FISHERIES RESEARCH BOARD OF CANADA

MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

No.

623

Title

Life history studies of Triaenophorus

at Heming Lake, Manitoba

Part III. Studies on eggs, coracidia and procercoids of Triaenophorus at Heming Lake.

Author

G. H. Lawler

Biological Station, Winnipeg

December 28, 1956

INTRODUCTION

Measurements of the eggs and coracidia of _Triaenophorus_ have been made in Canada by Ekbaum (1937) and Miller (1943). Observations of hatching have been carried out by the same authors, by Libin (1951) and by Rawson and Wheaton (1950). As part of the investigation of the life history of _Triaenophorus_ at Heming Lake in North Central Canada, eggs and coracidia were measured and experimental infections were made to determine whether the _Triaenophorus_ parasites there differed from those in other regions either in morphology or in their copepod hosts. Adequate descriptions of the eggs and embryos have been given by Miller (1952).

ACKNOWLEDGMENTS

The measurements of the coracidia and procercoids were made by Mr. A. B. McBurney.

MATERIALS

On May 11, 1,953 eggs of _Triaenophorus crassus_ and of _Triaenophorus nodulosus_ extruded from pike, _Esox lucius_, were placed in jars of lake water which were sunk in the muskeg where the temperature remained fairly consistent. The water in the jars remained at temperatures between 38° and 40° F and daily aeration was provided. By the end of May no hatching had occurred and it was assumed that the eggs had died. The jars containing the eggs were removed from the ground and kept in the laboratory where the surrounding temperature fluctuated above and below an average of

50° F. On June 9 the cultures were examined and the eggs were seen to be hatching and live coracidia were observed. Hatching probably started about June 7th or 8th. The cultures were then returned to the ground and examined daily. Under these temperature conditions the incubation period was approximately 30 days. Apparently the low temperatures in the muskeg retarded the rate of development.

The eggs were placed in petri dishes filled with fresh lake water and hatching continued. Copepods were placed in the dishes containing both coracidia and eggs and the cultures were again incubated in the muskeg and examined at 2 day intervals.

Measurements of eggs and coracidia were made with an occular micrometer.

EXPERIMENTAL RESULTS

Size of Eggs and Coracidia

Representative samples of eggs and coracidia of *T. crassus* and *T. nodulosus* were measured and the results are given in Table I. Data on the dimensions of the eggs and coracidia of the parasites at Heming Lake are in general agreement with the data published by Miller (1943). The Heming Lake measurements appear slightly lower than those for Lesser Slave but this could be due to the vagaries of sampling or to consistently low measurements by the investigator. In any case there does not appear to be any distinct differences in size of eggs and coracidia between the widely separated areas.

Rate of Hatching

The egg cultures of both species of *Triaenophorus* were examined every second day and counts of the relative numbers of hatched eggs were made, as well as counts of the numbers of living

Table I. Range in length and width of eggs and coracidia of T. crassus and T. nodulosus.

	Eggs		Coracidia	
	Length u	Width u	Length u	Width u
T. crassus				
Heming Lake	46.5 - 62.0	31 - 40.3	58.9 - 83.7	46.5 - 65.1
Lesser Slave Lake[1]	53 - 68	38 - 44	67 - 80	58 - 80
T. nodulosus				
Heming Lake	46.5 - 65.1	31 - 40.3	43.4 - 92	44.1 - 83.7
Lesser Slave Lake[1]	58 - 67	38 - 44	67 - 85	58 - 80

[1] Data from Miller, 1943.

Table II. The duration of hatching and length of life of coracidia observed in cultures of T. crassus and T. nodulosus.

Days after hatching first observed	T. crassus		T. nodulosus	
	Eggs Hatched (per cent)	Active Coracidia (av. no. observed)	Eggs Hatched (per cent)	Active Coracidia (av. no. observed
1	62	86.5	...	...
2	...	...	76	38.6
3	71.5	33.3	85	18.6
4	...	...	...	...
5	92.5	6.5	92.5	9.3
6	...	...	...	...
7	77.6	6.3	80	2.3
8	...	...	...	...
9	...	...	...	...
10	86.6	4.6	95.7	2.0
11	...	...	...	...
12	97.6	2.0	99.6	1.5
13	...	...	...	...
14	98.4	1.5	100	3.0
15	...	...	...	...
16	99.5	0	...	...

coracidia. Usually 3 subsamples from each culture were examined and counts were made and the average of the 3 counts recorded. The results of these observations are shown in Table II. In both species hatching continued for at least 15 days after it began. It appeared that the eggs of T. nodulosus hatched somewhat faster than T. crassus. As explained before, it was not until June 9 that we realized that the eggs that had been incubating were viable so the actual date that hatching began is somewhat obscure, however it probably began June 7th or 8th. On June 10, the day after hatching was first noticed, the average number of active coracidia in the samples was 86.5 and on June 11 the average number in the samples had decreased to 33.3. The number rapidly decreased to June 23 when no live coracidia were seen. These observations apply to T. nodulosus in most respects although the coracidia of T. nodulosus might have a slightly longer life span than T. crassus. The results of the experiment indicate that the majority of coracidia probably live from 2 - 3 days while others may live only a few hours.

DEVELOPMENT OF PROCERCOID IN THE FIRST INTERMEDIATE HOST

Experimental Infection

Plankton from Heming Lake was inoculated with egg and coracidia cultures of T. crassus and T. nodulosus. No procercoids developed or were even observed in Cyclops vernalis, Cyclops edax, Epischura lacustris and Daphnia longispina. In one Diaptomus minutus a small procercoid appeared but the plankter died before the procercoid had grown to any appreciable extent. Miller (1943) found that Diaptomus ashlandi became infected with Triaenophorus but in none of the experiments was he able to see the procercoid reach maturity or grow to any extent. Libin (1953) found that in the laboratory procercoids developed to the cercomere stage in Cyclops strenuus, C. bicuspidatus, C. vernalis, C. fimbriatus and C. phaleratus. Procercoids developed in C. serrulatus and C. vericans but cercomeres did not appear before growth ended. Procercoids also developed in C. prasinus, C. fuscus, C. leukarti and Diaptomus reighardi but little growth occurred. He was not successful in his attempts to infect C. albidus, C. bicolor, and C. viridis. In the Heming Lake experiment only Cyclops bicuspidatus readily became infected with T. crassus and T. nodulosus. As many as six procercoids were observed in a single C. bicuspidatus. No difficulty was experienced in maintaining C. bicuspidatus in the laboratory and so it was possible to follow the growth and development of both T. crassus and T. nodulosus from the coracidium to the mature procercoid in the body of C. bicuspidatus.

The number of copepods in the various cultures ranged from 2 to 12 and the number of procercoids in individual copepods from 1 to 5. The growth and development of the parasites T. crassus and T. nodulosus is shown in Table III. These data represent the average size of all procercoids in the respective samples and do not indicate the range in size nor the individual difference in size between animals. It is seen that the growth is very slow and that the ultimate size attained is considerably less than that shown by Miller (1943) for procercoids of the same species. For example, the average size of a procercoid of T. crassus after 8 days in the

Table III. The growth of T. crassus and T. nodulosus from the coracidium to the mature procercoid in Cyclops bicuspidatus (average length of procercoids in u).

Age of Procercoids (days)	T. crassus u	T. nodulosus u
2	...	...
4	43	...
6	...	57
8	73	49
10	80	55
12	91	70
14	93	78
16	104	91
18	...	90
20	154	...
22	157	116
24	179	128
26	...	129
28	165	...
30	...	156
32	153	...
34	...	147
36	157	...
38	...	138
40	160	...
42	...	136
44	153	...
46	...	132

Heming Lake experiment was 73 u while Miller (1943) found procercoids as large as 300 u. The present investigation has shown, as have previous investigations by others, that the number of parasites per host has some effect on the growth rate; the growth rate of procercoids in heavily infected copepods is retarded and the ultimate size is never as large as those in lightly infected copepods. This factor however, does not explain the obvious size difference between Heming Lake and Lesser Slave Lake experimental procercoids. Consideration was given to the effect of temperature on the rate of development and it is believed that this factor was responsible for the retarded growth observed in the Heming Lake experiment. The cultures were, as explained previously, kept in very cold ground where the temperature remained fairly constant at 39 to 40° F. for over a month. Only when the cultures were examined every second day were they subjected to higher or fluctuating temperatures. By the end of July the temperature in the cooler did reach 58° F. In contrast to this Miller (1943) carried out his experiments when the room temperature varied from 55° to 70° F. Libin (1953) showed that the growth of the procercoids in C. bicuspidatus and C. strenuus was considerably accelerated in cultures held at 56 to 60° F. compared to those held at 42 to 50° F. This difference in developmental temperature coupled with the fact that it has already been shown that the constant cold temperature retarded the development of the eggs and coracidia probably accounts for the diminutive size of the Heming Lake procercoids.

In one culture a copepod which had been found in the lake with a developing procercoid inside it was added to an already established T. crassus culture and the parasite development in naturally and laboratory infected copepods was followed. The growth is shown in Table IV. The growth of the naturally infected copepod increased for about 10 days, levelled off and showed no further sign of growth, probably having reached its maximum size. The procercoids which were developing in the laboratory culture showed a very progressive increase in size until the culture died over a month later, never having attained the size of the procercoid in the naturally infected host.

The cercomere or caudal appendage, according to Miller (1943), is an indication that the procercoid is mature and that after the cercomere is established the procercoid within stops growing or grows very slowly. Cercomeres were observed in procercoids as small as 144 u. The majority of procercoids which continued to grow slowly for such a long time before dying did not exhibit cercomeres.

The presence of procercoids did not seem to affect the longevity or activity of the Cyclops appreciably. Many of the parasitized Cyclops lived 52 days after the infections were noted.

Natural Infection

To determine the incidence of infection of Triaenophorus in the copepod population, plankton tows were made daily and the plankton was examined for evidence of Triaenophorus parasites. When parasites were observed the copepod was isolated and the procercoid was then measured. It was assumed that the parasite found in the copepod was Triaenophorus because although other cestodes

Table IV. Comparison of the growth of procercoids in a naturally infected host and an experimentally infected host.

	Natural Infection Length of procercoid u	Experimental Infection Length of procercoid u
June 17	269	43.
21	258	73.
23	301	80.3
25	...	91.
27	290	93.6
29	322	114.
July 3	...	154.
5	322	157.6
8	322	157.
11	322	165.
15	322	153.
20	322	157.
23	322	161.
27	322	155.

utilize *Cyclops* as an intermediate host none were prevalent during the sampling period. It was not possible to distinguish between the various species of *Triaenophorus* on the basis of size or shape so all references will be to *Triaenophorus* spp. of which *T. crassus*, *T. nodulosus* and *T. stizostedionis* are the members present in the lake. Pertinent data on the procercoid in its plankton host in nature are given in the Appendix. In natural populations of plankton, parasites were noted in one species only, *C. bicuspidatus*. It was thought at the onset of the study that the size distribution of the procercoids over the total sampling period might provide a clue to their possible identity. As the eggs of the three species of *Triaenophorus* are not believed to be shed at the same time it was thought that the parasites would appear more abundantly in the plankton at three separate intervals. Analysis of the infection rates in plankton throughout the spring and early summer showed that at least two peaks of parasite abundance are usually prominent and indications are that a third peak may exist. These peaks are not evident in the size distribution of the procercoids which means that the growth rates of the three species of *Triaenophorus* are comparable and tend to overlap. There is considerable variation in the size of individuals observed, the range extending from 129 u to 600 u. Very few procercoids were found under 270 u and only two over 500 u occurred. The average length for the total period of observation was 300 u. Some doubt exists as to whether those over 500 u actually are *Triaenophorus* parasites or are procercoids of *Dibothriocephalus latus*. Evidence that the unusually large procercoids could be D. latus is afforded by Wardle and McLeod (1952) who reported that the procercoid of D. latus is full grown at the end of 16 - 18 days, and is 500 - 600 u long. Miller (1952) and others have shown that the procercoids of T. crassus are mature at a length of 300 - 350 u. On the other hand Essex (1927), Vergeer (1936), Humes (1950), and others were unable to infect C. bicuspidatus with D. latus yet this is the only species in which procercoids were found in plankters in Heming Lake. Further experimental infections at Heming Lake with D. latus may clarify the problem.

Cercomeres were observed in procercoids as small as 161 u and were not observed in some specimens 386 u in length.

There did not appear to be any relationship between the size of procercoid and the state of maturity of the plankton host, mature and immature C. bicuspidatus appearing equally susceptible. Libin (1953) reported that C. bicuspidatus was somewhat resistant to invasion in the mature stage but was susceptible in the fourth and fifth copepodite stages so it is possible that the mature Cyclops observed at Heming had received the parasite at an earlier stage in development.

All procercoids appeared to be situated in the body of the Cyclops with the cercomere towards the posterior end of the plankter. In nature the infection per Cyclops is low and of all infected Cyclops examined only one contained more than one procercoid -- that Cyclops carried 2 procercoids. This differs greatly from the experimental results where upwards of 6 were observed living successfully, particularly striking when compared with Miller's (1943) experimental results where the number of parasites ranged from 1 to 32. Procercoids were not observed in C. bicuspidatus in nature after July 5.

SUMMARY

1. Measurements of the eggs and coracidia of T. crassus and T. nodulosus generally agreed with those made by investigators in other regions.

2. Incubation of the eggs at low temperatures retarded the development of the parasites and hatching did not begin until the egg cultures had been exposed to increased temperatures.

3. Experimental infections of several copepod hosts with eggs and coracidia of Triaenophorus showed that C. bicuspidatus was the susceptible host in Heming Lake.

4. The growth of procercoids in the copepod host was followed and although the parasites grew they did not grow as large as those grown by other investigators in the laboratory they appeared to survive for an unusually long time. Many of the parasitized Cyclops lived 52 days after the infections were noted. The slow growth was attributed to the affect of low temperatures during development.

5. Measurements were made of procercoids in Cyclops bicuspidatus which had become infected naturally. The procercoids observed were much larger than those in laboratory cultured Cyclops.

6. The possibility that some of the procercoids found in Cyclops might be Dibothriocephalus latus was discussed and evidence for and against was presented.

LITERATURE CITED

Ekbaum, E. 1937. On the maturation and hatching of the eggs of the cestode, Triaenophorus crassus Forel, from Canadian Fish J. Parasitol. 23 (3) 293-295.

Essex, H.E. 1927. Early development of Diphyllobothreum latum in northern Minnesota. J. Parasit. 14: 106-109.

Humes, A.G. 1950. Experimental copepod hosts of the broad tapeworm of man, Dibothriocephalus latus (L). J. Parasitol. 36 (6): 541-547.

Libin, M.L. 1951. Laboratory experiments on the control of the tapeworm, Triaenophorus crassus. (Unpublished M.Sc. Thesis, University of Alberta, April, 1951.)

1953. Laboratory infection of copepods with Triaenophorus crassus. Fish. Res. Bd. Canada, Ann. Rept. of Central Fisheries Research Station for 1953, App. 15, pp. 46-47 (Mimeo).

Miller, R.B. 1943. Studies on cestodes of the genus Triaenophorus from fish of Lesser Slave Lake, Alberta. II. The eggs, coracidia and life in the first intermediate host of Triaenophorus crassus Forel and T. nodulosus (Pallas). Canadian J. Res. D, 21, 284-291.

1952. A review of the Triaenophorus problem in Canadian lakes. Bull. 95, Fish. Res. Bd. Canada.

Rawson, D.S. and R.R. Wheaton, 1950. Studies of Triaenophorus crassus in Nesslin Lake, Saskatchewan, 1950. Fish. Res. Bd. Canada, Ann. Rept. of Central Fisheries Research Station for 1950, App. 4, pp. 18-21 (Mimeo).

Vergeer, T. 1936. The eggs and coracidia of *Diphyllobothreum latum*. Paps. Mich. Acad. Arts, Sci. and Letters 21: 715-726.

Wardle, R.A. and J.A. McLeod, 1952. The Zoology of Tapeworms. Univ. of Minn. Press. 780 pp.

APPENDIX

APPENDIX

Observations (including length and width of procercoids) on *Cyclops bicuspidatus* found in nature with *Triaenophorus* parasites.

Date	Procercoid Length u	Procercoid Width u	Comments
May 20	322	43	
	601	107	
	258	86	Dark brown
	386	64	Very brown, covered with algae
26	258	64	Cercomere
28	193	64	
30	537	64	Cercomere
June 1	215	54	
	279	86	
3	473	64	Heavily covered with algae
	322	64	Covered with algae
	483	129	Covered with algae
	279	75	Cercomere
	129	32	
5	193	64	
7	301	86	Covered with brown algae
	301	64	Cercomere
	301	64	Cercomere
	172	43	Immature *C. bicuspidatus*
9	290	64	Cercomere
11	269	54	Cercomere, immature *C. bicuspidatus*
	376	54	
	269	54	Cercomere
	301	64	Cercomere
13	258	64	Cercomere, immature *Cyclops*
	215	64	Cercomere, immature *Cyclops*
	215	64	Cercomere, immature *Cyclops*
	301	64	Cercomere, immature *Cyclops*
	301	54	Cercomere, immature *Cyclops*
	344	43	Cercomere, immature *Cyclops*
	269	54	Cercomere, immature *Cyclops*
	322	64	Cercomere, immature *Cyclops*
	322	54	Cercomere, immature *Cyclops*
	279	64	Cercomere, immature *Cyclops*
	301	64	Cercomere, immature *Cyclops*
	322	54	Cercomere, immature *Cyclops*
	301	54	Cercomere, immature *Cyclops*
	279	54	Cercomere, immature *Cyclops*
	322	64	Cercomere, immature *Cyclops*
15	269	64	Cercomere, immature *Cyclops*
	269	54	Cercomere, immature *Cyclops*
17	161	54	No cercomere, immature *Cyclops*
	365	54	Cercomere, immature *Cyclops*
	301	75	Cercomere, mature *Cyclops*
	301	54	Cercomere, mature *Cyclops*

Date	Procercoid Length u	Procercoid Width u	Comments
June 17 cont'd	237	54	Cercomere, immature Cyclops
	279	64	Cercomere
	344	64	Cercomere
	354	64	Cercomere
	161	75	Cercomere
	258	64	Cercomere
	344	64	No cercomere
	193	54	No cercomere
	279	54	Cercomere
19	258	54	Cercomere, immature Cyclops
	322	64	Cercomere, mature Cyclops
21	290	75	Cercomere, mature
	258	64	Cercomere, mature
	322	64	Cercomere
	365	86	No cercomere
23	290	64	Cercomere, mature Cyclops
	344	64	Cercomere, mature Cyclops
	301	64	Cercomere, mature Cyclops
	376	64	Cercomere, mature Cyclops
	322	64	Cercomere, mature Cyclops
	301	64	Cercomere, mature Cyclops
	129	86	Cercomere
	237	64)	Cercomere, in same Cyclops
	344	64)	
	301	64	No cercomere, small Cyclops
	322	64	Cercomere, mature Cyclops
	269	64	Cercomere, mature Cyclops
	279	75	Cercomere, mature Cyclops
	376	54	Cercomere, mature Cyclops
	301	54	Cercomere, mature Cyclops
	344	64	Cercomere, mature Cyclops
	269	64	Cercomere, mature Cyclops
25	237	64	Cercomere, immature Cyclops
	322	64	Cercomere, mature Cyclops
	269	75	Cercomere, mature Cyclops, some algae
	269	75	Cercomere, mature Cyclops, some algae
27	279	64	Cercomere, mature Cyclops
	258	64	Cercomere, mature Cyclops
29	182	54	Cercomere, immature Cyclops
	365	64	Cercomere, mature Cyclops
	322	75	Cercomere, mature Cyclops
	311	64	Cercomere, mature Cyclops, some algae
July 1	344	64	Cercomere, mature Cyclops
	386	64	No cercomere, mature Cyclops
	354	86	Cercomere, mature Cyclops
	301	64	Cercomere, mature Cyclops, some algae
	301	75	Cercomere, immature Cyclops, some algae
	279	64	Cercomere, mature Cyclops, some algae
	344	64	Cercomere, mature Cyclops