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Pomphorhynchus laevis in its intermediate host *Echinogammarus stammeri* in the River Brenta, Italy

B.S. Dezfuli[1],*, E. Rossetti[1], C.M. Bellettato[1] and B.J. Maynard[2]

[11]Department of Biology, University of Ferrara, Via Borsari, 46, 44100
Ferrara, Italy: [2]Department of Biology, Colorado State University,
Fort Collins, USA

Abstract

Infection rates of larval *Pomphorhynchus laevis* Müller, 1776 (Acanthocephala) were studied in its intermediate host *Echinogammarus stammeri* S. Karaman, 1931 (Amphipoda). Crustaceans were collected monthly, from July 1990 to July 1997, at two sites on the River Brenta (northern Italy). Of over 50,000 *E. stammeri* examined, more than 24% were parasitized with *P. laevis* larvae. The differences in the prevalence of infection between the two stations were significant. The intensity of infection varied from one to 15 acanthocephalans per host. Amphipods with multiple infections constituted 28–32% of each monthly sample. During the 7 years of investigation, the prevalence of infection typically reached its highest and lowest levels in the summer and spring months, respectively. Acanthocephalan larvae showed no preference for host sex. Infected ovigerous females of *E. stammeri* were found carrying eggs or juveniles in their brood pouches, but had fewer and smaller eggs. *Pomphorhynchus laevis* larvae co-occurred with *Acanthocephalus clavula* (Dujardin, 1845) and also with the tapeworm *Cyathocephalus truncatus* (Pallas, 1781) in the same host.

Introduction

Acanthocephalans are parasites exclusively of the digestive tract of vertebrates, and use arthropods as their intermediate host. The arthropod becomes infected by eating the mature egg, from which the acanthor larva is freed into the lumen of the host's alimentary canal. Later, this larva bores into the gut wall and enters the haemocoel. Here, development of the acanthocephalan proceeds through the acanthella stage up to the cystacanth stage, which can infect the vertebrate host when the arthropod is ingested. *Pomphorhynchus laevis* is a common parasite in some species of freshwater fish from Italy (Aisa & Gattaponi, 1981).

Larvae of acanthocephalans are known to have a variety of effects on arthropod intermediate hosts. These effects include changes in: host reproduction (Hynes & Nicholas, 1958; Dezfuli *et al*., 1991), integumental pigmentation (Oetinger & Nickol, 1981), respiration (Rumpus & Kennedy, 1974; Bentley & Hurd, 1996), behaviour (Moore & Gotelli, 1996; Zohar & Holmes, 1998), and immune reactivity (Lackie & Holt, 1988).

Variability in acanthocephalan prevalence in its intermediate host, even at different sites along one river, is due to several factors (Hine & Kennedy, 1974; Kennedy, 1985). To the best of our knowledge, this is the first long-term (7 years), uninterrupted study on population dynamics of an acanthocephalan in its intermediate host. This work was designed to determine: (i) the population dynamics of *P. laevis* larvae in *E. stammeri* over 7 years at two sampling sites, including a comparison of the two sites; (ii) any seasonal cycle of infection in the River Brenta; and (iii) the effect of infection on host reproduction. Data on the co-occurrence of *P. laevis* larvae with the larvae of other acanthocephalan species, as well as with tapeworm larva, will be provided.

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*Fax: 0039 532 249761
E-mail: dzb@dns.unife.it

Materials and methods

From July 1990 to July 1997, monthly samples of 120 to 600 specimens of *Echinogammarus stammeri* were collected from two stations on the River Brenta (Northern Italy, Province of Padua): Grantorto (upstream site) and Carturo (downstream site), 2 km apart. Amphipods were sampled with a dip net (mesh size 3 mm) in shallow waters near the bank, both from overhanging vegetation and below the waterline. They were then fixed in 6% formaldehyde for study within 12 days. In the laboratory, amphipods were cleared in lactic acid to facilitate the identification and counting of parasites. An analysis of variance was used to compare the rates of infection at the two stations.

Monthly subsamples of amphipods were collected from the Grantorto site for one year (March 1996 to February 1997) for comparison with the intensity of infection in male versus female amphipods. Sex-related differences in parasite loads were assessed using a chi-square test. To assess the effect of infection with *P. laevis* on reproduction by female amphipods, separate samples were taken from the Grantorto station each month from December 1995 through December 1996. Females in these samples were preserved and cleared as above. Each female was examined for total length (nearest mm), number of parasite larvae harboured, and number of eggs in the brood pouch. Multiple regression analysis was used to determine the relationship between the number of eggs carried and amphipod length and the number of larvae harboured.

To assess the relationship between infection and size of eggs carried, 30 infected and 30 non-infected ovigerous amphipods were collected from the Grantorto site. The infected group of females was restricted to those carrying only one parasite larva. From each of these females, three eggs of a similar developmental stage (selected eggs were not very small, nor did they have an embryo inside of them) were chosen and their diameters measured under a microscope using a millimetric glass slide. For each female, the mean diameter of three eggs was calculated. A Mann-Whitney U-test was used to compare the mean egg sizes of infected and non-infected female amphipods.

Results

In the present work, *P. laevis* larvae were classified as acanthella or cystacanth stages. This distinction was based on the shape and dimensions of each larva, and the development of its internal organs. The above features were compared with those of larvae of other acanthocephalan species studied by other investigators (see Schmidt, 1985).

In the upstream site at Grantorto, the monthly change in prevalence and intensity of infection by *P. laevis* in its intermediate host are shown in table 1. Over 7 years, 27310 specimens of *E. stammeri* were examined, of which 9897 (36%) were infected; a total of 14092 *P. laevis* larvae were found (table 1). In the downstream site at Carturo, the monthly change in prevalence and intensity of infection by *P. laevis* in its intermediate host are shown in table 1. Over 7 years, 26047 individuals of *E. stammeri* from this station were examined and 3375 (12%) were parasitized with *P. laevis*. A total of 4618 acanthocephalan larvae were recorded (table 1). The prevalence of infection at the upstream site was significantly greater than that recorded downstream (table 1; $F = 54.2$, d.f. = 1, 11, $P < 0.0001$).

From July 1994 to July 1997, we quantified the number of *P. laevis* larvae in infected amphipods from each of the two sites was quantified (table 2). In each month, a high percentage of amphipods harboured one *P. laevis* larva. Infected amphipods collected in subsamples at the Grantorto site from March 1996 to February 1997 harboured one to five larvae per host. The intensity of infection did not differ significantly between parasitized male and female amphipods ($\chi^2 = 3.604$, d. f. = 4, n = 376 male, 445 female).

The effect of infection on reproduction by male amphipods was not assessed quantitatively, but infected males were able to obtain mates. During each monthly sampling, many infected males of *E. stammeri* were seen in pre-copula with parasitized or uninfected females.

The number of eggs carried by female amphipods was a factor of both amphipod length and intensity of infection (multiple regression, $F = 248$, d.f. = 2, 593, n = 596 amphipods, $P < 0.000001$). The number of eggs carried in the brood pouches of uninfected females was directly proportional to amphipod length (fig. 1; regression coefficient = 2.8, $P < 0.000001$). Small females (4 mm) rarely carried more than five eggs; large females (11 mm) carried no fewer than 19. The number of eggs carried was negatively correlated with the number of *P. laevis* larvae harboured (fig. 2a; regression coefficient = –2.9, $P < 0.000001$). Females infected with four larvae did not carry more than five eggs. The length of amphipod and number of larvae were not significantly correlated in this sample (fig. 2b; Spearman's Rho = – 0.081, n = 596, $P = 0.05$). Female amphipods infected with one *P. laevis* larva carried significantly smaller eggs than did non-infected females (fig. 3; Mann-Whitney U, adjusted Z = –6.32, n = 30, $P < 0.000001$).

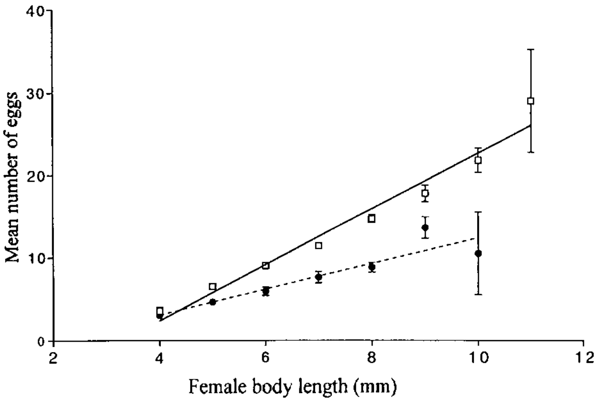

Fig. 1. The number of eggs carried by female amphipods is positively correlated with body length, and negatively with infection. Points represent the mean number of eggs carried (□, uninfected; ● infected females), error bars represent ± 1 standard error.

Table 1. The prevalence and intensity of infection of *Echinogammarus stammeri* by *Pomphorhynchus laevis* in Grantorto and Carturo.

	Grantorto				Carturo			
Month	No. *E. stammeri* examined	No. infected (prevalence)	No. of worms	Mean no. of worms ± S.D	No. *E. stammeri* examined	No. infected (prevalence)	No. of worms	Mean no. of worms ± S.D
Jul (90)	212	200 (94.33)	259	1.3 ± 0.6	300	16 (5.33)	24	1.5 ± 0.7
Aug	141	138 (97.87)	190	1.4 ± 0.7	253	14 (5.53)	19	1.4 ± 0.5
Sep	301	289 (96.01)	445	1.5 ± 0.8	350	35 (10.00)	54	1.5 ± 1.4
Oct	325	320 (98.50)	558	1.7 ± 1.2	313	50 (15.97)	76	1.6 ± 1.0
Nov	204	163 (79.90)	273	1.7 ± 1.1	314	29 (9.23)	45	1.6 ± 1.0
Dec	324	100 (31.48)	152	1.5 ± 0.8	313	50 (15.97)	73	1.5 ± 0.8
Jan (91)	334	35 (10.47)	62	1.8 ± 1.6	324	43 (13.27)	64	1.5 ± 0.9
Feb	218	64 (29.35)	103	1.6 ± 0.9	367	88 (23.97)	157	1.8 ± 1.6
Mar	388	72 (18.06)	104	1.4 ± 0.8	314	18 (5.73)	21	1.2 ± 0.4
Apr	338	162 (41.75)	285	1.8 ± 1.2	261	22 (8.56)	31	1.4 ± 0.8
May	325	16 (4.09)	27	1.7 ± 1.5	302	0 0	0	
Jun	300	65 (21.66)	70	1.1 ± 0.3	308	5 (1.63)	5	1.0 ± 0.0
Jul	300	14 (4.66)	17	1.2 ± 0.4	332	7 (2.10)	10	1.4 ± 0.5
Aug	234	35 (14.95)	37	1.1 ± 0.2	383	12 (3.13)	13	1.1 ± 0.3
Sep	330	33 (10.00)	39	1.2 ± 0.5	312	29 (9.29)	35	1.2 ± 0.8
Oct	301	50 (16.61)	58	1.2 ± 0.4	328	35 (10.67)	42	1.2 ± 0.4
Nov	300	162 (54.00)	218	1.3 ± 0.6	343	185 (53.93)	241	1.3 ± 0.8
Dec	302	56 (18.54)	69	1.2 ± 0.5	184	13 (7.06)	15	1.2 ± 0.4
Jan (92)	408	87 (21.32)	122	1.4 ± 0.7	425	53 (12.47)	70	1.3 ± 0.6
Feb	398	133 (33.41)	197	1.5 ± 0.8	340	22 (6.47)	27	1.2 ± 0.5
Mar	346	180 (52.03)	263	1.5 ± 0.9	360	11 (3.05)	18	1.6 ± 1.4
Apr	450	29 (6.44)	45	1.5 ± 0.9	340	42 (12.35)	58	1.4 ± 0.7
May	347	313 (90.20)	436	1.4 ± 0.8	288	28 (9.72)	36	1.3 ± 0.8
Jun	410	3 (0.73)	3	1.0 ± 0.0	440	2 (0.45)	4	2.0 ± 1.0
Jul	440	279 (63.40)	384	1.4 ± 0.6	410	289 (70.48)	354	1.2 ± 0.5
Aug	438	427 (97.48)	541	1.3 ± 0.6	368	29 (7.88)	29	1.0 ± 0.0
Sep	373	294 (78.82)	392	1.3 ± 0.6	387	42 (10.85)	44	1.0 ± 0.2
Oct	403	129 (32.00)	205	1.6 ± 0.9	422	67 (15.87)	89	1.3 ± 0.6
Nov	250	159 (63.60)	231	1.4 ± 0.7	546	69 (12.63)	79	1.1 ± 0.4
Dec	380	219 (57.63)	293	1.3 ± 0.6	373	36 (9.65)	44	1.2 ± 0.5
Jan (93)	482	242 (50.20)	354	1.5 ± 0.9	517	20 (3.86)	24	1.2 ± 0.5
Feb	353	260 (73.65)	381	1.5 ± 0.8	321	6 (1.86)	9	1.5 ± 1.2
Mar	396	43 (10.85)	71	1.6 ± 1.1	330	4 (1.21)	4	1.0 ± 0.0
Apr	480	92 (19.16)	162	1.8 ± 1.3	484	4 (0.82)	4	1.0 ± 0.0
May	373	25 (6.70)	30	1.2 ± 0.4	337	1 (0.29)	1	1.0 ± 0.0
Jun	326	24 (7.36)	26	1.2 ± 0.3	308	19 (6.16)	20	1.0 ± 0.0
Jul	300	190 (63.3)	224	1.2 ± 0.5	300	48 (16.00)	64	1.3 ± 0.6
Aug	312	210 (67.3)	333	1.6 ± 1.0	346	113 (32.6)	142	1.3 ± 0.5
Sep	338	328 (97.04)	516	1.6 ± 0.8	343	273 (79.6)	370	1.4 ± 0.7
Oct	442	242 (54.75)	413	1.7 ± 1.1	267	190 (71.16)	312	1.6 ± 0.9
Nov	298	132 (44.30)	208	1.6 ± 1.0	240	93 (38.75)	131	1.4 ± 0.8
Dec	268	133 (49.63)	230	1.7 ± 1.1	336	135 (41.37)	255	1.8 ± 1.2
Jan (94)	382	199 (52.10)	369	1.9 ± 1.3	310	191 (61.61)	364	1.9 ± 1.1
Feb	447	48 (10.74)	60	1.3 ± 0.6	267	3 (1.12)	3	1.0 ± 0.0
Mar	352	46 (13.07)	79	1.7 ± 1.5	375	13 (3.50)	21	1.6 ± 1.2
Apr	390	10 (2.56)	20	2.0 ± 1.5	453	17 (3.75)	19	1.1 ± 0.3
May	387	5 (1.29)	8	1.6 ± 1.3	247	3 (1.21)	3	1.0 ± 0.0
Jun	333	24 (7.20)	29	1.2 ± 0.4	345	36 (10.43)	41	1.1 ± 0.3
Jul	300	202 (67.33)	257	1.3 ± 0.6	320	10 (3.13)	11	1.1 ± 0.3
Aug	280	209 (74.64)	283	1.4 ± 0.7	290	154 (53.10)	199	1.3 ± 0.6
Sep	320	215 (67.19)	310	1.4 ± 0.8	294	44 (14.97)	59	1.3 ± 0.6
Oct	290	214 (73.79)	318	1.5 ± 0.8	284	28 (9.86)	35	1.3 ± 0.6
Nov	270	136 (50.37)	264	1.9 ± 1.1	274	9 (3.28)	10	1.1 ± 0.3
Dec	190	118 (62.11)	176	1.5 ± 0.7	270	4 (1.48)	5	1.3 ± 0.5
Jan (95)	293	84 (28.67)	93	1.1 ± 0.3	282	12 (4.26)	15	1.3 ± 0.9
Feb	260	86 (33.08)	151	1.8 ± 1.3	305	23 (7.54)	37	1.6 ± 0.8
Mar	305	1 (0.33)	3	3.0 ± 0.0	220	5 (2.27)	7	1.4 ± 0.5
Apr	290	2 (0.69)	5	2.5 ± 2.1	221	3 (1.36)	3	1.0 ± 0.0
May	253	17 (6.72)	20	1.2 ± 0.4	273	3 (1.10)	3	1.0 ± 0.0
Jun	291	1 (0.34)	1	1.0 ± 0.0	72	0 (0.0)	0	0.0 ± 0.0
Jul	297	221 (74.41)	297	1.3 ± 0.7	297	11 (3.70)	14	1.3 ± 0.5
Aug	307	217 (70.68)	285	1.3 ± 0.7	300	7 (2.33)	7	1.0 ± 0.0

Table 1. *Continued.*

	Grantorto				Carturo			
Month	No *E. stammeri* examined	No. infected (prevalence)	No. of worms	Mean no. of worms ± S.D	No. *E. stammeri* examined	No. infected (prevalence)	No. of worms	Mean no. of worms ± S.D
Sep	300	241 (80.33)	311	1.3 ± 0.6	300	6 (2.00)	6	1.0 ± 0.0
Oct	300	257 (85.66)	301	1.2 ± 0.5	300	7 (2.33)	8	1.1 ± 0.4
Nov	275	144 (52.36)	175	1.2 ± 0.6	292	15 (5.13)	16	1.1 ± 0.3
Dec	280	62 (22.14)	71	1.1 ± 0.4	280	7 (2.50)	7	1.0 ± 0.0
Jan (96)	250	54 (21.6)	59	1.1 ± 0.3	250	2 (0.80)	2	1.0 ± 0.0
Feb	295	71 (24.07)	84	1.2 ± 0.5	290	6 (2.07)	7	1.2 ± 0.4
Mar	300	50 (16.67)	67	1.3 ± 0.7	160	8 (5.00)	8	1.0 ± 0.0
Apr	305	49 (16.07)	62	1.3 ± 0.5	216	7 (3.24)	8	1.1 ± 0.4
May	300	42 (14.00)	49	1.2 ± 0.4	300	5 (1.67)	11	2.2 ± 2.7
Jun	300	54 (18.00)	63	1.2 ± 0.4	202	7 (3.46)	8	1.1 ± 0.4
Jul	300	189 (63.00)	220	1.2 ± 0.4	300	248 (82.67)	285	1.2 ± 0.4
Aug	300	93 (31.00)	106	1.1 ± 0.3	300	10 (3.33)	12	1.2 ± 0.4
Sep	300	29 (9.67)	32	1.1 ± 0.3	250	39 (15.76)	48	1.3 ± 0.5
Oct	300	87 (29.00)	105	1.2 ± 0.5	260	68 (26.15)	83	1.2 ± 0.5
Nov	300	68 (22.67)	79	1.2 ± 0.4	220	38 (17.27)	50	1.3 ± 0.7
Dec	275	42 (15.27)	49	1.2 ± 0.5	180	7 (3.89)	7	1.0 ± 0.0
Jan (97)	300	52 (17.33)	67	1.3 ± 0.6	170	9 (5.29)	10	1.1 ± 0.3
Feb	300	47 (15.66)	67	1.4 ± 0.8	180	11 (6.11)	16	1.5 ± 0.9
Mar	300	31 (10.33)	36	1.2 ± 0.4	180	12 (6.67)	14	1.2 ± 0.4
Apr	310	17 (5.41)	18	1.1 ± 0.2	320	8 (2.5)	8	1.0 ± 0.0
May	277	12 (4.33)	13	1.1 ± 0.3	336	1 (0.30)	1	1.0 ± 0.0
Jun	360	2 (0.56)	2	1.0 ± 0.0	373	12 (3.22)	13	1.1 ± 0.3
Jul	358	2 (0.56)	2	1.0 ± 0.0	260	19 (7.31)	21	1.1 ± 0.3
Total	27310	9897 (36.31)	14092		26047	3375 (12.22)	4618	

In each month, the most infected amphipods harboured cystacanth stages; nonetheless, mixed infections of acanthellae and cystacanths were common.

The co-occurrence of *P. laevis* and *Acanthocephalus clavula* (table 3) was recorded over 3 years of sampling at both sites. At the Grantorto site, 0.4–4.3% of hosts harboured mixed infections while at the Carturo site, 1.3–3.2% of amphipods were infected with larvae of both acanthocephalan species. *A. clavula* larvae were found alone in a low number of *E. stammeri*; the intensity of infection in this case in both sites varied from one to six larvae per host, with *A. clavula* larvae being more frequent in the Carturo site. From July 1990 to July 1997 in 53357 *E. stammeri* of both stations, 47 (0.08%) were infected with *Cyathocephalus truncatus* (intensity of infection = one larva per host). Twenty-eight of these 47 tapeworm larvae were found co-occurring with *P. laevis.*

Discussion

Pomphorhynchus laevis is a widespread acanthocephalan of freshwater fish in the palaearctic region (Hine & Kennedy, 1974). In the River Brenta, *P. laevis* occurs in many species of fish of different families (Dezfuli, 1994); this is likely to be due to the abundance of the crustacean intermediate host, which appears to be one of the preferred items in the diet of fish in this river (Dezfuli, 1994). In 1990, three species of amphipods, namely *Niphargus elegans*, *Synurella ambulans* and *Echinogammarus stammeri* were examined for the presence of helminth larvae. Only *E. stammeri* was found to harbour acanthocephalan larvae (Dezfuli *et al.*, 1991). Thus, the specificity of *P. laevis* for its intermediate host in the study area accords well with reports published for other members of this phylum (Hynes & Nicholas, 1958; Van Maren, 1979; Measures & Bossé, 1993). Accordingly, acanthocephalans are very specific for their intermediate host within a location, but they are able to use different species of intermediate host in different parts of their range (Kennedy *et al.*, 1978; Kennedy, 1985).

The prevalence and intensity of *P. laevis* infection in its intermediate host varies along the length of the river. At both stations, the prevalence of infection with *P. laevis* showed irregular fluctuations. In 72 out of 85 months, the upstream station (Grantorto) had significantly higher prevalences than did the downstream site and this is likely to be due to differences in the fish fauna between the two sites. At the downstream site (Carturo), the preferred definitive hosts of *P. laevis*, fish such as *Leuciscus cephalus* and *Barbus barbus*, are not abundant; rather, smaller species of fish such as *Phoxinus phoxinus*, *Padogobius martensii*, *Cottus gobio* and *Alburnus alburnus* are common here (Dezfuli, 1994). Nevertheless, on several occasions from two to 25 extraintestinal specimens of *P. laevis* were found in these smaller fish (Dezfuli, 1994). It is well known that *Pomphorhynchus* occurs in the mesenteries of several small species of fish which act as paratenic hosts of acanthocephalans (Paperna & Zwerner, 1976; Moravec & Scholz, 1991). However, paratenic hosts frequently lead to termination of the parasite's life cycle

Table 2. The distribution of *Pomphorhynchus laevis* larvae among *Echinogammarus stammeri* collected during 1994–1997 in Grantorto and Carturo.

	Grantorto				Carturo			
Month	1 larva (%)	2 larvae (%)	3 larvae (%)	> 3 (%)	1 larva (%)	2 larvae (%)	3 larvae (%)	> 3 (%)
Jul (94)	78.22	17.33	3.46	0.99	90	10	0	0
Aug	74.64	17.22	6.7	1.44	76.62	18.83	3.9	0.65
Sep	70.23	17.21	9.3	3.26	72.73	22.72	2.27	2.27
Oct	65.89	26.17	3.27	4.67	82.14	14.29	0	3.57
Nov	47.79	22.79	20.59	8.82	88.89	11.11	0	0
Dec	68.64	18.64	9.32	3.39	75	25	0	0
Jan (95)	90.48	8.33	1.19	0	91.66	0	0	8.33
Feb	61.63	18.6	9.3	10.47	52.17	39.13	4.35	4.35
Mar	0	0	100	0	60	40	0	0
Apr	50	0	0	50	100	0	0	0
May	82.35	17.65	0	0	100	0	0	0
Jun	100	0	0	0	0	0	0	0
Jul	76.02	16.29	6.33	1.36	72.73	27.27	0	0
Aug	80.18	10.6	7.37	1.84	100	0	0	0
Sep	77.18	18.67	2.49	1.66	100	0	0	0
Oct	85.6	12.06	1.95	0.39	85.71	14.29	0	0
Nov	84.03	12.5	2.08	1.39	93.33	6.67	0	0
Dec	87.1	11.29	1.61	0	100	0	0	0
Jan (96)	90.74	9.26	0	0	100	0	0	0
Feb	84.51	12.68	2.81	0	88.33	16.67	0	0
Mar	76	16	6	2	100	0	0	0
Apr	75.51	22.45	2.04	0	85.71	14.29	0	0
May	85.71	11.9	2.38	0	80	0	0	20
Jun	85.19	12.96	1.85	0	85.71	14.29	0	0
Jul	85.71	12.17	2.12	0	86.69	11.69	1.61	0
Aug	86.02	13.98	0	0	80	20	0	0
Sep	89.66	10.34	0	0	79.49	17.95	2.56	0
Oct	81.61	16.09	2.3	0	80.88	16.18	2.94	0
Nov	86.76	10.29	2.94	0	76.32	18.42	2.63	2.63
Dec	88.1	7.14	4.76	0	100	0	0	0
Jan (97)	75	23.08	1.92	0	88.89	11.11	0	0
Feb	70.21	21.28	6.38	2.13	72.73	18.18	0	9.09
Mar	83.87	16.13	0	0	83.33	16.67	0	0
Apr	94.12	5.88	0	0	100	0	0	0
May	91.67	8.33	0	0	100	0	0	0
Jun	100	0	0	0	91.67	8.33	0	0
Jul	100	0	0	0	89.47	10.53	0	0

before reproduction can occur (Nickol, 1985). In constrast, at the upstream station, smaller species of fish are rare and chub and barbel are abundant throughout the year (Dezfuli, 1994). The same finding was reported by Hine & Kennedy (1974) in their long-term survey on the distribution of *P. laevis* both in intermediate and definitive hosts in the River Avon. They suggested that the abundance of *P. laevis* was associated with variation in the abundance of acanthocephalans in invertebrate and vertebrate hosts.

Despite the lack of a seasonal cycle of *P. laevis* infection in *E. stammeri* of the River Brenta, the occurrence of acanthellae in every month over 7 years confirms that the infection of *E. stammeri* does in fact take place throughout the year. Likewise, the year-round presence of cystacanths in amphipods revealed that the acanthocephalan infects fish throughout the year. With regard to *P. laevis* distribution, Rumpus (1973) and Hine & Kennedy (1974) suggested that under environmental conditions that allow eggs to be long-lived and produced and shed throughout the year, the transmission of infection can take place at all times of the year and there should be no seasonal cycle in population levels in the intermediate host. Reasons and detailed information regarding the presence or lack of seasonal cycles of infection with some acanthocephalan species have been provided by Kennedy (1985).

Many reports have been published on the co-existence of two acanthocephalan species in the same crustacean host (Awachie, 1966; Van Maren, 1979). In contrast, records on the co-occurrence of tapeworm and acanthocephalan larvae in the same intermediate host are very rare (DeGiusti & Budd, 1959; Awachie, 1966; Dezfuli & Scholz, 1995).

It is well known that acanthocephalans probably do not alter the reproduction of male crustaceans (Hynes & Nicholas, 1958). In contrast, they can affect the reproductive activity of females; sometimes inducing either complete (Ward, 1986; Oetinger, 1987) or partial sterility (Hynes, 1955; Kennedy, 1985). Le Roux (1933) observed

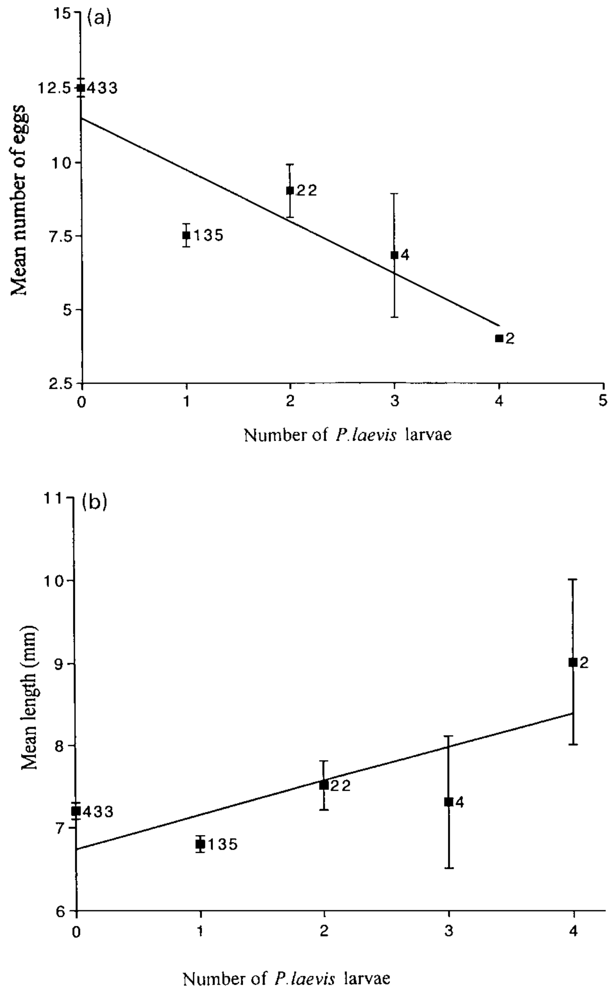

Fig. 2.a. The number of eggs carried by female amphipods is inversely correlated with the number of parasite larvae harboured. Error bars represent ± 1 standard error. Numbers beside the symbols are sample sizes. b. The length of female amphipods is not correlated with the number of parasites harboured. Error bars represent ± 1 standard error. Numbers beside the symbols are sample sizes.

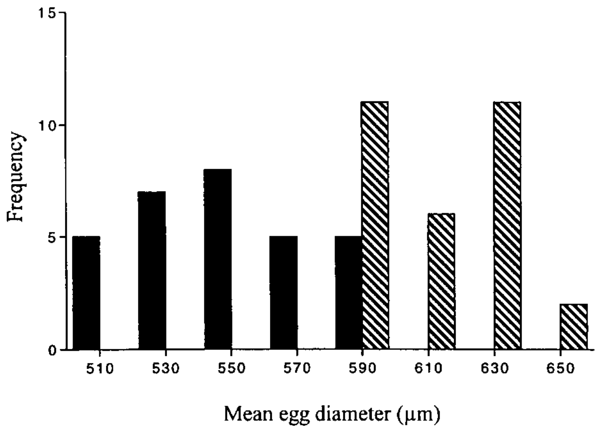

Fig. 3. Eggs carried by infected female amphipods (■) are smaller than those carried by non-infected females (▧).

Table 3. The co-occurrence of *Pomphorhynchus laevis* and *Acanthocephalus clavula* larvae collected during 1994–1997 in Grantorto and Carturo.

	Grantorto		Carturo	
Month	Mixed infection (%)	*A. clavula* (%)	Mixed infection (%)	*A. clavula* (%)
Jul (94)	0.5	0.5	0	10
Aug	0.96	0	3.21	1.28
Sep	1.4	0	0	0
Oct	1.86	0.47	0	15.15
Nov	1.46	0.73	0	0
Dec	0	0	0	20
Jan (95)	1.19	0	0	0
Feb	1.16	0	0	0
Mar	0	0	20	0
Apr	0	0	0	0
May	0	0	0	0
Jun	0	0	0	0
Jul	0.45	0	0	8.33
Aug	0	0	0	12.5
Sep	0.83	0	0	0
Oct	0	0	0	22.22
Nov	0.69	0	0	0
Dec	0	0	0	0
Jan (96)	0	0	0	71.43
Feb	0	0	0	14.29
Mar	3.92	1.96	0	11.11
Apr	2.04	0	0	22.22
May	0	4.55	0	0
Jun	0	0	0	0
Jul	0.53	0	0	0
Aug	0	0	0	16.67
Sep	3.13	9.38	0	0
Oct	4.3	6.45	1.35	8.11
Nov	0	0	2.63	0
Dec	2.38	0	0	12.5
Jan (97)	0	0	0	0
Feb	0	0	0	15.38
Mar	0	0	0	7.69
Apr	0	5.56	0	0
May	0	0	0	0
Jun	0	0	0	0
Jul	0	0	0	5

that females of *G. pulex* parasitized with the larvae of *Polymorphus minutus* do not reproduce. Later, Goodwin (1952) postulated a reason for this dysfunction. The parasitic larvae within the crustacean haemocoel absorb the carotenoids as well as other substances from the haemolymph. The carotenoids are involved in arthropod vitellogenesis and the absence or depletion could induce host sterility. Furthermore, the larvae of *P. minutus* prevent the sexual maturation, development of gonads, and copulation in female *G. pulex* and *G. lacustris* (Hynes, 1955; Hynes & Nicholas, 1958, 1963). Concerning *P. laevis*, Rumpus (1973) found that on average, infected females copulated less, produced fewer eggs and were less likely to mature fully. During the present survey, in each month of sampling, infected ovigerous females of *E. stammeri* were observed in the amplexus with parasitized or uninfected males (Dezfuli *et al*., 1991). However, this group of females produced fewer eggs than did uninfected ovigerous individuals (Dezfuli *et al*., 1991). In a recent comparison of the diameter of eggs carried by parasitized and uninfected *E. stammeri* with the same total body length, infected amphipods produce significantly smaller eggs (Dezfuli, unpublished data). In contrast, Awachie (1966) and Uznanski & Nickol (1980) reported that larvae of *Echinorhynchus truttae* and *Leptorhynchoides thecatus* do not produce apparent effects on the reproductive activity of their intermediate hosts, respectively *G. pulex* and *Hyalella azteca*.

With reference to the parasite load and length of intermediate host, there is a considerable body of evidence suggesting that infection is most prevalent in medium-sized crustaceans (Awachie, 1966; Hine & Kennedy, 1974; Uznanski & Nickol, 1980). Hine & Kennedy (1974) and Anderson & May (1978) provided good explanations for this phenomenon.

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