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Title: THE BIOLOGY OF TWO SPECIES OF *ECHINORHYNCHUS* (ACANTHOCEPHALA) FROM MARINE FISHES IN OREGON

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Two species of acanthocephalans which parasitize marine fishes along the Oregon coast were studied during 1972-73. *Echinorhynchus gadi* infections were observed in Dover sole, *Microstomus pacificus* and Rex sole, *Glyptocephalus zachirus* and constituted new host records for the parasite. The adult worm was redescribed since the acanthocephalan from the two pleuronectid flatfishes is identical to *E. gadi* as described in the literature, except for the regular occurrence of 17 rows of longitudinal hooks on the proboscis.

Echinorhynchus sp., found only in the Pacific staghorn sculpin, *Leptocottus armatus*, is an undescribed species and resembles only *E. laurentianus* which parasitizes pleuronectid flatfishes on the east coast of North America. The adult worm was described.

Echinorhynchus sp. was observed in *L. armatus* from all sampling sites and in Yaquina Bay fish throughout the year. The prevalence and intensity of infection in Yaquina Bay fish greater than

100 mm total length were similar in all size classes and substantially higher than in young fish.

A non-seasonal parasitic cycle was operating and restricted in Yaquina Bay and Echinorhynchus sp. was being acquired and lost from L. armatus throughout the year. The lack of a seasonal cycle was indicated by: little variation in monthly prevalence and intensity of infection; the monthly consistency of average worm length; the occurrence of small immature worms all months and the presence of all stages of sexual development in female worms each month.

Echinorhynchus gadi infections were found in all samples of M. pacificus and G. zachirus. Although the prevalence and intensity of infection of E. gadi generally increased with fish length and age for both hosts, no apparent seasonality of infection was observed. However a seasonal maturation cycle was evident by the growth of worms and the sexual development of the worm population. Only small immature parasites of both sexes were found in the spring and only large mature individuals were collected in the fall and winter.

The seasonal cycle of E. gadi in M. pacificus and G. zachirus was as follows: a single period of new infection acquisition during the spring onshore mirgration, followed by growth and mating during the summer and maturation, egg production and eventual elimination of spent adult worms during the fall and winter. The seasonal cycle is the first reported for a marine acanthocephalan in which migration of the definitive host and the restricted distribution of the unknown intermediate host (zone of infection) are primary factors controlling the distribution of the parasite.

Differences were observed between *M. pacificus* and *G. zachirus* in prevalence, intensity, parasite growth, parasite sex ratios and number of unisexual infections. The observed differences are probably related to habitat preferences and physiological factors affecting the host-parasite relationship of *E. gadi* and *M. pacificus*.

The life cycle of *Echinorhynchus* sp. was completed experimentally in the laboratory. Eggs ingested by the amphipod intermediate host *Anisogammarus confervicolus* hatch and the acanthor penetrates the intestine within two days. The developing acanthella, surrounded by a thin capsule of amphipod hemocytes, remain attached to the intestinal serosa for about ten days and then drop free into the hemocoel. The acanthella becomes infective in 33 days at 23°C. Acanthors of *E. lageniformis* and *E. gadi* were always encapsulated and melanized in *A. confervicolus* and did not develop.

The Biology of Two Species of *Echinorhynchus* (Acanthocephala) from Marine Fishes in Oregon

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

THE BIOLOGY OF TWO SPECIES OF ECHINORHYNCHUS (ACANTHOCEPHALA) FROM MARINE FISHES IN OREGON

INTRODUCTION

According to Golvan (1969) Echinorhynchus gadi (Zoega) Muller 1776 is a cosmopolitan parasite of marine and anadromous fishes and has been reported from over 50 hosts. Along the Oregon coast an acanthocephalan identified as E. gadi has been reported from the staghorn sculpin, Leptocottus armatus Girard in Yaquina Bay (Burreson, 1973) and in Coos Bay (Dunlap, 1951). Species resembling E. gadi have also been found in two offshore pleuronectid flatfishes, Dover sole, Microstomus pacificus (Lockington), and Rex sole, Glyptocephalus zachirus Lockington (R. E. Olson, personal communication).

Shulman (1958) stated that the definitive or final host range in acanthocephalans was determined ecologically rather than genetically. The close proximity of a fish population residing mainly in an estuarine habitat and putatively infected with E. gadi to those restricted to an open ocean habitat and also infected with E. gadi posed several interesting questions.

First are two separate life cycles involving the same species of parasite in operation? In this situation one cycle would be restricted to the estuary with L. armatus as the dominant host and the other would be restricted to offshore areas with M. pacificus and G. zachirus as the dominant hosts. Burreson (1973) reported small, immature specimens of E. gadi during all seasons from L. armatus in the upper regions of Yaquina Bay suggesting that the life cycle operates in that region. Since M. pacificus and G. zachirus never enter estuaries, the acanthocephala parasitizing these species must operate offshore.

Could there be one life cycle involving the same species of parasite but with two alternate intermediate hosts, one in the estuary and one offshore? Burreson (1973) observed that 47.2% of the L. armatus collected from offshore waters near Yaquina Bay were infected with E. gadi indicating the possibility of parasite acquisition offshore by these sculpins. Or are there two life cycles involving two different species of Echinorhynchus that are completely independent of each other?

With these questions in mind, the purpose of this study was to determine: (1) if one or two species of Echinorhynchus were infecting the estuarine (L. armatus) and offshore (M. pacificus and G. zachirus) fish populations based on morphological data, (2) the intermediate host(s), the life cycle and the larval development of the acanthocephalans, and (3) when and where the life cycle(s) are most efficient, how the prevalence and intensity changes seasonally and with the size of the fish and if the parasite life cycles are restricted to either the estuary or to the ocean.

Literature Review

Although the Phylum Acanthocephala is a small helminth group consisting of over 600 species, the taxonomic status of the group is quite unsettled. When comparing the monographs of Meyer (1932-33), Petroschenko (1956), Yamaguti (1963), and Golvan (1969), one observes a variety of classification schemes especially at the higher taxonomic levels. The actual names of these groups are not agreed upon nor is there a consensus on whether they are to be considered orders or classes.

Three orders are now generally recognized (Crompton, 1970). These are: Palaeacanthocephala, Eoacanthocephala and Archiacanthocephala. The genus Echinorhynchus is a member of the Palaeacanthocephala, an order which can be differentiated from the others by the following characteristics: definitive host habitat, mainly aquatic; two to eight multinucleated cement glands; sub-cuticular nuclei as numerous amitotic fragments or few highly branched nuclei; closed proboscis receptacle with two muscle layers; a single ligament sac which ruptures in mature female worms and is attached posteriorly inside the uterine bell; and crustacean intermediate hosts.

The species in the genus Echinorhynchus have been revised several times. For example, Petroschenko (1956) recognized 11 species, Yamaguti (1963) 20 species and Golvan and Hovin (1963) 29 species. However, using only cement gland morphology and definitive host location, Golvan (1969) removed 17 species from Echinorhynchus and assigned them to the genera Metaechinorhynchus or Pseudo-echinorhynchus. He also created three new species of Echinorhynchus, species which formerly were included with E. gadi.

These revisions were usually based on one or two morphological characteristics (proboscis armature, cement glands), on only a few specimens and on measurements given in the literature. Critical information on life cycles, genetic variations within a species and detailed morphological data were not considered. One of the major problems in acanthocephalan systematics is that such critical information is usually unavailable.

All known acanthocephalan life cycles involve an arthropod as an intermediate host in which larval development takes place and a vertebrate final host in which worm maturation and sexual reproduction takes place. Eggs are released from the body cavity of female worms into the intestine of the final host and are discharged with feces into the environment. After ingestion by an appropriate intermediate host, the egg hatches to liberate the acanthor larva, which moves out of the intestinal lumen into the host hemocoel there developing into the acanthella. When development of the acanthella is completed, it becomes a resting infective stage, called the cystacanth by some authors and an infective juvenile by others, that remains dormant until its host is eaten by the appropriate final host. When a suitable final host ingests the infective juvenile, the parasite is activated and an immature worm becomes established in the intestine.

Only 12 Palaeacanthocephalan life cycles have been completed experimentally (Crompton, 1970) including two species of Echinorhynchus. The life cycle and larval development of E. truttae Schrank was described by Awachie (1966). The freshwater amphipod Gammarus pulex L. was the intermediate host and the final host was the brown trout Salmo trutta L. Olson and Pratt (1971) completed the life cycle and described the larval development of E. lageniformis Ekbaum in Yaquina Bay, Oregon. The bay amphipod Corophium spinicorne Stimpson and the starry founder, Platichthys stellatus (Pallus) and juvenile English sole, Parophrys vetulus Girard were the intermediate and final hosts of the parasite.

The intermediate hosts for only six of Echinorhynchus have been determined (Yamaguti, 1963; Olson and Pratt, 1971): E. clavula (Dujardin, 1834) nec Hamann, 1892, E. gadi, E. salmonis, Muller, 1784, E. truttae and E. lageniformis.

Nybelin (1923, 1924) studied the life history of E. gadi infections in Gadus pollachius L. in European waters and found the following amphipods serving as intermediate hosts: Amphithoe rubricata (Mont.), Calliopius rathkei (Zaddach), Gammarus locusta (L.) and

Pontoporeia femorata Kroyer while Ekbaum (1938) found the amphipod *Cyphocaris challengeri* Stebbing to be the intermediate host for *E. gadi* infections in salmonids off British Columbia.

Ecological studies involving seasonal cycles of prevalence and intensity of infection, host specificity and parasite distribution have been conducted for *E. gadi* (Polyanski, 1958; Shulman, 1958), *E. truttae* (Awachie, 1966), *E. salmonis* (Tedla and Fernand, 1970), *E. clavula* (Chubb, 1964) and *E. lageniformis* (Barnes, 1967; Olson and Pratt, 1973).

METHODS

Most samples of staghorn sculpin, Dover sole and Rex sole were collected with a 16-foot otter trawl in Yaquina Bay and in the nearby open ocean. Some sculpins were collected with hook and line. Most specimens were kept alive and held in tanks of circulating sea-water at Oregon State University Marine Science Center and were examined within five days after capture. The total length was recorded, the stomach and intestine removed, placed in bowls, silt longitudinally and examined for acanthocephalans under a dissecting microscope.

The number and sex of the acanthocephalans were recorded after carefully removing them from the host intestine. The worms were washed in saline and left in distilled water until they became turgid and the proboscis was not withdrawn when touched. The specimens were then fixed in AFA for several hours, punctured with a sharp needle in several places to allow proper fixation, staining and clearing, stained with Semichon's acetocarmine, dehydrated in alcohol, cleared in methyl salicylate and mounted in Harleco Synthetic Resin.

The following measurements and data were recorded for each specimen: sex; total length (tip of extended proboscis to gonopore); body width (widest point); length and width of proboscis, proboscis receptacle, anterior and posterior testes, cement glands, and mature and immature embryos; length of lemnisci, uterine bell, uterus and vagina; position of ganglion; the proboscis armature which is the number of longitudinal rows of proboscis hooks, the number of proboscis hooks per row and hook length; and stage of development of the reproductive system.

Because the proboscis armature is a major acanthocephalan taxonomic characteristic, all hook counts involved at least two complete counts of the longitudinal rows around the proboscis. Only those specimens in which the proboscis was fully evaginated were included in these counts. Similarly, hooks in five to six rows on each proboscis were counted to ensure an accurate count of hooks per row. Hook length measurements were made only on hooks that could be studied in full side view and included hooks from the apical, median, basal, dorsal and ventral regions of the proboscis.

After the sex of the worm was determined, the females were classified according to the following criteria:

Ligament stage--ovary consisting of a ligament within the pseudocoel with ovarian balls undifferentiated, pre-fertilization;

Ovarian ball stage--ovary ruptured and individual ovarian balls scattered throughout pseudocoel, pre-fertilization;

Immature acanthor stage--developing embryos scattered throughout pseudocoel, ovarian ball number decreasing, embryos surrounded by one to three membranes, post-fertilization;

Mature--acanthor or fully embryonated egg surrounded by four enveloping membranes usually present in uterus.

Mature and immature embryos were measured either in the body cavity after teasing them from fixed and stained mature females or after teasing them from live females and placing them in Ringer's solution. Eggs for life cycle studies were teased from live gravid females, placed in filtered seawater and stored under refrigeration (5°C). Eggs stored by this method were used in infection experiments within two weeks.

Life Cycle Experiments

Three species of bay amphipods, Corophium spinicorne, Eohaustorius estuarius Bosworth and Ansiogammarus confervicolus (Stimpson) and three offshore amphipod species, Ampelisca brevisimulata Barnard, Photis sp. and Rhachotropis sp. were tested as potential intermediate hosts. Only amphipods were used because amphipods serve as intermediate hosts in all known life cycles of Echinorhynchus spp.

The amphipods were exposed by allowing them to feed on large numbers of eggs of Echinorhynchus lageniformis collected from

Platichthys stellatus and of Echinorhynchus spp. collected from L. armatus, M. pacificus and G. zachirus. After 6-8 hours, the exposed amphipods were transferred to aerated aquaria and held at 12°C and 23°C for the duration of the experiments. The amphipods were examined at regular intervals for developing parasites. This was accomplished by pulling the head from the body exposing the gut to which the early acanthella stages were attached. Older acanthellae were usually free in the hemocoel.

When recovered, the developing larvae were studied alive in 0.5% saline and made into permanent mounts following a fixing and staining procedure similar to that described for adult acanthocephalans. Drawings of the immature stages and adult worms were made with the aid of a camera lucida.

The time required for the development of an infective juvenile was determined by feeding larvae of various ages to young L. armatus.

All measurements were obtained with the aid of an occular micrometer and are reported in micrometers (μm) unless otherwise indicated.

RESULTS

Collection of Samples

Samples of *Leptocottus armatus*, *Microstomus pacificus* and *Glyptocephalus zachirus* were collected during most months from January, 1972 to July, 1973. A total of 577 *L. armatus* were examined: 432 from Yaquina Bay; 46 from offshore waters near Newport, Oregon; 30 from Coos Bay; 20 from Winchester Bay (Umpqua River); 13 from the Siletz River; 12 from the Siuslaw River, and 24 from the Columbia River estuary, near Astoria, Oregon. A total of 190 *M. pacificus* and 302 *G. zachirus* were collected between 7 and 20 kilometers offshore from Newport, Oregon in depths of 40 to 120 meters (Figure 1).

Identification of Parasites

A total of 2286 acanthocephalans were collected during the study: 770 specimens (321 males, 449 females) from *L. armatus*; 967 specimens (405 males, 562 females) from *M. pacificus*; and 549 specimens (276 males, 273 females) from *G. zachirus*. The specimens were identified as members of the order Palaeacanthocephala based on the following characteristics: a fish definitive host; cylindrical proboscis, directed ventrad; proboscis hooks many, arranged in alternating radial rows; closed proboscis receptacle with

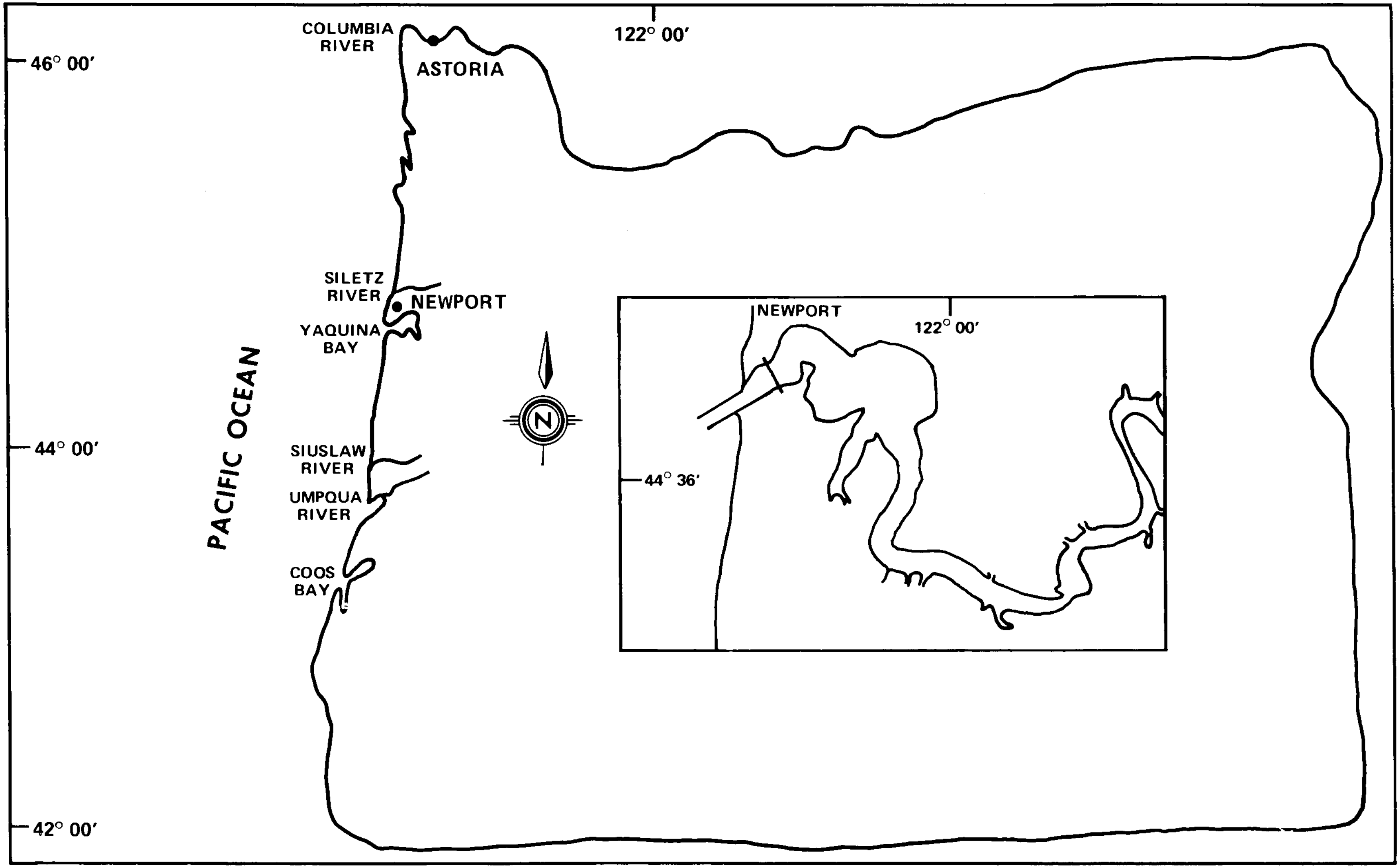


Figure 1. Location of sampling sites on the Oregon Coast and Yaquina Bay (inset).

two distinct muscle layers; single ligament sac which ruptures upon sexual maturation of females; males with two to eight multinucleate cement glands; lateral lacunar system; and subcuticular nuclei present as numerous amitotic fragments. They were assigned to the genus Echinorhynchus because of the aspinose trunk; proboscis of medium length (0.5-0.8 mm); ganglion located near middle of proboscis receptacle; clariform lemnisci; six pyriform cement glands arranged in a single chain along the trunk midline; fertilization membrane of the egg with distinct polar elongations and armed acanthor.

To determine if all the specimens from the three hosts were of the same species of Echinorhynchus, dimensions of 300 male worms (Table 1), 150 immature female worms (Table 2) and 150 mature female worms (Table 3) were obtained. The measurements from the immature and mature male specimens were combined because sexual maturity was difficult to determine. The average number of longitudinal rows of proboscis hooks from 600 specimens and the number of proboscis hooks per longitudinal row from 400 specimens were also determined.

Morphologically the acanthocephalans from all three fish hosts were similar except for the length of mature eggs (Table 3) and the number of longitudinal rows of proboscis hooks.

The lengths of the eggs from specimens infecting L. armatus averaged 84.7 (range 70-98), and the combined egg lengths from

Table 1. Dimensions of immature and mature male Echinorhynchus sp. from Leptocottus armatus and Echinorhynchus gadi from Microstomus pacificus and Glyptocephalus zachirus (average measurements with ranges in parentheses). *

	Leptocottus armatus		Microstomus pacificus		Glyptocephalus zachirus	
No. of worms	100		100		100	
Length (mm)	11.6	(4- 22)	13.1	(4.5- 23)	10.1	(4.5- 23)
Width	694	(383-1097)	667	(364-1199)	675	(383-1070)
Proboscis length	554	(437- 657)	584	(454- 670)	593	(505- 689)
Proboscis width	155	(128- 189)	196	(168- 214)	192	(166- 222)
Proboscis receptacle length	1185	(765-1658)	1290	(667-1785)	1259	(747-1734)
Proboscis receptacle width	198	(154- 244)	218	(202- 242)	216	(196- 238)
Lemnisci length	916	(551-1326)	980	(535-1450)	953	(525-1581)
Anterior testes length	1018	(535-1658)	1118	(424-2295)	928	(474-1964)
Anterior testes width	308	(188- 475)	350	(162- 707)	308	(181- 606)
Posterior testes length	977	(407-1454)	1072	(404-2290)	865	(424-1810)
Posterior testes width	308	(188- 475)	344	(151- 656)	295	(181- 556)
Cement glands length	341	(98-1045)	356	(102-1275)	322	(90- 832)
Cement glands width	264	(87- 757)	275	(72- 778)	251	(90- 606)

*Measurements in micrometers unless otherwise indicated.

Table 2. Dimensions of immature female Echinorhynchus sp. from Leptocottus armatus and Echinorhynchus gadi from Microstomus pacificus and Glyptocephalus zachirus (average measurements with ranges in parentheses). *

	Leptocottus armatus	Microstomus pacificus	Glyptocephalus zachirus
No. of worms	50	50	50
Length (mm)	13.4 (5- 25)	16.9 (5- 34)	14.1 (5- 24)
Width	716 (383-1479)	861 (424-1270)	635 (373-1096)
Proboscis length	604 (534- 727)	657 (555- 717)	630 (535- 767)
Proboscis width	170 (153- 196)	234 (199- 252)	211 (178- 244)
Proboscis receptacle length	1137 (867-1632)	1527 (765-1938)	1302 (757-1785)
Proboscis receptacle width	204 (172- 212)	231 (182- 272)	225 (182- 265)
Lemnisci length	900 (561-1250)	1030 (714-1326)	986 (510-1657)
Uterine bell length	234 (167- 303)	312 (172- 384)	273 (181- 454)
Uterus length	682 (371-1363)	772 (445-1020)	678 (444-1020)
Vagina length	182 (152- 212)	215 (151- 252)	198 (150- 273)

*Measurements in micrometers unless otherwise indicated.

Table 3. Dimensions of mature female *Echinorhynchus* sp. from *Leptocottus armatus* and *Echinorhynchus gadi* from *Microstomus pacificus* and *Glyptocephalus zachirus* (average measurements with ranges in parentheses). *

	Leptocottus armatus	*Microstomus pacificus*	*Glyptocephalus zachirus*
No. of worms	50	50	50
Length (mm)	27.9 (15- 45)	36.1 (24- 55)	31.7 (12- 52)
Width	1218 (765-1938)	1455 (1224-1734)	928 (757-1402)
Proboscis length	612 (525- 687)	681 (626- 778)	667 (638- 696)
Proboscis width	171 (143- 206)	230 (202- 253)	203 (186- 232)
Proboscis receptacle length	1453 (1173-2040)	1628 (1398-1960)	1671 (1409-1887)
Proboscis receptacle width	210 (180- 222)	232 (191- 282)	227 (192- 273)
Lemnisci length	1176 (765-1581)	1350 (871-1645)	1370 (841-1632)
Uterine bell length	360 (262- 556)	530 (313- 646)	380 (303- 454)
Uterus length	1128 (808-2333)	1188 (1010-1353)	689 (515-1091)
Vagina length	227 (212- 272)	275 (252- 303)	273 (202- 282)
Egg length	84.7 (70- 98)	104.8 (89- 120)**	104.8 (89- 120)**
Egg width	22 (18- 25)	26 (23- 30)**	26 (23- 30)**

*Measurements in micrometers unless otherwise indicated.

**Egg length and width measurements combined.

worms infecting M. pacificus and G. zachirus averaged 104.8 (range 89-120). The egg measurements were obtained from fully embryonated eggs as judged by the presence of the four enveloping membranes described by West (1964).

An important morphological characteristic in the taxonomy of the Acanthocephala is the proboscis armature (Van Cleave, 1952). Both male and female worms infecting L. armatus had an average of 15.0 longitudinal rows of proboscis hooks with a range of 13 to 16 rows (Table 4). The proboscis armature was the same in both sexes and 76% of the worms had 15 or 16 rows of hooks. The number of proboscis hooks per longitudinal row averaged 11.6 hooks and ranged from 10 to 13 (Table 5).

The specimens infecting M. pacificus had an average of 18.3 longitudinal rows of proboscis hooks with a range of 17 to 20 rows (Table 6). The male worms averaged 17.8 rows and 82% had 17 or 18 rows. The female worms averaged 18.7 rows and only 33% had 17 or 18 rows while 67% had 19 to 20 rows. Sexual dimorphism in proboscis armature has been reported in other groups of acanthocephala. Van Cleave (1952) observed that females generally have larger holdfasts than males.

The acanthocephalans infecting G. zachirus had an average of 17.9 longitudinal rows of proboscis hooks with a range of 17 to 20 rows (Table 7). The average number of rows observed in female

Table 4. Number of longitudinal rows of proboscis hooks in male and female Echinorhynchus sp. from Leptocottus armatus.

	Number of Worms Examined	Number of Rows of Hooks 13	14	15	16	Average
Male	100	4	24	50	22	14.9
Female	100	2	18	48	32	15.1
Total	200	6	42	98	54	15.0

Table 5. Average number and range of proboscis hooks per longitudinal row in male and female Echinorhynchus sp. from Leptocottus armatus and Echinorhynchus gadi from Microstomus pacificus and Glyptocephalus zachirus.

Host	Acanthocephalan Sex	Number of Worms Examined	Average	Range
Leptocottus armatus	Male	100	11.5	10-13
	Female	100	11.7	10-13
	Total	200	11.6	10-13
Microstomus pacificus and Glyptocephalus zachirus	Male	100	11.4	10-14
	Female	100	11.8	10-15
	Total	200	11.6	10-15

Table 6. Number of longitudinal rows of proboscis hooks in male and female Echinorhynchus gadi from Microstomus pacificus.

	Number of Worms Examined	Number of Rows of Hooks 17	18	19	20	Average
Male	100	46	36	12	6	17.8
Female	100	12	21	49	18	18.7
Total	200	58	57	61	24	18.3

Table 7. Number of longitudinal rows of proboscis hooks in male and female Echinorhynchus gadi from Glyptocephalus zachirus.

	Number of Worms Examined	Number of Rows of Hooks 17	18	19	20	Average
Male	100	56	31	12	1	17.6
Female	100	20	47	26	7	18.2
Total	200	76	68	38	8	17.9

worms was slightly greater than the number from male specimens. This difference was, however, not as great as the difference observed between male and female worms from M. pacificus. Eighty-seven percent of the male worms from G. zachirus contained 18 hooks or less while 80% of the female worms contained 18 or more hooks.

The average and range of the proboscis hooks per longitudinal row from the worms infecting M. pacificus and G. zachirus were similar (Table 5). There was little difference between the sexes except that the range in female specimens included 15 hooks.

Substantial differences were evident when the proboscis armature and egg lengths of the estuarine and offshore populations of acanthocephalans were compared. The characteristics of the acanthocephalans infecting L. armatus were: armature equal in both sexes; 13 to 16 longitudinal rows of proboscis hooks, averaging 15.0 rows; 10 to 13 proboscis hooks per row, averaging 11.6 hooks; and fully embryonated eggs 84.7 long by 22 wide. The characteristics of the acanthocephalans infecting M. pacificus and G. zachirus were: 17 to 20 longitudinal rows of proboscis hooks, averaging 18.1 rows; 10 to 15 proboscis hooks per row, averaging 11.6 hooks; fully embryonated eggs 104.8 long by 26 wide.

It was concluded that two species of Echinorhynchus were involved, one in M. pacificus and G. zachirus and one in L. armatus.

The morphological characteristics of the *Echinorhynchus* species that have been listed by Yamaguti (1963) and Golvan (1969) were compared to the characteristics of the *Echinorhynchus* species infecting *L. armatus* and the two pleuronectid flatfishes. The characteristics used to determine if the two species had previously been described were body length, proboscis armature and egg measurements.

Eight species of *Echinorhynchus* (Table 8) were similar to the specimens infecting *M. pacificus* and *G. zachirus* but only four (*E. gadi*, *E. ekbaumi*, *E. van cleavi*, and *E. yamaguti*) matched all or most of the criteria. The last three species were formerly *E. gadi* but were renamed by Golvan (1969). The specimens from the two pleuronectid flatfishes were identical to *E. gadi* which has 18-22 longitudinal rows of hooks except that the offshore worm population has 17-20 rows of hooks. The 17 rows were observed in 33.5% of all worms and in 51% of the male worms. This species cannot be distinguished from *E. gadi* on the basis of published reports and will be considered *E. gadi* here with the flatfish hosts constituting new host records.

The description of *E. gadi* according to Golvan (1969) is cursory. The data collected during this study adds additional information. Therefore, the adult worm is redescribed as follows:

Table 8. Characteristics of the eight species* of *Echinorhynchus* which most closely resembled the specimens infecting *Microstomus pacificus* and *Glyptocephalus zachirus*.

Species	Body Length (mm)		Proboscis Armature		Eggs (μm)	
	Male	Female	Hook Rows	No. of Hooks/Row	Length	Width
E. gadi (Zoega) Muller 1776	20	45-80	18-22	10-15	76-100	13-22
E. ekbaumi nom. nov. (= *E. gadi sensu* Ekbaum 1938)	10-25	10-25	18-20	12-13	70	30
E. vancleavei nom. nov. (= *E. gadi sensu* Van Cleave 1924)	8-20	50	18-22	10-13	76	13
E. yamaguti nom. nov. (= *E. gadi sensu* Yamaguti 1939)	5.8-7.5	10-20	18-20	12-14	-	-
E. cotti Yamaguti 1939	4.7-8.3	5.9-10	16-20	11-13	114-132	20-22
E. leidyi Van Cleave 1924	7-12	10-20	16-18	13	115-165	20-25
E. lotellae Yamaguti 1939	4.5-8.9	10-24	16-19	16	108-132	30-34
E. sevani Dinnik 1932	3.5-5.5	6.5-14	18-20	8-9	-	-
E. gadi (collected during this study)	4.5-23	12-55	17-20	10-15	89-120	23-30

*Reference Yamaguti (1963), Golvan (1969).

Order Palaeacanthocephala: With characters of the genus <u>Echinorhynchus</u>. Trunk cylindrical, elongate no marked swelling near anterior end. Sexual dimorphism of size apparent. Proboscis long cylindrical, directed ventrad. Armature slightly more developed in females: 17 to 20 longitudinal rows of 10 to 15 hooks; dorsal and ventral hooks similar. First 8 to 13 hooks in each row with strong roots; posterior row, hooks spiniform, rootless. Apical hooks 37 to 58 long, median hooks 49 to 64 long, basal hooks 31 to 49 long. Neck short. Proboscis receptacle cylindrical, double walled with ganglion near middle. Trunk unarmed, slightly curved ventrally. Lemnisci clariform, nearly always equal in length, bound distally to body wall by ligaments, rarely reaching beyond proboscis receptacle. Fragmented hypodermal nuclei numerous.

Male: Description from 200 immature and mature specimens: 4.5 to 23.0 mm long, 364 to 1199 greatest width. Neck about 100. Proboscis 454 to 689 long, 168 to 222 greatest width. Proboscis receptacle 667 to 1785 long, 196 to 242 wide. Lemnisci 535 to 1581 long. Testes, two, ovate, tandem, lying in anterior half of body, anterior testes slightly larger, 424 to 2295 by 162 to 707, posterior testes 404 to 2290 by 151 to 656. Total length of male reproductive system regardless of sexual maturity, occupying 47 to 68% of trunk length. Testes followed by 6 pyriform cement glands of uniform size, arranged one behind each other. Cement glands size dependent upon degree of maturation, 90 to 1275 by 72 to 778. Cement ducts winding posteriorly coming together in area of bursal cap. Saefftigen's pouch greatly variable in size, after extending from level of cement glands to bursal cap. Penis mammiliform surrounded by a ring of bursal papillae. Genital pore terminal.

Female: Since large size differences were observed between immature and mature specimens, the characteristics of 100 mature females are described. Trunk cylindrical 12.0 to 55.0 mm long, 757 to 1734 greatest width. Proboscis 626 to 778 long, 186 to 253 greatest width. Proboscis receptable 1398 to 1960 long, 191 to 282 wide. Lemnisci 841 to 1645 long. Female reproductive system from anterior edge of uterine bell to genital pore occupies 7.2% of trunk length. Uterine bell 303 to 646 long. Uterus 515 to 1353 long. Vagina 202 to 303 long. Eggs with polar elongation at each end and with four distinct membranes, 89 to 120 long by 23 to 30 wide in live specimens.

The acanthocephalan infecting _L. armatus_ was also compared to those described in the literature. This parasite has been reported to be _E. gadi_ by Dunlap (1951) and Burreson (1973) who also found it in _L. armatus_. However, based on proboscis armature data, the specimens infecting _L. armatus_ from the Oregon coast differed from _E. gadi_ and resembled only _E. laurentianus_ Ronald (Table 9). _Echinorhynchus laurentianus_ parasitizes pleuronectid flatfishes on the

Table 9. Characteristics of the five species* of Echinorhynchus which most closely resembled the specimens infecting Leptocottus armatus.

Species	Body Length (mm)		Proboscis Armature		Eggs (μm)	
	Male	Female	Hook Rows	No. of Hooks/Row	Length	Width
E. abyssicola Dollfus 1931	-	45-46	13	11	60-80	16
E. gomesi Machado Filho 1948	10-12	20-22	13	14	168	21
E. laurentianus Ronald 1957	5-11	10-15	14-16	11-13	--	--
E. paranense Machado Filho 1959	7-10	9-12	14	11	--	--
E. salmonis Muller 1784	3-4	6-7	12-16	9-11	90	23
Echinorhynchus sp. (collected during this study)	4-22	15-45	13-16	10-13	70-98	18-25

*Reference Yamaguti (1963), Golvan (1969).

east coast of North America, ranges in size from 5-15 mm and has 14-16 rows of proboscis hooks. Echinorhynchus sp. differs in the type of host, geographic location, body size (substantially larger) and number of rows of proboscis hooks (13-16). Therefore, it was concluded that the acanthocephalan infecting L. armatus was an undescribed species of Echinorhynchus and is hereafter referred to as Echinorhynchus sp.

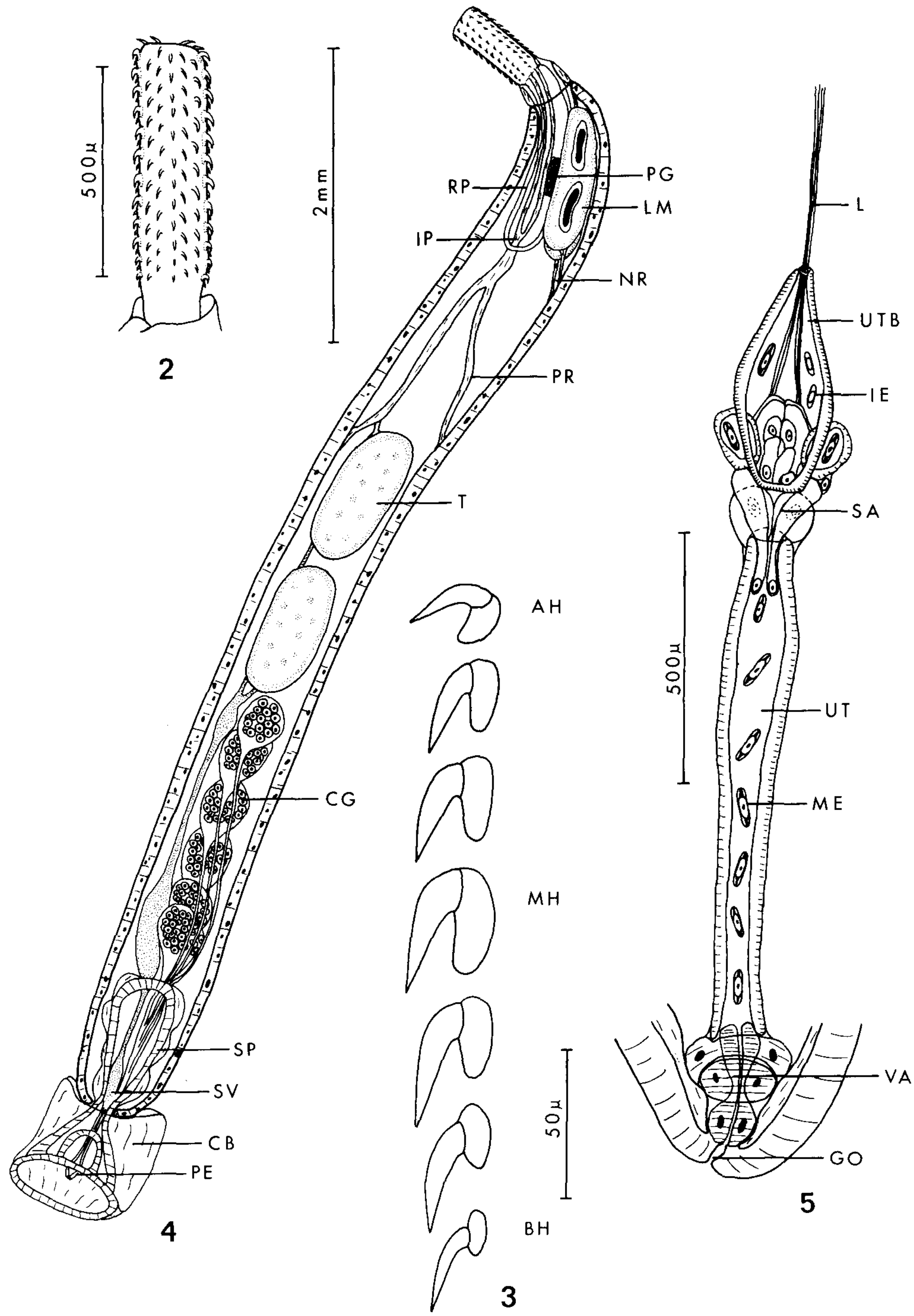
The description of the adult Echinorhynchus sp. is as follows:

Order Palaeacanthocephala: with characters of the genus Echinorhynchus. Trunk cylindrical, elongate, no marked swelling near anterior end. Sexual dimorphism of size apparent. Proboscis long, cylindrical, directed ventrad. Armature equal in both sexes: 13 to 16 longitudinal rows of 10 to 13 hooks, dorsal and ventral hooks similar (Figure 2). First 8 to 11 hooks in each row with strong roots, posterior hooks spiniform, rootless. Apical hooks 31 to 54 long, median hooks 39 to 60 long, basal hooks 30 to 49 long (Figure 3). Neck short. Proboscis receptacle cylindrical double walled with ganglion near middle. Trunk unarmed, slightly curved ventrally. Lemnisci clariform, nearly always equal in length, bound distally to body wall by ligaments, rarely reaching beyond proboscis receptacle. Fragmented hypodermal nuclei numerous.

Male: Description from 100 immature and mature specimens (Figure 4): 4.0 to 22.0 mm long, 383 to 1097 greatest width. Neck about 100. Proboscis 437 to 657 long; 128 to 189 greatest width. Proboscis receptacle 765 to 1658 long; 154 to 244 wide. Lemnisci 551 to 1326 long. Testes, two, ovate, tandem lying in anterior half of body; anterior testes slightly larger 538-1658 by 188-475; posterior testes 407-1454 by 188-475. Total length of male reproductive system regardless of sexual maturity occupying 59 to 69% of trunk length. Testes followed by 6 pyriform cement glands, arranged one behind each other. Cement gland size dependent upon degree of maturation, 98 to 1047 by 87-757. Cement ducts winding posteriorly coming together in area of bursal cap. Saefftigen's pouch greatly variable in size, often extending from level of cement glands to bursal cap. Penis mammiliform surrounded by a ring of bursal papillae. Genital pore terminal.

Female: Since large size differences were observed between immature and mature specimens, the characteristics of 50 mature females are described. Trunk cylindrical 15.0 to 45.0 mm long, 765 to 1938 greatest width. Proboscis 525 to 687 long, 143 to 206 greatest width. Proboscis receptacle 1173 to 2040 long, 180 to 222 wide. Lemnisci 765 to 1581 long. Female reproductive system (Figure 5) from anterior edge of uterine bell to genital pore occupies 6.8% of trunk length. Uterine bell 262 to 556 long, uterus 808-2333 long, vagina 212-272 long. Eggs with polar elongation at each end and 4 distinct membranes, 70 to 98 long by 18 to 25 wide in live specimens.

Figures 2-5. Adults of Echinorhynchus sp. from Leptocottus armatus. 2. Proboscis. 3. Apical, middle and basal proboscis hooks. 4. Mature adult male. 5. Female reproductive system. Abbreviations: AH, apical hooks; BH, basal hooks; CB, copulatory bursa; CG, cement glands; GO genital opening; IE, immature embryos; IP, proboscis invertor muscle; L, ligament; LM, lemnisci; ME, mature embryos; MH, middle hooks; NR, neck retractor; PE, penis; PG, proboscis ganglion; PR, proboscis retractor; RP, proboscis receptacle; SA, selector apparatus; SP, Saefftigen's pouch; SV, seminal vesicle; T, testes; UT, uterus; UTB, uterine bell; VA, vagina.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Prevalence and Intensity of Infection

Echinorhynchus sp. in Leptocottus armatus

The number, average length and size range of L. armatus by month and the prevalence of Echinorhynchus sp. by size class of fish collected are given in Table 10. Leptocottus armatus was collected from Yaquina Bay and adjacent offshore waters each month except during November, 1972, and May, 1973. The October, 1972 and January, 1973, collections consisted exclusively of offshore fish. From February through April, 1973, a large number of young of the year fish, from 25 to 99 mm, (Jones, 1962) were collected.

A total of 432 L. armatus were collected and examined from Yaquina Bay and 46 from offshore waters near Newport, Oregon. The fish were divided into four size classes that approximate age classes (Burreson, 1973; Jones, 1962) as follows: 229 young of the year fish, with a size range of 25-99 mm; 129 one year fish, with a size range of 100-149 mm; 76 two year fish, with a size range of 150-199 mm; and 44 three year and older fish, with a size range of 200-265 mm. Fish from at least three of the four size classes were collected during each sampling period except for the March, 1973 collection when no fish over 147 mm were collected. An additional 99 L. armatus were collected and examined from five areas both north and south of Yaquina Bay along the Oregon coast (Table 11).

Table 10. Number, average length and size range of <u>Leptocottus armatus</u> collected from Yaquina Bay and offshore waters near Newport, Oregon in 1972-73 and the number infected with <u>Echinorhynchus</u> sp. by size class of fish.

Month	No. of Fish	Average Fish Length (mm)	Range (mm)	Size Class (mm) 25-99	100-149	150-199	> 200
				N* I*	N I	N I	N I
March 1972	26	114	38-202	7-4	15-10	3-2	1-0
April	24	105	55-230	15-5	5-4	2-1	2-2
May	20	138	75-215	3-2	8-5	8-4	1-1
June	30	152	70-222	2-1	11-5	13-9	4-3
July	23	148	105-263		16-9	4-4	3-3
Aug	31	150	110-205		15-3	15-10	1-1
Sept	27	142	87-260	1-0	18-6	4-4	4-1
Oct	13	168	115-230		6-2	2-1	5-4
Dec	18	158	110-220		9-6	5-3	4-2
Jan 1973	17	200	105-260		3-0	3-1	11-7
Feb	56	68	25-230	49-2	1-0	4-1	2-2
March	77	53	30-147	75-0	2-2		
April	80	73	30-201	74-2	2-2	3-3	1-1
June	19	142	95-250	2-1	11-10	5-5	1-0
July	17	159	95-265	1-1	7-3	5-3	4-2

*N = Number of fish examined.
I = Number of fish infected.

Table 11. Location, collection date, number, average length and size range of Leptocottus armatus from five additional sites along the Oregon coast.

Location	Date	No. of Fish	Average Length of Fish (mm)	Range (mm)
Coos Bay (South Slough)	July, 1972	30	152	110-205
Winchester Bay (Umpqua River)	July, 1972	20	167	110-260
Siuslaw River*	August, 1972	12	144	125-158
Siletz River*	August, 1972	13	127	87-210
Columbia River Estuary (Astoria)	December, 1972	24	169	110-220

*Collections provided by Mr. M. Hosie, Oregon Department of Fish and Wildlife.

The prevalence and intensity of Echinorhynchus sp. in L. armatus increased with the length of the fish and therefore with age (Table 12). The prevalence of Echinorhynchus sp. was 7.9% in fish smaller than 100 mm total length, and 59% in larger fish. The intensity of infection averaged 2.3 parasites per young of the year fish and five parasites per fish in older L. armatus.

The monthly variations in prevalence and intensity of Echinorhynchus sp. in fish one year and older are presented in Table 13. Although the prevalence ranged from 42.8% to 100% ($\bar{x}$ = 64.1, S.E. = 5.02) over the study period, the extremes were associated with low sample sizes and no obvious seasonality of infection was evident from these data. The monthly average intensity ranged from 3.2 parasites in April, 1973, to 10.1 parasites in January, 1973. The high intensity value in January may be attributed to a combination of a small sample size (8 fish) and the highest intensity (43 worms) recorded during the study. The monthly prevalence of infection of the 229 young of the year fish ranged from 2.7% to 100% over the study period and the monthly average intensity ranged from one to six parasites.

Ten or less worms were found in 90% of the infected sculpins and 72% of them contained five or less parasites.

The prevalence and intensity of Echinorhynchus sp. in L. armatus collected from the other sites along the Oregon coast are

Table 12. Prevalence, intensity of infection and range of intensity of Echinorhynchus sp. in Leptocottus armatus collected from Yaquina Bay and offshore waters near Newport, Oregon in 1972-73.

Size Class (mm)	Total Number	Number Infected	Prevalence (%)	Average Intensity	Range
0-99	229	18	7.9	2.3	1-6
100-149	129	67	51.9	4.3	1-41
150-199	76	51	67.1	5.7	1-43
> 200	44	29	65.9	5.2	1-15
Total	478	165	34.5	4.7	1-43

Table 13. Monthly prevalence, intensity of infection and range of intensity of Echinorhynchus sp. in Leptocottus armatus larger than 100 mm total length collected from Yaquina Bay and offshore waters near Newport, Oregon in 1972-73.

Month	Total Number	Number Infected	Prevalence (%)	Average Intensity	Range
March 1972	19	12	63.2	4.2	1-7
April	9	7	77.8	5.6	1-13
May	17	10	58.8	3.6	1-9
June	28	17	60.7	6.3	1-41
July	23	16	69.6	4.4	1-16
Aug	31	14	45.2	4.6	1-17
Sept	26	11	42.3	5.4	1-21
Oct	13	7	53.8	4.3	2-12
Dec	18	11	61.6	4.3	1-7
Jan 1973	17	8	47.1	10.1	1-43
Feb	7	3	42.9	7.3	1-10
March	2	2	100	4.0	1-7
April	6	6	100	3.2	1-5
June	17	15	88.2	4.5	1-11
July	16	8	50.0	3.8	1-14

shown in Table 14. The parasite was recorded from all the sites and except for the lower intensity in the Siletz River fish, the prevalence and intensity of infection in these fish are similar to the levels observed in the Yaquina Bay.

Table 14. Prevalence, intensity of infection and range of intensity of Echinorhynchus sp. in Leptocottus armatus collected from five additional sites along the Oregon coast in 1972.

Location	Total Number	Number Infected	Prevalence (%)	Average Intensity	Range
Coos Bay	30	15	50.0	5.1	1-17
Umpqua River	20	16	80.0	6.4	1-21
Siuslaw River	12	10	83.3	3.2	1-6
Siletz River	13	6	46.2	1.7	1-5
Columbia River	24	16	66.7	4.6	1-13

Data on the prevalence of Echinorhynchus lageniformis in L. armatus was also recorded. This parasite was recorded in only four of the 432 (0.9%) L. armatus collected from Yaquina Bay but was present in six of the 13 (46.2%) L. armatus from the Siletz River. The amphipod Corophium spinicorne is the intermediate host of E. lageniformis (Olson and Pratt, 1971) and was observed in 10 of the 13 stomachs from the Siletz River staghorn sculpins. This amphipod was not frequently observed in the stomachs of the Yaquina Bay sculpins.

Echinorhynchus gadi in Microstomus pacificus

The number, average length and size range of M. pacificus by month and the prevalence of E. gadi by size class of fish collected are listed in Table 15. Microstomus pacificus was collected each month except July, 1972, and February-March, 1973. A total of 190 M. pacificus were captured during the study and because of the winter offshore migration of this species (Demory, 1971), few fish were collected during the winter and early spring. The fish were divided into five size classes that approximate age classes (Demory, 1975; Hagerman, 1952) as follows: 44 one year fish, with a size range of 50-119 mm; 40 two year fish, with a size range of 120-159 mm; 31 three year fish, with a size range of 160-199 mm; 30 four year fish, with a size range of 200-239 mm; and 45 five year and older fish, with a size range from 240-460 mm.

The prevalence and intensity of E. gadi generally increased with fish length and therefore with age (Table 16). The prevalence of E. gadi was 36.4% in one year old fish, and 76% in older fish. The intensity of infection averaged 2.4 parasites in one year old fish and 8.4 parasites in the larger individuals.

Changes in the prevalence and intensity of E. gadi by month are presented in Table 17. Although the prevalence ranged from 40% to 100% ($\bar{x}$ = 70.9, S.E. = 5.03) and the monthly average intensity of

Table 15. Number, average length and size range of Microstomus pacificus collected off central Oregon in 1972-73 and the number infected with Echinorhynchus gadi by size class of fish.

Month	No. of Fish	Average Fish Length (mm)	Range (mm)	Size Class (mm) 50-119 N* I*	120-159 N I	160-199 N I	200-239 N I	>240 N I
Feb 1972	5	171	165-188			5-2		
March	1	239	239				1-1	
April	25	192	60-401	14-6		2-2	2-2	7-5
May	10	174	65-450	5-4	1-0		2-2	2-0
June	22	185	80-290	2-1	6-5	6-4	4-4	4-4
Aug	10	242	150-357		1-1	3-3	1-0	5-4
Sept	16	215	111-392	2-0	4-1		3-3	7-7
Oct	28	174	115-290	3-1	11-6	4-4	5-3	5-5
Nov	13	217	100-460	3-1	5-1	1-1	2-2	2-2
Dec	6	233	182-272			2-2		4-4
Jan 1973	16	166	100-232	3-0	3-2	6-4	4-4	
April	14	138	50-250	7-3		1-1	2-2	4-2
May	13	124	55-200	5-0	5-4	1-0	2-2	
June	7	215	135-320		3-2		2-2	2-2
July	4	229	135-285		1-1			3-3

*N = Number of fish examined.
I = Number of fish infected.

Table 16. Prevalence, intensity of infection and range of intensity of Echinorhynchus gadi in Microstomus pacificus collected off central Oregon in 1972-73.

Size Class (mm)	Total Number	Number Infected	Prevalence (%)	Average Intensity	Range
50-119	44	16	36.4	2.4	1-6
120-159	40	23	57.5	5.4	1-24
160-199	31	23	74.2	7.7	1-28
200-239	30	27	90.0	8.2	1-27
> 240	45	38	84.4	10.7	1-43
Total	190	127	66.8	7.6	1-43

Table 17. Monthly prevalence, intensity of infection and range of intensity of *Echinorhynchus gadi* in *Microstomus pacificus* collected off central Oregon in 1972-73.

Month	Total Number	Number Infected	Prevalence (%)	Average Intensity	Range
Feb 1972	5	2	40	8.0	1-15
March	1	1	100	2.0	2
April	25	15	60	11.1	1-31
May	10	6	60	5.8	1-22
June	22	18	81.8	9.7	1-28
Aug	10	8	80	6.3	2-17
Sept	16	11	68.8	4.5	1-15
Oct	28	19	67.9	9.4	1-24
Nov	13	7	53.9	3.7	1-7
Dec	6	6	100	2.8	1-8
Jan 1973	16	10	62.5	3.7	1-10
April	14	8	57.1	9.3	1-24
May	13	6	46.2	4.5	1-13
June	7	6	85.7	12.7	1-43
July	4	4	100	9.8	5-20

infection ranged from two to 12.7 parasites over the study period, the extremes were associated with low sample sizes or the highest intensity and no obvious seasonality of infection was observed.

Ten worms or less were found in 76% of the infected Dover sole and 56% of them contained five or less parasites. The highest intensity observed was 43 worms.

Echinorhynchus gadi in Glyptocephalus zachirus

The number, average length and size range of G. zachirus by month and the prevalence of E. gadi by size class of fish collected are listed in Table 18. Glyptocephalus zachirus was collected each month except July, November and December, 1972, and February-March, 1973. A total of 302 G. zachirus were captured during the study. This species also exhibits a winter offshore migration (Demory, 1971) and samples collected during this period were small. The fish were divided into five size classes that approximate age classes (Hosie, 1975) as follows: 23 one year fish, with a size range of 70-119 mm; 35 two year fish, with a size range of 120-159 mm; 41 three year fish, with a size range of 160-189 mm; 101 four year fish, with a size range of 190-239 mm and 102 five year and older fish, with a size range of 240-384 mm.

The intensity of E. gadi increased with fish length and therefore with age, however the prevalence was similar in all but the

Table 18. Number, average length and size range of <u>Glyptocephalus zachirus</u> collected off central Oregon in 1972-73 and the number infected with <u>Echinorhynchus gadi</u> by size class of fish.

Month	No. of Fish	Average Fish Length (mm)	Range (mm)	Size Class (mm) 70-119 N* I*	120-159 N I	160-189 N I	190-239 N I	> 240 N I
Jan 1972	4	205	130-238		1-0		3-3	
Feb	4	184	124-223		1-0	1-0	2-0	
March	3	159	130-180		1-1	2-1		
April	64	177	70-287	17-3	9-5	8-4	20-17	10-6
May	40	214	100-310	3-1	3-0	3-1	17-11	14-9
June	5	212	150-260		1-1	1-0	1-0	2-2
Aug	26	193	120-350		7-5	6-5	9-8	4-4
Sept	35	262	154-384		2-1	2-2	8-7	23-15
Oct	26	192	100-310	1-0	6-3	4-3	11-7	4-0
Jan 1973	49	233	175-340			6-0	19-7	24-11
April	7	207	145-235		1-1		6-2	
May	17	203	90-310	2-1	3-1	5-3	2-1	5-0
June	7	250	165-320			1-0	2-2	4-2
July	15	260	165-320			2-0	1-1	12-2

*N = Number of fish examined.
I = Number of fish infected.

smallest size class (Table 19). The prevalence of E. gadi was 21.7% in one year old G. zachirus and 55.2% in older fish. Fish under 160 mm contained an average of 1.9 parasites per fish and the average for larger fish was 3.7 parasites.

Monthly changes in the prevalence and intensity of E. gadi in G. zachirus are given in Table 20. Although the prevalence ranged from 20 to 84.6% ($\bar{x}$ = 54.6%, S.E. = 4.95) and the average intensity ranged from one to 12.7 parasites over the study period, no obvious seasonality of infection was observed.

Ten worms or less were found in 96% of the infected Rex sole and 83% of them contained five or less parasites. The highest intensity observed was 31 worms.

Growth of Echinorhynchus spp. in Fish Hosts

Echinorhynchus sp. in Leptocottus armatus

Both male and female Echinorhynchus sp. occurred throughout the year. The average length of the 271 male worms collected from Yaquina Bay fish was 11.2 mm (range 4-22 mm) and changed very little from month to month (Figure 6). The mean length of the parasites ranged from 9.9 mm to 12.2 mm and small immature male worms were collected every month. The average length of the 50 male worms from offshore sculpins was 14.4 mm (range 7-21 mm).

Table 19. Prevalence, intensity of infection and range of intensity of Echinorhynchus gadi in Glyptocephalus zachirus collected off central Oregon in 1972-73.

Size Class (mm)	Total Number	Number Infected	Prevalence (%)	Average Intensity	Range
70-119	23	5	21.7	1.8	1-3
120-159	35	18	51.4	1.9	1-5
160-189	41	19	46.3	2.6	1-10
190-239	101	66	65.3	3.2	1-14
> 240	102	51	50.0	4.8	1-31
Total	302	159	52.7	3.5	1-31

Table 20. Monthly prevalence, intensity of infection and range of intensity of Echinorhynchus gadi in Glyptocephalus zachirus collected off central Oregon in 1972-73.

Month	Total Number	Number Infected	Prevalence (%)	Average Intensity	Range
Jan 1972	4	3	75	1	1
Feb	4	0	0	0	0
March	3	2	66.7	1	1
April	64	35	54.7	1.9	1-12
May	40	22	55	5.2	1-18
June	5	3	60	2.0	1-4
Aug	26	22	84.6	4.6	1-10
Sept	35	25	71.4	4.5	1-15
Oct	26	13	50	3.2	1-14
Jan 1973	49	18	36.7	2.0	1-5
April	7	3	42.9	3.7	2-5
May	17	6	35.3	2.0	1-3
June	7	4	57.1	1.5	1-3
July	15	3	20	12.7	1-31

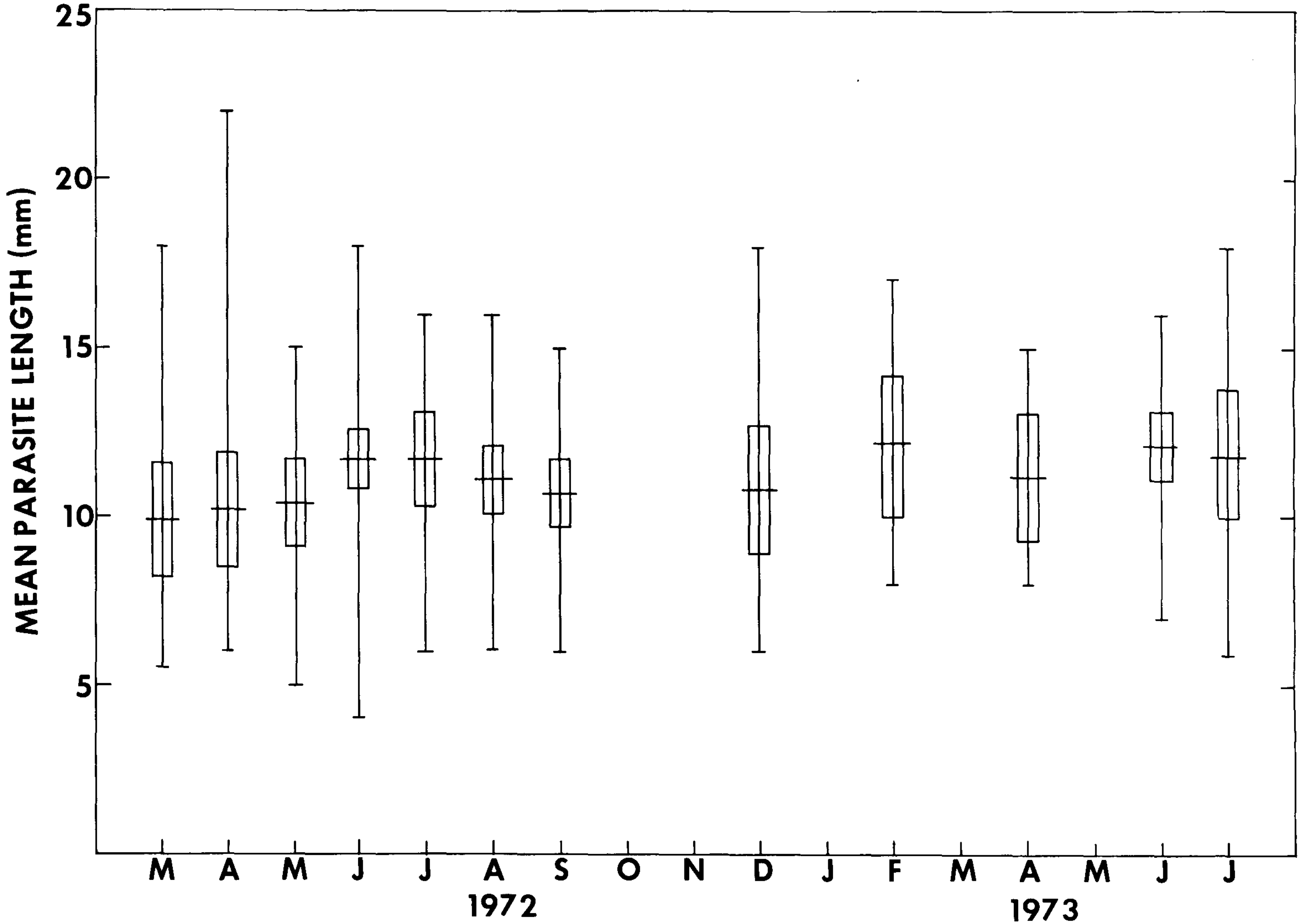


Figure 6. Seasonal changes in mean length of male Echinorhynchus sp. in Leptocottus armatus collected from Yaquina Bay, Oregon in 1972-73. Central horizontal line, mean; hollow bar, 95% confidence intervals for the mean; vertical line, observed range.

The average length of the 364 female worms collected from Yaquina Bay fish was 19.3 mm (range 5-45 mm) and as in the male specimens, averages changed very little from month to month (Figure 7). The mean length of the parasites ranged from 16.3 mm to 22.6 mm and immature female worms less than 10 mm were collected every month. The average length of the 85 female worms from offshore sculpins was 26.9 mm (range 10-41 mm).

The constancy of average worm length each month and the presence of small immature Echinorhynchus sp. in all months indicated that a non-seasonal cycle was operating in Yaquina Bay and the parasites were being acquired by and lost from L. armatus throughout the year.

Echinorhynchus gadi in Microstomus pacificus

Both male and female E. gadi were present in all samples. The 405 male worms collected averaged 13.1 mm long (range 4.5-23 mm). The average length of the male worms from M. pacificus increased substantially through the year (Figure 8). Small immature worms averaging less than 11 mm long were recovered during the months of February through April during both years. After April, the average length of male worms increased and was 18 mm (range 11-23 mm) from September, 1972 through January, 1973. Small male worms appeared again in April, 1973 (average length 8.2 mm) and the

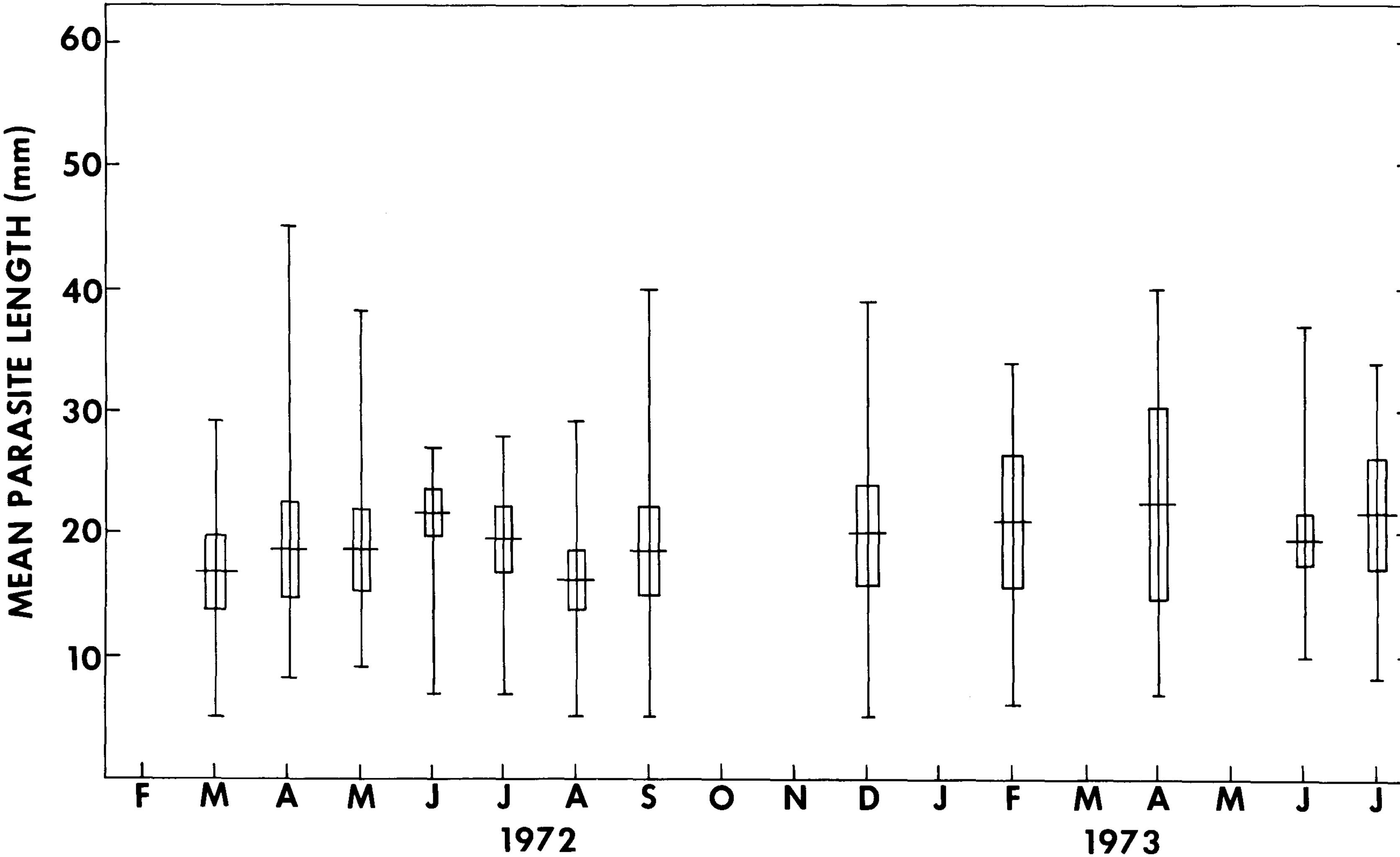


Figure 7. Seasonal changes in mean length of female Echinorhynchus sp. in Leptocottus armatus collected from Yaquina Bay, Oregon in 1972-73. Central horizontal line, mean; hollow bar, 95% confidence intervals for the mean; vertical line, observed range.

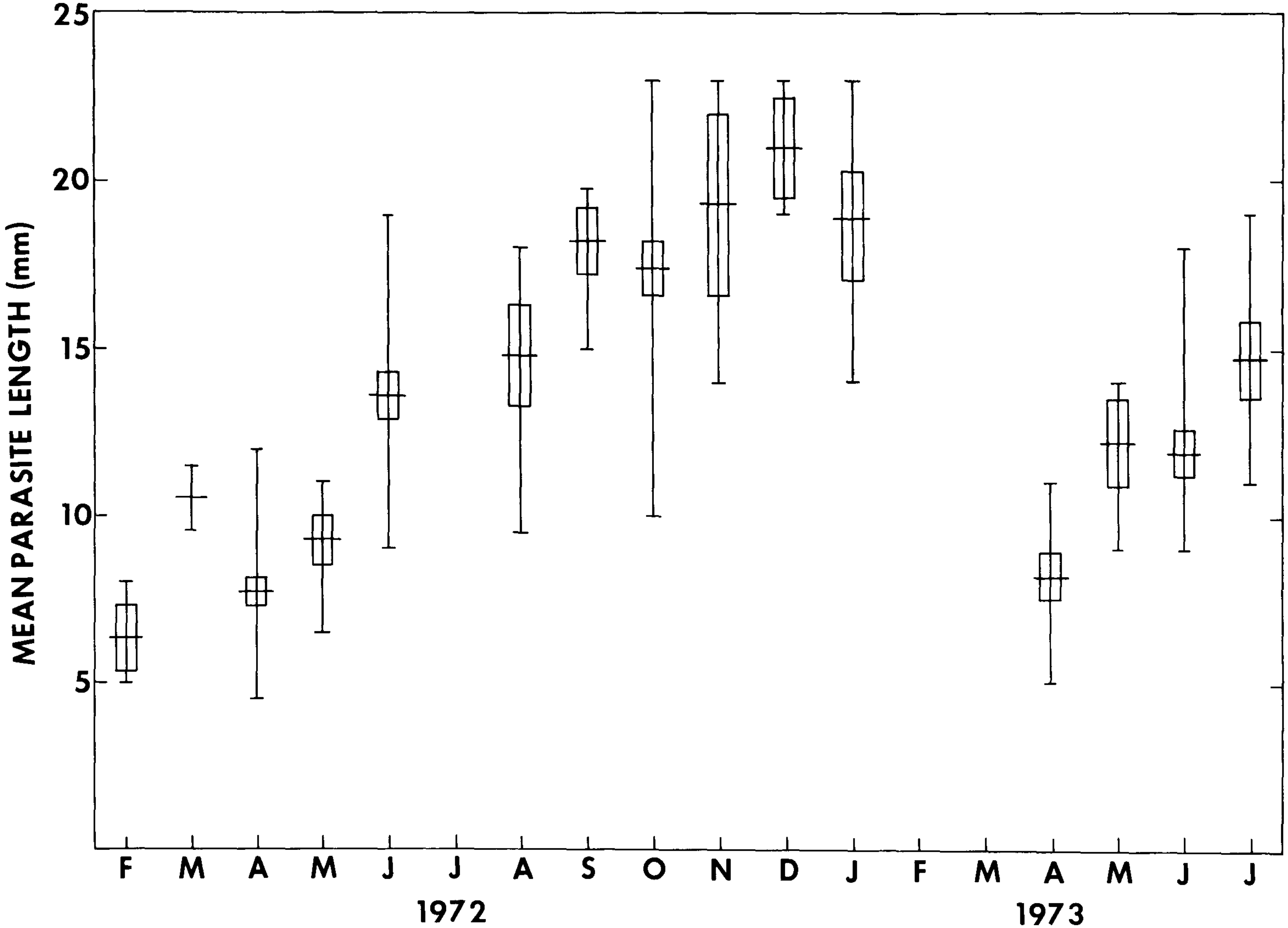


Figure 8. Seasonal changes in mean length of male Echinorhynchus gadi in Microstomus pacificus collected off Central Oregon in 1972-73. Central horizontal line, mean; hollow bar, 95% confidence intervals for the mean; vertical line, observed range.

average length increased to 14.7 mm by July, 1973.

A total of 562 female worms averaging 24.5 mm long (range 6-55 mm) were collected. The average length of the female worms increased substantially through the year (Figure 9). The average length of female worms was 8.7 mm in Feburary, 1972, and increased to a peak of 39.4 mm in December, 1972. Small female worms appeared again in April, 1973 (average length 11.8 mm) and increased in average length to 28.6 mm by July, 1973. The largest females observed were over 50 mm long and were found from September, 1972 through January, 1973.

Echinorhynchus gadi in *Glyptocephalus zachirus*

Both male and female *E. gadi* were collected throughout the year except in February and March, 1972. The 276 male worms collected averaged 10.1 mm (range 4-23 mm). The average length of the male worms from *G. zachirus* increased through the year (Figure 10). Small worms averaging 8.4 mm long (range 4.5-17 mm) were recovered during the months of March through August, 1972. The average length of male worms increased and was 13.0 mm (range 9-23 mm) from September, 1972 through January, 1973. Small male worms appeared again in April, 1973 (average length 7.9 mm) and increased in average length to 11.2 mm by July, 1973. Small male worms less than 11 mm in length were collected each month.

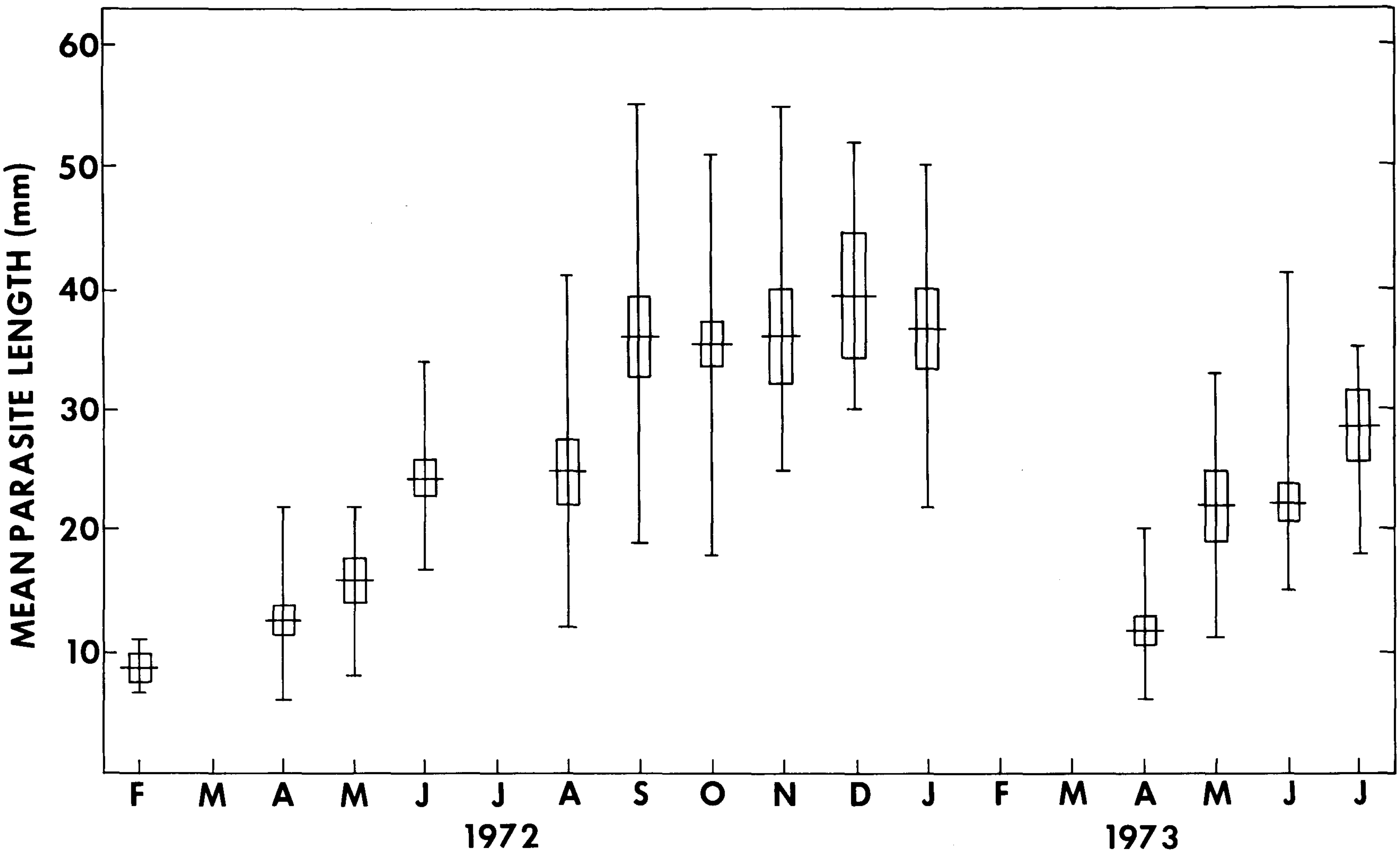


Figure 9. Seasonal changes in mean length of female Echinorhynchus gadi in Microstomus pacificus collected off Central Oregon in 1972-73. Central horizontal line, mean; hollow bar, 95% confidence intervals for the mean; vertical line, observed range.

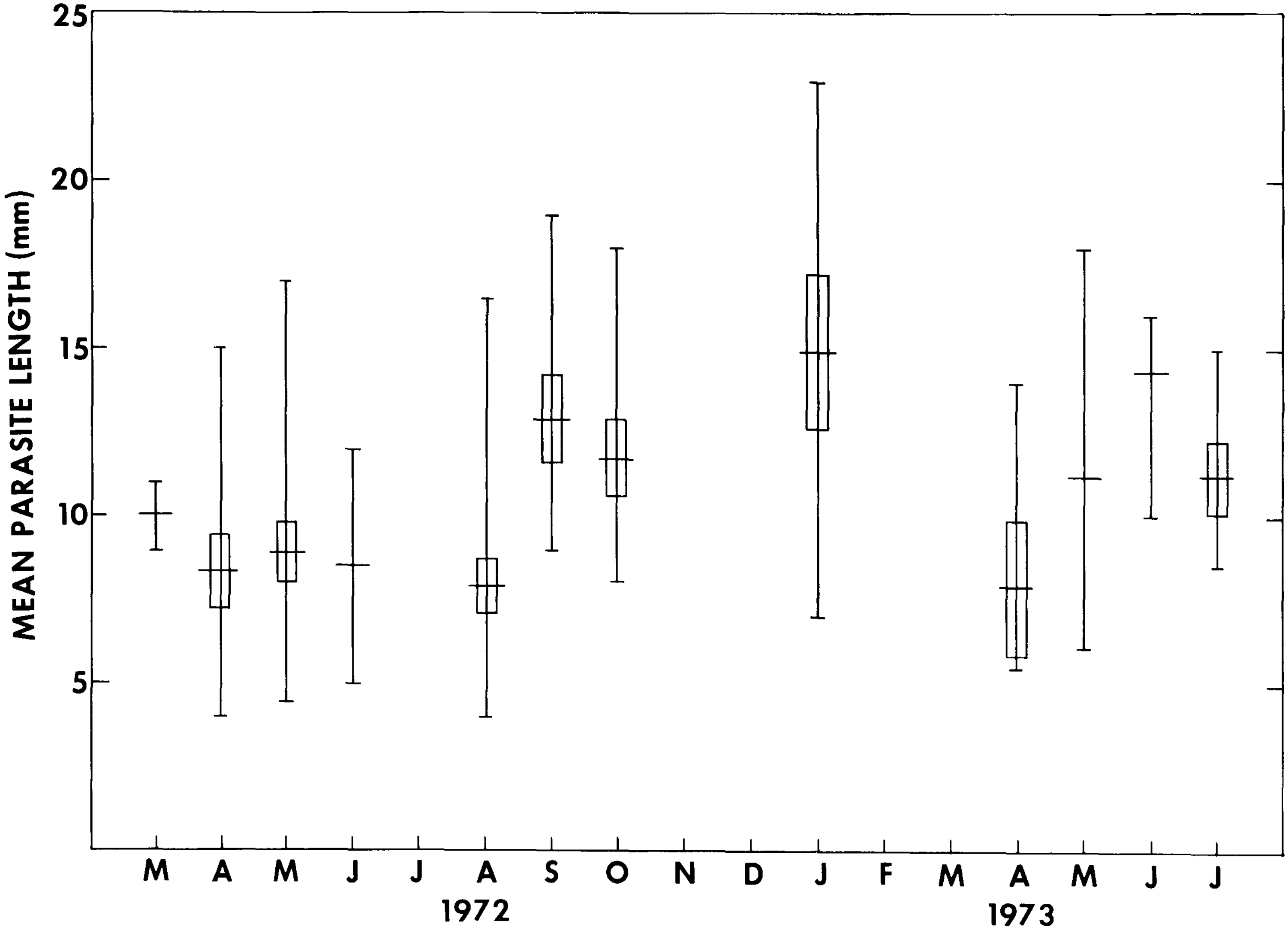


Figure 10. Seasonal changes in mean length of male Echinorhynchus gadi in Glyptocephalus zachirus collected off Central Oregon in 1972-73. Central horizontal line, mean; hollow bar, 95% confidence intervals for the mean; vertical line, observed range.

A total of 273 female worms averaging 18.2 mm (range 5-47 mm) were collected. The average length of female worms increased through the year (Figure 11). The average length of female worms was 11.4 mm in April, 1972 and increased to a peak of 27.9 mm in September, 1972. Small female worms appeared again in May, 1973 (average length 11.9 mm) and increased in length to 17.4 mm by July, 1973. The largest females observed were over 40 mm long and were found from September, 1972 through January, 1973.

Parasite Sex Ratios

The relative proportions of male to female worms are indicated by the sex ratio. The female *Echinorhynchus* sp. in *L. armatus* outnumbered the male worms throughout the study period and represented 58.3% of the population. The sex ratios by month ranged from 1:1.1 to 1:2.1 and the ratio over the entire year was 1:1.4 (Table 21). Immature males and females were found throughout the year in about equal numbers (56 males; 57 females). Female *E. gadi* also outnumbered the males in *M. pacificus*. The female parasites represented 58.1% of the population and the sex ratio over the entire year was 1:1.4 (Table 22). In *G. zachirus* the relative proportion of males to females was about equal over the entire period although variation from month to month was observed (Table 23). No consistent pattern

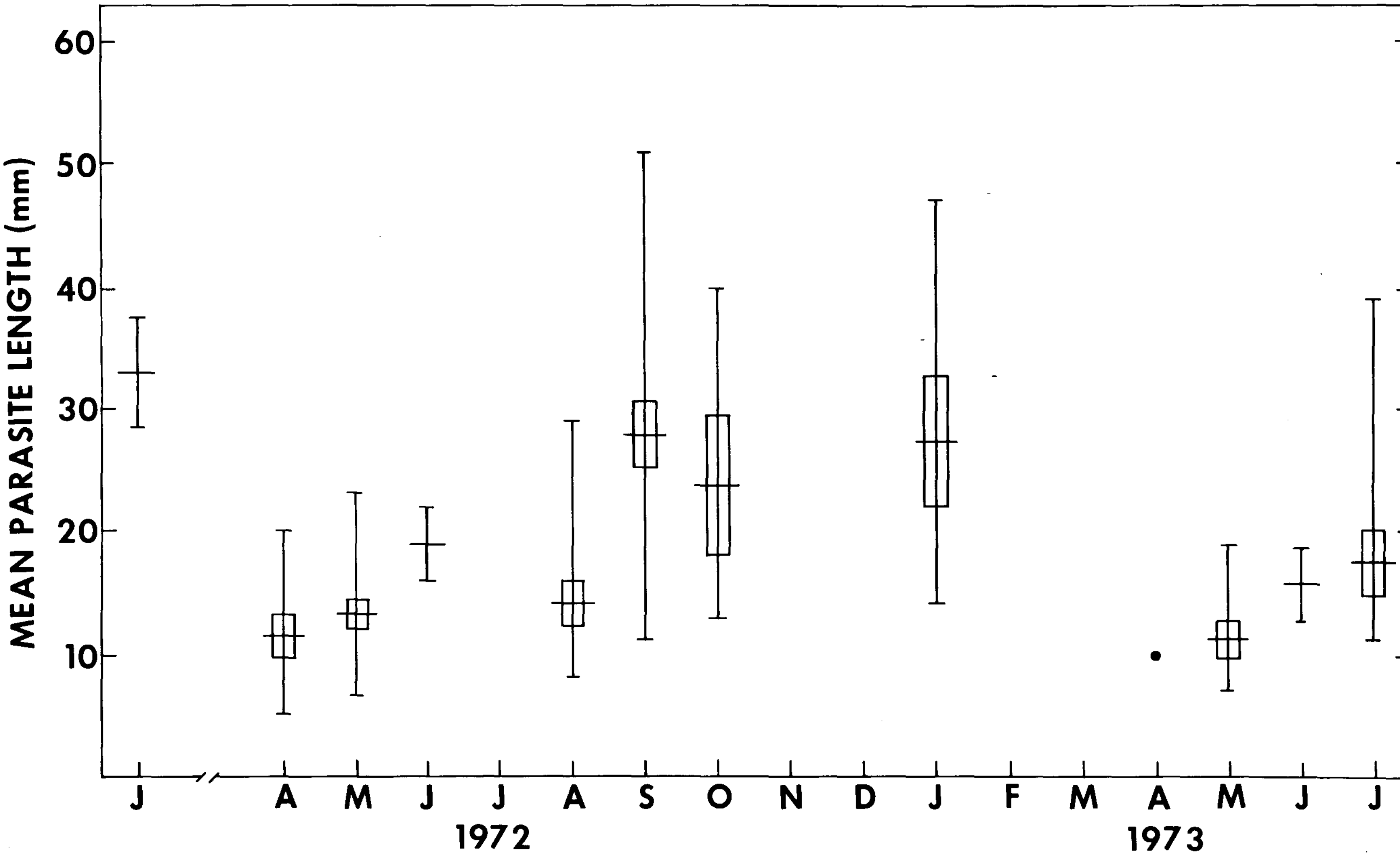


Figure 11. Seasonal changes in mean length of female Echinorhynchus gadi in Glyptocephalus zachirus collected off Central Oregon in 1972-73. Central horizontal line, mean; hollow bar, 95% confidence intervals for the mean; vertical line, observed range.

Table 21. Sex ratios of *Echinorhynchus* sp. in *Leptocottus armatus* collected from Yaquina Bay and offshore waters near Newport, Oregon in 1972-73.

Date	No. of Males	Percent Males	No. of Females	Percent Female	Sex-Ratio # Females/# Males
March 1972	24	40.7	35	59.3	1.5
April	20	39.2	31	60.8	1.6
May	17	42.5	23	57.5	1.4
June	52	47.7	57	52.3	1.1
July	25	35.7	45	64.3	1.8
Aug	28	43.8	36	56.2	1.3
Sept	24	40.7	35	59.3	1.5
Oct	13	43.3	17	56.7	1.3
Dec	19	40.4	28	59.6	1.5
Jan 1973	26	32.1	55	67.9	2.1
Feb	11	44.0	14	55.0	1.3
March	3	37.5	5	62.5	1.7
April	9	42.9	12	57.1	1.3
June	34	46.6	39	53.4	1.2
July	16	48.5	17	51.5	1.1
Total	321	41.7	449	58.3	1.4

Table 22. Sex ratios of *Echinorhynchus gadi* in *Microstomus pacificus* collected off central Oregon in 1972-73.

Date	No. of Males	Percent Males	No. of Females	Percent Female	Sex-Ratio # Females/# Males
Feb 1972	7	43.7	9	56.8	1.3
March	2	100.0	0	--	-
April	66	39.8	100	60.2	1.5
May	14	40.0	21	60.0	1.5
June	87	49.7	88	50.3	1.0
Aug	16	32.0	34	68.0	2.1
Sept	14	28.6	35	71.4	2.5
Oct	79	44.4	99	55.6	1.3
Nov	9	34.6	17	65.4	1.9
Dec	5	29.4	12	70.6	2.4
Jan 1973	16	43.2	21	56.8	1.3
April	31	41.9	43	58.1	1.4
May	10	37.0	17	63.0	1.7
June	29	38.2	47	61.8	1.6
July	20	51.3	19	48.7	0.95
Total	405	41.9	562	58.1	1.4

Table 23. Sex ratios of _Echinorhynchus gadi_ in _Glyptocephalus zachirus_ collected off central Oregon in 1972-73.

Date	No. of Males	Percent Males	No. of Females	Percent Female	Sex-Ratio # Females/# Males
Jan 1972	1	33.3	2	66.7	2.0
March	2	100	0	0	0
April	30	45.4	36	54.6	1.2
May	53	46.5	61	53.5	1.2
June	2	33.3	4	66.7	2.0
Aug	53	52.5	48	47.5	0.9
Sept	59	52.2	54	47.8	0.9
Oct	24	58.5	17	41.5	0.7
Jan 1973	17	47.2	19	52.8	1.1
April	10	90.9	1	9.1	0.1
May	5	41.7	7	58.3	1.4
June	4	66.7	2	33.3	0.5
July	16	42.1	22	57.9	1.4
Total	276	50.3	273	49.7	0.99

of changes in parasite sex ratio with season was observed in any of the hosts studied.

Unisexual Infections

Echinorhynchus spp. infections that consisted of all males or all females (unisexual infections) were regularly observed in all three hosts. Female worms from unisexual infections were examined for evidence of fertilization. If fertilization had occurred, as evidenced by developing embryos, it was assumed that male worms had been present; unfertilized females were an indication that males had not previously been present.

From *L. armatus* 59 unisexual infections of *Echinorhynchus* sp. were recorded; 38 female-only and 23 male-only infections (Table 24). Fertilized worms were collected from two of these infected fish and the 36 other female-only infections contained 52 unfertilized worms which represented 11.6% of the total female population observed in all sculpins. The male unisexual infections found in 23 *L. armatus* consisted of 29 worms representing 9% of the total male population observed.

A total of 30 unisexual infections of *E. gadi* were recorded in *M. pacificus*; 17 all-female and 13 all-male (Table 25). Fertilized worms were collected from three of these infected fish while the remaining 14 contained 18 unfertilized worms which represented 3.2%

Table 24. Unisexual infections of Echinorhynchus sp. recorded from Leptocottus armatus.

Month	No. of Infected Fish	Unisexual Infections Male	Unisexual Infections Female
March, 1972	16	1	4
April	12		4
May	12		3
June	18	3	3
July	16	2	3
Aug	14	3	4
Sept	11	1	5
Oct	7	2	
Dec	11	1	1
Jan, 1973	8		4(1M)*
Feb	5	2	1
March	2	1	
April	8	2	3(1M)*
June	16	4	1
July	9	1	2
Total	165	23	38

*(M) = Mature Female Infection.

Table 25. Unisexual infections of Echinorhynchus gadi recorded from Microstomus pacificus.

Month	No. of Infected Fish	Unisexual Infections	
		Male	Female
Feb, 1972	2	1	
March	1	1	
April	15	1	1
May	6		2
June	18	1	1
Aug	8	1	1
Sept	11	1	1(M)*
Oct	19		2
Nov	7		2(1M)*
Dec	6	2	1(M)*
Jan, 1973	10	3	1
April	8		2
March	6		2
June	6	2	1
July	4		
Total	127	13	17

*(M) = Mature Female Infection.

of the total female population observed. The male-only infections in 13 fish contained 15 worms which represented 3.7% of the total male population observed.

Nearly half of the E. gadi infections recorded in G. zachirus were unisexual infections; 38 female-only and 41 male-only (Table 26).

Fertilized worms were collected from two of these infected fish while 36 infections contained 43 unfertilized females; this represented 15.8% of the total female population. The 41 male-only infections totaled 51 worms which represented 18.5% of the male population.

The female worms from the unisexual infections were not new infections. The ovary ligament had ruptured and ovarian balls were scattered throughout the pseudocoel. Fifty-three percent of the females were greater than 25 mm in length. Likewise many of the largest males (> 20 mm) collected during the study were from unisexual infections.

Sexual Development and Maturation of Female Echinorhynchus spp.

To follow the course of female Echinorhynchus spp. maturation during the year, the worms were examined and classified by stage of maturity using the following criteria: presence of unruptured ligament; ovarian balls; immature acanthors; and mature eggs. The size

Table 26. Unisexual infections of *Echinorhynchus gadi* recorded from *Glyptocephalus zachirus*.

Month	No. of Infected Fish	Unisexual Infections	
		Male	Female
Jan, 1972	3	1	2
March	2	2	
April	35	13	13
May	22	1	4
June	3		2
Aug	22	5	3
Sept	25	3	4
Oct	13	7	2
Jan, 1973	18	4	6(2M)*
April	3	2	
May	6	1	1
June	4	2	1
July	3		
Total	159	41	38

*(M) = Mature Female Infection.

of the worms at the various stages of maturity according to these criteria are given in Table 27. Generally the worms from M. pacificus were larger at a comparable stage than those from either G. zachirus or L. armatus.

The sexual development of Echinorhynchus sp. in L. armatus from Yaquina Bay exhibited a non-seasonal pattern. All stages of maturity were observed in worms each month during the study (Figure 12). Gravid females made up 43.4% of the worm population. The 85 females collected from offshore samples contained only ovarian ball stage and older worms. Gravid females made up 68.2% of the worms from offshore collections.

The sexual development of E. gadi in both M. pacificus and G. zachirus exhibited a definite seasonal pattern of maturation in contrast to the continuous (non-seasonal) developmental pattern observed for Echinorhynchus sp. in L. armatus (Figures 13, 14). The ligament stage was observed in worms from February through May both in 1972 and 1973 and in both fish hosts. The ovarian ball stage in young worms was observed from April to August in both M. pacificus and G. zachirus. Large worms from unisexual infections were also in the ovarian ball stage in these hosts from October through January. These large females were probably unfertilized individuals.

The presence of immature acanthors indicated that fertilization had taken place. Worms in this stage of development first appeared

Table 27. Number of parasites, average length and size range of sexual maturity stages of Echinorhynchus sp. from Leptocottus armatus and Echinorhynchus gadi from Microstomus pacificus and Glyptocephalus zachirus.

Stage of Sexual Maturity	Echinorhynchus sp. from Leptocottus armatus			Echinorhynchus gadi from Microstomus pacificus			Echinorhynchus gadi from Glyptocephalus zachirus		
	No. of Parasites	Average Length (mm)	Range (mm)	No. of Parasites	Average Length (mm)	Range (mm)	No. of Parasites	Average Length (mm)	Range (mm)
Ligament	57	7.2	5-11	78	8.6	5-12	36	8.0	5-11
Ovarian ball	86	11.9	7-18	137	16.0	12-22	96	13.4	7-22
Immature acanthor	90	18.2	11-25	128	23.1	18.34	79	17.7	7-24
Mature acanthor	216	27.7	14-45	219	36.1	24-55	62	31.7	12-52

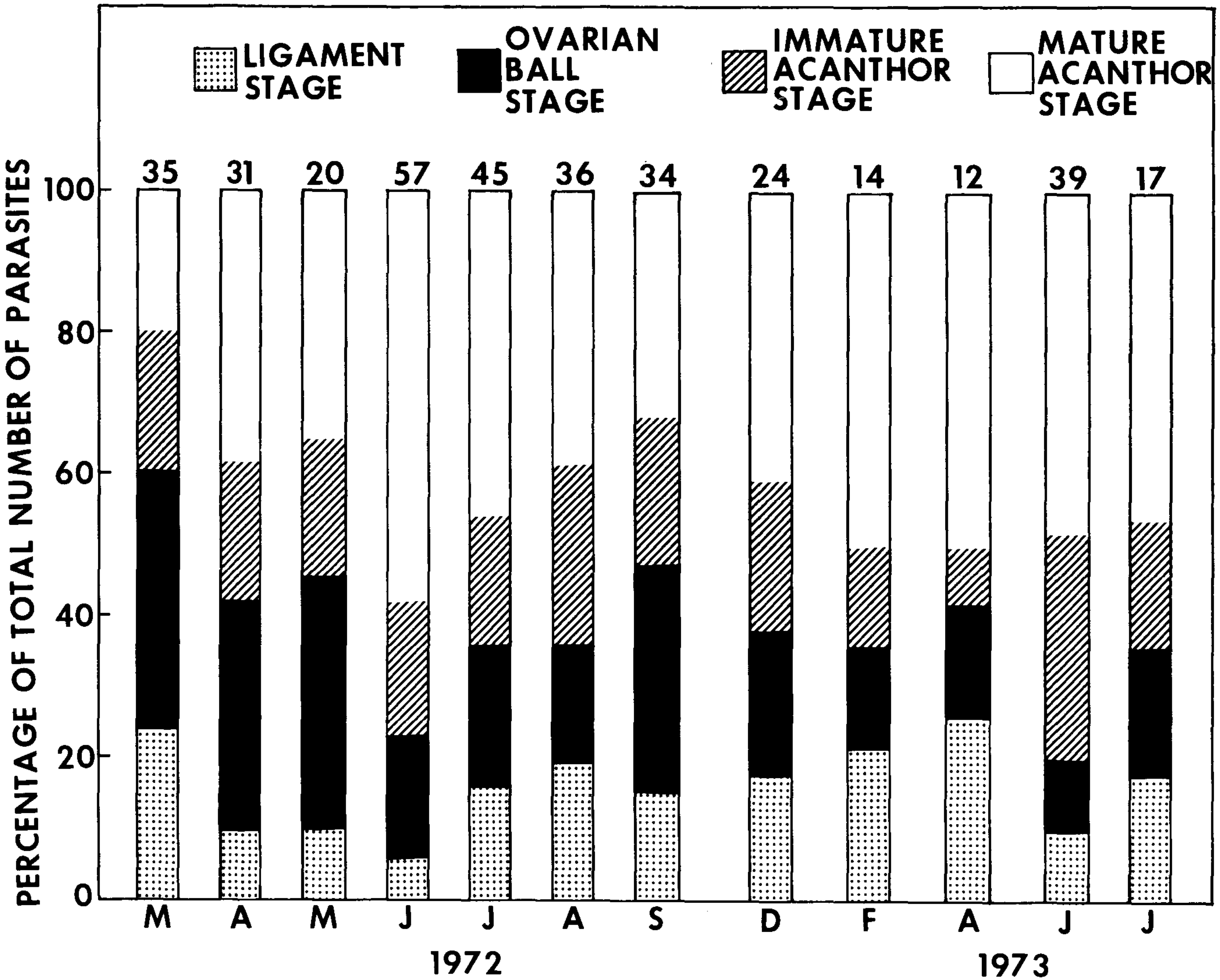


Figure 12. Seasonal differences in the percentage of Echinorhynchus sp. females with ligament, ovarian ball, immature acanthor and mature acanthor stages in Leptocottus armatus collected from Yaquina Bay, Oregon in 1972-73. Number of specimens examined is above each column.

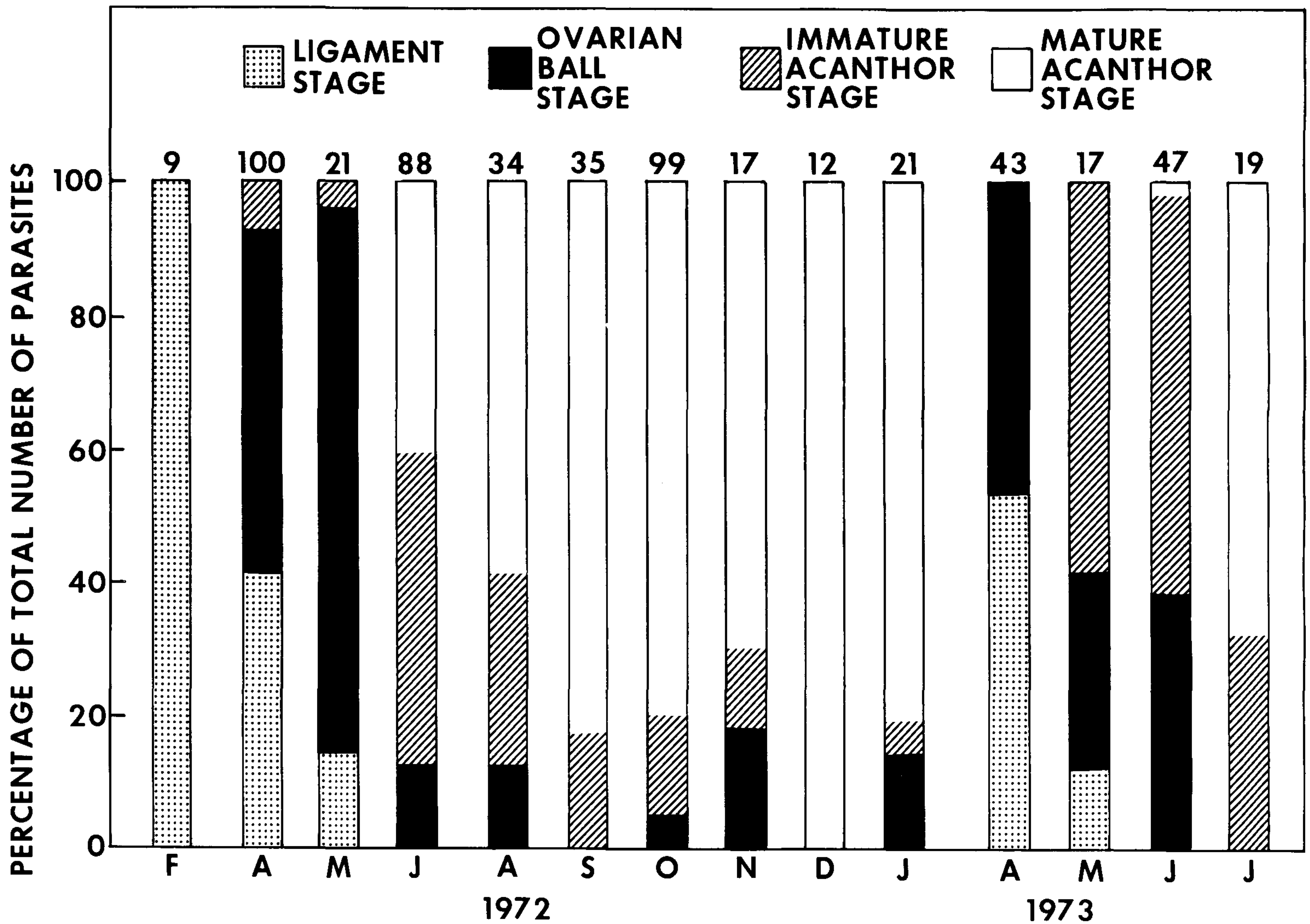


Figure 13. Seasonal differences in the percentage of Echinorhynchus gadi females with ligament, ovarian ball, immature acanthor and mature acanthor stages in Microstomus pacificus collected off Central Oregon in 1972-73. Number of specimens examined is above each column.

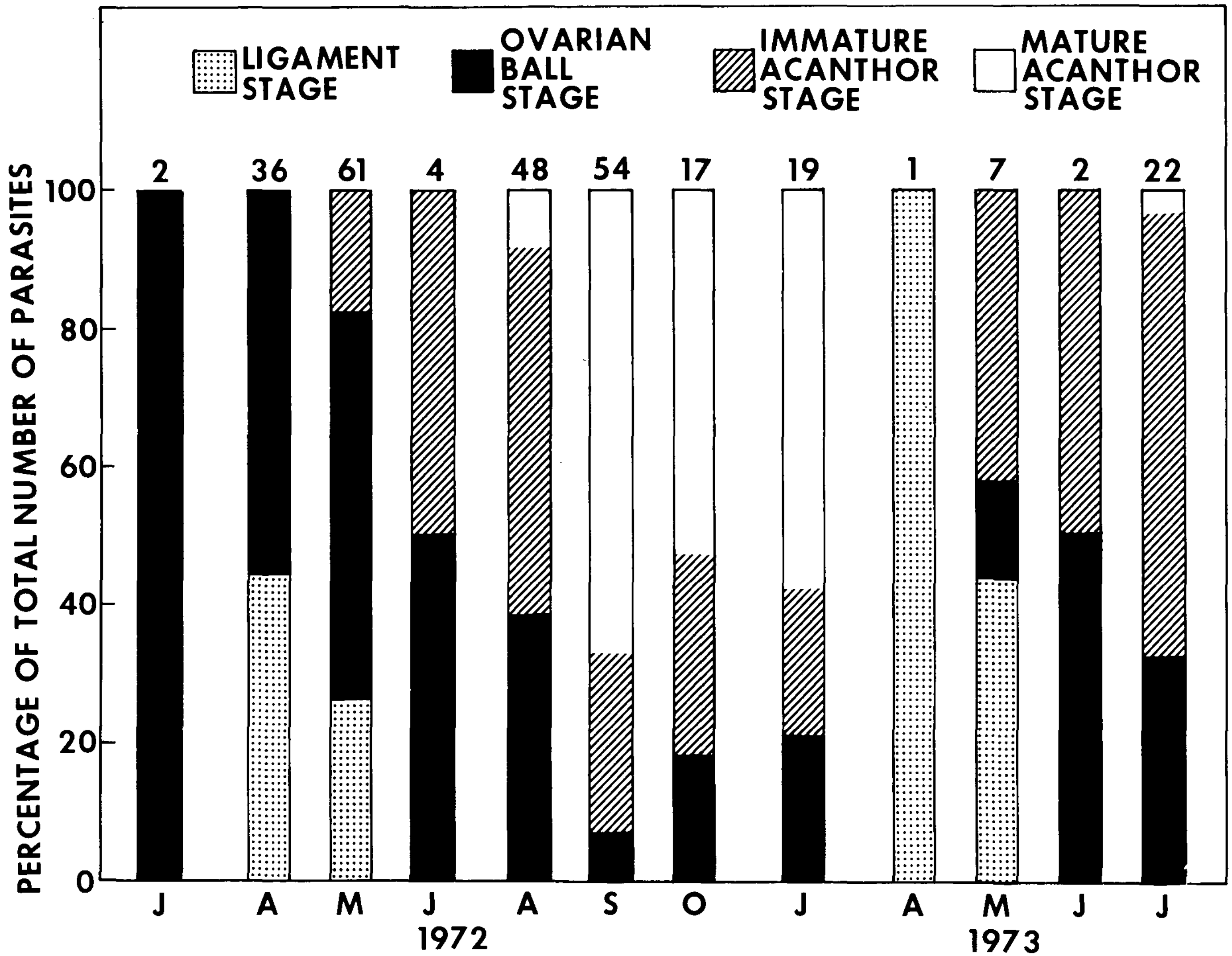


Figure 14. Seasonal differences in the percentage of Echinorhynchus gadi females with ligament, ovarian ball, immature acanthor and mature acanthor stages in Glyptocephalus zachirus collected off Central Oregon in 1972-73. Number of specimens examined is above each column.

in April in M. pacificus and in May in G. zachirus and could be found in both hosts until January. Copulation and fertilization occurred in E. gadi from M. pacificus from mid-March through August. The ovarian ball and immature acanthor stages were present in April and the ovarian ball stage was no longer present in the parasite population by September with the exception of 11 worms observed between October through January from unisexual infections (Figure 13).

Gravid females were first observed in June in M. pacificus and made up 81% of the E. gadi population from September through January. The remainder of the population consisted of unisexual ovarian ball (6%) and immature acanthor (13%) stages. A similar developmental pattern was observed for E. gadi in G. zachirus with 62% of the parasite population from September to January classified as gravid females.

Estimate of Longevity

The longevity of Echinorhynchus sp. in L. armatus could not be estimated because of the non-seasonal cycle. However, the longevity of E. gadi in both M. pacificus and G. zachirus was approximately one year.

Life Cycle and Larval Development

Life Cycle Experiments

The intermediate host of Echinorhynchus sp. was determined by a series of feeding experiments using six species of amphipods and mature eggs from three species of Echinorhynchus (Table 28). Positive infections were observed only with the combinations of E. lageniformis eggs and C. spinicorne and Echinorhynchus sp. eggs and A. confervicolus. The remaining combinations including all experiments involving E. gadi eggs were negative as were over 1000 individuals of each of the six amphipod species that were examined for natural infections. The three offshore amphipod species were selected for experimentation because they occurred in over 75% of the stomach samples of M. pacificus and G. zachirus.

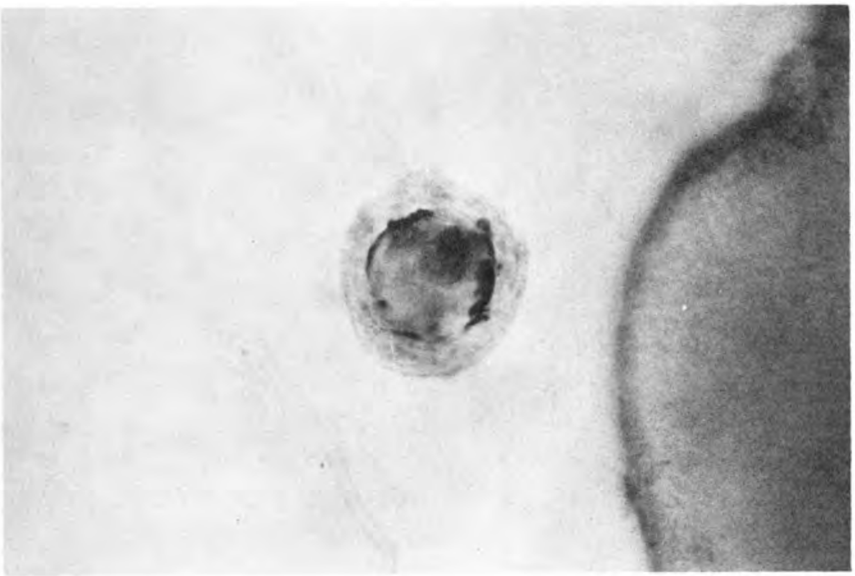
Large scale feeding experiments were conducted at 12°C and 23°C using the combination of Echinorhynchus sp. eggs and A. confervicolus to obtain developing larvae for detailed study. Over 600 larvae were recovered from experimentally infected amphipods. The reproductive system in 390 larvae was developed to a stage so that the sex of the worm could be determined. Of these, 197 larvae were identified as females and 193 as males with a resulting male to female sex ratio of 1:1.02.

Table 28. The various combinations of feeding experiments using six species of amphipods and eggs from three species of *Echinorhynchus* (+ equals positive infection, - equals no infection).

Amphipod Species	Collection Site	Egg Source		
		Echinorhynchus sp.	*E. lageniformis*	*E. gadi*
Anisogammarus confervicolus	Yaquina Bay	+	-	-
Corophium spinicorne	Yaquina Bay	-	+	-
Eohaustorius estuarius	Yaquina Bay	-	-	-
Ampelisca brevisimulata	Offshore	-	-	-
Photis sp.	Offshore	-	-	-
Rhachotropis sp.	Offshore	-	-	-

Some mortality of exposed A. confervicolus was observed possibly due to overinfection. Exposed amphipods usually contained two to six larval Echinorhynchus sp., and 12 larvae was the maximum number observed in any experimental infection. The rate of development appeared to be highly temperature dependent. Parasite development in A. confervicolus held at 12°C was slower than in those held at 23°C. After 35 days at 12°C, the parasites were at a stage of development equivalent to that at 12 days at 23°C. Development for 53 days at the lower temperature resulted in an acanthella with the proboscis everted prior to hook development. This was similar to the stage of development of acanthella found after 28 days at 23°C.

The early development of Echinorhynchus sp. occurred in close association with the serosa of the intermediate host intestine. The larva was surrounded by a thin layer of connective tissue and amphipod hemocytes while attached to the intestine. After the 10th day of development, the parasites became free in the amphipod hemocoel and were surrounded by a thin, transparent capsule of host connective tissue. Occasionally black structures of varying sizes were attached to the serosa of A. confervicolus and the other amphipods used in the feeding experiments. The smallest objects were recognizably acanthors and on some, the characteristic acanthor hooks were often visible. This darkening was definitely in and not on the tissues of the smaller parasites (Figure 15). In other cases the deposition of a



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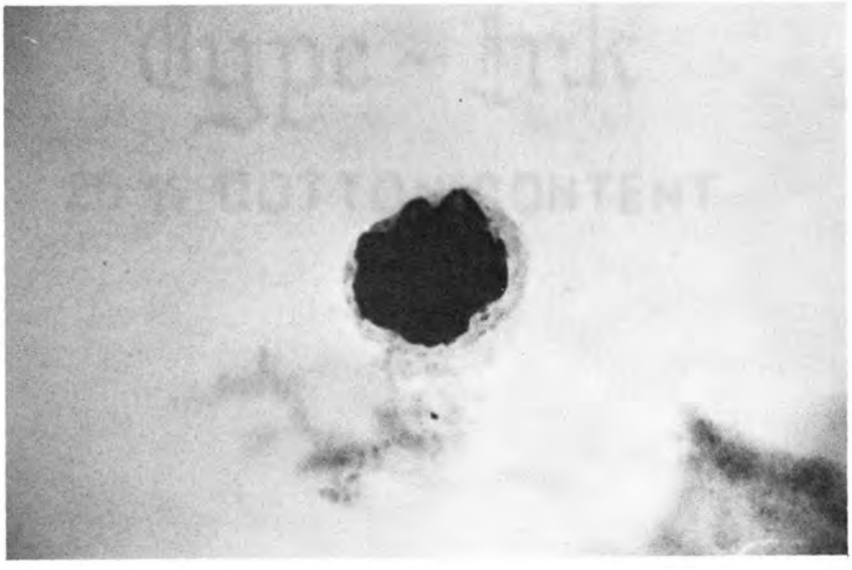

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Figure 15. Three-day acanthella of <u>Echinorhynchus</u> sp. removed from hemocoel of <u>Anisogammarus</u> <u>confervicolus</u>. A. Acanthella (A) surrounded by several layers of amphipod hemocytes (H) showing initial formation of melanin-like material (M). Semichon's acetocarmine stain. 10X. B. Acanthella (A) showing extensive deposition of melanin-like material (M). Semichon's acetocarmine stain. 10X.

melanin-like material or a large accumulation of hemocytes was seen on the outside of the acanthella.

Development of Echinorhynchus sp. in Anisogammarus confervicolus

The description of the larval development of Echinorhynchus sp. was based on the measurement and observation of over 600 larvae. As reported for other acanthocephalan larvae, there was variation in the size of the larvae recovered after identical periods of development, even for larvae developing in the same amphipod (Figure 16). For this reason, average measurements are reported and typical developmental stages for illustration were chosen.

Eggs (Figure 17) passed by the final host, L. armatus, into the water and measuring 88 to 20 are surrounded by four membranes. The outer membrane consists of a thin covering which is the membrane that surrounds the unfertilized egg. Beneath this is the prominent fibrillar coat. The most characteristic feature of the egg is the fertilization membrane which appears as a rigid shell with pronounced polar elongations. The fourth and innermost membrane is a thin structure which is found in close association with the body surface of the acanthor. Within the membranes, the acanthor measures 47 by 15 and the inner nuclear mass or entoblast is very evident. The entoblast is surrounded by a cortical area containing small nuclei that eventually become the giant nuclei of the acanthella stage.

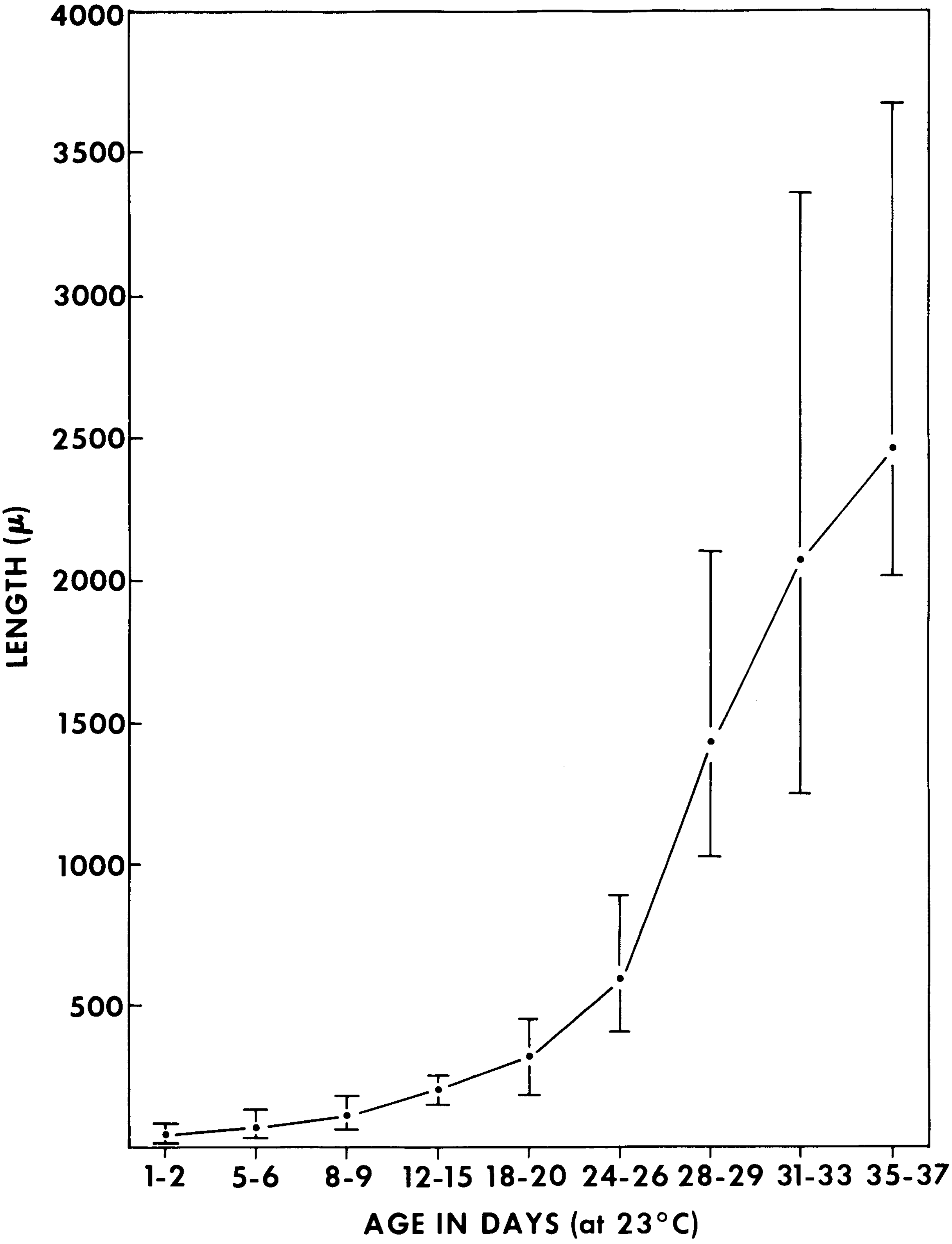


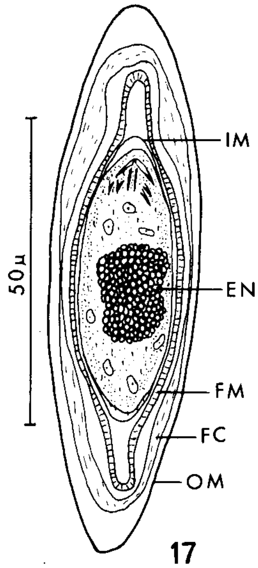
Figure 16. The growth (length) of Echinorhynchus sp. in its intermediate host Anisogammarus confervicolus (vertical line, observed range).

The exact number of these nuclei could not be determined in this early stage, but in later development the number was always 20 nuclei.

Acanthor. When ingested by the amphipod intermediate host, A. confervicolus, the acanthor hatches in the lumen of the intestine, penetrates the intestinal wall in 6 to 24 hours and attaches to the serosa. The anterior end of the acanthor is distinguished by three prominent rows of hooks and several smaller rows (Figures 17 and 18). The location and shapes of the hooks correspond to that described by Grabda-Kazubska (1964) for several Echinorhynchus spp.

Acanthella. The study of acanthella development was described from specimens obtained only from amphipods held at 23°C. After penetrating the gut, the attached acanthella stage is usually surrounded by crustacean hemocytes which eventually form an envelope or capsule (Figure 18). The acanthella has grown slightly after three days (Figure 19) averaging 70 by 44. The 20 giant nuclei are clearly visible ranging from 9 to 12 in diameter. The acanthor spines are still visible and were observed up to 23 days in the acanthella stage. After 5 to 6 days, the attached acanthella has become a bladderlike structure measuring 78 by 60 (Figure 20). This stage is characterized by the presence of four large apical nuclei which arise from the entoblast and recognizable proboscis, muscle and gonadal primordium.

Figures 17-22. Stages of development of Echinorhynchus sp. 17. Egg. 18. One-day acanthella. 19. Three-day acanthella. 20. Six-day acanthella. 21. Twelve-day acanthella. 22. Eighteen-day female acanthella. Abbreviations: AC, acanthor; AN, apical nuclei; C, capsule; EH, embryo hooks; EN, entoblast; FC, fibrillar coat; FM, fertilization membrane; GN, giant nuclei; GP, nuclei of genital primordium; H, hemocytes; IM, inner membrane; IPN, proboscis invertor nuclei; L, ligament; LN, lemniscal giant nuclei, MP, nuclei of muscle primordium; NR, neck retractor; O, ovary; OM, outer membrane; PG, proboscis ganglion; PN, posterior proboscis nuclei; PP, proboscis primordium; PR, proboscis retractor; RP, proboscis receptacle; UB, uncinogenous bands; UT, uterus; VA, vagina.



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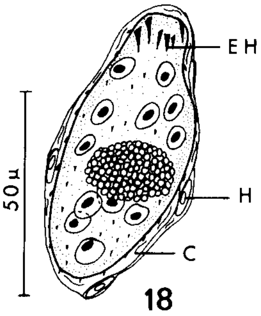

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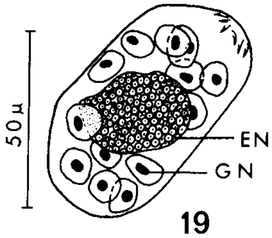

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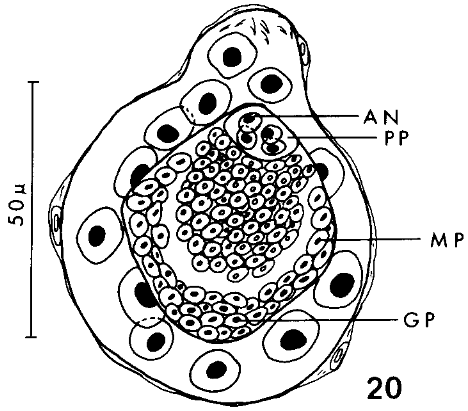

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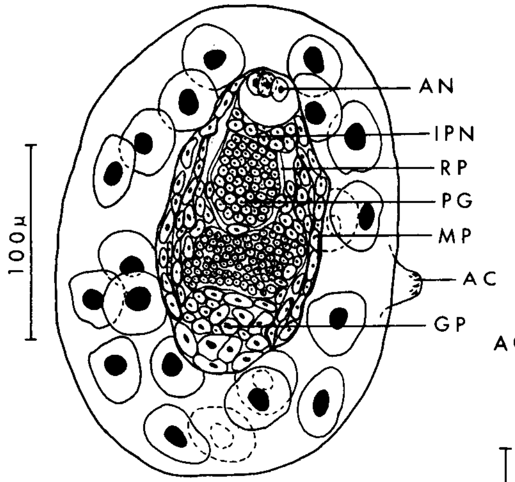

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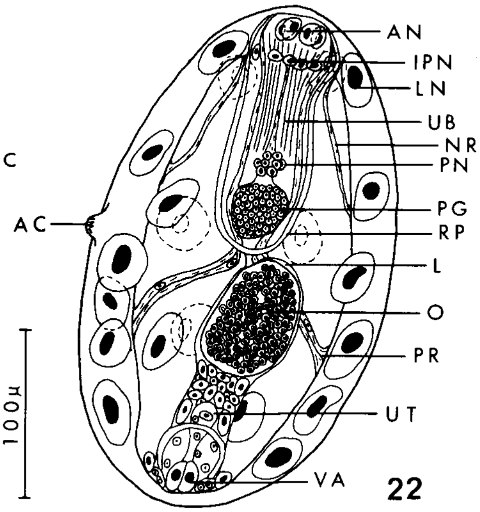

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Development proceeds very rapidly and by the 10th day the acanthella is free within the amphipod hemocoel. During this rapid growth period, development has centered around differentiation of the proboscis. The acanthella at 12 to 15 days (Figure 21) measures about 245 by 173 and contains an entoblast measuring 158 by 84. A small remnant of the original acanthor can be observed as larval hooks projecting from the acanthella tegument. The cerebrial ganglion primordium is a distinct cluster of cells while the proboscis invertor nuclei are clearly visible posterior to the developing proboscis. The proboscis receptacle ligaments which will form the doubled wall receptacle have developed to a point where the proboscis region can be distinguished from the rest of the developing acanthella. At this stage, the reproductive system is not differentiated and the sex of the developing organism cannot be determined. The 20 giant nuclei measure about 32 by 27 and are still randomly arranged in the cortical area. The muscle primordia are more clearly distinguished as longitudinal bands.

Between 18 and 20 days the shape of the acanthella has become oblong and measures 309 by 178 with the giant nuclei located in the periphery of the cortex (Figure 22). A ring of four of these giant nuclei, the lemniscal nuclei, has formed around the anterior end and marks the division of the presoma and metasoma. Eventually the presoma will consist of the proboscis, neck, proboscis receptacle with its associated nervous and muscular systems and the lemnisci while the metasoma will consist of the body proper including the reproductive organs.

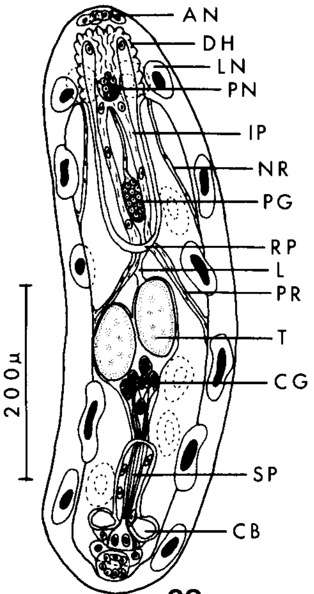
The two neck and the two proboscis retractor muscles are well defined. The neck retractor muscles are made up of a single binucleated tissue band attached to the body wall near the lemniscal nuclei and to the body wall posteriorly near the developing proboscis ganglion. The proboscis retractors each consist of two mononucleated tissue bands running diagonally from the base of the proboscis receptacle to the body wall. These paired bands develop in close association and later fuse into the binucleated rectractor muscles.

The reproductive system has developed to a point where the sex of the future adult can be distinguished. As is characteristic of the order Palaeacanthocephala, the reproductive system is enclosed by a single ligament sac attached to the posterior end of the proboscis receptacle. The reproductive system of an 18 day female acanthella (Figure 22) consists of a large ovary as well as the developing uterus-uterine bell complex and vagina. A 20 day male acanthella shows symmetrically located testes as well as the developing cement glands, Saefftigen's pouch and copulatory bursa.

Within the proboscis receptacle the ganglion, uncinogenous bands on which the future hooks will develop, eight posterior proboscis nuclei, the proboscis invertor nuclei and the four apical nuclei are distinct. A conspicuous feature at this stage of development is the circle of the eight posterior proboscis nuclei located within and near the posterior end of the proboscis receptacle. These nuclei correspond to the proboscis nuclear ring of Cable and Dill (1967). From these nuclei the uncinogenous bands, apparently in the form of a cylinder, extend anteriorly and terminate at the base of the four apical nuclei. The four apical nuclei are still located within the proboscis receptacle. These nuclei will go into the formation of the apical sense organ of the proboscis. The proboscis invertor nuclei will eventually form the invertor muscles.

A male acanthella measured 596 by 175 after 26 days of development (Figure 23) and the cortical area containing the giant nuclei has been forced to the periphery by the growth of the internal structures, resulting in the elongated shape characteristic of the fully developed acanthella The proboscis invertor muscles are now well developed as multinucleated tissue bands and extend posteriorly to pass over the ganglion where they contain nuclei larger than those of the ganglion. Posteriorly the invertors penetrate the wall of the proboscis receptacle and continue posteriorly as the proboscis retractors which attach to the body wall. The four apical nuclei have broken through the proboscis receptacle and are located at the anterior end of the acanthella. As the nuclear ring of the proboscis moves anteriorly, the uncinogenous bands arch posteriorly from the nuclear ring and

Figures 23-26. Stages of development of <u>Echinorhynchus</u> sp. 23. Male acanthella 26 days postinfection. 24. Acanthella after 28 days of development showing everted proboscis. 25. Anterior of male acanthella after 31 days of development showing inverted proboscis. 26. Infective juvenile female after 36 days of development in <u>Anisogammarus</u> <u>confervicolus</u>. Abbreviations: AN, apical nuclei; C, capsule; CB, copulatory bursa; CG, cement glands; DH, developing hooks; GN, giant nuclei; IP, proboscis invertor muscle; IPN, proboscis invertor nuclei, L, ligament; LM, lemnisci; LN, lemniscal giant nuclei; NR, neck retractor; O, ovary; PG, proboscis ganglion; PH, proboscis hooks; PN, posterior proboscis nuclei, PR, proboscis retractor; R, retinacula; RP, proboscis receptacle; SA, selector apparatus; SP, Saefftigen's pouch; T, testes; UT, uterus; UTB, uterine bell; VA, vagina.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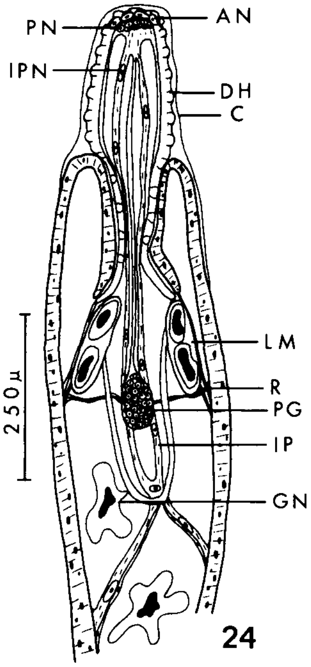
23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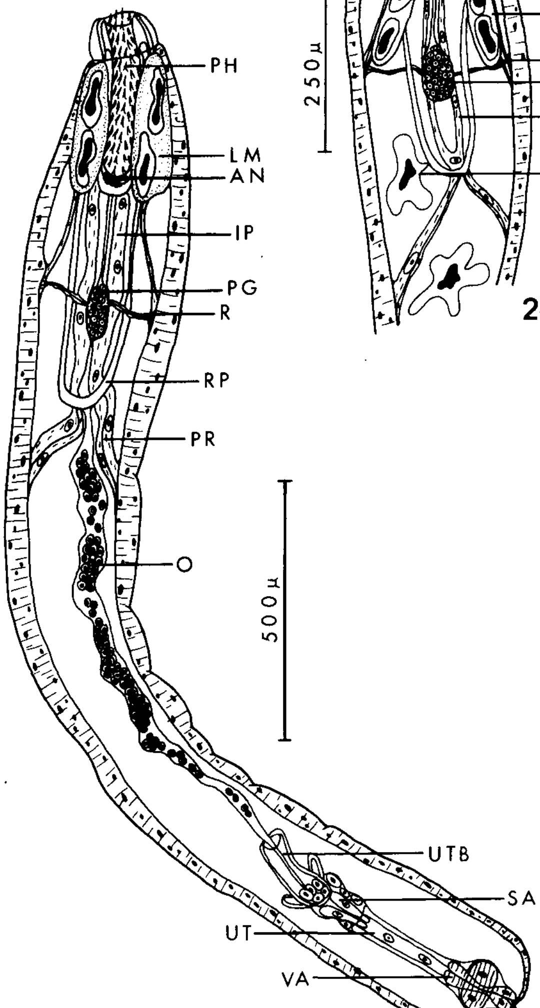
24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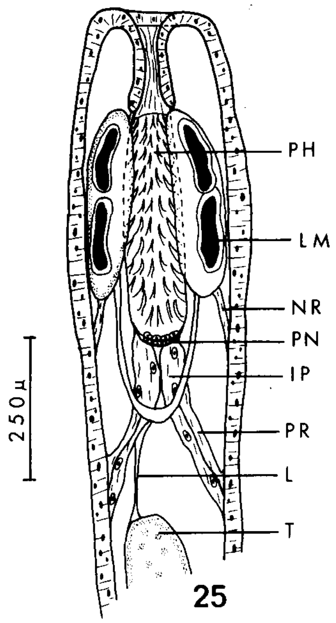
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25

locate beneath the proboscis wall. The developing hooks first appear as blunt conical projections from the bands and are first seen as the developing proboscis starts to evert.

Several large nerve trunks begin to appear extending from the ganglion. At this stage of development the two testes are transitional between a symmetrical and a tandom position. The eight cement glands have their ducts well developed.

The developing acanthella by days 28-29 measured 1440 by 180 (Figure 24). The proboscis has continued to evert and the developing hooks have increased in size although they are still completely imbedded in the proboscis wall. The apical nuclei are now located beneath the developing hooks and the proboscis nuclei have moved to the anterior tip of the everted proboscis. The two lemnisci develop as lateral evaginations of the hypodermal layer into the body cavity and the four lemniscal nuclei move into them, two into each lemniscus. The remaining giant cortical nuclei become dendritic and begin to break up to form the condensed nuclear fragments in the hypodermis of the infective juvenile and adult. Just anterior to the developing lemnisci, a slight constriction of the body wall separates the proboscis from the trunk. The lateral nerves or retinacula are very conspicuous running from the ganglion to the body wall. The circular muscular layer of the body wall appears and is lined internally with a layer of large round nuclei that will contribute to the formation of the longitudinal muscles. In male specimens, the testes are now in the final tandem position.

By the 31st day (Figure 25) the proboscis begins to invert as the acanthella completes its development. The neck retractor muscles completely pull the neck inward, with the result that the entire presoma rests inside the trunk. The proboscis hooks complete their development by breaking through the cuticle and the proboscis is capable of inversion and eversion. The length of worms at this stage ranged from 1200 to 3300 depending upon the position of the proboscis.

A female infective juvenile at 35 days of development with the proboscis slightly everted is shown in Figure 26. The length of larvae at this stage ranged from 2000 to 3700 and larval development is essentially completed. Successful completion of the life cycle and determination of the age at which juveniles become infective were accomplished by feeding experiments. This consisted of allowing young of the year L. armatus to feed on infected amphipods 30-37 days after the intermediate hosts were exposed to acanthocephalan eggs. The fish were held in tanks of circulating seawater free of the intermediate host for 30 days prior to the feeding experiments. Intermittent dissections revealed that juveniles 33 days of age or older were capable of producing infections in sculpins. Immature adult worms doubled their size, reaching 5-6 mm after 35 days in the final host.

DISCUSSION

Of the three species of Echinorhynchus in marine fishes along the Oregon coast two were studied here: Echinorhynchus gadi in Microstomus pacificus and Glyptocephalus zachirus, and Echinorhynchus sp. in L. armatus which was reported as E. gadi by Burreson (1973) and Dunlap (1951).

Echinorhynchus gadi has not previously been reported from M. pacificus and G. zachirus; these constitute new host records for the parasite. Ekbaum (1938) reported E. gadi from five Oncorhynchus spp. off British Columbia. However, Golvan (1969) renamed this acanthocephalan E. ekbaumi. The acanthocephalan from the two pleuronectid flatfishes is identical to E. gadi as described in the literature except for the regular occurrence of 17 rows of longitudinal hooks on the proboscis. Echinorhynchus gadi has been reported to have a minimum of 18 rows. Echinorhynchus sp., found only in L. armatus, is an undescribed species and resembles only E. laurentianus which parasitizes pleuronectid flatfishes on the east coast of North America.

Since acanthocephalans are endoparasites during all stages of their life history, the parasites host range is directly dependent on the ecology and distribution of the intermediate and definitive hosts. Factors affecting the host range of the acanthocephalans found in

L. armatus, M. pacificus, and G. zachirus would include: the life histories of the parasites, crustacean and fish hosts; the diet of the fish hosts at different times in their life history; the host environments; and host migrations.

Leptocottus armatus, a benthic euryhaline member of the cottid family, is found in coastal waters of the eastern Pacific Ocean and connecting bays and estuaries from Kodiak Island, Alaska to San Quentin Bay, Baja, California (Jones, 1962). It is extremely abundant in bays and estuaries along the Oregon coast including the Yaquina Bay estuary (Beardsley and Bond, 1970) and also occurs in offshore shallow water throughout most of the year (Burreson, 1973).

Leptocottus armatus is well adapted to estuarine life. They spawn during the winter and the young of the year are present in estuaries during the early spring when salinities are generally low. The small sculpins exhibit a wider tolerance to salinities than adult fish and occasionally migrate into fresh water (Jones, 1962). Staghorn sculpins occur in Yaquina Bay and offshore waters during most the year, but movements within the estuary and between the estuary and ocean are not well defined (Burreson, 1973).

Food of L. armatus consists of a wide variety of marine invertebrates and small fishes (Clemens and Wilby, 1961; Jones, 1962). Predominant in the diet of Yaquina Bay sculpins in this study was the gammarid amphipod, Anisogammarus confervicolus. This contrasts with the staghorn sculpin diet in Tomales Bay observed by Jones (1962) to consist of 88% of the tubiculous amphipods of the genus Corophium.

Leptocottus armatus was present in Yaquina Bay during all sampling periods and young of the year fish were most abundant from February through April. Echinorhynchus sp. was also observed in sculpins throughout the year. Young of the year fish had the lowest prevalence and intensity of infection probably because Anisogammarus confervicolus, the intermediate host of Echinorhynchus sp., was not a preferred food item for fish of this size. The prevalence and intensity of infection in fish greater than 100 mm total length were similar in all size classes and substantially higher than in young fish. The presence of Echinorhynchus sp. in 65.9% of L. armatus greater than 200 mm total length indicated that these fish feed on A. confervicolus throughout their adult life.

This contrasts with observations of E. lageniformis infections in starry flounders. Barnes (1968) reported that young Platichthys stellatus (30 to 150 mm in total length) in Yaquina Bay had the highest prevalence and intensity of E. lageniformis while older fish were rarely infected. He concluded that older fish no longer utilized amphipods as a major food source.

According to Jones (1962), the staghorn sculpin is rather unselective in its food habits and feeds largely on what is available.

This does not appear to hold true in Yaquina Bay where Corophium spinicorne, the intermediate host of E. lageniformis, is abundant but infections of this parasite in staghorn sculpins are rare. In the Siletz River estuary, L. armatus may feed more heavily on C. spinicorne as evidenced by the high prevalence (46.2%) of E. lageniformis in the small sample of 13 fish collected there.

The lack of variation in the prevalence and intensity of Echinorhynchus sp. infections throughout the year indicates a lack of seasonality in the parasite life cycle. The lack of a seasonal cycle is also clearly evident when age structure of the parasite population is examined. Immature and mature worms of both sexes were observed in fish of all size classes during all seasons of the year. Although the parasite population did not show a seasonal maturation cycle, female worms did predominate (58.3%) in the collections. The sex ratio of Echinorhynchus sp. was similar to the ratios of other acanthocephalan studies. Since fertilization occurs only once in acanthocephala (Hyman, 1951), and male worms do not usually live as long as females, a sex ratio in favor of female worms is usually observed in natural infections of acanthocephalans (Crompton, 1970).

Studies on Polymorphus minutus in ducks have demonstrated that a sex ratio of 1:1 exists during the first part of the infection (Nicholas and Hynes, 1958). The sex ratios change after copulation. Crompton and Whitfield (1968) observed in experimental infections with

P. minutus that the worms were found in the definitive host intestine in approximately equal numbers for the first 16 days of the infection. However, the rate of loss of males was about four times that of females and no males remained after 36 days although some females continued to live in the host for 20 more days. Similarly, Awachie (1966) reported that male E. truttae began to be discharged from trout more rapidly than female worms 45 days after the start of the experimental infection.

Female worms in the ligament stage of development and/or female worms 11 mm or less in length were considered new Echinorhynchus sp. infections regardless of time of year. Such infections were observed throughout the year in Yaquina Bay. Male worms 4 to 8 mm in length were likewise considered to be newly acquired infections and were also observed throughout the year.

The observed sex ratio of about 1:1 for young worms indicates that an equal number of males and females develop from eggs. This is further supported by the observation of a sex ratio of one male to 1.02 females in the intermediate host. Parenti, Antoniotti and Beccio (1965) reported that the sex ratio of E. truttae developing in natural populations of Gammarus pulex was 1:1 irrespective of the sex and age of the host and of the size of infection. The sex ratio of Acanthocephala in the intermediate host therefore appears to be under direct genetic control and is not influenced by the environment provided by the intermediate host (Crompton, 1970).

Approximately 12% of the female Echinorhynchus sp. collected were from unisexual infections. These worms could represent a segment of the parasite population that does not contribute to the production of new individuals if male worms never appear. This is unlikely in Echinorhynchus sp. in L. armatus since new infections occurred throughout the year and unmated females are never excluded from potential contact with a male.

The parasite-host system of Echinorhynchus sp. in L. armatus is similar to the model for endoparasites with non-seasonal cycles proposed by Kennedy (1970, 1972) in which host diet and feeding behavior of the fish are the major controls upon the flow of parasites through the system. In Yaquina Bay a dynamic equilibrium may be postulated where there is a variable but continuous level of infection of one year and older L. armatus by Echinorhynchus sp. infective juveniles and an equally variable and continuous loss of mature worms. The population of Echinorhynchus sp. at any given time will be the result of the interaction of these two variables.

Although natural infections of A. confervicolus were not recorded, breeding pairs of amphipods were observed throughout the year. Thus a constant supply of amphipods of all sizes were available for infection. Infected A. confervicolus were probably present in Yaquina Bay throughout the year since newly acquired infections in

L. armatus were recorded during all seasons. The offshore collections of L. armatus lacked immature worms probably because these fish had migrated from the source of infection.

The lack of a seasonal cycle of Echinorhynchus sp. infections in L. armatus is not unusual. Awachie (1966) found no seasonal cycle of prevalence of infection in E. truttae from trout in North Wales. This was due to the presence of infective juveniles in Gammarus spp. and of acanthocephalan eggs in trout throughout the year. The seasonal changes which he observed were correlated with the seasonal variation in the feeding activity of the fish. Similarly Chubb (1964) and Pennycuick (1971) working with Acanthocephalus clavula (Dujardin) and Polyanski (1958) with E. gadi found no seasonal cycles in prevalence or maturation cycles in the parasite population and all concluded that host feeding behavior was probably the most important factor in determining the level of parasitic infections.

Microstomus pacificus and Glyptocephalus zachirus are important components of the commercial demersal fishery of the Northwestern Pacific Ocean (Alverson, et al., 1964). The biology of these two species is similar, probably accounting for the fact that they are both parasitized by E. gadi. The observed differences in the host-parasite relationship between these flatfishes and E. gadi could reflect differences in host biology.

Microstomus pacificus occurs from Baja, California to the eastern Bering Sea and are abundant from northern California to the Columbia River. Adults are rarely found inside 20 fathoms and range in depth to at least 600 fathoms (Frey, 1971). Dover sole have a strong preference for mud and muddy-sand bottoms (Day and Pearcy, 1968). Tagging experiments off Oregon and California suggest the existence of several subpopulations of M. pacificus adults (Demory, 1975, Frey, 1971). Tag returns indicate limited coastwise movement, but extensive seasonal inshore-offshore movement dominated by female individuals. Demory (1971) reported that in February 80% of small Dover sole (less than 18 cm) occurred on the continental shelf between 50 and 100 fathoms and that from May through August approximately 95% of the small fish occurred between 20 and 40 fathoms. In November they were again found in deeper water from 50 to 70 fathoms. Large Dover sole (greater than 18 cm) were generally captured with smaller fish indicating that the seasonal migrations are not restricted to the younger fish.

The migration pattern of M. pacificus appears to be closely linked to the seasonal reproductive cycle. Hagerman (1952) and Demory (1975) reported that spawning in Dover sole occurs apparently at specific sites at depths of 200 and 400 fathoms over a three month period from December through February although a few spent fish were captured as early as November. By February about 30% of the mature females had shed their eggs and by April approximately 98% of the mature females were spent. After spawning most females return to shallower water and summer feeding grounds while large numbers of males remain in deeper waters.

Larval Dover sole spend approximately one year in the water column before they metamorphose and settle to the bottom. During this time they feed on plankton. Off the Columbia River, the larvae are about 64 mm in length whe "settling out" occurs in January and February near the edge of the continental shelf (Demory, 1975). Once on the bottom, M. pacificus feed almost exclusively on small mud-dwelling invertebrates such as gammarid amphipods and polychaete worms. Stomach contents of year old fish indicate they have the same feeding habits as the adults (Hagerman, 1952).

Glyptocephalus zachirus range from southern California to the Bering Sea and are most abundant from San Francisco northward to the Queen Charlotte Islands off Canada. Adults occupy a wide range from 10 to 400 fathoms, and show little preference for bottom sediment type, being equally distributed over sand or mud bottoms (Demory et al., 1976).

Like Dover sole, Rex sole also exhibit a seasonal inshore-offshore movement. Demory (1971) captured small Rex sole (less than 18 cm in length) in depths ranging from 20 to 140 fathoms. In February small fish were not found inshore of 40 fathoms but in May and August they were commonly found between 20-29 fathoms. Larger individuals also showed a similar pattern of movement.

Unlike M. pacificus, G. zachirus apparently has no specific spawning localities off the Oregon coast. Most spawning occurs in depths of 50 to 150 fathoms from January through June (Hosie, 1976). Like larval Dover sole, larval Rex sole feed on plankton and spend approximately one year in the water column before they metamorphose and settle to the bottom. Most larvae are about 50 mm in length when they assume a bottom life (Hosie, 1976). One-year and older Rex sole feed primarily on sand and/or mud dwelling invertebrates including polychaete worms and amphipods. The small mouth of this species precludes it eating bigger prey (Hosie, 1976).

During the course of study, M. pacificus and G. zachirus were regularly collected off Newport, Oregon except during winter and early spring when fish were in deeper water and rough ocean conditions prevented frequent sampling. Although other species of flatfishes were commonly collected during this study, (Citharichthys sordidus (Girard), C. stigmaeus Jordan and Gilbert, Eopsetta jordani (Lockington), Isopsetta isolepis (Lockington), Parophrys vetulus, and Psettichthys melanostictus Girard), E. gadi infections were found only in M. pacificus and G. zachirus.

When the monthly prevalence and intensity of infection in these two species was examined, there was no apparent seasonality of infection. However, when the parasite population within the hosts was examined, a seasonal maturation cycle was evident. Small immature worms of both sexes were collected only during the early spring and by September the entire parasite population consisted of sexually mature individuals in both hosts. Female E. gadi collected from M. pacificus in April, 1972 averaged 12.6 mm in length and 96% were classified as immature. The female worm population from September, 1972 through January, 1973 averaged 36 mm in length and 94% were classified as mature and 81% of these were gravid. In April, 1973 the female E. gadi population in M. pacificus consisted of parasites averaging 12.0 mm in length and 100% were classified as immature. A similar cycle was observed for male worms in M. pacificus and for E. gadi of both sexes in G. zachirus.

These data indicated that a seasonal maturation cycle was operating in the two pleuronectid flatfishes and recruitment into the parasite population did not occur throughout the year. The data also suggests that the longevity of E. gadi in these hosts was about one year.

The host-parasite relationship is strongly influenced by three interrelated factors: the distribution of infected intermediate hosts and the feeding preferences and seasonal migration patterns of the fish hosts.

During the spring onshore migration, M. pacificus and G. zachirus apparently acquired new infections of E. gadi when infected amphipods were consumed. This is supported by the fact that only immature parasites were found in the spring and only mature parasites in the fall and winter. The undetermined intermediate host of E. gadi is probably an amphipod found on muddy sediment type bottoms located in depths greater than those sampled during the study. During the fall offshore migration fully embryonated E. gadi eggs may be deposited in areas inhabited by the intermediate host, thus initiating a new generation of larval E. gadi. Based on the time required for the development of acanthocephalan infective juveniles as reported in the literature (Crompton, 1970) especially under the constraint of low water temperatures, larval developmental period for E. gadi may require 2 to 3 months.

The seasonal cycle of E. gadi in Dover sole and Rex sole can be summarized as follows: a single period of acquisition of new infections in the spring, followed by growth and mating during the summer and maturation, egg production and eventual elimination of spent adult worms during the fall and winter. Because a substantial number of adult male Dover sole do not undergo an onshore migration it is possible that a non-seasonal infection and maturation cycle similar to the one observed in Yaquina Bay occurs in deeper waters since the fish may not migrate from the area inhabited by infected amphipods.

A parasite cycle in which fish migration determined the distribution of the parasite was reported by Olson and Pratt (1973) for E. lageniformis in Yaquina Bay. They reported that 29.9% of young of the year English sole were infected with the acanthocephalan during summer months while older fish in offshore waters were rarely infected. Following the fall emigration to offshore waters, the young English sole leave the estuarine source of infected amphipods (Corophium spinicorne) so that the parasite is eventually lost from the offshore population.

Seasonal maturation cycles have been described for some freshwater acanthocephalans such as Neoechinorhynchus rutili (Walkey, 1967), Leptorhynchoides thecatus (DeGusti, 1949) and Acanthocephalus lucii (Komarova, as cited in Chubb, 1964). In all of these studies, the high degree of similarity suggested that these cycles were not controlled by specific physiological or behavioral mechanisms but were more probably the result of environmental factors, with the seasonal temperature changes the main influence.

The annual seasonal maturation cycle of E. gadi observed for M. pacificus and G. zachirus is the first reported for a marine acanthocephalan in which the migration of the definitive host and the restricted distribution of the unknown intermediate host (zone of infection) are the factors controlling the distribution of the parasite. Since acanthocephalans show a remarkable lack of specificity at the definitive host level (Nicholas, 1967) it is possible that other pleuronectid flatfishes along the Oregon coast are potential hosts for E. gadi but are not infected either because the habitats of the flatfish and the intermediate host do not overlap or because these other species of flatfishes do not feed on the intermediate host when it is available.

Although the host-parasite relationship between E. gadi and M. pacificus and G. zachirus are similar, differences were apparent when infections in the two hosts were compared. Intensity and prevalence of E. gadi infections generally increased with fish length and age for both hosts but the average intensity of E. gadi in M. pacificus (7.6 parasites) was twice that observed in G. zachirus (3.5 parasites) and over 83% of Dover sole greater than 160 mm total length were infected compared with only 56% of Rex sole of a similar size range. The prevalence of infection was similar in smaller individuals of both host species. Growth of the parasites in M. pacificus was greater than in G. zachirus. The lengths of female worms in the ovarian ball, immature acanthor, and mature acanthor developmental stages were significantly greater at the 95% confidence level. Based on these observations it appears that M. pacificus is a better host for E. gadi than is G. zachirus.

The sex ratio of the population of E. gadi in Dover sole was 1:1.4, similar to the ratio observed for Echinorhynchus sp. in Leptocottus armatus. The sex ratio for new infections in M. pacificus (ligament stage females/male worms 4 to 8 mm in length) was 1:1 and changed in favor of females upon the sexual maturation of the parasites and the loss of male worms. The population sex ratio of E. gadi in Rex sole was 1:1. Reasons for the different ratio in G. zachirus are not obvious. Small immature worms were collected only in the spring, but small mature worms of both sexes were observed most months. It may be that for unknown reasons male worms are not eliminated from Rex sole as they are in Dover sole.

The number of infected fish containing both male and female worms is related to the recruitment rate of parasites and not the genetically controlled sex ratio of infective juveniles in intermediate hosts (Crompton, 1970). Almost 16% of the female E. gadi population (43 worms) from G. zachirus were recorded from unisexual (female only) infections while in M. pacificus 3.2% of the females (18 worms) were from female only infections. Since the two pleuronectid flatfishes are infected only during their onshore migration and further recruitment into the worm population does not occur, the female unisexual infections represent a segment of the parasite population not contributing to egg production. Some unisexual infections containing large unmated female worms were observed in spring collections when most fish contained only small worms. These large worms must represent carryovers from the previous year's infection and could indicate that unmated worms live longer than those that produce eggs.

The differences observed between M. pacificus and G. zachirus in prevalence, intensity, parasite growth, parasite sex ratios and number of unisexual infections indicates that M. pacificus is probably the preferred host of Echinorhynchus gadi. The prevalence and intensity values are possibly related to habitat preferences of Dover sole that are similar to those of the intermediate host, but the growth and parasite sex ratio differences are probably related to physiological factors that affect the host-parasite relationship. The lower intensity of E. gadi in Rex sole may be related to the habitat preferences of the fish which could result in a decrease in the percentage of infected intermediate hosts consumed.

The life cycle and larval development of Echinorhynchus sp. is similar to that of other Palaeacanthocephalans that have amphipod intermediate hosts.

The retention of the acanthor hooks in the acanthella stage after 23 days of development is similar to the situation in the development of E. lageniformis (Olson and Pratt, 1971), E. truttae (Awachie, 1966) and Prosthorhynchus formosus (Schmidt and Olsen, 1964). In Echinorhynchus sp., the original acanthor becomes free from the host serosa and the developing acanthella drops into the amphipod hemocoel and is surrounded by host connective tissue. This differs from

Leptorhynchoides thecatus (DeGiusti, 1949) where the acanthor remains embedded within the gut wall of the amphipod. A stalk which is formed between the developing acanthella and the acanthor breaks leaving the acanthor attached to the host serosa.

The number of giant cortical nuclei (20) is constant during the development of Echinorhynchus sp. A constant nuclear number was reported for E. lageniformis and L. thecatus but varied in Polymorphus minutus (Nicholas and Hynes, 1963). Four of these nuclei are involved in the formation of the lemnisci of Echinorhynchus sp. and most Palaeacanthocephala. In the order Eoacanthocephala three cortical nuclei enter the lemnisci while some Archiacanthocephala such as Macracanthorhynchus spp. have 12 or more nuclei involved in lemniscal development (Cable and Dill, 1967).

A characteristic of a 5 to 6 day acanthella of Echinorhynchus sp. is the presence of four large apical nuclei which arise from the entoblast. In E. truttae the four apical nuclei arise from the breakup of one apical giant nucleus. The apical nuclei of Enchinorhynchus sp. remain within the proboscis receptacle during early acanthella development but after 26 days the nuclei break through the receptacle and are located at the anterior end of the proboscis. During proboscis development, the apical nuclei pass through the proboscis nuclear ring and presumably form the apical organ as reported for Paulisentis fractus (Cable and Dill, 1967).

The developmental rate for Echinorhynchus sp. is similar to that of other acanthocephala at similar temperatures. The infective stage is attained after about 33 days at 23°C, while E. lageniformis (Olson and Pratt, 1971) and L. thecatus (DeGuisti, 1949) larvae become infective in 30 days at 23°C and 30 to 32 days at 20-25°C respectively. Echinorhynchus truttae required 82 days to reach the infective stage at 17°C according to Awachie (1966). Hynes and Nicholas (1957) found that the development of Polymorphus minutus to the juvenile stage took 60 days at 17°C and Prosthorhynchus formosus developing in isopods became infective after 60 to 65 days at 20-23°C (Schmidt and Olsen, 1964). Factors other than temperature affecting the larval development rate could include the crowding effect of multiple infections (Lackie, 1971), the intensity of infection of individual hosts (Uglem and Larson, 1969) and the food supply of the intermediate host.

The larval stages of some Palaeacanthocephala and Archiacanthocephala may become encapsulated in the hemocoel by the cellular reaction of the arthropod intermediate host. In the order Eoacanthocephala, the larval stages generally remain non-encapsulated (Nicholas, 1973). During the larval development of Echinorhynchus sp. two types of capsules were observed. First was the formation of a thin envelope or capsule consisting of amphipod hemocytes which surrounded the acanthor stage after it penetrated the gut of

Anisogammarus confervicolus. This capsule was maintained throughout the growth and development of acanthellae and infective juveniles and was similar to the capsules observed during larval development of P. minutus (Crompton, 1964), Macracanthorhynchus ingens (Bowen, 1967), Moniliformis clarki (Crook and Grundmann, 1969) and E. truttae (Awachie, 1966).

The second capsule type was a multilayered structure with or without the deposition of a melanin-like pigment. Acanthors and/or early acanthella surrounded by this capsule perished within three days after penetration of A. confervicolus. These dead parasites appeared as dark encapsulated objects usually surrounded by a laminated structure of hemocytes loosely attached to the hemocoelic side of the amphipod gut. In most A. confervicolus which contained morbid parasites, "normally" developing acanthella either attached to the gut wall or free within the hemocoel were also observed. Occasionally a deposition of a melanin-like pigment and/or an accumulation of host hemocytes (multilayered) was observed surrounding only a portion of older acanthella of Echinorhynchus sp.

Arthropods generally encapsulate foreign objects in their tissues (Salt, 1970). Potential parasites invading the tissues of unsuitable hosts may be treated as a foreign object and "melanized." However in the appropriate host, the parasite may remain non-encapsulated or a non-melanized thin envelop unlike that formed around foreign objects may surround them (Nicholas, 1973).

Crompton (1964, 1970) observed that capsule formation in P. minutus and probably other Acanthocephala originates from the wound healing activities of host hemocytes involved in restoring the stretched serosa to its normal thickness after penetration by the acanthor. The capsule around P. minutus resembled the connective tissue laid down by hemocytes around the internal organs of gammarid amphipods and in plugging of superficial wounds. After the acanthella of P. minutus became free in the hemocoel, it was enveloped in a connective tissue capsule and hemocytes appeared to be responsible for the growth and repair of the capsule during the subsequent development of the parasite.

When the capsule was removed or damaged, a typical foreign object reaction occurred and a melanized cellular capsule was formed (Crompton, 1967). Apparently a living parasite within its envelope is treated like healthy host tissue.

Acanthors of E. lageniformis and E. gadi were always encapsulated and melanized in Anisogammarus confervicolus demonstrating a host specific relationship with regard to the intermediate host. However, studies by Lakie (1971) suggested that the intensity of experimental infections may cause unnatural physiological changes in the intermediate host which may account for the normal larval development of some Echinorhynchus sp. individuals and the encapsulation with melanin deposition of others in the same amphipod.

Acanthocephalans are commonly occurring, cosmopolitan parasites of vertebrates including marine and freshwater fishes that are of economic importance. Despite the fact that acanthocephalans perforate the intestine of their hosts with an imposing, spiny proboscis, pathological damage to host tissue is usually restricted to the site of penetration and gross effects on the host are detectable only in massive infections. Although histological studies were not conducted here, no obvious affects on the host fishes were observed, even in fish infected with as many as 43 worms. It is concluded that neither E. gadi in the commercially important Dover sole and Rex sole nor Echinorhynchus sp. in staghorn sculpins pose a serious threat to the host populations.

Information collected in this study on the prevalence, intensity and seasonality of E. gadi infections in Dover sole and Rex sole could prove useful in the identification of subpopulations and migration patterns of these hosts if further research provides data from other areas along the Pacific coast.

This study corroborates the work of others concerning the high degree of acanthocephalan host specificity at the intermediate host level. The two species of Echinorhynchus studied here are difficult to distinguish morphologically and have host habitat ranges that overlap. Nevertheless, laboratory experiments show that E. gadi will not develop in the intermediate host of *Echinorhynchus* sp., due either to physiological incompatibility between parasite and host or to the triggering of the amphipod host defense mechanisms. The cursory observations made here on encapsulation of incompatible larval acanthocephalans by amphipods indicate the potential of using these or similar host-parasite relationships to study the mechanisms of crustacean immune systems.

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