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Seasonal variation of *Hysterothylacium aduncum* (Nematoda: Raphidascarididae) infestation in sparid fishes in the Northeast Mediterranean Sea

Mustafa KALAY[1,*], Ahmet Erdem DÖNMEZ[2], Cafer Erkin KOYUNCU[2],
Ercüment GENÇ[3], Güzin ŞAHİN[4]

[1]Department of Natural Sciences, Faculty of Fisheries and Aquaculture,
Mersin University, Yenişehir Campus, 33169 Mersin - TURKEY

[2]Department of Aquaculture, Faculty of Fisheries and Aquaculture,
Mersin University, Yenişehir Campus, 33169 Mersin - TURKEY

[3]Department of Aquaculture, Faculty of Fisheries and Aquaculture,
Mustafa Kemal University, Tayfur Sökmen Campus, 31034 Hatay - TURKEY

[4]Department of Parasitology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY

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Abstract: The presence of a Raphidascarid parasitic nematode *Hysterothylacium aduncum* (Rudolphi, 1802) in the sparid fish *Sparus aurata* and *Diplodus vulgaris* was investigated in this study. A total of 471 individuals (208 *Sparus aurata* and 263 *Diplodus vulgaris*) collected for this purpose from the Gulf of Mersin between May 2003 and April 2004 were examined. Seasonal variation in the intensity (MI: mean ± SD) and prevalence (P: %) of the parasite were also determined and the highest P and MI values were found in June 2003 (3.5 ± 0.71 and 8.70%) for *S. aurata* and March 2004 (4.0 ± 0 and 19.5%) for *D. vulgaris*.

Key words: *Hysterothylacium aduncum*, sparidae, Gulf of Mersin, seasonal variation

Kuzey-Doğu Akdeniz'deki sparid üyelerinin *Hysterothylacium aduncum* (Nematoda: Raphidascarididae) enfestasyonunda mevsimsel değişim

Özet: Araştırmada, bir Raphidascarididae (Nematod) türü olan *Hysterothylacium aduncum* (Rudolphi, 1802)'un *Sparus aurata* ve *Diplodus vulgaris* türlerindeki varlığı araştırılmıştır. Bu amaçla Mayıs 2003 - Nisan 2004 tarihleri arasında Mersin Körfezi bölgesinden toplam 471 adet Sparidae bireyi (208 adet *Sparus aurata,* 263 adet *Diplodus vulgaris*) incelenmiştir. *H. aduncum*'un infestasyon oranı (OI: ortalama infestasyon ± SD) ve yoğunluğunun (Y: %) *S. aurata* ve *D. vulgaris* için en yüksek oranları sırasıyla Haziran 2003'de (3,5 ± 0,71 ve % 8,70) ve Mart 2004'de (4,0 ± 0 ve % 19,5) belirlenmiştir.

Anahtar sözcükler: *Hysterothylacium aduncum*, sparidae, Mersin körfezi, mevsimsel değişim

* E-mail: mkalay@mersin.edu.tr

The superfamily Ascoridoidea (families: Anisakidae and Raphidascarididae), commonly named anisakids, consists of numerous parasites that may infect a wide variety of water organisms. The genera of Anisakidae living in the aquatic environment of biological and economic importance are *Anisakis*, *Pseudoterranova*, and *Contracaecum*. Sea mammals and birds can be the definitive hosts of the members of these genera. *Hysterothylacium* is the other important anisakid genus in the aquatic environment and it belongs to the family Raphidascarididae. Both larvae and adult forms of the genus *Hysterothylacium* can also be present in teleosts (1). *Hysterothylacium aduncum* (Rudolphi, 1802) is one of the nematodes that may cause anisakiasis (2). It lives as sexually mature adults in the digestive tracts of marine teleosts and its larvae are known to occur in marine invertebrates and in fish. The 3rd stage larvae of *Hysterothylacium aduncum* have been found encapsulated in the mesentery and viscera of a wide range of fish that act as transport hosts (3). *Hysterothylacium* and related genera have an intestinal cecum and a short esophageal ventricle with a fairly large posterior appendix. However, the cecum increases the intestine's inner surface so that the size of the ventricular appendix may indicate the species' ability to bore into prey (4). The larva hatching from the egg is assumed to be 2nd stage, but it was considered an early young 3rd stage by Berland (5). Furthermore, 3rd stage larvae, "bearing teeth", are known to be a host in many invertebrates and they are encapsulated in the viscera of teleosts. Larval size appears important, with larvae developing in an invertebrate host smaller than 3 mm will encapsulate in a fish as 3rd stage; however, those bigger than 3 mm remain in the fish gut and grow and molt twice until the adult 5th stage of the host. Larvae entering the body of young fish cause harm and even death. (5). *H. aduncum* (Rudolphi, 1802) can occur both as encapsulated 3rd stage larvae in the viscera and as freely moving larvae, preadults (4th stage), and adults in the gut of teleosts (6). *H. aduncum* and related species have 3 lips and an esophagus that has a posterior ventricular appendix; their intestine has also an intestinal cecum, the opposed appendix and cecum that explains the old name *Contracaecum*. The excretory pore, on the other hand, is at the base of the nerved ring. The conical "cactus tail" may give them a "toe-grip" when "swimming" in the gut contents. In a dead fish it is quite common to see the stomach worms leave the host via the mouth and gills, and those in the intestine wriggle out through the anus (4). *H. aduncum* has been reported in fish collected in the Northeastern Atlantic and northern Europe (7), the Southwestern Mediterranean Sea (8), the Adriatic Sea (9), and the northeastern part of the Mediterranean Sea (10). Anisakiasis agents (*Anisakis simplex*, *Pseudoterranova decipiens*, *Contracaecum osculatum,* and *Hysterothylacium aduncum*) are responsible for human infections caused by the consumption of raw, undercooked (11), and not adequately salted, pickled, or smoked seafood (12). Nematodes are one of the most important agents for financial losses in the marketing value of fish (13). Therefore, these infections should be taken into serious consideration for wild and cultured marine fish. This study was designed to investigate the anisakid infections in 2 sparid fish species, *Sparus aurata* (gilthead sea bream) and *Diplodus vulgaris* (two banded bream), distributed along the south coast of Turkey (northeast Mediterranean Sea). In total, 208 specimens of gilthead sea bream (*Sparus aurata*) and 263 specimens of two banded bream (*Diplodus vulgaris*) were collected between May 2003 and April 2004 along the southern coast of Turkey (northeast Mediterranean Sea), located at 36.65°-36.8° N, 34.55°-34.8° E (Figure). Samples were obtained from commercial catches and individual fishermen. The fish were measured for total length and weight. The visceral organs and body muscles were also examined (cranial and post-cranial). Recovered parasites were then washed in physiological saline solution, fixed in 4% buffered formalin, and cleared in glycerin for examination. After examination, specimens were preserved in 70% ethanol (14). They were then identified using a phase contrast Nikon microscope (H550L). Nematode

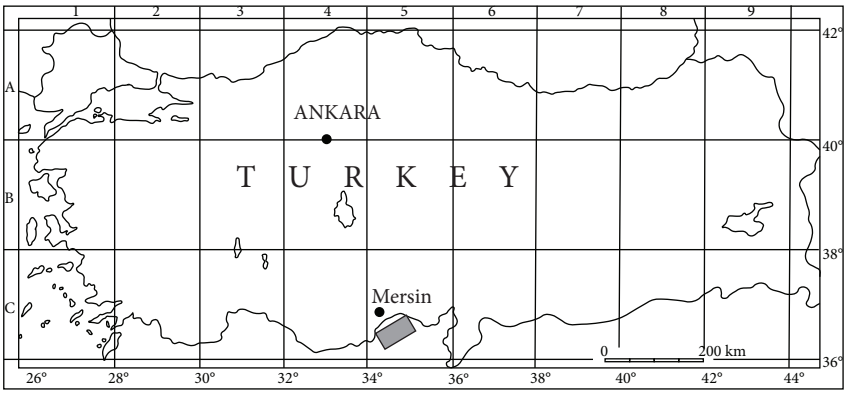

Figure. Sampling area.

specimens were counted in each infected fish and were identified according to their morphologic features (4,5,6,15). The prevalence (P) and the mean intensity (MI) of *H. aduncum* were measured as defined by Bush et al. (16). The Kruskal-Wallis non-parametrical test for heterogeneity was used to analyze the prevalence data.

Endoparasitic examination of the sparid individuals showed that the raphidascarid nematodes were only present in the stomach and intestine. The identified nematodes were classified as the adult form of *Hysterothylacium aduncum* (Rudolphi, 1802). Numbers of *H. aduncum* worms per gilthead sea bream and two banded bream were 2-7 and 1-6, respectively. Results of the present study for gilthead sea bream and two banded bream indicated that highest P and MI values were found in June 2003 (P = 8.70%), (MI = 3.5), May 2003 (P = 19.5%), and March 2004 (MI = 4.0), respectively. Moreover, the overall mean P and MI values for gilthead sea bream and two banded bream were in the warm seasons 6.25% (P) and 1.92 (MI), and 6.8% (P) and 2 (MI), respectively.

The overall prevalence (P) and mean intensity (MI) are listed in Tables 1 and 2. The data showed seasonal variations, with the highest prevalence in warm seasons. There was a positive relationship between warm seasons and the prevalence values of *H. aduncum* in the sparid fish. There was no significant difference between seasons or prevalence of *H. aduncum* in the gilthead sea bream and two banded bream ($P < 0.05$) (Table 3).

Anisakids characteristically occur in meso or benthopelagic species living in deep sea waters and are typically found in predator fishes. Natural transmissions also take place in specific habitats where the hosts find their characteristic nutrients (14,17). According to a previous study on teleosts helminth parasites, *Hysterothylacium* sp. was found in sparid fish with 1.74% prevalence level in İskenderun bay (10). In the present study, we only detected the anisakiasis agent *H. aduncum* in gilthead sea bream and two banded bream.

Abollo et al. (13) noted that in temperate waters, anisakid parasites are a natural part of the tropic web of marine ecosystems. Many parasite species, especially helminthes, possess complex life cycles involving trophic transmission from one host to the next by consumption of infected intermediate hosts. Smith (18) reported that seasonality might not be expected because anisakid agent eggs are shed by the final hosts, possibly throughout the year, and they may develop and hatch at any time.

Results of the present study demonstrated that the overall mean P and MI values for gilthead sea bream and two banded bream were in the warm seasons 6.25% (P) and 1.92 (MI), and 6.8% (P) and 2 (MI), respectively.

Table 1. Samples of gilthead sea bream, *Sparus aurata,* examined for *Hysterothylacium aduncum.*

Year	Sampling Month	N	W (g)	TL (cm)	Ni	Wi (g)	TLi (cm)	Nn	P	MI
2003	May	23	79.27 ± 4.05 (54.76-118.43)	17.63 ± 0.32 (15-21)	2	78.92 ± 8.13 (70.79-87.05)	18.5 ± 0.5 (17.5-18.5)	3	8.70	1.5
	Jun.	23	91.83 ± 4.95 (50.82-142.6)	18.44 ± 0.36 (15-22.5)	2	82.24 ± 23.42 (65.68-98.8)	17.75 ± 1.25 (16.5-19)	7	8.70	3.5
	Jul.	23	88.80 ± 2.07 (69.30-107.05)	18.57 ± 0.17 (17-20.5)	2	86.91 ± 10.63 (76.28-97.53)	18.95 ± 0.45 (18.5-19.4)	3	8.70	1.5
	Aug.	23	100.34 ± 3.18 (70.46-133.96)	19.26 ± 0.23 (16.5-21.5)	2	113.20 ± 17.32 (95.88-130.52)	20 ± 1.0 (19-20)	5	8.70	2.5
	Sep.	23	94.54 ± 3.02 (64.52-133.49)	18.13 ± 0.2 (16.5-20)	2	107.37 ± 3.31 (104.06-110.67)	20.10 ± 1 (20-20.2)	2	8.70	1.0
	Oct.	21	104.50 ± 4.51 (57.72-129.4)	19.35 ± 0.3 (16.5-20.5)	ND	ND	ND	ND	ND	ND
	Nov.	24	104.62 ± 4.29 (81.46-190.12)	19.32 ± 23 (17.6-23.5)	ND	ND	ND	ND	ND	ND
	Dec.	8	80.63 ± 4.58 (57.72-92.93)	16.36 ± 0.27 (15.3-17)	2	68.66 ± 0.96 (67.70-69.62)	15.2 (15-15.3)	2	25.0	1.0
2004	Jan.	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Feb.	10	94.09 ± 9.02 (61.90-161.48)	18.66 ± 0.59 (16-22.5)	ND	ND	ND	ND	ND	ND
	Mar.	22	93.07 ± 5.51 (22.33-151.8)	18.72 ± 0.35 (15.3-20.7)	1	61.17	16.5	3	4.55	3.0
	Apr.	8	108.11 ± 15.29 (62-183.36)	18.65 ± 0.83 (15.5-21.5)	ND	ND	ND	ND	ND	ND
Total		208	94.53 ± 9.42 (22.33-183.36)	18.46 ± 0.87 (15-23.5)	13	85.50 ± 19.05 (65.68-130.52)	18.16 ± 1.78 (15.3-20.1)	25	6.25	1.92

Sample size (N), body weight (W: mean ± SD; range), total body length (TL: mean ± SD; range), infected samples (Ni), body weight of infected fish (Wi: mean ± SD; range), total body length of infected fish TLi: mean ± SD; range), total number of nematode (Nn) Prevalence (P: %), mean intensity (MI: mean ± SD), not detected (ND)

Table 2. Samples of two banded bream, *Diplodus vulgaris,* examined for *Hysterothylacium aduncum.*

Year	Sampling Month	N	W (g)	TL (cm)	Ni	Wi (g)	TLi (cm)	Nn	P	MI
2003	May	21	65.62 ± 6.91 (32.69-159.53)	15.70 ± 0.5 (13-21)	4	53.71 ± 5.12 (46.30-68.84)	13.88 ± 0.31 (13-14.5)	6	19.5	1.5
	Jun.	24	55.55 ± 2.63 (32.84-79.8)	14.70 ± 0.25 (13-17)	1	85	17.5	2	4.17	2.0
	Jul.	15	55.60 ± 5.31 (29.24-88.13)	14.99 ± 0.45 (12-17.6)	ND	ND	ND	ND	ND	ND
	Aug.	24	69.70 ± 4.72 (34.91-116.23)	15.67 ± 0.33 (12.5-19.2)	1	99.94	18	3	4.17	3.0
	Sep.	25	85.77 ± 2.98 (55.67-117.68)	16.60 ± 0.21 (14.1-19)	ND	ND	ND	ND	ND	ND
	Oct.	25	84.56 ± 6.74 (50.76-176.52)	16.83 ± 0.48 (14-23)	ND	ND	ND	ND	ND	ND
	Nov.	25	82.36 ± 1.54 (68.06-96.51)	16.90 ± 0.11 (16-18)	ND	ND	ND	ND	ND	ND
	Dec.	24	74.93 ± 1.57 (61.31-90.63)	15.30 ± 12 (14.2-16.3)	1	77.62	15.2	1	4.17	1.0
2004	Jan.	11	59.20 ± 2.83 (40.25-70.22)	14.25 ± 0.17 (12.9-15.2)	ND	ND	ND	ND	ND	ND
	Feb.	20	76.54 ± 3.52 (51.66-115.13)	15.66 ± 0.22 (14-17.6)	ND	ND	ND	ND	ND	ND
	Mar.	24	85 ± 4.65 (54.33-124.64)	16.25 ± 0.36 (14.1-18.9)	1	97.79	16.8	4	4.17	4.0
	Apr.	25	75.14 ± 6.5 (35.90-177.12)	14.91 ± 0.38 (12-19.6)	ND	ND	ND	ND	ND	ND
Total		263	72.50 ± 11.32 (29.24-177.12)	15.65 ± 0.86 (12-21)	8	82.81±18.68 (46.30-99.94)	16.28 ± 1.71 (13-17.5)	16	6.08	2

Sample size (N), body weight (W: mean ± SD; range), total body length (TL: mean ± SD; range), infected samples (Ni), body weight of infected fish (Wi: mean ± SD; range), total body length of infected fish TLi: mean ± SD; range), total number of nematode (Nn) Prevalence (P: %), mean intensity (MI: mean ± SD), not detected (ND)

Table 3. Seasonal variations in gilthead sea bream, *Sparus aurata,* and two banded bream, *Diplodus vulgaris,* examined for *Hysterothylacium aduncum.*

Species	Season	P
Sparus aurata (gilthead sea bream)	Spring (Mar., Apr., May)	4.41 ± 4.35 a
	Summer (Jun., Jul., Aug.)	8.70 ± 0.00 a
	Autumn (Sep., Oct., Nov.)	2.90 ± 5.02 a
	Winter (Dec., Jan., Feb.)	12.50 ± 17.68 a
Diplodus vulgaris (two banded bream)	Spring (Mar., Apr., May)	7.74 ± 10.01 b
	Summer (Jun., Jul., Aug.)	2.78 ± 2.41 b
	Autumn (Sep., Oct., Nov.)	0.00 ± 0.00 b
	Winter (Dec., Jan., Feb.)	1.39 ± 2.41 b

Prevalence (P: mean ± SD), Letters a and b show differences of prevalence among seasons for each sparid species and significant at $P < 0.05$ level.

H. aduncum larvae and adults were also reported in several native fishes or marine cage farm fishes along the South Atlantic Ocean such as in Chilean waters (19). The survey of this nematode in the fauna associated with cage-reared salmonids in Chile showed that the maximum values for the prevalence and mean intensity (79% and 4.9, respectively) of this nematode occurred in the warm springtime. These values are similar to those found in the wild fish *Merluccius australis* that constitutes their natural definitive host (13). In addition, the highest prevalence and mean intensity (54.8% and 1.40, respectively) for this nematode in whitings, *Merlangius merlangus euxinus*, was also in the warm months of July/August from the Turkish coast of the Black Sea (20). In comparison, a much lower prevalence value and a similar mean intensity for *H. aduncum* were observed in wild sparid fish in this study.

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