*, *Parvicardium ovale*, *Venus verrucosa* (Bartoli, 1967), metacercariae of *P. brevicolle* in *Cardium edule* and *Mytilus edulis* (Looss-Frank, 1968) and metacercariae of *Caecicola parvulus* in Centrarchid fish (Walter, 1941). Furthermore, Bartoli (1987) claimed that the cercariae of *R. fractum*, a species which the life cycle is still unknown, encyst on algae because the adults of the family Gylienchidae are harvested in herbivorous fish. Timon-David (1933, 1934 and 1936) had found the metacercariae of Zoogonidae species in the muscles of urchins (*Panacentrotus lividus*, *Sphaenechinus granularis*, *Anbacia aequituberculata*, *Echinus acutus*, *Psammechinus micnotuberculatus*) but never in mollusks.

The study of the monthly distribution of parasites (Tables 2 and 3) showed that *G. fossarum* and *Curtuteria australis* were present throughout the year at all sampling sites. Infection by *G. fossarum* reaching its highest level (parasitism rate reaches 100 % and mean intensity 534.25 specimens) in southern Tunisia is significantly lower in Rades. The highest prevalence (69.16 %) in this last site was recorded in June and the highest mean intensity in December (31.31 specimens). *Curtuteria australis* was more frequent in Rades than that of port of Sfax, with highest levels of infection in February and January respectively and reached its main prevalence in April at Gargour site. The highest mean intensity value was elsewhere noted at these last localities (27.8 specimens). However *Cercaria lata* has been detected only during 5 months (November 2009 – March 2010) in the site of Gargour and has been signalled in 4 seasons in the other localities. The highest values of parasitism rate were recorded in September at Rades, in October at port of Sfax and in November at Gargour. The lower frequency values were recorded in June at Rades and in May at port of Sfax. The decrease of this frequency during these two months is, undoubtedly, due to the mortality of the miracidia or the very infected clams following the increase of the temperature of water. Indeed, others authors (Evans & Gordon, 1983; Evans, 1985) think that the life-span of infected intermediate hosts may be shorter because of higher temperatures. Moreover, Desclaux *et al.* (2004), in their study about the effect of digenean parasite *Himasthla quissetensis* on the population death of the Cockle, *Cerastoderma edule*, found that a high mortality rate of heavily infected cockles is recorded during periods with high temperatures. The variation of frequency according to sampling localities for these three species can be related with salinity, temperature and abundance in the prospected area of the intermediate and the final hosts allowing the completion of their heteroxenic life cycle. *Acanthoparyphium* sp. has been harvested only for 3 or 4 months, respectively, at the sites of Rades and port of Sfax, and it was more common at Gargour. The highest frequency has not exceeded 5.15 % in January at port of Sfax, reached 23.14 % during August and 88 % in April respectively at Rades and Gargour. However, the highest value of mean intensity was noted at Rades (38.8 specimens). *G. rebecqui* was absent from the site of Rades and was present only in winter at Gargour or in winter and spring at port of Sfax. The highest values of prevalence were recorded in December for two sampling localities; those of mean intensity were noted elsewhere in December at Gargour and during February at port of Sfax.

For the most frequent digenean species (*Gymnophallus fossarum*, *Curtuteria australis*, *Cercaria lata*, *Acanthoparyphium* sp., *Gymnophallus rebecqui*), we noted a low monthly variability for the frequencies. Except for *Curtuteria australis* ($\chi^2=15.58$, $df=1$, $p<0.05$), the variations of the distribution according to the months are generally not significant statistically in the three sampling localities. However, the variation became more noticeable when we compare the sites. Indeed, for *G. fossarum* and *Cercaria lata*, the difference was significant statistically between Rades and port of Sfax ($\chi^2=142.86$, $df=1$, $p<0.05$ and $\chi^2=23.16$, $df=1$, $p<0.05$ for *G. fossarum* and *Cercaria lata* respectively) and elsewhere between Rades and Gargour ($\chi^2=111.74$, $df=1$, $p<0.05$ and $\chi^2=37.38$, $df=1$, $p<0.05$ for *G. fossarum* and *Cercaria lata* respectively). Otherwise,

Table 3. The monthly variation in the mean intensity of digeneans species in *Rudilapes decussatus* from sampling sites

Sampling Sites	Parasites	O		N		D		J		F		M		A		M		J		J		A		S		O	
		2009		2009		2009		2010		2010		2010		2010		2010		2010		2010		2010		2010		2010	
Rades	<i>Gymnophallus fossarum</i>	0		1.4		31.31		5.41		5.64		3.63		3.19		5.95		5.09		6.25		7.33		20.38		4.44	
	<i>Curtuteria australis</i>	9.89		11.35		5.4		3.59		9.08		4.49		4.35		3.55		4.3		2.84		7.7		9.05		6.24	
	<i>Acanthoparyphium</i> sp	0		0		0		0		0		0		0		0		0		0		14.32		38.8		2.66	
Port of Sfax	<i>Gymnophallus fossarum</i>	90.92		306.8		543.25		274.5		429.6		99.94		152.5		86.97		64.37		178.4		84.18		288.6		103.5	
	<i>Curtuteria australis</i>	0		12.86		8.88		8.18		4.57		2.33		4		3.2		0		4		3.31		3.75		3	
	<i>Acanthoparyphium</i> sp.	0		0		16		2.28		0		0		1.33		0		0		0		0		2		0	
	<i>Gymnophallus rebecqui</i>	0		0		6.62		10.5		13.33		5		7		2.75		0		0		0		0		0	
Gargour	<i>Gymnophallus fossarum</i>	-		165.0		232.42		154.3		48.07		103.6		4.27		101		118.6		171.5		185.4		235.5		233.8	
	<i>Curtuteria australis</i>	-		27.85		16.6		24.1		2.5		8.53		11.9		1.54		2.45		3.92		5.17		19.0		13.3	
	<i>Acanthoparyphium</i> sp	-		0		4.26		2.45		0		0		4.27		2.75		2.83		2.33		2		5.86		11.66	
	<i>Gymnophallus rebecqui</i>	-		0		3.9		3.6		2		0		0		0		0		0		0		0		0	

this difference was not significant between the port of Sfax and Gargour for the first species and between Rades and Gargour for the second species. Concerning *Curtuteria australis*, this difference was usually significant between Rades and port of Sfax ($\chi^2=94.07$, $df=1$, $p<0.05$) and elsewhere between port of Sfax and Gargour ($\chi^2=68.26$, $df=1$, $p<0.05$) throughout the year. It became less significant between Rades and Gargour. For *Acanthoparyphium* sp., this difference was usually statistically significant between Rades and Gargour ($\chi^2=38.19$, $df=1$, $p<0.05$) and between the port of Sfax and Gargour ($\chi^2=35.42$, $df=1$, $p<0.05$) whereas it was not significant between Rades and port of Sfax. No significant difference was found between the two localities of harvest (Port of Sfax and Gargour) for *G. rebecqui*. So if we examine the variations of the distribution according to the sampling localities of the most frequent parasite (*G. fossarum*, *Cercaria lata*, *Curtuteria australis* and *Acanthoparyphium* sp.) it is possible to consider them as biological indicators of clams stocks. We think that parasitic fauna could be used to predict the capture area for unknown samples. This result allows us to control the harvesting of shellfish from polluted sites such as the port of Sfax where fishing is prohibited. Parasites were elsewhere used for distinction of fish stocks in the countries of the northern Mediterranean coast (Arthur & Albert, 1993; Margolis, 1993; Mackenzie, 2002; Power *et al.*, 2005; Timi, 2007).

The digenean fauna of clams has been the subject of research in the lagoon of Tunis (Gargouri Ben Abdallah, 2001). The comparison of the digenean fauna from this lagoon with other from Tunisian coasts (Rades, port of Sfax and Gargour) showed a low diversity. Only 3 species were harvested in this lagoon (*Bucephalus labracis*, *Cercaria lata*, *Lasiotocus longicystis*). The last species seems to be specific to the lagoon of Tunis and the others are common in all prospected Tunisian localities. The digenean fauna of clams from the Tunisian coasts compared to that of the Arcachon Bay (Dang, 2009; Dang *et al.*, 2009) showed that in our coasts the diversity is higher. *R. decussatus* from Arcachon Bay is colonized only with 4 species (*Curtuteria arguinae*, *Himasthla* sp., *M. fossarum*, unknown metacercariae). Compared to *Ruditapes philippinarum* from Arcachon Bay (Dang, 2009) and from the French Atlantic shore (Lassalle *et al.*, 2007), *R. decussatus* from our coasts is elsewhere more infected; manila clams were respectively infected only by 3 species (*Curtuteria arguinae* and *Himasthla continua*, *Psilostomum brevicole*) and 5 species (*Himasthla quissetensis*, *H. interrupta*, *H. elongate*, *Curtuteria arguinae*, *Psilostomum brevicole*). The digenean diversity of *R. decussatus* from Tunisian coasts, relatively more important, is undoubtedly related to the strategic situation of Tunisia, that representing a transition zone between the western and eastern Mediterranean, undergoes the influence of the Atlantic through the straits of Gibraltar and of the Red Sea via the Suez canal. The temperature, relatively higher on our coasts, can influence diversity and frequencies of the intermediate and final hosts. Furthermore, Flasch and Leborgne (1992) think that Manila clams, recently introduced to European waters, are probably free of their natural enemies.

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References

- ALLISON, F.R. (1979): Life cycle of *Curtuteria australis* n.sp. (Digenea: Echinostomatidae: Himasthlinae), intestinal parasite of the South Island pied oystercatcher. *N.Z. J. Zool.*, 6: 13 – 20
- ARTHUR, J.R., ALBERT, E. (1993): Use of parasites for separating stocks of Greenland halibut (*Reinhardtius hippoglossoides*) in the Canadian northwest Atlantic. *Can. J. Fish. Aquat. Sci.*, 50 (10): 2175 – 2181. DOI: 10.1139/f93-243
- ATTIA EL HILI, H., BEN SALAH, C., AYARI, W., BEN AMOR, N. (2007): A descriptive study of the infestation clam (*Ruditapes decussatus*) with *Perkinsus olseni* in Lake Bizerte in Tunisia. *Rapp. Comm. Int. Mer Médit.*, 38: 421
- BARTOLI, P. (1965): Experimental development of a new metacercaria of Gymnophallid family Morozov, 1955 (Trematoda: Digenea), a parasite of marine bivalves. *Extrait Bull. Soc. Zool. France*, XC (5 – 6): 571
- BARTOLI, P. (1967): Study of the life cycle of a little known trematode: *Lepocreadium pegorchis* (M. Stossich 1900) (Trematoda, Digenea). *Ann. Parasitol. Paris*, 42 (6): 605 – 619
- BARTOLI, P. (1973): The microbiotopes occupied by metacercariae of *Gymnophallus fossarum* P. Bartoli, 1965 (Trematoda, gymnophallidae) in *Tapes decussatus* L. *Bull. Mus. Natl. Hist. Nat.*, 117: 335 – 349
- BARTOLI, P. (1981): Demographic phenomenon and intraspecific competition of Gymnophallidae Morozov at the second intermediate hosts (Trematoda, Digenea). *Ann. Parasitol. Paris*, 56: 33 – 44
- BARTOLI, P. (1984): Distomatoses des lamellibranches marins sur le littoral méditerranéen Français. *Haloties*, 14: 98 – 107
- BARTOLI, P. (1987): Les Trématodes digénétiques parasites des poissons Sparidés de la Réserve Naturelle de Scandola. *Trav. Sci. Parc Natur. Région. Réserv. Natur. Corse*, 10: 1 – 158
- BOWERS, E.A., BARTOLI, P., RUSSELL-PINTO, F., JAMES, B.L. (1996): The metacercariae of sibling species of *Meiogymnophallus*, including *M. rebecqui* comb. nov. (Digenea: Gymnophallidae), and their effects on closely related *Cerastoderma* host species (Mollusca: Bivalvia). *Parasitol. Res.*, 82: 505 – 510
- BUSH, A.O., LAFFERTY, K.D., LOTZ, J.M., SHOSTAK, A.W. (1997): Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *J. Parasitol.*, 83: 575 – 583. DOI: 10.2307/3284227
- DANG, C. (2009): Population dynamics of Manila clams (*Ruditapes philippinarum*) in the Arcachon basin and consequences on the management of exploited populations. PhD thesis, France, Bordeaux: Bordeaux University
- DANG, C., DE MONTAUDOUIN, X., BALD, J., LANCELEUR, L., PAUL-PONT, I., CAILL-MILLY, N. (2009): Trematode parasites in the non indigenous Manila clam *Ruditapes philippinarum*. *Hydrobiologia*, 630: 139 – 144
- DESCLAUX, C., DE MONTAUDOUIN, X., BACHELET, G. (2004): Cockle (*Cerastoderma edule*) population mortality: the role of the digenean parasite *Himasthla quissetensis*. *Mar. Ecol. Prog. Ser.*, 279: 141–150
- DE MONTAUDOUIN, X., KISIELEWSKI, I., BACHELET, G., DESCLAUX, C.

- (2000): A census of macroparasites in an intertidal bivalve community, Arcachon Bay, France. *Oceanol. Acta*, 23: 453 – 468. DOI: 10.1016/S0399-1784(00)00138-9
- DE MONTAUDOUIN, X., THIELTGES, D.W., GAM, M., KRAKAU, M., PINA, S., BAZAIRI, H., DABOUINEAU, L., RUSSELL-PINTO, F., JENSEN, K.T. (2009): Digenean trematode species in the cockle *Cerastoderma edule*: identification key and distribution along the North-East Atlantic shoreline. *J. Mar. Biol. Assoc. U.K.*, 89: 543-556. DOI: 10.1017/S0025315409003130
- DHRAIEF, M.N., HITONORI, N., ZOUARI, M., GUERGEJ, H., LYMAYEM, Y., M'RABET, R., MISSAOUI, H., KRAÏEM, M.M. (2009): Juvenile rearing of *Ruditapes decussatus* (L., 1758), on the foreshore of Zarat (Gulf of Gabes, Tunisia), in experimental conditions. *Bull. Inst. Natl. Sci. Tech. Mer de Salammbo*, 36: 93 – 106
- DRIRA, Z. (2009): Contribution to the understanding the operation of the Gulf of Gabes: Study of dynamic and structural characteristics of phyto-zooplankton communities in relation to environmental variability and hydrographic characteristics of coastal and ocean areas. PhD thesis, Tunisia, Sfax: Sfax University
- EVANS, N.A. (1985): The influence of environmental temperature upon transmission of the cercariae of *Echinostoma liei* (Digenea: Echinostomatidae). *Parasitology*, 90: 269 – 275
- EVANS, N.A., GORDON, D.M. (1983): Experimental studies on the transmission dynamics of the cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology*, 87: 167–174
- FISCHER, W., BAUCHOT, M.L., SCHNEIDER, M. (1987): Fiches FAO d'identification des espèces pour les besoins de la pêche. (Révision 1). Méditerranée et mer Noire. Zone de pêche 37. Vol. I (Végétaux et Invertébrés). FAO, Rome, 760 pp.
- FLASCH, J.P., LEBORGNE, Y. (1992): Introduction in Europe, from 1972 to 1980, of the manila clam (*Tapes philippinarum*) and effects on aquaculture production and natural settlement. *ICES J. Mar. Sci.*, 194: 92 – 96
- FREDENSBORG, B.L., RAMIREZ, J.J., PARTIDA, L. (2013): *Acanthoparyphium* sp. and other metazoan symbionts of the American oyster, *Crassostrea virginica*, from South Texas. *J. Parasitol.*, 99(6):1129 – 32. DOI: 10.1645/12-164.1
- GAM, M., BAZAIRI, H., JENSEN, K.T., DE MONTAUDOUIN, X. (2008): Metazoan parasites in an intermediate host population near its southern border: the common cockle (*Cerastoderma edule*) and its trematodes in a Moroccan coastal lagoon (Merja Zerga). *J. Mar. Biol. Assoc. U.K.*, 88: 357 – 364. DOI: 10.1017/S0025315408000611
- GARGOURI BEN ABDALLAH, L. (2001): Les trématodes parasites de deux poissons d'intérêt commercial le loup *Dicentrarchus labrax* (Linnaeus, 1758) et l'anguille *Anguilla anguilla* (Linnaeus, 1758). Etude comparative et cycles évolutifs des espèces du genre *Bucephalus*. Thèse de Doctorat. Tunisie, Tunis: Faculté des Sciences de Tunis
- GARGOURI BEN ABDALLAH, L., MAAMOURI, F. (2002): Life cycle of *Bucephalus anguillae* Spakulova, Macko, Berrilli & Dezfuli, 2002 (Digenea, Bucephalidae) parasite of *Anguilla anguilla* (L.). *Syst. Parasitol.*, 53: 207 – 217
- GARGOURI BEN ABDALLAH, L., MAAMOURI, F. (2005): The life cycle of *Bucephalus labracis* (Paggi & Orecchia, 1965) parasite of *Dicentrarchus labrax*. *Bull. Eur. Assoc. Fish Pathol.*, 25: 297 – 301
- GARGOURI BEN ABDALLAH, L., TRIGUI EL MENIF, N., MAAMOURI, F. (2009): The morphology and behaviour of *Cercaria lata* Lespes, 1857 (Digenea, Faustulidae) from the Mediterranean clam *Tapes decussata* (L.). *J. Helminthol.*, 83: 69 – 76. DOI: 10.1017/S0022149X08125160
- HAMIDA, L. (2004): Reproduction of *Ruditapes decussatus* in a natural environment (southern Tunisia) and controlled locality (experimental hatchery): relationship with the immune system. PhD thesis, France, Brest, Western Brittany University
- HANAFY, M.H., GAB-ALLAH, A.F.A., HASSANINE, R.M.E. (1997): Larval trematode (Digenea: Lepocreadiidae) infection in the gonads of the commercial bivalve *Venerupis decussata* from lake Timsah, Suez Canal. *J. Egypt. Germ. Soc. Zool.*, 24: 167 – 181
- KIM, Y., POWELL, E. N. (2006): Relationships among parasites and pathologies in sentinel bivalves: NOAA Status and Trends "Mussel Watch" Program. *Bull. Mar. Sci.*, 79: 83 – 112
- KRAKAU, M., THIELTGES, D.W., REISE, K. (2006): Native parasites adopt introduced bivalves of the North Sea. *Biol. Invasions*, 8: 919 – 925. DOI: 10.1007/s10530-005-4734-8
- LASSALLE, G., DE MONTAUDOUIN, X., SOUDANT, P., CHRISTINE, P. (2007): Parasite co-infection of two sympatric bivalves, the Manila clam (*Ruditapes philippinarum*) and the cockle (*Cerastoderma edule*) along a latitudinal gradient. *Aquat. Living Resour.*, 20: 33 – 42. DOI: 10.1051/alr: 2007013
- LE TREUT, Y. (1986): La palourde. Anatomie - Biologie - Elevage - Pêche - Consommation - Inspection sanitaire. Thèse de Doctorat, France, Nantes: Ecole Nationale Vétérinaire, Université de Nantes.
- LEUNG, T.L.F., POULIN, R. (2007): Recruitment rate of gymnophallid metacercariae in the New Zealand cockle *Austrovenus stutchburyi*: an experimental test of the hitch-hiking hypothesis. *Parasitol. Res.*, 10: 281 – 287. DOI: 10.1007/s00436-007-0479-x
- LEUNG, T.L.F., POULIN, R. (2011): Intra-host competition between co-infecting digeneans within a bivalve second intermediate host: Dominance by priority-effect or taking advantage of others? *Int. J. Parasitol.*, 41: 449 – 454. DOI: 10.1016/j.ijpara.2010.11.004
- LOOS-FRANK, B. (1968). Der entwicklungzyklus von *Psilostomum brevicolle* (Creplin, 1829) [Syn. *P. platyurum* (Mühling, 1896)] (Trematoda, Psilostomatidae). *Zeitschrift für Parasitenkunde*, 31: 122 – 131
- MACKENZIE, K. (2002): Parasites as biological tags in population studies of marine organisms: an update. *Parasitology*, 124: 153 – 163
- MARGOLIS, L. (1993): A case of forensic parasitology. *J. Parasitol.*, 79: 461 – 462. DOI: 10.2307/3283591
- MOORE, J. (2002): Parasites and the behavior of animals. Oxford University Press, 315 pp
- MOURITSEN, K.N., POULIN, R. (2002): Parasitism, community structure and biodiversity in intertidal ecosystem. *Parasitology*, 124: 101 – 117. DOI: 10.1017/S0031182002001476
- MOURITSEN, K.N., POULIN, R. (2003): Parasite induced trophic facilitation exploited by a non host predator: a manipulator's nightmare. *Int. J. Parasitol.*, 33: 1043 – 1050. DOI: 10.1016/S0020-7519(03)00178-4
- POTASMAN PAZ, A., ODEH, M. (2002): Infections outbreaks associated with bivalve shellfish consumption: A worldwide perspective. *Clinical infectious Diseases*, 35: 921 – 928. DOI: 10.1086/342330

- POULIN, R. (1995): Adaptative changes in behaviour of parasitized animals: a critical review. *Int. J. Parasitol.*, 25: 1371 – 1383. DOI: 10.1016/0020-7519(95)00100-x
- POULIN, R., HECKER, K., THOMAS, F. (1998): Hosts Manipulated by One Parasite Incur Additional Costs from Infection by Another Parasite. *J. Parasitol.*, 84 (5): 1050 – 1052
- POULIN, R. (2013): Parasite manipulation of host personality and behavioural syndromes. *J. Exp. Biol.*, 216: 18 – 26. DOI: 10.1242/jeb.073353
- POWER, A.M., BALBUENA, J.A., RAGA, T. (2005): Parasite infracommunities as predictors of harvest location of bug (*Boops boops* L.): a pilot study using statistical classifiers. *Fish. Res.*, 72: 229 – 239. DOI:10.1016/j.fishres.2004.10.001
- OIE, H.K. (2006): Word organisation for animal health. Manual of Diagnostic tests for aquatic Animals. Fifth Edition, Paris, France, 265pp
- POPPE, G.T., GOTO, Y. (1991): European seashells: 1. (Polyplacophora, Caudoveata, Solenogastrea, Gastropoda). Christa Hemmen: Wiesbaden, Germany. 352 pp
- RUSSELL-PINTO, F., GONÇALVES, J.F., BOWERS, E. (2006): Digenean larvae parasitizing *Cerastoderma edule* (Bivalvia) and *Nassarius reticulatus* (Gastropoda) from Ria de Aveiro, Portugal. *J. Parasitol.*, 92: 319 – 332
- SKIRNISSON, K., GALAKTIONOV, K.V., KOZMINSKY, E.V. (2004): Factors influencing the distribution of digenetic trematode infections in a mudsnail (*Hydrobia ventrosa*) population inhabiting salt marsh ponds in Iceland. *J. Parasitol.*, 90(1):50 – 9
- THIELTGES, D.W., REISE, K. (2006): Metazoan parasites in intertidal cockles *Cerastoderma edule* from the northern Wadden Sea. *J. Sea Res.*, 56: 284 – 293.
- THIELTGES, D.W., REISE, K. (2007): Spatial heterogeneity in parasite infections at different spatial scales in an intertidal bivalve. *Oecologia*, 150: 569 – 581. DOI: 10.1016/j.seares.2006.06.002
- THIELTGES, D.W., KRAKAU, M., ANDRESEN, H., FOTTNER, S., REISE, K. (2006): Macroparasite community in molluscs of a tidal basin in the Wadden Sea. *Helgol. Mar. Res.*, 60, 307 – 316. DOI: 10.1007/s10152-006-0046-3
- TIMI, J.T. (2007): Parasites as biological tags for stock discrimination in marine fish from South American Atlantic waters. *J. Helminthol.*, 81: 107 – 111. DOI: 10.1017/S0022149XD7726561
- TIMON-DAVID, J. (1933): Contribution à l'étude du cycle évolutif des Zoogonidés (Trématodes). *C. R. Acad. Sci.*, 196: 1923 – 1924
- TIMON-DAVID, J. (1934): Recherches sur les Trématodes parasites des Oursins en Méditerranée. *Bull. Inst. Oceanogr.*, 652: 1 – 16
- TIMON-DAVID, J. (1936): Sur l'évolution expérimentale des métacercaires de *Zoogonus minus* Looss, 1901 (Trématodes, Famille des Zoogonidae). *C. R. Assoc. Franç. Avan. Sci., Marseille*, 1936: 274 – 276
- TRIGUI-EL MENIF, N. (1995): *Ruditapes decussatus* (Linnaeus, 1758) from the Tunisian coast: biometrics, reproductive and environmental impact on the bioaccumulation of trace metals. PhD thesis, Tunisia, Tunis: Tunis II University
- WALTER, S.L. (1941): Life history of *Caecicola parvulus* Marshall and Gilbert (Cryptogonimidae, Trematoda) and the development of its excretory system. *Trans. Am. Microsc. Soc.*, 60 (4): 461 – 484
- ZARRAD, R., EL ABED, A., M'RABET, R., MISSAOUI, H., ROMDHANE, M. S. (2003): Distribution spatiale de l'ichtyoplancton en été et en automne et conditions environnementales dans le golfe de Tunis. *Bull. Inst. Natl. Sci. Tech. Mer de Salammbo, Tunis*, 30: 39 – 47
- ZAMOURI-LANGAR, N. (1991): Le cycle sexuel de *Tapes decussatus*, Linnaeus 1758 (Bivalve/Veneridae) dans le canal de Tunis. *Bull. Inst. Natl. Sci. Tech. Mer de Salammbo, Tunis*, 18: 5 – 22