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EFFECTS OF INFECTION OF THE FRESHWATER AMPHIPOD *GRAMMARUS PULEX* LINNAEUS 1758 WITH THE TAPEWORM CYATHOCEPHALUS TRUNCATUS (PALLAS, 1781) (CESTODA : SPATHEBOTHRIDEA)

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INTRODUCTION

Gammarus pulex serves as an intermediate host of the salmonid fish tapeworm. *Cyathocephalus truncatus* and harbours the larva (procercoid) in the haemocoel from the onchosphere stage till it becomes the infective stage (Wisniewski, 1932; Okaka 1989). Although several authors (Wisniewski, 1932; Vik, 1958; Awachie, 1966) have reported on the occurrence of the unusually large procercoid in the body cavity of the amphipod, no study has been conducted on the effects of the infection on the amphipod.

The present investigation was carried out on laboratory infected gammarids, over a 20-months period, to study the effects of the length and weight of the parasite on the host and the condition factor (k) of infected gammarids compared with uninfected ones. Histochemical and histopathological studies, using tissue sectioning and staining technique, were also conducted on infected and non-infected gammarids to study possible structural damages on tissues and organs in the body cavity (location of the parasite) of the infected amphipods.

MATERIALS AND METHODS

Infected fish (rainbow trout, *Salmo giardneri*) from which eggs of the tapeworm were obtained for infecting the gammarids (*Gammarus pulex*) were caught at the Southern Beck tributary of the River Hull in Driffield, Yorkshire, England. 2500 young specimens of *G. pulex* freshly released from the brood sac of the female gammarids were exposed to infection as already described by Okaka (1984, 1989) and maintained in the laboratory. 100 gammarids were then examined every month for 20 months from the infected stock to monitor their lengths and weights and general development.

The lengths of the amphipods were noted before they were teased open to release the percercoid larva on a dried and weighed Whatman filter paper. The length of the procercoid was then taken after which the set up (specimens on filter paper) was put in the oven for drying. After 24 hours of drying, the dry weights of the procercoid and its gammarid host were determined using a sensitive balance. The same method was used to determine the length and dry weight of the

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uninfected gammarids. Data obtained from the length and weight measurements were used to calculate the condition factor (k) using the formular k = $wx100/l^3$ where w = weight in grams and l = total length in centimeters (Bagenal and Tesch, 1978; Odum and Oradiwe, 1995).

For histopathological studies some infected and non-infected gammarids were fixed in Bouin's fluid and processed for routine paraffin wax (m. p. 60°C) sectioning employing hematoxylin/eosin and Masson's staining techniques (Humason, 1962). Longitudinal and transverse sections (10µm thick) of the specimens were critically studied. Whole mount preparations of infected amphipods made using the technique of Chubb (1962) were also studied. For histochemical studies, the specimens were fixed in 10% neutral buffered formalin at 4°C for 12 hours and then processed for paraffin wax sectioning using a low melting point wax (m.p. 41%°C) as stated by Pearse (1972). The 10µm thick sections obtained were prepared for histochemical studies as follows : Periodic Acid Schiff's (PAS) staining technique was used for glycogen detection and Alcian blue staining technique for acid mucopolysaccharide determination : "Tween 66" incubation medium was used for Protein detection; oil-red-O staining technique was used for lipids localisation while Naphthol-AS-B1 phosphate method was used for alkaline and acid phosphatases enzyme activities at substrate pHs 8.3 and 5.2 respectively; 0-acetyl-5-bromoindoxyl was the substrate used for non-specific estarases localization. The above methods have been previously described by Arme (1966), Pearse (1972), Hassall and Jennings (1975) and Okaka (1984).

RESULTS

Longevity of Infection

Gammarids that harbour more than one of the tapeworm larvae in the body cavity tend to die within one year of infection and only those with single tapeworm larva infection tend to tolerate the infection for some longer months and by the 20th month, most gammarids had died (Table 1). In some extra experimental tanks maintained in the laboratory most gammarids were found to live with the infection only for about 2 years and all infected amphipods died at a maximum of 2 years and 4 months of infection.

Length/weight variations and Condition factor

The relationship between the lengths and dry weights of the procercoid and those of the gammarid host showed a direct one in which the lengths and weights burden of the parasite were seen to increase as host also grows in length and weight (Table 2). The maximum length of the procercoid was 18mm while that of the infected amphipod was 14mm (Table 1). Such large procercoids were seen to fold over once or twice within the body cavity of the host (Fig. 1). The mean dry weights obtained for the procercoid and the infected gammarid host were 0.00070 g and 0.01209 mg respectively (i. e. a ratio of 1 17.30). The dry weights of the non-infected *G. pulex* were also seen to be generally higher (maximum dry weight of 0.059 g) than those of infected *G. pulex* of the same length (Table 2). Uninfected gammarids were found to have a significantly higher mean condition factor (k) of 2.10 than the infected gammarids with mean "k" value of 1.59. Among infected gammarids, the "k" value was also observed to decrease with increase in length and weight of the tapeworm procercoid (Table 2).

Histological studies

Histological transverse sections of the infected and non-infected amphipods (Fig. 2a, 2b and 3) show clearly that the location of the worm in the ventral space results in the a displacement of the intestine and hepatic caeca to dorso-lateral positions. Folding of the worm results in the displacement of the organs to a greater extent (Fig. 2b). There were, however, no structural damages of the displaced organs when compared to those of the uninfected amphipod. A high power study of the histological section of the procercoid within the haemocoel of *G. pulex* show the presence of a thin surface coat or sheath over the teguments of the tapeworm and encloses the tapeworm procercoid within the host's body cavity.

The surface coat in tissue sections stained for histochemical studies, demonstrated strongly positive reaction to tests for glycogen and acid mucopolysaccharides but weakly positive reaction for lipids while test for enzyme activity for acid and alkaline phosphatases and non-specific esterases were negative. However, within the tegument and parenchyma of the procercoid, all these histochemical tests and enzyme localisation were positive as shown in Table 3. In both the infected and non-infected *G. pulex*, positive reactions were demonstrated for glycogen acid mucopolysaccharides and lipids in the hepatopancrease, intestine, gonads and fat bodies in the hemocoel. The organs also gave positive tests to enzyme activities for acid and alkaline phosphatases and non specific esterases (Table 3).

Observations on histological sections of infected amphipods show gonads that are structurally normal like those of uninfected gammarids. Infected male amphipods maintained in the laboratory were observed to copulate normally like the non-infected ones while the infected females were also observed to copulate normally and carry their brood like the non-infected females.

DISCUSSION

The study on the lengths and weights variations further confirm earlier reports of Wisniewski (1932) and Okaka (1989) that gammarids are mostly infected when young and the parasite and gammarid grow together until the larva attains the infective stage. This may be significant in that the amphipod host probably gets used to tolerating the infection early enough as to be able to carry on with normal life activities just like the non-infected individuals for not more than $2^{1}/_{3}$ years after which the host dies. The large size of the mature procercoid, the weight burden which the larva exerts on the gammarid host and the fact that infected amphipods have a lower condition factor than uninfected amphipods are all evidence of heavy physiological dependence of the parasite on the host for growth and development. The sheer weight burden of the parasite on the host is likely to reduce the host's locomotory and feeding activities thereby making it easy prey for fish and this is probably how fish, the definitive host of the parasite, acquire the infection in large numbers. Moore (1984) made a similar observation on gammarids heavily infected with *Polymorphus minutus* and stated that it was the reduction of the locomotory activities of the gammarids due to the infection that make them stay by the water surface and thus become easy prey for the ducks (the definitive host).

From the histopathological studies it is clear that the absence of tissue damages of the infected gammarids is probably due to the possession by the parasite of a surface coat which apparently

allows permeability of nutrient substances (the sheath being positive to test for glycogen mucopolysaccharides and lipids) but not a zone for enzyme or metabolic activities (the sheath being negative to alkaline and acid phosphatases and nonspecific esterases). This surface coat usually referred to as glycocalyx, has been reported from other tapeworm larvae (Arme, 1966; Lumsden, 1975) and its ultrastructure in *C. truncatus* procercoid was described by Okaka (1990).

Scott and Bullock (1974) reported lack of gonads and possible sterility in amphipods infected by the tapeworm *Bothrimonus sturionis* but *C. truncatus* infected amphipods (*G. pulex*) were seen in the present study to have well developed gonads and engage in reproductive activities. The shielding effect of the surface coat of the parasite may still be responsible for the lack of structural destruction of the amphipods gonads.

SUMMARY

Laboratory infected *Gammarus pulex* harbouring multiple infections of *Cyathocephalus truncatus* larvae tend to die within the first year of infection while those with single larva infection can live with the infection only for a maximum of about 2 years. A full grown prodercoid after 11 months of infection is unusually larger in size than its amphipod host and it is folded within the body cavity displacing organs like the hepatopancrease and intestine of the host from their normal positions but with no obvious histopathological damages to the host tissues. The mean condition factor (k) of non-infected gammarids (K = 2.10) was found to be significantly higher ($P < 0.05$) than that of infected amphipods (k = 1.59).

Index key words Tapeworm, *Cyathocephalus truncatus*; Procercoid; Amphipod; *Gammarus pulex*; histopathology; condition factor.

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Table 1 : Infections of *G. pulex* with *C. truncatus* procercoid following exposure of the amphipods to infective onchosphere of the tapeworms. 2500 *G. pulex* were exposed and 100 specimens examined monthly for 20 months.

Period	No. of *G. pulex* dead	No. of *G. pulex* alive	No. of *G. pulex* examined	No. infected	No. of procercoids retrieved	No. of *G. pulex* remaining Expt. tank	State of the procercoid
After 1 month	24	2476	100	16	48	2376	Immature
After 2 months	30	2346	100	30	38	2246	Immature
After 3 months	20	2226	100	28	32	2126	Mature
After 4 months	18	2108	100	26	26	2008	Mature
After 5 months	15	1993	100	24	24	1893	Mature
After 6 months	17	1876	100	25	25	1776	Mature
After 7 months	12	1764	100	25	26	1664	Mature
After 8 months	10	1654	100	26	26	1554	Mature
After 9 months	14	1540	100	29	30	1440	Mature
After 10 months	15	1425	100	32	32	1325	Mature
After 11 months	17	1308	100	35	35	1208	Mature & Egg laying
After 12 months	28	1180	100	43	45	1080	do
After 13 months	29	1051	100	38	38	951	do
After 14 months	26	925	100	34	34	825	do
After 15 months	28	797	100	35	35	697	do
After 16 months	32	665	100	20	20	565	do
After 17 months	30	535	100	17	17	435	do
After 18 months	34	411	100	23	23	301	do
After 19 months	68	223	100	37	37	133	do
After 20 months	120	13	13	12	12	-	do
Total	**587**		**1913**	**575**	**603**		

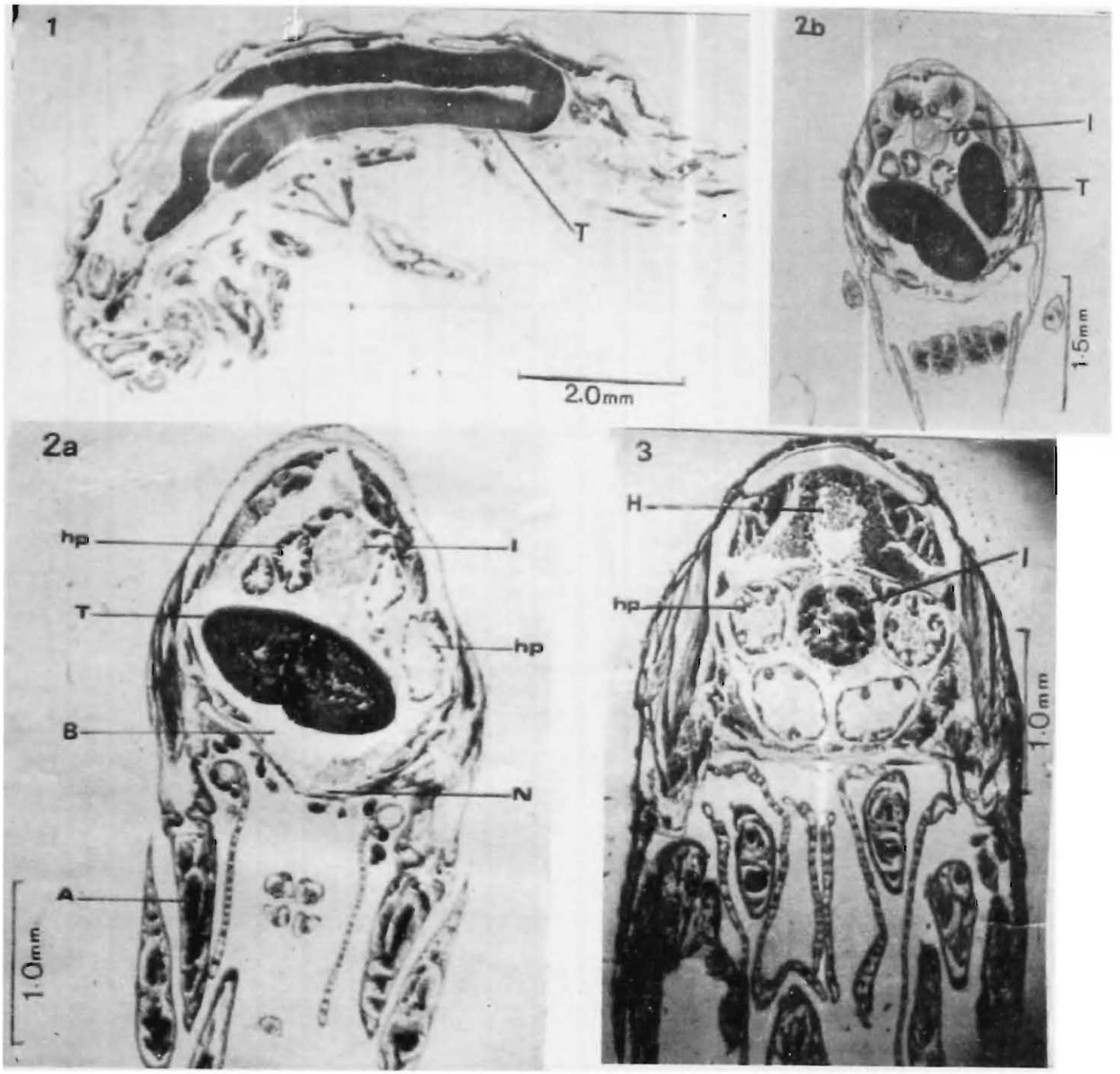

Fig. 1 : Longitudinal section of infected *Gammarus pulex* showing the folded tapeworm.
Fig. 2a : Transverse section of infected *Gammarus pulex* showing the tapeworm cut at mid-body.
Fig. 2b : Transverse section of infected *Gammarus pulex* showing two sections of the folded tapeworm.
Fig. 3 : Transverse section of an uninfected *Gammarus pulex* showing the normal positions of internal organs.

Labels :

A ..... Limb Appendages
B ..... Hemocoel (body cavity)
H ..... Heart
hp ..... Hepateopancrease
I ..... Intestine
N ..... Nerve cord
T .....Tapeworm (*Cyathocephalus truncatus*) procercoid.

Table 2 : The lengths, dry weights and condition factors of non-infected and infected *Gammarus pulex* and the length and dry weights of *Cyathocephalus truncatus* procercoid harboured by the amphipod (10 infected and 10 uninfected gammarids were used for each length).

S/NO	INFECTED *Gammarus pulex*					NON-INFECTED *Gammarus pulex*		
	Length (cm)	Mean Dry Weight (g)	Mean Condition Factors (k)	Mean length of *C. truncatus* larvae harboured (cm)	Mean Dry weight of *C. truncatus* larvae harboured (g)	Length (cm)	Mean Dry weight (g)	Mean condition factors (k)
1.	0.3	0.000564	2.08	0.4±0.2	0.00026	0.3	0.000566	2.09
2.	0.4	0.001320	2.06	0.6±0.3	0.00029	0.4	0.001322	2.06
3.	0.5	0.002586	2.06	0.6±0.2	0.00032	0.5	0.002650	2.12
4.	0.6	0.004322	2.00	0.8±0.2	0.00055	0.6	0.004498	2.08
5.	0.7	0.006703	1.95	0.8±0.2	0.00058	0.7	0.007232	2.10
6.	0.8	0.007686	1.50	0.9±0.2	0.00066	0.8	0.010760	2.10
7.	0.9	0.009210	1.26	0.9±0.2	0.00074	0.9	0.015452	2.11
8.	1.0	0.012792	1.27	1.0±0.3	0.00085	1.0	0.021005	2.10
9.	1.1	0.016763	1.25	1.1±0.2	0.00096	1.1	0.028216	2.11
10.	1.2	0.021765	1.25	1.2±0.2	0.00105	1.2	0.036973	2.13
11.	1.3	0.027456	1.24	1.4±0.2	0.00109	1.3	0.058994	2.14
12.	1.4	0.034000	1.23	1.6±0.2	0.00113	1.4	0.058994	2.14
Mean		0.012097	1.59	0.9±0.2	0.00070		0.019556	2.10

Table 3 : Distribution of some enzymes and reactions to certain histochemical stains in the alimentary tract and some organs in the body cavity of *Gammarus pulex* and in the larva of *Cyathacephalus truncatus*.

S/No	Tests	Infected *Gammarus pulex*				Non-infected *Gammarus pulex*				Larva of *C. truncatus*			
		Intestine	Hepatic caeca	Fat bodies in body cavity	Gonads	Intestine	Hepatic caeca	Fat bodies in body cavity	Gonads	Sheath	Tegu-ment	Parenchyma	Genital
1.	Alkaline phosphatase	**	**	*	**	**	***	**	**	-	***	**	**
2.	Acid phosphatase	**	**	*	**	***	***	**	**	-	***	**	**
3.	Non-specific esterase	*?	**	*	**	*	**	***	***	-	***	*	*
4.	Lipid (oil-Red-O)	*?	**	**	**	**	***	***	**	*	**	**	**
5.	Glycogen (PAS)	**	**	*	**	***	**	**	**	***	**	**	**
6.	Acid mucopolysaccharide (Alcian blue)	**	**	*?	**	***	**	**	**	***	***	*	*
7.	Protein (Tween 66)	*	**	*?	**	**	**	**	**	*	**	**	**

Key : *** Strongly positive
** Positive
* Weakly positive
- Negative
*? Reaction uncertain