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Influence of season, size and sex on the dynamic of gill metazoan parasite infesting the *Balistes capriscus* (Teleostei: Balistidae) of the Gulf of Gabès (Tunisia)

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PEER REVIEW

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Comments

Generally, this is an important research work, in which authors have demonstrated that a pioneering investigation on the effects of season, size and sex on the parasitological indices of the ectoparasite, infesting *B. capriscus* of the Gulf of Gabès. It is showed that host size is a more important indicator than host sex in infestation with ectoparasites. Details on Page 288

ABSTRACT

Objective: To investigate the effects of season, size and sex on the dynamic of gill metazoan parasite infesting the *Balistes capriscus* (Gmelin, 1788) (Teleostei: Balistidae) (*B. capriscus*) of the Gulf of Gabès (Southern Tunisia, Central Mediterranean Sea).

Methods: A parasitological survey of the grey triggerfish *B. capriscus* from the Gulf of Gabès (Southern Tunisia, Central Mediterranean Sea) was conducted monthly from June 2011 to June 2012. A total of 1080 fish were collected from commercial catches by pelagic trawl net at different fishing ports at Chebba (34°14' N, 11°06' E), Kerkennah (34°45' N, 11°17' E) and Zarzis (33°41' N, 11°48' E). The weight, size, sex, date and area of capture of each specimen were recorded. Then, *B. capriscus* was examined to search for ectoparasites. For each parasite species, parasitological indices were calculated.

Results: The parasite species are indentified as two copepods: *Naobranchia variabilis*, *Taneacanthus ballistae* and a monogenean: *Ancyrocephalus balisticus*. The parasitological indices depend significantly on seasonality; the highest prevalence of *Naobranchia variabilis*, *Taneacanthus ballistae* and *Ancyrocephalus balisticus* (28.89%, 35.93% and 55.56% respectively) was recorded during summer season (June–August), while the lowest prevalence of each (6.3%, 4.44%, 8.15%) recorded during winter season (December–February). Furthermore the parasitological indices depend significantly on the host size but not on host sex.

Conclusions: Our data suggest that the dynamic of gill metazoan parasite infesting *B. capriscus* is a result of a complex of biotic and abiotic factors. It is the first study on the effects of season, size and sex on the dynamic of gill metazoan parasite infesting *B. capriscus* (Teleostei: Balistidae) of the Gulf of Gabès (Southern Tunisia, Central Mediterranean Sea).

KEYWORDS

Dynamic, Gill metazoan parasite, Balistes capriscus, Gulf of Gabès

1. Introduction

Grey triggerfish, Balistes capriscus (Gmelin, 1789) (Teleostei:

Balistidae) (*B. capriscus*), belonging to the Balistidae family, is frequently associated with coral reefs, wrecks, outcroppings, artificial structure and hard bottom areas[1]. It is a species that

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occurs in the tropical and temperate zones in both eastern and western parts of the Atlantic Ocean^[2]. Indeed, they are found in the Eastern Atlantic, from the British Isles to Angola, including the Mediterranean Sea, and in the Western Atlantic from the Gulf of Mexico to Argentina^[3]. This species is common in the Southern Mediterranean Sea and appears to have extended its distribution area northwards due to global warming^[4].

Parasites are considered as good biological indicators that can be used as an effective tool to solve the problems related to the environment and the life cycle of their host. In fact, the abundance of the parasites depends strongly on the distribution, migration paths and biology of the host populations^[5]. It also appears that these organisms can be used as bioindicators of pollution and climate change^[6,7].

Several studies have been carried out on the parasites of *B. capriscus* captured from the Atlantic Ocean, but data in the Mediterranean Sea was only reported by Kacem *et al.*^[8], and Kacem and Neifar^[9].

Hence, this work is a pioneering investigation on the effects of season, size and sex on the parasitological indices of the ectoparasite infesting *B. capriscus* of the Gulf of Gabès (Tunisia).

2. Materials and methods

The investigation of parasites was performed on 1080 specimens of *B. capriscus* having a size range from 139 to 427 mm. *B. capriscus* was caught, using specific gill nets and hand lines, between June 2007 and June 2009 from different areas of the Gulf of Gabès: Chebba ($34^{\circ}14'$ N, $11^{\circ}06'$ E), Kerkennah ($34^{\circ}45'$ N, $11^{\circ}17'$ E) and Zarzis ($33^{\circ}41'$ N, $11^{\circ}48'$ E) (Figure 1). Fish were stored individually in sealed plastic bags at 4 °C.



Figure 1. Map of the Gulf of Gabès.

In the laboratory, fish were identified using the method described by Fisher *et al.*[10] and Whitehead *et al*[11]. The weight, size, sex, date and place of capture of each specimen were recorded, and the fish were examined for presence of ectoparasites.

The external surface of each sample was examined thoroughly using hand lens. Areas around the fins, nostril, operculum and the buccal cavity were examined for external parasites. The operculum of each specimen was opened and the inner side was examined under the dissecting microscope. Each gill arch was removed and placed in Petri dishes containing filtered seawater.

Monogeneans were detected using a stereomicroscope, detached from the gills and operculum, and then transferred to a dish containing filtered seawater. They were studied either alive or fixed between slide and coverslip in 70% alcohol. Fixed specimens were stained with Semichon's acetic carmine. Other specimens were double-stained with light green and Semichon's acetic carmine to study the morphology of the clamps. After dehydration through a graded ethanol series, specimens were cleared with clove oil and mounted in Canada balsam. Some fixed specimens were mounted in Berlese's fluid in order to study the haptoral sclerites and the genital armature.

Copepods attaching to the gill filaments were fixed and preserved in ethanol (70%). Before being dissected, they were cleared and stained in lactophenol. Copepods were studied using stereo and light microscopy. Parasites species identification was conducted based on morphological features described by Ho and Kim^[12].

For each parasite species, prevalence (proportion of the population infected) and mean intensity (mean number of parasites of infected hosts) were calculated according to Margolis *et al*[13]. The data were transformed to ln(x+1) where necessary to meet the assumption of homogeneity of variances (homogeneity confirmed by non-significant Cochran's C-test).

The nonparametric equivalent of a One-way ANOVA was used to test the significant effect of season on the epidemiologic index. This was done using MINITAB Statistical Software Release 14. The statistical significance was set at 0.05. When ANOVA shows a significant difference, a posteriori Student–Newman–Keuls (SNK) test was used to determine which means are significantly different at the 0.05 level of probability[14]. Pearson correlations between the number of parasite and the temperature, on the one hand, and the size of fish, on the other, were carried out using SPSS.

The relationship between prevalence and host size was analyzed by Spearman's rank correlation coefficient, and the relationship between prevalence and host sex by the Wilcoxon's signed rank test (both tests were performed using Statistical Graphic System, version 6.0).

3. Results

Three species of metazoan ectoparasites were detected, among which there are two copepods, *Naobranchia variabilis* (N. variabilis), Taneacanthus ballistae (T. ballistae) and one monogenean, Ancyrocephalus balisticus (A. balisticus).

The prevalence and the mean intensity of the ectoparasite fixed on the gill of host during the investigation period from June 2007 to June 2009 were shown in Table 1. The results revealed that the prevalence of Monogenea was higher than that obtained for each copepod (29.26%, 17.50% and 19.26% for *A. balisticus*, *N. variabilis* and *T. ballistae*, respectively). However, the mean intensity for parasite of infected fish was 9.13 for *A. balisticus*, 3.74 for *N. variabilis* and 3.51 for *T. ballistae*.

Table 1

Monthly variation of prevalence and mean intensity of ectoparasite infesting the gills of *B. capriscus* at gulf of Gabès.

Month	A. bali	sticus	N. vari	abilis	T. ballistae		
	Prevalence	Mean	Prevalence	Mean	Prevalence	Mean	
	%	intensity	%	intensity	%	intensity	
December	12.22	4.27	8.89	1.38	6.67	1.33	
January	7.78	4.00	5.56	1.40	4.44	1.25	
February	4.44	3.75	4.44	1.25	2.22	1.00	
Winter	8.15	4.09	6.30	1.35	4.44	1.25	
March	27.78	9.04	16.67	3.60	18.89	3.18	
April	28.89	8.50	17.78	3.94	22.22	3.20	
May	38.89	9.60	22.22	4.35	23.33	3.38	
Spring	31.85	9.10	18.89	4.00	21.48	3.26	
June	47.78	9.16	23.33	4.81	32.22	4.76	
July	52.22	9.57	27.78	3.88	34.44	3.10	
August	66.67	11.53	35.56	5.88	41.11	5.00	
Summer	55.56	10.24	28.89	4.95	35.93	4.32	
September	26.67	8.33	18.89	2.41	23.33	2.81	
October	20.00	8.17	15.56	2.07	12.22	2.55	
November	17.78	8.13	13.33	2.00	10.00	2.22	
Autumn	21.48	8.22	15.93	2.19	15.19	2.61	
All	29.26	9.13	17.50	3.74	19.26	3.51	

As shown in Table 1, Figures 2 and 3, each species of parasite changes in terms of prevalence or mean intensity depending on the season. The prevalence of the *A. balisticus* reaches a maximum value of 55.56% in the summer and a minimum value of 8.15% during the winter. Besides, the mean intensity of *A. balisticus* increased from 4.09 during winter to a maximum value of 10.24 during summer.



Figure 2. Correlation between mean water temperature (°C) of four seasons and parasitic prevalence (%) of gill metazoan parasite infesting *B. capriscus*.



Figure 3. Correlation between mean water temperature (°C) of four seasons and parasitic mean intensity of gill metazoan parasite infesting *B. capriscus.*

The same evolution is observed for the copepods. In fact, the seasonal data revealed that the highest prevalence of *N. variabilis* and *T. ballistae* (28.89%, 35.93%) are recorded during summer, while the lowest prevalence (6.30%, 4.44%) was recorded during winter.

The SNK test showed that the prevalence of *A. balisticus* in summer was higher than the other seasons (Table 2). However, for the *N. variabilis* and *T. ballistae*, the prevalence parameters of the summer and spring were equal, but higher than that in the winter (Tables 3 and 4).

Table 2

Analysis of prevalence and mean intensity of *A. balisticus* collected from *B. capriscus*.

Source of variation	df	Prevalence			Intensity		
		MS	F	Р	MS	F	Р
Season	3	1204.39	28.07	0	21.33	42.97	0
Residual	11	42.90			0.49		
Cochran's C-test		C=0.443 (P<0.05)			C=0.320 (P<0.05)		
Transformation		None			None		
SNK test		Su>Sp=Au>Wi			Su=Sp; Au=Sp>Wi		

Su: Summer; Sp: Spring; Au: Autumn; Wi: Winter.

Table 3

Analysis of prevalence and mean intensity of *N. variabilis* collected from *B. capriscus*.

Source of variation	df	Prevalence			Intensity		
		MS	F	Р	MS	F	Р
Season	3	1.09	23.77	0	0.52	50.38	0
Residual	11	0.05			0.10		
Cochran's C-test		C=0.63	C=0.836 (P<0.05)				
Transformation		L	Ln(x+1)				
SNK test		Su=Sp;	Su=Sp>Au>Wi				

Su: Summer; Sp: Spring; Au: Autumn; Wi: Winter.

Table 4

Analysis of prevalence and mean intensity of *T. ballistae* collected from *B. capriscus*.

Source of variation	df		Prevalen	ce	Intensity			
		MS	S F P		MS	F	Р	
Season	3	2.1	21.58	0	0.42	28.53	0	
Residual	11	0.1			0.01			
Cochran's C-test			C=0.616	5#	$C = 0.892^{\#}$			
Transformation			Ln(x+1)	Ln(x+1)			
SNK test		Su=	Sp; Au=S	Sp>Wi	Su=Sp; Au=Sp>Wi			

Su: Summer; Sp: Spring; Au: Autumn; Wi: Winter. #: Not significant.

Nevertheless, the mean intensity is equal in summer and spring and higher than that found in the winter for the 3 parasites similarly (Tables 2, 3 and 4).

The correlation between the water temperature and mean intensity of the ectoparasites was determined according to the output of the Person correlation coefficient. Indeed, as shown in Figure 3, the mean intensity increased with the increase in water temperature and its coefficient was found to be significant (P=0.262).

Weak positive correlations were observed between intensities of each parasite species and host body length (Figure 4). The prevalence and mean intensity of infection was monitored for seven body size classes of B. capriscus collected during the 24 months of sampling. There was a tendency for both prevalence and mean intensity to increase with the growth of host body size (Table 5). The prevalence of A. balisticus grew from 0% in the lowest size inferior to 150 mm to 72.06% in the highest body size group of 400 to 450 mm. In addition, the prevalence of copepod N. variabilis and T. ballistae grew from 0% in the lowest size inferior to 150 mm to 57.35% and 54.41%, respectively, in the highest body size group of 400 to 450 mm. The mean intensity of A. balisticus increased up to 24.84 in the largest body size class. Compared to the fish size, this increase in the intensity of infection was significant (r=0.98, P<0.01). Similarly, the mean intensity of N. variabilis and T. ballistae increased up to 5.41 and 6.78, respectively, in the largest body size class. Such increase in the fish size was significant (r=0.96, P<0.01; r=0.99, P<0.01).

Table 5

Comparison of infection of *A. balisticus*, *N. variabilis* and *T. ballistae* on *B. capriscus* by host age (size).

Host size (mm)	A. balisticus		N. vari	iabilis	T. ballistae	
	P (%)	Ι	P (%)	Ι	P (%)	Ι
<150	0	-	0	-	0.00	-
150-200	12.42	1.00	0.00	-	0.00	-
200-250	22.89	1.49	3.52	1.00	7.39	1.33
250-300	34.78	3.80	19.78	1.69	21.98	1.80
300-350	35.03	6.35	25.89	3.06	38.07	2.96
350-400	57.47	15.82	57.47	5.02	40.23	4.49
400-450	72.06	24.84	57.35	5.41	54.41	6.78
rs	0.97^{*}	0.98^*	0.96^{*}	0.96^{*}	0.99^{*}	0.99^{*}

P: Prevalence; I: Mean intensity; rs: Spearman's rank correlation coefficient, *: Significant at 0.01 level.

Concerning the prevalence and mean intensity of the three metazoan ectoparasites species according to the host sex, the male and female fish displayed no differences. Actually, the fish sex was not determinative in the parasitological indices of the ectoparasites (Table 6).

Table 6

Comparison of infection of *A. balisticus*, *N. variabilis* and *T. ballistae* in *B. capriscus* and host sex.

	A. balisticus			N. variabilis				T. ballistae				
Female		Male		Female		Male		Female		Male		
	P (%)	Ι	P (%)	Ι	P (%)	Ι	P (%)	I	P (%)	I	P (%)	Ι
	29.80	9.15	29.38	8.92	17.33	3.69	17.49	3.91	19.60	3.71	19.10	3.59
Ζ	0	0.40	0	0.40	0.62	0.36	0.62	0.36	0.63	0.53	0.63	0.53

P: Prevalence, I: Mean intensity, Z: Wilcoxon's test.

4. Discussion

The present parasitological study is a pioneering work representing an investigation on the effects of season, size and sex on the parasitological indices of the ectoparasite, infesting *B. capriscus* (Teleostei: Balistidae) of the Gulf of Gabès (Tunisia)

With respect to *A. balisticus* (Monogenea, Ancyrocephalidae), it was reported on the gills of *B. capriscus* fished from the Gulf of Mexico^[15]. It is for the first time that this parasite is reported in *B. capriscus* caught from the Gulf of Gabès, Mediterranean Sea.



Figure 4. Number of A. balisticus, N. variabilis and T. ballistae harbored by B. capriscus with different body lengths at Gulf of Gabès.

N. variabilis is commonly found in the Atlantic Ocean in many fish species belonging to several families such as Tetraodontidae (*Lagocephalus laevigatus*); Serranidae (*Centropristes striatus*, *Diplectrum formosum*); Pomadasyidae (*Haemulon plumieri*); Monacanthidae (*Ceratacanthus schoepfi*); Diodontidae (*Chilomycterus spinosus*, *Chilomycterus atinga*, *Chilomycterus schepfi*); Ogcocephalidae (*Ogcocephalus* sp., *Ogcocephalus radiatus*) and Clupeidae (*Brevoortia patronus*)[16,17]. However, to the best of our knowledge, it is the first time that it is reported in the Mediterranean Sea on the gill filaments of *B. capriscus*.

Taeniacanthus balistae was found for the first time on the gill filaments of Balistes sp. by Claus^[18]. This copepod was also reported in Alutera monoceros, Stephanolepis setifer, Stephanolepis hispidus, Diodon hystrix, Canthidermis rotundatus, Cantherhines pullus, Balistes vetula, Aluterus heudelotii in different regions of the world such as Florida, Lebanon, Haiti, Philippine Islands, in Alabama, the Gulf of Tunis and Belize^[19].

Due to the simple life cycle, Monogeneans, which are of great interest to the ecologists, are considered as one of the most important and sensitive parasites to any changes in water quality. The statistical analysis has revealed the presence of significant changes in the parasitological indices of *N. variabilis*, *T. ballistae* and *A. balisticus* among seasons conditions. Several possibilities exist which might account for the seasonal fluctuations in the prevalence and mean intensity of parasitic infection. Seasonality related host feeding habits, immunological alterations, availability of infected intermediate hosts, hormonal changes, and temperature are the most frequent causes suggested for seasonal fluctuation in prevalence and mean intensity of parasitic infections.

In fact, the prevalence and mean intensity increase during the summer when the temperature is high coinciding with spawning season of the grey triggerfish^[1], and decrease during the winter when the temperature is low, thus suggesting that the transmission rates increase with the increase in temperature. The latter is perhaps the most important biotic factor with measurable effects on all aspects of the monogenean life-cycle^[20,21]. It is worthwhile to mention that similar tendency was also observed for both copepods.

The obtained results have shown that the parasitological indices of ectoparasites depend significantly on the host size, which is in good agreement with research works undertaken on three reef fish species: *Stegastes nigricans*, *Dascyllus aruanus* and *Cephalopholis argus*^[20]. The positive correlation can be explained by the fact that the larger gill surface and an increased volume of water passing over the gills in larger fish might

increase the probability of contact with ectoparasite, and result in a higher infection level.

In our study we observed that host sex was not an important factor influencing the parasitism of *B. capriscus* in the Gulf of Gabès. According to Luque *et al.*[21] and Alves *et al.*[22], the absence of correlations in parasite prevalence and mean intensity with the sex of the host fish is a widely documented pattern, which is interpreted as a consequence of the absence of sexual differences in some biological aspects of the fish. This may be due to the similar sizes of male and female hosts.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

The rich monogeneans parasitofauna found in the gills, especially when present in large numbers, can cause severe damage to the gill and impair normal respiratory function, leading to the death of the host by hypoxia. This study is a pioneering investigation on the effects of season, size and sex on the parasitological indices of the ectoparasite, infesting *B. capriscus* of the Gulf of Gabès (Tunisia). This study has an important significancy about special gill parasites on fish.

Research frontiers

The manuscript describes that the parasitological indices of ectoparasites depend significantly on the host size, which is in good agreement with other researches. Also, the study reports that host sex is not an important factor influencing the parasitism of *B. capriscus* in the Gulf of Gabès.

Related reports

Monogeneans parasites on many fish species belonging to several families were reported by earlier workers. The literature data shows that monogeneans which are of great interest to the ecologists due to their simple life cycle are considered as one of the most important and sensitive parasites to any changes in water quality. They can cause severe damage to the gill.

Innovations and breakthroughs

This scientific study is a pioneering investigation on the parasites infesting *B. capriscus* in the Gulf of Gabès for the first time.

Applications

The manuscript shows that parasitological indices of ectoparasites depend on its host, but no host sex.

Peer review

Generally, this is an important research work, in which authors have demonstrated that a pioneering investigation on the effects of season, size and sex on the parasitological indices of the ectoparasite, infesting *B. capriscus* of the Gulf of Gabès. It is showed that host size is a more important indicator than host sex in infestation with ectoparasites.

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