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Metazoan parasite species in cultured mussel *Mytilus galloprovincialis* in the Thermaikos Gulf (North Aegean Sea, Greece)

Abdalnasser Rayyan[1], George Photis[2], Chariton C. Chintiroglou[1,*]

[1]Department of Zoology, School of Biology, Box 134, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece

[2]Laboratory of Ichthyology and Fish Pathology, Veterinary Faculty, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece

ABSTRACT: This is the first study on parasites of cultured *Mytilus galloprovincialis* L. in Greek waters, and is based on samples collected every 2 to 3 mo between September 2000 and November 2001 at 3 stations in the Thermaikos Gulf. Each sample comprised 40 mussels. We found 4 metazoan parasite species: hydroid *Eugymnanthea inquilina*, gill turbellarian *Urastoma cyprinae*, trematode *Proctoces maculatus* and gut copepod *Mytilicola intestinalis*. Of 840 mussels examined, 406 (48.3%) mussels were harbouring hydroids of *E. inquilina*, 278 (33.1%) were infested with *U. cyprinae*, 94 (11.2%) were infested with *M. intestinalis*, and only 7 (0.8%) were infested with *P. maculatus.* The prevalence and intensity of these parasites were related to temperature and pollution. Mussels infested with these parasites had significantly lower condition indices than non-infested mussels; larger mussels were more often infested than smaller ones.

KEY WORDS: *Mytilus galloprovincialis* · Parasites · Metazoa · Condition index

INTRODUCTION

Bivalve culture in Greek waters is almost entirely limited to the culture of the mussel *Mytilus galloprovincialis.* In 1996, production reached 37 000 t (Le Breton & Chintiroglou 1998). The farming of *M. galloprovincialis* in the Thermaikos Gulf is a very important commercial industry (Chintiroglou et al. 2002). Mussel culture markedly affects the ecosystem of the Thermaikos Gulf, since the high density of mussels can aid the spread of epizootic diseases. The present study was conducted to determine: (1) the species of metazoan parasites associated with farmed *M. galloprovincialis* in the Thermaikos Gulf; (2) the prevalence, intensity and monthly distribution of such parasites; (3) their effect on mussel condition and (4) evaluate the effects of various environmental parameters and of mussel size on parasite occurrence. Ours is the first study of metazopan parasites on *M. galloprovincialis* in Greek waters.

MATERIALS AND METHODS

Sampling was carried out between September 2000 and November 2001. Samples of *Mytilus galloprovincialis* L. were collected every 2 or 3 mo at 3 stations from longline mussel culture sites in the Thermaikos Gulf, along the boundaries of Thessaloniki Bay (Fig. 1). Sampling stations differed in terms of distance from main pollution sources in the area as well as in terms of abiotic parameters. Thus, Stn 2 was close to industrial pollution sources (Nikolaides & Moustaka 1990), whereas Stns 1 and 3 were influenced by the currents dominating the outer part of the Thermaikos Gulf (Krestenitis et al. 1997). Anticyclonic currents in the area can alter the water circulation from one season, or year, to another (Balopoulos & Friligos 1993).

A total of 840 mussels were collected during the study period. Each sample consisted of 20 mussels taken from the top (1 m depth) and 20 from the bottom (3 m depth)

*Corresponding author. Email: educreian@yahoo.com

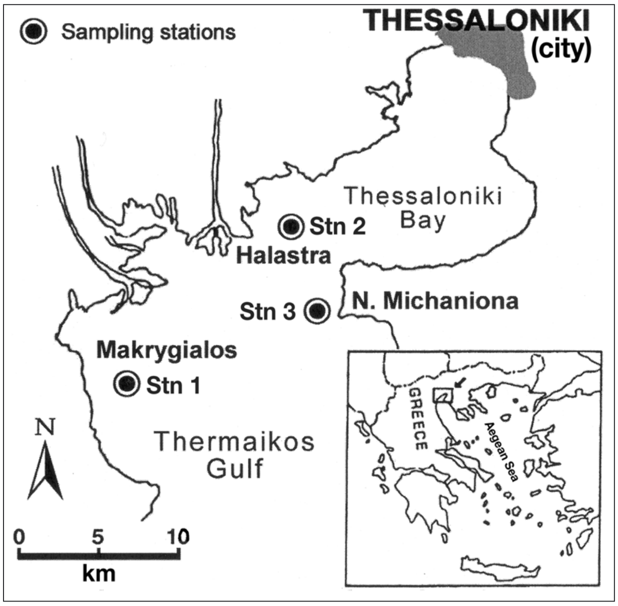

Fig. 1. Locations of the 3 sampling stations in the Thermaikos Gulf (North Aegean Sea)

of the hanging ropes (4 m in length). All mussels above 50 mm in shell length (commercial size) were stored without water in an icebox (ca. 4°C) for a maximum of 4 d. The water temperature was measured at each sampling location. In the laboratory, mussels were cleaned of mud and biofouling organisms and shell length was measured to the nearest mm, and total weight (TW), wet meat weight (MW) and shell weight (SW), to the nearest gram. The mussels were then opened and intervalve water, gills, mantle and visceral mass were examined for parasites. The condition index (CI) was calculated using the formula (Aguirre 1979):

$$CI = [MW/(TW - SW)] \times 100$$

Abundance of the hydroid *Eugymnanthea inquilina* (Palombi, 1935) in the mussels was classified as Class I: (1 to 50 polyps), Class II: (51 to 100 polyps) or Class III (>100 polyps); specimens of the other parasite species were counted individually. Mussels were separated into 4 size classes: 51 to 60 mm, 61 to 70 mm, 71 to 80 mm and 81 to 94 mm.

A 1-way analysis of variance (ANOVA) was used to compare the CI of infested and non-infested mussels. Fisher's least-significance difference (LSD) test was used to analyse the effect each parasite had on the CI of mussels, as well as to identify the effect of any possible combination of parasites on the mussels (herein designated 'level of parasitism'). The relation of parasite infestation to station, mussel size and the position of mussel on the rope (top or bottom) was evaluated by a χ^2 test. Monthly fluctuations of parasite infestation at each station were expressed as a coefficient of variation (cv, i.e. ratio between standard deviation and arithmetic mean of the monthly percent infestation of a parasite at each station).

RESULTS

We found 4 metazoan parasite species associated with *Mytilus galloprovincialis*: *Eugymnanthea inquilina* (Palombi, 1935), a bivalve-inhabiting hydroid; *Urastoma cyprinae* (Graff, 1913), a gill turbellarian; *Proctoeces maculatus* (Looss, 1901), a trematode, and *Mytilicola intestinalis* (Steur, 1902), an intestinal copepod.

Table 1. *Mytilus galloprovincialis* infested with *Eugymnanthea inquilina*. Percentage of infested mussels and abundance of hydroid during sampling period at 3 stations in Thermaikos Gulf (north Aegean Sea) as a function of mussel length (ML) and position on culture ropes (top and bottom). Hydroid abundance divided into 3 classes: I = 1–50, II = 51–100 and III = >100 polyps. N: number of mussels infested with hydroids; NT: number of infested mussels. NT, NB: number of infested mussels on top and bottom of culture ropes, respectively

Stn	Date (mo/yr)							Total prevalence		% No. with different classes of hydroid abundance											
										I				II				III			
no.	9/00	11/00	2/01	5/01	7/01	9/01	11/01	%	(N)	%	(N)	NT	NB	%	(N)	NT	NB	%	(N)	NT	NB
1	21	27	29	24	17	14	30	57.9	(162)	26.5	(43)	13	30	38.3	(62)	33	29	35.2	(57)	30	27
2	15	31	13	8	10	10	0	31.1	(87)	43.7	(38)	16	22	32.2	(28)	13	15	24.1	(21)	8	13
3	24	27	31	25	16	12	22	56.1	(157)	25.5	(40)	19	21	40.8	(64)	33	31	33.7	(53)	22	31
Total	60	85	73	57	43	36	52	48.3	(406)	29.8	(121)	48	73	37.9	(154)	79	75	32.3	(131)	60	71

ML	No. mussels	No. infested		I		II		III	
(mm)		%	(N)	%	(N)	%	(N)	%	(N)
50–60	87	32.2	(28)	42.8	(12)	39.3	(11)	17.9	(5)
61–70	429	42.6	(183)	36.1	(66)	44.3	(81)	19.6	(36)
71–80	286	60.5	(173)	24.3	(42)	31.2	(54)	44.5	(77)
81–94	38	57.9	(22)	4.5	(1)	36.4	(8)	59.1	(13)
Total	840	48.3	(406)	29.8	(121)	37.9	(154)	32.3	(131)

Occurrence of *Eugymnanthea inquilina*

Of the 840 mussels examined, 406 (48.3%) were harbouring the hydroid *Eugymnanthea inquilina* (Table 1). Prevalence and hydroid abundance was significantly lower at Stns 2 than at Stns 1 and 3 ($\chi^2 = 10.6$, p = 0.0315). During the hottest months (May to September), the percentage of mussels infested by hydroids was at its minimum at all 3 stations (Fig. 2). The coefficient of variation was lower at Stns 1 and 3 (0.105 and 0.107, respectively) than at Stn 2 (0.282). The branched hydroid forms were common throughout the year, but asexual budding of polyps sharply decreased in February, and formation of a medusa bud occurred in every host associated with polyps in July, with 50% of polyps in September 2000 and 2001, and a sharp decrease in November. No medusa bud formation occurred in February or May. High hydroid abundance (Class III) increased with increasing mussel length, whereas low hydroid abundance (Class I) decreased with increasing size ($\chi^2 = 38.3$, $p < 0.001$) (Fig. 3).

No significant difference ($\chi^2 = 3.69$, p = 0.1577) was found in the abundance of hydroids between mussels from the top or bottom of the ropes. The mean CI of infested and non-infested mussels differed significantly (ANOVA; $F = 66.26$, $p < 0.001$). Non-infested mussels had the highest CI (Fig. 4). Among infested mussels, those with high hydroid abundance (Class III) had the lowest CI (Fig. 4).

Occurrence of *Urastoma cyprinae*

Of the 840 mussels examined, 278 (33.1%) were infested with *Urastoma cyprinae*, with a mean of 3 worms infested mussel^{-1} (Table 2). The prevalence and intensity of *U. cyprinae* differed between stations ($\chi^2 = 16.03$, $p < 0.0003$). Highest prevalence and intensity

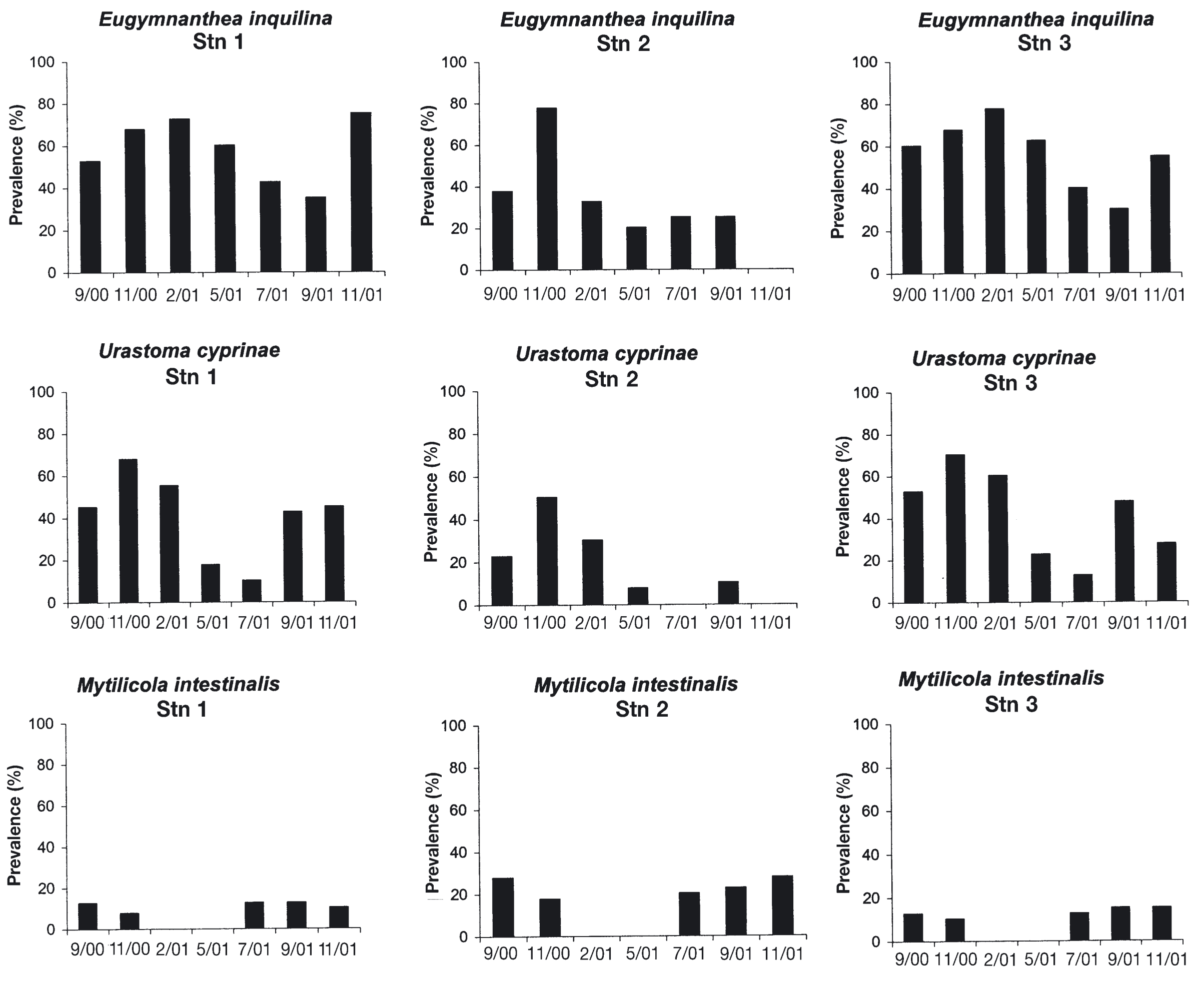

Fig. 2. *Mytilus galloprovincialis*. Monthly prevalence of 3 common metazoan parasites at sampling stations. Abscissas show mo/yr (e.g. 9/00 = September 2000)

was at Stn 3, where 117 (41.7%) mussels were infested, with a mean intensity of 3.7 worms infested mussel^{-1}, while the lowest prevalence and intensity was recorded at Stn 2 (Table 2), with a mean of 1.6 worms infested mussel^{-1}.

Prevalence and intensity of turbellarians were higher during the cold months (November 2000 to February 2001; temperatures 12 to 16.2°C), while the lowest prevalence was recorded in July (Table 2, Fig. 2). The monthly prevalence of *Urastoma cyprinae* had a low cv at Stns 1 and 3 (0.201 and 0.204, respectively). In contrast, the cv was higher at Stn 2 (0.428) as the parasite was absent from this station in July and November 2001. Infested mussels from the bottom of the ropes had a higher parasite prevalence and intensity than infested mussels from the top of the ropes ($\chi^2 = 3.10$, $p < 0.10$). In addition, larger mussels were more intensely infested ($\chi^2 = 9.81$, $p = 0.0203$), with mussels 71 to 80 mm length showing maximum infestation (Fig. 3).

Occurrence of *Proctoeces maculatus*

The prevalence of *Proctoeces maculatus* was very low at all stations. Only 7 of the 840 mussels were infested by this parasite. The intensity of infestation by sporocysts was very high at Stn 1; 1 mussel contained thousands of sporocysts producing cercaria ready for emission from the sporocysts. Because of the low prevalence of this trematode, no statistical analysis was undertaken.

Occurrence of *Mytilicola intestinalis*

Generally, the infestation level and intensity of *Mytilicola intestinalis* was low. At the 3 stations, only 94 (11.2%) mussels were infested with *M. intestinalis* with a mean intensity of 1.1 copepods infested mussel^{-1} (Table 3). Prevalence and intensity were significantly ($\chi^2 = 11.29$, $p = 0.0025$) higher at Stn 2, where 16.4% of the examined mussels were infested (some of them harbouring 4 copepods). The cv for the monthly prevalence of *M. intestinalis* was similar at all 3 stations (0.287, 0.287 and 0.283 for Stns 1, 2 and 3, respectively). *M. intestinalis* was not found during February or May 2001 at the 3 stations (Fig. 2). The total male:female ratio of the copepods collected was 6.4:3.6, and most females had egg-sacs. Bottom-rope mussels were more highly infested, with some bearing 4 copepods, while top-rope mussels had no more than 1 copepod ($\chi^2 = 15.28$, $p < 0.01$). Infestation level increased with increasing mussel size ($\chi^2 = 9.81$, $p < 0.05$, Fig. 3).

Mussel CI and parasite level

The mean CI of infested and non-infested mussels differed significantly (Fig. 4). In addition, the mean CI was dependent on the level of parasitism (ANOVA; $F = 38.87$, $p < 0.01$). No significant differences were observed in the mean CI between mussels infested by 2 parasites (i.e. *Eugymnanthea inquilina* [Class III] + *Mytilicola intestinalis* or *E. inquilina* [Class III] + *Urastoma cyprinae* or *M. intestinalis* + *U. cyprinae*) and those infested by 3 parasites (LSD test: $p > 0.05$) (Fig. 4). These results indicate that the effects of different parasites on mussels are not equal, i.e. the CI is affected more by certain combinations of infestations (especially double and triple infestations with the combination of *E. inquilina* [Class III], *U. cyprinae* and *M. intestinalis*, Fig. 4).

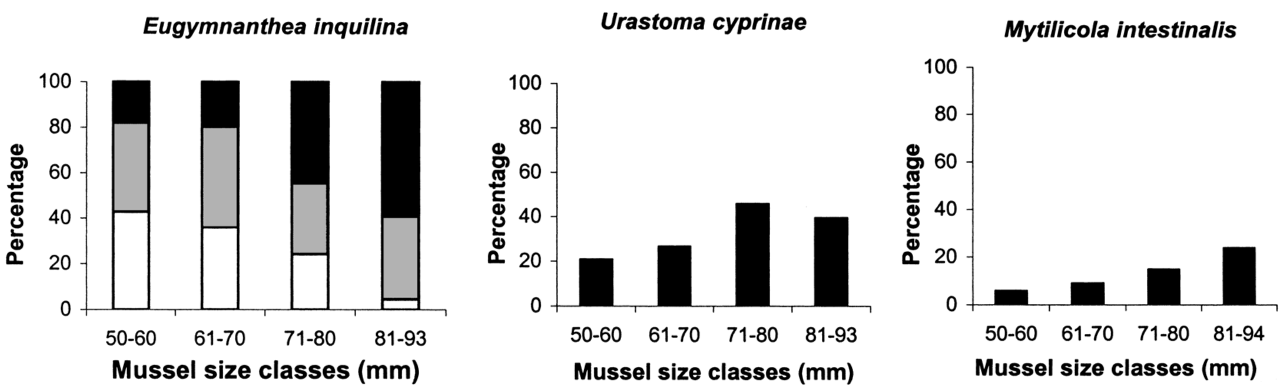

Fig. 3. *Mytilus galloprovincialis*. Percentage of infestation by 3 metazoan parasites as a function of mussel size. For *Eugymnanthea inquilina*, white, grey and black shading indicates low (Class I), medium (Class II) and high (Class III) hydroid abundance, respectively

Table 2. *Mytilus galloprovincialis*. Prevalence and intensity of *Urastoma cyprinae* on mussels during sampling period at the 3 stations, as a function of mussel length (ML) and position on culture ropes (top or bottom). N: number of infested mussels. NT, NB: number of infested mussels on top and bottom of culture ropes, respectively; NW: number of worms; NWT, NWB: number of worms in top-rope and bottom-rope mussels, respectively; MI: mean intensity infested mussel^{-1}

Stn	Prevalence in (mo/yr)							Total prevalence		Intensity						Mussel size class				
	9/00	11/00	2/01	5/01	7/01	9/01	11/01	%	N	NT	NB	NW	MI	NWT	NWB	ML (mm)	%	N	NW	MI
1	18	27	22	7	4	17	18	40.3	113	48	65	307	2.7	136	171	50–60	20.7	18	31	1.7
2	9	20	12	3	0	4	0	17.1	48	19	29	80	1.6	31	49	61–70	26.5	114	301	2.6
3	21	28	24	9	5	19	11	41.7	117	48	69	439	3.7	126	313	71–80	45.8	131	412	3.1
																81–94	39.4	15	82	5.4
Overall	48	75	58	19	9	40	29	33.1	278	115	163	826	3	293	533					

Table 3. *Mytilus galloprovincialis*. Prevalence and intensity of *Mytilicola intestinalis* on mussels during sampling period at 3 stations as a function of a mussel length (ML) and position on culture ropes (top or bottom). N: number of infested mussels; NC: number of copepods; NT, NB: number of infested mussels on top and of culture ropes, respectively; MI: mean intensity infested mussel^{-1}. N♂, N♀: number of males and females, respectively

Stn	Prevalence in (mo/yr)							Total prevalence				Intensity				Mussel size class		
	9/00	11/00	2/01	5/01	7/01	9/01	11/01	%	N	NT	NB	NC	MI	N♂	N♀	ML (mm)	%	N
1	5	3	0	0	5	5	4	7.9	22	4	18	22	1.0	14	8	50–60	5.7	5
2	11	7	0	0	8	9	11	16.4	46	15	31	53	1.2	34	19	61–70	8.8	38
3	5	4	0	0	5	6	6	9.3	26	7	19	26	1.0	15	11	71–80	14.7	42
																81–94	23.7	9
Overall	21	14	0	0	18	20	21	11.2	94	26	68	101	1.1	63	38			

DISCUSSION

Little is known about parasites and the diseases of mussels compared to other cultured bivalves such as oysters (Bower 1992). Research at Aristotle University of Thessaloniki on parasitic organisms associated with cultivated mussels in the Thermaikos Gulf began 3 yr ago, and was integrated within a larger programme aimed at assessing the condition of the natural stock and cultivated populations of *Mytilus galloprovincialis*.

Except for *Eugymnanthea inquilina*, which has only been reported from Italian (Cerruti 1941, Kubota 1989, Piraino et al. 1994) and Greek (Rayyan et al. 2002), waters, the other 3 metazoan parasites have been recorded from a broad geographical range (NE Atlantic coast, North Sea, Mexican waters, South African waters, western Mediterranean and Black Seas: Dethlefsen 1974, Machkevski 1985, Cáceres-Martinez et al. 1996, Villalba et al. 1997, Calvo-Ugarteburu & McQuaid 1998).

The prevalence of *Eugymnanthea inquilina* reported in this study (31.1 to 57.9%) is higher than that reported for the Gulf of Naples, but lower than that reported for other areas (Table 4). In the present study, Stn 2 was characterised by a lower prevalence than the other 2 stations. The abundance of polyps was related to mussel size (i.e. large mussels had more polyps), which is in agreement with the results of Crowell (1957), Kubota (1983) and Piraino et al. (1994).

The reproduction of this polyp in the Thermaikos Gulf is similar to that reported by Piraino et al. (1994), in that the asexual budding of a daughter polyp of *Eugymnanthea inquilina* occurred throughout the year, although it decreased during the cold months. The formation of medusa buds occurred mostly during the warm season and very rarely in the cold months.

With regard to the host-hydroid interaction, although Cerruti (1941) supposed a ciliary loss from the mantle cells of inhabited mussels, Mattox & Crowell (1951) and Kubota (1983) referred to hydroids living within bivalves as typical commensal organisms. Rees (1967) and Piraino et al. (1994) proposed that mutualism may occur, with the hydroid receiving some food and a sheltered environment in return for protecting the bivalve against other intruders. Despite previous suggestions of a commensal interaction between hydroids and mussels, in this study the hydroid-infested mussels, especially those with high hydroid abundance (Class III) displayed a signifi-

Table 4. *Mytilus galloprovincialis*. Prevalence of the 3 parasites in different areas of the world

Parasite Country	Location	Prevalence (%)	Source
Eugymnynthea inquilina			
Italy	Gulf of Naples	8	Crowell (1957)
	Pozzuoli, Taranto, La Spezia	61.9–86.2	Kubota (1989)
	Coastal sound of Taranto	73.9	Piraino et al. (1994)
Urastoma cyprinae			
Russia	Black Sea	70	Murina & Solonchenko (1991)
Spain	Galician Rias, NW Spain	≈100	Villalba et al. (1997)
Mexico	Baja California, NW Mexico	56–100	Cáceres-Martinez et al. (1996)
Mytilicola intestinalis			
England	SW coast	100	Davey & Gee (1976)
Germany	North Sea	100	Dethlefsen (1974)

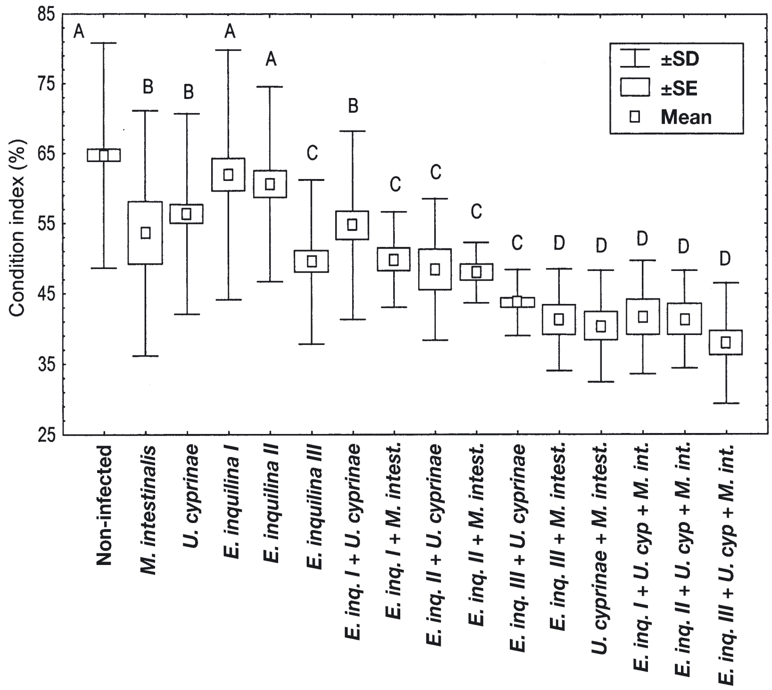

Fig. 4. *Mytilius galloprovincialis*. Box-whisker plots of CI and level of parasitism. Data bearing same letters do not differ from each other significantly (Fisher's LSD, $p < 0.05$). Full parasite names in Fig. 3

cantly lower CI than mussels with no hydroids. In addition, during dissection, in contrast to mussels with no hydroids, the meat of mussels with large numbers of hydroids was very watery and soft, and had an unpleasant smell.

With regard to factors affecting the prevalence and intensity of infestation of *Mytilus galloprovincialis* by *Urastoma cyprinae*, our study confirmed the results of Murina & Solonchenko (1991) for the same host from the Black Sea, in that prevalence and intensity were higher in larger hosts and in mussels from the bottom portions of the ropes. Furthermore, *U. cyprinae* intensity was higher in winter. Murina & Solonchenko (1991) suggested that the rate of infestation shows a seasonal pattern directly related to water temperature, with lower temperature resulting in a higher prevalence as well as an increased number of worms mussel^{-1}. The prevalence (17.1 to 41.7%) recorded in this study was still lower than that reported from the Black Sea or Spanish and Mexican waters (Table 4). The significantly lower prevalence and intensity of *U. cyprinae* from mussels at the polluted station (Stn 2) agrees with the results of Cáceres-Martinez et al. (1996, 1998). The presence of flatworms can greatly reduce feeding capacity in heavily infested mussels (Robledo et al. 1994). Our study also revealed that mussels infested by *U. cyprinae* had a lower CI when compared to non-infested ones.

Proctoeces maculatus has not been detected in *Mytilus galloprovincialis* in South African waters (Calvo-Ugarteburu & McQuaid 1998), and Villalba et al. (1997) recorded low prevalence of this parasite in mussels from Rias of Galicia (NW Spain). Results obtained from our study thus further confirm the low prevalence of *P. maculatus* in *M. galloprovincialis*.

In this study, prevalence and intensity of *Mytilicola intestinalis* in cultured *Mytilus galloprovincialis* was very low compared to reports from the English south coast and the German North Sea coast (Table 4). Describing monthly fluctuations in the infestation of *M. intestinalis*, Davey et al. (1978) reported that females of *M. intestinalis* breed twice, and that 2 generations of parasites coexist for most of the year, with recruitment taking place in summer and autumn. Dethlefsen (1974) recorded that the percentage of mussels infested by *M. intestinalis* is high in summer and low in winter, in agreement with our results. In the present study, male *M. intestinalis* were numerically dominant, and females with egg-sacs were present throughout the year, confirming the results of Hockley (1951) and Dethlefsen (1974). Our study also indicated that larger mussels were more often infested than smaller ones and that bottom-rope mussels were more infested than those near the surface. These findings agree with those of Hockley (1951), Hrs-Brenko (1967) and Davey & Gee (1976), who suggested that the negative phototropism displayed by *M. intestinalis* larvae is responsible for their strong invasion of mussels from the bottom part of the rope and the weaker invasion of mussels attached to buoys, and that larger hosts attract more parasites than smaller individuals because of the higher filtration rate of the former. The significantly higher prevalence of *M. intestinalis* in the polluted Stns 2 in the present study agrees with data from Caspers (1939), who suggested that this parasite might be indicative of polluted water. In the present study, mussels infested with *M. intestinalis* had a significantly lower CI than non-infested mussels, confirming results of earlier studies (Durfort et al. 1982, Theisen 1987, Tiews 1988).

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