SOME ASPECTS OF THE DEVELOPMENT OF *PHILONEMA* (NEMATODA:DRACUNCULOIDEA) IN *CYCLOPS BICUSPIDATUS* CLAUS

by

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ABSTRACT

Development of *Philonema oncorhynchi* and *Philonema agubernaculum* in *Cyclops bicuspidatus* were followed and compared by experimentally infecting the copepods with larvae obtained from gravid female worms in spawning salmon and trout. Larvae were dissected from copepods for examination at an interval of two to three days. They were found to undergo two moults in the copepods. Three larval stages are described. All the larval stages of the two species of *Philonema* are morphologically identical. The rates of development of the larvae were observed to be directly proportional to temperatures between 4° to 15°C. The Q_{10} for the range was calculated as 4. The rates of development at 10° were the same in the two species. The effects of temperature on larval development are discussed. An attempt is made to correlate the rates of development of *Philonema* larvae in the copepods under laboratory conditions and the infection of sockeye salmon in Cultus Lake, British Columbia.

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ERRATA

Abstract	line 11	change "to" to "and" between 4° and 15°C.
P. 1	last line	Salvelinus for Salvelinius.
P. 2	para. 3 line 5	tributary for tributory.
P. 3	para. 1 line 6	tributary for tributory.
P. 8	para. 3 line 1	bicuspidatus for bicupidatus.
P. 11	para. 3 line 3	haematoxylin for haemotoxylin.
P. 14	para. 1 line 6	had for have.
P. 15	Frequency distribution of sizes should be read to the left of the solid base line for the trout strain and the right of the dash line for the salmon strain.	
P. 16	para. 1 line 3	died for dies.
P. 18	para. 2 line 11	conspicuous for consipicuous.
P. 22	last line	conspicuous for consipicuous.
P. 32	para. 2 line 6	show for shows.
P. 40	para. 2 line 5	show for shows.
P. 42	para 4 line 6	analagous for analogous.
P. 48	para. 3 last line	add "to" after "are".
P. 54	para. 1 line 3	"of" for "to" the first young instar.

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ACKNOWLEDGMENT

The author wishes to express his sincere gratitude to Dr. J. R. Adams for suggesting and supervising this study. The author is very much indebted to Dr. G.G. Gibson for his many thought-stimulating suggestions and for his assistance in many ways throughout the early phase of the research. The patient technical assistance of Miss Wendy Woo is gratefully acknowledged. Thanks is also due to Mr. A.K.M. Bashirullah for his assistance in field collections.

Sincere gratitude is especially extended to the author's sister, Mrs. Marilyn Yip, without whose encouragement and sound advice, this study would never have been finished.

INTRODUCTION

Two species of the genus Philonema (Nematoda: Dracunculoidea) have been reported in North America. These nematodes are parasites of the body cavities of salmonoids.

Kuitunen-Ekbaum (1933) found that adult sockeye salmon, Oncorhynchus nerka, from English Bay, British Columbia were heavily infected with nematodes belonging to the superfamily Dracunculoidea. She described these dracunculoids as Philonema oncorhynchi. In the same year, Smedley (1933) described the same species of helminth from sockeye salmon caught in Cultus Lake, British Columbia. Later, Bangham and Adams (1954) reported P. oncorhynchi in several species of salmonoids throughout the freshwaters of British Columbia. This nematode also has been reported from Alaska, the State of Washington, and the North Pacific Ocean as far west as the Okhotsk Sea (Margolis, 1963).

Simon and Simon (1936) described Philonema agubernaculum which they distinguished from P. oncorhynchi on the basis of smaller size and different ratio of the length of anterior to posterior oesophagus. This parasite was found in the body cavity and muscle of the abdominal wall of Prospium williamsoni (Girard), Salmo shasta (Jordan) and Salvelinius fontinalis (Mitchell) from the waters of Wyoming

National Forest. Bangham (1951) reported the species from Snake River, Wyoming, which is a part of the Columbia River system.

However, Baylis (1947) considered P. oncorhynchi to be identical with P. agubernaculum. He suggested that the difference in size between the two species might be due to the influence of different hosts or the degree of maturity.

Platzer (1964) compared P. oncorhynchi with Philonema from Kootenay Lake, British Columbia. The latter were presumed to be P. agubernaculum because of their occurrence in one of the type hosts from a river system which, like the type locality, is a tributory of the Columbia. Platzer also examined the type specimens of P. agubernaculum from the U.S. National Museum. He concluded that there is no significant morphological difference between the mature adults of the two species.

Meyer (1958,1960) and Vik (1964) attempted to solve the life history of P. agubernaculum by experimentally infecting Cyclops sp. with larvae from gravid female worms. Meyer was able to keep the infected copepods alive for one month before feeding them to hatchery-reared fingerling, Salmo salar. No worm, however, could be found in the fish when they were autopsied several months later. Vik fed larvae to

Cyclops scutifer but the copepods died before any development of the larva could take place.

The life history of *P. oncorhynchi* was solved by Platzer (1964). He found that *Cyclops bicuspidatus* serves as an intermediate host for the parasite. First-stage larvae were obtained from gravid female worms taken from the coelomic cavity of spawning salmon, *Oncorhynchus nerka*, caught in Cultus Lake, British Columbia. The larvae were introduced into cultures of the copepods which became infected by ingesting the larvae. The larvae moulted twice in the haemocoel. Infective third-stage larvae were recovered from *Cyclops* seventy and seventeen days after infection when the copepods were maintained at 8°C and 12°C respectively. Infected copepods were later fed to hatchery-reared sockeye fingerlings. Third-stage larvae were obtained from the peritoneal tissues and tunica adventitia of the swim-bladder 4 to 10 days after infecting the fish. In naturally infected fish, fourth-stage larvae were found in the peritoneal tissue when the fish were 26 months old, and in the body cavity when the fish were 32 months old. The worms became mature only in salmon returning to freshwater to spawn in the 4th year.

Although there is no morphological difference between adult *Philonema* from the freshwater host in Kootenay

Lake and the anadromous sockeye of Cultus Lake, there is evidence that the life cycles in these two situations are different. Bashirullah (unpublished data) found mature P. agubernaculum in the body cavities of rainbow trout, Salmo gairdnerii, caught in May in the Lardeau River which is a tributory of Kootenay Lake. Obviously these worms had developed to maturity in less than 12 months. This suggests that there are two distinct "biological species" of Philonema differing in developmental rate, one adapted to resident freshwater hosts, the other to anadromous hosts with a four year spawning cycle.

A similar conclusion was reached by Akhmerov (1955) who restudied the several species of dracunculoids described from salmonoid fishes of the U.S.S.R. On the basis of morphology, he synonymized Philonema agubernaculum Simon and Simon, 1936, Philonema elongata Fujita, 1940, Coregonema sibirica Bauer, 1946, and Philonema oncorhynchi Kuitunen-Ekabaum, 1933. But he suggested that the ecology of P. oncorhynchi may be different from that of P. agubernaculum because the biology of their hosts is different.

The foregoing brief review gives a general picture of the life history of Philonema. But a number of aspects of the development of the parasite in the copepod need

clarification. Platzer (1964) did not make any detailed morphological study of the developmental stages of *P. oncorhynchi* in the crustacean host. There was no critical analysis of the effects of temperature on development. The morphological development of *P. agubernaculum* in the intermediate host also needs to be studied.

Therefore, the present study was undertaken to obtain detailed information on the developmental stages of *P. oncorhynchi* in the copepod host, to establish the moulting intervals and the rate of development to temperatures and finally, to determine experimentally whether *P. agubernaculum* from trout differs in these features from *P. oncorhynchi* in salmon.

MATERIALS AND METHODS

The problem was approached by experimentally infecting copepods collected from Cultus Lake with *Philonema* larvae. Copepods infected with sockeye strain (*P. oncorhynchi*) were maintained in incubators at 4°C, 10°C and 15°C; those infected with trout strain (*P. agubernaculum*) were kept only at 10°C. Development was followed by extracting and examining larvae at least three times a week. A criterion for comparing the rates of development at the different temperatures was established. This was the interval required for 50% of the larvae in a sample to show evidence of the first or second moult, as evidenced by loosening of the cuticle.

Sources of Philonema larvae.

Infective *Philonema oncorhynchi* larvae were obtained from gravid female worms freshly recovered from the body cavity of infected prespawning sockeye salmon caught at Sweltzer Creek, Cultus Lake in November. *Philonema agubernaculum* larvae were obtained in a similar manner from rainbow trout caught in May in Trout Lake, British Columbia.

The worms were placed in dechlorinated water where they burst, releasing the first-stage larvae. The broken pieces of the females were removed by filtering through a

fine mesh screen. The larvae were transferred to a 100ml graduated cylinder to which lake water was added making 100ml of inoculum. One ml of inoculum was then pipetted to a petri dish ruled in squares. The number of larvae in the dish were counted using a hand-counter. This was done under an Olympus dissecting scope, thus giving a rough estimation of the concentration of larvae in the inoculum.

Collection and Maintenance of Copepods.

Plankton was collected from different areas in Cultus Lake by means of a #10 bolting silk plankton net towed by a row-boat. Both horizontal and vertical tows were made. The plankton was emptied into two 3-gallon liver cans, three quarters full of lake water. An ice-pack was added to each can to keep the water cool. Six gallons of lake water were also transported back to the laboratory.

On arrival at the laboratory, the small and large plankters were separated immediately by siphoning the water first through a brass sieve of 1 mm pore size, then a piece of #12 bolting silk. During filtration, the water in the funnel was maintained at a constant level by regulating the rate of flow. The plankton so separated was stored overnight at 10°C in 3-gallon museum jars.

One hundred random samplings of copepods were made by a fine pipette. They were transferred with a drop of water to a 3 x 2" concavity slide for identification. Half of the sample was dissected to determine whether they were naturally infected with Philonema.

Infection of copepods.

Approximately one thousand copepods were concentrated into each of several 4" finger-bowls by filtering through a piece of #12 bolting silk. Philonema larvae were added to produce a concentration of three larvae per copepod. Following Platzer's methods (1964), the finger-bowls were kept at 10°C for 24 hours before the cultures were transferred to 8" stacking dishes. The concentration of copepods was about 250 per dish. Each dish, half-full of water, was covered with a piece of glass to reduce evaporation. The water level in the dishes was maintained by adding lake water from time to time.

Using the above method, adult Cyclops bicupidatus, both males and females, were found to be 30 to 40% infected with an average of two larvae each. Later experiments showed that the percentage of infection could be increased to 80 to 90% without altering much the overall degree of infection. This was done by allowing the copepods to remain in the

finger-bowls for 48 instead of 24 hours before being transferred to the 8" stacking dishes. A longer exposure to a more concentrated quantity of larvae provides a better chance of being infected.

Roughly 12,000 copepods were infected in three different groups. About 8,000 were infected in the first batch; 2,000 six days afterwards; another 2,000 fifteen days after the first infection.

Copepods infected with Philonema oncorhynchi larvae were maintained at 15°C and 10°C in controlled-environment rooms and at 4°C in a refrigerated incubator. Two culture dishes were transferred from 10°C to 15°C and 4°C respectively when 50% of the larvae from the copepods were found undergoing the first moult.

Copepods infected with Philonema agubernaculum larvae were maintained at 10°C in the controlled-environment room.

Copepods were fed weekly by adding 5 ml of hay-infusion to each dish. The infusion was prepared by steeping clover-hay in hot water and setting it aside to cool. The infusion was used only after it was one week old when the population of algae, bacteria and protozoans had reached a considerable size. It was discarded three weeks after

preparation because by this time it was usually too full of algae. Copepods were observed to ingest the ciliates but algae may also have served as food as green-pigmented granules were found in the intestine of copepods from the culture.

Extraction and examination of larvae from copepods.

Samples of copepods were examined three to four times a week. The culture dish was placed on a piece of black cloth under a cool-white flourescent lamp. The copepods were transferred by a fine pipette to a syracuse dish with two ml of chilled Ringer's solution for cold-blooded invertebrates. From this syracuse dish they were finally transferred to a well-slide when they were dissected under an Olympus dissecting scope with two N0.000 insect pins mounted in applicator sticks. Extracted larvae were examined with a Leitz compound microscope using a 10X ocular and 45X objective.

Fixing, mounting, staining and morphological study of larvae.

After examining the larvae with a compound microscope, they were fixed and preserved in formalin-acetic acid (1:1.25) and stored in small vials.

Goodey's lactophenol-cotton blue (Franklin and Goodey, 1949) was modified for staining the larvae in the

following manner:- Larvae were transferred from the vials to a slide by a dropper. They were caused to adhere to the surface by pressing with a human eyelash glued to the end of an applicator stick. Excessive fluid was withdrawn before the addition of a drop of 0.0004% lactophenol-cotton blue. Glass-wool filaments were arranged on four sides of the drop to serve as a support upon which an 18 mm square NO.O coverslip was gently laid with a pair of fine forceps. The coverslip was finally sealed with Gurr's glyceel.

Cotton-blue was used solely because it is a good nuclear stain. Besides cotton-blue, methylene-blue, haemotoxylin-eosin, fast-green and mixtures of fast-green and cotton-blue were also tried for staining. Fast-green (0.12% in 70% alcohol) is a poor nucleus stain. However, it stained the outline of structures clearly. It was more permanent than cotton-blue which faded away in one to two months time.

Measurements of the larvae were made on a Leitz compound microscope with an ocular micrometer. Living larvae as well as stained specimens were studied to trace the details of morphological development. A series of diagrams was drawn with the aid of a Leitz microprojector.

RESULTS

(1) Some general observations on the infection and maintenance of copepods.

(a) Some *Philonema* larvae from gravid female worms survived as long as twenty-five days in lake water at 10°C.

(b) No infection was found in nauplii, or in first, second and third copepodid stages. However, fourth and fifth copepodid stages were susceptible to infection. This is similar to the results of Watson and Price (1960) working with the coracidium of the cestode, *Triaenophorus crassus*.

(c) *Cyclops* ingested free-swimming larvae in the water and more passive ones in the bottom of the culture dish. Similar observations were made by Leiper (1907) on the guinea worm, *Dracunculus medinensis* and by Li (1935) on *Procamallanus fulvidraconis*.

(d) Dissection of infected copepods at half-hourly intervals following infection showed that 5 to 5½ hours are required for the larvae to penetrate the intestine and reach the body cavity.

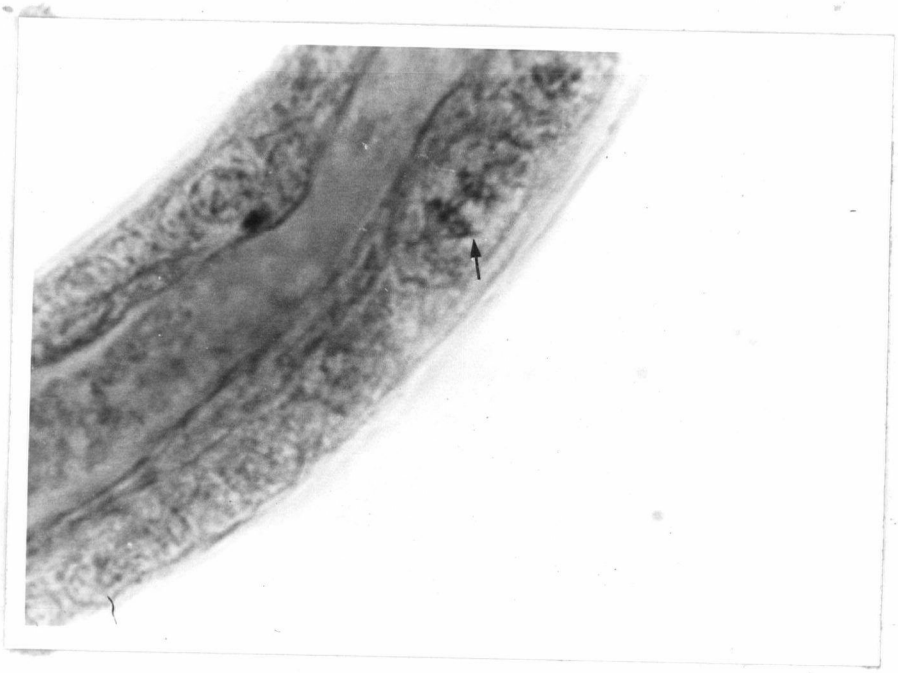
(e) The nutritional state of lightly infected (1 to 2 larvae) and heavily infected (5 or over) *Cyclops* seemed to be normal. Oil droplets were present in both cases.

Figure 1. Chromosomes of hypodermal cells of moulting First-stage larvae.

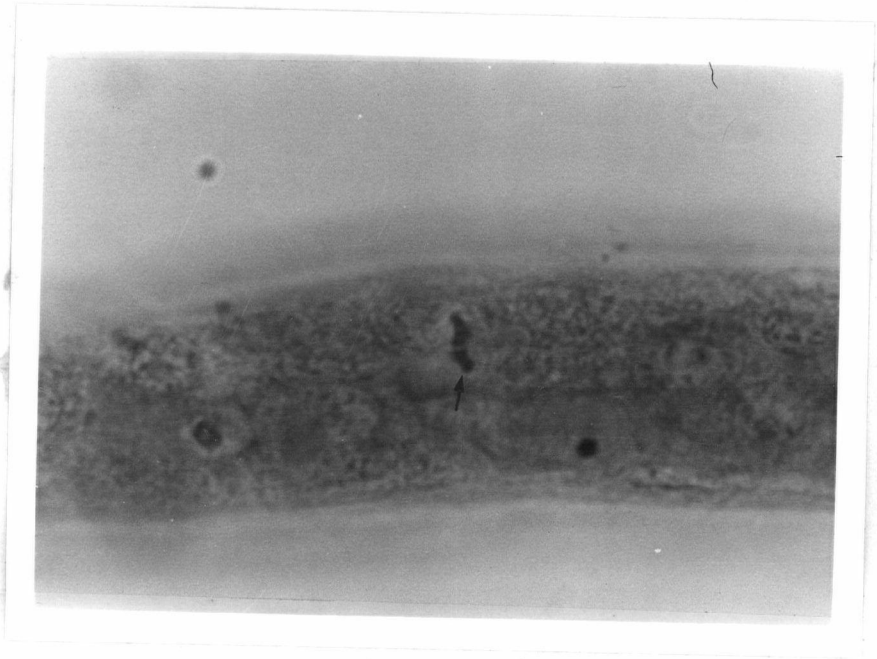
(a) *Philonema oncorhynchi*

(b) *P. agubernaculum*

Magnification - 1000X



(A)



(B)

Moorthy (1938) reported that Dracunculus medinensis infection had a deterrent effect on the reproductive activity of Mesocyclops sp. But in the present experiments, young copepods with 5 to 7 larvae were found to carry egg-sacs some time after infection. Nauplii and copepodid stages usually appeared in cultures that have been maintained for three to four weeks.

Reduction in agility, was the only obvious detrimental effect on heavily infected copepods. Heavily infected copepods usually moved sluggishly and tended to remain motionless on the bottom of the culture dish for long periods. No copepod infected with ten or more larvae could be found two to three weeks after infection, whereas those infected with one to two larvae survived for a much longer period of time. Sluggish movement may adversely affect survival because such animals are likely to be eliminated by competition in the long run.

No definite conclusion can be made on the effect of heavy infection on the development of larvae. In some cases, larvae of different stages were recovered from the same copepod, whereas in others, all the larvae from a copepod were the same stage. Often, third-stage larvae could be found along with the procercoids of Proteocephalus sp. which the copepods acquired under natural conditions.

Fig. 2. Comparison of the sizes of larvae between salmon and trout strains from *Cyclops bicuspidatus* maintained in 10°C.

(Sample size=20 for each stage)

Range of size of trout strain.

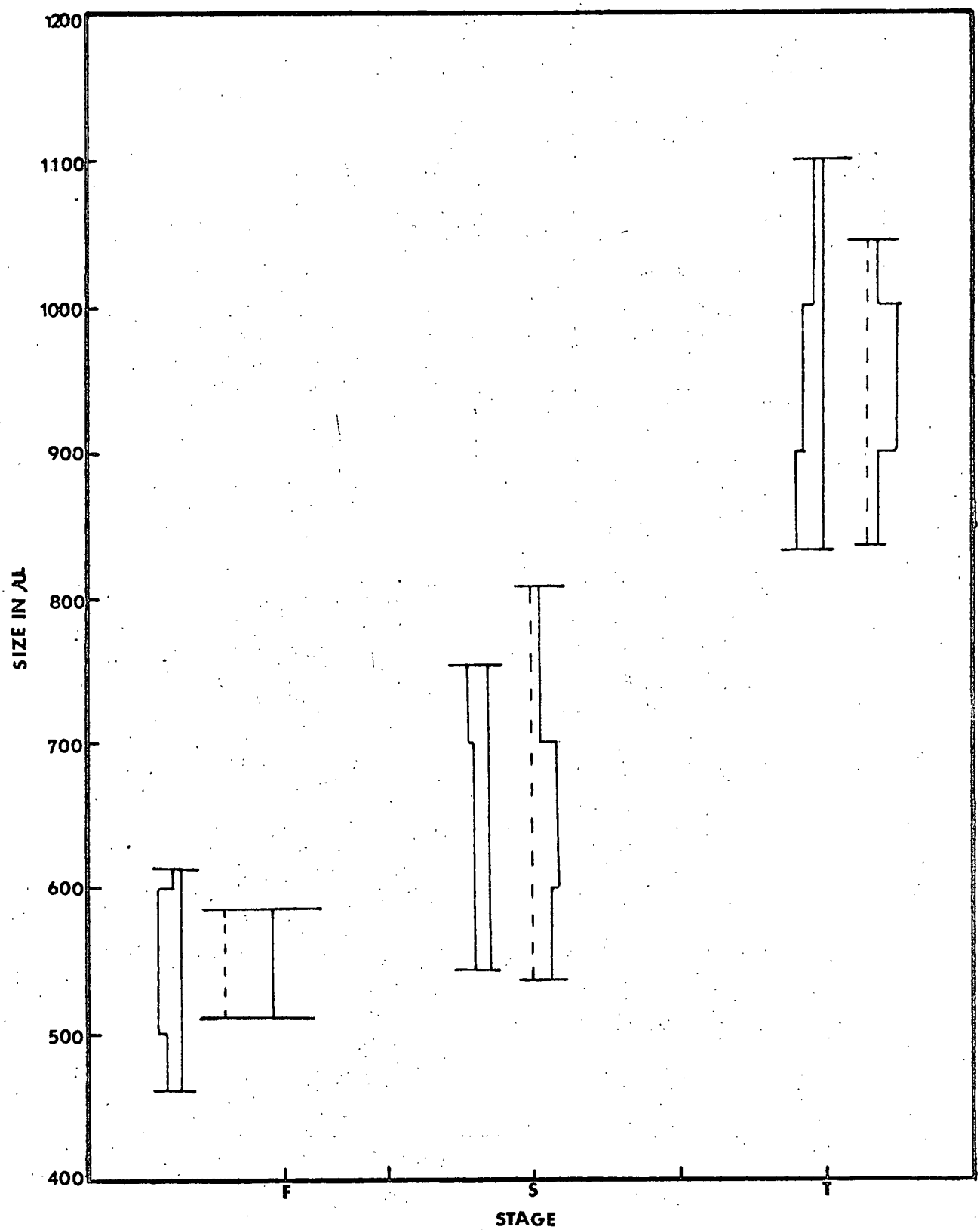
Range of size of salmon strain.

Frequency distribution of size.

F = First-stage

S = Second-stage

T = Third-stage


1200
1100
1000
900
800
700
600
500
400
SIZE IN µL
F
S
T
STAGE

(f) The infective third-stage of Philonema appeared unable to leave its cyclopoid intermediate host even after the latter dies. This is different from the procercoids of the cestode, Spirometra mansonoides which can liberate themselves from intact copepods (Mueller, 1959).

After twenty copepods were killed by anoxiation as a result of withdrawing a large quantity of water, the larvae inside were observed with a compound microscope for 12 hours. They moved along the haemocoel, into the antennae, appendages or caudal rami, attempting to leave the hosts. None could make its way out.

(g) The mortality of infected copepod cultures was more than 50% at 10°C and 15°C, three to four months after infection. At 4°C, the mortality was 30 to 40%. Some 200 to 300 copepods remained alive at 4°C for more than eight months.

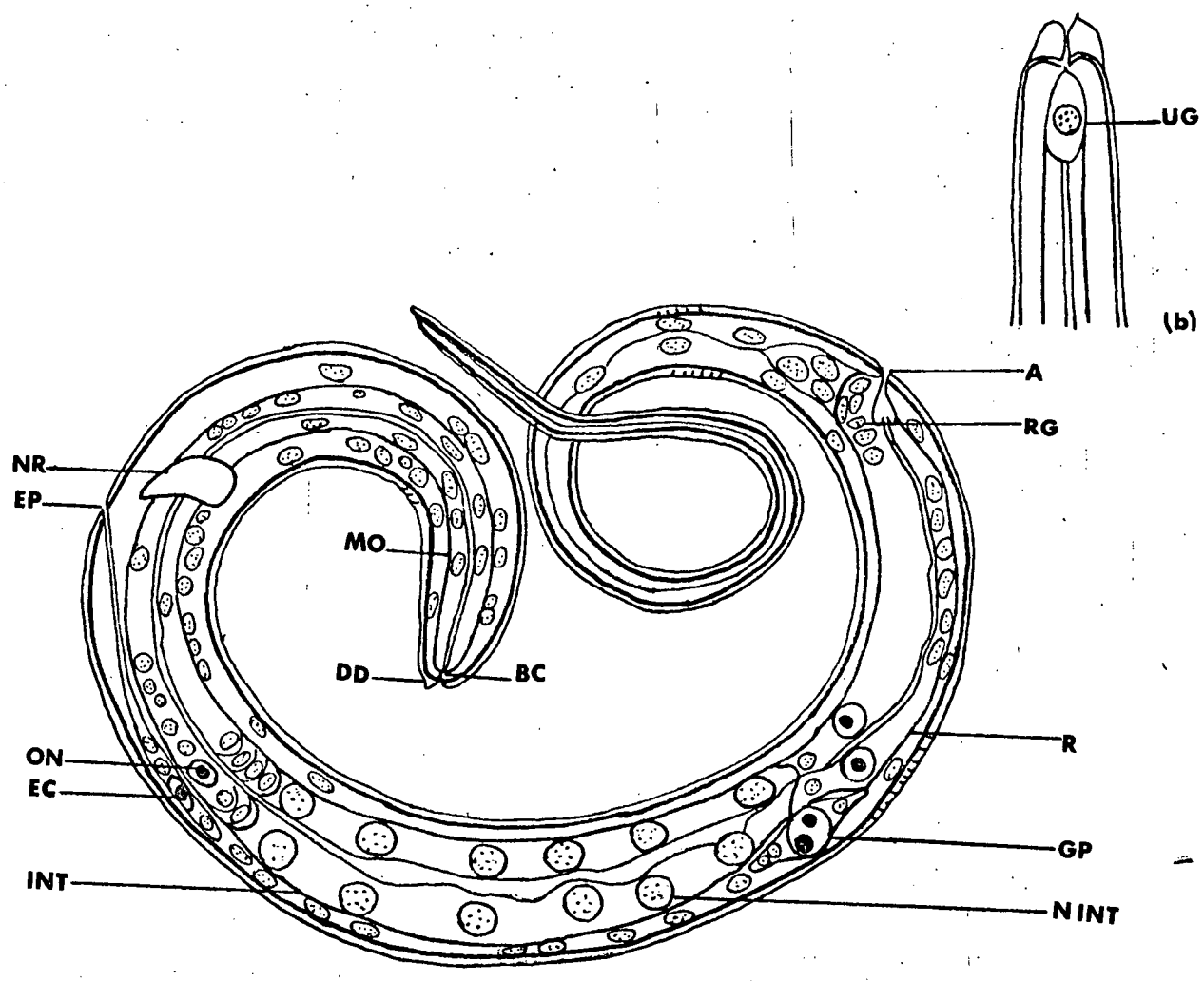
(2) Morphological description of developmental stages of Philonema sp. from Cyclops bicuspidatus.

Larvae of Philonema from both trout and salmon were found to undergo two moults in Cyclops bicuspidatus. In all stages, there was no morphological difference between the trout and salmon strains. Chromosome counts were also identical, 5 pairs per nucleus (Figure 1). A comparison of

Fig. 3. (a) Lateral view of First-stage larva.

(b) Dorsal view of the anterior extremity of a moulting First-stage larva showing the unicellular gland.

For legend see p. 70.



(a)

25 μ

the sizes of the various stages between the two strains is presented in Figure 2. A two-ways analysis of variance (Table VII) comparing the lengths of the different stages of these two strains at 10°C (sampled from different periods within each stage) shows that at the 99% interval of confidence there is no significant difference.

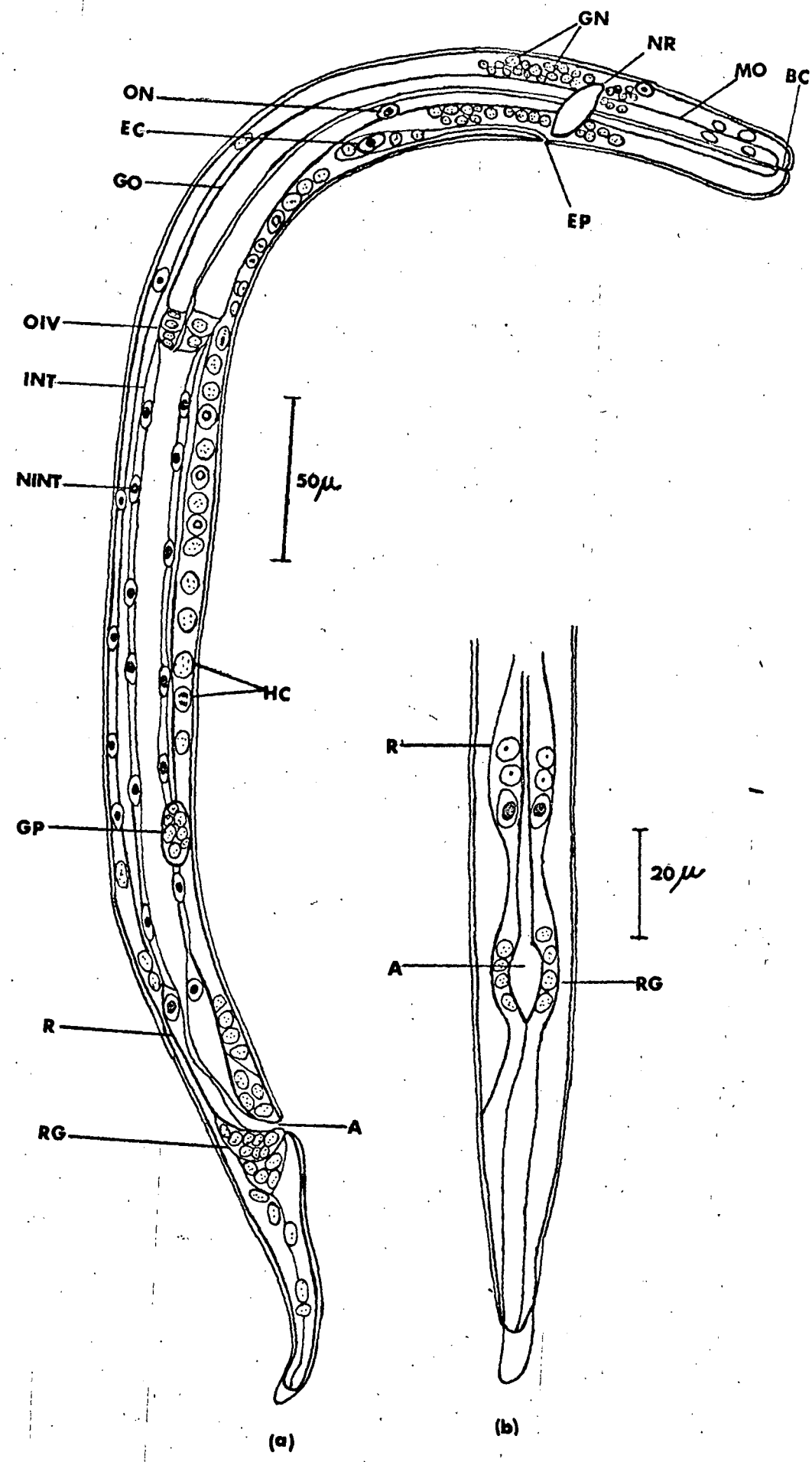
First-stage larvae from gravid female worms.

(Figures 3, 6a, 7a, 8, Table I)

Body length 490.3 μ (416.3 - 527.3 μ); body width at region of nerve ring 15.9 - 24.6 μ; body width at anus 13.6 - 18.5 μ. Cuticle thick, expanding at dorsal buccal region to form conical denticle. Amphids not discernible. Buccal cavity short. Nerve ring situated 44 to 61.8 μ from cephalic end. Excretory bladder (Figure 7a) 22.8 - 23 μ long, 4 μ wide ending posteriorly at oesophageal-intestinal junction on ventral side of pseudocoel; consisting of four cells. Comparatively large excretory cell with conspicious round nucleus, 5.13 - 6 μ long, located slightly posterior to a large and consipicious nucleus in muscular oesophagus. Excretory duct narrow, 26.69 - 27 μ long, 1 μ wide, leading anteriorly from excretory bladder and terminating posterior to nerve ring, 58.9 - 82.7 μ from anterior extremity. Muscular oesophagus 98.4 - 126.7 μ long,

Fig. 4. (a) Lateral view of Second-stage larva.

(b) Ventral view of the posterior extremity of Second-stage larva at anal region.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(a)
(b)

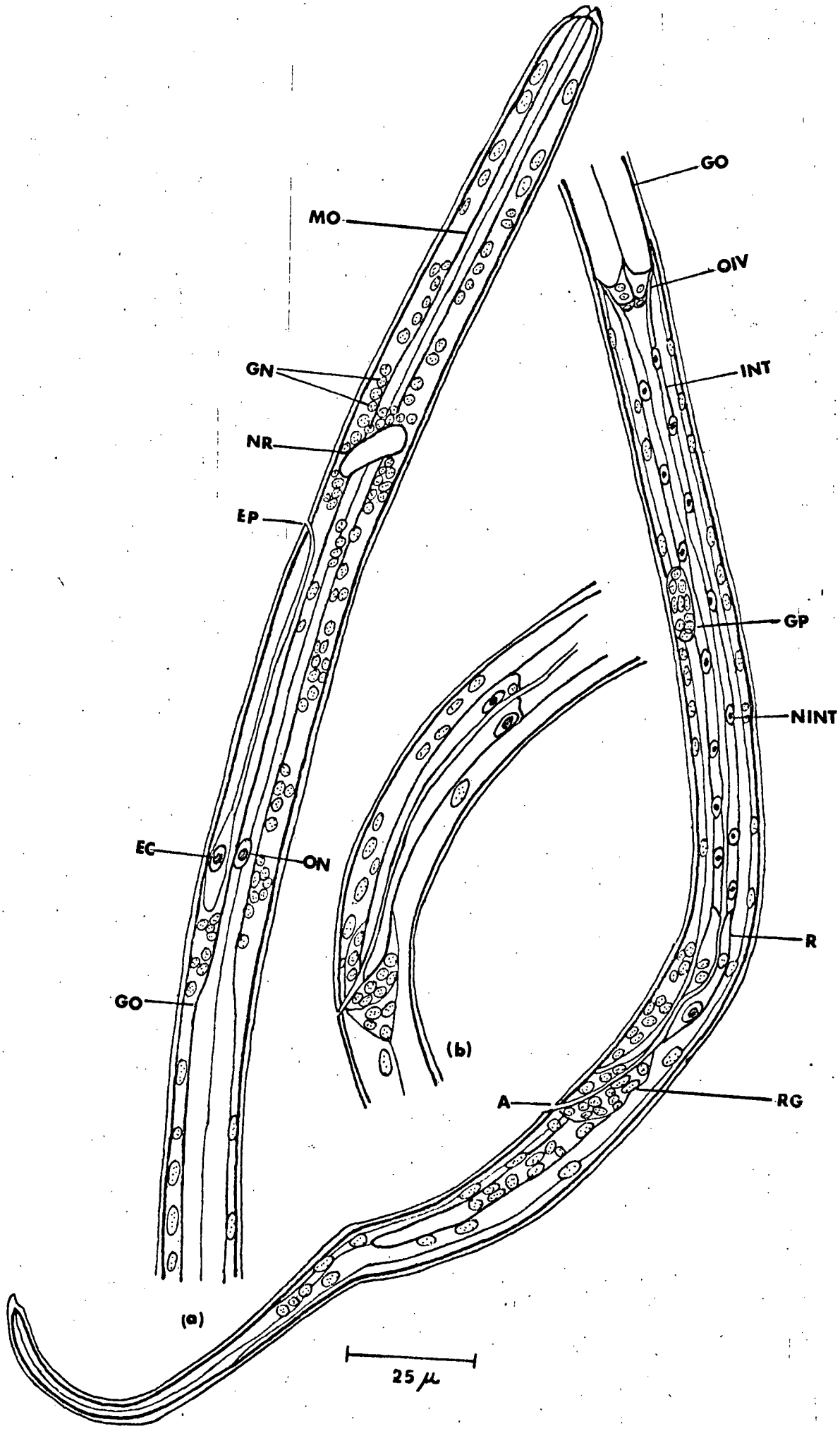
7.38 - 11.1 μ wide, immediately behind buccal cavity; expanding gradually posterior to nerve ring. Oesophagus terminating in crescent-like cells, four dorsal and two ventral, each with conspicious nucleus (Figure 6a). Thick walled intestine 88.6 - 135.3 μ long, consisting of ten to twelve cells; widest region 12.3 - 14.8 μ; narrowest at intestinal-rectal region, 6.2 - 12.3 μ. Lumen narrow, ending anterior to intestinal-rectal junction. Genital primordium composed of four cells (Figures 7, 8 and Table II), 18.5 - 20.9 μ long, situated ventrally 39 - 52.4% of body length from anterior extremity; adjacent to intestinal-rectal junction. Third cell from anterior largest, 13.5 - 14.8 μ in diameter, with prominent round nucleus. Rectum 49.2 - 71.3 μ long, 9.8 - 12.3 μ wide. Two large prominent nuclei, each 10 μ in diameter, located near intestinal-rectal junction, one on each side of narrow lumen. Rectal lumen expanding to form a heart-shaped chamber before opening to exterior. Intestinal and rectal lumens not connected. Poorly developed rectal gland near anal region. Phasmids not seen. Tail 58.9 - 82.7 μ long, filiform and tapering towards extremity.

First-stage larvae from Cyclops bicuspidatus.

First-stage larvae from the copepods resemble those from female worms except for the following minor differences:-

Fig. 5. (a) Lateral view of Third-stage larva.

(b) Lateral view of the rectal and anal regions of Early Third-stage larva.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25 μ

Total body length longer, 579.6 μ (499.5 - 638.5 μ). Unicellular gland 11 - 12 μ long, 6 - 7 μ wide (Figure 3), located dorsal to buccal cavity. Nucleus of gland round, with abundant chromatin granules. Intestinal and rectal lumens connected in larvae from haemocoel. Genital primordium situated 30.3 - 44.2% of body length from anterior extremity, immediately anterior to intestinal-rectal junction.

Second stage. (Figures 4, 6b, 7b, 8, Table I)

Shape stumpy, with blunt head and tail. Body length 492 - 809.3 μ; body width at anus 12.3 - 19.7 μ. Dorsal denticle absent. Amphids not discernible. Buccal cavity short. Nerve ring situated 59.4 - 74 μ from anterior extremity. Ganglionic nuclei concentrated around nerve ring. Excretory bladder 28.5 - 30 μ long, 5 - 6 μ wide; posterior extremity ending at anterior portion of glandular oesophagus. Excretory duct 34.2 - 35.4 μ long, opening posterior to nerve ring, 74 - 87.3 μ from anterior end. Oesophagus 196.8 - 359.2 μ long, poorly demarcated into anterior muscular and posterior glandular regions; width behind buccal cavity 4.9 - 6.2 μ. Oesophageal-intestinal valve present; with four dorsal and two ventral nuclei (Figure 6b). Hypodermal nuclei round and conspicious, showing mitotic division. Intestine

Fig. 6. Oesophageal-intestinal valves of

(a) First-stage larva.

(b) Second-stage larva.

(c) Third-stage larva.

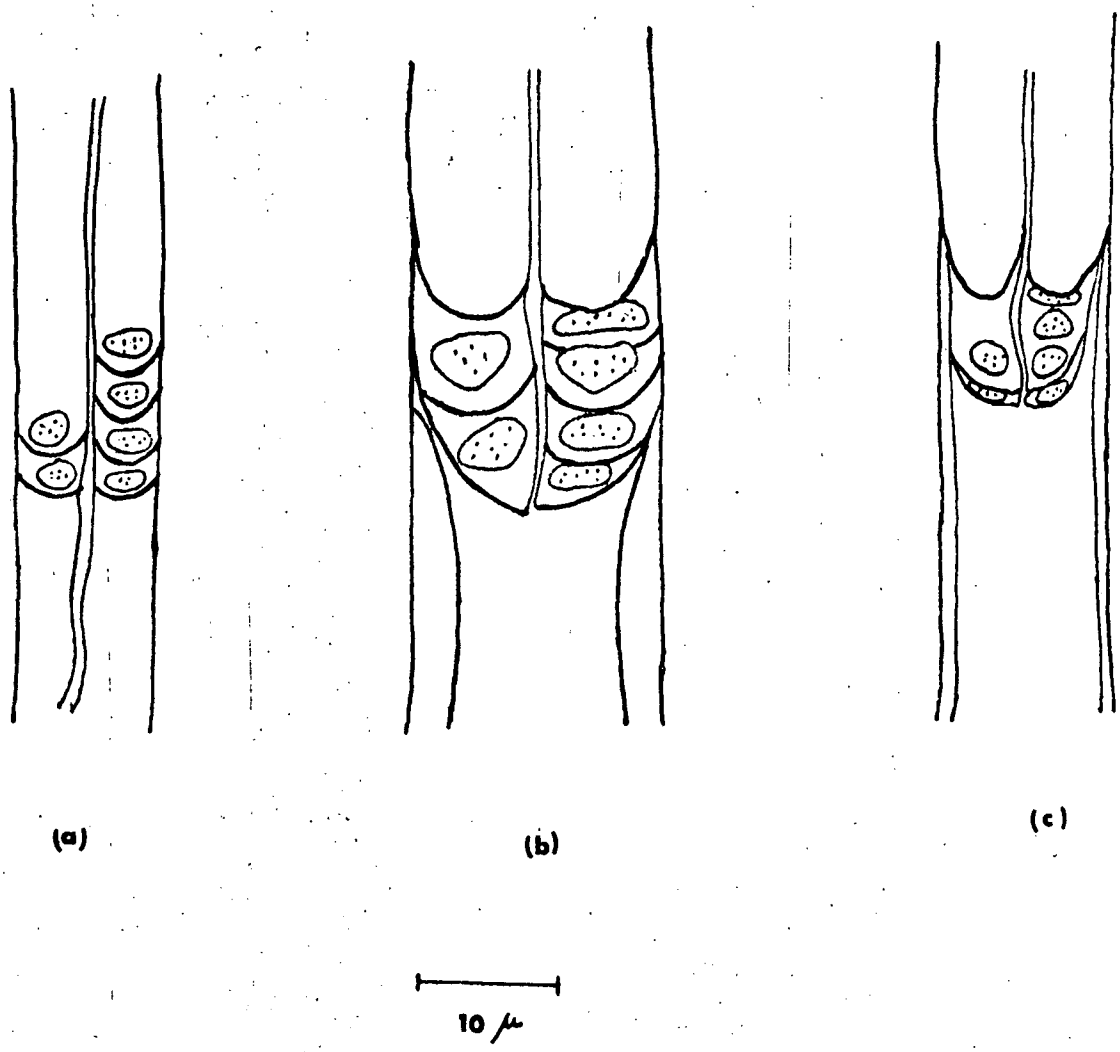

(a)
(b)
(c)
10 μ

TABLE I. Measurements of larval stages (in μ) (salmon strain)

(sample size - 20 for each stage)

Stage	First		Early second*	Second	Early third*	Third
	From female worms	From copepods				
TL	490.3 (416.3-527.3)	579.6 (499.5-638.3)	564.2 (492-666)	732.3 (625.4-809.3)	876.2 (788-964.3)	992.2 (915.8-1037.9)
NRG	53.2 (47.1-61.8)	59.7 (49.5-65.5)	61 (53.2-65.5)	64.77 (59.4-74.1)	79.5 (70.4-90)	91.3 (81.5-99.9)
LMO	105.2 (98.4-126.7)	123 (103.3-159.9)	240.3 (102.6-267.9)	294 (196.8-359)	190.1 (137.7-273)	206.64 (150.1-350.6)
LGO					321.1 (198-386.2)	476.9 (350-532.8)
WMO	6.8 (7.38-11.1)	7 (6.2-8.6)	6.47 (6.2-6.8)	5.5 (4.9-6.2)	5.5 (4.9-6.5)	4.92
WBNR	18.45 (15.9-24.6)	18.5 (17.2-18.45)	17.22 (15.99-18.5)	17.22 (14.8-18.5)	13.53 (11.1-14.8)	13.53 (11.1-14.8)
WBA	13.63 (15.3-18.5)	14 (13.5-15.4)	15.2 (13.5-20.9)	14.76 (12.3-19.7)	12.8 (12.3-16)	12.3 (11.1-12.3)
LI	118.7 (88.6-135.3)	113.3 (79.9-159.9)	166.6 (129-258.3)	259 (209-314.9)	196.8 (153.8-258.3)	132.4 (104.6-205.4)

TABLE I. (Continued)

Stage	First		Early second*	Second	Early third*	Third
	From female worms	From copepods				
WIw	13.63 (12.3-14.8)	13.4 (12-14.8)	11.5 (10.8-12.3)	11.7 (9.8-15.3)	9.1 (8.6-11.1)	8.61 (7.38-9.84)
LR	55.35 (49.2-71.3)	56.8 (43.1-59.8)	51.5 (36.9-67.6)	59.4 (51.7-68.9)	46.74 (43.1-55.4)	46.74 (36.9-61.5)
WR	9.64 (9.84-12.3)	9.9 (7.38-11.4)	9.84 (7.38-11.1)	10.3 (9.84-12.3)	7.38 (6.2-9.84)	7.9 (7.38-8.61)
DAT	221.2 (190.7-270.6)	286.5 (233.7-337)	107.2 (73.8-123)	119.9 (86.1-135.3)	121.5 (98.4-129.2)	130.8 (98.4-131.4)
LGP	18.45 (18.45-20.9)	18.45 (18.45-20.9)	14.5 (12.3-17)	18.45 (17.2-19.7)	18.45 (17.2-20.9)	18.45 (17.2-20.9)
GPR	43.7% (39-52.4)	38.4% (30.3-44.2)	43.9% (32.3-58.2)	51.3% (27.3-65.9)	47%(43.2-50.5) 63%(61.3-65.9)	
EXP†	71.84 (58.9-82.7)	78.8 (74-82.7)	86 (74-87.3)	86 (74-87.3)	100 (100-114)	100 (100-114)

* soon after larvae cast off old cuticle.

† six specimens for each stage.

209 - 314.9 μ long, consisting of ten to twelve cells; widest region 9.8 - 13.5 μ; narrowest at intestinal-rectal junction, 6.2 - 8.6 μ; lumen wide; intestinal walls thin. Genital primordium 17.2 - 19.7 μ long, closely packed with eight cells; situated 27.3 to 65.9% of body length from anterior extremity, usually posterior to middle of intestine (Figures 7b, 8). Rectum swollen; with thick walls and narrow lumen; 51.7 - 68.9 μ long; 9.8 - 12.3 μ wide; two large nuclei at intestinal-rectal junction. Rectal gland well developed, comprising twelve cells circumscribing anal region. Phasmids not seen. Tail 86 - 135.3 μ long, terminating typically in a cap formed by thin cuticle.

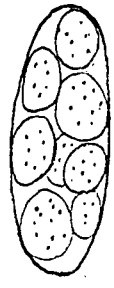
The average size (564 μ) of recently moulted second-stage larvae is smaller than that of the first-stage larvae from the copepods.

Third-stage (Figures 5a, 6c, 7c, 8, Table I)

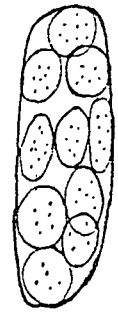
Body filiform in shape, with conical head and tapering tail. Body length, 992 μ (915.8 - 1037.9 μ); body width at region of nerve ring 11.1 - 14.8 μ; body width at anus 11 - 12.3 μ. Cuticle thick, forming four lip-like structures at buccal region. Amphids not discernible. Buccal cavity absent. Nerve ring 81.5 - 99.9 μ from anterior extremity. Ganglionic nuclei concentrated around nerve ring. Muscular oesophagus

Fig. 7. (a), (b) and (c) showing the genital primordium of First, Second and Third-stage larva respectively; (d) Excretory bladder of First-stage larva.

(a)

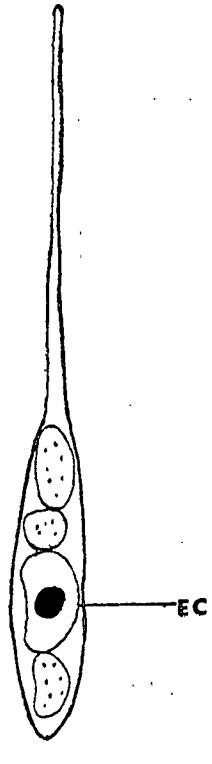


(b)



(c)

10 μ



(d)

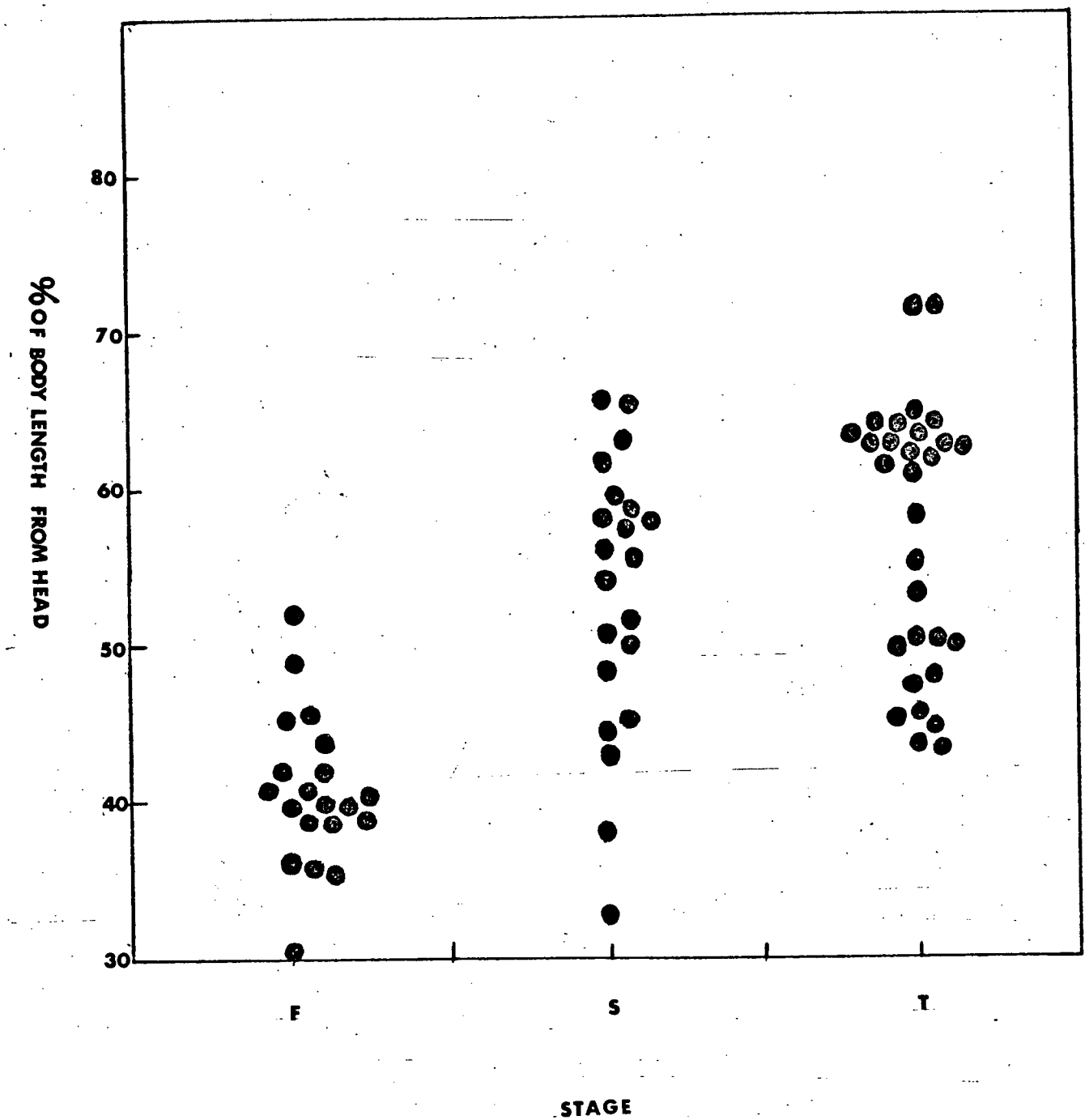
slender, 150 - 350.6 μ long, 4.92 μ wide. Glandular oesophagus well developed, 350 - 532.8 μ long; widening gradually to fill pseudocoel completely at intestinal-oesophageal junction. Oesophageal-intestinal valve well developed; a prominent structure with four dorsal and two ventral nuclei, protruding into lumen of intestine (Fig. 6c). Excretory bladder 20 - 22 μ long, 4 μ wide; ending posteriorly not far from muscular-glandular oesophageal junction. Excretory duct 60 - 62.7 μ long; excretory pore, 100 - 114 μ from anterior extremity. Intestine narrow, 104.6 - 205.4 μ long; anterior width 7.4 - 9.8 μ; posterior width 6.2 - 7.4 μ; fourteen to sixteen intestinal cells; lumen narrow. Genital primordium (Figs. 7c, 8) 17.2 - 20.9 μ long; ten closely packed cells; located in some specimens adjacent to glandular oesophagus, 43.2 - 50.5% of body length from anterior extremity in glandular oesophageal region; in other specimens at anterior portion of intestine, 61.3 - 65.9% from anterior extremity. Rectum 36.9 - 61.5 μ long; anterior region, thick walled, lumen narrow; posterior region, a fine tube leading to exterior. Rectal gland well developed, with fourteen to sixteen cells surrounding posterior tubular portion of rectum. Phasmids not seen. Tail 98.4 - 131.4 μ long. Cuticle at extremity of tail expanding dorsally to form a short protrusion (Fig. 5).

Fig. 8. The distribution of genital primordium in the larval stages.

F = First-stage.

S = Second-stage.

T = Third-stage.


% OF BODY LENGTH FROM HEAD
80
70
60
50
40
30
F
S
T
STAGE

Recently moulted third-stage larvae differ from the above description of third-stage in the following ways:-

(1) Small piece of sheath attached to tail.

(2) Body length shorter, 876.2 μ (788 - 964.3 μ).

(3) Glandular oesophagus shorter, 198 - 386.2 μ.

(4) Intestine longer, 153.8 - 258.3 μ.

(5) Rectal gland, not so well developed (Fig. 5b).

(3) Manner of moulting.

Philonema larvae undergo two moults in *Cyclops bicuspidatus*. The events of moulting were observed to take place in the following sequence:-

(i) Wrinkling of cuticle, thus indicating a shortening of the body.

(ii) Formation of new cuticle, most evident at tail region.

(iii) Loosening of old cuticle. During the first moult, loosening of the old cuticle was apparent first at the posterior extremity. In the second moult, the corresponding process was first evident at the anterior extremity.

(iv) Shedding of old cuticle. In the later phase of moulting, a sheath enclosing the anterior end breaks off in the form of a cap anterior to the excretory pore region.

Fig. 9. The distribution of larval stages (salmon strain) collected at different time intervals from *Cyclops* maintained in 15°C.

First-stage.

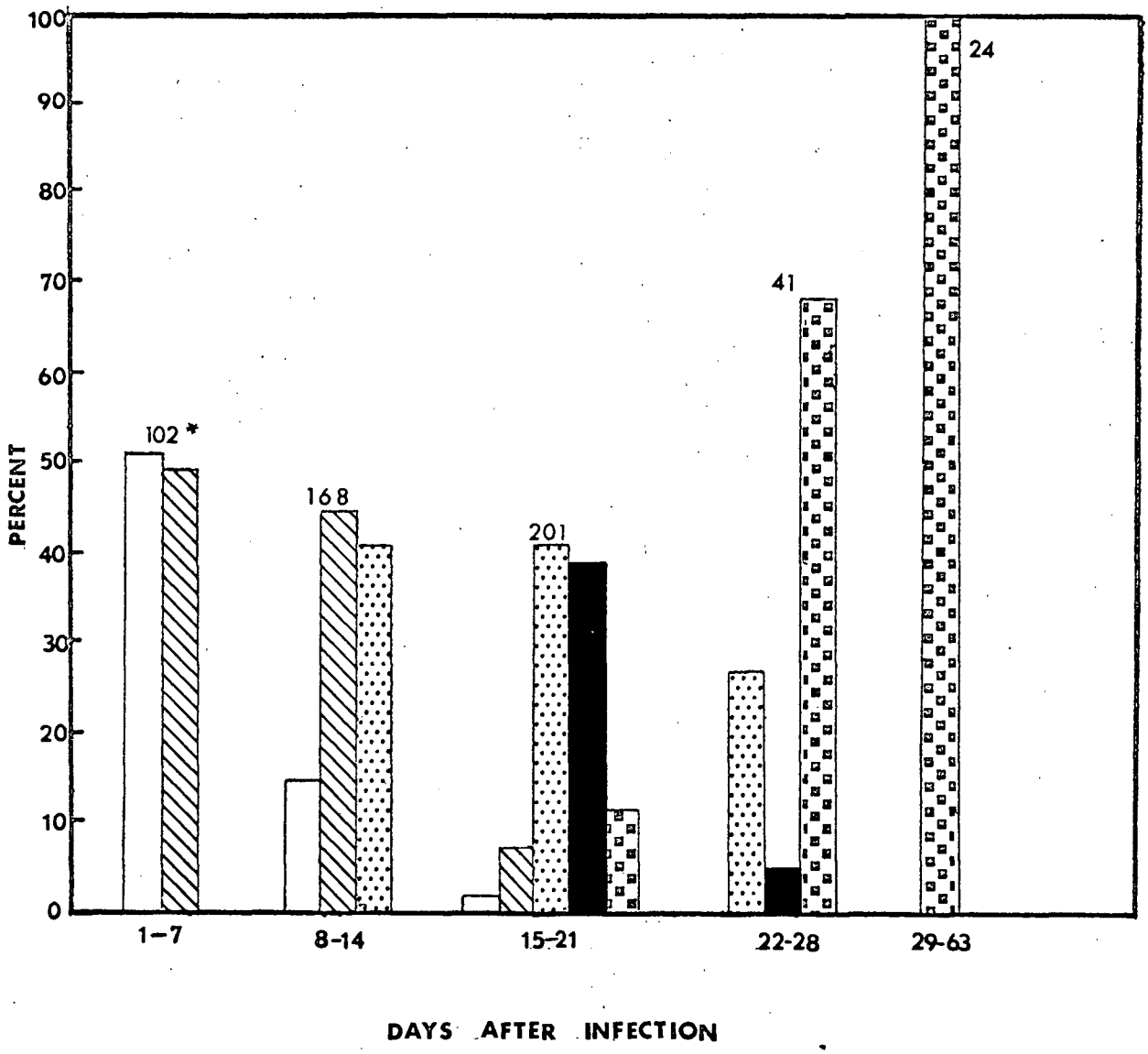
Moulting First-stage.

Second-stage.

Moulting Second-stage.

Third-stage.

* sample size of the interval.


100
90
80
70
60
50
40
30
20
10
0
PERCENT
102 *
168
201
41
24
1–7
8-14
15-21
22-28
29-63
DAYS AFTER INFECTION

(A sheath in form of a cap was found attaching inside-out to the head of the larva by the lining of the oesophagus.) Larvae wriggle free of the remaining sheath still attached to the posterior portion of the body.

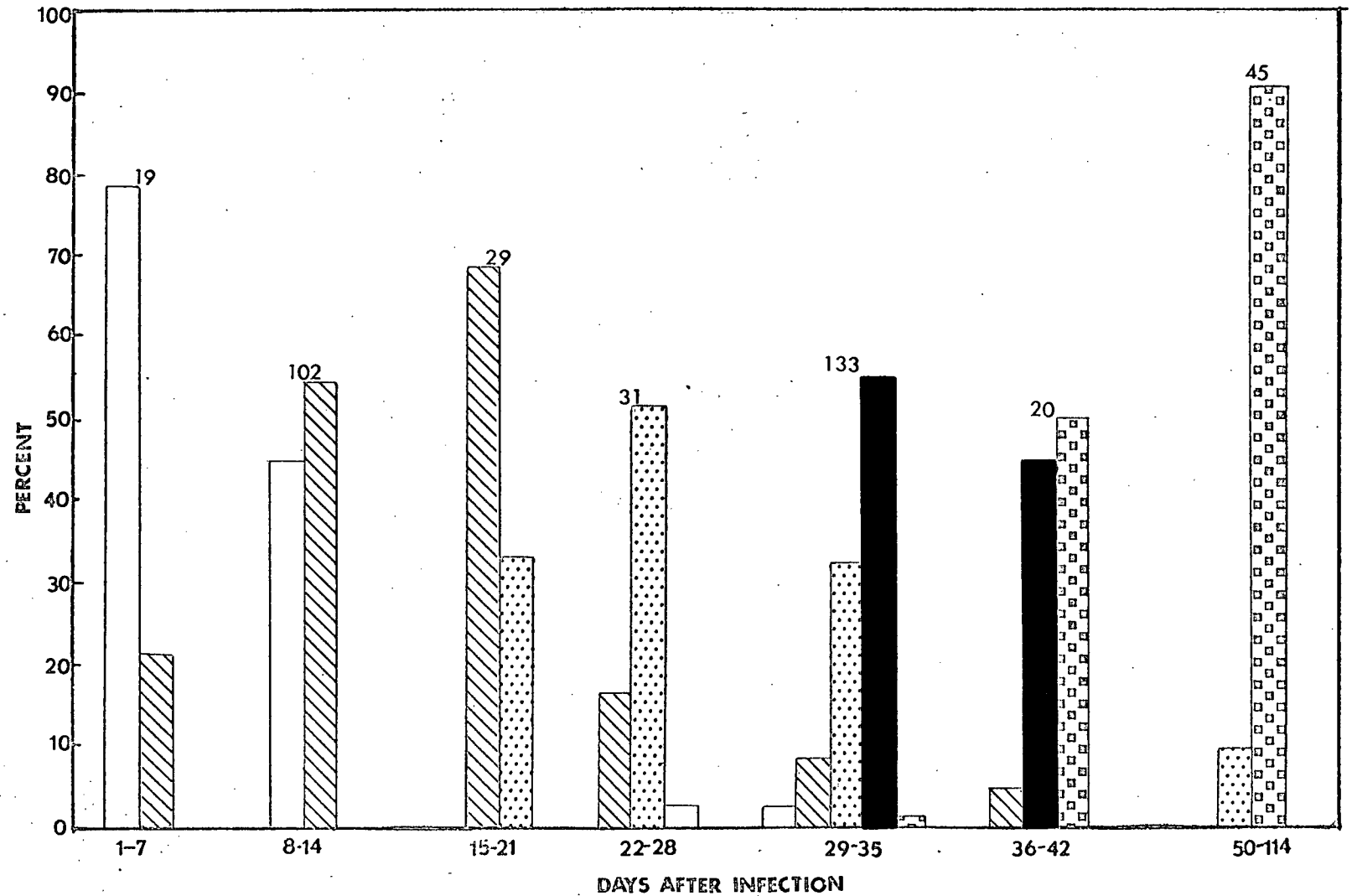
(4) Effect of temperature on rate of development.

The frequency distribution of various larval stages obtained from copepods maintained at 15°C, 10°C and 4°C at different intervals is presented in Fig. 9, 10, 11 respectively. (For raw data see Table III) Sample sizes are not uniform, due mainly to the difference in infections among copepods. The data shows that at all temperatures, complete transition in the population from one stage to another is slow. A specific stage usually extends over a period of three to four weeks. (For example, at 15°C, moulting first-stage larvae extended from 5 to 21 days after infection.)

The frequency distribution of larval stages of salmon and trout strains obtained from copepods maintained at 10°C is also shown in Fig. 10a, b. The slight difference in distribution between the two strains may be caused by the unequal sample size. Nevertheless, the overall frequency distribution is more or less the same.

Fig. 10. (a) The distribution of larval stages (salmon strain) collected at different time intervals at 10°C.

Legend - see Fig. 9


100
90
80
70
60
50
40
30
20
10
0
PERCENT
19
102
29
31
133
20
45
1-7
8-14
15-21
22-28
29-35
36-42
50-114
DAYS AFTER INFECTION

The effect of temperature on the rate of moulting is summarized in Table IV. The criterion for comparison is the time required for 50% of the sample to show signs of moulting.

(5) Effect of temperature on growth.

Figure 12 compares the range and frequency distribution of sizes of the three larval stages at three different temperatures.

A two-ways analysis of variance was performed on the sizes of the three larval stages (covering different periods of each stage) from 15°C, 10°C and 4°C (Table VI. Total sample size = 180). At the 99% confidence interval, it is found that (i) there is no significant difference between the lengths of the same stage from the three different temperatures, (ii) the response to temperature (interaction) is the same in all stages.

Finally, Figure 13 (Table V) summarizes the pattern of growth of *Philonema* larvae (salmon strain) in different temperatures.

10 (b) The distribution of larval stages (trout strain) collected at different time intervals at 10°C.

Legend - see Fig. 9.

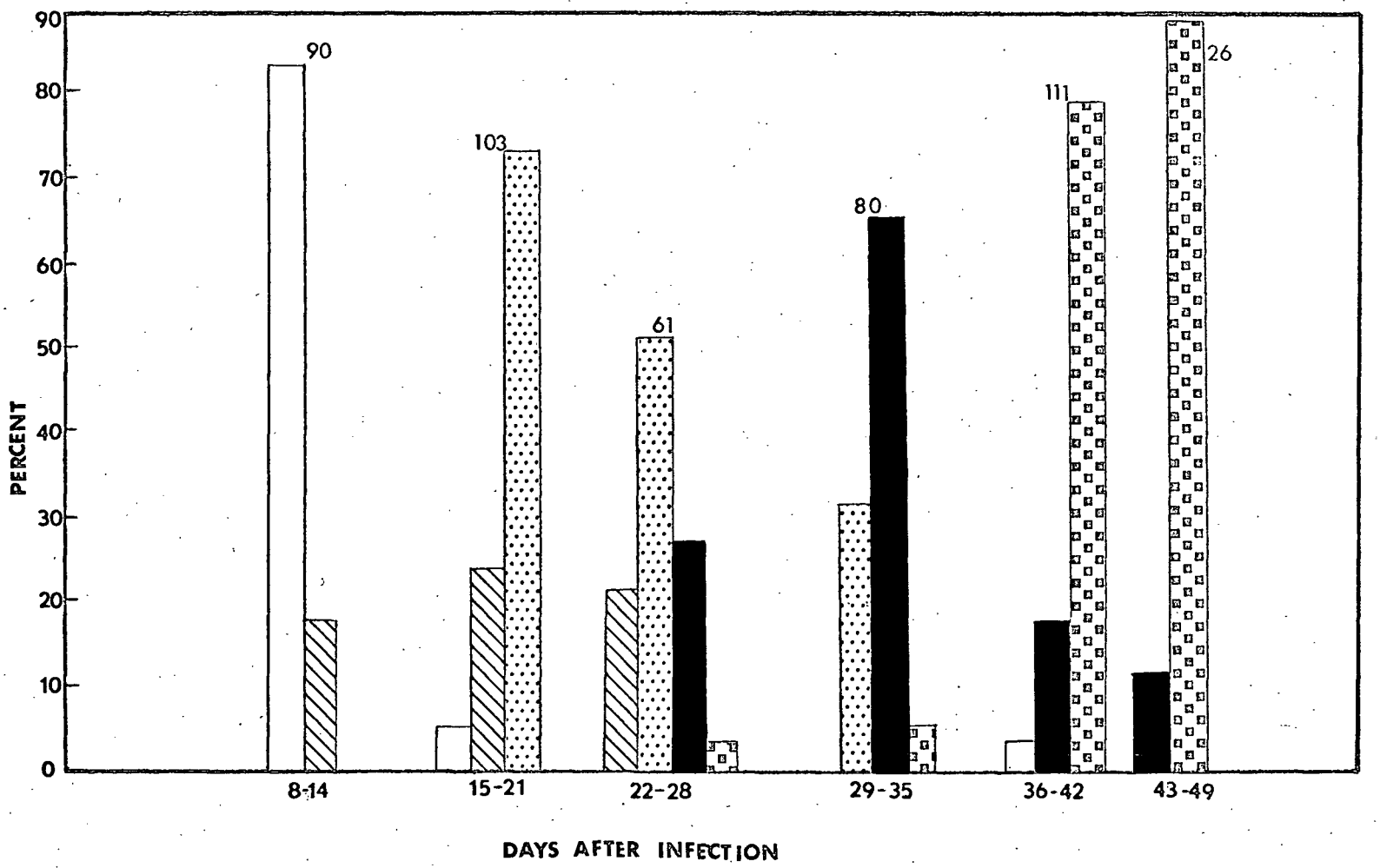

90
80
70
60
50
40
30
20
10
0
PERCENT
90
103
61
80
111
26
8-14
15-21
22-28
29-35
36-42
43-49
DAYS AFTER INFECTION

TABLE IV. Summary of the rates of moulting at different temperatures

Temperature	Strain	Time at which 50% of the samples collected are moulting First-Stage larvae	Time at which 50% of the samples collected are moulting Second-Stage larvae
15°C	Salmon	6 days after infection	17 to 19 days after infection
10°C	Salmon	13 days after infection	30 to 31 days after infection
10°C-15°C	Salmon	13 days after infection	12 days after change
10°C-4°C	Salmon	13 days after infection	47 days after change
10°C	Trout	12 to 15 days after infection	30 to 34 days after infection
4°C	Salmon	23 to 25 days after infection	74 to 78 days after infection

(For more information see Table III)

Fig. 11. The distribution of larval stages (salmon strain) collected at different time intervals from *Cyclops bicuspidatus* maintained in $4^{o}C$.

Legend - see Fig. 9

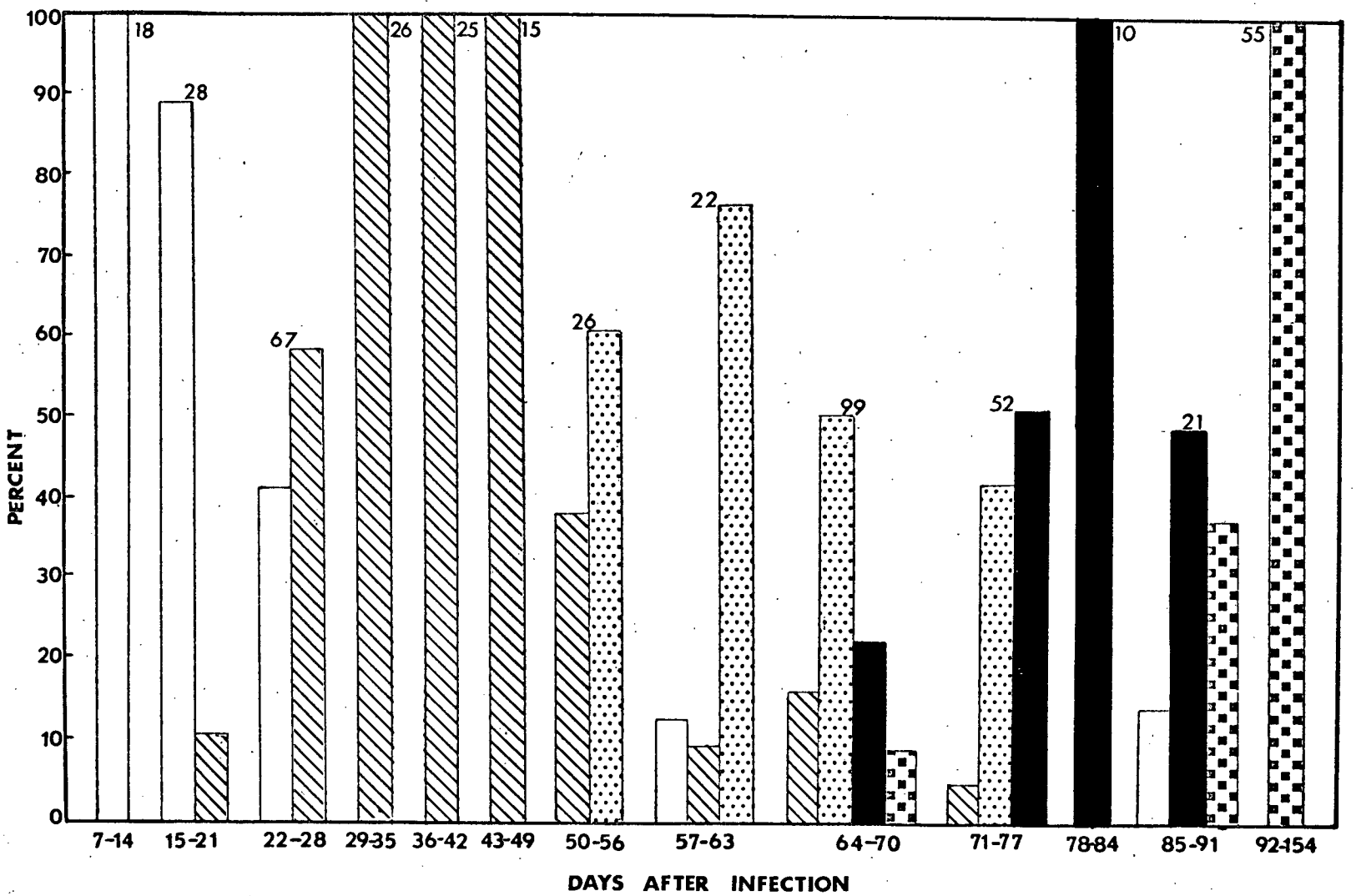

PERCENT
100
90
80
70
60
50
40
30
20
10
0
18
28
67
26
25
15
26
22
99
52
10
21
55
7-14
15-21
22-28
29-35
36-42
43-49
50-56
57-63
64-70
71-77
78-84
85-91
92-154
DAYS AFTER INFECTION

Fig. 12. Comparison of the range of sizes of the larval stages obtained from copepods maintained in $15^{o}C$, $10^{o}C$, and $4^{o}C$.

(Sample size = 20 for each stage)

Frequency distribution of First-stage

Frequency distribution of Second-stage

Frequency distribution of Third-stage

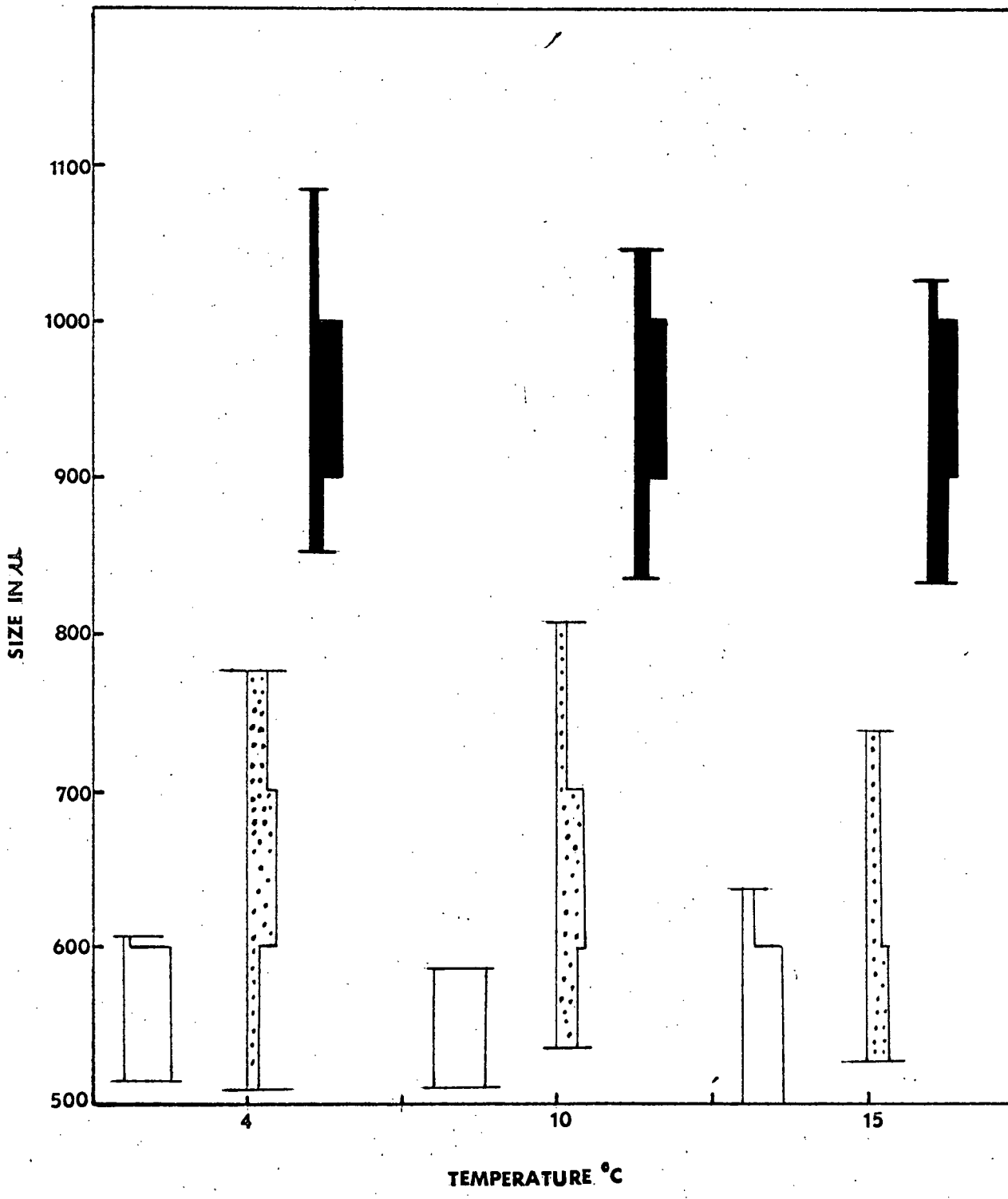
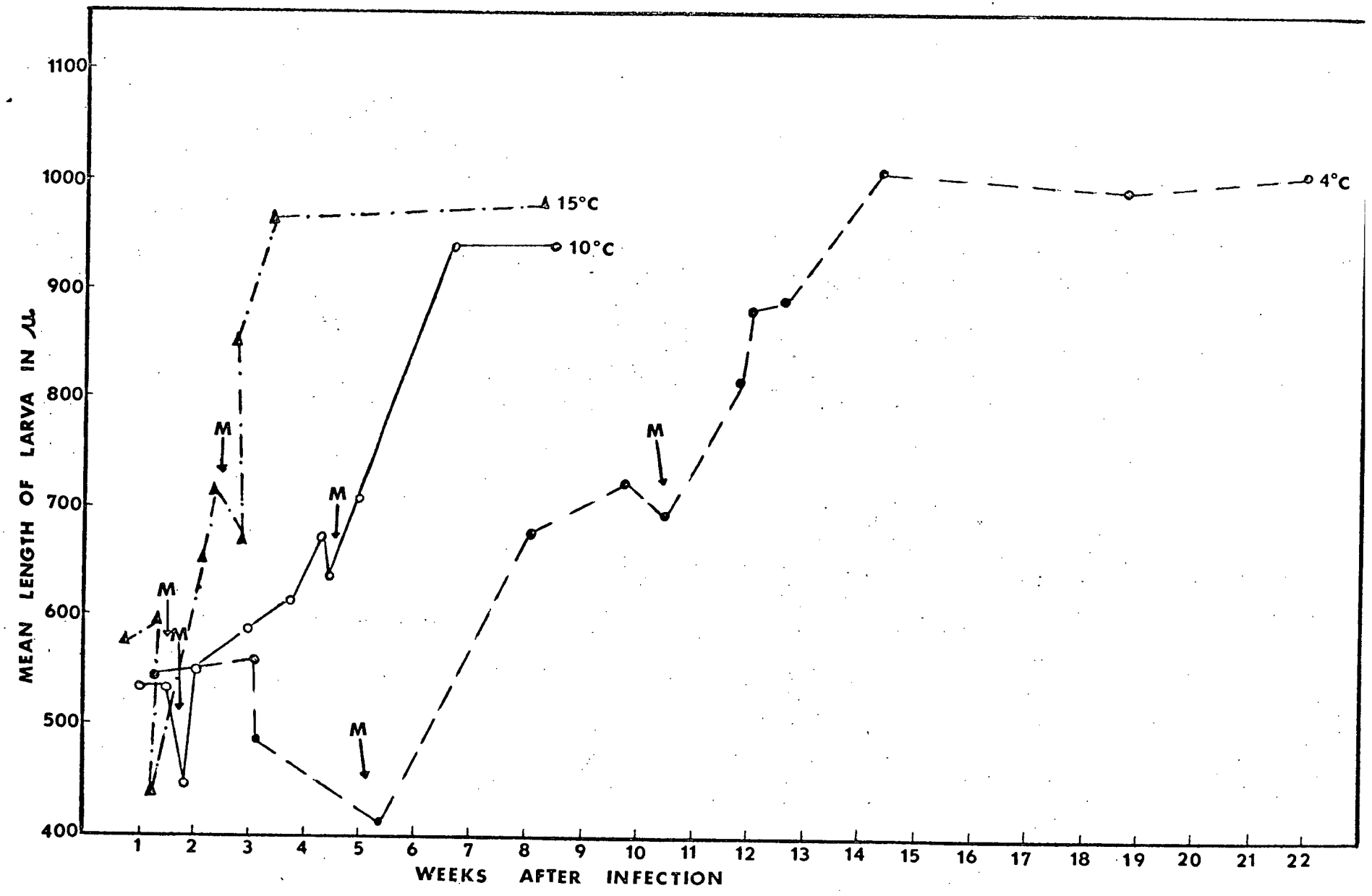

1100
1000
900
800
700
600
500
SIZE IN μ
4
10
15
TEMPERATURE °C

Fig. 13. Comparison of the growth of larvae recovered from *Cyclops bicuspidatus* maintained in 15°C, 10°C and 4°C.

M = Moulting


MEAN LENGTH OF LARVA IN μ
1100
1000
900
800
700
600
500
400
15°C
10°C
4°C
M
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
WEEKS AFTER INFECTION

DISCUSSION

Morphology:

During the process of dvelopment, prominent morphological changes in the larvae of *Philonema* take place mainly during moulting. Only increase in size and changes in proportion of different structures occur within each stage.

Difference in size is a major feature for distinguishing the larval stages. The third-stage larva is almost twice as long as the first (mean lengths, 992.2 vs. 490.34 μ). The second-stage is wider but shorter than the third. Data shows that there is an upper asymptote for the growth of third-stage larvae. (Figure 13. Table V) At 4°C the mean length of the larva recovered 96 days after infection is almost the same as that obtained 64 days later, slightly over 1 mm in both cases. Moreover, besides the cessation of growth, it appears that the third-stage larva may not be ingesting food. This observation is made on the basis that green-pigmented particles are absent in the intestine of such larvae. Green-pigmented particles not only are present in the intestines of the first and second-stage larvae but the intensity of these particles in the intestine changes with the intensity of algal growth in the culture dishes where

infected copepods are kept. This suggests that the larvae may be feeding on the intestinal contents of the copepods. The method by which they achieve this is not known.

These phenomena partially illustrate one of the features of Rogers' hypothesis. In this hypothesis, Rogers states that in parasitic species, the formation of the infective stage is usually preceded by a moult or partial moult. Whether the life cycle is direct or indirect, the infective stage is a "resting" stage in which the normal processes of development are suspended (Rogers, 1962). Here, in Philonema, the third-stage larva, like that of most nematodes, represents the "resting" stage in the life cycle.

Body forms also vary considerably between the three larval stages as a result of adaptation. The tail of the first-stage larva, which is long and filiform serves two purposes. The length of the tail allows it to be whipped around, coiled and uncoiled actively, thus functioning as a device to keep afloat. Observations under the microscope show that after being kept in the lake water for a fortnight, larvae show less vigorous movements and they gradually sink to the bottom of the dish. On the other hand, the whipping movement may act as a gesture which attracts the attention of

copepods leading to ingestion. Comparable suggestions were made by Li (1935) and Leiper (1907) for other species.

The second-stage is short and stumpy, due to the shedding of the long first-stage tail. The thin cuticle may permit an extensive increase in length during the second moult.

The head of the third-stage is conical while its body is filiform. Since it has to migrate from the intestine of the fish to the tunica adventitia of the swim-bladder after being ingested by the fish host (Platzer and Adams, manuscript in press), this type of body form may facilitate penetration. The conical head returns to a rounded form following the third moult in the fish. This was shown by a moulting third-stage larva recovered from an *in vitro* culture experiment carried out by the author.

Only the first-stage larva is equipped with the dorsal denticle. After infection, these larvae have to migrate from the intestine of the copepod to the body cavity where development to infective stage occurs. These facts suggest the possible function of this particular structure. It may be analogous to the hooks of the oncospheres of cestodes which act as a penetrative device (Thomas, 1937). The following observation made by Li (1935) confirms the presumption: "When ingested, *Procamallanus fulvidraconis*

larvae moved freely along the whole digestive tract. The larva coiled itself dorsally as far as it could go and then with a sudden springing action, the anterior portion was stretched and the dorsal spine struck on the intestinal wall of cyclops." Working with Dracunculus medinensis which also possesses a dorsal denticle, Isaev (1934) reported that in the intestine of copepod, the larva assumed the form of an elongate ring with the ends meeting. Then the larva straightened its cephalic end and pressed it against the intestinal wall. A similar behaviour was observed in Philonema.

A large light staining cell is located dorsally at the cephalic extremity of the first-stage larvae extracted from copepods. Though no connection between this cell and the dorsal denticle is visible, its location and its absence in subsequent larval stages lead to the belief that it may be a unicellular gland responsible for histolytic secretion associated with the penetration into the body cavity of the copepod.

If the assumption is correct, one should also expect to find the same structure in first-stage larvae from gravid female worms. But an examination of two hundred larvae yielded negative results. This may be due to the fact that

all the larvae examined were mounted laterally (because usually larvae recovered from female worms were coiled). The unicellular gland which is situated dorsally, therefore, could not be detected.

The oesophageal-intestinal valve of second and third-stage larvae is a thick muscular structure with five to six nuclei. It is similar in appearance to those of the larvae of Strongyloides stercoralis, Necator americanus and Ancylostoma canium, all of which have six to eight nuclei (Nicholas, 1956). The nuclei, three to four on the dorsal side and two on the ventral side, are always very prominent in both stages.

The excretory bladders of different stages differ only in size. The number of cells remains constant. The average lengths of the bladder and excretory duct measure 50 μ in the first-stage, 63 μ in the second and 80 μ in the third.

The genital primordium increases in number of cells but not in size (mean 18.54 μ) as the larva grows from the first to the third-stage. Figure 8 shows that in the first-stage, the genital primordium is generally located between 39 to 42% of body length from the anterior extremity. In the second-stage, its position varies considerably, from

43 to 66% whereas in the third-stage, two distinctive positions of genital primordia appear. One group ranges from 43 to 51%, the other from 61 to 66%.

There are two possible interpretations for the bimodal distribution of genital primordia in the third-stage. First, the difference in positions may be a form of sexual dimorphism. In nematodes, the female reproductive organs, especially the genital pores, are always located anterior to the corresponding organs in males. Applying this evidence to Philonema, the larvae whose genital primordia are situated between 43 to 51% must be the future females. The group whose organs are situated between 61 to 66% are the males. This supposition also explains satisfactorily the distribution of genital primordia in the second-stage. The wide range of positions may be an indication of the migration of genital primordia in this stage.

This interpretation is confirmed by the results of Schwartz and Alicata (1934) working with Necator americanus larvae. They observed that the genital primordia of six days old larvae from the lungs of infected guinea pigs were located in the last third of the intestine, whereas in younger third-stage they were anterior to the middle of intestine. Larvae with a more posterior genital primordium

were considered to be males because in female larvae of fourth-stage, the anterior and posterior ovaries branch from a point corresponding to the more central position of the genital primordium.

Nichols (1956) also mentioned that sexual dimorphism in Ascaris lumbricoides was first evident in middle third-stage larvae. Males are distinguished from females in that they have six cells in the rectal gland instead of three. No corresponding observation could be made in Philonema because the number of cells in the rectal gland is usually not well defined.

Secondly, the difference in position in genital primordium may be due to the presence of two species of larvae. Yoshida (1966) reported that difference in position in genital primordium is one of the diagnostic features to distinguish between the fourth-stage larvae of Ancylostoma duodenale and Necator americanus. This suggests the possibility that the difference in position in genital primordium indicates that trout and salmon strains of Philonema occur concurrently in the same lake. However, if the two strains really exist concurrently and could be distinguished in this manner, there must be also two distinctive groups of genital primordia in second-stage larvae.

But no such observation could be made. Moreover, the positions of the genital organs are the same in the adults of the two strains of Philonema.

Therefore, the first interpretation seems to provide a better explanation for the bimodal distribution of genital primordia.

Moulting:

Little is known about moulting in nematodes. Lee (1965) defined moulting as the formation of a new cuticle, the loosening of old cuticle and the rupture and ecdysis of the old cuticle with the ensuing escape of the larva. The significance of moulting in the life cycle is not clear. It may be a mechanism of growth related to the properties of the cuticle or it may be concerned with getting rid of nitrogenous waste materials. Rogers (1962) questioned the validity of the latter hypothesis (1) because he has demonstrated that ammonia and urea can be excreted even through the adult cuticle of Ascaris lumbricoides and (2) because nitrogenous wastes are excreted by larvae without difficulty (Weinstein and Haskins, 1955).

In Philonema, moulting probably functions partly as a physiological adaptation to a new environment. The assumption is based on the following observations which

indicate that the physiology of the larval stages is different. First-stage larvae from gravid female worms can survive in lake water whereas the second-stage from copepods bursts in the same medium. After the second moult, larvae, now being the infective stage, can again withstand an aquatic medium. So one may speculate that some physiological changes related to the properties of the cuticle have occurred during the second moult as a preparation for entering the forthcoming new environment.

Effects of temperature on development.

Information concerning the effects of temperature on the development of parasites is scanty. Most of the available literature deals only with superficial observations. Attempts were seldom made to correlate the physiological interactions between the host and its parasites, in response to ambient temperature changes.

As in all other animals, there are limits of tolerance to temperature for each parasite. Fluctuation of temperature within the limits will either decrease or increase the rate of metabolism. In general, the effects of temperature changes on parasites are:-

i) Shorten the duration of egg incubation.

A temperature increase to certain limits accelerates fission; a decrease, again to a certain extent, slows down the process. Maximal and minimal limits are characteristic for given species, e.g. for the branchiuran crustacean, *Argulus foliaceus*, it must not be less than 10°C; for the parasitic copepod, *Lernae cyprinacea*, not less than 14°C (Bauer, 1959).

At room temperature (22°C) the eggs of *Ascaris lumbricoides* take about 28 days to develop into second-stage larvae; at 31°C they require only 14 days (Fairbarn, 1955).

ii) Shorten the length of postembryonic development.

At 7.5°C, 21 to 28 days are required for the larvae of *Dochmoides stenocephala* (Ancylostomidae) to reach maturity in nutrient agar. At 27°C, they became mature in 51 hours (Gibbs and Gibbs, 1959).

iii) Increase oxygen consumption.

The third-stage larvae of the nematode, *Nippostrongylus brasiliensis*, have a high rate of oxygen consumption after being subjected to a temperature rise (Wilson, 1965).

iv) Increase the activity of invasive phases.

Free-swimming larvae of the trematode, *Diclybothrium armatum* are active for five to six hours at 13°C; at 24°C, they are active for one and a half hour (Bauer, 1959). The frequency of contraction of *Bucephalus elegans* cercariae increases from 0 - 102/minute when the temperature is increased from 0 to 28°C.

v) Change the size of larvae.

This aspect of temperature influence is not well documented. Ciliospores of the ciliate, *Icthyophthirius sp.* formed at 20 to 22°C have an average length of 30 μ; those at 7 to 8°C average 50 μ (Bauer, 1959). However, according to Vogt (1938), the procercoids of the cestode, *Triaenophorus nodulosus*, attained, after seventeen days in *Cyclops sp.* the length of 283 μ at 4°C; 326 u at 8.5°C; 345 μ at 14.5°C. Gibbs and Gibbs (1959) also observed that at 7.5°C the average length of the free-living stage of the nematode, *Dochmoides stenocephala*, was 532 μ as compared to 608 μ at 25°C.

vi) Increase the developmental rate of larval phases in the intermediate host.

A number of examples are available in this respect. DeGuisti (1949) found that the acanthocephalan, *Leptorhynchoides thecatus*, required at least two months to

develop to the infective stage, in Hyallela azteca maintained at 13°C. Conversely, full development was attained in 30 to 32 days if the amphipods were kept at 25°C.

Similar observations have been made in many trematodes (Vogel, 1934; Stadun, 1952; Dinnik, 1964; Olson, 1966). McCoy (1928) demonstrated that Cercaria hamata changed into metacercariae in "sun-fish", Eupotomus sp. after two to three weeks at 25°C and after six weeks at 14°C.

In oribatid mites, the development of cysticercoids of the cestode, Monococoestus americanus, was accelerated. At 15°C, development was completed in 81 days; at 20°C, 52 days; 25°C, 45 days (Freeman, 1952). In their work with Hymenolepis diminuta, Voge and Turner (1956) noted that larval development could be accomplished between 15°C (15 days required) and 37°C (5 days required) and that temperature below this range was unsatisfactory. Temperature higher than 37°C was lethal for the parasite, though not always for the host. Thus the tolerance span of the parasite and its host do not necessarily coincide.

Dracunculus medinensis, a nematode related to Philonema, undergoes its first moult between the fifth and seventh day and the second moult between the eighth and twelth day after infection of Cyclops in hot weather (90 to 102°F)

the corresponding moults take place respectively between the eighth and twelth day and between the thirteenth and sixteenth day after infection (Moorthy, 1938). Cystopsis acipenseris larvae become invasive 14 - 15 days after infection, in amphipods maintained at 18 to 20°C but only after 22 days at 8°C (Janicki and Rašin, 1929). Wuchereria bancrofti attains third-stage in mosquitoes at 54.4°F at 30 - 43°F development either fails to occur or the larvae die after reaching first-stage (Hu, 1934). Gibson (1965) noticed that third-stage larvae of Microfilaria sp. B could be recovered in 6.5 days if infected black flies were kept at 74°F. On the other hand, at 52 - 68°F, few third-stage were found eleven days after infection.

From the foregoing brief review, one generalization can be made on the effects of temperature on the development of parasites. Within the limits of tolerance, an increase in ambient temperature, under in vivo or in vitro conditions, will accelerate the rate function of parasites. A decrease in temperature will have the opposite effect.

In order to obtain a better picture of the effects of temperature on the development of larval stages under in vivo conditions, it is also necessary to understand the direct effects of temperature on the host itself.

Coker (1933, 1934) revealed that there was an inverse correlation between the size of copepods and the environmental temperature. The average size of female Cyclops vernalis which were maintained at 28 - 30°C was found to be 1.11 mm. In contrast, the value was 1.62 mm for those kept at 8°C. A similar correlation existed in Cyclops serrulatus and Cyclops viridis. But the validity of this investigation is questioned on the basis that (1) no statistical evaluation of data was made, and (2) the samples were not divided into different age groups.

According to Manfredi (1923), the entire developmental cycle of Cyclops bicuspidatus lasted about 1 - 1½ months at 13 - 15°C and about three weeks at 20 - 27°C. Ewers (1936) stated that increasing temperature would speed up the rate of development in most Cyclops until a certain optimum was reached for each species and further increase would slow down development. According to Rylov (1948), the eggs of Cyclops viridis develop in two to three days at 22 - 23°C but at low temperatures during winter, the process will take ten to fifteen days. At an optimum temperature, this species will complete the entire metamorphosis in only three to four weeks; at low temperatures, the process lasts several months.

As early as 1929, working with the cladoceran, Daphnia longispina, Brown demonstrated that the length of generation from the beginning to the first young instar to the end of first adult instar was affected by temperature. At 20°C, the time required is 187 days; at 25°C, the period is 138 days. The Q_{10} for the range is 1.31.

Oxygen consumption of the marine copepod, Calanus sp. is directly proportional to temperature. At 5°C, the oxygen used in ml/1000 copepods/hour is 0.26; at 10°C this value is raised to 0.38; at 15°C, it is 0.61 (Marshall, 1935).

Briefly, the above information reflects that the rate function of small crustaceans, like copepods, is also temperature dependent.

Coming back to Philonema, if the interval of time from infection to moulting (on a 50% sample basis) at one particular temperature is divided by that of a higher temperature (Table IV), we have the following values:- (1) For the first moult, 2.1 (15°C vs. 10°C); 1.6, 1.9 (10°C vs. 4°C); 3.8, 4.1 (15°C vs. 4°C). (2) For the second moult, 1.7, 1.6 (15°C vs. 10°C); 2.9, 2.5 (10°C vs. 4°C); 4.1,4.2 (15°C vs. 4°C).

If ignoring the slight deviations which may be due to the lack of uniformity of sample sizes, these values indicate that an increase of 5°C accelerates the rate of

moulting almost two times; an increase of 11°C shortens the interval required by as much as four times. That is to say, the response to temperature is the same in first and second-stage larvae and the Q_{10} for the rate of moulting for Philonema is 4.

This was confirmed by the experiment of transferring one dish from 10 to 15°C and 4°C after 50% of the larvae recovered were found to be moulting first-stage. The one transferred to 15°C was found undergoing the second moult twelve days after change; the one placed at 4°C, 47 days after change, four times as long.

Normally, Q_{10} values associated with thermochemical reactions range from 2 to 3. Therefore, the Q_{10} value of 4 obtained is relatively high. (But Vernberg and Vernberg (1965) working with the rediae of the trematode, Himasthla quissetensis, demonstrated that the Q_{10} was 9.2 for the metabolic rate of the warm acclimated forms between 12 and 18°C). With such a high Q_{10} value and the facts that the rate functions of parasites and copepods are temperature dependent, it may be justified to argue that the effects of temperature on the rate of development of Philonema in its intermediate host are two-fold, a direct and an indirect effect. Any increase in ambient temperature may influence the

metabolism of the parasite directly. This change in temperature also accelerates the metabolism of the crustacean host. Since many physiological requirements of a parasite are entirely host dependent, an increase in metabolism of the host will indirectly affect the metabolism of a parasite. Thus, the interaction of the direct and indirect effects on the rate functions of the parasite is responsible for producing a high Q_{10} value.

Bauer (1959) concluded that there is a characteristic optimal temperature typical for a given species. Species of the same genus, even parasitizing the same host, are often characterized by different temperature optima. Voge and Turner (1956) found that the rates of development of different cestode larvae varied considerably within the same species of intermediate host when kept at the same temperature. Because the rates of moulting as well as the morphology of trout and salmon strains Philonema larvae are alike, it is tempting to speculate that Philonema from trout and salmon belong to the same species. However, it may not be justifiable to establish such an identity merely based on the evidence of morphological resemblance and the similarity of the pattern of response to temperature between the larval forms.

There is no effect of temperature on the sizes of the larvae. Larvae recovered from 4°C appear to be larger than those from 15°C or 10°C (Figure 16, Table III). But two-ways analysis of variance comparing the lengths of the three different stages from 15°C, 10°C and 4°C gave highly insignificant F values at the 99% confidence interval (Table VI). This is contradictory to some of the observations concerning the effects of temperature on the size of larval stages which are summarized in the previous review. The probable explanation is that, as compared to the temperature ranges of Vogt (1933) and Gibbs and Gibbs (1959) (8 to 22°C; 7.5 to 25°C), the 4 to 15°C range adopted in the present experiment is quite narrow. A wider temperature range may have a more crucial effect on growth thus resulting in a prominent change of size.

Correlation of the rates of development of Philonema in Cyclops under laboratory conditions and the infection of Sockeye Salmon in Cultus Lake.

Finally, applying the information of the rates of development of *Philonema* in *Cyclops bicuspidatus* under laboratory conditions, an attempt is made to elucidate the infection of sockeye salmon in Cultus Lake.

Every year in Cultus Lake, spawning of sockeye salmon usually starts in mid November and lasts till mid December (Foerster, 1925). According to Platzer and Adams (manuscript in press), as the fish spawns female Philonema worms pass out with the eggs and first-stage larvae are released when the females burst in the lake.

Cyclops bicuspidatus, which are concentrated at a depth of 25 to 30 meters (Foerster, 1934) at this time of the year, become infected by ingesting the larvae. The average temperatures at 25 to 30 meters between the beginning of November and the end of December are calculated as 6.7°C and 6.3°C (from Ricker's (1937) temperature data taken between 1934 and 1936). From December onwards, the temperature of the lake gradually drops. In mid January, the average temperature at all depths is 5.8°C. It has been shown that development to infective third-stage under laboratory conditions could be completed one month after infection at 10°C; two and half months at 4°C. Therefore, with the above temperature conditions in the lake, by mid January, two months after infection, there are some larvae ready for infecting young fish.

According to Wolf (1905), Cyclops bicuspidatus is polycyclic i.e. capable of having more than two generations

during the year. Therefore, in mid December when the last batch of salmon arrives to spawn, young adult copepods as well as fourth and fifth copepodid stages are available for infection. Walter (1922) stated that adult Cyclops viridis can live for up to 10 to 14 months. He also assumed that smaller Cyclopidae live from 4 to 6 months while bicyclic species live for 10 to 12 months. Burckhardt (1900) suggested that Cyclopidae live from 8 to 14 months. In the laboratory, some Cyclops bicuspidatus infected with the larvae of Philonema were maintained for more than 8 months at 4°C.

But one question is: can the infected copepods survive the severity of winter when the minima vary from 2.6 to 5.2°C, considering the average of all depths (Ricker, 1937)? Cyclops bicuspidatus is a Palearctic eurythermal copepod which has been collected from lakes as cold as 4°C or as warm as 27°C (Rylov, 1948). Moreover, Roy (1932) reported that these copepods can survive over winter in the form of resting stage by covering itself with a secretion produced by the skin gland. A similar phenomenon was also observed by Birge and Juday (1908) in the bottom layers of many North American lakes. So, it is likely that some infected Cyclops can withstand the cold temperature of winter.

In the light of this information, together with the fact that Philonema larvae have no means of leaving their hosts, it is highly probable that copepodid stages and young adults infected in mid December could survive as long as five to six months till early May. During this time of the year, the fry become free-swimming in the lake. This offers a good opportunity for Philonema to infect a second age class of salmon, the first class, infected in January, having left the lake in April.

SUMMARY

(1) *Philonema* larvae were found to undergo two moults in *Cyclops bicuspidatus*.

(2) Three developmental stages are described. Larvae of trout and salmon strains are morphologically identical. A two-ways analysis of variance gave highly insignificant F values when the sizes of the larval stages of the two strains from copepods maintained at $10^{o}C$ were compared.

(3) The rates of development of *Philonema* larvae were directly proportional to temperature between 4 and $15^{o}C$.

(4) At $10^{o}C$, the rates of development were the same in salmon and trout strains.

(5) Temperature may have direct and indirect effects on the rates of development of *Philonema* larvae in copepods.

(6) Q_{10} for the range 4 to $15^{o}C$ was found to be approximately 4.

(7) Two-ways analysis of variance showed that (a) the extent of growth in the three stages from the three temperatures, $15^{o}C$, $10^{o}C$ and $4^{o}C$ were the same (b) the three stages have the same response (interaction) to temperature as far as growth was concerned.

(8) The experimental data indicate that it is possible for one age class of salmon in Cultus Lake to acquire infections with *Philonema* at two different periods of their lake residence (May after hatching and January to April before migration).

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LIST OF ABBREVIATIONS USED IN TABLES AND FIGURES

A - anus

BC - buccal cavity

DAT - distance from posterior extremity to anus

EC - excretory cell

EP - excretory pore

EXP - distance from cephalic end to excretory pore

GN - ganglionic nuclei

GO - glandular oesophagus

GP - genital primordium

GPR - distance from cephalic end to genital primordium

HC - nuclei of hypodermal cells

INT - intestine

LGO - length of glandular oesophagus

LGP - length of genital primordium

LI - length of intestine

LMO - length of muscular oesophagus

LR - length of rectum

MO - muscular oesophagus

NINT - nucleus of intestine

NR - nerve ring

NRG - the distance from cephalic end to middle of nerve ring

ON - nucleus of oesophagus

OIV - oesophageal-intestinal valve

R - rectum

RG - rectal gland

TL - total length of body

WBA - body width at anus

WBNR - body width at nerve ring

WIw - greatest width of intestine

WIn - width of intestine before joining rectum

WR - width of rectum

TABLE II. Positions of genital primordia of larvae

Stage	Lengths of larvae in μ	Lengths of genital primordia from cephalic end in μ	% of body length from cephalic end
First stage from female worms	416.00	190.65	45.8
	543.9	221.40	40.7
	471.75	217.71	46.1
	527.25	209.10	40.0
	499.50	196.80	49.6
	410.70	215.25	52.4
	527.5	221.55	42.0
	482.85	184.50	39.0
	471.75	196.8	41.7
	527.25	209.0	40.0
First stage from *Cyclops bicuspidatus*	588.3	178.35	30.3
	541.2	228.78	42.3
	549.81	221.40	40.3
	567.03	209.10	36.8
	547.35	196.80	36.0
	535.05	196.80	35.9
	531.2	235.00	44.2
	531.2	209.1	39.4
	553.5	225.1	40.8

TABLE II (Continued)

	721.5	196.8	27.3
	666.0	215.3	32.3
	666.0	320.8	48.2
	565.2	329.1	58.2
	527.3	268.1	50.8
	721.5	432.3	59.9
	527.3	240	45.5
	528.0	264	50.0
	527.5	305.3	57.8
	577.3	297.7	51.5
	699.0	378.0	54.0
	516.2	323.3	62.6
Second	616.1	402.3	65.2
	660.5	364.7	55.2
	538.4	311.7	57.8
	602.7	370.5	61.5
	721.5	274.5	38.1
	666.0	374.9	56.3
	699.3	409.62	58.5
	615.0	405.90	65.9
	723.24	314.9	43.5
	731.85	420.7	57.5
	781.1	343.4	44.0

TABLE II (Continued)

	888.0	570.2	64.2
	921.3	573.7	62.3
	1054.5	645.8	61.1
	915.0	652.7	71.3
	888.0	428.2	48.2
	893.6	639.5	71.5
	1010.1	507.9	50.3
	860.25	502.5	58.4
	959.4	599.0	62.5
	1025.8	648.2	63.2
	915.6	399.6	43.6
	893.5	399.8	44.7
	982.4	445.3	45.3
Third	899.1	388.5	43.2
	971.25	572.4	58.9
	943.5	608.8	64.5
	925.75	573.15	61.9
	838.05	476.0	55.5
	832.5	521.7	62.5
	1004.6	470.2	45.5
	972.5	632.4	64.9
	1043.4	526.5	50.5
	953.3	455.1	47.7
	1019.67	624.8	61.3
	923.73	575.6	62.3
	1014.2	647.1	12.0

TABLE II (Continued

	1113.8	674.3	53.5
	924.35	585.75	63.4
	869.2	439.0	50.5
	959.4	599.0	62.5
Third	1025.8	648.2	63.2
	915.86	399.6	43.6
	893.5	399.8	44.7
	982.4	445.3	45.3
	899.1	388.5	43.2

TABLE III. Proportion of larval stages present at different time intervals.

(a) At 15°C (salmon strain)

Time (days after infection)	1-7	8-14	15-21	22-28	35-63
Total sample size	102	168	201	41	24
Stages	50-M* 1st 52-1st	74-M 1st 25-1st 69-2nd	14-M 1st 4-1st 78-M 2nd 81-2nd 24-3rd	2-M 2nd 11-2nd 28-3rd	24-3rd
% of Stages present	49.02-M 1st 50.98-1st	44.05-M 1st 14.88-1st 41.07-2nd	6.97-M 1st 1.99-1st 38.8-M 2nd 40.29-2nd 11.94-3rd	4.87-M 2nd 26.80-2nd 68.29-3rd	100-3rd

* M - Moulting

TABLE III (Continued)

(b) The number of moulting First-stage larvae recovered 6 days after infection at 15°C (salmon strain)

Date when copepods infected	10/20/65	11/ 6/65
Date when larvae examined	10/26/65	11/12/65
Days after infection when larvae examined	6	6
No. of larvae examined	17	8
Stages present	10-M 1st 7-1st	3-M 1st 5-1st

Total No. of larvae examined = 25

No. of Moulting 1st = 13

% of Moulting 1st = 52

TABLE III (Continued)

(c) The number of moulting second-stage larvae recovered 17-19 days after infection at 15°C (salmon strain)

Date when copepods infected	11/ 6/65	10/20/65	11/12/65	11/ 6/65	10/24/65
Date when larvae examined	11/24/65	11/ 7/65	11/29/65	11/25/65	11/12/65
Days after infection when larvae examined	18	18	17	19	19
No. of larvae examined	13	6	7	19	32
Stages present	11-M 2nd 2-2nd	6-2nd	2-M 2nd 5-2nd	13-M 2nd 6-2nd	14-M 2nd 18-2nd

Total No. of larvae examined = 77

No. of moulting 2nd = 40

% of moulting 2nd = 50.6

TABLE III (Continued)

(d) At 10°C (salmon strain)

Time (days after infection)	1-7	8-14	15-21	22-28	29-35	36-42	43-112
Total sample size	19	102	29	31	133	20	45
Stages	4-M 1st 15-1st	56-M 1st 46-1st	20-M 1st 9-2nd	5-M 1st 16-2nd 1-M 2nd 9-3rd	11-M 1st 3-1st 74-M 2nd 43-2nd 2-3rd	1-M 1st 9-M 2nd 10-3rd	4-2nd 41-3rd
% of Stages present	21.05-M 1st 78.95-1st	54.8-M 1st 45.09-1st	68.97-M 1st 31.03-2nd	16.13-M 1st 3.23-M 2nd 51.61-2nd 29.03-3rd	8.27-M 1st 2.26-1st 55.64-M 2nd 32.33-2nd 1.50-3rd	5-M 1st 45-M 2nd 50-3rd	8.9-2nd 91.1-3rd

TABLE III (Continued)

(e) The number of moulting First-stage larvae recovered 13 days after infection at 10°C (salmon strain)

Date when copepods infected	11/ 6/65	11/12/65
Date when larvae examined	11/19/65	11/25/65
Days after infection when larvae examined	13	13
No. of larvae examined	33	10
Stages present	15-M 1st 18-1st	5-M 1st 5-1st

Total No. of larvae examined = 43

No. of larvae Moulting = 23

% of Moulting Stage = 53.5

TABLE III (Continued)

(f) The number of moulting second-stage recovered 30, 31 days after infection at 10°C (salmon strain)

Date when copepods infected	11/6/65	11/21/65	11/21/65
Date when larvae examined	12/7/65	12/21/65	12/22/65
Days after infection when larvae examined	30	30	31
No. of larvae examined	17	14	32
Stages present	11-M 2nd 6-2nd	6-M 2nd 8-2nd	15-M 2nd 17-2nd

Total No. of larvae examined = 63

No. of larvae moulting = 32

% of larvae moulting = 50.8

TABLE III (Continued)

(g) At 4°C (salmon strain)

Time (days after infection	1-14	15-21	22-28	29-35	36-42	43-49	50-56
Total sample size	18	28	67	26	25	15	26
Stages	All 1st	3-M 1st 25-1st	39-M 1st 28-1st	26-M 1st	25-M 1st	15-M 1st	10-M 1st 16-2nd
% of Stages present	100-1st	10.7-M 1st 89.3-1st	58.3-M 1st 41.8-1st	100-M 1st	100-M 1st	100-M 1st	38.5-M 1st 61.5-2nd

Time (days after infection	57-63	64-70	71-77	78-84	85-91	92-154
Total sample size	22	99	52	10	21	55
Stages	2-M 1st 3-1st 17-2nd	16-M 1st 51-2nd 23.1-M 2nd 9-3rd	3-M 1st 22-2nd 27-M 2nd	10-M 2nd	3-1st 10-M 2nd 8-3rd	55-3rd
% of Stages present	9.1-M 1st 13.6-1st 77.3-2nd	16.6-M 1st 51.5-2nd 23.2-M 2nd 9.09-3rd	5.8-M 1st 42.3-2nd 51.9-M 2nd	100-M 2nd	14.3-1st 47.6-M 2nd 38.1-3rd	100-3rd

TABLE III (Continued)

(h) The number of moulting First-stage recovered 23-25 days after infection at 4°C (salmon strain)

Date when copepods infected	11/ 6/65	11/12/65	11/6/65
Date when larvae examined	11/29/65	12/ 6/65	12/1/65
Days after infection when larvae examined	23	24	25
No. of larvae examined	11	17	14
Stages present	5-M 1st 5-1st	10-M 1st 7-1st	6-M 1st 8-1st

Total No. of larvae examined = 42

No. of larvae moulting = 21

% of larvae moulting = 50

TABLE III (Continued)

(i) The number of moulting second-stage recovered 74, 78 days after infection at 4°C (salmon strain)

Date when copepods infected	11/ 6/65	11/21/65	11/ 6/65
Date when larvae examined	1/19/66	2/ 3/66	1/23/66
Days after infection when larvae examined	74	74	78
No. of larvae examined	10	23	12
Stages present	6-M 2nd 4-2nd	10-M 2nd 13-2nd	6-M 2nd 6-2nd

Total No. of larvae examined = 45

No. of larvae moulting = 22

% of larvae moulting = 49

TABLE III (Continued)

(j) At 10°C (Trout strain)

Time (days after infection)	1-14	15-21	22-28	29-35	36-42	43-49 *
Total sample size	90	103	61	80	111	26
Stages	15-M 1st 75-1st	21-M 1st 6-1st 76-2nd	13-M 1st 31-2nd 16-M 2nd 1-3rd	52-M 2nd 25-2nd 4-3rd	19-M 2nd 4-2nd 88-3rd	3-M 2nd 23-3rd
% of stages present	16.67-M 1st 83.3-1st	20.4-M 1st 5.8-1st 73.7-2nd	21.3-M 1st 50.8-2nd 26.2-M 2nd 2.7-3rd	65-M 2nd 31.3-2nd 5-3rd	17-M 2nd 3.6-2nd 79.3-3rd	11.5-M 2nd 88.5-3rd

* due to the lack of specimen, it was impossible to follow the larval stages up to 112 days after infection as in the case of the salmon strain.

TABLE III (Continued)

(k) The number of moulting First-stage recovered 12,15 days after infection at 10°C (trout strain)

Date when copepods infected	5/29/65	5/29/65
Date when larvae examined	6/11/65	6/14/65
Days after infection when larvae examined	12	12
No. of larvae examined	30	30
Stages present	15-M 1st 15-1st	17-M 1st 13-1st

Total No. of larvae examined = 60

No. of larvae moulting = 32

% of larvae moulting ≅ 53.3

TABLE III (Continued)

(1) The number of moulting second-stage recovered 30-34 days after infection at 10°C (trout strain)

Date when copepods infected	5/29/65	5/29/65	5/29/65	5/29/65
Date when larvae examined	6/29/65	7/ 1/65	7/ 2/65	6/28/65
Days after infection when larvae examined	31	33	34	30
No. of larvae examined	8	9	28	5
Stages present	4-M 1st 4-1st	5-M 1st 4-1st	15-M 1st 13-1st	2-M 1st 3-1st

Total No. of larvae examined = 50

No. of larvae moulting = 26

% of larvae moulting = 52

TABLE V. The sizes of larvae collected at different time intervals

Temperature	Stage	Sample size	Mean length in μ	Range in μ	Time (days after infection)
$15^{\circ}C$	First	12	576.3	555 -593.9	5
		9	580.5	499.5	8 & 9
	Second	7	570.6	492 -666	12
		7	565.5	532.8-582.8	14
		6	650.4	621.6-693.8	16
		7	719.1	721.5-738.2	18
	Third	7	853.9	832.5-904.7	19
		12	967.1	899.1-1061.1	22
		8	975.3	932.4-1032.3	61
	Moulting 1 st	5	436.6	421.4-452.1	5
		7	483.3	402.9-541.2	8
		3	446.3	412.7-547.4	10
	Moulting 2 nd	5	655.8	405.2-743.7	14 & 19

TABLE V (Continued)

Temperature	Stage	Sample size	Mean length in μ	Range in μ	Time (days after infection)
$10^{o}C$		14	542.7	501.9-588.3	7
	First	5	545.0	504.3-574.4	11
		10	555.5	473.6-660.4	16
		8	590.8	544.9-721.5	21 & 23
		7	614.1	575.6-643.9	26
	Second	9	685.2	625.4-754.8	30 & 31
		5	741.7	538.74-809.34	34
		7	913.3	860.3-999.0	34
		6	972.2	943.5-993.5	46
	Third	7	976.9	888 -1037.9	60
		5	1009.0	993.5-1043.4	108
	Moulting 1st	6	439.2	300.1-527.3	13
	Moulting 2nd	5	592.0	333.0-732.6	25
		4	675.0	638.3-704.9	30

TABLE V (Continued)

Temperature	Stage	Sample size	Mean length in μ	Range in μ	Time (days after infection)
$4^{o}C$	First	10	543.4	516.6-608.9	12
		5	522.7	483.4-544.9	16
		4	563.9	537.5-605.2	23
	Second	10	675.9	589.2-788.1	55 & 53
		7	714.6	627.3-794.6	58 & 60
		9	721.3	572.7-794.6	69
	Third	15	896.7	725.7-1021.2	84
		11	886.0	788.1-964.3	87 & 89
		7	1005.7	932.3-1086.1	96
		5	991.7	923.7-1004.9	132
		5	1008.8	959.4-1029.3	160
	Moulting 1st	5	465.2	413.3-595.3	25
		6	403.1	335.8-451.4	39
	Moulting 2nd	4	688.2	602.7-830.3	74
		5	820.6	761.4-915	82 & 81

TABLE VI. Two-ways analysis of variance on the effect of temperature on the growth of larvae (salmon strain).

(a) Sizes of larvae at 15°C.

Stages		
First	Second	Third
593.85	528.90	860.25
611.50	527.25	838.05
582.75	532.80	843.60
582.75	584.25	860.25
555.00	611.50	832.50
555.00	666.00	838.05
582.75	638.25	925.78
555.00	621.60	954.60
582.75	666.00	1004.55
555.00	572.65	960.15
582.75	732.60	960.15
638.25	732.60	899.10
555.00	721.50	899.10
611.50	738.15	1061.05
627.15	721.50	1015.65
499.50	666.00	1026.75
572.65	593.85	987.90
582.75	666.00	1032.30
584.25	560.55	971.25
582.75	721.50	949.05
11592.90	12803.20	16806.18

TABLE VI (Continued)

(b) Sizes of larvae at 10°C

Stage		
First	Second	Third
588.3	551.04	832.50
541.2	547.35	860.25
512.9	544.89	860.25
549.81	552.27	838.05
526.44	575.64	888.00
536.28	578.69	976.80
501.84	643.88	965.70
567.03	666.00	971.25
549.81	611.31	982.35
533.82	621.74	987.90
547.35	691.85	971.25
533.82	651.26	999.00
522.75	809.34	1010.01
585.48	781.05	1026.75
535.05	785.97	1010.10
562.11	538.74	1037.85
531.20	793.50	1010.01
531.20	668.40	999.00
553.50	625.43	915.00
574.41	669.71	949.05
10884.30	12878.06	17043.12

TABLE VI (Continued)

(c) Sizes of larvae at 4°C

Stages		
First	Second	Third
520.29	510.45	882.45
530.13	622.38	865.80
608.85	589.17	876.90
516.60	634.68	854.70
552.29	567.03	876.90
523.98	664.20	870.84
544.89	651.90	940.95
483.39	664.20	970.47
571.95	699.30	953.25
537.51	666.00	908.97
541.04	627.30	964.30
605.16	645.75	976.60
569.49	694.95	918.87
553.50	725.70	1025.82
551.04	768.75	1019.67
516.60	745.58	1029.33
541.20	762.60	1004.91
542.43	794.51	1009.83
530.73	753.99	974.16
544.89	778.59	980.31
10885.94	13567.03	18904.65

TABLE VI (Continued)

(d) Cell sum table

	Stages			
	First	Second	Third	
Temp I_{15}	11592.90	12803.20	16806.18	41202.28
TempII_{10}	10884.30	12878.06	17043.12	40805.48
Temp III_{4}	10885.94	13567.03	18904.65	43357.62
	33363.14	39248.29	52753.95	125365.38

(e) Analysis of variance table

Source of variation	SS	df	MS
Instar SS	3294507.48	2	1647253.74
Temp SS	72894.38	2	36447.19
Interaction SS	93466.1	2x2	23366.52
Error	8517262.8	171	49808.5
Total	11978130.8	179	

$$F_{temp} = \frac{MS_{temp}}{EMS} = \frac{36447.19}{49808.50} = 0.73$$

At 0.01 level, for 2 and α df, tabulated F = 2.35, Therefore, temperature has no significant effect on the extent of growth of the different larval stages.

$$F \text{ interaction} = \frac{MS_I}{EMS} = \frac{23366.52}{49808.50} = 0.47$$

At 0.01 level, for 4 and α df, tabulated F = 1.99 Therefore, the interaction between temperatures and larval stages are highly insignificant i.e. the response to temperature is the same in the different stages.

TABLE VII. Two-ways analysis of variance on the sizes of salmon and trout strains Philonema larva from Cyclops maintained in 10°C.

(a) Sizes of larvae (trout strain) from the copepods maintained in 10°C.

Stages		
First	Second	Third
489.50	602.7	865.00
489.50	666.0	860.25
603.93	693.75	832.50
585.48	699.30	888.00
571.95	615.00	860.25
606.39	688.80	999.00
516.60	567.03	915.75
505.05	569.49	971.25
527.25	560.55	943.50
582.75	607.62	921.30
492.00	754.80	999.00
533.82	721.50	943.50
582.75	701.10	1010.10
555.00	723.24	1054.50
611.50	731.85	1110.00
473.55	738.00	1054.50
582.75	544.89	1054.50
571.95	582.75	949.05
605.16	560.55	943.50
585.48	606.39	888.00
11072.36	12935.31	19063.45

TABLE VII (Continued)

(b) Cell sum table

	Stages			
	First	Second	Third	
Salmon	10884.30	12878.06	17043.12	40805.48
Trout	11072.36	12935.31	19063.45	43071.12
	21956.66	25813.37	36106.57	83876.60

(c) Analysis of variance table

Source of variation	SS	df	MS
Instar SS	2675367.85	2	1337683.9
Location SS	26109.4	1	26109.40
Interaction SS	66900.8	2x1	33450.40
Error	3949525.15	114	34644.95
Total	6727902.34	119	

$$F \text{ location} = \frac{\text{MS location}}{\text{EMS}} = \frac{26109.4}{34644.95} = 0.75$$

At 0.01 level, for 1 and 114 df, tabulated F = 2.76

Therefore, there is no significant difference between the larval sizes of the two strains of Philonema.

$$F \text{ interaction} = \frac{MS_I}{\text{EMS}} = \frac{33450.40}{34644.95} = 0.96$$

At 0.01 level, for 2 and 114 df, tabulated F= 2.36

Therefore, the calculated F value for interaction is highly insignificant.