Studies on the biology, host-parasite interactions and distribution of *Lernaea* spp. in West Malaysia.

by

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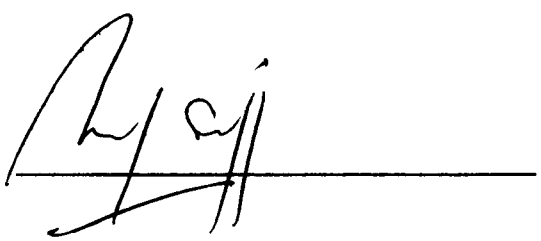
CONTAINS PULLOUTS

Declaration

The work presented in this thesis is the result of my own investigations and has neither been accepted; nor is being submitted for any other degree. All the sources of information have been duly acknowledged.



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CONTENTS

Abstract

Lernaea spp. was identified as a widespread problem in West Malaysia and various aspects of the parasite were studied. A survey of the distribution of Lernaea spp. in West Malaysia revealed its presence in all the 8 government owned fish breeding stations, 104 privately owned farms and 4 out of 5 consignments of imported fingerlings. The Lernaea spp. present in these ponds were identified as L. piscinae and L. cyprinacea "Asian" form (=L.elegans) and its morpha forms, L. ctenopharyngodonis and L.quadrinucifera.

The life cycles of L. cyprinacea and L. piscinae were determined under laboratory conditions.

Infection of C. auratus with the offspring of L. cyprinacea "Asian" form produced adult females similar to the maternal form and another form identified as L. ctenopharyngodonis. A. nobilis, infected with L. cyprinacea "Asian" form and L. piscinae produced only species identical to the maternal form. A high degree of polymorphism was revealed and was further investigated.
Morphometric studies on the larval stages and adult female parasites were investigated and reliable characteristics for the identification of Lernaea is discussed.

The distribution frequency of L. cyprinacea on 3 host species and L. piscinae on A. nobilis in aquarium tanks was tested for goodness of fit with the theoretical negative binomial

distribution. The bases of fins were found to be the preferred site of infection for both species.

Studies on growth performance of A. nobilis infected with L. piscinae in ponds, revealed a significant reduction in Specific Growth Rates. Infected fish had a higher mortality than uninfected fish. A decrease in parasite infection on the body surface after 3 months was associated with its appearance in the eye. A challenge infection did not establish and the fish were suspected of being immune.

Histopathological studies showed a typical inflammatory response with the formation of a granuloma. Eosinophilic granular cells, lymphocytes, and club cells which were identified in the hosts immune to the infection, were believed to play an important role in the rejection of parasites.

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CHAPTER 1.

GENERAL INTRODUCTION.

1.1. Potential of aquaculture and the importance of Lernae osis in West Malaysia.

Even though aquaculture was introduced to West Malaysia by Chinese immigrants 60 years ago, the industry in West Malaysia is still in its infancy. The sea that surrounds West Malaysia and to a lesser extent the streams, rivers and paddy fields, have been able to provide a rich source of fisheries products for the country.

Fish is the main source of protein in Malaysia. With a per capita fish consumption of over 40 kg., Malaysia ranks among the highest fish consumming nations in the world (Anon. 1984). The need for the development of aquaculture has arisen only during the last 2 decades as a response to the increase demand for fish through population growth and reduced fish catches. Decrease in fish harvest from the paddy fields is mainly caused by the introduction of double cropping, the spraying of pesticides and the increase in use of chemical fertilizers. The rivers, estuaries and the seas around West Malaysia are also not spared, pollution from industrial and agribased activities has resulted in decreases in fish catches. In 1980, 50% of the rivers were reported to have reached critical pollution levels at one point or another (Maheswaran 1980). Besides pollution overcapture of fisheries resources from the seas has resulted in the decline of the fish catch from the seas around West Malaysia.

With the rapid decline of natural fisheries resources, the Malaysian Government is presently promoting the expansion of aquaculture. In 1978, the total land area used for freshwater fish culture in West Malaysia had reached 5,507 hectres (Anon. 1979). There is an estimated 5,545 hectares of water surface that has not been utilized and has potential for the development of aquaculture. Progress in the expansion of aquaculture includes subsidising the initial cost of pond construction and supply of fish fry. The Government currently owns 8 breeding stations for the latter purpose. Fish are also being imported from the Far East (Hong Kong and Taiwan) by private dealers to meet the increasing demands. In 1983 Malaysia imported about 8 million food fish fry and about 7 million ornamental fish fry (Anon. 1984). The freshwater fish which are commonly cultured in West Malaysia include Cyprinus carpio Linnaeus, Aristichthys nobilis (Richardson), Puntius gonionotus (Bleeker), Osphronemus gouramy Lacepede, Trichogaster pectoralis (Regan), Ctenopharyngodon idella (Valenciennes) and species of tilapia which are as yet unidentified. These species are usually cultured together and reach marketable size of about 2 kilogram in 6-8 months.

A programme for the study of diseases of economically important fish in Malaysia was begun in 1978 by the author. So far the following fish diseases have been reported in West Malaysia; White Spot, Chilodonelliasis, Trichodinasis, Gyrodactylosis, Dactylogyrosis, Lernaeosis, Argulosis (Shariff 1980 & 1984) and Myxosporidosis (Shariff 1982).

Among these, Lernaeosis has been indicated to be the most economically important disease (Shariff and Vijiarungam in press). Lernaea is suspected to have been introduced with imported fish fry from the Far East (Hong Kong and Taiwan) with ornamental fish (Shariff 1980) and food fish (Suhairi et al. 1983). It is feared that, with the increase in imports of ornamental and food fish along with further expansion and intensification of aquaculture, there could be further economic losses due to Lernaeosis. The present study was thus initiated to investigate the biology, host-parasite interactions and distribution of Lernaea spp in West Malaysia with the hope that it might provide a base line for the management and control of the spread of the disease.

1.2. Genus Lernaea

Lernaea, a freshwater cyclopoid copepod parasite was founded by Linnaeus in 1758 and has since been documented from many parts of the world.

Infestation of the fish by lernaeid copepods occurs when the cyclopoid female penetrates the host integument. It then elongates and undergoes metamorphosis of the cephalic region to produce an anchor which is embedded in the host tissue. Hence it is also known as "anchor worm". Parasite burdens of as many as 1,426 parasites infecting carp of 520 mm length have been recorded (Tidd 1934).

Various publications on the genus Lernaea soon followed after its first report in 1758 by Linnaeus. A recent

publication by Kabata (1979) has reviewed the work on the history and systematics. It also included the geographic distribution of the different species of Lernaea.

1.3. Life cycle

Although over 40 species of the genus Lernaea have been described from specimens of adult female, knowledge of the life histories is almost totally lacking with the exception of Lernaea cyprinacea Linnaeus and to a more limited extent Lernaea barnimiana (Hartmann) and Lernaea chackoensis Gnanamuthu. The life cycle of the commonly known species L. cyprinacea has been studied by several workers, (Wilson 1917, Yashouv 1959, Lahav and Sarig 1964, Bauer et al. 1969, Al-Hamed and Hermiz 1973, Rukyani 1975 and Shields 1976) the most comprehensive and detailed description has been provided by Grabda (1963). The life cycle includes 3 naupliar, 5 copepodid stages and the cyclopoid stage. Kabata (1979) has referred to the female and male cyclopoid as pre-metamorphosis female and adult male. Copulation takes place during the cyclopoid stage. The male then dies and the adult female penetrates the skin of fish. Depending on the temperature, the complete life cycle requires 20 days at 25°C or 16 1/2 days at 30°C (Bauer et al. 1969).

Although the life cycle of L. cyprinacea has been successfully conducted under laboratory conditions without involving an intermediate host, Thurston (1969) found that in the natural environment L. barnimiana needed Bagrus

docmac Forskal as intermediate host before infecting Tilapia. The differences in the mode of development between L. cyprinacea and L. barnimiana cannot be justified on the basis of the different species, as Fryer (1968) found that in Lake Victoria L. cyprinacea lived on Tilapia before they infected B. docmac.

Although Kabata (1979) has suggested that most Lernaeids may not differ greatly from one another as far as ontogeny is concerned, the life cycle of L. chackoensis differs with the life cycle of L. cyprinacea. Gnanamuthu (1951) in his work on L. chackoensis suggested that the nauplius moulted only once to become metanauplius followed by the development of 6 copepodid stages. It would be of interest to investigate the life cycle of other species of parasite within the genus Lernaea to determine the extent of variation present, and these could be compared to the life cycle of the 3 species already described.

1.4. Morphology

The cephalic region of the pre-metamorphosis female embeds in the host tissue and develops into the anchoring structure. The matured female parasite develops a long unsegmented body measuring about 20 mm long. Anteriorly the head expands into 2 pairs of processes also known as the cephalic horn or the anchor. Since the shape of the anchor structure varies in different Lernaea species, it has thus been used as a constant character for the identification of Lernaea. A brief description of the commonly known

L. cyprinacea will be presented here. Among the 2 pairs of processes, the ventral horns are smaller and unbranched while the dorsal horns is Y or T shaped and divided into branches at some distance from the base (Fig. 1.1). The anterior process of the dorsal horns are longer than the posterior processes. The body possesses 4 pairs of biramous swimming legs which are small and fully formed, and a 5th leg which is uniramous and reduced . The pair of egg sacs, each with 200 to 500 eggs are attached to the posterior end.

Since the cephalic region of the cyclopoid female (pre-metamorphosis female) continues to develop within the host tissue to form the "anchor", its morphology may vary due to the different microhabitats available within different regions of the host and also due to differences amongst host species. Poddubnaya (1973) using different hosts was able to obtain 3 "species" of Lernaea from eggs hatched from one offspring. A simplified illustration of her work is presented in Table 1 A & B. She concluded that L. cyprinacea must be restricted to the parasite from Carassius auratus (Linnaeus) whereas all cyprinids carry Lernaea elegans Leigh-Sharpe. Poddubnaya's findings clearly suggested the necessity to re-examine all species of Lernaea.

1.5. Distribution

Currently out of the 40 nominal species, only 3 are present in Europe, 17 in Africa and 7 in North America (Kabata 1979). Poddubnaya (1978) suggested that the genus

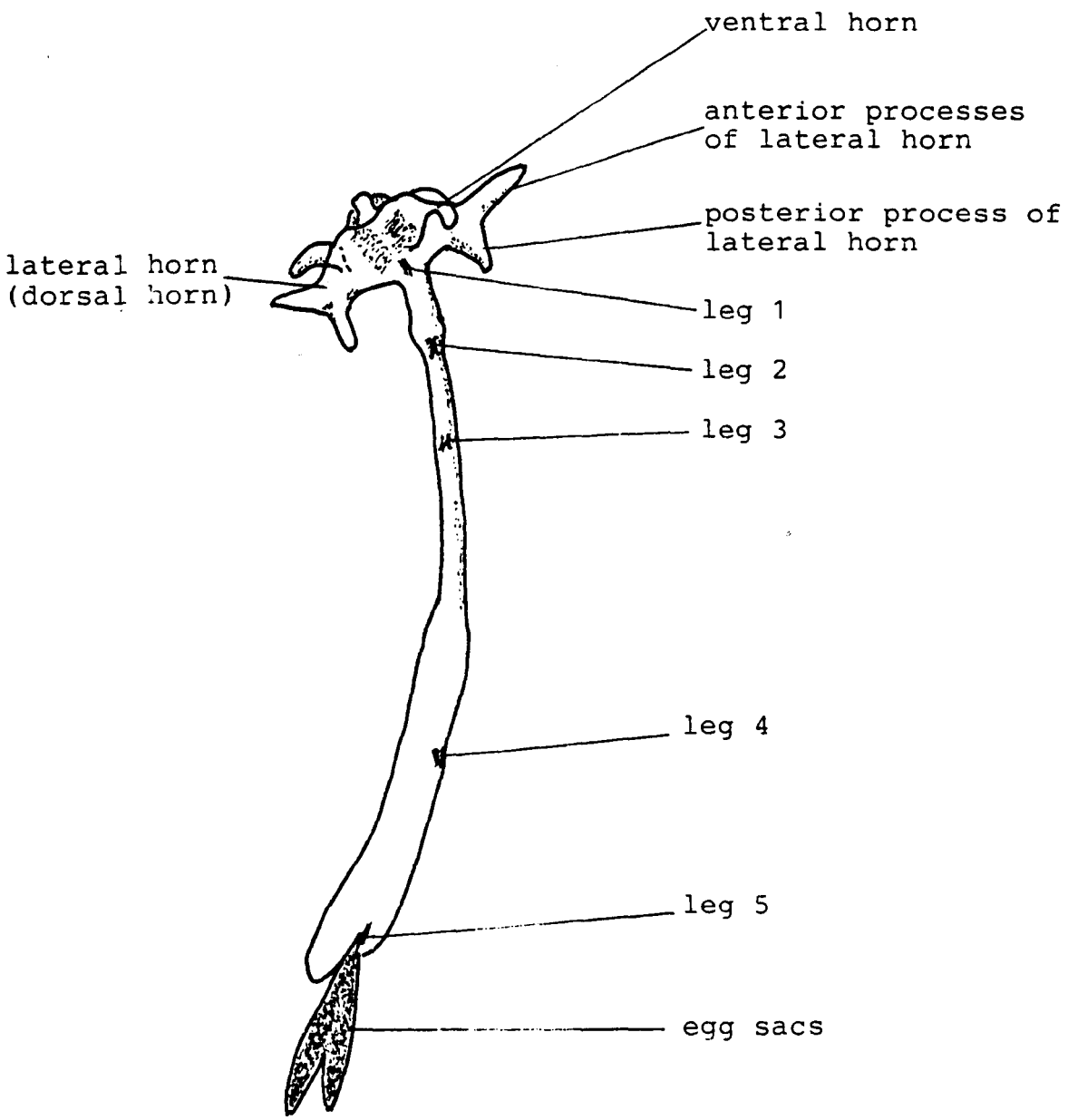


Figure 1.1 Body parts of L. cyprinacea.

Table 1.A

RESULTS OF PODDUBNAYA'S (1973) WORK ON LERNAEA SPP.

A) Lernaea species identified from various host.

Host	Lernaea sp.	Type
1. Cyprinus carpio	L. cyprinacea	a) "European" form 50% b) "Asian" form identical to L. elegans 50%
2. Ctenopharyngodon idella	i) L. Ctenopharyngodonis 50%	
	ii) L. quadrinucifera 25%	
	iii) L. cyprinacea	"Asian" form 25%
3. Carassius auratus	L. cyprinacea	"European" form
4. Ictiolobus bubalis	L. cyprinacea	"Asian" form
5. Aristichthys nobilis	L. cyprinacea	"Asian" form

Table 1.B

B) Result of Poddubnaya's experimental infection of different host species with different forms of *Lernaea*.

Infection with offspring of	Experimental host	*Lernaea* spp/Type obtained
1. *L. elegans*	*C. carpio*	*L. elegans*
2. *L. elegans*	*C. auratus*	*L. elegans*
3. *L. elegans*	*C. idella*	i) *L. elegans* 16.6% ii) *L. ctenopharyngodonis* 84.4%
4. *L. ctenopharygodonis*	*C. idella*	*L. ctenopharyngodonis*
5. *L. ctenopharygodonis*	*C. auratus*	i) *L. elegans* 82% ii) *L. ctenopharyngodonis* 18%

Lernaea was a Southern element from the Palaearctic regions which consist of Europe, North Africa and most of Asia. Poddubnaya also believes that since the marine fauna was unified, the distribution of Lernaea must have been wide spread. Subsequently, with the geological processes, it caused the disappearance of the old warm water fauna in moderate latitudes resulting to its survival only in South East Asia, and later they migrated back to the north. Poddubnaya suggested that L. cyprinacea "European" form was distributed to the north, Lernaea esocina Wilson to North-west and 2 other species, L. elegans (L. cyprinacea "Asian" form) and Lernaea parasiluri Yu were distributed to the East. L. elegans is found almost everywhere in USSR except for the Northern Regions and L. cyprinacea is quite rare. This indicates that L. elegans is replacing L. cyprinacea (Poddubnaya 1978). Poddubnaya concluded that more work has to be done to demonstrate the links between various Eastern branches of the genus.

1.6. Host-parasite relationship

The studies on host-parasite relationship normally includes the ecological and physiological aspects. Studies in these areas are currently receiving a considerable amount of interest, but very little information is available for studies on Lernaea.

The selection of site on the host by copepods reveals that the parasites are unevenly distributed on the host surface. Fryer (1968) found Lernaea to be restricted to

certain locations on the body where they occurred in greatest frequency. In Lake Victoria he found L. cyprinacea occurred most frequently near the jaws of the host, in Lake Bangweulu, Lernaea hardingi Fryer occurred most frequently around the vent of its major host, in Lake Nyasa, Lernaea lophiara Harding was almost exclusively a fin parasite and Lernaea bagri Harding occurred particularly in the various cavities of the head, mouth, gill chamber and operculum walls, but was not so restricted as certain other species. Lernaea palati Harding and Lernaea tilapiae Harding both of Lake Nyasa, occurred only in the mouth of the hosts. On the other hand L. barnimiana occurred almost anywhere on the body. The factors involved in the particular pattern of distribution shown by various species of Lernaea remain unknown. However, in the Victoria Nile, where the water was swift, L. barnimiana occurred in abundance in the mouth, but on the same host in still-water conditions, the infection of the buccal cavity was rare and most of the parasites were found at the base of the fins or flanks. On the basis of these findings, Fryer (1968) concluded that the distribution of L. barnimiana was influenced by environmental conditions.

Besides environmental conditions Fryer indicated that the same species when present in different areas may have different site preference on the host. His findings were based on the presence of L. cyprinacea in the mouth of fish from Lake Victoria but absence of such occurrence in fish from other places.

Fryer's work has revealed interesting results from a limited number of specimens but much more work needs to be done to elucidate and understand in greater detail the distribution of different species of Lernaea when present on different hosts and under different environmental conditions.

1.7. Effects on host

Lernaea has been known to be a serious pest of freshwater fish, particularly in hatcheries (Davis 1956 and Cressey 1983). High mortalities have occurred among cultured catfish, gold fish, bait minnows, carp, trout and other fish (Post 1983). As early as 1934 Tidd recorded losses of 18 tons of carp in the USA. Since then reports of losses to the aquaculture industry from Lernaea spp. infection have been numerous and cover a large area of the globe. It is clear that this parasite is of major economic importance in commercially important fish.

L. cyprinacea which was accidentally introduced into Indonesia from Japan in 1953 destroyed 30% of the main hatchery centres of North Sumatra, Java and North Sulawesi (Djajadiredja et al. 1983, Koesoemadinata 1979). It was estimated that in Java alone a total of 1.4 billion fish fry were lost during the epizootic (Rukyani (1975).

Most of the reports have been based on incidental observations, and thus there is a need to study the effect of Lernaeosis to elucidate the actual losses with reference to retardation in rates of growth and mortality rates.

1.8. Histopathology

Although the genus Lernaea was founded in 1758, the study of histopathological changes due to Lernaeosis began recently during the last decade. The parasite is capable of causing considerable tissue damage to its host.

Joy and Jones (1973) reported typical inflammatory response in Morone chrysops (Rafinesque) infected with Lernaea cruciata Le Suer. Their findings included mild necrosis, oedema and infiltration of neutrophils followed by macrophage infiltration with consequent phagocytosis of the dead neutrophils and other cellular debris. These events were followed by proliferation of fibroblasts, neovascularization and eventual maturation of fibroblasts and fibrocytes with collagen deposition. In addition to the findings of Joy and Jones, Khalifah and Post (1976) described encapsulation and occasional calcification in Pimephales promelas Rafinesque, Lepomis cyanellus Rafinesque and Catostomus commersoni (Lacepede) infected with L. cyprinacea. The penetration of parasites nearly parallel to the host surface resulting in a prolonged exposure to the integumental response was suggested as a contributing factor in the massive rejection of parasite L. cyprinacea on C. auratus (Shields and Goode 1978). Immunity was also considered by Shields and Goode as a factor contributing to the rejection of the parasites.

The involvement of an immune response in the rejection of the parasite needs further investigation as such

information might open up new ways of dealing with or controlling this parasite in aquaculture systems.

CHAPTER 2.

IDENTIFICATION AND DISTRIBUTION OF *LERNAEA* SPP. IN WEST MALAYSIA.

2.1. Introduction

Lernaea spp. has been present at the Freshwater Fisheries Research Institute, Malacca since 1960 (Anon. 1960). The author, during his extension services to the fish farmers, has also noted the presence of L. cyprinacea in many of the private fish farms. Lernaea piscinae Harding which was first reported by Harding (1950) was found in infected A. nobilis from Singapore (which was then part of Malaya). The occurrence of L. piscinae in A. nobilis was more recently reported by Shariff (1981) from the University Pertanian ponds at Serdang, Selangor. Incidental observations by the author suggested that Lernaea species was also being introduced into Malaysia with fish fry imported from Taiwan and Hong Kong. In view of the evidence that Lernaea has been reported to be responsible for serious losses to the aquaculture industry (Shilo et al. 1960, Bauer et al. 1969, Sarig 1971, Hoffman 1976, Kabata 1970,1979 and Djajadiredja et al. 1983) it would be useful to know the status of Lernaea spp., its distribution and its effects on the local aquaculture industry.

This study was thus conducted to survey the distribution of the parasite in West Malaysia, to identify the different species present and to attempt to trace the source of the infection.

2.2. Materials and Methods

2.2.1. Experimental design

The survey of the occurrence and species distribution

of Lernaea spp. involved the whole of West Malaysia and covered three different categories of ponds namely;

1) private ponds belonging to individual farmers.
2) ponds at the government breeding stations.
3) an importing agency which was centrally located and also the main importing agency in West Malaysia.

The farmers obtained their supply of fingerlings from both the importing agency and the government breeding stations.

2.2.2. Survey of the private fish ponds

One hundred and four out of the 13,852 privately-owned fish ponds from 10 different states were chosen for this study. All areas with aquaculture activities were represented and ponds were selected on a random basis from each area.

A. nobilis of 5-10 cm standard length were used to detect the presence of Lernaea in the fish ponds since preliminary observations revealed that this species was susceptible to both L. cyprinacea and L. piscinae. Fish known to be free of Lernaea infection, were obtained from the Freshwater Breeding Station in Malacca and were treated with formalin at 166 ppm for 30 minutes as a prophylatic measure to prevent the transmission of any disease to the study area) before they were used as test fish to detect the presence of Lernaea. The test fish were then transported in aerated plastic bags to the ponds selected for the study.

To avoid the problems associated with recovering the test fish from the ponds, they were introduced into cages (Fig. 2.1) measuring 106.6 cms long, 61 cms wide and 76 cms deep which were specially made for this study. In each of the selected ponds, 10 fish were released into the cages which were left to float in the ponds for a minimum of 4 days, during which time infection of the test fish would have taken place by the relatively advanced 5th copepodid stages or cyclopoid stage if they were present in the pond. Such stages would mature in 3-4 days (Chapter 3). This method of detecting Lernaea was adopted after successful preliminary trials in experimental ponds where Lernaea was known to be present. After 4 days the fish were removed and examined for the presence of adult Lernaea spp. The parasites were then removed by dissection and identified to species level based on the key prepared by Harding (1950) and the work of Poddubnaya (1973).

2.2.3. Survey of the Government breeding stations

The study involved all the 8 government-owned fish breeding stations in West Malaysia. Attempts to determine presence or absence of the parasite were made using the following sampling methods;

1) Introduction of 10 caged A. nobilis as test fish into 3 ponds as described above.

2) Examination of 5 brood stock and 20 fry each of C. carpio, Helostoma temmincki Bleeker,

Figure 2.1 The cage culture used for test fish (A. nobilis). The test fish were introduced into these cages and set afloat into the fish ponds to detect the absence or presence of Lernaea spp.

Tilapia spp., P. gonionotus, C.idella, A. nobilis and T. pectoralis which were already present in the ponds at the station.

The presence or absence of the parasite was recorded if it occurred on any one or all the three samples. The adult parasites obtained were identified to species level as described earlier.

2.2.4. Survey of the Importing agency

A. nobilis from 5 different consignments were examined from an importing agency in Kuala Lumpur. The fish were imported from the far East and the 5 different consignments arrived over a period of 4 months i.e. April - August 1982. On the arrival of each consignment 50 live fish were purchased and transported in oxygenated bags to the laboratory, where they were kept in aquarium tanks fitted with undergravel filters. The fish were examined for the adult parasite after being kept in the aquarium for 2 weeks. The longer duration was a precaution taken to allow for the possibility that the imported fish may have been infected with early larval stages of the parasite which would require a further 2 weeks to reach maturity (chapter 3).

2.2.5. Record of water quality and temperatures

The water temperature and pH of all the ponds were also recorded. The water temperatures were recorded by a minimum

and maximum thermometer and the pH measured with the HACH direct reading environmental laboratory kit.

2.3. Results

2.3.1. Private fish ponds

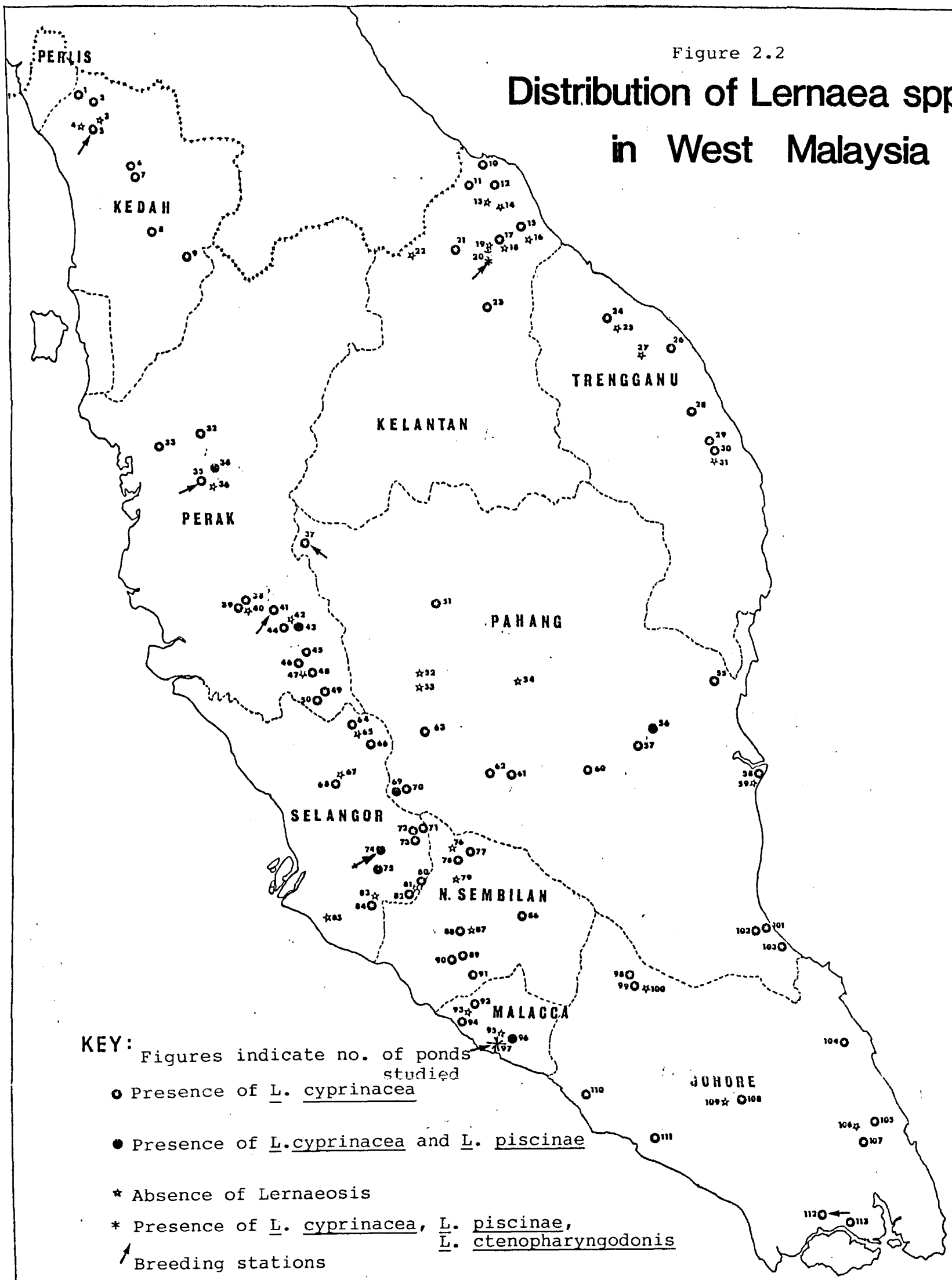
Figure 2.2 shows the 113 locations examined for the presence or absence of the parasite. The results of the survey revealed that Lernaea spp was present in all the 10 states where the study was conducted. Seventy two of the 104 private ponds studied (69.2%) revealed the presence of Lernaea (Table 2.1). L. piscinae was only found in 5 samples (4.8%) which came from Perak, Malacca, Pahang and Selangor and was always present together with L. cyprinacea "Asian" form. The remaining samples were identified as L. cyprinacea "Asian" form.

2.3.2. Government breeding stations

The results revealed that Lernaea was present in all the breeding stations studied. Infection by L. cyprinacea "Asian" form (Fig. 4.32) was present in C. carpio, A. nobilis, P. gonionotus, H. temmincki and O. gouramy (Table 2.2); L. piscinae (Fig. 4.33) was recorded from A. nobilis present at 2 stations i.e. at Bukit Tinggi and Batu Berendam. Lernaea ctenopharyngodonis Yin (Fig. 4.29) and Lernaea quadrinucifera Yin (Fig. 2.3) were only found on C. idella on 2 farms where the host was present i. e. at Malacca and Kelantan, Lernaea spp. was not found on Tilapia spp. or T. pectoralis.

Figure 2.2

Distribution of Lernaea spp. in West Malaysia



KEY:

Figures indicate no. of ponds studied

o Presence of L. cyprinacea

● Presence of L. cyprinacea and L. piscinae

☆ Absence of Lernaeosis

* Presence of L. cyprinacea, L. piscinae, L. ctenopharyngodonis

↗ Breeding stations

↗ Importing agency

✱ Presence of L. cyprinacea, L. ctenopharyngodonis L. quadrinucifera & L. piscinae

Table 2.1.

Results of the survey of the identification and distribution of *Lernaea* spp from private farms in West Malaysia.

States	No.of ponds studied	No. of ponds with		
		L.piscinae	*L.cyrinacea*	No. infection
Johore	12	0	9	3
Kedah	8	0	6	2
Kelantan	13	0	7	6
Malacca	5	1	3	2
Negeri Sembilan	10	0	7	3
Pahang	17	1	13	4
Perak	16	2	12	4
Selangor	15	1	10	5
Trenggannu	8	0	5	3
Total	104	5	72	32

Table 2.2.

Results of the examination of various host species for *Lernaea* spp. at the Government breeding stations in West Malaysia.

Station (State)	Host spp.	*Lernaea* spp.
Enggor (Perak)	*C. carpio*	*L. cyprinacea* "Asian" form
	T. pectoralis	-
	Tilapia. sp	-
	P. gonionotus	*L. cyprinacea* "Asian" form
Tapah (Perak)	*C. carpio*	*L. cyprinacea* "Asian" form
	P. gonionotus	*L. cyprinacea* "Asian" form
	H. temmincki	*L. cyprinacea* "Asian" form
	O. gouramy	*L. cyprinacea* "Asian" form
Tanah Rata (Pahang)	*C. carpio*	*L. cyprinacea* "Asian" form
Bukit Tinggi (Pahang)	*A. nobilis*	i) *L. cyprinacea* "Asian" form
		ii) *L. piscinae*
	P. gonionotus	*L. cyprinacea* "Asian" form
Jitra (Kedah)	*C. carpio*	*L. cyprinacea* "Asian" form
	P. gonionotus	*L. cyprinacea* "Asian" form
Kong-Kong (Johore)	*C. carpio*	*L. cyprinacea* "Asian" form
Batu Berendam (Malacca)	*A. nobilis*	i) *L. cyprinacea* "Asian" form
		ii) *L. piscinae*
	P. gonionotus	*L. cyprinacea* "Asian" form
	C. carpio	*L. cyprinacea* "Asian" form
	C. idella	i) *L. ctenopharyngodonis*
		ii) *L. quadrinucifera*
Machang (Kelantan)	*C. carpio*	*L. cyprinacea* "Asian" form
	C. idella	i) *L. ctenopharyngodonis*
		ii) *L. quadrinucifera*
	P. gonionotus	*L. cyprinacea* "Asian" form

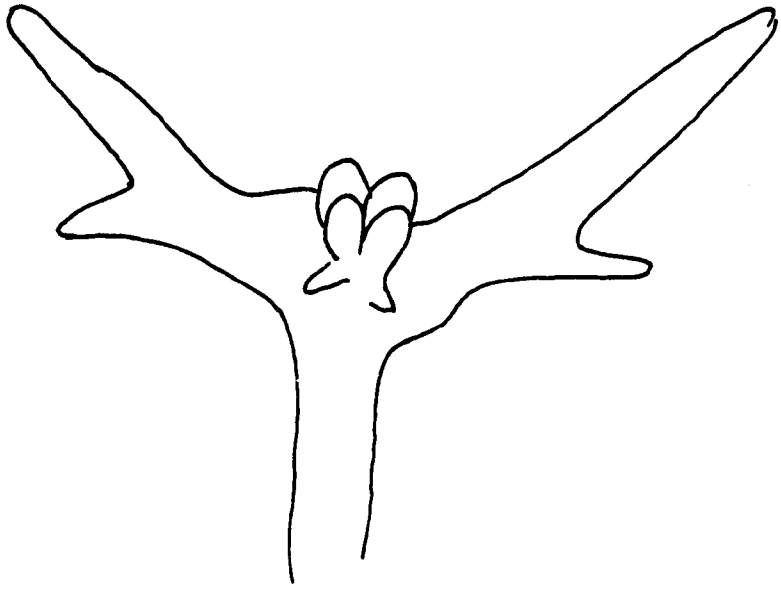


Figure 2.3 L. quadrinucifera from C. idella.
Note the 2 pairs of spherical lobes at the position of the ventral horns.

The brood stock at these stations revealed either the presence of the adult parasite on the body , or haemorrhagic lesions all over the body surface (Fig. 2.4). Examination of the fresh smears taken from gills and body surface of fish with these haemorrhagic lesions revealed the presence of the larval stages of Lernaea. In cases with severe infection, as many as 400 adult parasites were found on each individual fish (Fig. 2.5).

2.3.3. Importing agency

No adult parasites were seen on the newly purchased fish but by the 14th. day parasites were seen on the fish. Lernaea was present in 4 out of the 5 consignments (Table 2.3). The species identified were L. cyprinacea "Asian" form and L. piscinae. The average prevalence rate for the 5 consignments of L. piscinae was higher than L. cyprinacea which were 39.6% and 14.8% respectively. The mean intensity of infection was also higher for L. piscinae which was 1.81 parasites as compared to 1.47 for L. cyprinacea.

2.3.4. Water quality

The maximum and minimum diurnal water temperatures recorded from all the ponds studied were 23-32^{o}C, but lower temperatures of 17-24^{o}C were recorded from one breeding station which was sited on a hill. The pH of water ranged from 5.6-9.0. L. cyprinacea and L. piscinae were present in both extremes of pH (5.6-9.0) and from water temperatures of 23-32^{o}C . At the breeding station sited on a hill where

Figure 2.4 Haemorrhagic lesion over body surface of P. gonionotus

Figure 2.5 Lernaea infection in A. nobilis as many as 400 parasites were found in some fish.

Table 2.3.

Prevalence of L. cyprinacea "Asian" form and L. piscinae on A. nobilis obtained from the importing agency.

Consignment No.	No.of fish examined	Prevalence (%) of		Mean intensity	
		L. piscinae	L. cyprinacea	L. piscinae	L. cyprinacea.
1	50	52	16	1.68	1.37
2	50	28	14	2.00	2.50
3	50	60	4	1.60	1.00
4	50	58	40	3.78	2.50
5	50	0	0	0.00	0.00
Total	250	$\bar{X}$ 39.6	$\bar{X}$ 14.8	$\bar{X}$ 1.81	$\bar{X}$ 1.47

the lowest temperatures of 17-24°C, only L.cyprinacea was present.

2.4. Discussion

The distribution of the parasite Lernaea was common and wide spread in all the study areas. The source of the Lernaea found at the private fish farms could be traced to the breeding stations and the importing agency which are the sole suppliers of fish fingerlings to the farmers. There are no quarantine procedures to examine and ensure that the new stocks introduced into the breeding stations are free of the disease, nor are there any quarantine procedures for imported fish; similarly there are no measures for the examination and treatment of fish before they are supplied to the fish farmers. Thus the free flow of infected fish from the breeding stations and the importing agency has resulted in the wide spread of Lernaea in West Malaysia.

Although the study involved only one importing agency, there are more than 20 other known agencies which import fish from the Far East. Besides A. nobilis, C. idella which are cultured for food, various species of ornamental fish are also being imported. There is evidence that Lernaea spp. is also being brought into the country along with imported ornamental fish (Shariff 1980). Since many fish dealers handle both food fish and ornamental fish, this practice would further aggravate the spread of Lernaea amongst these fish.

During examination of the fish from the importing agency it was noted that no fish were seen to be infected with the adult parasite, but after maintaining the fish for two weeks the prevalence rate was found to be as high as 98%. Discussion with the personnel of the importing agency disclosed that fish infected with adult parasites are culled out before they are sold to customers. The agent is ignorant of the fact that the fish he sells are infected with the larval stages which are not visible to the naked eye. Thus the supposedly uninfected fish are habouring larval stages of the parasites and are being sold to unsuspecting farmers throughout the country. The culling of fish infested with the adult parasite before their distribution to fish farmers was also found to be a common practice at the breeding stations.

The study also revealed that L. cyprinacea "Asian" form, L. ctenopharyngodonis, L. quadrinucifera and L. piscinae were found in a wide range of pH, 5.6-9.0. This is considered to be a significant finding since Hoffman (1976) had reported that Lernaea has not been recorded from waters with pH lower than 7. Besides pH, Lernaea was also noted to be present at a wide range of water temperatures, that is 17-33^{o}C. According to Shields and Tidd (1968) the most successful laboratory cultures, L. cyprinacea were obtained at fluctuating temperatures of 24^{o}C to 29^{o}C and these temperatures are closely similar to the normal diurnal temperatures in West Malaysia. Thus, the tropical climate of Malaysia which experiences no seasonal temperature

changes could be considered favourable for the development of Lernaea throughout the year. In temperate climate L. elegans (Hoffman 1976), and L. cyprinacea (Bauer et al. 1969) are known to overwinter.

The results of the study showed the presence of 4 species of Lernaea; i.e. L. cyprinacea "Asian" form, L. piscinae, L. ctenopharyngodonis and L. quadrinucifera. A. nobilis was susceptible to L. piscinae while L. ctenopharyngodonis and L. quadrinucifera were restricted to C. idella amongst the host listed in Table 2.3. This host specificity could be one of the reasons for the lower number of private fish ponds (4.8%) found to be infected with L. piscinae; in contrast to L. cyprinacea which was found to be more common (69.2%) and had a wide range of host susceptibility. The wide range of host susceptibility of L. cyprinacea has also been reported by Hoffman (1967), Tidd (1934), Uzman and Rayner (1958), Fryer (1961a) Demaree (1967), Fratello and Sabatini (1972), Poddubnaya (1978), Kabata (1979) and Shariff et al. (in press). The wide spread occurrence of L. cyprinacea is also attributed to its ability to live on many unrelated fish hosts, as well as tadpoles, adult frogs and salamander and thus L. cyprinacea has been recorded to occur in Europe, Africa, India, South East Asia, Far East Asia, North and South America (Kabata 1979). Besides the broad range of host susceptibility of L. cyprinacea, the wide range of pH and temperature tolerance as noted are probably important factors that have enabled the parasite to be distributed world wide.

Although C. idella was cultured at 2 breeding stations and at all the private fish farms, L. ctenopharyngodonis and L. quadrinucifera were found only in the breeding stations. Failure to detect L. ctenopharyngodonis and L. quadrinucifera at the private farms could possibly be due to the use of different techniques of detection of Lernaea in the study areas. At the breeding stations all host species present were caught and examined, whereas at the private farms, only A. nobilis were introduced into cages as test fish. It is possible that A. nobilis is not susceptible to L. ctenopharyngodonis, since it has only been recorded from C. idella. Thus the failure to detect L. ctenopharyngodonis and L. quadrinucifera at the private farms does not indicates its absence. Acccording to Poddubnaya (1973) L. ctenopharyngodonis, L. quadrinucifera and Asian L. cyprinacea which is identical to L. elegans, belong to one polymorphic species. She was able to transmutate L. elegans (L. cyprinacea "Asian" form) into L. ctenopharyngodonis and vice versa. The relationship between L. cyprinacea "Asian" form L. quadrinucifera and L. ctenopharyngodonis has been examined and is presented in Chapter 4.

Lernaea polymorpha Yu (=L. piscinae, viz Chapter 4) which was noted to be host specific has been previously recorded in China on A. nobilis and Hypophthalmichthys molitrix (Cuvier and Valenciennes) (Yin et al. 1963). Restricted host specificity and distribution as shown in L. piscinae has also been recorded for L. palati which infects only Haplochromis chrysonotus (Boulenger) and has been

recorded to be present only in Lake Nyasa in Africa (Fryer 1968).

Heckmann and Farley (1973) reported the occurrence of L. piscinae in California from Hesperoleucus symmetricus symmetricus (Biard and Girard), but no description, illustration or measurements of the parasite were given. The possibility of its occurrence in a continent distant from the Far East (where it was first described) is unaccountable; for it has been reported to be found on a wild fish and as yet there has been no report of its presence in cultured fish. The only possible means of its occurrence in the new region could have been via transfaunation of susceptible host eg. A. nobilis or H. molitrix (which are cultured species) from the Far East, but in this case, it is likely that it would have first been detected on cultured fish such as those mentioned earlier. Neither are there any records of the release of the susceptible host into the rivers in California. The present findings are consistent with the suggestion of Kabata (1979) that the authors' identification is rather doubtful.

Kabata (1979) in his review of the geographic distribution of L. piscinae Harding 1950 has referred to it as an African form whereas the specimens of L. piscinae were actually obtained by Harding from Singapore situated in South East Asia. The distribution of L. piscinae therefore still appears to be limited to the Far East and its neighbouring region of South East Asia.

Poddubnaya (1978) suggested that the distribution of Lernaea was widespread since the sea was unified and subsequently with the geological processes, it caused the disappearance of old warm water fauna in the moderate latitudes resulting in its survival in South East Asia. Poddubnaya believes that L. esocina, L. elegans, L. cyprinacea "European" form and L. parasiluri were redistributed from this region. Although Malaysia is in South East Asia, the present study failed to reveal the presence of L. parasiluri and L. esocina in the farms and neither has Lernaea spp. so far been detected from fish in the wild (personal communication Dr. Mohsin and Susan Lim). Thus Malaysia may not have been in the geographical location where Lernaea spp. may have survived during the geological processes.

To what extent Lernaea has spread into the natural water bodies in West Malaysia is unknown considering the limited knowledge of Peninsular Malaysia parasitic fauna. It is possible that L. cyprinacea with its wide host range and pH and temperature tolerance may have reached the fish in the rivers and other water bodies with the effluent water from the fish ponds containing the parasitic larvae. However the spread of the disease to the natural water bodies may yet be restricted to limited areas around the fish farms present.

Mortality rates ranging from 42-100% due to lernaeosis have been recorded at the importing agency (per. communication Ban Lee). The average mortality rates due to

lernaeosis at the breeding stations for the period 1981 and 1982 have been reported to be 55.6% (Shariff and Vijiarungam in press). However at the private farms the effects of the disease may not be so obvious. Most of the private farms practise aquaculture on extensive scales and in these cases the management is at a lower level than in the intensive systems, thus records are not kept enough to assess the mortality rates.

Factors such as weight loss or slow growth rates caused by lernaeosis could reduce the income of the fish farmer. To date there has been no evidence available to elucidate the extent of the infection on growth performance. Even with a low burden of Lernaea infection, signs of stress reaction indicated by an increase in oxygen consumption has been reported by Srinivasachar and Shakuntala (1975) and thus even under natural conditions, low oxygen levels could be detrimental to the fish. Besides such effects, there is also a danger that the parasite may spread to the natural water bodies on a large scale due to the further development of aquaculture and the disease may become a threat to the natural fish population. Losses as a result of importation of L. cyprinacea and its subsequent distribution to the fish farms have also been documented in Indonesia. L. cyprinacea which was accidently introduced into Indonesia from Japan in 1953, destroyed 30% of the fish in its central hatcheries of North Sumatra, Java and North Sulawesi (Djajadiredja et al. 1983, Koesoemadinata 1979). It was estimated that in Java alone a total of 1.4 billion fish fry were lost during the

epizootic and according to Rukyani (1975) there are still more than 25% of the fish ponds in West Java heavily infected with Lernaea. Mortalities due to Lernaea infection has also caused much economic loss in Colorado after the transfer of a single unfortunate consignment of infested trout from Idaho. Besides the mortality, some of the fish have been unsalable and attempts to eliminate the parasite has also failed (Post 1983).

CHAPTER 3.

STUDIES ON THE LIFE CYCLE OF *LERNAEA PISCINAE* AND *LERNAEA CYPRINACEA*.

3.1. Introduction

Of the two Lernaea spp. identified in West Malaysia, only the life cycle of L. cyprinacea has been studied. The life cycle of L. cyprinacea has been described by Wilson (1918) and Shields (1976) from the United States, Yashouv (1959), Lahav and Sarig (1964) from Israel, Grabda (1963) from Poland, Bauer et al. (1969) from Russia, Al-Hamed and Hermiz (1973) from Egypt, and Rukyani (1975) from Indonesia. The life cycle of the less commonly known parasite L. piscinae has not been established to date.

One of the aims of the present study was to establish the life cycle of L. piscinae and to compare various aspects of its life history with that of L. cyprinacea. As well as morphological adaptation, many parasites exhibit physiological adaptations and it was considered that these might be reflected in the duration of the different stages of the life cycle at certain temperatures. Such information obtained might be useful in identifying larval forms to species level in the absence of adult stages or where they cannot be separated morphologically.

3.2. Materials and methods

3.2.1. Stock culture of L. cyprinacea and L. piscinae

3.2.1.1. Source of Lernaea

A constant supply of the parasites L. piscinae and L. cyprinacea was maintained at the Faculty of Fisheries

aquarium for the study. The original material was obtained from the university ponds where infected A. nobilis were collected. The parasites were identified to species level based on the key prepared by Harding (1950).

3.2.1.2. Establishment and maintenance of stock culture

L. piscinae was maintained on A. nobilis whilst L. cyprinacea, which has a wider range of host susceptibility was maintained on C. auratus. The choice of C. auratus as a host for L. cyprinacea was made because this fish is more hardy and therefore easier to maintain in the aquarium system than A. nobilis.

Before the initial infection, 30 fish of each species were examined and treated with 166 ppm formalin for 30 minutes to ensure that they were free of ectoparasitic infection. The treated fish were then closely examined visually for the presence of adult and larvae Lernaea and introduced into two separate glass tanks measuring 107 cms X 46 cms X 46 cms which were fitted with undergravel filtration systems and contained 200 litres of rain water where they were held in quarantine for a minimum period of 15 days. C. auratus were fed daily with freeze dried tubifex worms. A. nobilis is a filter feeder and these were fed with a mixture of ground, freeze dried tubifex worms mixed with wheat flour in equal proportions.

Eggs of L. piscinae and L. cyprinacea were removed from adult female parasites and hatched in groups of 30 in

separate petri dishes containing rain water. On hatching the naupliar stages were released into the tanks containing their respective hosts. Thirty fish were kept in each tank to maintain a constant culture of both species of the parasite. Dead fish were removed and replaced by new fish which were always treated as previously described to ensure they are free of Lernaea and other ectoparasites before introduction. Fish which recovered from the infection and were suspected of becoming immune to the infection (Shields 1976, Shariff 1981) were also removed and replaced with naive fish.

3.2.2. Experimental studies on the life cycle.

3.2.2.1. Experimental design

The experiment was designed to study the life cycle of L. piscinae on A. nobilis and of L. cyprinacea on C. auratus, H. temmincki and A. nobilis. Thirty fish of each species were used and the standard length of the fish was as follows; C. auratus 10-18 cms, H. temmincki 11.5-18 cms, and two batches of A. nobilis measuring 10-15 cms. The fish were examined, treated with 166 ppm formalin and quarantined as previously described to ensure that they are free of Lernaea and other ectoparasitic infection. Each group of fish was then introduced into glass tanks of 107 cms x 46 cms x 46 cms fitted with undergravel filt ration systems.

3.2.2.2. Collection and hatching eggs

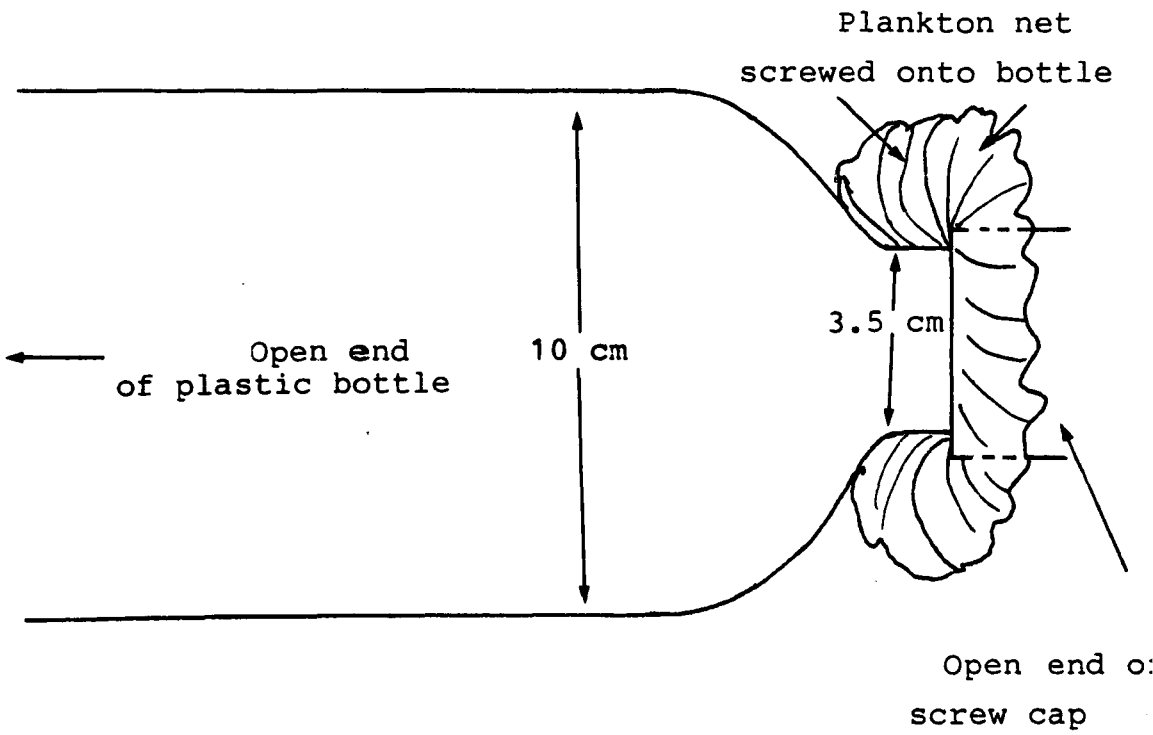
Eggs of L. piscinae and L. cyprinacea were

obtained from the stock culture. To ensure that the egg sacs used for the study of the life cycle of the parasite were of the same age group, the development of the eggs was synchronized by removing the second batch of egg sacs from all the parasites simultaneously as suggested by Shields (1976). Eggs were collected from the 3rd. batch of eggs of each parasite species and placed into petri dishes containing seasoned rain water. Thirty egg sacs were collected to establish each host-parasite system. Thirty egg sacs of L. piscinae were placed into one petri dish while a further 3 batches of 30 eggs sacs of L. cyprinacea were distributed into three petri dishes. The eggs were observed constantly and the hatching time recorded.

3.2.2.3. Establishment of *in vitro* and *in vivo* studies

When the majority of the eggs were hatched, the nauplii of L. piscinae were introduced into the tank containing A. nobilis. L. cyprinacea nauplii were introduced into the remaining three tanks containing H. temmincki, C. auratus and the second batch of A. nobilis. As described earlier each tank contained 30 fish.

Larvae of both parasite species hatched from 5 egg sacs were also kept in separate petri dishes to study the developmental stages of the parasite without a host. The petri dishes were left uncovered and kept in the aquarium shed along with the fish tanks. About 1/2 of the water in the petri dishes was removed with a Pasteur pipette daily and replaced with rain water.



Side view

Top view

Figure 3.1 Modified plastic bottle for sampling larval stages of Lernaea spp.

3.2.2.4. Collection of larval stages

To study the development of the parasite on the fish in the fish tanks, samples were taken twice daily, at 9 am. and 4 pm. for convenience. Collection of the larvae was made with a special bottle designed for the purpose (Fig. 3.1). The bottle was drawn gently across the fish tank to collect free swimming stages of the parasite. With the progressive development of the larval stages it became more difficult to obtain the copepodid by the above method. The copepodid stage normally attaches temporarily to the host for feeding (Kabata 1970) and were best collected by dipping the fish in 10% formalin and collected the released larvae. The larvae in the petri dishes were collected with a Pasteur pipette. The samples collected were kept in 10% formalin and later mounted on slides in formalin and examined under a microscope. The development of the preadult stage which was partially embedded in the host tissue was made by dissecting the parasite from the tissue. Four replicates of the experiment were conducted for each treatment.

The maximum and minimum diurnal water temperatures were recorded daily.

3.3. Results

The results of the experimental trials are presented in Tables 3.1, 3.2, 3.3 and 3.4. It can be seen from the tables that there were no differences between the duration of the various stages of the life cycle of *L. piscinae* and

L. cyprinacea obtained from their respective hosts used in this study. Hence, the life cycle of the two species will be described together.

The entire development of the parasite from hatching to adult took an average of 14-15 days at a mean temperature of 27°C. The daily mean temperature for this period was not very meaningful since the ambient temperatures fluctuated markedly on a diurnal basis. The diurnal temperature recorded during the study period was 24.3°C to 29°C.

There were three naupliar stages, nauplius I, II and nauplius III (Nauplius III is also known as metanauplius), and five copepodid stages, copepodid I-V. The copepodid female developed to the cyclopoid stage (pre-metamorphosis female) which embedded in host tissue and finally metamorphosed to the sedentary female parasite. The male cyclopoid died after 24 hours. The detailed morphological structure of the different stages has been described in Chapter 4.

Each nauplius stage lasted for only 24 hours, so that nauplius III or metanauplius was observed on the third day. The successive development of copepodid I to copepodid V took a further 7 days. Each copepodid stage lasted an average of 2 days.

The cyclopoid was obtained on the 12th or 13th day. At this stage the female parasites were seen to be firmly attached to the host tissue. The females underwent metamorphosis to form the young sedentary parasite within 12

Table 3.1

Development of L. cyprinacea on A. nobilis.

No. of days	Replicates A	B	C	D	Developmental stages
1	I	I	I	I	
2	II	II	II	II	Nauplius
3	III	III	III	III	
4	I	I	I	I	
5	I	I	I	I	
6	II	II	II	I & II	
7	II	III & II	III	II	Copepodid
8	III	III	IV	III	
9	III	III	IV & V	IV	
10	IV	IV & V	V	V	
11	V	V	V	V	
12	V	V & cyp.	V & cyp.	V	
13	cyp.	cyp.	cyp.	V & cyp.	Cyclopoid
14	cyp. & young female	cyp. & young female	cyp. & young female	cyp. & young female	(Premetamorphosis female and adult male)
15	young female	young female	young female	young female	(Postmetamorphosis female)

Table 3.2

Development of L. cyprinacea on C. auratus.

No. of Days	Replicates				Development stages
	A	B	C	D	
1	I	I	I	I	
2	II	II	II	II	Nauplius
3	III	III	III	III	
4	I	I	I	I	
5	I	I	I	I	
6	II	II	II	I & II	
7	III	II	II	II	
8	IV	III	III	III	Copepodid
9	IV & V	IV	IV	IV	
10	V	V	V	IV & V	
11	V	V	V	V	
12	V & cyp.	V & cyp.	V & cyp.	V	
13	cyp.	cyp.	cyp.	cyp.	Cyclopoid
14	young female	cyp. & young female	cyp. & young female	cyp. & young female	(Premetamorphosis female and adult male
15	young female	young female	young female	young female	(Postmetamorphosis female)

Table 3.3

Development of *L. cyprinacea* on *H. temmincki*

No. of days	Replicates				Development stages
	A	B	C	D	
1	I	I	I	I	Nauplius
2	II	II	II	II	
3	III	III	III	III	
4	I	I	I	I	Copepodid
5	I	I	I	I	
6	I & II	I & II	II	II	
7	II	II	II	III	
8	III	III	III	IV	
9	IV	IV	IV	V	
10	IV & V	IV & V	V	V	
11	V	V	V	V	
12	V	V	V & cyp.	V & cyp.	Cyclopoid (Premetamorphosis female and adult male)
13	cyp.	cyp.	cyp.	cyp.	
14	cyp. & young female	cyp. & young female	young female	young female	
15	young female	young female	young female	young female	(Postmetamorphosis female)

Table 3.4

Development of L. piscinae on A. nobilis.

No. of days	Replicates A	B	C	D	Development stages
1	I	I	I	I	Nauplius
2	II	II	II	II	
3	III	III	III	III	
4	I	I	I	I	copepodid
5	I	I	I	I	
6	I	II	II	I	
7	II	III	III	II	
8	III	III	III	III	
9	III	III	III	IV	
10	III & IV	III & IV	IV	IV	
11	V	V	V	V	
12	V & cyp.	V	V & cyp.	cyp.	cyclopoid (Premetamorphosis female and adult male)
13	cyp.	cyp.	cyp.	cyp.	
14	cyp. & young female	cyp. & & young female	young female	young female	
15	young female	young female	young female	young female	(Postmetamorphosis female)

hours. The young parasites were thin and transparent and difficult to observe without the aid of a microscope or a magnifying lens. A pair of small milky white egg sacs was seen on the recently attached parasite. The milky white eggs gradually turned to a green colour within 24 hours. At this stage the embryo was fully developed. After the formation of the embryo the eggs hatched within the next 12 hours. A second batch of eggs was seen within 12-24 hours after the first batch of eggs had hatched.

The results of the findings for the development of parasite *in vitro* are shown in Table 3.5 and 3.6. The eggs which hatched in the petri dishes without a host developed into the 3rd. nauplius stages and the 1st. copepodid stage within the same time period as described for the larval stages present on host (Table 3.1, 3.2, 3.3 and 3.4). No parasites were seen to develop further than the copepodid I stage in the absence of a host and died on the seventh day after hatching that is on the 3rd. day of the 1st. copepodid stage.

3.4. Discussion

The life cycle of both *L. cyprinacea* and *L. piscinae* were successfully completed in the experimental systems used. The life cycle of *L. cyprinacea* and *L. piscinae* were identical at mean temperature of 27°C. The need for an intermediate host species to complete the life cycle of *Lernaea* was initially reported by Wilson (1917). This was further indicated by Fryer (1961 a)when he found that the

Table 3.5

Development of *L. cyprinacea in vitro*.

No. of days	Replicates				Development stages
	A	B	C	D	
1	I	I	I	I	Nauplius
2	II	II	II	II	
3	III	III	III	III	
4	I	I	I	I	Copepodid
5	I	I	I	I	
6	Died	Died	Died	Died	

Table 3.6

Development of *L. piscinae in vitro*.

No. of days	Replicates				Development stages
	A	B	C	D	
1	I	I	I	I	Nauplius
2	II	II	II	II	
3	III	III	III	III	
4	I	I	I	I	Copepodid
5	I	I	I	I	
6	Died	Died	Died	Died	

copepodid stages of L. cyprinacea infected B. docmac which served as an intermediate host, before they infected Tilapia. Similarly, Thurston (1969) reported that L. barnimiana lived on Tilapia and Haplochromis as an adult and required Bagrus as an intermediate host in the life cycle. These reports were from studies involved fish in their natural habitat. However in the present study when only one host species was used in each individual tank to complete the life cycle of L. cyprinacea and L. piscinae, indicating that the use of the intermediate host is flexible and reveals the adaptabilty of the parasites. The use of one host species to complete the life cycle of L. cyprinacea under laboratory conditions has also been reported by Grabda (1963) and Shields (1976).

The result of the study on the development of the nauplius to cyclopoid stage agrees closely with that of Yashouv (1959) and Shields (1976). At 26-29.5°C Yashouv reported that development of L. cyprinacea from nauplius to cyclopoid stages is accomplished in 12-16 days and according to Shields at 25-32°C it takes between 11-15 days; which is closely related to the present study whereby at 24.3-29°C it took 12-15 days.

The result shows that, to complete the life cycle, that is from hatching of the egg to the emergence of the adult parasite with its eggs ready to hatch, took 16-17 days at 24.3-29°C. According to Lahav and Sarig (1964) and Shields (1976), the life cycle of L. cyprinacea takes 18-21 days at 25°C and 18-25 days at 25-32°C respectively, and Bauer

et al. (1969) has reported that at 20°C it takes 25 days, at 30°C in 16 1/2 days and Rukyani (1975) states that at 28°C it takes 21-23 days. The development rate is clearly sensitive to temperature but variation within the reported range 25-32°C is as great as 8 days. The large variation in the number of days it takes to complete the life cycle at closely related temperatures could be due to the differences in the mean and the durations of maximum and minimum temperatures at which the studies were conducted.

The findings for the life cycle of L. cyprinacea, of the present study is similar to those of other workers and Thurston (1969) found that L. barnimiana required 15 days to develop to cyclopoid stages at 21-26°C. This also closely resembles the findings for L. cyprinacea and L. piscinae in the present study, indicating an identical pattern of development within the genus Lernaea.

The in vitro study showed that in the absence of a host, copepodid I dies on the 3rd. day without undergoing further development. This is in agreement with Shields and Tidd (1968) although their experiment was conducted at 28°C. as compared to a wider range of temperatures of 24.3-29°C. recorded during the present study. The inability of the copepodid I to survive and moult indicates that it is an infective stage and is incapabable of surviving without a host. This information suggest the possibility that a satisfactory elimination of Lernaea from an infected site is possible by leaving the pond fallow for a minimum period of 7 days until all the infective stages have died.

The similarity in the life cycle of L. piscinae and L. cyprinacea makes it easier to treat the disease. It would not be necessary to identify the parasite to species level before preparing the treatment schedules since the similar life cycle would require a similar schedule for both L. cyprinacea and L. piscinae.

The treatment of the parasite with organophosphate compounds is not effective at the free swimming naupliar stages (Kabata 1970, Shariff et al. in press) or for the adult parasites which are embedded in the host tissue. The treatment schedule would therefore be directed to kill the free swimming copepodid stages. At mean temperatures of 27°C the copepods take 8-9 days before they develop into the cyclopoid stage and it would therefore be necessary to schedule the treatment before the 8th day. Thus, to ensure a successful treatment it would be advisable to introduce the drug at 7 days interval (providing a safety margin of one day before the parasite embeds in the host tissue), until the infection has cleared.

CHAPTER 4.

COMPARATIVE STUDY ON THE MORPHOLOGY AND MORPHOMETRICS OF *L. PISCINAE* AND *L. CYPRINACEA*.

4.1. Introduction

The identification of Lernaea is based on the shape of the cephalic horns, also known as the "anchor", and its processes. Harding (1950) and Fryer (1961b)reported that the shape of the attachment apparatus (anchor) of Lernaea is very variable even within species and its growth and orientation is affected by its encounter with bones and other hard tissue, during its growth within the host. Inspite of the variability of the anchor structure, both authors agreed that the structure of the anchor is a characteristic form for each species and still serves as a useful taxonomic character.

However the task of the taxonomist has been made more difficult since the publication of the results of the work of Poddubnaya in 1973 . Rearing larvae from the same batch of eggs and using them to infect different hosts, Poddubnaya was able to produce 3 different species of Lernaea from the offspring of one species, L. elegans.

The precise arrangement of the setae and spines in the adult parasite was expressed by Wilson (1917) as a character that could be used to distinguish the different species. However, Harding (1950) did not find any extraordinary differences between the setae and spines of the appendages of the adult parasites of different species.

The torsion of the body of the adult parasite also known as the flexion of the trunk, is acquired during the process of burrowing into the fish host (Wilson 1917).

This was indicated by Quidor (1913) to be constant for a given species. However, Wilson (1917, 1918), Leigh-Sharpe (1925) and Gnanamuthu (1951) dismissed the possibility that the torsion of the body could be used as a valid character since their findings on this character showed it to be inconsistent.

Fryer (1961b)examined the form of the pregenital prominence but found it unreliable since he found 2 or more specimens from the same host fish, Varcorhinus damascinus (Cuvier and Valenciennes) both with a single pregenital prominence but one bore a shallow cleft and the other a double and deep cleft.

Fratello and Sabatinis (1972) (cited by Kabata 1981) examined the chromosomes of Lernaea from C. carpio, C. auratus, Lepomis gibbosus (Linnaeus) and Gambusia affinis (Baird and Girard). They found the chromosomes to be identical in all specimens and concluded that these hosts harbour the same species of Lernaea. However, Kabata cautioned the study of chromosome number as a criterion for the identification of Lernaea and stated that some fish species, even belonging to different genera, can have identical sets of chromosomes.

When investigations revealed the presence of Lernaea species in Malaysia, it was felt that the taxonomic confusion which has developed for the identification of Lernaea warranted detailed studies on the Lernaea species present in Malaysia. The study was aimed to examine the

morphology and biometrics of the parasite and attempt to establish a more reliable method of identification for distinguishing between L. piscinae and L. cyprinacea.

In order to carry out the study it was felt necessary to:

a. Compare the morphological characters of the larval and adult stages of L. piscinae and L. cyprinacea.
b. Compare the morphometrics of the larval and adult stages of L. piscinae and L. cyprinacea.
c. Make a comparison of the results obtained with other species described so far and attempt to establish a more reliable method of identification.

4.2. Materials and methods

4.2.1. Larval stages

4.2.1.1. Experimental design

Three host-parasite systems were established to study the morphometrics of the larval stages. These host-parasite systems were;

1) L. cyprinacea infection of A. nobilis
2) L. cyprinacea infection of C. auratus
3) L. piscinae infection of A. nobilis.

Eighty A. nobilis of 10-15cms and 40 C. auratus of 41-85 mm in standard length were used for this experiment. The fish ~~which~~ were purchased from a local dealer and treated for ectoparasites with formalin at 166 ppm for 30 minutes. The

fish were also examined carefully to ensure that they were not infected with Lernaea sp. After treatment, the A. nobilis were divided into 2 groups of 40 each and placed into 2 glass tanks each measuring 106.7 x 45.7 x 45.7 cms., the C. auratus were introduced into a 3rd. tank of similar dimensions. All the tanks were fitted with under gravel filters. The C. auratus were fed daily with freeze dried tubifex worms while the A. nobilis were fed with a mixture of ground freeze dried tubifex worms and wheat flour mixed in equal proportions. The fish were acclimated to the system for a week before they were infected with Lernaea.

4.2.1.2. Introduction of infection

To ensure that the parasite egg obtained from the stock culture were of the same age group, they were collected from the 2nd. batch of egg sacs as described earlier in chapter 2. The eggs in batches of 30 were placed into petri dishes containing seasoned water and allowed to hatch. Each host parasite system was established by introducing larvae hatched from 30 egg sacs into tanks which consisted of 40 fish each. The larvae of L. piscinae were introduced into the tanks containing A. nobilis, larvae of L. cyprinacea were introduced into the tank containing C. auratus and into the tank with a second group of A. nobilis.

4.2.1.3. Collection, identification and measurements of the larval stages

Sampling of the larvae from the fish tank was carried out as previously described in chapter 3. A minimum of 20

larvae were collected at each sampling. The larvae were kept in labelled bottles filled with 10% formalin. Subsequently the larval stages were identified and drawings of each stage were made with a camera lucida. Twenty larvae of each stage from each host-parasite system were measured under a calib rated microscope for the following:

a) Total length
b) Body length
c) Furcae length
d) Cephalothorax length and width
e) Genital segment length and width
f) Length of antennulae

The above characters are also shown in Figure 4.1. The numbers of bristles and spines of the antennulae were also counted. To avoid confusion in the terminology of the characters of larval stages, reference was made to Grabda's work of 1963 as this was considered to be the most comprehesive study on Lernaea

4.2.1.4. Morphometrics.

One way analysis of variance and least significant difference tests were used to determine the variability present amongst the measurements of the larval stages from the different host parasite systems. The results of the analysis of variance were expressed at 95% confidence level.

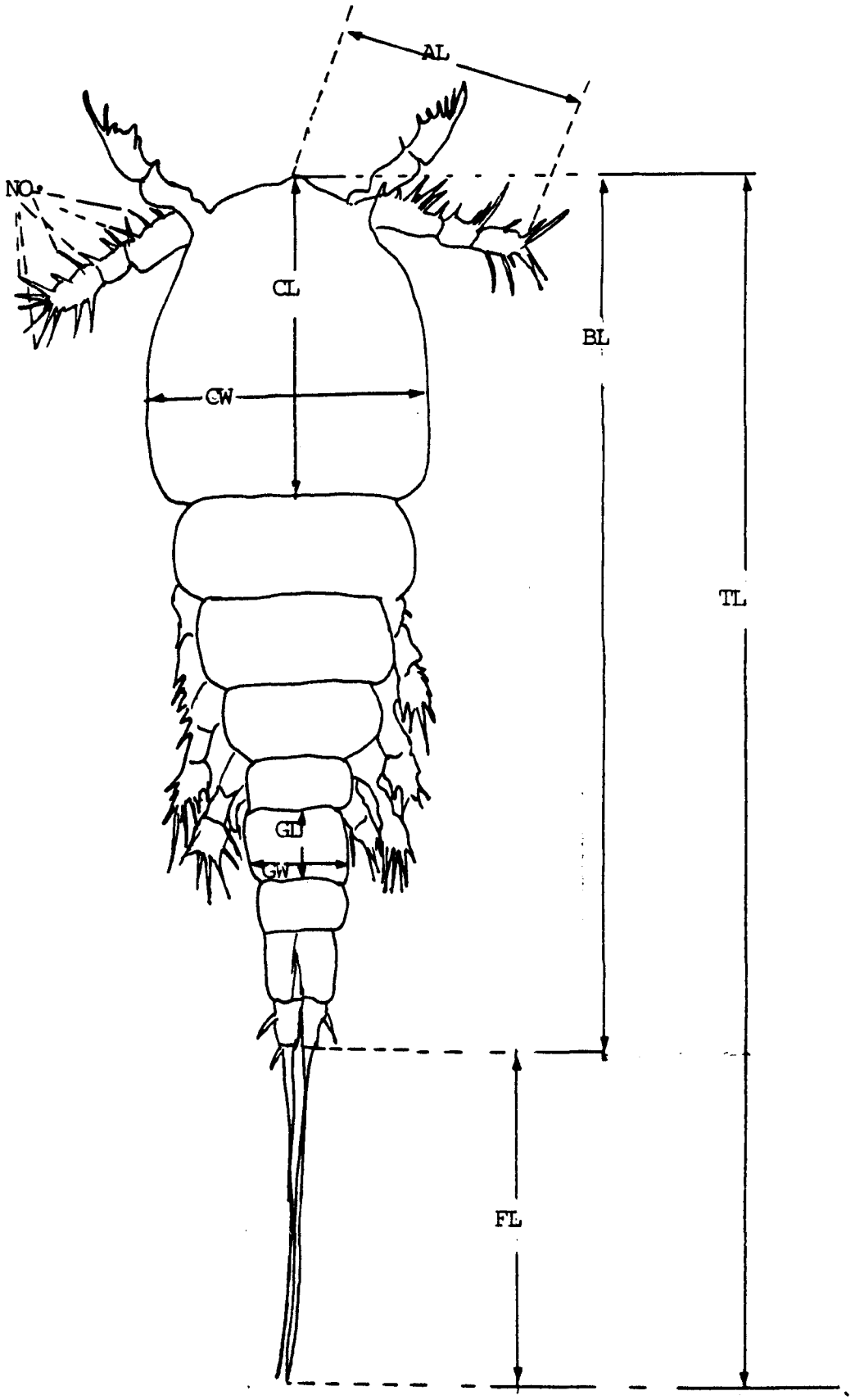
4.2.2. Adult female parasites

4.2.2.1. Experimental design

To study the morphological characteristics and the morphometrics of the adult parasites the 3 host parasites systems described earlier were used for this experiment. The fish were of the same stock and were treated for

Figure 4.1 Body parameters of larval stage of Lernaea sp.

AL - Antennulae length
BL - Body length
TL - Total length
CL - Cephalothorax length
CW - Cephalothorax width
GL - Genital segment length
GW - Genital segment width
NO - Number of spines and bristles of antennulae
FL - Furcae length.


AL
NO
CL
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GD
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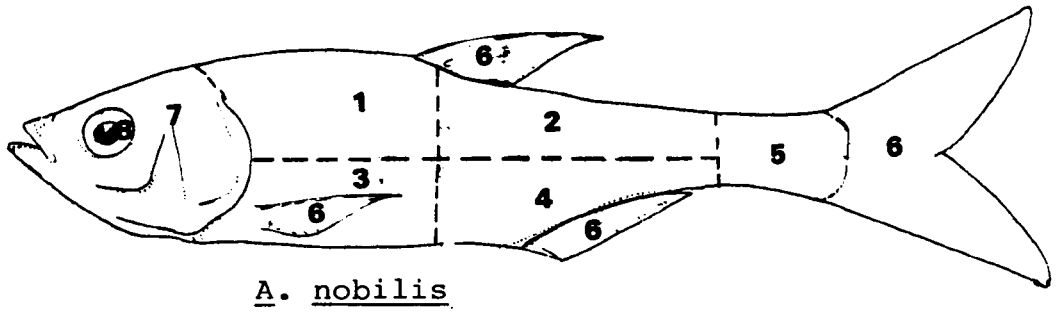
ectoparasites as described above. A large number of fish were used in this experiment and were maintained in groups of 40 making a total of 520 C. auratus and 1,040 A. nobilis. The 3 host parasites systems were established by introducing larvae hatched from 30 egg sacs into the tanks containing 40 fish each. The C. auratus were infected with L. cyprinacea and half the A. nobilis were infected with L. cyprinacea while the remaining half were infected with L. piscinae.

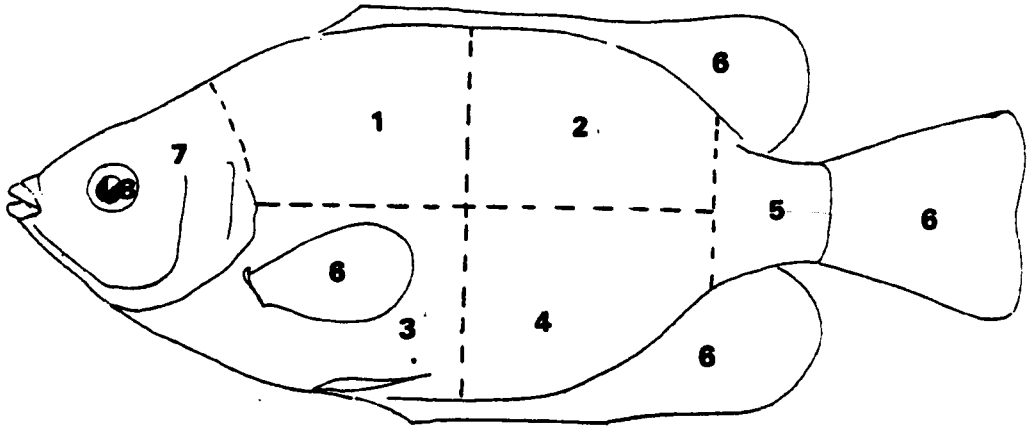
The fish were observed daily for the adult parasite to appear and after 4 days the fish were sacrificed by pithing. To assess the effect of the site-induced variation on the morphology of the parasite, the fish body was divided into 8 specific regions. The areas designated were as follows, region 1-4, body proper, 5 caudal peduncle, 6 fins, 7 head, 8 eyes (Fig. 4.2).

4.2.2.2. Removal of parasites from host tissue

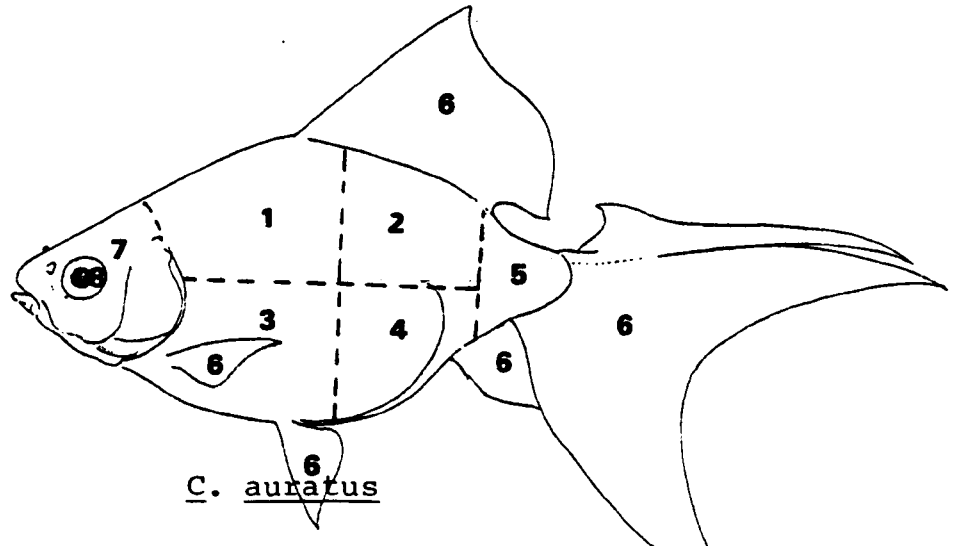
The parasites were removed from the tissue using a technique based on that described by Harding (1950). The tissue with the parasite embedded in it were removed by cutting, and these sections were placed into petri dishes containing potassium hydroxide to allow the tissue to soften so that the parasite could be removed easily without damage. After 12-24 hours the parasites were removed from the tissue with fine forceps and placed into labelled bottles filled with 10% formalin.



A. nobilis



H. temmincki



C. auratus

Figure 4.2 Demarkation of the different regions of the host species for comparative study on their effects on the morphology of Lernaea. Regions 1, 2, 3 and 4 - body proper, 5 - caudal peduncle, 6 - fins, 7 - head and 8 - eyes.

4.2.2.3. Sampling technique and measurements of the adult parasite.

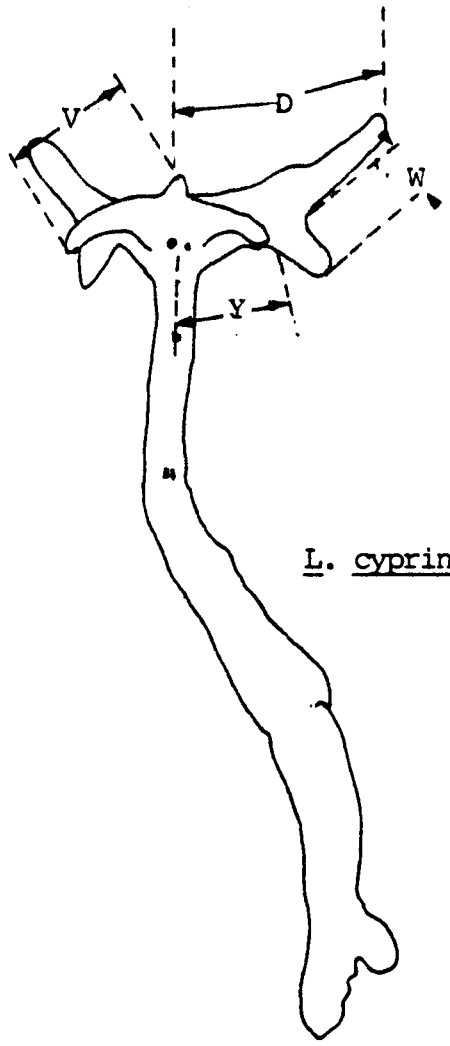
Since the morphology of the adult parasites has been reported to vary when they encounter mechanical obstruction such as cartilage or bony tissue (Fryer 1961b) the various body parts of the parasites obtained from the specific regions of the host (Fig. 4.2), which possibly may present different problems of attachment and anchorage of the parasite in the host were examined. Ten parasites were collected from each region of both the left and right sides of the host. The parasites collected from these regions were measured for the following:-

a) Length of dorsal horn
b) Distance between mid-body of parasite and the ventral process of dorsal horn
c) Length of the process of the dorsal horn
d) Length of the ventral horn
e) Position of swimming legs
f) Total length

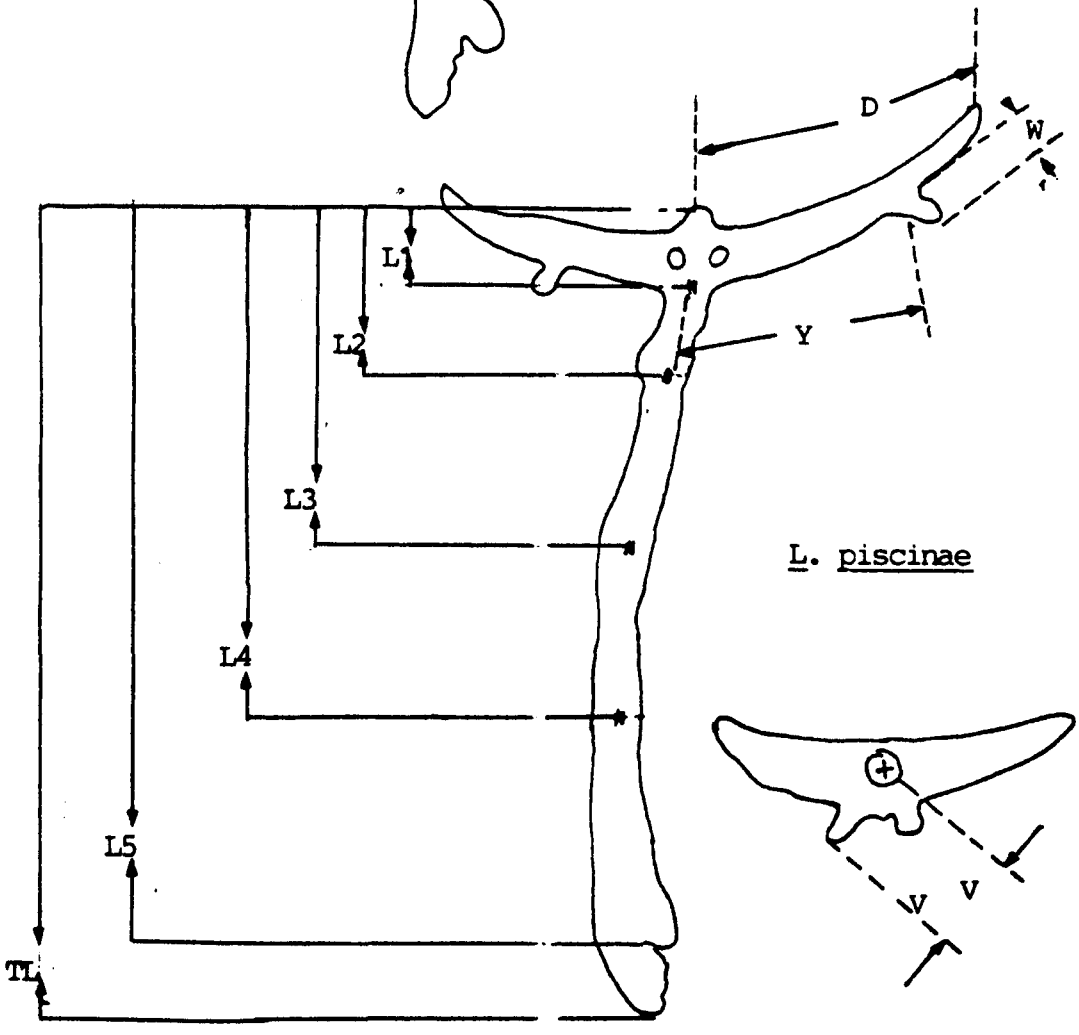
These are shown in Figure 4.3. Measurements of the left and right sections of the parasite were made separately for parasites obtained from the left and right sides of the host. All the above measurements were converted into percentage proportion in relation to the total length of the parasite. The measurement of the various parts of the parasites from the different regions of the host and also between the 3 host-parasite systems were compared to identify the presence of any consistency in the different

Figure 4.3 Figure shows the different characteristics of L. cyprinacea and L. piscinae which were measured.

D - Length of dorsal horn
V - Length of ventral horn
W - Length of process of dorsal horn
Y - Distance between mid-body and process of dorsal horn
TL - Total length.



L. cyprinacea



L. piscinae

characters which could be used in the identification of the parasite to species level.

4.2.2.4. Morphometrics.

Morphometric analysis was carried out to make the following comparisons:-

a) The left section of the cephalic processes (i.e. length of dorsal horns (D), distance between mid-body and process of dorsal horn (Y), length of the process of dorsal horn (W), length of the ventral horn (V)) as shown in Figure 4.3 were compared with parasites obtained from the left and right side of the host body proper (regions 1-4). The comparisons were made for each individual host-parasite system . Similarly comparison was also made of the right section of the cephalic section.

b) The cephalic processes (mean of pooled left and right section of parasites from body proper eg. (D1+D2)/2) were compared between parasites obtained from the left and right side for each host species.

c) The cephalic processes (mean of pooled left and right section from the left and right side of the host body proper) were compared between parasites obtained from the 3 host-parasite species.

d) The cephalic processes (mean of pooled left and right section from left and right side of the host) of

parasites obtained from regions 1, 2, 3 and 4 (body proper) were compared with each other for each host parasite system. Similar comparison were also made between parasites obtained from regions 5, 6, 7 and 8 (caudal peduncle, fins, head and eyes).

e) The position of the swimming legs of parasites obtained from the left side were compared to those from the right side of host body proper, for each host parasite system and also between the 3 host-parasite species.

f) The position of the swimming legs (mean of pooled data obtained from left and right side of the host) was compared between parasites from regions 1, 2, 3 and 4 (body proper) for each host parasite system and also between the 3 host parasite system. Similarly comparisons was made between parasites from regions 5, 6, 7 and 8 (the caudal peduncle, fins, head and eyes). Comparison were then made of parasites from body proper and those from regions 5, 6, 7 and 8 for each host parasite system.

g) Morphometric comparisons for the total length were treated similarly as in the position of swimming legs.

Insufficient parasites were located on the eyes of C. auratus infected with L. cyprinacea and A. nobilis infected with L. piscinae, therefore these were omitted from the morphometric analyses.

All statistical analyses were performed on the VAX

computer. The parasites were subsequently drawn with a camera lucida and identified to species level based on the key prepared by Harding (1940) and the work of Poddubnaya (1973).

4.3. Results

4.3.1. Morphology of the larval stages of L. cyprinacea and L. piscinae.

Since there were no morphological differences between the larval stages of L. cyprinacea and L. piscinae, they will be described together. The larval nauplius stage develops into the copepodid, the cyclopoid (which is also known as the pre-metamorphosis female or the adult male), the preadult or the post-metamorphosis female and finally the adult stage. There were 3 naupliar stages, 5 copepodid, and one cyclopoid stage, after which the post-metamorphosis female penetrated the host tissue to become a sedentary form.

4.3.1.1. Naupliar stages

4.3.1.1.1. Nauplius I (Fig. 4.4 and 4.5)

The body was transparent, greenish yellow in colour and elliptical in shape. The measurements varied in length from 142-165 um. About 80% of the body was filled with large numbers of vitelline globules. There was one pair

bristles arising from the posterior end of the body.

The antennulae have 3 segments, the basal segment was unarmed, whilst the second segment possesesed a long plumose bristle and a spine at its distal end. At the third segment there were 2 long plumose bristles and 3 spines. The antenna is biramous and the exopodite consisted of 4 segments, the first one was the largest and the second, third and fourth became smaller tapering to a fine point. Each segment had one long plumose bristle, whilst the fourth segment had an additional long bristle. The endopodite consisted of 1 segment which had 2 long plumose bristles and a small spine.

The mandibles which were smaller in size than the antennae were biramous and had the same number of segments as the antennae. The exopodite of the mandible was similar to that of the antennae with one long bristle on each segment whilst the final segment had an extra smaller bristle. The endopodite of one segment had 2 plumose bristles. The paired eyes were set close together forming an x-shape.

4.3.1.1.2. Nauplius II (Fig.4.6 and 4.7)

The body had an oval shape and the number of vitelline globules had decreased and now filled about 60% of the body. The bristles at the posterior end of the body had increased to 2 pairs. The number of appendages remained unchanged and were as seen in Nauplius I.

The antennule consisted of 3 segments as seen in Nauplius I but had 2 additional small spines on the final segment. The endopodite of the antenna increased its number of segments to 2 after the first moult but there was no change noted in the expodite. The mandibles were similar to those of nauplius I. The rudiment of the first maxillae with 2 spines was also present posterior to the mandible.

4.3.1.1.3. Nauplius III (Metanauplius) (Fig. 4.8 and 4.9)

The body had increased overall in size (0.1 X 0.2 mm) and the vitelline globules had decreased to 30% of the total body. At the posterior 1/3 of the body, segmentation was visible ~~present~~. The bristles present at the posterior end of the body had increased to 3 pairs. The number of appendages and segments did not change and were as seen in nauplius II. There was however, an increase in the number of spines on the antennulae from 3 to 6.

4.3.1.2. Copepodid stages

The body of copepod I to the cyclopoid stage was transparent and dark, peristaltic movements of the intestine became conspicuous in the mid body. The maxillae and maxillipedes remained unchanged and possessed 2 and 5 claws respectively throughout the developmental stages. The furcae maintained the 4 bristles each throughout the copepodid phase. The remaining characteristics varied with the developmental stages and are described below. The

measurements of the various body parts of the different copepodid stages and the pre-metamophosis female and the adult male stage and the arrangements of the bristles, spines and swimming legs are shown in Table 4.1 and 4.2.

4.3.1.2.1. Copepodid I (Fig. 4.10 and 4.11)

At the first copepodid stage, the body consisted of cephalothorax, 3 free thoracic and an abdominal segment, furcae, 2 pairs of biramous swimming appendages, uniramous endopodite and exopodite.

The antennae, antennule, mandibulae, maxillulae, maxillae, maxillipedes and the first pair of swimming appendages were borne on the cephalothorax.

The first free thoracic segment (posterior to the cephalothorax) was narrower than the cephalothorax. The other 2 thoracic segments gradually tapered and a pair of furcae protruded from the posterior abdominal segment. On each basic segment of the furca there were 3 bristles pointing to the external side and one pointing internally. The 2nd. pair of swimming appendages were attached to the 1st. free thoracic segment.

The antennulae consisted of 3 segments and were directed backwards. The central segment had 3 bristles and the distal segment had 11.

The antennae had 3 segments. The basic segment had a rudimentary exopodite in the form of a papilla with 3 small

bristles. The middle segment had no bristles, whilst the 3rd. segment had a short claw and 3 long bristles anterior to the claw. There were 2 to 3 additional small bristles on the same segment.

The maxillipedes with 2 segments were located posterior to the oral opening. The 2nd. segment was bent almost at a right angle and had 5 claws. The maxillae which cover the mandibulae and the maxillulae terminate with 2 hooks.

The 2 pairs of swimming appendages had 2 branches, the endopodite and exopodite, both consisting of one segment each. The arrangement and number of bristles and spines on the swimming appendages at individual stages are presented in Table 4.1 and 4.2.

4.3.1.2.2. Copepodid II (Fig. 4.12 and 4.13)

The body had 3 free thoracic segments and an addition of a 2nd abdominal segment. The first 2 pairs of swimming appendages had an exopodite and endopodite which consist of 2 segments each. The new 3rd. pair of biramous limbs which were attached to the 2nd. free thoracic segment had only one segment.

The antennulae had developed 4 segments, the basic segment being without bristles, the 2nd. segment had 3 bristles, the 3rd. segment had 3 smaller and one large bristle and the distal segment carried eleven bristles.

The first pair of swimming limbs had developed branches with 2 segments. On the coxopodite, one small bristle was present on the paracentral side while the basipodite has a claw on the paracentral side. The 2nd. pair of swimming limbs had a coxopodite with a single bristle on the internal side and the basipodite with one bristle on the external side. The exopodite and endopodite consisted of 2 segments each. The 3rd. pair had exopodite and endopodite on one segment. There was one bristle present on the internal side of the basipodite and another bristle on the external side

The rudimentary papillae with 3 small bristles present on the basic segment of the antennae seen at copepodid I were absent in copepodid II and there was an addition of a bristle present anterior to the claw.

The maxillipedes and maxillae increased in size without any other changes.

4.3.1.2.3. Copepodid III (Fig. 4.14 and 4.15)

At the copepodid III, the body had 4 free thoracic and 2 abdominal segments. The segments of the antennulae increased to 5 and there was an addition of a 4th. pair of biramous limbs. The first 3 pairs have branches consisting of 2 segments. The new 4th. pair of biramous limbs were attached to the 3rd. free thoracic segment, the exopodite and endopodite had one segment each.

The antennulae consisted of 5 segments. The basic segment was without bristles, the 2nd. had 6, the 3rd. had 3

bristles, the 4th. had 2 to 3 bristles and the last segment had 11 bristles.

The antennae now consisted of 3 segments and the last segment had 3 small spines on the paracentral side.

The maxillipedes, furcae and maxillae remain unchanged but increased in size.

4.3.1.2.4. Copepodid IV (Fig. 4.16 and 4.17)

The body consisted of 7 segments; 4 free thoracic and a new 3rd abdominal segment. There was a new 5th. pair of swimming appendages and a rudimentary 6th. pair. The endopodite and exopodite of the 4th. pair of swimming appendages developed 2 segments, the 5th. was uniramous and consisted of 2 segments, the 6th. pair was in the form of papillae with 2 spines each. The 5th. and 6th. pair of swimming appendages were attached to the 4th. free thoracic and the 1st. abdominal segments respectively.

The antennule had 6 segments, the basic segment was without bristles, the 2nd. had 4-5, the 3rd. had 5, the 4th. had 2, the 5th. had 4 and the last has 10-11 bristles.

4.3.1.2.5. Copepodid V (Fig. 4.18, 4.19, 4.20 and 4.21)

The body now consisted of 8 segments, 4 free thoracic and/a new fourth abdominal segment. The endopodites and expodite of the first 4 pairs of swimming appendages had

increased to 3 segments and the 5th. pair of limb develops 2 segments.

The maxillipedes, furcae, antennae and maxillae increaased in size without any change.

Sexual differences between male and female were seen at this stage. The genital segment in the female was smaller and the antennulae were shorter and bore only 26 to 28 bristles and spines compared to 34 in the male. The antennulae had 6 segments, in the female the basic segment had 4 bristles, the 3rd. had 5 to 6 bristles, the 4th. had 3, the 5th. had 4 and the last segment had 11 bristles.

In the male, the antennulae had also 6 segments; the basic segment was without any bristles, the 2nd. had 5, the 3rd. had 6-7, the 4th. had 3-4, the 5th. had 6-8 and the last segment had 10-11 bristles.

In the male, the claw present on the basipodite of the first pair of swimming appendages developed a 2nd. hook.

4.3.1.3. Cyclopoid stage (Fig. 4.22, 4.23, 4.24 and 4.25) (Pre-metamorphosis female and adult male)

The body consisted of 9 segments; an additional 5th. abdominal segment and 4 free thoracic segments.

The structure of all the body appendages was in general the same as in copepodid V stage but the external features were differentiated much more than the copepodid stage.

The male cyclopoid was smaller and thinner than the female (Table 4.3). In the female, the conspicuous oviduct lay laterally along the genital segment causing it to appear conspicuously large. Whilst in the male, the pair of oval shaped spermatophores were visible within the genital segment.

The antennulae in the female consisted of 6 segments whereas in the male they were longer and consisted of 8 segments. The number of bristles on the antennulae in the male was 40 compared to 25-27 in the female.

The basic segment of the antennulae in the female had no bristles, the 2nd. segment had 4 bristles, the 3rd. had 4-5, the 4th. had 4, the 5th. had 4 and the last segment had 12.

The antennae increased in size but remained otherwise unchanged.

In the male, the 5th and 6th rudimentary legs had 6 and 3 bristles whereas in the female they had only 4 and 1 respectively.

The maxillipedes, furcae and maxillae increased in size without any other change.

The arrangement of the bristles and spines of the swimming limbs remain unchanged as shown in Table 4.1 and 4.2.

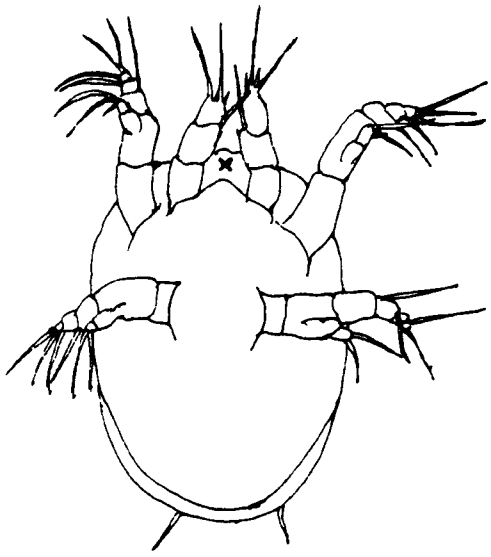


Figure 4.4 Nauplius I of L. piscinae

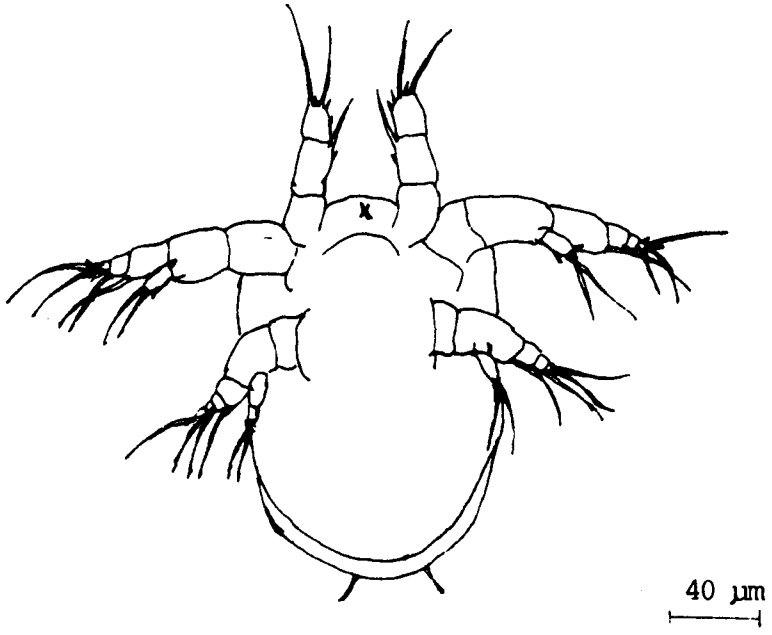


Figure 4.5 Nauplius I of L. cyprinacea

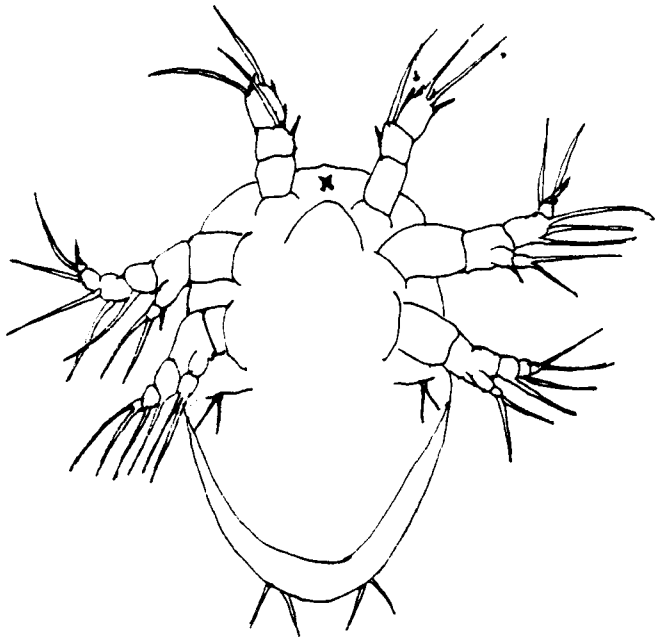


Figure 4.6 Nauplius II of L. piscinae

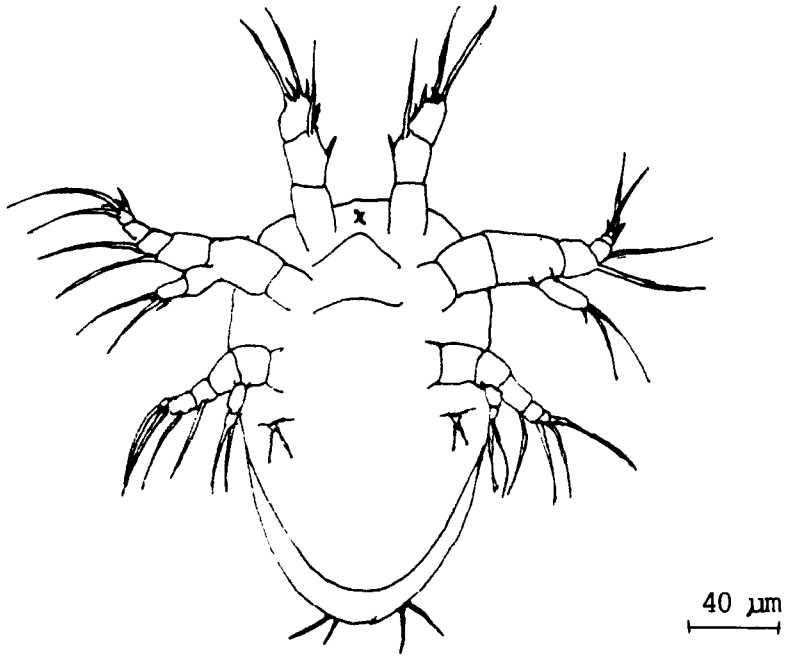


Figure 4.7 Nauplius II of L. cyprinacea

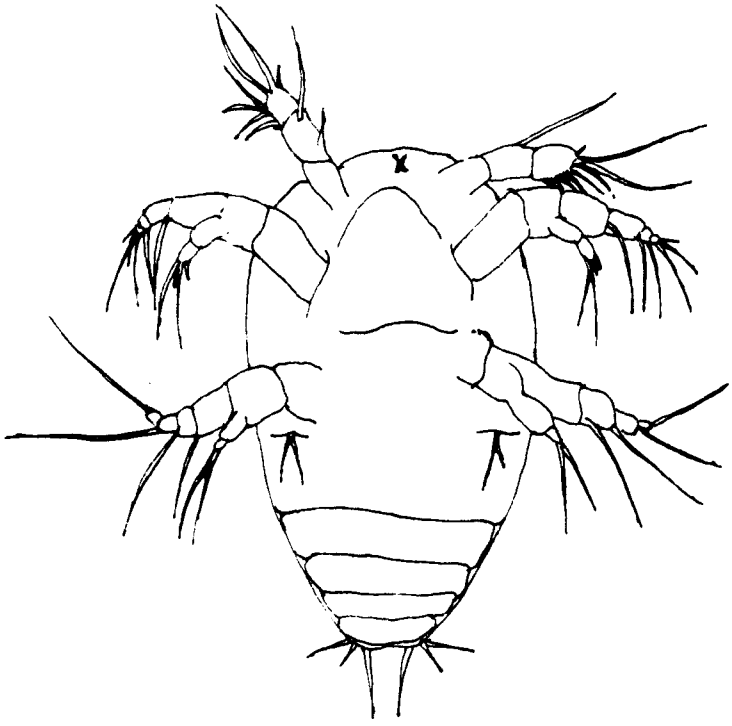


Figure 4.8 Nauplius III of L. piscinae

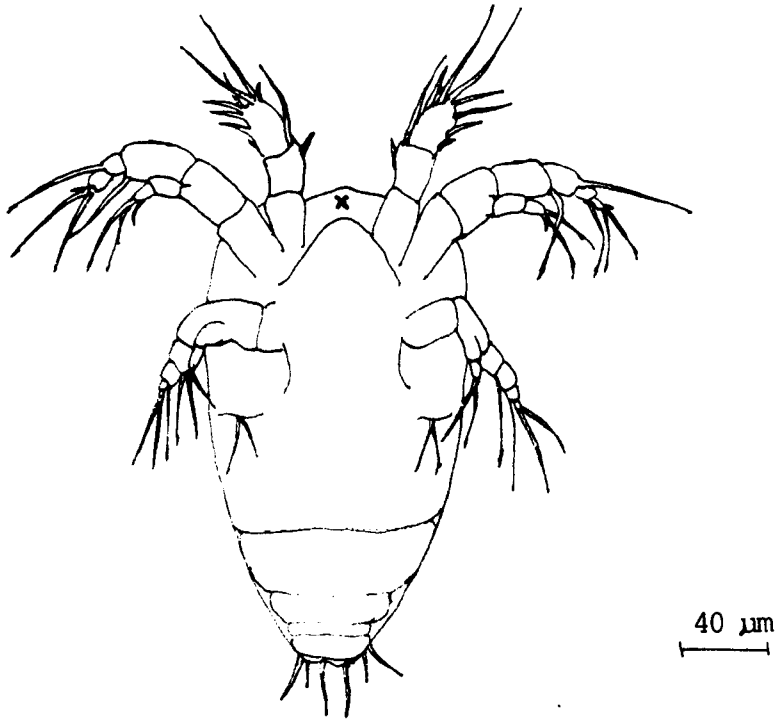


Figure 4.9 Nauplius III of L. cyprinacea

Figure 4.10 First copepodid larvae of L. cyprinacea.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) furcae

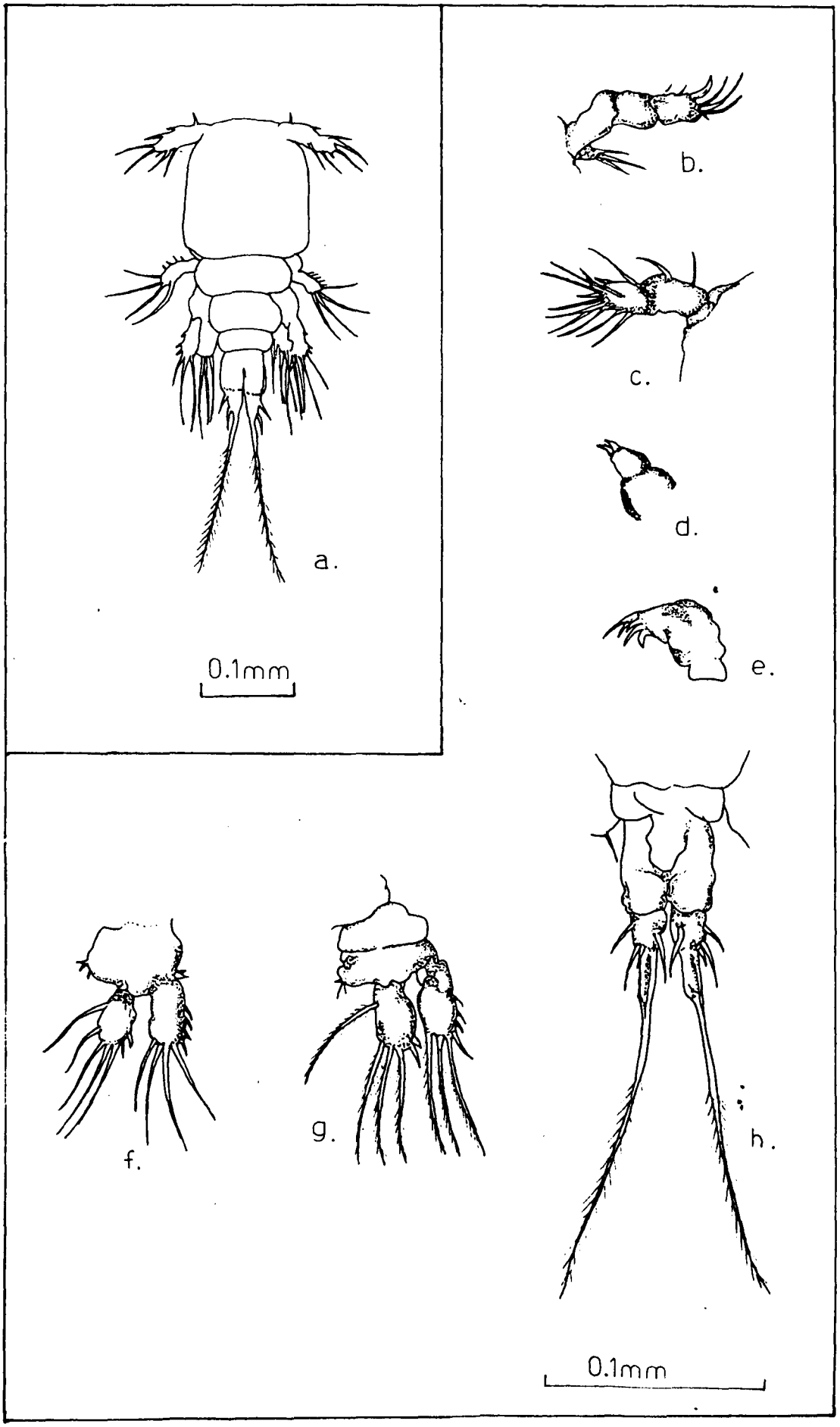

a.
0.1mm
b.
c.
d.
e.
f.
g.
h.
0.1mm

Figure 4.11 First copepodid larvae of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

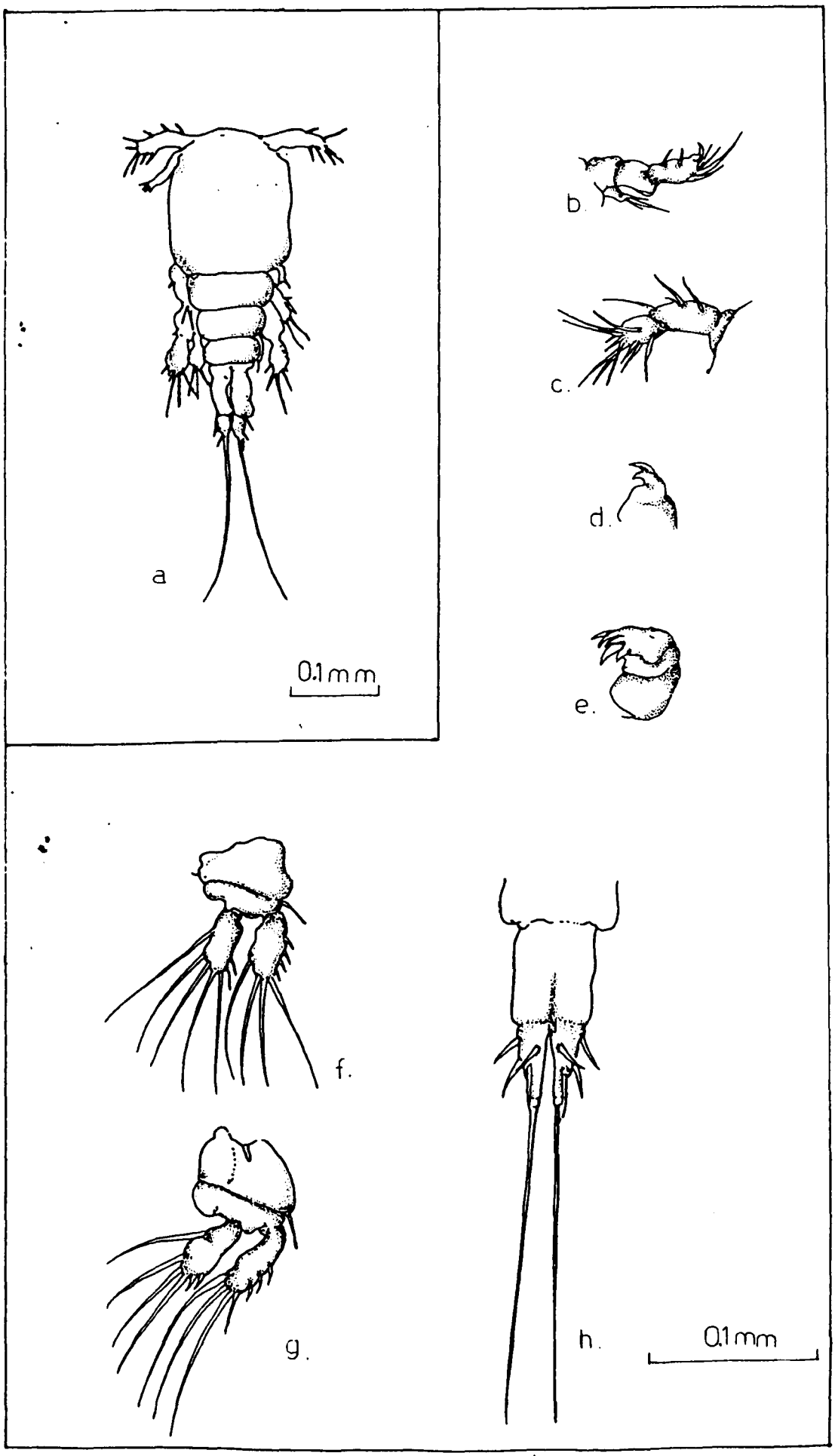
d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) furcae


a
0.1mm
b.
c.
d.
e.
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g.
h.
0.1mm

Figure 4.12 Second copepodid larvae of L. cyprinacea.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

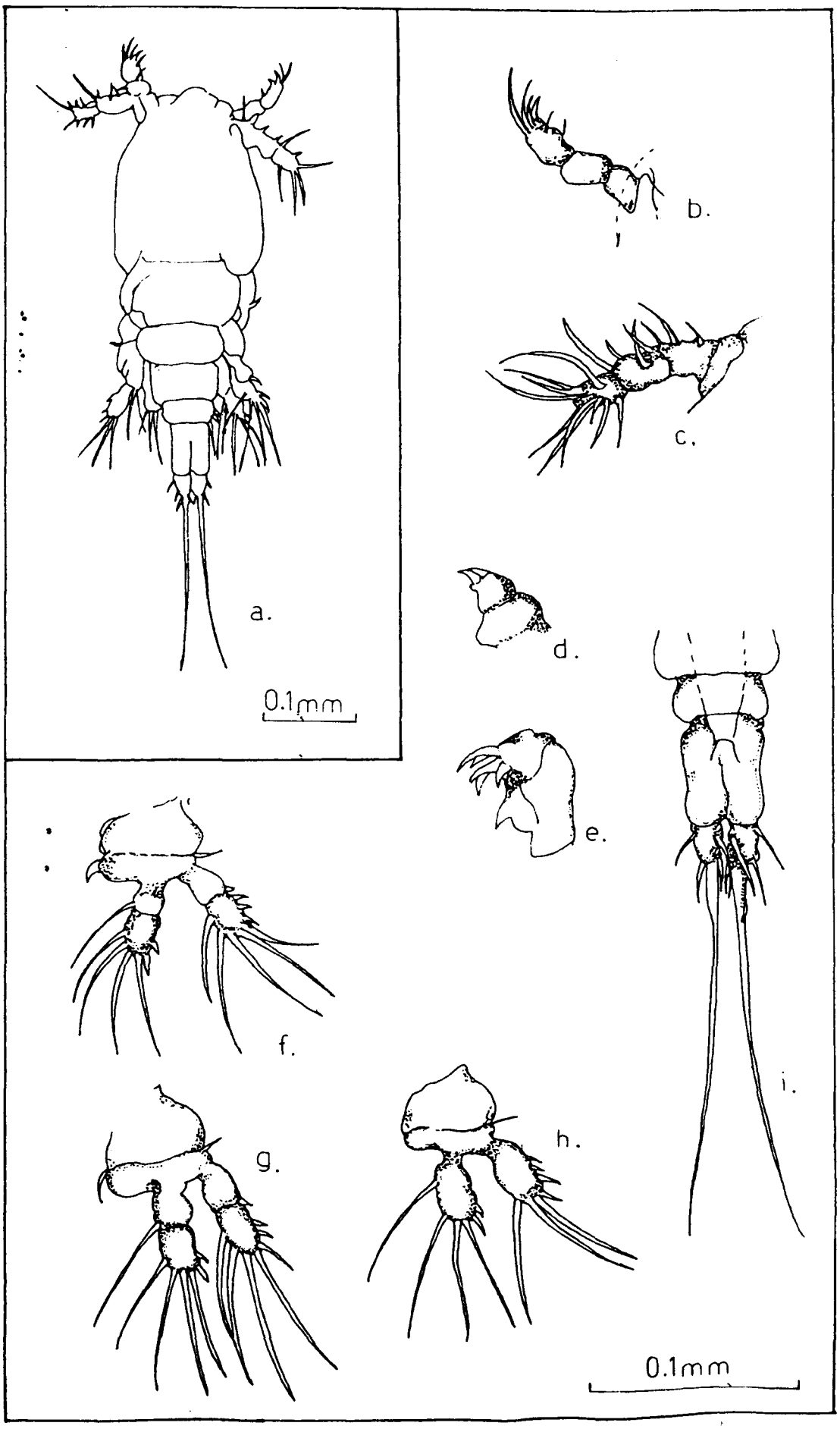
e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) furcae


a.
0.1mm
b.
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d.
e.
f.
g.
h.
i.
0.1mm

Figure 4.13 Second copepodid larvae of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

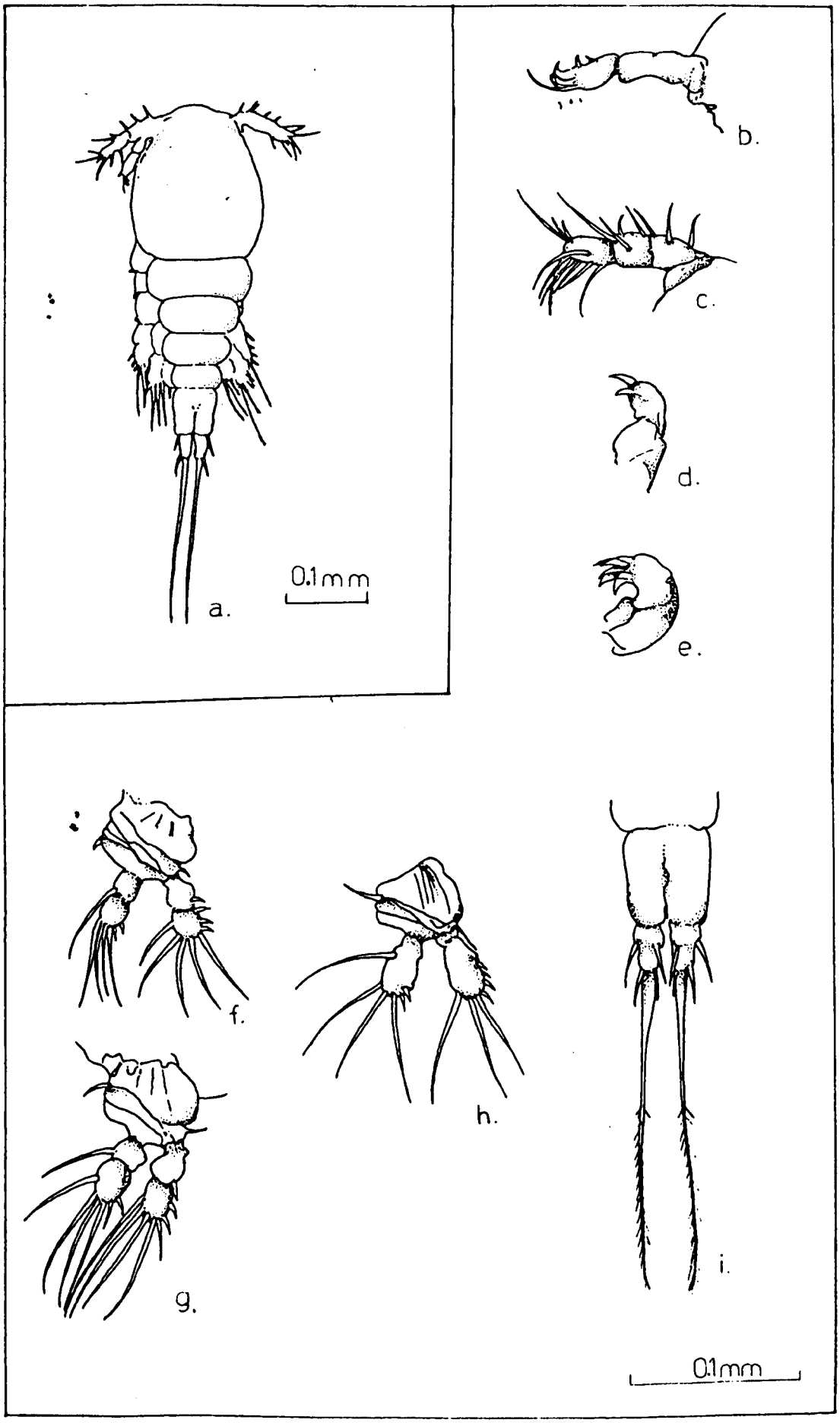
e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) furcae


a.
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b.
c.
d.
e.
f.
g.
h.
i.
0.1mm

Figure 4.14 Third copepodid larvae of L. cyprinacea;

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) furcae

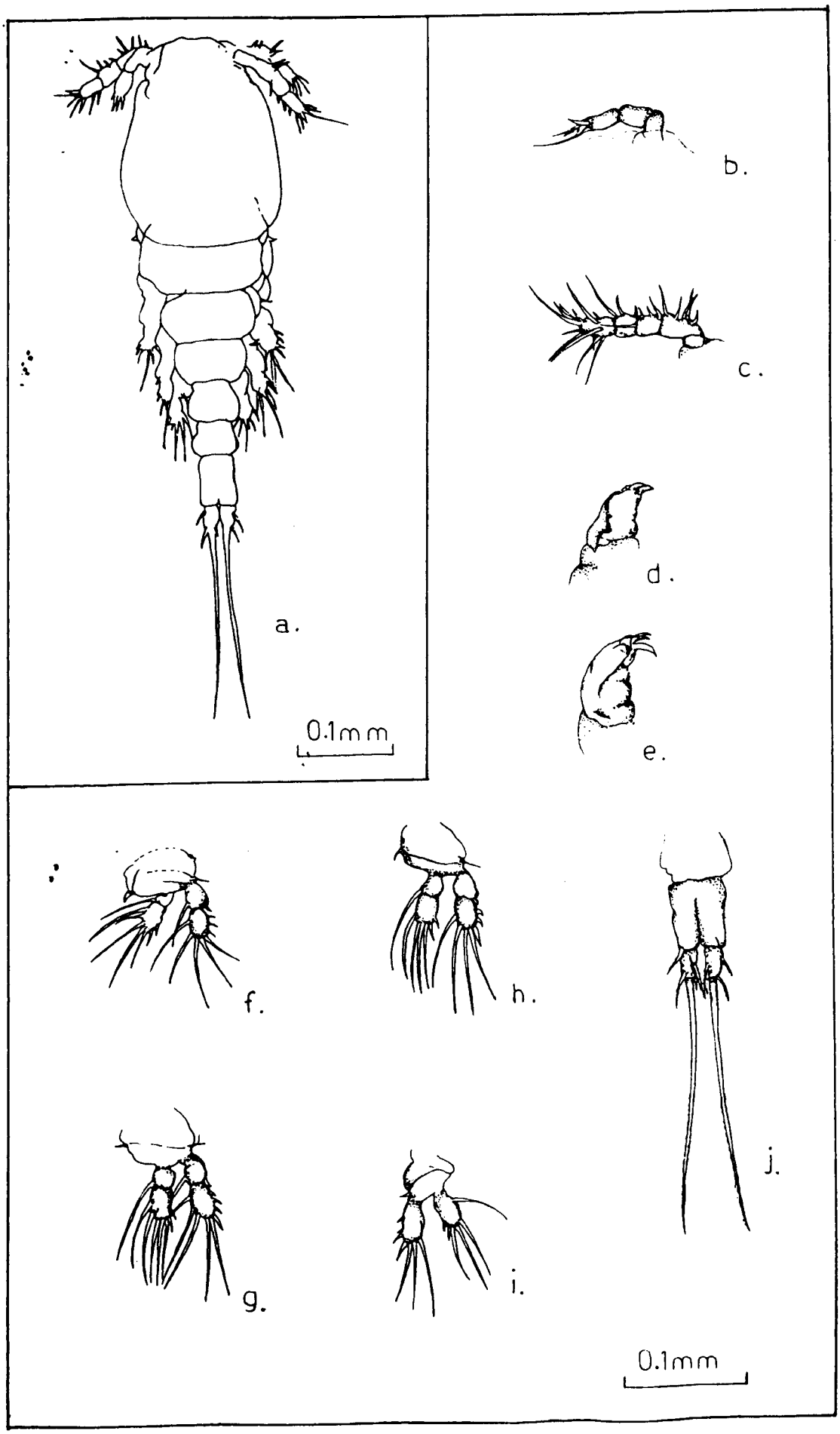

a.
0.1mm
b.
c.
d.
e.
f.
h.
g.
i.
j.
0.1mm

Figure 4.15 Third copepodid larvae of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) furcae

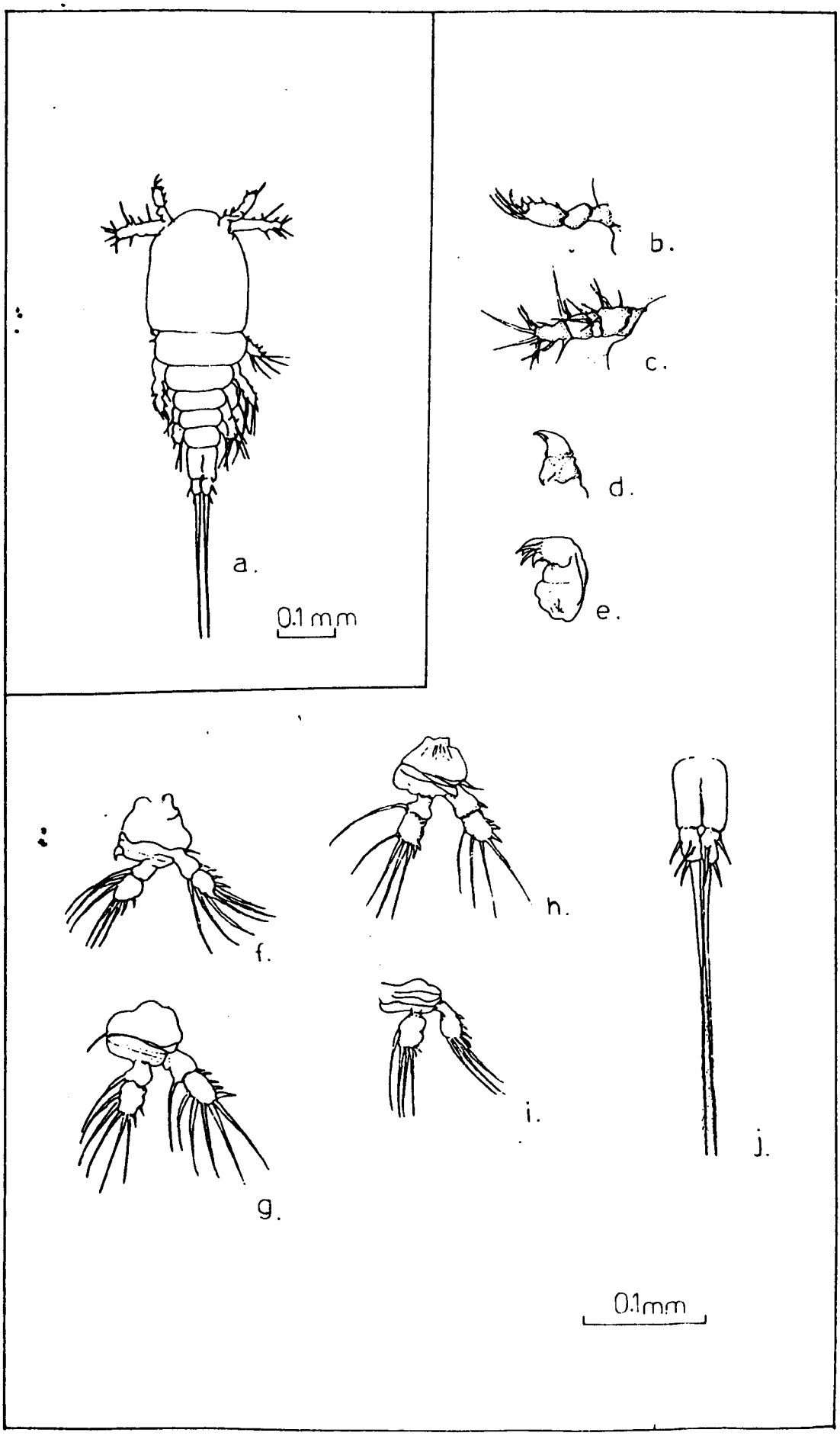

a.
0.1 mm
b.
c.
d.
e.
f.
g.
h.
i.
j.
0.1mm

Figure 4.16 Fourth copepodid larvae of L. cyprinacea.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae

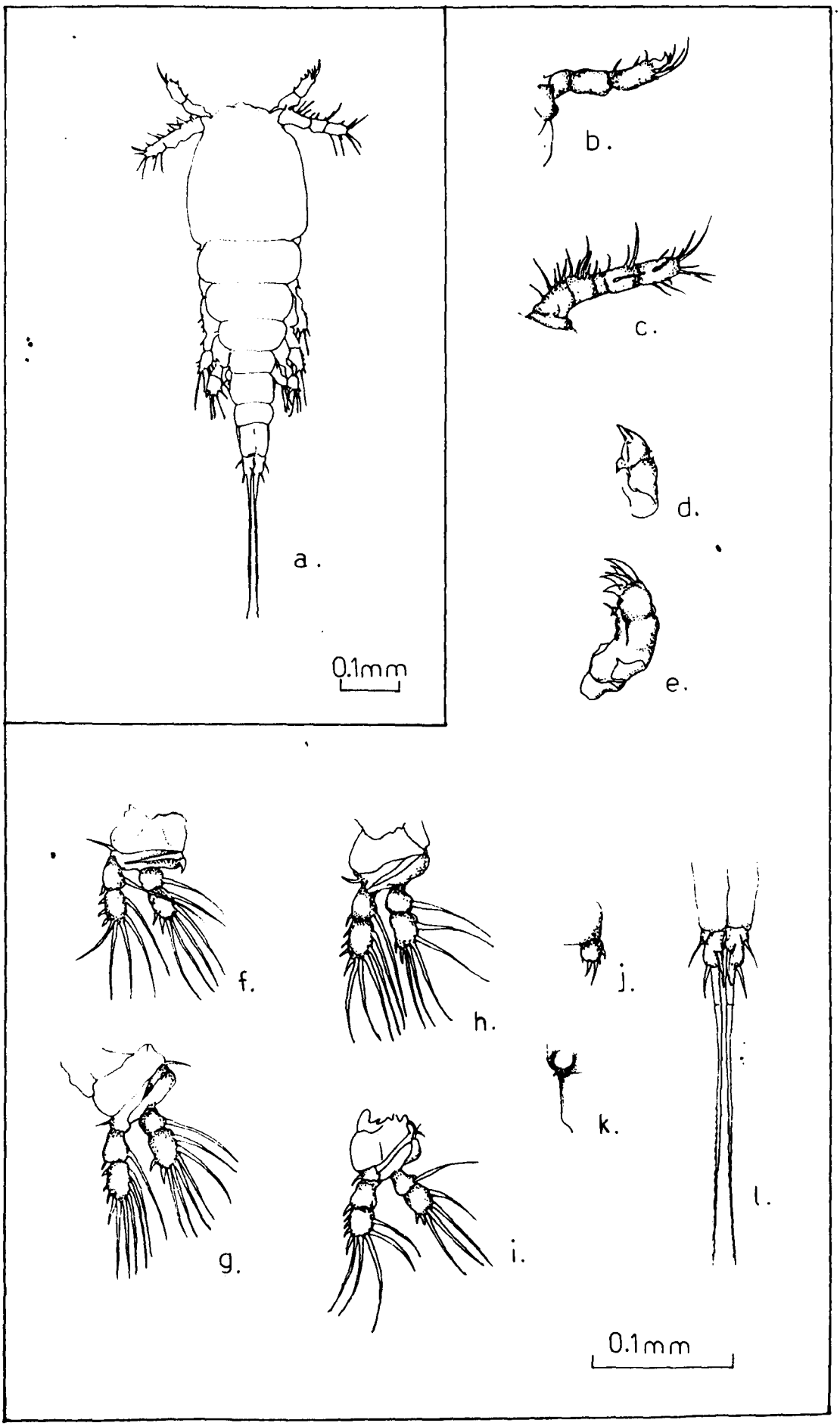

a.
0.1mm
b.
c.
d.
e.
f.
g.
h.
i.
j.
k.
l.
0.1mm

Figure 4.17 Fourth copepodid larvae of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae

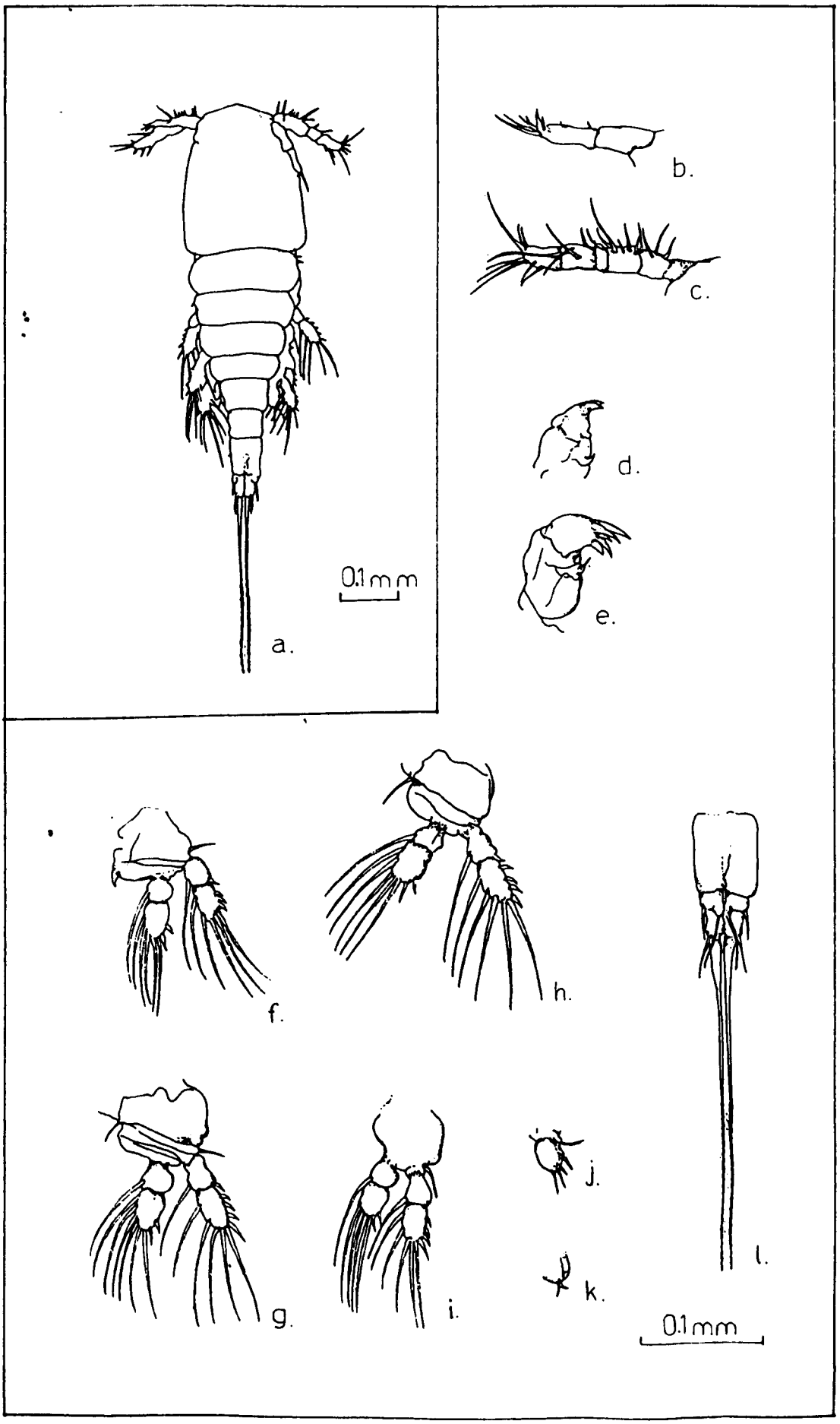

a.
0.1mm
b.
c.
d.
e.
f.
g.
h.
i.
j.
k.
l.
0.1mm

Figure 4.18 Male fifth copepodid larvae of L. cyprinacea.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae

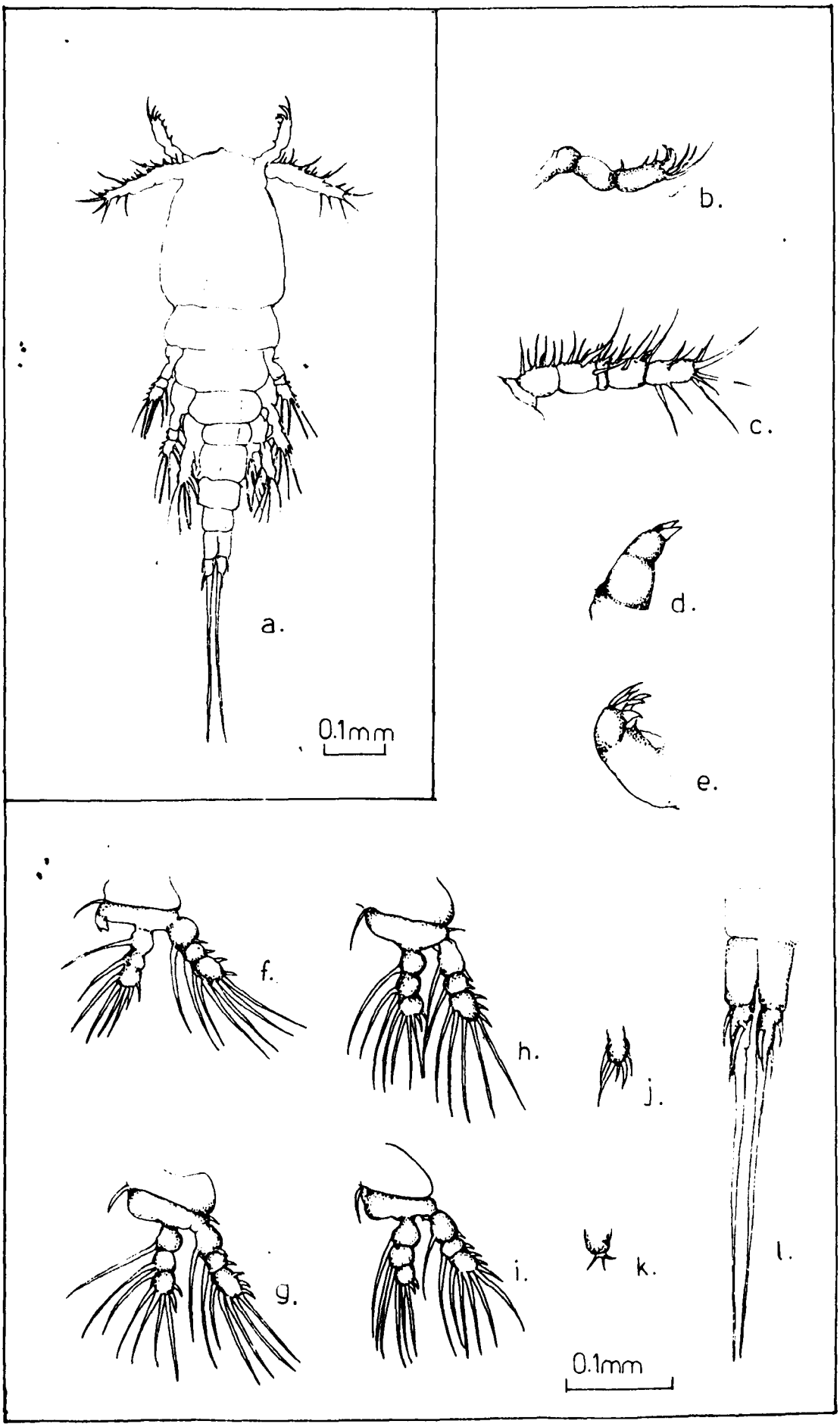

a.
0.1mm
b.
c.
d.
e.
f.
g.
h.
i.
j.
k.
l.
0.1mm

Figure 4.19 Male fifth copepodid larvae of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae

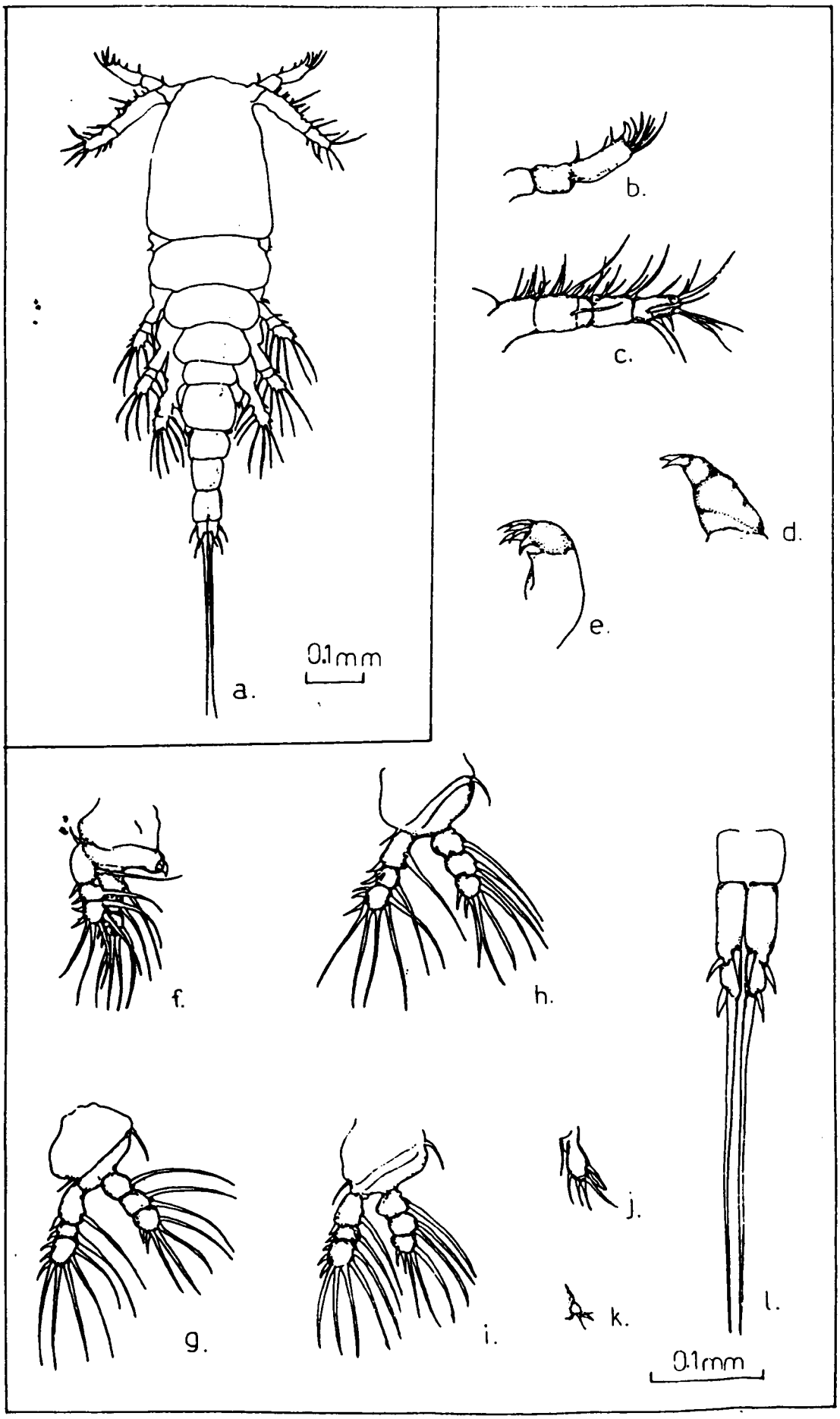

a.
0.1mm
b.
c.
d.
e.
f.
h.
g.
i.
j.
k.
l.
0.1mm

Figure 4.20 Female fifth copepodid larvae of L. cyprinacea.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

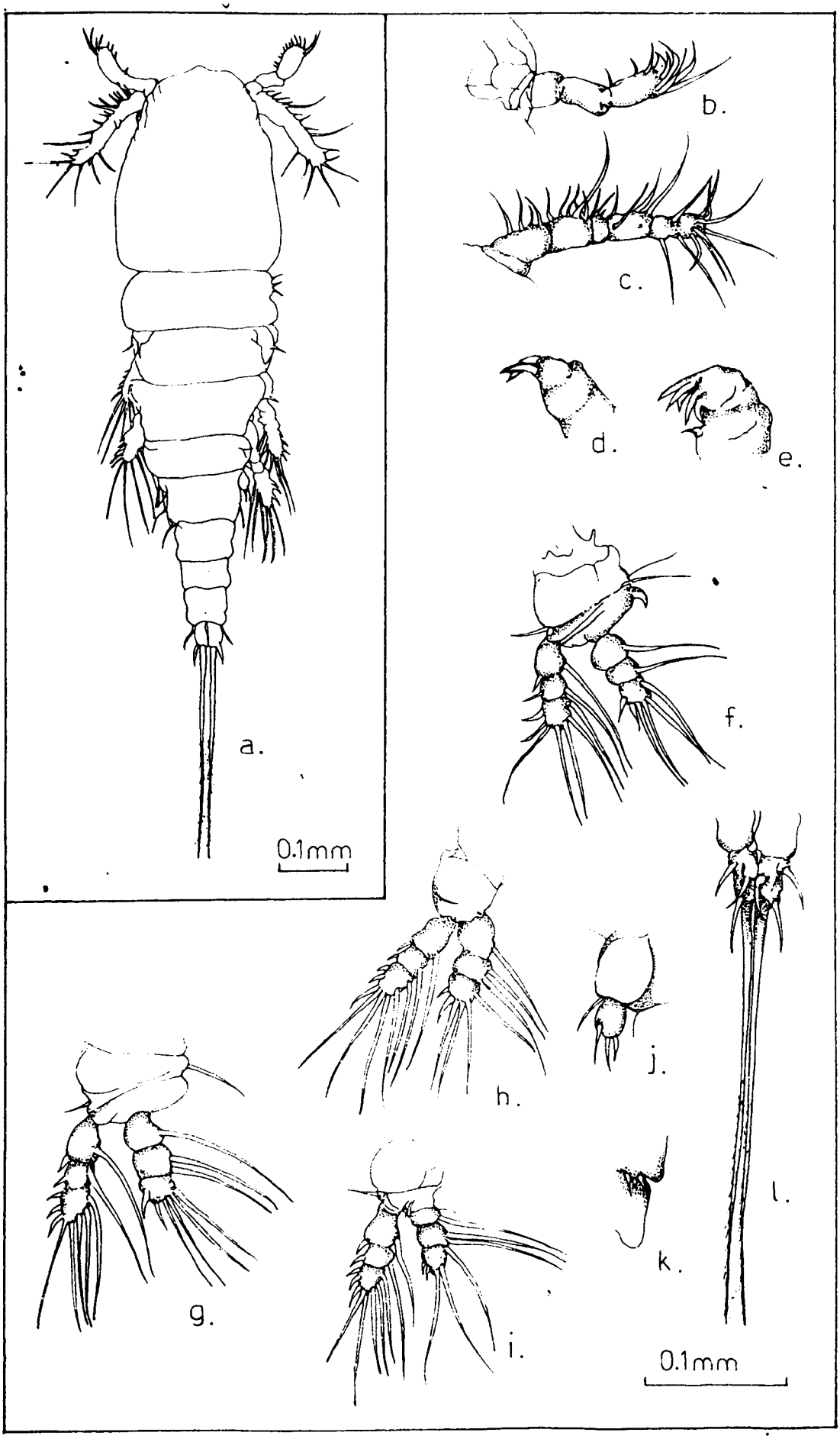
h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae


a.
0.1mm
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d.
e.
f.
g.
h.
i.
j.
k.
l.
0.1mm

Figure 4.21 Female fifth copepodid larvae of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

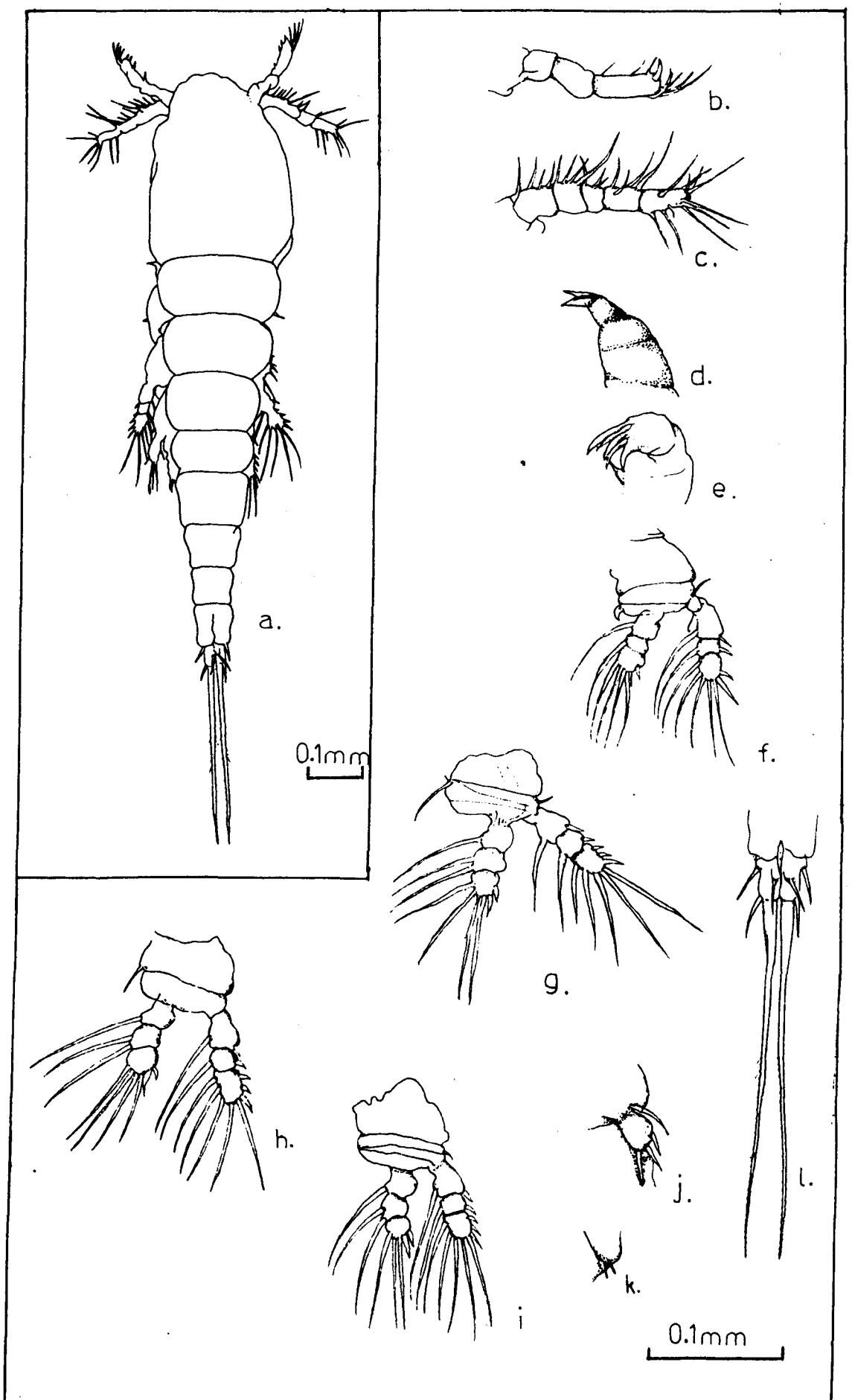
h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae


a.
0.1mm
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e.
f.
g.
h.
i
j.
k.
l.
0.1mm

Figure 4.22 Male cyclopoid stage of L. cyprinacea.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae

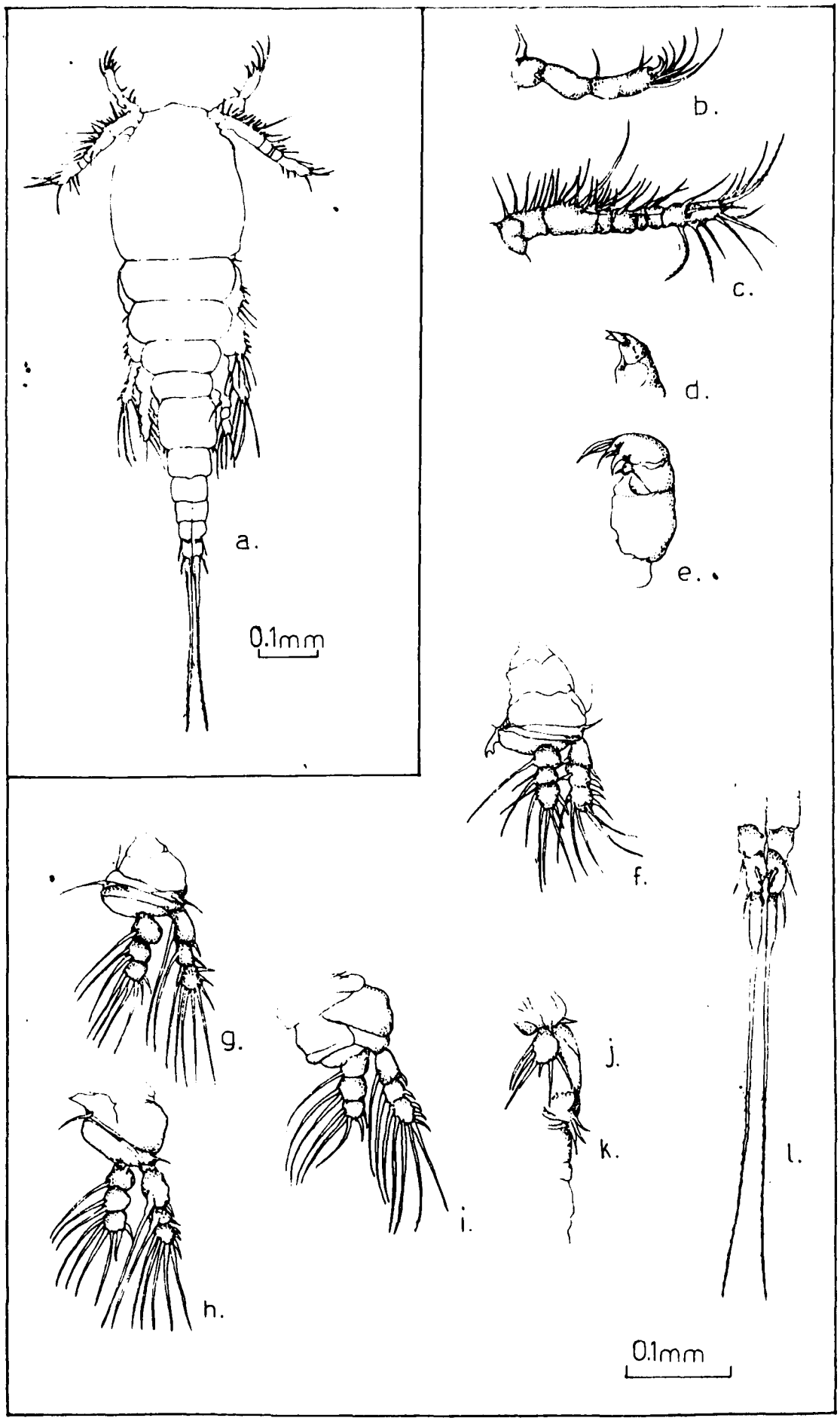

a.
0.1mm
b.
c.
d.
e.
f.
g.
h.
i.
j.
k.
l.
0.1mm

Figure 4.23 Male cyclopoid stage of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

g) second swimming leg

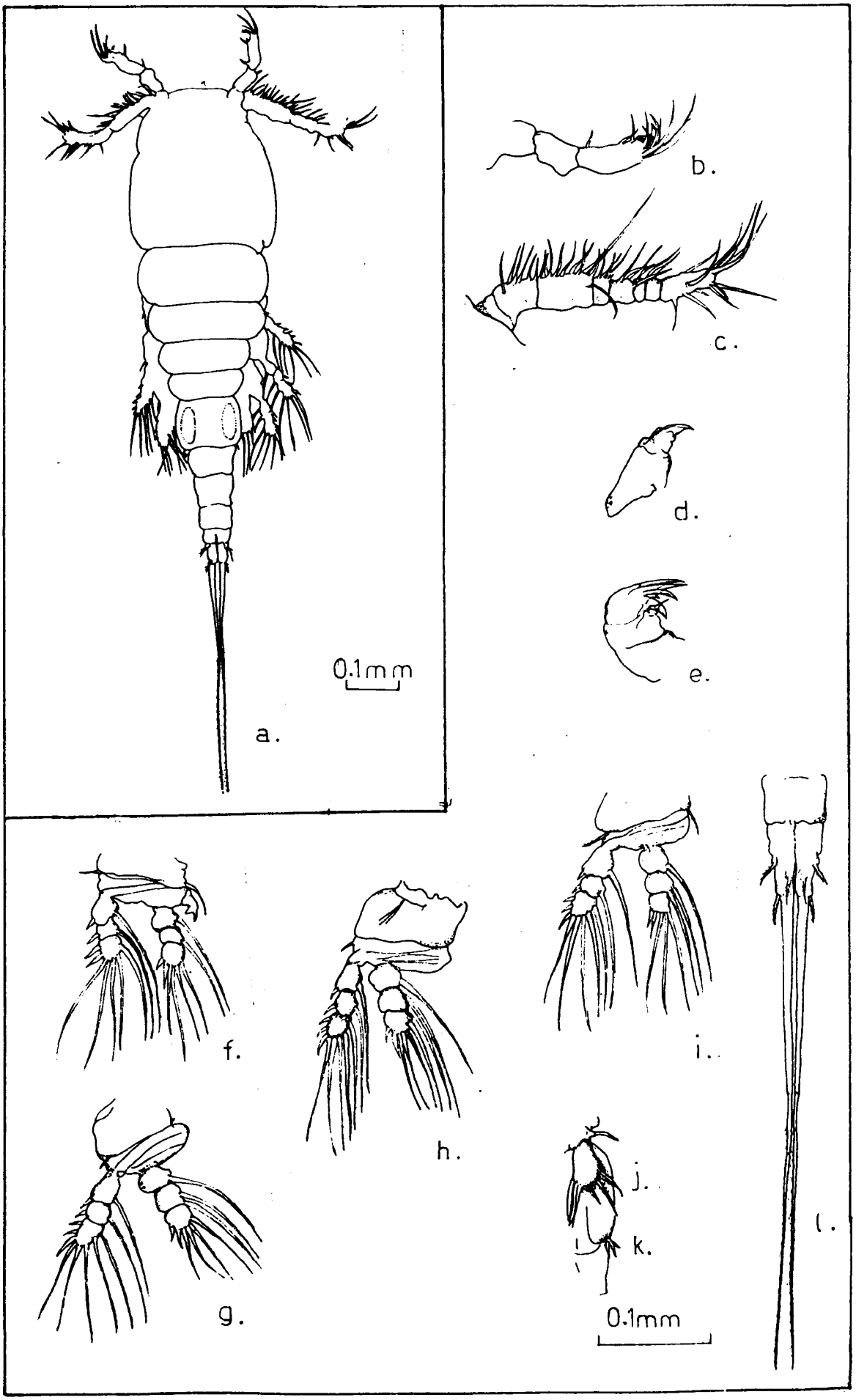
h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) sixth swimming leg

l) furcae


a.
0.1mm
b.
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d.
e.
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g.
h.
i.
j.
k.
l.
0.1mm

Figure 4.24 Female cyclopoid stage of L. cyprinacea.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

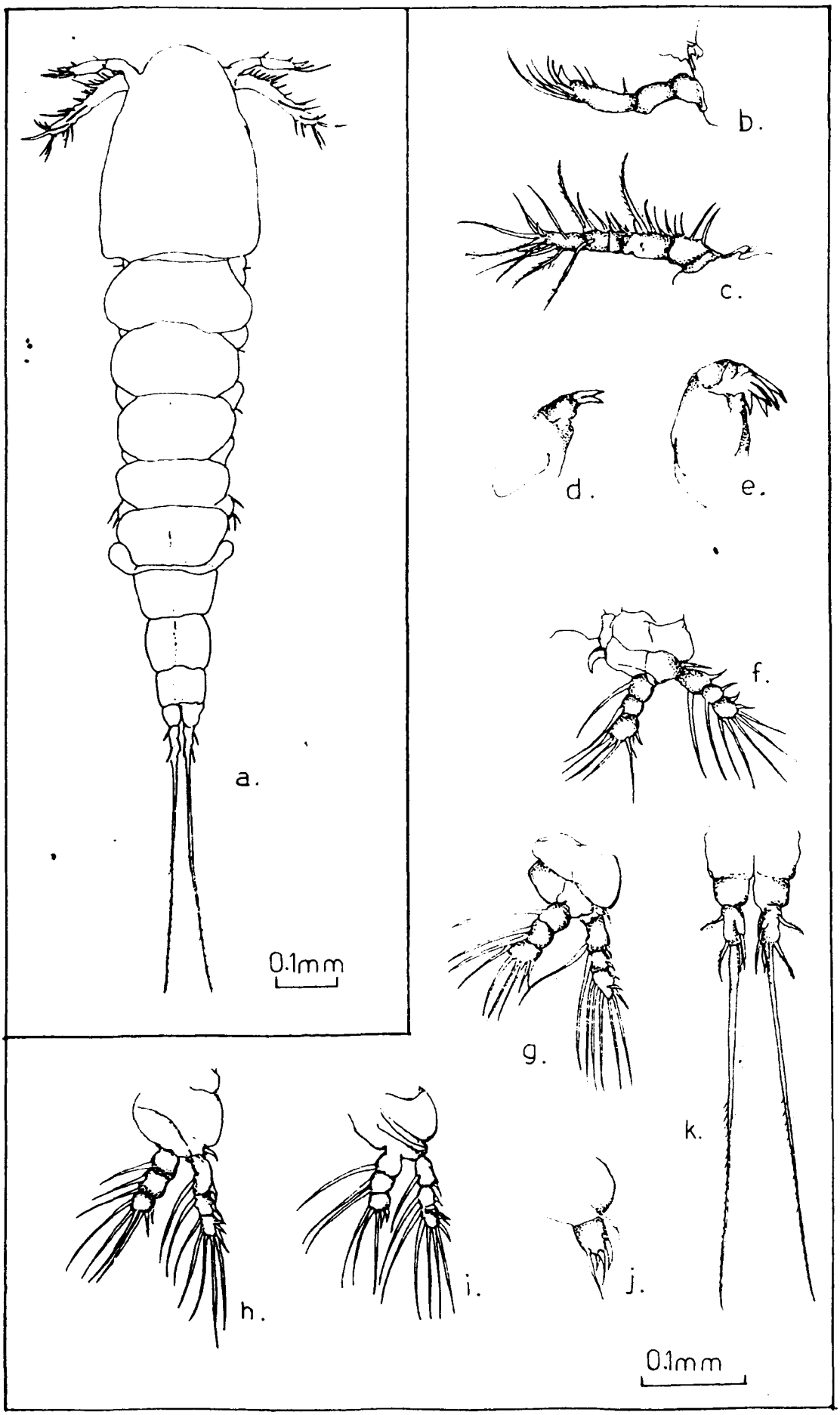
g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) furcae


a.
0.1mm
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c.
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g.
k.
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i.
j.
0.1mm

Figure 4.25 Female cyclopoid stage of L. piscinae.

a) dorsal view

b) antennae

c) antennulae

d) maxillae

e) maxillipedes

f) first swimming leg

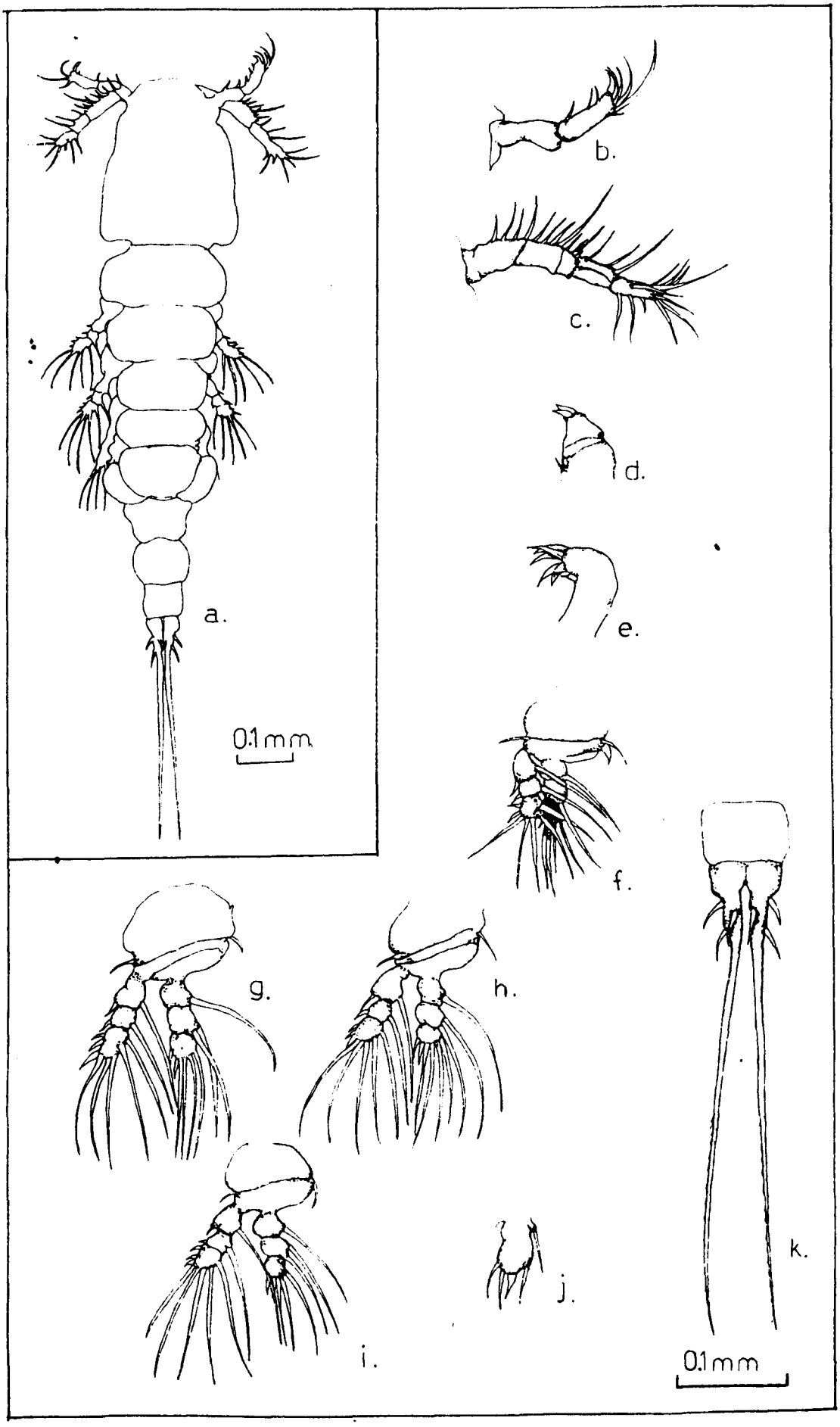
g) second swimming leg

h) third swimming leg

i) fourth swimming leg

j) fifth swimming leg

k) furcae


a.
0.1mm
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0.1mm

Table 4.1

Arrangement of spines and setae in swimming limbs of the 1st to 4th pair in copepodids (I-V) and cyclopod of *L. piscinae* on *A. nobilis*.

		Exopodite						Endopodite					
		Segm I		Segm II		Segm III		Segm I		Segm II		Segm III	
Copepodid Stage	Swimming Limbs	Spine	Setae	Spine	Setae	Spine	Setae	Spine	Setae	Spine	Setae	Spine	Setae
I	I Pair	4	4					2	5				
	II "	4	3					2	4				
II	I "	1	0	3	5			0	1	2	4		
	II "	1	0	3	4			0	1	2	4		
	III "	4	3					2	4				
III	I "	1	1	3	5			0	1	2	5		
	II "	1	1	4	5			0	1	2	5		
	III "	1	0	3	4			0	1	2	4		
	IV "	4	3					2	4				
IV	I "	1	1	3	5			0	1	2	5		
	II "	1	1	4	5			0	1	2	6		
	III "	1	1	4	5			0	1	2	5		
	IV "	1	0	4	5			0	1	2	4		
V(F)	I "	1	1	1	1	2	5	0	1	0	1	2	4
	II "	1	1	1	1	3	5	0	1	0	2	2	4
	III "	1	1	1	1	3	5	0	1	0	2	2	4
	IV "	1	1	1	1	3	5	0	1	0	2	2	3
V(M)	I "	1	1	1	1	2	5	0	1	0	1	2	4
	II "	1	1	1	1	3	5	0	1	0	2	2	4
	III "	1	1	1	1	3	5	0	1	0	2	2	4
	IV "	1	1	1	1	3	5	0	1	0	2	2	3
Cyclopoid Female	I "	1	1	1	1	2	5	0	1	0	1	2	4
	II "	1	1	1	1	3	5	0	1	0	2	2	4
	III "	1	1	1	1	3	5	0	1	0	2	2	4
	IV "	1	1	1	1	3	5	0	1	0	2	2	3
Cyclopoid Male	I "	1	1	1	1	2	5	0	1	0	1	2	4
	II "	1	1	1	1	3	5	0	1	0	2	2	4
	III "	1	1	1	1	3	5	0	1	0	2	2	4
	IV "	1	1	1	1	3	5	0	1	0	2	2	3

Table 4.2

Arrangement of spines and setae in swimming limbs of the 1st to 4th pair in copepodids (I-V) and cyclopod of *L. cyprinacea* on *C. auratus*.

		Exopodite						Endopodite					
		Segm I		Segm II		Segm III		Segm I		Segm II		Segm III	
Copepodid Stage	Swimming Stage	Spine	Setae	Spine	Setae	Spine	Setae	Spine	Setae	Spine	Setae	Spine	Setae
I	I Pair	4	4					2	5				
	II Pair	4	3					2	4				
II	I Pair	1	0	3	5			0	1	2	4		
	II Pair	1	0	3	4			0	1	2	4		
	III Pair	4	3					2	4				
III	I Pair	1	1	3	5			0	1	2	5		
	II Pair	1	1	4	5			0	1	2	5		
	III Pair	1	0	3	4			0	1	2	4		
	IV Pair	4	3					2	4				
IV	I Pair	1	1	3	5			0	1	2	5		
	II Pair	1	1	4	5			0	1	2	6		
	III Pair	1	1	4	5			0	1	2	5		
	IV Pair	1	0	4	5			0	1	2	4		
V(F)	I Pair	1	1	1	1	2	5	0	1	0	1	2	4
	II Pair	1	1	1	1	3	5	0	1	0	2	2	4
	III Pair	1	1	1	1	3	5	0	1	0	2	2	4
	IV Pair	1	1	1	1	3	5	0	1	0	2	2	3
V(M)	I Pair	1	1	1	1	2	5	0	1	0	1	2	4
	II Pair	1	1	1	1	3	5	0	1	0	2	2	4
	III Pair	1	1	1	1	3	5	0	1	0	2	2	4
	IV Pair	1	1	1	1	3	5	0	1	0	2	2	3
Cyclopoid Female	I Pair	1	1	1	1	2	5	0	1	0	1	2	4
	II Pair	1	1	1	1	3	5	0	1	0	2	2	4
	III Pair	1	1	1	1	3	5	0	1	0	2	2	4
	IV Pair	1	1	1	1	3	5	0	1	0	2	2	3
Cyclopoid Male	I Pair	1	1	1	1	2	5	0	1	0	1	2	4
	II Pair	1	1	1	1	3	5	0	1	0	2	2	4
	III Pair	1	1	1	1	3	5	0	1	0	2	2	4
	IV Pair	1	1	1	1	3	5	0	1	0	2	2	3

4.3.1.4. Young female (Fig. 4.26 and 4.27)
(Post-metamorphosis female)

After fertilization the female penetrates the host tissue and undergoes metamorphosis to become the adult parasite. The thoracic and abdomen regions became broader and elongated to form a cylindrical body and segmentation of the body gradually disappeared. Along with the growth of the body trunk, the cephalothorax was seen to broaden to form small nodules which gradually elongated to form the dorsal and ventral horns. With the development of the cephalic processes, the head became more prominent at this juncture.

In both L. piscinae and L. cyprinacea, the development of the dorsal horn took place first and was followed by the development of the ventral horns. The growth of the ventral horns in L. cyprinacea was from the dorsal region of the cephalothorax and the dorsal horns; whereas in L. piscinae the ventral horns developed from the bars of the dorsal horns ventrally.

4.3.1.5. Morphometrics of the larval stages of L. piscinae from A. nobilis and L. cyprinacea from C. auratus and A. nobilis.

The results of the morphometric study of the larval stages from the 3 host-parasite systems are presented in Table 4.3.

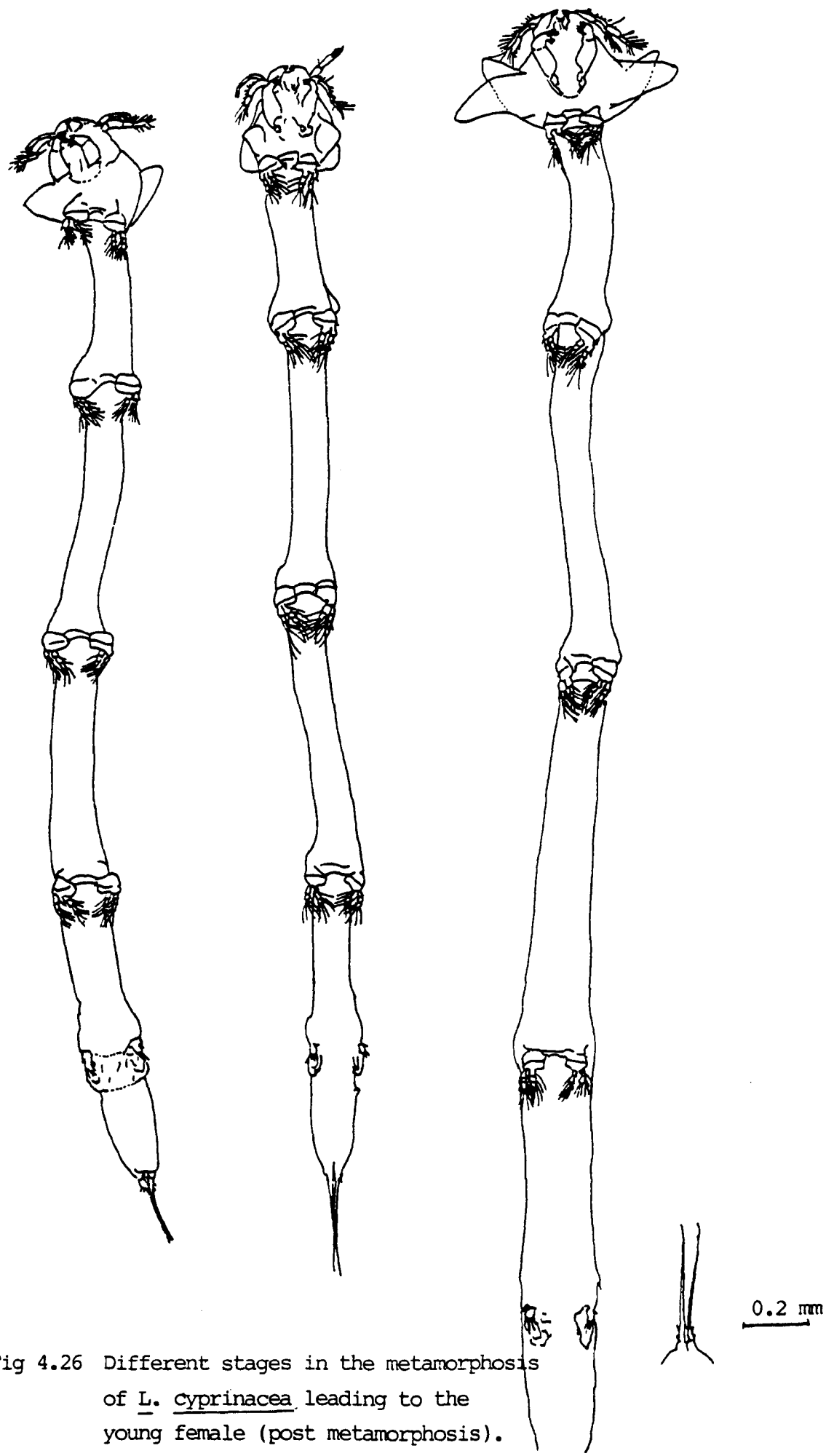


Fig 4.26 Different stages in the metamorphosis of L. cyprinacea leading to the young female (post metamorphosis).

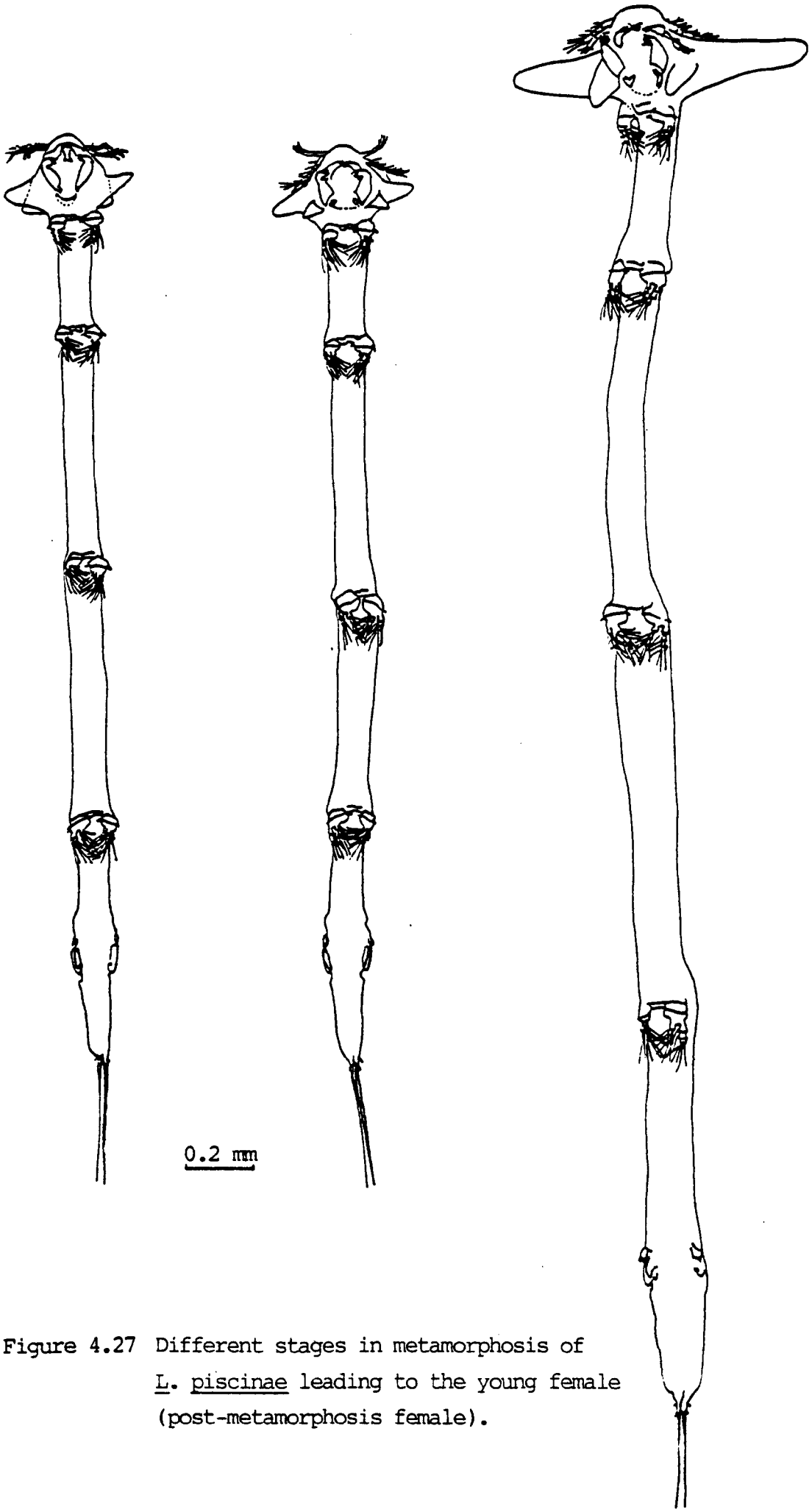


Figure 4.27 Different stages in metamorphosis of L. piscinae leading to the young female (post-metamorphosis female).

Table 4.3

Comparison of the measurements (at 5% level) of different characteristics of L. piscinae from A. nobilis and L. cyprinacea from A. nobilis and C. auratus. All values in micron and values in parenthesis indicate standard error of mean.

Characteristics Parasite sp. & Host Larval Stage	Total length			Body length			Furca			Cephalothorax width		
	GF L. cyp.	BHC L. cyp.	BHC L. pis.	GF L. cyp.	BHC L. cyp.	BHC L. pis.	GF L. cyp.	BHC L. cyp.	BHC L. pis.	GF L. cyp.	BHC L. cyp.	BHC L. pis.
Female cyclopoid	1.530 ^{a} (0.033)	1.313 ^{b} (0.034)	1.411 ^{c} (0.015)	1.157 ^{a} (0.031)	0.961 ^{b} (0.029)	1.043 ^{c} (0.015)	0.372 ^{a} (0.006)	0.351 ^{b} (0.007)	0.368 ^{ab} (0.004)	0.302 ^{a} (0.004)	0.272 ^{b} (0.004)	0.258 ^{c} (0.003)
Male cyclopoid	1.265 ^{a} (0.018)	1.126 ^{b} (0.011)	1.284 ^{a} (0.012)	0.858 ^{a} (0.018)	0.742 ^{b} (0.010)	0.874 ^{a} (0.008)	0.404 ^{a} (0.002)	0.384 ^{b} (0.004)	0.410 ^{a} (0.004)	0.247 ^{a} (0.002)	0.246 ^{a} (0.005)	0.249 ^{a} (0.002)
Female 5th. copepod	1.208 ^{a} (0.013)	1.105 ^{b} (0.013)	1.212 ^{a} (0.015)	0.895 ^{a} (0.013)	0.809 ^{b} 0.011	0. ^{a} (0.015)	0.313 ^{a} (0.002)	0.296 ^{b} (0.001)	0.312 ^{a} (0.002)	0.281 ^{a} (0.004)	0.259 ^{b} (0.004)	0.241 ^{c} (0.005)
Male 5th. copepod	1.066 ^{a} (0.011)	0.955 ^{b} (0.019)	1.093 ^{a} (0.010)	0.772 ^{a} (0.008)	0.662 ^{b} (0.016)	0.792 ^{a} (0.010)	0.292 ^{a} (0.006)	0.291 ^{a} (0.007)	0.301 ^{a} (0.002)	0.231 ^{a} (0.002)	0.221 ^{ab} (0.005)	0.219 ^{b} (0.003)
4th. copepodid	0.915 ^{a} (0.015)	0.875 ^{a} (0.015)	0.969 ^{b} (0.014)	0.664 ^{a} (0.013)	0.622 ^{b} (0.013)	0.701 ^{c} (0.013)	0.250 ^{a} (0.004)	0.253 ^{a} (0.002)	0.267 ^{b} (0.003)	0.221 ^{a} (0.004)	0.217 ^{ab} (0.003)	0.210 ^{b} (0.003)
3rd. copepodid	0.734 ^{a} (0.004)	0.690 ^{b} (0.009)	0.783 ^{c} (0.012)	0.511 ^{a} (0.009)	0.479 ^{b} (0.009)	0.554 ^{c} (0.012)	0.222 ^{a} (0.004)	0.211 ^{b} (0.004)	0.235 ^{c} (0.001)	0.185 ^{a} (0.002)	0.195 ^{a} (0.016)	0.171 ^{a} (0.003)
2nd. copepodid	0.598 ^{a} (0.007)	0.626 ^{b} (0.007)	0.663 ^{c} (0.003)	0.420 ^{a} (0.006)	0.436 ^{b} (0.005)	0.474 ^{c} (0.004)	0.178 ^{a} (0.001)	0.190 ^{b} (0.002)	0.189 ^{b} (0.002)	0.152 ^{a} (0.002)	0.151 ^{a} (0.003)	0.145 ^{a} (0.002)
1st. copepodid	0.488 ^{a} (0.005)	0.496 ^{a} (0.003)	0.495 ^{a} (0.005)	0.327 ^{a} (0.004)	0.337 ^{a} (0.003)	0.324 ^{a} (0.005)	0.161 ^{a} (0.006)	0.159 ^{a} (0.002)	0.171 ^{a} (0.002)	0.120 ^{a} (0.001)	0.115 ^{a} (0.002)	0.115 ^{a} (0.001)

Cephalothorax length			Genital Segment length			Genital Segment width			Number of bristles and spines on antenulae			Length of antennulae		
GF L. cyp.	BHC L. cyp.	BHC L.pis	GF L. cyp.	BHC L. cyp.	BHC L. pis.	GF L. cyp.	BHC L. cyp.	BHC L. pis.	GF L. cyp.	BHC L. cyp.	BHC L. pi	GF L. cyp.	BHC L. cyp.	BHC L. pis.
0.359 ^{a} (0.005)	0.331 ^{b} (0.008)	0.312 ^{c} (0.002)	0.10 ^{a} (0.00)	0.09 ^{a} (0.00)	0.09 ^{a} (0.000	0.182 ^{a} (0.004)	0.190 ^{a} (0.024)	0.166 ^{a} (0.001)	27.0 ^{a} (0.2)	26.7 ^{a} (0.3)	26.7 ^{a} (0.1)	0.266 ^{a} (0.003)	0.251 ^{b} (0.007)	0.249 ^{b} (0.000)
0.280 ^{a} (0.002)	0.263 ^{b} (0.002)	0.290 ^{c} (0.002)	0.08 ^{a} (0.00)	0.08 ^{a} (0.00)	0.09 ^{b} (0.00)	0.109 ^{a} (0.002)	0.110 ^{a} (0.007)	0.111 ^{a} (0.001)	38.4 ^{a} (0.2)	38.1 ^{a} (0.3)	38.0 ^{a} (0.1)	0.266 ^{a} (0.007)	0.259 ^{a} (0.002)	0.281 ^{b} (0.001)
0.319 ^{a} (0.004)	0.287 ^{b} (0.002)	0.303 ^{c} (0.002)	0.08 ^{a} (0.08)	0.07 ^{a} (0.00)	0.09 ^{b} (0.00)	0.135 ^{a} (0.002)	0.120 ^{b} (0.002)	0.110 ^{b} (0.002)	24.5 ^{a} (1.2)	26.4 ^{a} (0.01)	25.9 ^{a} (0.1)	0.239 ^{a} (0.002)	0.228 ^{b} (0.001)	0.240 ^{a} (0.003)
0.273 ^{a} (0.003)	0.243 ^{b} (0.006)	0.277 ^{a} (0.002)	0.07 ^{a} (0.00)	0.09 ^{a} (0.02)	0.08 ^{a} (0.001)	0.092 ^{a} (0.001)	0.091 ^{a} (0.001)	0.093 ^{a} (0.001)	35.1 ^{a} (0.3)	34.6 ^{a} (0.3)	34.5 ^{a} (0.3)	0.247 ^{a} (0.002)	0.228 ^{b} (0.002)	0.235 ^{a} (0.001)
0.241 ^{a} (0.007)	0.240 ^{a} (0.004)	0.258 ^{b} (0.004)	0.08 ^{a} (0.02)	0.08 ^{a} (0.02)	0.09 ^{a} (0.02)	0.075 ^{a} (0.022)	0.074 ^{a} (0.001)	0.078 ^{a} (0.016)	25.9 ^{a} (0.3)	25.4 ^{a} (0.3)	24.5 ^{b} (0.1)	0.195 ^{a} (0.001)	0.185 ^{b} (0.003)	0.197 ^{a} (0.003)
0.210 ^{a} (0.003)	0.196 ^{b} (0.003)	0.217 ^{a} (0.002)							21.3 ^{a} (0.2)	21.7 ^{ab} (0.2)	21.9 ^{b} (0.2)	0.157 ^{a} (0.001)	0.151 ^{b} (0.001)	0.154 ^{ab} (0.001)
0.181 ^{a} (0.002)	0.185 ^{a} (0.001)	0.194 ^{b} (0.001)							16.7 ^{a} (0.3)	16.2 ^{ab} (0.2)	16.1 ^{b} (0.1)	0.128 ^{a} (0.001)	0.130 ^{a} (0.001)	0.126 ^{a} (0.001)
0.154 ^{a} (0.003)	0.155 ^{a} (0.001)	0.152 ^{a} (0.001)							13.8 ^{a} (0.1)	13.0 ^{b} (0.0)	13.8 ^{a} (0.3)	0.097 ^{a} (0.001)	0.103 ^{b} (0.001)	0.100 ^{c} (0.000)

GF - Gold Fish (C. auratus).
BHC - Big Head Carp (A. nobilis)
L. cyp. - L. cyprinacea
L. pis. - L. piscinae

Values with different letters indicates they are significantly different from each other. Values with letters in common are not significantly different.

There were no consistent differences seen in any of the parameters measured throughout the various developmental stages amongst the 3 host-parasite systems. In addition, there were no consistent differences that could separate the 2 parasite species, L. piscinae and L. cyprinacea of the 3 host-parasite systems throughout the different larval stages. However, there were significant differences noted amongst the female cyclopoid stages from the 3 host-parasite systems. These were evident for the following characteristics:-

a) Total length

b) Body length

c) The length and width of the cephalothorax

The differences were most consistent for the cephalothorax length and a significant difference was noted even at the 5th. female and male cyclopoid stages.

The study of the morphometrics also revealed that there were no significant differences noted between the parasites L. piscinae from A. nobilis and L. cyprinacea from C. auratus at the following stages for the different characteristics noted below.

Characteristics	larval stage
a) Total length and b) Body length	male cyclopoid, 5th. female copepodid & 5th. male copepodid
c) Furcal length	5th. copepodid male & male cyclopoid

d)	Cephalothorax Length	5th. copepodid male & 3rd. copepodid
e)	Length of antennae	5th. copepodid male & 5th copepodid female

L. cyprinacea from A. nobilis and C. auratus were found to be similar at the following stages for the different characteristics but they differed significantly with L. piscinae from A. nobilis for these characteristics.

	Characteristics	Larval stages
a)	Total length	4th. copepodid
b)	Furcal length	4th. copepodid
c)	Cephalothorax length	2nd. copepodid & 4th. copepodid
d)	Genital segment length	5th. female copepodid & male cyclopoid
e)	Number of bristles	4th. copepodid
f)	Length of antennae	cyclopoid male

The width of the genital segment showed no significant differences at any larval stage within the 3 host-parasite systems. The total number of bristles was the same for all stages except for the 5th. male and female copepodid stage and for the male and female cyclopoid stages. There were similarities noted for the other characteristics among the 3 host-parasite systems (Table 4.3) but these were only

evident for 1 or 2 larval stages and thus a consistent pattern did not emerge.

4.3.3. Morphology of the adult female parasite.

4.3.3.1. Morphology of the adult female parasite from *A. nobilis* and *C. auratus* infected with *L. cyprinacea*("Asian" form)

There were 2 forms of *Lernaea* identified from *C. auratus* in the present study which were infected with *L. cyprinacea* of the Asiatic type. These were *L. cyprinacea* identical to the maternal form and *L. ctenopharyngodonis* in the proportion of 32 % and 68 % respectively.

L. cyprinacea of the Asiatic type had the dorsal arm of the anchor giving a Y or T shape and was divided into 2 branches at some distance from the base, the posterior process and the anterior process (Fig 4.28). The anterior process was larger than the posterior process. The 2nd. pair of arms, the ventral horns were unbranched and grew outwards and away from the base of the cephalothorax. The cephalothorax was prominently seen as a small semispherical structure situated in the middle of the dorsal and ventral horns.

The other form identified from *C. auratus* was identical with the description given for *L. ctenopharyngodonis* by Poddubnaya (1973). It was similar to *L. cyprinacea* described above but in addition it possessed a pair of rudimentary lobes, one on each side of the cephalothorax

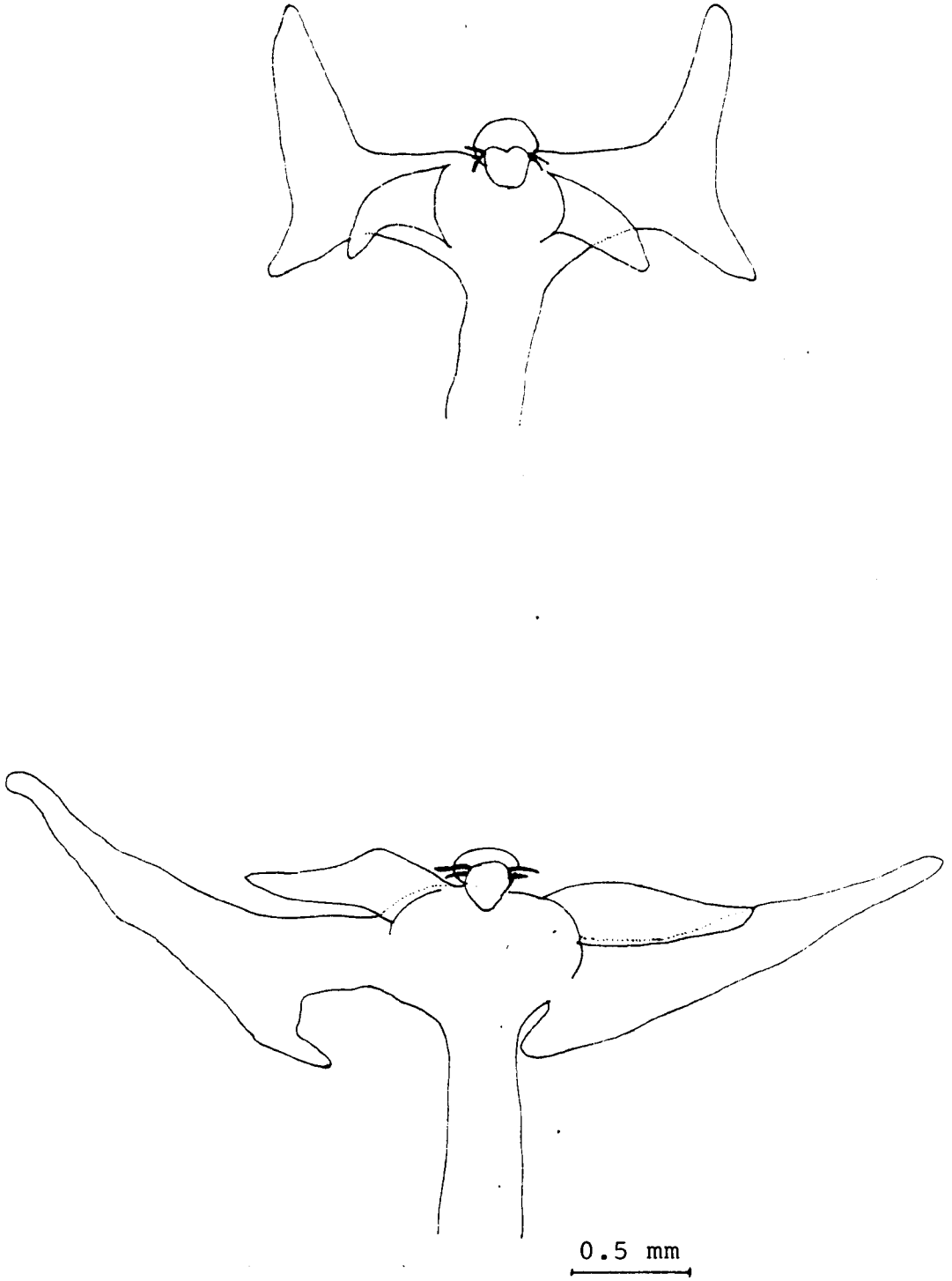


Figure 4.28 Forms of L. cyprinacea "Asian" from C. auratus

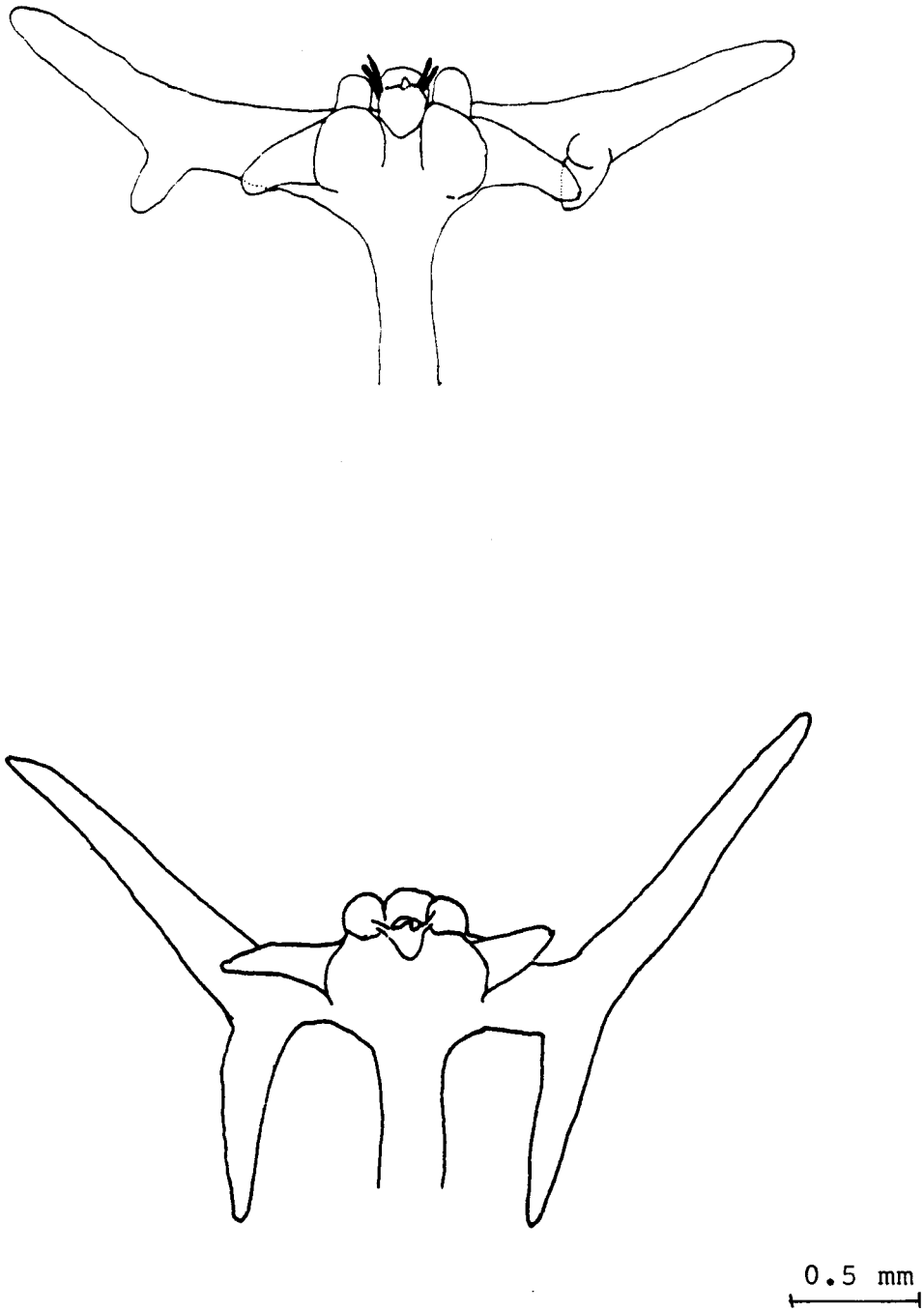


Figure 4.29 Forms of L. ctenopharyngodonis from C. auratus. Note the rudimentary lobes arising from the cephalothorax

(Fig. 4.29). On some specimens the dorsal horn and its anterior and posterior processes were more slender and longer compared to the majority of specimens (Fig. 4.29)

There was only one form of Lernaea identified from A. nobilis and it was L. cyprinacea "Asian" form and thus identical to the maternal form. As described earlier, it was seen to possess both the Y and T shape dorsal arms (Fig. 4.30).

Along with the normal forms of L. cyprinacea described above, abnormal forms of the parasites were obtained from both host species. The abnormal forms from C. auratus were noted to have a double process on the ventral horn (Fig. 4.31), rudimentary posterior processess of the dorsal horns (Fig. 4.31); with unequal proportion of ventral and dorsal horn with its processes. Similar abnormal forms were also noted from A. nobilis (Fig. 4.32).

The abnormal forms from C. auratus represented 38 % from a total of 120 parasites and in A. nobilis the the abnormal forms represented 17% from a total of 140 parasites. In C. auratus 46 % of the abnormal forms were from the caudal peduncle, fins and head and representing regions 5, 6, 7 and only 32% from the body proper (region 1-4). In A. nobilis 36% of the abnormal forms were from the caudal peduncle, fins, head and the eyes (representing regions 5, 6, 7 and 8) and 12.5% located on the body proper (region 1-4).

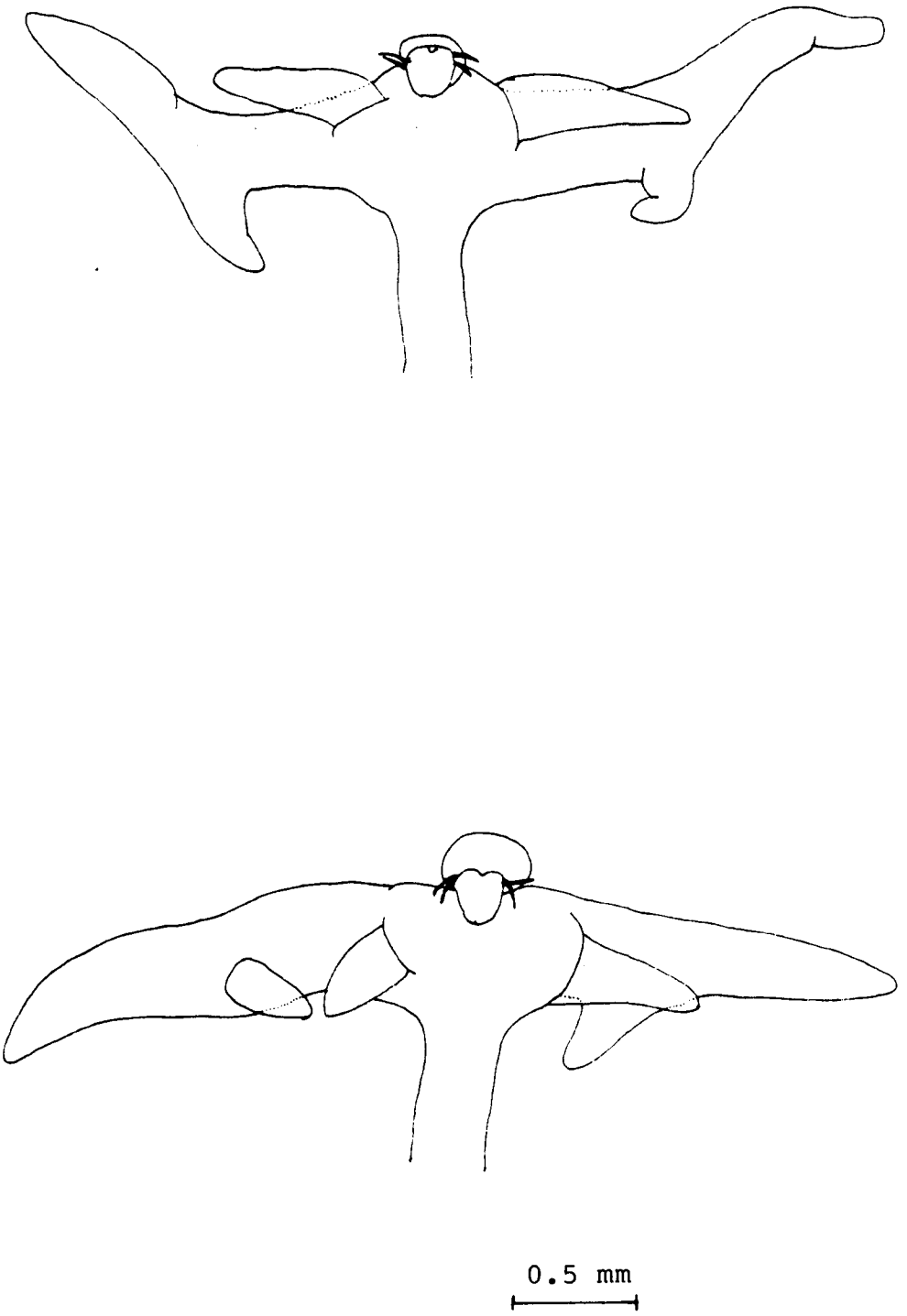


Figure 4.30 Forms of L. cyprinacea "Asian" from A. nobilis

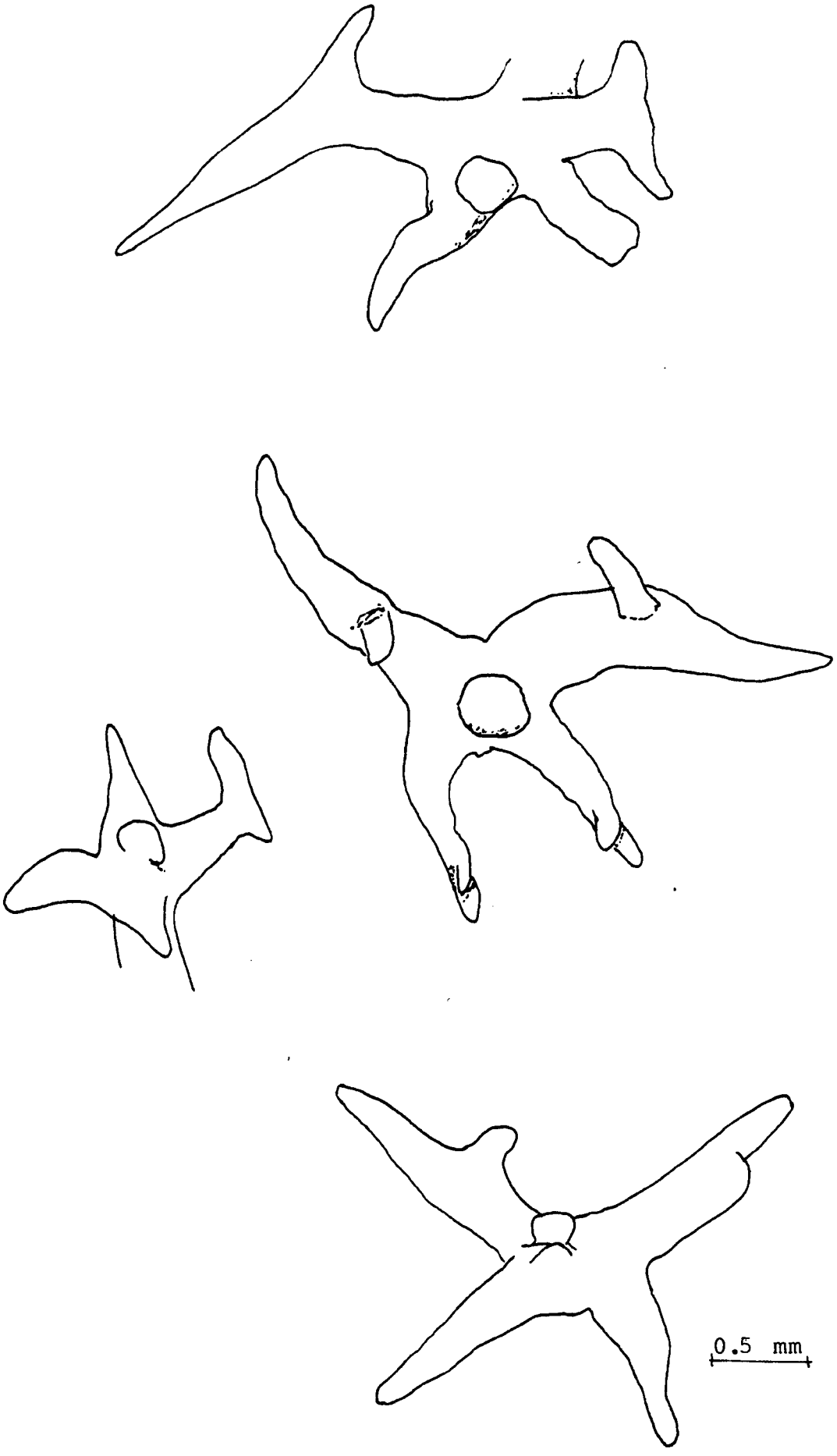


Figure 4.31 Abnormal forms of L. cyprinacea from C. auratus

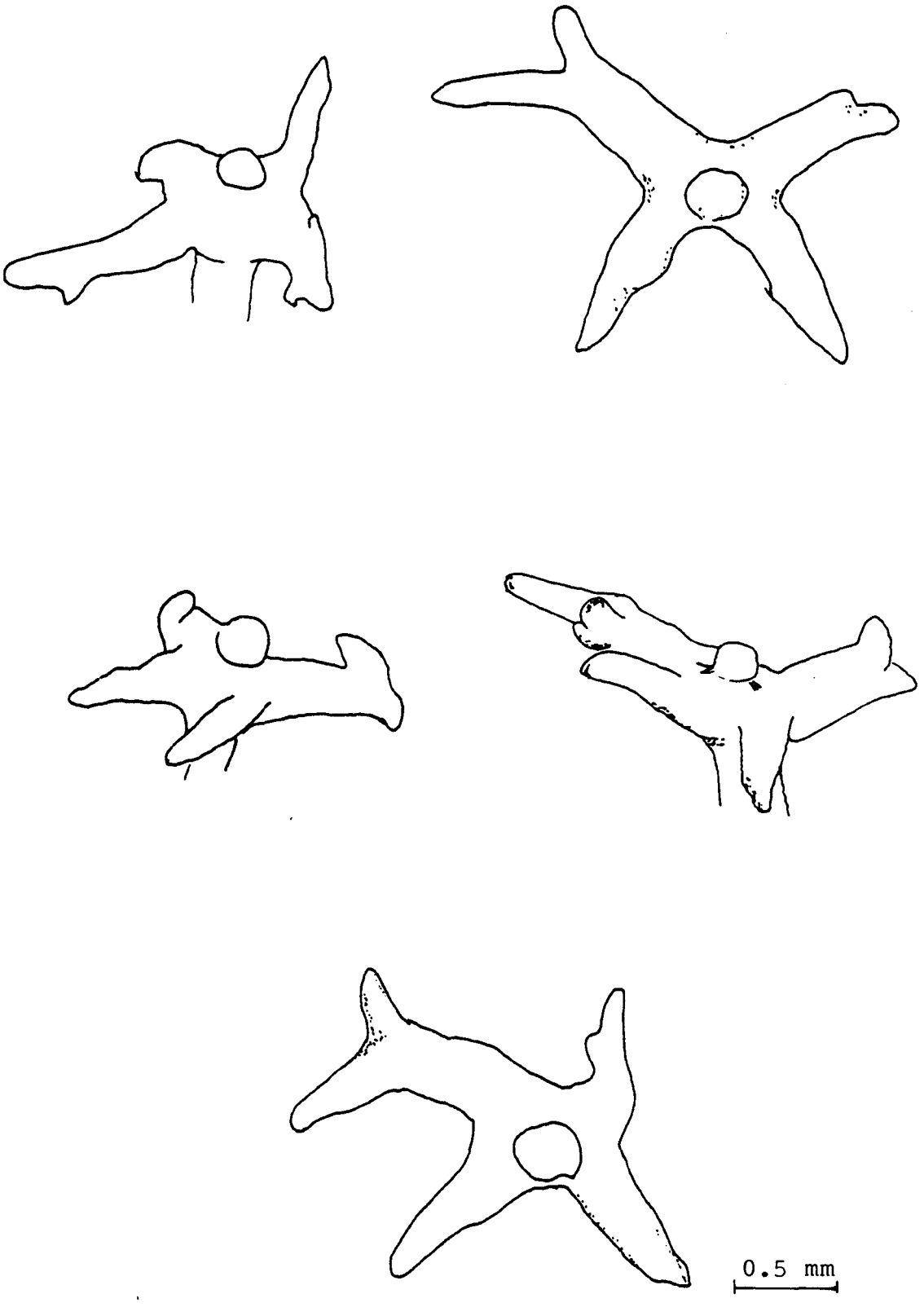


Figure 4.32 Abnormal forms of L. cyprinacea from A. nobilis

4.3.3.2. Morphology of the adult female L. piscinae from A. nobilis.

The parasites collected from A. nobilis infected with L. piscinae did not reveal any morphological changes from the maternal form. The parasite possessed a typical T shaped bar of the dorsal horns and the posterior processes were perpendicular to the dorsal horn (Fig. 4.33). The ventral horns were relatively shorter compared to L. cyprinacea described earlier, and they grew facing each other forming an acute angle.

In L. piscinae the abnormal forms were also found to have rudimentary dorsal horns (Fig. 4.34) or the posterior process was absent on one or both sides of the dorsal horns. An extra process on the dorsal horn was also seen in some specimens. The total number of abnormal types represented 19% from a total of 120 parasites. The abnormal types found from body region 1-4 represented 16% and 21.5% were collected from the caudal peduncle, fins and the head regions (5, 6 and 7).

4.3.4. Morphometrics of the adult female L. piscinae from A. nobilis and L. cyprinacea from C. auratus and A. nobilis in relation to site of attachment and penetration.

Inspite of the variations in the morpha forms of L. cyprinacea it was considered as a single species for the study of morphometrics.

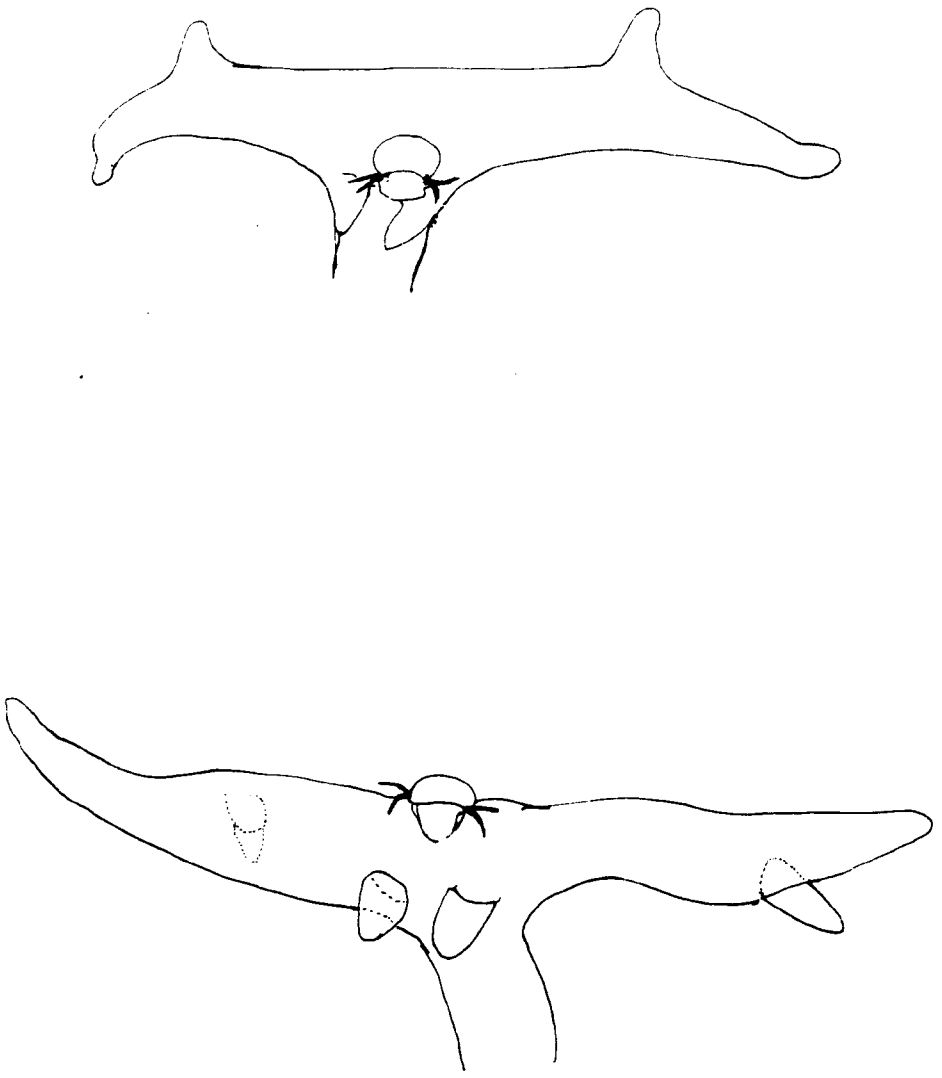


Figure 4.33 L. piscinae from A. nobilis

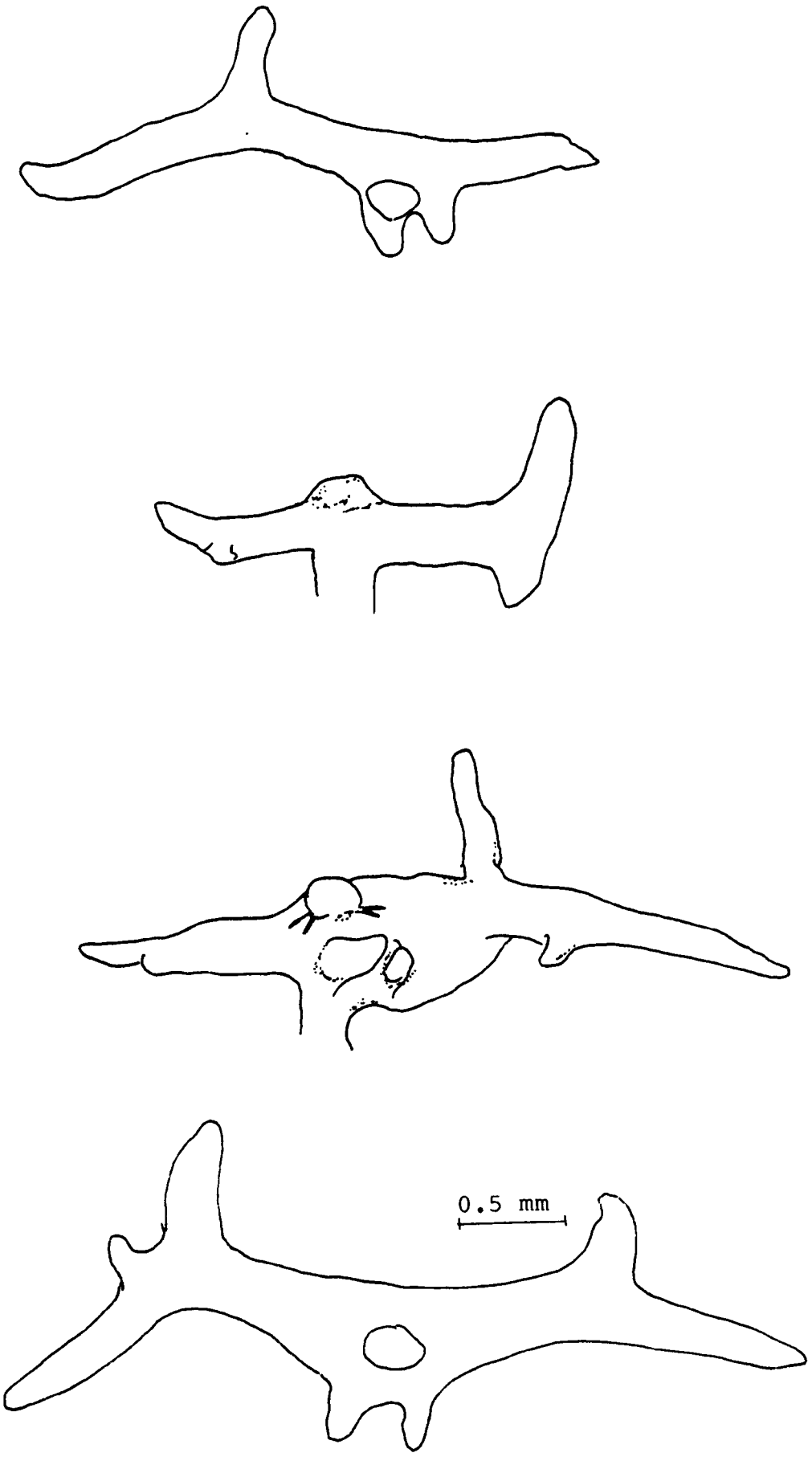


Figure 4.34 Abnormal forms of L. piscinae from A. nobilis

4.3.4.1. Dorsal horn ('D')

Table 4.4 shows the results of the morphometrics of the dorsal horn. Both the left and right dorsal horns of L. cyprinacea from A. nobilis were significantly different when the parasites were obtained from the left and right sides of the host body proper. There were significant differences in L. cyprinacea from C. auratus and A. nobilis when the mean of the pooled ('D'1+'D'2)/2 values for the length of the left and right sections of the dorsal horns were compared from the left and right side of host. There was no difference between any of the values measured for the dorsal horns of L. cyprinacea from C. auratus and L. piscinae from A. nobilis.

The mean length of the dorsal horns in parasites from the body proper differed significantly between the 3 host-parasite systems; in the descending order L. piscinae had the longest dorsal horn followed by L. cyprinacea of C. auratus and L. cyprinacea of A. nobilis (Table 4.4).

Table 4.5 contains the comparisons of the different parts of the cephalic processes. The first part shows the comparison of the length of the dorsal horn of parasites obtained from the different regions of the body proper (regions 1-4). From the table it can be seen that no significant differences could be demonstrated between the lengths of the dorsal horn from the body regions of any of the 3 host-parasite systems (Table 4.5). Similarly, parasites from the regions 5, 6, 7 and 8 were indistinguishable statistically with the exception of L. cyprinacea from C. auratus as shown in Table 4.6 which was very variable.

When the values for the dorsal horn length from the body proper (regions 1-4) were pooled and the mean compared to those from regions 5-8 pooled, there were significant differences between parasites (Table 4.7). These differences were consistent in all the 3 host-parasite systems.

4.3.4.2. Distance between mid-body of the parasite and the process of dorsal horn ('Y')

Table 4.8 shows the results on the study of the morphometrics of the distance between the mid-body of the parasite and the process of dorsal horn indicated as 'Y' value in Fig. 4.3. There were significant differences in the 'Y' value for the right hand section of L. piscinae when obtained from the left and right side of the host's body proper. However the differences were significant in both L. cyprinacea and L. piscinae when the mean of the pooled values of the left and right sections of 'Y' were compared from the left and right side of A. nobilis.

Among the 3 host-parasite systems, 'Y' values were similar for L. cyprinacea from A. nobilis and those from C. auratus and these both differed significantly from L. piscinae from A. nobilis (Table 4.8). The value of 'Y' for L. piscinae was bigger than for L. cyprinacea from both C. auratus and A. nobilis.

The 'Y' values for L. cyprinacea taken from regions 1-4 differed significantly even for parasites on the same host species. For example L. cyprinacea from region 1 were significantly larger than those from region 2 on A. nobilis (Table 4.5). L. cyprinacea from C. auratus and L. piscinae

from A. nobilis showed variations with regions 5, 6, 7 and 8. In L. cyprinacea the parasites from regions 5 & 7 differed significantly from all those from region 6 while in L. piscinae the variation was noted in the parasites from regions 5, 6, and 7 (Table 4.6).

Comparison of the 'Y' values between parasites of the body proper (pooled regions 1-4) and those from the caudal peduncle, fins and head (pooled regions 5, 6 and 7), showed significant differences for L. cyprinacea from C. auratus and also L. piscinae from A. nobilis (Table 4.7).

4.3.4.3. Length of the process of the dorsal horn ('W').

The results of the analysis of the measurements of 'W' are presented in Table 4.9. The left section of 'W' from the right side of the host did not differ significantly from 'W' from the left side of the host but when the values of 'W' from the left and right section were pooled and the mean values compared, they were found to be significantly different in L. cyprinacea from A. nobilis (Table 4.9). The length of the process of the dorsal horns was different among the 3 host-parasite system. In the descending order, the length of the process of the dorsal horn was greater in L. cyprinacea from C. auratus, followed by that of L. piscinae of A. nobilis and L. cyprinacea of A. nobilis.

The value of the 'W', for L. cyprinacea taken from regions of the body proper (regions 1-4) of A. nobilis, were found to be significantly different from the same value for L. cyprinacea from C. auratus; and in both these cases the regions 1, 3 and 4 differed significantly with those from regions 2 (Table 4.5). The value of 'W' for parasites

from regions 5, 6, 7 & 8 was found to vary for L. cyprinacea from C. auratus and for L. piscinae from A. nobilis (Table 4.6). In the former, the value of 'W' for L. cyprinacea from C. auratus, regions 5 and 6 varied significantly from those of region 7. In L. piscinae, variation was present in parasites from regions 5 and 7 compared to those from region 6.

The mean of pooled values of 'W' for parasites obtained from regions 1-4 differed significantly with the mean of pooled values of those from regions 6-8 in all 3 host-parasite systems (Table 4.7).

4.3.4.4. Length of ventral horn ('V')

The length of the ventral horn ('V') in L. cyprinacea from A. nobilis varied when they were obtained from the left and right side of the host body proper (Table 4.10). This was true for the left and right value of 'V' ('V'1 and 'V'2). The mean values of 'V' ('V'1+'V'2)/2 were significantly different in L. cyprinacea from A. nobilis when comparisons were made between parasites from the left and right sides of the host body. Considering the 3 host-parasite systems, the length of the ventral horn was greatest in L. cyprinacea from A. nobilis followed by that of L. cyprinacea from C. auratus and these both differed significantly with the smaller ventral horns of L. piscinae from A. nobilis.

The value of 'V' from regions 1-4, was found to vary for L. cyprinacea and L. piscinae from A. nobilis (Table 4.5). In L. cyprinacea the length of the ventral horns were only similar in parasites of regions 3 and 4, whereas in

L. piscinae the ventral horns of regions 1, 2 and4 differed significantly with the parasites from region 3.

The variation was found in L. cyprinacea from C. auratus and L. piscinae from A. nobilis located in regions 5, 6, 7 and 8. In both cases, the parasites from regions 5 and 7 differed from those from region 6 (Table 4.6).

Comparison of the ventral horn of the parasites from the body proper with those from the caudal peduncle, fins, head and eyes revealed significant differences in L. cyprinacea of A. nobilis and also in L. piscinae from A. nobilis (Table 4.7).

4.3.4.5. Position of the swimming legs

The results of the morphometric studies of the position of swimming legs of parasites from the 3 host-parasite systems are shown in Tables 4.11, 4.12, 4.13 and 4.14.

There was no significant difference in the position of the swimming legs when comparisons were made between parasites obtained from the left and right sides of the host body proper (Table 4.11). However there was variation seen when the position of the legs was compared between the parasites of the 3 host-parasite systems. The variations were seen in Legs 1 to 5. The position of Legs 1 and 4 varied between all the 3 host-parasite systems, whereas Legs 2 and 3 were found to be similar amongst L. cyprinacea from C. auratus and A. nobilis but they differed from those of L. piscinae from A. nobilis. The position of Leg 5 of L. cyprinacea from C. auratus was similar to that of

L. piscinae and they both differed from those of L. cyprinacea from A. nobilis.

In the region of the body proper, variation in the position of the swimming legs was only noted in Legs 1 and 4. In Leg 1 , the variation was found in L. cyprinacea of A. nobilis and L. piscinae of A. nobilis (Table 4.12). In L. cyprinacea the position of the legs of the parasites from regions 1, 2 and 3 differed significantly from those of region 4, whilst in L. piscinae regions 1 and 3 differed from those of 2 and 4. In L. piscinae from A. nobilis, parasites from regions 1 and 3 differed from those of regions 2 and 4.

The position of the swimming legs of parasites from regions 5, 6, 7 and 8 (caudal peduncle, fins, head and eyes), varied in Leg 1 and Leg 2 of L. cyprinacea from A. nobilis. Leg 3 differed in all the 3 host-parasite systems (Table 4.13). The position of Leg 1 of L. cyprinacea from A. nobilis, differed significantly in parasites from region 5 and 6 with those from 7 and 8, but in leg 2, the parasites from regions 8 differed significantly with those from regions 5, 6 and 7. In L. cyprinacea from C. auratus, variation in the position of Leg 3 was found in parasites from region 5 when compared with those from region 6 and 7. In L. cyprinacea from A. nobilis, the difference was between parasites from region 8 and those from region 5, 6, and 7. In L. piscinae, a difference was seen between parasites from region 7 and those from region 5 and 6.

Position of Legs 2 and 4 of L. cyprinacea from A. nobilis and Leg 4 of L. piscinae of A. nobilis were found to be significantly different between parasites from region 1-4 and those from regions 5, 6, 7 and 8 (Table 4.14).

4.3.4.6. Total length

Among the body proper regions, there were significant differences in the total length of L. cyprinacea from A. nobilis, parasites from region 2 differed significantly from those of regions 1, 3 and 4, whereas those from region 4 varied from region 3 (Table 4.15). The values for total length of parasites from regions 5, 6 and 7 differed in L. cyprinacea from C. auratus; parasites from regions 5 and 6 differed significantly from region 7 (Table 4.16).

Significant differences in the total length were seen in L. cyprinacea from A. nobilis when parasites were compared for the left and right side of the host (Table 4.17).

The total length of parasite did not show any significant differences amongst the 3 host-parasite systems.

Comparison of the total length between parasites of the body proper and those from the caudal peduncle, fins and head showed significant differences in L. piscinae from A. nobilis; parasites from region 6 differed significantly from those of region 7 (Table 4.18).

4.3.4.7. Summary of morphometric studies on the adult female parasites

The left or right sections of the cephalic processes ('D', 'Y', 'V' and 'W') were found to be significantly different in parasites obtained from the left and right sides of the host in some cases; these differences were also noted when the mean of pooled value of the left and right section was compared in parasites from the left and right side of the host.

Table 4.4

Comparison of the length of dorsal horn 'D' in relation to its position on the parasite and on the host.

	Comparison of D1 from left and right side of host				Comparison of D2 from left and right side of host				Comparison of D from the left and right side of host (D1+D2)/2				Comparison of D between host		
	left	right	T value	Prob.	left	right	T value	Prob	Left	Right	T value	Prob	Between Host	F value	Prob.
Host/parasite	n=40	n=30			n=40	n=30			n=80	n=60			5		
C. auratus/	17.66	18.74			17.01	18.34			17.34	18.54			17.94 [a]		
			1.36	>0.18			1.65	>0.01			2.14*	<0.03			
L. cyp.	(0.52)	(0.59)			(0.52)	(0.62)			(0.37	(0.42)			(0.28)		
A. nobilis/	13.77	15.80			14.08	15.64			13.92	15.72			14.82 [b]	88.24*	<0.00
			2.58*	<0.01			2.01*	<0.07			3.13*	<0.00			
L. cyp.	(0.46)	(0.59)			(0.47)	(0.68)			(0.33)	(0.42)			(0.28)		
A. nobilis/	19.50	20.24			19.16	19.92			19.33	20.08			19.70 [c]		
			1.04	>0.30			1.0	>0.33			1.31	>0.19			
L. pis.	(0.33)	(0.55)			(0.45)	(0.59)			(0.28)	(0.40)			(0.24)		

D1 - Left section of dorsal horn
D2 - Right section of dorsal horn
* - Indicates significant difference at probability level shown.
Values with different letters indicates they are significantly different from each other.
Values with letters in common are not significantly different.
Value in parenthesis indicates standard error of mean.

Table 4.5

Comparison of the different parts of the cephalic processes of Lernaea obtained from the different regions of body proper (regions 1-4).

Cephalic processes	Host-parasite	Regions				F value	DF
		1	2	3	4		
Length of dorsal horn 'D'	C. auratus/L. cyp.	18.32 (0.69)	18.12 (0.63)	17.10 (0.42)	18.06 (0.42)	0.97	156
	A. nobilis/L. cyp.	15.02 (0.49)	13.73 (0.49)	15.35 (0.47)	15.18 (0.71)	1.81	156
	A. nobilis/L. pis.	19.92 (0.54)	19.66 (0.44)	19.62 (0.45)	19.62 (0.49)	0.31	156
Distance between mid-body and the processes of dorsal horn 'Y'	C. auratus/L. cyp.	9.15 (0.49)	9.30 (0.39)	9.07 (0.25)	8.95 (0.40)	1.21	156
	A. nobilis/L. cyp.	9.29 [a] (0.27)	7.67 [b] (0.39)	8.94 [a] (0.24)	8.84 [a] (0.26)	5.68*	156
	A. nobilis/L. pis.	11.47 (0.26)	11.47 (0.27)	11.48 (0.30)	11.64 (0.20)	0.10	156
Length of the process of dorsal horn 'W'	C. auratus/L. cyp.	4.20 [a] (0.40)	6.21 [b] (0.41)	4.07 [a] (0.23)	4.47 [a] (0.30)	8.77*	156
	A. nobilis/L. cyp.	3.52 [a] (0.28)	2.56 [b] (0.26)	3.64 [a] (0.29)	3.36 [a] (0.26)	3.24*	156
	A. nobilis/L. pis.	3.53 (0.28)	4.01 (0.21)	3.46 (0.32)	3.60 (0.16)	0.95	156
Length of the ventral horn 'V'	C. auratus/L. cyp.	11.47 (0.66)	11.49 (0.44)	11.76 (0.30)	11.14 (0.47)	0.36	156
	A. nobilis/L. cyp.	12.97 [a] (0.38)	11.12 [b] (0.66)	13.28 [c] (0.27)	11.07 [c] (0.63)	5.27*	156
	A. nobilis/L. pis.	6.49 [a] (0.26)	6.04 [a] (0.15)	7.53 [b] (0.21)	7.59 [ab] (0.23)	8.68*	156

* - Indicates significant difference at 5% level.
Values with different letters indicates they are significantly different than each other. Values with letters in common are not significantly different. Values in parenthesis indicate standard error of mean.

Table 4.6

Comparison of the different parts of the cephalic processes of Lernaea obtained from the caudal peduncle, fins, head and eyes (regions 5, 6, 7 & 8) from 3 host-parasite systems.

Cephalic processes	Host-parasite	Regions				F value	DF
		5	6	7	8		
Length of the dorsal horn 'D'	C. auratus/L. cyp.	15.09[a] (0.51)	13.95[b] (0.58)	17.40[c] (0.91)		6.23*	116
	A. nobilis/L. cyp.	14.32 (0.65)	13.68 (0.66)	13.49 (0.52)	14.21 (0.56)	0.34	96
	A. nobilis/L. pis.	19.33 (1.03)	18.26 (0.93)	17.98 (0.68)		0.64	57
Distance between mid-body and process of dorsal horn 'Y'	C. auratus/L. cyp.	8.67[a] (0.45)	7.60[b] (0.37)	9.73[a] (0.40)		5.24*	116
	A. nobilis/L. cyp.	9.18 (0.34)	8.02 (0.43)	8.71 (0.35)	9.22 (0.77)	1.71	96
	A. nobilis/L. pis.	11.08[a] (1.24)	12.53[b] (0.68)	10.77[c] (0.92)		5.29*	57
Length of process of dorsal horn 'W'	C. auratus/L. cyp.	4.19[a] (0.29)	3.76[a] (0.31)	5.21[b] (0.50)		3.60*	116
	A. nobilis/L. cyp.	2.72 (0.33)	3.01 (0.34)	2.69 (0.40)	1.77 (0.24)	2.22	96
	A. nobilis/L. pis.	2.15[a] (0.46)	3.80[b] (0.55)	1.88[a] (0.23)		5.66*	57
Length of the ventral horn 'V'	C. auratus/L. cyp.	11.28[a] (0.41)	12.22[b] (0.46)	10.48[a] (0.45)		3.16*	116
	A. nobilis/L. cyp.	10.34 (0.43)	10.62 (0.44)	11.43 (0.59)	11.03 (0.56)	0.84	96
	A. nobilis/L. pis.	5.92[a] (0.24)	7.08[b] (0.26)	5.32[a] (0.36)		9.32*	57

*-Indicates significant difference at 5%
Values with different letters indicates they are significant different than each other. values with letters in common are not significantly different

Table 4.7

Comparisons of the different parts of the cephalic processes between parasites from body proper (regions 1-4) and the caudal peduncle, fins, head and eye (regions 5, 6, 7 & 8).

Cephalic processes	Host-parasite	regions 1-4	regions 5,6,7 & 8	T value	Prob.	DF
Length of the dorsal horn 'D'	C. auratus/L. cyp.	17.85 (0.28)	15.09 (0.37)	5.97*	<0.00*	238
	A. nobilis/L. cyp.	14.82 (0.28)	13.95 (0.33)	2.02	<0.04*	278
	A. nobilis/L. pis.	19.70 (0.24)	18.52 (0.51)	2.32	<0.2*	218
Distance bet. mid-body and processes of dorsal horn 'Y'	C. auratus/L. cyp.	9.10 (0.19)	8.46 (0.26)	2.04	<0.04*	238
	A. nobilis/L. cyp.	8.69 (0.15)	8.72 (0.23)	0.13	>0.90	278
	A. nobilis/L. pis.	11.52 (0.13)	10.37 (0.60)	2.71	<0.03*	238
Length of the process of dorsal horn 'W'	C. auratus/L. cyp.	4.81 (0.19)	4.22 (0.20)	2.09	<0.03*	238
	A. nobilis/L. cyp.	3.27 (0.13)	2.57 (0.18)	3.13	<0.00*	278
	A. nobilis/L. pis.	3.65 (0.12)	2.61 (0.27)	3.99	<0.00*	218
Length of the ventral horn 'V'	C. auratus/L. cyp.	11.46 (0.22)	11.49 (0.26)	0.09	>0.92	238
	A. nobilis/L. cyp.	12.11 (0.27)	10.73 (0.24)	3.69	<0.00*	278
	A. nobilis/L. pis.	6.91 (0.12)	6.11 (0.19)	3.35	<0.00*	218

* - indicates significant difference at probability level shown.
Values in parenthesis indicate standard error of mean.

Table 4.8

Comparison of distance between mid-body and the process of dorsal horn 'Y' in relation to its position on the parasite and on the host.

	Comparison of Y1 from left and right side of host				Comparison of Y2 from left and right side of host				Comparison of Y from the left and right side of host (Y1+Y2)/2				Comparison of Y between host		
	left	right	T value	Prob.	left	right	T value	Prob.	Left	Right	T value	Prob.	Between Host	F value	Prob.
Host/parasite	n=40	n=30			n=40	n=30			n=80	n=60					
C. auratus/	9.32	8.87			9.93	8.73			9.63	8.76			9.19 [a]		
			0.97	>0.33			1.18	>0.24			1.53	>0.13			
L. cyp.	(0.35)	(0.43)			(0.35)	(0.44)			(0.24)	(0.31)			(0.19)		
A. nobilis/	8.26	9.09			8.32	9.07			8.29	9.09			8.69 [b]	95.71*	0.00
			1.84	>0.07			1.66	>0.11			2.62*	<0.00			
L. cyp.	(0.31)	(0.19)			(0.31)	(0.29)			(0.22)	(0.21)			(0.15)		
A. nobilis/	11.20	11.89			11.10	11.87			11.15	11.88			11.52 [c]		
			1.83	>0.07			2.04*	<0.04			2.76*	<0.00			
L. pis.	(0.20)	(0.30)			(0.20)	(0.31)			(0.14)	(0.21)			(0.13)		

Y1 - Left section of the distance between mid-body and the process of dorsal horn.
Y2 - Right section of the distance between mid-body and the process of dorsal horn.
* - Indicates significant difference at probability level shown.
Values with different letters indicates they are significantly different from each other.
Values with letters in common are not significantly different.
Value in parenthesis indicates standard error of mean.

Table 4.9

Comparison of the length of process of dorsal horn 'W' in relation to its position on the parasite and on the host.

	Comparison of W1 from left and right side of host				Comparison of W2 from left and right side of host				Comparison of W from the left and right side of host (W1+W2)/2				Comparison of W between host		
	left	right	T value	Prob.	left	right	T value	Prob.	Left	Right	T value	Prob.	Between Host	F value	Prob.
Host-parasite	n=40	n=30			n=40	n=30			n=80	n=60					
C. auratus/	4.74	4.98			4.59	5.03			4.67	5.01			4.84[a]		
			0.44	>0.66			0.82	>0.42			0.89	>0.37			
L. cyp.	S.E (0.34)	(0.45)			(0.32)	(0.45)			(0.23)	(0.32)			(0.18)		
A. nobilis/	3.07	3.43			3.19	3.40			3.13	3.42			3.27[b]	27.32*	<0.00
			0.86	>0.39			0.46	>0.65			0.94	>0.94			
L. cyp.	S.E (0.25)	(0.29)			(0.26)	(0.30)			(0.18)	(0.21)			(0.14)		
A. nobilis/	3.38	4.10			3.28	3.84			3.33	3.97			3.65[c]		
			2.26*	<0.29			1.60	>0.12			2.72*	<0.01			
L. pis.	S.E (0.24)	(0.24)			(0.23)	(0.27)			(0.17)	(0.18)			(0.12)		

W1 - Left section of the length of the process of dorsal horn.
W2 - Right section of the length of the process of dorsal horn.
* - Indicates significant difference at probability level shown.
Values with different letters indicates they are significantly different from each other.
Values with letters in common are not significantly different.
Value in parenthesis indicates standard error of mean.

Table 4.10

Comparison of the length of ventral horn 'V' in relation to its position on the parasite and on the host.

Host-parasite	Comparison of V1 from left and right side of host			Comparison of V2 from left and right side of host			Comparison of V from the left and right side of host (V1+V2)/2			Comparison of V between host		
	left n=40	right n=30	T value Prob.	left n=40	right n=30	T value Prob.	Left n=80	Right n=80	T value Prob.	Between Host	F value	Prob.
C. auratus/	11.62	11.32		11.55	11.27		11.59	11.30		11.46		
			0.47 >0.64			0.44 >0.66			0.64 >0.52			
L. cyp.	(0.44)	(0.46)		(0.44)	(0.45)		(0.31)	(0.32)		(0.22)		
A. nobilis/	11.41	12.95		11.14	12.95		11.28	12.95		12.11	184.82*	<0.00
			2.10* <0.04			2.45* <0.01			3.30* <0.00			
L. cyp.	(0.50)	(0.53)		(0.51)	(0.53)		(0.36)	(0.37)		(0.14)		
A. nobilis/	6.83	6.99		6.83	6.99		6.83	6.99		6.91		
			0.45 >0.65			0.45 >0.45			0.64 >0.52			
L. pis.	(0.25)	(0.22)		(0.26)	(0.22)		(0.18)	(0.16)		(0.12)		

V1 - Left section of ventral horn
V2 - Right section of ventral horn
* - Indicates significant difference at probability level shown.
Values with different letters indicates they are significantly different from each other.
Values with letters in common are not significantly different.
Value in parenthesis indicates standard error of mean.

Table 4.11

Comparison of the position of swimming legs of Lernaea obtained from the left and right side of body proper (region 1-4) from 3 host-parasite systems.

Leg	Host-parasite	Left	Right	T value	Prob.	DF	Between Host-parasite	F value	DF
1	C. auratus/L. cyp.	6.74 (0.18)	6.81 (0.17)	0.29	>0.78	68	6.76 [a] (0.12)		
	A. nobilis/L. cyp.	5.89 (0.18)	6.45 (0.17)	1.49	>0.14	78	6.18 [b] (0.13)	13.41*	227
	A. nobilis/L. pis.	5.77 (0.13)	6.06 (0.14)	1.42	>0.16	68	5.92 [c] (0.09)		
2	C. auratus/L. cyp.	17.23 (0.30)	17.63 (0.34)	0.89	>0.38	68	17.40 [a] (0.22)		
	A. nobilis/L. cyp.	17.21 (0.21)	16.69 (0.22)	1.59	>0.12	68	16.95 [a] (0.15)	115.63*	227
	A. nobilis/L. pis.	13.75 (0.21)	14.07 (0.21)	0.95	>0.35	78	13.91 [b] (0.15)		
3	C. auratus/L. cyp.	41.29 (0.36)	41.65 (0.37)	0.68	>0.50	68	41.44 [a] (0.25)		
	A. nobilis/L. cyp.	40.40 (0.41)	40.88 (0.38)	0.90	>0.37	40	40.94 [a] (0.28)	157.19*	227
	A. nobilis/L. pis.	35.68 (0.32)	35.47 (0.29)	0.45	>0.65	40	35.58 [b] (0.22)		
4	C. auratus/L. cyp.	71.25 (0.31)	71.81 (0.44)	1.08	>0.28	68	71.49 [a] (0.26)		
	A. nobilis/L. cyp.	69.92 (0.48)	70.50 (0.24)	1.02	>0.31	40	70.21 [b] (0.27)	12.18*	227
	A. nobilis/L. pis.	69.61 (0.44)	69.46 (0.41)	0.81	>0.42	68	69.53 [c] (0.30)		
5	C. auratus/L. cyp.	91.04 (0.18)	91.29 (0.25)	0.81	>0.42	68	91.14 [a] (0.14)		
	A. nobilis/L. cyp.	90.16 (0.45)	90.81 (0.13)	1.35	>0.18	40	90.49 [b] (0.23)	4.67*	227
	A. nobilis/L. pis.	91.38 (0.13)	90.97 (0.24)	1.59	>0.12	40	91.17 [a] (0.13)		

* - Indicates significant difference at 5% level.
Values in parenthesis indicate standard error of mean.

Table 4.12

Comparisons of the position of swimming legs of Lernaea obtained from the body proper (regions 1-4).

Leg	Host-parasite	Regions 1	2	3	4	F value	DF
1	C. auratus/L. cyp.	6.81 (0.37)	6.48 (0.24)	6.43 (0.19)	7.07 (0.24)	1.06	66
	A. nobilis/L. cyp.	6.34 [a] (0.24)	6.11 [a] (0.21)	6.74 [a] (0.21)	5.52 [b] (0.27)	4.68*	76
	A. nobilis/L. pis.	5.72 [a] (0.27)	6.19 [b] (0.12)	5.56 [a] (0.16)	6.21 [b] (0.17)	3.02*	76
2	C. auratus/L. cyp.	17.23 (0.37)	17.27 (0.45)	17.79 (0.50)	17.20 (0.40)	0.41	66
	A. nobilis/L. cyp.	17.09 (0.35)	17.30 (0.28)	17.04 (0.29)	16.37 (0.29)	1.75	76
	A. nobilis/L. pis.	13.77 (0.38)	14.14 (0.33)	13.71 (0.24)	14.03 (0.26)	0.43	76
3	C. auratus/L. cyp.	41.07 (0.58)	41.62 (0.49)	41.08 (0.41)	41.80 (0.58)	0.50	66
	A. nobilis/L. cyp.	40.34 (0.52)	41.38 (0.62)	40.83 (0.59)	40.00 (0.46)	1.19	76
	A. nobilis/L. pis.	35.90 (0.57)	34.90 (0.66)	35.72 (0.47)	35.77 (0.39)	1.07	76
4	C. auratus/L. cyp.	70.97 (0.54)	71.61 (0.54)	71.25 (0.30)	71.86 (0.61)	0.48	66
	A. nobilis/L. cyp.	69.74 (0.28)	70.93 (0.49)	70.16 (0.79)	70.02 (0.46)	0.48	76
	A. nobilis/L. pis.	70.60 [a] (0.87)	68.23 [b] (0.23)	69.69 [a] (0.63)	69.61 [a] (0.38)	2.80*	76
5	C. auratus/L. cyp.	91.25 (0.29)	91.00 (0.22)	91.16 (0.35)	91.20 (0.32)	0.13	66
	A. nobilis/L. cyp.	90.57 (0.16)	90.93 (0.19)	89.81 (0.83)	90.64 (0.36)	1.04	76
	A. nobilis/L. pis.	91.31 (0.25)	91.04 (0.26)	91.02 (0.35)	91.33 (0.24)	0.35	76

* - Indicates significant differences at 5% level.
Values with different letters indicates they are significantly different than each other. Values with letters in common are not significantly different.

Table 4.13

Comparison of the position of swimming legs of Lernaea obtained from the caudal peduncle, fins, head and eyes (regions 5, 6, 7 & 8) of 3 host-parasite systems.

Leg	Host-parasite	Regions 5	6	7	8	F value	DF
1	C. auratus/L. cyp.	6.97 (0.22)	6.32 (0.20)	7.38 (1.05)		1.51	47
	A. nobilis/L. cyp.	5.72 [a] (0.25)	5.84 [a] (0.24)	7.15 [b] (0.35)	7.81 [b] (0.79)	6.84*	56
	A. nobilis/L. pis.	5.97 (0.41)	6.47 (0.43)	5.39 (0.31)		1.92	27
2	C. auratus/L. cyp.	17.49 (0.34)	17.53 (0.34)	16.96 (0.56)		0.48	47
	A. nobilis/L. cyp.	16.86 [a] (0.23)	17.82 [a] (0.48)	16.81 [a] (0.98)	19.38 [b] (0.42)	4.36*	56
	A. nobilis/L. pis.	14.26 (0.39)	14.03 (0.42)	14.56 (0.52)		0.34	27
3	C. auratus/L. cyp.	41.51 [a] (0.62)	41.99 [b] (0.38)	39.38 [b] (0.56)		4.83*	47
	A. nobilis/L. cyp.	41.26 [a] (0.46)	41.53 [a] (0.35)	41.54 [a] (0.31)	43.94 [b] (0.47)	6.39*	56
	A. nobilis/L. pis.	34.85 [a] (0.59)	35.37 [a] (0.69)	37.75 [b] (0.86)		4.58*	27
4	C. auratus/L. cyp.	71.51 (0.52)	71.98 (0.38)	70.81 (0.71)		1.50	47
	A. nobilis/L. cyp.	71.43 (0.70)	71.37 (0.32)	70.08 (0.49)	72.32 (0.53)	1.75	56
	A. nobilis/L. pis.	68.95 (0.70)	66.06 (0.98)	68.00 (1.80)		1.38	27
5	C. auratus/L. cyp.	91.53 (0.31)	91.21 (0.17)	91.15 (0.61)		0.43	47
	A. nobilis/L. cyp.	90.04 (0.40)	91.01 (0.12)	90.34 (0.24)	91.11 (0.43)	1.72	56
	A. nobilis/L. pis.	90.53 (0.32)	91.22 (0.32)	90.96 (0.37)		1.01	27

* - Indicates significant difference at 5% level.
Values with different letters indicate they are significantly different from each other. Values with letters in common are not significantly different. Values in parenthesis indicate standard [illegible]

Table 4.14

Comparision of the position of swimming legs of Lernaea obtained from the body proper(regions 1-4) and caudal peduncle, fins, head, eyes (regions 5, 6, 7, & 8) of the 3 host-parasite systems.

Leg	Host-parasite	(regions 1-4)	(regions 5,6,7,8)	T value	Prob	DF
1	C. auratus/L. cyp.	6.77 (0.12)	6.79 (0.24)	0.09	>0.93	118
	A. nobilis/L. cyp.	6.18 (0.13)	6.35 (0.21)	0.75	>0.46	138
	A. nobilis/L. pis.	5.92 (0.10)	5.94 (0.23)	0.12	>0.91	108
2	C. auratus/L. cyp.	17.40 (0.22)	17.40 (0.22)	0.0	>1.00	118
	A. nobilis/L. cyp.	16.95 (0.16)	17.59 (0.27)	2.15*	<0.03	138
	A. nobilis/L. pis.	13.91 (0.15)	14.29 (0.25)	1.26	>0.21	108
3	C. auratus/L. cyp.	41.45 (0.26)	41.27 (0.34)	0.42	>0.68	118
	A. nobilis/L. cyp.	40.63 (0.27)	41.20 (0.22)	1.63	>0.10	178
	A. nobilis/L. pis.	35.57 (0.22)	35.99 (0.47)	0.91	>0.37	108
4	C. auratus/L. cyp.	71.49 (0.26)	71.47 (0.30)	0.04	>0.97	118
	A. nobilis/L. cyp.	70.21 (0.27)	71.33 (0.29)	2.80*	<0.005	138
	A. nobilis/L. pis.	69.53 (0.30)	71.37 (0.32)	2.93*	<0.004	98
5	C. auratus/L. cyp.	91.15 (0.15)	91.33 (0.18)	0.77	>0.44	118
	A. nobilis/L. cyp.	90.49 (0.23)	90.59 (0.17)	0.34	>0.74	138
	A. nobilis/L. pis.	91.17 (0.14)	90.91 (0.20)	1.04	>0.30	108

* - Indicates significant difference at probability level shown.
Values in parenthesis indicate standard error of mean.

Table 4.15

Comparison of the total length of Lernaea obtained from the different regions of the body proper(region 1-4) of 3 host-parasite systems.

Regions	1	2	3	4	F value	DF
Host-parasite						
C. auratus/L. cyp.	8.47 (0.32)	8.56 (0.22)	8.32 (0.20)	8.47 (0.21)	0.21	69
A. nobilis/L. cyp.	8.30 [a] (0.18)	9.33 [b] (0.27)	7.97 [a] (0.25)	8.77 [c] (0.20)	6.50*	79
A. nobilis/L. pis.	8.48 (0.20)	8.38 (0.26)	8.56 (0.20)	8.10 (0.21)	0.80	79

Values in parenthesis indicate standard error of mean.
* - Indicates significant difference at the probability level shown.
Values with different letters indicates they are significantly different than each other. Values with letters in common are not significantly different.

Table 4.16

Comparisons of the total length of Lernaea obtained from regions 5, 6, 7 & 8 of the 3 host-parasite systems.

Regions	5	6	7	F value	DF
Host-parasite					
C. auratus/L. cyp.	8.51[a]	8.83 [b]	7.92 [ab]	3.13*	49
	(0.22)	(0.17)	(0.33)		
A. nobilis/L. cyp.	7.86	8.14	8.32	0.90	39
	(0.18)	(0.28)	(0.33)		
A. nobilis/L. pis.	7.89	7.61	7.61	0.21	29
	(0.33)	(0.38)	(0.37)		

Values in parenthesis indicate standard error of mean.
* - Indicates significant difference at 5% level.
Values with different letters indicates they are significantly different than each other. with letters in common are not significantly different.

Table 4.17

Comparisons of total length of *Lernaea* obtained from the left & right sides of body proper (regions 1-4) from 3 host-parasite systems.

Host-parasite	Left	Right	T value	Prob	DF	Between host-parasite	F value	DF
C. auratus/*L. cyp.*	8.54 (0.16)	8.34 (0.18)	0.82	>0.41	68	8.46 (0.13)		
A. nobilis/*L. cyp.*	8.86 (0.20)	8.33 (0.14)	2.12*	<0.03	78	8.61 (0.13)	0.89	229
A. nobilis/*L. pis.*	8.32 (0.14)	8.44 (0.17)	0.49	>0.63	78	8.38 (0.11)		

Values in parenthesis indicate standard error of mean.
* - Indicates significant difference at the probability level shown.

Table 4.18

Comparison of the total length of *Lernaea* obtained from body proper (regions 1-4) and fins, head & eyes (regions 5, 6, 7 & 8) of 3 host-parasites systems.

Host-parasite	Body proper	Fins, head & eyes	T value	Prob	DF
C. auratus/*L. cyp.*	8.46 (0.11)	8.52 (0.14)	0.38	>0.00	118
A. nobilis/*L. cyp.*	8.60 (0.13)	8.04 (0.14)	2.66*	<0.01	118
A. nobilis/*L. pis.*	8.38 (0.11)	7.70 (0.20)	3.08*	<0.00	108

Values in parenthesis indicate standard error of mean.
* - Indicates significant difference at the probability level shown.

There was less variation amongst parasite from within the body regions (1, 2, 3 and 4), however amongst parasites from the caudal peduncle, fins, head and eyes (5, 6, 7 and 8), there was a greater number of incidences of significant variations.

The values for the sections of the cephalic processes ('D', 'Y', 'V' and 'W') of parasites from the body region (1-4) were in most instances found to be significantly greater in size than those from the caudal peduncle, fins, head and eyes.

The distance between the mid-body and the process of the dorsal horn ('Y') and the length of the ventral horn (V) were found to be constant among L. cyprinacea when these were obtained from C. auratus and A. nobilis and they differed significantly with L. piscinae from A. nobilis. The values of 'Y' and 'V' were significantly greater in L. cyprinacea than L. piscinae. The dorsal horn ('D') and the length of the process of dorsal horn ('W') were found to be significantly different between L. cyprinacea from A. nobilis and L. cyprinacea from C. auratus and cannot therefore be used as taxonomic indications.

The position of swimming legs did not differ amongst parasites from the left and right side of the host body proper. The position of swimming legs 2 and 3 were found to be constant in L. cyprinacea when obtained from C. auratus and A. nobilis and they differed significantly with those of L. piscinae from A. nobilis. There was a greater frequency

of variation in the position of swimming legs amongst parasites in regions 5, 6, 7 and 8 and variation was less common amongst parasites from regions 1, 2, 3 and 4 .

4.3.4.8. Correlation of the total length of the parasite with the length of individual parts of the body

Tables 4.19, 4.20 and 4.21 demonstrate the relationships between the rate of growth in length of the body and the rate of growth of the individual body parts. These relationships are presented in the table as the length of the individual parts expressed as a percentage of the total body length. For convenience four (4) size classes of total body length are used.

From Table 4.19, it can be seen that L. cyprinacea on A. nobilis increased in total length and at the same time, there was also an increase in the length of all body parts except for Leg 1.

The correlation coefficent for the length of the dorsal horns and the body length (Table 4.19) was as low as 0.23, The maximum growth increments in relation to that of the total body were obtained for legs3, 5, 4and 2 in that order. The dorsal horn ('D') and the distance from the mid-body to the dorsal horn process ('Y') both showed a positive correlation with the total length of the parasite of 0.35 and 0.38 respectively indicating a high growth rate in proportion to that of the total body.

Table 4.19

Relationship between total lengths and length of individual parts of Lernaea cyprinacea of A. nobilis.

Total length	No. of specimen	D	Y	W	V	Leg 1	Leg 2	Leg 3	Leg 4	Leg 5
6-7	25	13.97	7.85	2.73	11.12	6.39	16.37	39.61	68.35	88.44
7-8	35	14.32	7.43	2.75	10.43	6.25	16.44	41.19	70.63	89.80
8-9	21	13.74	8.38	3.44	12.04	5.88	16.82	40.09	70.20	90.39
9-10	18	14.11	8.23	3.41	13.17	6.01	16.74	41.34	71.87	90.50
10-11	8	15.36	9.45	3.88	13.21	6.63	17.44	43.42	70.57	90.46
DF 105	r=	+ 0.35*	+0.38*	+0.23*	+0.32*	-0.01	+0.26*	+0.44*	+0.28*	+0.31*

D - Length of dorsal horn
Y - Distance between mid-body and process of dorsal horn
W - Length of the process of dorsal horn
V - Length of ventral horn
DF- Degrees of freedom
r - Correlation coefficient
* - Indicates a significant difference at 5% level.

Table 4.20

Relationship between total lengths and length of individual parts of *Lernaea cyprinacea* of *C. auratus*.

Total length	No. of specimen	D	Y	W	V	Leg 1	Leg 2	Leg 3	Leg 4	Leg 5
6-7	12	18.84	10.46	5.88	10.99	7.09	18.68	41.77	71.91	91.60
7-8	25	17.60	9.09	5.13	12.31	6.69	17.69	41.64	71.14	90.92
8-9	29	16.17	0.03	5.15	11.07	6.39	17.59	41.74	71.79	91.23
9-10	18	16.21	9.66	4.96	12.20	6.98	16.52	41.81	70.62	90.85
10-11	4	16.07	6.42	4.22	10.79	5.80	16.70	40.68	74.75	91.85
DF	86	r= -0.42*	-0.37*	-0.28*	0.04	-0.12	-0.34*	-0.24*	0.01	0.07

D - Length of dorsal horn
Y - Distance between mid-body and process of dorsal horn
W - Length of the process of dorsal horn
V - Length of ventral horn
DF- Degrees of freedom
r - Correlation coefficient
* - Indicates a significant difference at 5% level.

Table 4.21

Relationship between total lengths and length of individual parts of *Lernaea piscinae* of *A. nobilis*.

Total length	No. of specimen	D	Y	W	V	Leg 1	Leg 2	Leg 3	Leg 4	Leg 5
6-7	47	20.10	11.77	4.03	7.59	6.12	14.03	36.07	69.24	90.24
7-8	21	20.50	11.55	3.85	7.37	6.07	14.19	36.38	70.25	91.11
8-9	23	19.62	11.32	3.44	6.55	5.69	13.44	34.99	69.19	91.34
9-10	14	19.47	11.21	3.28	6.02	5.67	13.27	34.93	69.14	91.66
10-11	7	18.88	10.97	3.03	6.38	5.74	12.90	33.58	67.13	90.23
DF	110	r -0.42*	-0.27*	-0.24*	-0.36*	-0.20*	-0.33*	-0.22*	-0.28*	0.18

D - Length of dorsal horn
Y - Distance between mid-body and process dorsal horn
W - Length of the process of dorsal horn
V - Length of ventral horn
DF- Degrees of freedom
r - Correlation coefficient
* - Indicates a significant difference at 5% level.

In L. cyprinacea from C. auratus, most of the body parts revealed a negative correlation coefficient when compared to the growth in the total length of the parasite. The length of the dorsal horns ('D'), distance from mid-body to the dorsal horn process ('Y') and the dorsal horn process ('W') and position of Leg 2 and Leg 3 showed an increase in the total length of the body (Table 4.20). The remaining parts showed no correlation.

In L. piscinae on A. nobilis, there was a negative correlation between the growth of all body parts and the growth in total length of the parasite except for Leg 5 (Table 4.21).

4.4. Discussion

The study on the morphology of the larval stages of L. cyprinacea (from C. auratus and A. nobilis) and L. piscinae (from A. nobilis) showed them to be identical and closely resembling the descriptions of larval stages of L. cyprinacea given by Grabda (1963) and those of L. elegans given by Nakai (1927). The differences noted in the present study from the descriptions of Nakai and Grabda were in the arrangement of spines and setae in the swimming limbs at the various larval stages. According to Grabda the 2nd. endopodite segment of the 3rd. pair of swimming legs of the IV copepodid stage had 6 setae while the 2nd. exopodite segment of the 4th. swimming leg had 4 setae. In contrast to the present study there was one seta less (5) for the former and one seta more (5) in the latter. Comparison of the

present work with Nakai's study on L. elegans revealed more differences and for convenience these are presented in Table 4.22. The number of free body segments, number of segments, bristles and spines of the antennula of L. piscinae and L. cyprinacea were identical with the report of Grabda on L. cyprinacea (Table 4.23).

Inspite of the minor variations between the morphology of the parasites in the present study with those of other workers (which were thought to occur due to the misunderstanding by the various workers as Grabda (1963) has reported), it is not surprising that the morphology of the larval stages of L. cyprinacea was identical to that of L. piscinae as it has been reported by Kabata (1979) that it is very probable that most lernaeids do not differ greatly from one another as far as ontogeny is concerned. Thus the morphology of the larval stages of the genus Lernaea cannot be used for a taxonomic purposes.

The study of the morphometrics of the larval stages of L. cyprinacea however revealed that there were variations in the total length, body length, length of the furcae, the cephalothorax length, length of the antennae and the length and width of the genital segments at various stages when they were obtained from C. auratus and A. nobilis. The study also showed that some of the measurements of the larval stages of L. cyprinacea and L. piscinae were similar when present on different hosts, C. auratus and A. nobilis respectively. These similarities were noted for the total length, body length, furcal length, length and width of the

Table 4.22

Comparison between spines and setae of the swimming limbs of L. cyprinacea from C. auratus (present study) with the work of Nakai (1972) on L. elegans from C. carpio. Figures in brackets represent Nakai's findings.

Copepodid stage	swimming limbs (pair)	Exopodite	Endopodite
II	I III	segment I setae 0 [1] spines 4 [3]	
III	III IV	spines 4 [3]	segment II spines 2 [1]
V male	I II		segment III setae 4 [5] spines 2 [3] setae 4 [3]

Table 4.23 Comparison of the present findings with the work of others on the different larval stages of Lernaea.

Lernea spp.	L. cyp.	L. cyp.	L. elegans	L. barnimiana	L. pis.	No. of free body segments (thoracic & Abdomen)		No. of Antennula Segments		No. of bristles & spines	
Reference	Present Study	Grabda (1963)	Nakai (1927)	Thurston (1969)	Present Study	Present study	Grabda	Present study	Grabda	Present study	Grabda
Host	C. auratus	C.carassius	C. carpio	Tilapia sp.	A. nobilis	C. auratus	C. carassius	C. auratus	C. carassius	C. auratus	C. carassius
Copepodid Stage											
I	0.32	0.31-0.33	0.29	0.33-0.40	0.32	4	4	3	3	14	14
II	0.42	0.43-0.47	0.36	0.50-0.55	0.47	5	5	4	4	18	18
III	0.51	0.56-0.67	0.45	0.55-0.60	0.55	6	6	5	5	23	22-23
IV	0.66	0.60-0.62	0.56	0.62-0.80	0.70	7	7	6	6	22-25	22-23
V (M)	0.77	0.88	0.71	0.70-0.85	0.79	8	8	6	6	34	34
V (F)	0.90	0.73-0.74	0.89	0.82-0.95	0.90	8	8	6	6	26-28	27
cyclopoid (M)	0.86	0.90-1.10		0.85-1.00	0.87	9	9	6	6	25-27	
cyclopoid (F)	1.57	0.72		0.92-1.20	1.04	9	9	8	8	40	37

genital segment, number of bristles and spines on antennulae and the total length of antennae. In addition to the above differences and similarities it was also noted that L. piscinae from A. nobilis showed a greater difference from L.cyprinacea of A. nobilis than L. cyprinacea from C. auratus.

The study demonstrated that the morphometric data revealed variation in the larval stages of the same species (L. cyprinacea) when it occured on different hosts. It also identified variation in different species (L. cyprinacea and L. piscinae) when present on the same (A. nobilis) or different host (C. auratus and A. nobilis). But these variations were not consistent for any one character for the different larval stages. However some of the characteristics for L. cyprinacea were not influenced by the host speciesand these characteristics were distinct from of those of L. piscinae. These characteristics were the length of genital segment at the 5th female copepodid and the length of the antennae of the cyclopoid stage of both sexes. Thus these consistent differences between the two species of Lernaea could be used to identify the parasite at these stages. This has only been tested for the parasite and host species involved in this study and may not be constant for other hosts or parasite species.

Comparison of the larval stages of L. piscinae and L. cyprinacea of the present study with the work of Thurston (1969) on L. barnimiana revealed that there were differences in the body length. L. barnimiana was of larger size than

L. cyprinacea at the copepodid II and III but were smaller at the female cyclopoid stage (Table 4.23). L. barnimiana was larger in size than L. piscinae at the II copepodid stage. On the other hand, the body length of L. cyprinacea in the present study also differed from that reported by Grabda (1963) for the same species. The male V copepodid and cyclopoid stages were smaller whereas the female V copepodid stage and female cyclopoid were larger than those of Grabda's study. Nakai's (1927) findings on L. elegans (although now considered to be a synonym of L. cyprinacea) was found to be smaller than all the others listed in Table 4.23.

Much more has to be done to elucidate the morphometry of the larval stages when present on their respective hosts before it can be concluded that this method could be widely used to identify the larval stages of other species of the genus Lernaea.

The study also showed that larval stages of L. cyprinacea when present on C. auratus were larger in size compared to the same parasite larvae from A. nobilis. Similarly the larvae from C. auratus were also found to be larger in size (female cyclopoid stage) when compared with the findings of Grabda (1963) for L. cyprinacea on crucian carp, Carassius carassius (Linnaeus). A similar situation was noted for the adult parasite whereby L. cyprinacea specimens from C. auratus were bigger than their counterparts from A. nobilis. This may well be an

indication of host adaptability and is discussed at greater length below.

When C. auratus were infected with larvae of L. cyprinacea ("Asian" form) two forms of adult female were identified, that is L. cyprinacea (identical to the maternal form) and L. ctenopharyngodonis, whereas in A. nobilis under similar exposure to L. cyprinacea, the adult parasite obtained did not vary in its morphology and was identical to the maternal form. The "Asian" form of L. cyprinacea obtained from C. auratus and A. nobilis was identical with the descriptions provided by Harding (1950), Poddubnaya and illustrations by Yin et al. (1963). L. ctenopharyngodonis was also identical to the description by Poddubnaya (1973) and illustrations by Yin et al. (1963).

Poddubnaya (1973) looked at the morphological variation of L. cyprinacea ("Asian" form) and found that when C. auratus were exposed to infection, she obtained adult parasites all identical to the maternal form, i.e. L. cyprinacea ("Asian" form) and none of L. ctenopharyngodonis as was found in the present study. However, Poddubnaya was able to obtain L. ctenopharyngodonis when she infected the host Ctenophayngodon idella with larvae of L. cyprinacea, although it was in the proportion of 84.4% and the remaining 16.6% resembled the maternal form (viz Page 9). Poddubnaya was able to reverse the process to obtain L. cyprinacea by infecting C. auratus with L. ctenopharyngodonis, and found

82% L. cyprinacea. It is apparent from the above that L. cyprinacea ("Asian" form) with its polymorphism characteristic is able to change its morphology and this ability may vary in different hosts. Thus L. ctenopharyngodonis is identical to L. cyprinacea and should be considered a morpha of L. cyprinacea . This is in agreement with Poddubnaya's work. Further evidence that L. ctenopharygodonis and L. cyprinacea ("Asian" form) are the same, was seen from the study of the position of swimming legs. A t-test on the position of the swimming legs from these 2 species revealed no significant difference (Table 4.24). The role of the swimming legs in the identification of parasite species of the genus Lernaea is discussed below.

On the other hand L. piscinae remained consistent with its maternal form and did not exhibit the polymorphic character as was seen in L. cyprinacea; this may possibly be related to the narrow host specificity of L. piscinae. Besides A. nobilis, L. piscinae has only been reported from silver carp, H. molitrix (Yin et al. 1963).

The investigation to elucidate the influence of infection site on parasite morphology revealed that there were variations in the measurements of the anchor and its processes from different regions. Such variations were common among parasites from regions 5, 6, 7 and 8 (regions of the caudal peduncle and eyes, fins and head). Variation was also noted when the mean of pool value of regions 5, 6,

Table 24

Comparison of the position of swimming legs of L. cyprinacea "Asian" form and its morpha L. ctenopharyngodonis obtained from C. auratus

Leg	L. cyp. ("Asian" form)	L. cteno.	T. value	Prob.	DF
1	7.08 0.22	7.17 0.16	0.33	>0.74	29
2	17.52 (0.51)	17.39 (0.22)	0.20	>0.20	29
3	42.76 (0.41)	41.06 (0.32)	0.43	>0.86	29
4	70.52 (0.61)	70.96 (0.35)	0.63	>0.53	29
5	90.90 (0.38)	91.65 (0.25)	1.98	>0.06	29

7 and 8 were compared with the mean of pool value of regions 1-4 (making up the body proper). Variation amongst the different regions of the body (1-4) was less common.

The morphometric study defined the variation indicated by the description of morphological structures of the parasites which were obtained from the different regions of the host. There was a higher proportion of morphological variation amongst the parasites obtained from the , caudal peduncle, fins, head and eyes (regions 5, 6, 7 and 8) than in those obtained from regions body proper (region 1-4). Regions 5, 6, 7 and 8 consist mainly of bony or cartilaginous structures and it may be that, during the growth of the parasites in these regions, the anchor and its processes encounter mechanical obstructions and the hard tissue influences the shape and size of the anchor and its processes, thus giving rise to the greater degree of morphological variations described above. Morphological variation due to the mechanical obstruction was suggested by Leigh-Sharpe (1925), Harding (1950), Fryer (1961b) and Demaree (1967) for lernaeids but these reports were not substantiated. Using morphometry, the present study has confirmed this phenomenon.

Morphological variations occurred less frequently in parasites from the body proper (region 1-4) than in parasites from the caudal peduncle, fins, head and eyes; it would thus, be highly desirable that the collection of parasites for taxonomic studies should be made from the body proper where possible. Although there is likely to be some

degree of some morphological variation among the parasites from the body proper regions, a larger number of specimens would reveal the measurements characteristic of the parasite species. The use of the morphometric technique on constant characters would enhance the degree of confidence in the identification, thus avoiding to a large extent the confusion which surrounds this genus.

Parasites with obvious abnormalities of different parts of the body, such as the absence of the left or right horn should not be considered as specimens for taxonomic studies considering the high proportion of abnormalities reported here eg. 46% for L. cyprinacea from C. auratus. The choice would seem necessary to use a larger number of specimens than is frequently reported especially if these are from the regions 5 to 8 where abnormalities are most frequent.

This study has shown that some characters are more consistent than others i.e. the distance from mid-body to the process of the dorsal horn, the position of the swimming legs 2 and 3 and the length of the ventral horns of L. cyprinacea. These remained unchanged and therefore were not influenced by its presence on different host species (C. auratus and A. nobilis) and the morphometric data of these features also differed significantly from that of L. piscinae. Thus together with the morphological studies, morphometry should be incorporated as a component in the identification of this much confused genus Lernaea.

The position of the legs of L. cyprinacea taken from A. nobilis and C. auratus are within the range of values reported by Poddubnaya (1973) for L. cyprinacea and they also fall within the range of values for L. cyprinacea of C. auratus as reported and of A. nobilis (Harding 1950) (Table 4.25).

The comparative study of the position of the swimming legs 1, 4 and 5 of L. cyprinacea from C. auratus and A. nobilis however showed variations in the position depending on the host species. Poddubnaya (1973) found no influence of the host on the position of the swimming legs of the parasite she used but the use of statistical method for comparison here has revealed otherwise.

The values found for the position of the swimming legs of L. piscinae from A. nobilis fall within the range of values reported by Harding (1950) and even with L. parasiluri Ho (1961) (Table 2.25). The similarity of the position of swimming legs of L. parasiluri of Ho (1961) with that of L. piscinae is not surprising since they were both shown subsequently to be identical species (Ho's identification of L. parasiluri is here considered invalid) as discussed below. Comparison with the authentic description of L. parasiluri by Yu (1938) and subsequently by Yin et al. (1963) with respect to the position of the swimming legs revealed that the position of leg 2 is comparatively closer to leg 1 than in L. piscinae. Indeed it is closer than any other species shown in Table 4.25.

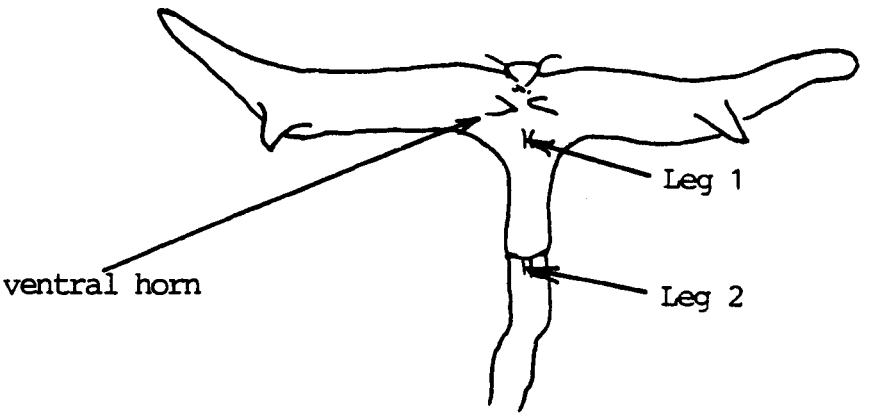
Table 4.25

Position of swimmming legs in perentage (%) of total body length of varicus species of Lernaea.

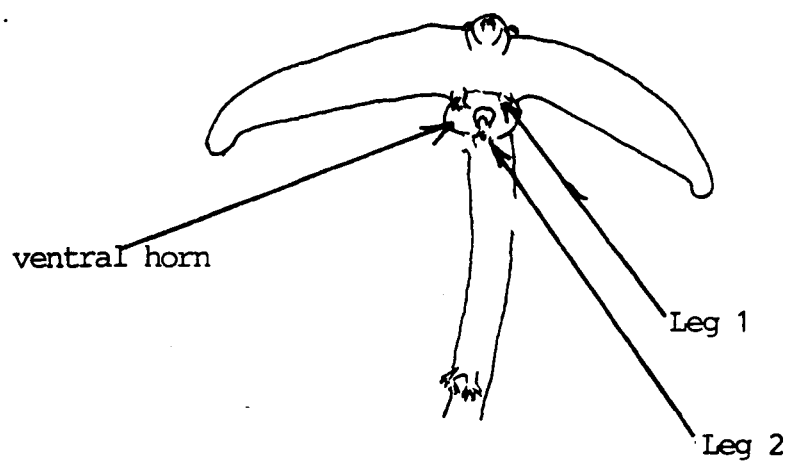
Lernaca spp. Host Reference	L. piscinae A. nobilis Present study	L. piscinae A. nobilis Harding 1950	L. polymorpha A. nobilis Yin et al 1963	L. parasiluri A. nobilis Ho 1961	L. parasiluri A. nobilis Yin et al 1963	L. cyprinacea C. auratus Present study	L. cyprinacea A. nobilis Present study	L. ctenopharyngodonis C. auratus Present study	L. ctenopharyngodonis C. idella Yin et al 1963	L. cyprinacea C. auratus Present study	L. cyprinacea A. nobilis Harding 1950	L. cyprinacea A. nobilis Poddubnaya 1973
Leg 1	5.5-6.4 5.9	5-7	6.7-7.9	5.7	5-6	6.5-7.2 6.8	5.9-6.9 6.2	5.6-7.8 6.9	5.0-10.6	5.9-9.8 7.1	6.9	4.8
Leg 2	13.4-14.6 13.9	13-14	14-16	14.4	7-9	16.4-18.5 17.4	16.4-17.7 17.0	16-18 17.4	16.7-22	16.3-19 17.5	16-20	16-20
Leg 3	34.7-36.9 35.5	31-38	34-38	34.2	33-40	40.2-42.4 41.4	40.1-41.7 41.4	39.7-42.2 41.0	44.0-47 41.5	39.6-43.7	42.5	40-46
Leg 4	67.4-70.6 69.1	69-74	69-73	68.1	63-68	70.4-73.0 71.4	69.6-71.5 70.2	68.2-73.5 70.9	69.0-75	69.5-73.7 71.3	73-74	72-76
Leg 5	90.7-91.7 91.1	91-93	91-92	91.7	89-91	90.7-92.1 91.1	89.6-91.2 90.5	90.4-93.4 91.7	89.0-94	89.4-92.0 90.9	90-92	90-94

L. cyprinacea *C. auratus* Boddubnaya 1973	*L. quadrinucifera* *C. idella* Present study	*L. tilapiae* *T. squamipinnis* Harding 1950	*L. tuberosa* *E. sardella* Harding 1950	*L. palati* *H. chrysonotus* Harding 1950	*L. barilli* *B. microlepis* Harding 1950	*L. bernimiana* *L. forskalii* Harding 1950	*L. longa* *L. niloticus* Harding 1950	*L. diceracephala* *C. mossambicus* Harding 1950	*L. octocornus* *C. punctatus* Yin *et al* 1963	*L. lonhiara* *L. lethrinus* Harding 1950	*L. bistricornis* *C. schoutedeni* Harding 1950	*L. esocina* *C. idella* Yin *et al* 1963
4-6	6.3	8	7.8	8.7	8.4	7.2-9.8	6.3	10	7.7-8.7	6	8	11.0-13
16-20	17.4	25	18.0	26.0	20.0	19-24	14.0	23	18.7-18.8	16	21	24.3-24.5
42-46	39.8	50	42.0	55.0	47.0	41-51	36.0	50	40.0-41.3	42	45	42.3-46.8
72-76	70.8	77	72.0	80.0	77.0	73-79	64.0	71	69-71	76	76	67-72
92-94	90.6	90	91.0	93.0	92.0	89-92	77.0	92	88-90	93	94	90-92

Ho (1961) synonymized L. parasiluri with L. piscinae but a detailed study of his illustrations (Fig. 4.35) and description in his report on L. parasiluri indicates that Ho was actually describing L. piscinae and not L. parasiluri. The misconception is evident from the following point. L. parasiluri as first described by Yu (1938) possesses a pair of ventral horns set between the first and second pair of legs (Fig. 4.35). However Ho's description of L. parasiluri states that the ventral horns which he refers to as "another kind of process paired rather short, stout and directed inwards towards one another or anteriorly projection out of the ventral surface of the main bar" lies between the head and leg 1. The differences separating L. parasiluri (as first described by Yu 1938) and Ho's subsequent description is the position of the ventral horns, which according to Yu's description and illustrations lies between legs 1 and 2 and not between the head and leg 1 as described by Ho. It is possible that Ho did not refer to Yu's work, but in his comparison with Yamaguti's (1939) description of L. parasiluri, the presence of the ventral horns (described as auricle like processes) situated between leg 1 and 2 was ignored and considered abnormal as he states Yamaguti had provided his description based on only one specimen. The unique difference in the position of the swimming leg 2 of the authentic L. parasiluri with the description given by Ho (1961) has already been noted. Had Ho referred to Yu's illustrations he would have seen the difference in the position of the ventral horns and leave little doubt that Ho's specimen were actually not L. parasiluri but L.



L. parasiluri according to Ho 1961



L. parasiluri Yu 1938.

Figure 4.35 Comparison between L. parasiluri Yu 1938 and L. parasiluri as described by Ho 1961.

In L. parasiluri Yu 1938, the ventral horn lies between the 1st and 2nd pair of legs, thus a small distance separates the legs. In L. parasiluri (Ho's 1961 description) the ventral horns lie above Leg 1 and the distance between the 1st and 2nd pair of legs is greater (Table 4.25). Also note the similarities of L. parasiluri of Ho with L. piscinae of the of the present study (Fig. 4.33).

piscinae and thus his reason for synonymizing it with L. piscinae.

Another species which revealed similarity in the position of the swimming legs with L. piscinae was L. polymorpha as described by Yu 1938 (Table 4.25), and a detailed study of the description and illustration of the reports of L. polymorpha reveal that both these species are identical. The species L. piscinae was established by Harding (1950) after he had examined specimens collected from A. nobilis that were obtained from Singapore. It is unfortunate that Harding was apparently unaware of Yu's (1938) work, for it is clear that the morphological structure of the parasite, the position of the swimming legs and the host (only A. nobilis and silver carp have been the recorded host of L. polymorpha) are all identical. Furthermore, the origin of the specimens Harding described could be traced back to China where the description of L. polymorpha orginated . The Singapore imports of A. nobilis are from China. From the above evidence it could thus be concluded that L. piscinae is a Junior synonym of L. polymorpha. Ho, who had earlier (1961) synonymized L. piscinae with L. parasiluri has now agreed to the above change (personal communication through Dr. Z. Kabata).

The name L. polymorpha could be misleading, as it would mean that this particular species shows more polymorphism as compared to other species within the genus Lernaea. As seen in this study, the polymorphism was more obvious in L. cyprinacea which was identified as 2 forms, i.e. L.

cyprinacea ("Asian" form) and L. ctenopharyngodonis and Poddubnaya's (1973) work identified 3 different forms from the offspring of L. cyprinacea. The morphometric study of the morphology of the adult female also support the above i.e. that variation in L. cyprinacea occurred more frequently than in L. piscinae (= L. polymorpha).

A comparison of the variation in the position of the swimming legs 2, 3 and 4 of L. piscinae with that of L. cyprinacea (either of A. nobilis or C. auratus) reveals that they could be easily distinguished from one another (Table 4.25). Further comparison of the position of the swimming legs 2, 3 and 4 of L. piscinae with those of L. tilapiae, Lernaea tuberosa Harding, L. palati, Lernaea barilli Harding, L. barnimiana, Lernaea longa Harding, Lernaea diceracephala Harding, L. lophiara and Lernaea bistricornis Harding (all reported by Harding 1950), Lernaea octocornua Yin, L. esocina and Lernaea rhodei Hu, reported by Yin et al. (1963) and L. cyprinacea by Poddubnaya (1973), also showed that L. piscinae could be easily distinguished from all the species mentioned by their differences in the position of the swimming legs.

Table 4.25 reveals that the position of legs of L. cyprinacea and its other "forms" i. e. L. ctenopharyngodonis , L. quadrinucifera are identical. Table 4.25 also reveals that L. cyprinacea or any other species could be easily distinguished from each other if the mean values particularly of the position of legs 2, 3 and 4, are used as comparison. However the use of morphometric methods

would prove to be more helpful for such comparison and when a large number of species are involved. The position of legs of L. octocornua closely resemble those of L. cyprinacea. It is possible that it is another morpha of L. cyprinacea due to its presence in a different host, Ophicephalus punctatus Bloch as recorded by Yin et al. (1963). If it is not a morpha of L. cyprinacea, then it is the only species that shows similarities in the position of legs with L. cyprinacea and its morpha forms.

The correlation of growth of the individual body parts of the body with the total length of the body of L. cyprinacea varied in parasites from the two hosts. In A. nobilis there was a positive correlation between in growth of all the body parts, except for leg 1, in relation to the increase in total length of the parasite (Table 4.19, 4.20, 4.21) However L. cyprinacea of C. auratus revealed a lower growth rate in proportionto that of the total body length, especially of the dorsal horn and the ventral horn. A negative correlation was seen between the position of all the five pairs of swimming legs in relation to the total length. Comparatively, L. cyprinacea of A. nobilis had a higher growth rate in proportion to the total body. The dorsal horns and swimming legs 2 to 5 of L. cyprinacea from A. nobilis had a comparatively high growth rate in proportion to the total body.

When correlations were established between the total length of the parasite and its individual parts of the body, a progressive growth trend was seen in the position of the

legs of L. cyprinacea from A. nobilis. The growth of leg 2 to leg 4 has been justified by Poddubnaya (1971) in order to cater for the reproductive function, as it is in this region that eggs are produced and are then moved through the oviducts to the genital segment, where these are stored prior to their deposition in egg sacs.

A negative correlation was seen in L. cyprinacea from C. auratus indicating that the growth is achieved more rapidly in this host compared to L. cyprinacea from A. nobilis. Growth of the body parts other than the total length occurred most rapidly during the initial growth period, whereas the growth rate of the total body is slowed. When the maximum size of the individual body parts is reached, the growth of the trunk continues and its proportion in relation to the body parts is consequently greater as the parasite matures. A similar pattern of rapid growth of the body parts during the initial stages of attachment was also seen in L. piscinae on A. nobilis, revealing a negative correlation which indicates a faster growth rate of body parts than total body length.

In addition to the rapid growth, the results of the mean values of the measurements of the different body parts of the adult parasite (Table 4.4, 4.8, and 4.9) and those of the larval stages (Table 4.3) reveal that L. cyprinacea from C. auratus is of bigger size (in proportion to total length for the adult parasite and in actual size for the larval stages) than L. cyprinacea from A. nobilis. There is a possibility that the rapid early growth of L.

piscinae on A. nobilis and L. cyprinacea on C. auratus in addition to the larger size of the latter compared with L. cyprinacea in A. nobilis may possibly be related to the suitability of the host. Inspite of the wide range of host susceptibility to L. cyprinacea (chapter 2) C. auratus is the most common host (Eisen 1977, Kabata 1979, Bauer et al. 1981). According to Dogiel (1962), when a parasite occurs in more than one host, it is almost always possible to observe that in one of them it more frequently grows to a larger size, reaches maturity more rapidly, produces the greatest number of eggs and generally appears to be the best adapted. This suggests that C. auratus is the most susceptible host for L. cyprinacea. An egg count (from 10 pairs of egg sacs) of L. cyprinacea from C.auratus and A. nobilis revealed that the number of eggs in the parasites from C. auratus was significantly greater in number (154) than in parasites from A. nobilis (115).

A similar situation of host preference has also been reported by Tedla and Fernando (1969) whereby the parasite Ergasilus centrarchidarum Wright was found to be larger in size and produced a greater number of eggs when parasitic on rock bass, Ambloplites rupestris (Rafinesque) as compared to its other host smallmouth bass, Micropterus dolomieui Lacepede. Tedla and Fernando concluded that the larger size of the parasite and its higher reproductive rate on rockbass was a measure of the degree of adaptation and thus considered it to be a preferred host of E. centrachidarum. The rapid initial completion of growth of the anchor and its

processes in L. piscinae on A. nobilis as compared to L. cyprinacea on the same host could also be based on the best adapted host theory; as L. piscinae has a narrower range of host specificity (Chapter 2). The narrow range of host specificity is an indication of an old evolutionary process leading to a stable host parasite relationship as suggested by Sprent (1959). Comparison of egg counts from L. piscinae with that of L. cyprinacea both from A. nobilis revealed that L. piscinae produced a significantly larger number of eggs (188) as compared to L. cyprinacea (115).

CHAPTER 5.

HOST-PARASITE RELATIONSHIP; FREQUENCY DISTRIBUTION, SITE SELECTION AND PARASITE BURDEN IN RELATION TO HOST LENGTH.

5.1. Introduction

The study of the host-parasite relationship which involves ecological and physiological factors could contribute towards a greater understanding of some of the basic biological problems.

According to Fryer (1968) the distribution of L. barnimiana was influenced by environmental conditions, when he found its occurrence in the mouth of fish in swift flowing rivers and on fins or flanks in fish from still waters. The influence of water currents on the distribution of Lernaea on its host has also been expressed by McNeil (1961) and Bulow et al. (1979). In contrast to the above, Fryer's (1968) findings on the distribution of L. hardingi, L. Lophiara and L. bagri revealed differences in site preference among all 3 species. The distribution of L. palati and L. tilapiae was similar, and L. barnimiana was reported to occur almost anywhere on the body. Besides environmental factors, the reasons for the different pattern of distribution shown by the various species of Lernaea remain unknown.

More recently the quantitative approach towards host-parasite relationship has received considerable interest. As explained by Anderson (1976) a more formal mathematical framework is ideally required in order to achieve quantitative insight into the dynamics of animal populations, and it is generally accepted that genuine

scientific advances in a diffuse area of investigation such as ecology, will be slow and uncertain if conducted entirely in intuitive and verbal terms.

The mathematical approach towards the host-parasite relationships of Lernaea has only been studied by Eisen (1977). In his study on the distribution of L. cyprinacea on C. auratus Eisen found it fitted the negative binominal distribution indicating that the parasite tended to be clumped i.e. a large number of the parasites occur in a small proportion of the host population than would be expected by random distribution.

Since L.cyprinacea and L.piscinae were found to have diverse characteristics with reference to host susceptibility, the former revealed a wide range of fish host while the later only infected A. nobilis, it was thus decided to study the frequency distribution, hosts length relationship to parasite burden and variation of infection levels among different hosts by the 2 parasite species.

5.2. Materials and methods

5.2.1. Experimental design

The data on the distribution of the adult female parasite L.cyprinacea on C. auratus and A. nobilis and of L.piscinae on A. nobilis, were recorded from fish used for experiment in Chapter 4 (pg. 56 and 58). In addition to these 3 host-parasite systems, data were also collected from H. temmincki infected with L. cyprinacea.

Five hundred and twenty H. temmincki of 50-130 mm standard length were purchased from the local aquarium fish dealer and were treated with formalin at 166 ppm for 30 minutes for ectoparasites. The fish were then acclimatized in aquarium conditions for one week. They were then exposed to L. cyprinacea infection using the same method as used for the other 3 host-parasite systems. Three hundred and ninety egg sacs of L.cyprinacea were hatched and distributed equally among groups of 40 fish kept in each tank. On the appearance of the adult parasites their distribution on the body of the host was recorded (parasites from the left and right side of the host were recorded separately) from the different regions of the body as illustrated in figure 5.1.

5.2.2. Sampling technique

To describe the location of the parasite, the body was divided into eight major regions, region 1-4 the body proper, 5 - the caudal peduncle, 6 - the fins, 7 - head, 8 - eyes. The major regions were further sub-divided into regions as shown in Figure 5.1. The different fins on the body and its base measuring about 2 mm were recorded separately. If the location of the fins occupied more than one region of the body as for example, the dorsal fin of H. temmincki covered almost the whole of the dorsal region, it was divided into three sections, anterior, middle and posterior. The periorbital region around the eye was also considered a separate region. The data on the distribution of the parasites were collected from 500 fish.

Figure 5.1 Regions of fish body identified as locations for *Lernaea* sp.

Body proper
1) Anterior dorsal
2) Posterior dorsal
3) Anterior ventral
4) Posterior Ventral

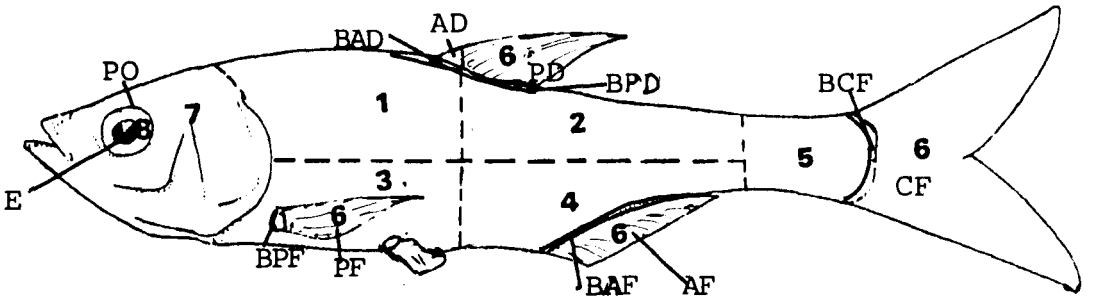
Caudal peduncle - 5

Fins - 6
AD - Anterior dorsal
BAD - Base of anterior dorsal
MD - Mid-dorsal
BMD - Base of mid-dorsal
PD - Posterior dorsal
BPD - Base of posterior dorsal
PF - Pectoral fin

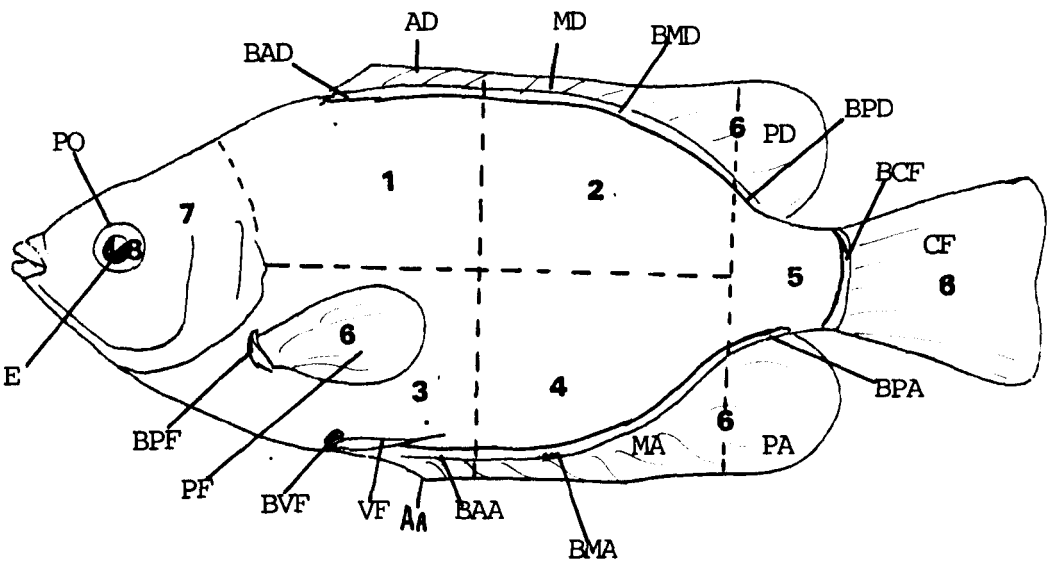
Fins
AA - Anterior anal
BAA - Base of anterior anal
MA - Mid anal
BMA - Base of mid-anal
PA - Posterior anal
BPA - Base of posterior anal
CF - Caudal fin
BCF - Base of caudal fin
VF - Ventral fin
BVF - Base of ventral fin
BPF - Base of Pectoral fin

Eye - 8
E - Eye

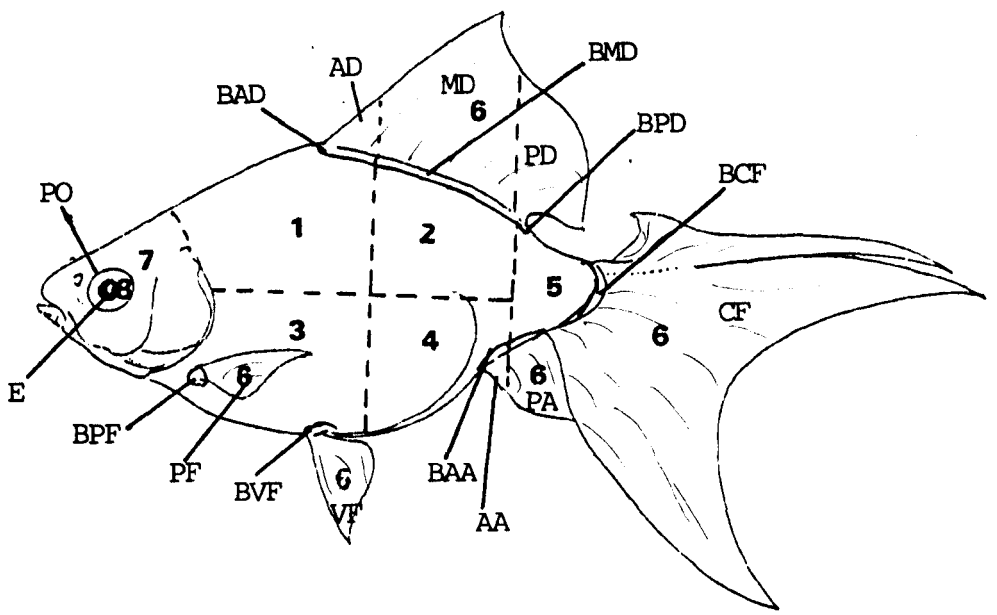
Head - 7
PO - Periorbital



A. nobilis



H. temmincki



C. auratus

5.2.3. Measurement of host body surface area

The surface area of the major regions 1-8, including the base of all fins, of the four-hosts were measured with the aid of a graph paper. The individual sections of the separate regions were cut and placed on a graph paper, the area covered by the section was marked off and the number of squares counted within the area covered and multiplied by two (for left and right side). The average surface area of the three host species was derived by taking measurements from 3 fish of each species which were within the average standard length of the respective group.

5.2.4. Statistical analyses, theoretical negative binomial and poisson distributions.

All statistical analyses were carried out on the Vax computer at the University of Stirling. The frequency distributions of the parasites were compared with the theoretical negative binomial and poisson distributions on a computer programme as designed by Davies (1971).

Definitions such as Mean Intensity, Density and Prevalence were used according to the recommendations of Margolis *et al.* (1982).

5.3. Results

5.3.1. Frequency distribution

The frequency distribution of *L. cyprinacea* on

H. temmincki, C. auratus, A. nobilis, and of L. piscinae on A. nobilis in comparison with the theoretical negative binomial distribution are shown in Table 5.1, 5.2, 5.3 & 5.4. The frequency distribution of L. cyprinacea in H. temmincki, C. auratus and A. nobilis, conforms to the theoretical negative binomial distribution. The distribution frequency of L. cyprinacea from the three host species could not be fitted by a poisson distribution.

The distribution frequency of L. piscinae on A. nobilis revealed that it could neither fit the theoretical negative binomial or the poisson distribution (Table 5.4).

In C. auratus and H. temmincki infected with L. cyprinacea and A. nobilis infected with L. piscinae the 0 class size was the greatest in distribution and generally in large amounts that is 63.2%, 40.6% and 65.4% of the three hosts population respectively (Fig. 5.2, 5.3 and 5.4). In all the 4 host-parasite systems, there was a sharp reduction in the class 1 size distribution frequency. In H. temmincki it was 18.2%, C. auratus 10.8%, while in A. nobilis infected with L. piscinae it was 7.8%. The 0 size class frequency distribution of L. cyprinacea from A. nobilis was lower compared to the other host-parasite systems and was 24% (Fig. 5.5). In general, after the class 1 size, the frequency distribution was seen to decline gradually in all 4 host-parasite systems. However, in A. nobilis infected with L. piscinae, the frequency distribution of class size 8 was noted to be higher than for class size 5(2.4%), 6(2.4%) and 7(1.8%) as compared to 2.8%

Table 5.1

Comparison of the observed frequency with negative binomial and poisson distribution of L. cyprinacea infection in H. temmincki.

class number	class size	observed frequency	fitted negative binomial	fitted poisson distribution
1	0	203	200.83	56.75
2	1	91	93.31	123.48
3	2	57	58.64	134.34
4	3	40	39.64	97.44
5	4	27	27.99	53.01
6	5	18	20.21	23.07
7	6	12	14.81	8.37
8	7	9*	10.97	2.60
9	8	9	8.18	0.72
10	9	9	6.14	0.17
11	10	8	4.63	0.04
12	>11-20	7	3.51	0.01
	>20	10	11.39	0.00
		E 500	E 500	E 500
			X 5.99	X 1706.01
			P>0.05	P<0.001
			DF 10	DF 6
			K=0.702	

* Chi-squared test for poisson distribution was calculated by adding the classes after this point.

Table 5.2

Comparison of the observed frequency with negative binomial and poisson distribution of L. cyprinacea infection in C. auratus.

class number	class size	observed frequency	fitted negative binomial	fitted poisson distribution
1	0	316	311.23	128.07
2	1	54	67.52	174.44
3	2	31	35.71	118.79
4	3	22	22.60	53.93
5	4	17	15.47	18.36
6	5	15	11.08	5.00
7	6	10*	8.16	1.14
8	7	10	6.13	0.22
9	8	9	4.68	0.04
10	9 - 30	6	3.61	0.01
11	>30	10	13.80	0.00
		E 500	E 500	E 500
			X 14.43	X 3104.83
			P>0.05	P<0.001
			DF 7	DF 5
			K=0.40	

* Chi-squared test for poisson distribution was calculated by adding the classes after this point.

Table 5.3

Comparison of the observed frequency with negative binomial and poisson distribution of L. cyprinacea infection in A. nobilis.

class number	class size	observed frequency	fitted negative binomial	fitted poisson distribution
1	0	120	118.74	29.40
2	1	96	98.13	83.29
3	2	77	75.30	118.02
4	3	54	56.31	111.49
5	4	43	41.55	78.99
6	5	32	30.41	44.77
7	6	24	22.14	21.15
8	7	11	16.06	8.56
9	8	7*	11.61	3.03
10	9 - 19	5	8.38	0.96
11	20 - 29	6	6.03	0.27
12	30 - 39	9	4.34	0.07
13	>39	16	10.98	0.02
		E 500	E 500	E 500
			X 12.56	X 840.22
			P>0.05	P<0.001
			DF 10	DF 7
			K=1.25	

* Chi-squared test for poisson distribution was calculated by adding the classes after this point.

Table 5.4

Comparison of the observed frequency with negative binomial and poisson distribution of L. piscinae infection in A. nobilis.

class number	class size	observed frequency	fitted negative binomial	fitted poisson distribution
1	0	327	321.05	90.07
2	1	39	55.86	154.38
3	2	22	29.95	132.31
4	3	17	19.68	75.59
5	4	16	14.12	32.39
6	5	12	10.64	11.10
7	6	12	8.27	3.17
8	7	9*	6.58	0.78
9	8	14	5.31	0.17
10	9	4	4.38	0.03
11	10	5	3.59	0.01
12	11-20	10	2.99	0.00
13	>20	13	17.59	0.00
		E 500	E 500	E 500
			X 43.08	X 4100.95
			P<0.001	P<0.001
			DF 10	DF 5
			K=0.19	

* Chi-squared test for poisson distribution was calculated by adding the classes after this point.

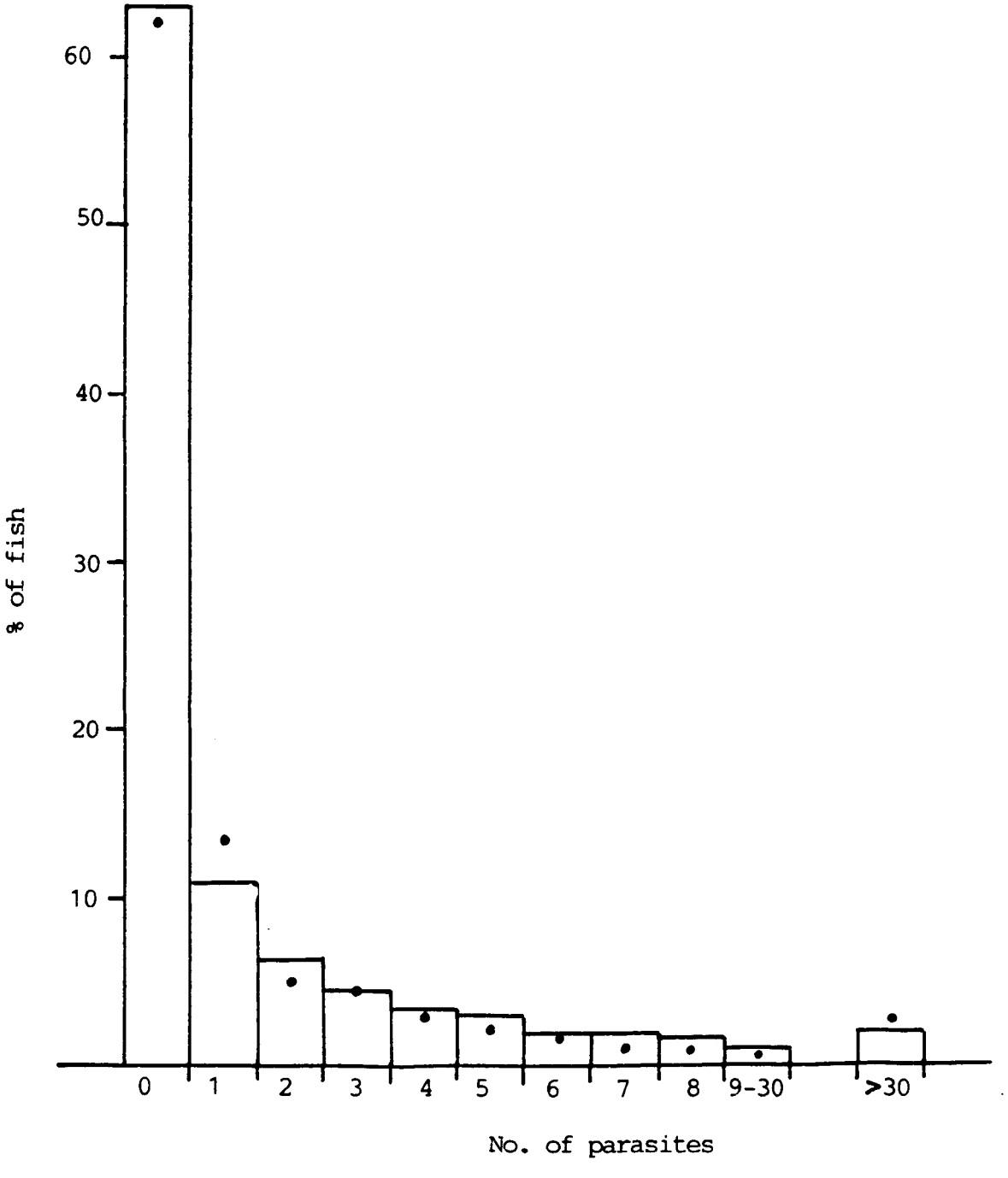


Figure 5. 2 Frequency distribution of L. cyprinacea in C. auratus

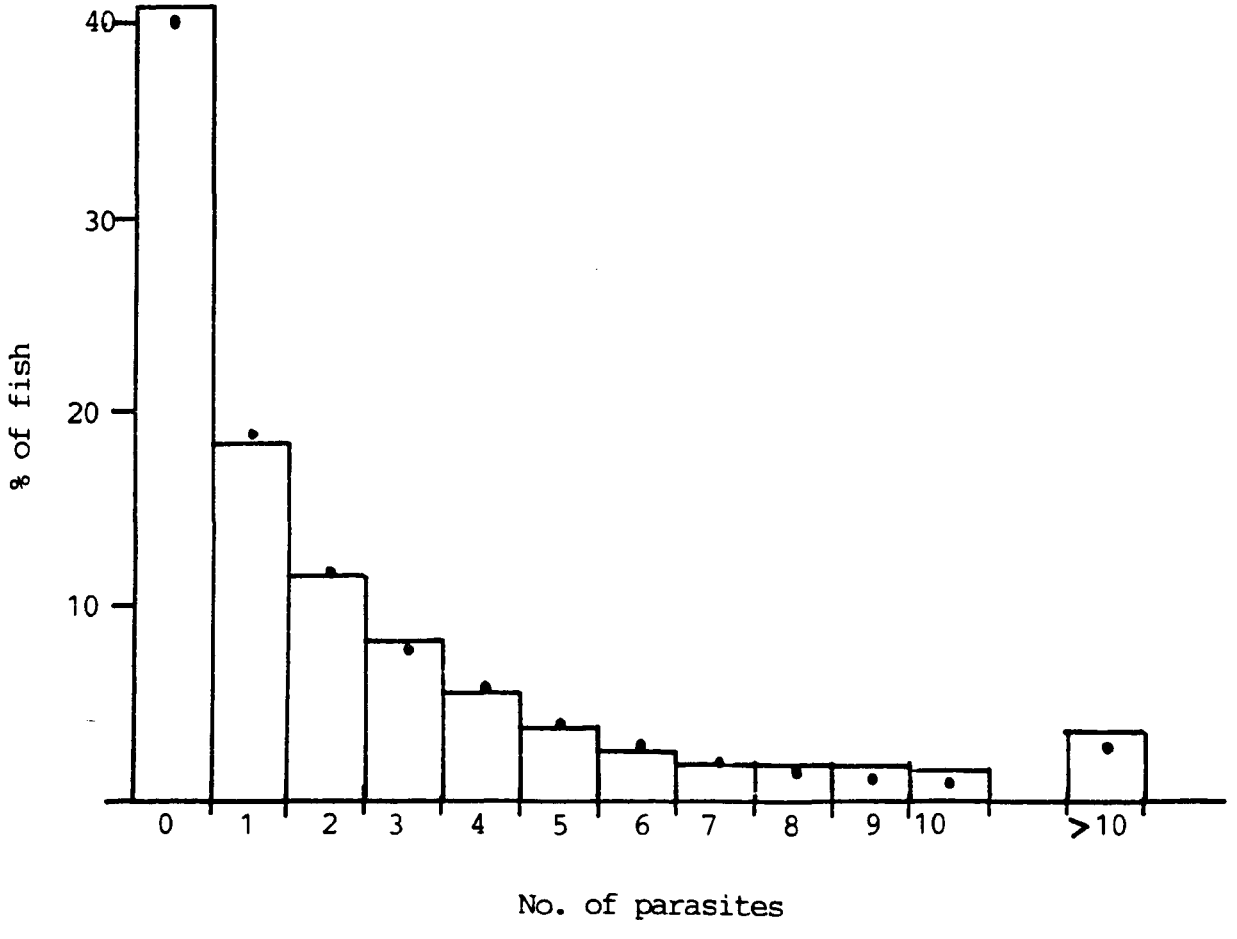


Figure 5.3 Frequency distribution of L. cyprinacea in H. temmincki

The histogram represents the original data and the spots (•) the fitted negative binomial distribution.

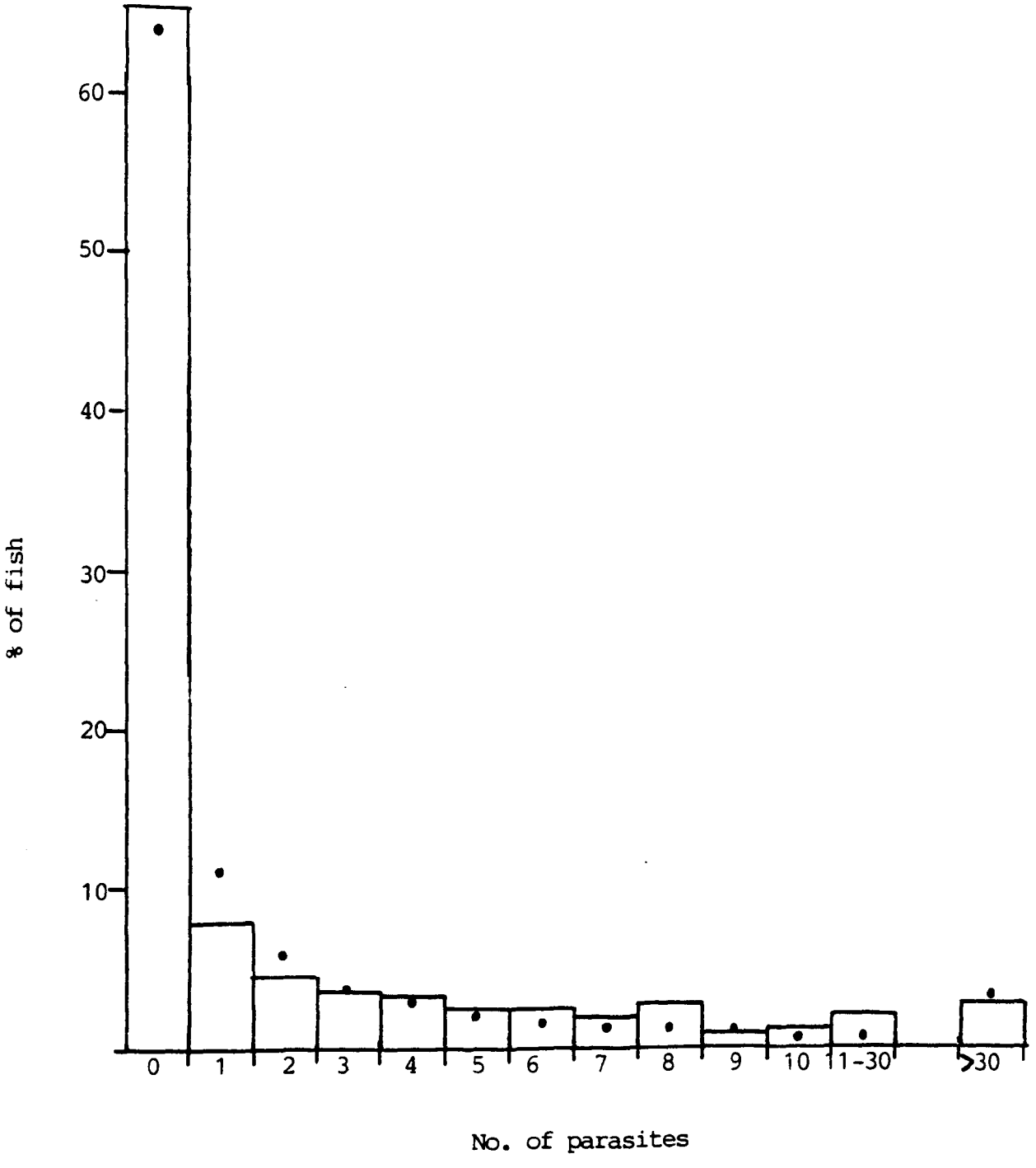


Figure 5.4 Frequency distribution of L. piscinae in A. nobilis. The histogram represents the original data and the spots (•) the fitted negative binomial distribution.

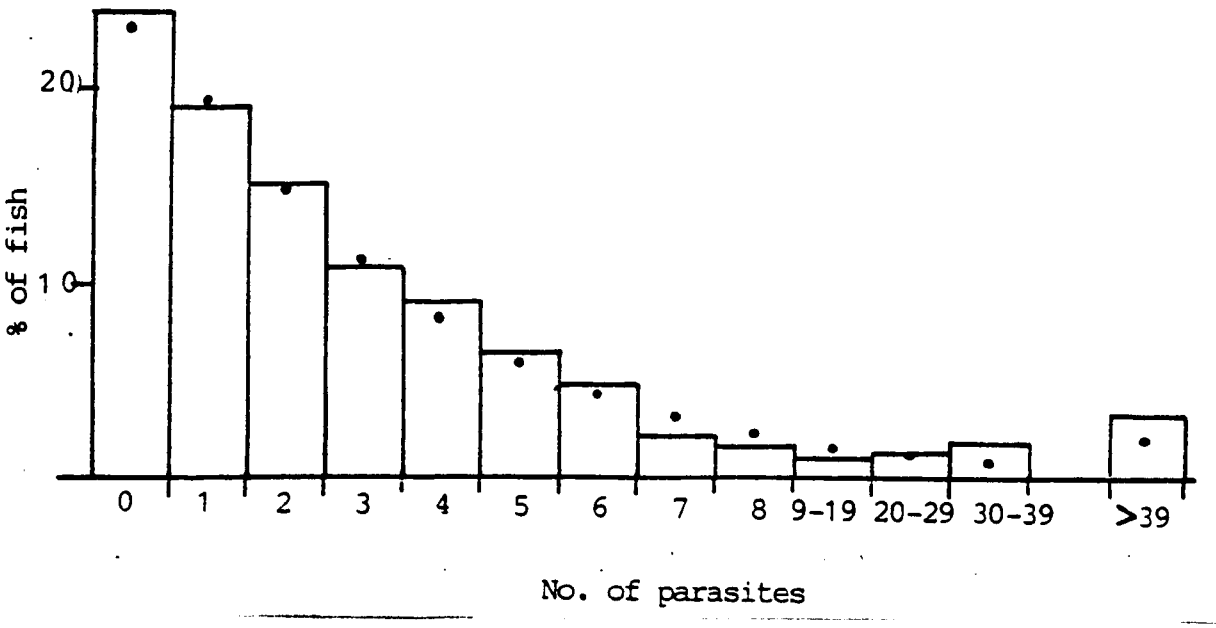


Figure 5.5 Frequency distribution of L. cyprinacea in A. nobilis
The histogram represents the original data and the spots (•) the fitted negative binomial distribution.

of the former (Fig. 5.4). Class size 10 was found to be greater than class 9 by 2%.

The distribution frequency of class size 9 and above was small in all the 4 host-parasite systems. In H. temmincki, C. auratus, and A. nobilis infected with L. cyprinacea, it was 6.8%, 3.2% and 7.2% respectively; while in A. nobilis infected with L. piscinae it was 6.4%.

The maximum number of L. cyprinacea from H. temmincki, C. auratus and A. nobilis, was 298, 115 and 81 parasites respectively recorded from a single host; whilst in L. piscinae the maximum number of parasites was 90 from a single host. The variance in all 4 host parasite systems was larger than the mean. In C. auratus, H. temmincki and A. nobilis infected with L. cyprinacea the variance was 111.55, 342.63, 168.04 and the variance to mean ratio was 42.4, 86.06 and 32.13 respectively. In A. nobilis infected with L. piscinae, the variance wa s 93.41 and the variance to mean ratio was 32.13

5.3.2. Host-length parasite relationship

In general the prevalence (expressed in percentage) of infection was noted to increase from fish of small size class to fish of middle size class in each case, followed by a decrease among the fish of larger size class (Fig. 5.6, 5.7, 5.8 and 5.9). In H. temmincki of 5-13 cm size range, the highest prevalence of L. cyprinacea infection were found to be among the 9-10 cm class size (Fig. 5.6).

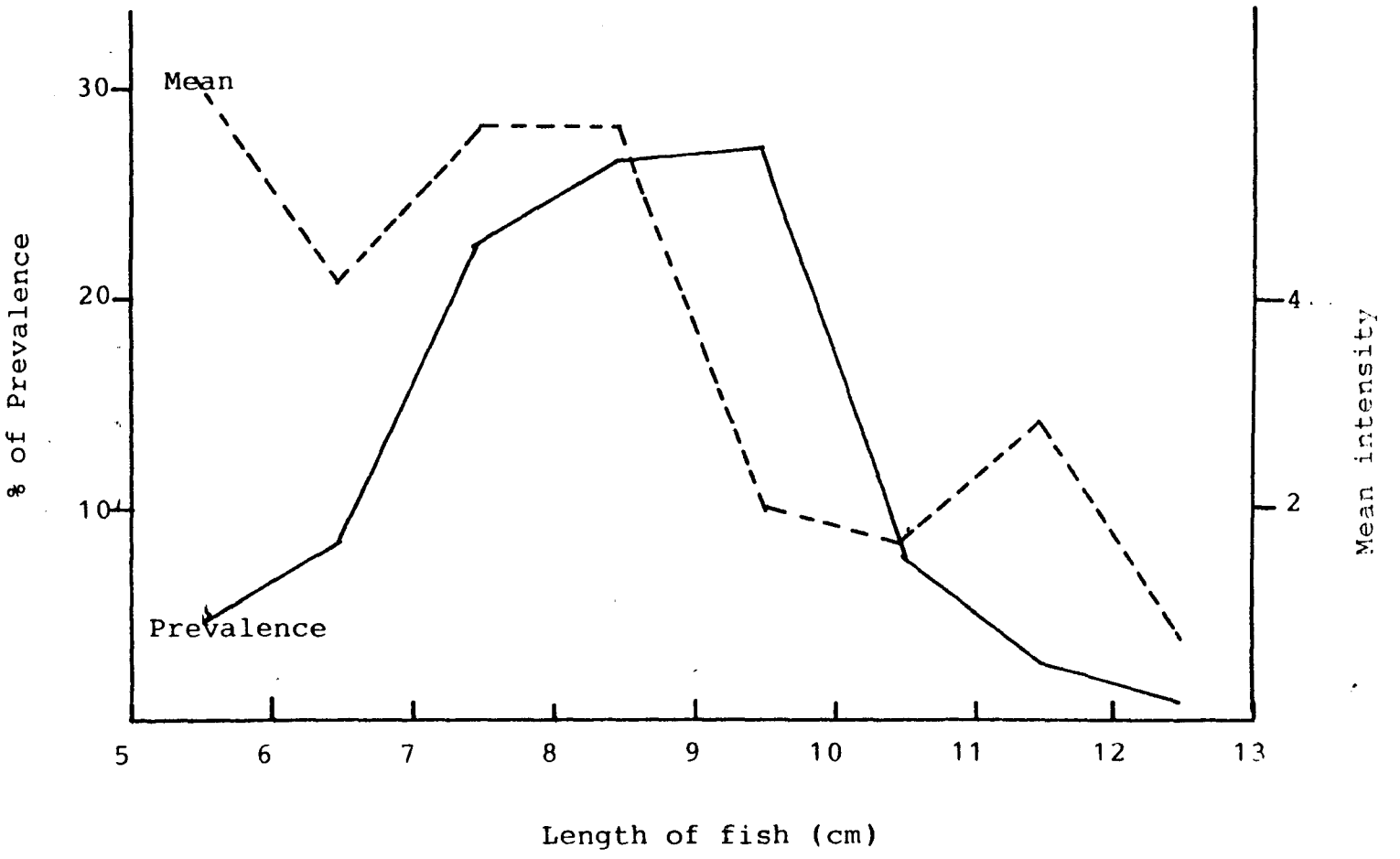


Figure 5.6 Percentage prevalence and mean intensity of L. cyprinacea H. temmincki in relation to length of fish.

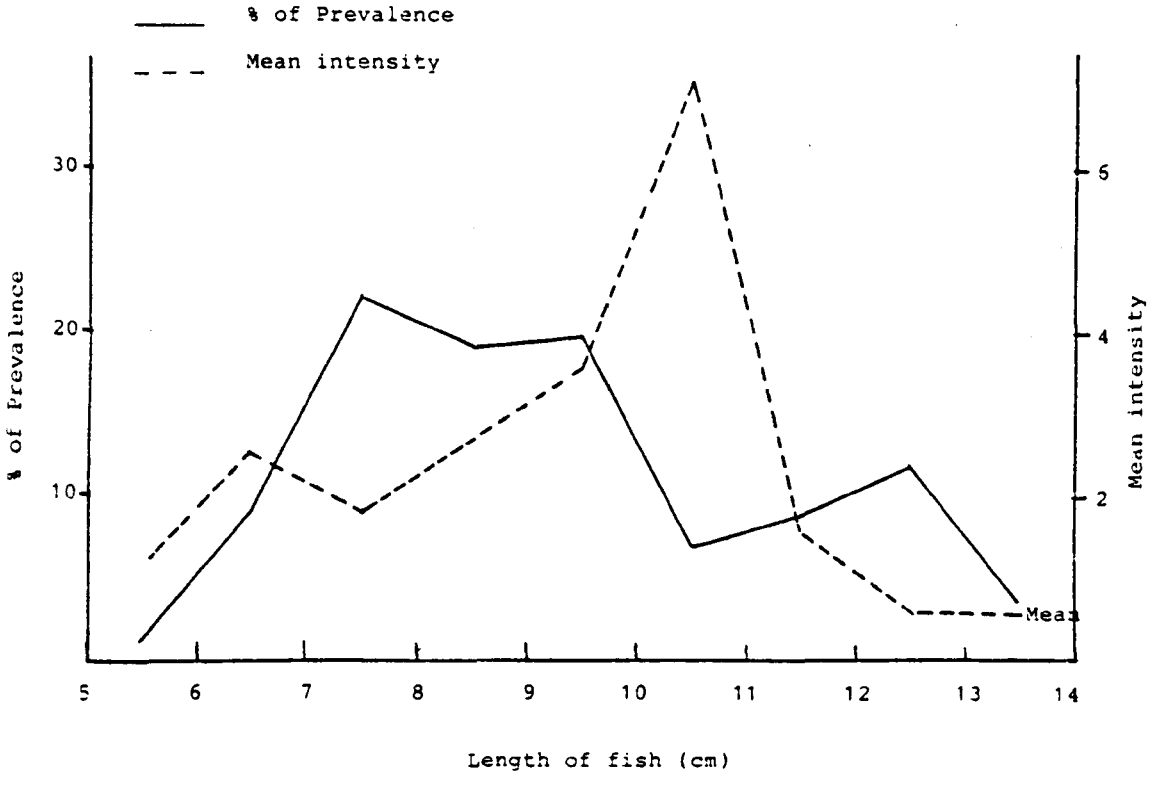


Figure 5.7 Percentage prevalence and mean intensity of L. cyprinacea in C. auratus in relation to length of fish.

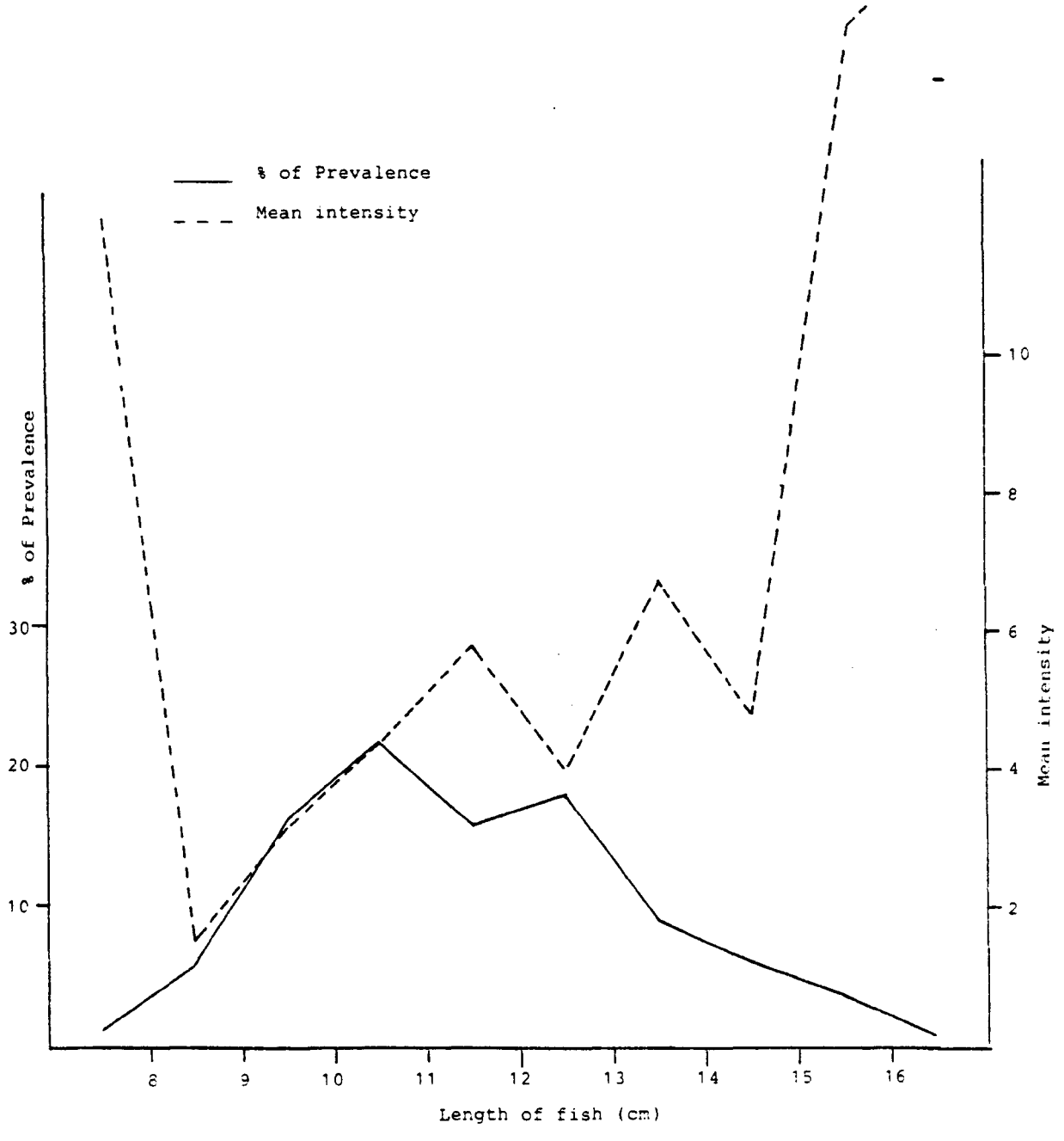


Figure 5.8 Percentage prevalence and mean intensity of L. cyprinacea in A. nobilis in relation to length of fish.

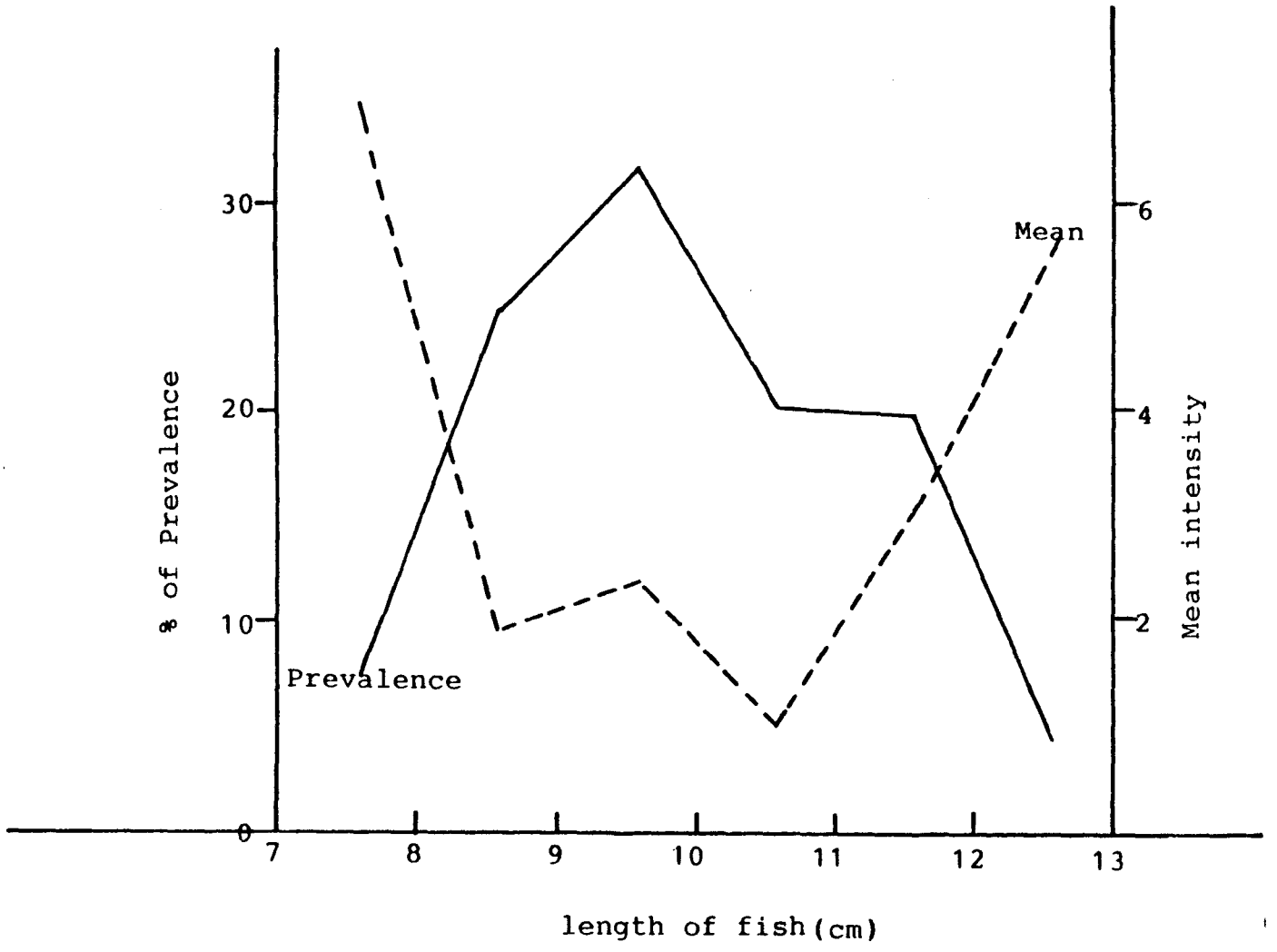


Figure 5.9 Percentage prevalence and mean intensity of L. piscinae in A. nobilis in relation to length of fish.

In C. auratus of 5-14 cm size range, the peak of prevalence was in the 7-8 cm class size (Fig. 5.7). In A. nobilis of 8-16 cm size range and infected with L. cyprinacea, the peak of the prevalence of infection was in the 10-11 cm size class (Table 5.8). The highest intensity was also in this size class (32 parasites per infected fish) among the 4 host-parasite systems. In A. nobilis of 7-13 cm size range, infected with L. piscinae, the prevalence was highest at the 9-10 cm class size (Fig. 5.9).

The mean intensity of infection in relation to size class of host did not reveal any general pattern amongst the 4 host-parasite systems. In H. temmincki infected with L. cyprinacea, the mean intensity was highest in the smallest size class (5-6 cm) and lowest in fish of the largest class size of 12-13 cm (Fig. 5.6). In C. auratus infected with L. cyprinacea, the Mean Intensity was highest 10-11 cm class size while the lowest intensity was seen in 12-13 cm class size. In A. nobilis infected with L. cyprinacea, the highest peak of intensity was seen in the 16-17 cm class size which was also found to be the highest (i.e. 16.25%) among the 4 host-parasite systems (Fig. 5.8). The 8-9 cm class size revealed the lowest mean intensity. In A. nobilis infected with L. piscinae, the higher mean intensity infection was noted at the extreme ends of both the smallest and highest class size; while the 10-11 cm class size had the lowest intensity of infection (Fig. 5.9).

5.3.3. Site-selection

Table 5.5, 5.6, 5.7, 5.8, 5.9 and Figure 5.10 shows the

Table 5.5

Distribution of *Lernaea cyprinacea* on *A. nobilis*

Site of Infection	No.of Parasites	Surface area (sq.mm/host)	Density (No.of parasites/ sq.mm)
Body Proper			
Anterior dorsal	306	554	0.55
Posterior dorsal	288	313	0.92
Anterior ventral	414	720	0.58
Posterior ventral	249	295	0.84
Peduncle	331	245	1.35
Head	121	1087	0.11
Eye	8	89	0.09
Periorbital	5	1	5
Fin Dorsal			
Anterior dorsal	11	24	0.45
Base	115	16	7.19
Posterior dorsal	5	188	0.03
Base	127	20	6.35
Fin Ventral	13	404	0.03
Base	78	8	9.75
Fin Anal	5	249	0.02
Base	180	7	25.71
Fin Pectoral	72	673	0.10
Base	170	30	5.67
Fin Caudal	17	602	0.03
Base	103	21	4.90
Total	2618	5545	

Table 5.6

Distribution of *Lernaea piscinae* on *A. nobilis*.

Site of Infection	Total No. of Parasites	Surface area (sq.mm/host)	Density (No.of parasites/ sq.mm)
Body proper			
Anterior dorsal	224	388	0.58
Posterior dorsal	123	199	0.62
Anterior ventral	399	567	0.70
Posterior ventral	159	188	0.85
Peduncle	106	194	0.55
Head	146	755	0.19
Eye	5	68	0.07
Periorbital	21	1	21
Fin Dorsal			
Anterior dorsal	0	18	0.00
Base	24	12	2.00
Posterior dorsal	0	148	0.00
Base	20	14	1.43
Fin Ventral	0	168	0.00
Base	36	8	4.50
Fin Anal	1	118	0.01
Base	32	8	4.00
Fin Pectoral	8	381	0.02
Base	42	18	2.33
Fin Caudal	1	388	0.00
Base	106	24	4.42
Total	1453	3664	

Table 5.7

Distribution of Lernaea cyprinacea on C. auratus.

Site of Infection	Total No. of Parasites	Surface area (sq.mm/host)	Density (No.of parasites/ sq.mm)
Body proper			
Anterior dorsal	92	281	0.33
Posterior dorsal	83	156	0.53
Anterior ventral	244	459	0.53
Posterior ventral	153	236	0.65
Peduncle	133	74	1.79
Head	68	417	0.16
Eye	4	28	0.14
Periorbital	15	1	15.00
Fin Dorsal			
Anterior dorsal	5	53	0.09
Base	19	10	1.90
Mid-dorsal	3	314	0.01
Base	27	22	1.22
Posterior dorsal	0	66	0.00
Base	29	7	4.14
Fin Anal			
Anterior Anal	0	79	0.00
Base	40	16	2.50
Posterior Anal	0	160	0.00
Fin Pectoral	3	266	0.01
Base	63	14	4.50
Fin Caudal	186	2818	0.07
Base	123	17	7.23
Fin Ventral	1	402	0.00
Base	24	18	1.33
Total	1315	5913	41.99

Table 5.8

Distribution of *Lernaea cyprinacea* on *H. temmincki*.

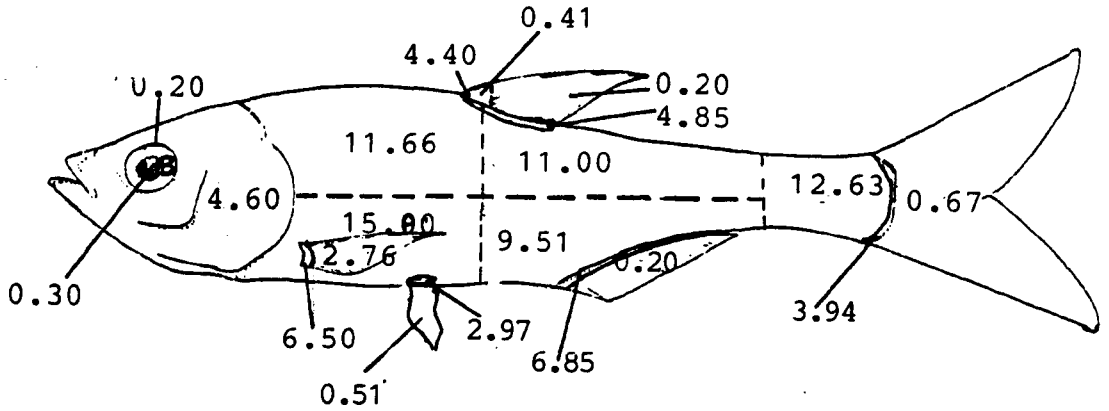
Site of Infection	Total No. of Parasites	Surface area (sq.mm/host)	Density (No.of parasites/ sq.mm)
Body proper			
Anterior dorsal	78	396	0.20
Posterior dorsal	60	344	0.17
Anterior ventral	82	491	0.17
Posterior ventral	32	252	0.13
Peduncle	55	236	0.23
Head	208	609	0.34
Eye	53	87	0.62
Periorbital	250	1	250
Fin Dorsal			
Anterior dorsal	8	146	0.05
Base	244	38	6.42
Mid-dorsal	34	158	0.21
Base	235	28	8.43
Posterior dorsal	3	108	0.03
Base	8	1	8.00
Fin Anal			
Anterior anal	19	58	0.33
Base	115	13	8.85
Mid-anal	9	188	0.05
Base	78	17	4.58
Posterior-anal	1	88	0.01
Base	46	3	15.33
Fin Pectoral			
Fin	17	428	0.04
Base	174	16	10.88
Fin Ventral			
Fin	4	164	0.02
Base	40	4	10.00
Fin Caudal			
Fin	96	550	0.17
Base	40	16	2.50
Total	1991	4439	

Table 5.9

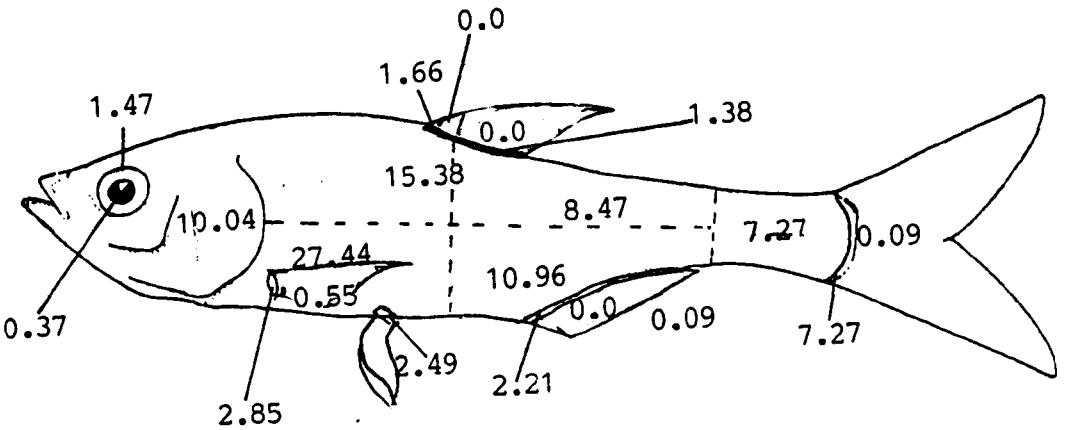
Descending order of density of L. cyprinacea infection from different regions of C. auratus, A. nobilis and H. temmincki and of C. piscinae from A. nobilis.

Lernaea cyprinacea infection of			Lernaea piscinae infection of
C. auratus	H. temmincki	A. nobilis	A. nobilis
Periorbital	Periorbital	Base of anal fin	periorbital
Base of caudal fin	Base of posterior anal fin	Base of ventral fin	Base of ventral fin
Base of pectoral fin	Base of pectoral fin	Base of anterior dorsal fin	Base of caudal fin
Base of posterior dorsal fin	Base of ventral fin	Base of posterior dorsal fin	Base of anal fin
Base of anterior dorsal fin	Base of anterior anal fin	Base of pectoral fin	Base of Pectoral fin
Base of anal fin	~~Base of anterior anal fin~~	Periorbital	Base of anterior dorsal fin
Peduncle	Base of posterior dorsal fin	Base of caudal fin	Base of posterior dorsal fin
Base of ventral fin	Base of anterior dorsal fin	Peduncle	Body posterior ventral
Base of mid-dorsal fin	Base of mid-anal fin	Body posterior dorsal	Body anterior ventral
Body posterior dorsal	Base of caudal fin	Body posterior ventral	Body posterior dorsal
Body posterior ventral	Eyes	Body anterior ventral	Body anterior dorsal
Body anteror ventral	Head	Body anterior dorsal	Peduncle
Body anterior dorsal	Fin anterior anal	Fin anterior dorsal	Head
Head	Peduncle	Head	Eye
Eye	Fin mid-dorsal	Fin pectoral	Fin pectoral
Fin anterior dorsal	Body anterior dorsal	Eye	Fin anal
Fin caudal	Fin caudal	Fin ventral	Fin caudal
Fin pectoral	Body posterior dorsal	Fin caudal	Fin posterior dorsal *
Fin	Body anterior ventral	Fin posterior dorsal	Fin ventral *
Fin ventral	Body posterior ventral	Fin anal	Fin anterior dorsal *
Fin anterior anal *	Fin anterior dorsal		
Fin posterior dorsal*	Fin mid-anal		
Fin posterior anal *	Fin pectoral		
	Fin posterior dorsal		
	Fin ventral		
	Fin posterior anal		

* with zero infection

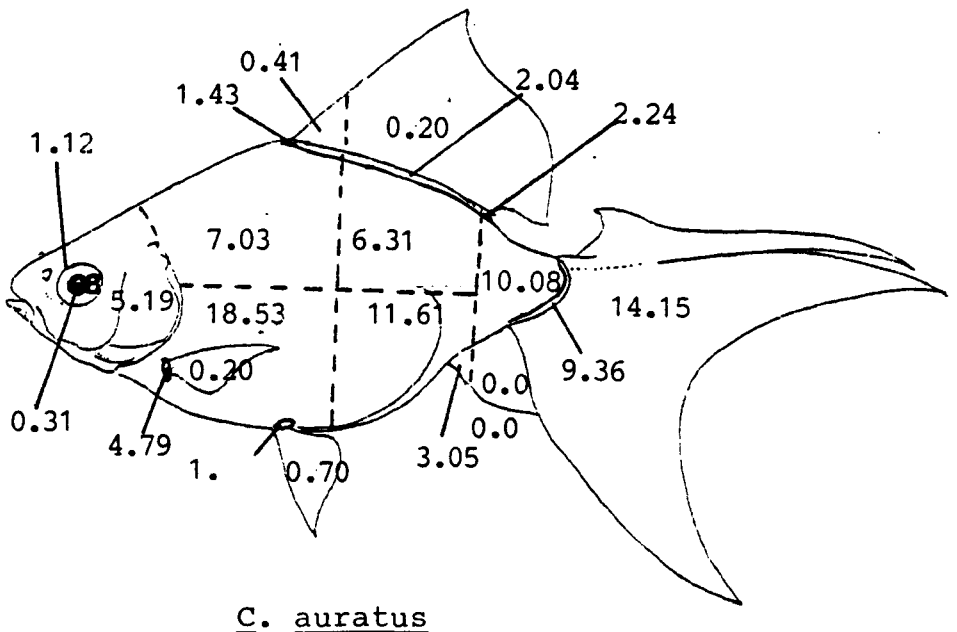


A. nobilis infected with L. cyprinacea

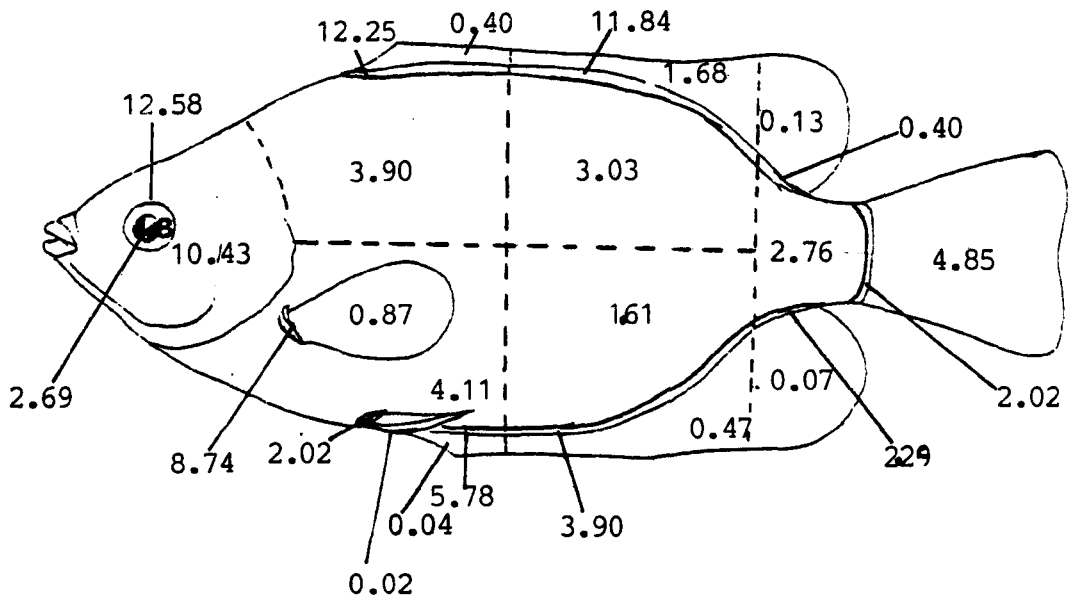


A. nobilis infected with L. piscinae

Figure 5.10 a Distribution of L. piscinae and L. cyprinacea A. nobilis (in percentage of total number).



C. auratus



H. temmincki

Figure 5.10 b Distribution of L. cyprinacea in C. auratus and H. temmincki (in percentage of total number).

site selection of L. piscinae and L. cyprinacea on their respective hosts. The term site selection refers to part of the host in which the parasite was found. Site selection was expressed in terms of density i.e. number of parasites per sq. millimeter.

The site selection by the adult parasite in the 4 host parasite systems revealed that the density of distribution, was greatest in the periorbital region except in A. nobilis infected with L. cyprinacea (Table 5.5, 5.6). Although the sequence of distribution at the base of all the fins was not similar in the 4 hosts, the bases of fins were found to have the second highest density of parasites except in A. nobilis where it was the highest. In C auratus infected with L. cyprinacea, the highest density of these parasites was found at the base of the caudal fins, whereas in H. temmincki and A. nobilis it was at the base of anal fins. In A. nobilis infected with L. piscinae the highest density was at the base of the ventral fins. The fin proper, that is total fin area exclusive of base, showed the lowest density of infection in all the 4 host parasite systems (Table 5.9 and Fig. 5,10).

Considering the fin proper (exclusive of fin base), the anterior dorsal was found to have the highest density of infection in C.auratus and A. nobilis infected with L. cyprinacea, whereas in A. nobilis infected with L. piscinae and H. temmincki infected with L. cyprinacea it was the pectoral and the anterior anal fins respectively (Table 5.9).

In H. temmincki infected with L. cyprinacea the fin proper revealed a higher density of infection than the body regions and the reverse was seen in A. nobilis with L. piscinae and in H. temmincki with L. cyprinacea.

Within the body proper regions (taking into consideration both the anterior and posterior regions), the ventral region showed higher density of infection in 2 host parasite systems, that is in C. auratus infected with L. cyprinacea (Table 5.7) and in A. nobilis infected with L. piscinae (Table 5.6); the reverse was seen in A. nobilis and H. temmincki infected with L. cyprinacea where the dorsal region revealed a higher density of infection (Table 5.8 and 5.9). The posterior ventral region of the body proper was also found to be infected with the highest density in host parasite systems with the exception of H. temmincki and A. nobilis infected with L. cyprinacea. With the exception of L. piscinae in the other 3 host parasites systems, the peduncle region had a higher intensity of infection as compared with the body proper regions 1-4.

The density of the parasite in the remaining regions varied in different hosts. The head region had a higher density of infection compared to the eyes in all host parasite systems with the exception of H. temmincki infected with L. cyprinacea (Table 5.9).

5.4. Discussion

The frequency distribution of L. cyprinacea on

A. nobilis, H. temmicki and C. auratus did not fit poisson distribution but conformed with the theoretical negative bionomial distribution. This means that the parasite distribution within the host population was not random but overdispersed; indicating that a large number of parasites were accommodated in a small number of hosts. According to Crofton (1971a, 1971b) such a pattern with high number of parasites in a small number of host is a regulatory mechanism to prevent unlimited exponential growth of the parasites. He postulated that a higher number of parasites in a small number of host may result in host mortalities and this in turn reduces the parasite population which is an essential regulatory role in the population dynamics of host-parasite interactions. Another obvious advantage of this pattern is that it will increase the probability of a male and female parasite occurring together on the same fish (Boxshall 1974). Overdispersed distributions have also been recorded from numerous other host-parasites systems (Kennedy 1968, Kennedy and Hine 1969, Walkey et al. 1970, Pennycuick 1971, Crofton 1971, Boxshall 1974, Anderson 1974, Mackenzie and Liversidge 1975, Kennedy 1975, Shotter 1976, Jarrol 1979 and others). The distribution of L. cyprinacea on C. auratus predicted by the negative binomial distribution has also been reported by Eisen (1977). Although most of the previous studies were conducted on naturally infected host-parasite systems, the results of the present study closely agree with the other workers on the frequency

distribution of parasite in the host population inspite of the fact that the infection of the host was conducted under laboratory conditions which provided an equal opportunity for every parasite to infect a host. The distribution of L. piscinae in A. nobilis did not fit the negative binomial distribution, but the value of K was below zero indicating that the distribution was overdispersed. Tanner et al. (1980) suggested that overdispersion is very likely to be under genetic control and similarly Wakelin (1978) suggested that there is convincing evidence to indicate that susceptibility of host animals to a wide variety of parasites is under genetic control.

There was apparently no indication that fish of longer standard length had a higher burden of infection. Previous studies on the intensity of infection of Lernaea in relation to host length in fish from the wild do not suggest any significant trend, although there were indications that smaller host tended to have a higher burden of infection. The work of Timmons and Hemstreet (1980) showed that L. cyprinacea infection in largemouth bass, Micropterus salmoides (Lacepede) was restricted to small fish of less than 100 mm, although the authors noted that they had previously observed infection in adult M. salmoides of 225 mm. Amin et al. (1973) reported that although there was no strong correlation of infection with host size, smaller fish tended to be more heavily infected than larger ones, for example amongst a total of 105 fish, total length of 2-9 mm,

29 of the infected fish were from the 2-3 mm group range. The above reports indicate that the higher level of infection is amongst smaller fish in the natural population, and it is possible that the lower burden of parasites in larger host could be a result of a build up of immunity in older group host or mortality. The possibility the development of an immune response is investigated in Chapter 6. Under laboratory conditions when fish have only been exposed to a single infection as in this study, the results would apparently differ from those in the natural population where infection may have been persistent for some time enabling the fish to develop immunity.

A comparison between the average body surface areas amongst the 4 groups of fish species and the density of infection also failed to reveal any relationship. Although C. auratus had a larger body surface area, 38% more than the body surface area of A. nobilis, but the intensity of infection by L. piscinae was greater in A. nobilis with a Mean of 8.3 parasites, compared to 7.14 in C. auratus even though they were exposed to the same number of larvae. On the other hand H. temmincki which had a body surface area of 19.9%, less than that of A. nobilis with L. cyprinacea showed almost the same intensity of infection i.e. 6.7 as compared to 6.89 parasites in the former.

Between C. auratus and A. nobilis, the former was revealed to be the preferred host for L. cyprinacea (viz Chap 4). The mean intensity of infection was found to be higher in C. auratus 7.14 parasites as compared to 6.89 in

A. nobilis infected with L. cyprinacea. Similarly, the maximum number of parasites from a single host was also higher in C. auratus, 115 as compared to 81 parasites in A. nobilis infected with L. cyprinacea.

The study of the site selection of the adult parasite on the host failed to show any major differences between the two species of parasites, L. piscinae and L. cyprinacea, and neither was there any differences among the 4 host-parasites system used in the experiment. High density of infection was confined to the periorbital region and the base of the fins. Although the surface area of the body proper (region 1-4) was larger than the base of fins in all 4 hosts, the total parasite population was always higher at the base of fins. For example in H. temmincki where the surface area of body proper was 33.4%, the total parasite population was only 12.6% whereas the base of fins which was represented by a small surface area of 13.1% had a parasite population of 49.1%. The base of the fins as the major site of infection of the adult parasite of L. cyprinacea has also had been reported by Bulow et al. (1979), from a stream population, in pond fish by Haley and Winn (1959) and in a hatchery, rainbow trout by McNeil (1961). According to McNeil L. cyprinacea seemed to prefer locations which offered greatest protection from water currents and Bulow et al. (1979) agreed with McNeil and added that, in addition to the protection from stream currents, the site at the base of fins would also provide the parasite protection from the effects of the scraping action of the host. The influence

of environmental conditions on site selection has also been reported by Fryer (1968) where he found the occurrence of L. barnimiana in the mouth of Barbus altianalis radcliffi Boulenger from a swift flowing river. The base of the fin as the site of attachment of another copepod, Lepeophtheirus pectoralis (Muller) has been reported by Boxshall (1974). Shimura (1983) in his study on the location of Argulus coregoni Thorell from Oncorhynchus masou (Brevoort) found that the skin around the fins, particularly the pectoral and pelvic fins served as the preferred site of attachment. He then compared his findings with those of Bazal et at. 1969, where Argulus foliaceus (Linnaeus) revealed a different pattern of distribution; the caudal fins and posterior half of the peduncle were the preferred sites. Shimura suggested that the difference in ditribution between the 2 species on different host was due to the differences in swimming pattern of the host; the host of A. coregoni is a swift swimmer thus it selected a site sheltered from water flows while the other a carp was an inactive swimmer therefore there was less influence of water currents on site selection by the parasites. He further concludes that the rubbing action of the fish body against the pond surface may also have an influence on the site selection in A. coregoni.

The results of this study, demonstrated clearly that the location of parasites was mainly at the bases of the fins, even though conducted in static aquarium tanks in the absence of the strong currents found in streams or hatchery conditions. However there have been no previous reports of

high intensity of infection in the periorbital region as was seen in this study. There are no other reports of high levels of infection in the periorbital region. This could have been due to the fact that the data collected on site selection by previous workers was from natural populations or in hatcheries, and in such cases the influence of water current may have resulted in the low or absence of infection in the periorbital region.

The results of the present study show that the periorbital region was the site with the highest density of infection. It was also noted that this region also lacked scales. There is thus a possibility that the site preferrence by Lernaea might also be related to the physical nature of the tissue as well as the influence of water currents. Kabata and Cousens (1977) in their study on the location of Salminicola californiensis Wilson on the body of Oncorhynchus nerka (Walbaum) showed that, at the larval stages, the attachment was mainly concentrated on the fins; i.e 37% of the population was found on the fins as compared to 31.5% at the base of the fins. However very few of the adult parasites in their study were found on the fins as compared to 65.2% at the base of the fins. The authors concluded that the movement of the parasites from the fins and the scale covered skin was in search for firmness and penetrable tissues such as that at fins axillae and base. Therefore besides the protection from water currents and protection from scraping action of the host, the selection

of the major sites of infection by the parasite could also be influenced by the physical nature of the tissue.

In the present study the fins proper were found to have the lowest intensity of infection, Demaree (1967), Fryer (1968), Sanderson (1974), Timmons and Hemstreet (1980) recorded that the favoured region for Lernaea infection was around the dorsal fin. In the present study, the base of the fins were regarded as the favoured sites, however the highest intensity of infection in C. auratus was in the base of caudal fins, the base of the posterior anal fin in H. temmincki and the base of the anal fin in A. nobilis; for L. piscinae on A. nobilis it was the base of ventral fin.

CHAPTER 6.

EFFECTS OF *L. PISCINAE* ON THE GROWTH OF *A. NOBILIS*.

6.1. Introduction

The adult female parasite Lernaea penetrates the host and feeds on the host tissue fluids. Depending on the severity of infection, it may cause severe mortalities as reported by Nakai (1927), Tidd (1934), Bauer (1969), Al-Hamed and Hermiz (1973) and Shariff and Vijiarungam (in press). Bauer (1959) and Sarig (1971) suggested that infection with L. cyprinacea results in loss of weight in the infected fish but these reports were based on qualitative observations made on the infected and the uninfected fish and were unsupported by quantitative data.

The occurrence of Lernaea spp. was found to be common in fish ponds in West Malaysia (Chapter 2). Lernaea infection was detected in more than 60% of the ponds studied and as yet there is no information on the quantitative effect of the disease on growth rates.

The aim of the present study was to quantify the effects of L. piscinae on the growth of A. nobilis, one of the food fishes of major importance.

6.2. Materials and methods

6.2.1. Experimental design

The study was conducted at the Freshwater Fisheries Research Station in Malacca where 6 ponds of 0.27 hectare each were used for the experiment. Six hundred A. nobilis fingerlings which were bred at the research station and were

known to be free of Lernaea infection were first weighed and measured for standard length and treated with 166 ppm formalin for 30 minutes to remove any ectoparasites that might be present. After the treatment, a batch of 100 fish was introduced into each of the 6 ponds. The ponds were divided into 3 categories for separate treatments, i.e. 1) low infection 2) high infection and 3) control. Fish in ponds designated as high infection were to receive double the number of parasites introduced to the low infection ponds. Two ponds were allocated for each treatment A and B. To enable the fish to acclimatize to the new ponds, Lernaea infection was not introduced until 2 weeks after the fish were released into the ponds.

6.2.2. Feeding and maintenance

As A. nobilis is a filter feeder, all experimental ponds were fertilised with 40 pounds of triple phosphate bimonthly. This is standard practice in the culture of A. nobilis and sufficient primary production is maintained for a good growth.

6.2.3. Introduction of Lernaea infection.

The source of the Lernaea used to infect the ponds was from a stock of A. nobilis which was maintained in the aquarium in the Faculty of Fisheries, Universiti Pertanian. The infected fish were transported in oxygenated plastic bags to Malacca, and 10 fish infected with a total of 20 adult parasites and another 10 fish infected with 40 adult

parasites were introduced into ponds catergorised as Light and Heavy infection respectively. The infected fish were placed in floating cages in the experimental ponds. Equal numbers of uninfected fish were similarly introduced into the control ponds. The fish were removed from the cages after 14 days. The 2 week period would have allowed the parasites from the fish in the cages to hatch, develop and infect the fish in the ponds.

6.2.4. Sampling of fish

Sampling of the fish in the ponds was carried out approximately once in every 3 weeks to monitor the level of infection and its effects on the growth rates. The first sampling was done 2 weeks after the parasites were first introduced into the ponds. A seine net was dragged across the ponds and 30 fish out of the 100 fish in each pond were picked at random for sampling. These fish were anaesthetised with quinalidine sulfate at 40 mg/l, before the fish were weighed and measured for the standard and total length. The number of adult parasites present on the body of the fish were recorded and the fish was then released back into their respective ponds. Specific Growth Rates were calculated from the difference between the initial sampling and the 9th. sample that is 26 weeks after infection.

The Specific Growth Rate is defined by the percentage weight gain per day and was calculated using the following equation.

$$\text{SGR (\%/day)} = \frac{\text{Log}_e\, w_2 - \log_e w_1 \times 100}{T_2 - T_1}$$

where w_2 is the weight in grams at time T_2

w_1 is the weight in grams at time T_1

$T_2 - T_1$ is the time interval between weighing w_2 and w_1 in days

6.2.5. Challenge of infection

At the 9th. sampling (6 months after the fish were first infected) no parasites were found on the body surface but some were seen on the eyes of the fish from ponds thus indicating the possibility of the development of resistance to the parasite. A new batch of parasites was then introduced into one of the Low and one of the High infection ponds as a challenge infection. The parasite was introduced into these 2 ponds following the technique described earlier and using an equal number of 35 adult female parasites was introduced into each pond. Infection was also introduced into one of the Control ponds (previously uninfected). The last sampling was carried out 3 weeks after the parasites were reintroduced. To determine the mortality rate, a count was made of all the fish from the individual ponds during the last sampling.

6.3. Results

6.3.1. Level of infection on body surface

The results of the counts of adult parasites on the

body surface of fish from 2 treatments and the Control at each sampling are presented in Table 6.1.

The level of infection (expressed as total number of parasites from 30 fish) on fish from the treatment ponds was low at most of the samplings except at samples 4 and 5 when a higher burden of parasites was recorded from both the High treatment ponds.

There was no significant differences between the level of infection in the ponds categorised as High and Low infection (Table 6.2). Subsequently the data were accumulated for both treatments and the results are presented in Figure 6.1. The level of infection (expressed as the total number of parasites from 2 replicates of both treatments) on the body surface was seen to increase from 3 parasites at the initial infection to 12 parasites by the 2nd. sampling (Fig. 6.1), followed by a decrease to 2 parasites by the 3rd. sampling, that is 9 weeks after the infection was first introduced. The 4th. sampling revealed the maximum number of 26 parasites that is 12 weeks after the infection was first introduced into the ponds. The level of infection subsequently decreased to zero by the 9th. sampling, that is 6 months after the infection was introduced.

There was no infection seen on fish from the control ponds during this period.

6.3.2. Infection of the eye

Infection of the eye was evident from the 6th. sampling

Table 6.1

Total number of adult female L. piscinae found on the body surface of A. nobilis in each pond. Haemorrhagic lesions (in parenthesis) and the number of parasites found on eyes (underlined) recorded from 30 fish from each pond at every sampling. (A and B are replicates).

Sample No.	Time Interval (week)	Treatments: Light Infection A	Light Infection B	High Infection A	High Infection B	Control A	Control B
1	2	0(10)	0(9)	1(18)	2(16)	0	0
2	5	5(4)	1(5)	6(15)	0(6)	0	0
3	9	1(9)	0(13)	0(35)	1(14)	0	0
4	12	3(20)	0(25)	8(17)	14(13)	0	0
5	14	3(9)	1(7)	5(1)	9(5)	0	0
6	17	4(4)	1(10)	3(11)	1(8)1	0	0
7	20	1(19)	3(15)	1(23)	0(9)1	0	0
8	23	0(3)2	2(0)1	1(0)2	0(2)	0	0
9	26	0(0)2	0	0	0(2)	0	0
10	29	0	0(19)*	0	0(5)2*	0	14(11)*

* - Re-introduction of parasites into previously infected ponds and the Control pond for the first time.

Table 6.2

Comparison of the number of a) adult parasites L. piscinae and b) haemorrhagic lesions from A. nobilis infected at high and low level of infection.

a) Adult Parasites

	Mean No.	S.E	t test	Probability
	Between replicates A and B			
Low infection A	1.89	0.63	1.38	>0.21
Low infection B	0.89	0.35		
High infection A	2.78	0.97	0.19	>0.85
High infection B	3.00	1.67		
	Between Treatments			
Low infection	1.39	0.37	1.59	>0.13
High infecion	2.89	0.94		

b) Haemorrhagic lesions

	Mean No.	S.E	t test	Probability
	Between replicates A and B			
Low infecion A	8.67	2.32	0.55	>0.59
Low infection B	9.33	2.61		
High infection A	13.33	3.93	1.82	>0.11
High infection B	8.33	1.71		
	Between treatments			
Low infection	9.00	1.70	0.93	>0.37
High infection	10.83	2.17		

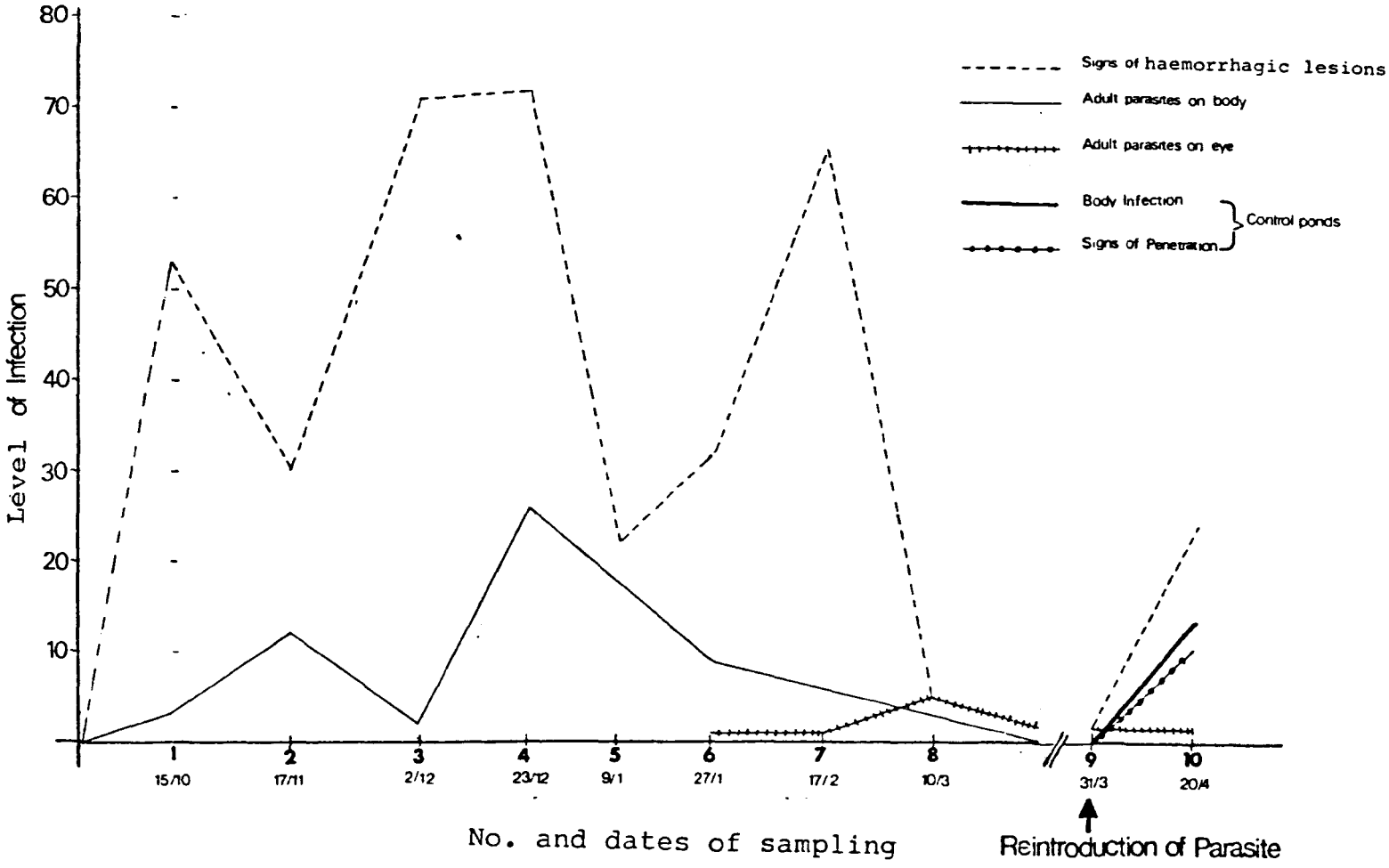


Figure 6.1 Graph showing the level of infection of L. piscinae on A. nobilis from low and high treatment ponds (pooled) and the fish from the control ponds.

onwards, that is 4 months after the infection was first introduced. The level of infection of the eye increased from 2 to 5 parasites by the 8th. sampling, that is 5 months after the infection was introduced. The infection subsequently decreased to 2 parasites during the 9th. sampling.

6.3.3. Haemorrhagic lesions

Haemorrhagic lesion associated with the parasite infection were noted from the first sampling and counted subsequently. Table 6.1 records the number of haemorrhages associated with parasitic infection. Since there was no significant differences in the data from the ponds categorised as High and Low treatment ponds (Table 6.2), the data were subsequently combined and is presented in Fig. 6.1.

The peaks of haemorrhagic lesions were seen during the 1st., 3rd., 4th., and 7th. sampling that is 2, 9, 12, and 20 weeks after the parasite was first introduced into the ponds. After the 7th. sampling the lesions decreased sharply by the 8th. sampling and was reduced to 2 lesions by the 9th. sampling.

6.3.4. Growth rate

The results of the Specific Growth Rate are presented in Table 6.3. There was a significant difference in the Specific Growth Rates seen between the replicates of fish

Table 6.3

Specific growth rates of A. nobilis after 26 weeks' infection with high and low numbers of L. piscinae and the control

a) Between Replicates A and B

Treatments	Mean weights (g) Initial	S.E.	Final	S.E	Specific growth rate Mean wt(g) gain	S.E.	t value	Probability
Control A	73.65	0.18	218.83	8.24	0.55	0.02	0.86	>0.40
Control B	74.22	0.20	235.83	10.08	0.57	0.02		
Low A	73.88	0.15	152.50	11.96	0.33	0.04	1.03	>0.31
Low B	73.92	0.13	137.17	8.81	0.28	0.04		
High A	74.51	0.13	162.30	7.77	0.39	0.03	2.20*	<0.03
High B	74.50	0.23	136.90	6.28	0.30	0.02		

b) Between Treatments (result of pooled values of replicates A & B)

	Weight increment(g)	S.E.	specific growth rate	S.E.	F value	Prob.	
Control	153.40	6.55	0.56	0.01			A
Low	70.94	7.44	0.31	0.03	48.05*	<0.00	B
High	75.55	5.22	0.34	0.02			B

(from ponds A and B) from the high treatment ponds (Table 6.3 a). When the replicates were pooled and comparisons were made between the three treatments, there was a significant difference when the Control was tested with both the Low as well as with the High treatment, but on the other hand there was no difference seen between the Low and High treatments (Table 6.3 b).

Figure 6.2 and Table 6.4 present the mean weights of fish from the light and heavy infections and control fish recorded during the seven months sampling period. Although the mean weights of the three treatments were not significantly different (Table 6.4) when the fish were first introduced, the mean weight of the lightly infected fish was significantly higher than both the High infection or the Control at the 1st. sampling. From the 2nd. sampling onwards the Control showed the highest mean weight and the fish with High infection showed the lowest mean weights (Fig. 6.2 and Table 6.4), and these were significantly different among the 3 treatments till the 8th. sampling. The difference between the Light and Heavy infection was seen to decrease during the 9th. sampling, and they were both significantly different from the Control fish. The difference between the High and Low infections was gradually reduced until, by the end of the experiment there was no significant differences in weight between them. However, the difference between the Control and infected fish was maintained

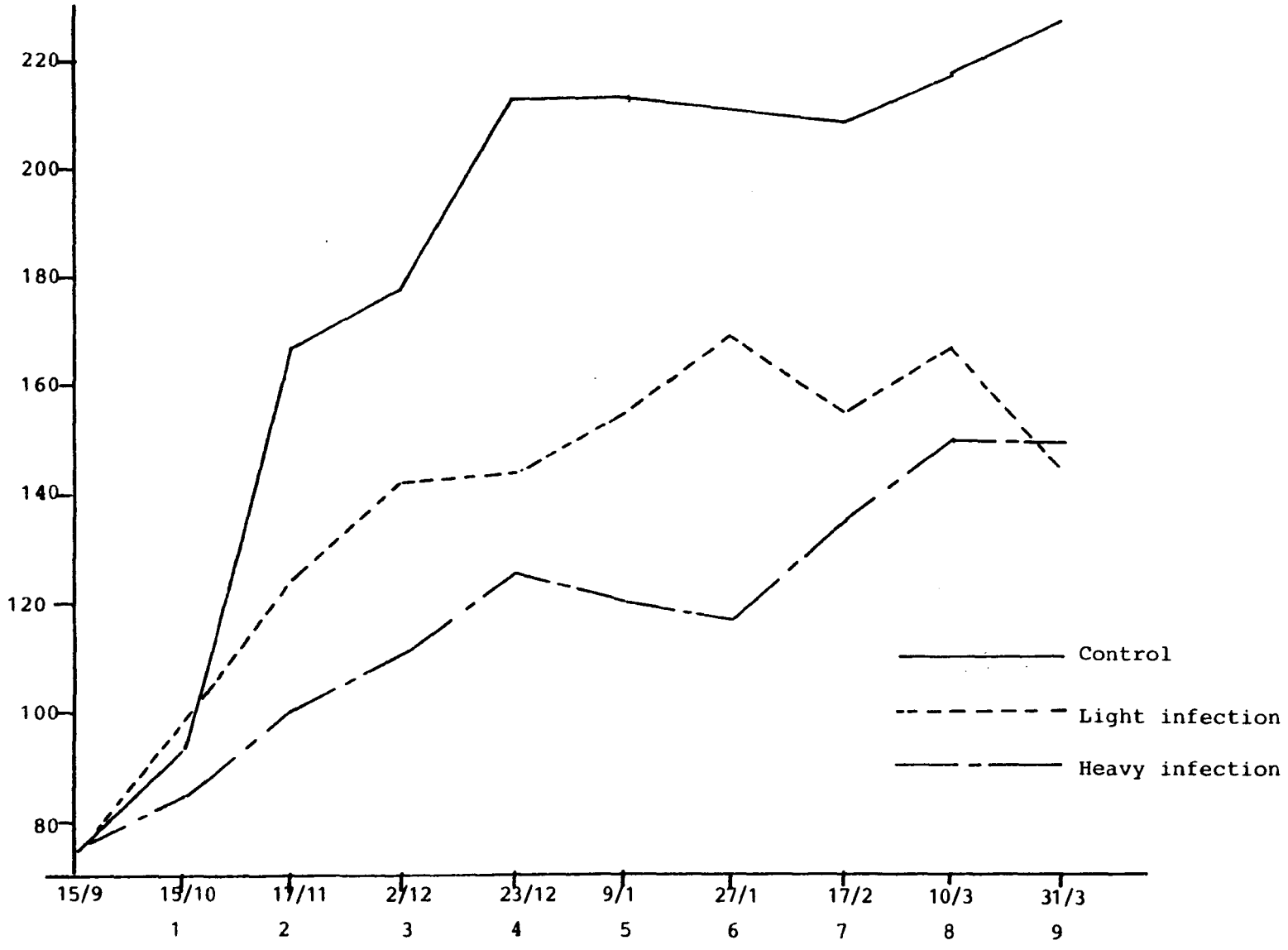


Figure 6.2 Mean weight (gm) of A. nobilis from the control ponds and those infected with Low and High levels of L.piscinae.

Table 6.4

Comparison of the mean weights (grams) of A. nobilis infected with High and Low levels of L. piscinae and the Control (standard mean error values in parenthesis).

Sample	Control	low	high	F value
	n=60	n=60	n=60	
0	73.93	73.90	74.06	0.41
	(0.1)	(0.1)	(1.1)	
1	93.09 a	98.83 b	84.67 c	5.19 *
	(2.8)	(3.1)	(3.4)	
2	167.7 a	124.8 b	100.8 c	45.26 *
	(5.1)	(5.4)	(4.6)	
3	178.6 a	142.9 b	110.3 c	44.85 *
	(4.5)	(5.2)	(5.5)	
4	212.9 a	144.8 b	126.3 c	68.77 *
	(5.6)	(5.9)	(4.9)	
5	213.6 a	155.4 b	120.3 c	55.19 *
	(5.5)	(7.7)	(5.5)	
6	211.2 a	168.9 b	116.9 c	64.55 *
	(5.4)	(7.3)	(4.6)	
7	209.7 a	155.1 b	135.4 c	40.42 *
	(5.9)	(6.3)	(6.0)	
8	217.3 a	167.7 b	150.3 c	26.15 *
	(5.8)	(7.8)	(6.7)	
9	227.3 a	144.8 b	149.6 b	51.31*
	(6.5)	(7.4)	(5.2)	

Table 6.5 shows the difference in mean weights of infected fish expressed as a percentage of the mean weight of the Control fish (i.e. Light and Heavy infection pooled). From the initial 0% difference, the mean weight difference increased to 32.7% by the 2nd. sampling and then attained a maximum of 35.5% at the 5th. sampling. After the 5th. sampling it gradually decreasedto 26.8% by the 8th. sampling and at the final sampling it was 35.2%.

6.3.5. Mortality

The mortality in the ponds were as following;

Treatment	Pond A	Pond B
Low infection	21%	23%
High infection	19%	24%
Control	13%	11%

6.4. Discussion

Generally the level of infection was considered to be low in both the Low and and High infection ponds. At the peak of infection, the relative density of infection (number of parasites divided by the number of fish sampled) was 0.2 in the low treatment ponds and 0.73 in the high treatment ponds. However the figures on the mean intensity of infection may not reflect the true burden of parasites on the host as, in addition to the infection by the adult female parasites, there will also be the presence of parasitic larval stages on the host. The number of parasites was recorded every 3 weeks, however, the study of

Table 6.5

Mean weight (grams) of A. nobilis infected with L. piscinae (Light and Heavy infection pooled) and compared with those from the control ponds (also pooled).

Sample No.	Time Interval (weeks)	Control	Infected (Light & Heavy)	Percentage Differences (%)
	Initial wt.	73.93	73.90	0
1	2	93.08	91.74	1.4
2	5	167.26	112.55	32.7
3	9	178.65	126.60	29.1
4	12	212.85	146.30	31.3
5	14	213.55	137.83	35.5
6	17	211.22	142.91	32.3
7	20	155.41	135.38	12.7
8	23	217.28	158.99	26.8
9	26	227.33	147.21	35.2

the life cycle (Chapter 3) showed that it takes 2 weeks for the parasite to develop and penetrate the host tissue and the parasite may have been more numerous on the fish in the intervening weeks at a different stage in the life history.

The pin point haemorrhages noted during the 1st. sampling (2 weeks after the introduction of the parasites) may have been lesions caused by the parasites attempting penetration of the host. However there is also a possibility that some of these lesions may have been caused by an earlier infection from the preadult stages i.e. cyclopoid stages, which would have been present on the fish at the initial stage of the experiment, that were introduced into the ponds to serve a source of infection. In this case the infection would have taken place in 1-3 days and the haemorrhages seen during the 1st. sampling may also have been lesions left behind after these parasites had dropped off the host. Under laboratory conditions parasites were noted to survive on the host for 1 to 3 weeks. Haemorrhagic lesions seen during the remaining sampling could have been either indications of attempts by the parasites to penetrate the host tissue or lesions left by parasites that had dropped off the host body.

The haemorrhagic lesions did not reveal a closely related pattern to the number of adult parasites and may indicate that the number of parasites may have been different during the intervening weeks. However Fig. 6.1 does show a decrease in the number of haemorrhagic lesions

with a decrease of adult parasites from the 8th. sampling onwards.

Although the number of parasites introduced into ponds categorised as High infection was double (40 parasites) the number of parasites introduced into ponds categorised as Low infection (20 parasites), there were no significant differences either in the number of adult parasites or haemorrhagic lesions on the fish from these ponds.

The level of Lernaea infection varied during the first 13 weeks but after the 4th. sampling, that is 18 weeks after the infection was first introduced, a general decrease in the level of infection was observed. There were no parasites observed during the 9th. sampling, that is 28 weeks after the infection was first introduced. At the same time as the decrease in the number of parasites found on the body surface, infection of the eye was noted from the 6th. sampling onwards. The infection of the eye, together with the presence of haemorrhagic lesions seen on the body surface of the fish indicated that Lernaea was still present in the ponds inspite of the zero infection on the body surface. There was an increase in the haemorrhagic lesions at sample 7, followed by a decrease and subsequent absence of infection on the body surface. The appearance of the parasites in the eye indicates that the parasites were no longer able to penetrate the body but were limited to establishment in the eye. The eye is generally considered to be a privileged site in terms of immunity and it suggested that the fish had become immune to the infection,

the fish were thus tested by a challenge with a new batch of parasites. The challenge infection was also carried out on Control fish which were naive and never exposed to infection before. The result of the challenge infection revealed a higher number of haemorrhagic lesions in the previously infected fish but did not result in any establishment of adult females on the body proper. The naive fish which were exposed to infection for the first time showed a substantial number of parasites on the body proper. The results of the infection in naive fish (in the control ponds) also ruled out the possibility of any relationship with the physical change of host skin tissue which could have prevented Lernaea infecting the body surface in fish from the treated ponds as both these fish stocks were from the same age group.

The above study, together with the incidental observations made at the breeding stations where brood stock revealed haemmorhagic lesions on the body surface but no infection by adult parasites (Chapter 2) all supported the suggestion that the host develops immunity to the parasite with time period. The occurrence of eye infection in association with a decrease in body infections was thought to result from the presence of antibodies on the body surface. Fletcher and Grant (1969) and Hines and Spira (1973) have reported that specific antibodies which develop within the body can pass through epidermal layers into mucus covering the body surface. The cornea which is avascular and also devoid of a mucus covering, remains free of any

immune factors which may have developed within the body, thus offering a new location for parasite infection. A similar situation whereby the greatest frequency of infection was in the cornea of fish which had already demonstrated immunity towards the monogenean Benedenia (=Epibdella) melleni has been reported by Nigrelli (1937). The decrease in the number of adult L. cyprinacea on C auratus due to an immune response has been reported by Shields and Goode (1978) but infection of the eye was not indicated. The development of an immune response towards other species of parasites in fish has also been recorded by several workers. Bauer 1953, Beckert and Allison 1964, Bradshaw et al. 1971 and Hines and Spira (1973) reported varying degrees of resistance to subsequent infections of Ichthyophthirius multfiliis Fouquet; whilst Hines and Spira (1974), Goven et al. 1980 and Wolf & Markiw (1982) were able to demonstrate protective immune response to the same parasite. Molnar and Berczi (1965) and Harris (1972) demonstrated precipitous sera of fish to Ligula intestinalis Pallas, a cestode, Pomphorhyncus laevis (Müller) an acanthocephalan respectively whilst Cottrell (1977) and McArthur (1978) showed precipitous fish sera to digenean infections. Resistance to challenge infection of Gyrodactylus species has been reported by Lester and Adams (1974) and Scott and Robinson (1984). Kennedy and Walker (1969) suggested that the cestode Caryophllaeus laticeps Pallas was rejected by dace, Leuciscus leuciscus Linnaeus after the establishment of an acquired immunity.

The author in his earlier report (Shariff 1981) recorded a similar finding of a decrease of L. piscinae infection with an increase in eye infection in A. nobilis cultured in cages. However in this case, infection of the eye was seen after a 3 week period as compared to a 4 month period seen in the present study.

The difference in the time period noted within the two studies could possibly be due to the difference in the burden of parasites on the host. The relative density of infection during the initial sample was 2 parasites as compared to the lower infection level of 0.73 at the peak of infection seen for high infection fish in the present study. The higher burden of parasite recorded by Shariff (1981) would have stimulated a more rapid immune response in contrast to the present study when the burden of parasite was relatively lower and thus resulting in a slower immune response. Fish has been shown to respond rapidly to higher concentrations of antigens (Anderson et al. 1979)

The studies on growth rates showed that there was a significant difference between the Specific Growth Rate of fish from the High and the Low infection ponds compared to those from the control ponds. The percentage difference between the control and treated fish (both High and Low infection pooled) was as high as 35%. The difference in Growth Rate seen between the control and treated fish is clearly the consequence of L. piscinae infection.

At the first sampling, the lightly infected fish had a higher Mean Weight than those of the Control and High infection. This could possibly be due to the stimulation effects suggested by Kabata (1958, 1981) which he reported to occur during the initial stages of infection to copepods. Kabata suggested that this effect resulted in a higher metabolic rate and more energetic feeding activity which promotes growth. However such an increase in growth was not evident amongst fish with High infection (which had 55% more parasites than the fish from the Light infection ponds) and it is possible that the transitory stimulation effect in fish with a higher burden of parasites is shorter and is succeeded by an early retardation of growth. The stimulation effect reported by Kabata (1981) is a transitory stage followed by severe retardation.

Fish with High and Low levels of Lernaea infection showed a higher mortality compared to those from the control ponds. This suggests that besides the effect on growth rates, the chances of survival of the fish with Lernaea infection are also reduced. Infection of the eye could also have resulted in higher mortality. The loss of lens material due to Lernaea infection (Shariff 1981) could lead to blindness and thus make the fish more susceptible to predation. Goff and Green (1978) found that blind fish do not exhibit the same behaviour as normal fish and neither do they seek shelter as quickly as normal fish when light intensity is increased to levels at which potential predators are active (Goff 1977). The mortality was not

assessed until the end of the experiment in this case but it would be interesting to assess the stage at which mortality is most likely to occur. This would then throw some light on the actual causes of mortality.

CHAPTER 7.

THE HISTOPATHOLOGY OF *L. PISCINAE* INFECTION IN *A. NOBILIS*

7.1 Introduction

The skin forms the primary barrier against the environment and thus has been a subject of great interest in the study of its histopathological changes in many disease processes during the last two decades. In warm water fish ponds, external parasites form the largest group of pathogenic organisms (Sarig 1971) amongst which the crustacea are the largest and can cause considerable damage to the host tissue (Smith 1975, Kabata 1970). The parasite Lernaea spp. has been the cause of great economic concern in many parts of the world and it is surprising that there have been to date very few reports on its pathogenic effects on its host. Infestation of fish by lernaeid copepods occurs when the cyclopoid female penetrates the host surface. It then undergoes metamorphosis of the cephalic region to form the anchor process which is embedded in the host tissue.

A typical inflammatory response in M. chrysops infected with L. cruciata was reported by Joy and Jones (1973). In addition to Joy and Jones' findings, Khalifah and Post (1976) described encapsulation and occasional calcification in P. promelas, L. cyanellus and C. commersoni infected with L. cyprinacea. It has been previously shown (Chapter 6) that there is a marked reduction in the number of parasites with time period followed by a subsequent absence of infection and it was suggested that this is an indication of the development of an immune response. Rejection of Lernaea by the host has only been reported by Shields and Goode

(1978). They suggested that the penetration of the parasite nearly parallel to the surface resulting in a prolonged exposure to the integumental response was a contributing factor in the rejection of L. cyprinacea from C. auratus.

A major objective of the present investigation was to describe the histopathology of L. piscinae through acute and chronic stages and compare the responses of naive fish with fish suspected of being immune. Such a study would provide a better understanding of the disease processes and the capacity of the fish to overcome infection.

7.2. Materials and methods

One hundred and fifty A. nobilis of 60 - 110 mm total length, which were examined and found to be free of the adult female Lernaea infection, were treated with formalin at 166 ppm for 30 minutes to remove any other ectoparasites. The fish were then maintained in 5 tanks, each containing 30 fish which were fed with fine ground tubifex worms mixed with an equal proportion of flour. After acclimatizing the fish for 1 week, larvae of L. piscinae hatched from 10 egg sacs were introduced into each of the 5 tanks. The fish were then observed regularly. The temperature of the water in the aquarium tanks was 23-29^{o}C. To study the sequential histopathological changes, the fish were sacrificed at 30 minutes, 1, 2, 4, 16, 32 hours etc up to 21 day period. The duration of infection was assessed from the time of the establishment of the parasite at the metamorphosed cyclopoid

stage, as it is at this stage that the parasite embeds in the host tissue. Since there was high fish mortality due to the infection during the experiment, fish with a higher number of parasites were sacrificed first, whilst fish with less than 3 parasites were maintained to study the histopathological changes over a longer period.

At each sampling, 4 fish were sacrificed. The tissue around the embedded parasite was carefully removed and fixed in 10% buffered formalin for several days. The tissue was then decalcified in 4N-formic acid, for 12 hours and subsequently processed using routine histological procedures. ~~as presented in Appendix 1~~. The sections were embedded in paraffin wax, sectioned at 5um, and subsequently stained with Haematoxylin and Eosin (H+E), Periodic acid and Schiff (PAS), MSB.

Fish which had previously been exposed to infection for more than 2 months in the tanks and were believed to be immune were challenged with infection. Only 10 immune fish were available and tissue sections were removed at 2 and 4 days. The 4 days time period would have provided an opportunity for the parasite to embed in the host tissue (Chapter 3). Tissue sections were also made from fish exposed to natural infection in the university ponds and which were believed to be immune. These fish showed haemorrhagic lesions (Fig. 7.1) but the adult female parasite was absent. Tissue sections from fish suspected to be immune were processed in the same manner as for those described above.

Figure 7.1 A. nobilis from the pond, with haemorrhagic lesions but no parasites. These fish were suspected of being immune.

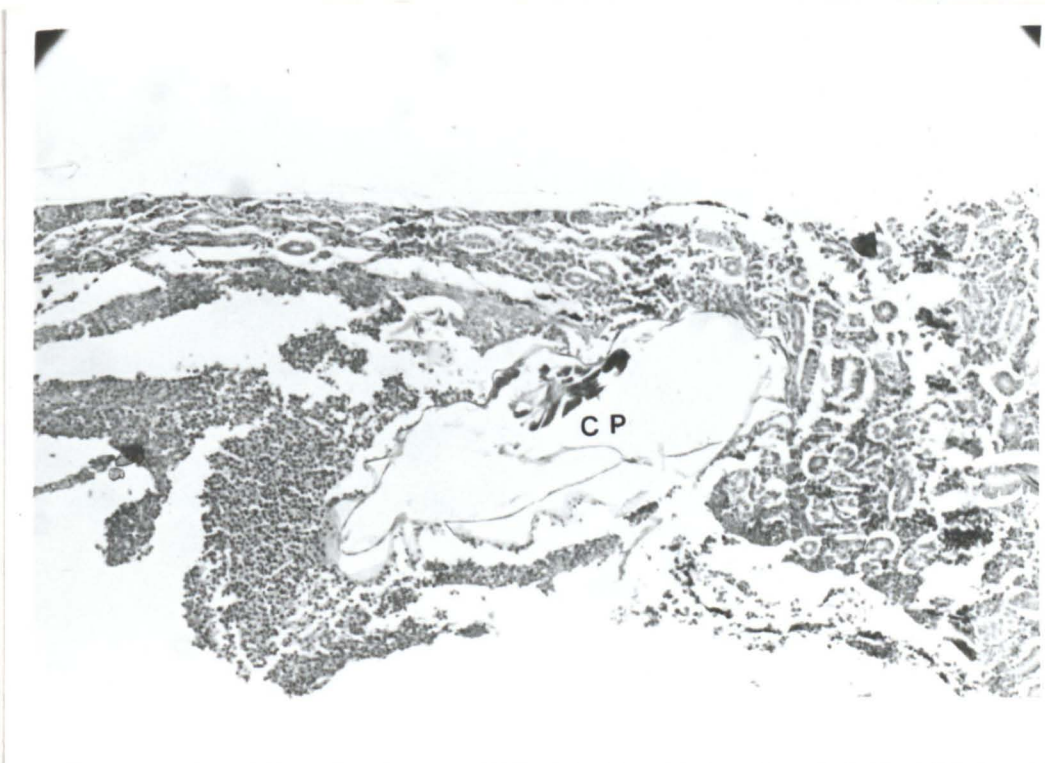


Figure 7.2 Transverse section of the cephalic process (CP) seen in the kidney tissue. H&Ex100.

7.3. Results

7.3.1. Clinical observations

Four days after the introduction of nauplius I, the fish were seen to be in constant swift movements with short resting periods. Even during the resting periods, the fins made swift movements. On the 13th. day, pin point haemorrhages were noted, i.e. approximately 8 - 24 hours after the metamorphosis of the copepodid V larvae to cyclopoid stage. Occasionally the fish were seen to rub the body against the gravel bed of the tank. About 24 hours after the pin point haemorrhages were noted, the cylindrical transparent body of the parasite could be seen under the stereomicroscope. On the 15 -16th. day, the body of parasite further elongated and took up a milky colour which gradually turned to ivory.

Four days after the emergence of the adult parasite, dissection of several specimens from the body of the host revealed that the cephalic region along with 2-4 mm of the parasite body were embedded in the host tissue. In some small fish the parasites had penetrated the relatively thin dermal layer and the cephalic region of the parasite was found in the organs of the visceral cavity in some fish. Fish with large number of parasites were gradually seen to become sluggish and make no attempt to move. A high mortality occurred at this stage due not only to high parasite burdens but also in the smallest fish where the parasite size was large in relation to the size of the fish.

Fish from the ponds which were suspected of being immune only revealed haemorrhagic lesions but no adult female parasites were found on the body.

In fish that were challenged with infection, adult parasites were found on the 2nd. day, but on the 3rd. day parasites were no longer seen on the host. In others only haemorrhages were noted on the body surface (Fig. 7.1).

7.3.2. Histopathological observations

In the histological sections, penetration of the parasite into the host tissue was evident 16 hours after the development of the cyclopoid stage, that is 13 days after the newly hatched larvae were first introduced. About 1/8th. of the anterior portion of the cylindrical body was embedded in the host tissue whilst the remaining 7/8th. remained suspended externally. In most cases the parasites had penetrated the tissue at an angle, sliding in between the layers of the overlapping scale and disrupting the stratum spongiosum and compactum layers. The anterior portion of the parasite, the cephalic region was always seen to develop in the dermal layer but in some instances was seen in the visceral organs (Fig.7.2). Host muscle tissue was noted within the anterior alimentary canal which forms a lumen in the cephalic region of the parasite (Fig. 7.3).

At 16 hours there were severe haemorrhages all along the path of entry and around the site of location of the parasite (Fig. 7.4 & 7.5). Haemorrhages were also seen

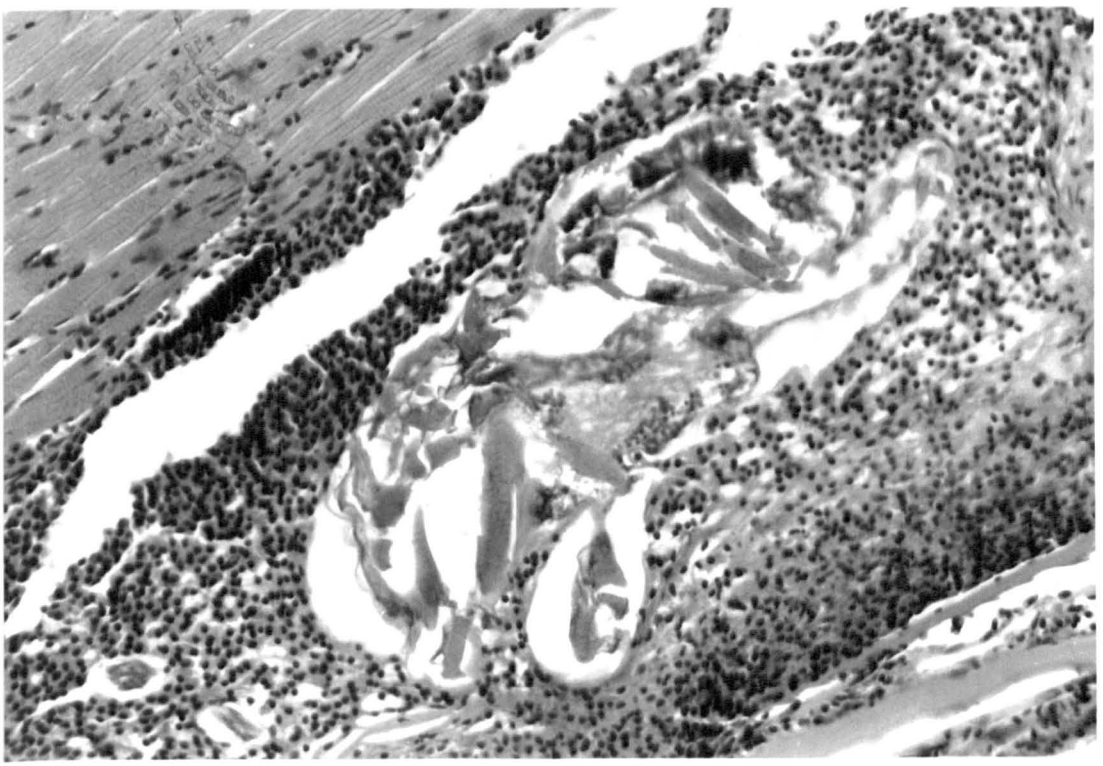


Figure 7.3 Muscle tissue seen in the lumen of the anterior alimentary canal within the cephalic processes. H&Ex400.

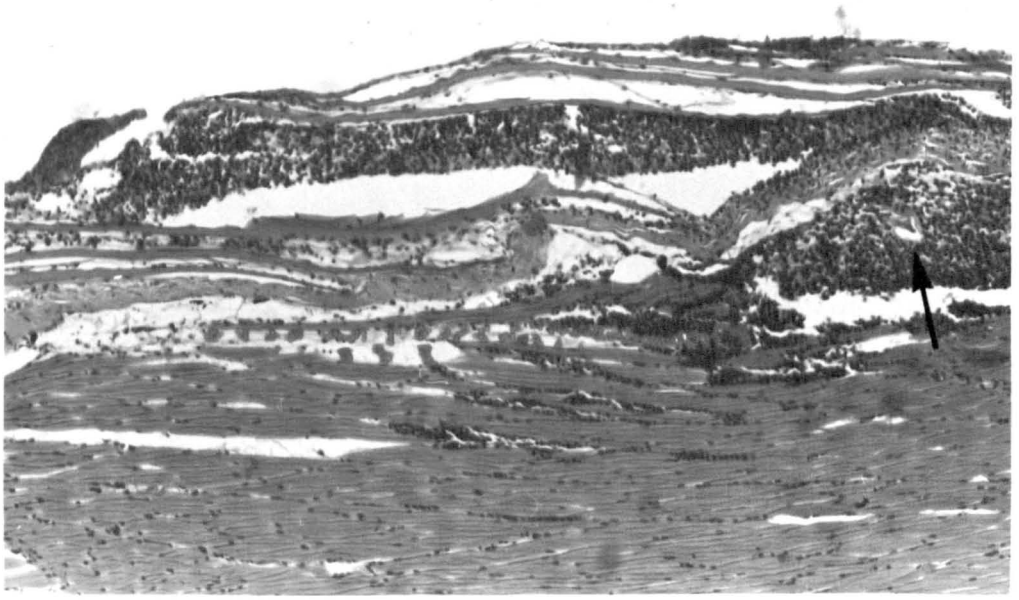


Figure 7.4 Haemorrhages along the path of entry of parasite and site of location of parasite. A small section of the lumen (arrowed) can be seen surrounded by red blood cells. H&Ex100.

under the scales and also in between muscle bands lying close to the parasite. Haemorrhages from underneath the scales spilled out to the external surface, resulting in the formation of clusters of red blood cells at the tip of the scales (Fig. 7.6). Red blood cells and neutrophils were also found interdispersed under the scales and the periphery of the haemorrhagic lesions. Large aggregations of melanin were seen within the epidermal layer, whilst in some, the granules (melanosomes) were being released to the surface.

Adjacent to the parasite body, the breach made by the parasite was covered by several layers of epithelial cells in an attempt to seal off the haemorrhagic lesion from the external surface (Fig. 7.7), whereas in others, clusters of red blood cells were seen exposed to the external medium, surrounded by collars of hyperplastic epithelial cells (Fig. 7.5).

Many of the red blood cells in the haemorrhagic lesions were undergoing degenerative changes, revealing pyknosis and karyolysis of the nucleus (Fig. 7.8a and b). The blood vessels were congested with leucocytes and in some cases, forming margination. The collagen bundles of the compactum layer along the site of penetration were also disrupted and infiltrated with red blood cells and it was also oedematous. The sarcoplasmic fibers adjacent to the parasite were undergoing necrosis.

At 2 1/2 days, (64 hours) after infection, massive numbers of mononuclear leucocytes and neutrophils were found

Figure 7.5 Haemorrhages along path of entry of parasite. Arrow shows part of the parasite's cylindrical body lying at point of entry. A collar of hyperplastic epithelial cells surrounding the red blood cells adjacent to the parasite. H&Ex100

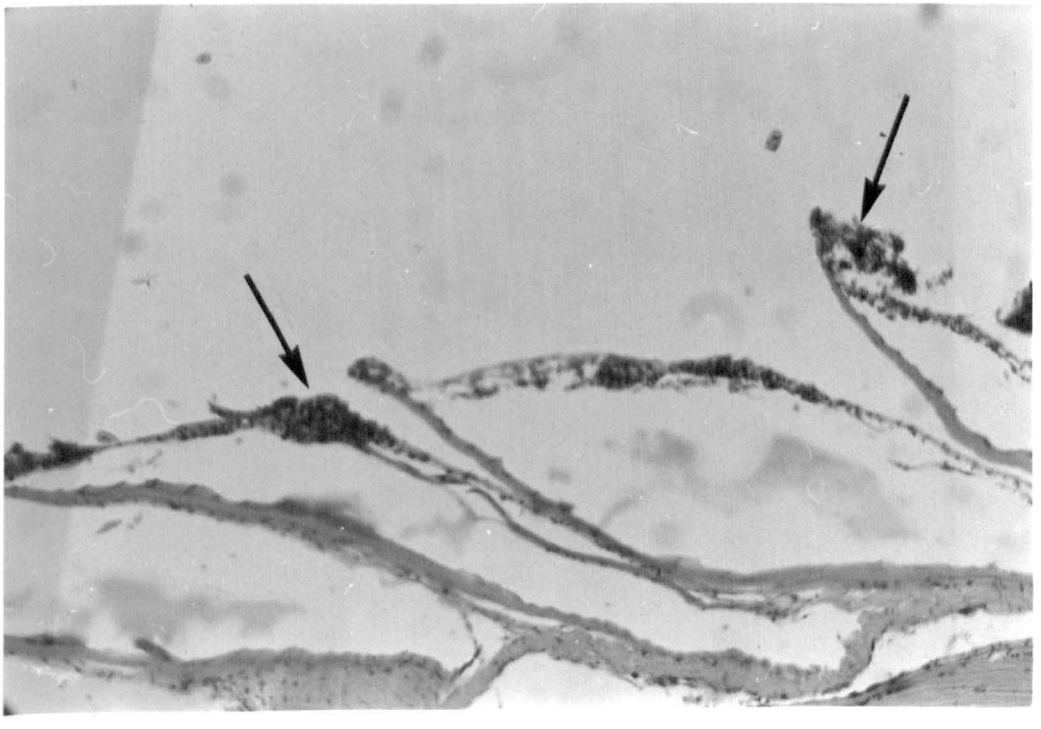
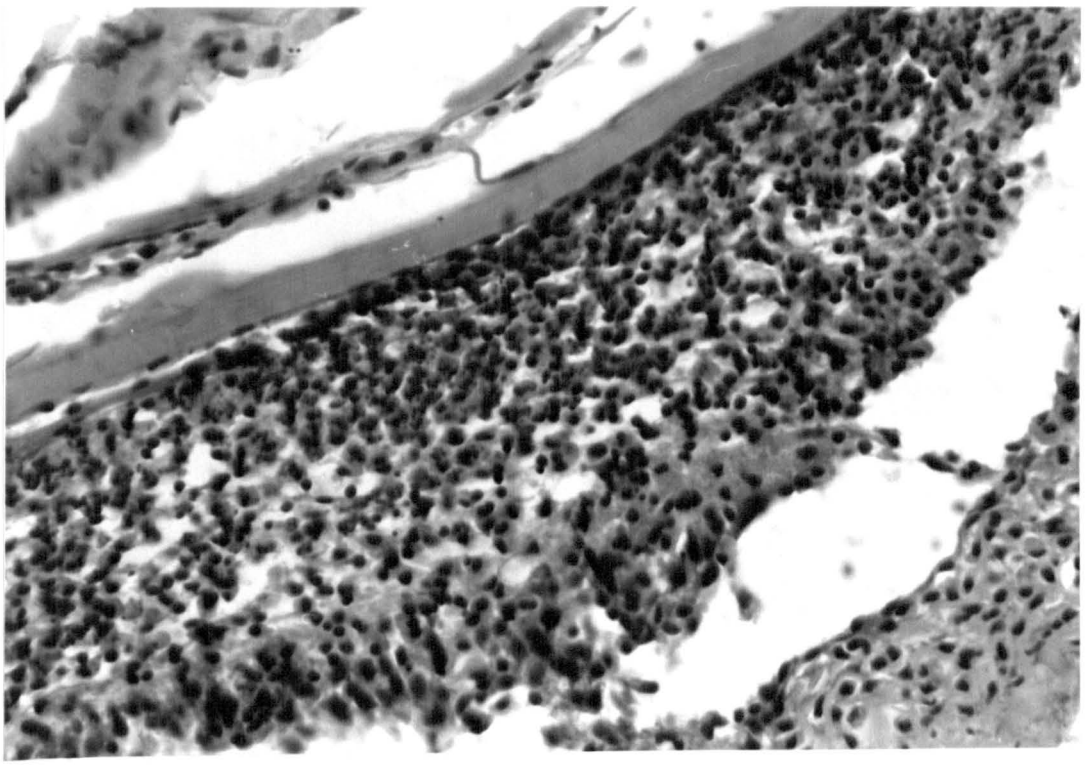
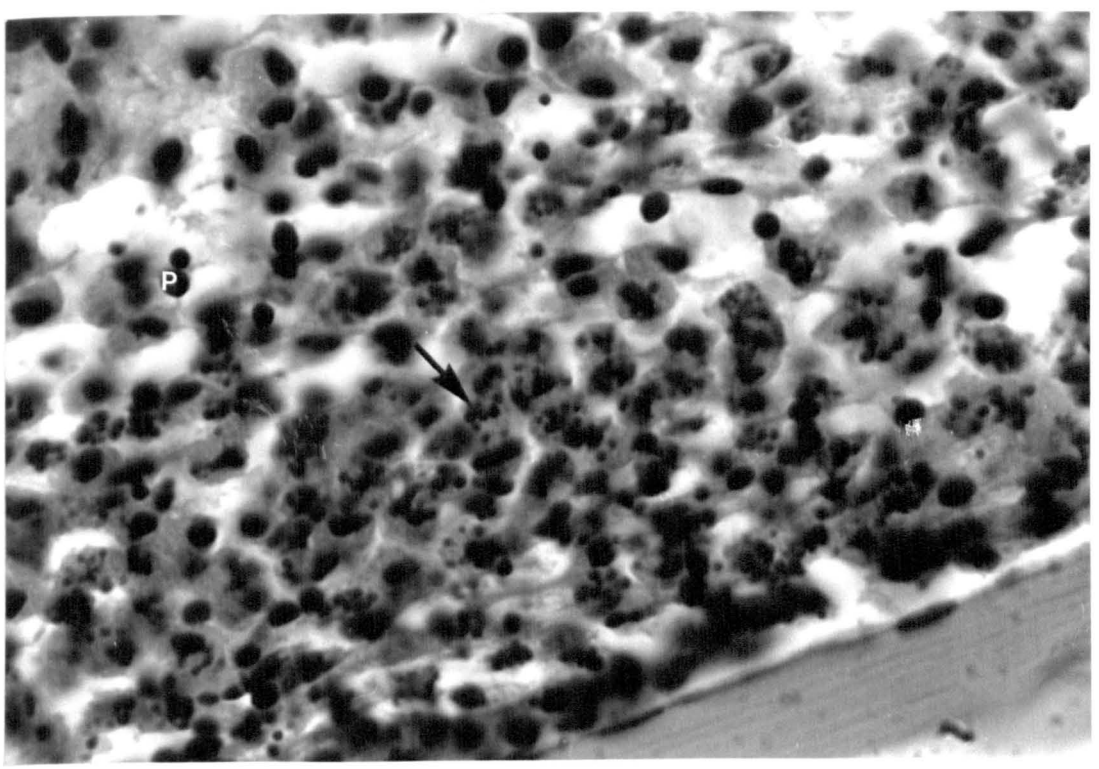


Figure 7.6 Clusters of red blood cells (arrowed) at the tips of scales. H&Ex250

Figure 7.7 Adjacent to the parasite body, the part of breach (arrowed) was sealed off by layers of hyperplastic epithelial cells. H&Ex250.



a)



b)

Figure 7.8 a&b Red blood cells undergoing degenerative changes, note the pyknotic (P) and karyolyse stages (arrowed) a) H&Ex400 b) H&Ex1000

interdispersed in exudate all along the site of penetration and at the location of the parasite. Some of these cells were pyknotic. In some areas the cells were less abundant and only exudate was present. The vessels around the lesion remained congested with mononuclear cells. Some of the muscle fibers were undergoing lysis and mononuclear cells were found around them. Muscle tissue was still seen in the anterior alimentary canal which forms the lumen of the cephalic region of the parasite. A thin cyst possibly composed of host and parasite material had developed around the cephalic region of the parasite. The breach point was plugged with a nodule formed of inflamatory exudate and cells and was exposed to the external medium (Fig. 7.9).

Around the periphery of the lesion, a few fibroblast cells were noted, but most prominent was the massive vascularization of the region (Fig.7.10). Large clumps of melanin were seen beneath the epidermis and among the fragmented layers of the dermal region (Fig. 7.11).

At the 5th. day, (128 hours after infection) large number of inflamatory cells were undergoing degenerative changes. Similarly the muscle fibers were also undergoing degenerative changes accompanied by myophagic activity by monocytes. The melanin deposits underneath the epithelial cells and fragmented dermal layers had increased in thickness, some of them were above 7um thick. Thick clumps of melanin were also found interdispersed among the cells undergoing degenerative changes. Adjacent to the point of of entrance of the parasite, fibroblast cells were seen to

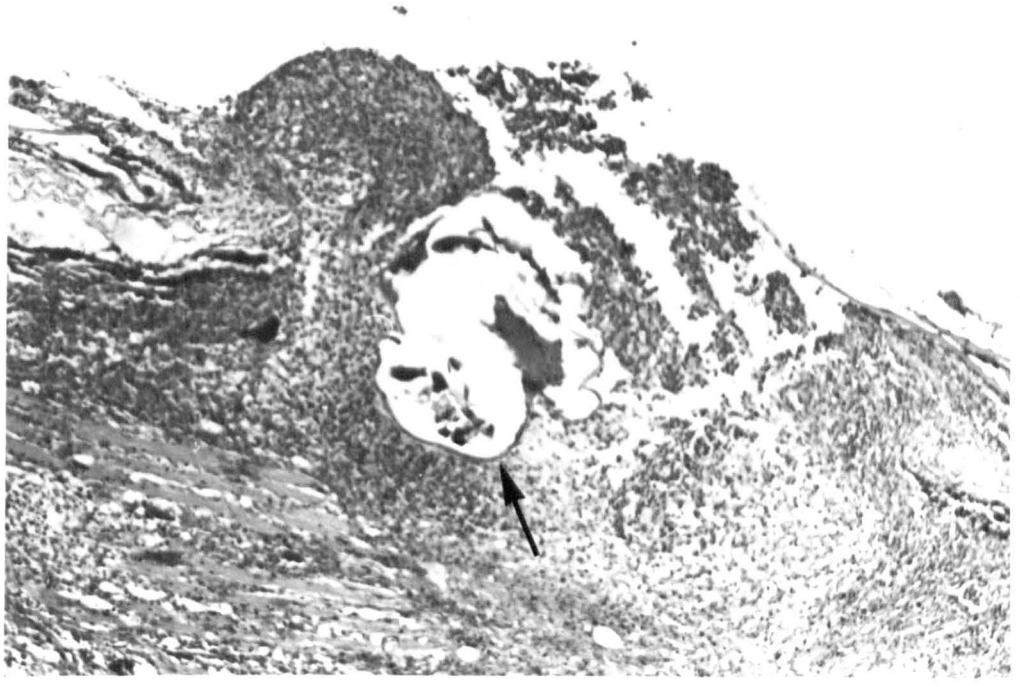


Figure 7.9 Thin cyst is seen around the parasite (arrowed) and the inflammatory exudate and cells were seen exposed to the external medium. H&Ex100

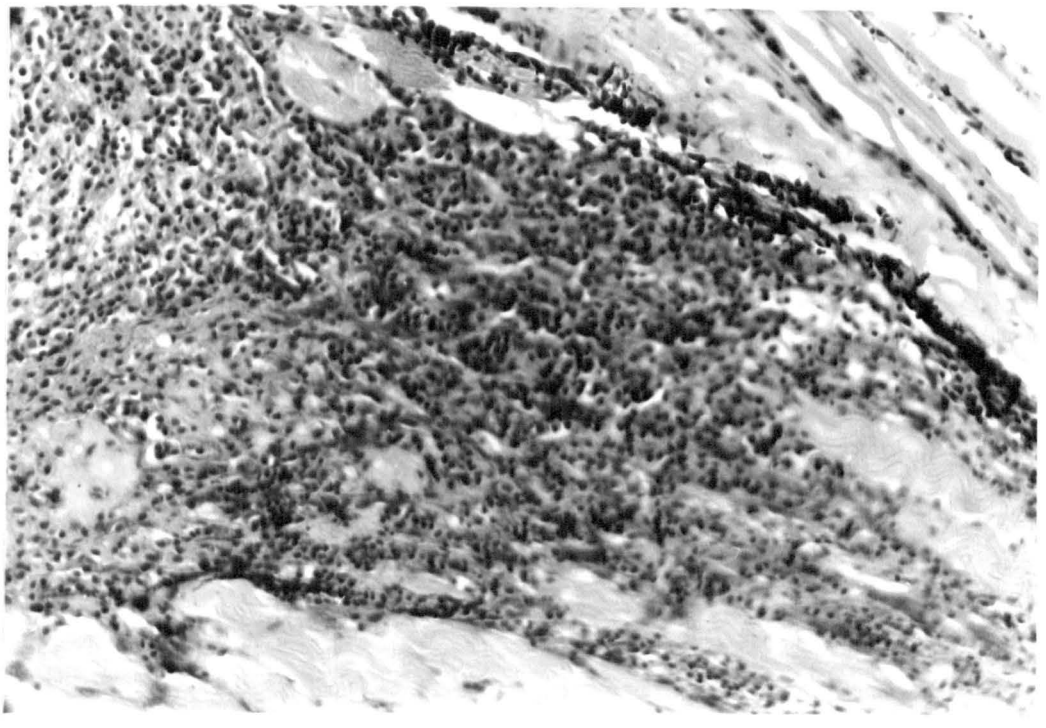


Figure 7.10 Fibroblast cells with massive vascularization of the region around the periphery of the lesion. H&Ex250.

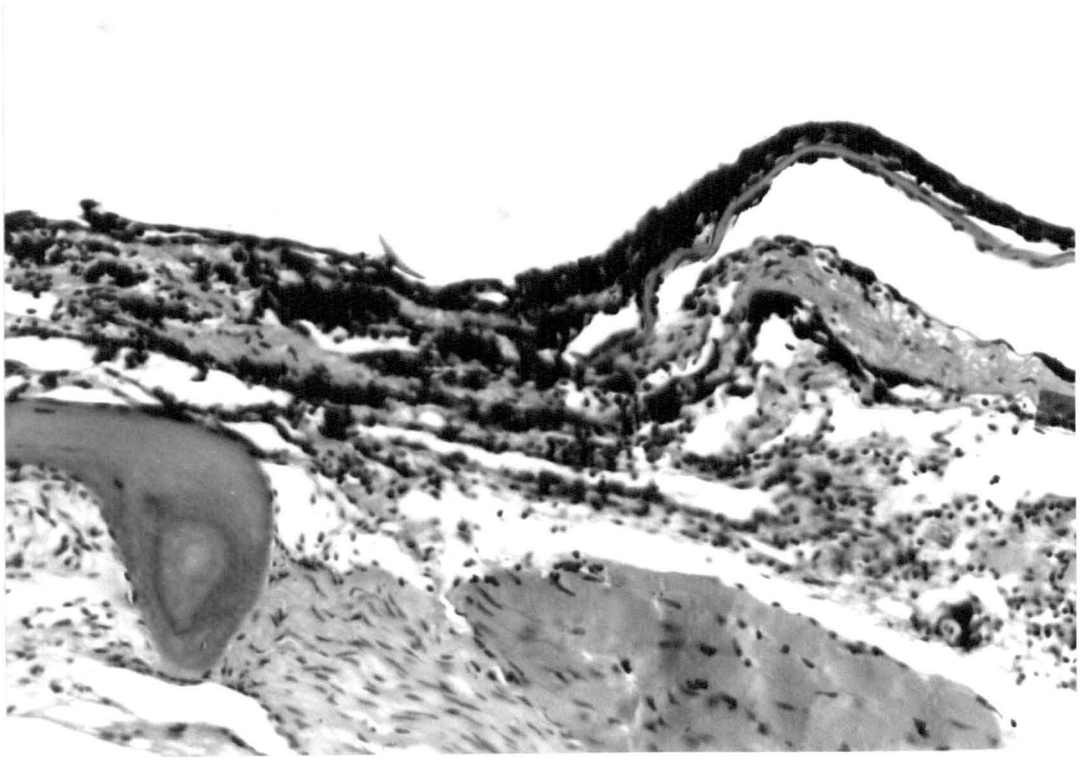


Figure 7.11 Large clumps of melanin were seen beneath the epidermis and among the fragmented layers of dermal region. H&Ex400.

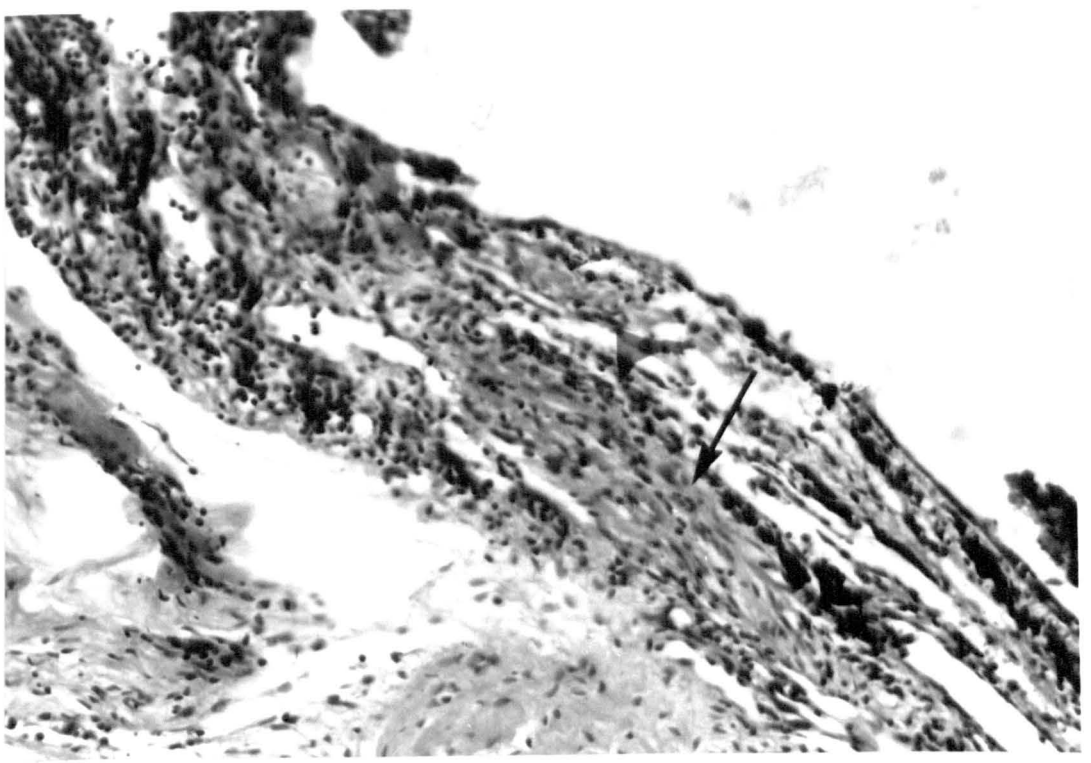


Figure 7.12 Adjacent to the point of penetration fibroblast cells (arrowed) were seen laying collagen tissue to seal off the breach. H&Ex400

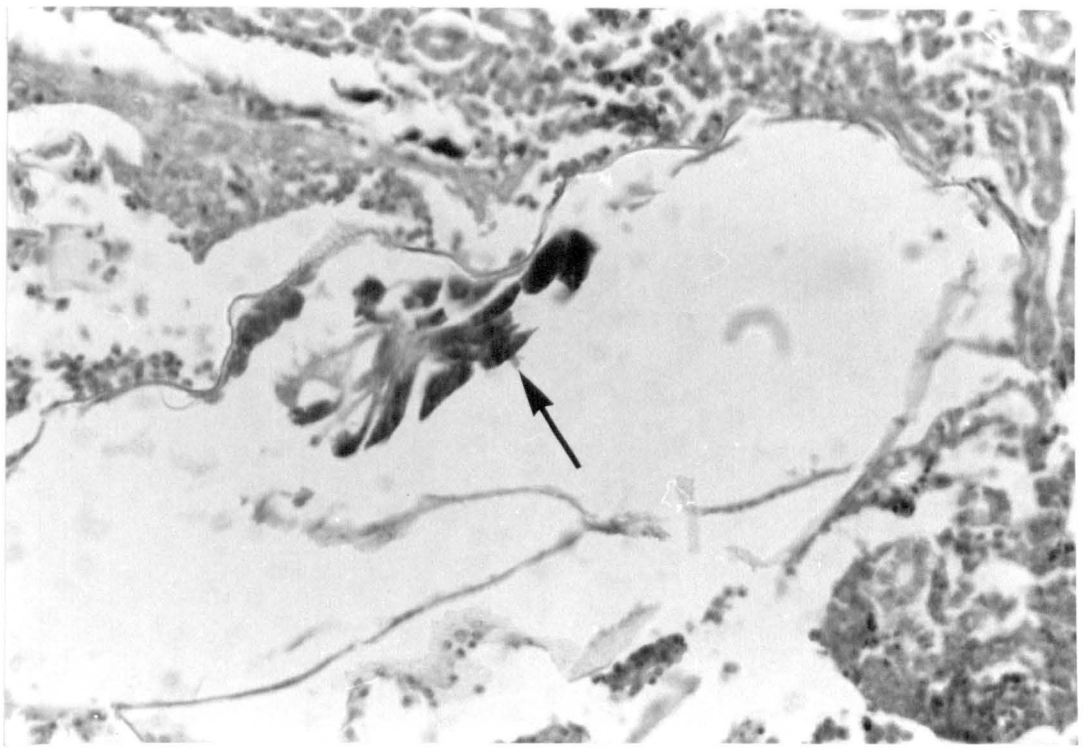


Figure 7.13 Trypanosoma sp. (arrowed) were seen in the anterior alimentary canal in the cephalic region of the parasite. H&Ex400

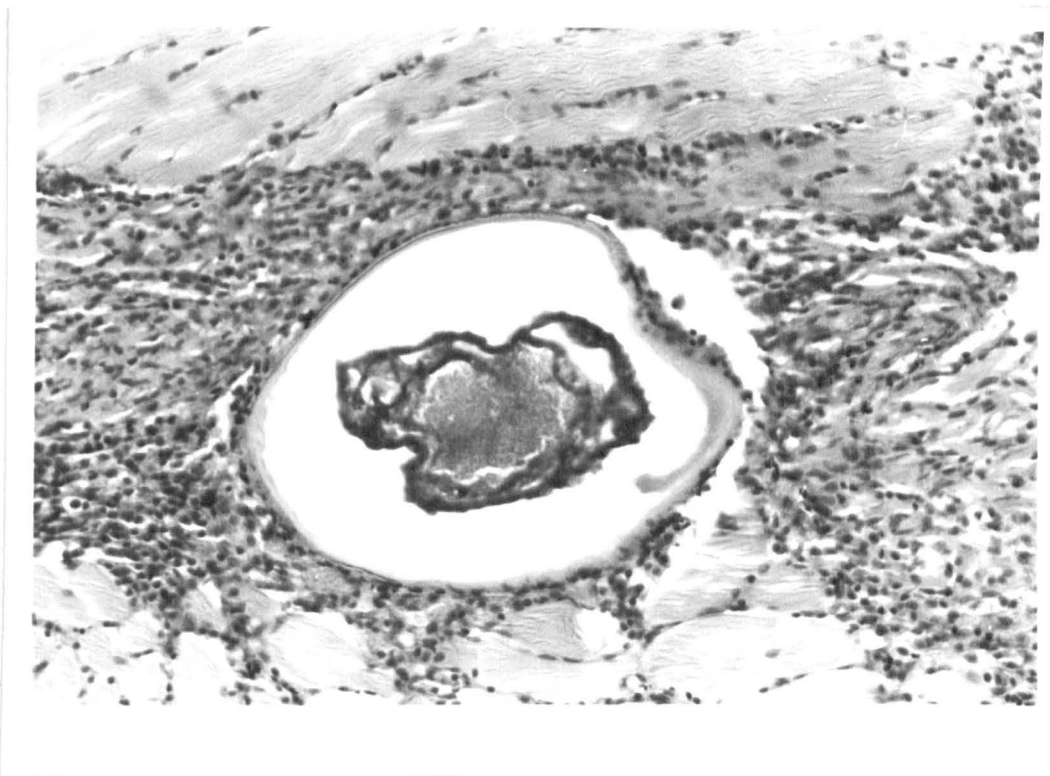


Figure 7.14 Formation of cyst around the smaller horns of the cephalic processes. H&Ex40

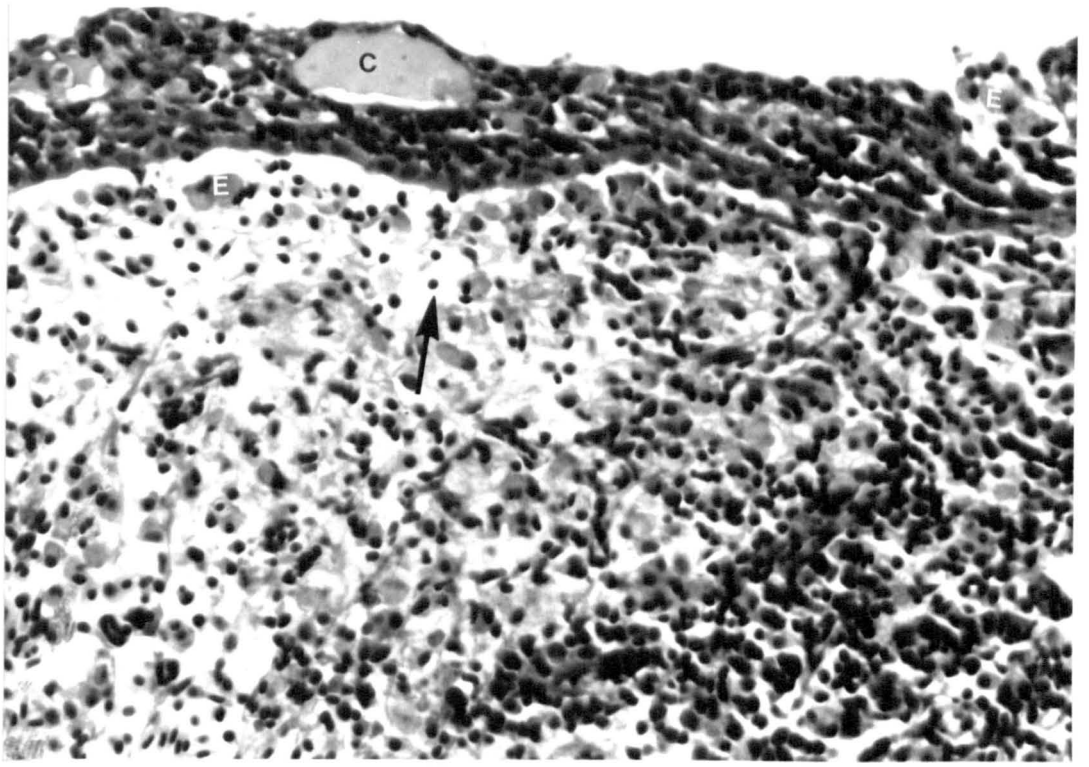


Figure 7.15 Eosinophilic granular cells (E) seen in the epidermal layer and in the inflammatory lesion. Note the presence of large club cells (C) in the epidermal layer and the lymphocyte like cells (arrowed) in the inflammatory lesion. H&Ex400

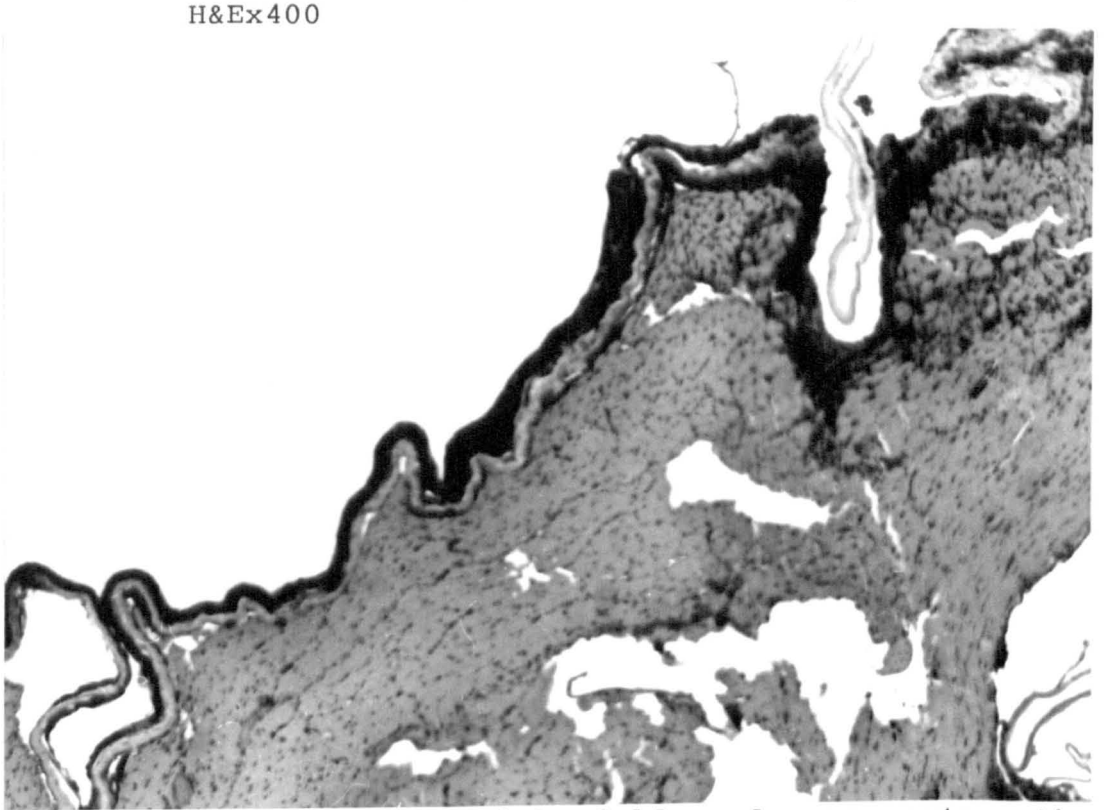


Figure 7.16 Skin was thrown into folds and some regions the underlying muscle tissue was hollow. H&Ex250

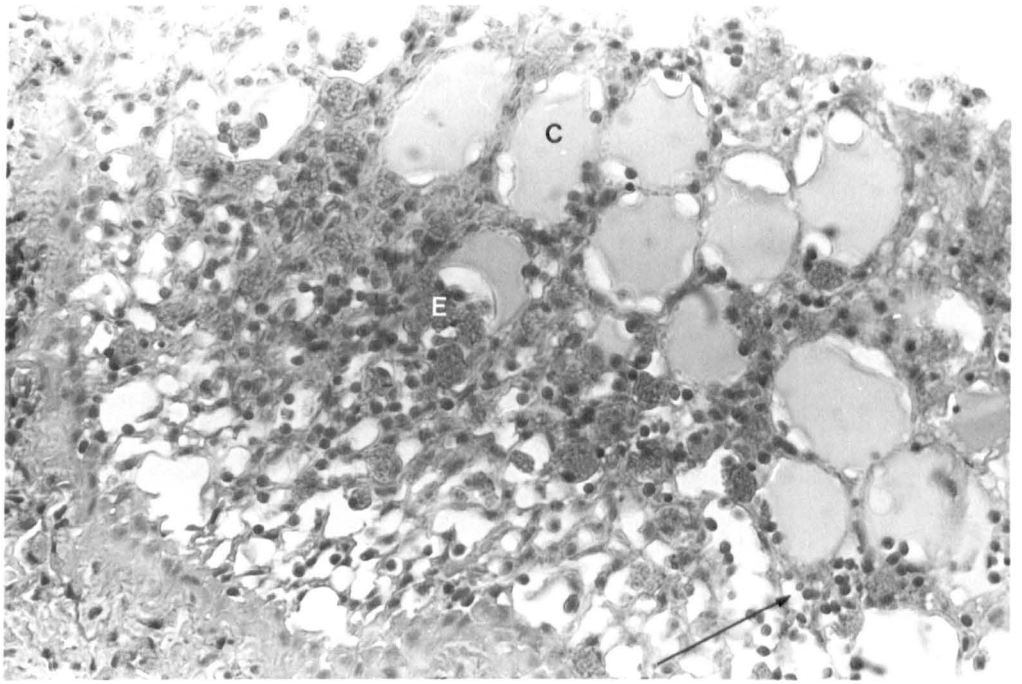


Figure 7.17 In immune fish from the ponds, the epidermal layer was greatly thickened due to the presence of large numbers of club cells (C). Eosinophilic granular cells (E) and lymphocyte like cells (arrowed). H&Ex400

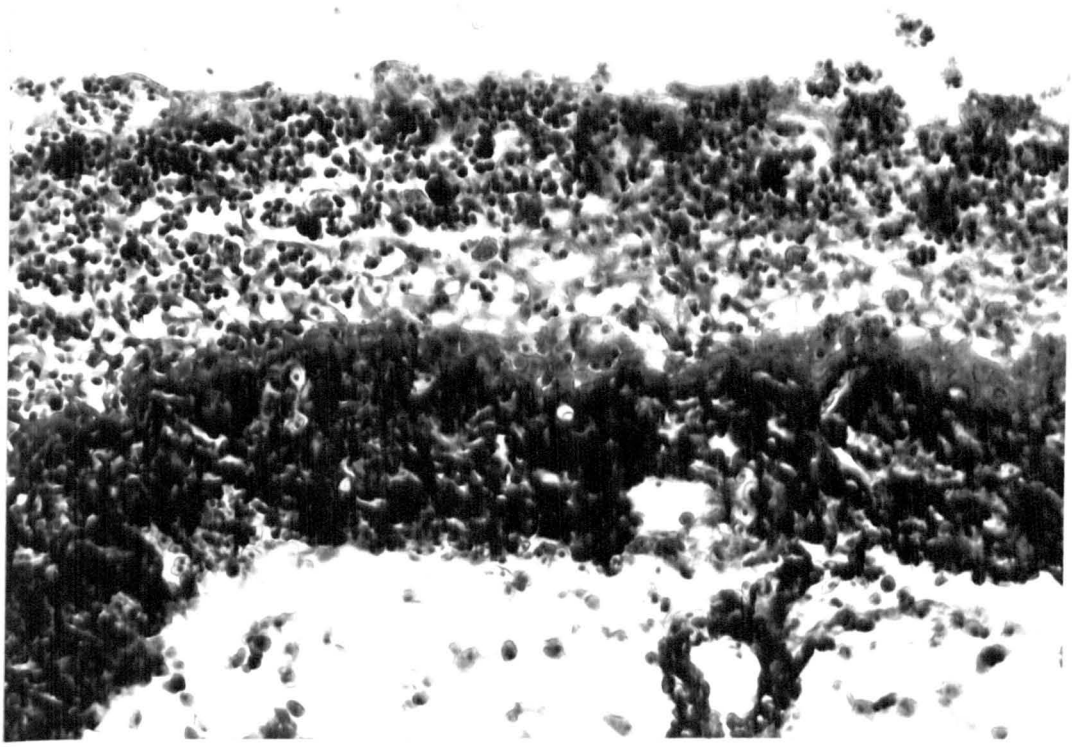


Figure 7.18 In immune fish from the ponds, some regions of the epidermal layer were devoid of club cells. MSBx400

lay collagen tissue to seal off the breach (Fig. 7.12). In those cases where the parasite had penetrated into the kidney, the protozoan Trypanosoma sp. was seen in the lumen of the anterior alimentary canal in the cephalic region of the parasite.

Mononuclear cells were abundant around the lesion. There was an increase in vascularization and more fibrotic tissue was seen around the periphery of the lesion. The cyst around the parasite had increased in thickness and this was only seen around the smaller horns of the cephalic processes (Fig. 7.14).

At 10 days (256 hours) after infection, the epithelial cell layers were thickened due to spongiosis and with the presence of large club cells interdispersed between them. More fibrous tissue was seen around the parasite and underneath the point of breach. Mononuclear cells were now more abdundant. The area immediately surrounding the parasite at the body surface and the underlying tissue remained exposed to the external medium.

At 3 weeks (512 hours), the picture did not differ markedly from that described above. In addition to the mononuclear cells commonly dispersed among the necrotic cells around the lesion, eosinophilic granular cells (EGC) were first noted together with cells resembling lymphocytes(Fig. 7.15). These EGC's had a strong affinity for eosin (acidophilic). The EGCs were found interdispersed among the inflammatory cells in the lesion and also within

the epidermal layer. The EGCs had an eccentric nucleus and the cell measured 4.5 um, however cells that were within the epidermal layer were larger and measured 5 um to 5.7 um. In some specimens the skin layer was thrown into folds and some regions of the underlying muscle tissue were hollow (Fig. 7.16).

The epidermal layer around the lesions of immune fish collected from the ponds was greatly thickened and was spongiotic. It contained ~~of~~ large club cells, eosinophilic granular cells and lymphocyte like cells (Fig. 7.17). The large number of eosinophilic granular cells and club cells nearly filled the whole of the epidermal layer in. In some sections of the epidermal layer, the club cells were absent (Fig. 7.18). The EGC's in the epidermal layer measured 7 um while those present within the oedematous dermal layer were 5.7 um in diameter. In addition, the EGCs which were in the epidermal layer had larger granules as compared to cells that were found in the dermal layer. Large aggregates of red blood cells were also seen within the epidermal layer.

The stratum spongiosum and stratum compactum were severely oedematous and could not be differentiated. Red blood cells and EGCs were seen dispersed within these oedematous layers (Fig. 7.19).

Fish from the tanks which were suspected to be immune, showed a less intense response to the challenge. The epidermal layer was spongiotic but there were fewer club

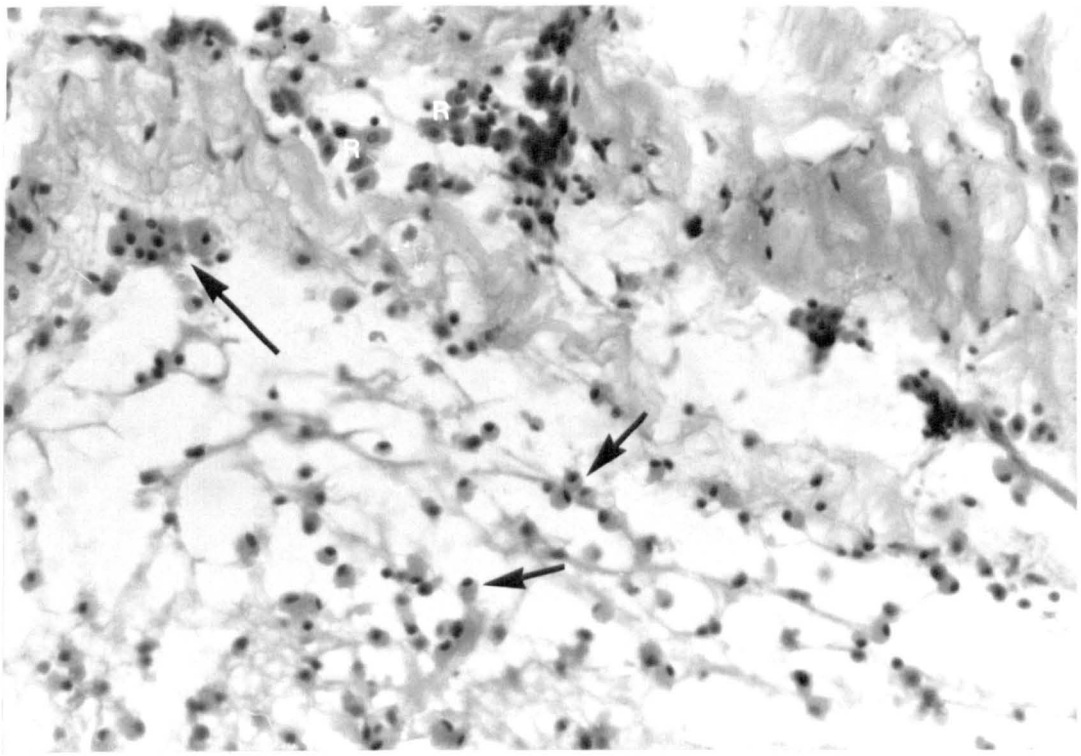


Figure 7.19 Red blood cells (R) and Eosinophilic granule cells (arrowed) were seen dispersed within the oedematous layers of the stratum spongiosum and compactum (from immune fish collected from the ponds). H&Ex400

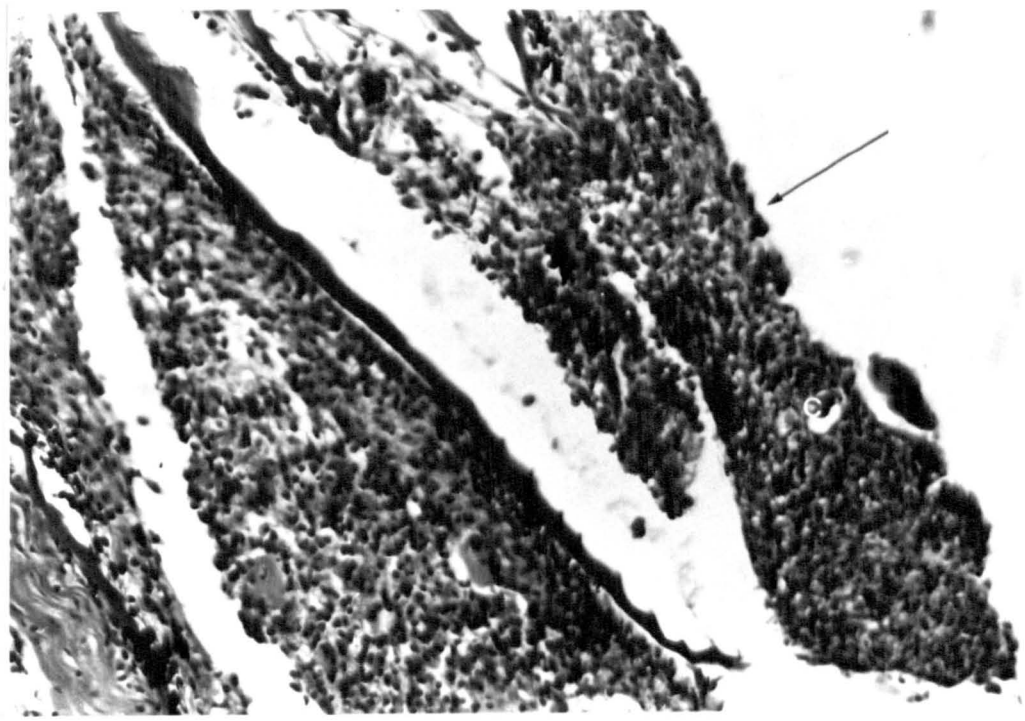


Figure 7.20 Spongiosis of epidermal layer with the presence of large numbers of eosinophilic granular cells (arrowed) and occasional club cells (C). (Immune fish from tanks) H&Ex250

Figure 7.21 Parasite surrounded by eosinophilic granular cells (arrowed) in fish that were challenged to infection. Note the shallow depth of the parasite in the host tissue.

cells although EGCs were present in large numbers (Fig. 7.20). In some sections, the parasites were only seen to penetrate under the scales and they were surrounded by large numbers of EGCs (Fig. 7.21).

7.4. Discussion

Infection of the skin with Lernaea, resulted in a marked disruption of the epidermal and dermal layers of the host tissue at the site of penetration by the parasite. This was followed by a severe acute and chronic inflammatory response.

In the initial stages muscle tissue was noted in the lumen of the parasite and thus it could be concluded that the parasite may have entered the tissue at least partly by mechanical action. The route of penetration was, in most cases between the overlapping scales indicating the choice of an easy path of entry by the parasite. In smaller fish, the anchor of the parasite was found in the internal organs, and in such cases the fish did not survive long and were dead within a week. Thus the high mortalities recorded in hatcheries could be related to such conditions where the penetration of the parasite may have caused traumatic damage to the vital organs. The penetration of Lernaea into the vital organs resulting in death has also been recorded by other workers. Otte (1965) recorded the penetration of Lernaea resulting in peritonitis and death, Khalifah and Post (1976) reported instant death in several cases of cranial penetration.

The inflammatory lesions seen in the present study was a typical granulomatous response. Granuloma formation in fish has been described by Timur G. (1975), Timur M. (1975), Timur et al. (1977) and Sommerville (1981).

Neutrophils were present around the periphery of the lesion during the acute inflammatory response; they did not reveal any indications of phagocytosis activity. The precise role of neutrophils in fish remains poorly defined. Ellis (1982) has suggested that, since they possess most of the enzymes found in mammalian neutrophils and they are usually present in many inflammatory lesions, this indicates that they play an active role in the defence mechanism. The presence of neutrophils in the inflammatory response to Lernaea has previously been documented by Joy and Jones (1973) and Shariff (1981).

The presence of an hyperplastic epithelial plug adjacent to the site of penetration was noted in the first instance the parasite was first found in the tissue. According to Anderson and Roberts (1975), the rapid epidermal covering ofthe wound must be a major survival advantage in the aqueous environment, as the difference in osmotic pressure of the surrounding water would cause fluid, protein and ion losses by outflow and would be very significant. They concluded that the healing epidermis would not only help reduce these losses but would also act as a barrier against potential pathogens. In the present study, the specimens did not reveal a complete coverage of the breach by epithelial cells, but instead, inflammatory

cells in exudate were exposed directly to the external surface. The limitation of the epithelial covering might be the result of the constant movement of the distal parts of the parasite were suspended in the external medium. It is likely that the movement of the parasites with water currents might have caused constant irritation and damage to the epithelium preventing a sufficiently stable platform to form a stronghold for the cells to grow.

Fish that were immune in tanks showed only haemorrhages or the development of parasites which were rejected on the 3rd. day. In such cases the parasites did not penetrate below the stratum compactum. This aspect would be interesting to investigate further. Further investigation of the possible immune response of A. nobilis to L. piscinae which in this case appears to be very effective, would greatly enhance the general knowledge of defence mechanism against parasites of fish.

An attempt was made to study the process of infection in fish which were injected with serum from suspected immune fish. The results were inconclusive and large numbers of treated fish died. This was thought to be due to experimental technique. However, from the small number of fish surviving there was evidence of rejection of the parasite on the day following exposure to infection; whereas the control fish remained infected for in excess of 2 weeks. This experiment should be repeated once the technical difficulties have been overcome. Specific immune responses

have only been demonstrated for a small number of the parasites affecting fish (Harris 1972, Hines and Spira 1973, Cottrell 1977, and McArthur 1978).

Large numbers of eosinophilic granular cells, lymphocyte like cells and large club cells were found to be associated with the rejection of the parasite in chronically infected fish and those suspected of being immune. Eosinophilic granular cells have often been associated particularly with parasite induced pathological conditions. Chaicharn and Bullock (1967) suggested that their function was concerned with the physiological defence mechanism when he found a number of these granular cells in parasite induced lesions in catostomids. Blackstock and Pickering (1980) reported a large number of eosinophilic granular cells in association with irritation of the salmonid epithelium, either by the ectoparasite Ichthyobodo sp. or by repeated formalin treatment. Smith (1975) also reported finding many distinct eosinophilic granular cells in marine fish infected with copepod Lernaeenicus spp. and the caligoid Sphyrion sp. Roberts and Bullock (1976) reported that eosinophilic granular cells are more abundant under pathological conditions. The precise role of the eosinophilic granular cell is still unknown. An active Golgi region, accumulation of granules, increase in cell size as it matures and the exposure of the typical cytoplasm, all suggest that this cell type is secretory. Blackstock and Pickering (1980), Roberts (1972), Baldo and Fletcher (1975) and Ellis (1977) speculated that

eosinophilic granular cells in fish play the role of mast cells in mammals.

In mammals, mast cells have been associated with expulsion of parasites from the gut of rats (Askenase 1980), or have a direct effect on the parasite (Rothwell et al. 1974, Kelly and Dineen 1976) or may act indirectly by promoting the pathotopic transfer of immunoglobulins (Barth et al. 1966, Murray 1972). King and Miller (1984) have shown that there is an increase in mucosal mast cell protease in the gut lumen occurring rapidly after a challenge in rats immune to Nippostrongylus sp.

If it is true that eosinophilic granular cells play the role of mast cells of mammals as suggested by Roberts (1978), Baldo and Fletcher (1975) and Ellis (1977) then there is a possibility that these cells might have been involved in the rejection of the parasite. The presence of lymphocytes and the increase in size and number of club cells might also have been related to this phenomenon. The club cells of fish has been indicated to produce toxic substances to predators (Pfeiffer and Pletcher 1964) or other aquatic animals and are only produced when the fish are under stress (Thomson 1969) and it is thought that their presence in the Lernaea induced lesion is possibly related to a specific immune response to the parasite.

CHAPTER 8.

GENERAL DISCUSSION AND CONCLUSION

Poddubnaya (1978) suggested that as a result of the geoglogical processes Lernaea cyprinacea "European" form, L. elegans (=L. cyprinacea "Asian" form), L. esocina and L. parasiluri survived only in South East Asia. Later L. cyprinacea "European" form migrated back to the North, L. esocina to North West and L. elegans and L. parasiluri to the East. The present study found only L. piscinae, L. cyprinacea "Asian" form (=L. elegans) and morphae L. ctenopharygodonis and L. quadrinucifera None of the other species mentioned by Poddubnaya were found although Malaysia lies in the geographical region of South East Asia. In the light of the present study, this hypothesis needs to be revised. Even in Thailand and Indonesia which also lie in South East Asia, only L. cyprinacea has been recorded though the morphological details have not been reported. Hence much more work has to be done to demonstrate the links between the various Eastern species of the genus Lernaea.

The study has confirmed the presence of L. piscinae in South East Asia and its neighbouring countries. On the basis of the arguments presented in Chapter 4 the name L. piscinae is considered here to be a junior synonym of L. polymorpha Yu. Harding (1950) who erected L. piscinae was unaware of L. polymorpha and its description by Yu (1938). L. piscinae can no longer be considered an "African" form as reported by Kabata (1979). Kabata agrees with the author on these points (pers. communication).

The morphometric studies have also made it clear that L. parasiluri which was synonymized with L. piscinae by

Ho (1961) was not justified and Ho's specimens which he referred to as L. parasuluri were actually L. piscinae (=L. polymorpha). Ho agrees with the author on this (pers. communication).

The investigation did not reveal the presence of the "European" form, the oldest branch of L. cyprinacea in West Malaysia. Poddubnaya (1978) suggested that L. cyprinacea "Asian" form (=L. elegans) is the youngest branch of L. cyprinacea and is still undergoing development and providing a starting point for numerous species. The present study has shown that L. ctenopharygondonis is a morpha of L. cyprinacea "Asian" form. The suggestion by Poddubnaya (1978), that to avoid confusion, the name L. cyprinacea should be restricted only to the "European" form which occurs on C. auratus and the name L. elegans be used for L. cyprinacea "Asian" form seems reasonable. However, before a definite conclusion could be made it would seem to be desirable to carry out experimental infection of other cyprinids with L. cyprinacea "European" form . On this basis, distribution of L. cyprinacea and L. elegans and its morpha form would need re-examination.

The use of morphometrics on the study of the larvae and adult female parasite has produced significant findings. The adult female of L. cyprinacea showed constancy in some characteristics of the cephalic processes i.e. the ventral horn and the distance between mid-body and process of dorsal horns, which were not affected when the parasite was obtained from 2 different host species. These

characteristics were significantly different than those of L. piscinae. More work has to be done to establish these parameters for the other species of Lernaea to elucidate whether these characteristics vary amongst all the species of this genus.

The position of the legs was another characteristic which showed interesting results. L. cyprinacea and L. piscinae could be distinguished from several other species, although in some cases the range of values reported for the position of legs were rather large. The present study showed similarities between the position of the swimming legs of L. cyprinacea "Asian" form and in those of L. quadrinucifera indicating that the latter may be a morpha of L. cyprinacea. This supports Poddubnaya's (1973) findings whereby she obtained L. quadrinucifera from the offspring of L. cyprinacea "Asian" form. It may well be that the position of the swimming legs is a very strong taxonomic character within the genus and the position of the legs in other Lernaea spp. would also require further examination to determine the extent of variability present amongst them.

The use of morphometrics would provide more precise values and thus with the use of the position of the legs and the cephalic processes, identification of Lernaea could possibly be made easier.

The morphological studies revealed that parasites from the body proper were less variable in size and morphology than those obtained from the caudal peduncle, fins, head and

eyes. It was also shown that in most cases the cephalic processes of parasites obtained from the body proper were significantly different in size than those of parasites obtained from the caudal peduncle, fins, head and eyes. It is therefore suggested that parasites be collected from the body region for taxonomic purposes.

The left or right section of the cephalic processes may also vary between parasites obtained from the left and right side of the host. This could most probably be due to the influence of water currents on the exposed portion of the parasite body which may have its subsequent effects on the development of the attachment organ in the host tissue. Thus, in addition to the influence of microhabitat within the host tissue, studies on the effect of the external environment might help provide a better understanding of the influence of these factors on the genus Lernaea.

It would not be advisable to use the morphology of the cephalic processes as the sole taxonomic characters for the identification of Lernaea. Using these characters alone has already given rise to much confusion and resulted in the erection of invalid species. For example L. ctenopharygondonis and L. quadrinucifera was shown in the present study to be a morpha of L. cyprinacea "Asian" form when experimental infections were carried out. Poddubnaya demonstrated by experimental infection that L. quadrinucifera is also a morpha of L. cyprinacea "Asian" form and this is supported here by the morphometric analysis. Further experimental infections might elucidate

similar relationships between species in the genus. As seen from the past references, L. ctenopharyngodonisand L. quadrinucifera were identified as separate species, whereas the fact that it was obtained from the offspring of L. cyprinacea "Asian" form reveals otherwise. L. ctenopharyngodonisand L. quadrinucifera are actually morphae of L. cyprinacea "Asian" form.

Attempts to identify the larval stages of Lernaea to species level based on morphology failed, as no differences were seen between L. piscinae and L. cyprinacea larvae. However morphometrics did show some significant differences between the two species at certain larval stages. Though morphometric analysis is useful when applied to adult female parasite forms its use at the larval stage is less satisfactory. The study showed that there are very few variations between species at the larval stages and when these values were compared with the findings of other workers on the same species of parasites they showed an unacceptably wide range of measurements.

Besides morphometrics, the use of other techniques used to identify parasites of the genus to species level should be explored. The use of morphometrics requires large numbers of parasites and is very time consuming, thus an alternative simple method would prove to be valuable and would ideally be used in conjunction with experimental infection.

The studies on frequency distribution of

L. piscinae and L. cyprinacea revealed an overdispersed pattern. Since the fish were sacrificed for collection of parasites, studies could not be made to determine whether the mortality within the overdispersed population was dependent on the clump size. The experiment on histopathological study showed that the penetration of a parasite into the internal organs of a small size host was sufficient to cause mortality. Thus, in addition to the mortality caused by clumping of parasites within a small number of host in the population, mortality occurs in small fish with low infection. Further investigations would be required to examine the maintenance of equilibrium of Lernaea population among its hosts.

A. nobilis experimentally infected with L. piscinae revealed a complete absence of Lernaea infection on the body surface after 6 months and was followed by its appearance on the eye. The eye is considered to be an immunologically privileged site due to the absence of a mucous lining and the avascular nature of the cornea. Thus, it would be devoid of any antibodies that may be present on the rest of the body surface along with the mucus. The presence of the parasite solely on the eyes suggests the development of an immune response which requires further investigations.

The histopathological studies indicated that eosinophilic granular cells, lymphocyte like cells and club cells could possibly be involved in the rejection of L. piscinae in immune fish. Although previous worker have indicated that eosinophilic granular cells are secretory, it

would be interesting to discover their precise role. The answer could further elucidate the defence mechanism of fish against parasitic infection. Further work on the immune response could lead to an immunization programme which would be of great value to the aquaculture industry.

Lernaea has the potential to cause great economic losses to the aquacultural industry. The study on growth rates clearly indicated a difference of 35% in the mean weight of the infected fish when they were compared to the uninfected controls at the end of a 6 months period. In West Malaysia more than 50% of the ponds studied were infected, thus the total losses to the fish farmers must be substantial. It is most likely that fish farmers with infected fish may have to wait for a longer growing period before harvesting their fish. This is because of the low growth rate of fish infected with Lernaea. Besides the low growth rates, mortalities due to Lernaea could also reduce the fish harvest. If the problem is left unchecked, there is also a threat to the natural fish population as it was seen that L. cyprinacea demonstrated a wide range of host susceptibility and pH tolerance. Thus, Lernaea could easily be distributed to the indigenous fish population through expansion of fish culture. There is an urgent need for the development of control measures to check the spread of the disease in Malaysia and at the same time to eradicate it from farms where it is known to be present. Ideally it should be controlled before it spreads to the natural water bodies where it would become a more persistent problem.

The study on growth rate was terminated after 6 months, when the fish had recovered from Lernaea infection. Further work on the Specific Growth Rates following recovery period would provide interesting data on the length of time the fish would require to reach marketable size compared to the control fish. Since a normal growth cycle is from 8-12 months, the investigation would provide more realistic figures in terms of actual economic losses.

Another aspect to examine would be the effect of supplementary feeding. Could fish that are provided with supplementary feed gain immunity at a faster rate than fish that depended only on primary production in the ponds? Such an answer would be of great benefit to farmers who have no alternative method of eradication of the disease by reducing the losses by other means.

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