THE LIFE HISTORY OF *PHILONEMA ONCORHYNCHI* IN SOCKEYE SALMON FROM CULTUS LAKE AND THE MORPHOMETRIC VARIATION OF THE ADULT NEMATODES

by

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Abstract

The life cycle of Philonema oncorhynchi was studied in sockeye salmon, Oncorhynchus nerka, from Cultus Lake, British Columbia. Gravid female worms from the coelom of sockeye spawners burst in lake water releasing living first-stage larvae. These were ingested by Cyclops bicuspidatus and developed to the infective third stage in the haemocoele. Development required 17 days at 12°C or 70 days at 8°C. Each of six hatchery-reared sockeye fingerlings were fed 14-70 copepods infected with third-stage larvae. Fourth-stage larvae were recovered from the peritoneal tissues of four fingerlings when examined four to ten days after infection. The later stages of development were studied by maintaining naturally infected sockeye salmon for two years in freshwater. These had early fourth-stage larvae in the parietal peritoneum and tunica adventitia of the swim bladder when captured as downstream migrants at Cultus Lake. When the fish were 26 months old, late fourth-stage larvae were found in the peritoneal tissues. These moved into the coelom when the fish were 32 months old and moulted to the preadult stage. The comparative morphology of mature worms collected from B.C. salmonids was studied. The type species, Philonema oncorhynchi Kuitunen-Ekbaum, 1933 was obtained from the type host, Oncorhynchus nerka, in the type locality, Vancouver, B.C. Philonema were also obtained from salmonids with a freshwater life cycle in a landlocked area, Kootenay Lake, which was once contiguous with the type locality of Philonema aqubernaculum Simon and Simon, 1936. The morphology was constant for worms found in different hosts and geographical areas. Size was an unreliable characteristic and appeared to be a host-dependent varia-

tion. The type specimens of Philonema agubernaculum Simon and Simon, 1936 were examined and no differences in morphology found.

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INTRODUCTION

Kuitunen-Ekbaum (1933) found that adult sockeye salmon, Oncorhynchus nerka (Walbaum), from English Bay, British Columbia, were heavily infected with specimens of an undescribed nematode which were found free in the body cavity or entwined amongst the pyloric caeca. The nematode had the characteristics of the Camallanata family Dracunculidae but differed sufficiently from the other dracunculoid genera, Dracunculus and Philometra, to justify the erection of a new genus and species, Philonema oncorhynchi Kuitunen-Ekbaum, 1933. Kuitunen-Ekbaum described these infections as harmless to sockeye salmon since no worms were found in the muscles. She found also that mature female worms burst after immersion in sea water and discharged thousands of living larvae. The larvae died after two days in the sea water. The rupture of the female worms was ascribed to the contraction of muscle bands. In the same year, Smedley (1933) described the same species of worm from sockeye salmon collected at Cultus Lake, British Columbia, but she adopted the name given by Kuitunen-Ekbaum when she learned on the eve of publication that the species had already been described and named.

Simon and Simon (1936) described another new species, Philonema agubernaculum, which they found in the body cavity and muscle of the abdominal wall of Prosopium williamsoni (Girard), Salmo shasta (Jordan) and Salvelinus fontinalis (Mitchell) from the waters of Wyoming National Forest. They justified the description of this new species chiefly by its much smaller size and the difference in ratio of anterior to posterior oesophagus. However, unlike P. oncorhynchi, it was noted that pathological changes

of the gonads accompanied infections with P. agubernaculum.

Salvelinus fontinalis from Lake Edward, Quebec, were found to have severe multiple adhesions of the viscera which Richardson (1937) decided were the consequence of a nematode infection. He named this nematode Philonema salvelini, but did not give any description of the specimens beyond the range of total length of several immature encysted worms.

Fujita described a species from Japan, Philonema ochotense, in 1937 (Fukui, 1961). Fujita (1939) described three more species of Philonema from salmonids taken from Kamchatka: Philonema kondai from Oncorhynchus keta; Philonema tenuicauda from Oncorhynchus nerka and O. keta; and Philonema salvelini from Salvelinus leucomaenis. The descriptions contained full measurements of the nematodes which differed in their respective size and in the number of caudal papillae in the male. He was apparently unaware that the trivial name "salvelini" had been used by Richardson (1937) and therefore his P. salvelini was invalid. In 1940 he added a fifth species, Philonema elongata, from the body cavity of Oncorhynchus kawamurae taken in Lake Tazawa, Akita Prefecture, Japan. This species differed from the the others only in size and number of caudal papillae in the male.

Baylis (1948) described several specimens of Philonema which were collected from Salvelinus alpinus subspecies taken at the mouth of the Strindberg River, East Greenland. In most respects the specimens were intermediate between P. oncorhynchi and P. agubernaculum and Baylis identified them as P. oncorhynchi since he considered P. agubernaculum to be identical with P. oncorhynchi despite the generally smaller measurements. These he suggested might be due to the influence of different hosts or degree of maturity. Baylis also found that, even though cephalic papillae had not

been described previously in Philonema, conspicuous submedian papillae were present. He also noted that preanal papillae were present though neither Kuitunen-Ekbaum nor Smedley had mentioned them.

Akhmerov (1955) declared that Philonema agubernaculum Simon and Simon, 1936, Philonema elongata Fujita, 1940, and Coregonema sibirica Bauer, 1946 were synonyms of Philonema oncorhynchi Kuitunen-Ekbaum, 1933. Akhmerov's decision was based on a study of specimens from sockeye and chum salmon, Salvelinus leucomaenis and Arctic grayling, Thymallus arcticus. He did not synonymize the other four species described by Fujita because the descriptions were not available. The dracunculoid genus Coregonema was erected by Bauer in 1946 because it possessed a gubernaculum (Akhmerov, 1955). Akhmerov did not find a gubernaculum in these worms from Arctic grayling and said they were morphologically identical with P. oncorhynchi. However, since the Arctic grayling was a freshwater fish he proposed that the nematodes in their coelom were a subspecies of P. oncorhynchi which he named Philonema oncorhynchi sibirica.

Spasskii and Rakova (1958) agreed with Akhmerov's synonyms but published a further description of the average measurements and morphology of Philonema oncorhynchi.

In 1960, Meyer proposed the binomen Philonema caballeronense, nom. nov., to replace Philonema salvelini Fujita, 1939, since this binomen was preoccupied by its earlier use by Richardson (1937) and hence, as a primary homonym, was unavailable.

Fukui (1961) published a summary of the characteristics of Fujita's specimens of Philonema. He also misinterpreted Akhmerov's synonym and said that Philonema agubernaculum Simon and Simon, 1936, was synonymous with

Philonema elongata Fujita, 1940. Fukui admitted that the worms he examined from salmon were badly damaged and though they were identified as P. oncorhynchi and P. ochotense on the basis of size, he was not certain of their identity.

Table I records the occurrence of Philonema sp. compiled from the literature.

Two attempts at solving the life history of P. agubernaculum have been reported from Maine (Meyer, 1958, 1960; Vik, 1964). Meyer found that freshwater Cyclops sp. ingested the larvae released by the rupture of mature P. agubernaculum. Later, one or more larvae were found to be present in the body cavity of the copepods. Infected copepods were kept alive for a month and then fed to hatchery-reared fingerling Salmo salar. These fish were autopsied several months later but were not infected. Meyer suspected that Osmerus mordax was involved as an intermediate host since it was a forage fish for landlocked Atlantic salmon. He examined a large number using the pepsin-HCl digestion method but the results were negative. Vik (1964) reported that nine Philonema larvae were recovered from 450 O. mordax digested by pepsin-HCl. These were fed to a single hatchery trout from which six small P. aubernaculum specimens were recovered two months later. Vik also fed larvae to Cyclops scutifer but they died before any development took place. He suggests that the copepod is the first intermediate host and the smelt may be a second intermediate host in the life history of P. agubernaculum, even though the infection level is low. He also observed that in several infected Salmo salar and Salvelinus fontinalis, perforations of the body wall were present which might have been the method by which the P. agubernaculum females expelled their larvae. However,

Meyer (1960) suggests that gravid females of P. agubernaculum are passed out with the roe at spawning.

Adult Philonema are found in the coelom but the larval site has not been reported in the literature. Bangham and Adams (1954) reported finding larval nematodes in the swim bladder walls of salmonids during a parasite survey of British Columbia fresh-water fishes. These larvae were called Philonema because of the characteristic oesophagus and tail.* Margolis (1963) also reported finding the same nematode larvae in young sockeye and likewise suggested it was an early stage of P. oncorhynchi.

The foregoing review on the taxonomy and life history of the genus Philonema indicates that a number of aspects need clarification, namely the complete solution of the life history, investigation of host-dependent variation, host specificity, and pathology.

The comparative morphology of adult worms found in British Columbia salmonids and the life cycle of Philonema oncorhynchi in Cultus Lake sockeye salmon are presented in this thesis. The study of the adult morphology was enhanced by the favorable situation in British Columbia because Philonema occurs in a number of anadromous and freshwater salmonids, some of which occur in the same geographical regions. Cultus and Kootenay Lakes were the main collecting areas because of the availability of infected fish. The characteristics that have been used to describe species of Philonema could be evaluated by studying adult worms from different hosts and geographical areas.

The life cycle was elucidated by using two approaches: experiment-

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* Personal communication from Dr. J.R. Adams.

ally infecting young fish and following the development in a group of naturally infected fish. For the former, first-stage larvae of Philonema oncorhynchi were obtained from gravid female worms taken from Cultus Lake sockeye salmon. Wild-caught copepods were infected experimentally with the first-stage larvae and maintained until the larvae developed to the infective third stage. Young hatchery-reared sockeye fingerlings were fed infected copepods. Thus, the route of infection was established for Cultus Lake sockeye salmon. With the second approach, the development of larvae in the swim-bladder peritoneum was followed by maintaining a group of naturally-infected sockeye salmon from Cultus Lake in freshwater and examining the fish periodically.

This thesis is presented in two parts, each treated as a separate entity. Part I contains the work on the life history of Philonema oncorhynchi in Cultus Lake sockeye salmon. Part II contains the comparative study of the adult morphology.

PART I

Life Cycle of *Philonema oncorhynchi* in Cultus Lake Sockeye Salmon

The life history of *Philonema oncorhynchi* in Cultus Lake sockeye salmon, *Oncorhynchus nerka*, was elucidated in the following ways. Infective larvae of *P. oncorhynchi* from spawning sockeye were used to infect copepods, *Cyclops bicuspidatus*, which were obtained from Cultus Lake. This copepod was used because it is the major food source for young sockeye (Foerster, 1925). Mueller's methods (1959) for obtaining, infecting, and maintaining copepods were used with modifications. Young fish were infected by artificial feeding and killed within two weeks to try to determine the route of migration of the larvae. The later development of *P. oncorhynchi* in sockeye was followed for two years by autopsying naturally-infected sockeye from Cultus Lake at intervals. With this approach the relationship of the *P. oncorhynchi* life cycle to the sockeye life cycle was recorded.

Methods and Materials

Collection of P. oncorhynchi Larvae. Fresh larvae were obtained from female sockeye salmon from the Sweltzer Creek fish trap at Cultus Lake, B.C. (operated by the International Salmon Commission). The fish were killed by a blow on the head and brought back to the parasitology laboratory at the Department of Zoology, University of British Columbia. Fish, held in a vertical position with the tail up, were opened by a clean incision from the anus to the pelvic girdle. Each fish was then inverted and the ripe eggs allowed to flow into a pan. The incision was then continued to the posterior base of the pericardial cavity. Female worms were

recovered from amongst the eggs in the pan and from the body cavity of the fish where they were threaded amongst the viscera. They were transferred to Ringer's solution in a Petri dish.

Only gravid females containing moving first-stage larvae were selected for infection of copepods. Five gravid females were transferred to a 1000 ml Erlenmeyer flask containing 500 ml of cold (10°C) dechlorinated tap water. After one minute the worms burst, releasing the larvae. After one hour the remains of the female worms were removed and the flasks stored in a refrigerator set at 8°C.

Collection of Copepods. Plankton was collected in Smith Bay in Cultus Lake with a Wisconsin plankton net, #12 bolting silk screen. The net was towed with a rowboat and all plankton collected was poured into a three-gallon liver tin, about three-fourths filled with lake water. This was transported back to the parasitology laboratory. On arrival the plankton was aerated overnight in a refrigerated room at 10°C.

Infection and Culture of Copepods. The plankton collected from Cultus Lake contained the following crustaceans:

(a) cladocerans

(b) *Epischura nevadensis*

(c) *Cyclops bicuspidatus*

Epischura nevadensis and the cladocerans were present in low numbers and died during the first two days when the plankton was concentrated. *C. bicuspidatus*, the major plankter, was amenable to culture and proved to be a ready intermediate host. A sample of the copepods was dissected before the experiment to ensure that they were uninfected.

The copepods were concentrated by filtering the contents of the liver tin through #12 bolting silk. The copepods were equally divided into three four-inch stacking dishes, each dish containing approximately 1000 copepods. Each dish was inoculated with fresh P. oncorhynchi larvae until there were three larvae to each copepod and placed in the refrigerator for 24 hours. After this time a small sample of copepods was examined.

If 70 per cent of the copepods were infected then the dish was diluted in one of three ways:

(1) The contents of a dish were poured into an eight-inch stacking dish which was then filled with one liter of filtered lake water and kept in the refrigerator.

(2) The contents of a dish were poured into a 16" x 12" x 6" polyethylene wash basin, containing one gallon of filtered lake water, and kept at 12°C on a water table.

(3) The contents of a dish were poured into a three gallon museum jar, filled with filtered lake water, aerated, and kept at 12°C on a water table.

If fewer than 70 per cent of the copepods were infected on the first exposure, the dishes were reinoculated with larvae and left for another 24 hours. This was repeated until the standard was met or until too many copepods had high multiple infections.

The jars were maintained under the above conditions and the copepods were fed with a mixed culture of Paramecium bursaria, P. caudatum and Euglena sp. Samples of the copepods were examined at intervals to follow the development of the larvae.

Infection and Maintenance of Young Fish. Young hatchery-reared *O. nerka* (eight months old) were used as experimental hosts. They were anesthetized with MS 222 (Sandoz) and fed copepods through a polyethylene stomach tube. The fish were kept in a small aquarium for 24 hours and then removed to a large holding aquarium (12°C) with running water. The water in the small aquarium was filtered to recover regurgitated copepods or larvae which might haved passed through the digestive tract in this interval.

The number of infective larvae fed to each fish was calculated in the following manner:

(1) A small number of copepods was examined from the lot to be fed to fish and the mean number of infective larvae per copepod determined.

(2) Each fish was fed an exact number of copepods. Regurgitated copepods were subtracted from the total and the dose of infective larvae found by multiplying the number of copepods ingested by the mean infection per copepod.

Experimental fish were killed at intervals by pithing. The organs and parietal peritoneum were removed and shredded in separate dishes for examination with a dissecting microscope.

Naturally Infected Young Sockeye Salmon. These were obtained from Cultus Lake, April 1962, as downstream migrants. All fish examined at this time were infected with fourth-stage *P. oncorhynchi* larvae. The smolts were maintained in the hatchery at the University of British Columbia and examined at intervals to determine the development of *P. onchrhynchi* larvae.

Fixation, Staining and Measurement of Larvae. Larvae were fixed

overnight in formalin-acetic acid before staining.

The following modification of Goodey's lactophenol-cotton blue stain was used for staining larvae (Franklin and Goodey, 1949):

(1) A drop of 0.0025 per cent cotton blue lactophenol was placed on a clean slide and spread so that it covered one-half the area of the coverslip to be used.

(2) Two glass wool fibers were arranged outside the drop for cover-glass support.

(3) Larvae were transferred from the fixative to the lactophenol-cotton blue with an eyelash glued to an applicator stick.

(4) The slide was heated to 70°C on an electric plate until the larvae were a dark blue color.

(5) The coverslip was applied and sealed with Gurr's Glyceel. The larvae were measured on a Leitz binocular microscope (interpupillary distance set at 66 mm) with an ocular micrometer.

Sections of Larvae In Situ. Infected copepods were fixed in Palades osmic acid, pH 7.4 (Palade, 1952) for one hour at 0°C, then rinsed in distilled water and dehydrated through successive alcohols. They were embedded in Maraglas epoxy resin (Freeman and Spurlock, 1962). Sections were cut at 1.5 μ with a Porter-Blum ultramicrotome and stained with alkaline toluidine blue, 0.1 per cent (Trump, 1961).

Infected swim bladders from sockeye smolts were prepared in the following manner for light microscopy:

(1) The fish were killed by pithing and the swim bladder was exposed by a mid-ventral incision.

(2) The swim bladder was injected with egg albumin from one end and

the gas removed simultaneously by another syringe (#30 needle) from the other end.

(3) After the swim bladder was inflated it was fixed with alcohol-formalin-acetic acid and embedded in paraffin for sectioning.

Results

A. Experimental Infection and Maintenance of *Cyclops bicuspidatus*.

In a longevity test *P. oncorhynchi* larvae released by gravid females remained active in lake water for 17 days at 8°C. Some larvae died the first day after release from female worms but this may have been a result of overcrowding in the stock infection flasks. Larvae remain suspended in water for a long time because their active movements serve to maintain their position.

Shallow stacking dishes ensured high contact between concentrated copepods and larvae. The copepods swam about aimlessly, recoiling on contact with each other or with larvae. On contacting larvae, copepods were observed to ingest them head first. Copepods also ingested dead larvae.

Infected copepods usually contained only one or two larvae in their haemocoele though as many as seven first-stage larvae were found in some experimentally infected copepods. No difference in swimming behaviour was noted between uninfected and infected copepods.

Three methods were used to maintain infected copepods. Maintenance of the copepods in the refrigerator at 8°C was the best method with about 150 to 200 infected copepods surviving in each stacking dish for 150 days. The polyethylene dishpan method yielded 100 copepods per gallon of water after two months at 12°C. The museum jars yielded only 10 copepods per gallon of water after three months at 12°C and were invaded by cladocerans.

In all maintenance methods a mixed culture of Paramecium and Euglena was used as food and the copepods retained the iridescent globules of oil which are present when the copepods are taken from Cultus Lake. Unfed copepods lost the highly coloured oil globules becoming white and translucent. Reproduction may have occurred under the maintenance conditions but propagation did not since no nauplii or copepodids were found after three weeks.

B. Description of the Early Developmental Stages of *P. oncorhynchi* in *C. bicuspidatus*.

Three developmental stages were recognised in the infected copepods. They were designated first-, second-, and third-stage larvae respectively. The first stage in the copepods was found to be the same form that escapes from the female. Two moults in the copepod are therefore indicated.

1. First-stage larva.

The measurements of 28 larvae released from a mature female worm are presented in Table II. Figure 1 shows the characteristic features of the first-stage larva. The oral opening is conspicuous and surrounded by papillae. Amphids are present posterior to the papillae but are inconspicuous. A triangular denticle is situated at the dorsal aspect of the oral opening. The anus is clearly demarcated on the body wall. Phasmids are slightly posterior to the anus and are inconspicuous. The body is attenuated sharply into a very long and filiform tail which tapers into a sharp point and contains numerous nuclei.

The oesophagus is long and dilated, containing a hyaline substance with numerous refractile particles. The nerve ring is pale staining and situated half-way along the undivided oesophagus. A glandular oesophagus

is not present. The excretory pore and cell could not be located. The intestine consists of 18-30 cells with prominent nuclei and it does not appear to be connected to the rectum. The genital primordium consists of four cells which are located mid-way between the termination of the intestine and the anus. The cells of the genital primordium contain nuclei with very large clumps of chromatin. The rectum is a thin-walled tube.

First-stage larvae recovered from the haemocoele of copepods infected for two days were found to have the intestine united to the rectum and the denticle was still present. First-stage larvae moved actively about in the haemocoele of the copepods and were observed to writhe actively when removed from the copepod.

2. Second-stage larva.

The measurements of 10 specimens are recorded in Table II. Twenty-five days after exposure to first-stage larvae, second-stage larvae of *P. oncorhynchi* were recovered from the haemocoele of *C. bicuspidatus* maintained at 8°C. When maintained at 12°C, second-stage larvae were recovered after 17 days.

This stage of *P. oncorhynchi* is characterized by a sheath which is the cast cuticle of the first larval stage. At the anterior end of the exuviae, the triangular denticle and the moulted oesophageal lining of the first larval stage can be seen distinctly (Fig. 2).

Two types of second-stage larvae were found. One was shorter than the first-stage larva and designated as an early second-stage larva, while the second was equivalent in length to the first-stage larva but had a muscular and glandular oesophagus, and therefore was designated as the late second-stage larva.

Early second-stage larva. Six specimens were measured, one from a copepod maintained at 12°C for 17 days and five from copepods maintained at 8°C for 58 days (Table II). The following differences were noticed between this stage and the first-stage larva.

(a) The dorsal denticle is not present.

(b) The intestine is narrow and the nuclei bulge from the intestine's external surface.

(c) The rectum is thick and swollen.

(d) The tail is short and tapers rapidly into a fine sharp point.

(e) A sheath is present.

(f) The larvae do not move actively when in the copepod's haemocoele or when they are removed from the copepod.

(g) The genital primordium could not be located.

Late second-stage larvae. Three specimens were recovered from *C. bicuspidatus* maintained for 17 days at 12°C. The fourth specimen was obtained from a *C. bicuspidatus* maintained at 8°C for 74 days. The differences from the previous stage are:

(a) The glandular oesophagus is present and is thicker and slightly longer than the muscular oesophagus.

(b) There is an increase in total body length.

3. Third-stage larva.

Measurements for 17 third-stage larvae are presented in Table II and this stage is illustrated in Figure 3. Third-stage larvae were found after 70 days in *C. bicuspidatus* maintained at 8°C. In *C. bicuspidatus* maintained at 12°C, the third stage was found after 17 days. The third

stage differs from the previous stage in the following ways:

(a) No sheath is present.

(b) The glandular oesophagus is a very prominent structure in the larvae, occupying one-half the length of the larvae and filling the pseudocoelom with its great width.

(c) The tail has rounded shoulders near the rounded tip.

(d) The larvae move actively in the copepod's haemocoele and also when released.

(e) The rectum is a narrow, thin-walled tube.

(f) A prominent oesophago-intestinal valve is present which protrudes into the lumen of the intestine.

Third-stage larva in situ. Sections showed that the third-stage larvae occupy the haemocoele of *C. bicuspidatus*. The larvae are intertwined with the copepod's organs and also extend into the most posterior abdominal segments. Figures 4 and 5 show sagittal sections of an infected copepod. Cross and tangential longitudinal sections through a larva are present. There is a close association of the larva with the organs of the copepod. Some sections show larvae in close association with large droplets of oil present in the copepod's haemocoele.

C. Experimental Infection of Young Salmon.

1. Results of experimental infection.

Experiment I was started on January 14, 1964, using three sockeye as described in the section on "Methods". Table III shows the details of the number fed and recovered. One fish was autopsied after four days and yielded five fourth-stage larvae from the scrapings of the parietal peritoneum. The larvae were fixed, stained and measured (Table IV). The last two

fish were autopsied after nine days. One was negative while the other contained two fourth-stage larvae which were recovered from the parietal peritoneum.

Experiment II was started on January 27, 1964, using four fish, three sockeye and one coho (Table III). They were maintained overnight as before and removed the following morning. A total of 30 copepods were recovered from the filtered water and all were fed to one of the sockeye on January 29. This fish was maintained overnight as before and marked by clipping the dorsal fin before transferring to the holding tank with the other fish. One sockeye was autopsied 17 hours after feeding to see if larvae could be recovered from the intestinal tissue but the fish was negative. The next fish was autopsied at five days and seven fourth-stage larvae were found in the swim-bladder peritoneum and three in the parietal peritoneum. These larvae were fixed, stained and measured (Table II). The marked sockeye and the coho were autopsied on February 8, 1964, 12 days after infection. Three fourth-stage larvae were found in the parietal peritoneum and 11 in the swim bladder peritoneum of the marked sockeye. One fourth-stage larva was found in the swim bladder peritoneum of the coho. The larvae were fixed, stained and measured except for the larva from the coho which was lost (Table II).

2. <u>Description of fourth-stage larvae from experimentally infected salmon</u>.

Twenty three of the 33 fourth-stage larvae recovered were measured. It was found that the four to five day infections yielded larvae that were much smaller than those from the nine to ten day infections. The differences were expressed in the total body length and in length of the glandular

oesophagus. These lengths in the younger fourth-stage larvae were significantly smaller than the measurements for the third-stage larvae and for the fourth-stage larvae from older infections (Table IV). The measurements of the older larvae were not significantly different from those of the third-stage larvae. The significant reduction in size of the larvae directly after infection of the fish indicated that a moult had probably occurred and on this basis the larvae in the swim bladder were designated fourth-stage larvae. No change in organ structure was observed in the transition from third to fourth larval stages.

Four to five day and nine to ten day larvae occurred both in the parietal peritoneum and tunica adventitia of the swim bladder; however, the older larvae were found predominantly in the tunica adventitia of the swim bladder, whereas the younger larvae were distributed evenly between the two sites (Table III).

D. Description of the Developmental Stages of *P. oncorhynchi* in Naturally Infected *O. nerka* from Cultus Lake.

Yearling downstream migrants were obtained from Cultus Lake in May, 1962. These fish were maintained in fresh water at the Department of Zoology aquarium, University of British Columbia, until March, 1964. According to Foerster (1938) downstream migrants are eleven and one-half months old. These fish were sampled at intervals to determine the developmental course of *P. oncorhynchi* (Table V and VI).

Two developmental stages of *P. oncorhynchi* were found in sockeye maintained in freshwater. The earliest stage, which corresponded to the fourth-stage larva in experimental infections, was found in the swim-bladder peritoneum. This stage was also designated fourth stage. The older stage

was found only in the coelom and designated preadult because maturing gonads were present.

The morphology of the fourth stage is similar to that of the third stage, differing only in the increase in length of the body, tail, muscular and glandular oesophagus, and intestine. The genital primordium could not be found in any of the specimens examined. It was noticed during dissections that the larvae were capable of re-penetrating the tunica adventitia of the swim bladder after being dissected out. They could also move actively through the tunica adventitia of the swim bladder when disturbed by poking with a dissecting needle.

No growth occurred in the fourth-stage larvae sampled during the first six months. Thereafter there was rapid growth so that eight months later large fourth-stage larvae were found. These were designated as late fourth-stage since the sexes could be recognized through the presence of rudimentary female and male gonads. The genital tract in the female was didelphic amphidelphic and the vulva was not completely formed. The uteri and ovaries appeared as a double row of cells. The male genital tract was joined to the ventral wall of the cloaca and consisted of a double row of cells. The morphology of the other body organs remained the same except for their increased length and width. This stage was later found in the coelom along with preadult worms.

Four preadult worms were found during the early part of the study. They did not appear abundantly until the fish were 32 months old or 20 months after the study was initiated. Few females were found. They differed from the late fourth stage in that the vulva was well developed and open, the uteri were large tubes and the ovaries were recognizable. No

moult was observed and this stage was designated preadult on the basis of morphological changes. Preadult males were more numerous. They had spicules, anal papillae, a tridentate telamon, and a well differentiated genital tract.

Several male tails were removed from live preadults, mounted in Ringer's solution and studied under an oil immersion lens. Accurate counts of anal papillae could be made only when the tail was ventral side up. The preanal papillae were sessile with clearly demarcated nerve endings while the postanal papillae protruded from the cuticle and also had distinct nerve endings. The papillae occurred in pairs or singly and their positions were numbered from the posterior extremity. The number of postanal and preanal papillae ranged from 17 to 19 each. One arrangement is shown in Figure 7 and the variability listed in Table VII.

The sockeye which had been maintained in freshwater for 20 months exhibited severe pathology of the coelom associated with the presence of late fourth and preadult stages. Adhesions of the pyloric caeca, liver, stomach, gonads and intestine to the body wall were present in almost every fish regardless of sex. When adhesions were teased apart, preadult males were found encased in fibrous capsules. Figure 8 shows an adhesion of the pyloric caeca to the body wall with which *P. oncorhynchi* preadult males were associated.

During the period of this study, the sockeye grew from an average fork length of 8.5 cm to 21.0 cm. The weight also increased on the average from 8.0 gm to 120 gm.

1. The tissue phase of *Philonema oncorhynchi* in *O. nerka*.

Fourth-stage *P. oncorhynchi* larvae were found primarily in the con-

nective tissue of the tunica adventitia of the swim bladder and the pneumatic duct (Fig. 9). Sections of whole fish revealed that the fourth-stage larvae were also in the loose connective tissue of the parietal peritoneum. The collagenous fibers of the loose connective tissue appeared to allow free movement for the larvae. When inflated swim bladders were removed from infected sockeye smolts, larvae could be seen moving slowly through the tunica adventitia. Larvae that were freed by dissection from the tunica adventitia were capable of re-entering the tissue if they came in contact with it. In tissue sections large clear areas were noted about many larvae. The density of fibroblasts was greatest in the region of the larvae, indicating a tissue reaction to their presence.

Discussion

The observed life history of *Philonema oncorhynchi* is similar to that reported for other dracunculoids which have a cyclopoid intermediate host. *Philometra fujimoti* Furuyama, 1932, encysted in the fins of *Ophiocephalus argus* Cantor was found to utilize five species of *Cyclops* as intermediate hosts, and two weeks were required for development of the infective larvae. *Dracunculus medinensis* (Linnaeus), the human guinea worm, utilizes *Mesocylops leukarti* as an intermediate host and requires 12-18 days to develop to the infective third-stage larva (Moorthy, 1938; Onabamiro, 1956). *Dracunculus ophidensis* Brackett, 1938, is found in *Thamnophis sirtalis*. The infective larvae develop in 15 days in *Cyclops viridis*, the intermediate host. Tadpoles were found to be second intermediate hosts (Brackett, 1938). *Micropleura indiva* Khera, 1951, found in *Lissemys punctata*, used *Cylops* species as an intermediate host and infective larvae are formed in six days (Siddiqi and Jairajpuri, 1963). Meyer (1958, 1960) and Vik (1964) reported

the infection of Cyclops sp. with P. agubernaculum larvae; however, they were unable to complete the life cycle in this fashion. Vik found larvae in pepsin-HCl digests of Osmerus mordax and fed them to young hatchery-raised Salvelinus fontinalis. He recovered preadults after several months and suggested O. mordax was an intermediate host but the very low prevalence may indicate that the larvae he recovered from the smelt was an accidental infection.

P. oncorhynchi differs from other dracunculoids in having a much longer developmental period both in the intermediate cylopoid and in the salmonid hosts. This fundamental difference is probably a result of the adaptation of the life history of P. oncorhynchi to that of the sockeye salmon, O. nerka. In Cultus Lake, sockeye spawn at Lindell Beach in one to six meters of water (Foerster, 1925), beginning usually the first week of November and continuing until mid-December (Foerster, 1929). The eggs hatch during the winter and the fry are found free-swimming in the lake in early May (Foerster, 1938). They remain in the lake for eleven and one-half months. Occasionally they remain two years but rarely zero or three years (Foerster, 1929). The sockeye migrate to sea near the end of April (Foerster, 1938), remaining there two years and then return in the fall of their third year.

Gravid P. oncorhynchi females are passed out into the redd along with the roe and burst after one minute in the lake water. The active first-stage larvae are infective for C. bicuspidatus which is abundant throughout the lake all year (Ricker, 1937). Analysis of temperature data from 1932 to 1936 for the period November first to December 30 from 1932 to 1936 (Ricker, 1937) has shown that the mean temperature for this period

is 7.7°C (average for 0, 10, 20, 30 and 40 meters). Infective third-stage larvae of P. oncorhynchi maintained experimentally at 8°C develop in seventy days. From this it is suggested that infection of sockeye fingerlings takes place during the month of January. Foerster (1925) demonstrated that C. bicuspidatus is the major food supply for young sockeye in Cultus Lake during their lacustrine residence. Probably only the third-stage larva is infective since second-stage larvae were passed by salmon fingerlings in the experiments. It has also been shown that infected C. bicuspidatus can survive at least 150 days at 8°C when fed Paramecium. However, it is not known how long they could survive under the natural conditions of their environment, but since their swimming ability is not affected by the presence of one to seven P. oncorhynchi larvae it is suggestive that their survival is similar to that of uninfected copepods.

The parietal peritoneum and the tunica adventitia of the swim bladder are the sites of the fourth larval stage. The larvae appear most abundantly in the tunica adventitia. The route of migration has not been shown. However, the larvae must migrate into the intestinal walls after the copepod has been broken down by the digestive processes. Four migratory routes are suggested. (1) The larvae may enter the deep visceral lymphatics and proceed to the posterior cardinal veins via the coeliaco-mesenteric and subvertebral lymph trunks. (2) Alternately, the larvae may enter the hepatic portal system and once in the circulatory system, the larvae could be transported to the swim bladder by the coeliaco-mesenteric artery where they could penetrate the vessel walls and enter the tunica adventitia. The parietal peritoneum is supplied by extensions of the segmental vessels from the dorsal aorta. (3) Another route may involve an

active migration through the intestinal wall and into the loose connective tissue of the mesentery. The larvae could disperse throughout the peritoneum in this manner since the loose collagenous tissue would not restrict the migrations of the larvae. It has been shown that the fourth-stage larvae are spread throughout the peritoneum in naturally infected fish though the majority are in the tunica adventitia of the swim bladder. (4) The fourth migratory pattern may involve penetration through the wall of the intestine into the coelom and then penetration into the parietal peritoneum or tunica adventitia.

Observations on naturally infected sockeye from Cultus Lake have shown that the fourth-stage larva does not grow for six months after the fish migrate downstream. At this time the fish is 18 months old. Thereafter growth is rapid and when the fish is 26 months old late fourth-stage larvae are found in the peritoneal tissues with rudimentary gonads. This stage is found in the coelom when the fish is 32 months old and preadults also have appeared in abundance.

The life cycle of P. oncorhynchi is harmonious with that of the Cultus Lake sockeye. Preadult worms begin to appear one year before the sockeye is due to spawn. The mechanism by which the life cycle of the worm is coordinated with that of the sockeye is not known but a relationship to the gonadal growth or maturity of the sockeye is suggested. The sockeye maintained in fresh water during the study grew substantially during the last year and their size was comparable to that reported for kokanee (Vernon, 1957). The development of pathological adhesions in male and female fish appeared to be a tissue reaction to the presence of P. oncorhynchi. This is in contrast to andromous sockeye which show tissue reactions

only in the male fish. Whether the gonadotrophic, gonadal hormones, or increased growth of the fish affects the nematode is not known. Other cases in which maturation of a parasite occurs with the host have been documented by several research workers. Stunkard (1959) found that Polystoma stellae matures at the same time as its host, Hyla. Self (1963) found that gravid Nematobothrium texomensis are only found in gravid buffalo fish, Ictiobius bubalus. Triaenophorus nodulosus in Esox lucius is well known (Miller, 1952) and Hopkins (1959) suggested that the maturation of T. nodulosus may be related to temperature changes. However, Chubb (1963) suggested that other host physiology related to maturation may be involved.

PART II

Morphometric Analysis of *Philonema* from British Columbian Fishes

The number of caudal papillae and the oesophageal and total body lengths have been the main criteria used for taxonomic discrimination of *Philonema* spp. However, the amount of variation has not previously been studied in a number of salmonid hosts. This study investigates the variability of *Philonema* collected from British Columbia salmonids.

The type species, *Philonema oncorhynchi* Kuitunen-Ekbaum, 1933 was obtained from the type host, *Oncorhynchus nerka*, in the type locality, Vancouver, British Columbia. Concurrently, *Philonema* were obtained from salmonids with a freshwater life cycle in a landlocked area, Kootenay Lake, which was once contiguous with the type locality of *Philonema agubernaculum* Simon and Simon, 1936. In this manner, the variation of *Philonema* found in different hosts which occur in the same or different geographical regions could be assessed.

The study of *Philonema* in British Columbia salmonids was augmented by specimens obtained from the Helminthological Collection, United States National Museum (U.S.N.M.). This material included the types of *Philonema agubernaculum* Simon and Simon, 1936 from *Salvelinus fontinalis* and *Prosopium* sp. In this way, the material collected from British Columbia salmonids could be compared directly with specimens collected throughout temperate North America.

Methods and Materials

Fish infected with *Philonema* were collected from the Fraser River system and Kootenay Lake region. The specific localities are listed in

Tables VIII and IX with the dates of capture.

Fish were autopsied in the following way: the fish was opened with a mid-ventral incision from the anus to the base of the pectoral fins, and the coelom examined for roundworms. The pyloric caeca were teased apart since worms were often found intertwined amongst them and the associated fat deposits. The ovaries were also teased apart since worms were occasionally found in this site. The swim bladder was not inspected in mature fish unless worms were not found in the coelom. In this case the swim bladder was dissected out along with its peritoneum and teased apart in saline. The tissues and saline were then examined for larvae.

Philonema females were dipped in 95 per cent ethanol and transferred to 70 per cent ethanol immediately. This procedure was used because female Philonema burst if immersed first in 70 per cent ethanol. Prolonged immersion in 95 per cent ethanol caused shrivelling and loss of the cuticle of the females. Philonema males were placed in Ringer's saline and steaming 70 per cent ethanol poured over them. All specimens were stored in glycerine-alcohol (one part glycerine to 20 parts 70 per cent alcohol) and were cleared by allowing the alcohol to evaporate slowly at room temperature until the specimens were in pure glycerine.

Total lengths of Philonema males were found by projecting glycerine mounts of the worms with a Bausch and Lomb Microprojector. The images were projected onto paper and a pencil line traced along the midline of each worm. A stage micrometer (2.0 mm) was projected in the same manner and its length traced on the paper. A Brunning chartometer was calibrated with the micrometer tracing and was used to measure the lengths of the males. Philonema females were projected with an Omega enlarger (135 mm enlarging

lens). Tracings were made in the same manner as for the males except that a centimeter rule was projected to calibrate the chartometer. All measurements other than total lengths were made with an ocular micrometer in a Leitz binocular microscope at an interpupillary setting of 66 mm.

All head mounts, both male and female, were temporary. The head was cut off as close as possible to the papillae and mounted in glycerine with the coverslip supported by glass rods. The mounts were examined with an oil immersion lens. Male tails were cut off just anterior to the preanal papillae, mounted, and examined in the same way.

Worms taken from salmon were designated as Philonema oncorhynchi in the results whereas worms taken from other hosts were designated as Philonema sp. The reasons will be presented in the discussion.

Results

The analysis of morphological variation in nematodes and other soft-bodied organisms is hindered by the absence of hard parts which would be free from distortion caused by fixation. Therefore, a constant method of fixation was used so that one might assume the degree of variation would remain constant.

The comparative study of adult morphology was based primarily on sexually mature males and gravid female worms collected from spawning fish. If only immature worms were available, then these were used for comparison with sexually mature worms from other fish. Large numbers of Philonema were recovered from kokanee, sockeye salmon, and Kamloops trout so that comparisons could be made between worms from individual fish of the same species and worms from different fish species.

Head papillae were found both in male and female worms. Their ar-

rangement was constant in all worms examined from different hosts.

Caudal papillae were shown to be quite variable in preadult male P. oncorhynchi (Part I). It was found that the papillae could not be examined properly in a lateral view because the tail was too thick in the mature adult. The strong coiling of the male tail resisted all attempts to prepare proper mounts. Counts of caudal papillae were made nevertheless with lateral views and the same degree of variation was encountered when male Philonema from all fish were examined. Therefore, it was assumed that caudal papillae were not a reliable characteristic for differentiation of species.

Philonema infections were not found in the following fish: steelhead trout (Salmo gairdnerii), pink salmon (O. gorbuscha), spring salmon (O. tshawytscha), coho salmon (O. kisutch) (Table VIII), and Yellowstone cutthroat trout (Salmo clarkii lewisii) (Table IX).

Specimens from Oncorhynchus nerka.

Sockeye salmon were sampled from Cultus Lake, the mouth of the Fraser River, and Great Central Lake on Vancouver Island. The prevalence of P. oncorhynchi was 100 per cent, 20 per cent and 100 per cent, respectively (Table VIII).

The worms were usually intertwined amongst the pyloric caeca or lying loose in the coelom of spawning fish (Fig. 10). Live P. oncorhynchi were also found in spawned-out female sockeye which were "water-logged." No host reaction was found in female sockeye except for two encysted female worms in two fish.

In the male sockeye, many adhesions and cysts were present (Fig. 11). When the cysts were opened, P. oncorhynchi were found. The pyloric caeca

were attached to the parietal peritoneum by fibrous tissue. Thin strands of fibrous tissue were also found between the ventral surface of the liver and the parietal peritoneum. Two precocious sockeye males (jacks) were examined and the same situation found.

P. oncorhynchi females taken from female sockeye in the mouth of the Fraser River had eggs in their uteri. Female worms taken from female sockeye entering Cultus Lake had many developing and first-stage larvae in their uteri. Female worms taken from spawning female sockeye in Cultus Lake had only first-stage larvae in their uteri. The sequence of development of the P. oncorhynchi larvae appears to be correlated with the progressive gonadal maturation of the female sockeye.

In contrast to the female sockeye, male and precocious sockeye spawners produced P. oncorhynchi females with only eggs present or with nothing in the uteri.

Male P. oncorhynchi measured from sockeye salmon showed a great variation in the total body and organ lengths amongst individual hosts (Figs. 12-17, Table XI). Examination of the figures reveals that the measurements for male worms from females, males and precocious sockeye form separate groups except for spicule lengths, which are similar. In general, male worms from jacks were the smallest while male worms from normal male sockeye were almost as large as those from female sockeye.

Female P. oncorhynchi also showed considerable variability in size when worms from individual hosts were compared (Figs. 18-20, Table X). The total body lengths of female worms from jacks and male sockeye were smaller than those from female sockeye. Organ lengths were similar in the female worms regardless of the host's sex. Extreme variations are prob-

ably due to the small sample size from each host which was a result of the difficulty in obtaining whole worms when they were entangled amongst the pyloric caecae.

One female worm collected by Rausch from Oncorhynchus nerka in Alaska, U.S.N.M., No. 56177--590A, was studied and measured. Other worms were in the collection but their poor condition did not allow study. The female worm was immature, full of unfertilized eggs and fell into the size range of P. oncorhynchi collected from male sockeye. No differences between this worm and the P. oncorhynchi in the author's collection could be found.

Specimens from Oncorhynchus nerka kennerlyi (Kokanee).

One female kokanee was obtained from Cultus Lake which was three years old and preparing to spawn. On autopsy it was found that the internal organs were adhered firmly to the body wall with fibrous tissue. The organs were dissected away from the body wall and Philonema males were found amongst the adhesions. Dead Philonema females were found encysted on the lateral parietal peritoneum.

Minute Philonema were found coiled on the swim bladder, ovaries and testes, pyloric caecae, and body wall of spawning and spawned-out kokanee examined from the Kootenay Lake region (Table IX). Occasionally they were found encysted in egg membranes. Adhesions were found only in one female and one male kokanee.

Philonema found were in either of two stages, late fourth-stage larva or preadult with the exception of two maturing female worms found in an immature female kokanee. No differences were noted in infections of male and female kokanee.

Late fourth-stage larvae recovered from kokanee were longer than those from anadromous sockeye maintained in freshwater but the organ lengths were similar except for the glandular oesophagus length (Figs. 22-26, Table XII).

Male preadult Philonema from kokanee were smaller than the preadults from sockeye salmon but the relative proportions appeared to be the same (Figs. 22-26, Table XII).

Female preadult Philonema were also much smaller than preadults from sockeye salmon except for tail length (Figs. 22-26, Table XII). The maturing Philonema females contained developing embryos and they were similar in size to those recovered from sockeye males (Figs. 18-21, Table XII).

Specimens from Oncorhynchus keta.

Four chum salmon spawners were captured in Cultus Lake (Table VIII). Only five male P. oncorhynchi were found in the male chum and no host reaction was seen. The two infected chum females had only encysted dead P. oncorhynchi females on the pyloric caeca and ovaries and the worms were calcified in some cases. One female had extensive adhesions of the liver and stomach which were attached to the ventral parietal peritoneum.

The measurements of the male worms from the male chum were similar to those of the male P. oncorhynchi recovered from female sockeye (Figs. 12-17, Table XI). No measurements were made on the calcified female worms since the structural features were obliterated.

Specimens from Salmo gairdnerii.

The prevalence of Philonema in Kamloops trout from Kootenay Lake

was low (Table IX). Immature trout had few infections and made up the major part of the sample (35 out of 46 fish). All spawning trout were infected. Adhesions were present only in two infected female trout.

One larval Philonema was recovered from the swim bladder of an immature trout at Gerrard (Table IX).

Male Philonema from female trout were similar to male P. oncorhynchi from jack sockeye with respect to all measurements made (Figs. 12-17, Table XIII). The nerve ring distance was larger and the spicule length was shorter but considerable overlap of the ranges occurred.

Female Philonema from female trout were equivalent to female P. oncorhynchi from male sockeye with respect to total body length but the organ lengths were generally smaller though considerable overlap of ranges occurred with all measurements in sockeye salmon (Figs. 18-21, Table XIII).

Shaw (1947) deposited Philonema oncorhynchi from Oregon steelhead trout in the U.S.N.M., No. 40004--3273A. These were obtained and the measurements of two males taken (Table XIII). The morphology and length measurements correspond with those from Kamloops trout. The head papillae were present and the number of caudal papillae was variable as in other Philonema examined.

Specimens from Salmo salar.

Philonema agubernaculum collected by Dr. Meyer from landlocked Atlantic salmon, Lake Sebago, Maine were obtained from the U.S.N.M., No. 46160--4225D. No males were found in the collection.

Eight females were complete. Only one contained first-stage larvae while the rest contained eggs or had empty uteri. The females were smaller

in total body and glandular oesophagus length than female P. oncorhynchi from jack sockeye but the nerve ring distance and muscular oesophagus lengths were similar (Table XIII).

Specimens from Salvelinus malma.

Only one infected Dolly Varden was obtained from Cultus Lake (Table VIII). The infected female was immature and adhesions of the liver to the parietal peritoneum were present.

The prevalence of Philonema infections in Kootenay Lake Dolly Varden was variable (Table IX). Mature worms were found in a spawning male which had adhesions and cysts containing only female Philonema. Dolly Varden in the main lake had few infections whereas more infections were found in fish taken from Meadow Creek. Several males and females had adhesions and cysts as described above.

The male Philonema from the Cultus Lake infection were similar in size to male P. oncorhynchi from jack sockeye (Figs. 12-17, Table XIV). The spicule lengths differed but were equivalent to those found in male Philonema from Kamloops trout. Male Philonema from Dolly Varden in Meadow Creek were classified as preadults since no fertile worms were found. The measurements agreed with those of P. oncorhynchi preadults from sockeye (Figs. 22-26). Fourth-stage larvae were also found in these fish (Meadow Creek) and corresponded to the late fourth-stage larvae recovered from the coelom of sockeye salmon (Figs. 22-26).

The female Philonema found in the Cultus Lake Dolly Varden was smaller than any P. oncorhynchi recovered from spawning sockeye (Figs. 18-21, Table XIV). The adult female Philonema from the Cooper Creek Dolly Varden were equivalent to those from jack sockeye (Figs. 18-21). Preadult

females were found in the Meadow Creek Dolly Varden and were equivalent to the preadults recovered from Kootenay Lake kokanee (Figs. 22-26, Table XIV).

Specimens from *Salveninus fontinalis*.

Type specimens of *Philonema agubernaculum* were deposited in the U.S.N.M., No. 8908--M373A, by Simon and Simon (1936). One complete male and the anterior portion of another male were present along with one complete and one headless female.

The male measurements corresponded to those of *P. oncorhynchi* from male sockeye and Kamloops trout (Table XIII). Contrary to the description of *Philonema agubernaculum* males by Simon and Simon (1937), head papillae were present and also pre- and post-anal papillae.

The females contained larvae which were not completely matured. Except for total body length, the females were smaller than both *Philonema oncorhynchi* in sockeye and *Philonema* in Kamloops trout (Table XIV).

Specimens from *Prosopium williamsonii*.

Only three uninfected whitefish were obtained from Cultus Lake (Table VIII).

A large number of whitefish were examined from Gerrard (Table IX). All the infected fish were approaching maturity. Abdominal adhesions were present in four whitefish but were not associated with the *Philonema* infection.

The measurements of the male worms were variable but good comparisons were made with male worms from Kamloops trout and jack sockeye salmon (Figs. 17-17, Table XV). As in male worms collected from other species of salmonids, the spicule length was slightly variable (Fig. 17).

The total body length of female worms was comparable to that of female worms from male sockeye; however, the nerve ring distance and muscular oesophagus length were similar to those for female worms from Kamloops trout and jack sockeye (Figs. 18-21, Table XV). The glandular oesophagus length was shorter than that for other fertile females (Fig. 21).

Philonema aqubernaculum collected by Simon and Simon, 1936 from Prosopium sp. Montana, was obtained from the U.S.N.M., No. 38004--M590E. One complete female was present and the length measurements were comparable to those of female worms from jack sockeye (Table XV).

Discussion

Eight species of Philonema have been described on the basis of total body length and ratio of muscular to glandular oesophagus in both the male and female worms. The number of caudal papillae in the male worm was also used. Baylis (1948) was first to suggest that the species were morphologically identical and that the size differences were probably due to host differences. Akhmerov (1955) made Philonema aqubernaculum Simon and Simon, 1936 and Philonema elongata Fujita, 1940 synonyms of Philonema oncorhynchi Kuitunen-Ekbaum, 1933. In his survey of parasites of Kamchatka fishes it was found that size was an unreliable characteristic and that the morphology was constant for worms found in different hosts. This study supports Akhmerov's conclusions with respect to the morphological variation in Philonema oncorhynchi.

Analysis of the morphological variation in P. oncorhynchi has brought to light some interesting characteristics, e.g. the extreme variation in size of worms from male and female sockeye salmon. The female worms from male sockeye salmon were much smaller than those from female

fish and characteristically did not contain embryos. The infertility of the female worms in male sockeye salmon is due either to the fact that the males are sterile or that the numerous eggs of the female are incapable of development. This may be a result of the environment produced by the male sockeye salmon. The infertility of P. oncorhynchi in male sockeye salmon is probably correlated with the fact that the worms in the coelom of the male cannot be released during spawning as are fertile worms in the coelom of female fish. In the latter, the ovarian membranes disintegrate, releasing the roe into the coelom from whence it is expelled by body contractions through the female urogenital pore. In the male fish the testes remain intact and the milt is expelled via the gonoducts. It is evident that P. oncorhynchi infections in male sockeye have reached a dead-end in their life cycle.

The wide variation in length measurements made on P. oncorhynchi from the type host, O. nerka, were found to overlap and encompass most measurements on Philonema taken from salmonids in two geographically separated areas, Cultus and Kootenay Lakes. Adult worms taken from whitefish, Dolly Varden and Kamloops trout were generally smaller than those from sockeye but comparisons of total body length, distance to nerve ring, muscular and glandular oesophagus, tail and spicule length revealed the tremendous variability of these morphometric aspects in the genus Philonema. Examination of the type specimens for Philonema agubernaculum Simon and Simon (1936) revealed no detectable morphological differences or morphometric characteristics which can be used to set the specimens apart from Philonema oncorhynchi. Simon and Simon (1936) described P. agubernaculum on the basis that the worms they collected from Rocky Mountain whitefish,

Eastern Brook and rainbow trout were much smaller than P. oncorhynchi and that the ratio between the anterior and posterior parts of the oesophagus was 1.0:2.7 whereas the ratio in P. oncorhynchi was 1.0:1.1. Examination of oesophageal ratios in specimens in the present collection showed extreme variability. Many authors hesitate to use ratios of this or any body part as a criterion of species differentiation in nematodes (Barraclough and Blackith, 1962). The difference in final size of adult Philonema in the various salmonids is most likely a reflection of suitability of the environment of the parasite. It has been shown in this paper that there is considerable difference in size between worms recovered from male and female fish of the same species. Westbrook and Scott (1955) found that Litomosoides carini grew to different lengths in male and female cotton rats. The effect of host hormones appears to be the causative agent since they would probably be the primary effectors of a difference in environment in fishes of different sex. It is reasonable to assume that, in the various salmonids that Philonema infects, a difference in hormones and body chemistry exists and the extent of this is reflected in the growth of Philonema in their body cavities. From this point of view, the sockeye is the host in which Philonema attains its largest possible size, whereas, growth in other salmonids ceases at a lower level in the growth curve of Philonema. This interpretation is based on the assumption that length of infection has no bearing on the size of the parasite since worms in jack sockeye are comparable to those from male sockeye one year older. This assumption leads to the hypothesis that maturation of Philonema is correlated with host maturation and consequently this is the factor which determines that all Philonema are physiologically alike since mature worms were found only in salmonids

which were in spawning condition.

In the sockeye salmon, host tissue reactions to P. oncorhynchi were found mainly in the male fish. This fact may be related to the fact that the male host is a "dead-end" for the parasite. Alternately or at the same time the hormonal differences may cause an immune response to the presence of the worms in the male's coelom. The tissue reaction in the male but not the female sockeye salmon may be related to the difference in blood cortisol and cortisone levels in the salmon, the female having double the concentration found in males (Idler, 1959). Cross (1963) has shown that immune responses to Nematospiroides dubius in rats is prevented by the administration of cortisone.

Fibrosis in the form of extensive abdominal adhesions were found in other salmonids, mainly Dolly Varden. The pathology was associated with the presence of Philonema in most cases though adhesions were found in a few fish which were uninfected. This may have been caused in these cases by Diphyllobothrium infections. MacLulick (1942) has reported the pathology of Philonema sp. in lake trout, Cristovomer namaycush, while Meyer (1960) and Vik (1964) have found massive adhesions in brook trout and landlocked Atlantic salmon from Maine. The pathology of Philonema in these salmonids may be related to their different life histories, e.g. they do not die after spawning as do Pacific salmon.

Vernon (1957) demonstrated that three distinct populations of kokanee were present in Kootenay Lake. Two populations spawned when three years old while the third spawned at four years. Kokanee were sampled from all populations in this study and mature Philonema were not found. The worms may fail to mature because some stimulus present in anadromous

sockeye is missing in the freshwater form, kokanee. The lack of an appropriate stimulus may prevent the larvae from growing to maturity. The infection of the kokanee must come from the inoculum introduced into their feeding area by spawning trout.

SUMMARY

The life cycle of Philonema oncorhynchi was found to be similar to other dracunculoid life cycles. Sockeye salmon release gravid female P. oncorhynchi into the water with the roe. The female worms burst, releasing larvae which are ingested by the intermediate host, Cyclops bicuspidatus. After two moults in the copepods haemocoele, the larvae are infective to young sockeye salmon. The larvae migrate to the tunica adventitia of the swim bladder, undergoing a third moult during the migration. In naturally infected sockeye salmon, the larvae move to the coelom when the fish are 26 months old. Here the larvae moult and preadults are formed.

The adult morphology of Philonema found in British Columbia salmonids was compared. The variability of the type species, Philonema oncorhynchi from the type host, Oncorhynchus nerka, encompasses most of the variability found in Philonema from other hosts. Examination of the types of Philonema agubernaculum reveals no morphological differences. The differences in size are attributed to host-dependent variation.

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List of Abbreviations Used in Tables and Figures

F - female

FLF - fork length of fish

GP - distance to the genital primordium from the anterior end

L - length of worm

LGO - length of glandular oesophagus

LGp - length of the genital primordium

LI - length of intestine

LMO - length of muscular oesophagus

LSh - length of sheath

LTe - length of testis

Lv - larva

M - male

No. - sample number

NR - distance to the nerve ring from the anterior end

PM - precocious male (jack)

S* - length of spicule

SF - sex of fish

SW - sex of worm

T - distance from the anus to the tip of the tail

V - distance from the anterior end to the vulva

W - number of worms measured

WBA - body width at the anus

WBNR - body width at the nerve ring

WBOV - body width at the oesophago-intestinal valve

WCal - width of calomus

WCap - width of capitulum

WGO - width of glandular oesophagus

WI - width of intestine

WMO - width of muscular oesophagus

* In Figs. 22-26, "S" indicates that the fourth-stage larvae are from sockeye salmon in Table VI.

Table I. Records of *Philonema* spp., compiled from the literature.

Author	Date	Species	Host	Locality
Kuitunen-Ekbaum	1933	*Philonema oncorhynchi*	*Oncorhynchus nerka*	British Columbia
Smedley	1933	*P. oncorhynchi*	*O. nerka*	" "
Simon and Simon	1936	*P. agubernaculum*	*Salvelinus fontinalis*, *Salmo shasta*, *Prosopium williamsonii*	Wyoming
Richardson	1937	*P. salvelini*	*S. fontinalis*	Quebec
Fujita *	1937	*P. ochotense*	--	Japan
"	1939	*P. kondai*	*Oncorhynchus keta*	Sea of Okhotsk
"	1939	*P. tenuicauda*	*O. nerka*, *O. keta*	" "
"	1939	*P. salvelini*	*Salvelinus leucomaenis*	" "
"	1940	*P. elongata*	*Oncorhynchus kawamurae*	Japan
MacLulich	1942	*Philonema* sp.	*Cristovomer namaycush*	Ontario
Bauer **	1946	*Coregonema sibirica*	*Thymallus arcticus*	Kamchatka
Shaw	1947	*P. oncorhynchi*	*Salmo gairdnerii*	Oregon
Baylis	1948	*P. oncorhynchi*	*Salvelinus alpinus*	Greenland
Munroe	1949	*Philonema* sp.	*S. fontinalis*	Labrador

continued -

Table I, cont'd.

Author	Date	Species	Host	Locality
Bangham	1951	P. agubernaculum	Salvelinus namaycush, Salmo trutta, P. williamsonii	Wyoming
Haderlie	1953	P. oncorhynchi	S. gairdnerii	California
Bangham and Adams	1954	P. oncorhynchi	Salmo clarkii, S. gairdnerii, Salvelinus malma, O. nerka, Oncorhynchus kisutch	British Columbia
" "	1954	Philonema sp.	Prosopium cylindraceum, P. williamsonii, Acrocheilus alutaceus	" "
Meyer	1954	P. agubernaculum	S. fontinalis, Salmo salar	Maine
Akhmerov	1955	P. oncorhynchi	O. nerka, O. keta, S. leucomaenis	Kamchatka
Dombroski	1955	P. oncorhynchi	O. nerka	British Columbia
Spasskii and Rakova	1958	P. oncorhynchi	O. nerka	Kamchatka
Spasskii and Roitman	1959	P. oncorhynchi	Thymallus thymallus, T. arcticus	Kamchatka
Fukui	1961	P. oncorhynchi	--	Japan
"	1961	P. ochotense	--	"
Margolis	1963	P. oncorhynchi	O. nerka	British Columbia, Alaska

continued -

Table 1, cont'd.

Author	Date	Species	Host	Locality
Vik	1964	*P. agubernaculum*	*S. fontinalis*, *S. salar*	Maine

* Fukui, 1961.

** Akhmerov, 1955.

Table II. Measurements of Philonema oncorhynchi larvae from experimental infections. Measurements are in microns.

Stage	First (28)	Second		Third (17)	Fourth	
		Early (6)	Late (4)		Young (11)*	Older (12)**
L	609 (559-656)	502 (376-564)	615 (552-684)	961 (901-1020)	896 (841-1003)	975 (909-1066)
NR	69 (63-80)	70 (57-83)	64 (54-68)	100 (88-105)	91 (74-103)	91 (91-108)
LMO	135 (114-148)	169 (148-185)	133 (114-171)	213 (137-242)	203 (160-237)	205 (165-228)
WMO	7 (5-14)	10 (7-11)	7 (5-9)	6 (4-8)	4 (3-6)	3 (3-4)
LGO	-	-	148 (125-180)	436 (314-547)	386 (308-456)	454 (388-570)
WGO	-	-	12 (10-13)	10 (8-13)	8 (7-10)	10 (9-12)
WBNR	22 (20-29)	25 (23-26)	23 (22-25)	15 (14-17)	14 (13-21)	16 (14-17)
WBOV	-	27 (23-30)	23 (20-24)	15 (13-16)	14 (13-14)	16 (14-18)
WBA	18 (15-23)	20 (17-22)	17 (13-19)	13 (11-14)	12 (11-13)	14 (12-15)

continued -

Table II, cont'd.

Stage	First (28)	Second		Third (17)	Fourth	
		Early (6)	Late (4)		Young (11)*	Older (12)**
LI	113 (92-131)	212 (120-257)	221 (140-285)	207 (171-322)	202 (131-305)	212 (171-228)
WI	15 (10-23)	10 (7-14)	7 (6-9)	6 (3-9)	6 (3-6)	6 (3-7)
T	282 (245-311)	120 (103-137)	113 (98-128)	109 (88-128)	107 (83-120)	105 (74-125)
LSh	-	668 (599-721)	732	-	-	-
GP	269 (237-309)	-	-	-	-	-
LGP	37 (14-48)	-	-	-	-	-

* Young larvae are from 4-5 day old infections.

** Older larvae are from 9-10 day old infections.

Table III. Infection of hatchery-reared sockeye salmon with *Philonema oncorhynchi*.

	No. of copepods ingested	Estimated no. of third-stage larvae	Duration of infection (days)	Location			Larvae recovered (per cent)
				Parietal peritoneum	Swim bladder wall	Kidney	
Experiment A							
Fish 1.	17	37	4	5	-	-	13.5
Fish 2.	17	37	9	-	-	-	0
Fish 3.	14	31	9	2	-	-	6.5
Experiment B							
Fish 1.	40	68	0.75	-	-	-	0
Fish 2.	40	68	5	3	7	1	16.2
Fish 3.	70	91	10	3	11	-	15.4
Fish 4.*	25	32	10	-	1	-	3.1

* Fish 4 was a coho, others are hatchery-reared sockeye.

Table IV. Size differences between third, recently moulted 4th and 4th stage larvae.

	Larvae	Total Length	Glandular Oesophagus Length
A.	3rd stage from haemocoele of C. bicuspidatus	N = 17 $\bar{x}$ = 961.4μ $S_{\bar{x}}^2$ = 59.3	N = 17 $\bar{x}$ = 435.7μ $S_{\bar{x}}^2$ = 174.7
B.	Recently moulted 4th stage larvae from experimentally infected O. nerka (4-5 day infection)	N = 11 $\bar{x}$ = 897.7 $S_{\bar{x}}^2$ = 386.6	N = 11 $\bar{x}$ = 386.3 $S_{\bar{x}}^2$ = 120.8
C.	4th stage larvae from 9-10 day infection	N = 12 $\bar{x}$ = 975.3 $S_{\bar{x}}^2$ = 149.6	N = 12 $\bar{x}$ = 453.9 $S_{\bar{x}}^2$ = 171.1
	A compared with B	t = 3.44**	t = 2.64*
	A compared with C	t = 1.01 n.s.	t = 0.95 n.s.
	B compared with C	t = 3.43**	t = 3.91**

Weighed "t" test used.
n.s. - not significant.
** - significant at 0.01 level.
* - significant at 0.05 level.

Table V. Developmental stages of *P. oncorhynchi* found in naturally infected Cultus Lake sockeye maintained in freshwater for two years.

Date fish were examined	Age of fish	Hypothetical age of infection	Number of fish examined	4th stage Larvae present		Preadult stages present	
				Early	Late	F	M
May, 1962	12	4	10	+	-	-	-
June	13	5	4	+	-	-	-
July	14	6	4	+	-	-	-
August	15	7	10	+	-	-	-
September	16	8	3	+	-	-	-
October	17	9	17	+	-	-	+*
November	18	10	4	+	-	-	-
February, 1963	21	13	5	+	-	-	-
March	22	14	7	+	-	-	+*
July	26	18	6	-	+	+*	
August	27	19	6	-	+	+*	-
January, 1964	32	24	7	-	+	-	+
February	33	25	2	-	+	-	+
March	34	26	6	-	+	-	+

* Only one found.

Table VI. Measurements of *Philonema oncorhynchi* from Cultus Lake sockeye salmon maintained in freshwater.

Stage	Fourth			Preadults	
	Early (20)	Late			
		F (8)	M (9)	F (2)	M (10)
L	1.07 (.96-1.20)	4.80 (3.85-6.33)	3.90 (3.63-4.44)	42 (26-59)	18 (11-23)
NR	99 (88-105)	151 (141-203)	153 (135-185)	375 (344-406)	233 (185-264)
LMO	223 (205-262)	349 (308-431)	313 (283-338)	748 (725-770)	450 (264-517)
WMO	5 (5-6)	15 (10-21)	12 (10-12)	62 (43-80)	39 (25-48)
LGO	485 (419-581)	995 (830-1384)	923 (725-1058)	1500	1286 (722-1637)
WGO	14 (10-16)	45 (31-71)	41 (37-49)	164 (155-172)	121 (61-205)
WBNR	17 (16-20)	54 (46-84)	46 (38-60)	333 (222-444)	156 (111-205)
WBOV	18 (15-19)	55 (47-80)	52 (48-63)	347 (222-472)	195 (105-250)

continued -

Table VI, cont'd.

Stage	Fourth			Preadults	
	Early (20)	Late			
		F (8)	M (9)	F (2)	M (10)
WBA	14 (11-15)	33 (31-37)	40 (33-44)	145 (105-185)	133 (50-222)
V	-	1.93 (1.36-2.87)	-	12.1 (5.5-18.7)	-
LTe	-	-	1.65 (1.28-2.05)	-	-
S	-	-	-	-	350 (314-387)
WCap	-	-	-	-	14 (12-17)
WCal	-	-	-	-	7 (6-7)
T	121 (91-134)	245 (227-258)	259 (221-283)	660 (515-805)	394 (332-455)

Total length, distance to vulva, testis length are given in millimeters.
Other measurements in microns.

Table VII. Anal papillae of male preadult *Philonema oncorhynchi*.

Position	Variability
Postanal	
1	Always median and single.
2	Median and single, or closely paired.
3	" " " " " "
4	Median and single; paired either close or wide.
5	Always paired, either close or wide.
6	Median and single; paired either close or wide.
7	Always paired either wide or partially fused.
8	Always median and single.
9	" " " "
10	Always paired, either separated or partially fused.
11	Always paired and separate.
Preanal	
12	Always paired and separate.
13	" " " "
14	May be paired, separate or partially fused or right member absent.
15	Paired, right member may be absent.
16	" " " " " "
17	Paired, either right or left member may be absent.
18	" " " " " " " " "
19	Paired, right member may be absent.
20	Paired, left member may be absent.
21	" " " " " "
22	Paired or completely absent.

Table VIIJ. Prevalence of *Philonema oncorhynchi* in the Fraser River System and Vancouver Island.

Host	Date	Locality	# of fish examined*	# of fish infected*	Prevalence
Oncorhynchus nerka	Aug., 1962	Steveston	55 (26,29)	11 (6,5)	20%
" "	Nov., 1962	Cultus Lake	11 (9,2)	11 (9,2)	100%
" "	Nov., 1963	Cultus Lake	11 (5,6)	11 (5,6)	100%
" "	Aug., 1963	Great Central Lake	2 F	2 F	100%
Kokanee	June, 1963	Cultus Lake	1 F	1 F	100%
Oncorhynchus keta	Dec., 1962	" "	1 M	1 M	100%
" "	Nov., 1963	" "	3 F	2 F	66%
Oncorhynchus gorbuscha	Nov., 1963	" "	1 F	0	0
Oncorhynchus kisutch	Nov., 1963	" "	1 F	0	0
Oncorhynchus tshawytscha	Nov., 1963	Steveston	1 F	0	0
Salmo gairdnerii	Nov., 1963	Steveston	10 (4,6)	0	0
Salvelinus malma	June, 1962	Cultus Lake	3 (2,1)	1 F	33%
" "	June, 1963	" "	1 M	0	0

continued -

Table VIII, cont'd.

Host	Date	Locality	# of fish examined*	# of fish infected*	Prevalence
Prosopium williamsonii	June, 1962	Cultus Lake	2 F	0	0
" "	June, 1963	" "	1 F	0	0

* The first numbers in parentheses are female fish and the second are male fish.

Table IX. Prevalence of *Philonema* in the Columbia River System.

Host	Date	Locality	#of fish examined*	# of fish infected*	Prevalence
Kokanee	Sept., 1962	Meadow Creek	90 (41,49)	58 (29,29)	65%
"	Sept., 1963	" "	10 (5,5)	8 (5,3)	80%
"	Sept., 1962	Redfish Creek	15 (7,8)	4 (2,2)	27%
"	May, 1963	Kootenay Lake	12 (10,2)	1 F	8%
"	May, 1963	Duncan Lake	22 (12,10)	0	0
"	Sept., 1963	Kokanee Creek	6 (3,3)	3 (2,1)	50%
"	Sept., 1963	Goat Creek	9 (4,5)	9 (4,5)	100%
"	Sept., 1963	Lardeau River	10 (4,6)	6 (1,5)	60%
"	Sept., 1963	Pass Creek	1 M	1 M	100%
Salmo gairdnerii	May, 1963	Kootenay Lake	46 (26,20)	13 (10,3)	28%
" "	Sept., 1963	Gerrard	1 M	1 M	100%
Salmo clarkii	May, 1963	Kiakho Lake	13 (10,3)	0	0
Salvelinus malma	Sept., 1962	Cooper Creek	1 M	1 M	100%

continued -

Table IX, cont'd.

Host	Date	Locality	# of fish examined*	# of fish infected*	Prevalence
Salvelinus malma	May, 1963	Kootenay Lake	49 (30,19)	1 M	2%
" "	Sept., 1963	Meadow Creek	7 (5,2)	4 (2,2)	18%
Prosopium williamsonii	Sept., 1962	Lardeau River	1 F	0	0
" "	May, 1963	Gerrard	40 (22,18)	8 (4,4)	20%
" "	Sept., 1963	Meadow Creek	19 (11,8)	0	0

* The first numbers in parentheses are female fish and the second are male fish.

Table X. Female *P. oncorhynchi* from *O. nerka*.

No.	SF	FLF (mm)	W	L (mm)	NR (μ)	LMO (μ)	LGO (μ)
1	F	-	6	219 (199-238)	383 (360-415)	787 (720-860)	1822 (1660-2020)
2	F	580	5	274 (186-322)	384 (360-420)	798 (720-890)	1972 (1770-2330)
3	F	555	3	223 (212-244)	360	796 (750-860)	1830 (1720-2020)
4	F	550	8	209 (181-234)	345 (300-420)	720 (610-800)	1841 (1550-2100)
5	F	570	5	246 (212-289)	388 (360-400)	805 (750-830)	1914 (1380-2220)
6	F	560	6	236 (221-258)	350 (330-360)	688 (550-780)	1708 (1500-1910)
7	♂ PM	430 445	6	168 (138-195)	356 (278-500)	685 (500-833)	1685 (1360-2000)
8	M	590	10	123 (81-205)	365 (305-415)	750 (666-888)	1728 (1520-1840)
9	M	600	10	117 (83-143)	404 (362-455)	793 (722-944)	1789 (1600-1968)

Table XI. Male *P. oncorhynchi* from *O. nerka* and *O. keta*.

No.	SF	FLF (mm)	W	L (mm)	NR (μ)	LMO (μ)	LGO (μ)	T (μ)	S (μ)
1	F	-	19	30 (22-38)	325 (178-400)	615 (425-820)	1665 (1230-2250)	385 (282-470)	327 (283-381)
2	F	580	18	38 (33-42)	358 (340-390)	650 (560-740)	1810 (1610-2080)	480 (390-600)	368 (330-530)
3	F	555	8	40 (37-47)	369 (344-412)	705 (627-812)	1956 (1776-2414)	535 (480-597)	356 (314-418)
4	F	550	11	35 (27-46)	347 (308-387)	665 (554-775)	1845 (1443-2331)	477 (320-547)	345 (326-369)
5	F	570	4	34 (31-39)	354 (326-375)	663 (603-713)	1922 (1721-2303)	518 (492-541)	347 (326-381)
6	F	560	6	34 (33-37)	-	-	-	-	-
7	PM	430 445	11	18 (9-32)	236 (172-320)	469 (320-670)	1355 (932-1626)	356 (295-517)	360 (332-381)
8	M	590	10	31 (25-39)	322 (271-369)	583 (523-677)	1698 (1443-2109)	455 (406-504)	350 (332-381)
9	M	600	10	26 (16-33)	305 (246-357)	573 (517-713)	1596 (1249-1748)	463 (394-554)	324 (295-363)
10	M*	730	5	34 (32-35)	358 (340-370)	676 (550-770)	1908 (1660-2140)	458 (420-490)	374 (320-470)

* *O. keta*

Table XII. *Philonema* from *O. nerka kennerlyi*.

No.	SF	FLF (mm)	SW	W	L (mm)	NR (μ)	LMO (μ)	LGO (μ)	T (μ)	S (μ)
11	F	195	F	2	58 (55-61)	292 (278-305)	528 (500-555)	1277	-	-
12*	F	395	Lv	6	8 (5-10)	180 (154-209)	326 (295-357)	676 (583-777)	312 (228-369)	-
	M	380								
	F	217								
	M	215	F	8	19 (11-30)	225 (197-271)	419 (344-517)	974 (733-1166)	489 (344-578)	-
	F	235								
			M	17	12 (5-14)	206 (166-246)	365 (271-424)	871 (638-1110)	291 (234-351)	284 (191-326)

* Sample 12 - measurements of worms from five kokanee were pooled.

Table XIII. *Philonema* from *S. gairdneri* and *S. salar*.

No.	SF	FLF (mm)	SW	W	L (mm)	NR (μ)	LMO (μ)	LGO (μ)	T	S
13	F	500	F	27	149 (107-181)	306 (250-360)	585 (470-720)	1355 (1050-1750)	-	-
			M	22	21 (17-26)	304 (246-418)	510 (394-715)	1240 (890-1830)	329 (295-375)	311 (264-363)
14	F	790	F	23	129 (101-148)	298 (250-372)	545 (472-638)	1510 (1193-1832)	-	-
			M	10	19 (17-24)	264 (240-295)	436 (381-535)	1342 (1082-1832)	342 (283-406)	313 (234-369)
15*	-	-	M	2	24 (22-25)	295	529 (492-566)	1208 (1177-1238)	317 (301-332)	305 (301-308)
16**	-	-	F	8	41 (32-46)	313 (250-361)	583 (472-611)	935 (777-1110)	-	-

* Steelhead trout, Oregon; U.S.H.M. Coll. No. 40004--3273A.

** Landlocked Atlantic salmon; U.S.H.M. Coll. No. 46160--4225D.

Table XIV. *Philonema* from *S. malma* and *S. fontinalis*.

No.	SF	FLF (mm)	SW	W	L (mm)	NR (μ)	LMO (μ)	LGO (μ)	T (μ)	S (μ)
17*	F	310	F	1	57	170	280	1050	-	-
			M	4	10 (6-13)	207 (180-246)	341 (330-360)	892 (760-1010)	308 (250-390)	275 (230-300)
18*	M	450	F	2	74 (67-81)	292 (250-333)	569 (500-638)	1578 (1499-1637)	-	-
19*	M F	380 340	Lv	2	10 (9-10)	185 (154-215)	329 (289-369)	988 (833-1143)	474 (332-615)	-
			F	5	14 (12-19)	194 (148-221)	363 (295-424)	827 (583-1000)	371 (314-449)	-
			M	6	12 (10-15)	204 (178-228)	362 (283-412)	932 (833-1000)	255 (221-289)	313 (283-338)
20**	-	-	F	1	67	233	416	1000	888	-
			M	1	19	258	406	1176	369	332

* No. 17 is from Cultus Lake, 18 and 19 from Kootenay Lake region.

** Brooktrout; U.S.H.M. Coll. No. 8908--M373A, type for *Philonema agubernaculum* Simon et Simon, 1936.

Table XV. *Philonema* from *Prosopium williamsonii*.

No.	SF	FLF (mm)	SW	W	L (mm)	NR (μ)	LMO (μ)	LGO (μ)	T (μ)	S (μ)
21	F	202	F	5	95	280	506	951	-	-
	F	243			(78-105)	(222-316)	(444-583)	(816-1249)		
	F	236								
	F	246	M	3	13	221	365	836	318	340
					(13-14)	(209-246)	(326-387)	(805-888)	(301-344)	(326-363)
22*	-	-	F	1	85	305	638	1360	916	-

* *Prosopium* sp.; U.S.N.M. Coll. No. 38004--M590E, designated *Philonema agubernaculum*.

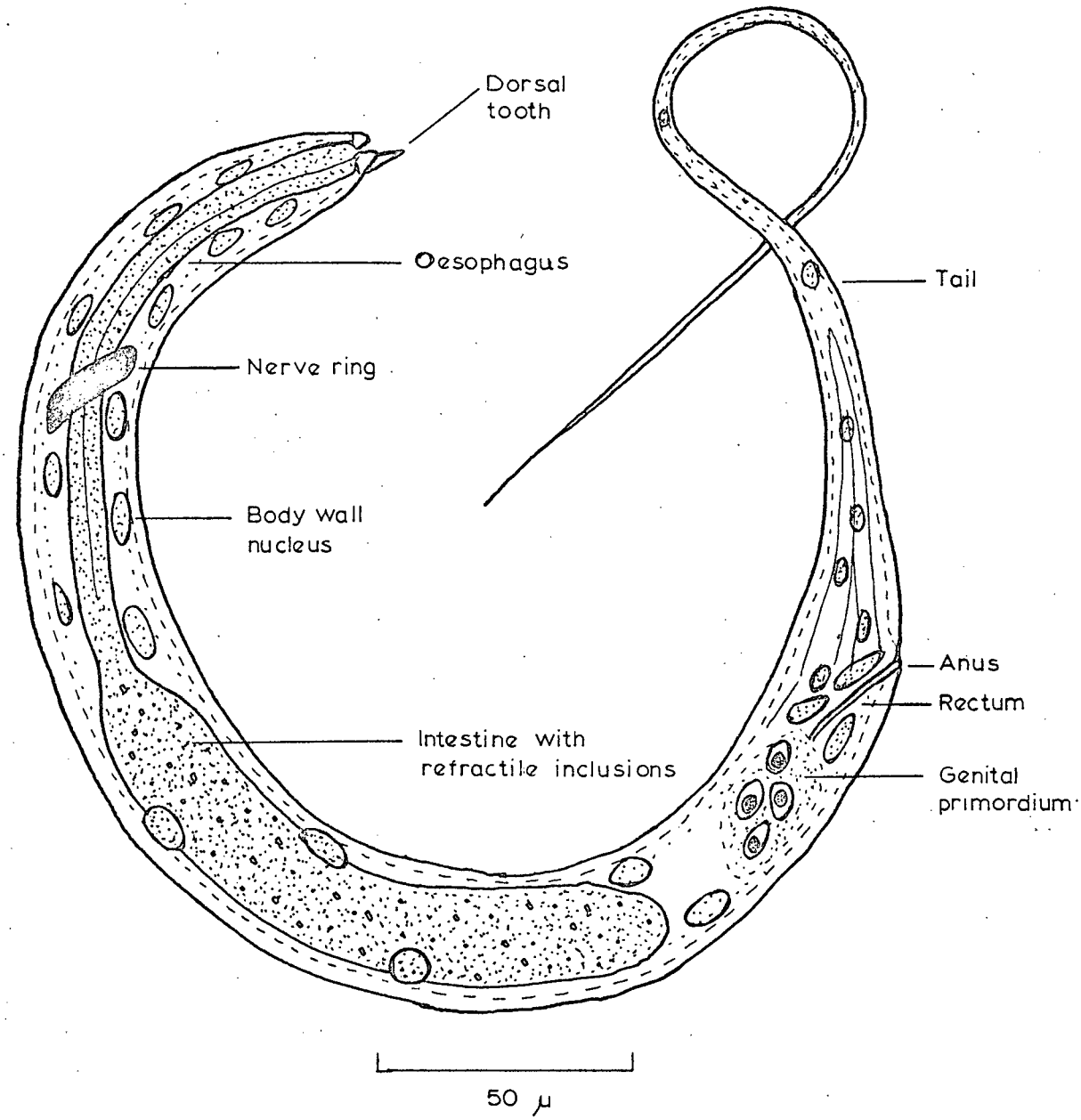

Fig. 1. First-stage larva of Philonema oncorhynchi from a gravid female P. oncorhynchi.

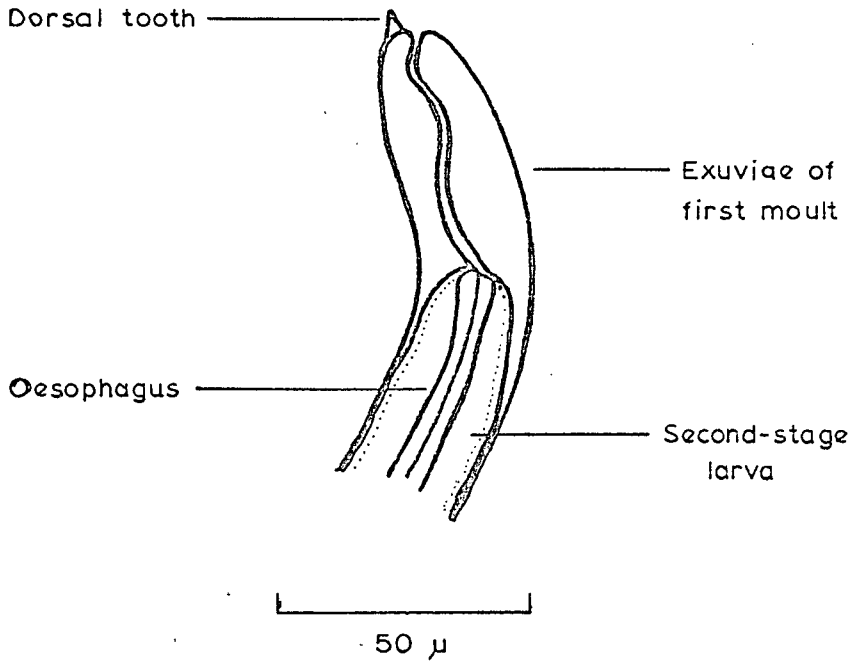

Fig. 2. Anterior end of a second-stage larva of P. oncorhynchi showing the exuviae of the first moult. The tooth of the first-stage larvae is lost with the exuviae.

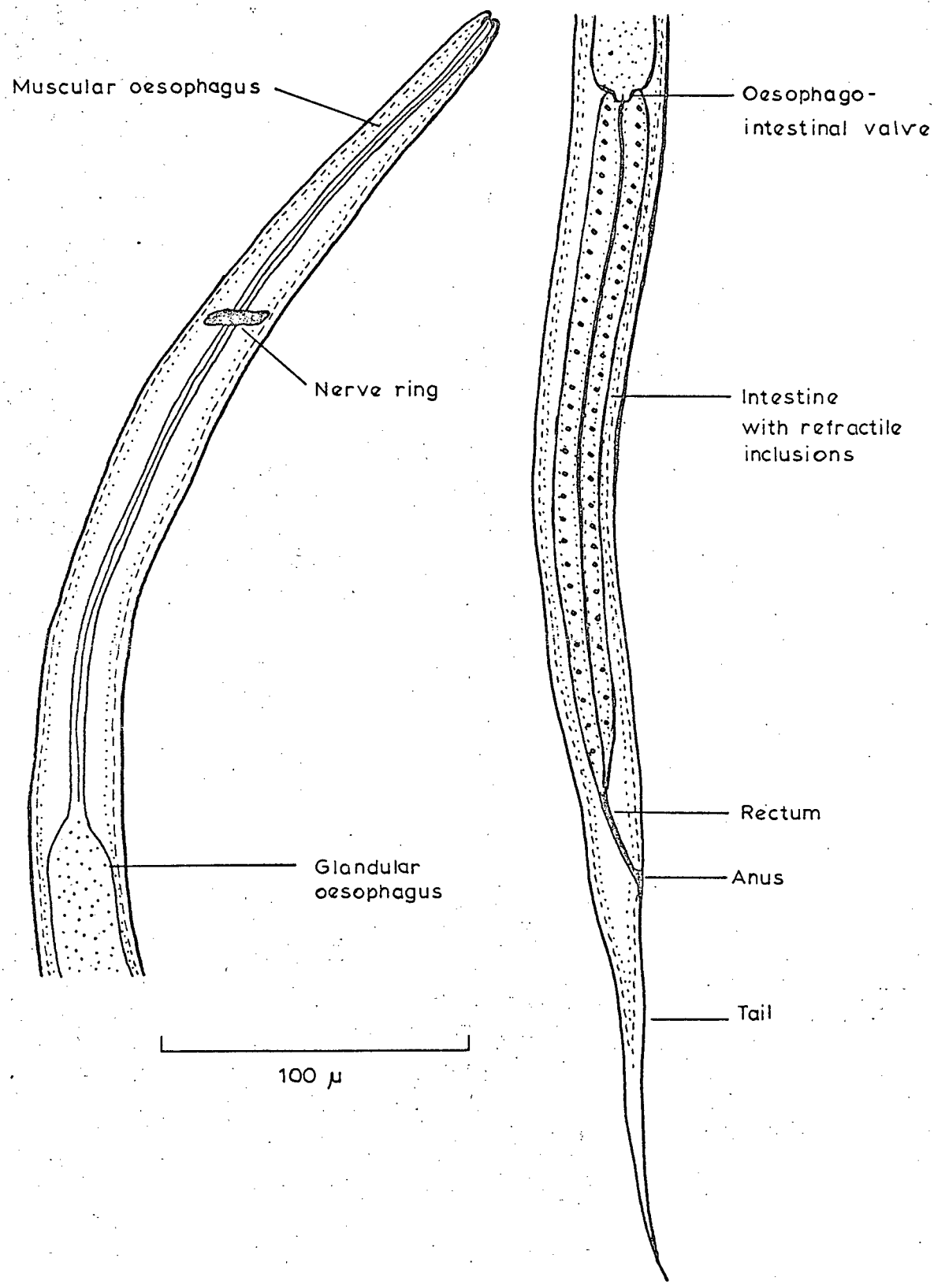

Fig. 3. Third-stage larva of P. oncorhynchi from the haemocoele of C. bicuspidatus. Anterior and posterior regions of larva are shown.

Fig. 4. Saggital section of C. bicuspidatus infected with a third-stage larva of P. oncorhynchi, X400.

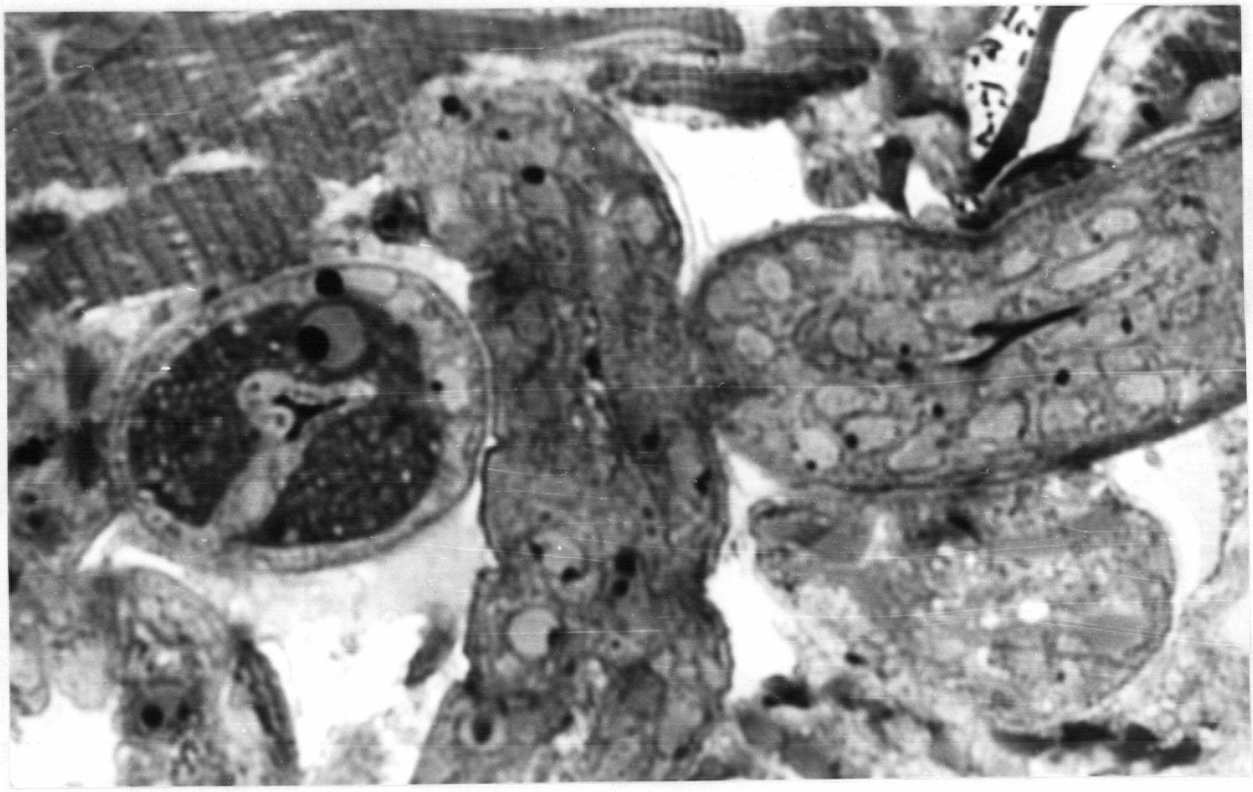

Fig. 5. Higher magnification of larva in Fig. 4. Note Y-shaped lumen of glandular oesophagus, X2000.

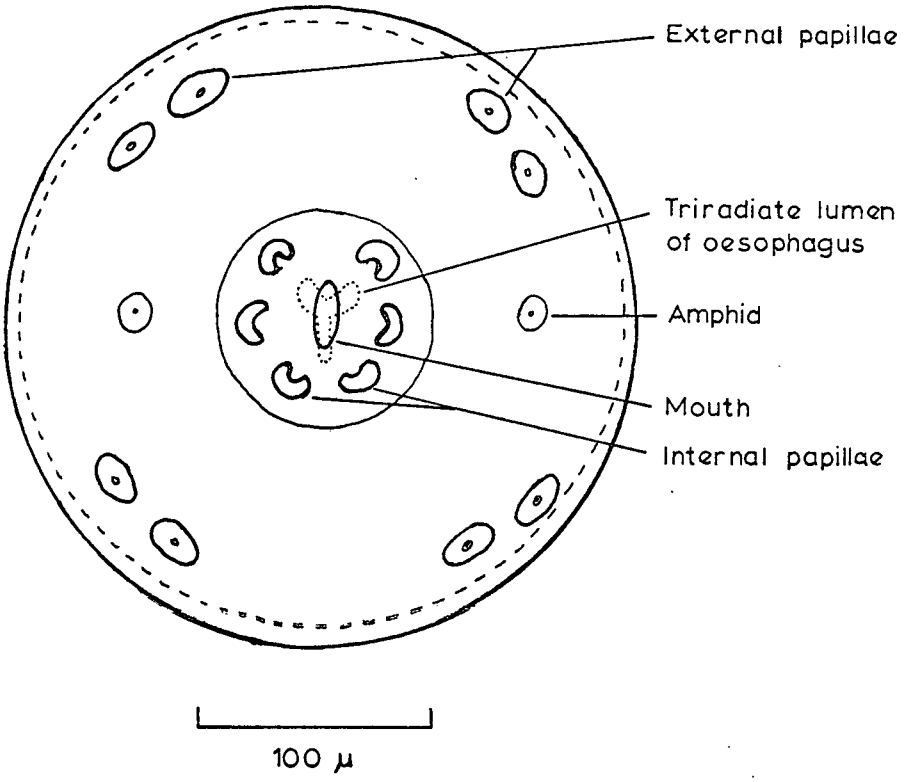

Fig. 6. Cephalic papillae of a preadult male Philonema oncorhynchi from sockeye salmon maintained in freshwater.

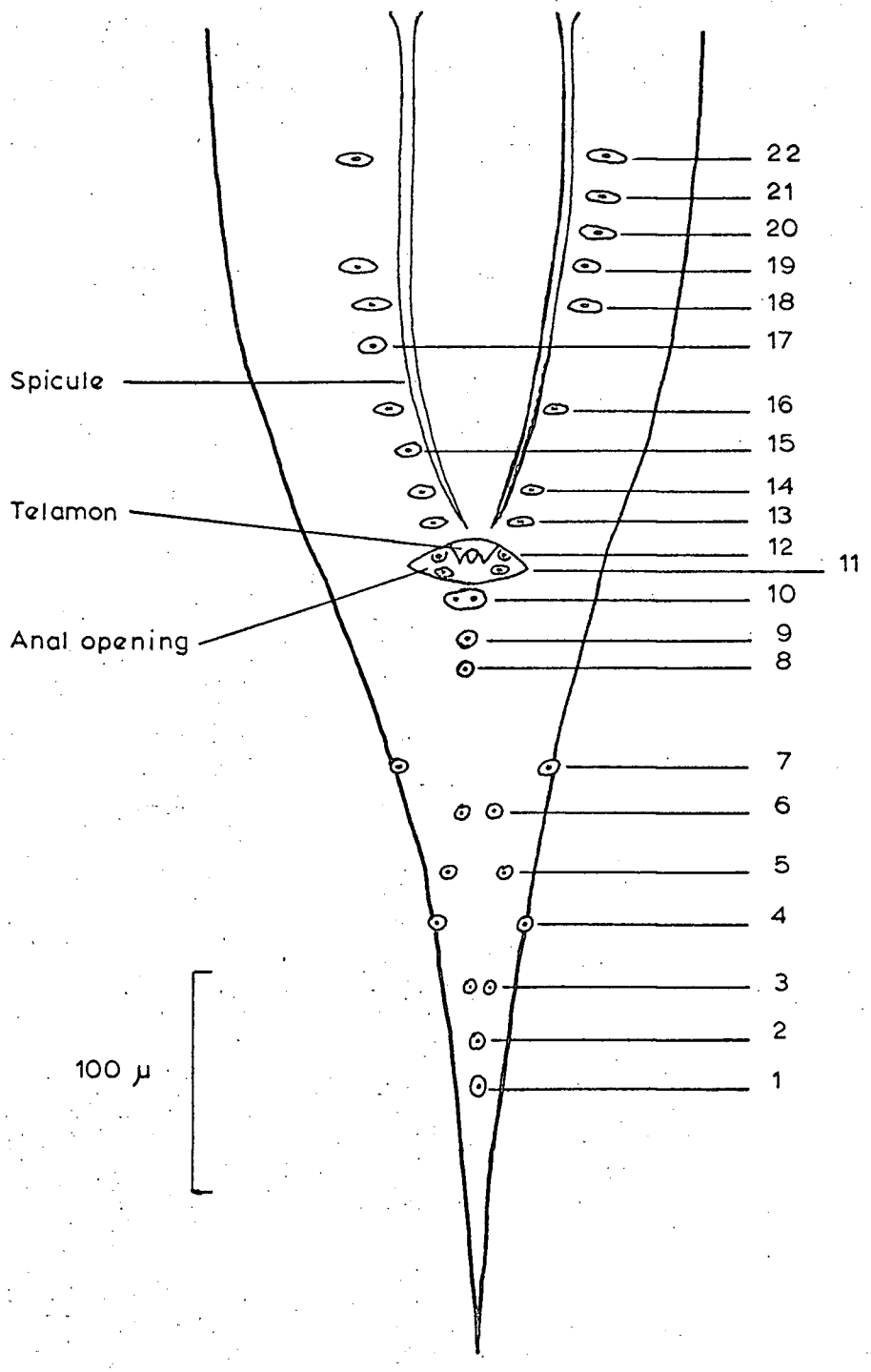

Fig. 7. Caudal papillae of a male preadult Philonema oncorhynchi from sockeye salmon maintained in freshwater. Variability of the papillae is listed in Table VII.

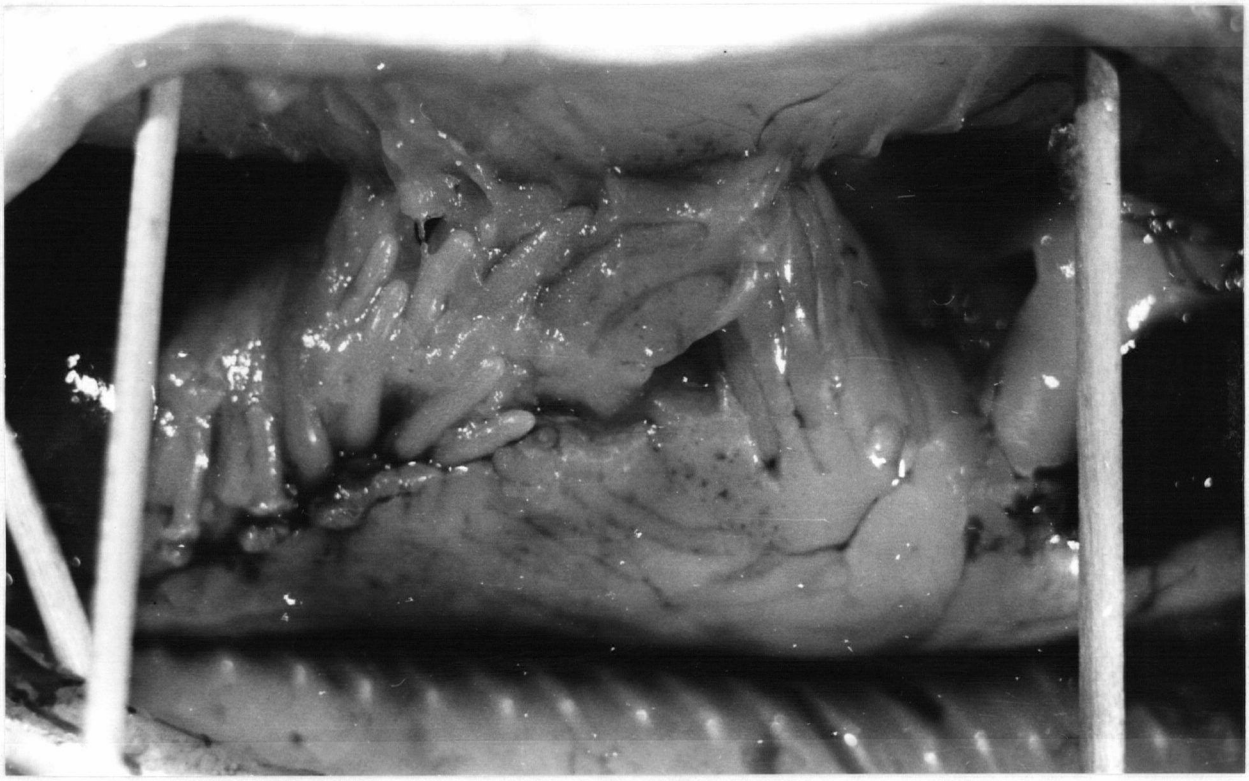

Fig. 8. Adhesions of the pyloric caeca to the body wall of a 34-month old infected sockeye salmon which was maintained in fresh water for two years, X1.5.

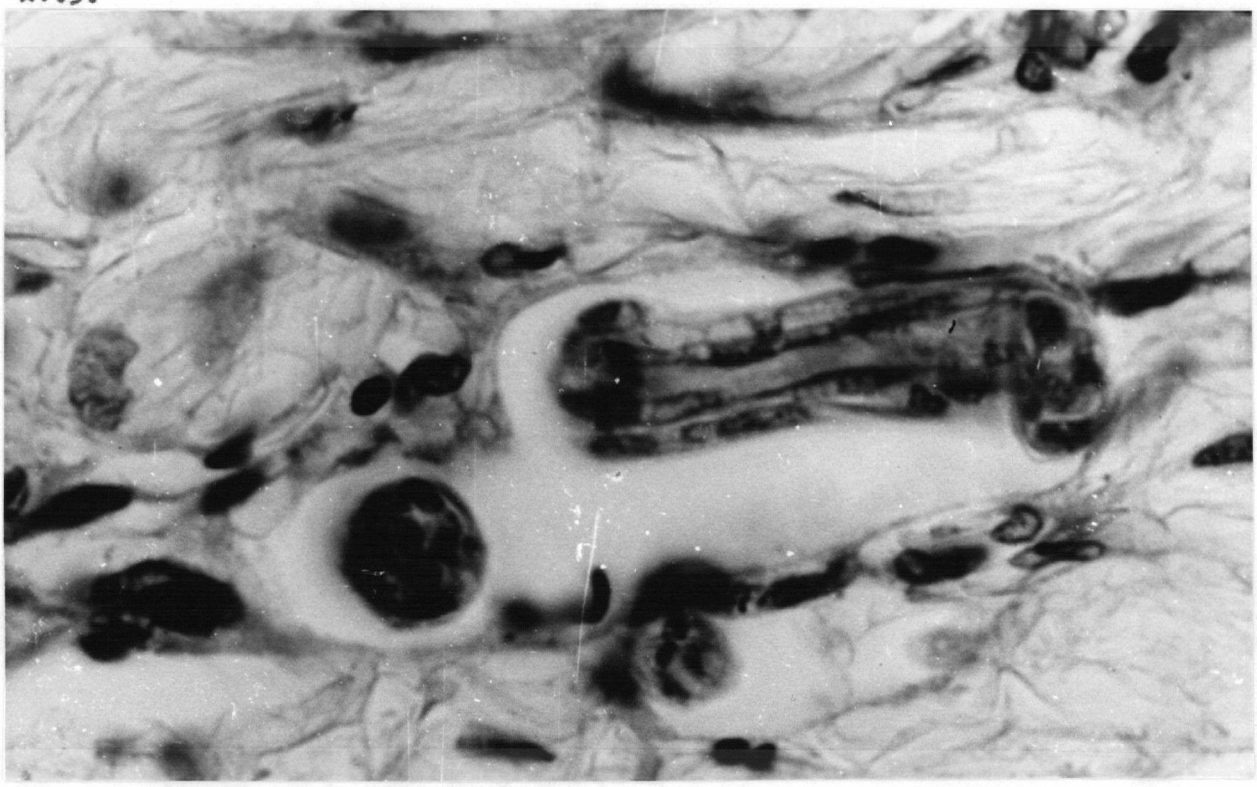

Fig. 9. Fourth-stage larva in the tunica adventitia of the swim bladder from a Cultus Lake yearling sockeye salmon, X800.

Fig. 10. Gravid female P. oncorhynchi in the body cavity of a spawning Cultus Lake sockeye salmon, X2.

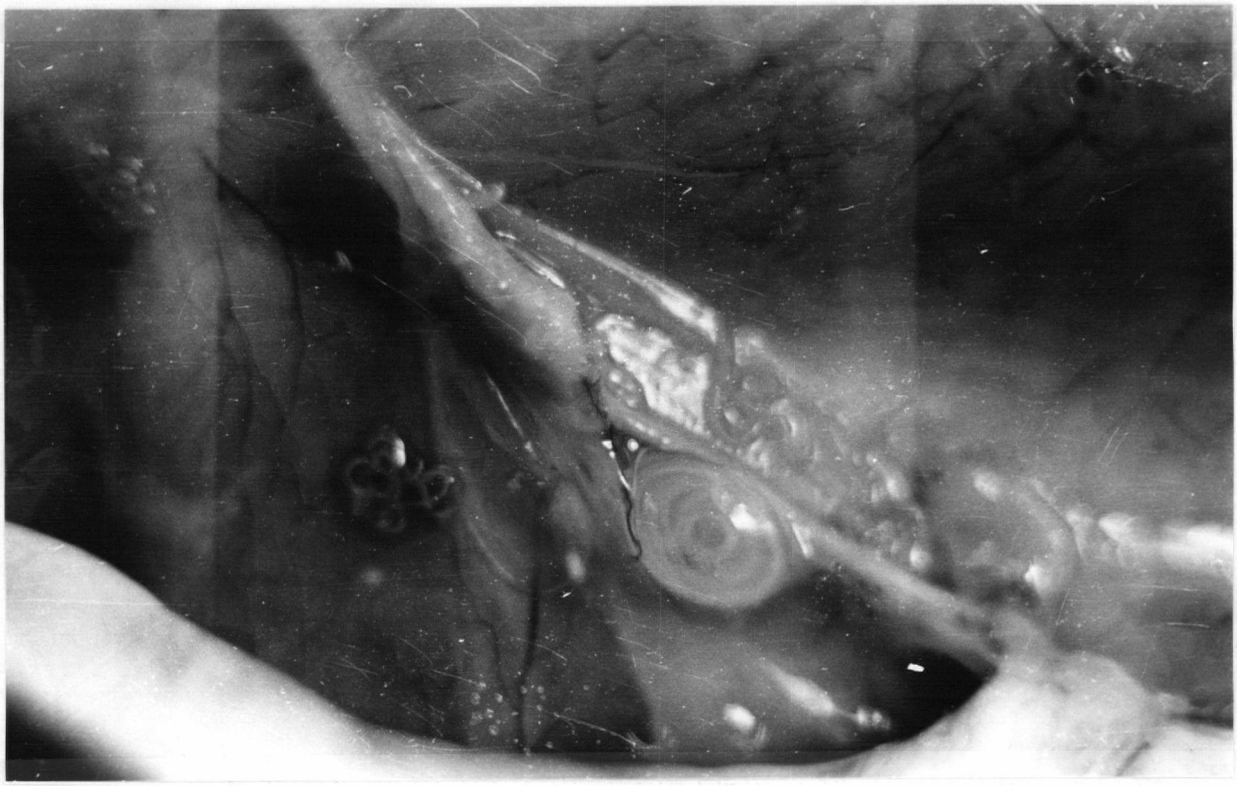

Fig. 11. Encysted P. oncorhynchi and long fibrous adhesions in spawning male sockeye salmon (Cultus Lake), X2.

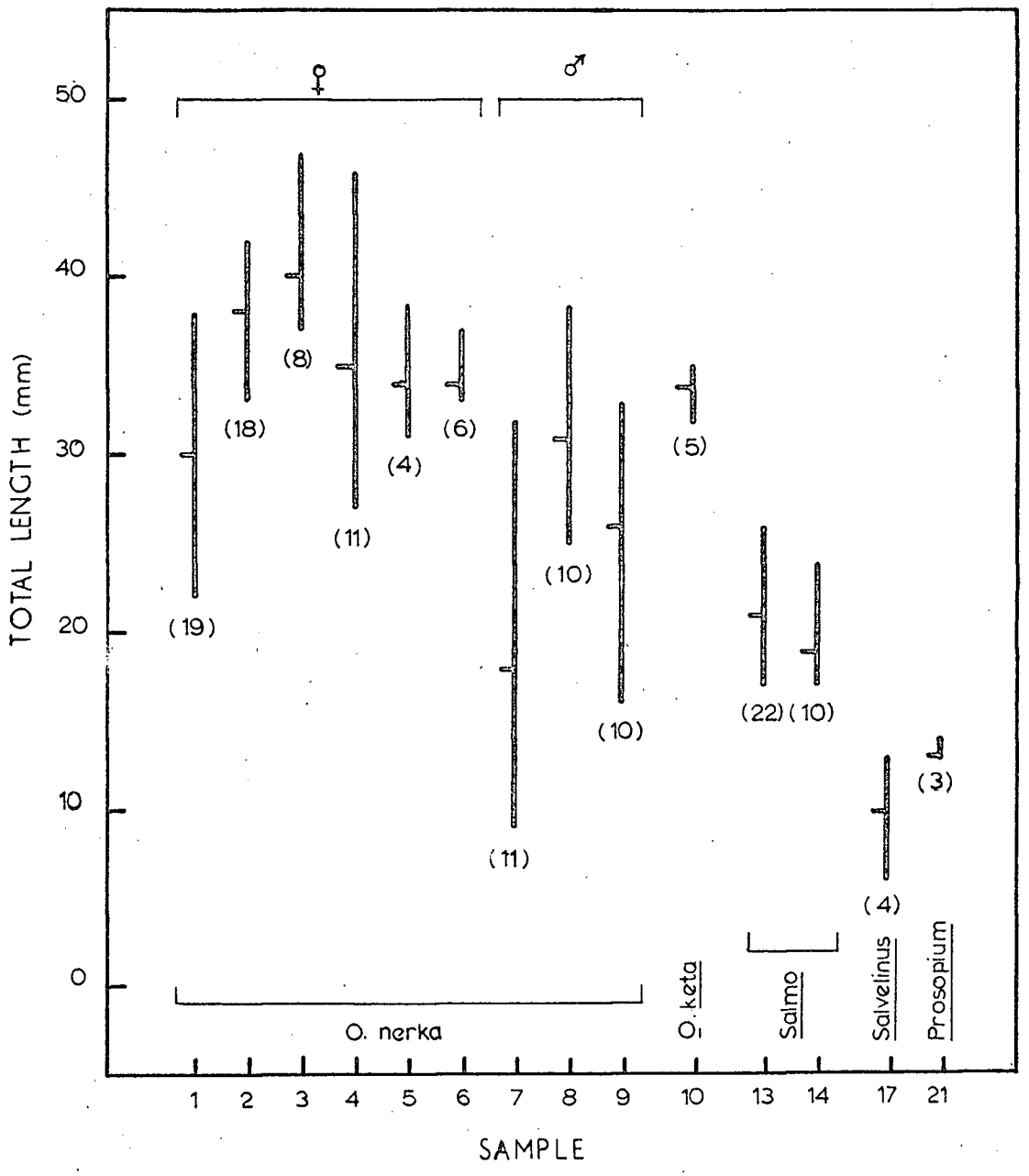

Fig. 12. Total lengths of male Philonema.

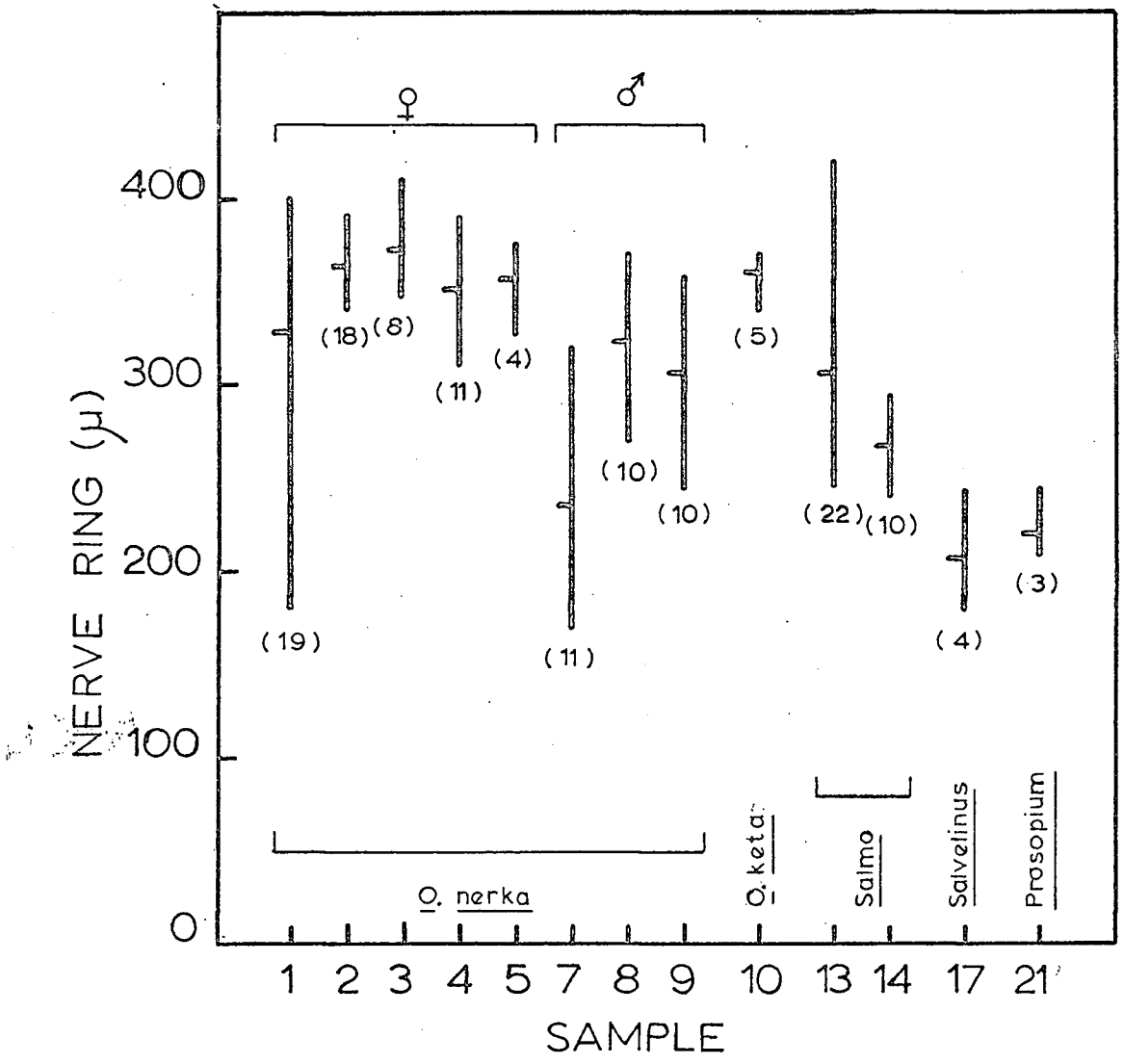

Fig. 13. Distance of nerve ring from the anterior end of male Philonema.

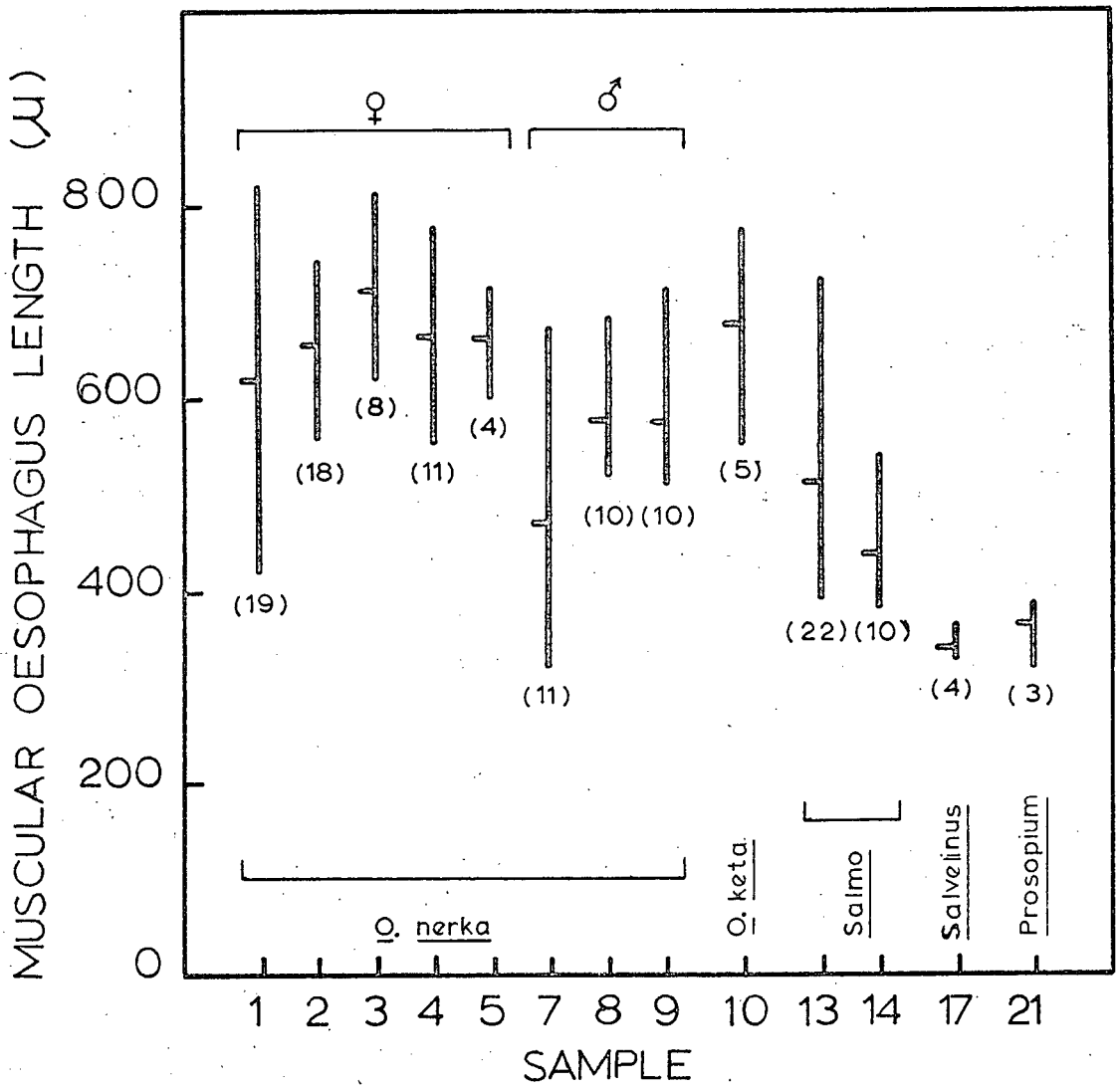

Fig. 14. Length of muscular oesophagus in male Philonema.

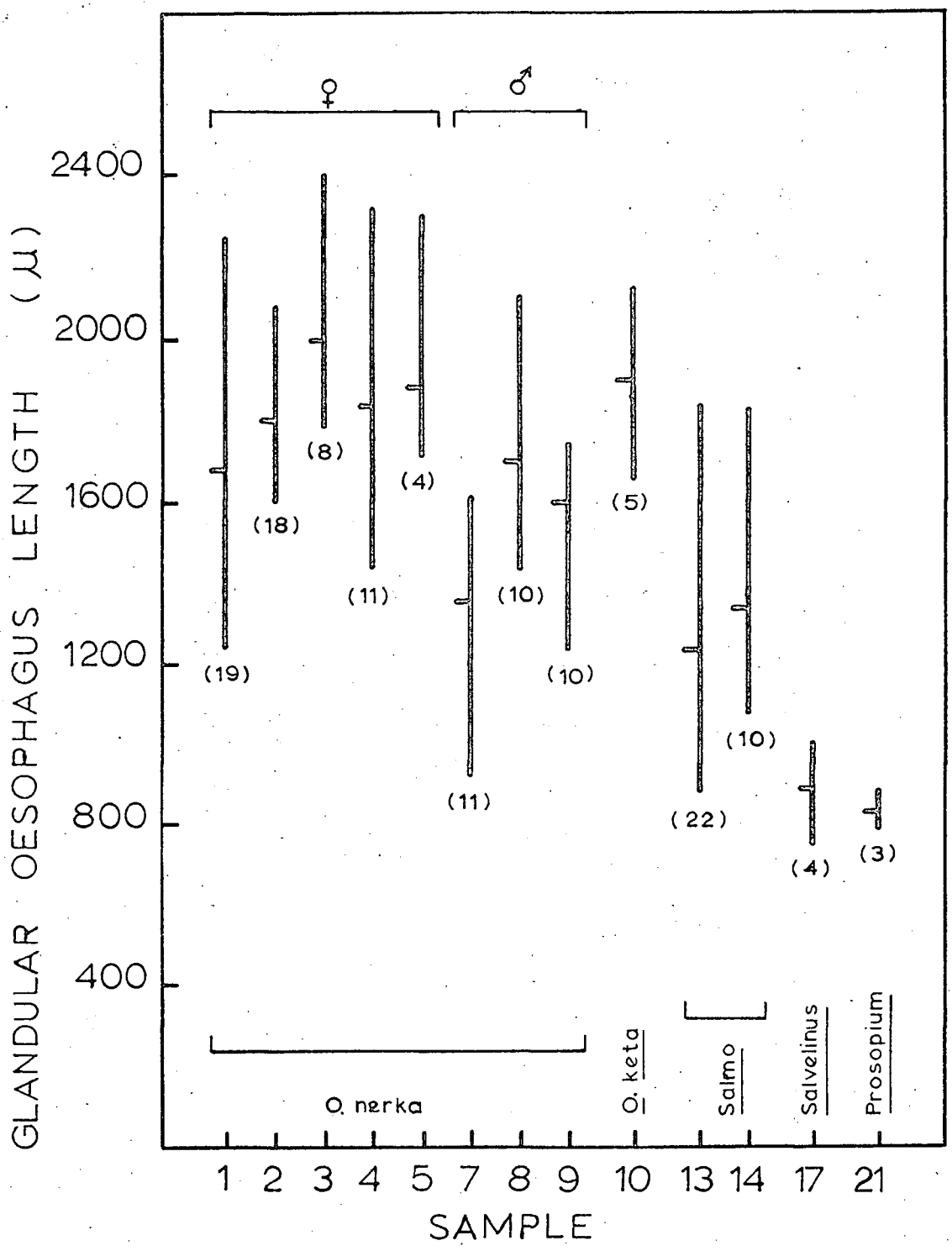

Fig. 15. Length of glandular oesophagus in male Philonema.

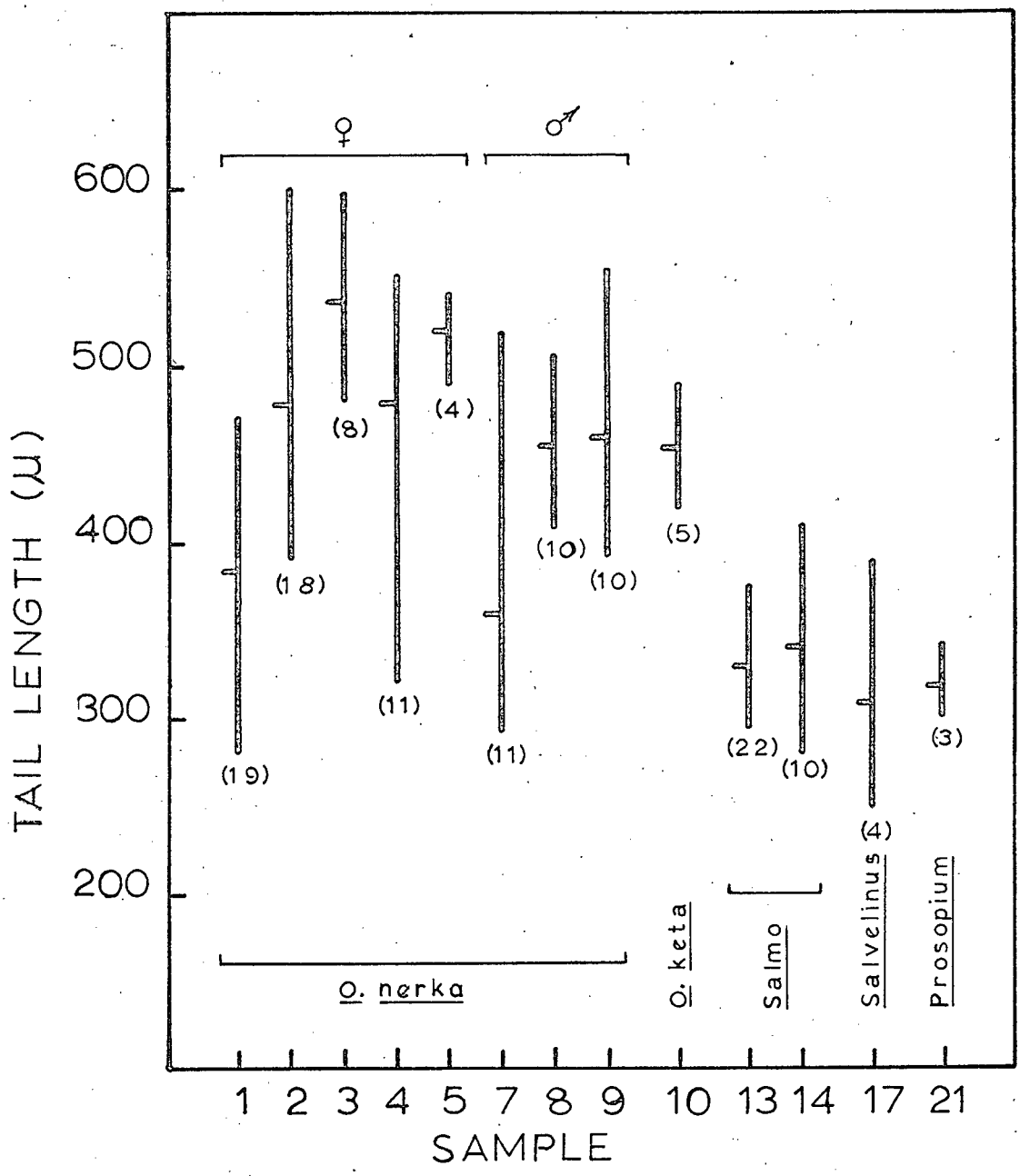

Fig. 16. Tail lengths of male Philonema.

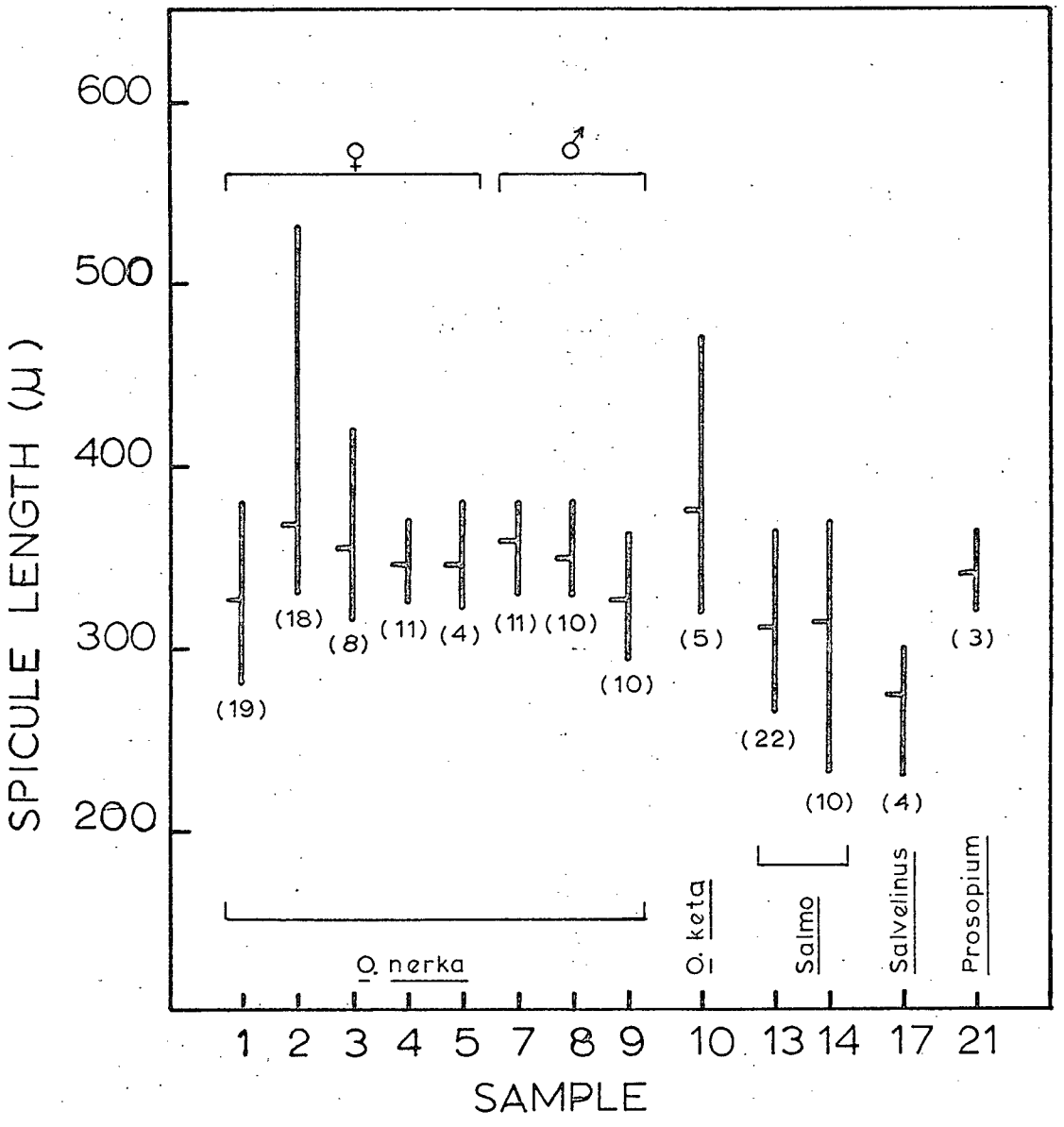

Fig. 17. Spicule lengths of male Philonema.

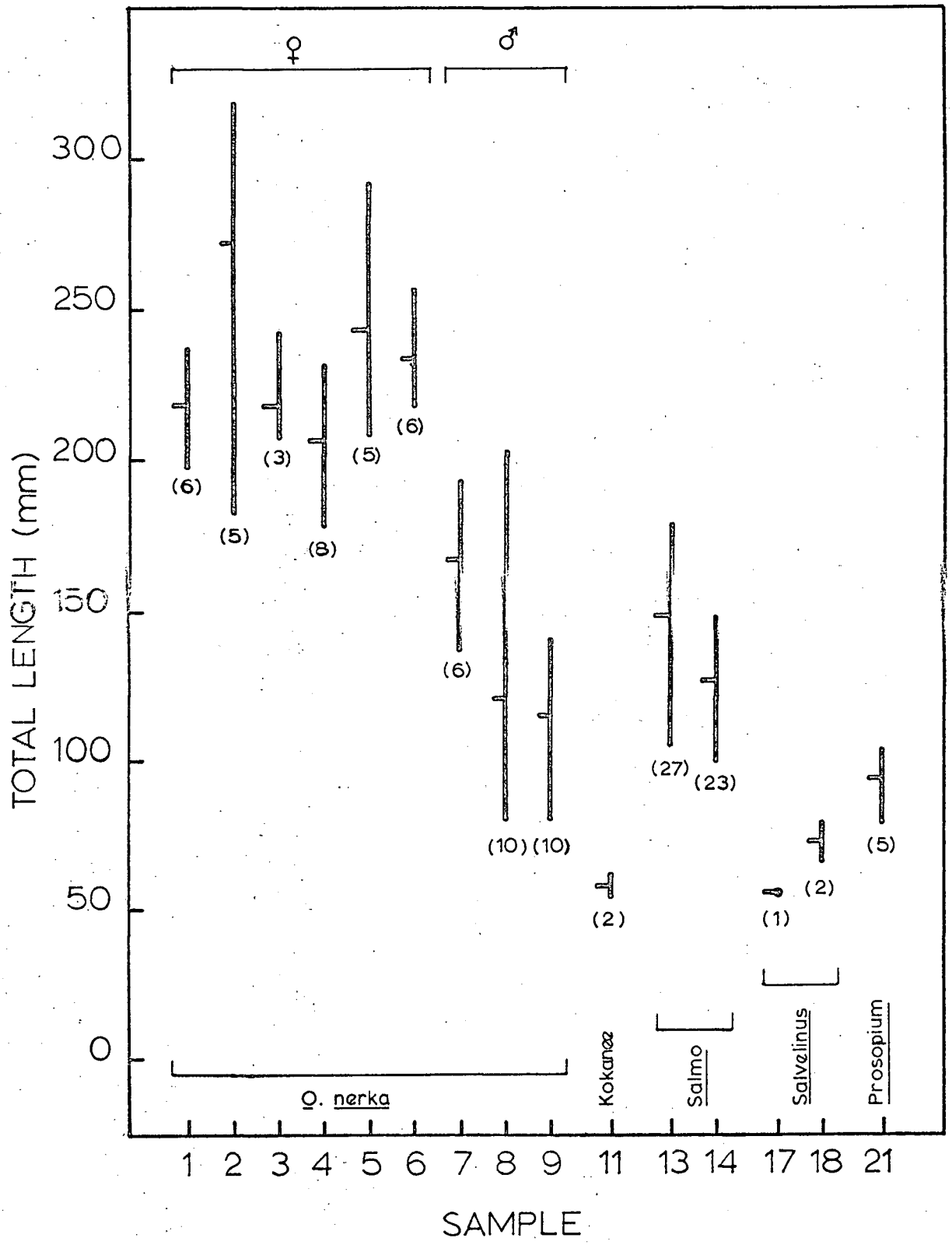

Fig. 18. Total body length of female Philonema.

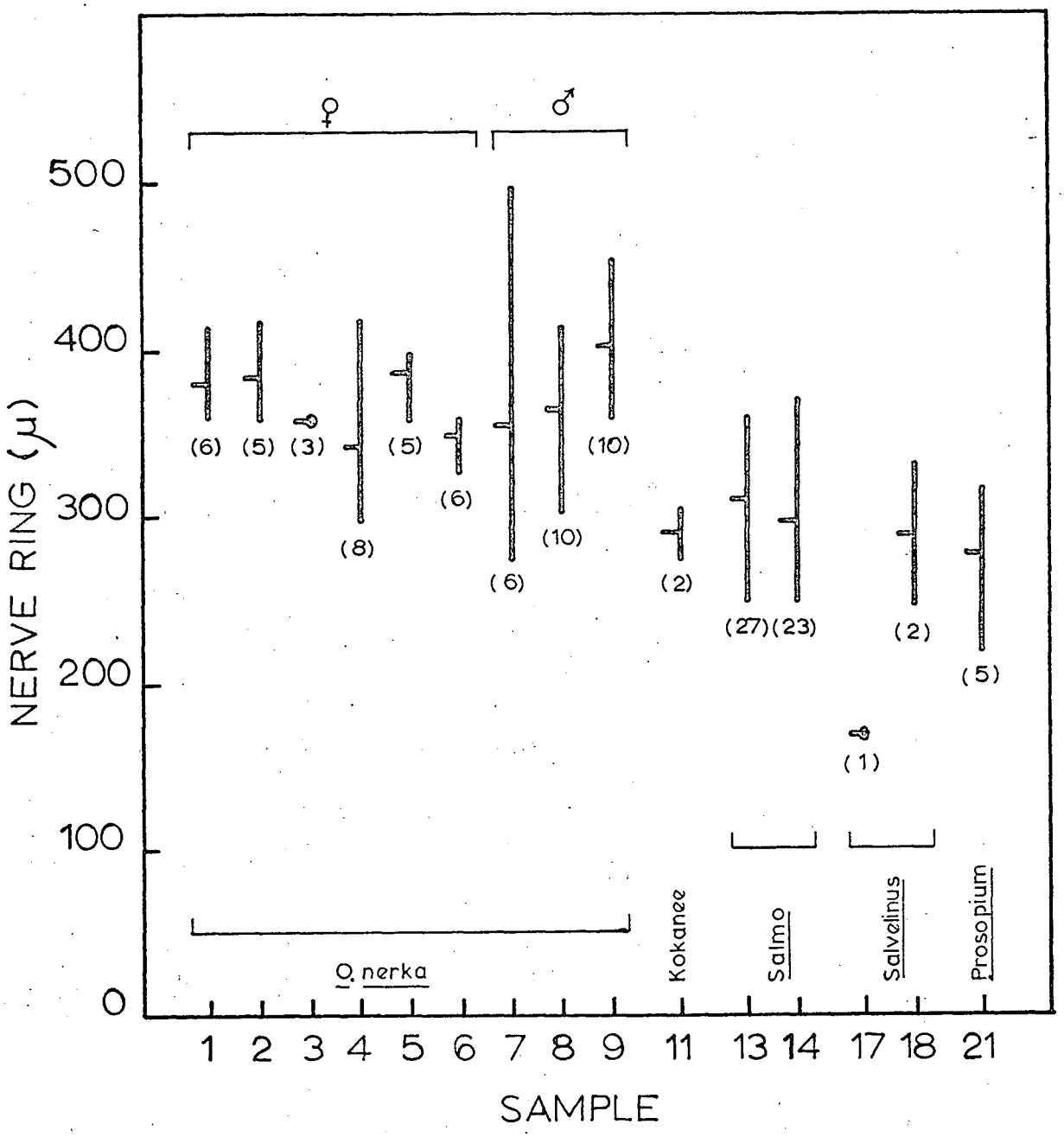

Fig. 19. Distance to nerve ring in female Philonema.

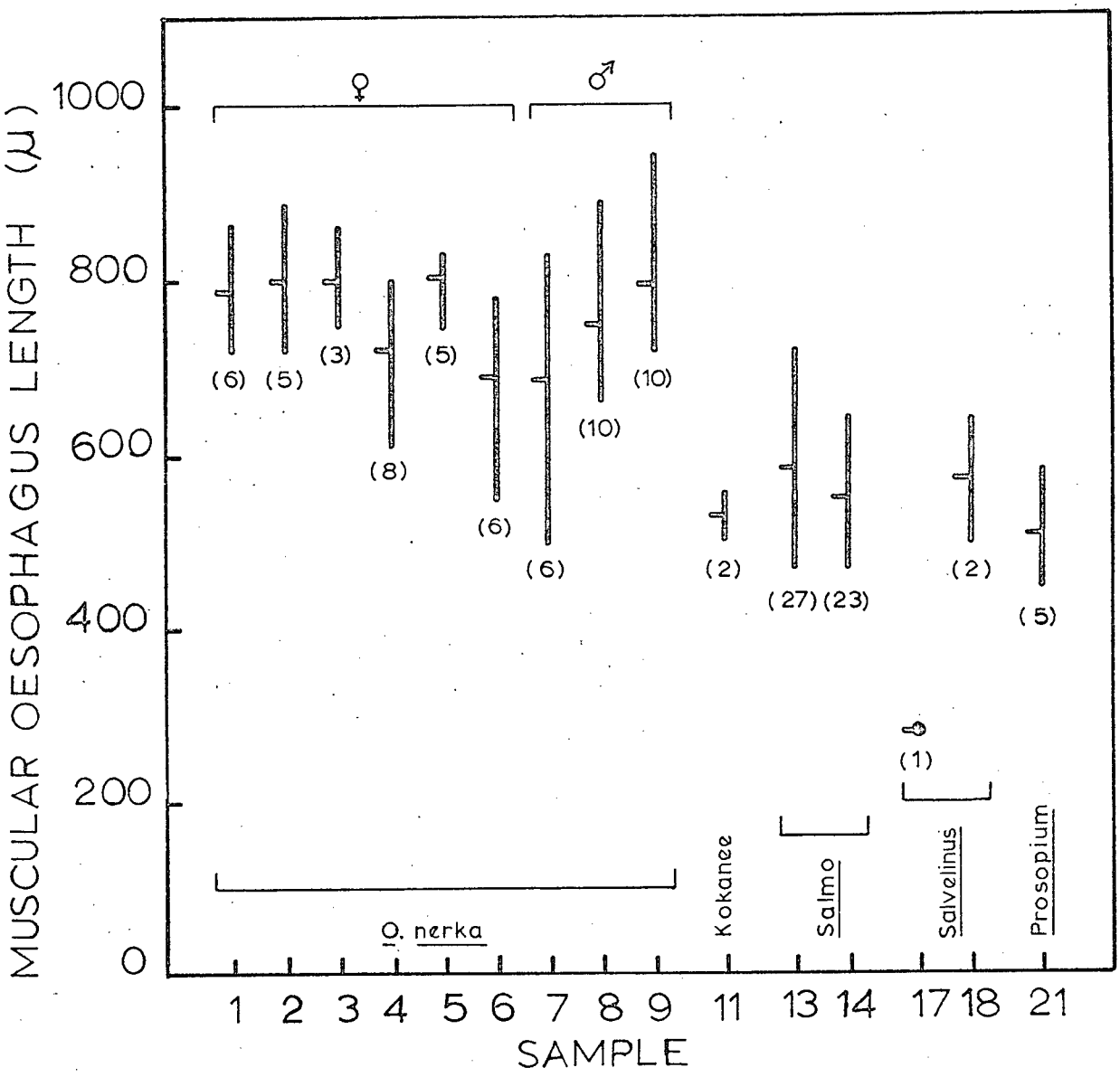

Fig. 20. Muscular oesophagus lengths of female Philonema.

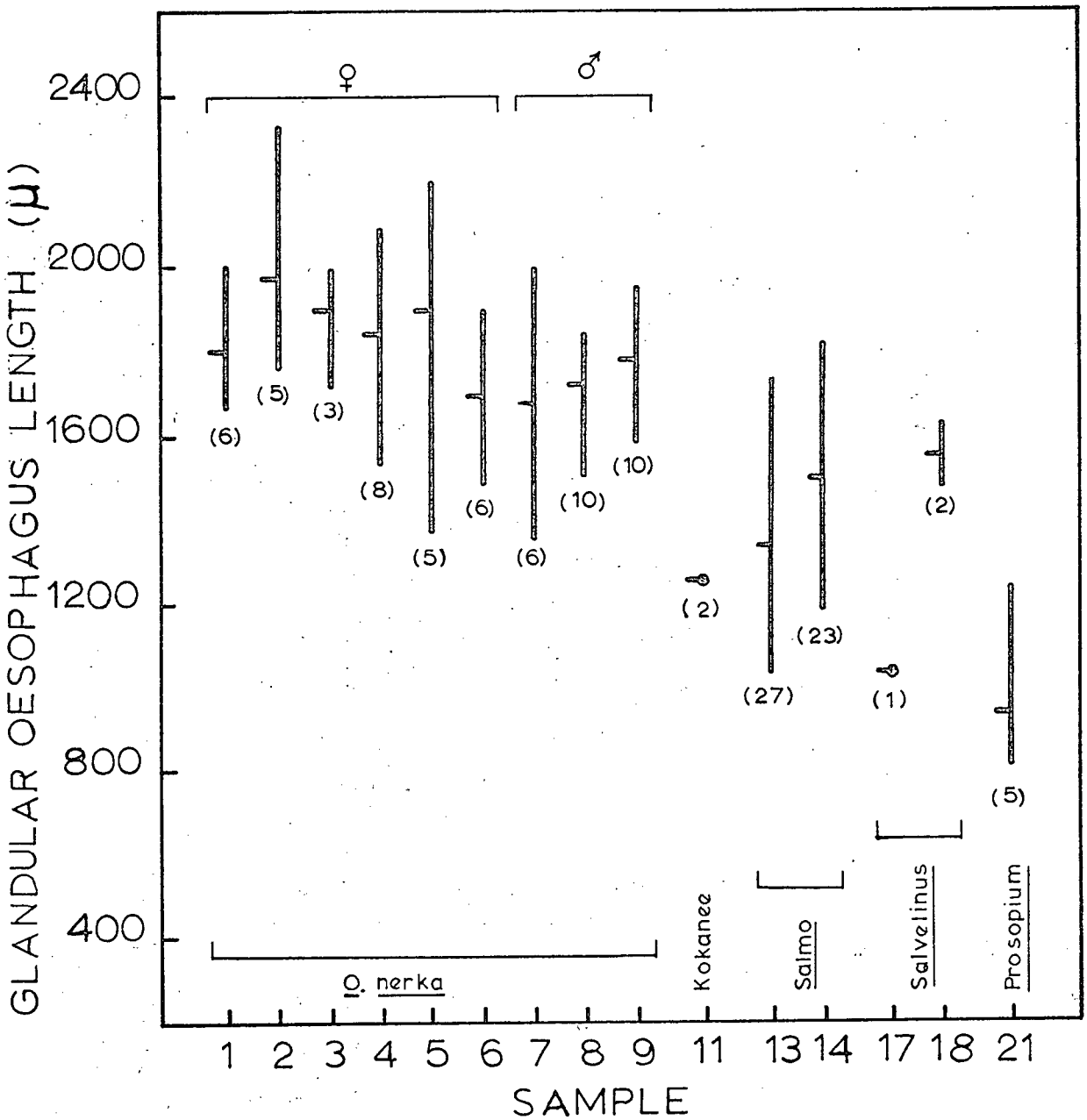

Fig. 21. Glandular oesophagus length of female Philonema.

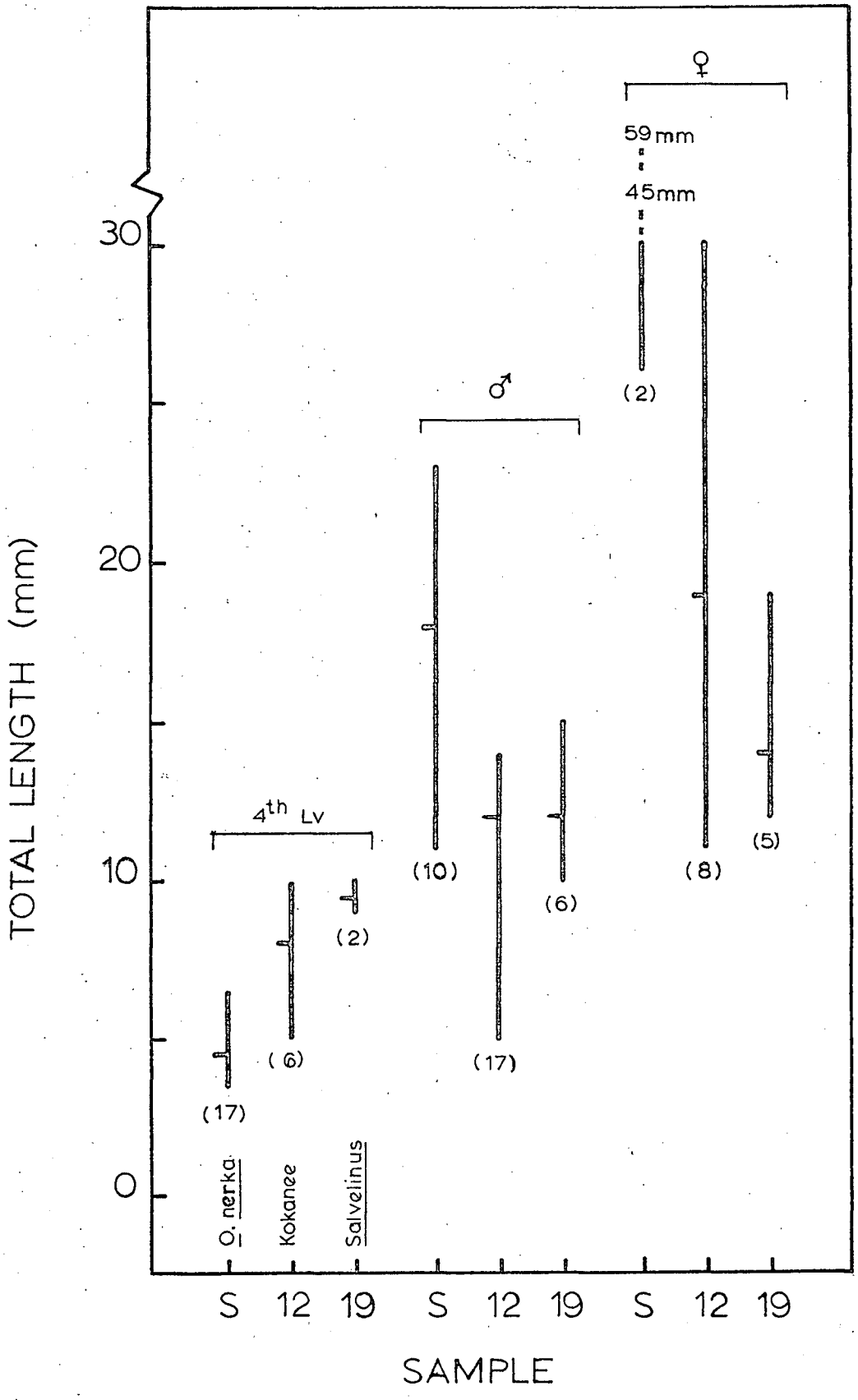

Fig. 22. Total body length of fourth-stage larvae and preadult Philonema.

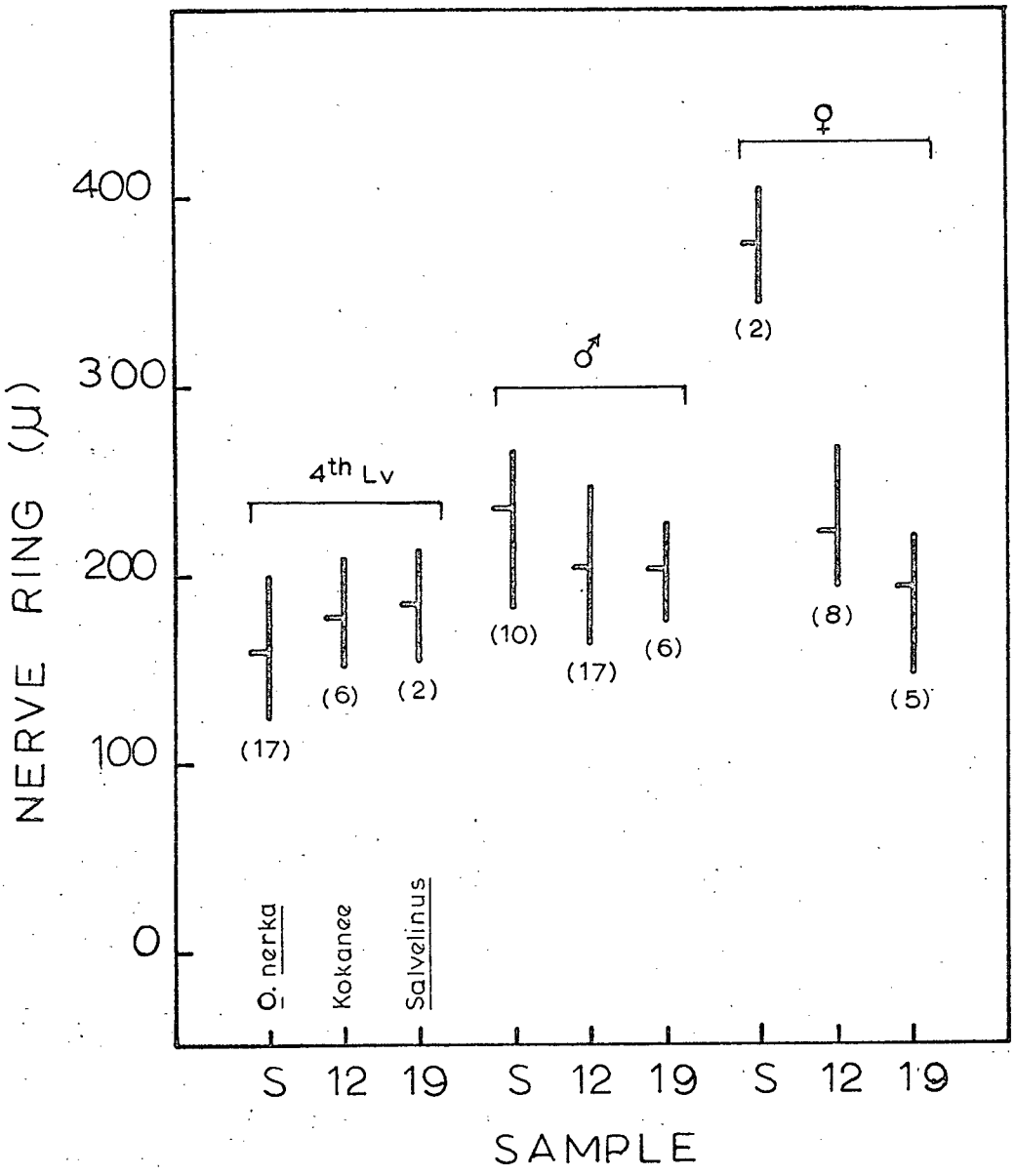

Fig. 23. Nerve ring distance of fourth-stage larvae and preadult *Philonema*.

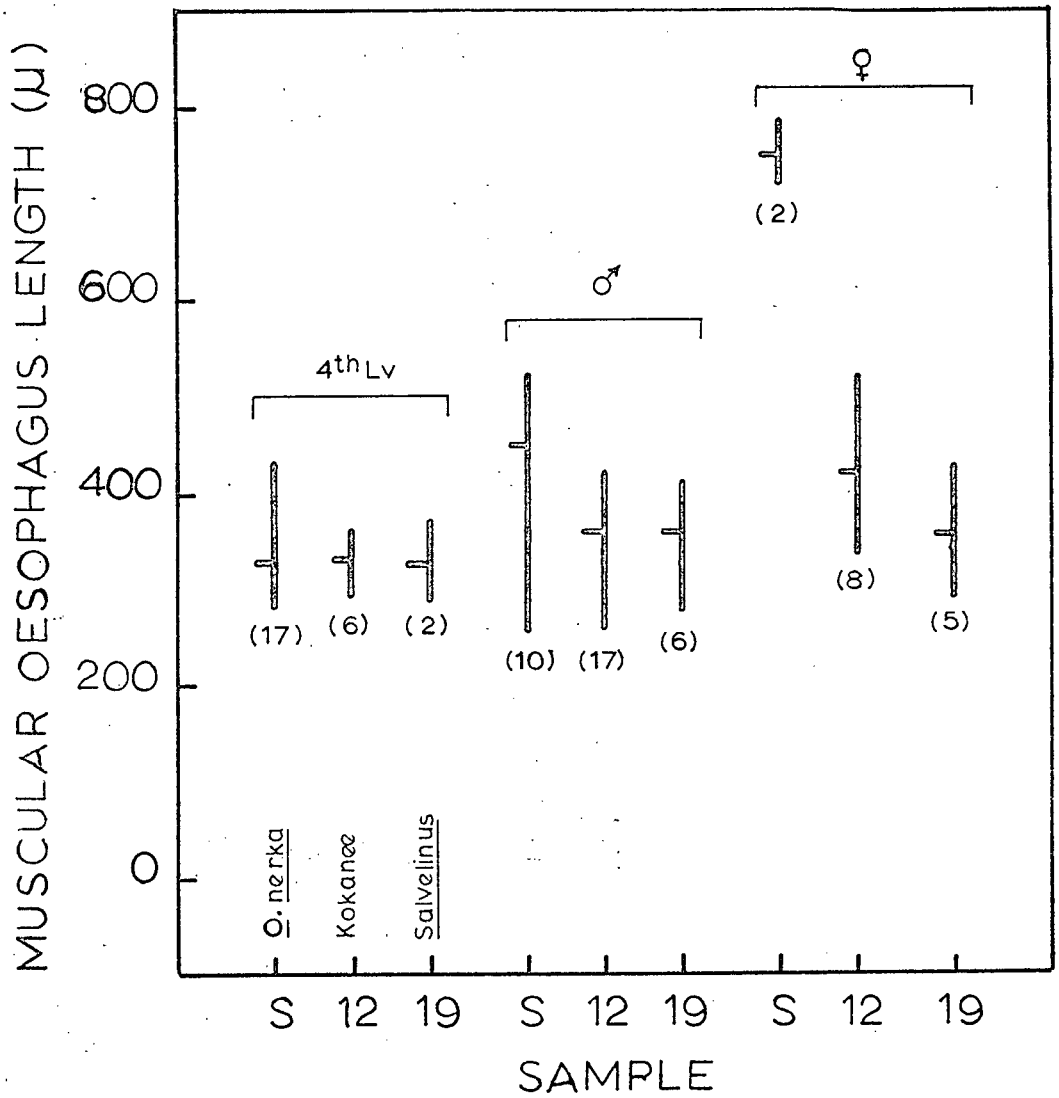

Fig. 24. Muscular oesophagus lengths of fourth-stage larvae and preadult Philonema.

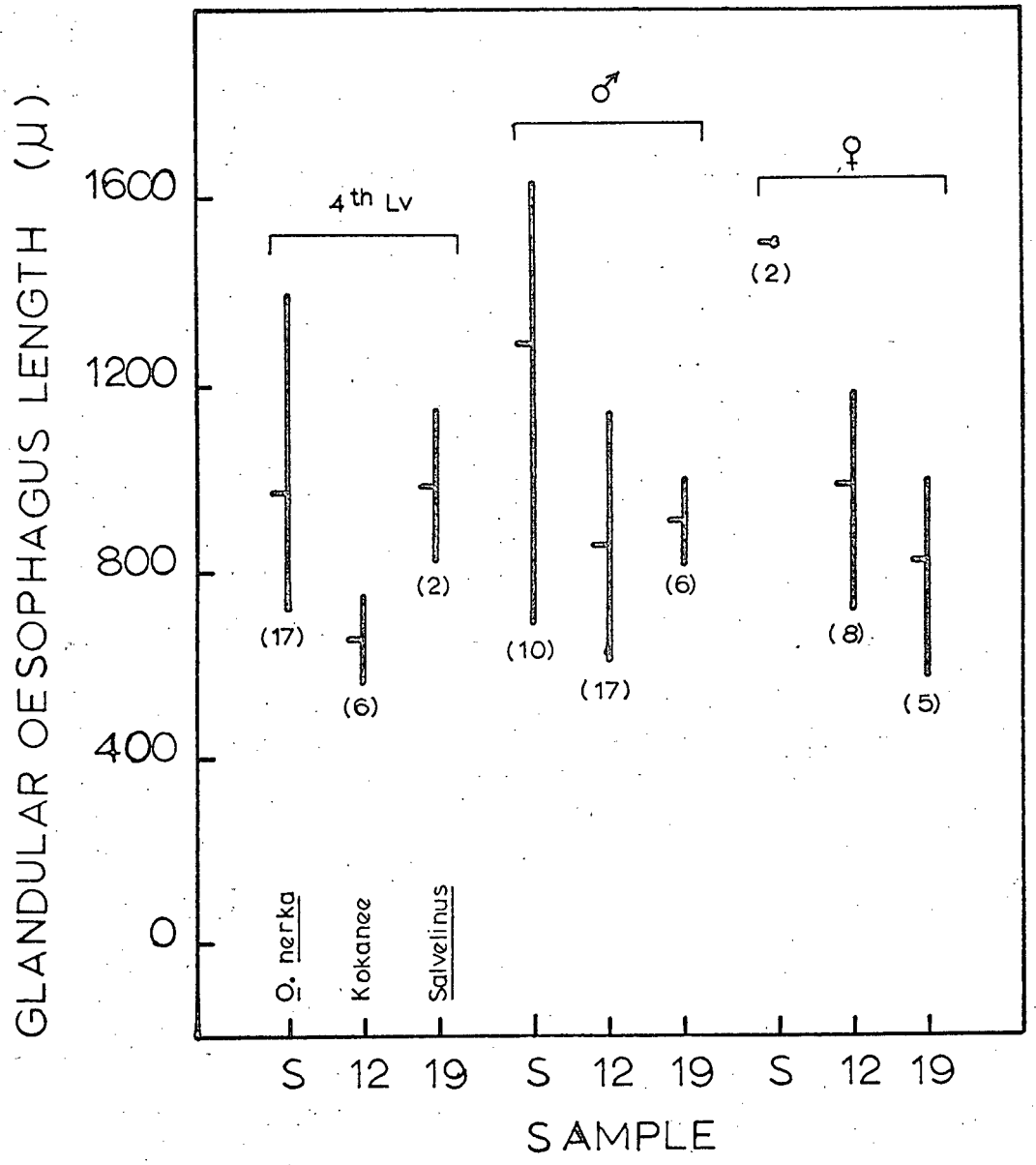

Fig. 25. Glandular oesophagus lengths of fourth-stage larvae and preadult Philonema.

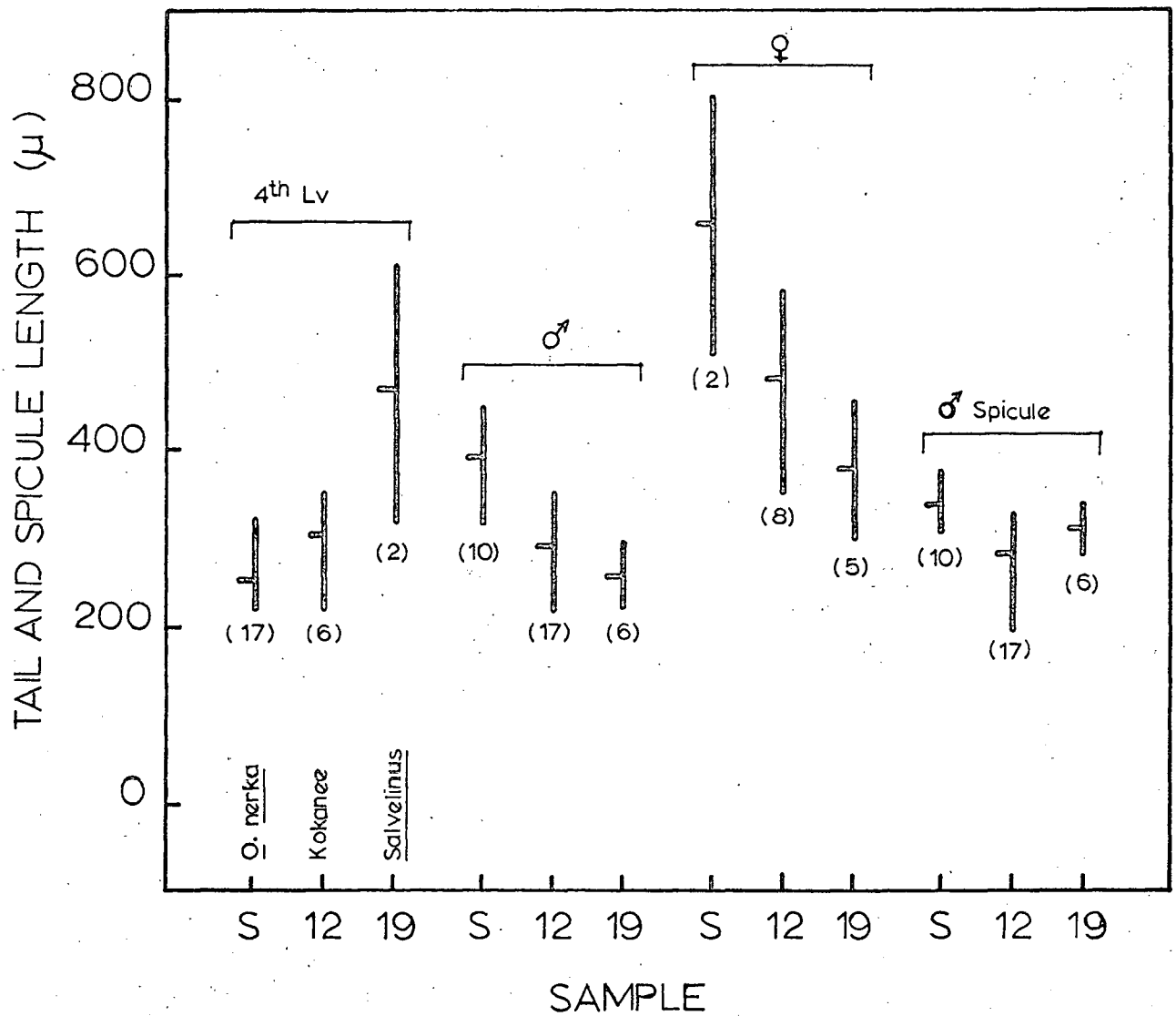

Fig. 26. Tail lengths of fourth stage larvae and preadult Philonema and also spicule lengths of male preadults.