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Title THE MORPHOLOGY AND ECOLOGY OF ECHINORHYNCHUS LAGENIFORMIS EKBAUM, 1938 (ACANTHOCEPHALA)

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The acanthocephalan Echinorhynchus lageniformis Ekbaum, 1938, is a common intestinal parasite of the starry flounder, Platichthys stellatus (Pallas), of Yaquina Bay, Lincoln Co., Oregon. Three hundred and sixty-one flounders were examined and 146 (40.5 percent) were found to harbor infections of E. lageniformis.

Cystacanths of E. lageniformis were found encysted in the body cavity of the starry flounder; this is probably the result of the fish feeding on larval infections not yet mature enough to attach to the intestinal mucosa.

As the size of the flounder increased, the percent infection decreased. The smaller fish with the higher incidence of infection were found to be of the size range that ate amphipods, which may be the intermediate host, as the main part of their diet. The older fish do not feed on amphipods.

A correlation could not be found between the sex of the starry flounder and the incidence and degree of parasitism.

A peak of adult worms was found in April, 1966 - 198 mature females as compared to a low of 30 mature females in December, 1965. This indicates a seasonal periodicity in the *E. lageniformis* population of Yaquina Bay. Possible explanations for this seasonal occurrence were discussed.

It is postulated that *E. lageniformis* lives in its final host for about a year. The one-year-old plus fish were found to have a high incidence of infection, whereas the two-year plus fish had a low incidence of infection.

Twenty-six percent of the worms collected were males and 74 percent were females. Of the 146 infections, 74 were unisexual and 72 were mixed (containing both sexes). In April, 1966, the percent infection was the highest - when there were 20 mixed infections and two unisexual infections. This indicates that all the females have a good chance of being fertilized.

The starry flounder intestine reacts to *E. lageniformis* at the point of attachment. It was observed that the larger the worm, the more severe the reaction.

No crowding effects were observed in single species infections of *E. lageniformis* or in concurrent infections with trematodes.

The shelled acanthor larva was found to have four enveloping membranes.

Data indicates that when the proboscis reaches a certain length and width, it does not increase in size, although the neck and trunk of the worm do increase in size.

THE MORPHOLOGY AND ECOLOGY OF ECHINORHYNCHUS LAGENIFORMIS EKBAUM, 1938 (ACANTHOCEPHALA)

by

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THE MORPHOLOGY AND ECOLOGY OF ECHINORHYNCHUS LAGENIFORMIS EKBAUM, 1938 (ACANTHOCEPHALA)

INTRODUCTION

The acanthocephalan, Echinorhynchus lageniformis Ekbaum, 1938, is a common intestinal parasite of the starry flounder, Platichthys stellatus (Pallas), of Yaquina Bay, Lincoln Co., Oregon. However, there is no report in the literature of Echinorhynchus lageniformis from the West Coast of the United States (California, Oregon, and Washington). Echinorhynchus lageniformis has been cited in the literature as from the starry flounder and occasionally from the two-lined flounder, Lepidopsetta bilineata (Ayres), caught from the inshore waters of Departure Bay, Vancouver Island (Ekbaum, 1938).

In the description of Echinorhynchus lageniformis, Ekbaum (1938) did not include a detailed account of the proboscis morphology nor the criteria used in making proboscis measurements. Also, she did not establish the correct number of egg membranes enveloping the acanthor larva. West (1964) has shown that the acanthor of Echinorhynchus gadi has four enveloping egg membranes instead of three, the number usually cited in the literature for acanthocephalan eggs.

The morphological portion of this thesis will describe the egg membranes and give an account of the proboscis morphology. Proboscis measurements comparable with those of the original description will be included.

When studying the population ecology of acanthocephalans, the following factors should be considered: The incidence of infection in the host, whether or not there is a seasonal periodicity (a peak in adult acanthocephala incidence at one time during the year); relationship of size and sex of host to the infection rate; the longevity of the parasite in the host; the pathological effects of the parasite on the host; the effect of concurrent infections with other helminths and multiple single species infections on the size and number of parasites; the sex structure of the population.

Data on the occurrence of Echinorhynchus lageniformis in the starry flounder of Departure Bay was presented by Ekbaum (1938). Chubb (1964) studied the occurrence of Echinorhynchus clavula in the fish of Llyn Tegid (Bala Lake), Merionethshire, Wales, and found no seasonal periodicity. Neither Polyanski, as cited in Chubb (1964), studying Echinorhynchus gadi in the fishes of the Barents Sea, nor Bauer and Nicol'skaya, as cited in Chubb (1964), in their work on Echinorhynchus salmonis, found a cyclic fluctuation in the acanthocephalan population.

Van Cleave (1916) reported a seasonal occurrence of some acanthocephalans from North American freshwater hosts. He stated that the main causes of seasonal changes are the length of the life cycle of the parasite in the final host, the length of development in the intermediate host, and seasonal changes in the food of the final host. Steinstrasser (1936) cited a seasonal occurrence of the acanthocephalan Neoechinorhynchus rutili from the rainbow trout, Salmo irideus Gibbons. Leptorhynchoides thecatus, a parasite of freshwater fishes in the United States, showed a seasonal periodicity (De Giusti, 1949). Komarova, as cited in Chubb (1964), reported that

Acanthocephalus lucii in the percid fish of the river Dnepr occurred seasonally. Echinorhynchus gadi likewise varied seasonally in the fishes of the White Sea (Shulman and Shulman-Albova, as cited in Chubb, 1964).

Awachie (1965) in studying the ecology of Echinorhynchus truttae in a trout stream in North Wales, found the incidence of the worm is more or less uniform in its definitive host, Salmo trutta, throughout the year, but the degree of parasitism fluctuated irregularly and could be correlated with seasonal feeding by the trout. He did find the larval stages showing a seasonal periodicity in the intermediate host, Gammarus pulex. No correlation between the sex of trout and the incidence of parasitism was found, although a relationship was established between the intensity and between the incidence and the age of the trout.

Ekbaum (1938) and Chubb (1964) both reported more female than male acanthocephala from their respective studies. Styczynska (1958), Van Cleave (1952, 1953), and Nicholas and Hynes (1958) also found that there were more females than males.

The interactions of acanthocephalans with other species of helminths during concurrent infections has been studied by Chubb (1964), Beck (1951), and Holmes (1961, 1962a, b). Although Chubb (1964) observed concurrent infections of Echinorhynchus clavula and other species of parasitic helminths, no interaction was noted between the species of parasites. Beck (1951) reported that there might be a crowding effect between concurrent infections of Moniliformis dubius Meyer, 1933, and the cestode Hymenolepis diminuta Rudolphi,

1819. Recently, Holmes (1961, 1962a, b) confirmed Beck's observations with experimental results, showing that both Hymenolepis diminuta and Moniliformis dubius interacted with one another in that both were reduced in size when compared to single species infections.

Nicholas and Hynes (1958), using single species infections of the acanthocephalan Polymorphus minutus, have examined the effect of crowding. They concluded that the number of worms required to stunt growth was not known, although they suggested that many hundreds would be required. Read (1951) reported that crowding does exist in single cestode infections - that is, the larger the number of cestodes infecting a host, the smaller the worms.

Hyman (1951) stated that the acanthocephala are undoubtedly the most injurious of helminth parasites. Baer (1952) noted that while the proboscis of acanthocephalans is deeply imbedded in the intestinal mucosa, there is hardly any trace of inflamation. In his work with Echinorhynchus clavula, Chubb (1964) found no evidence of harm to the host other than the injury caused by the hooks in the wall of the gut. Nicholas and Hynes (1958) stated that the number of Polymorphus minutus necessary to cause symptoms of disease in the host birds may be in the hundreds.

Prakash and Adams (1960) found Echinorhynchus lageniformis caused intestinal lesions and extensive damage to the intestinal wall of the starry flounder. They reported that the female may penetrate more deeply than the male and induces formation of a chronic nodular outgrowth on the coelomic side of the intestinal wall.

A group of Russian workers (Bauer, Shulman and Shulman-Albova, as cited in Bykhovskaya-Pavlovskaya et al., 1962) have reported that members of the genus Echinorhynchus may cause lesions, ulcerations, inflamation, tumors, and connective tissue hypertrophy where the proboscis penetrates the intestinal mucosa. They have also reported heavy mortality in fish populations with heavy acanthocephalan infections.

Bullock (1963) studied the histology of the post-cecal intestine and the rectum of two species of salmonid fishes, Salvelinus fontinalis and Salmo gairdneri, heavily infected with Acanthocephalus jacksoni. The main effects were found to be damage to the epithelium and proliferation of the connective tissue.

The ecological portion of this thesis will consider the population of Echinorhynchus lageniformis found in the starry flounder of Yaquina Bay.

MATERIALS AND METHODS

Specimens of the starry flounder, Platichthys stellatus, were caught with a 16-foot gulf try net (a small otter trawl) towed slowly along the bay bottom by the R/B CERCARIA. All fish were collected from the vicinity of Sally's Slough or Hinton Point (along the three dolphins) in Yaquina Bay, Lincoln Co., Oregon. After removal from the net, the fish were placed in plastic buckets, without water, for transportation to the Oregon State Marine Science Center. Upon arrival at the laboratory, the fish were placed in a cold room at 4° C for two to three hours. Before examination, each fish was assigned a number corresponding to a number on a "posting" sheet.

The total length of each fish was recorded in centimeters. Next, the intestine was removed, cut into convenient lengths, and placed in a quart jar containing tap water. Determination of the sex of the starry flounder was made by examination of the gonads following Orcutt's method (1950). The pieces of intestine were cut lengthwise. As each piece was cut, it was examined for acanthocephalans, which were carefully removed from the intestine with a pair of fine jewelers' forceps.

The worms were placed in a numbered vial filled with distilled water. At this time, the number of male and female worms was recorded. The vials with the worms were placed in a cold room at 4° C overnight. This relaxed the worms so the proboscis remained extended when fixed. Van Cleave's fixative was used (85 parts of

85 percent ethyl alcohol, 10 parts commercial formalin, and 5 parts glacial acetic acid).

In the preparation of stained and cleared acanthocephalans, a technique described by Chubb (1962) was followed. The acanthocephalans were taken from the fixative and placed directly in the stain (3 parts of 45 percent acetic acid and 1 part Ehrlich's hematoxylin) for fifteen minutes. Dehydration was carried out in glacial acetic acid for six hours. The worms were cleared in successive mixtures of glacial acetic acid and methyl salicylate in proportions of 3:1, 1:1, and 1:3, followed by pure methyl salicylate. When the worms were placed in the above mixtures, they first floated, but when penetration was complete, they sank to the bottom. When sinking occurred, they could be moved to the next solution. The worms were now stored in pure methyl salicylate, where they may be kept indefinitely.

Although other techniques were tried, this technique was found to be the most satisfactory in terms of less shrinkage and shriveling of the worm, and also the time involved was much less than the method outlined by Van Cleave (1953).

Whole mount slides were made in the following way: From the pure salicylate, the specimens were washed in xylol for two to three minutes and then mounted in picolyte.

All female worms were examined to determine their state of sexual maturity, following the criteria set forth by Chubb (1964). Mature worms were those with shelled acanthor stages (even though some ovarian balls were present) and immature ones were those with only ovarian balls in the body cavity.

Morphological examinations were carried out with the worms on temporary slides in pure salicylate.

All measurements are in millimeters and were taken with an ocular micrometer from unmounted specimens.

Measurements of the proboscis and hooks were made using the following criteria: The length of the proboscis was considered as the straight line distance between the free anterior extremity and the base of the thorn of the most posterior series of hooks. Measurement of the diameter of the proboscis was taken at the widest point and did not include the thorns of the hooks which extended beyond the proboscis wall (Van Cleave, 1953).

Areas of normal and infected intestine of the starry flounder were sectioned in order to determine the effect of the parasite on the intestinal tissue. The whole intestine was fixed in Bouin's and then areas of normal intestine and areas where the worm had been attached were removed and embedded in tissuemat and sectioned at ten microns. All sections were stained with Harris Hematoxylin and Eosin Y and then mounted in permount. Photographs were made with a Zeiss Photomicroscope.

MORPHOLOGICAL RESULTS AND DISCUSSION

The following is an amended description of Echinorhynchus lageniformis Ekbaum, 1938, after Ward, 1951: Males, fusiform in shape; length 1.5 - 5.0 mm, width 0.5 - 0.8 mm. Females 2.5 - 6.5 mm in length; bulbous swelling posterior part 1.2 - 2.2 mm in diameter, diameter of anterior cylindrical region 0.4 - 0.7 mm. Proboscis 0.25 - 0.40 mm long, cylindrical in shape. Hooks 14 - 16 longitudinal rows of eight to ten each. Apical hooks 0.045 - 0.050 mm long, median 0.050 - 0.055, basal 0.035 - 0.040. Proboscis sheath 0.8 - 1.2 mm long; lemnisci slightly longer. Testes placed obliquely, 0.30 - 0.50 x 0.20 - 0.35 mm. Six pear-shaped cement glands 0.20 - 0.25 x 0.10 - 0.15 mm. Eggs 0.065 - 0.080 mm x 0.015 - 0.020 mm.

This is an acanthocephalan with very marked sexual dimorphism, and Ekbaum (1938) stated that this morphological difference is already apparent in individuals 1.5 mm long. I was able to detect differentiation in female worms which were 1.0 mm to 1.1 mm long.

Ekbaum (1938) did not tell how she made the proboscis measurements. Using Van Cleave's (1953) method, the following measurements were obtained: Thirty females varying in length from 1.2 - 5.5 mm had proboscis lengths of 0.34 - 0.44 mm and widths of 0.18 - 0.24 mm. Measurement of twelve males varying in length from 1.7 - 4.6 mm had proboscis lengths of 0.36 - 0.40 mm and widths of 0.22 - 0.46 mm.

If the measurement of total length is plotted against proboscis width and length (Figure 4, p. 39), it appears that while the rest of the worm grows, the proboscis length and width remain fairly constant. Thus, it seems that when the females reach 1.2 - 1.4 mm in length, the proboscis has reached a size that is large enough and with hooks of sufficient size to serve as an adequate organ of attachment for the growing worm.

Van Cleave (1952) pointed out that for a number of Echinorhynchus species, the proboscis of the juvenile or cystacanth inside the arthropod host had attained all features of a fully mature worm located in the final vertebrate host. The cystacanth stage also included the same number of proboscis hooks as found in the adult.

Although E. lageniformis exhibits variability in the number of hooks per row (Ekbaum, 1938), Van Cleave (1952) pointed out that many investigators had used this as false evidence of an increase in number of hooks during ontogeny. He stated that:

> "Every particle of the available evidence seems to contradict the assumption the number and size of hooks and the proboscis size increases with the growth of the whole animal. In the first place, no one has ever recorded the discovery of hook rudiments on the proboscis of any adult or juvenile acanthocephalan. In assuming that hook numbers increased with the growth of the worm, one loses sight of the possibility that proboscis size and hook number may be linked in a predetermined pattern of heredity in which both the size of the proboscis and the number of hooks are correlated and are genetically determined."

In 1952, Van Cleave pointed out that the most conspicuous changes or modifications of body form are adaptations as accessory structures for attachment. An exception to this is the formation of the bulbous posterior end of the female E. lageniformis, which does not function as a holdfast organ, but primarily serves as a device for increasing the storage area or capacity for developing eggs (Van Cleave, 1952).

Along with her original description, Ekbaum (1938) included a figure of the egg showing the acanthor with two enveloping membranes. West (1964) found that *Echinorhynchus gadi* has four membranes enveloping the acanthor - an outer, thin membrane which was the original one surrounding the unfertilized egg; beneath this, a fibrillar coat or membrane; next, the fertilization membrane, which appears as a rigid shell with pronounced polar elongations; the fourth and inner-most membrane, a thin one in close association with the acanthor. The shelled acanthors of *E. lageniformis* were also found to have four membranes, which were all similar in properties and appearance to those described by West for *E. gadi*.

ECOLOGICAL RESULTS

During the period of June, 1965, to April, 1966, a total of 361 starry flounder from Yaquina Bay were examined for the acanthocephalan Echinorhynchus lageniformis. One hundred forty six, or 40.5 percent, were found to be infected. A total of 1,506 worms were recovered from the intestines, with the mean number of E. lageniformis per infected fish being 10.3 (Table 1, p. 33).

Table 2 (p. 34) includes monthly summations for the period of observations. The number of fish examined in June, 1965, was higher than other months because two samples were taken. There was only one sample taken for the October-November period; it was collected approximately halfway between the preceeding and following samples. The range of percent infection varied from 68 percent in April, 1966, to a low of 14 percent in December, 1965, whereas the mean number per infected fish ranged from 19.5 (April, 1966) to 3.3 (October-November, 1965). Figure 1 (p. 36) shows the relationship between the percent infection and mean number over the entire observational period. The wide variation and separation of the two lines is due to the fact that even though the percent infection is low (as in December, 1965), one or two fish may have a large number of parasites, and thus greatly skew the mean upward. The range of worms infecting the fish was from 0 to 136.

The majority of worms was found attached in the upper part of the intestine, immediately behind the junction with the stomach. In heavy infections, the first quarter to fifth of the intestine had worms evenly distributed along this area. Some were found attached as far back as half the length of the intestine. Worms attached this far back were always old, large females. Males were occasionally found in the food material in the intestine.

Cystacanths were observed in 18 infections encysted on the outside of both the liver and intestine and in the intestinal mesenteries.

Multiple infections (infections of different age groups) of E. lageniformis were found in 12 of the 146 infections, or in 8.2 percent. The two age groups could be recognized one from the other on the basis of size and were randomly intermingled in all but two infections. In the two instances where the groups were not associated, the old infection consisted of one old female, which was attached farther back in the intestine than the younger group. The older groups of worms consisted entirely of females in 10 of the 12 multiple infections; whereas the young infections consisted of both males and females. In 11 of the 12 multiple infections, the younger infection outnumbered the older one.

Concurrent intestinal infections with E. lageniformis and other helminths were observed only twice. Both concurrent infections were with trematodes. Nematodes and trematodes were quite common in the stomachs of infected starry flounders. Trematodes were found in the intestines of 23 fish not infected with E. lageniformis. The most common helminth found in the digestive system of the starry flounder was E. lageniformis. It was the only acanthocephalan observed.

The size of fish examined ranged from 10 to 51 cm. Figure 2 (p. 37) shows that there were two peaks in the size frequency, one at 21 cm and the other at 30 cm. This would indicate that the majority of fish examined belonged to two different year classes. From June, 1965, to January, 1966, the monthly fish samples showed a wide variation in mean length. The mean decreased from 28 cm, in January, to 16 cm, in April. This indicates that smaller fish were appearing in the sample, causing the mean to decrease. The majority of smaller fish from February to April had younger infections and larger numbers of parasites per individual than the larger fish examined during the same time period.

No quantitative studies were made of food found in the flounder intestines, but since amphipods are assumed to be the intermediate host of E. lageniformis (no isopods were observed in the stomach contents), their presence was recorded. Amphipods were found in 51 intestines, or 14.5 occurrences per 100 intestines examined. The size of fish found with amphipods ranged from 12 to 29 cm, with the majority (41) being 12 to 21 cm in length. Intestinal contents of fish 20 cm or longer consisted mostly of small Cancer magister, Macoma, and other pelecypods, polychaetes, and shrimps.

As can be seen from Figure 2 (p. 37), as the size of the fish increased, the percent infection decreased. No E. lageniformis was found in fish longer than 36 cm. The smaller fish with the higher incidence of infection were of the same size range as those with amphipods as the main part of their diet. No infected amphipods were observed.

No correlation could be found between the sex of the starry flounder and the percent infection (Table 3, p. 35). Although monthly figures varied, the yearly figures were not significantly different. Over the entire collection period, 38 percent of the males and 41 percent of the females were found to be infected, and the males had a mean number of 9.3 worms per infection while the females had a mean of 9.8 worms per infection.

Of the total of 1,506 worms collected, 396 (26.2 percent) were males and 1,110 (73.8 percent) were females (Table 1, p. 33). Table 2 (p. 34) includes the number and percent of each sex for each month's collection. The ratio of males to female worms increased during early spring and decreased in late summer (Figure 1, p. 36).

A total of 899 female worms was examined to determine if they were sexually immature or mature. Two hundred and six (23 percent) were found to be immature, and 693 (77 percent) were mature (Table 1, p. 33). Table 2 (p. 34) has similar information for each month, with the number of mature females varying during the collection period, but reaching a peak in April, 1966.

Of the 146 infections, 74 were unisexual (consisting of only one sex) - 69 being all females and 5 all males. Seventy-two of the infections were mixed (containing both females and males). Except in April, 1966, and September, 1965, there were more unisexual than mixed units (the acanthocephala from a single infected fish were considered one unit). In the April collection, 20 mixed infections were found to 2 unisexual ones. June, 1965, showed an almost equal number of unisexual to mixed infections, and after June the number of unisexual to mixed units increased - except in September (Figure 5, p. 40). The increase of mixed units in the months of January, February, March, and April corresponds to the increase in ratio of males to females.

The starry flounder intestine reacts to *Echinorhynchus lageniformis* at the point of attachment. Two reactions can be seen: (1) a polypoid protrusion on the outside of the intestinal wall and (2) a similar protrusion in the lumen of the intestine. The reaction on the outside of the intestine varies in size from a small, barely visible bump, to a protrusion 1 to 2 mm long. The reaction on the inside also varies in size. It may be just barely visible among the rugae of the intestine, or it may be taller than the rugae, with a diameter of 1 to 2 mm, and surrounding the neck of the worm. The larger reactions are associated with large female worms. Male worms cause only small protrusions, if any at all.

Since the males do not cause as severe reactions as the females, the following comparison is based on the reactions caused by females. Comparison of normal intestine (Figure 6, p. 42) to infected intestine (Figure 7, p. 42) showed that the female attached above the submucosa region. A detailed study of the cell types of the reaction was not made. In the middle of the reaction area, there is a hole made by the proboscis of the worm (Figure 7 and Figure 8, p. 42). As the reaction grows towards the serosa, the cells of the reaction invade the submucosa and muscularis externa and cause them to be reduced (Figure 9, p. 42). As the reaction cells grow toward the lumen, they surround the neck of the animal and the rugae around the point of attachment become reduced.

DISCUSSION

In this study, attachment of most *Echinorhynchus lageniformis* in the starry flounder intestine was along the same length as described by Ekbaum (1938). She also stated that occasionally the parasite was found throughout the length of the intestine. This was not observed in this Yaquina Bay study.

Finding cystacanths encysted outside the intestine of the vertebrate host is not uncommon in acanthocephalan infections. Besides *E. lageniformis* encysted in the body cavity of the starry flounder, I observed *Echinorhynchus gadi* cystacanths encysted in the intestinal mesenteries of *Leptacottus armatus* collected from Yaquina Bay. De Giusti (1949), working on the life cycle of *Leptorhynchoides thecatus*, found cystacanths encysted in the body cavity of the rock bass *Ambloplites rupestris*. When he conducted experimental infections (feeding the infected amphipod intermediate host to fish known to be free of the parasite), he found the following: The cystacanth in some cases became encysted in the fish mesenteries. If fish were fed amphipods containing juveniles at least 30 days old, the parasites were able to establish themselves in the intestine. If the ingested amphipods contained juveniles up to 28 days old, the worm was unable to establish itself in the intestine, but was found to encyst in the intestinal mesenteries. Acanthella stages were unable to establish themselves in either the intestine or the body cavity. De Giusti concluded that cystacanths encysted in the body cavity of bass represent larvae not sufficiently mature when ingested by the fish to be able to establish themselves in the intestine. The appearance of *E. lageniformis* cystacanths in the body cavity of the starry flounder is probably due to the fish feeding upon amphipods carrying the worms at various ages.

Ekbaum (1938) found 26.7 percent of the starry flounders from Departure Bay inshore waters infected with *E. lageniformis*. This is lower than the 40.6 percent infection found in the starry flounder of Yaquina Bay. Possibly the discrepancy between these two studies is due to availability of the intermediate host. Another significant factor may be that Ekbaum's sample was less complete than the samples in this Yaquina Bay study. She examined a total of 116 fish as compared to 361 in this investigation, and her samples were from four collections in 1934 and three in 1936.

The starry flounder showed an infection range of 14 to 68 percent through the year (Figure 1, p. 36), indicating a seasonal trend. The value for the October-November sample is not considered representative because of the small sample size. Throughout the year, Awachie (1965) found a 76.2 to 100 percent infection of *Echinorhynchus truttae* in the brown trout. He concluded that the consistently high incidence was a result of continuous feeding by the trout upon the intermediate host. Chubb (1964) studied the ecology of *Echinorhynchus clavula* in four fish (the grayling, *Thymallus thymallus*; pike, *Esox lucius*; roach, *Rutilus rutilus*; and eel, *Anguilla anguilla*) and found the total infection rate for these species to be 46.0, 11.5, 16.1, and 27.7 percent respectively. He was able to correlate the degree of infection with the feeding upon the intermediate host. The grayling, which had the highest infection rate of the four species examined, contained the intermediate host in its stomach 50 percent of the time. Its yearly range was from 33 percent to 55 percent - again indicating a fairly constant feeding on the intermediate host. The large monthly range of infection found in this study indicates that the starry flounder was not consistently feeding on the intermediate host.

From the work of Chubb (1964) and Awachie (1965), it becomes clear that to understand the dynamics of a parasite population, one must also understand the biology of the final host. Chubb (1964), in discussing the occurrence of E. clavula in the fishes of Llyn Tegid, points out two important concepts that are commonly overlooked: (1) The fish acquire direct infections by feeding upon the intermediate host and (2) seasonal variation in feeding intensity or changing of food habits by a species affects its chances of acquiring the parasite. Thus, to interpret the wide variance in percent infection of E. lageniformis over the year, it becomes necessary to understand the biology of their final host, the starry flounder.

Orcutt (1950) studied the life history of the starry flounder of Monterey Bay, California. Upon analysis of the stomach contents of 250 specimens from 1.5 cm to 53.6 cm in length, he found changes in diet correlated with an increase in specimen size. His results indicated that fish in the 1.0 to 3.9 cm size range feed mainly on copepods; amphipods are the main diet of fish 4.0 cm to 17.9 cm long; above 18.0 cm they feed principally on clams, clam siphon tips, and small cancer crabs. Orcutt also computed the age from scale analyses and was able to show an age-length relationship (Figure 3, p. 38). Comparison of Orcutt's data with the length frequency graph of fish observed in this Yaquina Bay study disclosed two peaks - a two-year-old peak at 21 cm and a three-year-old peak at 30 cm.

In looking at the variance of monthly incidence of infection (Figure 1, p. 36), the month with highest percent infection (68 percent) was April, 1966, although only two months before, in February, the infection rate was only 20 percent. It is important to note that mean fish length in February was 28 cm, whereas in April it was 16 cm. The March and April samples included smaller fish than in previous months. These smaller fish were of the size that Orcutt (1950) reported as having amphipods as the main part of their diet. The high percent infection in April and mean number per infected fish can be correlated with the fact that these smaller fish were eating mainly amphipods, and thus were obtaining the infection.

Observations of the change in food habits with an increase in size in both this study and Orcutt's 1950 work explain why the larger fish are not parasitized (Figure 2, p. 37). The small fish, with high infection rates, are the ones which feed mainly on amphipods. As the fishes grow, their food habits change and the intermediate host is no longer fed upon. This results in the individual fishes eventually losing their infections. Larger fish found with acanthocephalan infections occurring after the intermediate host is no longer a main part of their diet may have picked up this infection near the time of change in food habits, or they may occasionally feed on an amphipod and thus be reinfected.

Awachie (1965) found an opposite situation in his study, noting a general increase in incidence and intensity of trout infection by Echinorhynchus truttae with increased length and age of the fish. Also, the mean number of parasites per age group rose from 49 in the one-plus year age group to 201 in the four-plus year group. He was able to explain these observations by showing that the larger fishes eat larger numbers of the intermediate host.

In a study of the relation between the sex of brown trout and the intensity and incidence of E. truttae in these fish, Awachie (1965) found no apparent correlation between the sex of the trout and the incidence and degree of parasitism. His monthly figures fluctuated, but the total infection for the year of male and female fish was very similar in percent and mean number per infected fish. The males had an 89 percent infection with a mean of 14.3 parasites per fish for the year; whereas the females had an 86.8 percent and 19.3 mean number for the year. When comparing Awachie's data with similar data from this Yaquina Bay investigation (Table 3, p. 35), one would have to conclude that there is no correlation in either case between the sex of the fish and the incidence and intensity of the acanthocephalan infection.

In determining whether or not E. lageniformis shows a seasonal periodicity, Chubb (1964) stated that where a seasonal periodicity has been reported, there has been a peak in adult acanthocephala incidence at one time of the year. He found that development of E. clavula was uniform and no peak incidence was observed, although the level of occurrence was irregular throughout the year. This irregularity, he felt, fell within a range which is probably determined by the feeding intensity on and the availability of the intermediate host.

There is a peak in April, 1966, of mean number of parasites and the percent infection in Yaquina Bay (Figure 1, p. 36). At this time, there was an abundance of adult worms, demonstrated by the presence of 198 mature females (with shelled acanthors) as compared to 30 in December and 20 in October-November (Table 2, p. 34). The late winter and early spring rise in intensity of infection and number of mature worms is correlated with the appearance of young, one to one-plus year age fish with high infections. The main food of this group was amphipods. It could be that this peak of incidence is a sampling phenomenon. The infection may be higher in the winter than shows because of the inability of the net to catch infected starry flounder smaller than 10 cm. Perhaps a sample of small starry flounder would clear this picture up.

There is another possible explanation for the seasonal occurrence. The two-plus year class fish caught in April contained few parasites, whereas the one and one-plus year class caught at the same time contained a large number of worms. These smaller fish carried very young infections as determined by the small body size of the acanthocephalans. During the winter when the intensity and mean number were lowest, the mean length of the fish being caught was the highest, and the number of mature worms was low. These larger (two and two-plus year) fish were no longer using the intermediate host as part of their diet, and thus had lost or were losing acanthocephalan infection. Then in late winter and early spring, the one and one-plus year class was becoming infected by feeding on the intermediate host, causing the percent infection, the mean number, and the number of mature worms to increase at this time. These phenomena could then combine to cause the seasonal fluctuation in *E. lageniformis* populations.

Thus, it would seem that *E. lageniformis* lives in the final host for about one year, infecting the fish only during the time in their life history when they feed upon the intermediate host (one to one-plus year class or earlier) and then dying out by the time the fish are of the two-plus year class. Hence, the parasites seasonal periodicity may be a result of change in food habits by the final host.

The above conclusions contrast with Chubb's 1964 study, which postulated a dynamic equilibrium with a variable, but continuous level of infection of the fish by newly ingested cystacanths, and an equally variable, continuous loss of mature worms. He concluded that the population of worms in the fish at any given time is the result of interaction between these two variables. Awachie (1965) found a similar dynamic equilibrium with *E. truttae*. There was a high continuous rate of feeding upon the intermediate host by the final host throughout the year.

De Giusti (1949), in his study of the *Leptorhynchoides thecatus* life cycle, observed a seasonal periodicity. In May and June, the fish contained large numbers of immature worms, but, as the season progressed, the number of immature acanthocephalans decreased until the majority present were fully mature males or females. In October and November, few immature individuals were present, and also in December the worm number declined. By the end of February, the acanthocephalan was found only occasionally. De Giusti also concluded that the parasite life span may be one year.

There has been some discussion as to the role of temperature in regulating acanthocephalan infections. Shulman and Shulman-Albova, as cited in Chubb (1964), found a seasonal periodicity of E. gadi in the fishes of the White Sea, which freezes over during the winter. Shelled acanthors began appearing at the end of July and the worm incidence decreased in August. In September, the incidence of the worm began to increase again, owing to the appearance of the new generation of parasites in the fish. Polyanski, as cited in Chubb (1964), found no cyclical fluctuation of E. gadi in the Barents Sea, which does not freeze over in the winter. The development of the worm was distributed uniformly throughout the year, which situation compares well with Chubb's 1964 findings in the fish of Llyn Tegid lake, which also does not freeze over in winter. Awachie (1965) discovered no seasonal cycle in the incidence of E. truttae from a stream in North Wales which is geographically in the same area as Llyn Tegid. Chubb (1964) and Awachie (1965) both postulate that temperature may play a major role in the determination of the presence or absence of a well-defined seasonal periodicity of some acanthocephala. Since Yaquina Bay does not freeze over during the year and there is a seasonal periodicity of E. lageniformis, this would seem to indicate that temperature is not a significant controlling factor in the infection by E. lageniformis of the starry flounder.

In examining the sexual composition of acanthocephalan populations, workers have found the female worm to be far more abundant than the male worm (Awachie, 1965; Chubb, 1964; Ekbaum, 1938). Ekbaum (1938) found 18 percent male E. lageniformis. Hoffman, as cited in Chubb (1964), reported the occurrence of 21.7 percent male worms of E. truttae in the trout Salmo trutta. In this investigation, 26.2 percent of the worms were males (Table 1, p. 33). Van Cleave (1952, 1953) and Nicholas and Hynes (1958) have reported that male acanthocephalans tend to disappear from the host sooner than the female, thus leaving a population chiefly or exclusively of females. Van Cleave (1952) pointed out that these findings have led many investigators to think that there are only a few males, that these few males are in only a small number of infections, and thus the chance for females to become fertilized is low. Nicholas and Hynes (1958) reported that the males leave the final host after copulation with the female worms.

Styczynska (1958) in looking at the sex proportion of larval forms of Acanthocephalus lucii in an isopod, reported close to 40 percent males - which corresponds to the higher percentage of males in the April-June period of this Yaquina Bay study (Table 2, p. 34). Thus, between April and July probably is the period when the sex proportions of the population are most nearly equal. This was the time of greatest occurrence of the younger infections, which have the highest male to female ratios (Figure 1, p. 36). At this same time, greater numbers of mixed units appeared in the fish (Figure 5, p. 40), again caused by the younger infections containing more males than found in older infections. The July and August drop in sex ratio (Figure 1, p. 36) would indiciate the males have copulated and are passing out of the host. The high number of mixed units in the April sample suggests that there is a good chance for the majority of females to become fertilized. Ekbaum (1938) found that, when the parasites of only one sex occurred, these were always females and in all infections the females always outnumbered the males. In this Yaquina Bay work, five infections were found to consist entirely of males, and in three of the mixed infections males were in the majority.

A crowding reaction between parasites of a single species has been shown by Read (1951) experimentally. He found that the cestode Hymenolepis diminuta was progressively smaller with increasingly heavier infections; but with the decreasing size, the surface area per unit of weight became larger. He postulated that as the number of worms increased, the ability of each worm to meet its biological requirements decreased. Thus, by reducing their size, these crowded worms can survive by maintaining a higher than usual relative surface area per unit of weight. Nicholas and Hynes (1958), using single infections of the acanthocephalan Polymorphus minutus, did not observe any stunting of growth, but thought that perhaps an infection of many hundreds of worms might result in reduced size of individuals. No crowding reactions were noted in the single species infections of E. lageniformis.

Although only two concurrent infections of E. lageniformis with other helminths, both trematodes, were observed in the intestine of the starry flounder, this does not necessarily mean that there is an interaction taking place nor that the acanthocephalans are outcompeting the trematodes for space and food. Since 23 intestinal infections consisting only of trematodes were found with a majority in the two-plus year class of fish, it may be that the flounders' changing food habits cause a succession in the parasite fauna. Chubb (1964) found concurrent infections of Echinorhynchus clavula and other species of helminths, but observed no interactions between the species.

Holmes (1961, 1962a, b), using experimental infections of the acanthocephalan Moniliformis dubius and the cestode Hymenolepis diminuta, found that when in the same host, both helminths have smaller length to average weight ratios than when in single infections. They also are found in different parts of the intestine.

It should be pointed out that, although crowding and competition in single and concurrent infections of helminths can be shown experimentally, it is difficult to identify these conditions in naturally occurring parasite populations, just as it is hard to determine in non-parasitic populations of animals.

Prakash and Adams (1960) also studied the reaction of E. lageniformis in the intestine of the starry flounder. They reported the polypoid extrusion on the serosal side of the intestine, but did not mention the degeneration of the muscularis externa, muscularis mucosae, and rugae. This may be because the infection they studied was a young one. The picture in their paper that shows a female attached in the submucosa is of a very young female, as the posterior swelling does not extend above the rugae. Old females cause large reactions and extend well above the rugae.

A complete cellular description of the reaction area was made by Prakash and Adams (1960). Quoting from them:

> "A critical examination of this polypoid formation reveals that it is not a neoplastic tissue but a case of chronic granuloma as evidenced by the presence of irregularly scattered small round cells, mononuclear histiocytes and fibroblasts. The granulation tissue at the site of the lesions consists of small round cells (lymphocytes) and mononuclear histocytes. An abundance of fibroblasts is also noticed which in the immediate vicinity of the parasite tend to form a collagenic tissue."

The lesions that they observed were deficient in eosinophils and even in advanced necrosis no bacteria were found.

They concluded that since the most damage is associated with female worms, and they are known to penetrate deeper than the males, that the severity of the infection is largely a matter of degree of penetration by the parasite rather than due to the action of pathogenic bacteria. Also, it can be concluded, from this Yaquina Bay study, that the larger the female, the more severe the reaction. Other than the damage caused by the worm in the intestine, nothing else was noticed that would indicate that worms were detrimental to their host.

SUMMARY

The ecology of Echinorhynchus lageniformis from the starry flounder of Yaquina Bay has been discussed.

Three hundred and sixty-one starry flounders were examined and 146 (40.5 percent) were found to be infected. The range of infection varied from a high of 68 percent in April, 1966, to a low of 14 percent in December, 1965.

The total length of males and females was plotted against proboscis length and width. It indicated that as the neck and trunk of the worm grow, the proboscis length and width remain fairly constant.

The shelled acanthor larva was found to have four enveloping membranes.

Cystacanths were found encysted in the body cavity of the starry flounder; this is probably due to the fish feeding on an intermediate host with larval infections not yet mature enough to attach to the intestine.

The starry flounders were found to undergo a change in food habits with an increase in size. It was observed that as the size of the fish increased, the percent infection decreased. The smaller fish with the higher incidences of infection were found to be the same size range as those with amphipods as the main part of their diet.

No correlation could be shown between the sex of the starry flounder and the incidence and degree of parasitism.

A seasonal periodicity was found for the *Echinorhynchus lageniformis* of Yaquina Bay. A peak of adult worms was found in April, 1966 - 198 mature females in 32 fish as compared to a low of 30 mature females in 36 fish in December, 1965. Possible explanations for this seasonal occurrence were discussed.

It is postulated that *E. lageniformis* lives in its final host for about a year. The one-year-old fish were found to be heavily infected while the two and two-plus-year-old fish had a low incidence of infection.

Of the total worms collected, 26.2 percent were males and 73.8 percent were females. Of the 146 infections, 74 were unisexual and 72 were mixed. In April, 1966, when the percent infection was the highest, there were 20 mixed infections to 2 unisexual infections. This indicated that all females have a good chance of being fertilized.

The starry flounder intestine reacts to *E. lageniformis* at the point of attachment. It was observed that the larger the worm, the more severe the reaction.

No crowding effects were observed in single species infections of *E. lageniformis* or in concurrent infections with trematodes.

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APPENDIX

Table 1. The occurrence of *Echinorhynchus lageniformis* in the starry flounder of Yaquina Bay. The results for the complete period of observation, June 1965 to April 1966, are summed.

Total Number Fish Examined	361
Total Number Fish Infected	146
Percent Fish Infected	40.5
Total Worms	1506
Mean Number of *E. lageniformis* per Infected Fish	10.3
Number of Each Sex of *E. lageniformis*	
Male	396
Female	1110
Percent of Each Sex of *E. lageniformis*	
Male	26.2
Female	73.8
Number Female Worms Examined for Sexual Maturity	899
1. Ovarian Ball Stages	206
2. Shelled Acanthor Stages	693
Length Range of Fish Examined in cm	10-51

Table 2. The occurrence of *Echinorhynchus lageniformis* in the starry flounder of Yaquina Bay, Lincoln County, Oregon. The results are summed for each month.

Month	Year	Number Fish Examined	Number Fish Infected	Percent Fish Infected	Mean Number *E. lageniformis* per Infected Fish	Observed Numbers		Percent		Number Females Examined for Stage of Sexual Maturity	1. Ovarian Ball Stages	2. Shelled Acanthor Stages
						Males	Females	Males	Females			
June	65	83	32	39	9.2	113	182	38	62	116	20	96
July	65	50	20	40	6.5	37	94	28	72	39	7	32
August	65	27	12	45	6.8	12	70	14.5	85.5	63	23	40
September	65	21	9	43	9.5	15	69	17.9	82.1	54	6	48
October November	65	15	9	60	3.3	1	29	3.3	96.7	29	9	20
December	65	36	5	14	9.4	11	36	23.4	76.6	36	6	30
January	66	26	10	38	7.6	15	61	19.7	80.3	58	14	44
February	66	30	6	20	10.8	15	61	19.5	80.5	53	11	42
March	66	41	21	51	12.2	46	209	19	81.0	189	46	143
April	66	32	22	68	19.5	131	299	30.5	69.5	262	64	198

Table 3. The relation between the sex of the starry flounder and the incidence of infection.

Month	MALE				FEMALE			
	Number Examined	Number Infected	Percent Infected	Mean Number Parasites per Infected Fish	Number Examined	Number Infected	Percent Infected	Mean Number Parasites per Infected Fish
June	41	14	34	8	21	7	33	7.8
July	27	10	34	4.9	20	8	40	9.5
August	14	7	50	9.8	12	4	33	3.0
September	11	4	36	8.5	10	5	50	10.0
October November	9	5	55	1.2	6	4	66	6.0
December	22	2	9	2.5	14	3	20	6.6
January	11	4	36	5.25	11	6	54	3.0
February	17	3	18	16.0	13	4	31	7.0
March	22	14	64	6.6	19	7	40	23.3
April	14	9	64	29.9	14	10	71	11.9
May								
Totals	188	72	38	9.26	140	58	41	9.8

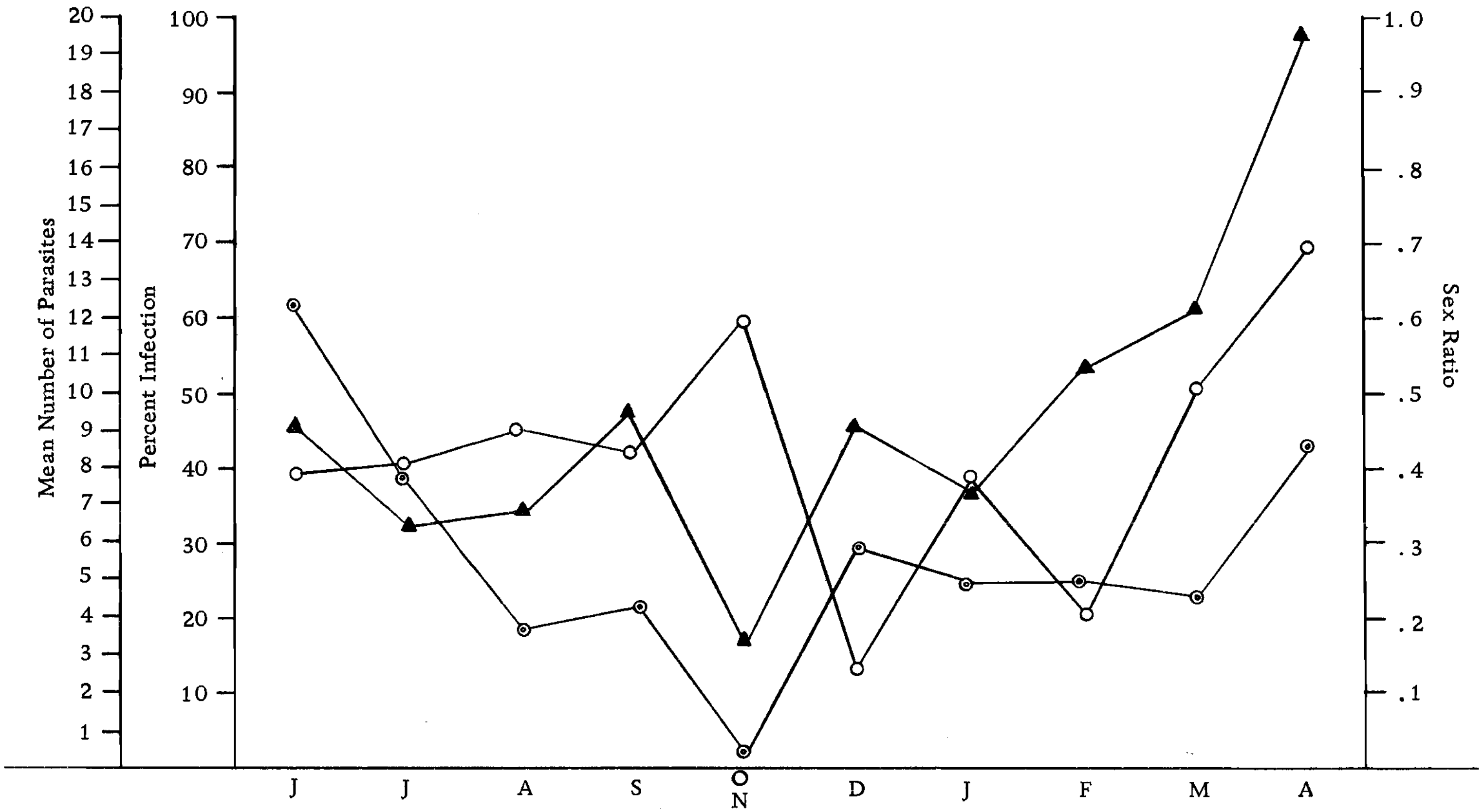

Figure 1. Summarizing the occurrence and seasonal trends in the composition of the population of <u>Echinorhynchus lageniformis</u> in the starry flounder of Yaquina Bay. ◎—◎ sex ratio (No. of male worms/No. of female worms); ○—○ percent infection; ▲—▲ mean number of parasites.

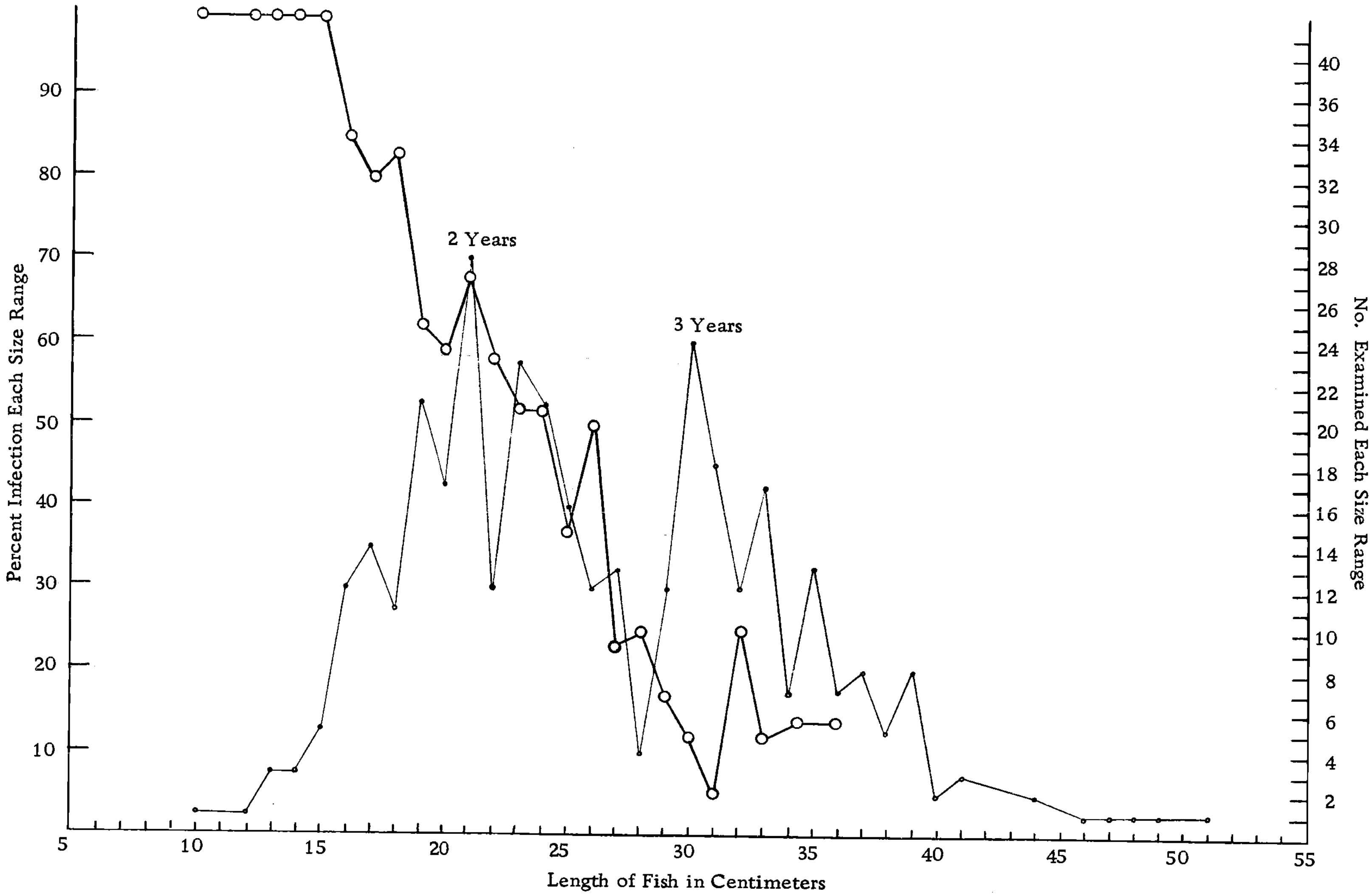

Figure 2. The relationship between the length and age of the fish and percent infection. o—o No. fish examined for each size range, O—O percent infection of each size range of fish.

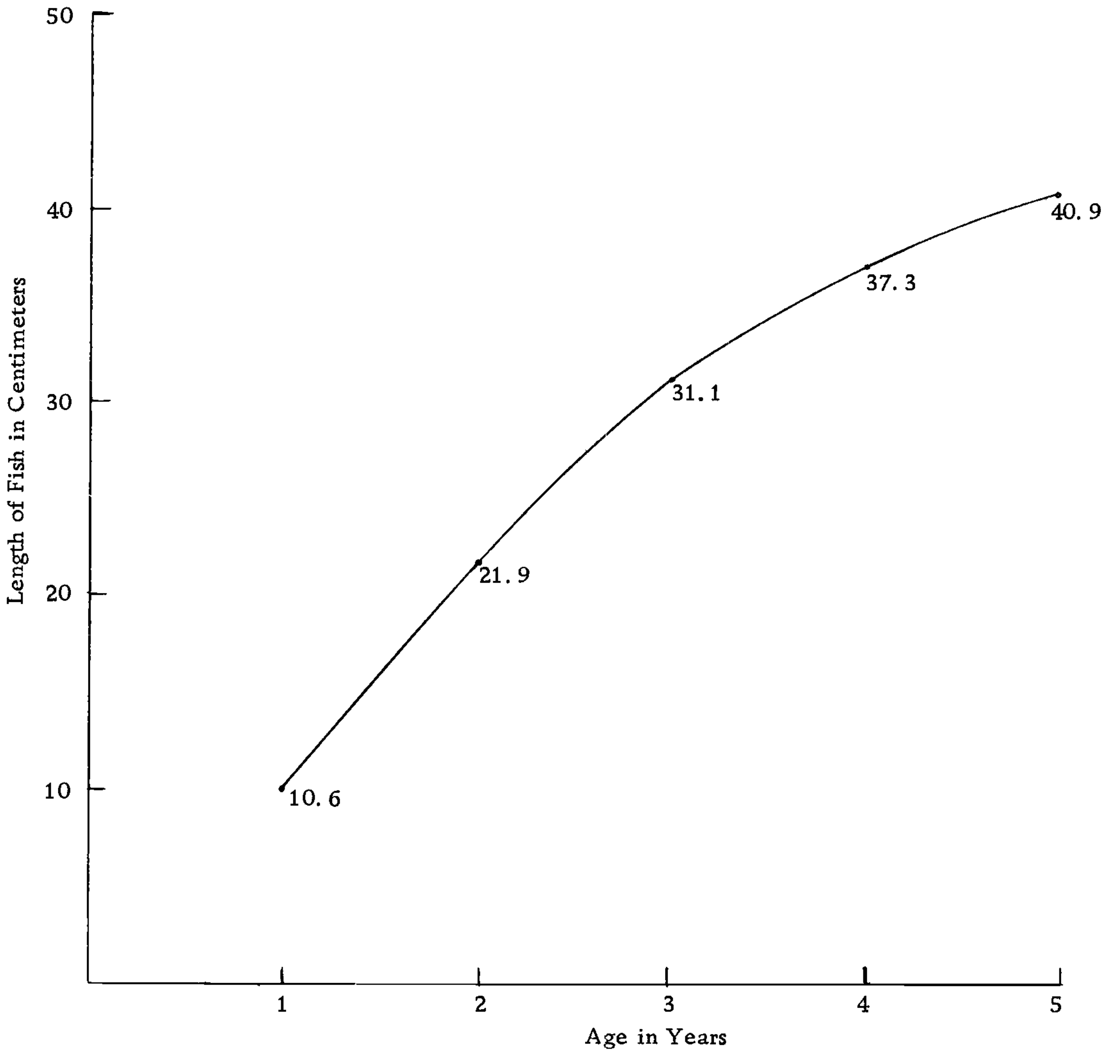

Figure 3. Age-length relationship of the starry flounder. Combined male and female lengths. After Orcutt (1950).

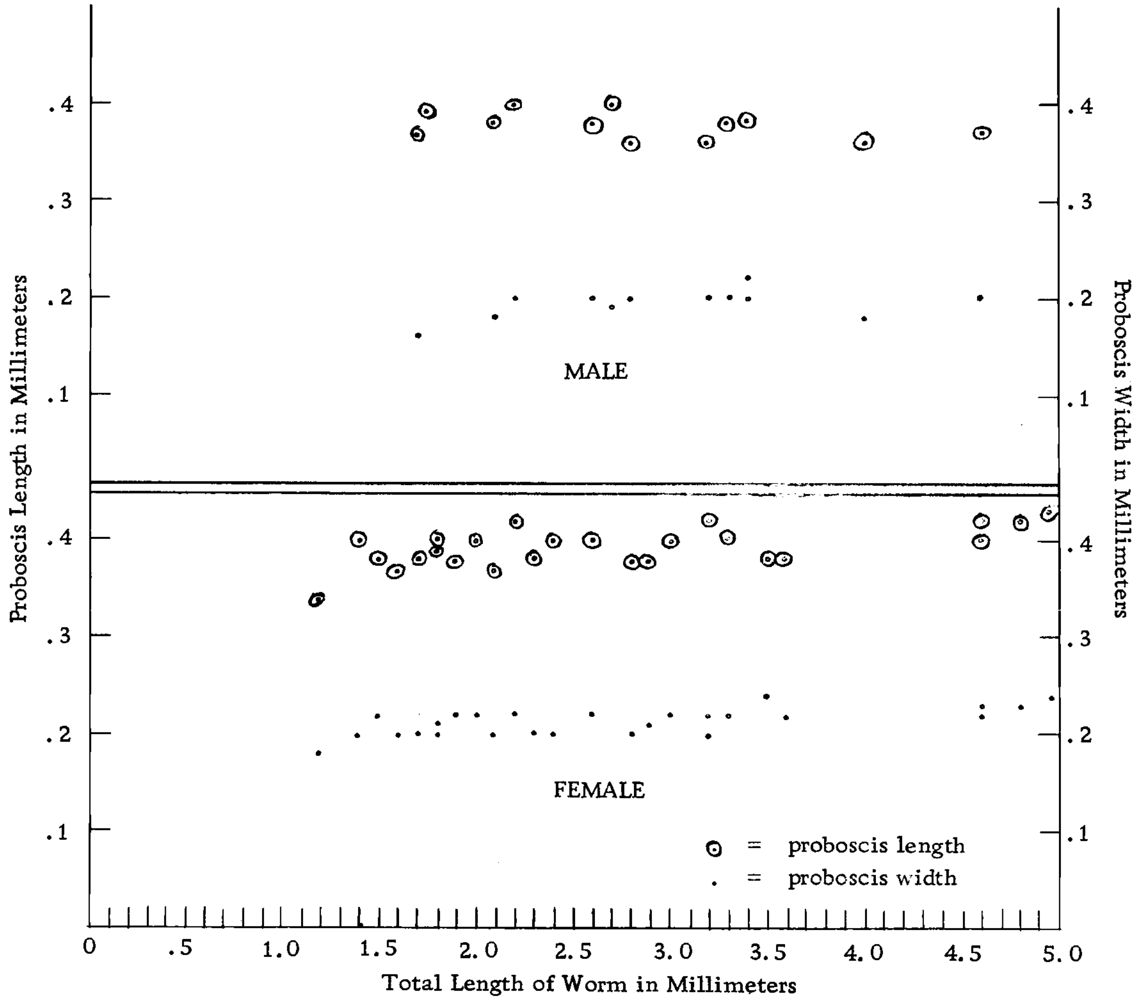

Figure 4. The relationship between the total length of the worm and the length and width of the proboscis. Based on 35 females and 12 males.

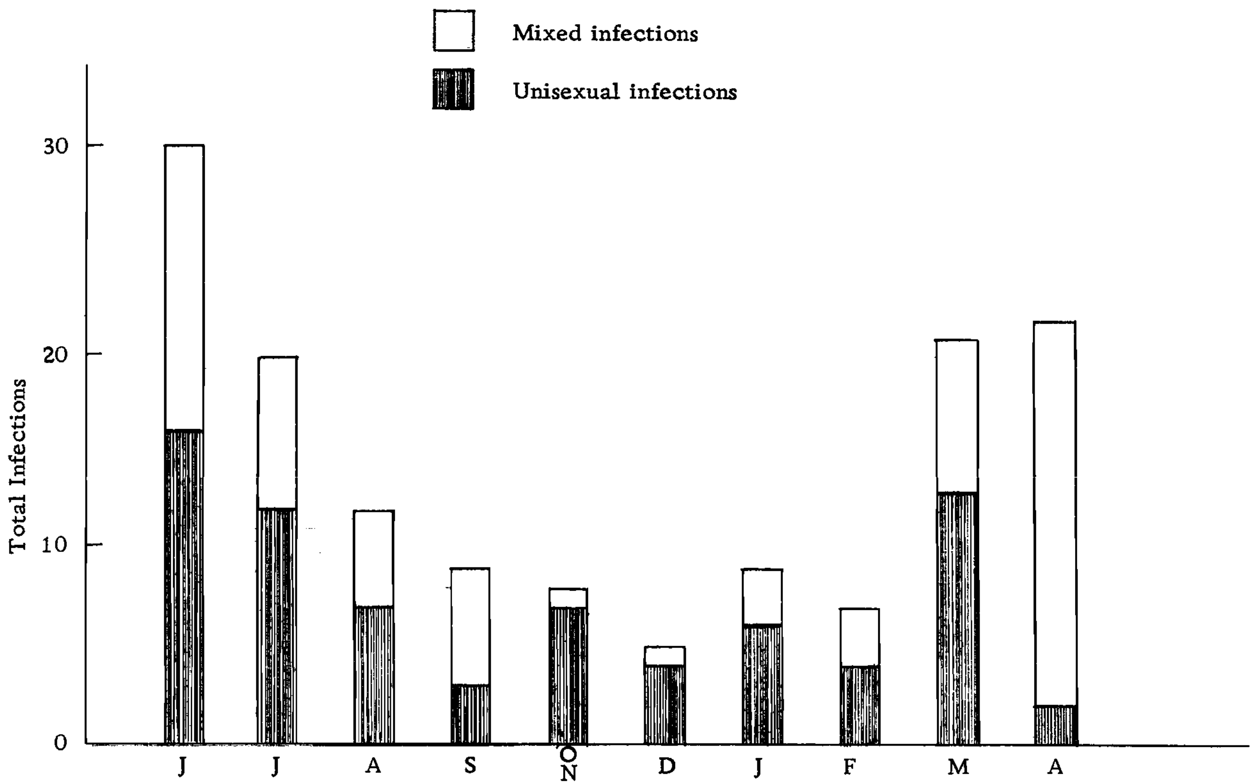

Figure 5. The number of mixed and unisexual infections observed in each sample.

Figure 6. Cross section of normal intestine of the starry flounder *Platichthys stellatus*. 12x. H and E stain.

Figure 7. Cross section of starry flounder intestine showing reaction and hole made by proboscis of *Echinorhynchus lageniformis*. 25x. H and E.

Figure 8. Cross section of starry flounder intestine showing proboscis of *Echinorhynchus lageniformis* surrounded by reaction. 10x. H and E.

Figure 9. Cross section of starry flounder intestine showing reduction of submucosa and muscularis mucosae in area of reaction cells. 32x. H and E.

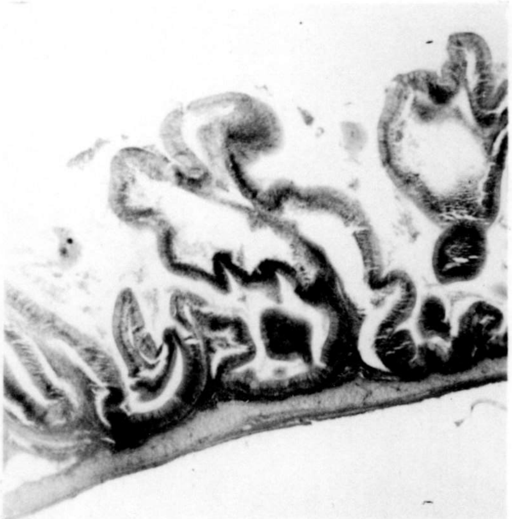

FIG. 6

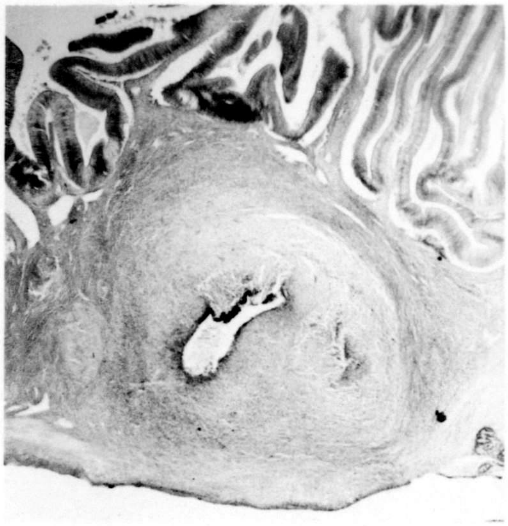

FIG. 7

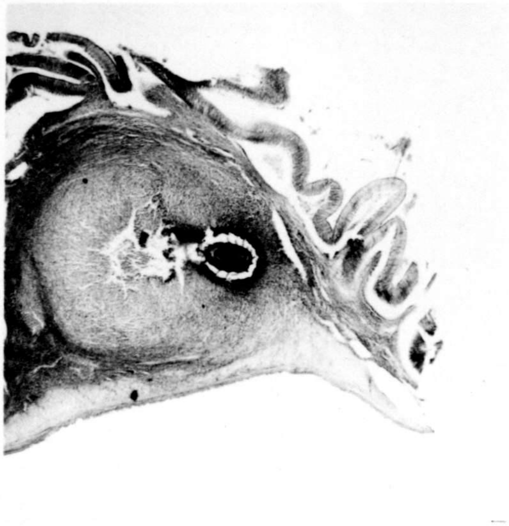

FIG. 8

FIG. 9