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Seasonal Variation and Pathology Associated with Helminthes Infecting Two Serranids (Teleostei) of Iskenderun Bay (Northeast Mediterranean Sea), Turkey

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Abstract

This study presents a detailed record of the philometrid nematode, *Philometra lateolabracis* (Nematoda: Philometridae) and trypanorhynch pleurocercoid, *Grillotia* sp. (Cestoda: Trypanorhynchia) in white grouper, *Epinephelus aeneus*, and dusky grouper, *E. marginatus*, from Iskenderun Bay, Turkey, during 2000-2003. A total of 885 (418 white and 467 dusky grouper) serranids were sampled during the research period. Of these fish, 130 (31.1%) white and 118 (25.3%) dusky groupers were found infected with helminthes. In order to estimate the influences of helminthic infestation on fish growth, the length-weight relationship was determined. Trypanorhynch cestodes larvae were collected only from the external mesenteries of the internal organs, whereas philometrid nematodes were found in the ovary of the infected fish. Severe pathologic changes were detected in the ovary tissues of white groupers infected with *P. lateolabracis*.

Key Words: Philometra lateolabracis, Nematoda, *Grillotia* sp., Cestoda, *Epinephelus aeneus*, *Epinephelus marginatus,* Grouper, Mediterranean Sea, Iskenderun Bay

Introduction

Groupers (Serranidae; Perciformes) are protogynous hermaphrodites (Heemstra and Randall, 1993; Gokce *et al*., 2001). Since groupers have a high commercial value, both fishing and culturing of these species have become more popular throughout the world. However, the members of the genus *Epinephelus* were particularly found at risk, probably due to their large body size, long lifespan and slow reproduction (Morris *et al.*, 2000). Therefore, exploratory research attempts to assess serranid species and their parasites have been given an increasing importance in recent years. Philometrid nematode and trypanorhynch cestode are important helminthic disease agents for serranids. Whereas *Philometra* sp. is known as a serious pathogen of groupers (Cengizler and Sarihan, 1995; Genc, 2002; Moravec *et al*., 2003; Moravec and Genc, 2004; Moravec, 2004), no clear evidence of pathologies of encysted trypanorhynch cestode (Diesing, 1863) pleurocercoids has been proved yet (Scholz *et al*., 1993; Genc, 2002). Moreover, factors that influence the prevalence of parasites have not been documented. This study was designed to investigate the condition of groupers, *E. aeneus* and *E. marginatus* with regards to helminth infections in the Northeast Mediterranean Sea.

Material and Methods

During the 36 month-sampling period, fish were collected monthly between June 2000 and May 2003 using fish pots (spherically, 76x55 cm and 61x48 cm) in Iskenderun Bay (35°54'09"E - 36°30'05"N, 35°54'09"E - 36°25'04"N), in the Northeast Mediterranean Sea, Turkey. Specimens were all females because the sampling was only conducted by using fish pots. Groupers turn into males later in their life (after spawning as a female for one or more years, the grouper changes sex, functioning as a male during future spawning events) and usually move slowly and find a cave or sheltering canyon to hide. Due to this reason, male groupers are generally caught using harpoons or tridents, not fish pots.

A total of 885 (mean 24.6 fish/month) groupers were examined for philometrid nematodes and trypanorhynch cestodes. Water temperature was monthly recorded and all sampled fish were measured for both weight and length using a digital scale with an accuracy of ±0.01g and a millimetric ruler, respectively. Following these measurements, fish were examined in terms of occurrence of helminth infestations. All individuals were recorded separately as the infected ones with helminths and the non-infected individuals. Encysted cestode larvae were removed from outer membranes of the hepatopancreas, intestine, and gonads of fish using a tiny pointed tweezers. The encysted trypanorhynch pleurocercoids were identified as *Grillotia* sp. (Scholz *et al.*, 1993; Campbell and Beveridge, 1994; Palm and Overstreet, 2000; Knoff *et al*., 2004). Philometrid nematodes (Nematoda, Drancunculoidae) were isolated from the ovarian tissues and ovary ducts of serranids, and identified as *Philometra lateolabracis* Yamaguti, 1935 (Moravec and Genc, 2004). The parasites were fixed in labelled tubes containing 10 ml 70% ethyl alcohol and the ovary specimens were placed into separate tubes with 10% buffered formalin (Frimeth, 1994; Bush *et al.*, 1997; Palm, 1997; Beveridge *et al.*, 1999). After 24 hours, once all fixed tissue specimens were manually processed for histopathological examination, they were embedded in paraffin wax. Tissue sections were, then, cut at 5µm thickness (Leica), mounted on lam, and stained with Mayer's haematoxilyn and eosin. The stained sections were examined with a microscope (Olympus trinocular BX50).

In order to illustrate the influences of parasitic infestation on fish growth, the length-weight relationship was calculated using the following formula; $W=aL^b$, where "*W*" indicates fish weight, "*L*" refers to fish length, and "*b*" is an exponent. According to Garcia *et al*., (1998), in the equation of length-weight relationship, no clear-cut explanation exists for biological interpretation of the numerical values of the parameters "*a*" and "*b*". However, there can be an exception of isometric growth pattern. In these cases, "*a*" can be interpreted as a condition factor. In statistical analyses, exponent "*b*" was measured repetitively. Estimated exponents were, then, compared using independent *t*-test with a 95% confidence. Both linear and curvilinear (quadratic) models were tested in order to estimate the relationship between water temperature and infection rate. Due to the fact that zero-catch synthetically increased the variation, it was disregarded during the statistical analysis. Statistical analyses were performed using, Statistica 2000 for Windows release 5.0J (Statsoft Inc.) and Excel 2002 (Microsoft Corp.).

Results

Two species of helminth parasites *Philometra lateolabracis* (Nematode) and *Grillotia* sp. (cestode pleurocercoid) were detected from white and dusky groupers. Prevalence of *P. lateolabracis* was 22.0% in white groupers (n=418) and 14.8 % in dusky groupers (n=467) with a range of 5-83 parasites per fish (Table 1). The parasite infection showed seasonal variations with the highest prevalence in summer season (Figures 1 and 2). There was a positive relationship between water temperature and prevalence of *P. lateolabracis* in white groupers (r=0.90) or dusky groupers (r=0.57).

Prevalence of *Grillotia* sp. was 17.7% in white groupers and 18.4% in dusky groupers with a range of 3-12 parasites per fish (Table 1). There was a relatively low correlation between temperature and the prevalence rate of *Grillotia* sp. infection of white (r=0.43) or dusky (r=0.38) groupers. The parasite had no apparent seasonal changes in the prevalence of infection, and was found in all seasons except for winter of 2001-2002 in dusky groupers (Figures 1. and 2.).

There was no significant difference in the mean length and weight of white and dusky groupers between *P. lateolabracis*-infected and un-infected groups (p>0.05) (Table 2).

The histopathological observations indicated that *P. lateolabracis* infection caused severe damages in the ovary of groupers (Figure 3). Hyperemia, congestion, fusion, and edema were observed in the epithelium of ovary infected with nematodes. In addition, the parasite disturbed egg releases while blocking the ovarian ducts.

Discussion

Many parasite species, especially helminthes, possess complex life cycles involving trophic transmission from one host to the next by the consumption of infected intermediate hosts. Results of the present study indicated that the prevalence of only nematode-infected and only cestode-infected and both nematode and cestode-infected (Nn, Nc, Nn+c) white

Table 1. The monthly helminth infection status of groupers* (June 2000 to May 2003)

	The number of grouper		Un-infected Nui (%)		Nematode Nn (%)		Cestode Nc (%)		Nematode+Cestode Nn+c (%)	
Months	W	D	W	D	W	D	W	D	W	D
Jan.	29	36	26 (89.7)	34 (94.4)	0	0	2 (6.9)	2 (5.6)	1 (3.4)	0
Feb.	30	40	27 (90)	32 (80.0)	0	0	2 (6.7)	8 (20.0)	1 (3.3)	0
Mar.	36	35	25 (69.4)	24 (68.6)	4 (11.1)	5 (14.3)	4 (11.1)	4 (11.4)	3 (8.3)	2 (5.7)
Apr.	38	46	28 (73.7)	28 (60.9)	3 (7.9)	5 (10.9)	5 (13.2)	7 (15.2)	2 (5.3)	6 (13.0)
May	43	56	27 (62.8)	50 (89.3)	6 (14)	2 (3.6)	4 (9.3)	3 (5.4)	6 (14)	1 (1.8)
June	40	41	21 (52.5)	24 (58.5)	10 (25)	2 (4.9)	2 (5)	6 (14.6)	7 (17.5)	9 (22)
July	38	38	19 (50)	25 (65.8)	10 (26.3)	6 (15.8)	5 (13.2)	2 (5.3)	4 (10.5)	5 (13.2)
Aug.	33	36	18 (54.6)	19 (52.8)	10 (30.3)	5 (13.9)	0	6 (16.7)	5 (15.2)	6 (16.7)
Sep.	30	38	19 (63.3)	25 (65.8)	6 (20)	6 (15.8)	1 (3.3)	3 (7.9)	4 (13.3)	4 (10.5)
Oct.	35	38	22 (62.9)	29 (76.3)	7 (20)	3 (7.9)	3 (8.6)	3 (7.9)	3 (8.6)	3 (7.9)
Nov.	38	33	32 (84.2)	27 (81.8)	0	0	6 (15.8)	5 (15.2)	0	1 (3.0)
Dec.	28	30	24 (85.7)	30 (100)	0	0	4 (14.3)	0	0	0
Total	418	467	288 (68.9)	349 (74.7)	56 (13.4)	32 (6.9)	38 (9.1)	49 (10.5)	36 (8.6)	37 (7.9)

*Nui: The number of un-infected grouper, Nn: The number of infected grouper with nematodes (*P. lateolabracis*), Nc: The number of infected grouper with cestodes (*Grillotia* sp.), Nn+c: The number of infected grouper with nematodes+cestodes, (%): Prevalence W: White grouper, D: Dusky grouper

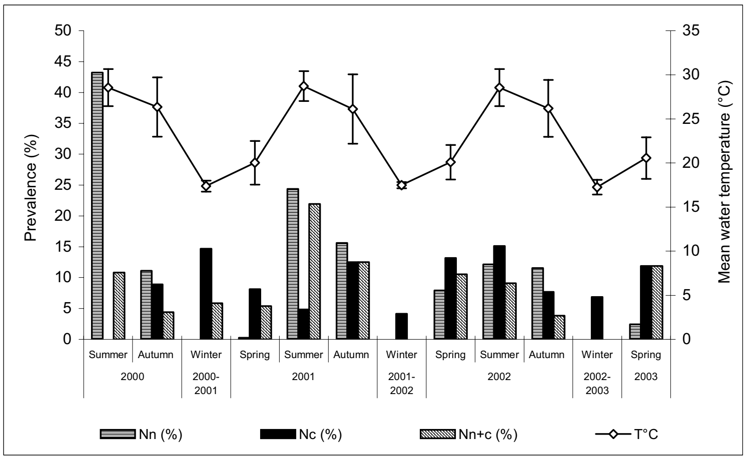

Figure 1. Seasonal parasite changes in the prevalence of *Philometra lateolabracis* (Nn) and *Grillotia* sp. (Nc) in white groupers.

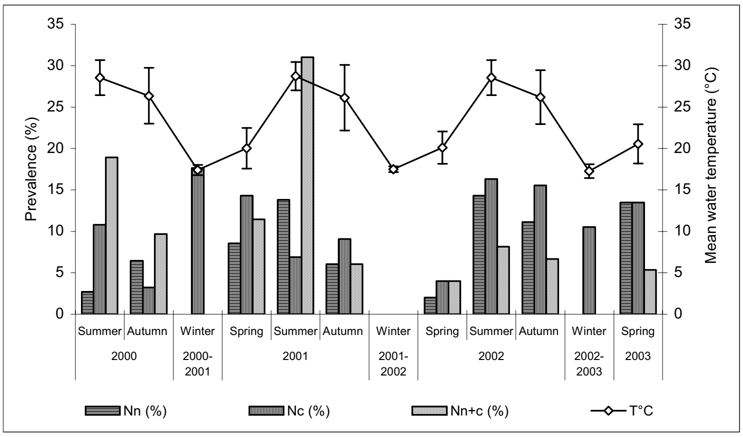

Figure 2. Seasonal parasite changes in the prevalence of *Philometra lateolabracis* (Nn) and *Grillotia* sp. (Nc) in dusky groupers.

Table 2. The relationship between growth and infected/non-infected groupers

Groupers		$W= aL^b$	*TL*±Sd. (TL_{min}-TL_{max})	*W*±Sd. (W_{min}-W_{max})
Un-infected	White	$W= 0.13L^{2.30}$ $r^2= 0.87$	29.98±7.28 (16.8-69.7)	376.69±251.60 (140-2123)
	Dusky	$W= 0.19L^{2.23}$ $r^2= 0.87$	32.27±9.75 (16.1-67)	509.16±362.34 (183-2832)
Nematode	White	$W= 0.09L^{2.39}$ $r^2= 0.92$	31.22±7.14 (20-63.7)	378.14±242.30 (197-1985)
	Dusky	$W= 4.04L^{1.34}$ $r^2= 0.87$	32.26±5.77 (23-45.3)	438.09±113.38 (251-682)
Cestode	White	$W= 0.02L^{2.73}$ $r^2=0.90$	34.97±8.61 (19.9-56.8)	494.73±330.50 (156-1717)
	Dusky	$W= 0.15L^{2.29}$ $r^2= 0.88$	32.51±8.91 (19.6-65)	509.40±388.65 (177-2580)
Nematode +Cestode	White	$W= 0.05L^{2.56}$ $r^2= 0.96$	38.09±11.13 (23.6-69)	638.97±573.77 (208-2350)
	Dusky	$W= 0.52L^{1.95}$ $r^2= 0.81$	32.61±5.43 (22-44.7)	491.37±177.83 (183-1086)
Total	White	$W= 0.09L^{2.39}$ $r^2= 0.90$	31.30±8.16 (16.8-69.7)	410.21±307.76 (140-2350)
	Dusky	$W= 0.19L^{2.23}$ $r^2= 0.87$	32.32±9.15 (16.1-67)	502.91±342.40 (177-2832)

and dusky groupers, during a three-year sampling period, were 13.4%, 6.9% 9.1% and 10.5%, 8.6%, 7.9%, respectively. Amundsen *et al*. (2003) indicated that parasite carrying capacity might be higher in predator hosts. Since Serranids are predators, the prevalence of infestations in groupers is worth being taken into account. These prevalences were in consistency with Amundsen *et al*. (2003)'s notion, excluding of the prevalences of nematode infections in wild fish.

Warm water conditions resulted in an unusually heavy infestation of philometrid nematodes (parasitic nematodes decreased the spawning capacity of serranid fish during hot seasons). The nematode prevalence records of the current study showed that one out of every four-serranid samples in Iskenderun Bay was infected with philometrid nematodes. This high prevalence of nematodes clearly underlines the potential threat to the grouper populations.

It is continuously reported that helminth

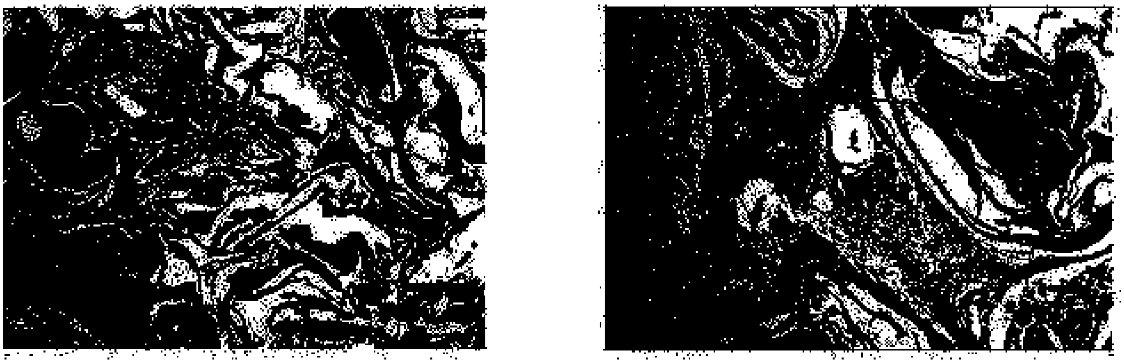

Figure 3. Histological sections of the ovary of white and dusky groupers infected with *Philometra lateolabricis* that cause seriously destruction (A: White grouper (x100), B: Dusky grouper (x200), Nu: nucleus, P: Body of the nematode, POF: Post-ovulatory follicle, St6: Oocyt stage six, Zr: Zona radiata, H&E)

infections are of concern for sea fish aquaculture (Berland 1987; Sunders 2003). Histological observations in this study indicated varying degrees of histopathological degenerative changes in the ovary of serranids.

The present study is the first report and large-scale investigation of the effects of philometrid nematodes and trypanorhynch cestodes on serranid species in Iskenderun Bay, the Northeast Mediterranean Sea, Turkey. Considering current findings, we should inform that *P. lateolabracis* is a serious threat to white grouper, *E. aeneus* and dusky groupers *E marginatus*. Future studies should make it possible to accumulate enough biological information to find out control procedures to reduce these parasite infections. It would be a very important attempt to address what we could do to maintain our natural environments, to create sustainable natural fish stocks and aquaculture development in near future.

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