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THE MORPHOLOGY AND LIFE CYCLE OF TANAORHAMPHUS AMBIGUUS, VAN CLEAVE, 1921(ACANTHOCEPHALA)

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THE MORPHOLOGY AND LIFE CYCLE OF *TANAORHAMPHUS AMBIGUUS* VAN CLEAVE, 1921 (ACANTHOCEPHALA)

by

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A THESIS

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To My Beloved Parents

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ABSTRACT

THE MORPHOLOGY AND LIFE CYCLE OF TANAORHAMPHUS AMBIGUUS VAN CLEAVE, 1921 (ACANTHOCEPHALA)

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Tanaorhamphus ambiguus Van Cleave 1921 is redescribed on the basis of more than 100 worms. Included are descriptions of immature and mature females, fully formed eggs and males. The worms used in this study were from the Oyster River, New Hampshire (eels and tomcods) and from Chesapeake Bay, Maryland (eels only). In the present study marked intraspecific variations have been encountered and described. The variations are manifested in the number of proboscis hooks, number of giant nuclei in the subcuticula, lemnisci and cement gland. These variations are unlike those usually noticed in other species of Eoacanthocephala and are characteristic of the species.

Experimental demonstration of the life cycle of T. ambiguus and an account of development in the arthopod intermediate host are reported. This is the first life cycle report of a brackish water species of Acanthocephala. The intermediate host is an amphipod, Gammarus tigrinus. Hatching takes place in the intestine of the amphipod host within 1 to 6 hours after exposure to the shelled acanthors. Acanthors

are free in the hemocoel of the amphipod within 20 to 96 hours after initiating infection. The development of the parasite is completed in two distinct phases: The first phase of development (preacanthella, lasting from the 2nd day of development until about 14th day) is characterized by slow growth accompanied by few internal changes. The second phase of development (acanthella, lasting from about the 13th day of development to the 27th day) is characterized by rapid elongation and extensive differentiation. By the 27th day, development of the parasite in the amphipod host apparently is complete and by the 28th to 32nd day the juvenile is infective to the definitive host as determined by feeding experiments. No transport host is required to carry the juvenile to the definitive host. At room temperature (22-25^{o}C) T. ambiguus in the amphipod host required 28-32 days to complete development and reach the infective stage, and the juveniles in the definitive host reached sexual maturity and passed eggs approximately one month after infection. The development of the parasite at 13 ± 2^{o}C in both the intermediate and definitive hosts was impeded.

From the incidence of intensity of infections, infection experiments and the life cycle studies, it is concluded that T. ambiguus is primarily a parasite of the eel, (Anguilla rostrata) and is of brackish water origin. However,

the worms may be found in other fishes in localities where the infected intermediate hosts are available, but the worms do not reach sexual maturity.

I. INTRODUCTION

The acanthocephalans are interesting animals and are well known for their extreme adaptations to a parasitic mode of existence. Jensen (1952) aptly described acanthocephalans as "a sac of reproductive structures with a crown of hooks at the anterior end and a genital opening at the posterior end". They are of widespread occurrence; as adults in all classes of vertebrates, and as juveniles usually in a variety of arthropod hosts.

The family Tenuisentidae Van Cleave, 1936, one of the families of Eoacanthocephala (Van Cleave, 1936) consists of four genera: Tenuisentis Van Cleave, 1936, Tanaorhamphus Ward, 1918, Pandosentis Van Cleave, 1920 and Microsentis Martin and Multani, 1966; and five species: Tenuisentis niloticus Van Cleave, 1936, Tanaorhamphus longirostris (Van Cleave, 1913), T. ambiguus Van Cleave, 1921, Pandosentis iracundus Van Cleave, 1920 and Microsentis wardae Martin and Multani, 1966. The position of the genus Pandosentis is uncertain. Golvan (1959) assigned Pandosentis to Gracilisentinae Petrotschenko, 1956, one of the subfamilies of Neoechinorhynchidae Ward, 1918, whereas Yamaguti (1963) considers it under the family Tenuisentidae. Very little is known either on distribution or life cycles

of any of the members of Tenuisentidae.

Since Van Cleave's (1921) original description of Tanaorhamphus ambiguus, there have been no published reports of this species, neither on its incidence nor its life cycle. Linton (in an unpublished manuscript) extended the host range of the species, and Huizinga (personal communication to Dr. W. L. Bullock) found natural infections of T. ambiguus in Gammarus mucronatus* (Bousfield, 1969) and in eels from Chesapeake Bay.

Van Cleave's (1921) description of T. ambiguus was based on only three female specimens from the U.S. National Museum. The male has not been described. The original description given by him has been found incomplete and in error in some respects during the course of my studies. A full redescription of the species, therefore, seems warranted.

In the present study, the species has been re-examined and redescribed on the basis of more adequate material.

Numerous studies bearing on the life cycles of acanthocephalans have been reported in literature. All the life cycle studies made thus far are of acanthocephalans of either fresh water fishes or of terrestrial and aquatic birds and mammals. It is of interest to note that no life histories of brackish water or marine fish acanthocephalans have hitherto been traced experimentally.

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* Identified by Dr. R. A. Croker, (Zoology Department, U.N.H.)

The present study, in addition to taxonomic and morphological description, includes the natural and experimental observations made on the life cycle of T. ambiguus. This is the first life cycle ever traced experimentally in a brackish water form.

Since Moore (1962) pointed out that there is a need for more information about acanthor types and their development to aid in establishing phylogenetic relationships, it is hoped that this study, and many more to come of acanthocephalan parasites, might help clear the confusions that exist in taxonomy, and aid in a better understanding of the phylogenetic relationship of the group.

II. LITERATURE REVIEW

The Genus Tanaorhamphus Ward, 1918

H. B. Ward (1918) established the genus Tanaorhamphus with Tanaorhamphus longirostris (Van Cleave, 1913) as type species. Van Cleave (1913) ranked the new species under the genus Neorhynchus when he first described it from the gizzard shad (Dorosoma cepedianum) from the Illinois River at Havana, Illinois. Though Van Cleave (1913) was aware of the fact that the form of the proboscis of his new species indicated the possibility of establishing a separate genus, he did not do so. On the basis of his study of other aspects of the morphology of this species, he concluded that this form did not reveal a deviation beyond the purely specific level of variation. Meanwhile the family Neoechinorhynchidae Ward, 1918 was established and since Neorhynchus Hamann, 1892 and Eorhynchus Van Cleave, 1914 were preoccupied for other groups, both these names were rejected and the first established name Neoechinorhynchus Stiles and Hassall, 1905 was proposed as the type genus and to designate all the species described then under the former names. Ward's (1918) basis of establishing a new genus was mainly on (a) the form of proboscis and number and nature of hooks, (b) the number of nuclei in the cement gland.

Tanaorhamphus was allocated along with other genera under the family Neoechinorhynchidae.

The only other species described under the genus Tanaorhamphus is T. ambiguus Van Cleave, 1921. The occurrence of this species in eels was noticed by Linton (1901) although he believed it to be the European species Echinorhynchus globulosus. Van Cleave (1921) by his study of the specimens which bore the name E. globulosus from the U.S. National Museum, (the specimens were from the collection of the U.S. National Museum, catalog number 6471, with Linton's identification as E. globulosus) and another specimen deposited in the U.S. National Museum under the catalog number 6301 of the Hassall Collection (1891), demonstrated that they represent an undescribed species of the genus Tanaorhamphus Ward, 1918, and described it as T. ambiguus. In his paper on the acanthocephalan parasites of eels, Van Cleave (1921) designated the eel (Anguilla chrysypa= Anguilla rostrata) as the type host of T. ambiguus. Later Linton (unpublished manuscript) reported T. ambiguus from the following fishes: Pomolobus pseudoharengus (Alosa pseudoharengus) alewife - Clupeidae; Morone americana (Roccus americana) bass - Serranidae; Zygonectes diaphanus (Fundulus diaphanus?) killifish - Cyprinodontidae. Huizinga (personal communication to Dr. W. L. Bullock) found this species in eels from Chesapeake Bay and turned over a collection of this species to Dr. W. L. Bullock.

The genus Tenuisentis was established in the year 1936 by Van Cleave for specimens that Meyer (1932) had described as Rhadinorhynchus niloticus. Since the genus Tenuisentis failed to agree with any recognized family, he (1936) proposed the recognition of a new family, Tenuisentidae, with the characters of the genus Tenuisentis. With the establishment of the family Tenuisentidae, Tanaorhamphus has been allocated to this family because of its possession of characters which suggested closer relationship to Tenuisentis. Thus far, no other species has been added to the genus Tanaorhamphus.

Life History Studies

A very useful and comprehensive historical review of studies having a direct bearing upon life cycles has been given by Ward (1940). Hopp (1954) discussed more detailed studies of acanthocephalan development since Ward. Jensen (1952) reviewed the life cycle studies of fish acanthocephalans since 1862. Awachie (1966) brought together subsequent contributions since Hopp (1954). It is the purpose of the writer in dealing with historical review to provide a review of known acanthocephalan life cycles, particularly of those belonging to Eoacanthocephala.

Only a few life cycles have been demonstrated experimentally in the group Eoacanthocephala. The life cycles that have been elucidated are of the following

species: Neoechinorhynchus cylindratus by Ward (1940); N. emydis by Hopp (1954); N. rutili by Merritt and Pratt (1964); Octospinifer macilentis by Harms (1965); and Paulisentis fractus by Cable and Dill (1967).

The first acanthocephalan life cycle studies made in any detail were by Ward (1940) for N. cylindratus from fresh water fishes. The first intermediate host is an ostracod, Cypria (Physacypria) globula. She found experimentally that 28 days are required for the embryos to develop to a stage closely resembling the adult worm except in size and sexual maturity to become infective to the second intermediate host. The second intermediate host is a bluegill, Lepomis pallidus, and the juvenile acanthocephalans localize and encyst in the liver of these fish. Though Ward had no experimental evidence, she believed that bluegills serve in a more essential capacity than that of a mere transport host.

Hopp (1954) reported the life cycle of N. emydis, a parasite of the map turtle. This is the only turtle acanthocephalan parasite thus far reported. The ostracod, Cypria maculata forms the first intermediate host. Hopp found that the eggs ingested by ostracods hatch in the intestine, releasing a typical acanthor which enters the body cavity of the ostracod and develops to an unencysted juvenile in 21 days without a period of attachment to the host's intestine. He was of the opinion that ostracods

containing juveniles are ingested by snails (Campeloma rufum) in which the worms enter and become encysted in the tissues, especially of the foot in which cysts are seen as opaque spots. Juveniles of this species had been reported earlier in other species of snails by Whitlock (1939) and by Lincicome and Whitt (1947). Hopp believed that the snail serves in a more essential capacity than that of a transport host although he presented no experimental proof of this.

Merritt and Pratt (1963) demonstrated experimentally the life history of N. rutili, a fresh water fish parasite. The intermediate host is an ostracod (Cypria turneri). Within the intestine of the ostracod the acanthor hatches, penetrates the wall of the intestine, and in 6 to 12 days is found free in the hemocoel where it metamorphoses through the acanthella to juvenile stage in 48 to 57 days. Merritt and Pratt have also compared the life cycle of this species with those of N. cylindratus and N. emydis. Villot (1885) reported finding Echinorhynchus clavaeceps (=N. rutili) encysted in the adipose tissue of the larvae of Sialis niger. The larva of S. niger does not function in the life cycle of this species (Walkey, 1967).

Another member of the family Neoechinorhynchidae, whose life cycle has been explored experimentally, is Octospinifer macilentis, parasite of fresh water catostomid fishes. Harms (1965) showed experimentally that Cyclocypris

serena (Koch) was the only ostracod species that sustained infection until cystacanth larvae (juvenile) developed. He found that the eggs hatched inside the intermediate host within 4 hours and liberated acanthors with anterior spines. By day 30, the infective stage was reached in the intermediate host. Unlike N. cylindratus and N. emydis, no transport host was required to carry the infective juvenile to a definitive host. In the definitive host, worms were established in the intestine within 4 hours. Maturity was reached after approximately 8 to 10 weeks for males and 16 weeks for females.

Cable and Dill (1967) added another genus to the list of life cycle studies of Eoacanthocephala when they reported the experimental determination of the life cycle of Paulisentis fractus, a fresh water fish parasite. The life cycle of this species is interesting in that it deviated from the previously assumed pattern, for the first time, by having a copepod (Tropocyclops prasinus) as the intermediate host. They also found the development in the intermediate host to be surprisingly rapid. It required only 13 days to develop to the juvenile stage in copepods.

The information available regarding the life cycle of Tanaorhamphus ambiguus is that of Huizinga (personal communication to Dr. W. L. Bullock) who found natural infections of T. ambiguus in Gammarus mucronatus from Chesapeake Bay.

III. MATERIALS AND METHODS

The eels (Anguilla rostrata), which serve as the definitive host in these studies were collected from the Oyster River in Durham, N. H. The Collections were made at a narrowly restricted point just below the dam on the Newmarket Road, where the water depth is about 5 to 6 ft. The salinity of brackish water at the Oyster River was found to range from 4-8 parts per thousand at low tides to 14.7-27.6 at high tides.

Measurements of salinity were made of surface water at low tide and high tide as well as near the bottom at high tide. To collect deep water samples when the tide was high, a water sample collector was used. This was a P.V.C. (Polyvinyl chloride) plastic water bottle of 2 liter capacity, 12 inches long and 4 inches in diameter with a lid. The bottle was attached to a long rope by which it could be placed at desired depths. A heavy metallic ring when released, closed the lid at the desired depth. Salinity measurements were made with a salinity tester. The purpose of measuring the salinity at Oyster River was to know the salinity range, and thus choose a medium in which eels and amphipods (the definitive and the intermediate host) could be maintained in the laboratory.

Collection of eels was made with an aid of a trap made of 0.5 sq. inch mesh hardware cloth, 4 ft. long and 1½ ft. in diameter.

Collections were made during the summer months of 1966, during the summer and fall of 1967 and 1968. The trap was usually set during the noon hours and checked for eels the same night (7 or 8 P.M.) and/or left overnight to be checked the next morning (8 A.M.). Usually, the following fishes were trapped at the Oyster River: Eels (Anguilla rostrata), Killifish (Fundulus heteroclitus) and Tomcods (Microgadus tomcod).

Eels collected were usually kept in a cold room ($13\pm2^{\circ}C$) in the Zoology Department at the University of New Hampshire and autopsied the same or the next day for observation of parasites. Before autopsy, the eels were relaxed in MS 222 (Tricaine Methane Sulfonate, 1 gram in 4 liters of water). The eels were relaxed or narcotized within a one half to one hour time period.

All eels were examined for parasites by removing the intestine and the stomach and laying them out on a paper towel. They were moistened with 0.9% saline before opening. The stomach contents were also observed, and if necessary, preserved in 5% buffered formalin. Only on two occasions did the writer come across eels that were heavily infected with Tanaorhamphus ambiguus. In these two instances, the acanthocephalans were localized at the posterior part

of the duodenal region of the intestine. In both instances, the worms appeared to be in clusters, the mature ones especially localized at one point. In very light infections, the parasites occupied any level of the intestine. The large parasites were removed from the intestine with the aid of a forceps by grasping delicately the body of the parasite and quickly removing them from the intestinal wall of the host. This technique resulted in worms being removed entirely without damage and with no mucus attached to the proboscis. Small immature worms were removed by scraping the mucosa with a scalpel. The contents were then poured into a small jar containing 0.9% saline. The jar was closed with a cap and shaken well. This caused the worms to separate from the mucus and concentrate at the bottom of the jar after a few minutes. The liquid in the jar was decanted and the worms were removed in fresh 0.9% saline and cleared of remaining mucus with the aid of entomological minuten needles. Specimens thus obtained were washed in 0.9% saline and left overnight at room temperature in distilled water to become turgid with the proboscis well everted. Specimens were then fixed in Demke's (formalin-alcohol-acetic acid) fixative for several hours and stored in glycerine alcohol mix. Various stains such as chrome alum-gallocyanin, Grenacher's alcoholic borax carmine, Harris' hematoxylin were tried. Chrome alum-gallocyanin was used frequently for staining as the stain

seemed to bring out the features of the worms remarkably well. The counter stain used for chrome alum-gallocyanin was chromotrope-2R. Attempts were made to apply Chubb's method of staining, using acetic acid as the dehydrant, especially for the developmental stages of larvae obtained from artificial infection of amphipods (Chubb, 1962). The results were not satisfactory especially for the adult worms which showed heavy staining and much shrinkage. Before staining, specimens were pricked with an entomological minuten needle for proper staining and exchange of fluids. Specimens were gradually cleared in successive 2:1, 1:1, 1:2 mixture of absolute alcohol and terpineol, followed by pure terpineol, and mounted in permount.

A series of longitudinal and transverse sections of both immature females and male worms were prepared and stained with chrome alum-gallocyanin, using chromotrope-2R as a counter stain. Before embedding the worms in paraffin, they were stained in chromotrope-2R (0.001% in absolute alcohol) to make them visible in the paraffin blocks.

The majority of the mature worms collected, were stored in 0.9% saline at approximately $4^{o}C$ in a refrigerator (following Jensen, 1952). The worms were observed at regular intervals of one week to see whether they were active and alive. Survival was generally for periods of 6 to 8 weeks. Beyond this period the worms were usually inactive and presumably dead. Female worms were dissected during

this period as eggs were needed for experiments on life cycle studies. Some of the dead worms were left for a few more weeks to check the viability of eggs obtained from them. Eggs from 36 live mature female worms were concentrated by centrifugation (2 minutes at about 1,800 R.P.M.) in 12% sea water in centrifuge tubes and stored in the refrigerator at approximately 4°C (Harms, 1964). The stored eggs were kept for 4 months when they were used to infect 135 amphipods with good results (Table IX, Expt. 8). It was found that it was better to have eggs stored than to try to keep the worms alive for longer periods.

For infection experiments, eels measuring not more than 35 cm were usually used. Occasionally, when this size eels was not available, eels larger than the specified size were used. From collection data, it was determined that eels of this size were not naturally infected. The eels used for the experiments were maintained live in aquaria in 12% sea water at two different temperatures or just one according to the nature of experiments. Thus, the aquaria were kept in a 'Cold room' (13 ± 2°C) and/or 'Office room' (22-25°C) in the Zoology Department at the University of New Hampshire. For the aquaria in the cold room, aeration was provided by a permanently fixed air circulating system and for aquaria in the 'Office room', aeration was provided with the aid of a small air pump. Filtration of

the water was by means of plastic filters. The water was changed once every 2 to 4 weeks depending on the type of food provided and the condition of the water.

Before subjecting the eels to infection, the fecal contents were examined for at least a week to make certain that they were free of parasites. Examination of fecal contents consisted in looking for the mature eggs of T. ambiguus and/or exoskeletal plates and appendages of amphipods. The presence of exoskeletal plates and/or appendages of host amphipods in fecal matter gave a clue to the possible occurrence of immature T. ambiguus. Such eels were suspect and were, therefore, avoided.

The eels proved to be hardy laboratory animals, and could be kept with ease in the artificial environment. However, much care would have to be taken to keep them the desired length of time, because eels frequently jump out even through small openings.

Aquarium confined eels were, however, difficult animals to feed and infect with parasites. The majority of eels were indifferent to food and remained so for some time. Forced feeding usually resulted in regurgitation of any kind of food material given. Initially, eels were offered various foods. If they fed on any of these foods, their feeding was immediately taken advantage of for infecting purposes. Otherwise a forced feeding method was adopted.

Out of the many eels that were offered different food materials, only two eels began feeding on small pieces of meat. This was taken advantage of in infecting these two eels with naturally infected amphipods. The method adopted to infect eels, was to first crush the head of the infected amphipod in order to immobilize it. This was then wrapped in the meat and given to the eels, by dropping it in the aquarium and observing until the eel swallowed it. This method enabled me to determine the two host life cycle of T. ambiguus, and the time the infective larvae took to reach sexual maturity at different temperatures in the definitive host.

To determine the stage at which the larvae reached the infective stage in artificially infected amphipods, and to determine the growth and development of the parasites in the definitive host, larvae of different stages and immature worms obtained from tomcods were fed to eels. Forced feeding was usually necessary and different methods of forced feeding were tried. The use of a dropper to force feed larvae or immature worms was not found to be a successful method. Since the size of the dropper was small, the dropper could only reach the oesophagus of the eel. This often resulted in regurgitation after the eel regained consciousness. (The eels were always narcotized in MS 222 before forced feeding). Even the use of a long plastic tube (6" by ¼") with a rubber bulb at one end or

hypodermic syringe attached to it at one end, resulted in regurgitation. Infection through the anus was also tried with the aid of a dropper but with negative results.

Another method attempted was to wrap the live larvae or immature worms in small pieces of meat or the dissected stomach of a tomcod and thrust them into the stomach of eels with the aid of long plastic tube (6" by ¼"). The eels then were allowed to regain consciousness either after keeping them narcotized for some time or for a day or two at 4°C in a refrigerator with constant observation to determine whether regurgitation occurred. If regurgitation occurred, the experiment was abandoned, if not, the eels were maintained for a desired length of time, and dissected according to schedule for the recovery of parasites. Out of a limited number of experiments, a few certainly gave good results, and will be discussed later.

Finally, an attempt was made to inject immature worms directly into the fish intestine. The method consisted of making an incision (about ½ inch) in the skin with a scalpel on the ventral side slightly toward the right side of the eel. This position allowed the intestine to be lifted out with the aid of a curved needle without disturbing any of the blood vessels. Meanwhile immature worms in 0.9% saline were aspirated into the sterile injection tube without the injection needle. The injection needle of the size that permitted the passage of the parasites (size 14) was

inserted on to the injection tube and the parasites injected into the intestine of eel. The incision was then sewed with cotton thread. Surgical clips were used in the initial experiments, but they were less effective in closing the wound because of the slimy skin. Of a total of three operations, one was successful; the eel remained alive for about 2 months.

Usually large numbers of worms (72 - 200) were used in both forced feeding and surgical experiments. It was later found that the smaller the number of parasites used, the better the results.

The potential intermediate hosts, amphipods, were collected from the same habitat where eels were collected. The collecting periods for amphipods were the same as those mentioned previously for eels. Collections were made with the aid of a collecting net that was dragged across the bottom near the shore when the tide was low. Stones, water plants and other debris were gathered while the collecting net was dragged. Usually amphipods and a few isopods and sometimes small fishes (Killifish and very small eels) were found among the material dragged. Sometimes, only water plants with large numbers of amphipods were collected.

Sorting and cleaning of amphipods was accomplished with 12% sea water prepared in laboratory. They were maintained in the same medium. Usually about 350 to 500 amphipods were collected at one time. On one occasion, however,

about one thousand or more were collected. A reserve supply of amphipods was isolated in plastic containers and maintained in the 'Cold room' in 12% sea water with constant aeration. No attempt was made to feed amphipods that were to be dissected for determination of incidence of infection or subjected to infection experiments.

Several hundred amphipods were dissected initially to determine the incidence of infection. Later, the naturally infected amphipods were separated by superficial observation and the incidence of infection noted. Superficial identification of the infected amphipods was accomplished by observing a white streak on the lateral sides of amphipods. This was effectively done by observing a few (about 20-25) amphipods in a finger bowl with light directed through the sides. Some uninfected amphipods also showed a white streak on the lateral side. Such suspected ones and the really infected ones were separated into another finger bowl and each one observed under the dissection microscope for confirmation. While observing, a slight pressure was exerted on the cover slip by a needle or forceps which compressed the body of the amphipod slightly, and made it more transparent. The larvae were then very clearly seen. Both sides of the amphipods were examined in this way.

Naturally infected amphipods were fixed in Demke's and stained in various stains and mounted with the

procedure already described. Before fixing in Demke's fixative, the infected amphipods were left in distilled water overnight so that the larvae were completely extended. Another method attempted was to put the live infected amphipod in hot water before fixing. This resulted in the larvae contracting and curling to a considerable extent. This method was found to be unsatisfactory. The distilled water stretching gave very good results. The majority of the infected amphipods were used for infection experiments with eels.

Since a microscopic observation of the eggs of a mature female worm indicated the presence of both immature and mature eggs, the method of infection adopted was to cut a worm into as many as 10 to 12 pieces and offer them to amphipods. Usually 10-15 amphipods were placed in each stender dish containing about 20-25 ml. 12% sea water. The amphipods readily ate the worm fragments in 5 to 10 minutes. Though this method resulted in definite infection, it resulted in a heavy mortality rate of amphipods. This method was found to be satisfactory especially for the study of early development because the number of acanthors or pre-acanthellae observed were usually from 4-8 per infected amphipod.

The usual method (following Ward,1940, Jensen, 1952, and others) also was tried by exposing amphipods to eggs for about 3 hours, in small stender dishes containing 15

to 20 amphipods in 12% sea water. This method was found to be satisfactory for the study of the development of later stages (acanthellae), because the acanthellae observed were usually from 1 to 4 per infected amphipod. Though the percent infection was found to be less, it certainly resulted in lower mortality rate.

After 3 hours of exposure, the amphipods were then removed, washed in 12% sea water with the use of 'mesh sieve' and transferred to a plastic container with constant aeration, and maintained at room temperature (22-25^{o}C) for the duration of the experiment with a change of water at regular intervals of 3 to 4 days. Uninfected control amphipods were also maintained for each experiment conducted.

Periodic dissections of the amphipods were made in 0.4% saline under a dissecting microscope. The head was carefully pulled out to a certain extent until it became separated from the rest of the body. The last 3 posterior segments were then removed. This allowed for easier removal of the complete alimentary canal and the digestive glands. Later the head and the gland were also removed. The exposed alimentary canal was stained with Azure A in 0.4% saline. When acanthors were found, the alimentary canal with the acanthors were fixed in Demke's fixative and stained by the usual procedures for later critical studies. For the preacanthellae and acanthellae in the hemocoel, merely opening the hemocoel with the dissecting needle followed by

a slight pressure in a sort of squeezing fashion at the posterior end and anterior end of the animal brought out the larvae. For microscopic examination and observation, the larvae were stained with Azure A in 0.4% saline. For early and late acanthellae, whole mounts were prepared using a acetic stain (Heron's trichrome) and dehydrated with glacial acetic acid/methyl salicylate mixtures. (Chubb 1962). Drawings were made by camera lucida for preacanthellae and early acanthellae and by microprojection for late acanthellae.

IV. MORPHOLOGY

General Redescription

The species is redescribed here. Included are descriptions of immature and mature females, fully formed eggs and males. The following description is based on a study of over 100 worms. The worms include those collected from eels from the Oyster River in New Hampshire and Chesapeake Bay in Maryland, (those from Chesapeake Bay eels are from Huizinga's collection donated to Dr. W. L. Bullock) and tomcods at Oyster River, N.H. Both whole mounts and serial sections were used for study. All measurements are given in microns unless otherwise indicated.

The worms are white and curled when observed alive in the intestine. The extended specimens are curved ventrally. Body elongate, round to oval in cross section. Trunk long, aspinose, slightly enlarged anteriorly in either sex. General shape similar in both the sexes. Worms exhibit sexual dimorphism in body size, in size of proboscis and number and size of hooks. Males are shorter than females. Fully extended proboscis cylindrical and elongate usually enlarging very slightly toward the tip; armed with 14 to 22 longitudinal rows with 11/12 to 16/17 hooks per row. In both the sexes the rows of hooks are regular in their

arrangement and appear evenly spaced in quincuncial pattern. Hooks are prominent, long, strongly recurved tapering to a fine point, similar in size except for the anterior-most hooks and the posterior three basal circles of hooks which show very slight reduction in length and width. The hooks in the middle region of proboscis are rather broad; hooks of the basal circle are less curved (arcuate). Proboscis cuticle thick. Neck very short. Proboscis receptacle thick walled, single layered and elongate. The single muscle layer of proboscis receptacle continues into the proboscis up to the 5th circle of basal hooks. Inverter muscles slender and proportionally elongate. Proboscis ganglion in posterior region of receptacle. Apical organ extends up to the 4th or 5th circle of anterior hooks. Proboscis retractors long. Lemnisci usually subequal, about two or two-half times the length of receptacle. Number of nuclei in lemnisci is variable. Hypodermal giant nuclei several and variable in both sexes; 0 to 4 in ventral and 8 or less to 16 or more in dorsal line. Some nuclei in the dorsal line appear to be in pairs with additional overlapping nuclei. Of the dorsal giant hypodermal nuclei, many are in the mid-dorsal line and some slightly dorsolateral in position.

Adult female (Fig. 4)

Total body length 4.40 to 13.72 mm; Worms with fully developed embryos 8.2 to 13.72 mm; Those with incompletely

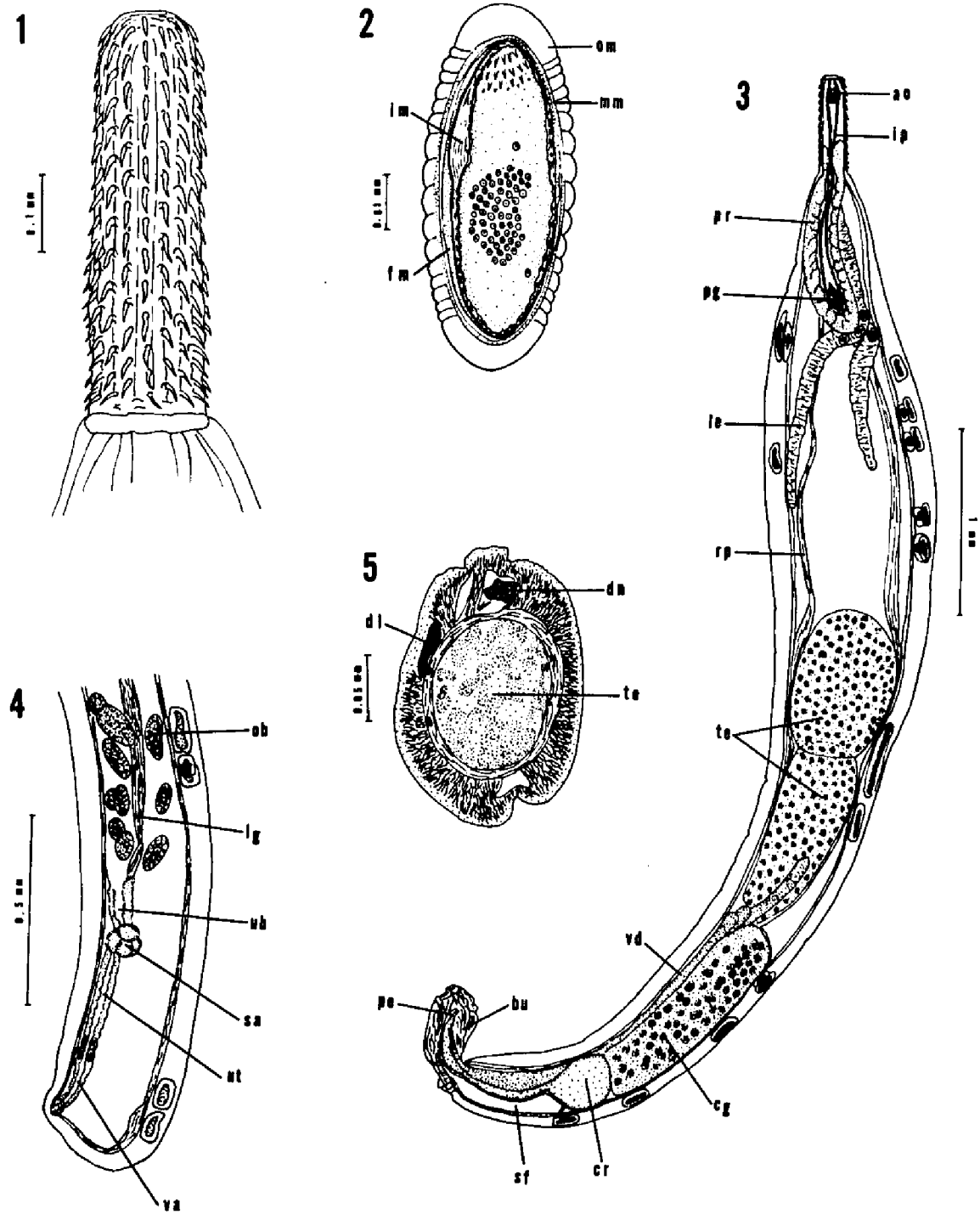
developed embryos or germ balls 4.40 to 9.6 mm. Maximum width in the region posterior to receptacle (within the lemniscal region) 410 to 1,520. External presoma 620 to 840 long by 120 to 210 wide. Proboscis length 580 to 770. Hooks larger than in male. Anterior hooks 40 to 55 long by 8 to 11 wide. Middle hooks 40 to 53 long by 8 to 13 wide. Posterior hooks 40 to 48 long by 5 to 8 wide. Proboscis receptacle 390 to 1,200, as long as or longer than presoma. Long lemniscus 860 to 2,490 long. Short lemniscus 750 to 2,100 long.

Genital pore ventral, distinctly subterminal, about 60 to 290 distance from the posterior tip of the worm. Vagina with a sphincter at each end; Shorter than the uterus in both immature and mature specimens. Vagina 130 to 210 long. Uterus 140 to 390 long. Selective apparatus 70 to 140 long. Uterine bell short, muscular; measures (along with selective apparatus) 130 to 520. Total length of reproductive tract from anterior opening of uterine bell to genital pore 440 to 1,180 or approximately one-tenth to one-twelfth of the total body length. Ligament sac distinctly separated; Uterine bell in direct communication with the ventral sac.

Figures 1 - 5. Tanaorhamphus ambiguus

1. Proboscis of male
2. Mature egg (shelled acanthor) drawn from living specimen.
3. Mature male
4. Posterior portion of female
5. Transverse section through the region of testis

Abbreviations: ao, apical organ; bu, bursa; cg, cement gland; cr, cement reservoir; dl, dorsolateral nucleus; dn, dorsal nucleus; fm, fertilization membrane; im, inner membrane; ip, inverter of proboscis; le, lemniscus; lg, ligament; mm, middle shell membrane; ob, ovarian ball; om, outer shell membrane; pe, penis; pg, proboscis ganglion; pr, proboscis receptacle; rp, retractor of proboscis receptacle; sa, selector apparatus; sf, Saefftigen's pouch; te testes; ub, uterine bell; ut, uterus; va, vagina; vd, vasdeferens.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Mature egg or shelled acanthor (Fig. 2)

Living, fully formed, mature eggs are ovoid and measure 62 to 72 by 26 to 31. Mature eggs preserved in formalin measure 48 to 58 by 20 to 28. Four membranes enclose the acanthor as was observed by West (1963, Ph.D. Thesis, University of New Hampshire) in mature eggs of Acanthocephalus jacksoni and Echinorhynchus gadi. They are: Outer thick shell membrane, middle thin shell membrane, fertilization membrane and an inner membrane. The outer thick shell membrane shows characteristic encircling folds which extend almost the entire length of acanthor; a feature which may be useful in identification of the species. Outer shell membrane is much thicker at the two polar ends.

Adult male (Fig. 1, 3)

Total body length 2.73 to 8.14 mm; Maximum width in the region posterior to receptacle 300 to 1,010. External presoma 440 to 680 long by 100 to 170 wide. Proboscis 410 to 630 long. Anterior hooks 43 to 50 long by 8 to 11 wide, middle hooks 42 to 50 long by 8 to 12 wide and posterior hooks 38 to 47 long by 5 to 7 wide. Proboscis receptacle 330 to 950 long. Long lemniscus 630 to 2,730 long and short lemniscus 590 to 2,640 long.

Anterior testis 220 to 850 long, posterior testis 210 to 1,040 long; both testes as wide as the body cavity. Single syncytial cement gland with several nuclei; Cement

reservoir globular to pyriform; immediately posterior to or slightly overlapping cement gland; 70 to 350 long. Saefftigen's pouch club shaped; as long as cement duct. Total length of genitalia (from anterior end of the anterior testis to the posterior end of the trunk) 1,290 to 4,640 or approximately one third to two third of total length of body. Copulatory bursa simple without digital structures.

Intraspecific Variation

Sexual dimorphism

Some of the many aspects of sexual dimorphism in Acanthocephala was summarized by Van Cleave (1920). Bullock (1962) dealt with sexual dimorphism and variability in Acanthocephalus jacksoni from different hosts and different geographical locations. At present, there is increasing evidence of sexual dimorphism in many other acanthocephalan species. Similar to other species of Acanthocephala, T. ambiguus exhibits marked sexual dimorphism.

T. ambiguus exhibits sexual dimorphism in all of the following features: 1) general body size; 2) Proboscis dimensions; 3) number of proboscis hooks and 4) to a certain extent, size of hooks.

Variation in hooks

All hook counts involved counts of 6 to 8 rows of hooks around the proboscis. The results of a count of the

number of hooks on the proboscides of 41 females and 33 males of T. ambiguus are shown in Table 3. The usual number of longitudinal rows in females seems to be 20, although deviation from this usual number was seen in 6 individuals which had 18, 19 and 22 rows. In the males the usual number of longitudinal rows was found to be 16. Only 3 individuals deviated from the normal pattern by having 15 and 18 rows. Only 4 females and 2 males had 18 longitudinal rows; hence overlap was very limited.

The number of hooks per longitudinal row showed more variation at the same time that this feature exhibited marked sexual dimorphism (Table 3). In the females the number of hooks per row ranged from 13/14 to 16/17; in the males from 11/12 to 14/15. Though it is not possible to indicate the typical range in both males and females, one could notice the remarkable differences between males and females in this regard. Six out of 33 males and 12 out of 41 females overlapped in possessing 14/15 hooks per longitudinal row.

There were no situations where the individuals had irregular arrangement or abnormal hooks.

Subcuticular giant nuclei

The present study indicated the number of subcuticular nuclei to be highly variable. The count that is presented in Table 2 is based on superficial observation. Dorsal subcuticular giant nuclei vary from 8 to 15 (Table 2).

TABLE 1. VARIATION IN THE NUMBER OF NUCLEI IN LEMNISCI

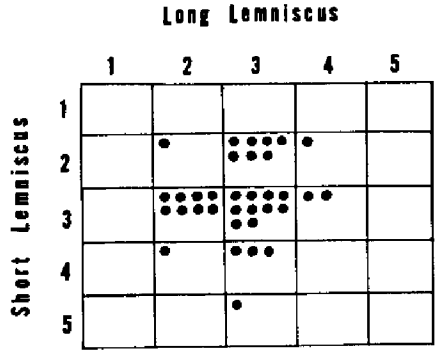


TABLE 2. VARIATION IN THE NUMBER OF NUCLEI IN SUBCUTICULA

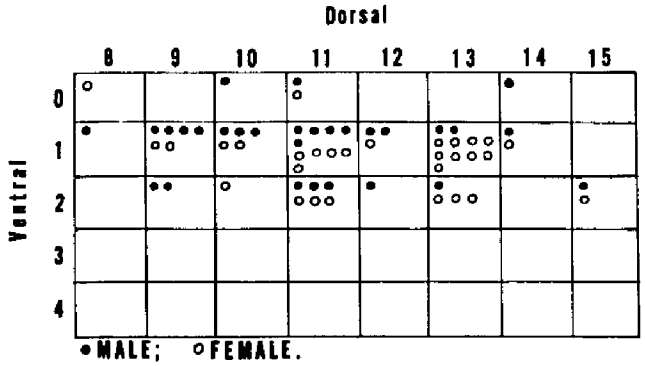
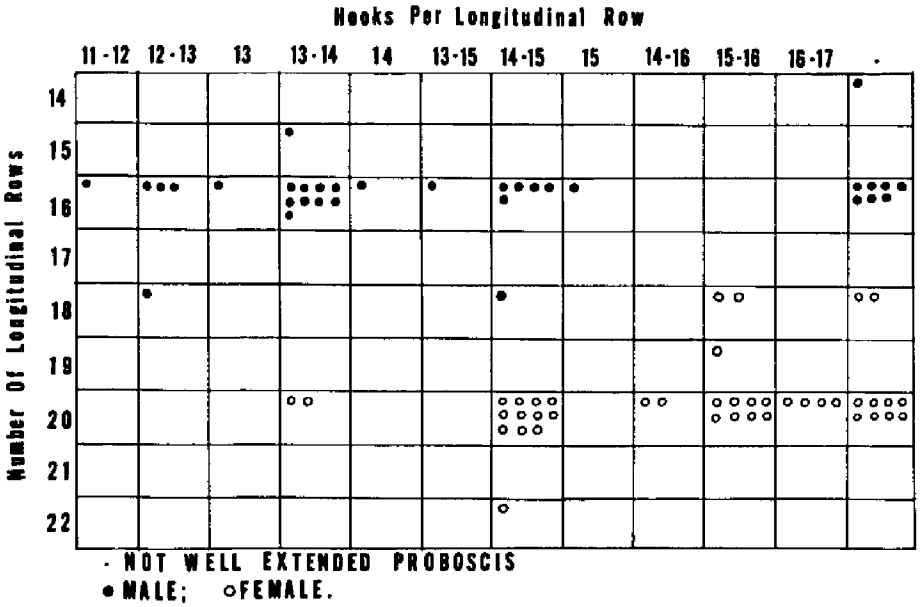


TABLE 3. VARIATION IN THE NUMBER OF HOOKS IN PROBOSCIS



Out of the 68 males and mature and immature females observed, 59 revealed 8 to 15 dorsal subcuticular nuclei. The remaining 9 mature females possessed 2 to 7 nuclei (not shown in Table 2.). Whether the presence of ovarian balls and mature and immature eggs obscured the nuclei because of a slight shift in the dorso-ventral axis of the worms in whole mount, or whether the number represented extreme variation is not clear. Observation of the larval stages (see Fig. 8, 11.) with only 6 nuclei seems to indicate the occurrence of a lesser number of nuclei to be a possibility.

The number of ventral subcuticular nuclei varied from 0 to 2. Out of 68 male and mature and immature female specimens observed, 38 had 1; 17 had 2 and 12 had none.

Most of the dorsal subcuticular nuclei appear to be in pairs (Fig 3.). A careful examination indicated some nuclei to have overlapping or very closely located additional nuclei (Fig. 3.). These additional nuclei seem to be slightly dorsolateral in position. Cross section studies substantiated this fact (Fig. 5). Even the ventral subcuticular nuclei seem to show this feature (Fig. 3). If these additional nuclei were considered, the number would be larger than was reported above. Very careful examination is required to observe these additional overlapping dorsolateral nuclei.

This variation in the number of subcuticular nuclei encountered in T. ambiguus is apparently a characteristic

feature and is unusual. There is no sexual dimorphism exhibited in this feature.

Giant nuclei in the lemnisci

Bullock (1962) in discussing the variation in lemnisci in Acanthocephalus jacksoni, dealt primarily with the variations in shape and size. In the present study of T. ambiguus, the lemnisci were frequently observed to be elongate and subequal with very little size difference. In none of the individual worms observed did I come across any deviation from the normal shape, although they seemed to be slightly curled in a few individuals. The chief variation that was observed in the lemnisci was in the number of nuclei.

Thirteen worms (males and females) were dissected alive at the anterior end to expose the lemnisci along with the proboscis, and were then mounted for study. In addition, 21 whole mounts of males and females were observed in which nuclei were clearly seen. It was found that the number varied remarkably from individual to individual (Table 1). The number of nuclei in short lemnisci varied from 2 to 5, and in the long lemnisci from 2 to 4 presenting varied patterns of (short/long) 2/2, 2/3, 3/2, 3/3, 3/4, 4/2, 2/4, 4/3 and 5/3. Patterns of 4/4 and 5/5 were not observed. The most frequently occurring patterns were 2/3, 3/2, and 3/3. Juveniles obtained from naturally infected amphipods also revealed similar type of variation. This variation

seems to be characteristic of the species.

Nuclei in the cement gland

Attempts to count the number of nuclei in the cement gland proved futile because of the presence of several nuclei. Only a few worms (juveniles and adults) permitted a rough estimate of the number. It was found to vary; the number ranged from 26 to 47 or more.

Comparison of T. ambiguus with T. longirostris

Comparison of T. ambiguus with T. longirostris is presented in Table IV. For T. ambiguus, descriptions given by Van Cleave (1921), Linton (unpubl.) and the writer have been included. Additional discussion is presented later.

Table IV

Comparison of Tanaorhamphus longirostris (Van Cleave, 1913) and T. ambiguus Van Cleave, 1921

(All measurements in microns unless otherwise indicated)

		T. longirostris	T. ambiguus		
			Van Cleave's description (Female only)	Linton's description	Present description
Body length	(M)	4 mm*		2 to 6 mm	2.73 to 8.14 mm
	(F)	6.2 mm*	7.9 mm	3 to 8 mm	4.4 to 13.72 mm
Body width (Max)	(M)	470*		370 to 420	300 to 1,010
	(F)	630*	670	390 to 670	410 to 1,520
Proboscis length	(M)			440 to 680	560 to 720
	(F)		770	620 to 840	700 to 840
	(M&F)	500*			
Proboscis width	(M)			100 to 170	100 to 130
	(F)		190	120 to 210	130 to 220
	(M&F)	150*			
Hook arrangement	(M&F)	Irregular		Regular	Regular
No. of longitudinal rows	(M)			16	14-18 (16^{+})
	(F)		20	18 or 20	18-22 (20^{+})
	(M&F)	20^{+}			

(Continued)

		T. longirostris	T. ambiguus		
			Van Cleave's Description (Female only)	Linton's description	Present description
No. of hooks per row	(M)			15, 16	11/12 to 15
	(F)		16	16, 17 to 18	13/14 to 16/17
	(M&F)	6 to 10			
Anterior hooks	(M&F)	54*		45	40 to 55
Middle hooks	(M&F)		41 to 47	40 to 45	40 to 53
Posterior hooks	(M&F)	16*	24	36 to 45	38 to 48
Proboscis receptacle	(M&F)	540*	960	510 to 1,120	330 to 1,200
Lemnisci	(M&F)	half length of body	2,300	950 to 1,680	(sub equal) two or two-half times of receptacle
No. of nuclei in lemnisci	(M&F)	2, 1	3, 3	2, 1	constantly variable
Subcuticular nuclei: dorsal/ ventral	(M&F)	5/1+	5/1	5 and 10/1	8-15 / 0-2

(Continued)

	T. longirostris	T. ambiguus		
		Van Cleave's description (Female only)	Linton's description	Present description
Female				
Genitalia length	490			440 to 1,180
Vagina	127			130 to 210
Uterus				140 to 390
Genital orifice	near posterior end			subterminal
Mounted embryos	without hooks 27/8 to 10			with hooks 30 to 44/11 to 18
Male				
Genitalia length				1,290 to 4,640
Anterior testis	440 to 600			220 to 850
Posterior testis	275 to 600			210 to 1,040
Cement gland	270*			230 to 1,390
No. of nuclei in cement gland	16			Large and variable number

M - Male
F - Female
* - Average measurements
+ - Usual number

Discussion

Van Cleave's (1921) description is unfortunately based upon the study of a few (3) mounted specimens and is very general. A comparison of his original description with the present one (Table IV) agrees in most of the specifications given by him for the species. The features that differed significantly were the size of the basal hooks and the number of subcuticular nuclei.

Linton in an unpublished manuscript, reported T. ambiguus from a few fishes and included incomplete descriptions of the specimens collected. A comparison of his description with the present one especially in regard to the nature and size of proboscis and number, arrangement and size of hooks closely agrees with the present description (Table IV). His basal hook measurements were 36 to 45 microns for the female and 30 to 36 microns for the male, which are close to my measurements. Van Cleave (1921) found the basal hooks to be 24 microns long which may be an error, because in all of the 85 specimens examined none measured as small as 24 microns.

The only other species of the genus is Tanaorhamphus longirostris. The pattern and nature of the proboscis hooks in T. ambiguus are distinctly different from those of T. longirostris in that they are regularly arranged in definite longitudinal rows. The hooks are almost similar in size,

except for the most anterior and the basal hooks which are slightly smaller in length and breadth. The number of subcuticular nuclei is highly variable, while Van Cleave (1913) described 5 dorsal and 1 ventral nucleus for T. longirostris. Lemnisci are long with a variable number of nuclei in contrast to a typical neoechinorhynchid pattern of two and one for T. longirostris. The nuclei in the cement gland are several and variable. The female genital orifice is distinctly subterminal, about 60 to 290 microns distant from the posterior tip of the worm. The embryos (acanthors) have anterior hooks (Fig. 6), in T. ambiguus, while the embryos of T. longirostris appear to be without anterior hooks. Finally T. ambiguus is a brackish water species having the migratory eel as a definitive host and a brackish water amphipod as an intermediate host.

Van Cleave's basis for assigning this species to Tanaorhamphus was probably due to the following features: 1) elongate proboscis; 2) number of longitudinal rows of hooks which he found to be 20; 3) measurement of the basal hooks which he found to be 24 microns long, believing that there was a gradual decrease in the size of hooks from anterior to posterior of the proboscis as was seen in T. longirostris. He apparently overlooked the regular arrangement of the proboscis hooks of his species. Other morphological features were omitted in his descriptions and the

male was not studied. Much of this omission is undoubtedly because of the poor condition of the specimens he had to work with.

T. ambiguus differs from Tenuisentis niloticus, the only species under that genus, in many of the morphological details, and in its geographic distribution. The differences are seen in the following features: 1) length of lemnisci; 2) length of the genital apparatus; 3) length of the cement gland and 4) the number of subcuticular nuclei. In Tenuisentis niloticus, the lemnisci are shorter than proboscis receptacle; testes are elongate and nearer to the anterior extremity of the trunk than to the posterior; syncytial cement gland very long ranging from 2.7 to 4.2 mm in length (Van Cleave, 1936). The number of subcuticular nuclei are fewer in number. It is a fresh water species and so far reported only from Egyptian Nile. The only similarity T. ambiguus has with Tenuisentis niloticus appears to be in the body size, form of proboscis and arrangement of hooks which are regular and in definite longitudinal rows with several in a row, subcuticular nuclei occurring in pairs, and presence of dorso-lateral nuclei. (In Tenuisentis niloticus lateral in position).

There are reports in literature (Luhe, 1912; Van Cleave, 1938, 1941; Bullock, 1957, 1962, 1966 and others) dealing with variations. In the present study, variations have been encountered and described. The variations are

manifested in the number of proboscis hooks, number of giant nuclei in the subcuticula, lemnisci and cement gland. These variations are unlike those usually noticed in other species of Acanthocephala and are characteristic of the species. The number of nuclei in subcuticula, lemnisci and cement gland are usually primary characters in the taxonomy of Acanthocephala by presenting a typical pattern (number). Deviating from the normal pattern, T. ambiguus appears to be unique by showing inconsistency in these features of taxonomic importance.

In the light of the morphological evidence discovered in the present study, it would seem best to erect a new genus for this species.

In his paper on the acanthocephalan parasites of eels, Van Cleave (1921) indicated that there was no evidence of marine species of Acanthocephala inhabiting the intestine of Anguilla chrysypa(=Anguilla rostrata). The present studies on the life cycle indicate T. ambiguus to be primarily associated with eels in brackish water and to have a brackish water amphipod as intermediate host. Whether this species is carried either to marine or fresh water situations is not known.

Linton, in his unpublished manuscript, reported T. ambiguus from alewives, bass and killifish. The size of the worms collected from these fishes ranged from 3 mm to 8 mm for females and 2 to 6 mm for males (Table IV, Linton's description) indicating immaturity of the worms.

Linton also stated that he did not observe embryos in females of his collections. In the present study, the range of size of the mature females was 8.2 to 13.72 mm. Large numbers of the species were collected from tomcods both by Dr. W. L. Bullock and myself from the Oyster River. There was not, however, a single sexually mature worm. Study of the specimens from tomcods indicated the size range to be 5.17 to 7.95 mm for females, and 3.96 to 5.96 mm for males. Van Cleave and Mueller (1934) state that, in a number of hosts, the adjustment of parasites may be obviously imperfect as indicated by the complete lack of gravid females. The adjustment of T. ambiguus seems to be imperfect in alewives, bass, killifish and tomcods.

On the basis of the collection data and of the life cycle studies, it is concluded that T. ambiguus is primarily a parasite of eel (Anguilla rostrata), and is of brackish water origin. However, it may be found in other fishes in localities where the infected intermediate hosts are available, but the worms do not reach sexual maturity.

V. GENERAL OBSERVATIONS

Natural Infections

Definitive host

During various months of the years 1966-1968, several collections of eels were made from time to time to note the incidence of infection of Tanaorhamphus ambiguus. Data in Table V. shows the total incidence of natural infection of eels with T. ambiguus during this period. Only 28 out of 125 eels examined from the Oyster River were infected with T. ambiguus, the incidence of infection being 22.4%. (Incidence of natural infection of T. ambiguus in eels during each year is given in Appendix 2, a, b, and c.) The number of worms recovered from these eels ranged from 1 to as many as 272 per host with an average of about 21 (Appendix 1.) One collected on July 28, 1966, measuring 41.5 cm. yielded about 120 mature and immature worms and the other collected on June 25, 1968, measuring 56 cm., yielded about 272 mature and immature worms (Appendix 1.) The rest of the infected eels (26) had only a few worms, mostly immature; the number ranging from 1 to 18 with an average of 7.

The eels were often (58%) infected with a species of Neoechinorhynchus. These neoechinorhynchids from eels

Table V

Incidence of Natural Infection of *T. ambiguus* in Eels During Various Months Of The Years 1966 - 1968

	Total Number Examined	Total Number Infected	Total Percent Infection
January	-	-	-
February	-	-	-
March	-	-	-
April	3	0	0
May	4	3	75.0
June	26	6	23.1
July	18	1	5.55
August	25	6	24.0
September	25	10	40.0
October	19	2	10.05
November	4	0	0
December	1	0	0
Total	125	28	22.4

have been identified in the past (by Van Cleave and by Linton) as N. cylindratus, but this identification is questionable. Records of Dr. W. L. Bullock and myself show that the liver of Fundulus heteroclitus (killifish) is often infected with this Neoechinorhynchus indicating this fish's probable role as the second intermediate host in the life cycle of this worm.

It was noted that eels measuring 28-33 cm. were not usually infected with T. ambiguus. Of the 29 eels measuring in the specified range, only 3 were found to be infected with T. ambiguus, the number of worms ranging from 1 to 3 per host.

Small eels ranging in length from 7 to 13.5 cm. were collected from Great Bay, South Newington, N. H. About 8 were examined, but none were found to be infected with T. ambiguus. Experiments where these eels were fed infected amphipods, and which they ate immediately, were unsuccessful.

T. ambiguus has been found in tomcods, especially during the fall. Of the 52 examined, 31 were found to harbor T. ambiguus (59.62%) (Appendix 3.). The number ranged from 1 to as many as 117 per host, with an average of about 21. All the worms examined were found to be immature ones. Records and observations of Dr. W. L. Bullock confirm this observation. The worms were found to occupy various positions in the intestine. Except for a few worms, especially at the anterior region of the intestine immediately below the

pyloric caecae, most were unattached indicating that they could be easily voided along with the fecal content. Infected tomcods left in the aquarium for 2 or 3 days apparently voided their parasites, since I found several worms at the bottom of the aquarium along with the fecal material. The adjustment of T. ambiguus therefore seems to be imperfect in tomcods. Tomcod collecting certainly proved to be a convenient method of obtaining T. ambiguus for morphological studies.

Intermediate host

Observations were also made of the stomach contents of eels. On one occasion, about 150-200 amphipods were recovered from the stomach of an eel that measured 41.5 cm. The same eel yielded 120 mature and immature adults of T. ambiguus. The amphipods were in various stages of digestion. Usually arthropods (either ostracods, amphipods or copepods) are suspected to be the intermediate host for aquatic species of Acanthocephala. Having this suspicion in mind, about 75 undigested amphipods obtained in this way were dissected, and 5 were found to be infected with juveniles which were identical to the adults obtained from the intestine of the host eel. No cyst or sheath was observed surrounding the parasites. A few juveniles were obtained from the anterior part of the stomach. These worms had apparently come out of digested amphipods. A number of juveniles were found in the anterior part of the duodenum, and most of the mature worms were localized in a cluster at the posterior aspect

of duodenum. The amphipods were identified as Gammarus tigrinus (a brackish water species) by Dr. R. A. Croker, Zoology Department, U.N.H.

Since this observation indicated the probable intermediate host, a subsequent step was to collect amphipods from the same point where eels were collected and to check for natural infection. On August 1, 1966, about 1,000 amphipods were collected from among the water plants. About 568 amphipods were dissected, and 11 were found to be infected with juveniles. From then on, subsequent collections were made and the incidence of infection noted. (Appendix 4.) Table VI shows the total incidence of natural infection of T. ambiguus in amphipods during various months of the years 1966-1968. From a total of 5,794 amphipods that were examined (earlier by dissection method and later by superficial observation, see Methods and Materials), 67 were found to be infected (1.155%). There was not a single occasion where an amphipod harbored more than one juvenile. Incidence of infection of T. ambiguus in amphipods during various months of the years, 1966, 1967 and 1968 is given in Appendix 5, a, b, c.

Table VI

Incidence of Natural Infection of *T. ambiguus* in Amphipods During Various Months Of The Years 1966 - 1968

	Total Number Examined	Total Number Infected	Total Percent Infection
January	300	1	0.33
February	-	-	-
March	-	-	-
April	-	-	-
May	418	2	0.48
June	548	0	0
July	1,355	4	0.295
August	825	19	2.3
September	1,044	22	2.1
October	627	13	2.07
November	364	4	1.1
December	313	2	0.64
Total	5,794	67	1.155

Experimental Infection

Definitive host

Experimental infection of eels with naturally infected amphipods. Two eels collected from Oyster River were isolated and checked for eggs of T. ambiguus in feces at regular intervals for about a month. Since no eggs were found, they were believed to be uninfected. Each of the infection-free eels was maintained alive in an aquarium in 12% sea water and at different temperatures; one at 22-25°C and the other at 11°C. Since they ate small pieces of meat, this was taken advantage of in infecting them with naturally infected amphipods (see Methods and Materials). Because collections of amphipods were made at various intervals of time, and because of the availability of a limited number of naturally infected amphipods, the infections of eels were attempted whenever naturally infected amphipods were obtained. Thus each of the eels was fed with 5 infected amphipods during a period of 18 days. Fecal examinations were begun 2 weeks after infection to find the first date of passage of eggs. The results of the experiments are presented in Table VII.

An eel maintained at a temperature 22-25°C passed out eggs in feces approximately one month after infection. It was autopsied the day eggs were first found in feces.

Table VII

Experimental Infections of Eels with Naturally Infected Amphipods

No. of Experiments	Eel size in cm.	Temp. eels maintained	Date of Infection	No. of infected amphipods fed	Date of autopsy	No. of worms recovered
1	29	22-25°C	Oct. 9 to Nov. 6, 1967	5	Dec. 10 1967	2 F, 1 M (3)
2	29	(13±2°C)	"	5	Dec. 11 1967	2 F, 1 M (4)

Controls 2 - negative

F - Female

M - Male

Three worms were recovered; 2 females and 1 male from the posterior aspect of the duodenum. Both females had embryonated eggs.

No eggs were found in feces of the eel maintained at $13\pm2^{o}C$ at this time. Since it jumped out of the aquarium and died approximately one month after infection, it was also autopsied. Four worms were recovered from it; 2 females and 2 males. The worms had only ovarian balls. In both cases the worms were established in the posterior aspect of the duodenum.

The experiments confirmed that the juveniles were directly infective to the eels. That temperature affects development was also evident.

Experimental infection of Fundulus heteroclitus with naturally infected amphipods. Fundulus heteroclitus is abundantly available in the Oyster River. The observation of the stomach content of the eels indicated that eels feed on Fundulus. Since a previous survey of parasites in Fundulus had indicated one to be infected with two juveniles of T. ambiguus, the following experiment was conducted to determine whether Fundulus sustained infection of T. ambiguus.

One killifish collected from Oyster River measuring 6 cm. in length was isolated in a finger bowl containing brackish water. It was maintained at the room temperature

(22-25°C). It was fed with uninfected amphipods during the one month period. It was observed that 4 to 5 hours after eating amphipods, fecal pellets were passed which consisted mainly of undigested exoskeleton of amphipod. Two naturally infected amphipods were fed to the same fish and after 5 hours, the finger bowl was checked for fecal pellets. Two fecal pellets containing amphipod exoskeleton and one infective juvenile (T. ambiguus) were recovered from the finger bowl. Two months later the fish was autopsied and no parasites were found in the intestine. Probably the other infective larva escaped observation. This experiment and the observations made both by Dr. W. L. Bullock and myself for the incidence of T. ambiguus in killifish confirm that killifish do not play a part in the life cycle of T. ambiguus.

Infection of eel with juveniles (28 day stage) obtained from artificially infected amphipods. Out of a limited number of experiments conducted (which involved forced feeding) only one was successful, which demonstrated that the juveniles were infective for the definitive host after 28 days of development.

Three 28 day stage juveniles obtained from artificially infected amphipods were force-fed to an infection free eel measuring 30 cm. in length. Eleven days later 2 juveniles were recovered in the intestine of the fish. The worms were attached to the wall of the intestine in the

anterior aspect of the duodenum.

Experimental infection of eels with immature worms of T. ambiguus obtained from tomcods. Because immature worms of T. ambiguus were available in tomcods, several attempts were made to infect eels using different forced feeding methods and sur ical techniques (see Methods and Materials). The number of experiments conducted and the results obtained are summarized in Table VIII.

Four experimental eels (Table VIII, Expts. 1-4) died 2 to 10 days after infection probably due to forced feeding or surgical methods that might have caused injury to the fish. The number of worms recovered in these experimental eels ranged from 7.7 to 49.5%.

Of the 6 eels, only 2 (Table VIII, Expts. 5 and 6) lived for two months when maintained at ($13\pm2^{o}C$); one that was force fed and the other in which infection was carried out by the surgical method. The percent recovery of worms was found to be 23.6 and 12.32% respectively. The worms recovered from these eels were firmly attached to the intestine and though they had grown in size, they were not gravid. Since sexual maturity was not attained in these worms, even after two months infection at temperatures of $13\pm2^{o}C$, the effect of temperature is once again evident.

The loss of worms by forced feeding means was mainly by regurgitation and in surgery infection the worms came out through anus.

Table VIII

Experiments with Laboratory Forced Feeding or Surgical Infection of Eels With Immature Worms of *T. ambiguus* Obtained from Tomcods ($13 \pm 2^{o}C$)

Expt. No.	Eel size	Method of Infection	No. of worms used for Infection	Time recovery of worms (days)	No. worms recovered	Per cent recovered
1	30 cm.	Forced feeding by a capillary tube	117	3	9	7.7
2	60 cm.	Forced feeding along with meat pieces	200	10	60	30.0
3	30 cm.	Surgical method	138	2	50	36.2
4	30 cm.	Surgical method	186	2	92	49.5
5	43 cm.	Forced feeding along with dissected stomach of tomcod	72	65	17	23.6
6	30 cm.	Surgical method	81	62	10	12.3

Intermediate host

Amphipods (G. tigrinus) used for experimental infections were those collected from the Oyster River. The mature worms used for the infection experiments were those obtained from two eels that were heavily infected (Appendix 1.) In the experiments conducted, eggs from fresh live worms, eggs from worms that were preserved alive for about 6 weeks at 4°C in 0.9% saline and eggs obtained from mature worms, centrifuged and stored in 12% sea water at 4°C for about 4 months, were used. At varying periods after exposure, the amphipods were examined for larval stages. The results of a number of experiments conducted are summarized in Table IX. The incidence of experimental infections varied from 42.8 to 83.3%. Of 288 experimental amphipods examined, 173 or 87.8% became infected. Controls were run for all experiments. A total of 350 amphipods in the controls were found to be negative. In the experiments (Table IX, Expts. 1-8) where amphipods were exposed to eggs, usually 1-4 parasites were present in any infected amphipod and the per cent infection was found to vary from 42.8 to 71.4 and the mortality rate was found to be comparatively low. In the experiments (Table IX, Expts. 9-11) where amphipods were exposed to bits of worms containing both immature and mature eggs, usually 4-8 parasites were present in any infected amphipod.

Table IX

Experimental Infection of Amphipods

No. of Expts.	Method of Infection	No. amphi-pods ex-posed	No. examined	No. infected	No. died	Percent infected
1	Exposure to eggs	40	38	26	2	68.4
2	"	20	16	10	4	62.5
3	"	10	7	3	3	42.8
4	"	10	8	4	2	50.0
5	"	10	10	5	-	50.0
6	"	7	7	5	-	71.4
7	"	10	8	4	2	50.0
8	"	135	106	52	29	49.1
9	Exposure to bits of worm containing eggs	72	32	26	40	81.25
10	"	100	24	20	76	83.3
11	"	100	32	18	68	56.25
Totals		514	288	173	226	87.8

The usual medium in which cultures were maintained, was 12% sea water. However, a few experiments were conducted in different salinity media (25% and 50% sea water) to determine the effect of salinity on development (Table IX, Expts. 2 and 3). Salinity did not seem to affect development.

Development of T. ambiguus in the amphipod host was found to be normal. By the 27th day after exposure to embryonated eggs, amphipods maintained at room temperature (22-25°C) contained apparently fully developed larvae of T. ambiguus. By the 28th day or after, they were infective for the definitive host.

The evidence obtained by natural observation and by successful infection experiments where G. tigrinus sustained infections with ease until juveniles developed, were adequate to prove that G. tigrinus is an intermediate host. It is also evident that the life cycle of T. ambiguus does not require a second intermediate host.

VI. LIFE CYCLE

Stages in the Life Cycle

Mature egg or Shelled acanthor (Fig. 2)

A detailed description of the mature egg is already given in dealing with morphology (See p. 28).

Acanthor (Fig. 2, 6)

Living unhatched acanthors are elongate and measure 51 to 57 by 17 to 20 microns (subsequent measurements are in microns). They maintain a characteristic shape almost like a pear; broader at the posterior half and narrower at the anterior half (Fig. 2). Within the membranes, the cuticula of the acanthor appear to be folded throughout its length except at the anterior region where there are hooks.

Hatching takes place within 1 to 6 hours in the intestine of amphipod after ingestion of embryonated eggs. The mechanism of hatching is not clear. Probably it is aided by the action of the gastric mill and the digestive juices of the amphipod, as well as by the movements of the acanthor itself (Awachie, 1966). Within the intestine of the amphipod, the outer and the middle membranes develop a split at a point one third the distance from the posterior pole (Fig. 7). The split is seen only on one side of the

egg. The fertilization membrane and the inner membrane were not observed either in the empty shell of egg or on the immediately hatched out acanthors. They probably become lost at the time of hatching. Harms (1965) observed only 3 membranes in eggs of Octospinifer macilentis and indicated that following hatching the motile acanthor was still enclosed by the two membranes, middle and fertilization membrane, (which may be fertilization and inner membrane) and that they become lost after the initial phase of hatching. Following hatching, living acanthors measure 37 to 45 by 17 to 19 and mounted specimens measure 30 to 44 by 11 to 18, indicating that they become reduced in size.

The acanthor becomes active soon after hatching in the intestine of amphipod. It appears to assume a heart shape with an anterior broader rounded end and a posterior narrower pointed end. The anterior end bears 4 circlet of 10 to 11 needle-like hooks each. The cuticular folds become very prominent so that the body of the acanthor appears to be encircled with numerous rings of folds, like those found on the outer shell membrane. The anterior region with hooks is capable of retraction into the body. The acanthor displays a characteristic movement which indicates the presence of contractile fibrils near the outer surface of the body of acanthor. The fibrils, however, were not observed. Meritt and Pratt (1964) reported the presence of thin sheets of muscle in the acanthors of Neoechinorhynchus

rutili. When the acanthor moves the folds appear to move as a wave from the posterior toward the anterior end with a resulting retraction of the anterior region with hooks.

Internally similar to that of an unhatched acanthor, the central embryonal nuclear mass, or entoblast containing the closely grouped nuclei, is seen either as a single or bilobed mass. The peripheral zone, which appears to be granular, contains 6 to 10 or more slightly larger vesicular nuclei which become more evident in the preacanthella stages. These represent the nuclei of the subcuticula and lemnisci. Vesicular, future giant nuclei seem to appear while the acanthor is still unhatched. The future number appears to be established after the acanthor reaches preacanthella stage.

The acanthors appear to pass through the intestinal wall of the amphipod mainly by its own mobility. Penetration seems to begin immediately upon hatching. The actual penetration of acanthors was not observed, but it appears that penetration is accomplished within 20 to 96 hours after initiating infection. DeGiusti (1949) and Jensen (1952, Ph.D. Thesis, University of Minnesota) have observed the actual penetration of the acanthor in the wall of the intestine of amphipods for Leptorhynchoides thecatus, and Pomphorhynchus bulbocolli, respectively. The time the acanthors take to pass through the intestinal wall of the amphipod probably depends on the position of the acanthor

in the intestine (near or away from the intestinal wall), the direction of movement of acanthors, and the intestinal contents. If the direction of movement of acanthors is along the length of intestine, penetration may not be accomplished at all. If the direction is toward the wall of the intestine a shorter time may be needed. Acanthors have been observed in the intestine without much change in structure until about 4 days after infection.

Preacanthella (Figs. 8-10)

This stage represents the first phase of development, and lasts from the 2nd day until about the 14th day. It is a stage of slow growth accompanied by little internal change.

Once the acanthor penetrates the intestine, it begins to differentiate in shape in the hemocoel. By the 2nd to 5th day following exposure of amphipods to shelled embryos, the parasite is found lying free and immobile in the hemocoel. The preacanthella assumes a nearly spherical to oval shape. Internally it shows the embryonal nuclear mass in the form of a single mass occupying a central position. The giant nuclei are vesicular and become more evident. The number is remarkably variable (6 to 19 or more). They seem to be scattered in the syncytium which appears to be granular. Of these giant nuclei, usually 2 or 3, much larger and more conspicuous than the others, usually occupy an anterior position; one or two of these become the giant ventral nuclei of the trunk. Others represent the future giant

Figures 6-13. Tanaorhamphus ambiguus (continued)

6. Acanthor from intestine of amphipod 4 days after infection.

7. Empty shell of egg

8. Two-day preacanthella from hemocoel of amphipod

9. Seven-day preacanthella

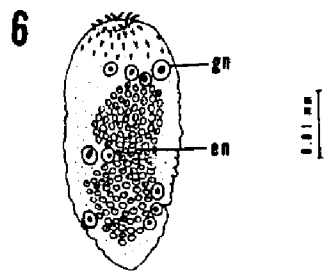
10. Nine-day preacanthella

11. Thirteen-day acanthella

12. Sixteen-day male acanthella

13. Eighteen-day female acanthella

Abbreviations (others given with explanation of Figures 1-5): an, apical nuclei (only two represented; usual number is three) ap, apical organ primordium; en, embryonal nuclear mass; gn, giant nucleus; gp, genital primordium; ln, nucleus of lemnisci; ov, ovary; pn, proboscis nuclear ring; rs, uncinogenous bands;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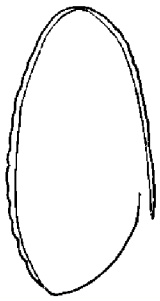
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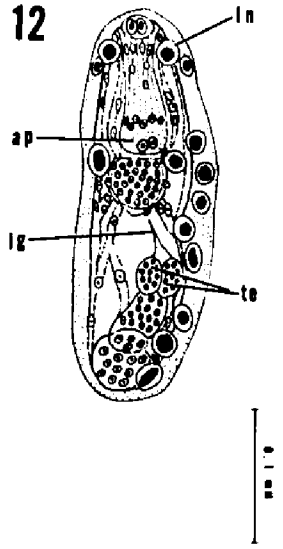
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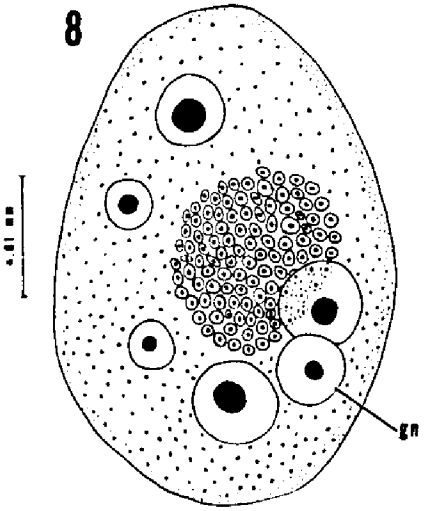
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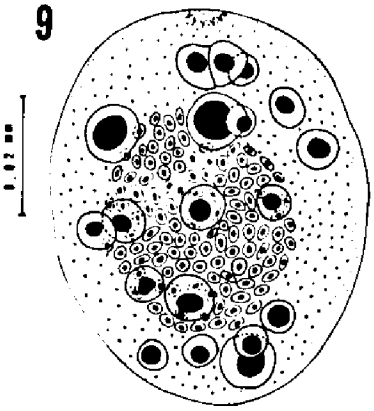
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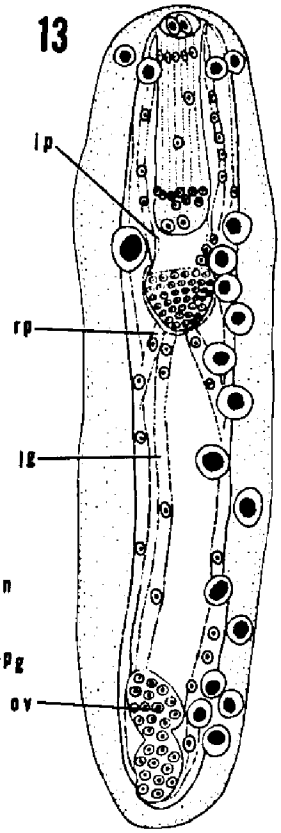
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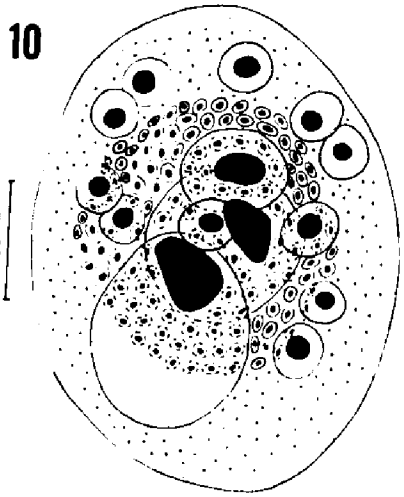
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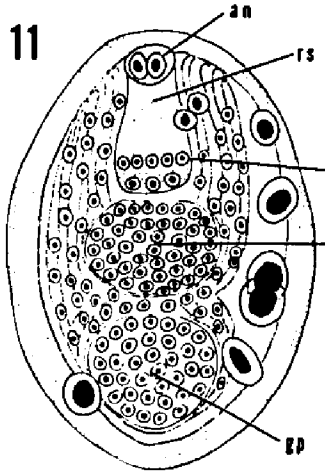
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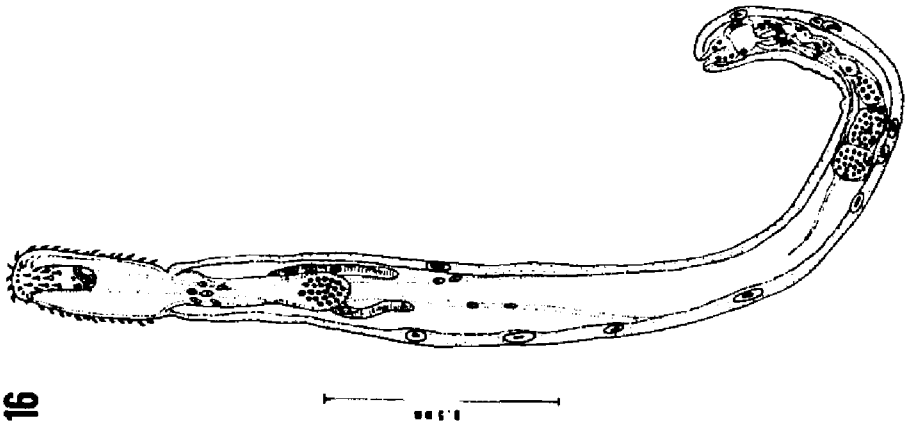
Figures 14-16. Tanaorhamphus ambiguus (continued)

14. Twenty-day female acanthella

15. Twenty-seven day female acanthella

16. Twenty-seven day male acanthella

Abbreviations (given with explanation of Figures 1-13.)



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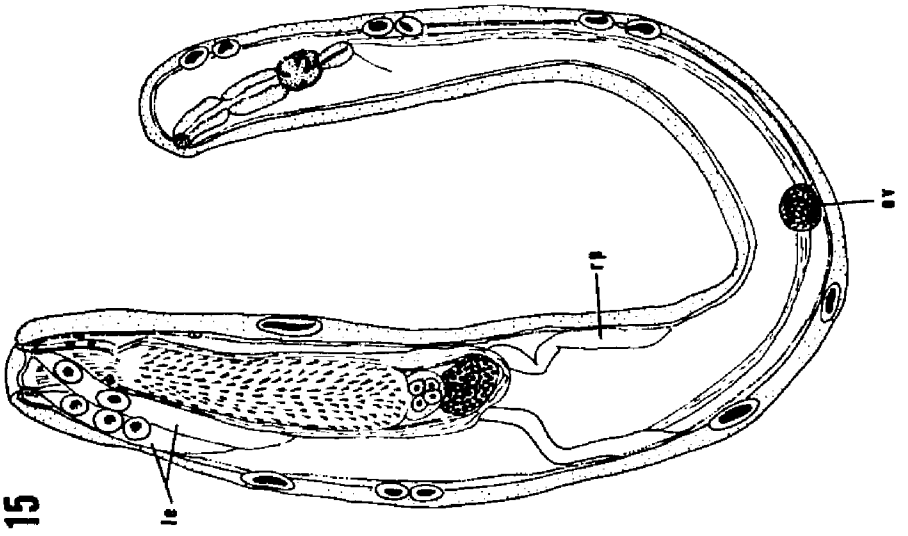

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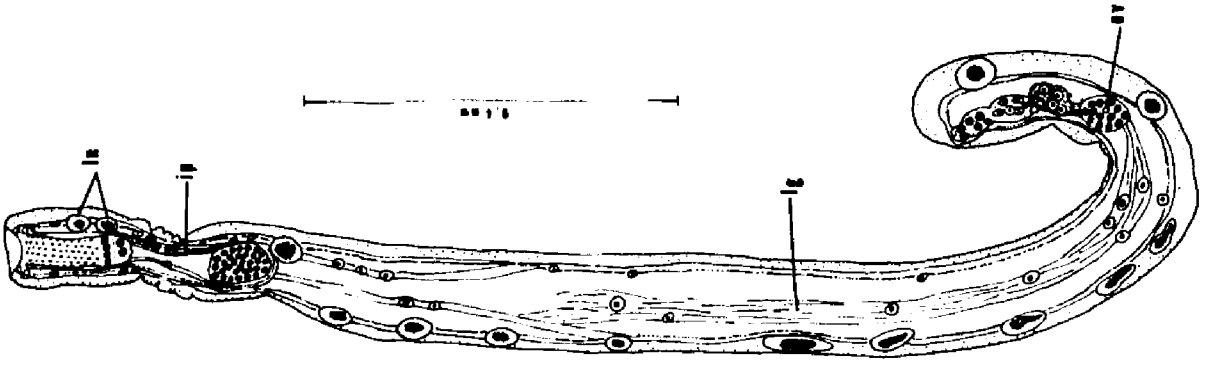

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dorsal nuclei of the trunk and nuclei of lemnisci. Apical nuclei have not been observed in any of the living preacanthellae studied. They seem to become evident only in the acanthellae after about the 13th or 14th day of development. Usually hooks become atrophied in the early preacanthellae. However, one preacanthella (Fig. 9) obtained on the 7th day of development showed a single circlet of hooks, presumably on the verge of becoming atrophied. There is a steady increase in size from an early to late preacanthella. Second to 5th day living preacanthellae measured 40 to 48 by 37 to 43; by the 7th to 9th day, preacanthellae reached 65 to 80 by 50 to 67 and by 11th to 14th day they were 100 to 165 by 90 to 96.

Acanthella (Figs. 11-16)

This stage represents the second phase of development and lasts from about the 13th day up to 27th day. It is a stage of rapid elongation and extensive differentiation.

By the 13th to 14th day following exposure of amphipods to shelled embryos, the larva becomes an acanthella which is indicated by the extensive differentiation in structure and size. The body of the early acanthella has shown increase in length and width. A few whole mounts of early acanthellae recovered from the 13th to 14th day measured 138 to 175 by 100 to 125. In the living specimens,

the internal structures are not clear. The central embryonal nuclear mass appear to be segregated into two portions in tandem arrangement with variable number of vesicular giant nuclei (6 to 19). In the stained specimens, (Fig.11) the embryonal nuclear mass seems to show further differentiation. There is the establishment of the future brain indicated by a cluster of nuclei occupying a central position in the body. The future muscle layers of the body wall, proboscis receptacle, and the proboscis retractors become established and are seen as striations with numerous scattered myoblastic nuclei. The apical nuclei, usually 3 in number, occupy the anterior extremity of the acanthella. Their origin was difficult to determine. Two or four giant vesicular nuclei located at the anterior region near the proboscis apparatus are believed to be lemniscal nuclei. The future proboscis apparatus is also evident with plasmic uncinogenous bands (Cable and Dill, 1967), the proboscis nuclear ring, and the future apical organ. The genital primordium is generalized and is approximated toward the posterior aspect of the body. The sex is difficult to determine at this age.

By the 15th to 18th day, the acanthellae increase in length and the sex is clearly differentiated. Differences in size of the sexes are also obvious. Male acanthellae of this age measured 306 to 320 by 103 to 110 and female acanthellae measured 505 to 1,510 by 110 to 162.

The vesicular giant nuclei appear to take up their future positions; there is a variable number in the dorsal region and one or two in the ventral. Most of the dorsal giant nuclei appear to be in pairs; in a pair usually one takes up a mid-dorsal and the other a dorsolateral position. The proboscis apparatus and its receptacle are well differentiated. The proboscis nuclear ring with several nuclei (usually about 10 or more) is near the posterior end of the proboscis apparatus. From that ring narrow plasmic bands, the uncinogenous bands, as observed by DeGiusti (1949), Cable and Dill (1967) and others, extend anteriorly and terminate at the base of the group of three apical nuclei. Scattered nuclei are observed in the proboscis apparatus between the apical nuclei and the proboscis nuclear ring. They probably represent the nuclei of the muscle layers of the lateral body wall. The future apical organ appear to have usually 2 nuclei.

The early acanthella presents the basic architecture of the adult. The development in the late acanthella is simply an elaboration of structure already established in rudimentary form within the early acanthella. Hence, further discussion will be in terms of development of organs or organ systems.

The proboscis begins its development inverted as is observed by Degiusti (1949), Cable and Dill (1967) and others for other acanthocephalan species. There seems

to be no doubt about the hooks being developed from the uncinogenous bands. Prior to 20th day, the uncinogenous bands appear as a plate with numerous ridges. The ridges represent the future hooks. After the 20th day of development, the proboscis become distinctly demarcated and begins to evert with accompanying growth in length in the anterior region (Fig. 14). By this time the ridges have differentiated in the form of conical processes. By about the 24th day of development the eversion is completed and hooks erupt to the surface of the proboscis while it is in the everted state. By 27th day stage, the proboscis is fully developed and is retracted into the proboscis sheath (Fig. 15). The complete development of the proboscis appears to be similar to that observed for Leptorhynchoides thecatus (DeGiusti, 1949).

At about 24 days of development, the proboscis nuclear ring is seen to be scattered at the anterior tip along with the 3 apical nuclei, after the complete eversion of the developing proboscis. By the 27th day, they seem to disappear much as has been observed by Cable and Dill (1967). Observation of a few acanthellae of the 24th day stage, showed the apical nuclei in the process of descending into the apical organ which is in the form of an invagination at the anterior end. By the 27th day, after inversion of the proboscis, the apical organ is seen to possess only 3 nuclei (Fig. 15 - shows 4 nuclei which is

an error). Observation of a few acanthellae of this stage confirmed this fact. The 2 nuclei of the apical organ found in the early stages of acanthella were not observed in the late acanthellae. Whether they remain in the apical organ or disappear, is not clear.

The brain develops as a very conspicuous structure in the early acanthella, and as development proceeds and other structures become elaborated, it slowly becomes inconspicuous in the late acanthella occupying a position at the base of the proboscis receptacle.

In the 15th to 18th day acanthellae, the lemniscal nuclei (2 or 4) are found near the anterior region of the proboscis apparatus. Prior to 20 days of development, the primordia of the lemnisci seem to appear. By the 24th day, they are clearly seen as two slightly elongated sacs with the usual number of nuclei that may be present in the juvenile or adult. Since the variable number of nuclei in the lemnisci are always seen close together, it is possible that nuclear fragmentation takes place after the lemniscal nuclei move into the primordia of lemnisci. By the 27th day the lemnisci are much elongated.

The pseudocoel first becomes visible as a clear visible area immediately posterior to the primordium of proboscis receptacle after about 14 days of development. It becomes more and more spacious as the larva increases in length and approaches the late acanthella.

In the 15th to 18th day acanthellae, the muscle layers of the body wall, the proboscis retractors and proboscis receptacle are more evident and contain variable numbers of myoblastic nuclei. In females of this age, the ligament which divides the pseudocoel into the dorsal and ventral sacs is apparent; the ligament usually contains a variable number of myoblastic nuclei (Fig. 13). These features, established at this stage, are maintained through acanthella, juvenile and adult. The musculature of the proboscis develops simultaneously with the proboscis. The inverters are indistinct in the 15th to 18th day acanthellae. After 20 days of development, the inverters (dorsal and ventral) become more evident, when the proboscis begins to evert with the developing hooks. By the 27th day of development, they seem to begin functioning, when the proboscis is retracted into the proboscis receptacle.

The 16th day male acanthella shows two testicular masses at the anterior end of the genital primordium. Further differentiation of the genital primordium is indistinct (Fig. 12). The entire future genital apparatus appears to be enclosed by a genital ligament. After 20 days or more, most organs of the genital apparatus are delineated. By the 24th day Saefftigen's pouch becomes evident, the genital opening is complete, and all of the elements of the male reproductive system are clearly identifiable.

The 15th to 18th day female acanthellae show the genital primordium to be in the form of a bilobed mass containing genital nuclei (Fig. 13). The anterior lobe of the genital primordium is believed to be the ovarian primordium which in later acanthellae (Figs. 14 and 15) is pinched off and appears to occupy any position in the pseudocoel, probably attached to the ligament. The posterior lobe of the genital primordium gives rise to the main genital apparatus. By the 20th day of development, further differentiation of the posterior lobe of the genital primordium is evident (Fig. 14), and by the 24th day, the genital opening is complete and all the elements of the female reproductive system are distinctly delineated.

By the 27th day the acanthellae reach 1 to 2.88 mm. in length (proboscis inverted) and approach the juvenile in morphology.

Juvenile

By the 28th to 32nd day of development, the acanthella reaches the juvenile stage. A few juveniles obtained at this stage measured 2.06 to 3.58 mm. (proboscis everted). The juveniles continue to grow in length reaching a size that is ordinarily found in the juveniles obtained from naturally infected amphipods.

Juvenile males obtained from naturally infected amphipods measure 2.66 to 5.37 mm. in length and 0.33 to

0.56 mm. in width; juvenile females 4.16 to 6.2 mm. in length to 0.33 to 0.60 mm. in width, (proboscis everted in both the sexes). The size seems to vary depending on how long the juveniles have been in the amphipod host. In many of the females observed, the single ovarian sphere had fragmented into numerous ovarian balls.

Development in the eel

The description of development within the definitive host is incomplete because of the difficulties experienced in forced feeding. At a temperature varying between 22-25^{o}C the female worms seem to pass eggs in feces of the definitive host approximately one month after infection. This seems to indicate that at warmer temperatures the juveniles probably become mature immediately or within a week after infection. At low temperatures the juveniles seem to take a longer time to reach sexual maturity. At 13 ± 2^{o}C the worms do not seem to pass eggs in feces of the definitive host even after two months infection.

Discussion

The life cycle of T. ambiguus takes the same pattern of development as described for other acanthocephalan species. For clarity and ease of description, the development of the parasite is discussed from the standpoint of different stages. Since in the acanthella there is an elaboration of the structures that have been already established in the early acanthella, the discussion also includes the development of organs and organ systems.

It is interesting to note that the development of the parasite is completed in two distinct phases. The first phase of development (referred to here as the preacanthella) lasts from the 2nd day until about the 14th day. It is characterized by slow growth accompanied by few internal changes. The second phase of development (acanthella) lasts from about the 13th to the 27th day of development. It is characterized by rapid elongation and extensive differentiation.

There is no evidence that preacanthellae of T. ambiguus become attached to the serosa of the intestine in the amphipod as observed by DeGiusti (1949) for Leptorhynchoides thecatus, Hynes and Nicholas (1957) for Polymorphus minutus, and Awachie (1966) for Echinorhynchus truttae, nor is there an indication that the juveniles become encysted in their intermediate host. The writer

agrees with Cable and Dill (1967) in referring to the unencysted infective stage as juvenile.

Nuclear constancy (eutely) in T. ambiguus is not as rigid as in other eoacanthocephalans. This variability in the number of nuclei seems to be characteristic of this species. Only the apical nuclei seem to show consistency. As in the neoechinorhynchids, T. ambiguus possesses 3 apical nuclei (only two shown in all figures). There appears to be little constancy in the number of giant nuclei, number of nuclei in the proboscis nuclear ring, and in the myoblastic nuclei.

The effect of temperature on the development of T. ambiguus both in the intermediate and definitive host has been considered. Effect of temperature on the development has been experimentally demonstrated by DeGiusti (1949) and Awachie (1966) and others for other acanthocephalan species. At room temperature (22-25^{o}C) T. ambiguus required 28 to 32 days to complete development and reach the infective stage. At 13$\pm$2^{o}C though the complete development was not carried out, it was observed that on about 18th day of development the parasite seems to be still in the preacanthella stage. The effect of temperature was also evident when an infected eel maintained at a temperature 22-25^{o}C passed eggs in feces approximately one month after infection. No eggs were found in feces of the eels maintained at 13$\pm$2^{o}C even after

two months infection.

It is evident from experimental infections that the life cycle of T. ambiguus does not require a second intermediate host, although it seems likely from the food habits and large size of the eels that tomcods (abnormal host) may aid in the massive infections.

Table VI shows that the incidence of infection of T. ambiguus in amphipods reaches a peak during August, September and October. Eels collected during May through October show incidence of infection of T. ambiguus varying from 5.55 to 75% and with an average of 20 to 21 parasites per host (Table V). Tomcods collected during October and November harbored only immature specimens of T. ambiguus (Appendix 3). About 59.62% were found to be infected with an average of about 21 worms per host. These data indicate the possibility of seasonal variation of infection occurring in T. ambiguus.

SUMMARY

Tanaorhamphus ambiguus is redescribed on the basis of more adequate material. The worms used in this study were from the Oyster River, New Hampshire (eels and tomcods) and from Chesapeake Bay, Maryland (eels only). Marked intraspecific variability is noted and described. The variations are manifested in the number of proboscis hooks, number of giant nuclei in the subcuticula, lemnisci and cement gland.

History of the genus Tanaorhamphus is traced and a review of known eoacanthocephalan life cycles is given.

Comparison of T. ambiguus with T. longirostris is given and the relation of T. ambiguus to T. longirostris and Tenuisentis niloticus is discussed.

The life cycle of T. ambiguus is determined by means of observations on natural infections and by experimental demonstrations. An account of the development of the parasite in the arthropod intermediate host (Gammarus tigrinus) is given. This is the first life cycle report of a brackish water species of Acanthocephala.

The embryonated eggs hatch in the intestine of the amphipod host within 1 to 6 hours after ingestion. Acanthors are free in the hemocoel of the amphipod within 20 to 96 hours after initiating infection. The development is completed in two distinct phases. The first phase of development,

(preacanthella, lasting from the 2nd day of development until about 14th day) is characterized by slow growth accompanied by few internal changes. The second phase of development (acanthella, lasting from about the 13th day of development to the 27th day) is characterized by rapid elongation and extensive differentiation. By the 27th day, the development in the amphipod host apparently is complete and by the 28th to 32nd day, the juvenile is infective to the definitive host as demonstrated by feeding experiments. The rate of development of the parasite in the amphipod host was determined at room temperatures of 22-25^{o}C. No transport host is required to carry the juvenile to the definitive host.

Temperature effects on the development of the parasite both in the amphipod and definitive hosts have been considered.

Data on the incidence of natural infection both in amphipod host and eel host indicate the possibility of seasonal variation occurring in T. ambiguus. From the incidence of intensity of infections, infection experiments and the life cycle studies, it is concluded that T. ambiguus is primarily a parasite of eel (Anguilla rostrata) and is of brackish water origin. However, the worms may be found in other fishes in localities where the infected intermediate hosts are available, but the worms do not reach sexual maturity.

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Appendix 1

Incidence of Infection of *Tanaorhamphus ambiguus* in Eels Collected During the Years 1966 - 1968

	Date of Collection	Number Examined	Number Infected	Number of Worms Recovered
1	7-28-66	3	1	120
2	8-3-66	3	1	5
3	9-14-66	7	4	6
4	6-25-67	10	2	2
5	6-26-67	4	0	0
6	7-10-67	2	0	0
7	7-11-67	3	0	0
8	7-24-67	8	0	0
9	8-5-67	3	0	0
10	8-19-67	1	1	1
11	8-20-67	11	3	5
12	8-27-67	6	1	2
13	9-5-67	1	0	0
14	9-6-67	1	0	0
15	9-11-67	3	1	1
16	9-19-67	2	1	1
17	10-2-67	1	1	11
18	10-9-67	1	0	0
19	10-20-67	1	0	0
20	10-22-67	1	0	0
21	10-23-67	1	0	0
22	10-30-67	4	0	0
23	11-7-67	1	0	0
24	11-12-67	1	0	0
25	11-19-67	1	0	0
26	11-26-67	1	0	0
27	12-14-67	1	0	0
28	4-27-68	3	0	0
29	5-16-68	3	3	8
30	5-17-68	1	0	0
31	6-2-68	2	0	0
32	6-25-68	10	4	288
33	7-5-68	1	0	0
34	7-29-68	1	0	0
35	8-9-68	1	0	0
36	9-13-68	1	0	0
37	9-14-68	2	0	0
38	9-19-68	4	2	7
39	9-22-68	0	0	0
40	9-24-68	1	0	0
41	9-26-68	2	2	19

Appendix 1 (continued)

	Date of Collection	Number Examined	Number Infected	Number of Worms Recovered
42	9-29-68	1	0	0
43	10-2-68	2	1	4
44	10-3-68	1	0	0
45	10-8-68	1	0	0
46	10-9-68	3	0	0
47	10-10-68	1	0	0
48	10-21-68	1	0	0
49	10-22-68	0	0	0
50	10-24-68	0	0	0
51	10-25-68	0	0	0
52	10-29-68	1	0	0
		125	28	580

Total percent infection - 22.4

Average/host - about 21

Appendix 2

Table (a)

Incidence of Natural Infection of T. ambiguus in Eels During Various Months of a) 1966; b) 1967; c) 1968

	1966		
	Number Examined	Number Infected	Percent Infection
January	-	-	-
February	-	-	-
March	-	-	-
April	-	-	-
May	-	-	-
June	-	-	-
July	3	1	33.3
August	3	1	33.3
September	7	4	57.0
October	-	-	-
November	-	-	-
December	-	-	-
Total	13	6	46.1

Appendix 2 (continued)

Table (b)

	1967		
	Number Examined	Number Infected	Percent Infection
January	-	-	-
February	-	-	-
March	-	-	-
April	-	-	-
May	-	-	-
June	14	2	14.3
July	13	0	0
August	21	5	23.8
September	7	2	28.6
October	9	1	11.1
November	4	0	0
December	1	0	0
Total	69	10	14.5

Appendix 2 (continued)

Table (c)

	1968		
	Number Examined	Number Infected	Percent Infection
January	-	-	-
February	-	-	-
March	-	-	-
April	3	0	0
May	4	3	75.0
June	12	4	33.3
July	2	0	0
August	1	0	0
September	11	4	36.4
October	10	1	10.0
November	-	-	-
December	-	-	-
Total	43	12	27.9

Appendix 3

Incidence of Infection of *Tanaorhamphus ambiguus* in Tomcods Collected During the Years 1968 and 1969

	Date of Collection	Number Examined	Number Infected	Number of Worms Recovered
1	10-2-68	0	0	0
2	10-14-68	7	6	117
3	10-16-68	4	1	1
4	10-17-68	5	4	32
5	10-20-68	8	3	24
6	10-22-68	4	3	25
7	10-23-68	4	2	39
8	10-27-68	3	3	122
9	10-28-68	3	2	21
10	10-31-68	4	4	138
11	11-7-68	4	3	126
12	1-17-69	6	0	0
		52	31	645

Total percent infection 59.62

Average/host - about 21

Appendix 4

Incidence of Natural Infection of *Tanaorhamphus ambiguus* in Amphipods Collected During the Years 1966, 67 & 68.

	Date of Collection	Number Examined	Number Infected
1	8-1-66	568	11
2	11-28-66	292	4
3	12-17-66	63	1
4	5-15-67	18	0
5	6-7-67	138	0
6	6-26-67	260	0
7	7-15-67	335	1
8	7-29-67	620	3
9	8-14-67	257	8
10	9-4-67	219	6
11	9-11-67	225	6
12	9-17-67	300	6
13	10-8-67	325	6
14	12-24-67	250	1
15	1-2-68	300	1
16	5-9-68	400	2
17	6-2-68	150	0
18	7-7-68	400	0
19	9-26-68	300	4
20	10-8-68	302	7
21	11-24-68	72	0
		5,794	67

Appendix 5

Table (a)

Incidence of Natural Infection of *T. ambiguus* in Amphipods During Various Months of a) 1966; b) 1967; c) 1968

	1966		
	Number Examined	Number Infected	Percent Infection
January	-	-	-
February	-	-	-
March	-	-	-
April	-	-	-
May	-	-	-
June	-	-	-
July	-	-	-
August	568	11	1.94
September	-	-	-
October	-	-	-
November	292	4	1.37
December	63	1	1.59
Total	923	16	1.73

Appendix 5 (continued)

Table (b)

	1967		
	Number Examined	Number Infected	Percent Infection
January	-	-	-
February	-	-	-
March	-	-	-
April	-	-	-
May	18	0	0
June	398	0	0
July	955	4	0.42
August	257	8	3.11
September	744	18	2.42
October	325	6	1.84
November	-	-	-
December	250	1	0.4
Total	2,947	37	1.26

Appendix 5 (continued)

Table (c)

	1968		
	Number Examined	Number Infected	Percent Infection
January	300	1	0.33
February	-	-	-
March	-	-	-
April	-	-	-
May	400	2	0.5
June	150	0	0
July	400	0	0
August	-	-	-
September	300	4	1.33
October	302	7	2.32
November	72	0	0
December	-	-	-
Total	1,924	14	0.726