ECOLOGICAL STUDIES ON THE PARASITES OF *ETROPLUS SURATENSIS* (BLOCH) (PISCES: CICHLIDAE) WITH SPECIAL REFERENCE TO *ENTEROGYRUS* SPP. (MONOGENEA: ANCYROCEPHALINAE)

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for the degree of Doctor of Philosophy

by

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DECLARATION

I hereby declare that this thesis has been compiled by myself and is the result of my own investigations. It has neither been accepted, nor is being submitted for any other degrees. All the sources of information have been duly acknowledged.

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To my Parents

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Introduction

1. Introduction

1.1. Brief history and background of aquaculture and fish health in Sri Lanka

In the past, the Sri Lankan fish industry was mainly comprised of capture fisheries, with a heavy dependence on the marine section. Landings from inland fisheries increased enormously following the introduction of the tilapia *Oreochromis mossambicus* (Peters) in 1952. Sri Lanka has a high potential for the development of freshwater fisheries in its several thousand irrigation reservoirs. Therefore, in the last few decades, attention has been focused on reservoirs mainly for the following reasons; their high potential, there is less cost incurred in their development than in marine fisheries, and because of their great socio-economic importance with regard to food supply and employment opportunities.

Fernando (1971) pointed out the doubled increase in freshwater fish production to 800 metric tons from 1954 to 1958, then 8,000 metric tons by the year 1968. He attributed this success to the introduction and establishment of *O. mossambicus*. The increased fishing effort also enabled more successful exploitation of economically important native species. The deficiency of lacustrine fish amongst the indigenous fauna and the low yield of other indigenous species have been the main reasons for the spectacular success of *O. mossambicus* in the reservoirs (Fernando & Indrasena 1969; Fernando 1971, 1973, 1982; De Silva & Fernando 1980).

The introduction of food fishes to reservoirs commenced in 1948. Until 1952, six exotic species, *Ctenopharyngodon idella* Cuvier & Valenciennes, *Hypopthalamichthys molitrix* (Valenciennes), *Cyprinus carpio* L., *Tricogaster pectoralis* Regan, *O. mossambicus*, *Helostoma temmincki* (Cuvier and Valenciennes) and one native species from the coastal lagoons of Sri Lanka, *Etroplus suratensis* (Bloch) have been introduced into freshwater reservoirs (Fernando, 1971). The success of *O. mossambicus* in different reservoir types has led to a subsequent search for other exotic species that would allow the yields to be further increased. This led to the introduction of three further tilapia species, *Tilapia hornorum* Trewawas, *Tilapia*

rendalli (Boulenger) and *Tilapia zilli* Gervais in 1969. However, this has not been successful (Fernando 1973). The introduction of *Oreochromis niloticus* (L.) in 1975 again added some success to increasing the production.

There were virtually no aquaculture practices in Sri Lanka until the early eighties. The Ministry of Fisheries started community village pond culture (stocking and managing of seasonal tanks) in 1980, encouraging small scale farming (Siriwardena, 1989). Production of necessary and appropriate fish fry and fingerlings is one of the most important steps in this direction. This fish fry and fingerling production programme has been facilitated by the success of induced breeding of the exotic carps, such as the Chinese major carps; grass carp, *C. idella*; silver carp *H. molitrix*; and bighead carp, *Aristichthys nobilis* (Richardson); and the Indian major carps; rohu, *Labeo rohita* (Hamilton-Buchanan); mrigal *Cirrhinus mrigala* (Hamilton); catla, *Catla catla* (Hamilton) (Balasuriya, 1987).

The master plan for the development of fisheries in the eighties, called for an increase in per capita fish consumption from, 15 kg/year to 21-22 kg/year by 1989, an increase of 210,000 mt/year from 1982 to 330,000 mt/year by 1986 (Anon 1986, cited by Subasinghe & Balasuriya, 1987). As the maximum sustainable yield of the continental shelf fishery is approximately 250,000 mt/year, two thirds of the desired increase was to be generated by improving freshwater fisheries. In order to meet the projected production of freshwater fisheries, the government embarked upon an immense aquaculture programme in collaboration with various aid agencies. This intensification necessitated trained labour to handle management problems as well as disease outbreaks. Since aquaculture was a new activity, the important diseases and control measures had not been identified.

The disease problems arising with the intensification of aquaculture at the freshwater fisheries stations in Sri Lanka is documented by Balasuriya (1983, 1987). Three bacterial, two fungal, and thirteen parasitic diseases have been encountered. Of these, two bacterial and six parasitic diseases are identified as causing great losses to the aquaculture industry. Since 1982, a considerable effort had been made to control

these diseases, but these have met with only partial success. Mass mortalities of fish have been reported from time to time in open water bodies (Balasuriya, 1987). In May of 1983 mass mortalities of *O. mossambicus* and *O. niloticus* were observed at Padaviya reservoir and Pimburaththewa reservoir destroying about 100 tons of fish. During this period even fish captured in gill nets laid by fishermen during the night had died and spoiled rapidly, making the harvest worthless. There are occasional reports of mass mortalities due to oxygen depletions in organically polluted small lakes, e.g. Beira lake and Kandy lake. Ulcerative disease was first reported in Sri Lanka at the end of 1987 during the rainy period (Frerichs, 1988) and, since then, has reappeared at similar periods in subsequent years. The native snakehead, *Ophiocephalus striatus* Bloch was the fish most highly affected.

Complete parasite surveys of fish and life cycle studies of parasites are lacking in Sri Lanka. The records of identified fish parasites are of a few intestinal parasites, and a few parasitic crustaceans and gill monogeneans. Mendis & Fernando (1962), Fernando (1963, 1964, 1969, 1974) record the parasites reported in freshwater fishes with some descriptions and the host. Senadhira (1967a,b,c) gives a host check list for all helminths recorded in Sri Lanka. A list of all the parasite records up to date are given in the Table 1 together with their hosts.

Gussev (1963) described 20 new species of Monogenoidea from Sri Lanka including two species from *E. suratensis*, *Ancyrocephalus etropli* and *Ceylonotrema colombensis* from Colombo vicinity. Gussev & Fernando (1984), gave a brief report on 23 gill monogeneans, including the 20 species described previously. Gussev & Fernando (1973) redescribed *Enterogyrus globidiscus* from the stomachs of *E. suratensis*, which is described earlier by Kulkarni (1969) as *Urocledius globidiscus* from the gills of the same host. Gussev & Fernando (1973) described another species of *Enterogyrus*, *E. papernai* from the same host from the same locality, Parakrama Samudraya (reservoir).

Crusz & Sathananthan (1960) recorded the ectoparasitic digenean *Transversotrema patialense* (Soparkar, 1924) from a freshwater fish. Its life cycle has

Table 1 : The fish parasites recorded from inland fishes of Sri Lanka.
(The scientific names are those used in the original reports)

Parasite	Host	Reference
Protozoa		
Cryptobia sp.	*Labeo rohita*, *Cirrhinus mrigala*, *Ctenopharyngodon idella*	Balasuriya, 1983
Trichodina sp.	*L. rohita*, *C. mrigala*, *Cyprinus carpio* L., *Catla catla* (Hamilton), *Labeo dussumieri* Valenciennes	Balasuriya, 1983
Ichthyophthirius sp.	*C. idella*, *L. rohita*, *C. mrigala*	Balasuriya, 1983
Scyphidia sp.	*L. rohita*, *C. mrigala*, *C. carpio*	Balasuriya, 1983
Myxobolus sp.	*L. rohita*, *C. mrigala*, *C. carpio*	Balasuriya, 1983
Monogenea		
Dactylogyrus curiosus Gussev, 1963	*Rasbora daniconius* (Hamilton-Buchanan)	Gussev, 1963
Dactylogyrus daniconii Gussev, 1963	*R. daniconius*	Gussev, 1963
Dactylogyrus saranae Gussev, 1963	*Puntius sarana* (Hamilton-Buchanan)	Gussev, 1963
Dactylogyrus aequipinnati Gussev, 1963	*Danio aequipinnatus* (McClelland)	Gussev, 1963
Dactylogyrus dorsalis Gussev, 1963	*Puntius dorsalis* (Jerdon)	Gussev, 1963
Dactylogyrus fernandoi Gussev, 1963	*P. dorsalis*	Gussev, 1963
Dactylogyroides macracanthus (Tripathi, 1959)	*Puntius filamentosus* (Valenciennes)	Gussev, 1963
Dactylogyroides vittati Gussev, 1963	*Puntius vittatus* Day	Gussev, 1963
Dactylogyroides bimaculati Gussev, 1963	*Puntius bimaculatus* (Bleecker)	Gussev, 1963
Conudiscoides (=*Ancylodiscoides*) *jaini* (Gussev, 1963)	*Macrones keletius* (Valenciennes)	Gussev, 1963
Bifurcohaptor lanki Gussev, 1983	*M. keletius*	Gussev & Fernando, 1984
Ancyrocephalus esomi Gussev, 1963	*Esomus dandrica* (Valenciennes)	Gussev, 1963
Ancyrocephalus heteranchoris Gussev, 1963	*R. daniconius*	Gussev, 1963
Ancyrocephalus rasborae Gussev, 1963	*R. daniconius*	Gussev, 1963
Ancyrocephalus daniconii Gussev, 1963	*R. daniconius*	Gussev, 1963
Ancyrocephalus aequalis Gussev, 1963	*R. daniconius*	Gussev, 1963
Ancyrocephalus kirtisinghei Gussev, 1963	*R. daniconius*	Gussev, 1963
Ancyrocephalus tripathii Gussev, 1963	*R. daniconius*	Gussev, 1963
Ancyrocephalus danionis Gussev, 1963	*D. aequipinnatus*	Gussev, 1963
Ancyrocephalus etropli Gussev, 1963	*Etroplus suratensis*, *Etroplus maculatus* (Bloch)	Gussev, 1963
Ceylonotrema colombensis Gussev, 1963	*E. suratensis*	Gussev, 1963
Enterogyrus globidiscus (Kulkani, 1969)	*E. suratensis*	Gussev & Fernando, 1973
Enterogyrus papernai Gussev & Fernando, 1973	*E. suratensis*	Gussev & Fernando, 1973
Dactylogyrus sp.	*L. rohita*, *C. mrigala*, *C. catla*, *C. carpio*, *C. idella*, *L. dussumieri*, *Aristichthys nobilis*	Balasuriya, 1983
Gyrodactylus sp.	*L. rohita*, *C. mrigala*	Balasuriya, 1983

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Digenea		
Transversostrema patialense (Soparkar, 1924)	*Macropodus cupanus* (Cuvier & Valenciennes)	Crusz & Sathananthan, 1960
Digenean metacercaria	*L. rohita, C. mrigala, C. idella, A. nobilis, H. molitrix*	Balasuriya, 1983
Cestoda		
Bothriocephalus gowkongensis Yeh, 1955	*Puntius sarana* (Hamilton-Buchanan)	Fernando & Furtado, 1962
Senga lucknowensis Johri, 1956	*Mastacembelus armatus* Lacépèd	Fernando & Furtado, 1962
Gangesis bengalensis (Southwell, 1913)	*Wallago attu* (Bloch & Schneider)	Fernando & Furtado, 1962
Ligula sp.	*L. rohita*	Balasuriya, 1983
Acanthocephla		
Zeylanechinorhynchus longinuchalis Fernando & Furtado (1963)	*Macrones vittatus* (Bloch)	Fernando & Furtado, 1962
Pallisentis nagpurensis (Bhalerao, 1931)	*Ophiocephalus striatus* (Bloch)	Fernando & Furtado, 1962
Nematoda		
Camallanus ceylonensis Fernando & Furtado, 1964	*W. attu*	Fernando & Furtado, 1963
Zeylanema anabantis (Pearse, 1933)	*Anabas testudineus* (Bloch), *P. filamentosus, R. daniconius*	Kulasiri & Fernando, 1956
Zeylanema pearsei (Yeh, 1960)	*R. daniconius*	Yeh, 1960
Zeylanema kulasiri (Yeh, 1960)	*Ophiocephalus punctatus* (Bloch), *A. testudineus*	Yeh, 1960
Zeylanema fernandoi (Yeh, 1960)	*O. striatus, O. punctatus*	Yeh, 1960
Zeylanema sweeti (Moorthy, 1937)	*R. daniconius, Clarias teysmanni* (Gunther), *O. punctatus*	Kulasiri & Fernando, 1956; Yeh, 1960
Procamallanus planoratus Kulkarni, 1936	*C. teysmani, O. striatus, O. punctatus*	Kulasiri & Fernando, 1956
Procamallanus spiculogubernaculus Agarwal, 1958	*C. teysmani*	Kulasiri & Fernando, 1956
Procamallanus confusus Fernando & Furtado, 1964	*Heteropneustus fossilis* (Bloch)	Fernando & Furtado, 1963
Procamallanus sp.	*W. attu*	Fernando & Furtado, 1963
Spinitectus corti (Moorthy, 1938)	*Ophiocephalus gachua kelaarti* Munro, 1955	Costa & Wijekoon, 1966
Hedruris sp. (larva)	*W. attu, O. bimaculatus, Glossogobius giuris* (Hamilton-Buchanan)	Fernando & Furtado, 1963
Eustrongylides sp. (larva)	*H. fossilis, O. bimaculatus, W. attu*	Fernando & Furtado, 1963
Annelida		
Placobdella undulata Harding	*E. suratensis*	Mendis & Fernando, 1962

.....continued

Crustaceans		
Ergasilus ceylonensis Fernando & Hanek, 1972	*P. sarana, P. dorsalis*	Fernando & Hanek, 1972
Ergasilus mendisi Fernando & Hanek, 1972	*O. bimaculatus*	Fernando & Hanek, 1972
Paraergasilus brevidigitus Yin, 1954	among zooplankton	Fernando & Hanek, 1973
Sinergasilus major (Markevich, 1940)	*C. idella*	Balasuriya, 1983
Lamproglena chinensis sprostoni Kirtisinghe, 1964	*O. striatus*	Kirtisinghe, 1964
Lernaea cyprinacea chackoensis Gnanamuthu, 1951	*C. carpio, Osphronemus goramy, H. fossilis, G. giuris*	Mendis & Fernando, 1962; Kirtisinghe, 1964; Fernando & Hanek, 1973
Lernaea sp.	*C. carpio, L. rohita, C. mrigala, L. dussumieri, A. nobilis*	Balasuriya, 1983
Argulus foliaceus L.	*C. carpio*	Mendis & Fernando, 1962; Kirtisinghe, 1964
Alitropus typus Milne Edward, 1840	*R. daniconius, W. attu*	Mendis & Fernando, 1962; Ingle & Fernando, 1963

been studied in detail by Crusz, Ratnayake & Sathanandan (1964). Kulasiri & Fernando (1956) reported three species of camallanid nematodes. Yeh (1960) described five species of camallanid nematodes, out of which three were new. A new genus *Zeylanema* was erected and five species were placed in it. Fernando & Furtado (1963) described 3 species of cestodes, 2 species of acanthocephalans and 6 species of nematodes. Further, they included a tabular list of parasitic fish helminths recorded from Sri Lanka and their host species. The leech, *Placobdella undulata* Harding was recorded on *E. suratensis* by Mendis & Fernando (1962).

A review of parasitic crustaceans on fish recorded up to 1964 has been written by Kirthisinghe (1964). Fernando & Hanek (1972) described two new species of *Ergasilus* from freshwater, *E. ceylonensis* and *E. mendisi.* More have been added by the same authors in 1973. Detailed descriptions of *Paraergasilus brevidigitus* Yin, 1954 and *Lernaea cyprinacea chackoensis* Gnanamuthu, 1951 are provided together with brief descriptions of the two *Ergasilus* spp. described earlier, *Argulus foliaceus* L., *Lamproglena chinensis sprostoni* Kirtisinghe, 1964 and *Alitropus typus* Milne Edward, 1840.

All the fish parasites recorded up to 1962 were included in the guide to the freshwater fauna of Sri Lanka by Mendis & Fernando (1962). The supplements added later by Fernando (1963, 1964, 1965, and 1969) revise the lists.

It is very clear that other than recording some of the ecto- and intestine inhabiting parasites not much attention has been paid to systematic parasitic surveys concerning the possible impact on fisheries or aquaculture. It should be noted that this type of work has only been undertaken by Balasuriya (1983; 1987) on the diseases in carp hatchery and fingerling holding tanks.

The understanding of the environmental influence is of prime importance in forecasting the possible fluctuations of parasite populations and their possible effect on fisheries and aquaculture systems. This subject has been paid little attention in Sri Lanka as well as in the tropics in general. It was decided to undertake this project

paying particular attention to *E. suratensis*, selecting it as a suitable candidate for the study because of the following reasons;

1. its importance in fisheries and its potential for aquaculture as the one of the two native cichlids; the other species, *Etroplus maculatus* (Bloch) is important only as an ornamental fish,
2. owing to its introduction into freshwater reservoirs from coastal lagoons it was interesting to study the chances of survival of the parasitic fauna in a completely different habitat, and
3. its access to an understanding of the effect of environmental factors on the parasite population in two completely different types of waterbodies.

1.2. The biology of *Etroplus suratensis* (Bloch)

Order	-	Percomorpha
Suborder	-	Labroidei
Family	-	Cichlidae
Subfamily	-	Etroplines
Genus	-	*Etroplus*
Species	-	*suratensis*
Common names	-	Green chromide, Pearl spot (English)
		Gan koraliya, Mal koraliya (Sinhalese)

Etroplus suratensis is a euryhaline cichlid indigenous to South India and Sri Lanka (Munro, 1955). It primarily inhabits estuaries. In 1950 it was introduced by the department of Fisheries to man-made fresh water reservoirs from coastal lagoons where, they now flourish (Fernando & Indrasena, 1969; Costa, 1983). However, they have not been recorded from rivers that connect the freshwater habitats with the estuary (Radda, 1973), Probably the fish may not withstand flowing water conditions. The reason for this is unknown but may be related to the inability to withstand flowing water conditions.

In Sri Lanka, some aspects of the biology of this species have been studied by Costa (1983), in three different habitats, Negambo Lagoon (salinity 0.02-28 ‰),

Colombo Lake (salinity 0.02-2.5 ‰) and Parakrama Samudra (freshwater reservoir), and by De Silva, Maitipe & Cumaranatunge (1984), in Koggala Lagoon (salinity 2-6 ‰) and Udawalawa reservoir (freshwater). Ward & Wyman (1975, 1977), Ward & Samarakoon (1981) and Samarakoon (1983, 1985) have paid special attention to the reproductive biology of *E. suratensis* with an emphasis on breeding ethology and breeding patterns.

This is one of the most popular food fishes of brackish and inland waters in Sri Lanka, presently 5% of the fish caught in the major inland reservoirs consists of *E. suratensis* (Costa, 1983). According to Ward & Wyman (1975), the largest specimen of the green chromide was found in Negombo lagoon and was nearly 18 inches (46 cm) in length and nearly 1.5 lbs. (680 g) in weight. The largest specimens collected by Costa (1983) from Colombo lake, Negombo lagoon and Parakrama Samudraya (reservoir) have measured 15 cm, 16 cm and 22 cm long respectively. De Silva *et al.* (1984) estimated that it takes about 2-3 months for them to reach a size of 6 cm. According to this growth rate, it can be assumed that not more than one year is taken for the fish to attain about 14-16 cm.

Costa (1983), pointed out that, according to the food, feeding habit and the morphological adaptation of the mouth, *E. suratensis* mostly displays a benthic feeding habit. According to De Silva *et al.* (1984), the dentition of *E. suratensis* is well suited to exploitation of two types of food, macrophytes as well as invertebrates, especially molluscs; thus it is not a complete herbivore as suggested by Ward & Samarakoon (1981). The molariform pharyngeal teeth are used to shred (triturate) or physically break down the cellulose walls of macrophytes as well as to crush the molluscan shells.

The diet of *E. suratensis* seems to be very varied consisting of terrestrial and aquatic macrophytes, epiphytic and filamentous algae, phytoplankton, zooplankton, sponges, oligochaetes, molluscs, crustaceans and detritus (Schiemer & Hofer, 1983; Costa, 1983; De Silva *et al.*, 1984). According to Schiemer & Hofer (1983) soft mud habitats with a predominance of meio benthos did not provide a usable diet for

Etroplus.

De Silva *et al.* (1984) considered that there was a difference in food habit for mollusc and the macrophytes according to habitat; the reservoir fish had a high preference for macrophytes whilst the lagoon fish for molluscs. This was not the case in the study of Costa (1983) who found much similarity of food habit in brackish and freshwater habitats. It might be expected, therefore, that these differences or similarities are reflected in the parasite fauna.

E. suratensis seems to be a visual feeder; feeding in the early morning and late afternoon hours (De Silva *et al.* 1984).

According to Costa (1983) sexual maturity is first attained in the length range 8.0-9.0 cm in females in the brackish water habitat and fecundity ranged from 700-3900 eggs in mature specimens. *E. suratensis* in lagoons, nests and reproduces twice during the year, just after the north-east monsoon in February and in the drier part at the middle of the southwest monsoon season in July. At these times, water conditions were favourable for nest construction and maintaining visual contacts with offspring as it is accompanied by a decrease in the water turbidity and increase in salinity in Negombo lagoon (Ward & Wyman, 1975; Samarakoon, 1983). De Silva *et al.* (1984) found the same pattern in the Koggala lagoon. The same seems to occur in India too, where *E. suratensis* breeds in shallow confined waters twice a year in Kerala, one during June-July and another in November to March (Jayaprakas, 1980). According to Costa (1983) the spawning of *E. suratensis* in the freshwater reservoir Parakrama Samudraya takes place only once per year after the north-east monsoon in February.

E. suratensis is a substrate-brooder. Brood nests were in 30-110 cm deep in water and where the white sand was more apparent (Ward & Wyman, 1977; Ward and Samarakoon, 1981). The brood pits become the temporary home of the non-swimming larvae, for about a week. They then start to swim and swarm around their parents in tightly clustered schools probably until they attain sexual maturity. The protection of the school and its direction is carried out by both the parents (Ward & Wyman, 1975).

Multiple parental caring of schools was reported by the same authors.

De Silva *et. al.* (1984) reported the deficiency of the fish out of the range 5-11 cm in catches from the traps laid in the sub-littoral region, the habitat of young as well as breeding grounds for adults (Ward & Wyman, 1975; Ward & Samarakoon, 1981). They suggested the possibility of the fish beyond 11 cm length are recruited into the deeper regions of the lagoon. According to Ward & Samarakoon (1981), even in the sub-littoral region *E. suratensis* prefers sandy regions for breeding. Therefore, it may be that De Silva *et al.* (1984) did not lay their traps in these sandy regions and the fish were not available. It may be that young fish in schools are able to avoid traps due to the protective behaviour provided by the parents.

In Negombo lagoon, chromides are living and interacting with not less than 41 other species of estuarine and marine fish (Ward & Wyman, 1975). Ward & Wyman (1977) pointed out that there are about 11 species of piscivorous fish, eight species of piscivorous birds (divers and waders) and one aquatic snake (*Natrix* sp.) predating on *Etroplus* in Negombo lagoon. The information on the ecological relationships with other fish is meagre and, therefore, further studies on this aspect would be a great help in the parasite life-cycle studies.

As reported by Ward & Wyman (1975), 'Brush pile fishing' is the commonest fishing method for *Etroplus* in Negombo lagoon. The piles of mangrove branches mimic the mangrove swamp, which attracts the fish. The fish are caught in baited traps in Koggala lagoon, a box of rectangular shape made out of galvanized iron mesh and carries the food inside in a container. The trap is an aperture on one side wall made by cutting and turning the mesh inwards allows the fish to enter but not to escape due to the cut edge of the mesh. In Udawalawa, *Etroplus* is caught by the 'hook and line' method, using the decapod crustacean *Caridina* spp. as the bait. Usually the person fishing stands still in the waterbody in the littoral and sub-littoral regions whilst casting the hook.

There are few statistics concerning the *Etroplus* fishery and the significance

to the fishing communities or the economics involved. Ward & Wyman (1975) estimated that the annual production of *E. suratensis* from the 'brush-pile' fishery is 17,000 lbs. (7710 kg) per year which accounts for 11 % of the 'brush pile' harvest in Negombo lagoon. In Udawalawa, *E. suratensis* accounts for 10 % of the total catch (Candrasoma *et al.*, 1986) whilst it is about 5 % of the total catch, when the inland fishery of the whole country is considered (Costa, 1983).

The *Etroplus* fishery depends more on capture operations than fish culture, even in India (Jayaprakas, 1980). Whilst in both countries, India as well as in Sri Lanka, *E. suratensis* is considered as a good candidate for both brackish-water and freshwater fishery and culture. The potential for culture of *E. suratensis* in India and the cost-benefit analysis has been studied by Matondkar (1978), Jayaprakas (1980) & Sumithra-Vijayaraghavan, Krishna Kumari, Gopinathan & Dhawan (1981). In an experimental estuarine pond, an investment return of 33% was obtained by Sumithra-Vijayaraghavan *et al.* (1981). Jayaprakas (1980) has reported the ability of *E. suratensis* to breed freely in confined waters but an induced breeding method for *Etroplus* has not yet been established (Samarakoon, 1985). Jayaprakas (1980) suggests the possibility of transferring eggs attached to introduced substances into culture ponds.

As it is stated by Schiemer & Hofer (1983), that the cichlid *O. mossambicus* occupying the same localities as *E. suratensis*, unlike them, feeds continuously throughout the 24 hour cycle in the lower half of the water column during day time and near the surface during night. Food is taken up both by sucking up of the detrital aggregate of the upper sediment layers and by filtering suspended phytoplankton. According to Al-Hussaini & Kholy (1954), *O. niloticus* and *O. mossambicus* have similar feeding habits. The difference of the micro-habitats of *O. mossambicus* and *E. suratensis* reported in Parakrama Samudraya by Schiemer & Hofer (1983), may be due to the difference in food habit; *O. mossambicus* preferring all the micro-habitats available and *E. suratensis* preferring areas with submerged vegetation and the sandy littoral areas.

1.3. The climatic condition in Sri Lanka

Sri Lanka is an island situated 6°-10° N of the equator, and covers an area of 65,610 km^2. The morphological structure is characterized by a flat coastal area and a central highland.

The dominant characteristic of the climate of Sri Lanka is the monsoon, which occurs twice a year as the south-east monsoon and the north-east monsoon, according to the predominant wind direction. Together with the 'intermonsoon seasons', Domrös (1974) divided a year into four seasons:

March - mid-May (the first intermonsoon season),
mid-May - September (SW monsoon season),
October - November (second intermonsoon season),
December to February (NE monsoon season).

The 'aridity index' pattern presented by Domrös (1974) shows a high regional variation in the number of humid and arid months and, accordingly, the island is divided into a 'wet zone', a 'dry zone', and an 'arid zone'. The central highlands forms a climatic barrier which modifies the effect of two monsoons making an unequal distribution of rain throughout the country. During the SW monsoon the 'wet zone' towards the southwest receives more rain whilst during the NE monsoon the 'dry zone' towards the northeast receives more rain.

The temperature condition of Sri Lanka can be summarized by the term 'thermic diurnal climate', which means that the temperatures are characterized predominantly by differences in their diurnal course. The changes of temperature between day and night are greater than the seasonal differences, making a division of the year into temperature seasons scarcely possible and questionably useful.

In the lowlands up to an altitude of 150 m, the monthly mean temperature varies between 24.5°C and 28.5°C. The annual temperature range amounts to a maximum of 4.3°C in Trincomalee on the east coast and a minimum of 1.5°C in Rathnapura and Thalawakele. The warmest months are April-May and the coldest are

December-February (Dobesch, 1983). Consideration of the diurnal temperature ranges reveals considerable spacial differences. The coastal meteorological stations have the smallest amplitudes (Galle 4.7°C and Jaffna 4.8°C) whilst the stations situated further towards the interior (Badulla 9.8°C and Thalawakele 8.9°C) have the highest (Dobesch, 1983).

1.4. The study localities

Sri Lanka has a coast line of 1,760 km and along which are situated several lagoons. In addition, it has several thousand perennial and seasonal irrigation reservoirs in the dry zone. The study was mainly concentrated on two water bodies important for their fishery, further selected on the basis of the feasibility of collection of fish and transportation (Figure 1).

Koggala lagoon (Figure 1) is situated on the south-west coast. This is the largest lagoon in the southern Sri Lanka occupying 743 ha and a mean depth of around 2.4 m. The lagoon, and the river, Koggala oya flowing down to the lagoon, are under the direct influence of the south west monsoon. In general, there are plenty of aquatic macrophytes in the littoral region. During the shorter periods of inundations, terrestrial vegetation becomes a source of food for phytophagous fauna.

The Udawalawa reservoir across the river Walawe was constructed in 1965 (Figure 1). This is the largest and deepest perennial reservoir situated in the southern region of the country. As this is situated in the dry zone, the river, and especially the catchment above the dam, is under the influence of the north-east monsoon. At full supply level, the reservoir has a water spread of 3374 ha and the mean depth is around 15.3 m (Chandrasoma, Muthukumarana, Pushpakumara & Sreenivasan, 1986). According to the observations of the same authors, Udawalawa lacks submerged aquatic vegetation but the terrestrial vegetation grows on the bottom of the littoral region during the low water level periods and becomes a good source of food for a long period for macrophyte feeders. The fish *Etroplus* was stocked once, after the construction of the reservoir and was established without necessitating further stocking.

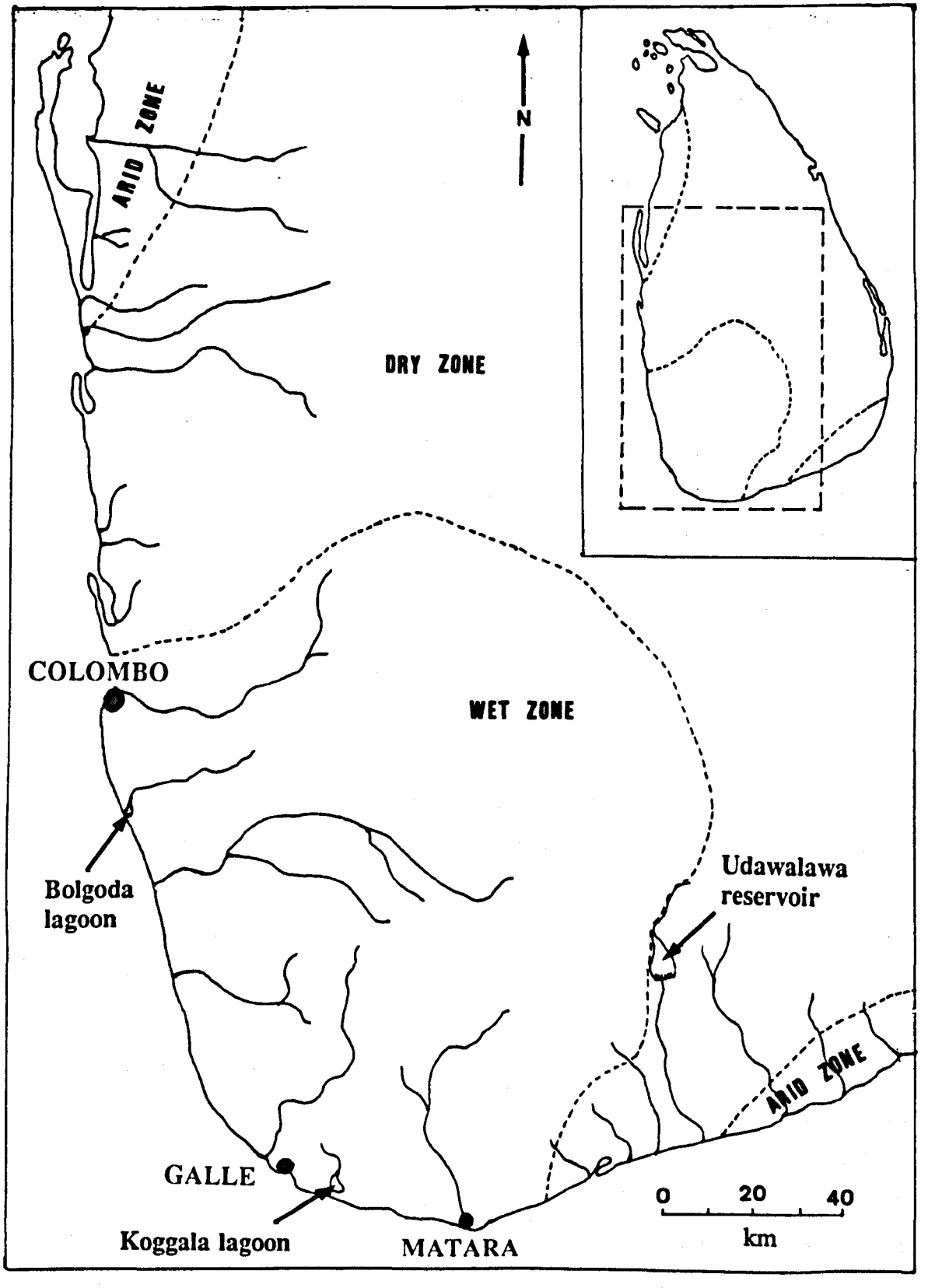


Figure 1: The location of the three localities sampled and major climatic zones.
Inset: Map of Sri Lanka showing the enlarged area.

1.5. Aims of the study

The work was mainly inaugurated with the hope of expanding the knowledge of the fish parasite fauna of Sri Lanka and provide information towards understanding the environmental factors which govern their population densities. Such information can assist in the assessment of the possible impact of these parasites on fish health in aquaculture and managed fisheries. Potential pathogens can be recognised and information necessary for preventative and control measures acquired.

The selected host, *E. suratensis* is euryhaline, the major environmental influence, in this case being the salinity. Thus, the effect of the introduction of *E. suratensis* from brackish lagoons to the completely different freshwater reservoir habitat on the parasite fauna it bears was investigated. In order to do this, the parasite fauna of the two different localities was surveyed and compared. The salinity can act directly on the parasite or restrict the distribution of final and intermediate host, thus life cycles were investigated where possible.

With its year round constant temperature and the influence of monsoons, the climate of Sri Lanka provides an ideal example of the tropical condition. Therefore, the ecological data of parasite populations were analyzed with reference to the climatic parameters to see whether these conditions influence the parasite population. In addition, the way the intermediate hosts are influenced by the climatic factors and their contribution to the fluctuations were considered.

Of the parasite fauna of *E. suratensis*, the monogenean genus *Enterogyrus* is unique due to its habitat, the hostile stomach. Two species of the same genus, *E. globidiscus* and *E. papernai* were found to occur simultaneously in the same stomachs of the fish occurring in both the fresh water and brackish water habitats during the initial surveys. The study was therefore subsequently focused on these two interesting parasites with the view to understanding their adaptation to such an unusual microhabitat, the influence of the external habitat and the inter-relationship between the two species.

Chapter 2

Ecological aspects of parasites of *Etroplus suratensis* (Bloch)

2.1 Introduction

The ecology of fish parasites, that is the relationships of the parasites to their environment, may be studied from two aspects, one in relation to the macro-habitat ie. the environment their hosts and free living larval stages experience and the other the micro-habitat ie. the environment they experience in or on the host. The parasitic stages of ectoparasites experience both these environments directly. The endoparasites, though they have no direct contact with the external environment, their physiology eg. the development and maturation, may be affected by its changes.

The relationships of fish parasites with the macro-habitat may be studied from three different aspects:

1. Waterbodies such as lakes, streams etc. may be considered as (whole) entities and the species composition and infection levels of parasites of the same host compared, their different hydrological (physical and chemical water parameters), and hydrobiological (faunistic composition) characters being the major reason for differences,
2. for migratory fish, the species composition and infection levels in the different habitats along their path may be compared, the salinity differences being the major influence if they are anadromous or catadromous, and
3. annual climatic (seasonal) changes may be considered to compare the changes in infection levels of fish in the same waterbody; the temperature being the major influence in this case.

The micro-habitat is the environment which has to supply all the requirements for a parasite. Therefore, providing it can supply all the nutritional requirements and a non-hostile surrounding, parasite relationships can develop with suitable hosts through the evolutionary process. Nonetheless the behaviour of a host is important as this governs the host-finding ability of a parasite. The host, being a living organism, reacts to the damaging effects caused by a parasite and develops appropriate defense mechanisms. The parasite must, therefore be able to overcome these eg. the immune

response which acts against its survival. An understanding of the ecological relations within a micro-habitat thus depends on the knowledge on the parasite's preference for a particular site in particular host of a particular age and the response exerted by the host.

2.1.1 Macro-habitat effects on the parasite species compositions and parasite infestation levels

(1) Comparison between water bodies

Several attempts have been made to characterise a habitat by its parasite fauna, and to predict the occurrence of species and level of infection. Wisniewski (1958) and his followers working on lake Drużno made an effort to characterise the parasite fauna of the different stages of lake succession, ie. oligotrophic, mesotrophic or eutrophic. The work on the parasites of the fish of Llyn Tegid by Chubb (1963) and Mishra & Chubb (1969) agreed with Wisniewski's (1958) postulate, however, the importance of the degree of host specificity of the parasites through out their life cycle was stressed. Halvorsen (1971) comparing the parasites of the same species of fish in limnologically as well as zoogeographically very different localities, reported that the similarity of the parasitic fauna was very high. Therefore, he concluded that the parasite fauna can contribute very little to characterization of a water-body.

Some attempts have been made to compare the richness of parasite fauna and the infection levels of same species of fish in standing and flowing water systems. The work of Chubb (1970) revealed that trout had a fewer number of parasite species in the river populations than in the lake populations, but roach and pike had a higher number in river populations. Essex & Hunter (1926, cited by Oliveira, 1986) and Mishra and Chubb (1969) found that the fluvial systems have a lower percentage of infected fish than those in lakes. However, Griffith (1953) reported that the fish of streams and rivers have a higher percentage of infection compared to the same species of fish in lakes.

The composition of the parasite fauna as well as the parasite burdens on same species of fish inhabiting even similar flowing water systems were found to be

different and reasons seems to be the different fish populations (resident and migratory) sampled, the different size of the fish sampled, the physical differences of the systems and the differences in local conditions (eg. bottom texture) creating a difference in habitat diversity favourable for intermediate host (Amin, 1977; Muzzall, 1982).

Khalil (1969) investigated the parasitic fauna of the river Nile crossing Sudan and compared it with the fauna of the same host fish or related species of other African countries. He attributed the differences in the Sudanese helminth fauna to hydrobiological factors which may limit the transmission of the parasites and the distribution of intermediate hosts.

Petrushevski and Bauer (1948 cited by Dogiel 1964) in their study of the distribution of fish parasites in Siberian rivers, distinguish three types of parasites: the parasites occurring along the entire course of the river, the parasites occupying the middle and lower reaches and the parasites occupying the upper parts of the river. Amongst the parasites restricted to a particular region, those which require intermediate hosts predominated. More than half of the parasites which occupied habitats all along the river belonged to species which develop without an intermediate host. The difference in distribution of intermediate hosts was attributed to the fact that the two parts of the river belonged to two climatic zones.

Izyumova (1988) discussed in detail, with appropriate examples from the U.S.S.R., reservoirs and the factors affecting parasite populations in reservoirs, identifying water level and current, temperature, turbidity and shallowness, higher aquatic vegetation and ichthyophagous birds as the main components.

After impoundment of newly made reservoirs, the parasite fauna of the parent stream tends to change to that of the lake or sluggish river type (Hoffman & Bauer, 1971). The monitoring of the parasite fauna in the parent streams and, after impoundment, in the lake by Stolyarov (1954 & 1955 cited by Dogiel, 1964) revealed that the parasitic protozoans and monogeneans involving direct life cycles showed no

great change from their previous levels during the period of formation the reservoir. They occasionally showed an increase in infection levels, particularly in the area adjacent to the dam where the water flow is more static. Here the conditions of poor flow resemble the lake and thus the parasite fauna is more similar to the lake. Copepods numbers drop sharply during the first year, probably as a result of unfavourable conditions for their free swimming copepod stage. Parasites with planktonic copepod intermediate host and parasites with benthic invertebrate oligochaetes and benthic crustacea reappeared after one or two years. The reappearance was held for many years for the parasites involving mollusc intermediate hosts, due to the very slow population build up of the mollusc. Therefore, it seems that the parasitic faunal changes are closely associated with the hydrological and hydrobiological changes (occurring at the same time).

In the present study, the parasite fauna of the fish *E. suratensis* inhabiting two standing water-bodies, the reservoir and the lagoon was compared. The differences and/or similarities in the prevalence and abundance levels of the common parasites in the two localities would be able to explain with the stated background information on the ecology of the fish parasites.

(2) Effect of fish migration on the fish parasite fauna

Permanent emigration from the natural habitat

Two interesting examples of change of parasite fauna due to permanent emigration of the fish species from its original habitat to one which is unusual for the species are stated by Dogiel (1964). The freshwater fish, *Leuciscus brandti* (Dybovskii), has emigrated into the sea, to return to the areas in the vicinity in the river mouth only during the spawning season. In contrast, *Siniperca chautsi* (Basilevskii), a member of the marine family Serranidae, has become fully adapted to life in the river which it never leaves. It appeared that both species have taken some of their parasites with them into their new habitat and, on the other hand, had acquired a set of parasites typical of their new habitat, alien to them.

Petrushevski (1961) and Dogiel (1964) discuss in detail with examples the

effect of acclimatization of fish to new habitats as a result of human activities, on the original fish fauna. This, in general, results in complete or partial loss of the parasites they had in their original habitat. They may acquire new parasites with wide specificity from local fish. The spread of a parasite from, or to, the newly introduced fish may be responsible for epizootics in the less resistant host. Amongst the original parasites of fish, it is predominantly those with the direct mode of development (particularly monogenoidea) which survive the acclimatization (Dogiel, 1964).

Fish migration along a salinity gradient

Fish of the same species have very diverse fauna depending on the different habitats in which they occur during different stages of life. This is well exemplified by the marine fishes, eg. gadoid fishes, mullets etc. whose larval stages develop in brackish water show great differences in the parasite fauna between their young and adult stages (Polyanski, 1955 & Reshetnikova, 1955; cited by Dogiel, 1964). This is evident in anadromous and catadromous fish also, with the periodical migration of fish. As cited by Dogiel (1964), Dogiel and Petrushevski (1934, 1935) were able to show the loss of freshwater parasites by salmonids with the migration to sea and vice versa. The loss of the ectoparasites was followed by the gradual loss of the intestinal fauna; the reduction in the number of species and the intensity of infestation being directly related to the time spent by the fish in the new environment. Stenohalinity of ectoparasites makes them good indicators for the migrations of their hosts into waters of differing salinities.

Rockicki (1975) working on the parasitic fauna of Gdānski bay, in the Baltic sea, found that the parasitic fauna of the bay is of mixed characters and thus divided it into marine, estuarine and freshwater. The author adopted the following criteria; the freshwater species are those which, apart from brackish water, do not occur in marine fishes, whilst species which, apart from brackish water, are not found in freshwater fish species, are marine parasites. As commented by Rockicki (1975), the proposed classification of Bauer & Shulman (1948) to divide fish into the categories fresh, brackish and marine, depends on the extent to which the life cycles of classified parasites have been explored and thus is impracticable. The investigations of the

parasite fauna of migratory fish in this study show the change in the parasite fauna during the course of their passage through brackish water to fresh water.

The work of Oliveira (1986) on the endoparasitic fauna of fish inhabiting Rio da Guarda, a river complex in Brazil, shows the non existence of freshwater forms other than the larvae of *Eustrongylides* sp. and the trematode *Saccocoelioides beauforti* (Hunter & Thomas, 1961) Overstreet, 1971 in the estuarine area, and the absence of marine forms (other than the parasites of migratory mugilids) beyond the estuarine area.

The brackish water environment is harsh for the existence of stenohaline parasite species. Ectoparasites that enter brackish water on euryhaline fish are in direct contact with the variations in salinity. Egg stages, the miracidia and cercaria of digeneans, and first stage larva of nematodes represent free living stages which can be affected by salinity. In addition, salinity can be responsible for the absence (or presence) of stenohaline intermediate hosts leading to the differences in the species of parasite present when compared with the adjacent habitats.

Collins (1987) investigated the responses of three parasitic copepods common on *Mugil cephalus* L. to changes between fresh and brackish water habitats and found that the faunal variation of a habitat was largely dependent on the salinity of the habitat. As cited by Möllar (1978), Möllar (1974, 1975) found that none of the European freshwater species of parasitic crustaceans or hirudineans were able to survive in salinities between 7 and 20-25 ‰. The inability to tolerate low salinities by *Lepeophtheirus salmonis* (Krøyer, 1837) on spawning salmon in freshwater was reported by Berger (1970) cited by Kabata (1981). Walkey, Lewis & Dartnall (1970) report the inability to tolerate saline conditions by the freshwater copepod *Thersitina gasterostei* (Pagenstecher, 1861) parasitic on euryhaline sticklebacks.

Sundnes (1970) discovering the fact that the intestinal contents of *Lernaeocera branchialis* (L.) are nearly isosmotic with sea water suggested that the copepod did not have any osmoregulatory ability and the parasite must be restricted in its

distribution to the part of the host range in which its osmotic needs can be met. On the other hand, a high range of salinity tolerance by both *Lernaea cyprinaceae* L. and *L. branchialis* was reported by Shields & Sperber (1974) and Panikkar & Sprotson (1941) respectively, possibly because a high proportion of the parasite is embedded within host tissue.

Möllar (1978) conducted experiments to find out the tolerance ranges of some marine and freshwater forms. The free living stages of the marine, endoparasitic *Cryptocotyle lingua* (Crepl., 1825) Fischoeder, 1903 and *Podocotyle atomon* (Rud., 1802) Odhner, 1950 were affected only at salinities less than 4‰. *C. lingua* metacercaria was less common in brackish water. In *Hysterothylacium* (=*Contracaecum*) *aduncum* (Rudolphi, 1802) the time of survival of larvae within the egg was reduced in fresh water. The salinity range of 0-32‰ had no effect on the survival of *Anisakis* larvae in-vitro. Unlike the endoparasites, the ectoparasitic crustaceans were more sensitive to salinity changes; the marine ectoparasitic copepods *Acanthochondria depressa* Scott, 1905 and *Lepeophtheirus pectoralis* (Müller, 1776) being three times more tolerant of 16‰ than the freshwater forms, *Piscicola geometra* L., and *Argulus foliaceus* L.

Möller (1978) showed that the intestinal parasites *H. aduncum* and *Echinorhynchus gadi* (Zoega in Müller, 1776) (Acanthocephala), were not able to stabilise their water balance when the osmolarity of the surrounding medium changed. It was also found that the osmolarity of water ingested by fish changed in the stomach to an average value (about 300 m osmol or 10.5 ‰ salinity) in spite of the external salinity, to make the osmotic pressure of the intestinal contents of a fish constant (Möller, 1978). Therefore, those parasites which are adapted to isosmotic conditions cannot stabilise their water balance when osmolarity of the surrounding medium changes.

As was shown by Möllar (1978), a reduction in water temperature increased the salinity tolerance of parasite larvae as well as of adult parasites.

(3) Seasonal variations of parasitic infections

Seasonal changes of nature are very sharply reflected in organic life. Therefore, even the parasites which have no direct ties with the external environment should not remain indifferent to annual cycle of climatic changes as it is not only the host itself that provides the environment for parasites, but also the external environment of the host.

Factors determining the seasonal changes are many and varied and they operate through the seasonal changes of the environmental conditions and in the type of feeding, as well as in the details of the life-cycles of the parasites as well as host. The most significant factor for the temperate zone seasonal patterns was thought to be water temperature variation (Aho, Camp & Esch, 1982; Camp, Aho & Esch, 1982; Esch, 1983; Granath & Esch, 1983a; Kennedy, 1977). The other factors considered to be important were the dietary and social host behaviour (Anderson, 1976; Kennedy, 1977; Smith, 1973) and parasite population density (Esch, 1983; Granath & Esch, 1983b; Holmes, Hobbs & Leong, 1977).

Information on seasonal fluctuations in the temperate zone;

It is shown that the parasites in the temperate zone are under the influence of seasonal changes of water temperature, the variation of which may be up to 20°C or even more. Temperature in these conditions is the chief factor causing seasonal cycling of parasites. All the other factors are normally secondary to temperature, although in certain conditions and seasons they may assume an importance equal to, but not eliminating the effect of temperature. Although there are different patterns, when the parasites species of a particular parasite group with similar life-cycle patterns are considered, the general trend of the seasonal biology of these groups can be summarised as follows. Some groups are however very difficult to summarize due to the complexity of their life-cycle patterns and the different type of abiotic and biotic factors acting on each of them.

Protozoans have a year round occurrence on their host, with or without exhibiting seasonal fluctuations. Being parasites with direct life cycles, they develop

extensively under crowded culture conditions. In addition, their infection levels increase during the periods when the ambient temperature is well suited for their reproduction. Thus, if there are (or are not) any fluctuations in infection levels, it is mainly due to the balance between these two factors.

Monogeneans in the temperate zone mostly undergo cyclic changes in incidence and abundance. Most of them die off during winter and others remain inactive. In spring, eggs hatch out or the overwintering adults produce eggs. With the increase of temperature each generation takes a shorter time to reach maturity, increasing the population rapidly. Therefore, the temperature is the major factor in determination of seasonal patterns of occurrence of the Monogenoidea (Chubb, 1977). The tolerance limits of some monogenean species differs from the common range or sometimes the degree of the aggregation of hosts vary from time to time leading to a change in the general seasonal pattern observed (Izyumova, 1953 cited by Dogiel 1964; Rawson & Rogers, 1972).

The metacercariae as well as adult digeneans parasitize fish. The metacercariae tend to accumulate in fish (if their survival time is long) whilst the adults are in a dynamic situation of parasite establishment and loss due to death. There is a minimum temperature below which the eggs will not hatch, development of the larvae in the snail host will not occur, cercaria will not be released or invade the fish host, and at which the formation of metacercariae within the fish will cease (Chubb, 1977).

According to Chubb (1980), cestode and nematode larvae are present in fish at all seasons, although the level of establishment can vary from season to season. The development of eggs liberated by the adults are controlled by the prevailing water temperature. Therefore, the eggs liberated at all times of the year retain their viability until the rapid rise in temperature in spring. Hatched larvae then get ingested by the invertebrate intermediate host. Development in the invertebrate host, the ingestion of them by the fish and the growth of larvae in fish tissue are facilitated at this time owing to warm water conditions. The season of invasion to fish host appears to be during the summer. In milder climatic zones, invasions can also continue over the

winter, but even then peak invasions occur during the warmer summer months.

There is a range of seasonal patterns of incidence and intensity exhibited by adult digeneans in freshwater habitats. Different parameters apply at each stage in the life cycle and temperature can influence them in different ways (Chubb, 1979). Seasonal maturation of adult worms, seasonal occurrence of first and/or second intermediate host, seasonal pattern of development and occurrence of metacercariae and difference in annual feeding habit of fish may contribute to these variations. Owing to this great variation in the patterns, Chubb (1979) using the seasonal calender for the life cycle of the trematode *Crepidostomum metoecus* (Braun, 1900) showed the difficulty in producing a simple hypothesis that will be applicable to all, or to identify some of the factors involved.

As stated by Chubb (1982), almost all the adult cestodes, nematodes and acanthocephalans have, at the longest, an annual turnover of occurrence in their host. There is usually no increase in the numbers of worms in the intestines to a maximum at any time of the year, therefore a dynamic equilibrium exists at all seasons between gain and loss of worms. The levels and periodicity of infection of the intermediate hosts, their availability to the definitive fish host, the feeding behaviour and migrations of the fish and the success of the parasite larvae in establishing, play a part in determining the ultimate seasonal biology of the parasite. Many larvae are available at all the times of the year, but this does not necessarily mean that the parasite is in an invasive condition. The annual changes in temperature seem to influence parasite recruitment owing to the changes in host feeding activity, effects on establishment in the host and rejection of already established parasites in the host. The annual changes of photoperiod seem to have a secondary effect influencing the invertebrate and vertebrate host.

The nonexistence of a seasonal occurrence of adult or larvae in the fish host does not necessarily mean the absence of any effect of temperature on any stage of parasite.

Some of the seasonal variations of parasite biology eg. seasonal maturation, are coincident with seasonal changes of temperature, and temperature may not have a direct cause but an indirect one mediated through some other way probably with other changes in host biology. Therefore, the direct causal influence remains to be shown by experiments in many instances (Chubb, 1988). The accumulated literature on the trematode *Bunodera luciopercae* (Müller, 1776) by Chubb (1988) shows the influence of water temperature on most of the biological processes such as hatching of the eggs, the development of sporocysts and rediae, the development of cercariae, the development of the metacercariae, and the establishment of the juvenile trematodes and accumulation of eggs and finally the release of worms at the end of their life span. However, the maturation of their gonads which occur during winter months are influenced by the hormones of the host (Forbes, Ensor & Chubb, 1989), the host is directly influenced by temperature but not the parasite.

As cited by Kabata (1981), Kuperman & Shulman (1972, 1977) experimentally showed the effect of seasons on the development of the egg sacs of *Ergasilus sieboldi* Nordmann, 1832. The level of ambient temperature before experimental transfer at equal photoperiod had influenced the accumulation of eggs in the ovary in the experiment done in spring and there was no egg accumulation, and therefore, empty ovaries in the experiment done in the autumn. The importance of photoperiod was then considered, a low percentage of egg sac formation had occurred in late summer and autumn compared to that in the spring and early summer.

Seasonal fluctuations in tropical zone

Information on seasonal fluctuations in tropical zones of the world is very limited (Madhavi, 1979; Chubb, 1988). Of the work published on seasonal occurrence of fish parasitic helminths only 4% come from tropics (Chubb, 1988). Of the few studies available, it is evident that seasonal variations occur in the tropics in spite of the absence of temperature as a triggering factor. It seems the factors such as seasonal rainfall, peak prevalence of intermediate host, behavioral changes of host etc. are in operation from the little information available.

The importance of protozoan diseases amongst the other fish diseases in the tropics in Southeast Asia is embodied in Arthur (1987). *Ichthyophthirius multifiliis* Foquet 1876, *Trichodina* sp. and *Ichthyobodo* sp. cause problems on fry in hatcheries and grow out ponds. Outbreaks of ichthyophthiriasis have tended to occur during the rainy seasons at the end of each year, around December-January in *Puntius gonionotus* (Valenciennes, 1842) in one breeding station in Malaysia (Wong & Leong, 1987). In Thailand, trichodiniasis was reported to increase during the rainy season (Tonguthai & Chinabut, 1987).

The work on the trematode, *Allocreadium fasciatusi* Kakaji 1969 in the tropics showed that the water temperature did not play an important role in the seasonal biology (Madhavi, 1979). Although recruitment, occurrence and maturation of the parasite occurred all year round, a seasonal periodicity of recruitment, growth, maturation and egg release occurred due to the peak prevalence of the copepod second intermediate host in September.

Bauer & Karimov (1990), working in a water body in which the temperature was constant during the whole year at 16°C, demonstrated that temperature does not play the chief role in seasonality as it does in temperate water bodies. In the group of parasites which infect fishes directly, the fluctuation in occurrence was minimal. In the second group in which the host was infected via the intermediate host taken as fish food, the occurrence increased when the fishes ate more food, ie. during the pre-spawning period.

Furtado & Tan (1973) investigating the incidence of infestations of two caryophyllid cestodes, *Lytocestus parvulus* Furtado, 1963 and *Lytocestus lativitellarium* Furtado & Tan 1973, and two camallanids, *Procamallanus clarias* Ali, 1956 and *Procamallanus parvulus* Furtado & Tan, 1973, attributed the variation in seasonal occurrence to the abundance of intermediate hosts in the diet during the peak periods. As the peaks coincided with the monsoon season of the year, the effect of season on the abundance of the intermediate host was also considered.

Zaman & Leong (1987) found a seasonal variation in the occurrence of the caryophyllid cestode, *L. lativitellarium* in the catfish *Clarias macrocephalus* (Gunther) in Malaysia, with high peaks around September. This was caused by the recruitment of parasites during the major spawning period of catfish, in May-June, which coincides with the rainy season.

Leong (1986) investigating the seasonal occurrence of *Dactylogyrus* spp., *Bothriocephalus acheilognathi* Yamaguti, 1934 and *Lamproglena minuta* Capart, 1943 in *Puntius binotatus* (Valenciennes, 1842) in Malaysia, found the peaks in prevalence and mean intensity, to occur in the wet rainy season except for the cestode. The peaks for the copepod intermediate host of *L. minuta* had occurred during the early part of the wet season, when there is a close proximity due to the congregations of fish in pools prior to spawning in November and December. However, this close proximity has not been sufficient to explain the observed seasonal trend in *Dactylogyrus* sp. Although the conditions for *B. acheilognathi* had been optimal throughout the year, their disappearance from March to September, could be due to many factors, such as change of diet of host, temperature and/or decrease in abundance of intermediate host.

It is clear from above mentioned studies that seasonal variations in fish parasites are not confined to the temperate climates. Due to the lack of data on seasonal occurrence in the tropics and the incomplete nature of the information in the few investigations which have been carried out, Chubb (1977, 1979, 1980, 1982 & 1988) has frequently pointed out the importance of future studies in this field in the tropics.

2.1.2. Micro-habitat effects on the parasite species

(1) Host species

Specificity is an evolutionary and ecologically conditioned phenomenon (Shulman, 1958). The physiological living conditions in or on a host play a major part in the adaptations of a parasite to it. Not less important are the ecological conditions under which the host lives, the dispersal method of the parasite and its behaviour, since these determine the possibility of contact with the parasite. A number of other

factors which make possible the completion of the life cycle are also important. Only when these requirements are met it is possible for a stable host-parasite system to exist.

Shulman (1958) grouped the parasites according to the degree of specificity a parasite has, as follows:

1. specific to a single species of fish
2. specific to several hosts of same genus
3. specific to a group of hosts belonging to related genera or to an entire family
4. specific to a historically combined faunistic complex
5. specific to ecologically related hosts.

According to Bychowsky (1957) the majority of Monogenoidea (74.1%) occur on one host only and most of the others in the fish belonging to the same genera. Rohde (1978, 1979) showed that marine monogenea exhibit a high degree of host-specificity and are restricted to a single family or a genus, and in some cases to a single species of host. Monogenea having direct life cycles, and therefore produce few eggs; the high degree of host and site specificity facilitates mating for their propagation (Rohde, 1978). The work of Bashirullah & Rado (1987) also agrees with this concept of the high specificity of monogeneans.

The degree of specificity of helminths may differ at various stages of its developmental cycle. At the stages during which relatively little development takes place (e.g. metacercariae of trematodes, encysting larvae of nematodes) as a rule parasites are loosely specific and have a wide range of host (Shulman, 1958).

The loosening of the bonds of specificity maybe associated with the influence of the external environment on one of the links of the host parasite system (Shulman, 1958). In some of these cases it may be due to the resultant improvement in the chances of infection if a large number of suitable hosts in the waters are involved. In yet other cases, this may related to the potential for the depression of the defensive immunological mechanisms, facilitating survival and development of parasites.

Bauer (1948 cited by Dogiel 1964) and Kennedy (1975) showed that the fish with related origins but with different food habits, one a pelagic form feeding on plankton and the other a bottom living benthos feeder, have a very different intestinal fauna composition but very similar ectoparasites. In contrast, unrelated host species having analogous living conditions and the same diet may tend to have a similar parasite fauna. Rohde (1979) showed this tendency even amongst monogeneans usually regarded as 'phylogenetically highly specific'.

Narrow specificity reduces the distribution of the parasite by restricting its host range, and may also decrease its intensities, since it may suffer mortality or reduced fertility in unsuitable hosts. Such instances may, however, be overcome by those features in the life cycle and the behaviour of the free-living infective stages which serve to increase the probability of contact (Kennedy, 1975).

(2) Age/size of fish

Gorbunova (1936), as quoted by Dogiel (1964), divided the fish parasite fauna into 3 groups according to influence of host age:

1. parasites independent for their incidence on the age of the host,
2. parasites decreasing in abundance with the age of the host- young feed mainly on plankton, and
3. parasites increasing in abundance with the age of the host, both prevalence and intensity may increase when parasites accumulate.

Most of the literature, Frankland (1955), Paling (1965), Hanek & Fernando (1978d, 1978e) and Buchmann (1989), provide evidence of a positive correlation between host size and monogenean parasitization level. The size of the surface area available for settling, the larger body surface area for the attachment of the invasive stage or the increased volume of water passing over the gills would increase the probability of host finding resulting in higher levels of infections in larger fish. However, Llewellyn (1962) showed that *Gastrocotyle trachuri* Ben & Hesse, 1863 and *Pseudaxine trachuri* Per. & Per., 1890 are more common on young fish, much less frequent on 2- and 3-year-old fish (*Trachurus trahcurus* L.). Paperna (1963a), Prost

(1963) and Molńar (1971) focused on the importance of the development of the gills of carp fry to a certain extent for them to be susceptible to monogenean infections. The gills should be of more than a minimum size for their haptors to be satisfactorily anchored between the secondary filaments.

Metacercariae, of the larvae of cestodes and nematodes frequently survive in the body of fish for a long time and, sometimes, even for the remainder of the life of the fish (Chubb, 1979; 1980). Because of the accumulation of these in the older fish they tend to have more larvae than younger fish. The work by Pennycuick (1971) on a *Diplostomum* metacercaria and the plerocercoid stage of a cestode illustrates this tendency. Where the reverse is found i.e. the greater intensity of infection in small young fish compared to older fish, may be explained by the probability that small fish feed more on the intermediate host eg. as was found for *Schistocephalus solidus* (Müller, 1776) in three spined sticklebacks, which feed more on copepod *Cyclops* in their young stage than do older fish.

The majority of adult endoparasitic helminths living in the alimentary canal tend to have an annual turnover of occurrence in their host (Chubb, 1979; 1982). Since there is no accumulation, the higher occurrence of them in adult fish compared to young fish (Pennycuick, 1971; Furtado & Tan, 1974) may be due to their having a higher proportion of food or to a gradual change in food habit such that they consume more of the organism which is the source of infection.

Greater prevalence and/or intensity of infection on larger (or older) hosts has been reported for a variety of natural parasitic copepod-fish associations (Cloutman & Becker, 1977; Bortone, Bradley & Oglesby, 1978, Hanek & Fernando, 1978d, 1978e). The findings of Poulin, Curtis & Rau (1991) indicated that the probability of the parasites contacting a host is correlated with surface area which leads to the higher infections of ectoparasitic copepods in larger fish.

Many authors (Chappell, 1969; Pennycuick, 1971; Furtado & Tan, 1973; Cloutman & Becker, 1977; Hanek & Fernando, 1978d, 1978e) found no significant

difference in incidence and intensity between male and female fish. Others found that females were less heavily infected with parasites than males (Paling, 1965; Batra, 1984), probably due to the presence of oestrogen (Thomas, 1964). During breeding, this trend tends to be reversed when oestrogen levels are lower (Thomas, 1964). In addition to this higher physiological resistance of females, the males being larger, presumably eat more food and are thus likely to ingest more infected intermediate host (Thomas, 1964; Batra, 1984).

(3) The site of infection

The microhabitat is the habitat the parasite species occupies within or on its host. The survival of a parasite in an infection site primarily depends on the satisfaction of its nutritional requirements and an harmonious physical and chemical environment. In addition, it must be able to overcome the defense mechanisms exerted by the host and the competition by other organisms occupying the same niche.

Environmental effects

Llewellyn (1956) reported that the site selection of polyopisthocotyleans is determined by the need to avoid the effect of the gill ventilating current. The existence of a higher affinity for certain regions of the gill in them has been reported by many; Frankland (1955), Llewellyn (1956), Llewellyn & Owen (1960), Owen (1963) and Suydam (1971). Wootten (1974), Shaharom-Harrison (1984) and Hanek & Fernando (1978a, 1978b, 1978c) all report the site selection of dactylogyrids. Rhode (1979), evaluating the intrinsic and extrinsic factors responsible for niche restriction, showed that the intrinsic factors can be more important than the external factors; an important factor responsible for niche restriction in monogeneans is site selection to increase intraspecific contact and thus mating.

Most copepods are known to favour specific sites on their host fishes. Copepods which are semi-mobile on the body surface of the host tend to congregate in areas that provide shelter and food compatible with their needs (Kabata, 1981). Hanek & Fernando (1978a, 1978c) found that *Ergasilus centrarchidarum* Wright 1882 was randomly distributed on the gills of *Lepomis gibbosus* (L.), showing no preference

for any special part, but was much more selective on the gills of *Ambloplites rupestris* (Raf.), the selectivity being to different areas of arches but no difference between arches.

Interspecific effects

Holmes (1973) reported that similar or closely related species of parasites in the same habitat either compete, tending towards exclusion of the inferior, or interact, leading to niche diversification. Pielou (1975) reports that species with similar requirements can coexist successfully provided none of their population is resource-limited.

There is a considerable body of literature on interspecific effects on parasites, some finding that parasite species co-exist in micro-habitats, whilst others report competition in concurrent infections in the same micro-habitat. Bashirullah & Rado (1987) report the co-occurrence of three species of the polyopisthocotylean *Choricotyle* in the grunt *Orthopristis ruber* (Cuvier) with no evidence of antagonism. Paperna (1964b) reports the competitive exclusion of one species *Dactylogyrus* by another. Yet co-occurrence of four species of *Dactylogyrus* and their preference to same areas of the gill complex were reported by Dzika & Szymański (1989). Fryer (1968) found site preference to occur in *Ergasilus flaccidus* Fryer, 1965, *E. latus* (Fryer, 1960) and *E. kandti* (Van Douwe, 1912) areas within the arches and among the arches of the same fish.

(4) The host response

Despite the behaviour and the nature of a host, perhaps the most important factor influencing host and site selection is the nature of the response employed by the host employs against the parasite. It is often the effect of the parasite and the effects of the host response on host's survival (eg. skin damage leading to osmotic imbalance, gill hyperplasia leading to suffocation) which is the most important aspect for fish culture rather than the effects on the parasite.

The host response can be an acute or chronic inflammatory response and/or the

delayed cell mediated response. The response by inflammation is a more widely studied area than the cell mediated response, and this provides sufficient clues to the pathogenicity of a parasite to its economically important host. There is little information on the development of the immune response to parasites, but this generally seems of less importance than cellular responses in effective elimination of the parasite or restricting tissue damage.

The infection levels of a parasite can sometimes be misleading when estimating the potential pathogenicity of a parasite as there are some parasites which elicit very harmful effects even at low levels. However, generally, the higher the number of parasites, the greater the effect. The tissue invading parasites are more pathogenic than intestine inhabiting parasites. Therefore, the study of the histopathology in this study was mainly concentrated on the common histozoic parasites.

Gill monogeneans seem to elicit varying degrees of tissue response which often appear to be species specific rather than broadly characteristic of a particular parasite group. It may result in a tissue response ranging from no reaction to severe inflammation (Cosgrove, 1975; Roubal, 1986). Some monogeneans, *Dactylogyrus vastator* Nybelin, 1924 (Paperna, 1964a), *Dactylogyrus lamellatus* Achmerov, 1952 (Molnár, 1972), seem to cause severe damage, inducing haemorrhages, marked hyperplasia of the gill epithelium and goblet cells and copious mucus secretion which ultimately develops into a severe gill pathology which may in turn kill the host. On the other hand, for some other monogeneans eg. small diplectinids *Lamellodiscus acanthopagari* Roubal, 1981 and *Lamellodiscus squamosus* Roubal, 1981 (Roubal, 1986), only a slight hyperplastic response was reported.

The pathology of copepods is generally localized if they are sedentary. The localized tissue reaction occurs around the point of attachment and near the site of feeding. Kabata (1984), Paperna & Zwerner (1984) and Roubal (1989) described the hyperplastic response associated with copepod parasites and reported the occasional fusion of neighbouring gill filaments. Roubal (1989) further reported the presence of lymphocytes as well as leucocytes in the constricted blood vessels. Neuhaus (1929

cited by Kabata 1970) reported the loss of mucous cells in the so-called capping tissue (on the inner sides of primary lamellae), occlusion of afferent blood vessels in some cases, hypertrophy of gill tissue and subsequent formation of swellings and fusion with the neighbouring filaments.

A fibrous layer of host origin delimiting the encysting parasite is the commonly reported histopathological change for encysted larvae of helminths. The encysted larvae directly damage the tissue in the migratory path and encyst in an organ thus occupying the space (Kennedy & Lie, 1976). The pathogenicity of encysting larvae depends on the tissue in which they are present, the extensiveness of the infection, the size of the encysted larval form and the parasite species. Many (Mitchell, Smith & Hoffman, 1982; Mitchell, Ginal & Bailay, 1983; Rosen & Dick, 1983; Weiland & Meyers, 1989) reported degenerative changes in surrounding tissue such as pressure atrophy, ascites, visceral adhesions, abdominal distension etc. in severe cases with encysting larvae.

The inflammatory process leading to encapsulation is common even for metacercariae (MaQueen, Mackenzie, Roberts & Young, 1973; Sommerville, 1981), or cestode larvae (Arme & Owen, 1970; Hoole & Arme, 1982; Joy & Madan, 1989; Sharp, Pike & Secombes, 1989) or nematode larvae (Elarifi, 1982). A sequential study of the involvement of inflammatory cells in the host response was carried out by Sommerville (1981) on the encystment of the metacercaria of *Stephanochasmus baccatus* (Nicoll, 1907) infecting flat fish. The cells first involve in the response were the macrophages. The description of histopathology of the encysting larva of the nematode *Pseudoterranova decipiens* (Krabbe, 1878) (Ascaridoidea) by Ramakrishna & Burt (1991) exhibited the same pattern.

2.1.3 Objectives of the Present study

In this study, ecological aspects of the parasites of *Etroplus suratensis* was investigated in attempt to understand the environmental factors governing their population levels to make it possible to forecast the possible fluctuations on fisheries and aquaculture.

The parasite population levels were monitored for a year in two localities, one representing their natural brackish water lagoon habitat, the Koggala lagoon, and the other a reservoir to which they had been introduced and were established.

The parasites found in the survey were identified as far as possible. The larval forms were used to experimentally infect a laboratory host to try to obtain the mature parasite needed for the identification. An attempt was made to find the intermediate host wherever possible.

The effect of the introduction of the fish to a completely new freshwater habitat from a brackish water habitat on its parasite fauna and their population levels were investigated with the pooled year round data on infection levels. The fluctuations in the samples within the year in both localities were analyzed and related to fluctuations in the seasonal environmental parameters.

An attempt was made to understand the host factors governing the parasite infection levels such as (i) host specificity (ii) the host age/size (iii) the site specificity and (iv) the degree of host response to the parasite's presence.

2.2 Materials and Methods

2.2.1. Parasitic survey of *E. suratensis* and *Oreochromis* species

2.2.1.1. Sampling Procedure

Live *Etroplus suratensis* from Koggala Lagoon were obtained from fishermen using baited (roasted coconut meal in wheat flour paste) trap cages in the sublittoral region of the lagoon. It was impossible to obtain live fish from fishermen of Udawalawa reservoir as the fish they land were moribund and died during transportation. Therefore, this was acquired with the help of the labourers of Udawalawa Fresh-water Fisheries Station Cage Culture Site. The fish were caught and left in cages until they could be collected. This was unsuccessful as fish died in the course of leaving them in the cages, resulting in very small number of fish in a sample. Then the fishing was done a day prior to collection. Subsequently after the closure of Fisheries Station (31.10.89), this was continued by two fisherman fishing in the same previous site and extreme care was taken to reduce deaths. Fish were sampled from one locality in alternative months.

Fish were brought to the laboratory at University of Ruhuna in oxygenated polythene bags and left in the aquarium of the Zoology Department Animal House in de-chlorinated tap water until the examination. Koggala lagoon fish were left in water with same salinity as the lagoon. The appropriate salinity was attained by mixing sea water with de-chlorinated tap water. Fish were kept in 200 l tanks in the density of 20 fish per tank. Aeration was supplied almost all the time with an aerator. Tanks were cleaned by siphoning and 1/3 of water exchanged every day. When disease outbreaks occurred, more water was changed or fish were transferred to new tanks to minimize deaths. No chemical treatments were given. Feeding was done with a fish feed or the *Hydrilla* according to their preference; *Hydrilla* was most preferred.

Two tilapia samples (one *Oreochromis mossambicus* sample from Koggala and one *Oreochromis niloticus* sample from Udawalawa) from each locality and one *E. suratensis* sample from Bolgoda lagoon were collected in a similar manner.

The sampling months, the number of fish examined in the samples, the mean lengths and weights of the fish in the samples were as follows:

Udawalawa reservoir

Month	Number of fish	Mean length ± SD	Mean weight ± SD
April	11	9.4 ± 1.4	39.7 ± 19.5
June	8	8.6 ± 1.8	33.6 ± 19.3
August	11	8.1 ± 1.5	31.1 ± 19.6
October	13	8.4 ± 2.6	37.0 ± 38.9
December	17	9.4 ± 2.5	43.6 ± 39.8
February	18	8.7 ± 2.8	37.0 ± 47.4

Koggala lagoon

Month	Number of fish	Mean length ± SD	Mean weight ± SD
May	10	9.9 ± 1.6	45.0 ± 21.8
July	12	9.9 ± 1.8	44.6 ± 24.6
November	20	8.8 ± 1.5	33.4 ± 16.9
January	16	9.8 ± 1.8	42.9 ± 23.0
March	20	9.2 ± 1.5	31.6 ± 19.6
May	16	9.0 ± 2.0	38.6 ± 26.8

2.2.1.2. Parasitic Survey

The protocol used for examination and quantification of parasites is given below, and the quantifying methods are summarized in the Table 2.15.

In a day, one to two fish were decapitated for survey. They were measured for standard lengths and weights. At the time of decapitation, blood was collected using a Pasture pipette, placed a drop on a slide and covered with a cover slip. The slide was left covered in a moistured petri-dish until examination.

Skin

The entire surface of fish was observed with a hand lens of magnification x10 to examine the macro-parasites attached, mainly the leeches and the large crustaceans. The number of each was counted per fish. For submicroscopic parasitic examinations scrapes were taken from five 1 cm^2 areas as follow;

head
dorsum above operculum
dorsum just posterior to mid-body
ventrum above rectum and
caudal fin.

Scrapings from both sides were taken on to a slide (each side separately) and observed under x100 magnification. The number of parasites present in the smears were counted. When flagellates were present in high numbers and obstructed counting by their movement approximations were done. As there were not much difference between the counts of two sides of body collective numbers were subjected to analysis.

Blood

Blood parasites in three microscopic fields of magnification x100 (objective x10; Olympus IMT2 microscope) was counted.

Gills

The total number of ergasilids present on all the arches of one side was counted under stereo microscope.

Since there were two species of gill monogeneans the opisthohaptor had to be seen in order to distinguish the species for counting purposes. Thus, the second gill arch of one side was placed in a small petri-dish (5 cm diameter) with autoclaved aquarium water and left aside at room temperature (28-31°c) for five hours. During this period the gill monogeneans detached themselves from the dying tissue and lay on the bottom of the dish. This petri-dish was kept on the microscope stage (Olympus IMT2 inverted microscope) and the numbers counted under x100 power.

Internal organs

a. abdominal cavity

An incision was made along the ventrum from the vent to the head. The abdominal wall was removed to expose the viscera. The alimentary canal and associated organs were removed by cutting across the oesophagus and around the anus, and placed in a petri-dish with physiological saline. The abdominal cavity, the surfaces of the excised alimentary canal and associate organs were examined carefully. The total numbers of different parasites present on the mesentery and organs were counted. Live and dead metacercarial cysts and cysticerci were identified under compound microscope and counted separately. As the fat tissue obscured the view of transparent nematodes, the gut was laid out with adhering mesentery and slightly pressed between two glasses and observed under x40 magnification of the inverted microscope.

b. alimentary canal and associate organs

Squash preparations of entire liver, gall bladder and spleen was made and checked for parasites. The alimentary canal was divided into oesophagus, stomach, fore-, mid- and hind-intestine, and rectum. The muscular rectum was first separated from the tubular portion and the rest was divided into three equal fore-, mid- and hind- intestinal portions. Pieces were placed on a glass, slit-open and the contents scraped out to a side and thinly spreaded. It was then covered with another glass and the contents as well as the gut wall were examined under x40 magnification. The number of different parasites present were counted.

c. other internal organs

Entire organs of heart, kidney, gonads, urinary bladder and swim bladder were dissected out and squash preparations were observed for any parasites. The eyes were dissected out, the lense, humor and the retina were examined separately. The cranial cavity was opened and smears of entire brain tissue were examined.

d. muscle

The skin was removed and muscle fillets of the entire fish was taken out. The

muscle was little by little pressed in between two glass slides and the total number of parasites present was counted.

N.B. Until the removal of gills, the surface of the fish was kept wet (left in a water bowl) and afterwards it was kept under refrigeration until examination.

2.2.1.3. Preservation and description of parasites

Protozoans

These parasites were observed in wet mounts and identified up to the generic level. Where the species identification was required, the smears made were not useful as practically it was difficult to obtain adequate number of parasites, due to their very low infestation levels.

Monogeneans

Freshly removed monogeneans were fixed in glacial acetic acid and stored in 5% formalin. The 5% formalin was unsatisfactory for fixation as this caused the worm to contract and curl, making it difficult to use them for taxonomy study. Later the worms were mounted in Malmberg's fluid (1970) for measurements. As the worms were stored in 5% formalin and this makes the tissues hard, it was difficult to flatten the stomach monogeneans properly for measurements of hard parts. The cover slips were therefore pressed destroying the internal organs to flatten the hard chitinous parts. However, in the case of small gill monogeneans with very thin tegument the mounting with Malmberg's fluid was not satisfactory to see the internal organs. Staining with Mayer's paracarmine was not satisfactory too.

Digeneans

a. metacercariae

First the cysts containing metacercariae were isolated from the tissue mechanically. The dimensions of the cysts mounted in saline were measured under slight coverslip pressure. Then the metacercariae were removed mechanically. The metacercariae with thin walls were liberated when a slight pressure was applied on the

coverslip. In the instances where this was impossible, when the metacercariae were having thick walls, cysts were placed in cavity blocks with saline and opened using surgical needles with the aid of a stereo microscope.

The mounting of the liberated live metacercariae was done as follows; the living specimens were placed on a cover slip by the aid of a capillary pipette with a drop of saline. The ventral side was turned towards the glass and left until it adhered on to the glass. Then the cover slip was adhered on to a slide, making the slide to contact with the saline drop. The slide with the specimen was inverted and the excess water was removed using a filter paper and when the metacercariae was pressed enough to cease its movement, the cover slip was sealed with paraffin wax and observed under the microscope. These live specimens were used to make the diagrams of excretory systems and observe the glands and their ducts. Whenever it was required to leave the live metacercariae, it was done by leaving with their cysts in saline under refrigeration.

The liberated metacercariae were then fixed in glacial acetic acid under slight cover glass pressure, added few drops of alcohol followed by glycerin. Then the measurements were taken. When glycerin was not giving a good contrast, the fixed specimens were stored in 80% ethanol and later, stained with Mayer's paracarmine.

b. adults

Fixed in glacial acetic acid with cover slip pressure to flatten them where necessary. The specimens were stained with Mayer's paracarmine and used for diagrams and measurements. When it was difficult to identify the structures, serial sections were made using unflattened specimens fixed in glacial acetic acid and stained in Haematoxylin and Eosin.

Cestode larvae

The measurements of the capsules were taken with live worms under slight coverslip pressure. Diagrams were made with live specimens, prepared similarly as in the case of metacercaria. The ,mechanically liberated larvae were fixed in glacial

acetic acid and stored in 80% ethanol. The measurements were taken with the fixed specimens mounted on glycerin.

Nematodes

Specimens were fixed in glacial acetic acid and stored in 80% alcohol. Measurements were taken with these fixed material mounted on glycerin.

Crustaceans

Fixed directly in 80% ethanol. Temporary mounts were made with Lactophenol and observed without applying a coverslip. When the detailed structures were required to observe under high magnification, a cover slip was applied.

All specimens were measured with a calibrated ocular. The measurements in all the descriptions are given in micrometers, as means with ranges in parentheses. All drawings were made with the aid of a drawing tube (Olympus).

2.2.2. Life cycle studies

2.2.2.1. Life cycle studies of digeneans

(a) Mollusc intermediate host

A search was made for the mollusc intermediate hosts of the metacercariae found in the fish examined. Infection of fish from the emitting cercaria can be used for obtaining large numbers of metacercariae for identification and further experimental infections of the final hosts.

At a time, approximately 1-3 hundreds of all types of gastropods found were collected from both types of habitats. Molluscs along the margins and in shallow water bodies were collected using nets attached to a triangular or round frames and the frame to long wooden broom handles. The pore size of the nets was large enough to drain most debris of the mud, but retained even very small molluscs. The molluscs collected were transported in wide-mouth plastic bottles together with water and vegetation. In the laboratory they were placed individually in vials filled with pond water or 8 ‰ saline water (mixed pond water and sea water), according to the habitat.

The water used was collected from ponds which were free from mollusc. The isolated molluscs were stimulated with cold water as well as bright light to release cercariae.

(b) infection of fish host

When any cercarial release was observed, the corresponding mollusc was left with 2-3 parasite-free tilapia, *Oreochromis niloticus*, fry reared in the University of Ruhuna, Zoology department fish ponds. After approximately 1-2 weeks, these fish were screened for metacercariae. *O. niloticus* was used as the experimental host mainly due to its availability, its being a cichlid and thus closely related to *Etroplus*. When the metacercariae recovered from the experimental infections of *O. niloticus* were similar to any of the metacercariae parasitizing *Etroplus*, these were then infected to *Etroplus* from the locality where the parasite was naturally absent. The metacercariae developed in these experimental infections of *Etroplus* were then recovered and measured in order to confirm that they were the same as the natural infection, by comparing with the measurements of the naturally infected parasites. Some metacercariae recovered experimentally were used for final host infection studies.

(c) Infection of final hosts

This was carried out mainly for the purpose of obtaining adults for identification. Laboratory rats and chicks were used as final hosts. Rats bred in the University of Ruhuna, Zoology department Animal House were readily available. When required, chicks were obtained by incubating eggs of the common domestic hen at 38°C. Percentage hatching was between 20-30 %. These rats and chicks were fed with the commercial feed available for egg laying hens.

Metacercarial cysts and larval *Contracaecum* nematodes with adhering tissue were collected from fish. These parasites were collected in physiological saline and the number collected was counted before feeding.

These larval stages were fed to chicks within three hours of dissection from the fish. They were wrapped in a piece of fish flesh and placed in the throat using

blunt forceps. When rat infections were carried out, parasites were quickly wrapped inside a ball of cheese and given to rats immediately. Rats greedily fed on these cheese balls and consumed them within a few minutes.

After appropriate time intervals (within 1 1/2 hours to 3 days, see Table 2.12) animals were killed using chloroform. The alimentary canal was removed and placed in a large petri dish containing a small amount of physiological saline. Portions of 3 cm were separated from the intestine in sequence, opened and the wall and the contents were thoroughly examined for adult parasites under a stereo microscope with the aid of dissecting needles. The gizzard of birds and the stomach of rats were also examined.

As no adults were recovered using the method above, rats and chicks were infected as above and killed after only 5 and 2 hours respectively to determine the fate of the fed parasites.

2.2.2.2. Life-cycle study of *Dermoergasilus amplectans*

Ripe egg sacs with eggs about to hatch were collected from adult copepods attached to the gills of fish brought from Koggala lagoon. These egg sacs were transparent and had acquired a dotted appearance due to compound eyes of the nauplii inside. Three trials were done subsequently to proceed the life-cycle the with the failure of the prior. These trials were carried under the temperature variation of 27.4-29.8° C, as follows:-

1. 10 egg sacs in filtered autoclaved lagoon water each in a small petri-dish of 5cm diameter (one egg sac/dish). The lids were kept open for aeration.
2. 25 egg sacs in one tank with 20 l of filtered Koggala lagoon water. A 30 μ net was used for filtering to remove all the zoo-plankton, especially the naturally occurring *D. amplectans* larvae and their predators. Five litres of water renewed every day.
3. 25 egg sacs in one tank with 20 l of filtered Koggala lagoon water with two uninfected fish from Udawalawa reservoir, where the parasite is

absent. Fish were introduced at day five post incubation since it was found that the copepodid stages appear in this time and the copepodid stages 1-3 died around the post incubation day 8 (it was suspected that fish need for their survival). From the day 6, a fish from the tank was examined every day to check the time of attachment, and this fish was replaced by another new fish. Five litres of water renewed every day.

At the time of water removal, the copepods in the removing water was retained by filtering the removing water through the 30 μ net; the eggs and the larvae were larger than this pore size. The copepods in the one litre of water was fixed and retained and the others were returned to the tank. As the number of egg sacs recovered was low (1-2 mature egg sacs per a fish), any replicates could not be tried out.

The copepods collected from one litre of sample were fixed in 80 % ethanol. The larvae from each day were kept in separate vials. Slides were prepared by mounting them with glycerin. The lengths of all the specimens were measured. The morphology of the different stages of the copepods were also studied.

2.2.3. Ecological survey

2.2.3.1. Data reporting and statistical analysis

The data on parasite infection levels were reported in terms of Prevalence (percentage), Range (intensity range), Mean intensity and Abundance. The number of fish observed are also included. The definitions for these ecological terms are as described in Margolis, Esch, Holmes, Kuris & Schad (1982).

Before performing any statistical test, the normality of data and homogeneity of variance were checked by the Chi-square test and Bartlett's test (Statgraphic Package) respectively. None of the data sets were agreed to this requirement. Therefore, appropriate square root transformations were selected to obtain normality of data and homogeneity of variance. The Two sample t-test or One-way analysis of variance (one-way ANOVA) was used for two sample testing and multi-sample analysis respectively. When F- values of one-way ANOVA test indicated significance

difference, Tukey test for multiple comparison was used to discern specific differences between the groups.

When normality and homogeneity of data were not obtained, Mann-Whitney non-parametric two sample analysis by ranks test or Kruskal-Wallis non-parametric one-way analysis of variance by ranks was used. In this case, Dunn test was used for multiple comparisons (Zar, 1984).

The size of the fish of the samples varied widely. However, since there was no signifiant difference between the lengths of the fish of the samples at 95 % level, at both localities, it was assumed that these two factors did not interact to modify the effect of the other. One-way analysis was thus applied on each factor separately.

The effect of these factors were first investigated independently, as describe above. Where there were significant differences due to seasons and significant differences due to the age of the fish, the transformed data were analyzed by the two-way ANOVA to see whether there were any interactions between these two factors. All the sets analyzed here could be normalized with transformations.

The data on the site preference of parasites, the number of parasites in the sites, were analyzed with a sign test. Since the data of one site was not independent from the others the above described tests could not be applied to see whether the sites in a selected catagory were significantly different from each other. The sign test was used therefore. This test could be applied to compare only the difference of the values of two sites at a time, when there were more than two sites to compare for the category, the combinations of two were considered and the probability obtained with each test was tested with

$$\frac{\text{the probability (0.05)}}{\text{number of combinations}}$$

for 95 % significance level.

2.2.3.2. The macro habitat effect on the parasite species composition and parasite infestation levels

(1) Comparison of the fauna between water bodies

The parasite infestation level data collected with all the samples within the period of one year were pooled together and the ecological parameters for parasite infections, prevalence, mean intensity and abundance were calculated for each parasite in each locality.

The parasites were categorized according to locality preference. Where they were found in both habitats the abundance values were compared with Mann-Whitney non-parametric test with untransformed data.

The single sample collected from Bolgoda was compared with the sample of the same month from Koggala lagoon, the two brackish water localities.

(2) The variation due to seasonal changes in the locality

The data collected in alternative months within the period of one year was statistically tested with one-way ANOVA or Kruskal-Wallis test for the two localities separately. The infected as well as uninfected fish ie. the data set used for calculating abundance values were used here. The significant differences were discovered by multiple range tests. These changes in abundance values were tired to explain in terms of seasonal changes and any other change of definite or intermediate hosts which can be induced by the seasonal changes.

(3) Environmental parameters

At the time the fish samples were collected from the two habitats, the temperature, salinity and pH of the water body were measured.

Surface temperatures of three stations 10, 20 and 30 meters away from bank were measured by collecting water into a 200ml beaker and dipping a mercury thermometer. The mean was taken as the surface temperature of the water body.

The salinity of the samples collected as above was measured by inserting the probe of the Beckman RS 5-3 Induction Salinometer into the beaker. The salinometer was calibrated according to the method given by Srtickland and Parsons (1965). The method is as follows; The concentration of precipitable halides in a graded series of water samples were estimated by titrating with silver nitrate of known strength using chromate as indicator. The chlorinity values thus obtained were converted to salinity values using the standard equation, salinity ‰ = 0.030 + 1.805 chlorinity ‰. This graded series of water samples, of which the salinities were accurately known, was used to calibrate the induction salinometer.

The same water samples were used for Ph measurements. Measurements were made with the aid of a portable Ph meter from an Environmental Multiprobe of W.P.A. Instruments Ltd., U.K. The Ph meter was calibrated using standard buffer solutions.

In all cases, these parameters were measured between 9.30 and 10.30 a.m. The monthly total rainfall data were obtained from the Meteorological Department at Colombo referring to the nearest station to these water bodies.

2.2.3.3. The micro-habitat effect on the parasites

(1) Host preference

The infection levels of the samples of tilapia, a sample of *O. mossambicus* from Koggala lagoon and *O. niloticus* which was predominant in Udawalawa reservoir, were compared with the samples of *Etroplus* collected in the same month from the same locality to find the host preference of the parasites. The *Oreochromis* species were identified by external characters.

(2) Host age (size) preference

The infection level data which consider both the infected and uninfected fish were pooled separately for the two localities. In order to determine the influence of host size, each set was catagorized into 3 groups according to the size of the fish. The length groups were as follows,

size 1 - length less than 8.0 cm

size 2 - length above or equal to 8.0 cm and less than 10.0 cm

size 3 - length above or equal to 10.0 cm

N.B. The length range of fish collected form Udawalawa reservoir was 6.0 - 15.5 cm and form Koggala lagoon was 6.4 - 13.5 cm.

The infection level data set for each parasite in each locality were analysed to find any significant difference due to the size of the fish.

(3) Site preference by the parasite

The data collection for parasite infestation levels was extended for detail counting of the parasites in relation to the site of the gill apparatus to investigate the site preference of the parasites. The distribution of gill and stomach inhibiting parasites were investigated.

The gill arches were assigned the names arch 1-4, the most external one was the arch 1. Each arch was divided into three segments, ventral, middle and dorsal, and the hemibranchs external was the one towards operculum and internal was the one towards interior (Figure 2.1).

Fifty fish collected from Koggala lagoon were examined for *Dermoergasilus amplectans*. Gills were separated from fish and examined under the stereo microscope. The site of attachment was noted on four gill arches on one of the sets of two sides, randomly selected. The site of attachment of parasite was noted as the arch, the segment of the arch and the hemibranch. Since all the copepods were concentrated into tips of the primary filaments the division of the primary lamellae was not considered. The sign test was applied separately on the data sets, total number of parasites in each arch, total number of parasites in each segment and total number of parasites in each hemibranch.

Data for gill arch preference of gill monogeneans were collected on the four arches of one of the two sides. The arches were left separately in small petri-dishes

(5 cm in diameter) for five hours and the number detached were counted. The preferred area of the gill arch was then investigated on the second gill arch of the other side. Here, the arch was placed on a glass slide and divided by cutting to the 3 segments of approximately equal size. The three segments were placed on three glass slides and further the primary lamellae were separated into 2 equal areas, the proximal and distal portions. Then the hard cartilaginous part was separated from the proximal part of the gill lamellae. All these six portions were placed on slides separately, spread thinly, covered with a cover glass and the number of worms of two gill monogenean species were counted separately. Since it was difficult to cut and separate the two hemibranchs this catagory was not attempted. The sign test (Daniel, 1990) was applied on the data sets, the number of parasites in each arch, the number of parasites in the segments of second arch and the number of parasites in the distal and proximal regions of the second gill arch.

The site preference study on stomach parasites, *Enterogyrus* species, is considered under Part 2.

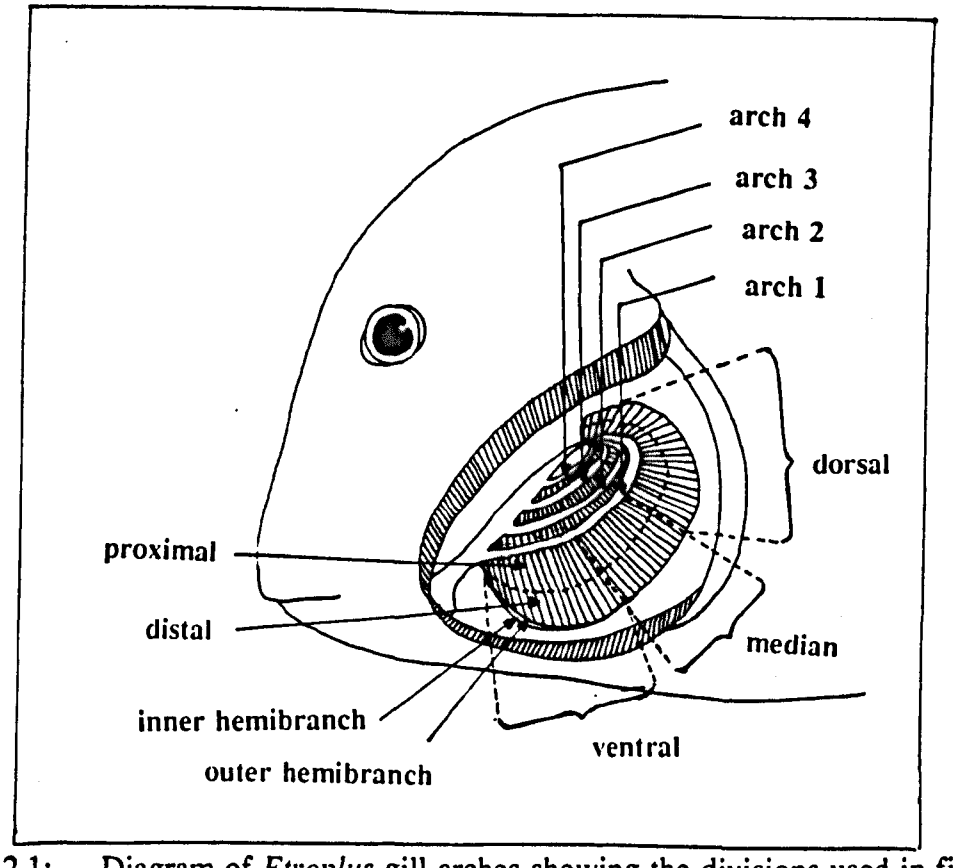


Figure 2.1: Diagram of *Etroplus* gill arches showing the divisions used in finding the distribution of gill parasites.

(4) The host tissue response

Tissue samples for histological examination were fixed in 10% buffered formalin and left refrigerated for 24 hours to minimize tissue degeneration prior to fixative penetration. The tissues with embedded encysting larvae were sampled unawaring their presence, from the fish and the areas of the tissues suspected to be infected.

All the tissues to be processed were cassetted, labelled and autoprocessed in a histokinete (HISTOKINETTE 2000). Tissues were positioned in suitably sized molds, blocked in molten wax and cooled rapidly on a freezing plate.

The blocks were trimmed to expose the tissue. Wherever necessary blocks were decalcified in RDC (rapid decalcifier from Histopath), for about 2 hours. The tissues which were not decalcified were soaked in water for a same time interval. Then the blocks were cooled on a cold plate before being sectioned. A Leitz-Wetzlar micrometer was used to cut 4-5 μm sections. The sections were floated on water maintained at 40°C in a water bath and collected on prewashed glass slides. The slides were then marked with a diamond pen, dried on a hot plate and the wax melted in a 60°C oven.

The sections were stained with haematoxylin and eosine (H&E). The photomicrographs were taken on Leitz-orthomat automatic photo microscope with black and white film Ilford Pan F-135 50 ASA.

2.2.4. Experimental environmental manipulation

From a sample of *Etroplus* brought from Udawalawa reservoir, a group of 40 fish having the size range 8.0-10.0 cm in standard lengths were selected and left together in a holding tank for one week to obtain a unform ecto-parasite distribution among them. After this period, fish were randomly selected placed in groups of eight in four tanks with 50 litres of water. Tanks carried the water of different salinities; the salinities used were 0, 8, 16 and 24 ‰. Declorinated tap water was mixed with sea water to attain the necessary salinity. The tanks were cleaned by suction under gravity

and a fourth of water was changed every day. Fish were fed *ad libitum* with the plant *Hydrilla*. At the end of the third week, they were decapitated and the number of external parasites present were investigated. The experiment had to be terminated at this time since the fish in low salinities were moribund due to the build up of some parasites.

Since the fish were from a sample brought from one time and left together in one tank for a week, it was assumed that the initial parasite burden of fish was similar. The parasite burden levels of the four salinities at the end of the experiment were compared by one-way ANOVA or Kruskal-Wallis test, then the multiple comprison tests were performed whenever necessary.

2.3. Results

2.3.1. Parasite survey

2.3.1.1. Parasites found in *Etroplus suratensis (Bloch)*

1. Protozoans

A. Family: Bodonidae Stein 1878.

***Ichthyobodo* sp.**

Host: *E. suratensis* (Bloch)

Site of infection: skin and gills

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Prevalence, Mean intensity for cm^2 of body surface area (Number of fish surveyed): Udawalawa reservoir *E. suratensis* 32.1 %, 110.1 (84): Koggala lagoon *E. suratensis* 6.0 %, 1.5 (100): Bolgoda lagoon *E. suratensis* 14.3 %, 1.5 (14)

Identification:

Very small, ovoid to pyriform body; non motile nature when attached to host and performance of spiral movement during swimming made it possible to identify them as *Ichthyobodo* sp. Only one species of *Ichthyobodo* has been described and this euryxenous parasite has a wide range of distribution (Becker, 1977). Hence, this probably belongs to the same species, *I. necator.*

B. Family: Trypanosomidae Doflein, 1911.

***Trypanosoma* sp.**

Host: *E. suratensis* (Bloch), *O. mossambicus* (Peters), *O. niloticus* (Linnaeus)

Site of infection: Blood

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Prevalence, Mean intensity in 3 microscopic fields of mag. X100 (Number of fish surveyed): Udawalawa reservoir *E. suratensis* 94.9 %, 15.0 (84); *O. niloticus* 100 %, 9.1 (14): Koggala lagoon *E. suratensis* 37.2 %, 2.9 (94); *O. mossambicus* 6.6 %, 2.0 (15): Bolgoda lagoon 42.9 %, 2.8 (14)

Identification:

Elongated body; presence of single flagellum with undulating membrane led to the identification as a *Trypanosoma* sp. In addition, the trypomastigotes were present in two forms, a few short stumpy trypomastigotes were present amongst the

long slender forms.

Trypanosomes seem to be host specific. However, according to Becker (1977), the same *Trypanosoma* species may occur in closely related fish host species and they may have a greater specificity for the invertebrate vector rather than for the vertebrate host. Since all the host fish under investigation belong to the family *Cichlidae* and inhabit the same localities, it may be the same *Trypanosoma* sp. occuring in all of them.

C. Family: Ophryoglenidae Kent, 1882, emend. Kahl, 1932

Ichthyophthirius multifiliis Fouquet, 1876

Host: *E. suratensis* (Bloch), *O. niloticus* (Linnaeus)

Site of infection: Skin and gills

Locality: Udawalawa reservoir

Prevalence, Mean intensity for cm^2 of body surface area (Number of fish surveyed): Udawalawa reservoir *E. suratensis* 40.5 %, 187.8 (84); *O. niloticus* 21.4 %, 1.3 (14)

Identification:

Ovoid to round uniformely ciliated trophozoites, older ones having the characteristic crescentic shaped macronucleus, made it possible to identify them as *I. multifiliis.*

D. Family: Urceolariidae Stein, 1867

***Trichodina* sp.**

Host: *E. suratensis* (Bloch), *O. niloticus* (Linnaeus)

Site of infection: Skin and gills

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Prevalence, Intensity for cm^2 of body surface area (Number of fish surveyed): Udawalawa reservoir *E. suratensis* 35.7 %, 5.1 (84); *O. niloticus* 14.3 %, 1.5 (14): Koggala lagoon *E. suratensis* 13.0 %, 1.3 (100): Bolgoda lagoon *E. suratensis* 21.4 %, 2.3 (14)

Identification:

The saucer- to bell-shaped body with a highly developed basal adhesive disc

identified them as a trichodinid. The shape of the denticles, the presence of moderately developed inner rays (Hoffman, 1978) and an adoral ciliary spiral turns completing an entire circle (Lom and Haldar, 1977) identified them as a *Trichodina* sp.

The very low number of parasites found on the fish made it practically impossible to make smears to stain with $AgNO_3$ according to the method of Klein for detailed measurements. However, the specimens from all three localities had 23-25 denticles with similar shape. Although they were similar in appearance, the parasites brought from Udawalawa reservoir were unable to survive in water of 8 ‰ salinity (see Section 2.3.4) indicating their stenohalinity, thus the parasites from the two different environments may belong to different species.

E. Family: Scyphidiidae Kahl, 1935

***Apiosoma (=Glossatella)* sp.**

Host: *E. suratensis* (Bloch)

Site of infection: Skin

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Prevalence, Intensity for cm^2 of body surface area (Number of fish surveyed): Udawalawa reservoir *E. suratensis* 4.8 %, 3.5 (84): Koggala lagoon *E. suratensis* 17.0 %, 4.4 (100): Bolgoda lagoon *E. suratensis* 14.3 %, 5.5 (14)

Identification:

Flask-shaped body with short narrow base for attachment and the compact macronucleus lying in posterior part of the body identified this parasite as a *Glossatella* sp. Although the size and the shape was similar in the freshwater and brackish-water specimens, the inability of the freshwater parasite species to tolerate 8 ‰ salinity experimentally indicates their stenohalinity. Therefore, the parasites from the two different environments may belong to different species.

2. Monogeneans

Family: Dactylogyridae Bychowsky, 1933

Subfamily: Ancyrocephalinae Bychowsky, 1937

***Enterogyrus* spp.**

Host: *E. suratensis* (Bloch)

Site of infection: Stomach

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Prevalence, Mean intensity (Number of fish surveyed): Udawalawa reservoir 88.5 %, 41.0 (78): Koggala lagoon 100 %, 76.4 (94): Bolgoda lagoon 100 %, 43.1 (14)

Note: It was only in a later stage of the ecological study that it became apparent that there were two species of *Enterogyrus* occurring in the stomachs. Thus, separate counts were not taken for the two species at this stage and counts included both species. *Enterogyrus globidiscus* was the most abundant with only low numbers of *Enterogyrus papernai* amongst the *E. globidiscus* worms. A small number of fish were sampled from Udawalwa reservoir (33 fish), Koggala (50 fish) and Bolgoda (14 fish) lagoons and analysed in detail. The following percentages of total counts, mean (range), for *E. papernai* populations are presented below.

Udawalawa reservoir: 17.17 (12.77 - 22.00)

Koggala lagoon: 16.24 (8.51 - 28.85)

Bolgoda lagoon: 16.78 (8.33 - 30.77)

***Enterogyrus globidiscus* (Kulkani, 1969) Gussev & Fernando, 1973** (Figure 2.2)

Description: (based on 16 specimens)

Body spindle-shaped, flattened dorsoventrally, 289 (214-348) long and 91 (77-118) in greatest width. Haptor distinctly separated from body, dome shaped, wider anteriorly, 46 (42-59) in length and 73 (61-82) in breadth. Body covered by tegument, 5 (4-7) thick in mid region. Two pairs of eye-spots; posterior pair larger, closer to each other and situated just anterior to pharynx. Pharynx 28 x 26 (18-31 x 18-30) leads to very short oesophagus and intestine in form of cyclocoel.

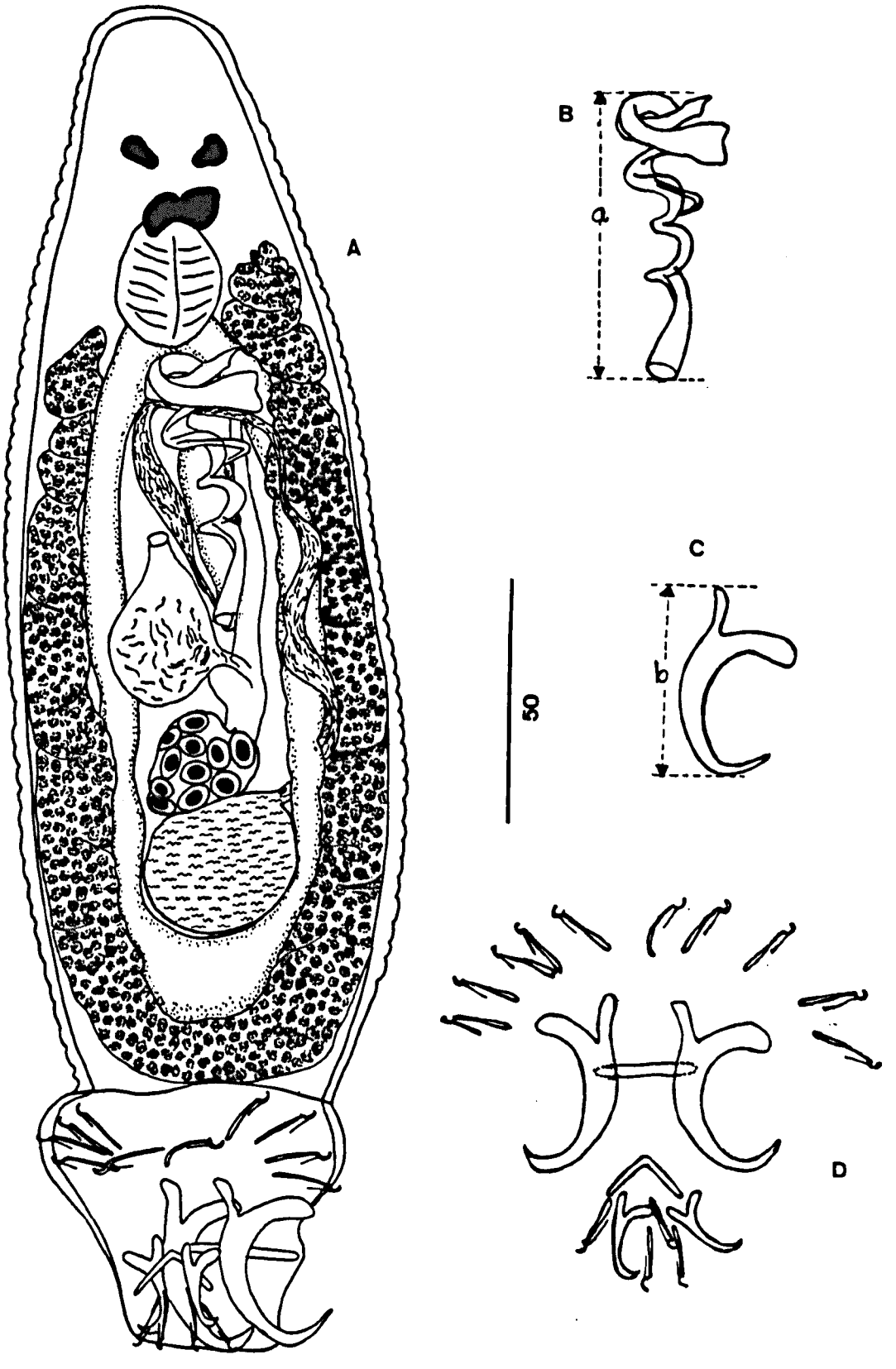


Figure 2.2: *Enterogyrus globidiscus*. (A) Entire worm. (B) Copulatory tube, a) length of the copulatory tube. (C) Dorsal hamulus, b) external hamuli length. (D) Opisthaptor armature.

Table 2.1: Measurements of *Enterogyrus globidiscus.*

Character	Kulkani (1969) n=4	Gussev & Fernando (1973) n=10	Fixed material *E. suratensis* n=16
Total length	465-543	350	214-398
Maximum width	111-143	120	77-109
Haptor	60-110 X 81-125	50 X 90	42-59 x 61-75
Marginal hooks	12-15	13-15	13-15
Dorsal bar	25-35	2-6 x 23-28	3-4 x 26-27
Dorsal hamulus			
Total length	51-65	34-41†	38-41†
Point		10-12	10-11
Ventral bar	21-25	1 x 18-20	1 x 18-22
Ventral hamulus			
Total length	25-30	18-20†	18-20†
Point		6	5-6
Copulatory organ		70-90	62-78
Testis	48-68 x 43-64		18-43 x 35-50
Ovary	31-38 x 27-36		19-31 x 20-38
Pharynx	28-33 x 32-37		18-31 x 18-30
Tegument thickness (mid body)		6	4-7

† external hamulus length
n - number of specimens measured

Two pairs of hamuli; larger dorsal pair 39 (38-41) in total length (external hamulus length[1]), point 10 (10-11); small ventral pair 19 (18-20) in total length (external hamulus length), point 5 (5-6). Straight dorsal bar 26 x 3 (26-27 x 3-4), thin, inverted, 'V-shaped'; ventral bar 20 x 1 (18-22 x 1) (both arms of 'V'). Seven pairs of marginal hooks; pairs 3-7 on anterior edge of haptor, 14.5 (14-15), of similar shape; first and second pairs between ventral hamuli directed posteriolateraly with thinner shafts 14 (13-15).

Testis 34 x 43 (18-43 x 35-50), lies at posterior end of area confined by intestinal loop. Vas deferens encircles left intestinal loop, proceeds anteriorly crossing body in oblique manner dorsally to copulatory tube, turns posteriorly and enlarges to form seminal vesicle and open into initial, enlarged part of copulatory tube. Prostrate gland situated dorsally and medially in area covered by vas deferens and also opens to initial part of copulatory tube. Copulatory organ, spiral tube with funnel-shaped enlarged initial part; has fringe present along entire length; final portion enlarged and has triangular expansion which looks like accessory piece; copulatory organ 74 (68-78) in length (see Figure 2.2B).

Vaginal armature absent. Vaginal opening situated little lateral to median line, in mid level of body portion (excluding haptor); this immediately opens to sac-shaped seminal receptacle. Vitellaria extensive, almost extracaecal, but slightly covering intestine ventrally. Ovary 25 x 32 (19-31 x 20-38), immediately anterior to testis. Oötype difficult to identify. Uterus, a tube extending anteriorly, opens via genital pore. Eggs eliptical 54 x 46 (48-60 x 35-42) (number measured = 30). Details of excretory system not clear.

Identification-

As all the morphological characters, and the measurements of the specimens (Table 2.1) and the host species involved agree with the description of *Enterogyrus globidiscus* by Gussev and Fernando (1973), these specimens were considered to

[1]distance between the furthest points of shaft and outer root portions (Figure 2.2C), the term outer refers to the root directed outward in relation to the point

belong to the same species. The Indian specimens measured by Kulkarni (1969) appear to be larger than Sri Lankan specimens.

***Enterogyrus papernai* Gussev & Fernando, 1973** (Figure 2.3)

Description (based on 16 specimens)

Smaller worms; body comparatively short and wide, 226 x 86 (154-275 x 64-118). Body not straight, curved with concave side ventrally. Haptor distinctly separated from body, dome-shaped, wider anteriorly, 64 x 76 (45-80 x 67-99). Body covered by extremely thick tegument; outer layer 3 (3-4) thick; inner layer 2 (2-3) thick in mid region of the body; thickness decreases anteriorly and increases posteriorly. Two pairs of eyes; posterior pair just anterior to pharynx. Pharynx 24 (19-27). Intestine in form of cyclocoel.

Two pairs of hamuli; larger dorsal pair 41 (40-42) in total length (external hamulus length); point 5.5 (5-6), bearing a "kink" at its middle. Roots of approximately similar lengths, 12 (12-13); inner root considerably bent towards point. Ventral pair similar to that of *E. globidiscus*, little shorter in total length 17 (16-18); roots 5 (5-6); point 5 (4-6). Ventral hamuli and two pairs of marginal hooks in between at pointed extreme posterior end of the haptor. Straight dorsal bar 15.5 x 4 (14-16 x 4); inverted'V'- shaped ventral bar, 14 x 1 (11-16 x 1) (both arms of 'V'); both much shorter than that of *E. globidiscus*. Seven pairs of marginal hooks; pair 3-7 on anterior edge of haptor, 13 (12-13); shafts 9 (8-9). Pair 1 and 2 little longer, 14 (13-14) shafts 10 (9-10).

The basic internal anatomy was similar to *E. globidiscus*. However, due to the short body, body curvature and comparatively long copulatory organ, the positions of the internal organs are different from *E. globidiscus*.

Testis lies mid ventrally, at level of the posterior end of the intestinal loop. Vas deferens encircles left intestinal loop, proceeds anteriorly crossing the body in oblique manner, turns posteriorly to form seminal vesicle, opens to initial part of copulatory tube. Prostrate gland situated medially and dorsally in mid level of total body length,

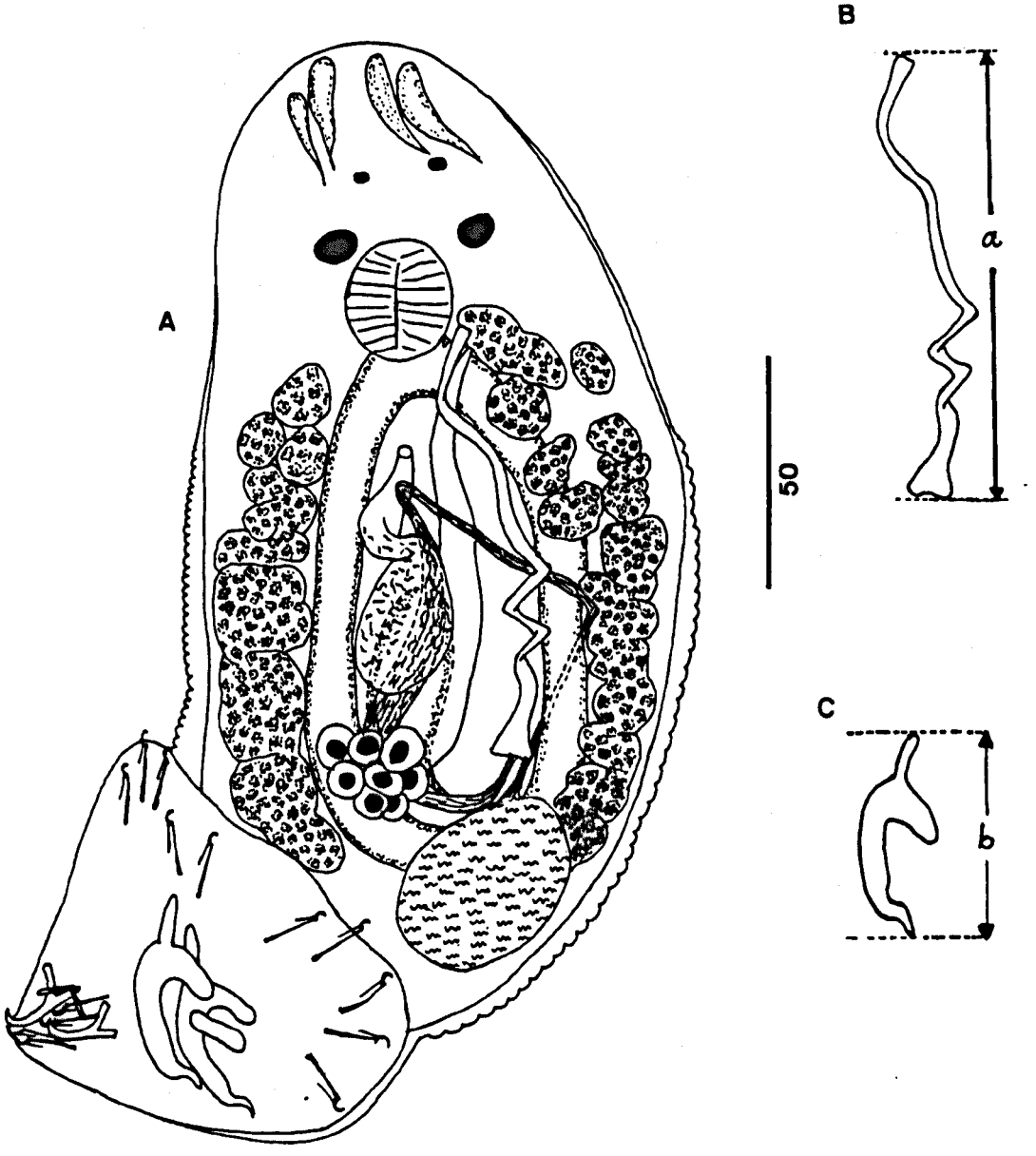


Figure 2.3: *Enterogyrus papernai*. (A) Entire worm. (B) Copulatory tube, a) length of the copulatory tube. (C) Dorsal hamulus, b) external hamuli length.

Table 2.2: Measurements for *Enterogyrus papernai.*

Character	Gussev & Fernando (1973) n= 15	Fixed specimens from *E. suratensis* n= 16
Total length	up to 250	154 - 275
Maximum width	up to 140	64 - 118
Haptor	70 x 70	45-80 x 67-99
Pharynx	20 x 20	19 x 27
Tegument thickness		
Outer layer	3	3 - 4
Inner layer	6	2 - 3
Hook pairs 2-6		
Total length	13	12 - 13
Handle	9	8 - 9
Hook pairs 1 & 7		
Total length	14	13 - 14
Handle	10	9 - 10
Ventral hamulus		
Total length	16 - 18	16
(external hamulus length) Roots	7	5 - 6
Point	6 - 7	4 - 6
Dorsal hamulus		
Total length	41 - 45	40 - 42
(external hamulus length) Suplimentary hook	5	5 - 6
(after the 'kink' in the point) Roots	12 - 13	12 - 13
Ventral bar	1 x 15	1 x 11-16
Dorsal bar	4-5 x 15-18	4 x 13-15
Copulatory organ	80 - 120	83 - 101

n - number of specimens measured

has longitudinally striated appearance. Copulatory organ, spiral tube, 93 (83-101) in length (see Figure 2.3B), longer than that of *E. globidiscus.*

Vaginal armature absent; vaginal opening in the mid level of body portion, slightly lateral to median line. Pore immediately opens to sac-shaped seminal vesicle; seminal vesicle proportionately larger and elongated compared to *E. globidiscus.* Vitellaria extensive, extracaecal, slightly covers intestine ventrally. Ovary lies above testis; oötype not clear; uterus opening via genital pore. Eggs elliptical 44 x 36 (32-54 x 26-42) (number measured = 30). Details of the excretory system not clear.

Identification:

As all the morphological characters, and the measurements of the specimens (Table 2.2) and the host species involved agree with the description of *E. papernai* by Gussev and Fernando (1973), these specimens must belong to the same species.

***Ancyrocephalus etropli* Gussev, 1963** (Figure 2.4)

Host: *E. suratensis* (Bloch)

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Site of infection: Gill filaments

Prevalance, Intensity for second gill arch of one side (Number of fish surveyed): Udawalawa reservoir 100 %, 108.4 (84): Koggala lagoon 100 %, 117.9 (100): Bolgoda lagoon 100 %, 91.6 (14)

Description: (based on 12 specimens)

Very small, spindle-shaped worms, 234 (196-255) long, 83 (50-102) wide. Haptor well demarcated from body, 46 (42-51) long and 76 (62-90) wide. Seven pairs of marginal hooks; first pair smallest 10 (8-12); pairs 2-5 measured 17 (14-18); pair 6 longest 19 (16-20) and pair 7 lies between the hamuli, 12 (11-13). Hamuli similar in size and shape. Dorsal pair 31.5 (30-32) in total length; length of shaft 27 (26-29); outer root 1.5 (1-2); inner root 7 (5-8); point 4. Ventral pair 29 (27-32) in total length; length of shaft 28 (26-29); outer root 2 (1-2); inner root 6 (4-7); point 4 (3-4). Bars are of similar shape and size, 29 x 3.5 (23-31 x 3-4).

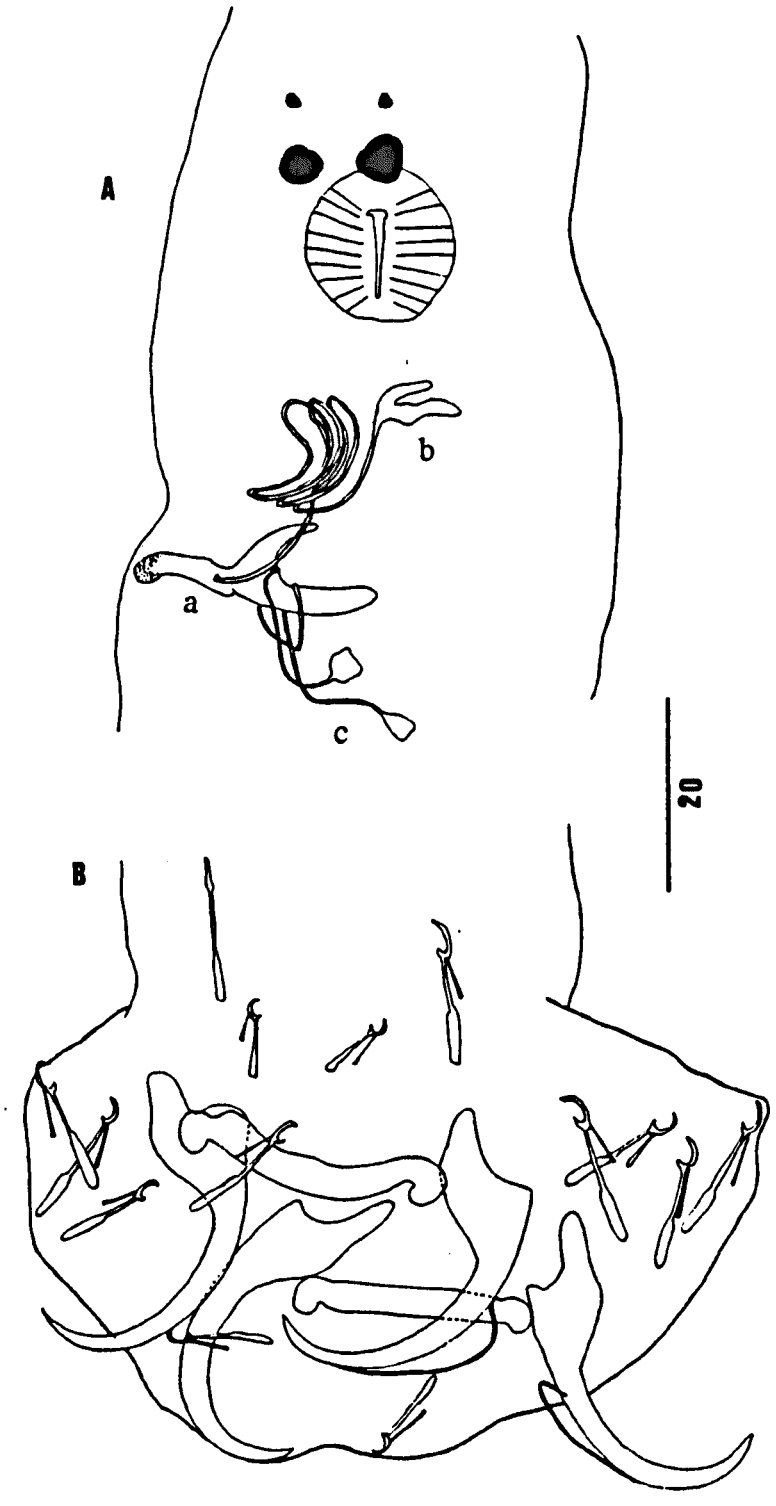


Figure 2.4: *Ancyrocephlus etropli.* (A) Anterior portion with copulatory complex and vaginal armature, a) accesory piece, b) funnel and the tube, c) vaginal armatur. (B) Opisthapter.

Table 2.3: Measurements of *Ancyrocephalus etropli.*

Character	Gussev & Fernando (1973) n=10	Fixed specimens from *E. suratensis* n= 12
Length	100 - 130	196 - 255
Width	60	50 - 102
Haptor	30 - 50	42-51 x 62-90
Hooks pair 1	11 - 17	8 - 12
pairs 2-4		14 - 16
pair 5		14 - 18
pair 6		16 - 20
pair 7		11 - 13
Dorsal hamulus, total	26 - 31	30 - 32
shaft	23 - 25	26 - 29
outer root	1 - 2	1 - 2
inner root	5 - 7	5 - 8
point	4	4
Ventral hamulus, total	26 - 30	27 - 32
shaft	23 - 26	26 - 29
outer root	2	1 - 2
inner root	5 - 6	4 - 7
point	4	3 - 4
Dorsal bar	3 x 23-25	3 x 23-31
Ventral bar		3-4 x 23-29
Tube diameter initial part	4	3 - 5
Tube diameter elsewhere	1	< 1
Accesory piece length	21	23 - 27

n - number of specimens measured

Copulatory organ consists of tube and accessary piece. Initial part of tube widened, 3.5 (3-5) diameter; tube diameter less than 1, spirally coiled; final part ending near accessory piece. Accessory piece, three branched plate of 25 (23-27) at its highest length. Just posterior to copulatory complex, coiled thin tube occurs with swollen ends, probably a vaginal tube.

Identification:

The description agrees with that of *Ancyrocephalus etropli* described by Gussev (1963) from the hosts *Etroplus suratensis* and *E. maculatus* from Colombo vicinity, Sri Lanka. But the present specimens are a little larger and show variation in marginal hook pair sizes, where Gussev has given one range for all pairs (Table 2.3); the first pair of hooks are shorter than the range given by Gussev. However, due to all of the other similarities, including the host species, these specimens are considered to belong to the species *Ancyrocephalus etropli.*

***Ceylonotrema colombensis* Gussev, 1963** (Figure 2.5)

Host: *E. suratensis* (Bloch)

Locality- Udawalawa reservoir, Koggala and Bolgoda lagoons

Habitat- Gill filaments

Prevalence, Mean intensity for second gill arch of one side (Number of fish surveyed): Udawalawa reservoir 91.7 %, 5.3 (84): Koggala lagoon 63.0 %, 3.1 (100): Bolgoda lagoon 92.9 %, 1.9 (14)

Description (based on 10 specimens)

Large worms, 384 (326-429) in length, 104 (90-109) in maximum width. Haptor well separated from body, 46 x 95 (42-51 x 77-106). Haptor contains 7 pairs of marginal hooks; first pair shorter 25 (22-27); second to fourth pairs 30 (28-32); fifth pair 28 (27-29); sixth pair 13 and of embryonal type; seventh pair thicker and shortest, thickness abruptly tapering towards sickle; sickle fixed to shaft and without filament loop, 22 (19-23) long. Hamuli of similar shape and measurements; points considerably long. Dorsal pair 23.5 (23-24) in total length; shaft 18 (17-18); external process 3; internal process 10 (9-10); point 11 (11-12). Ventral pair 20 (19-21); length

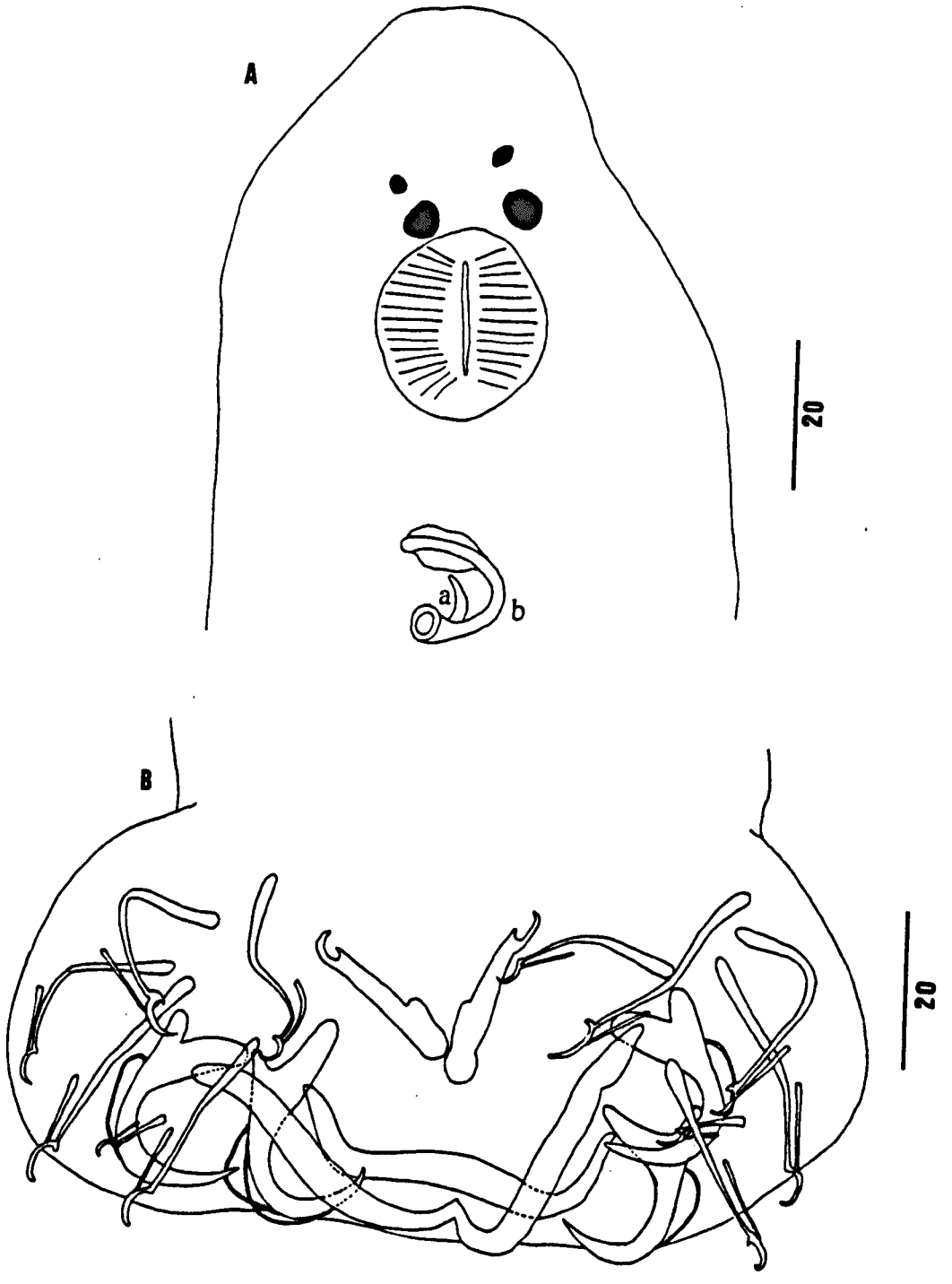


Figure 2.5: *Ceylonoterma colombensis*. (A) Anterior end with copulatory complex, a) accesory piece, b) funnel and the tube. (B) Opisthaptor.

Table 2.4: Measurements of *Ceylonotrema colombensis.*

Character		Gussev (1963) n= 3	Fixed specimens from *E. suratensis* n= 10
Total length		110 - 170	326 - 429
Maximum width		70 - 100	90 - 109
Haptor		50 x 140-170	42-51 x 77-106
Lengths of hooks	pair 1	30 - 50	22 - 27
	pairs 2-4		28 - 32
	pair 5		27 - 29
	pair 6	12	13
	pair 7	30 - 35	19 - 23
Dorsal hamulus, total length		30 - 35	23 - 24
shaft		20 - 25	17 - 18
outer root		5	3
inner root		17	9 - 10
point		20	11 - 12
Dorsal bar		6-8 x 70	3-4 x 46-51†
Ventral hamulus, total length		31	19 - 21
shaft		27	16 - 18
outer root		5	4 - 5
inner root		10	6 - 8
point		11	10 - 12
Ventral bar		6-9 x 55-70	4-5 x 72-81
Diameter of posterior eye		16 - 20	4 - 5
Tube,total length		22	28 - 29
diameter of initial part		7	5 - 6
diameter of middle part		2 (little over)	2 - 3

of shaft 17 (16-18); external process 4 (4-5); internal process 7 (6-8); point 11 (10-12). Dorsal bar wavy giving a 'W'-shape, middle thicker and ends tapering, 4 (3-4) thick, total length of 'W' 48 (46-51). A pair of bars join at their ends and form the 'V'-shaped ventral bar; ventral bar 5 (4-5) thick, 77 (72-81) in total lengths.

Copulatory organ consists of 'C'- shaped tube and accessory piece. Funnel-shaped initial part of tube diameter 5 (5-6); elsewhere 2 (2-3); ends with wing like expansions at its sides. Total length of tube 28.5 (28-29). Accessory piece lies inbetween ends of curved tube.

Identification:

The description agrees with that of *Ceylonotrema colombensis* described by Gussev (1963) from the host *Etroplus suratensis* from Colombo vicinity, Sri Lanka. However, the present specimens appear to be much larger than Gussev's specimens (Table 2.4). Many of the other measurements also disagree. Being larger, the present specimens have smaller marginal hooks, hamuli and dorsal bar; but the ventral bar is little longer than in Gussev's specimens. The discrepancy may due to the smaller number of specimens (3) used for the description by Gussev. He also reported a considerable shrinkage in specimens he used, which may have caused the disproportionate hook lengths compared to body size.

Even though most of the measurements disagree, the similarity of structure, especially the similarity of the haptoral sclerites of uncommon shape which caused *C. colombensis* to be placed in a different genus, and the similarity of host species, suggests that these specimens most probably belong to the species *Ceylonotrema colombensis.*

3. Digenea

A. Family: Acanthostomidae Poche, 1926

Acanthostomidae gen. sp.

Cercaria (Figure 2.6A)

Host: *Melanoides tuberculata* (Muller)

Locality: Koggala lagoon

Description: (based on 12 specimens)

Bi-ocellate, lophocercous, monostomatous cercaria. Body oblong pyriform, 223 (189-277) long, 107 (81-125) wide. Oral sucker terminal, 41.5 x 39 (37.5-47 x 34-47), protrusible, without normal appearance of sucker; surrounded by few rows of small spines. One pair of eye-spots, posterior to oral sucker. Pharynx, oesophagus and caeca not differentiated. Acetabulum represented by mass of cells lying anterior to excretory bladder.

Large penetration glands, 12-16 in number, massed together between eye-spots and excretory bladder; associated ducts open in 4 groups on oral sucker. In newly emitted cercaria, excretory bladder was circular, subsequently became 'V'-shaped, when cercaria contracted longitudinally compressed, bilobed appearance was evident.

Tail attached postero-ventrally, 434 (397-459) long and 33 (31-44) wide near base with well-developed fins. One pair of lateral fin folds in anterior third of tail. Median fin originates one fourth distance posterior to base on dorsal side, extending over tip to ventral side, ends one third anterior to tip. At rest, body curved at an angle of 45° to vertical tail.

Metacercaria (Figure 2.6B-E)

Host: *E. suratensis* (Bloch) and *O. mossambicus* (Peters)

Site of infection: within fin ray and gill filament cartilage, experimentally found to encyst on fins and scales

Locality: Koggala lagoon

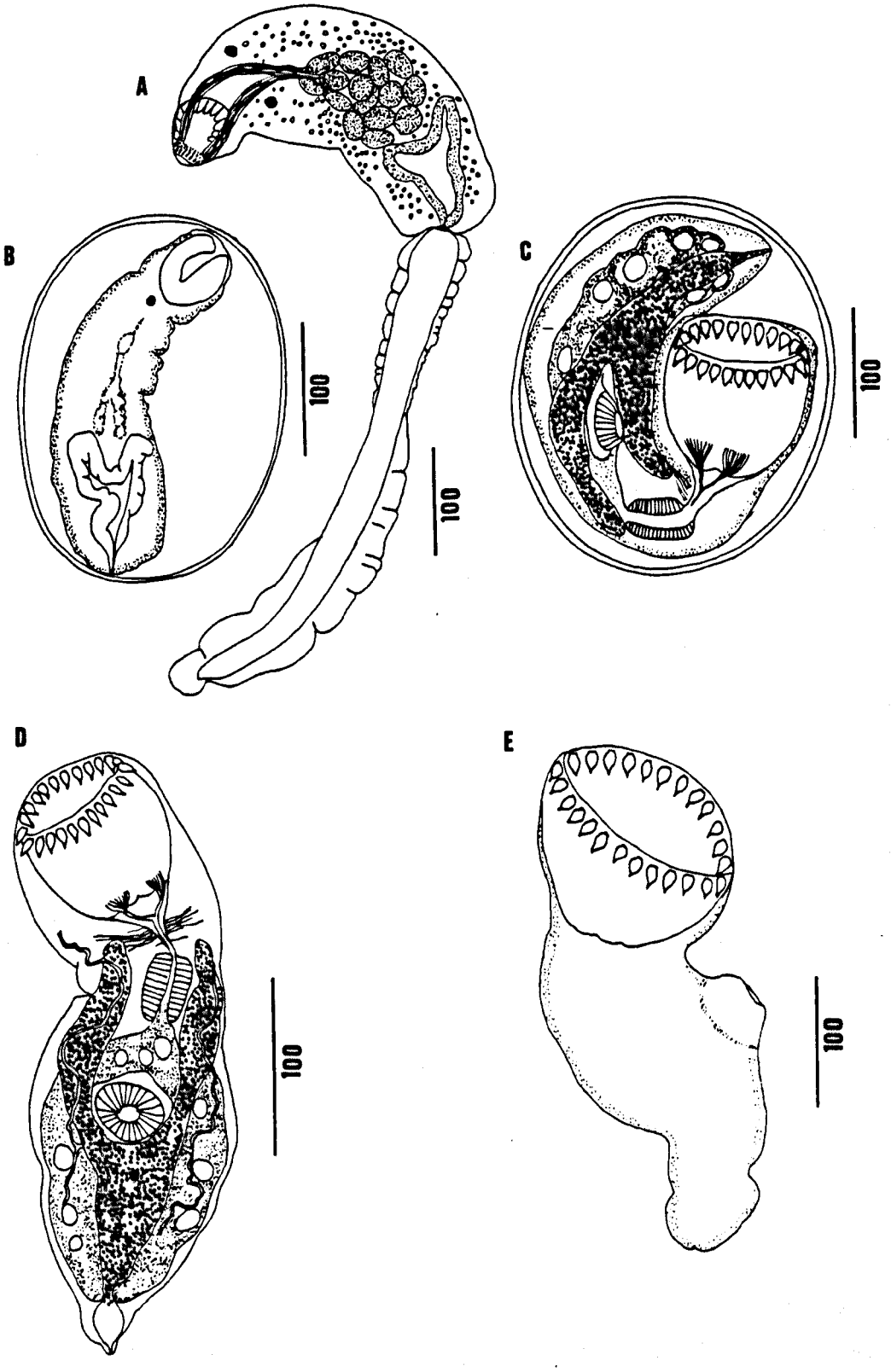


Figure 2.6: Acanthostomid cercaria and metacercaria. (A) Cercaria. (B) 1 - day old metacercaria. (C) Mature metacercaria in the cyst. (D) Excysted metacercaria. (E) Dead metacercaria.

Prevalence, Mean intensity for second gill arch of one side (Number of fish surveyed): *E. suratensis* 28.0 %, 2.1 (100); *O. mossambicus* 53.3 %, 2.1 (15)

Description: (based on 7 specimens)

Cyst of parasite origin elliptical to round, non pigmented, 286 x 262 (264-307 x 242-292); wall thin, approximately 8-10 thick. Host capsule absent. Worm bent on itself. Easily damaged when attempting to release metacercaria from host cartilage.

Body 356 (306-384) long, tapering posteriorly to more or less sharp point with maximum breadth in region of oral sucker or acetabulum; width 134(88-143) in region of acetabulum. Oral sucker well developed, cup-shaped, 156 (52-174), with single row of circumoral spines, 24-25 in number. Eye-spots absent. Acetabulum comparatively small, not well embedded in parenchyma, 38 (32-45) in diameter.

Pre-pharynx long, funnel-shaped. Pharynx 30 (26-34) long. Oesophagus very short. Caeca bifurcate just anterior to acetabulum, long, ending near posterior extremity.

Excretory bladder Y-shaped, bifurcating just posterior to acetabulum; arms wide, terminating near oral sucker. Genital organs not developed.

Dead worms with brown-yellow pigmentation sometimes found in the fin rays, oral sucker was open wide and internal structures had degenerated.

Identification:

The presence of an elongate slender body, circumoral coronet of spines, well-developed oral sucker, small unembedded acetabulum, and 'Y'-shaped excretory bladder with long excretory arms extending anterior to pharynx, indicate that these specimens probably belong to family Acanthostomidae, rather than the closely related family Cryptogonimidae.

Morphologically the specimens were very similar to the cercaria and

metacercaria of *Acanthostomum floridense* (McCoy, 1928) Price, 1940 as cited by Yamaguti, (1975). This was reported to encyst in fin rays and beneath the scales, occasionally in the muscles and branchial region, of almost any small reef fish in North America. There is also a resemblance in that these parasites were found in a similar site of infection and in brackish water fish. It is with reasonable confidence that these specimens are placed in the family Acanthostomidae, and it is considered likely that they belong to the genus *Acanthostomum*, although, on the evidence available it was not possible to determine that they belonged to the subfamily Acanthostomidae.

B. Family: Cryptogonimidae (Ward, 1917) Cirurea, 1933

***Exorchis* sp.**

Metacercaria (Figure 2.7)

Host: *E. suratensis* (Bloch) and *O. mossambicus* (Peters)

Site of infection: on scales, fins and gills

Locality: Koggala and Bolgoda lagoons

Prevalence and Mean intensity for second gill arch of one side (Number of fish surveyed): Koggala lagoon *E. suratensis* 67.0 %, 2.5 (100); *O. mossambicus* 26.7 %, 1.0 (15): Bolgoda lagoon *E. suratensis* 50.0 %, 2.4 (14)

Description: (based on 12 live specimens)

Cyst elliptical, 165 x 142 (115-225 x 90-175); wall with easily broken, transparent layer of 3.5 (3-5) thickness. Worm lays compressed in longitudinal axis with head bent back on ventral side.

Body pyriform, 254 x 176 (186-300 x 135-205), tapering at anterior end and bluntly oval at posterior end. Armed with spines all over body. No pigment granules scattered on body.

Eye-spots 2-3 lateral to pharynx, frequently 2 on one side. Cephalic glands about 40 in number, situated dorsally and laterally around oral sucker and opening into it. Oral sucker round to elliptical, 44 x 35 (35-50 x 28-45), subterminal. Pre-pharynx,

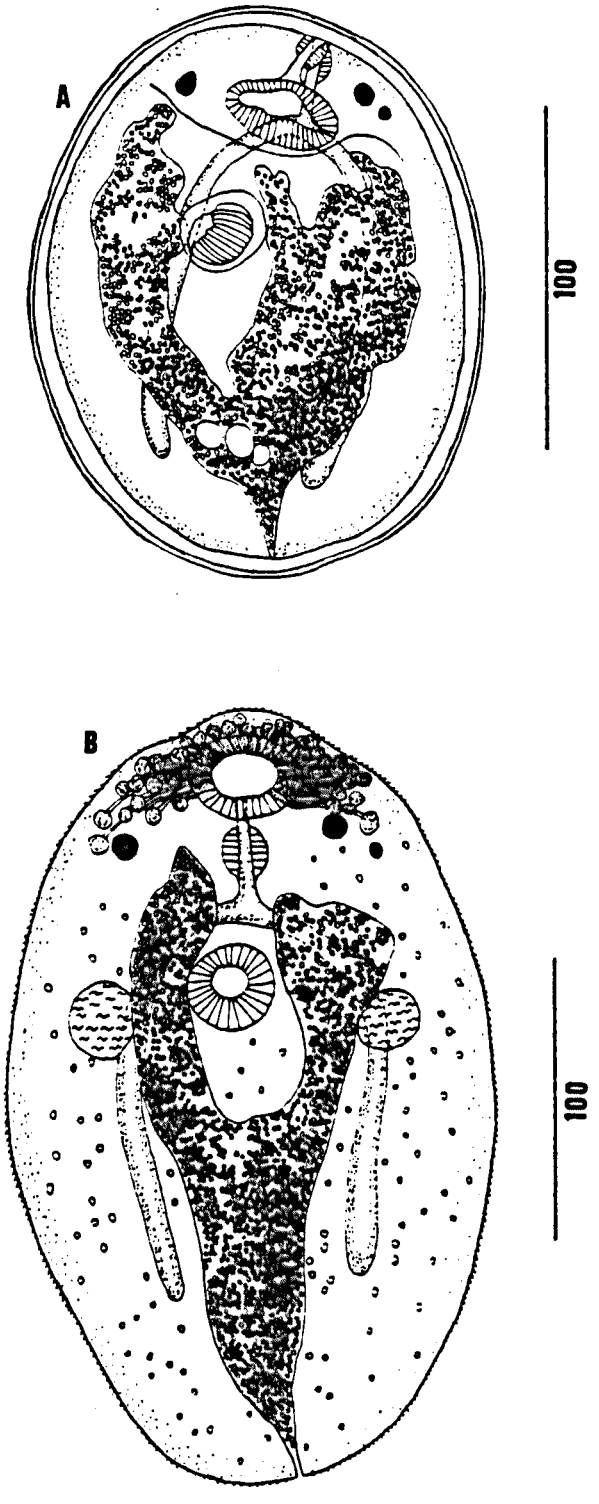


Figure 2.7: *Exorchis* sp. (A) Metacercaria in cyst. (B) Excysted metacercaria.

pharynx and oesophagus present. Pharynx 28 (25-32). Caeca terminating little anterior to posterior end of the body, containing disc-shaped substances. Acetabulum smaller than oral sucker, 32 (28-36) in diameter, lying little posterior to caecal bifurcation and embedded in parenchyma.

Testes elliptical, little smaller than acetabulum, lay extra-caecal, slightly posterolateral to acetabulum.

Excretory bladder 'V'-formed, both lateral arms running anterolateral to acetabulum, reach the level of oesophagus. Excretory corpuscles smaller, less sparsely distributed.

Identification:

Having no special characters, the key by Yamaguti (1971) to the families of adult worms could not be used to attribute this metacercaria even to a family. The description, however, largely agrees with the description of the subfamily Exorchiinae Yamaguti, 1938 of the family Cryptogonimidae. The very small body size, small acetabulum embedded in body parenchyma, symmetrical testes lateral to caeca in middle third of the body and 'V'-shaped excretory with bladder arms reaching near to pharynx place the specimens in agreement with the diagnosis given for the subfamily Exorchiinae Yamaguti, 1938.

Apart from the slight discrepancies in measurements and the presence of spines (easily lost in preserved specimens) all over the body surface, the description of the specimens from *Etroplus*, fits exactly with the one given for the metacercaria of *Exorchis oviformis* Kobayashi, 1915 (cited by Yamaguti, 1975). The sites of infection were also similar. Therefore, even if these specimens do not belong to the same species, they may belong to the same genus. Adult specimens are required for further identification.

C. Family: Renicolidae Dollfus, 1939

Renicolidae gen. sp.

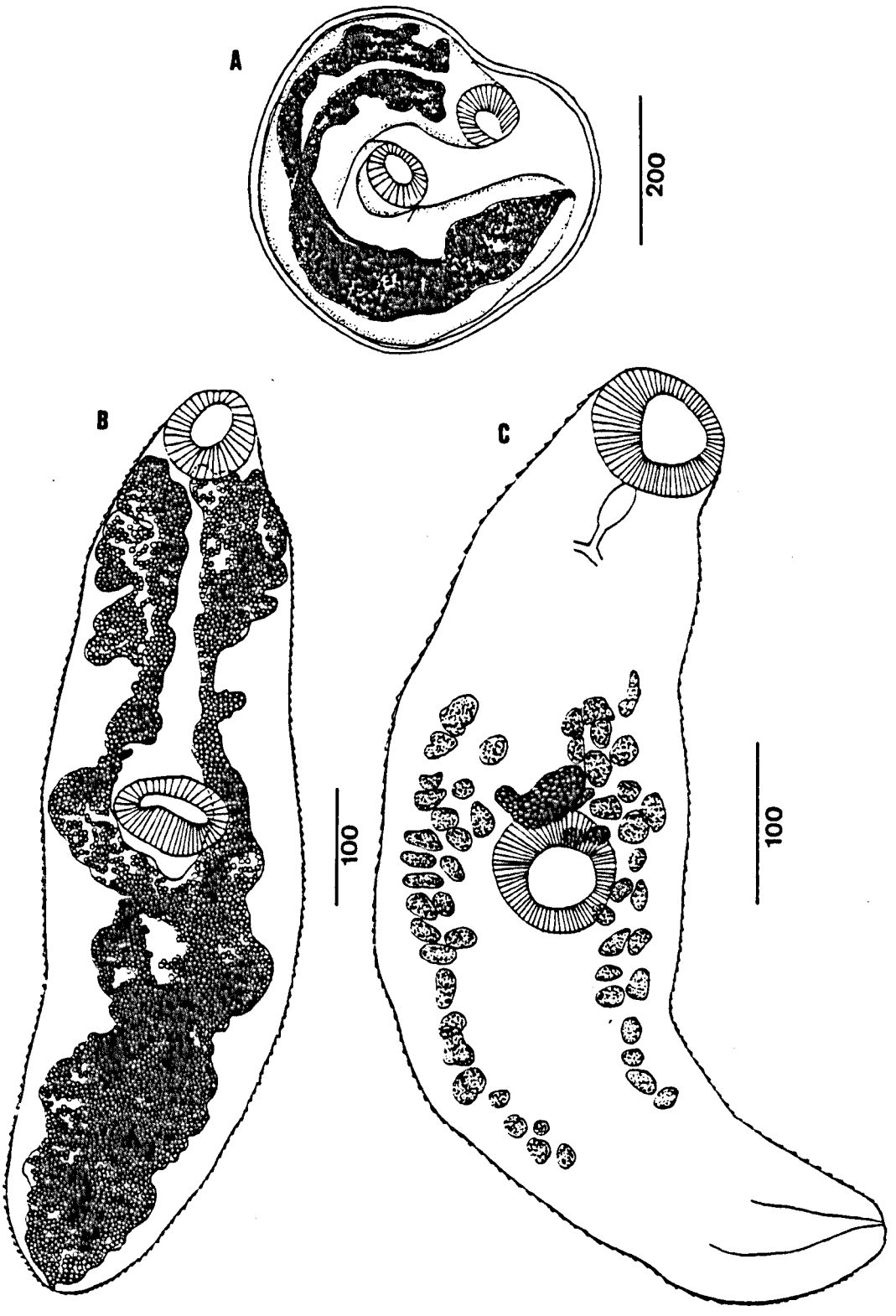


Figure 2.8: Renicolid metacercaria. (A) Metacercaria in cyst. (B) Metacercaria showing excretory vesicle. (C) Metacercaria (excretory granules removed by fixation with glacial acetic acid).

Cercaria: Although one infected gastropod of *Thiara* sp. was found, it died before the conclusion of experimental infection and before it was identified for certain as the cercarial stage of this metacercaria.

Metacercaria (Figure 2.8)

Host: *E. suratensis* (Bloch) and experimentally in *O. niloticus* (Linnaeus)

Site of infection: liver tissue

Locality: Udawalawa reservoir

Prevalence, Mean intensity (Number of fish infected): *E. suratensis* 15.4 %, 1.3 (78)

Description: (based on 8 specimens, which were all that were available)

Cysts round in shape, 520 x 504 (496-566 x 484-558); transparent cyst wall single layer of 14 (10-20), not enclosed in a layer of host origin. Metacercaria folded upon itself within cyst.

Metacercaria oblong in shape, 758 (563-1008) in length, 234 (142-282) in width, spinose. Oral sucker sub-terminal, 83 x 78 (74-93 x 64-99); leading to small pharynx of 26 x 15 (22-32 x 8-19). Oesophagus short, 26 (19-32). Intestine with 2 caeca, which could not be traced back. Acetabulum slightly smaller than oral sucker, 76 x 64 (58-99 x 64-99), almost equatorial and projecting on ventral surface.

Vitellaria in lateral fields of mid region of body. Ovarian anlagen pre-acetabula, about half of size of acetabulum, submedian.

Excretory bladder massive, 'Y'-shaped, branches at level of mid-body, with lateral diverticula; arms reaching level of oral sucker. Excretory corpuscles large, fill bladder.

Identification:

The two family characters, submedian immediately anterior acetabular ovary, 'Y'-shaped excretory bladder with long arms reaching to near oral sucker with numerous lateral diverticula, found in the specimens are the main characters which are

not common among digeneans, and possibly indicating that they belong to the family Renicolidae. In addition, the presence of vitellaria in the middle third of the body, an acetabulum slightly smaller than oral sucker and in the middle third of the body, are also in agreement with this designation. The metacercarial characters, encystment in liver of fish and that it is bent on itself in cyst also concur, since the metacercaria of *Renicola buchanani* Martin & Gregory, 1951 was reported to occur in the liver of the fishes *Fundulus p. parvipinnis* Girard and *Gillichthys mirabilis* Cooper (cited by Yamaguti, 1975) in North America.

The adults specimens are necessary to verify the family and to identify the species fully.

D. Family: Heterophyidae (Leiper, 1909) Odhner, 1914

***Centrocestus* sp.**

Metacercaria (Figure 2.9)

Host: *E. suratensis* (Bloch)

Site of infection: Gills

Locality: Koggala lagoon

Prevalence, Mean intensity for second gill arch of one side (Number of fish observed): *E. suratensis* 1.0%, 2.0 (100)

Description: (based on one cyst and one live metacercaria, which were all that were available)

Cyst of parasite origin elliptical, 208 x 165, hyaline, transparent; cyst wall 2 thick, covered by host capsule of 8.

Metacercaria pyriform, 310 in length and 162 in greatest breadth. Beset with cuticular spines. Oral sucker terminal, 30-38 in diameter. Head spines arranged in two rows around opening of oral sucker, of same size, 16 in each row. Acetabulum about as same size as the oral sucker, embedded in parenchyma, lying just posterior to middle of body.

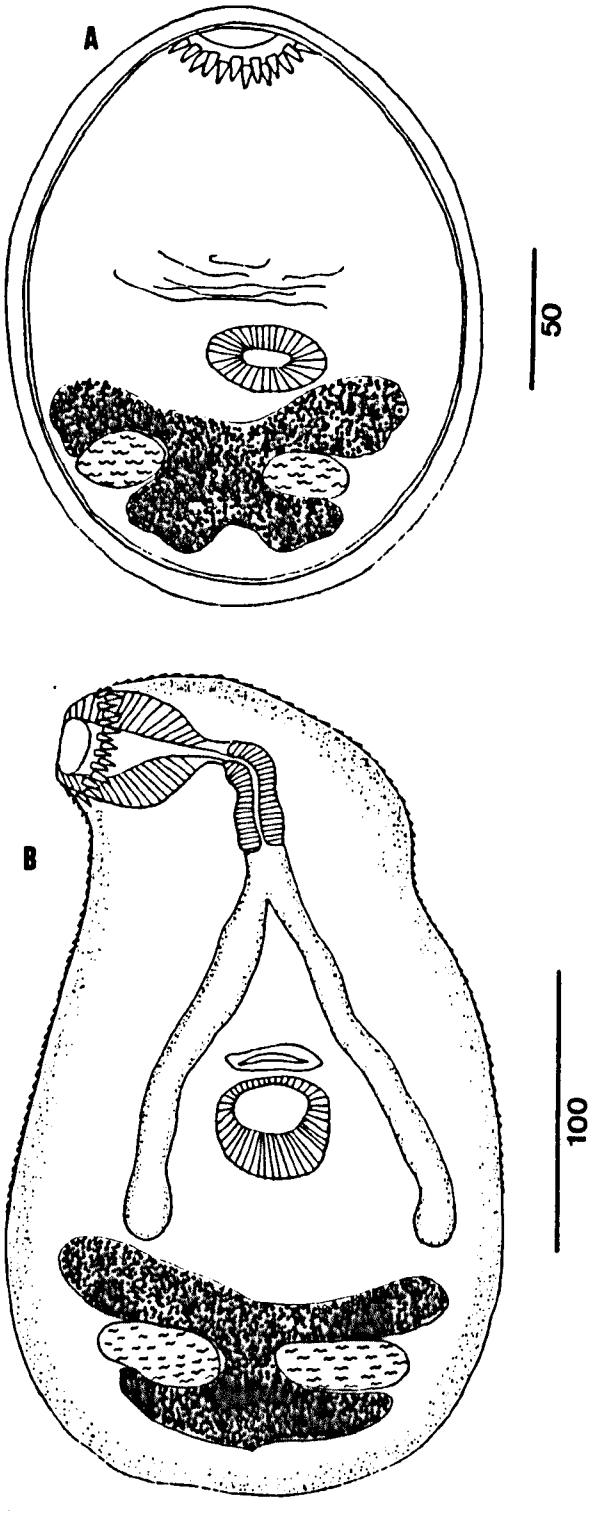


Figure 2.9: *Centrocestus* sp. (A) Metacercaria in cyst. (B) Excysted metacercaria.

Short pre-pharynx, long pharynx and short oesophagus present. Pharynx 30 long. Caeca terminating anterior to antero-lateral corners of excretory bladder; terminal ends are broader.

Testes elongate, elliptical, lying symmetrically lateral to excretory bladder; genital opening situated just anterior to acetabulum.

Excretory bladder 'V'-shaped; but pressure of testes give it an appearance of 'X'. Excretory corpuscles round in shape.

Identification:

Due to the lack of useful characters, the keys based on adult characters and family diagnoses (Yamaguti, 1971) were not helpful in ascertaining the identity of the specimens even to the family level.

The presence of: a small, flattened, pyriform, spined body; a terminal oral sucker with two alternate rows of circumoral spines; a pre-pharynx, short oesophagus and caeca terminating anterior to the testes; an acetabulum embedded in the parenchyma; symmetrical testes in posterior hindbody; a genital pore which is immediately pre-acetabular and 'V'-shaped excretory bladder compressed laterally suggest that this metacercaria belongs to the genus *Centrocestus* of the family Heterophyidae.

The characters of this metacercaria are very similar to the metacercaria of *Centrocestus formosanus* (Nishigory, 1924) Price, 1932 description given in Komiya (1964). Yamaguti (1975) considered *C. formsanus* as synonymous with *C. yokogawsi* Kobayashi, 1942. The equal cyst size, pyriform body shape, presence of 32 equal size circumoral spines in two rows and an oesophagus shorter than pharynx make the specimen most similar to the species descriptions cited by Yamaguti (1975). The glands in 5 longitudinal rows on the ventral side and opening outside through the tegument may have been overlooked. This species is reported from brackish waters of inland seashore in Japan (Nashigori, 1924 cited by Yamaguti, 1975). Nashigori

(1924) reported the first intermediate host as *Melanoides* (*Melania*) spp., while Chen (1950) cited by Yamaguti, (1975) reported them through *Melanoides* (*Melania*) *tuberculata chinensis* (Muller, 1774). *M. tuberculata* were very abundant in Koggala lagoon where the metacercariae were found.

Though it may not be *C. formosanus*, it can be concluded that this metacercaria belongs to the same genus. Adult specimens are necessary for further identification.

E. Family: Cyathocotylidae Poche, 1926

Cyathocotylidae gen. sp. A

Metacercaria A (Figure 2.10A-B)

Host: *E. suratensis* (Bloch) and *O. mossambicus* (Peters)

Site of infection: abundant in muscle, occasionally a few in liver and mesentery

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Prevalence, Mean intensity (Number of fish surveyed): Udawalawa reservoir, *E. suratensis* 98.7 %, 21.3 (78): Koggala lagoon *E. suratensis* 91.5 %, 8.4 (94); *O. mossambicus* 93.3 %, 3.1 (15): Bolgoda lagoon *E. suratensis* 85.7 %, 5.4 (14)

Description (based on 30 specimens; measurements of metacercaria for the three localities and two different hosts, *E. suratensis* and *O. mossambicus*, are given in the Table 2.5).

Cyst globular 385 x 375 (312-448 x 296-440); wall hyaline, double layered; outer layer 4-8 and inner layer 2 thick. Cyst enclosed in a cream coloured fibrous capsule of host origin, 406 x 396 (344-444 x 320-440). Between larva and cyst wall large space containing numerous excretory droplets of varying size.

Metacercaria dorsoventrally flattened, free in cyst, oval to pyriform, 423 (280-448) in length and 234 (164-256) at the greatest width. Lacks spines. Ventral concavity exists in the anterior two thirds of body. Oral sucker terminal, width higher than the length, 42 (32-50) in diameter and 25 (22-35) in length. Pre-pharynx absent. Pharynx 27 (24-32), similar in length to oral sucker. Oesophagus 36 (20-42). Caeca

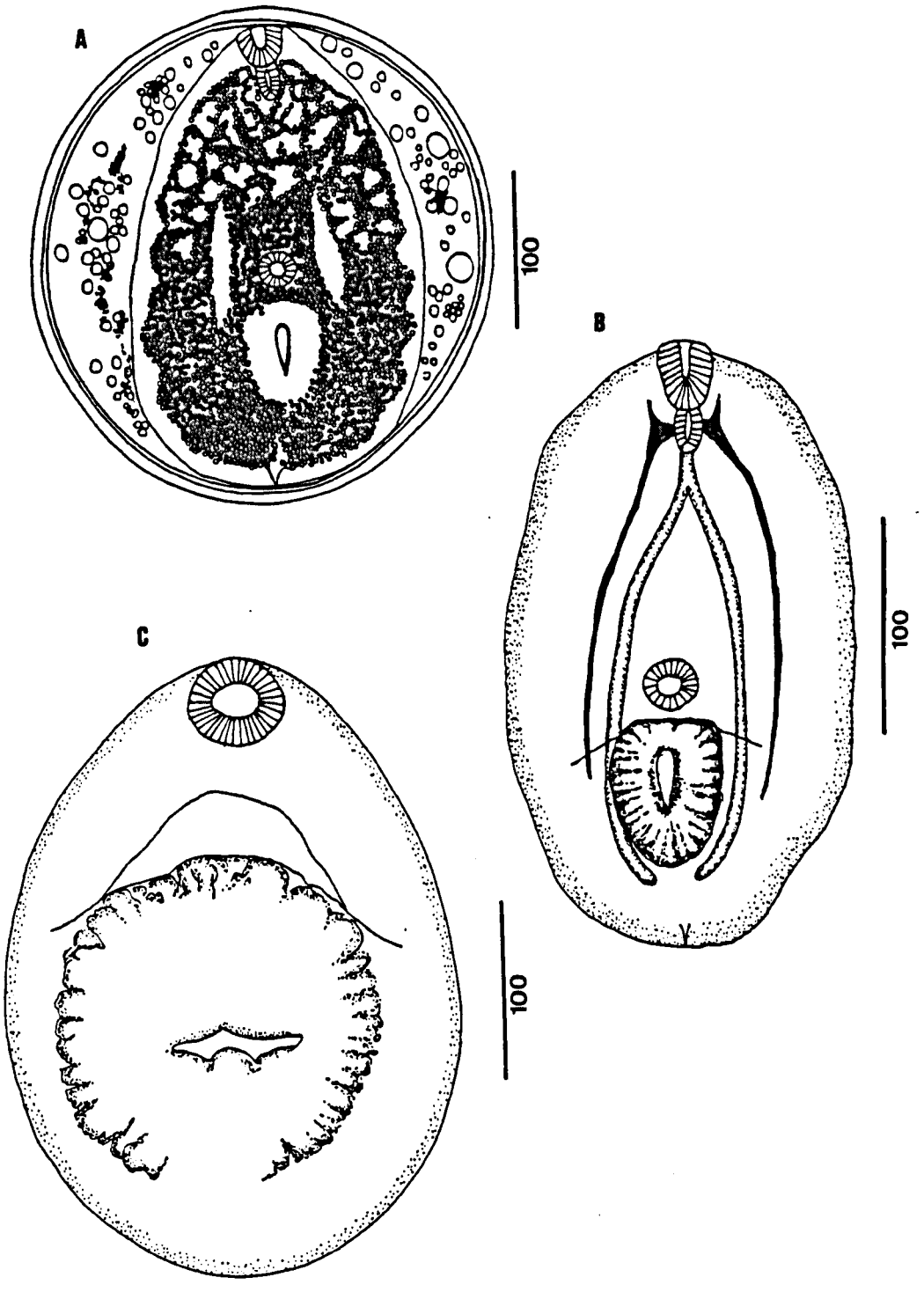


Figure 2.10: Cyathocotylid metacercaria A and B. (A) Metacercaria A in cyst. (B) Excysted metacercaria A (excretory granules removed by fixation with glacial acetic acid). (C) Excysted metacercaria B (excretory granules removed by fixation with glacial acetic acid).

narrow, terminate near posterior extremity. Acetabulum smaller than oral sucker, 28 (20-36), in diameter and lies at the anterior end of tribocytic organ. Tribocytic organ 78 x 81 (65-86 x 60-96), situated in posterior third of body, circular to rectangular in shape, when closed the slit is longitudinal, contains numerous radially arranged glandular cells. Genital anlage posterodorsally to tribocytic organ.

The ducts of the excretory system are very much widened and filled with round excretory corpuscles thus making one very conspicuous pair of ducts and one ill-defined pair around the tribocytic organ in the posterior two third of body. These form a network of anastomosing tubules in the anterior third of the body. Posteriorly these two pairs of tubes open into the arms of 'Y'-shaped very small excretory bladder and this opens outside with the pore on the posterodorsal side of the body. The tubules arising from the flame cells and opening to the wide ducts could not be observed. Nerve commissure dorsal to oesophagus, gives rise to two anterior and two posterior branches.

Identification:

The presence of a tribocytic organ, 'W'-shaped wide excretory ducts and the lack of the lappets suggests the Family Cyathocotylidae. According to Yamaguti (1971), this family has four subfamilies. The presence of pyriform shaped body and the small tribocytic organ place these specimens in the subfamily Prohemistominae. The absence of a strongly developed, massive tribocytic organ suggests a similarity with the genera *Prohemistomum* or *Mesostephanus*. The structure of adult specimens is required for further identification.

With only the structure of the metacercaria available, where even the traces of reproductive organs are absent, it is not wise to ascribe them to a genus. This is especially difficult in the cyathocotylid metacercaria since a bipartition can develop such that the posterior portion becomes enlarged with the development of reproductive organs in the later stages of development towards adult.

Cyathocotylidae gen. sp. B

Metacercaria B (Figure 2.10C)

Host: *E. suratensis* (Bloch)

Site of infection: muscle

Locality: Bolgoda lagoon

Prevalence, Mean intensity (Number of fish surveyed): *E. suratensis* 14.3 %, 1.0 (14)

Description (based on 2 specimens which were all that were available)

Cyst of parasite origin, globular, 318.5 x 295 (314-323 x 292-298); wall hyaline, appeared to be single layered, 3 thick; enclosed in fibrous capsule of host origin. With host capsule, 336 x 306 (320-352 x 300-312). Only very little space existed between larva and cyst wall and this contained numerous globular granules.

Metacercaria dorsoventrally flattened, oval to pyriform, 313 (262-364) in length and 246 (224-268) at the greatest width. Lacks spines on tegument. Oral sucker terminal 48 (41-55) in diameter. Pre-pharynx, pharynx, oesophagus not seen. No visible trace of acetabulum. Trybocytic organ occupying large area of ventral surface 172 X 160 (140-204 x 132-188), circular in shape, when closed slit is horizontal and contains numerous radially arranged glandular cells.

Excretory system similar to that of metacercaria A. Ducts of the excretory systems filled with round excretory corpuscles, thus enlarged to give two wide ducts, one distinct and other ill defined which fuse with each other in the anterior third with sparse network of joining tubes. Excretory bladder 'V'-shaped, reduced to simple bridge connecting posterior ends of wide tubes with dorsoterminal excretory pore.

Worm compressed in the longitudinal direction such that head bent ventrally. Absence of acetabulum (or hardly discernable) and presence of very large tribocytic organ, covering two thirds of ventral surface, makes metacercaria B different from metacercaria A.

Table 2.5 : Measurements of the cyathocotylid metacercariae from muscles of *Etroplus* and *Oreocromis* species.

Type of metacercaria	Metacercaria A				Metacercaria B
Host and Locality	*E. suratensis* Udawalawa	*E. suratensis* Koggala	*Oreocromis* spp. Koggala	*E. suratensis* Bolgoda	*E. suratensis* Bolgoda
Number of specimens measured	15	15	10	8	2
Capsule - length	398 (344 - 440)	403 (352 - 444)	418 (340 - 488)	402 (360 - 424)	336 (320 - 352)
- width	384 (320 - 440)	392 (332 - 440)	395 (328 - 456)	389 (324 - 424)	306 (300 - 312)
Cyst - length	376 (312 - 448)	384 (336 - 440)	397 (320 - 456)	388 (336 - 418)	319 (314 - 323)
- width	368 (296 - 424)	375 (320 - 440)	382 (320 - 448)	369 (320 - 384)	295 (292 - 298)
Cyst - outer wall	4 - 8	4 - 8	3 - 6	4 - 6	3
- inner wall	2	2	2	2	
Parasite - length	417 (280 - 448)	398 (328 - 440)	410 (300 - 456)	384 (288 - 416)	336 (262 - 364)
- width	237 (164 - 252)	227 (180 - 256)	235 (168 - 252)	228 (160 - 248)	306 (224 - 268)
Oral sucker - diameter	38 (32 - 44)	42 (36 - 50)	43 (38 - 48)	39 (32 - 45)	48 (41 - 55)
- length	29 (22 - 34)	30 (25 - 35)	28 (24 - 32)	28 (20 - 32)	54
Pharynx - length	28 (24 - 30)	27 (24 - 32)	28 (24 - 35)	26 (22 - 30)	-
Oesophagus - length	31 (20 - 35)	35 (25 - 42)	38 (24 - 50)	33 (25 - 35)	-
Acetabulum - length	26 (20 - 34)	26 (20 - 32)	28 (24 - 35)	27 (25 - 35)	-
- width	29 (28 - 36)	30 (24 - 36)	33 (30 - 40)	28 (24 - 32)	-
Adhesive organ - length	74 (68 - 86)	78 (65 - 82)	84 (65 - 90)	76 (72 - 85)	172 (140 - 204)
- width	82 (60 - 96)	82 (70 - 90)	83 (68 - 92)	80 (70 - 96)	168 (112 - 138)

Measurements of *E. suratensis* from Udawalawa and Koggala were combined in the description.

Identification:

The characters exhibited by these specimens were similar to those in metacercaria A. The round shape and the presence of an extremely large tribocytic organ led these specimens to the subfamily Cyathocotylinae. The large size of the tribocytic organ and the absence of an acetabulum suggests that this may belong to the genus *Cyathocotyle.* However, the adult structure is necessary for further identification.

F. Family: Diplostomidae Poirier, 1886

Diplostomatidae gen. sp.

Metacercaria (Figure 2.11)

Host: *E. suratensis* (Bloch)

Site: muscle

Locality: Udawalawa reservoir

Prevalence, Mean intensity (Number of fish surveyed): *E. suratensis* 2.6 %, 1.0 (78)

Description: (based on two specimens which were all that were available)

Cyst oval, thin walled, burst due to pressure applied on muscle.

Metacercaria leaf-shaped, 1127 x 406 (1072-1184 x 384-432), division of body into anterior and posterior parts not distinct. Long, narrow part posterior to adhesive organ however would elongate later to form the hind portion. Lappets on either side of oral sucker, feebly muscular, 88 (86-93) long when relaxed. Tegument lacks spines. Oral sucker terminal, diameter 47 (43-51), length 41 (40-42). Pre-pharynx absent. Pharynx barrel-shaped 39 (38-40), about same length as oral sucker. Oesophagus short, 18 (16-20). Caeca narrow, terminate at extreme posterior. Acetabulum 38 x 34 (33-44 x 31-37), lies at the mid point of the total body length.

Trybocytic organ longitudinally elongated, pyriform, 173 x 131 (163-184 x 125-138), with longitudinal median slit, lies little posterior to acetabulum. Anterior part and portion immediately around slit stained darker than posterior region with

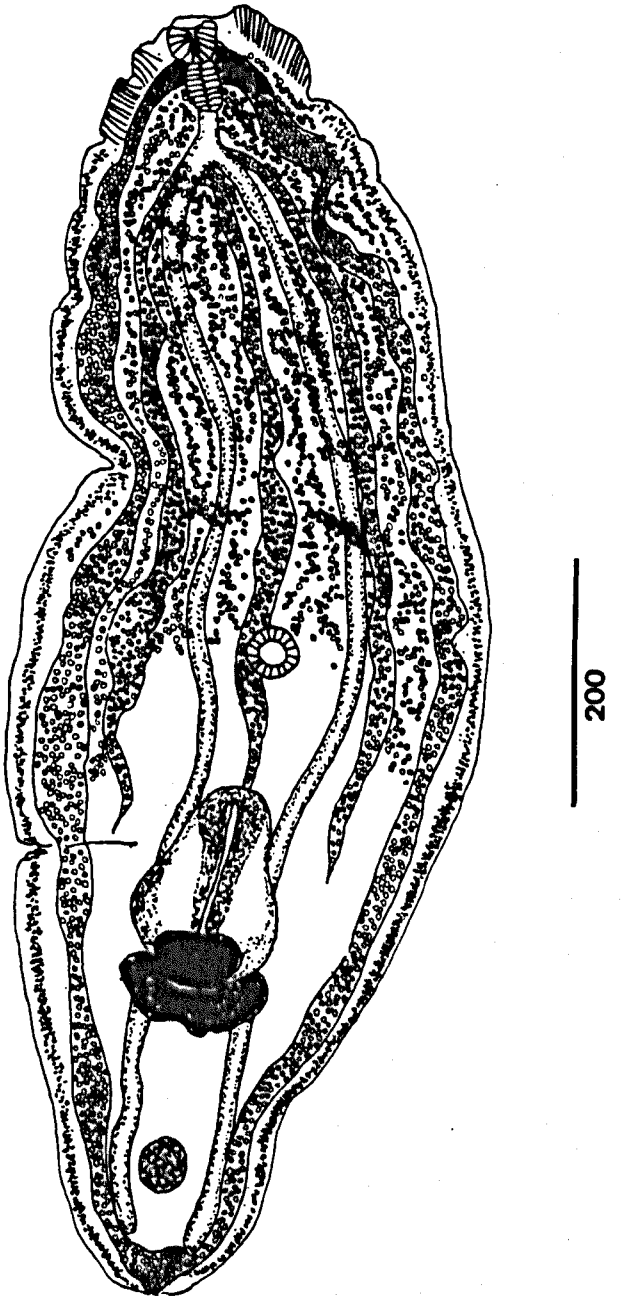


Figure 2.11: Diplostomid metacercaria, excysted.

Mayer's paracarmine stain. Tribocytic gland bilobed, tandemly arranged, both transversely elongated and lie at posterior end of tribocytic organ. Posterior end of organ being covered by first lobe of tandemly arranged tribocytic gland. Genital anlage present medially, just anterior to caecal termination.

Primary excretory tubules enlarged and five were found in pre-acetabular region (one median and two lateral pairs). These tubules joining in region of pharynx and oesophagus. Most lateral pair extended to posterior region and opened into excretory bladder. Median tubule extended into holdfast organ. Innermost pair seem to join with lateral pair anterior to acetabulum. In addition, tubules give rise to branches to form a peripheral reticular network in the anterior half of the body.

Identification

The presence of a leaf-shaped body, lappets, tribocytic organ with compact glands at its base and the excretory system with enlarged tubules which are reticular in the periphery, placed these metacercaria in the family Diplostomidae (Yamaguti, 1971). The metacercariae of the family Strigeidae are more common in muscles and show a similar structure, except that the excretory system in the Strigeidae is in the form of a network with prominent lacunae.

According to Yamaguti (1975), there are only a few species of diplostomids selecting muscle as the site of infection. Of the diplostomids possessing lappets and choosing muscle as a habitat, *Bolbophorous* seems to be the most likely genus to which these specimens can be ascribed. According to Yamaguti (1971) the metacercaria of this genus is characterized by a trilobate anterior end, five excretory tubules and a bipartite tribocytic organ. If the difference in staining characters represent bipartition of tribocytic organ, all these characters demonstrate the affinity to the genus *Bolbophorous*. However, the adult structure is necessary for a confirmation.

Bolbophorous confusus levantinus Paperna & Lengy, 1963 was used to experimentally infect *Tilapia nilotica* (*Oreochromis niloticus*) (Paperna & Lengy, 1963

cited by Yamaguti, 1971). *Bolbophorous indiana* Mehra, 1962 was first described in India in the bird *Anhinga melanogaster* (Gmelin, 1789) (cited by Yamaguti, 1971). This bird was seen occasionally in the vicinity of Udawalawa reservoir.

G. Family: Strigeidae Railliet, 1919

Strigeidae gen. sp.

Mesocercaria (Figure 2.12A-B). (The stage in between the entering into the second intermediate host and encysting).

Host: *E. suratensis* (Bloch)

Site of infection: Abdominal cavity, moving on the surface of the organs of the alimentary canal

Locality: Udawalawa reservoir

Prevalence, Mean intensity and (Number of fish surveyed): 7.9 %, 4.8 (101); none of the mesocercariae were found in the survey samples, but in a sample of 28 fish collected on 03.07.91 for tissue removal for histology, data for this sample was 34.8 %, 4.8 (28)

Description: (based on 3 small and 8 large specimens which were the only specimens available)

Smaller specimens oblong in shape, 824 x 368 (786-918 x 300-436), similar in structure to large specimens. Oral suckers 44 x 56 (42-51 x 54-58); pharynx 38 (34-43). Oesophagus short and caeca terminating near posterior end. Acetabulum equatorial, diameter 56 (51-58). Tribocytic organ oval, longer than wide, 173 x 135 (160-179 x 102-147). Tribocytic gland in posterior region of tribocytic organ, circular shape, 52 x 66 (58-68 x 60-74). Genital anlage present posteromedially to tribocytic organ. Excretory tubules very sparsely filled with excretory granules, not clear.

Larger specimens oval to pyriform, 1344 x 978 (1040-1536 x 880-1296), dorsoventrally flattened. Ventral concavity exists in anterior two third of body. Oral sucker terminal, higher than broad, 74 x 92 (48-83 x 61-106). Pre-pharynx absent. Pharynx long 65 (58-90), oesophagus of approximately same length. Caeca narrow, terminate near posterior extremity either side of genital anlage. Acetabulum slightly

larger than oral sucker, 86 x 80 (70-93 x 77-83), lies equatorially far above tribocytic organ. Tribocytic organ situated anteriorly in posterior third of body, oval in shape with slit opening horizontally, 215 x 196 (192-224 x 160-282). Tribocytic gland oval in shape, situated in position such that half of its full length overlapping posterior region of tribocytic organ, 85 (70-102) long and 107 (90-12) wide. Genital anlage present medially, posterior to tribocytic gland. Primary tubules of excretory system very sparsely filled with excretory granules, therefore edges not clear.

Metacercaria (Figure 2.12C-D)

Host: *E. suratensis*

Site of infection: body cavity, cysts attached to mesentery and gut wall

Locality: Udawalawa reservoir

Prevalence, Mean intensity (Number of fish surveyed): 41.0 %, 4.5 (78), excluding the metacercaria found in 03.07.91 sample

Description: (based on 12 specimens)

Cyst elliptical to oval shape, sometimes elongated, varied widely in size, unflattened measured 1375 x 895 (838-1764 x 705-1162). Outermost covering fibrous, semi-transparent, yellowish white and granular in appearance, 86 (60-125) in thickness. Inner to this layer gelatinous material deposited layerwise forming hyaline cyst of parasite origin with varying thickness of 409 (200-550).

Metacercariae oval in shape, not flattened dorso-ventrally, opaque white (due to presence of granules in the excretory system), sluggish, even when released mechanically they were inactive, 636 x 547 (454-856 x 405-608). When flattened by external pressure measured 853 x 596 (706-924 x 462-684).

Oral sucker terminal, diameter 58 (51-65), height 65 (58-75). Per-pharynx absent; pharynx elliptical; oesophagus short. Caeca slender, convergent behind tribocytic organ. Acetabulum equatorial, larger than oral sucker, 76 x 69 (65-103 x 64-97). Lappets posterolateral to oral sucker, 156 x 117 (123-185 x 96-137). Trybocytic organ elliptical 175 x 187 (129-226 x 148-226), with horizontal central opening.

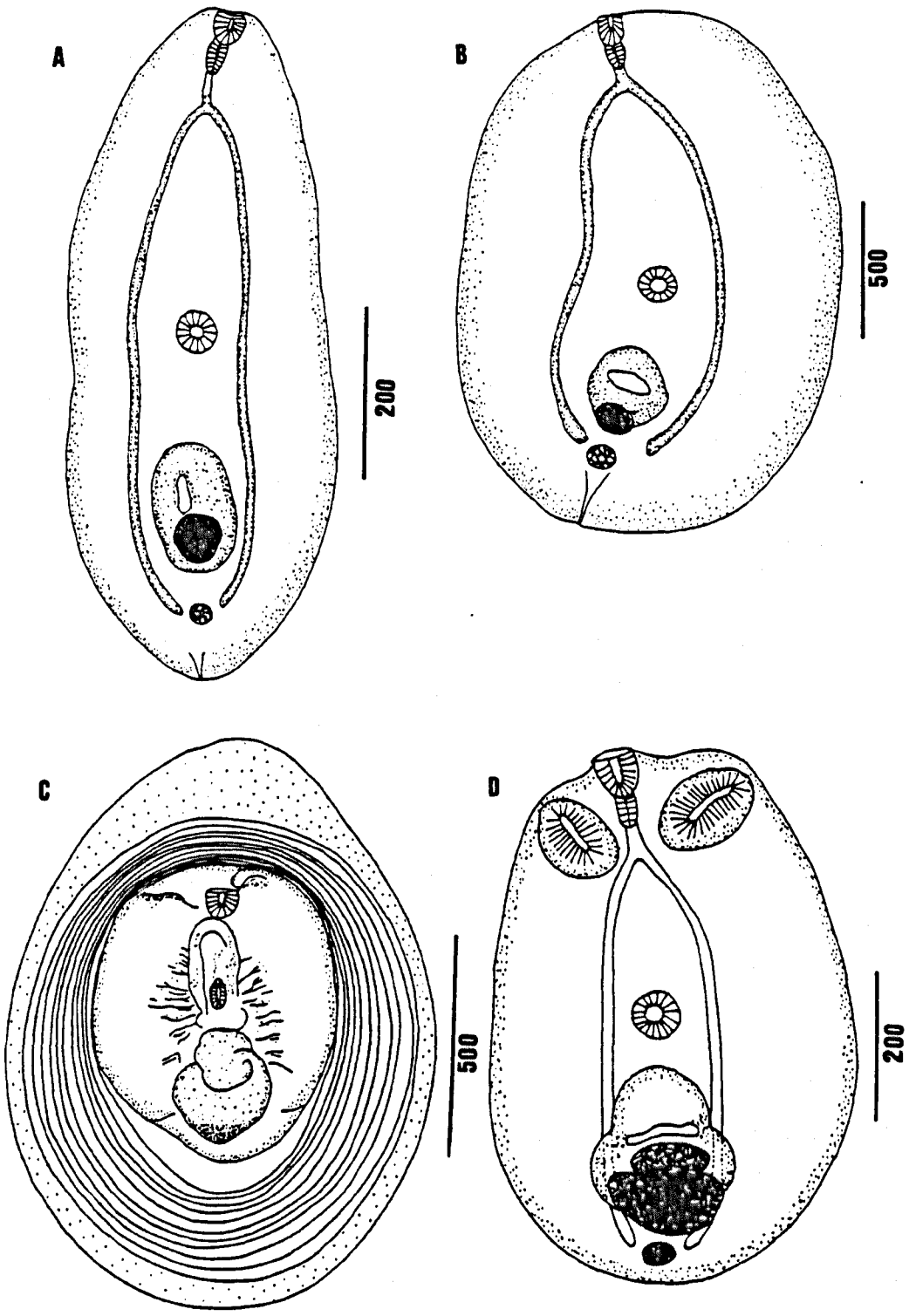


Figure 2.12: Strigeid mesocercaria and metacercaria. (A) Small mesocercaria. (B) Large mesocercaria. (C) Encysted metacercaria. (D) Excysted metacercaria.

Divided into two parts in region of slit to anterior smaller portion compared to posterior larger portion. Tribocytic gland consists of two distinct lobes arranged in tandem, oval, transversely elongated. Usually both or sometimes the anterior overlap with the tribocytic organ. Genital anlage medial, posterior to tribocytic organ and between two caecal ends. Excretory system consisting of extensive network and more or less prominent lacunae.

Identification:

Presence of acetabulum, trybocytic organ, two lappets on either side of oral sucker and excretory system forming extensive network and more or less prominent lacunae placed these metacercariae in the family Strigeidae. The metacercariae show similar characters to the Diplostomidae but differ in the structure of excretory system and the presence of prominent lacunae placing the specimens in the family Strigeidae. The absence of a distinct division of the body into anterior and posterior parts is considered to be one of the characters distinguishing larval metacercaria of Strigeidae (Bykhovskaya-Pavlovskaya, 1962), supporting this diagnosis identification.

Reproductive organs represented by a single mass of heavily staining cells posterior to the tribocytic organ is another. Also in some mesocercaria the cyst of parasite origin is hyaline and consists of a gelatinous material. The presence of these common features of strigeid larva in the metacercariae and mesocercariae suggests that the specimens belong to the same family. The presence of mesocercariae (free living stages in the second intermediate host) is a common feature of this family. Metacercariae were smaller than the mesocercariae, the possible reason being the production of a large quantity of secretion which makes up the thick cyst wall.

The body shape of the adult worms and the structure of reproductive organs are required for further identification.

H. Family: Transversotrematidae Yamaguti, 1954

Transversotrema patialense Soparkar, 1924

Cercaria (Figure 2.13 A-B)

Host: *Melanoides tuberculata*

Locality: Koggala lagoon

Description: (based on 12 specimens)

Cercaria bi-oculate, furcocercous, with precocious sexual development. Body wider than long, 348 x 641 (262-416 x 448-768), appearance similar to that of adult. Oral sucker absent. Pharynx diameter 55 (42-64). Acetabulum 108 (93-117) in diameter. Internal structure similar to that of adult, reproductive structures almost fully developed.

Tail attached postero-dorsally to posterior extremity, 470 (403-582) long, 111 (100-122) wide, equal in width, divided into 2 furcae of equal width. Furcae shorter than tail stem, 256 (224-283) long, 88 (77-105) wide. Pair of appendages at base of tail with adhesive pads at distal ends, 220 (205-238) long with pad, stalk 60 (51-69) wide. Cercaria rests with tail end directed vertically upward and body reflexed at base of tail to turn upwards too.

Adult (Figure 2.13C)

Host: *E. suratensis* (Bloch), *O. mossambicus* (Peters) and *O. niloticus* (Linnaeus)

Site of infection: Under scales of fish

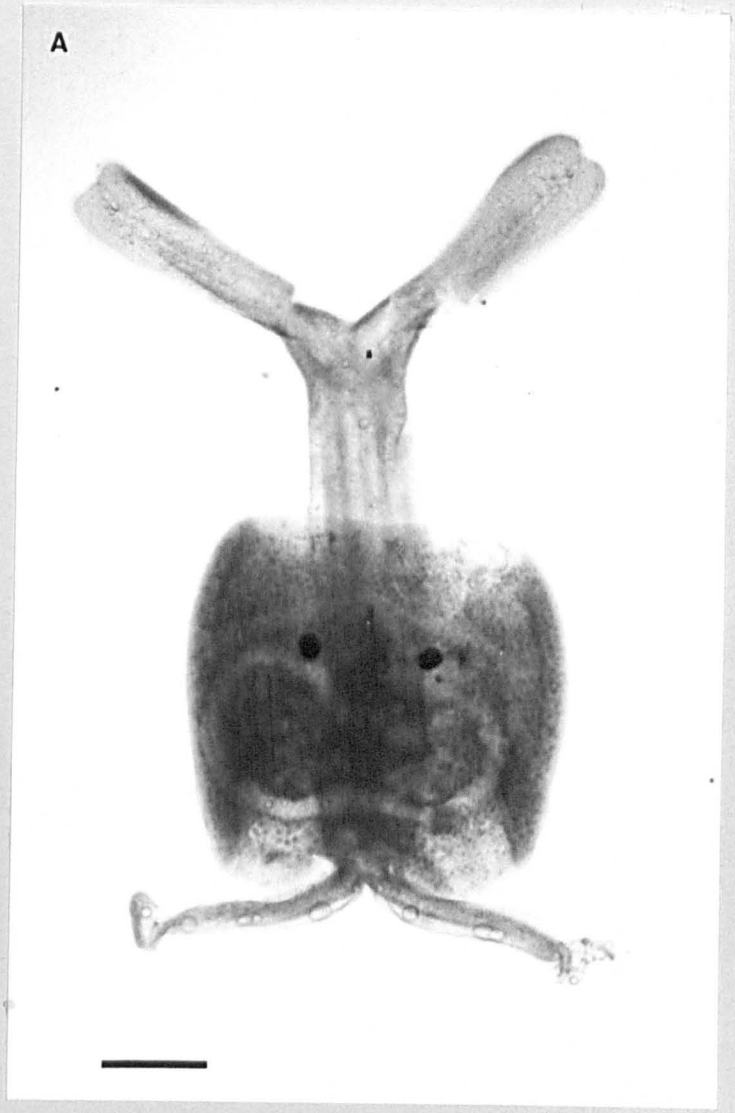
Locality: Koggala and Bolgoda lagoons, Udawalawa reservoir

Prevalence, Mean intensity for 10 cm^2 body surface area (Number of fish surveyed): Koggala lagoon *E. suratensis* 37.0 %, 1.7 (100); *O. mossambicus* 46.7 %, 2.4 (15): Bolgoda lagoon *E. suratensis* 28.6 %, 1.5 (14): Udawalawa reservoir *O. niloticus* 7.1 %, 1.0 (14)

Description (based on 10 specimens)

Leaf-like in form, transversely elongate with anterior margin straight or slightly convex and posterior margin highly convex, 429 x 878 (320-528 x 576-1200), with concave ventral and convex dorsal surfaces. Body covered with spines. Eye-spots 19 (13-26). Oral sucker absent, mouth opening directly to pharynx, 86 x 75 (61-112 x 51-99). Acetabulum pedunculate lying just posterior to pharynx, diameter 115 (86-125). Narrow oesophagus bifurcate, a little anterior to middle of body, giving rise to

Figure 2.13: *Transversotrema patialense.* (A) Cercaria in resting position. (B) Cercaria. (C) Adult. (Stained with Mayer's Paracarmine)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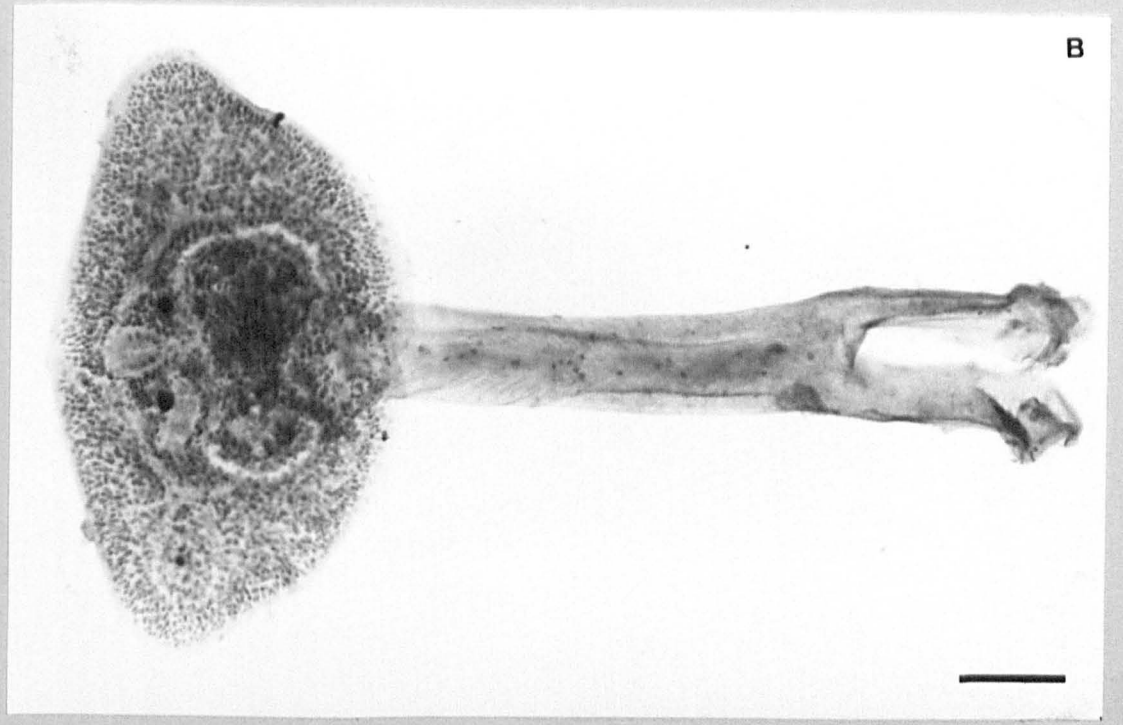
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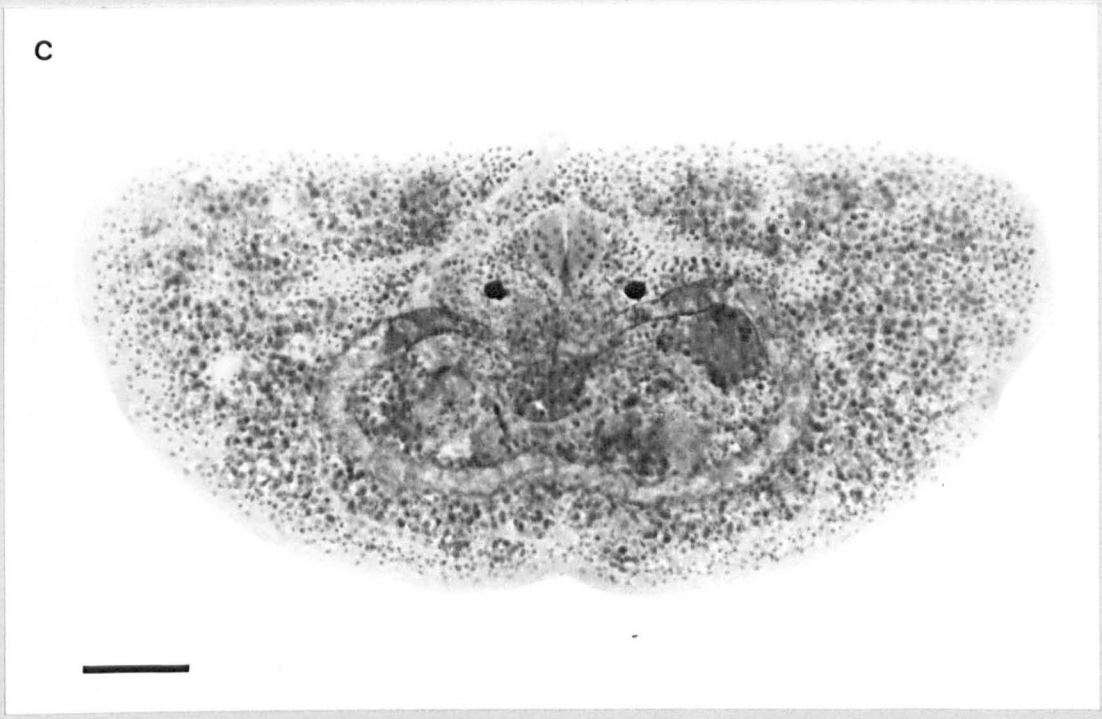

C

branches uniting posteriorly.

Lobed testes intercaecal, posterolateral, 72 x 146 (64-78 x 138-155). Seminal vesicle lies laterally to right eye-spots sometimes compressed by intestinal caecum. Vas deferens opens to common genital atrium present on the right side near anterior margin. Ovary lies in left anterolateral corner of intercaecal field, contains germinal follicles, 68 (58-86). Laurer's canal distinct, opens on ventral surface, left side, posterolaterally and intercaecally. Uterus with transverse and ascending parts; transverse part descends to inter-testicular area obliquely anterior to left testis then ascends anterior to right testis obliquely proceeding to common genital atrium laterally to vas deferens. Only one egg was found in proximal part of uterus of one specimen, 74 x 37. Vitellaria extracaecal covering most of area, few follicles remain median to lateral loops of intestine. Vitelline reservoir in middle of body.

Excretory system could not be traced in detail in fixed specimens. The collecting tube of the system connected anteriorly to bladder, ascends extracaecally, turning laterally at mid level to form a wide loop encircling vitellaria. Then it turned posteriorly at the mid level to open into bladder again. Before it turned posteriorly, it gave rise to a secondary tube which had three branches, one turning towards oral sucker being widest out of three, and with almost uniform breadth.

Identification:

The presence of: transversely elongated body; ventral acetabulum; no oral sucker; posteriorly united caecal branches; approximately median genital pore on the anterior margin of the body; vitellaria follicular, extensive, mostly extracaecal placed these specimens in the genus *Transversotrema* Witenberg, 1944.

These *Transversotrema* specimens differ greatly from *T. haasi* Witenberg, 1944 and *T. licinum* Manter, 1970 by their shape and distribution of vitelline follicles. In addition, the size of the acetabulum of *T. haasi* is equal in size to the pharynx. *T. chauhani* Agrawal & Singh 1981 does not have intercaecal vitelline follicles. Amongst the other species described, clear cut differences did not exist and proportions of their

Table 2.6 : Measurements of cercaria and adults of some *Transversotrema* spp.

	Transversotrema patialense	*Cercaria koliensis**	*Transversotrema laruei*	*Cercaria soparkari**	*Cercaria chackai**	Specimens from *E. suratensis* (10 specimens)
Cercaria	Soparkar (1924)	Olivier (1947)	Velasquez (1961)	Pandey (1971)	Nadakal et.al. (1969)	
Body length	374 - 459	240 - 310 (270)	280 - 430	200-330	440 - 590 (505)	262 - 416 (348)
Body width	697 - 782	260 - 490 (350)	480 - 700	390-490	720 - 1,020 (835)	448 - 768 (641)
Diameter of pharynx	58 - 78	45 - 55	50	40-50	50 - 80 (60)	42 - 64 (55)
Diameter of acetabulum	135	94 - 110	90 - 120	120-130	100 - 145 (130)	93 - 117 (108)
Length of tail-stem	580 - 595	370 - 430 (400)	400 - 430	320-510	500 - 630 (567)	403 - 582 (470)
Width of tail-stem	102	—	90 - 120	60-80	110 - 160 (136)	100 - 122 (111)
Furcal length	340 - 374	200 - 260 (240)	180 - 250	120-180	300 - 360 (313)	224 - 283 (256)
Furcal width	135	—	70 - 90	70-80	90 - 110 (103)	77 - 105 (88)
Caudal appendage length	220 - 306	160 - 180	120 - 180	120-180	240 - 280	205 - 238 (220)
Caudal appendage width	110 -130	—	30 - 50	20-60	60 - 90 (76)	51 - 69 (60)
Adult	Crusz *et al.*(1964)		Velasquez (1975)			
Body length	368 - 560		230 - 550 (390)			320 - 528 (429)
Body width	640 - 1072		460 - 900 (480)			576 - 1200 (878)
Diameter of pharynx	56 - 96		40 - 50 (45)			56 - 106 (75)
Diameter of acetabulum	96 - 112		70 - 90 (80)			86 - 125 (109)

* No adult specimens were described

body sizes and structure of the excretory system seemed little different from each other. However, according to Olivier (1947), it was unlikely that species so closely similar in all other internal characters would have excretory tubular patterns that differ markedly.

Nadakal, Mohandas & Sunderaraman (1969) used body sizes of the cercarial stage as one criterion for differentiating species. Measurements of cercaria released by *Melanoides tuberculata* from the same locality largely agreed with the measurements of *T. laruei* Velasquez, 1958 or were slightly larger or intermediate between *T. laruei* and *T. patialense* Soparkar, 1924. Only the measurement of the caudal appendage length directly disagreed with *T. laruei* and agreed with *T. patialense* (Table 2.6). In contrast to the cercaria, all the measurements of the adult specimens agreed with measurements of *T. patialense* adults, particularly the diameters of the pharynx and the acetabulum, which were more similar to *T. laruei* in cercarial stage. When the width to length proportions were considered, specimens from *E. suratensis* agreed with that of *T. patialense*, which is wider than *T. laruei.*

The mollusc intermediate host for the cercaria of *T. patialense* is *M. tuberculata* (Crusz *et al.*, 1964) and that for *T. laruei* is *Thiara riquetti* (Velasquez, 1975). Since *M. turberculata* released cercariae, there is a high probability that the specimens belong to *T. patialense.* As with *T. laruei* (Velasquez, 1961), *T. patialense* seems to tolerate slightly brackish conditions, behaving normally in water at a salinity of approximately 7 ‰ and below (Mills, 1979). The cercaria emitted by *M. tuberculata* from the lagoon, readily infected *E. suratensis* and *O. mossambicus* experimentally. The measurements of the adults obtained by the cercarial infection agreed with that of the adults found in the fish parasite survey (see Section 2.3.2.1(b)). Therefore it is reasonable to place the specimens of the present study under the species *T. patialense.*

I. Family: Waretrematidae Srivastava, 1937

Malabarotrema indica Zhukov, 1972

Adult (Figure 2.14)

Host: *E. suratensis* (Bloch) and *O. mossambicus* (Peters)

Site of infection: Intestinal cavity

Locality: Koggala and Bolgoda lagoons

Prevalence , Mean intensity (Number of fish surveyed): Koggala lagoon *E. suratensis* 78.7 %, 6.4 (94); *O. mossambicus* 26.7 %, 2.7 (15): Bolgoda lagoon *E. suratensis* 78.6 %, 7.2 (14)

Description: (based on 15 specimens)

Body elongate pyriform, length 845 (626-968), maximum width 298 (196-

f 2.14356) at equatorial level. Tegument armed with spines in anterior fourth of body. Eye-spots dispersed in neck region.

Oral sucker subterminal, 125 x 117 (94-134 x 107-126); pre-pharynx 134 (74-160), long and thin; pharynx enormous, 107 x 114 (86-135 x 106-127), strongly muscular, intestine without bifurcation, caecum relatively wide posteriorly, terminating anterior end of posterior third of body or above. Acetabulum very large, strongly muscular, diameter 143 (116-155), situated at anterior end of middle third of body.

Testis single, elliptical to oblong-shaped, 186 x 95 (120-240 x 74-118), situated in posterior third, slightly towards right side. Hermaphroditic pouch elongate, pyriform, extended dorsally to acetabulum to genital atrium which is situated anterior to acetabulum, enclosing internal seminal vesicle, prostatic complex, metraterm, and hermaphroditic duct. Seminal vesicle bipartite, anterior, internal part oval to round, 38 x 37 (32-48 x 22-43), external, posterior part round, 65 x 54 (38-80 x 35-77). Genital pore median, close to anterior border of acetabulum.

Laurer's canal present; seminal receptacle adjacent to ovary. Ovary globular, entire, 85 x 74 (45-106 x 32-96), median, near anterior third of testis. Uterus winding anterior to ovary and enters hermaphroditic pouch, contains 1-30 eggs in uterus. Eggs, oval, sculptured with ridges, unembryonated, 78 x 47 (56-94 x 30-55). Vitellaria follicular, extensive, compact, extending posteriorly from level of anterior end of

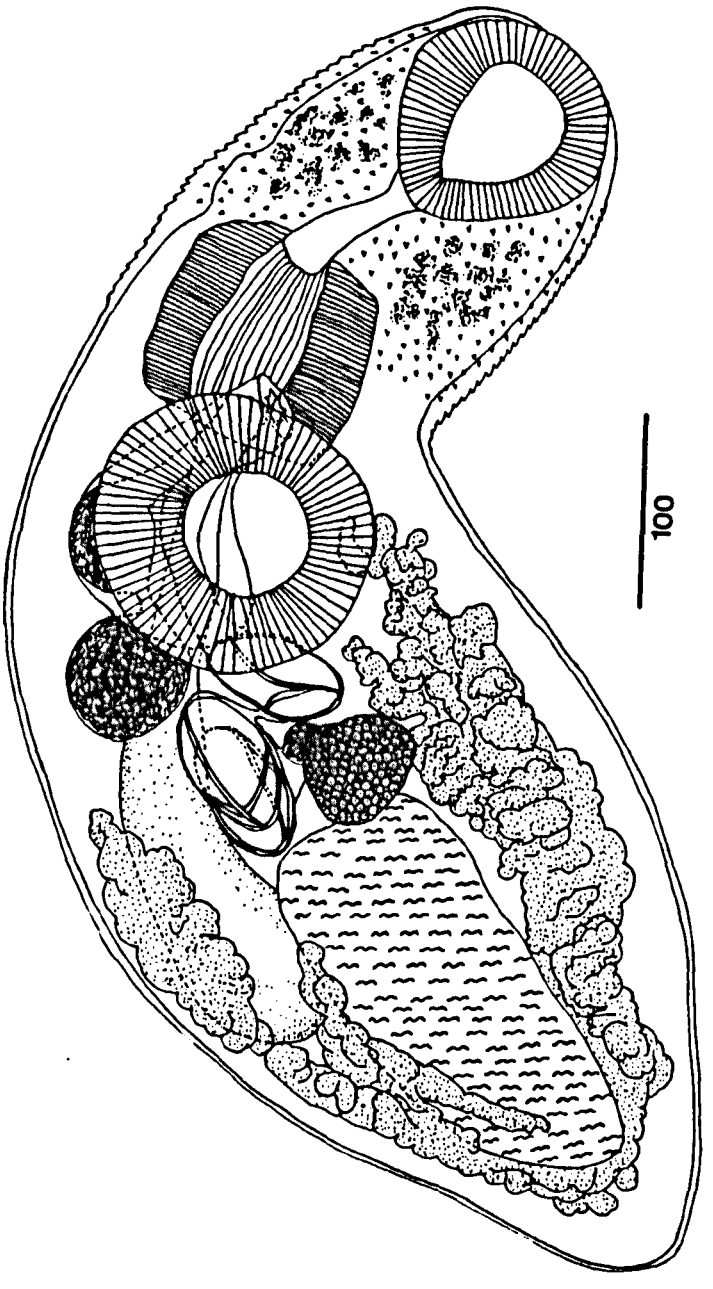


Figure 2.14: *Malabarotrema indica*, adult.

Table 2.7 : Measurements of *Malabarotrema indica.*

	Zhukov (1972)	Specimens from Koggala lagoon
Length	1000-1200	624-960
Width	280-370	192-352
Oral sucker	83-120 x 110-120	94-128 x 96-122
Pharynx	110-140 x 80-110	83-128 x 72-117
Acetabulum	120-150 x 140-170	113-150 x 106-154
Prepharynx	120-170	74-160
Caecum	250-370 x 120-160	220-416 x 48-112
Testis	210-300 x 120-140	120-240 x 74-118
Internal seminal vesicle	-	32-48 x 22-43
External seminal vesicle	-	38-80 x 35-77
Ovary (diameter)	62-83	45-106 x 32-96
Eggs	71-79 x 39-43	56-94 x 30-50

testis, confluent posterior to testis.

Identification-

According to keys and the family diagnoses given in Yamaguti (1971), the presence of: complex hermaphroditic pouch with internal seminal vesicle, prostatic complex, metraterm, and hermaphroditic duct; genital atrium opening between pharynx and acetabulum; external seminal vesicle; uterus between ovary and hermaphroditic pouch; unembryonated uterine eggs; and extensively diffuse tubular vitellaria placed these specimens in the family Waretrematidae. The presence of a single testis, tubular vitellaria confined to the posterior region of the body and the rather short caeca placed these in the subfamily Waretrematinae. The presence of a long pre-pharynx and the uterus reaching the testis did not correspond to any of the genera given in Yamaguti (1971).

Zhukov (1972) described a new genus and species, *Malabarotrema indica*, from *Etroplus suratensis* from India. Even though measurements for the Sri Lankan specimens were a little smaller than the Indian specimens of Zhukov (Table 2.7), they were morphologically identical in every other way. The similarity of the host with the morphological similarity suggested that these specimens belong to the species *Malabarotrema indica.*

4. Cestoda

Family: Dilepididae Railliet & Henry, 1909

Paradilepis scolecina (Rud., 1819)

Cysticercus - Live worms (Figure 2.15 A-B)

Host: *E. suratensis* (Bloch)

Site of infection: Mesentery around bile duct and gall-bladder

Locality: Udawalawa reservoir

Prevalence, Mean intensity (Number of fish surveyed): 69.2 %, 3.5 (78)

Description: (based on 10 specimens)

Cyst oval, 574 x 352 (406-780 x 245-518), wall 9 (6-11) thick, adjacent to larva. Transparent larva inside cyst, cysticercoid, occurs with four simple suckers and rostellum armed with two rows of hooks. Cysticercoids 498 x 354 (370-720 x 230-498); suckers 58 x 40 (53-66 x 36-42). Upper rows of hooks larger, 10 in number, length 107 (96-112). Lower rows of hooks smaller, 10 in number, length 74 (68-80). Excretory corpuscles present in body region. Two lateral canals of excretory system connected to excretory bladder. Rostellum invaginated.

Cysticercus - dead worms (Figure 2.15C)

Host: *E. suratensis* (Bloch)

Site of infection: Mesentery around bile duct and gall-bladder

Locality: Udawalawa reservoir

Prevalence, Mean intensity (Number of fish surveyed): 93.6 %, 11.3 (78)

Description: (based on 16 specimens)

Found in same site of infection as *P. scolecina*, in same fish. Mostly smaller than live cysts, 396 x 265 (260-700 x 140-500), non-transparent, yellowish brown in colour. Dead larval bodies not evident. Loosely arranged 20 hooks of two different sizes, long hooks 102 (96-106), small hooks 72 (66-75). Concretions of yellowish brown pigment, 1-5, found on surfaces.

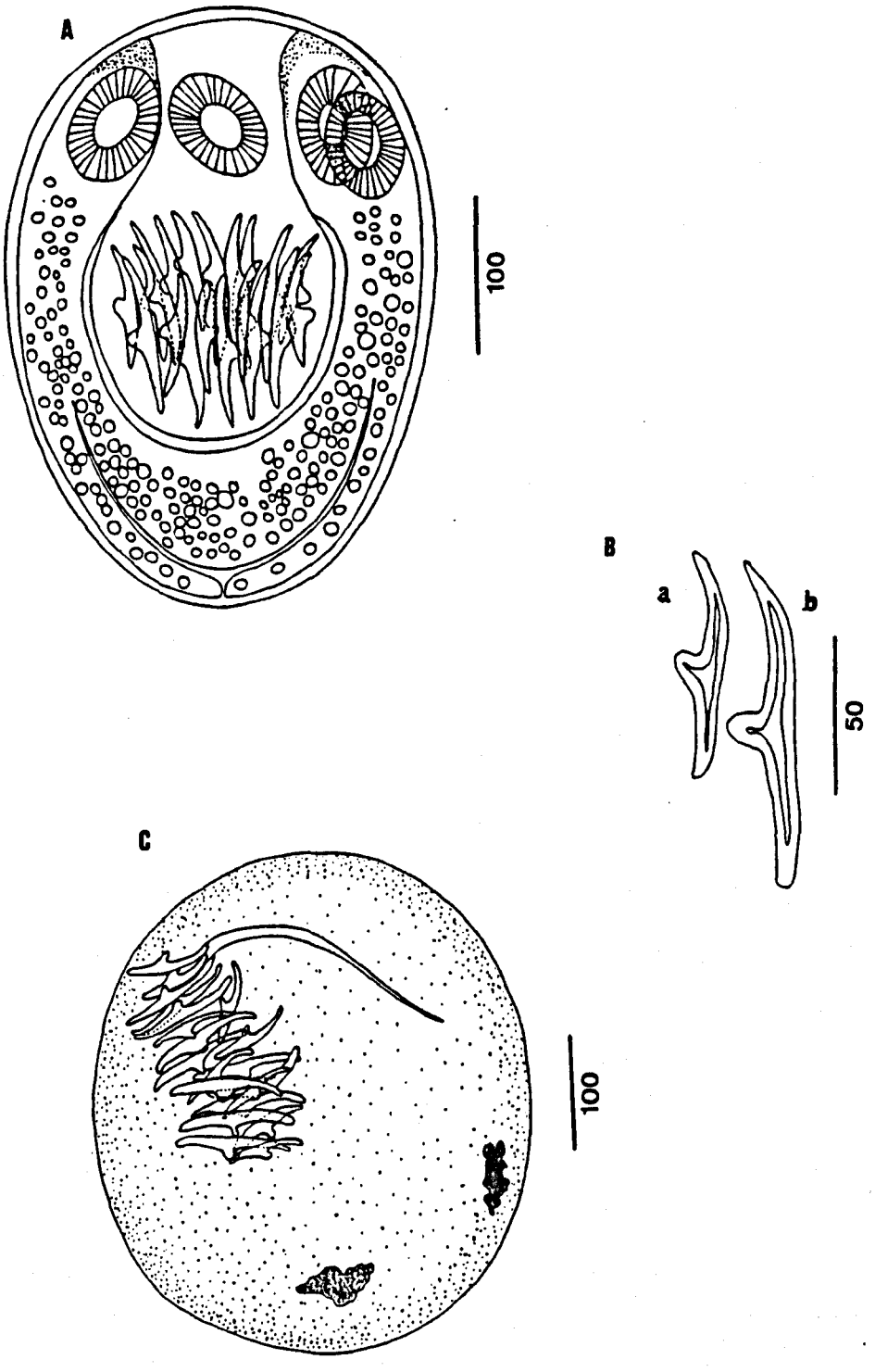


Figure 2.15: Cysticercus *Paradilepis scolecina*. (A) Encysted live cysticercus. (B) Hooks, a) small lower row hook, b) large upper row hook. (C) Dead cysticercus.

Identification:

According to the key provided for larvae of orders Tetrarhynchidea, Tetraphyllidea and Cyclophyllidea occurring in fishes of the Soviet Union by Bykhovskaya-Pavlovskaya (1962), the presence of four simple suckers on the head and a single terminal trunk armed with two rows of hooks placed the specimens in the order Cyclophyllidea. The larval types of this order are differentiated according to the size of hooks. The presence of 20 hooks with the upper row of hooks, not less than 100 in length and lower row of hooks not less than 50, agreed with the cysticercus of *Paradilepis scolecina*. The shape of the hooks also agreed with this species. The adult of this species (syn. *P. brevis*) was recorded in Sri Lanka by Burt (1940).

The measurements of the specimens are given with the values given for cysticercus *P. scolecina* by Bauer (1987) and, as can be seen in the Table 2.8, all the values for the specimens from *Etroplus* agree with that given for the cysticercus of *P. scolecina*. The site of infection of these specimens was also similar to the reported occurrence in Bykhovskaya-Pavlovskaya (1962), ie the body cavity, mesentery, walls of gall-bladder, and liver of numerous freshwater fish. Therefore it can be concluded that these specimens belong to cysticercus *P. scolecina*.

According to the key given by Joyeux and Bear (1950), the hooks sizes of adult *P. scolecina*, larger hooks 102-129 and smaller hooks 78-92, are nearly equal to the values of these specimens. *Paradilepis delachauxi* has small hooks of 80-87. The size of the small hooks of this specimen is somewhat similar to this value, too. The hooks of *P. delachauxi*, however, have guards which are absent in *P. scolecina*. For further identification experimentally obtained adult specimens are necessary.

Table 2.8: Measurements of cysticerci *P. scolecina*.

	Bauer (1987)	Measurements of *P. scolecina* from *E. suratensis*
Cyst	560 - 1310 x 300 - 700	406 - 780 x 245 - 518
Cysticercoid larva	300 - 750 X 170 - 560	370 - 720 x 230 - 498
Upper row of hooks	90 - 120	96 - 112
Lower row of hooks	60 - 80	68 - 80

5. Nematoda

A. Family: Rhabdochonidae Skrjabin, 1946

***Rhabdochona* sp.**

Host: *E. suratensis* (Bloch) and *O. mossambicus* (Peters)

Site of infection: duodenal wall tissue

Locality: Koggala lagoon

Prevelance, Mean intensity (Number of fish surveyed): *E. suratensis* 48.9 %, 5.8 (94); *O. mossambicus* 26.7 %, 5.5 (15)

Description:

Relatively small, filiform worms. Anterior end of body narrow, rounded with indistinct cephalic papillae. Buccal capsule funnel-shaped, with longitudinal thickenings projected anteriorly as teeth. Vestibule long and narrow. Oesophagus distinctly divided into short muscular portion and long glandular portion. Body cuticle with transverse striations throughout its length.

Male (based on 11 specimens) (Figure 2.16 A,D,E & F)

Shorter than female, 305 (278-400) in length, 14 (11-17) in breadth at begining of oesophagus. Buccal capsule (prostome and vestibule) 46 (42-53) in length. Entire oesophagus 691 (570-822), with anterior muscular portion taking 72 (68-91). Nerve-ring surrounding muscular oesophagus. Testis, 143 (102-186) posterior from anterior end of worm. Spicules unequal, dissimilar in shape, wall sclerotised with transverse striations. Long spicule 175 (157-191); short spicule 38 (34-42) in length. In both, posterior ends pointed and curved. Narrow caudal alae with supporting pedunculated papillae. Pre-anal papillae 3 pairs, post-anal 4 pairs.

Female (based on 10 specimens)(Figure 2.16 B,C & G)

Body length 560 (418-629), width 13.5 (12-17) at the beginning of oesophagus; width much greater posteriorly, unlike males. Buccal capsule 45 (38-53). Entire oesophagus 560 (533-824); anterior muscular portion 114 (88-136). Posterior end of body, pointed and straight. Anal pore subterminal. Vulva located 65-70 % of body length from anterior end. Vulval lips not elevated. One ovary located at each end of

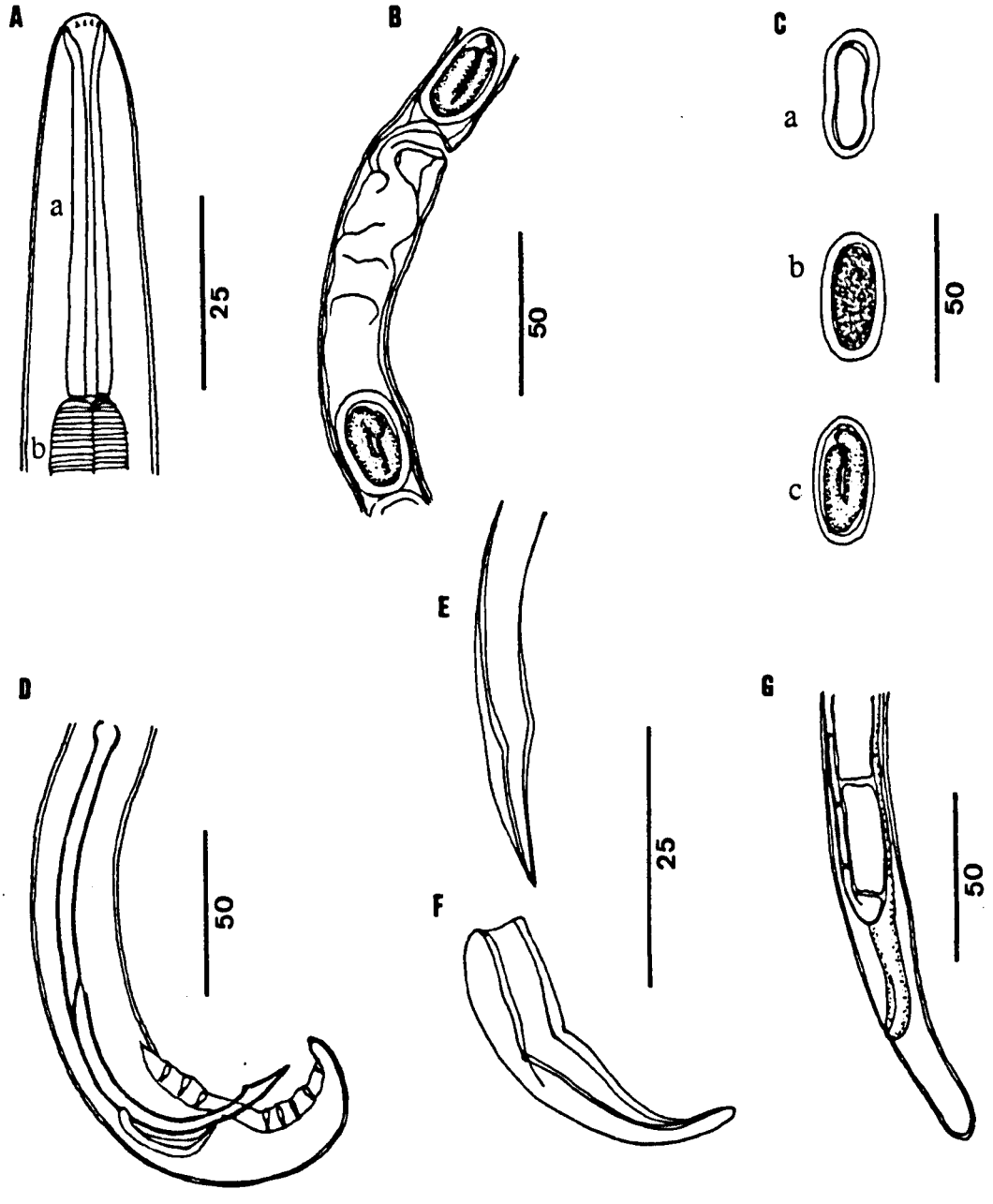


Figure 2.16: *Rhabdochona* sp. (A) Anterior end, a) buccal capsule, b) muscular pharynx. (B) Vulvar region of female. (C) Eggs, a) uncleaved, b) cleaved, c) egg with larva. (D) Posterior end of male. (E) Posterior end of long spicule. (F) Small spicule. (G) Posterior end of female.

body. Uteri opposed, containing numerous eggs arranged in a single row. Eggs smooth, thick-walled, barrel-shaped, sometimes narrow equatorially, with no polar plugs; mature eggs with developing larvae 33 x 19 (32-35 x 18.5-21.5); egg shell varies in thickness, from 3-5.5.

Identification

The presence of an oesophagus which is divisible into an anterior muscular portion and a posterior glandular portion places these specimens in the order Spirurida. A buccal capsule in the form of a long chitinized tube, which is slightly expanded in its anterior part, unequal spicules, absence of a gubernaculum and numerous caudal papillae place the specimens in the family Rhabdochonidae.

The presence of the main character which Moravec (1975) used to separate the genus *Rhabdochona* Railliet 1916, from other genera, ie. the funnel-shaped bucal capsule with longitudinal thickenings projecting anteriorly as teeth, made it possible to place these worms in this genus. According to the reconstructed definition for the genus *Rhabdochona* proposed by Moravec (1975) and the characters used in the key to the genera, the males lack caudal alae and have numerous pre-anal (at least 6 pairs) and 5-7 post-anal pairs of papillae. In this respect the specimens in the present study vary from the generic diagnosis.

Caudal alae are absent in males of the subfamily Rhabdochoninae, although rudimentary circum-anal alae can be present but rarely (Moravec, 1975). The other subfamilies of the Rhabdochonidae have similar caudal alae and sessile papillae. *Sterliadocona* Skrjabin, 1946 from the same subfamily as *Rhabdochona* (Skrjabin, 1949; Yamaguti, 1961) has 4 pre-anal (3 in the identifying species) and 4 post-anal sessile papillae supporting caudal alae but not teeth in the funnel-shaped buccal capsule. However, due to the nature of the buccal capsule, the specimens were placed in the genus *Rhabdochona*.

The specimens were mounted in glycerine, and were therefore not in a condition to observe the minute characters required for further identification. As the

worms were very thin, the *en face* view to see the structure of papillae, rudimentary lips and number of teeth and the ventral views of tails for proper counting of papillae, could not be prepared. Therefore, new specimens are required for further identification using Scanning Electron Microscopy.

B. Family: Anisakidae (Railliet & Henry, 1912) Skrjabin & Karokhin, 1945

***Contracaecum* larva**

Unencysted second stage larva (L_2)

Host: *E. suratensis* (Bloch)

Site of infection: Stomach & duodenal lumen, liver

Locality: Udawalawa reservoir

Prevalence, Mean intensity (Number of fish surveyed): 15.4 %, 2.6 (78)

Description: (based on 3 specimens which were all that were available)

Larva unencysted, actively wriggling, 384 (369-418) in length 7-8 in breath at level of ventriculus. Body covered with cuticle of 1 thickness; cuticle lightly striated; striations could be clearly seen at anterior end. Head with cuticular boring tooth. Mouth opened into 21 (19-24) long oesophagus. Ventricular appendix arising from ventriculus, 44 (38-51) long, not hollow. Intestinal caecum 4 (2-8) long, not developed in some. Intestine clearly visible, granular, opens through subterminal anus. Tail 46 (29-60) long.

Encysted ensheathed third stage larva (L_3)

Host: *E. suratensis* (Bloch) and *O. niloticus* (Linnaeus)

Site of infection: Mesentry, stomach wall, liver and spleen

Locality: Udawalawa reservoir, Koggala and Bolgoda lagoons

Prevalence, Mean intensity (Number of fish surveyed): Udawalawa reservoir *E. suratensis* 96.2 %, 6.4 (78); *O. mossambicus* 92.9 %, 2.9 (14): Koggala lagoon *E. suratensis* 3.2 %, 1.0 (94): Bolgoda lagoon *E. suratensis* 78.6 %, 2.5 (14)

Description: (based on 20 specimens)

Coiled and encysted in organs within thin capsule of host origin. Larvae ensheathed by second stage cuticle 6 (5-8) thick. Larvae measured 2323 (1535-3596) in length and 84 (46-133) in breadth at level of ventriculus. Covered with striated cuticle of 2-3 thick. Head with cuticular boring tooth. Nerve-ring occurs 63 (52-78) from anterior end. Oesophagus 84 (73-92) long, opens into 12 (10-14) long ventriculum. Hollow ventricular appendix 353 (255-480) long. Intestinal caecum well developed, 157 (90-190) long. Intestine simple tube. Anus subterminal, situated 60 (55-64) from posterior extremity. Reproductive organs undeveloped. Excretory pore opening base of sub-ventral lips.

Identification:

The presence of a ventriculus led these specimens to the family Anisakidae (Hartwich, 1974). The characters of the excretory system, the position of the excretory pore at the base of the lips led the specimens to the subfamily Anisakinae (Hartwich, 1974). Further, the presence of a ventriculus, posteriorly directed ventricular appendix and anteriorly projecting intestinal caecum led the specimens into two genera *Phocascaris* and *Contracaecum* belonging to the Anisakinae (Myers, 1975). The character, presence or absence of interlabia, could not be used to separate genera as these are not yet visible in L_3 larva (Huizinga, 1966, 1967). Of these two genera, the genus *Phocascaris* can be eliminated, as this occurs in marine mammals (Myers, 1975), and the final host therefore have no access to a freshwater reservoir and doubtfully to the two brackishwater lagoons studied. Therefore, these nematodes were identified as a species of *Contracaecum.*

Comments: The actively wriggling, unencysted, unensheathed larva were considered as second stage larva mainly referring to the life cycle studies of *Contracaecum rudolphii* = (*C. spiculigerum*) (Huizinga, 1966) *C. multipapillatum* (von Drasche, 1882) Lucker, 1941 (Huizinga, 1967) and a related species, *Rapidascaris acus* (Bloch, 1779) Railliet & Henry, 1915 (Smith, 1984). According to the these studies, the second stage and third stage larvae occur in the fish intermediate host (in *R. acus* also the fourth stage). The second stage was not observed to moult either in the haemocoel

of the copepod or as it migrated through the intestine of fish. The second stage larva was observed to moult, day 10 (*C. spiculigerum*) and day 18 (*C. multipapillatum*) post-infection after a size increase, and retained the second stage cuticle and eventually became encapsulated. Therefore, the absence of ensheathed moult cuticle around the actively wriggling larvae made it possible to identify them as second stage larva.

6. Crustaceans

A. Family: Ergasilidae Thotell, 1859

Dermoergasilus amplectens (Dogiel & Akhmerov, 1952) Ho & Do 1982 (Figure 2.17)

Host: *E. suratensis* (Bloch) and *O. mossambicus* (Peters)

Site of infection: tip of the gill filaments

Locality: Koggala and Bolgoda lagoons

Prevalence, Mean intensity for four gill arches of one side (Number of fish observed): Koggala lagoon *E. suratensis* 98.0 %, 24.8 (94); *O. mossambicus* 6.7 %, 1.0 (15): Bolgoda lagoon *E. suratensis* 100 %, 28.9 (14)

Description: (based on 10 ovigerous female specimens)

Body length 655 (593-702), width 187 (156-218). Cephalothorax twice long as wide. Antennae considerably larger in relation to body size, long and stout, permanently fixed into single structure. Urosome less than half of length of prosome. Metasomal somites abruptly narrow from cephalothorax, tapers towards urosome. First abdominal segment (genital somite) largest, second segment next largest, last two very small. Egg sacs about half of body length.

Antennules 6-segmented, setae on segments 4, 10, 4, 4, 2 and 6. Antennae 3-segmented and with terminal claw, attached to large anterolateral lobes on body. Third segments lay on one another to form a permanent circle which encircles primary gill filaments with help of loose cuticular membrane covering whole structure but not terminal claw. First segment smallest, about third of second. Third segment two thirds of second. Mouth parts of typical ergasilid type.

Swimming legs pairs 1-4 biramous, rami 3-segmented, except fourth exopod with two segments. Formula for spines (Roman numerals) and setae (Arabic numerals) as follows:

Leg 1	Coxa 0-0	Basis 1-0	Exopod I-0; 0-1; II,1,4
			Endopod 0-1; 0-1; II,4
Leg 2 & 3	Coxa 0-0	Basis 1-0	Exopod I-0; 0-1; 1,5
			Endopod 0-1; 0-1; I,4
Leg 4	Coxa 0-0	Basis 1-0	Exopod I-0; 1,4
			Endopod 0-1; 0-2; I,3

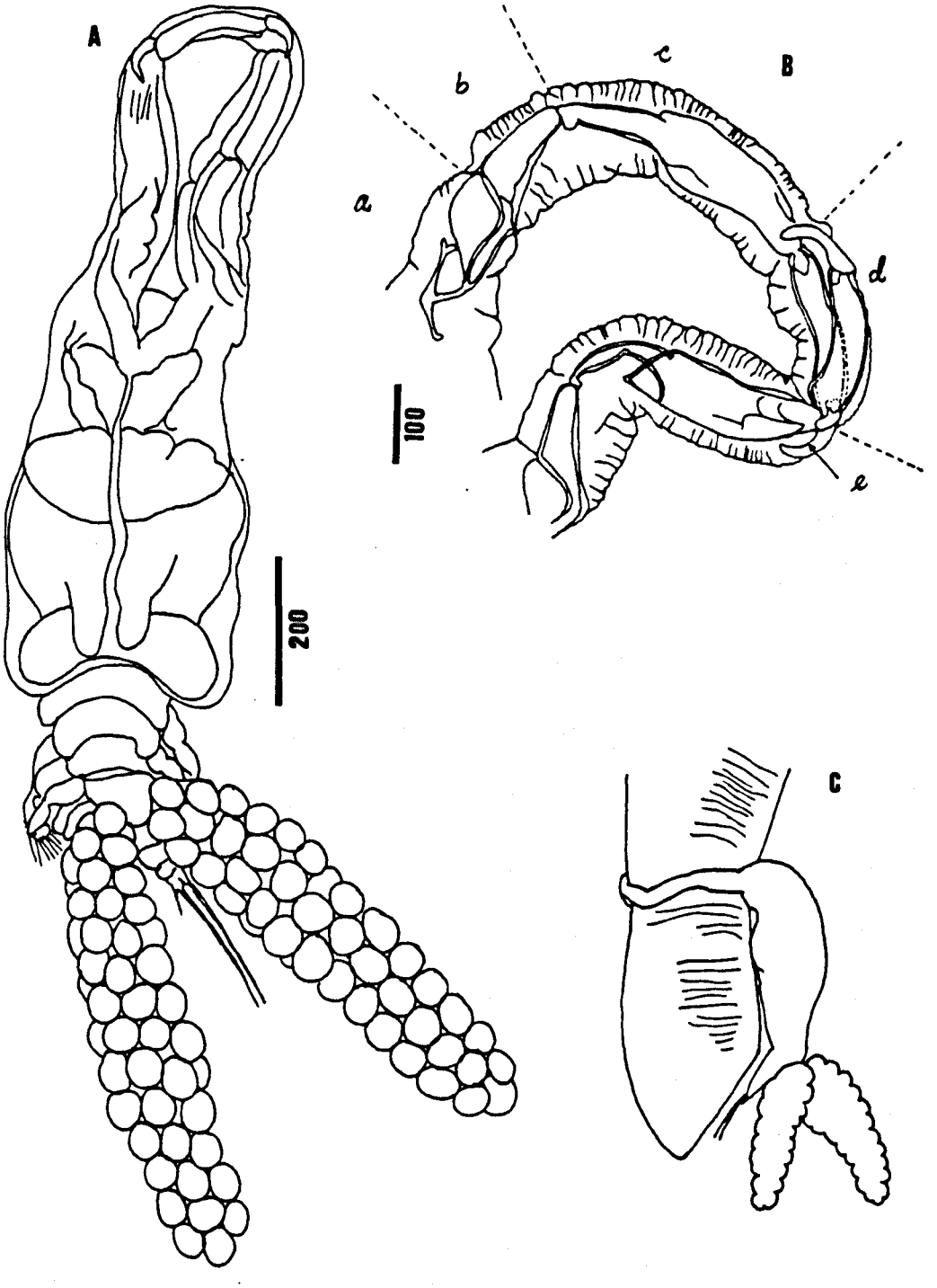


Figure 2.17: *Dermoergasilus amplectans*. (A) Adult copepod, female. (B) Second pair of antenna covered bya thin dermis, a) antennal lobe, b-d) segments 1-3 of antenna in order, e) the claw. (C) The way of attachment to the gill primary lamellae.

Leg 5 reduced to single segment with two long and one short setae. A seta present near base of last thoracic segment bearing fifth leg. Caudal rami carry one short and one very long seta.

Identification:

The presence of the pair of antennae fully covered with a cuticular membrane, except the terminal claw, and only one inner seta on the middle segment of the endopod of the second and third swimming legs confirm that these specimens belong to the genus *Dermoergasilus* Ho & Do, 1982.

The redescription of the *Dermoergasilus amplectens* by Ho and Do (1982) largely agrees with these specimens. However, in the description of Ho and Do (1982), the pair of antenules have five-segments with the fifth segment partially divided into two (in the diagram given) and the second segment of the antenna is equal to the third. In addition, the size is approximately double the size of the Sri Lankan specimens. The differences may be due to geographical and host variation as the specimens observed by them were from Kojima Bay in Japan on *Mugil cephalus* L. Therefore the specimens may be belong to the same species or a sub species of it.

***Ergasilus parvitergum* Ho, Jayrajan & Ramakrishnan (per. comm.)** (Figure 2.18)

Host: *E. suratensis* (Bloch)

Site of infection: Middle part of gill filaments

Locality: Koggala and Bolgoda Lagoons

Prevalence, Mean intensity for four gill arches of one side (Number of fish observed): Koggala lagoon *E. suratensis* 28.0 %, 1.5 (94): Bolgoda lagoon *E. suratensis* 14.3 %, 1.5 (14)

Description: (based on 8 ovigerous female specimens)

Body length 640 (577-671), greatest breadth 250 (234-281). Cephalothorax distinctly inflated, breadth two third of length. Metasomal somites abruptly narrowed from cephalothorax, decreasing in size posteriorly. Fourth pedigerous somite extremely reduced, equal to size of fifth. Genital segment considerably larger than other urosomal segments, egg sacs approximately double than body length.

Antennule 6-segmented, setae on segments 3, 10, 4, 3, 2 and 7. Antenna with 3 segments and terminal claw, strongly curved. First segment little less than half of length of second. Second and third of equal lengths. Mouth parts of typical ergasilid type.

Swimming leg pairs 1-4 biramous, rami 3-segmented, except fourth exopod with two segments. Formula for spines and setae as follow;

Leg 1	Coxa 0-0	Basis 1-0	Exopod	I-0; I-1; II,1,4
			Endopod	0-1; 0-1; II,4
Leg 2 & 3	Coxa 0-0	Basis 1-0	Exopod	I-0; 0-1; 1,5
			Endopod	0-1, 0-2; 1,4
Leg 4	Coxa 0-0	Basis 1-0	Exopod	I-0; I,1,4
			Endopod	0-1; 0-2; I,3

Leg 5 reduced to small process with one seta. Caudal rami with one very long, two moderately long and one short seta.

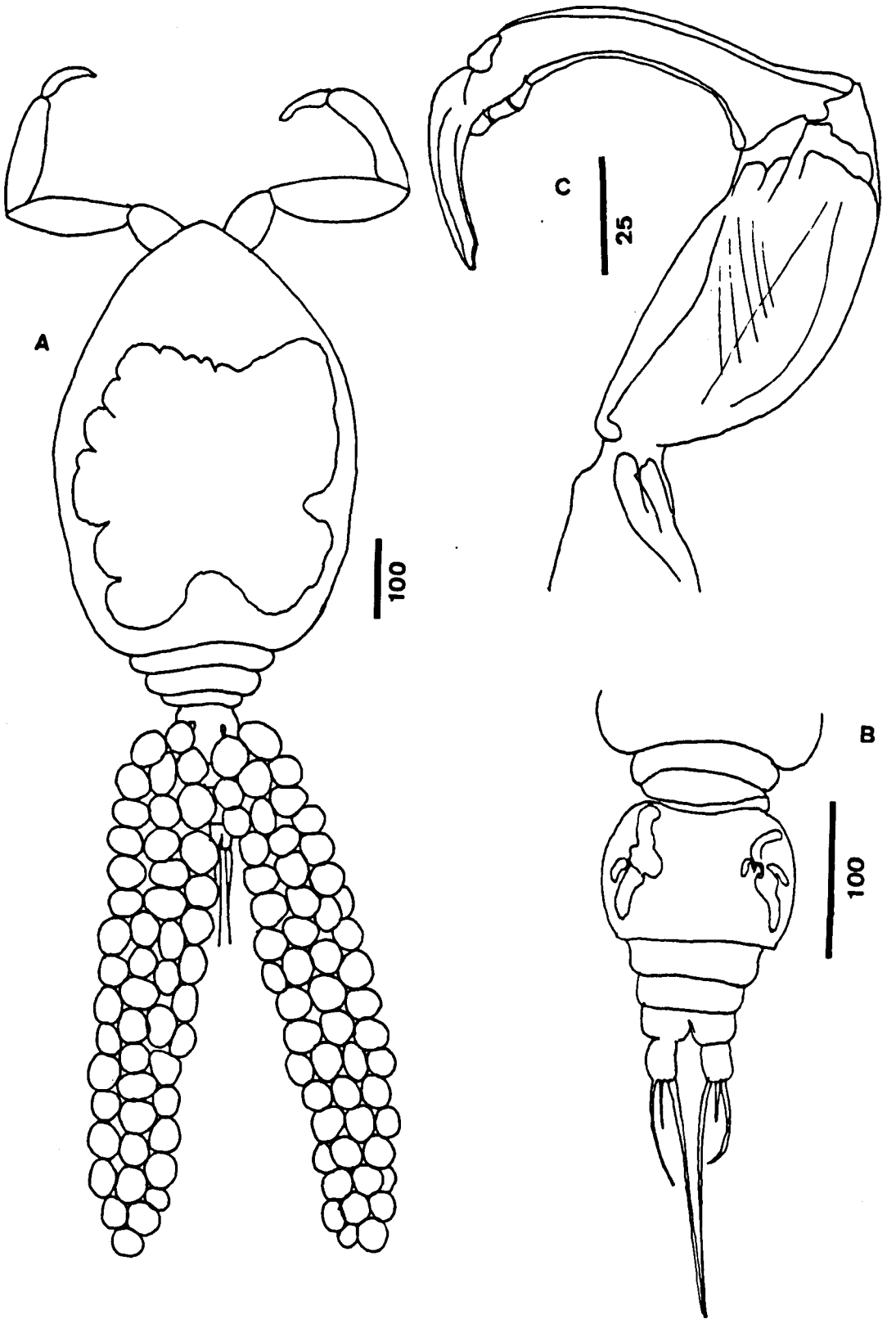


Figure 2.18: *Ergasilus parvitergum* (A) Adult copepod, female with egg sacs. (B) Thoracic and abdominal segments. (C) Antenna.

Identification:

The distinctly inflated cephalothorax and the extremely reduced forth pedigerous somite and all other characters make the specimens similar to the species described as *Ergasilus parvitergum* by Ho, Jayrajan & Ramakrishnan (in press) from the hosts *Etroplus suratensis* (Bloch) and *Caranx malabaricus* (Bloch & Schneider) from Trivandrum, India.

B. Family: Argulidae Müller, 1785

***Argulus* sp.**

Host: *E. suratensis* (Bloch)

Site of infection: Skin

Locality: Koggala lagoon

Prevalence, Mean intensity (Number of fish surveyed): *E. suratensis* 19.0 %, 1.6 (94)

Description:

Female (based on 3 specimens, Figure 2.19, A-H)

Body 4800-6900 long; length to breadth ratio 1. Carapace round, posterior lobes reaches posterior end of abdomen covering four fifths of it; posterior sinus reach posterior end of second thoracic segment leaving third and fourth free. Posterior sinus of abdomen very shallow. Cephalic area well separated by conspicuous anterolateral sinus, acquire about half of total length. Median eye in level of sucker attachment. Brown spots on lateral lobes of carapace except in respiratory areas.

Pair of median dorsal ribs, converge midway in region of eyes, curve outwards

at anterior ends. Anterolateral sinus arise lateral to compound eyes, run sideways, proceed backwards, join the transverse groove of cephalic region marking triangular cephalic area. Secondary sutures arise from this pair of sutures, join with another pair of sutures, run more laterally and backwards up to level of fourth thoracic segment. No sutures marked by any pigments.

On ventral side, spines occur on cephalic and anterolateral area of carapace; cephalic spines confined to thick rim in anterior margin and three small areas medially and laterally to antennae. Paired lateral eyes medium in size, located between antennae and edge of suction cups. Thoracic segments 4 in number, equal in size; fourth narrows towards abdomen. Respiratory areas well defined by brown pigment bands; anterior area completely separated from posterior, 3 times smaller than latter. The former, oval in outline, little compressed medially and laterally between the levels of suckers and maxillipeds. Reniform posterior area extends from level of first to third pair of swimming legs. Abdomen acquires posterior fourth of total length; length to width ratio 0.75; lobes round; posterior sinus in posterior eighth. Seminal receptacles small, kidney-shaped, close to anterior margin of abdomen. Eggs carried under the thoracic region, tightly packed, so four to six sided.

Inwardly directed posterior spine on basal segment of antennule stout, blunt; on next segment two small inwardly and backwardly curved spines, one in mid anterior and other in mid posterior margins; segment terminating in a strongly recurved claw; slender terminal segment with four minute spines. Antennae four-segmented; first with stout conical spine at its base; on an near small depression close

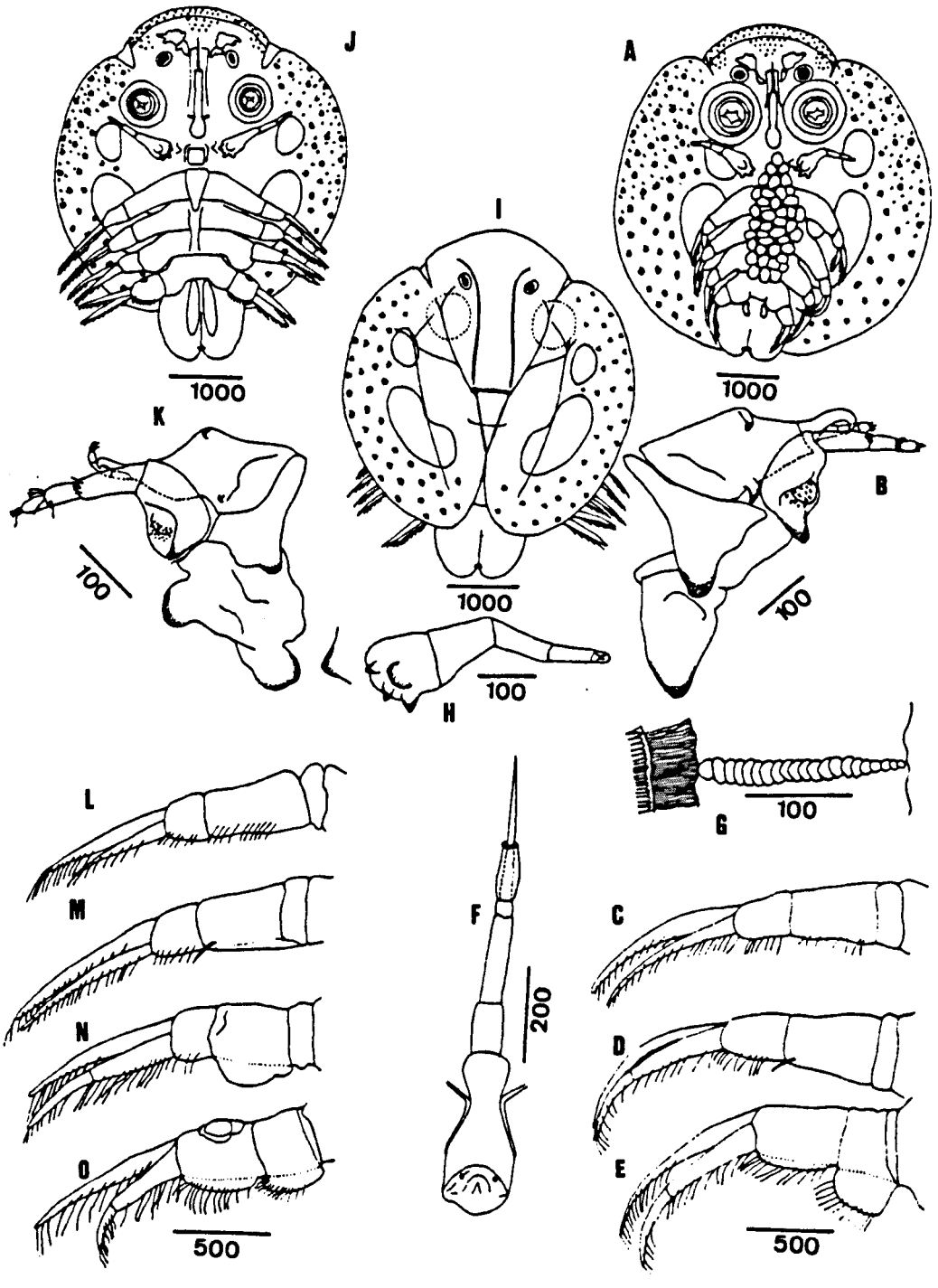


Figure 2.19: *Argulus* sp. (A) Female, ventral view. (B) Antennule and antenna of female. (C)-(E) Swimming legs 2-4 of female. (F) Oral tube. (G) A supporting rod of maxillary sucker showing the arrangement of plates. (H) Second maxilla. (I) Male, ventral view. (J) Male, dorsal view. (K) First and second antennae of male. (L)-(O) Swimming legs 1-4 of male.

to posterior margin has minute scales present; following segments diminishes in size compared to prior. Post-antennal spine triangular and stout.

Ribs of maxillary sucker made up of 13-22 imbricated sclerits; with 58-61 ribs; sucker pedunculated. Mouth tube and stylet simple, tube not spinous ventrally. Second-maxilla five-segmented; basal plate with three blunt teeth widely apart, nearly equal in size, seta present lateral to middle spine; semi-circular depression in basal plate carries many scales and few setae in its posterior margin. Last segment terminates with small protuberance, curved towards two small claws. Pair of blunt post-maxillary teeth present in the levels of maxillae.

Distal ends of rami of third and fourth pairs of swimming legs reach beyond carapace outline. No flagella on any of swimming legs. The third basipodite bears one seta. The fourth basipodite carry square-shaped cuticular expansion ventrally.

Male (based on two specimens, figure 2.19 F-O)

Body 3700-4500 long, smaller than the female. Carapace round, width to length ratio 0.75-0.80; the posterior lobes reach only anterior level of abdomen; thus posterior sinus less deep than in female, ends at posterior end of second thoracic segment. Fifth of abdomen covered by carapace lobes. Carapace pigmentation and pattern of sutures similar to female. Proportional length of cephalic area to its body length and carapace length remain same as in female. Unlike in female, large gap exists between the compound eyes and edge of suction cups.

The proportions of thoracic segments similar to female; but abdomen as same width as its length, and third of body length. Testes 4-times longer than width, taper towards ends. Posterior sinus of abdomen very shallow as in female reaching eighth of its length.

Antennal structures same as with female, but all spines less defined. Two knob like stout post-antennal spines; one posteromedially directed and other towards compound eye.

Maxillary sucker with 56-60 ribs. Ribs made up of 10-16 imbricated sclerites. The structure of second-maxilla similar to female, but semi-circular depression larger. Pair of post-maxillary spines similar to female.

The distal ends of rami of all 4 swimming leg pairs reach beyond outline of carapace. Thoracic legs modified. Basipodite of second pair with narrow expansion ventrally and one spine directed towards mid-line and one setae. Basipodite of third pair with wide bulge ventrally. Anterior margins of basipodite and coxapodite of fourth pair depressed to allow space for bulge on third pair; peg like process on anterior surface of coxopodite terminate with knob at its mid length; ventral expansion on basipodite similar to that of female; coxopodite broader compared to female.

Identification

The presence of a small respiratory area anterior to the larger one, the presence of blunt teeth on the basal plate of the maxillae and 13-22 imbricated plates on the

ribs, absence of flagella on swimming legs prevent the specimens being ascribed to any of the species given in the key by Wilson (1944).

Amongst the 11 species of *Argulus* described from India (Natarajan, 1982), *A. giganteus* Ramakrishna, 1951, *A. indicus* Ramakrishna, 1951, *A. quadristriatus* Devaraj & Ameer Hamsa, 1977 and *A. mangalorensis* Natarajan, 1982 have respiratory areas similar to the specimens under study. *A. scutiformis* Yamaguti & Yamasu, 1959 from Japanese fishes also have the same type of respiratory area.

Of the argulids, *A. giganteus*, *A. bengalensis* Ramakrishna, 1951 and *A. scutiformis* have a similar appearance (Yamaguti & Yamamasu, 1959) and all similar in appearance to the present specimens. The new specimens differ from *A. bengalensis* mainly in the structure of the respiratory areas. It does not belong to the other two species due to the absence of the flagellum in the first pair of swimming legs. The pigmentation is similar to *A. indicus* but it differs superficially from them all. Therefore, this is possibly a new species.

2.3.1.2. Parasites found only in *Oreochromis* spp.

1. Monogenea

Family: Dactylogyridae Bychowsky, 1933

Subfamily: Ancyrocephalinae Bychowsky, 1937

Cichlidogyrus bifurcatus Paperna, 1960 (Figure 2.20)

Host: *O. niloticus* (Linnaeus)

Site of infection: gill filaments

Locality: Udawalawa reservoir

Prevalence, Mean intensity for second gill arch of one side (Number of fish surveyed): 50.0 %, 1.6 (14)

Description: (based on 8 specimens which were all that were available)

Large worms, length 574 (429-692), maximum width 148 (74-176). Haptor comparatively very small, elliptical, 70 x 84 (54-80 x 61-106). Three pairs of head glands; two pairs of eyes; posterior pair larger, at anterior end of pharynx. Pharynx leads to very short oesophagus; intestine bifurcates and unites posteriorly.

Two pairs of hamuli of same size; ventral pair, attached to 'V'-shaped bar, total length 32 (29-36), shaft 29 (27-32), inner root 9.5 (9-10), outer root 6 (5-6) and point 9.5 (9-10). Dorsal pair with longer inner root, total length 30 (28-32), shaft 28 (26-30), inner root 11 (9-13), outer root 6 (5-6) and point 10 (9-11). 'V'-shaped bar total length 58 (56-60), inner margin of each arm with 3-4 tooth like projections. Dorsal bar, half moon shaped plate with thickened edges, total plate length 53 (46-56), pair of attached appendages of 12 (10-14) long give 'H'-shaped appearence. Seven pairs of marginal hooks; first pair 14 (12-16); second pair 16 (15-17); third to sixth 18 (17-18); seventh 14.5 (14-15). The sickles of marginal hooks round-shape, all with filament loops.

Single testis lies in posterior inter-intestinal space; vas deferens arising proceeds anteriorly, turns back and forms seminal vesicle which opens to funnel and copulatory tube (the ejaculator). Bilobed prostrate gland also opens to tube;

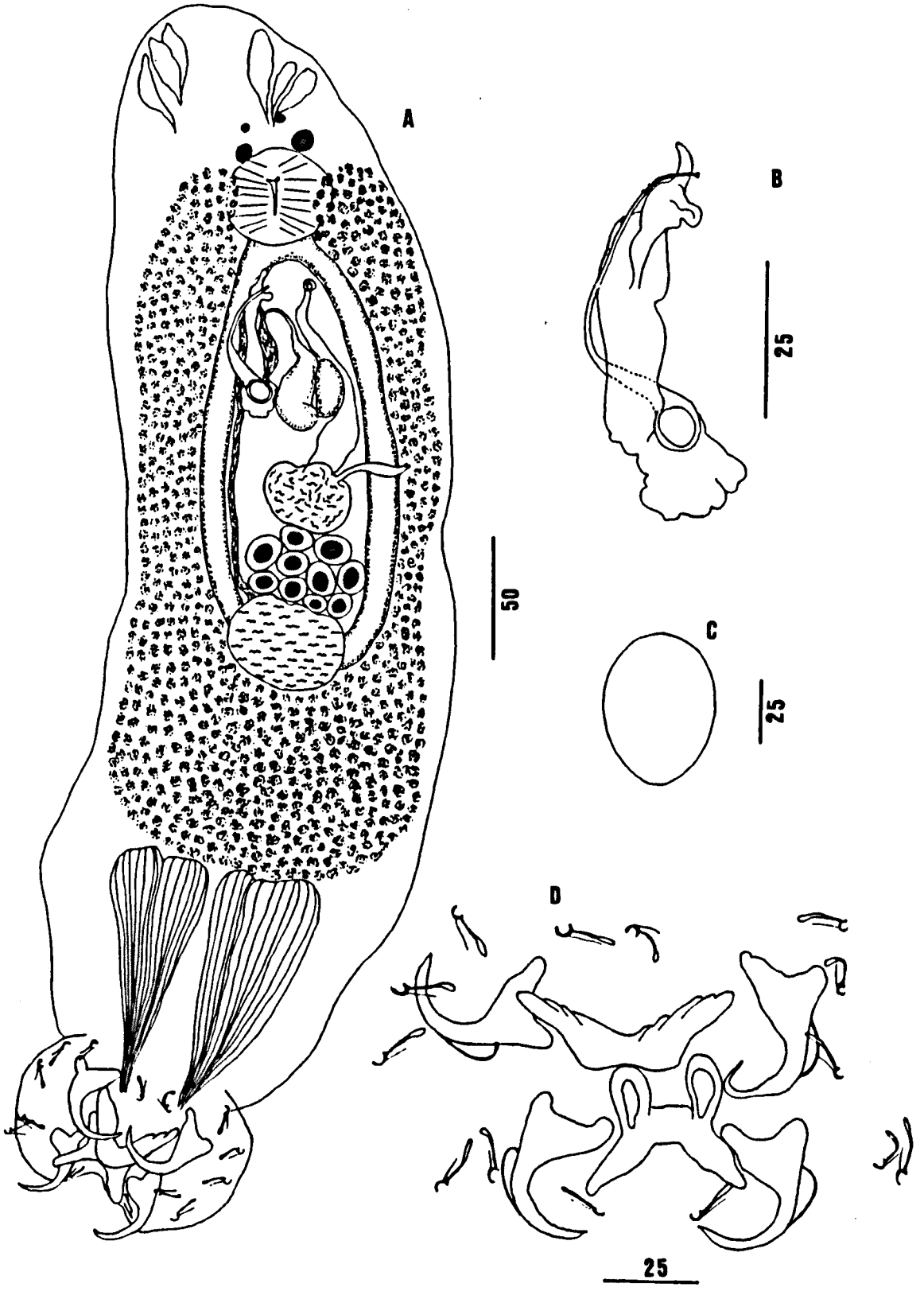


Figure 2.20: *Cichlidogyrus bifurcatus* (A) Entire worm. (B) Accessory piece with funnel and tube. (C) An egg. (D) Opisthaptor armature.

Table 2.9 : Measurements for *Cichlidogyrus bifurcatus*.

Character		Paperna (1960) n=7	Fixed specimens from *Oreochromis niloticus* n=8
Number of specimens observed		7	8
Total length		300 - 440	429 - 692
Maximum width		50 - 100	74 - 176
Haptor		40-60 x 60-100	54-80 x 61-106
Length of ejaculatory tube		40 - 60	48 - 63
Length of accessory piece		30 - 47	49 - 56
Lengths of 1st pair of hamuli	total	30 - 40	29 - 36
	inner root	7 - 13	9 - 10
	outer root	5 - 11	5 - 6
	shaft	25 - 34	27 - 32
	point	5 - 10	9 - 10
Lengths of 2nd pair of hamuli	total	30 - 40	28 - 32
	inner root	12 - 20	9 - 13
	outer root	4 - 19	5 - 6
	shaft	18 - 27	26 - 30
	point	5 - 10	9 - 11
Length of 'V'-shaped bar		40 - 70	56 - 60
Length of 'half moon'-shaped plate		25 - 36	46 - 56
Length of its appendage		6 - 14	10 - 14
Length of hooks	pair 1	12 - 17	12 - 16
	pair 2	17 - 20	15 - 17
	pair 3	20 - 25	17 - 18
	pair 4-5	23	17 - 18
	pair 6	30	17 - 18

two parts may represent gland and reservoir. A plate with deeply serrated posterior margin attached at base of funnel. On other side, accessory piece consists of elongated slightly curved plate which bifurcates into long sickle-shaped branch and short branch having round edge. Ovary irregularly oval, lies anterior to testis. Uterus arising opens beside the copulatory complex. Vitellaria extra-caecal, extensive posteriorly. Vaginal pore laterally at level of anterior margin of middle third of body, opens to seminal receptacle situated anterior to ovary. Posterior extremity of body occupied by two pairs of cement glands with narrow posterior ends in opisthaptor.

Identification-

The presence of the characteristic *Cichlidogyrus* type supporting bars and the host, make the specimens undoubtedly belong to the genus *Cichlidogyrus*. Due to the presence of the equal pair of hamuli, slender central marginal hook pair with filament loop and base continuous with the shaft, serrated plate like appendage attached to the funnel make the specimens most similar to *Cichlidogyrus bifurcatus*. When comparing the measurements given by Paperna (1960), most of the values agree with the values for the specimens from Udawalawa reservoir (Table 2.9). However, these specimens seem larger, the half moon-shaped plate longer, and third to sixth pairs of marginal hooks are smaller. Even though there are these differences, due to the similarity with the description given by Paperna (1960), these specimens should belong to *Cichlidogyrus bifurcatus*. Paperna has found this species on young fish of *Oreochromis niloticus*, which is the same host as in Udawalawa reservoir.

2. Digenea

Family: Echinostomatidae Poche, 1926

Echinostomidae gen. sp.

Cercaria (Figure 2.21A)

Host: *Gyrulus* sp.

Locality: Udawalawa reservoir

Description: (based on 10 specimens)

Non-oculate, lophocercous, diastomatous, pharyngeate metacercaria. Echinostome type, only collar but not spines were visible.

Body flattened dorso-ventrally, oblong pyriform shape, length 175 (168-188), width 78 (70-84). Spination conspicuous at anterior end. Tail single stem tapering towards end and with small fin little anterior to end. Tail 186 (176-194), approximately of same length as body, width 16 (14-19) near base. Oral sucker 23 (18-27); pre-pharynx absent; pharynx 13 (11-15) long; oesophagus extended up to acetabulum, bifurcate to give broad caeca terminating near posterior end. Acetabulum at anterior end of posterior third of body, protruded out of the ventral surface, 30 (28-36). Excretory arms filled with corpuscles evident at either side of oesophagus.

Metacercaria (Figure 2.21B-D)

Host: *O. niloticus* (Linnaeus)

Site: Skin and gills (experimentally on gills near the base of primary filaments, but not on skin)

Locality: Udawalawa reservoir

Prevalence, Mean intensity for 10 cm^2 area of body surface (Number of fish examined) 14.3 %, 1.00 (14)

Description: (based on 2 specimens which were all that were available)

Cyst globular, 148 x 122 (134-163 x 106-138), wall single-layered, transparent and thin, thickness 2. Easily broken with liberation of larva. Cyst not covered by layer of host origin. Cercaria folded inside leaving no space in cyst.

Body oblong or pyriform, length 339 (228-450), greatest breadth 116 (94-138). Anterior end provided with head collar. Head spines 24, dorsally uninterrupted; 3 marginal spines (end group spines) on either side at ends, larger; 3 middle spines shorter, arranged alternately. Body covered with cuticular spines;

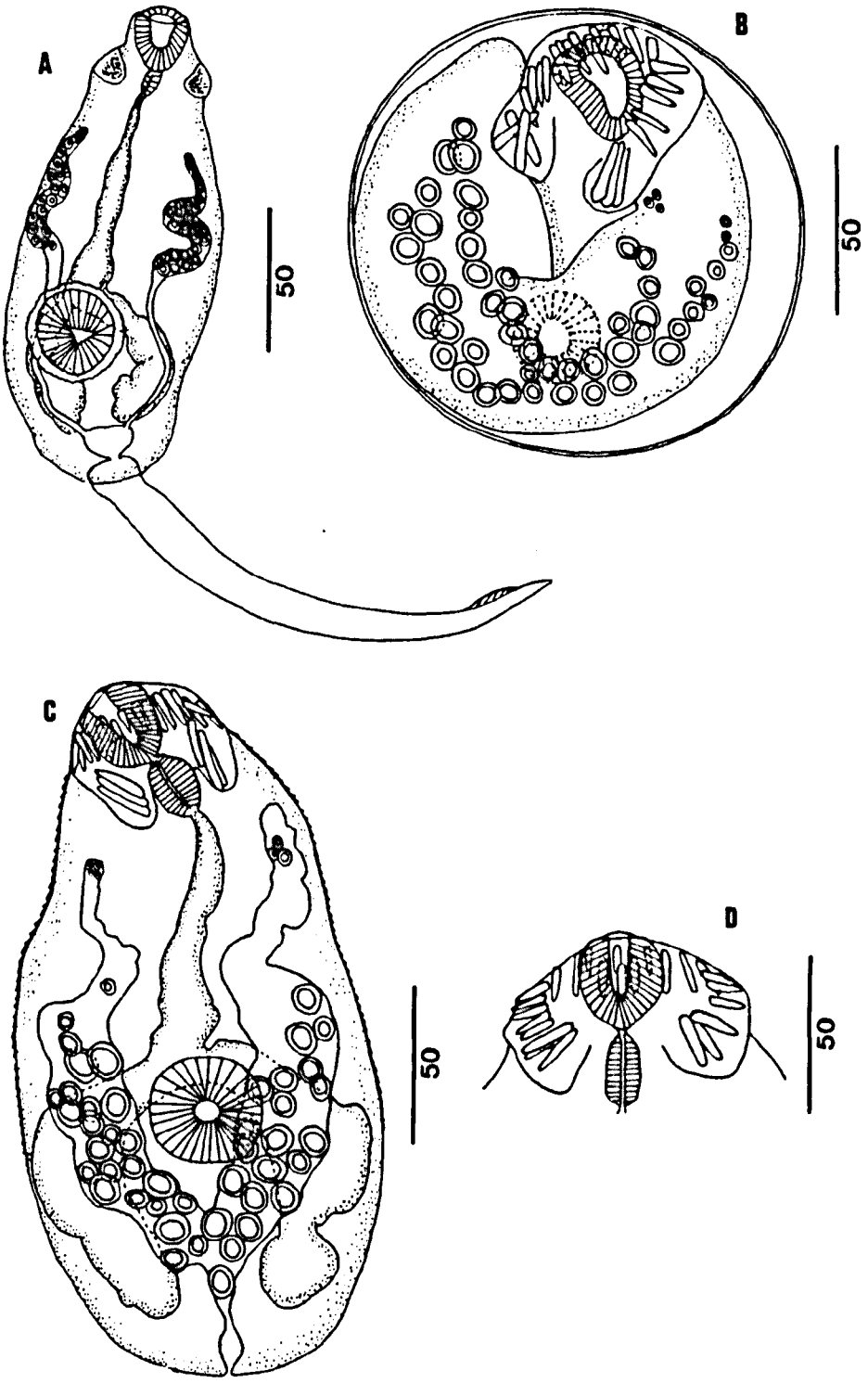


Figure 2.21: Echinostomid cercaria and metacercaria. (A) Cercaria. (B) Metacercaria in the cyst. (C) Excysted metacercaria. (D) Anterior end of metacercaria.

become less sparse posterior part of body.

Oral sucker 31 (27-35); opens to very short pre-pharynx; pharynx 24 (22-26); oesophagus very long, about third of body length, bifurcating in level of acetabulum to give wide caeca terminating at posterior end. Acetabulum at the anterior end of the posterior third of body, little larger than oral sucker, diameter of 40 (35-45). Excretory vesicles 'Y'-shaped, concretions present in arms of the excretory vesicle, 40-56 in total, 6-12 in diameter.

Identification-

The presence of head collar which is provided with a crown of spines lead these specimens to Family Echinostomatidae. Using the key by Yamaguti (1971) based on adult characters, it was difficult to identify these specimens beyond the family level. The Undivided, short body which was not attenuated posterior to acetabulum and uninterrupted collar spines made it possible for these specimens to belong to subfamily Echinostomatinae. The representatives of this subfamily usually have two rows of coronet spines and the acetabulum situated in the anterior body region. It is possible that the post-acetabular region enlarges with the develpoment of the reproductive organ as they become adults, thus giving an anterior position to acetabula.

3. Cestoda

Family: Dilepididae Railliet et Henry, 1909

Cysticercus *Gryporhynchus pusillum* (Nordmann, 1832) (Figure 2.22)

Host: *O. niloticus* (Linnaeus)

Site of infection: Duodenal wall

Locality: Udawalawa reservoir

Prevalence, Mean intensity (Number of fish surveyed): 92.9 %, 21.6 (14)

Description: (based on 12 specimens)

Cysticercoids unencysted, 625 x 268 (526-768 x 184-324), with four simple

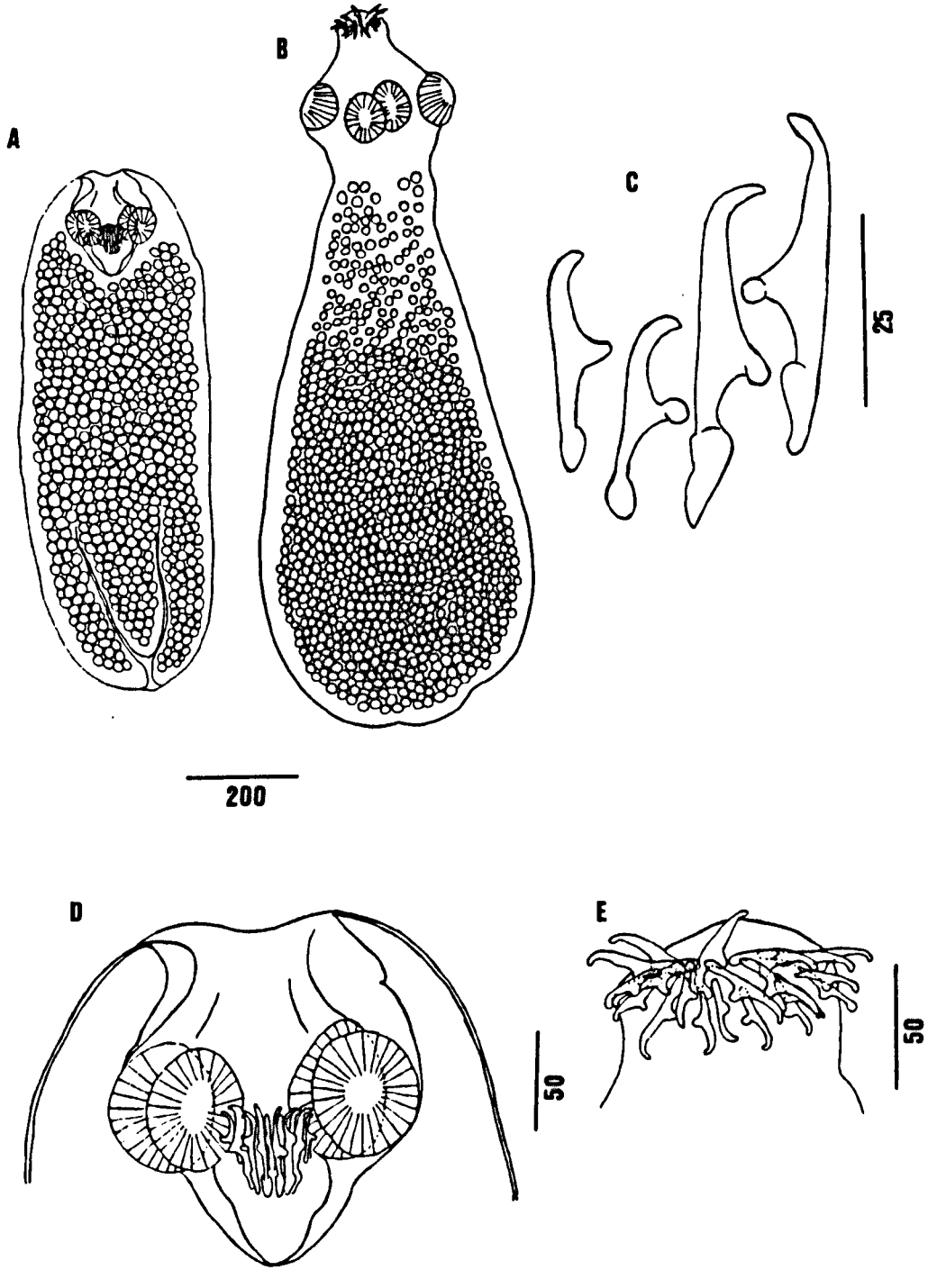


Figure 2.22: Cysticercus *Gryporhynchus pusillum.* (A) Cysticercus with invaginated scolex. (B) Cysticercus with evaginated scolex. (C) Hooks. (D) Invaginated scolex. (E) Rostellum of evaginated scolex.

suckers and rostellum armed with two rows of hooks. Scolex with suckers and hooks, invaginated, occasionally evaginated. Invaginated portion 154 x 107 (140-170 x 83-122). Suckers 56 x 34 (54-58 x 27-37). Upper row of hooks larger, 10 in number, length 44 (43-45). Lower row of hooks smaller, 10 in number, length 26 (26-27). Calcarieous corpuscles present in the body region. The two lateral canals of the excretory system connected to an excretory bladder.

Identification:

According to the key to the larvae of tapeworms orders Tetrarhynchidea, Tetraphyllidea and Cyclophyllidea occurring in the fishes of the Soviet Union by Bauer (1987), worms having two rows of hooks with upper row of hooks with 40-60 in length and lower rows of hooks with 20-30 in length, belong to Cysticercus *Gryporhynchus pusillus*.

In addition, these specimens closely agreed with the size measurements of cysticercus *G. pusillus*, 350-750 x 280-420, and the scolex width 115-122. The shape of the hooks was also similar to that given in Bauer (1987). However, according to the diagram given, cysticercus *G. pusillus* has a well defined neck and only the rostellum portion is invaginated. Also the body is wider proportionate to length, making it different from recent specimens. The site of infection in the host *O. niloticus* was the duodenal wall which is similar to that reported for tench, (the intestinal wall) by Bykhovskaya-Pavlovskaya (1962).

The hooks of these specimens superficially look like those of *Paradilepis delachauxi*. But, the hooks of adult *P. delachauxi* measure: large hooks 110-122 and smaller hooks 82-93 (Mahon, 1955), and are therefore, twice as large as the hooks of these specimens. However, *P. delachauxi* hooks have appendages on the guard and handle which resemble the hooks of these specimens. However, the specimens from *O. niloticus* do not have appendages, the guard and the handle is enlarged.

According to these features, these specimens belong to cysticercus *G. pusillus* and adults must be obtained experimentally for further identification.

2.3.2 Life cycle studies of the parasites found

2.3.2.1 Life-cycle studies of helminths

(a) Mollusc intermediate hosts of digeneans

The distribution of the molluscs in the two water bodies was very different. In Koggala lagoon, where *Melanoides tuberculata* was found abundantly whilst *Thiara* sp. was low in number, the both species were found near to the bank on the submerged vegetation and amongst detritus. In contrast, molluscs were lacking on the sparse submerged vegetation on the banks of Udawalawa reservoir and the bottom soil was hard, without any debris or mud. The mud in the deeper areas of the reservoir was collected using a grab and was very fine, sticky and devoid of molluscs. As the water level receded during the dry season (August-October) the molluscs, which are normally widely distributed, were concentrated in small pools or pits. Therefore, only in October could a good collection of, *Thiara* sp. and *Melanoides tuberculata* be obtained. Again, in February, even though the reservoir level was high, a different species of mollusc, *Gyraulus* sp., was found in a ditch near the bank.

During the investigation, four types of cercariae which could be related to the metacercarial parasites of *E. suratensis* and *Oreochromis* spp. were found and are listed in Table 2.10. The diagrams and descriptions of these cercariae are given in the Section 2.3.1, under the digeneans found in the study. Unfortunately, the only mollusc found infected with the larval stages of the renicolid metacercaria died before a diagram of the cercaria could be produced.

(b) Infection of fish host

All the cercaria identified were found to successfully infect *Oreochromis niloticus* and to prefer the same site of infection as observed in the natural infections of *Etroplus*. Cercaria of the acanthostomid metacercaria did not encyst inside the fin rays of *O. niloticus* fry perhaps due to the physical restriction ie. less space in fin ray segments, and was found to encyst on the surface of the fins. The cercaria infected adult *O. niloticus* without difficulty.

When the cercaria were found to successfully infect *O. niloticus* and were identified by their appearance as the metacercariae infecting *Etroplus* in the natural habitat, the cercariae were used again to infect *Etroplus*. For this naturally uninfected fish from the other uninfected locality were used. These infections to *Etroplus* was done to make sure that the metacercaria was indeed the same as those from the natural infection. The metacercariae/adult specimens obtained from the experimental infections of *Etroplus*, ie. the acanthostomid metacercaria and adult *Transversotrema patialense*, were measured and the data are given in Tables 2.11 A&B together with the measurements of the parasites from the naturally infected fish. The echinostomid metacercaria was found to occur naturally only in *O. niloticus* from Udawalawa reservoir, two specimens from the 14 fish investigated, thus, the cercarial infections were only attempted on *O.niloticus*. The measurements of the experimentally infected metacercaria are given with those of the naturally infected fish in the Table 2.11D. The measurements of the experimentally obtained metacercariae are within the ranges of the naturally obtained metacercariae, thus confirming that the cercariae used for experimental infections are indeed the larval stages of the naturally found metacercariae.

Due to the death of the infected mollusc only few specimens of the renicolid metacercaria could be obtained experimentally. Therefore, in this case only the metacercaria encysted in *O. niloticus* were measured and compared with those of the natural infections of *Etroplus* (Table 2.11C).

(c) Infection of the final host.

The trials carried out to establish and transform the metacercariae, and the third stage larva of *Contracaecum* sp., into adults in chicks and rats were not successful (Table 2.12). The discovery of cyathocotylid metacercaria and *Contracaecum* larvae in the contents of posterior large intestine and/or rectum in chicks and rats, 2 and 5 hours after feeding the parasites demonstrates that the parasites do not establish in these host, and hence their unsuitability. The cyathocotylid metacercariae found in the posterior intestine and rectum were still surrounded by the parasite capsule, further suggesting the unsuitability of the host by failing to stimulate excystment.

Table 2.10 : Infections in mollusc collected from two localities.

Month	Mollusc	Number collected	Number releasing cercariae		
Koggala lagoon					
July	*Melanoides tuberculata*	71	2	(2.8%)	Cercaria of *Transversotrema patialense*
November	*Melanoides tuberculata*	162	11	(6.8%)	Cercaria of *Transversotrema patialense*
			6	(3.7%)	Cercaria of Acanthostomid metacercaria
	Thiara sp.	15			
			0		
March	*Melanoides tuberculata*	236	12	(5.1%)	Cercaria of *Transversotrema patialense*
	Thiara sp.	21	3	(1.3%)	Cercaria of Acanthostomid metacercaria
May	*Melanoides tuberculata*	175	0		
			9	(5.1%)	Cercaria of *Transversotrema patialense*
	Thiara sp.	8	1	(0.6%)	Cercaria of Acanthostomid metacercaria
			0		
Udawalawa reservoir					
October	*Melanoides tuberculata*	19	0		
	Thiara sp.†	113	1	(0.9%)	Cercaria of Renicolid metacercaria
February	*Gyrulus* sp.	121	7	(5.8%)	Cercaria of Echinostomid metacercaria

† only the release of cercariae related to the metacercariae found in the study are given.

Table 2.11: The measurements of experimentally obtained metacercaria with naturally occuring metacercaria

A: Acanthostomid metacercaria

	Experimentally obtained	Naturally occuring
Number of specimens	10	7
Cyst length	254 (238-296)	286 (264-307)
width	242 (216-273)	262 (242-384)
Body length	342 (324-378)	356 (306-384)
width	123 (72-152)	134 (88-143)
Oral sucker diameter	138 (110-152)	156 (52-174)
Pharynx	34 (30-38)	38 (32-45)
Acetabulum diameter	33 (24-38)	30 (26-34)

B: *Transversotrema patialense*

	Experimentally obtained	Naturally occuring
Number of specimens	8	10
Body length	386(304-484)	429 (320-528)
width	772 (520-996)	878 (576-1200)
Pharynx length	75 (53-100)	86 (71-112)
width	68 (43-92)	75 (51-99)
Acetabulum diameter	113 (82-125)	115 (86-125)
Testes length	64 (58-69)	72 (64-78)
width	138 (128-148)	146 (138-155)
Ovary diameter	62 (53-79)	68 (58-86)

C: Renicolid metacercaria

	Experimentally obtained	Naturally occuring
Number of specimens	12	8
Cyst length	513 (475-546)	520 (496-566)
width	498 (475-546)	540 (484-558)
Body length	774 (578-885)	758 (563-1008)
width	214 (130-265)	234 (142-282)
Oral sucker diameter	69 (55-89)	78 (64-99)
Pharynx	26 (24-30)	26 (22-32)
Oesophagus	25 (16-30)	26 (19-32)
Acetabulum length	70 (50-86)	76 (58-99)
width	68 (56-82)	74 (64-99)

D: Echinostomid metacercaria

	Experimentally obtained	Naturally occuring
Number of specimens	10	2
Cyst length	155 (143-168)	148 (134-163)
width	127 (108-148)	122 (106-138)
Body length	357 (247-489)	339 (228-450)
width	129 (105-156)	116 (94-138)
Oral sucker diameter	32 (26-36)	31 (27-35)
Pharynx	25 (23-28)	24 (22-26)
Acetabulum diameter	44 (36-51)	40 (35-45)

Table 2.12: The results of the feeding trials of metacercaria to definite host.

A: The results of the feeding trials of Cyathocotylid metacercaria A to laboratory final hosts.

Chick / Rat	Date of Infection	Number of parasites fed	Hours/Days allowed for development	Number of parasites recovered
Chicks				
A	14.08.90	20	3 days	
	15.08.90	18	2 days	
	16.08.90	26	1 day	
				0
B	14.08.90	37	2 days	
	15.08.90	25	1 day	
				0
E	14.12.90	35	2 days	
	15.12.90	46	1 day	
				0
F	14.12.90	26	2 days	
	15.12.90	23	1 day	
				0
N	19.02.91	28	2 hours	5 in rectum
O	19.02.91	16	1½ hours	11 in rectum
Rats				
BB	16.12.90	34	2 days	0†
	17.12.90	23	1 day	
EE	25.02.91	16	6 hours	0†
FF	25.02.91	28	5 hours	4† in large intestine

† could not check the rectal and posterior large intestinal contents due to dehydration of contents.

B: The results of the feeding trials of Renicolid metacercaria to laboratory final hosts.

Chick / Rat	Date of infection	Number of parasites fed	Days allowed for development	Number of parasites recovered
D-Chick	10.12.90	25	2	0
AA-Rat	08.12.90	30	2	0

C: The results of the feeding trials of Acanthostomid metacercaria to laboratory final hosts.

Chick / Rat	Date of infection	Number of parasites fed	Days allowed for development	Number of parasites recovered
L-Chick	04.02.91	23	2	0
M-Chick	05.02.91	14	1	0
CC-Rat	08.01.91	38	2	0
DD-Rat	11.01.91	24	1	0

D: The results of the feeding trials of *P. scolecina* cysticerci to laboratory final hosts.

Chick	Date of infection	Number of parasites fed	Days allowed for development	Number of parasites recovered
H	19.12.90	1	4	
	20.12.90	5	3	
	22.12.90	6	1	
				0
J	23.02.91	9	4	
	24.02.91	18	3	
	26.02.91	11	1	
				0

E: The results of the feeding trials of *Contracaecum* spp. to laboratory final hosts.

Chick	Date of infection	Number of parasites fed	Hours/Days allowed for development	Number of parasites recovered
C	14.08.90	8	3 days	
	15.08.90	16	2 days	
	16.08.90	8	1 day	
				0
G	14.12.90	12	2 days	
	15.12.90	9	1 day	
				0
I	22.12.90	12	2 days	
	23.12.90	14	1 day	
				0
K	29.12.90	16	2 days	
	30.12.90	10	1 day	
				0
N	19.02.91	6	2 hours	2 in rectum
O	19.02.91	17	1½ hours	7 in rectum

2.3.2.2. Life-cycle study of *D. amplectens*

The numbers of each stage found in 1 litre of the total 20 litres of water over the 16 days of the experiment are given in the Table 2.13. According to results of trial 1, the life cycle stages could not develop beyond nauplius 2 stage, possibly without any food in the environment due to the use of filtered autoclave lagoon water, and in trial 2 beyond copepodid stage 3 without fish (probably the host fish) in the environment. Only the eggs placed in the tank with filtered lagoon water and fish (trial 3) developed into adults. The duration of the period which the eggs took to develop into first copepodid stage was approximately 4 days at temperature 29 ±1 °C (trials 2 and 3). The duration from first copepodid stage to the appearance of adults was 5 days at the same temperature (trial 3). The experiment was not successful in determining the duration of the free living stage of adult females. Only adult males were recorded from the tank. No attached specimens could be found on the gills of host fish, male or female, even up to day 16 which was the end of the experiment. At no time were any females found in the experimental tanks even after filtering the whole tank. The fate of the females were therefore unknown after stage 5 copepodids.

Six naupliar stages, five copepodid stages and adult males were found. The stages occurring in the water sampled were separated according to the structural morphology and length differences. Figure 2.23 shows the six naupliar stages and Table 2.14 gives details of the length of each stage. The lengths of *E. sieboldi* (Abdelhalim, Lewis & Boxshall, 1991) and *Neoergasilus japonicus* (Urawa, Muroga & Kasahara, 1980a, 1980b) are provided for comparison.

Nauplius stage 1 (Figure 2.23A)

Body oval; a knob like rostrum at the anterior end; hemispherical knob on the dorsal surface near the caudal end. Median eye just posterior to rostrum. Anus a transverse slit on ventral surface near posterior margin. Caudal rami represented by a pair of long setae. Antennule uniramous, 2-segmented; first segment with one seta; second with two setae. Antenna biramous; 2-segmented protopod; coxa short, with gnathobasic seta; endopod one segmented with 2 apical setae; exopod 5 segmented, first four carrying 1 seta and the last

with 2. Mandible biramous; unsegmented protopod bearing 1 stout seta; exopod 2-segmented; first bearing 1 seta; second bearing 2; endopod 2-segmented; fist bearing 2 seta medially; second segment small, with spatulate element and 3 slender setae.

Nauplius stage 2 (Figure 2.23B)

General appearance quite similar to that of stage 1, but larger. Differ in the following features: caudal organ migrated slightly onto posterodorsal surface, and on mandible, long setule added midway on inner seta of basal segment of endopod.

Nauplius stage 3 (Figure 2.23C)

General appearance similar to that of stage 2, but larger. Antennule with 4 apical setae. On caudal end, one pair of caudal setae newly appeared.

Nauplius stage 4 (Figure 2.23D)

Body resembling nauplius stage 3, but larger. Antennule with 6 setae on terminal segment. Small spine added to terminal segment of endopod of antenna. First maxilla newly appeared on ventral surface just posterior to mandible. On caudal end, third pair of short setae appeared.

Nauplius stage 5 (Figure 2.23E)

Body resembling the previous stage, but larger. Spine on the endopod of the second antenna increased in length. On caudal end, fourth pair of short setae added.

Nauplius stage 6 (Figure 2.23F)

Body elongate, showing traces of incipient thoracic segmentation ventrally; rudiments of first and second swimming leg pairs on ventral surface. Terminal segment of the antennule with 7 apical setae. Third and fourth caudal setae longer than in preceding stage.

The lengths of some copepodid stages were overlapping with each other (Table 2.15). Morphological characters helped, in addition to lengths, to distinguish these stages and are as given below. The urosomal development is illustrated in Figure 2.24.

Table 2.13 : Data of life cycle study of *D. amplectens.*

Set up	1. Filtered Autoclaved lagoon water		2. Filtered lagoon water		3. Filtered lagoon water with fish	
No. of egg sacs	12		25		25	
Day	Stage	No. in 1 liter	Stage	No. in 1 liter	Stage	No. in 1 liter
0	nauplius 1	65	nauplius 1	42	nauplius 1	34
1	nauplius 1	34	nauplius 1	24	nauplius 1	14
	nauplius 2	45	nauplius 2	37	nauplius 2	51
2	nauplius 2	17	nauplius 2	6	nauplius 2	12
			nauplius 3	53	nauplius 3	47
3	all dead*		nauplius 3	9	nauplius 3	4
			nauplius 4	66	nauplius 4	38
					nauplius 5	2
4			nauplius 5	12	nauplius 5	14
			nauplius 6	35	nauplius 6	40
			copepodid 1	25	copepodid 1	8
5			nauplius 6	0	nauplius 6	7
			copepodid 1	75	copepodid 1	42
6			copepodid 1	24	copepodid 1	5
			copepodid 2	14	copepodid 2	36
7			copepodid 1	23	copepodid 2	22
			copepodid 2	6	copepodid 3	13
			copepodid 3	18		
8			copepodid 1	13	copepodid 3	8
					copepodid 4 males	11
					females	4
					copepodid 5 males	7
					females	1
9			all dead*		copepodid 5 males	8
					females	3
					adult males	4
10					adult males	3
11					adult males	5
12					adult males	2
16					adult males	2*

* number collected from all 20 litres.

Table 2.14 : Measurements of lengths (in μm) of the life-cycle stages of Ergasilids. Data given as a range or Mean ± Standard error.

Stage	*D.amplectens*		*E. sieboldi*†		*N. japonicus*‡	
	Length	Number measured	Length	Number measured	Length	Number measured
Nauplius 1	58-68 (61 ± 2)	34	79 ± 19	29	92 (84-105)	235
Nauplius 2	86-95 (90 ± 3)	63	131 ± 41	41	109 (100-118)	105
Nauplius 3	99-110 (104 ± 3)	51	164 ± 46	46	123 (110-140)	150
Nauplius 4	115-134 (124 ± 5)	16	179 ± 38	38	142 (128-155)	136
Nauplius 5	140-148 (144 ± 3)	68	232 ± 26	57	155 (143-183)	169
Nauplius 6	153-170 (161 ± 5)	47	289 ± 40	27	180 (163-235)	123
Copepod 1	228-288 (246 ± 14)	55	321 ± 35	17	352 (277-418)	166
Copepod 2	262-307 (286 ± 10)	58	386 ± 47	39	423 (365-474)	120
Copepod 3	314-378 (351 ± 16)	21	546 ± 66	19	F. 480 (401-556) M. 450 (384-522)	34 33
Copepod 4	390-448 (388 ± 49)	15	711 ± 82	26	F. 546 (469-623) M. 508 (469-589)	43 31
Copepod 5	474-499 (485 ± 8)	20	M. 786 ± 34 F. 769 ± 50	47 56	F. 630 (528-760) M. 577 (460-639)	56 48
Adult	493-522 (506 ± 11)	16	871 ± 23	23	F. 670 (573-795) M. 624 (549-716)	20 75

In copepodid stages the lengths were measured from anterior end to the end of caudal ramii.
† Data from Abdelhalim *et al* (1991)
‡ Data from Urawa *et al* (1980a,b)
M. - male, F. - female

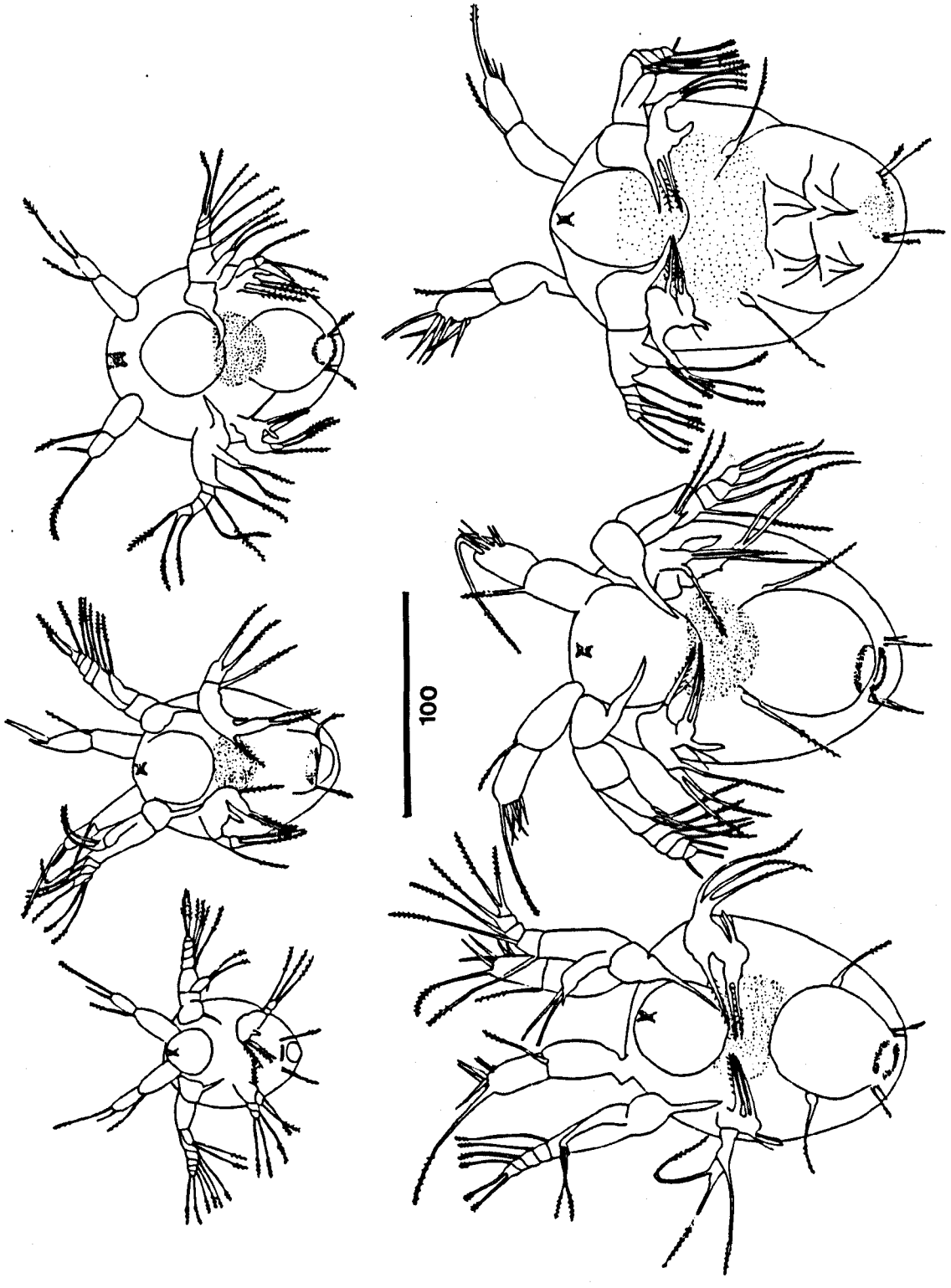


Figure 2.23: (A)-(F) The six naupliar stages of *D. amplectans*.

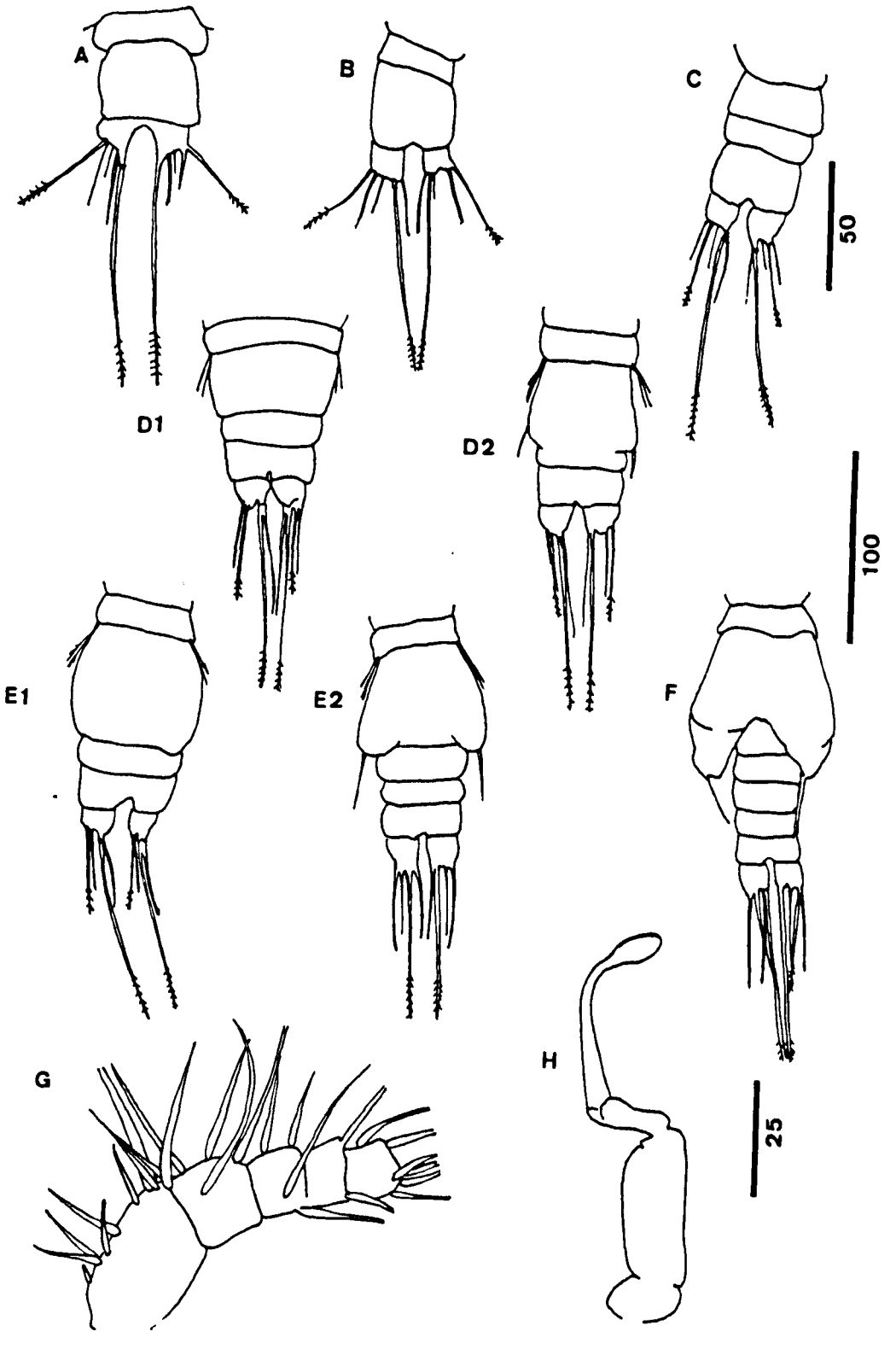


Figure 2.24: Development of the post-naupliar stages. (A)-(E) Urosome development of the copepodid stages 1-5, a) & c) females, b) & d) males. (F) Adult male. (G) The antennule of fifth copepodid stage. (H) Maxilliped of adult male.

First copepodid

Body six segmented, comprising cephalostome, 3 pedigerous somites and two limbless somites. Caudal rami armed with 2 short setae and one long inner setae, which is double with a short branch arising basally. At the middle there is another short seta. Antennule four segmented; first segment longest, setal formula 4: 2: 2: 6. Antenna 4-segmented. First and second are longer and of similar size. Exopod is completely lost. First and second swimming legs biramous; protopods 2-segmented and all rami 1-segmented. Armature of legs are as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	III,4	II,5
Leg 2	0-0	1-0	I,4	I,4

Third leg rudimentary, represented by a pair of bilobed processes.

Second copepodid

Body 7-segmented, comprising cephalostome, 4 pedigerous somites and two limbless somites. Antennule setal formula 9: 3: 2: 6. lengths. Antenna structure similar to the preceding stage. Three pairs of biramous legs present; leg 1 and 2 with 2-segmented rami, rami of third legs 1-segmented. Armature formula:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	I-0;II,5	0-1;II,4
Leg 2	0-0	1-0	I,0;1,4	0-1;I-5
Leg 3	0-0	1-0	I,4	I,4

Fourth legs developing, fifth thoracic segment devoid of appendages.

Third copepodid

Body 8-segmented, comprising cephalosome, four pedigerous somites, and three limbless somites forming urosome. First segment of antennule indistinctly divided into two segments. Antennule with the setal formula 9: 4: 2: 6. Antenna with

no change. Anal segment incised posteriorly to about third of its length. Four pairs of biramous legs present; leg 1 to 3 with 2-segmented rami; leg 4 with 1-segmented rami. Armature formula as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	I-0;II,5	0-1;II,5
Leg 2	0-0	1-0	I-0;1,5	0-1;I-5
Leg 3	0-0	1-0	I-0;1,5	0-1;I,4
Leg 4	0-0	1-0	I,4	I,4

One seta is present posterolaterally on the fifth thoracic segment.

Fourth copepodid

Body 9-segmented; urosome having one more segment than the preceding stage. Male and female fourth copepodids are distinguishable by the shape of genital somite; female genital somite unarmed and with convex lateral sides while male genital segment armed with a pair of setae, elongated, with posterolateral bulges, partially separated from the first abdominal segment behind. Antenna of female stout and longer than male but with same proportions. Antennules with five segments. The setal formula 10: 4: 4: 2: 6. The segmentation of rami of legs similar to the preceding stage. The rami of fourth leg separated. Armature formula of legs as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	I-0;II,6	0-1;II,5
Leg 2	0-0	1-0	I-0;1,6	0-1;I-5
Leg 3	0-0	1-0	I-0;1,6	0-1;I,5
Leg 4	0-0	1-0	I,5	I,5

Fifth leg represented by 3 pairs of setae on surface of somite. The short middle setae on caudal rami lost leaving a process there.

Fifth copepodid

Male with an additional abdominal somite in the urosome to proceeding stage a and total 10 segments in the body. With distinct boundaries between genital and first abdominal somite. Genital somite with well-developed posterolateral lobes bearing a pair of setae. Maxilliped at the developing stage.

Female does not have additional abdominal somite; therefore the same number of somites as the preceding stage. Genital somite with convex sides but with no lobes. Antenna stouter and longer than in male (Figure 2.24H). Maxillipeds absent. Otherwise similar to male.

Antennule setal formula 10: 4: 4: 2: 6 (Figure 2.24G). Leg 1-3 biramous with indistinctly 3-segmented rami; second and third segments of rami incompletely separated. Leg 4 with similar segmentation in endopod and indistinctly 2-segmented exopod. Armature formula is as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	I-0;(0-1;II,5)	0-1;(0-1;II,4)
Leg 2	0-0	1-0	I-0;(0-1;1-5)	0-1;(0-1;I,4)
Leg 3	0-0	1-0	I-0;(0-1;1,5)	0-1;(0-1;I,4)
Leg 4	0-0	1-0	(I-0;1,4)	0-1;(0-2;I,3)

Fifth leg comprising single lobate segment bearing two long and one short setae apically. Setation of caudal rami similar to previous stage.

Adult male (free swimming females were not found)

Body comprising 5-segmented prosome and distinctly 6-segmented urosome, with additional abdominal segment. Antennule 5 segmented; 7 setae on the terminal segment. Antenna with similar structure to the proceeding stage. Maxilliped 4-segmented (Figure 2.24I); first short; second segment stout and long; third segment slightly thinner and about third of the second and with process on distal end; fourth slender, longest, bears a terminal claw. Leg 1-4 with segmentation of rami distinct, all

rami 3-segmented except for 2-segmented exopod of leg 4. Setation is similar to preceding stage. Branch on long inner setae of caudal rami are lost.

2.3.3. The ecological survey

The parasites of *Etroplus* found during this study are listed in the Table 2.15 together with the locality, microhabitat, life cycle stage a summary of the quantification methods used to obtain the counts for intensity data.

Some ectoparasite data given do not reflect the situation in the natural habitat as the parasites *Ichthyobodo* sp., *Ichthyophthirius multifiliis* and the gill monogenean *A. etropli* multiplied in holding tanks. The protozoan parasites were present in hardly noticeable numbers in freshly collected samples and started to appear in skin smears within 8-12 days post collection in some samples (Figure 2.25 & 2.26). They then sometimes multiplied to levels which were lethal to the fish. Whenever this happened, steps were taken to increase survival of fish ie. quick removal of carcasses, more water changing and introduction to parasite - free stocking tanks, and this reduced the parasite numbers. Thus the graphs replects attempts to control parasite numbers in holding tanks.

In the case of *A. etropli* the situation was different. This parasite already had 100% prevalence on the hosts when brought in from the natural environment. The confinement favoured their multiplication and caused high densities. It was not necessary to control their populations as no detrimental effects were apparent. It is evident that in most of the samples, the high densities subsequently reduced. This is probably due to the action of some regulatory factors on high population densities, resulting in a reduction and thus regulation in the population size (Figure 2.27). The attempts taken to control the protozoans may also have reduced the monogenean numbers in those samples too. However, the population densities of the monogeneans

Table 2.15 : Parasites found in the survey with their stage, sex, micro habitat and the method of quantification.

Parasite	Locality U	Locality K	Micro Habitat	Stage and Sex	Quantifying Method
Protozoa					
Trichodina sp.*	P	P	Skin, Gill filaments	trophozoites	10 cm^2 body surface area
Apiosoma (=*Glossatella*) sp.*	P	P	Skin	trophozoites	10 cm^2 body surface area
Ichthyobodo sp.	P	P	Skin, Gill filaments	trophozoites	10 cm^2 body surface area
Ichthyophthirius multifiliis	P	A	Skin, Gill filaments	trophozoites	10 cm^2 body surface area
Trypanosoma sp.*	P	P	Blood	trypomastigote	total in 3 microscopic areas of mag. x100
Monogenea					
Ancyrocephalus etropli	P	P	Skin, Gill filaments	larvae, juveniles and adults	2nd gill arch of one side
Ceylonotrema colombensis	P	P	Gill filaments	juveniles, adults	2nd gill arch of one side
Enterogyrus globidiscus	P	P	Stomach	adults	entire stomach inner surface
Enterogyrus papernai	P	P	Stomach	juveniles,adults	(both species counted together)
Digenea					
Renicolid metacercaria	P	A	Liver	metacercaria	entire liver
Strigeid metacercaria	P	A	Mesentery	metacercaria	entire mesentery
Exorchis sp.	A	P	Skin, Gill filaments	metacercaria	2nd gill arch of one side
Acanthostomid metacercaria	A	P	Fin & Gill filament cartilage	metacercaria	2nd gill arch of one side
Cyathocotylid metacercaria A	P	P	Muscle, Mesentery and Liver	metacercaria	entire muscle, liver & mesentery
Transversotrema patialense	P[1]	P	Scale pockets	adult	10cm^2 body surface area
Malabarotrema indica	A	P	Intestine	adult	entire intestinal and rectal cavity
Cestoda					
Paradilepis scolecina (live & dead)	P	A	Mesentery	cysticercus	entire mesentery
Nematoda					
Rhabdochona sp.	A	P	Fore- & mid-intestine	sub adults, adults males & females	entire fore- and mid-intestinal walls
Contracaecum sp.*	P	P	Mesentery, Stomach	stage 3 larva	entire mesentery, spleen and liver
Annelida					
Leech (unidentified)	A?	P	Skin	adult	entire body surface
Crustacea					
Dermoergasilus amplectens	A	P	tip of gill filaments	adult female	four gill arches of one side
Ergasilus parvitergum	A	P	gill filaments	adult female	four gill arches of one side
Argulus sp.	A	P	Skin	adults	entire body surface

[1] One *Transversotrema patialense* specimen was found on one tilapia from Udawalawa

A - Absence of Parasite, P - Presence of Parasite

K - Koggala Lagoon, U - Udawalawa Reservoir

* - probably of different species between the localities

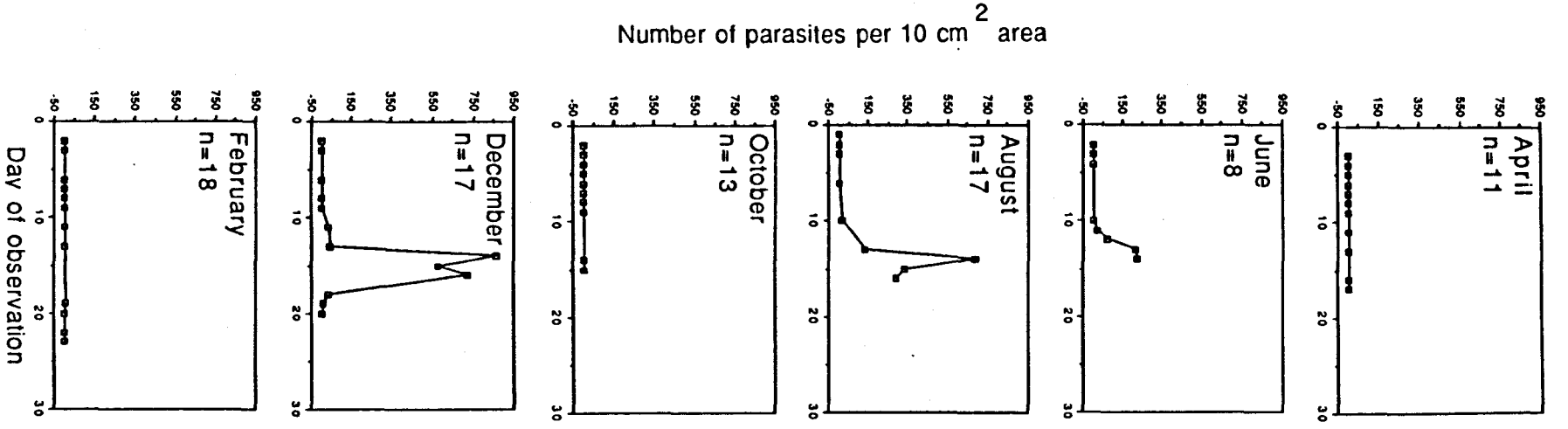


Figure 2.25: *Ichthyophthirius multifiliis* levels of fish; for fish brought from Udawalawa and left in holding tanks. Each point represents minimum of 1 to maximum of 3 fish. n = total number of fish observed.
Note: The environment was not constant and efforts were made to control the parasite when infection levels were high.

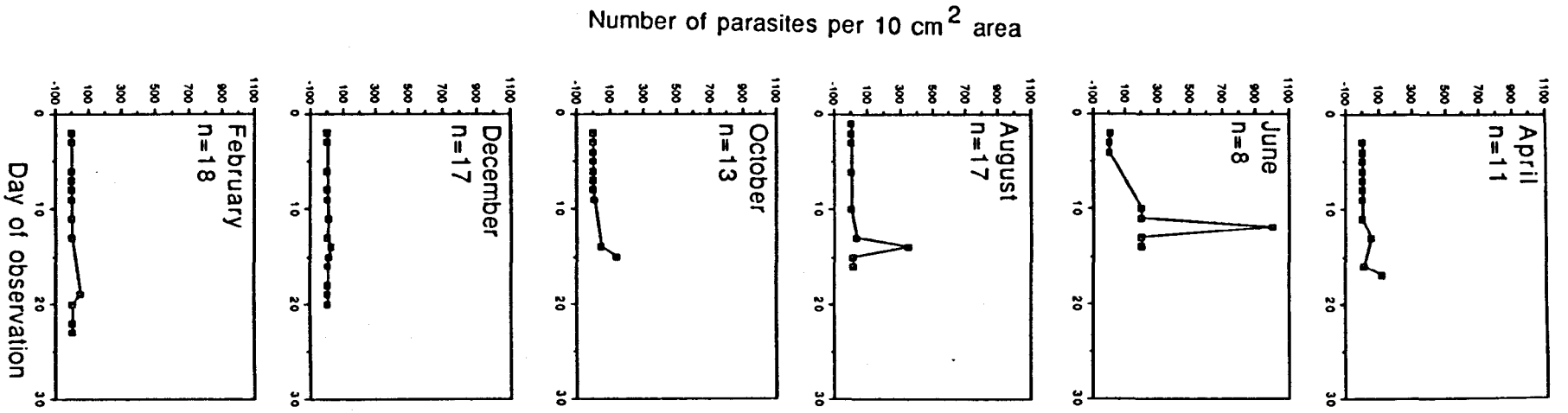


Figure 2.26: *Ichthyobodo* levels of fish; for fish brought from Udawalawa and left in holding tanks. Each point represents minimum of 1 to maximum of 3 fish. n = total number of fish observed.
Note: The environment was not constant and efforts were made to control the parasite when infection levels were high.

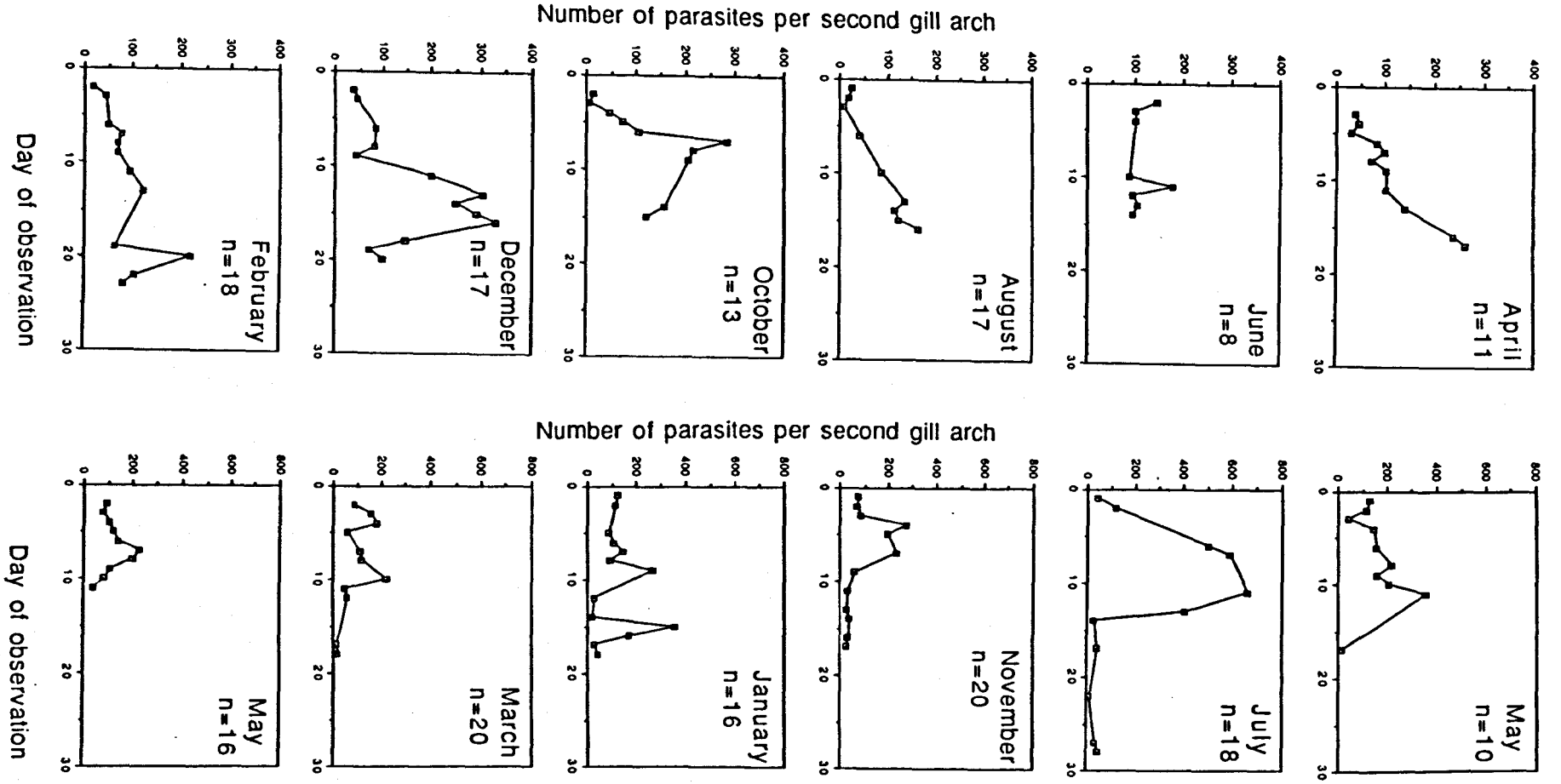


Figure 2.27: *Ancyrocephalus etropli* levels of fish; for fish brought from Udawalawa and left in holding tanks (the sacle between the two localities differ). Each point represents minimum of 1 to maximum of 3 fish. The fish size varied. n = total number of fish observed.

Note: The environment was not constant and efforts were made to control the protozoans, *I. multifiliis* and *Ichthyobodo.*

C. colombensis and *Enterogyrus* species did not seem to be multiplying rapidly, as did the other species, parasite densities remaining much uniform, during the holding period.

2.3.3.1. The macro habitat effect on the parasite species composition and parasite infection levels

(1) Comparison of the fauna between water bodies

A. Koggala lagoon with Udawalawa reservoir (brackish water fauna with fresh water fauna)

The parasite fauna of *Etroplus* is categorized according to habitat in the Table 2.16. The protozoan *I. multifiliis*, two species of metacercaria (renicolid metacercaria and strigeid metacercaria) and *P. scolecina* were absent in the lagoon fish, whilst two species of metacercaria (*Exorchis* sp. and acanthostomid metacercaria), the two adult digeneans (*T. patialense* and *M. indica*), the nematode *Rhabdochona* sp. and the crustacean parasites were absent from the fish from the reservoir.

Table 2.16: The parasites of *E. suratensis* according to the locality in which they were found.

Parasites exclusive to Udawalawa reservoir	Parasites exclusive to Koggala lagoon	Parasites occupying both localities
I. multifiliis	*Exorchis* sp.	Tricodinids*
Renicolid metacercaria	Acanthostomid metacercaria	Scyphidians*
Strigeid metacercaria	*T. patialense*	*Ichthyobodo* sp.
P. scolecina	*M. indica*	*Trypanosoma* sp.*
	Rhabdochona sp.	*A. etropli*
	D. amplectens	*C. colombensis*
	Ergasilus parvitergum	*E. globidiscus*
	Argulus sp.	*E. papernai*
		Cyathocotylid metacercaria
		Contracaecum sp.*

* It is not certain if more than one species is involved.
NB. Only one known species of *Ichthyobodo* exists ie. *I. necator* and this is known to be euryhaline.

Table 2.17: Year round ecological data on the parasites from Udawalawa reservoir and Koggala lagoon. Data categorized according habitat preference.

Table 2.17A: Parasites exclusive to Udawalawa reservoir.

Parasite	Locality	n	Prevalence	Intensity Range	Mean Intensity	Abundance
Ichthyophthirius multifiliis	Udawalawa	84	40.5	0-951	187.8 ± 289.3	76.0 ± 204.6
Renicolid metacercaria	Udawalawa	78	15.4	0-3	1.3 ± 0.7	0.2 ± 0.5
Strigeid metacercaria	Udawalawa	78	41.0	0-26	4.5 ± 5.0	1.8 ± 3.9
Paradilepis scolecina(Live)	Udawalawa	78	69.2	0-11	3.5 ± 2.6	2.4 ± 2.7
" (Dead)	Udawalawa	78	93.6	0-46	11.3 ± 8.9	10.6 ± 9.0

Table 2.17B :Parasites exclusive to Koggala lagoon.

Parasite	Locality	n	Prevalence	Intensity Range	Mean Intensity	Abundance
Exorchis sp.	Koggala	100	67.0	0-13	2.5 ± 2.3	0.6 ± 2.2
Acanthostomid metacercaria	Koggala	100	28.0	0-5	2.1 ± 1.2	0.6 ± 1.2
Transversotrema patialense†	Koggala	100	37.0	0-6	1.7 ± 1.3	0.6 ± 1.1
Malabarotrema indica	Koggala	94	78.7	0-56	6.4 ± 8.2	5.0 ± 7.7
Rhabdochona sp.	Koggala	94	48.9	0-31	5.8 ± 7.3	2.8 ± 5.8
Dermoergasilus amplectens	Koggala	94	98.0	0-68	24.8 ± 16.0	24.3 ± 16.2
Ergasilus parvitergum	Koggala	94	28.0	0-4	1.5 ± 0.9	0.4 ± 0.8
Argulus sp.	Koggala	94	19.0	0-4	1.6 ± 0.9	0.3 ± 0.7

Table 2.17C :Parasites common to both localities.

Parasite	Locality	n	Prevalence	Intensity Range	Mean Intensity	Abundance
(a) found in higher infection levels in Udawalawa reservoir, significantly different at 95 % level.						
Trichodina sp.*	Udawalawa	84	35.7	0-47	5.1 ± 9.1	1.8 ± 6.0
	Koggala	100	13.0	0-2	1.3 ± 0.5	0.2 ± 0.5
Ichthyobodo sp.	Udawalawa	84	32.1	0-1000+	110.1 ± 199.0	35.4 ± 122.8
	Koggala	100	6.0	0-2	1.5 ± 0.5	0.1 ± 0.4
Trypanosoma sp. *	Udawalawa	84	94.9	0-120	15.0 ± 19.1	14.2 ± 19.0
	Koggala	94	37.2	0-13	2.9 ± 2.6	1.1 ± 2.1
Cyathocotylid metacercaria A	Udawalawa	78	98.7	0-147	21.4 ± 27.1	21.1 ± 27.1
	Koggala	94	91.5	0-34	8.4 ± 6.7	7.7 ± 6.8
Contracaecum sp.* (L_3)	Udawalawa	78	96.2	0-18	6.4 ± 3.7	6.1 ± 3.9
" (L_4 & L_5)	Udawalawa	78	96.2	0-18	6.9 ± 4.2	6.6 ± 4.3
(L_5)	Koggala	94	3.2	0-1	1.0 ± 0.0	0.3 ± 0.2
Ceylonotrema colombensis	Udawalawa	84	91.7	0-27	5.2 ± 4.8	4.8 ± 4.8
	Koggala	100	63.0	0-24	3.1 ± 4.0	2.0 ± 3.5
(b) found in higher prevalence and infection levels in Koggala lagoon, significantly different at 5 % level.						
Apiosoma (=*Glossatella*) sp.*	Udawalawa	84	4.8	0-7	3.5 ± 2.5	0.2 ± 0.9
	Koggala	100	17.0	0-16	4.4 ± 3.7	0.8 ± 2.2
Enterogyrus spp.	Udawalawa	78	88.5	0-135	41.0 ± 35.5	36.2 ± 35.9
	Koggala	94	100.0	4-466	76.4 ± 86.1	76.4 ± 86.1
(c) found at similar levels in both localities, not significantly different at 95 % level.						
Ancyrocephalus etropli	Udawalawa	84	100.0	3-334	108.4 ± 82.5	108.3 ± 82.6
	Koggala	100	100.0	4-663	117.9 ± 122.6	117.9 ± 122.6

n = number of fish observed

† found in Udawalawa on *O. niloticus*

* not certain whether the same species is involved in both localities

Table 2.18: Year round ecological data on the parasites from Udawalawa reservoir and Koggala lagoon. Data categorized according to Prevalence and Mean intensity levels.

Table 2.18A: Parasites with high Prevalence (> 50 %) and Mean intensity levels (> 10 parasites per a unit area of quantification).

Parasite	Locality	n	Prevalence	Intensity Range	Mean Intensity	Abundance
Trypanosoma sp.	Udawalawa	84	94.9	0-120	14.9 ± 19.1	14.2 ± 18.9
Ancyrocephalus etropli	Udawalawa	84	100.0	3-334	108.3 ± 82.6	108.3 ± 82.5
	Koggala	100	100.0	4-663	117.9 ± 122.6	117.9 ± 122.6
Enterogyrus spp.	Udawalawa	78	88.5	0-135	41.0 ± 85.5	36.2 ± 35.9
	Koggala	94	100.0	4-466	76.4 ± 86.1	76.4 ± 86.1
Cyathocotylid metacercaria A	Udawalawa	78	98.7	0-147	21.4 ± 27.1	21.1 ± 27.1
	Koggala	94	91.5	0-34	8.4 ± 6.7	7.7 ± 6.8
Paradilepis scolecina(Live)	Udawalawa	78	69.2	0-11	3.5 ± 2.6	2.4 ± 2.7
" (Dead)	Udawalawa	78	93.6	0-46	11.3 ± 8.9	10.6 ± 9.0
Dermoergasilus amplectens	Koggala	94	98.0	0-68	24.8 ± 16.0	24.3 ± 16.2

Table 2.18B: Parasites with high Prevalence and low Mean intensity levels.

Parasite	Locality	n	Prevalence	Intensity Range	Mean Intensity	Abundance
Ceylonotrema colombensis	Udawalawa	84	91.7	0-27	5.2 ± 4.8	4.8 ± 4.8
	Koggala	100	63.0	0-24	3.1 ± 4.0	2.0 ± 3.5
Exorchis sp.	Koggala	100	67.0	0-13	2.5 ± 2.3	0.6 ± 2.2
Malabarotrema indica	Koggala	94	78.7	0-56	6.4 ± 8.2	5.0 ± 7.7
Contracaecum sp. (L_3)	Udawalawa	78	96.6	0-18	6.4 ± 3.7	6.1 ± 3.9
" (L_3 & L_4)	Udawalawa	78	96.2	0-18	6.9 ± 4.2	6.6 ± 4.3

Table 2.18C: Parasites with low prevalence.

Parasite	Locality	n	Prevalence	Intensity Range	Mean Intensity	Abundance
Trichodina sp.	Udawalawa	84	35.7	0-47	5.1 ± 9.1	1.8 ± 5.9
	Koggala	100	13.0	0-2	1.3 ± 0.4	0.2 ± 0.5
Apiosoma (=*Glossatella*) sp.	Udawalawa	84	4.8	0-7	3.5 ± 2.5	0.2 ± 0.9
	Koggala	100	17.0	0-16	4.4 ± 3.2	0.8 ± 2.2
Ichthyobodo sp.	Udawalawa	84	32.1	0-1000+	110.1 ± 199.0	35.4 ± 122.8
	Koggala	100	6.0	0-2	1.5 ± 0.5	0.1 ± 0.4
Ichthyophthirius multifiliis	Udawalawa	84	40.5	0-951	187.8 ± 289.3	76.1 ± 204.6
Trypanosoma sp.	Koggala	94	37.2	0-13	2.9 ± 2.6	1.1 ± 2.1
Renicolid metacercaria	Udawalawa	78	15.4	0-3	1.3 ± 0.7	0.2 ± 0.5
Strigeid metacercaria	Udawalawa	78	41.0	0-26	4.5 ± 5.0	1.8 ± 3.9
Acanthostomid metacercaria	Koggala	100	28.0	0-5	2.1 ± 1.2	0.6 ± 1.2
Transversotrema patialense	Koggala	100	37.0	0-6	1.7 ± 1.3	0.6 ± 1.1
Contracaecum sp.	Koggala	94	3.2	0-1	1.0 ± 0.0	0.3 ± 0.2
Rhabdochona sp.	Koggala	94	48.9	0-31	5.8 ± 7.3	2.8 ± 5.8
Ergasilus parvitergum	Koggala	94	28.0	0-4	1.5 ± 0.9	0.4 ± 0.8
Argulus sp.	Koggala	94	19.0	0-4	1.8 ± 0.9	0.3 ± 0.7

n = number of fish observed

Table 2.17 and Table 2.18 summarise the ecological data of parasite infection levels for the entire survey period.

The extent of the parasite burden is given in Table 2.17 under the categorization of parasites according to their locality. In the case of the parasites, common to both localities, significant differences in the parasite burdens were found (Table 2.17C). *Ichthyobodo*, *Trypanosoma*, *C. colombensis*, the cyathocotylid metacercaria and *Contracaecum* were found to be more prevalent and had higher intensities in Udawalawa compared to the lagoon. In contrast, the stomach monogeneans, *Enterogyrus* spp. and the ectoparasitic scyphidians were found to have higher values in the lagoon. Tricodinids and *A. etropli* were found to thrive with similar burdens in both localities. As population fluctuations of *A. etropli* occurred in the holding facility, the interpretation of the data on this parasite may be inconclusive.

In the Table 2.18, the parasites are categorized according to prevalences and infection levels. The highly prevalent (found in more than 50 % of fish observed) and numerous parasites (more than 10 parasites per fish or per quantifying area) were the monogeneans *A. etropli* and *Enterogyrus* spp. (*E. globidiscus* representing about 75-80 % of this) in both localities, the protozoan blood parasite *Trypanosoma* sp., the cyathocotylid metacercaria and the cestode *P. scolecina* in Udawalawa, and the crustacean *D. amplectens* in Koggala (Table 2.18A).

The monogenean *C. colombensis* in both localities, the digeneans *Exorchis* sp., cyathocotylid metacercaria and *M. indica* in Koggala, and the nematode *Contracaecum* sp. in Udawalawa were also highly prevalent, but were found in low intensities as seen in the Table 2.18B. No other species of parasite were found in more than half of the fish examined (Table 2.18C).

B. Bolgoda lagoon with Koggala lagoon (the two brackish water localities)

The ecological data on the sample collected in May'91 from Bolgoda lagoon are given with the data for May'91 sample from Koggala lagoon in the Table 2.19. The sample which was collected at the same time as the Bolgoda sample was selected

Table 2.19: Ecological data on the parasites of *E. suratensis* from Bolgoda lagoon and Koggala lagoon†.

Parasite	Locality	n	Preval.	Range	Mean intensity	Abundance
Protozoa						
Trichodina sp.	Bolgoda	14	21.4	0-3	2.3 ± 0.6	0.5 ± 1.0
	Koggala	16	18.8	0-2	1.3 ± 0.6	0.3 ± 0.6
Apiosoma (=*Glossatella*) sp.	Bolgoda	14	14.3	0-7	5.5 ± 2.1	0.8 ± 2.1
	Koggala	16	18.8	0-3	1.7 ± 1.2	0.3 ± 0.8
Ichtyobodo sp.	Bolgoda	14	14.3	0-2	1.5 ± 0.7	0.2 ± 0.6
	Koggala	16	6.3	0-2	2.0	0.1 ± 0.5
Trypanosoma sp.	Bolgoda	14	42.9	0-6	2.8 ± 1.9	1.0 ± 1.8
	Koggala	16	42.9	0-7	2.6 ± 2.1	1.1 ± 1.9
Monogenea						
A. etropli	Bolgoda	14	100.0	29-205	91.6 ± 50.3	91.6 ± 50.3
	Koggala	16	100.0	43-245	127.6 ± 57.4	127.6 ± 57.4
C. colombensis	Bolgoda	14	92.9	0-4	1.8 ± 0.9	1.7 ± 1.0
	Koggala	16	87.5	0-4	1.7 ± 0.9	1.5 ± 1.0
Enterogyrus spp.	Bolgoda	14	100.0	13-74	43.1 ± 19.7	43.1 ± 19.7
	Koggala	16	100.0	14-82	43.6 ± 20.3	43.6 ± 20.3
Trematoda						
Acanthostomid metacercaria	Bolgoda	14	21.4	0-4	2.0 ± 1.7	0.4 ± 1.1
	Koggala	16	18.8	0-2	1.3 ± 0.6	0.3 ± 0.6
Exorchis sp.	Bolgoda	14	50.0	0-4	2.4 ± 1.3	1.1 ± 1.5
	Koggala	16	50.0	0-5	2.1 ± 1.4	1.1 ± 1.4
Cyathocotylid metacercaria A	Bolgoda	14	85.7	0-18	5.4 ± 4.6	4.6 ± 4.7
	Koggala	16	81.3	0-34	8.0 ± 8.8	6.5 ± 8.5
T. patialense	Bolgoda	14	28.3	0-2	1.5 ± 0.6	0.4 ± 0.8
	Koggala	16	31.3	0-2	1.4 ± 0.5	0.4 ± 0.7
M. indica	Bolgoda	14	78.6	0-24	7.2 ± 6.5	5.6 ± 6.5
	Koggala	16	81.3	0-13	4.4 ± 4.1	3.6 ± 4.1
Nematoda						
Contracaecum sp. (L_3)	Bolgoda	14	78.6	0-5	2.5 ± 1.3	1.9 ± 1.5
	Koggala	16	0.0	0		0.0
Crustacea						
D. amplectans	Bolgoda	14	100.0	6-64	28.9 ± 17.3	28.9 ± 17.3
	Koggala	16	93.8	0-53	20.7 ± 12.7	19.4 ± 13.3
E. parvitergum	Bolgoda	14	14.3	0-2	1.5 ± 0.7	0.2 ± 0.6
	Koggala	16	31.3	0-2	1.4 ± 0.5	0.4 ± 0.7

† the data of the parasites found only in Koggala are not included

Environmental Parameters of the site at the time of sample collection

	Bolgoda lagoon	Koggala lagoon
Temperature °C	28.8	28.5
Salinity ‰	4.3	6.3
pH	7.8	8.0

for the comparison with the assumption that the prevailing climatic conditions (rainfall, temperature etc.) were similar. However, of the water parameters, the salinity of the sample collected in Bolgoda was little less than in Koggala lagoon (4.3‰ and 6.3‰ respectively).

When comparing these two samples, almost all the parasites found in Koggala lagoon were also present in Bolgoda, with the exception of *Rhabdochona* sp. and *Argulus*. This may be due either to their complete absence of the parasite on the host in Bolgoda, or the difference in the micro-habitat of the sampling area. Alternatively they may not be common and may have been missed due to the small sample size.

The ecological data for parasites populations (Table 2.19) showed remarkably similar infection level between Bolgoda and Koggala, with the exception of *Contracaecum* which showed a higher prevalence and infection levels in Bolgoda. Though *Contracaecum* were absent in the Koggala sample used for comparison, they were found in some other Koggala samples at very low levels. The slight differences in infection levels showed by *A. etropli*, the cyathocotylid metacercaria, *M. indica* and *D. amplectens* may be due to minor differences in the environments.

(2) The variation due to seasonal changes in the locality

There were no clear difference in prevalence values between the samples for most of the parasites and the data including fish uninfected by a particular parasite. The data used to calculate abundance values may give a better representation of the ecological picture and this data set was therefore analyzed in order to discover any variations in parasite abundance. The significance of the difference in abundance of the parasites in the samples collected during the period of survey was assessed for each parasite in each locality. The results of the statistical tests are given in the Table 2.21, along with all the ecological data for parasites.

Seasonal changes

In order to understand the difference between the monthly samples, seasonal differences were identified. The environmental parameters for both localities are given

Table 2.20: Environmental Parameters

	Parameter	April	May	June	July	Augu.	Sept.	Octo.	Nov	Dece.	Janu.	Febr.	Marc.	Apri.	May
Udawalawa															
	Total monthly Rainfall (mm)[2]	132	130	24	30	3	23	291	343	242	144	29	68	128	85
	Temperature °c[1]	30.2		29.6		30.8		30.5		28.5		29.5			
	Salinity ‰[1]	<---------- always less than one ---------->													
	pH [1]	7.4		7.8		8.1		7.6		6.5		7.3			
	Mean monthly air temperature °c [2]														
	Minimum	25.5	25.6	24.9	24.4	25.1	25.3	24.7	23.8	23.7	23.4	22.6	24.7	25.2	26.1
	Maximum	32.0	31.7	30.1	30.9	32.4	31.4	30.9	30.2	29.5	30.0	30.7	31.5	31.9	31.5
Koggala															
	Total monthly Rainfall (mm)[2]	345	322	185	249	18	32	105	286	161	127	83	52	106	454
	Temperature °c[1]		27.8		28.0		29.7		28.5		27.5		30.5		28.5
	Salinity ‰[1]		7.2		5.5		9.5		3.5		6.5		11.7		6.3
	pH [1]		8.2		7.2		7.9		7.1		7.3		8.0		7.6
	Mean monthly air temperature °c [2]														
	Minimum	25.3	26.1	25.3	24.8	25.3	25.6	24.7	24.0	23.1	23.2	22.8	24.6	24.9	25.7
	Maximum	31.0	30.3	28.8	28.5	28.2	29.6	30.0	29.6	29.9	29.7	30.9	32.3	31.1	30.5

[1] Parameters measured at the time of sampling, [2] Data obtained from the Meteorological Department at Colombo

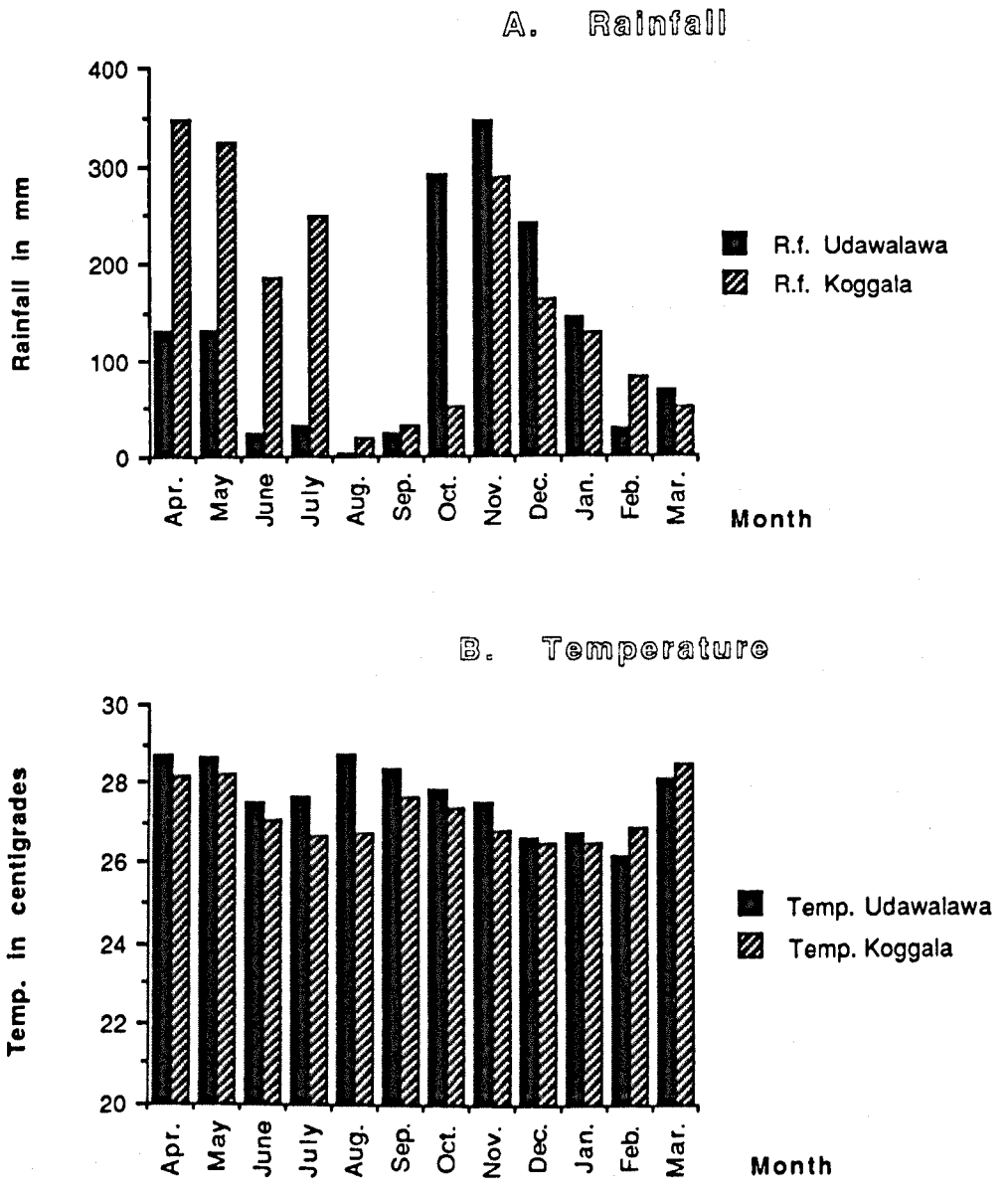


Figure 2.28: Environment parameters. (A) Monthly mean rainfall; (B) Monthly mean air temperatures (the average of the maximum and minimum of monthly mean temperatures) of the two localities.

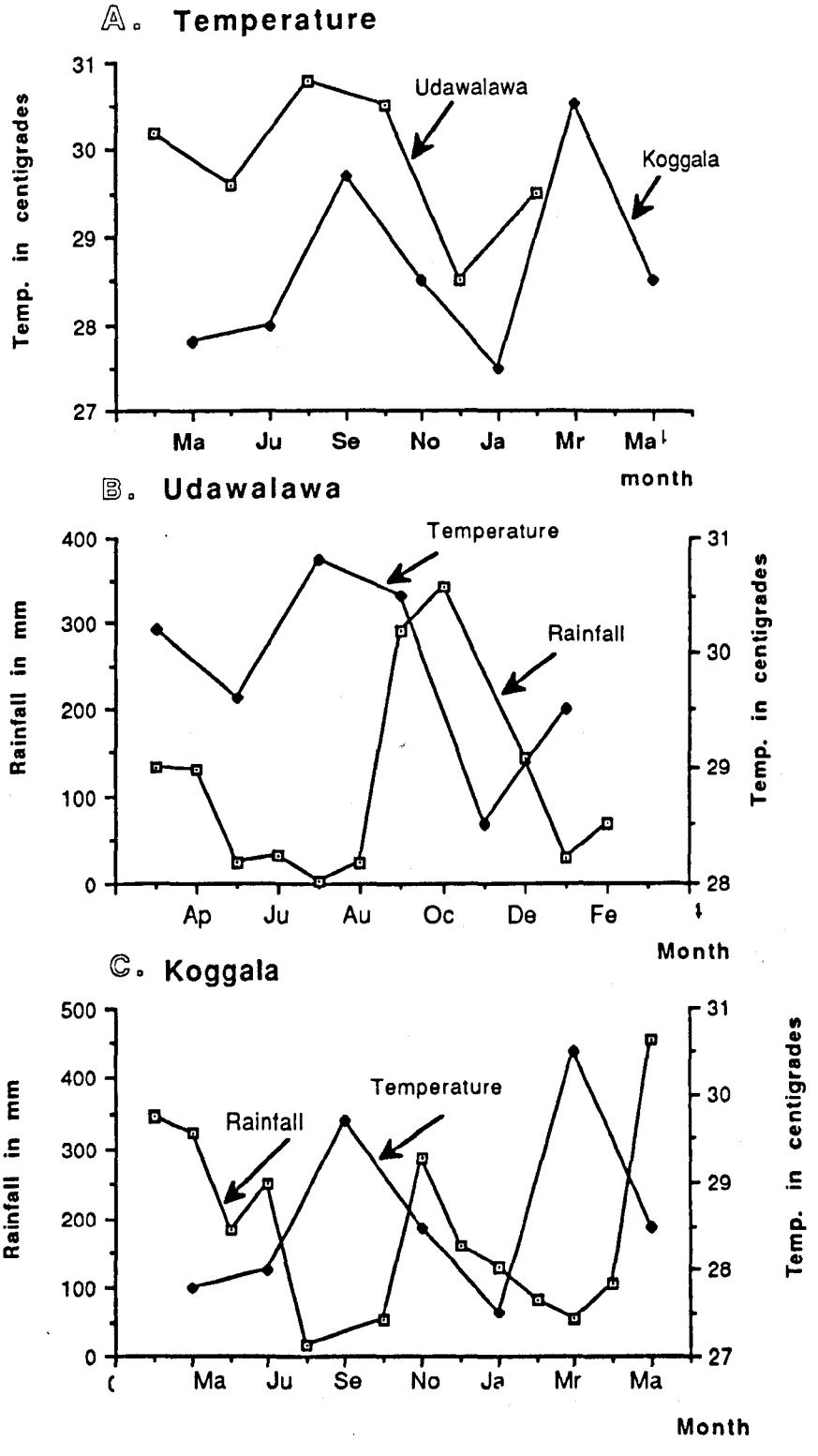


Figure 2.29: Environmental parameters and their relatioships. (A) The water temperature of the two localities (measured at 10.00 hour). (B) & (C) The relationship of water temperature to changes in the rainfall of the localities.

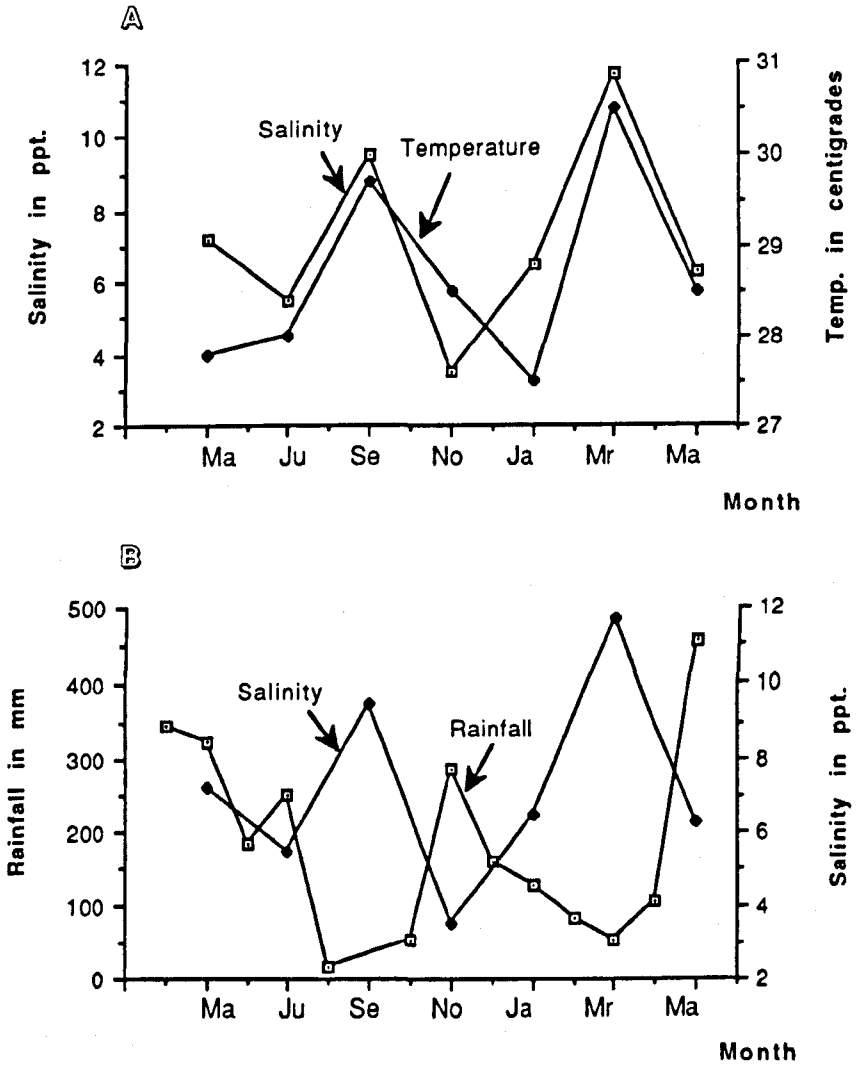


Figure 2.30: Relationships of environmental parameters. (A) The relationship of salinity with temperature at Koggala lagoon. (B) The relationship of salinity with rainfall at Koggala lagoon.

in Table 2.20. In the two localities the rainy periods coincided with each other. But, the South West monsoon has given more rain to Koggala lagoon from April to July and less rain to Udawalawa which lasted April to May. In contrast, the North East monsoon rain has given more rain to Udawalawa from October to January and less rain to Koggala from November to January (Figure 2.28A). The temperature values for Udawalawa were mostly a little higher than those of Koggala in air temperature (Figure 2.28B) or water temperature (Figure 2.29A), but followed a similar pattern. In both localities, the pattern of change of temperature reciprocally followed the rain fall pattern (Figure 2.29B & C). However, the rainy period from April to May did not lower the temperature in Udawalawa to any great extent (Figure 2.29B) probably due to the low rainfall experienced. In Koggala, the pattern of salinity change was similar to the pattern of change in temperature (Figure 2.30A) but was reciprocal with the rainfall (Figure 2.30B). As these parameters, showed a high correlation with each other, they may have collectively acted on the parasite fauna. However in the situations where they acted singly it would be difficult to separate out such effects.

Protozoans

Except for *I. multifiliis* the protozoan burden did not show any significant variation within the period of study (Table 2.21A). *I. multifiliis* infections peaked inside the holding tanks in the months of June, August and December. It appeared in very low numbers in other samples except in October where there were none (Figure 2.25). In the colder rainy December, the higher number of parasites found in aquarium maintained fish may reflect a higher number in the reservoir. In the June and August, the stress caused by the infections of parasite *Ichthyobodo* together with *Ichthyophthirius* may have enhanced the building up of these parasites (Figure 2.25 & 2.26).

Monogeneans

The most numerous gill monogenean, *A. etropli* showed no significance difference in abundance in Koggala samples throughout the study period, but there was a significant difference in the parasite burden in Udawalawa samples with a high burden in December and a low burden in August (Table 2.21B & Figures 2.31 A1 &

B1). The first few specimens surveyed in each sample, before the build up of populations within the aquarium, showed little variation in Koggala but a high variation in Udawalawa (Figure 2.27). Ideally the parasites with short, direct life-cycle should have been sampled at the site, as soon as the fish were collected. However, it was not possible in this study with the facilities available. In spite of this, in both localities the variation in abundance values of *A. etropli* followed the rainfall pattern fairly closely (Figure 2.31 A2 & B2), peaks immediately following the rainfall peaks. Even though these infections built up in stock tanks, it can be argued that this build up is proportional to the number of parasites brought in with the samples, since the time scale was the same.

The other two monogenean species did not show any apparent population changes in holding tanks and displayed significant seasonal variations. *C. colombensis* revealed that infections were high in June in Udawalawa and in July in Koggala (Table 2.21B Figures 2.32 A & B). It was difficult to correlate these peaks, which appeared only one time in the year, to any of the environmental parameters. If this was not due to a sampling error it may have been influenced by host factors or a factor relating to the biology of the parasites.

The seasonal pattern of abundance was different for *Enterogyrus* spp.; two peaks were evident in Koggala, one in July and one in January-March, whilst a steady increase was apparent in Udawalawa reservoir from December to February (Table 2.21B & Figures 2.33 A1 & B1) without a peak corresponding to the one in July at Koggala. The increase in abundance of *Enterogyrus* occured when the monsoon rainy periods receded at Koggala (Figure 2.33 B2). The low rainfall in the SW monsoon season in Udawalawa has not caused any increase but an increase was evident in December to February when the NE monsoon season receded (Figure 2.33 A2). The breeding seasons of *E. suratensis* occurred in July and February in the lagoons and in February in the reservoirs (Costa, 1983), which correspond with the times of increase in abundance. Therefore, the rainfall and/or breeding aggregations may have collectively or independently acted to enhance the population growth.

Table 2.21: Ecological data on Parasites of *Etroplus* in Samples.

Table 2.21A: Data on Protozoan Infections in two localities.
Since fish were left in holding tanks for up to 2-3 weeks the infection levels of direct life-cycle ectoparasites may have changed. Therefore, the data do not necessarily represent the original level of natural infection.

UDAWALAWA Parasite		April	June	August	October	Decem.	Februa.	Sig. Level
1. *Trichodina*sp.[2]	n(ran)	11(0-47)	8(0-23)	17(0-10)	13(0-7)	17(0-4)	18(0-4)	
	Preva.	45.45	75.00	29.41	30.77	23.53	27.78	
	Inten.	13.2± 19.1	6.5± 9.0	3.4± 3.8	2.8± 2.9	2.5± 1.3	1.8± 1.3	
	Abund.	6.0± 13.9	4.9± 8.2	1.0± 2.5	0.8± 2.0	0.6± 1.2	0.6± 1.1	n.s.
2. *Apiosoma* (=*Glossatella*) sp.[2]	n(ran)	11(0-1)	8(0-7)	17(0-3)	13(0)	17(0)	18(0)	
	Preva.	9.09	25.00	5.88	0.00	0.00	0.00	
	Inten.	1.0	5.0± 2.8	3.0	-	-	-	
	Abund.	0.1± 0.3	1.3± 2.5	0.2± 0.7	0.0	0.0	0.0	n.s.
3. *Ichtyobodo* sp.[2]	n(ran)	11(0-115)	8(0-1000)	17(0-349)	13(0-153)	17(0-35)	18(0-92)	
	Preva.	27.27	75.00	35.29	46.15	29.41	5.56	
	Inten.	56.3± 55.8	300.8±351.3	74.5±135.0	63.3± 65.4	10.8± 13.9	61.0± 43.8	
	Abund.	15.4± 36.3	225.6±327.9	26.3± 83.9	29.2± 53.5	3.2± 8.6	6.8± 22.4	n.s.
4. *Ichthyophthirius multifiliis*[2]	n(ran)	11(0-3)	8(0-220)	17(0-690)	13(0)	17(0-992)	18(0-7)	
	Preva.	18.18	62.50	58.88	0.00	70.59	27.78	
	Inten	2.5± 0.7	104.4±104.3	194.4±225.1	-	299.9±393.6	3.4± 2.3	
	Abund.	0.5± 1.0[a]	65.3± 95.6[ab]	114.4±195.5[b]	0.0± 0.0[a]	229.4±365.2[b]	0.9± 1.9[a]	***
5. *Trypanosoma* sp.[1]	n(ran)	11(0-24)	8(5-120)	11(2-64)	13(0-27)	17(1-82)	18(0-46)	
	Preva.	81.81	100.00	100.00	92.31	100.00	94.44	
	Inten.	10.2± 7.3	26.9± 37.9	20.9± 17.7	9.5± 7.3	11.3± 20.6	15.5± 14.7	
	Abund.	8.4± 7.7	26.9± 37.9	20.9± 17.7	8.8± 7.4	11.3± 20.6	14.7± 14.7	n.s.

KOGGALA Parasite		May	July	November	January	March	May	
1. *Trichodina* sp.[2]	n(ran)	10(0-2)	18(0)	20(0-2)	16(0-1)	20(0-1)	16(0-2)	
	Preva.	30.00	0.00	20.00	6.25	5.00	18.75	
	Inten.	1.3± 0.6	-	1.4± 0.5	1.0	1.0	1.3± 0.6	
	Abund.	0.4± 0.7	0.0± 0.0	0.4± 0.7	0.1± 0.3	0.1± 0.2	0.3± 0.6	n.s
2. *Apiosoma* (=*Glossatella*) sp.[2]	n(ran)	10(0-10)	18(0)	20(0-16)	16(0-2)	20(0-1)	16(0-3)	
	Preva.	30.00	0.00	30.00	18.75	5.00	18.75	
	Inten.	6.7± 3.1	3.5± 0.7	5.8± 5.8	1.7± 0.6	2.5± 2.1	3.0	
	Abund.	2.0± 3.5	0.4± 1.1	1.8± 3.8	0.3± 0.7	0.3± 0.9	0.2± 0.8	n.s.
3. *Ichtyobodo* sp.[2]	n(ran)	10(0-2)	18(0-1)	20(0)	16(0-1)	20(0)	16(0-2)	
	Preva.	20.00	11.11	0.00	6.25	0.00	6.25	
	Inten.	2.0± 0.0	1.0± 0.0	-	1.0	-	2.0	
	Abund.	0.4± 0.8	0.1± 0.3	0.0± 0.0	0.1± 0.3	0.0± 0.0	0.1± 0.5	n.s.
4. *Trypanosoma* sp.[2]	n(ran)	10(0-13)	11(0-3)	20(0-8)	16(0-5)	20(0-7)	16(0-7)	
	Preva.	20.00	25.00	30.00	43.75	45.00	43.75	
	Inten.	7.0± 8.5	2.8± 0.5	3.2± 2.5	1.9± 1.5	2.8± 2.2	2.6± 2.1	
	Abund.	1.4± 4.1	0.9± 1.4	1.0± 1.2	0.8± 1.3	1.3± 2.0	1.1± 1.9	n.s.

[1] transformed data tested with One Way Analysis of Variance, [2] untransformed data tested with Kruskal Wallies non parametric method
Significance Levels; * P(probability)< 0.05, ** P< 0.01, *** P< 0.001, n.s.- no significance difference at 95% level
n(ran) - number of fish examined (range of parasites)

Table 2.21B: Data on Monogenean Infections in two localities.

Since fish were left in holding tanks for up to 2-3 weeks the infection levels of direct life-cycle ectoparasites may have changed. Therefore, the data do not necessarily represent the original level of natural infection.

UDAWALAWA Parasite		April	June	August	October	Decem.	Februa.	Sig. Level
1. *Ancyrocephalus etropli*[3]	n(ran)	11(30-262)	8(87-178)	17(3-166)	13(5-304)	17(37-334)	18(15-218)	
	Preva.	100.00	100.00	100.00	100.00	100.00	100.00	
	Inten.	109.4± 76.1	113.1± 31.3	66.7± 57.2	137.2± 95.7	161.7±108.6	73.5± 47.9	
	Abund.	109.4± 76^{ab}	113.1± 31.3^{b}	66.7± 57.2^{a}	137.2± 95.7^{bc}	161.7±108^{c}	73.5± 47.9^{b}	**
2. *Ceylanotrema colombensis*[1]	n(ran)	11(1-13)	8(5-27)	17(0-13)	13(0-5)	17(0-13)	18(1-7)	
	Preva.	100.00	100.00	82.35	84.62	88.24	100.00	
	Inten.	4.2± 3.4	14.3± 7.0	5.1± 4.3	3.4± 0.9	5.1± 4.1	3.2± 1.8	
	Abund.	4.2± 3.4^{a}	14.3± 7.0^{b}	4.2± 4.4^{a}	2.8± 1.5^{a}	4.5± 4.2^{a}	3.2± 1.8^{a}	***
3. *Enterogyrus* spp.[1]	n(ran)	11(0-22)	8(0-21)	11(0-81)	13(2-68)	17(4-120)	18(12-135)	
	Preva.	72.73	87.50	54.55	100.00	100.00	100.00	
	Inten.	11.0± 7.4	8.9± 6.9	15.0± 32.3	26.5± 19.1	55.0± 29.6	72.6± 33.5	
	Abund.	8.0± 8.0^{a}	7.8± 7.1^{a}	8.2± 24.2^{a}	26.5± 19.1^{b}	55.0± 29.6^{c}	72.6± 33.5^{c}	***

KOGGALA Parasite		May	July	November	January	March	May	
1. *Ancyrocephalus etropli*[3]	n(ran)	10(13-355)	18(4-663)	20(12-436)	16(16-352)	20(9-213)	16(43-245)	
	Preva.	100.00	100.00	100.00	100.00	100.00	100.00	
	Inten.	152.0± 95.4	163.4±216.9	91.2±112.4	102.8± 94.2	90.9± 72.8	127.6± 57.4	
	Abund.	152.0± 95.4	163.4±216.9	91.2±112.4	102.8± 94.2	90.9± 72.8	127.6± 57.4	n.s.
2. *Ceylanotrema colombensis*[2]	n(ran)	10(0-5)	18(0-24)	20(0-2)	16(0-8)	20(0-2)	16(0-4)	
	Preva.	90.00	88.88	50.00	50.00	30.00	87.50	
	Inten.	3.4± 1.1	6.3± 6.8	1.2± 0.4	2.5± 2.3	1.3± 0.5	1.7± 0.9	
	Abund.	3.1± 1.5^{c}	5.6± 6.7^{c}	0.6± 0.7^{a}	2.5± 2.0^{ab}	0.4± 0.7^{a}	1.5± 1.0^{bc}	***
3. *Enterogyrus* spp.[1]	n(ran)	10(8-30)	12(12-466)	20(4-67)	16(8-371)	20(26-283)	16(14-82)	
	Preva.	100.00	100.00	100.00	100.00	100.00	100.00	
	Inten.	19.9± 6.9	108.8±142.6	27.1± 15.7	119.5±106.4	126.2± 70.8	43.6± 20.3	
	Abund.	19.9± 6.9^{a}	108.8±143^{bc}	27.1± 15.7^{a}	119.5±106.4^{c}	126.2± 70.8^{c}	43.6± 20.3^{ab}	***

Table 2.21C: Data on Digenean Infections in two localities.

UDAWALAWA Parasite		April	June	August	October	Decem.	Februa.	Sig. Level
1. Cyathocotylid		n(ran)	11(2-28)	8(0-33)	11(1-61)	13(5-49)	17(3-126)	18(4-147)
metacercaria[1]	Preva.	100.00	87.50	100.00	100.00	100.00	100.00	
	Inten.	12.6± 7.6	17.9± 14.3	12.6± 16.6	18.5± 13.2	34.1± 35.8	23.6± 37.6	
	Abund.	12.6± 7.6	15.6± 14.7	12.6± 16.6	18.5± 13.2	34.1± 35.8	23.6± 37.6	n.s.
2. Strigeid	n(ran)	11(1-13)	8(0-6)	11(0-5)	13(0-3)	17(0-12)	18(0-26)	
metacercaria[2]	Preva.	45.45	50.00	18.18	46.15	47.06	38.89	
	Inten.	2.2± 2.2	3.5± 1.3	2.0± 1.4	5.5± 4.2	7.4± 8.2	3.3± 3.0	
	Abund.	1.0± 1.8	1.8± 2.1	0.4± 0.9	2.5± 4.0	3.5± 6.6	1.3± 2.4	n.s.
3. Renicolid		n(ran)	11(0-1)	8(0)	11(0-1)	13(0-1)	17(0)	18(0-3)
metacercaria[2]	Preva.	18.18	0.00	9.09	7.69	0.00	44.44	
	Inten.	1.0± 0.0	-	1.0± 0.0	1.0± 0.0	-	1.5± 0.8	
	Abund.	0.2± 0.4^{ab}	0.0± 0.0^{a}	0.1± 0.3^{a}	0.1± 0.3^{a}	0.0± 0.0^{a}	0.7± 0.9^{b}	**

KOGGALA Parasite		May	July	November	January	March	May	
1. Cyathocotylid		n(ran)	10(1-12)	12(0-30)	20(0-14)	16(0-25)	20(3-25)	16(0-34)
metacercaria[1]	Preva.	100.00	91.67	85.00	93.75	100.00	81.25	
	Inten.	7.1± 4.6	12.3± 7.3	7.5± 5.4	11.3± 6.3	7.2± 5.8	8.0± 8.8	
	Abund.	4.1± 4.6^{a}	11.3± 7.8^{bc}	6.4± 5.7^{ab}	10.6± 6.7^{c}	7.2± 5.8^{abc}	6.5± 8.5^{a}	*
2. *Exorchis* sp.[3]	n(ran)	10(0-3)	18(0-3)	20(0-5)	16(0-13)	20(0-5)	16(0-5)	
	Preva.	50.00	58.33	50.00	87.50	95.00	50.00	
	Inten.	1.8± 0.8	1.8± 0.8	2.0± 1.5	4.6± 3.9	2.0± 1.4	2.1± 1.4	
	Abund.	0.9± 1.1^{a}	1.0± 1.1^{a}	1.1± 1.5^{a}	4.1± 3.9^{b}	1.9± 1.4^{ab}	1.1± 1.4^{a}	**
3. Acanthostomid		n(ran)	10(0)	18(0)	20(0-5)	16(0-4)	20(0-3)	16(0-2)
metacercaria[2]	Preva.	0.00	0.00	55.00	81.25	5.00	18.75	
	Inten.	-	-	2.0± 1.5	2.4± 1.0	3.0	1.3± 0.6	
	Abund.	0.0± 0.0^{a}	0.0± 0.0^{a}	1.1± 1.5^{ab}	1.9± 1.3^{b}	0.2± 0.7^{a}	0.3± 0.6^{a}	***
4. *Transversotrema*	n(ran)	10(0-3)	18(0-2)	20(0-2)	16(0-6)	20(0-2)	16(0-2)	
patialense[3]	Preva.	50.00	41.67	30.00	31.25	30.00	31.25	
	Inten.	2.0± 1.0	1.2± 0.5	1.2± 0.4	3.0± 2.7	1.2± 0.4	1.4± 0.6	
	Abund.	1.0± 1.2	0.9± 1.1	0.4± 0.6	0.9± 2.0	0.4± 0.6	0.4± 0.7	n.s.
5. *Malabarotrema*	n(ran)	10(0-13)	12(0-4)	20(0-26)	16(0-56)	20(0-24)	16(0-13)	
indica[1]	Preva.	80.00	75.00	95.00	62.50	75.00	81.25	
	Inten.	6.6± 4.6	2.1± 1.1	5.6± 5.8	12.7± 16.6	7.5± 7.6	4.4± 4.1	
	Abund.	5.3± 4.9	1.6± 1.3	5.3± 5.8	7.9± 14.3	5.6± 7.3	3.6± 4.1	n.s.

Table 2.21D: Data on Cestodes and Nematodes Infections in two localities.

UDAWALAWA Parasite		April	June	August	October	Decem.	Februa.	Sig. Level
1. *Paradilepis scolecina* (Live)[1]	n(range)	11(0-4)	8(0-4)	11(0-9)	13(0-6)	17(0-5)	18(0-11)	
	Preva.	72.72	75.00	72.72	76.92	64.71	61.11	
	Inten.	2.8± 1.2	2.3± 1.2	4.0± 2.6	2.7± 1.9	2.7± 1.9	5.6± 3.7	
	Abund.	2.0± 1.6	1.8± 1.5	2.9± 2.9	2.1± 2.0	1.8± 2.0	3.4± 4.0	n.s.
2. *Paradilepis scolecina* (Dead)[1]	n(ran)	11(0-9)	8(3-15)	11(0-46)	13(2-30)	17(0-22)	18(0-32)	
	Preva.	90.90	100.00	81.81	100.00	94.12	94.44	
	Inten.	6.2± 2.5	7.1± 3.8	17.2± 13.2	8.9± 8.6	11.6± 5.4	14.6± 10.7	
	Abund.	5.6± 3.0	7.1± 3.8	14.1± 13.7	8.9± 8.6	10.9± 6.0	13.8± 10.9	n.s.
3. *Contracecum* L_4[2]	n(ran)	11(0-8)	8(0-1)	11(0-2)	13(0-2)	17(0-4)	18(0-11)	
	Preva.	9.09	12.50	18.18	15.38	35.29	5.88	
	Inten.	8.0	1.0	1.5± 0.7	1.5± 0.7	2.2± 1.5	11.0	
	Abund.	0.7± 2.4	0.1± 0.4	0.3± 0.6	0.2± 0.6	0.8± 1.3	0.6± 2.6	n.s.
4. *Contracaecum* sp. (L_4 & L_5)[1]	n(ran)	11(0-15)	8(1-18)	11(2-16)	13(0-10)	17(2-13)	18(0-18)	
	Preva.	90.90	100.00	100.00	92.31	100.00	94.44	
	Inten.	7.3± 5.4	9.0± 5.0	7.0± 3.7	5.3± 2.7	7.2± 3.7	6.4± 4.6	
	Abund.	6.6± 5.6	9.0± 5.0	7.0± 3.7	4.9± 3.0	7.2± 3.7	6.1± 4.7	n.s.

KOGGALA Parasite		May	July	November	January	March	May	
1. *Contracaecum* sp.[2]	n(ran)	10(0-1)	12(0)	20(0)	16(0-2)	20(0)	16(0)	
	Preva.	10.00	0.00	0.00	12.50	0.00	0.00	
	Inten.	1.0	-	-	1.0± 0.0	-	-	
	Abund.	0.1± 0.3	0.0± 0.0	0.0± 0.0	0.1± 0.3	0.0± 0.0	0.0± 0.0	n.s.
2. *Rhabdochona* sp.[2]	n(ran)	10(0-31)	12(0-13)	20(0-13)	16(0-2)	20(0-7)	16(0-3)	
	Preva.	80.00	50.00	50.00	37.50	45.00	43.75	
	Inten.	17.1± 10.6	5.0± 4.1	4.2± 4.2	1.2± 0.4	4.0± 2.2	2.0± 0.8	
	Abund.	13.7± 11.8[b]	2.5± 3.8[a]	2.1± 3.6[a]	0.4± 0.6[a]	1.8± 2.5[a]	0.9± 1.1[a]	**

Table 2.21E: Data on Crustacean Infections in Koggala lagoon.

KOGGALA Parasite		April	June	August	October	Decem.	Februa.	Sig. Level
1. *Dermoergasilus amplectans*[1]	n(ran)	10(0-58)	18(1-43)	20(9-68)	16(5-43)	20(6-59)	16(0-53)	
	Preva.	90.00	100.00	100.00	100.00	100.00	93.75	
	Inten.	23.3± 17.9	15.7± 14.3	34.4± 16.1	20.3± 9.8	30.6± 17.1	20.7± 12.7	
	Abund.	21.0± 18.4[ab]	15.7± 14.3[a]	34.4± 16.1[e]	20.3± 9.8[abcd]	30.6± 17.1[bde]	19.4± 13.3[abc]	***
2. *Ergasilus parvitergum*[2]	n(ran)	10(0-2)	18(0-3)	20(0-4)	16(0-4)	20(0-1)	16(0-2)	
	Preva.	40.00	22.22	35.00	37.50	10.00	31.25	
	Inten.	1.3± 0.5	1.8± 1.0	1.6± 1.1	1.8± 1.3	1.0± 0.0	1.4± 0.5	
	Abund.	0.5± 0.7	0.4± 0.8	0.6± 1.0	0.7± 1.1	0.1± 0.3	0.4± 0.7	n.s.
3. *Argulus* sp.[2]	n(ran)	10(0-1)	12(0-4)	20(0-26)	16(0-56)	20(0-24)	16(0-13)	
	Preva.	10.00	25.00	20.00	31.25	5.00	37.50	
	Inten.	1.0	1.8± 1.3	1.8± 1.0	1.6± 0.9	1.0	1.3± 0.6	
	Abund.	0.1± 0.3	0.5± 1.0	0.4± 0.8	0.5± 0.2	0.1± 0.2	0.3± 0.6	n.s.

Digeneans

The abundance values for the metacercaria in Udawalawa did not show any statistically significant difference with the exception of the strigeid metacercaria found in the liver (Table 2.21C & Figure 2.34A). It increased in abundance significantly in April'90 and February'91. This may be a result of the temperature drop occuring only once a year in Udawalawa from December to January.

Cyathocotylid metacercarial abundance in Koggala showed two peaks which were significant, one in July and one in January. Even though insignificant, cyathocotylid metacercaria showed a small peak in abundance values in June and a high peak in December (Table 2.21.C & Figures 2.35 A1 & B1). In both localities these peaks correspond to the end of the rainy periods (Figures 2.35 A2 & B2).

Exorchis metacercaria and acanthostomid metacercaria exhibited significantly higher abundances in Koggala in January and March (Table 2.21C & Figures 2.34 B & C), which follows the cold period in December and January.

Adult digeneans, *Transversotrema* sp. and *M. indica*, infection levels were similar throughout the year (Table 2.21C).

Cestodes

Live as well as dead *P. scolecina* cysticerci did not show any significant difference in abundance during the study period (Table 2.21D). If live cysticerci represent recent infections, the similarity in presence of live worms throughout this period suggests a similar year round infection ability. Even though insignificant, the samples of the comparatively dry August and February months contained more *P. scolecina* (live and dead). During the dry months there may therefore be a higher chance for infection.

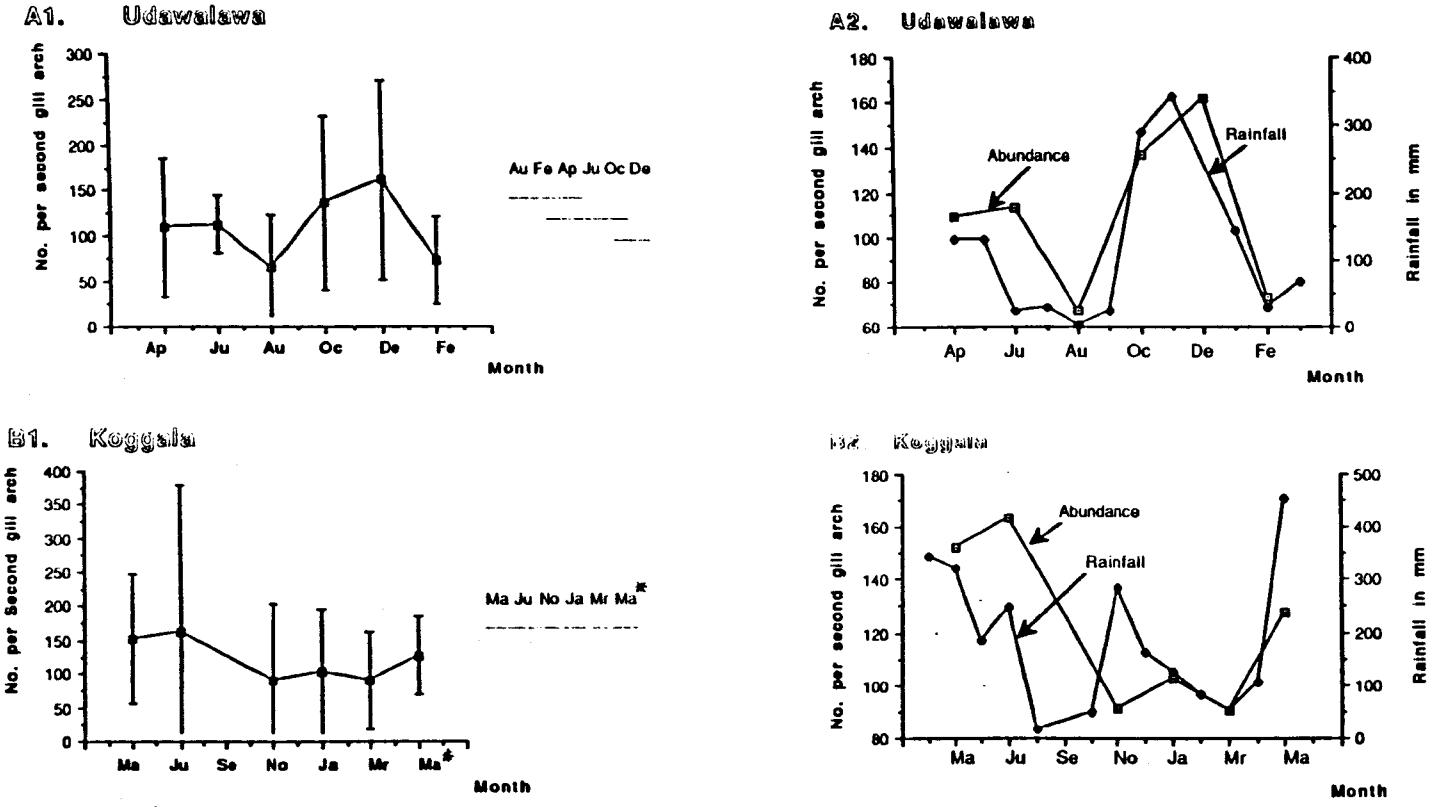


Figure 2.31: The variation of abundance of *A.etropli* at two localities and its relationship with rainfall.
A1, B1 - Abundance values (with standard deviations) in the two localities.
A2, B2 - Abundance values plotted with the rainfall of the locality.
The underline indicates the samples with no significant difference.

Since fish were left in holding tanks for up to 2-3 weeks the infection levels of direct life-cycle ectoparasites may have changed. Therefore, the data do not necessarily represent the original level of natural infection.

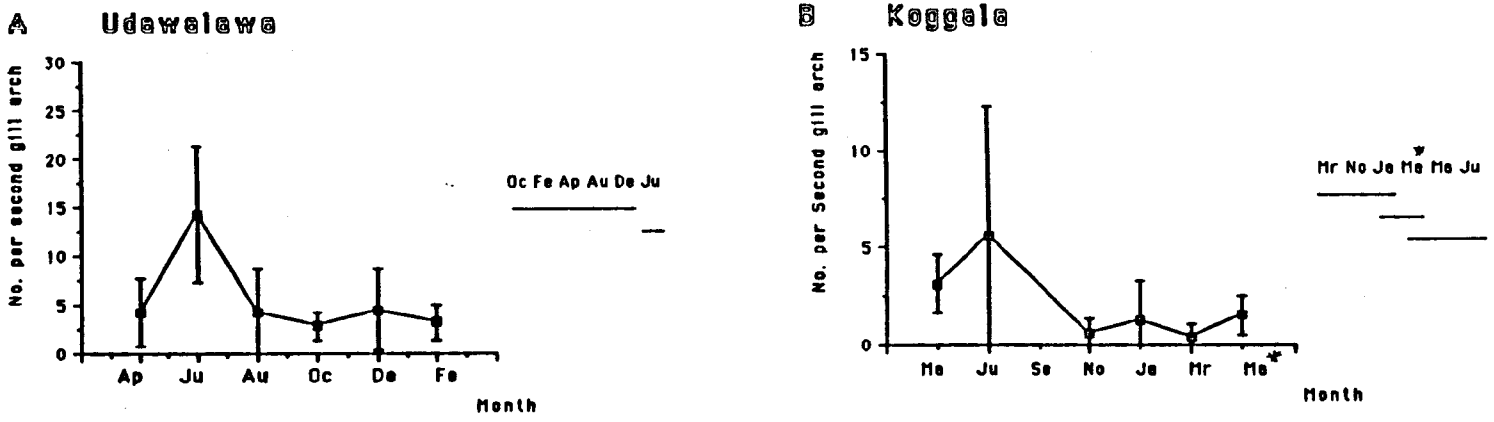


Figure 2.32: The variation of abundance of *C. colombensis* at two localities (with standard deviations). The underline indicates the samples with no significant difference.

Since fish were left in holding tanks for up to 2-3 weeks the infection levels of direct life-cycle ectoparasites may have changed. Therefore, the data do not necessarily represent the original level of natural infection.

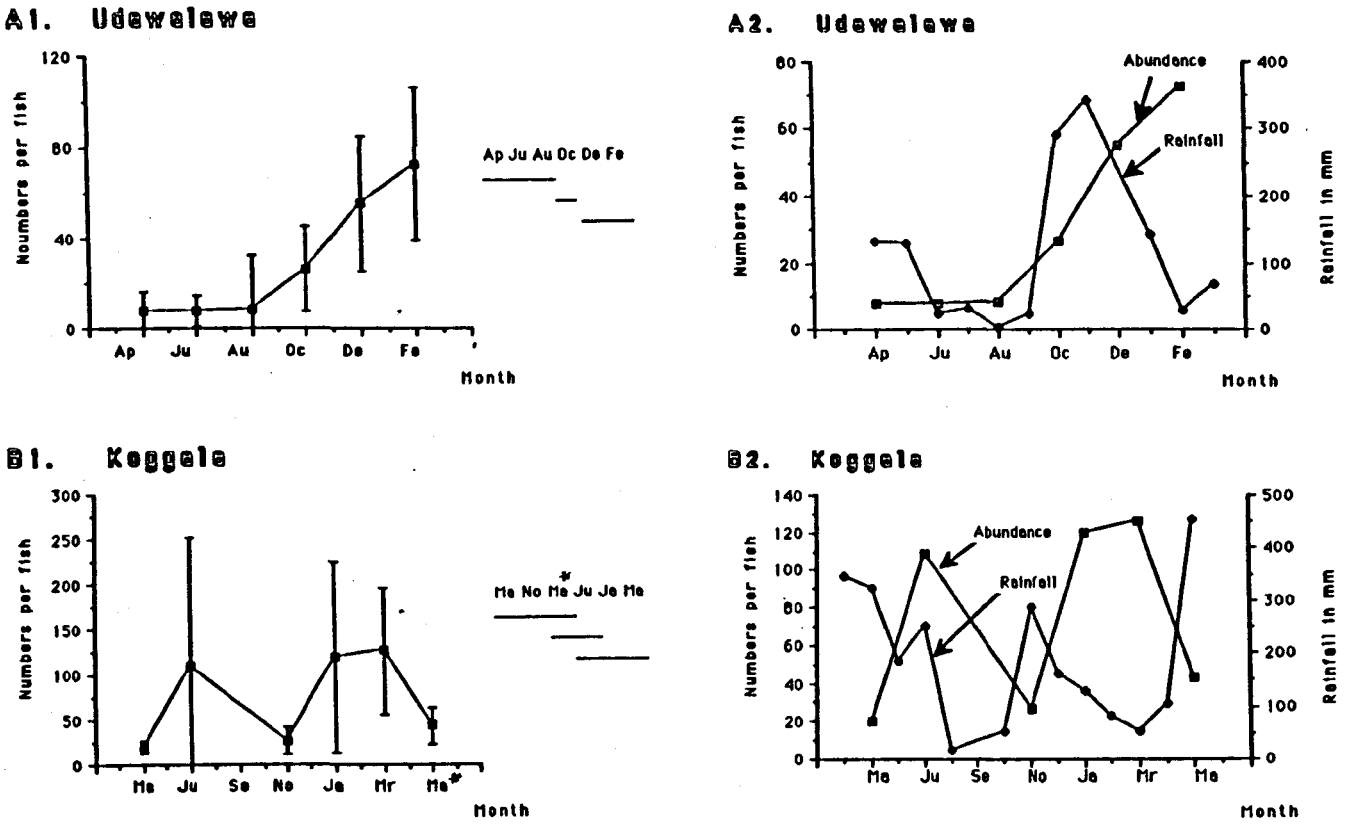


Figure 2.33: The variation of abundance of *Enterogyrus* spp. at two localities and its relationship with rainfall.
A1, B1 - Abundance values (with standard deviations) in the two localities
A2, B2 - Abundance values plotted with the rainfall of the locality.
The underline indicates the samples with no significant difference.

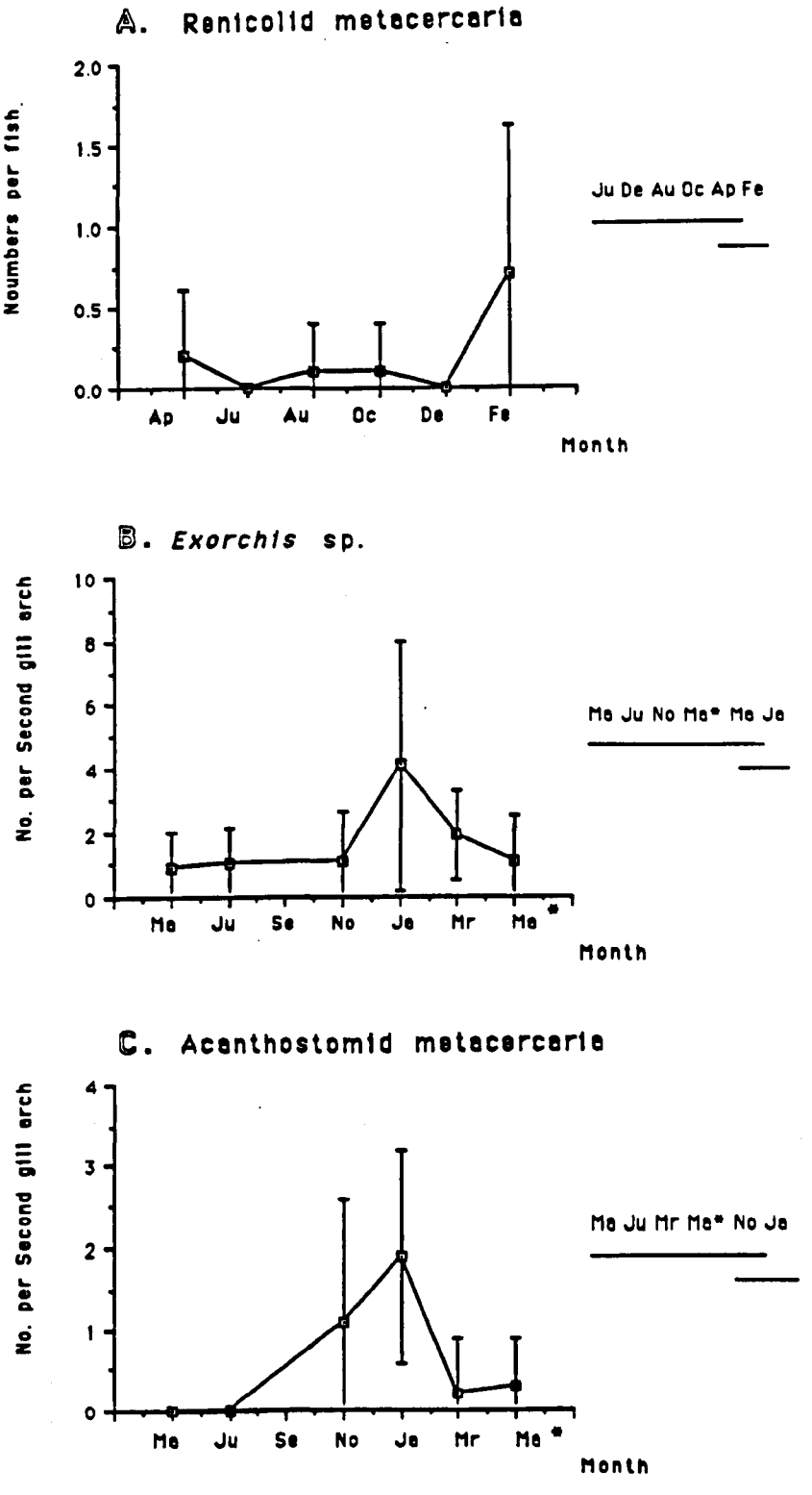


Figure 2.34: The variations of abundance values of, (A) Renicolid metacercaria, (B) *Exorchis* sp., and (C) Acanthostomid metacercaria (with standard deviations).
The underline indicates the samples with no significant difference.

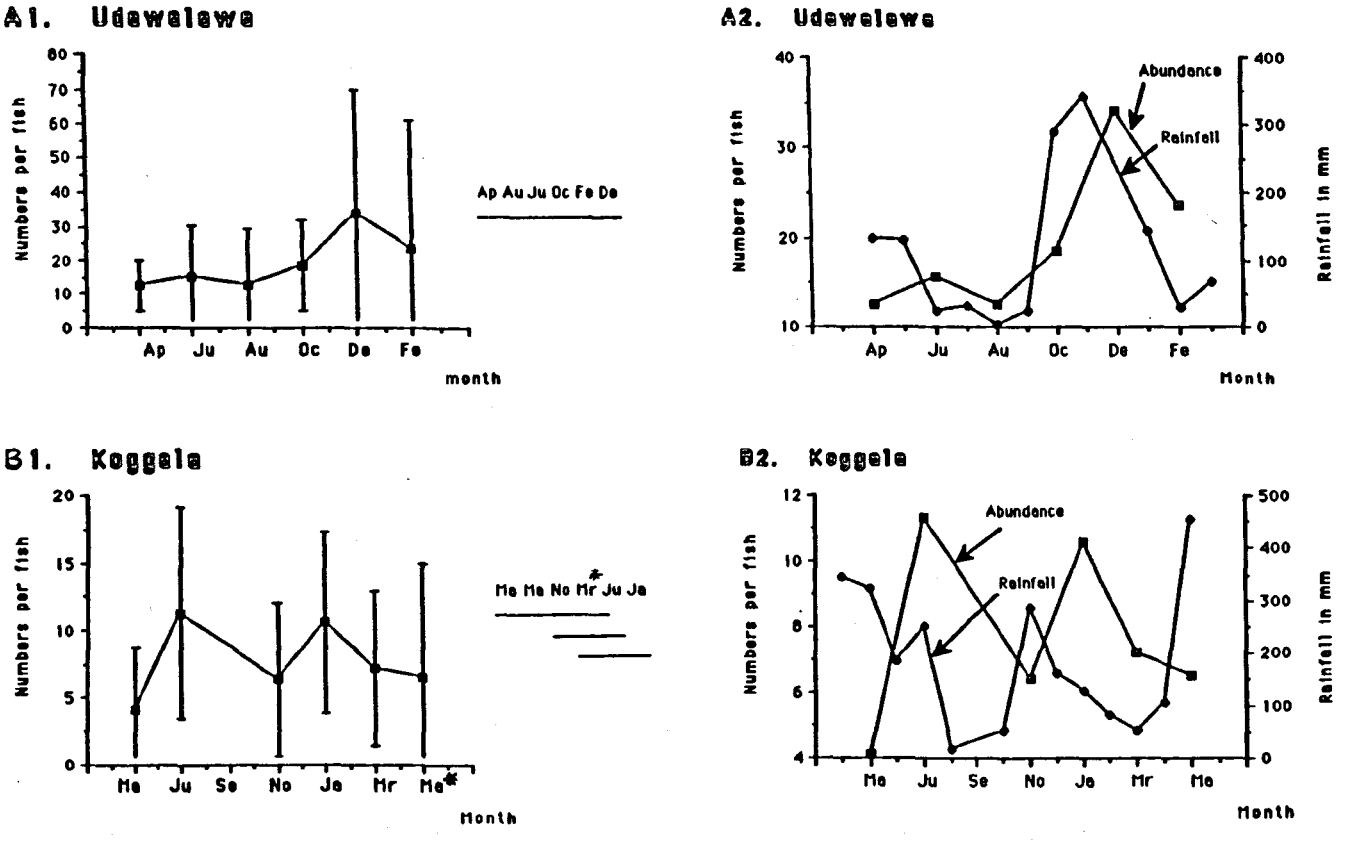


Figure 2.35: The variation of abundance of cyathocotylid metacercaria at two localities and its relationship with rainfall.
A1, B1 - Abundance values (with standard deviations) in the two localities
A2, B2 - Abundance values plotted with the rainfall of the locality.
The underline indicates the samples with no significant difference.

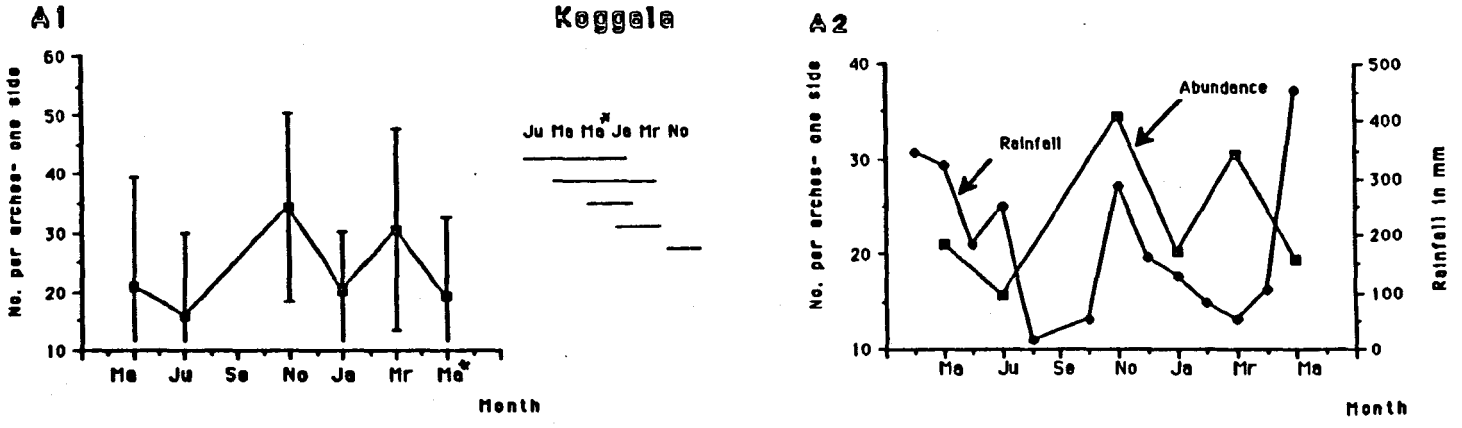


Figure 2.36: The variation of abundance of *Dermoergasilus amplectens* at Koggala (with standard deviations) and its relationship with the rainfall.
The underline indicates the samples with no significant difference.

Nematodes

Active second stage (L_2) and the encysted third stage (L_3) *Contracaecum* larvae did not show any year round significant difference in abundance values (Table 2.21D). Since the active *Contracaecum* larvae represent recent infections, their similar year round presence suggests the equal availability of the second stage larva to be present in plankton through out the year.

Rhabdochona worms showed a significantly higher burden in May'90 and the abundance declined considerably very much in the subsequent samples (Table 2.21D). The peak in May did not reappear in May'91. Later it was observed that a high number occurred a little later, at the end of July'91, in a sample collected for the work on *Enterogyrus*. The ecological data for this sample was as follows; Number of fish observed- 13, Prevalence- 76.9 %, Intensity range- 0-6, Mean intensity- 2.8 ± 1.7, Abundance- 2.2 ± 1.9.

Crustaceans

Of the crustacean parasites, the *Dermoergasilus amplectens* population fluctuated greatly with numbers declining significantly at the end of the rainy periods, in July and January (Table 2.21E & Figure 2.36 A & B). Thereafter, the numbers increased until the next rainy season. The other two crustacean parasites showed year round presence with no statistically significant fluctuations.

Influence of life cycle strategy

Monoxenous Parasites

When all the monoxenous parasites showing significant variations in abundance were considered, two peaks could be identified in most of them within the study period of approximately one year (Table 2.22A). These peaks occurred during pre-monsoonal, monsoonal or post-monsoonal periods. Of these parasites *A. etropli*'s peaks correspond to the heavy rainfall periods. Post-monsoonal periods are favoured by the parasites *Enterogyrus* and *D. amplectens*. Population increase of *D. amplectens* during post monsoonal period created peaks in the pre-monsoon of next rainy season.

Table 2.22: Higher burden periods of Monoxenous and Heteroxenous parasites.
Table 2.22A: Higher burden periods of Monoxenous Parasites*.

Parasite	Locality	1st peak	2nd peak
Protozoa			
I. multifiliis‡	Udawalawa	June, August	December
Monogenea			
A. etropli‡	Udawalawa	June	October, December
	Koggala†	May, July	January
C. colombensis‡	Udawalawa	June	-
	Koggala	May, July	-
Enterogyrus spp.‡	Udawalawa	-	Octo., Dece., Febr.
	Koggala	July	January, March
Crustaceans			
D. amplectans	Koggala	March	November

Table 2.22B: Higher burden periods of Heteroxenous Parasites*.

Parasite	Locality	1st peak	2nd peak
Metacercariae			
Renicolid metacer.	Udawalawa	-	Febr.'91, April'90
Cyathocot. metacer.	Udawalawa†	July	December
	Koggala	July	January
Exorchis sp.	Koggala	-	January
Acanthostomid metacercaria	Koggala	-	November, January
Nematodes			
Rhabdochona sp.	Koggala	May	-

* The size of letters indicates the height of the peak; higher peaks having larger letters.
† Abundance values between samples are not significantly different.
‡ These data do not necessarily represent the natural infection levels.

The small peaks or the non-appearance of peaks in Udawalawa during the South West monsoon from April to May, in *A. etropli*, *Enterogyrus* and probably *I. multifiliis* (*I. multifiliis* peaks in June and August were attributed to stress) may be due to the less rain in Udawalawa during this monsoonal period. The absence of peaks in *C. colombensis* during North East monsoon period in both localities is a complicated situation which cannot readily be explained.

Heteroxenous Parasites

When these parasites are considered, only one peak could be recognized with the exception of the cyathocotylid metacercaria, which showed two peaks at the end of each monsoon periods (Table 2.22B). The small peak obtained for cyathocotylid metacercaria in Udawalawa in the South West monsoon season could be due to less rain or/and a lesser drop in temperature.

The cercarial release in all these metacercaria seems to be influenced by the North East monsoon, even in Koggala where the rainfall of this season was not as high as in the South West monsoon. Therefore this rain may have acted in combination with the colder temperature in December-January to influence the increase in parasite abundance. The reason for the appearance of two peaks in cyathocotylid metacercariae abundance may due to the fact that this cercarial release is influenced only by rain. In contrast to all other parasites the abundance of values of *T. patialense* and the strigeid metacercaria did not show any significant variation, suggesting year round equal availability of cercaria. Later, a higher infection level of strigeid metacercaria was observed in a sample collected for tissue preservation for histology at the end of June 1991, suggesting a possible environmental influence on this parasite too. The data for the sample was; Number of fish observed- 28, Prevalence- 34.8, Range- 0-42, Intensity- 16.8 ± 11.1, Abundance- 6.00 ± 10.41.

All passively host finding parasites ie. those which rely on intermediate hosts or encyst in open water or on vegetation etc. and depend on the feeding of host for entering, *M. indica*, *P. scolecina* and *Contracaecum*, show year round abundance values which are not significantly different. This might be due to the fact that year

round equal presence of the larval stage or/and year round equal population levels of intermediate host.

Rhabdochona showed a peak in the South West monsoon season. This parasite may have shown this peak due to the increase of population levels of the intermediate host or/and due to a different life cycle pattern. More details about the biology of the parasite is required to explain this situation.

The influence of host size preference on seasonal infestation levels

If the parasites show a host size preference, the influence of this factor can lead to show or hide significant variations between monthly samples. Therefore, where there were significant variations in the parasite infections between the samples and the parasite shows a significance difference in host size preference, two way ANOVA test was performed in order to see whether the two factors interact to show the differences. Only in the case of Koggala cyathocotylid metacercaria an interaction was found, representing the fish size has an influence on the abundance values and vice versa.

2.3.3.2. The micro-habitat effect on the parasite

(1) Host preference

A comparison of the parasites found on *E. suratensis* and *Oreochromis* spp. in the two localities are given in the Table 2.23. Ecological data on parasites of both these fish, collected on same sampling date are given in the Tables 2.24 A & B.

In general, the protozoans, digeneans and nematodes found were not host specific while the monogeneans, cestodes and crustaceans found were host specific. In Udawalawa, two parasites; *Cichlidogyrus* sp., the echinostomid metacercaria and *P. delachauxi* were found to be exclusive to tilapia. However, the occurrence of the Echinostomid metacercaria was very low. *Oreochromis* spp. shared all the other parasites with *Etroplus*. However, the parasitic fauna (species composition) of *Etroplus* in both localities was richer when compared with *Oreochromis* spp. (Table 2.23).

Table 2.23: Comparison of Parasite fauna between *Etroplus suratensis* and *Oreochromis* spp. from same Locality.

Udawalawa reservoir

Parasites exclusive to *E. suratensis*	Parasites exclusive to *O. niloticus*	Parasites common to both hosts
Apiosoma (Glossetella) sp. ?	*Cichlidogyrus* sp.	*Trichodina* sp. *
Ichthyobodo sp. ?	*T. patialense*?	*I. multifiliis*
A. etropli	*P. delachauxi*	*Trypanosoma* sp. *
C. colombensis	*Echinostomid* metacercaria?	Renicolid metacercaria
Enterogyrus spp.		*Contracaecum* sp.
Strigeid metacercaria ?		
Cyathocotylid metacercaria		
P. scolecina		

Koggala lagoon

Parasites exclusive to *E. suratensis*	Parasites exclusive to *O. mossambicus*	Parasites common to both hosts
Trichodina sp. ?	None	*Trypanosoma* sp. *
Apiosoma (=*Glossatella)* sp ?		*Exorchis* sp.
Ichthyobodo sp. ?		Acanthostomid metacercaria
A. etropli		Cyathocotylid metacercaria
C. colombensis		*T. patialense*
Enterogyrus spp.		*M. indica*
Contracaecum sp. ?		*Rhabdochona* sp.
Leech (unidentified) ?		*D. amplectens*
E. parvitergum ?		
Argulus sp.?		

* probably of different species
? might not be host specific, the absence may be due to very low prevalence

Table 2.24: Ecological data on the parasites of *Oreochromis* sp. with that of *Etroplus* from the two localities collected at the same time.
Table 2.24A: Ecological data on the parasites of *O. niloticus* at Udawalawa reservoir with the data of Etroplus for the month of February for comparison.†

Parasite	Host	n	Prevalence	Intensity Range	Mean Intensity	Abundance
Protozoa						
Trichodina sp. *	*Etroplus*	18	27.8	0-4	1.8 ± 1.3	0.6 ± 1.1
	O. niloticus	14	14.3	0-2	1.5 ± 0.7	0.2 ± 0.6
I. multifiliis	*Etroplus*	18	27.8	0-7	3.4 ± 2.3	0.9 ± 1.9
	O. niloticus	14	21.4	0-2	1.3 ± 0.6	0.3 ± 0.6
Trypanosoma sp. *	*Etroplus*	18	94.4	0-46	15.5 ± 14.7	14.7 ± 14.7
	O. niloticus	14	100.0	0-30	9.1 ± 9.8	9.1 ± 9.8
Monogenea						
Cichlidogyrus sp.	*O. niloticus*	14	50.0	0-3	1.6 ± 0.8	0.8 ± 1.0
Digenea						
Renicolid metacercaria	*Etroplus*	18	44.4	0-3	1.5 ± 0.8	0.7 ± 0.9
	O. niloticus	14	7.1	0-1	1.0	0.1 ± 0.3
Echinostomid metacercaria	*O. niloticus*	14	14.3	0-1	1.0 ± 0.0	0.2 ± 0.4
T. patialense	*O. niloticus*	14	7.1	0-1	1.0	0.1 ± 0.3
Cestoda						
P. delachauxi	*O. niloticus*	14	92.9	0-60	21.6 ± 18.8	20.1 ± 19.0
Nematoda						
Contracaecum sp.	*Etroplus*	18	94.4	0-18	6.4 ± 4.6	6.1 ± 4.7
	O. niloticus	14	92.9	0-6	2.9 ± 1.5	2.7 ± 1.6

Table 2.24B: Ecological data on the parasites of *O. mossambicus* at Koggala lagoon with the data of *Etroplus* for the month of January for comparison.†

Parasite	Host	n	Prevalence	Intensity Range	Mean Intensity	Abundance
Protozoa						
Trypanosoma sp. *	*Etroplus*	16	43.8	0-5	1.9 ± 1.5	0.8 ± 1.3
	O. mossambicus	15	6.6	0-2	2.0	0.1 ± 0.5
Digenea						
Exorchis sp.	*Etroplus*	16	87.5	0-13	4.6 ± 3.9	4.1 ± 3.9
	O. mossambicus	15	26.7	0-1	1.0 ± 0.0	0.3 ± 0.5
Acanthostomid metacercaria	*Etroplus*	16	81.3	0-4	2.4 ± 1.1	1.9 ± 1.3
	O. mossambicus	15	53.3	0-5	2.1 ± 1.4	1.1 ± 1.5
Cyathocotylid metacercaria	*Etroplus*	16	93.8	0-25	11.3 ± 6.3	10.6 ± 6.7
	O. mossambicus	15	93.3	0-6	3.1 ± 1.8	2.9 ± 1.9
T. patialense	*Etroplus*	16	31.3	0-6	3.0 ± 2.7	0.9 ± 2.0
	O. mossambicus	15	46.7	0-7	2.4 ± 2.5	1.1 ± 2.1
M. indica	*Etroplus*	16	62.5	0-56	12.7 ± 16.6	7.9 ± 14.3
	O. mossambicus	15	26.7	0-7	2.8 ± 2.9	0.7 ± 1.8
Nematoda						
Rhabdochona sp.	*Etroplus*	16	37.5	0-2	1.2 ± 0.4	0.4 ± 0.6
	O. mossambicus	15	26.7	0-16	5.5 ± 7.1	1.5 ± 4.2
Crustacea						
D. amplectens	*Etroplus*	16	100.0	5-43	20.3 ± 9.8	20.3 ± 9.8
	O. mossambicus	15	6.7	0-1	1.0	0.1 ± 0.3

* probably of different species, † data for parasites exclusive to *Etroplus* are not given

Since the protozoan parasites were not identified up to the species level, it was difficult to affirm that the same species of protozoan parasites were present in both hosts. As trichodinids are often host specific, the Trichodina species found on the two hosts may differ. The situation with *Trypanosoma* is not clear, and they may also differ. The absence of scyphidians and *Ichthyobodo* on tilapia in both localities, *Contracaecum* and *Argulus* in Koggala lagoon may not be due to the complete host rejection. Since these parasites are generally not species specific, the absence of them in a small sample may be due to their low prevalence. In Koggala, both hosts were infected by the cyathocotylid metacercaria which was considered to be of the same species (measurements are given in the Table 2.5). In Udawalawa there were higher burdens of the cyathocotylid metacercaria on *Etroplus*, the tilapia specimens however, were not infected by this parasite. Probably *O. niloticus* may be resistant to their infection unlike *O. mossambicus*. *T. patialense* was not found on *Etroplus* from Udawalawa, but was found on tilapia from same locality. It was present on both hosts in Koggala.

The ecological data for parasites (Table 2.24A) showed that the protozoan infection levels were the same for both hosts in Udawalawa except for the intensity of *Trypanosoma* sp. where it was found to be higher for *Etroplus*. The intensity of *Contracaecum* and the prevalence and intensity of the renicolid metacercaria were lower in *O. niloticus* when compared with *Etroplus* in the same habitat.

In Koggala, the cyathocotylid metacercaria, *T. patialense* and the *Rhabdochona* sp. were found to occur at the same prevalence level on both *O. mossambicus* and *Etroplus* (Table 2.24B). However, the *Rhabdochona* sp. occurred at higher intensities on tilapia whilst the cyathocotylid metacercaria was lower. *T. patialense* had similar intensity levels in both hosts. *Trypanosoma* and the acanthostomid metacercaria showed a lower prevalence in tilapia but similar intensity levels. *Exorchis* sp., *M. indica* and *D. amplectens* were present in lower prevalence and lower intensities in tilapia than *Etroplus*. Only a single specimen of *D. amplectens* was found on one tilapia suggesting that it was not a preferred host for this parasite.

(2) Host age (size) preference

The sizes of the fish in the samples collected varied very much. The largest specimen from Udawalawa reservoir and Koggala lagoon measured 15.5 cm (167.10 g) and 13.5 cm (105.07 g) respectively in standard length, whilst the smallest measured 5.9 cm (11.91 g) and 6.4 cm (12.34 g) respectively. The mean weigths for samples from both localities varied between 31.0 and 45.0 g (Table 2.25) and the lengths of the samples were not significantly different from each other. The three size groups are however not represented equally in the samples for both localities (Figure 2.37).

Parasite preference for different host sizes, with an indication of the statistical significance is listed in Table 2.26. The preference of the host sizes are ranked in increasing order, probability levels are given and the underlines indicate where the host sizes are not significantly preferred at the 95% level. The different patterns of preferences found are as follow;

1. significantly high preference for eldest fish

preference for size 3 - Tricodinids and active *Contracaecum* worms in Udawalawa, *A. etropli* and *Enterogyrus* spp. in Koggala

preference for size 2 & 3 - *A. etropli* in Udawalawa

2. significantly higher preference for younger fish

preference for size 1 - *Trypanosoma* sp. in Udawalawa

preference for size 1 & 2 - live *P. scolecina* and *D. amplectens*

3. increasing preference with increasing size and/or accumulation

cyathocotylid metacercaria in both localities and *M. indica.*

Table 2.25: The mean weights of fish in the samples.

Udawalawa

Month	Number of fish	Mean weight ± SD
April	11	39.67 ± 19.54
June	8	33.57 ± 19.32
August	11	31.09 ± 19.57
October	13	37.02 ± 38.85
December	17	43.58 ± 39.81
February	18	37.04 ± 47.43

No significant difference in the fish weights in the samples at 95 & 99 % levels.

Koggala

Month	Number of fish	Mean weight ± SD
May	10	45.01 ± 21.84
July	12	44.64 ± 24.60
November	20	33.37 ± 16.94
January	16	42.86 ± 22.98
March	20	31.58 ± 19.58
May	16	38.60 ± 26.78

No significant difference in the fish weights in the samples at 95 & 99 % levels.

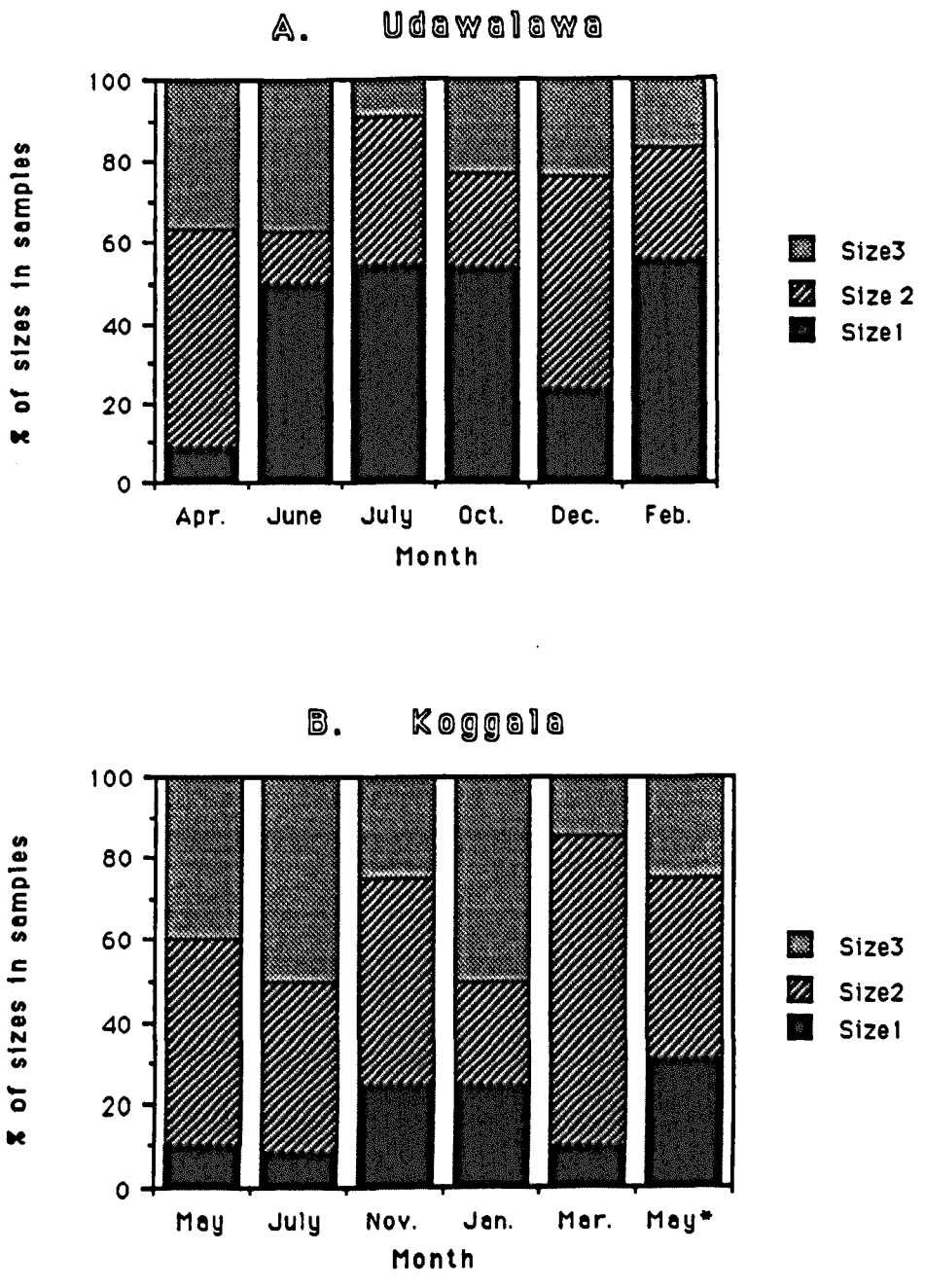


Figure 2.37: The size composition of the fish samples as a percentage of the total number of fish examined in each sample.

Table 2.26: Parasites with significant differences in host size preference.

Trichodina sp. (Udawalawa) [2]	Size	Size 1	Size 2	Size 3	
	X ± SD	1.28 ± 4.13	2.27 ± 8.59	2.22 ± 3.19	
	n (P)	36	30	18	(*)
Trypanosoma sp. (Udawalawa) [1]	Size	Size 3	Size 2	Size 1	
	X ± SD	6.89 ± 6.85	11.61 ± 16.89	21.19 ± 23.26	
	n (P)	18	28	32	(**)
A. etropli (Udawalawa) [1]	Size	Size 1	Size 3	Size 2	
	X ± SD	84.39 ± 65.89	107.44 ± 87.58	137.63 ± 90.40	
	n (P)	36	18	30	(*)
A. etropli (Koggala) [1]	Size	Size 1	Size 2	Size 3	
	X ± SD	65.40 ± 52.08	86.99 ± 75.92	200.04 ± 168.01	
	n (P)	19	50	31	(***)
Enterogyrus spp. (Koggala) [1]	Size	Size 1	Size 2	Size 3	
	X ± SD	39.22 ± 30.20	60.59 ± 55.14	122.87 ± 122.66	
	n (P)	18	46	30	(**)
Cyathocotylid metacercaria (Udawalawa) [1]	Size	Size 1	Size 2	Size 3	
	X ± SD	6.84 ± 4.44	17.43 ± 15.31	52.11 ± 38.55	
	n (P)	32	28	18	(***)
Cyathocotylid metacercaria (Koggala) [1]	Size	Size 1	Size 2	Size 3	
	X ± SD	2.56 ± 3.94	6.04 ± 4.74	13.23 ± 7.34	
	n (P)	18	46	30	(***)
P. scolecina (Udawalawa) [2] (live cysticerci)	Size	Size 3	Size 2	Size 1	
	X ± SD	0.56 ± 1.10	2.29 ± 1.88	3.53 ± 3.27	
	n (P)	18	28	32	(***)
Contracaecum sp. (L_3 worms) (Udawalawa) [2]	Size	Size 1	Size 2	Size 3	
	X ± SD	0.19 ± 0.78	0.54 ± 2.10	1.00 ± 2.03	
	n (P)	32	28	18	(*)
Malabarotrema indica (Koggala) [2]	Size	Size 1	Size 2	Size 3	
	X ± SD	4.11 ± 6.56	4.30 ± 8.69	6.73 ± 6.61	
	n (P)	18	46	30	(*)
Dermoergasilus amplectens (Koggala) [1]	Size	Size 3	Size 1	Size 2	
	X ± SD	15.29 ± 11.52	28.05 ± 14.30	28.42 ± 17.34	
	n (P)	31	19	50	(***)

X - means for the sizes, SD - Standard deviations, n - number of fish, (P) - Probability levels * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ the underlines indicate the sizes not significantly different, sizes are arranged according to the increasing order of means

(3) Site preference of the parasite

The mean numbers of *D. amplectens* in the different sections of the gill apparatus are given in the Table 2.27. The mean numbers for *A. etropli* and *C. colombensis* in different sections of the gill apparatus of *Etroplus* from Udawalawa reservoir and Koggala lagoon are given in the Tables 2.27A & B. The results of the sign tests carried out to find the preferred sites are also given; the sites with significantly different sites at 95 % levels are denoted with different letters.

For *D. amplectens* significant differences were found between the arch and the segments (Table 2.27C). Of the four arches, the copepods were more concentrated on the first two arches and the fourth arch was the least preferred. Of the three segments of the arches, the distal was the most favoured and the middle segment was favoured least. There was no difference between the internal and external hemibranchs.

A. etropli showed a similar preference for the sites over the gill apparatus in both the habitats. The first two arches were favoured equally and the fourth was the least favoured. The proximal region of the primary filaments were preferred to the distal region of the second gill arch and the parasites were concentrated more to the middle segment with no distinction between the other two regions.

C. colombensis in both habitats showed some difference in the gill arch preference, whilst the preference for the areas over the second gill arch was the same. Of the arches the first three were equally preferred in the Koggala lagoon fish. The preference in Udawalawa reservoir was similar to that of *A. etropi*; the first two were most preferred and the fourth was the least preferred. The proximal area was favoured over the distal area and, unlike *A. etropli*, the ventral and middle segments were equally and highly preferred.

Table 2.27 : The data on preference for gill sites.

Table 2.27A : The spatial distributuion of *A. etropli* over gill apparatus. The sites carrying a significantly different number of parasites at 95 % level are assigned with similar letters.

Koggala lagoon				
Arch	1	2	3	4
No. of worms observed (Mean ± SD)	105.4 ± 78.9	96.7 ± 68.0	84.5 ± 69.9	67.3 ± 60.4
Significant differences	a	a	b	c
Segments of arch	Poximal	Distal		
No. of worms observed (Mean ± SD)	69.7 ± 44.6	50.6 ± 35.1		
Significant differences	a	b		
Hemibranch	Middle	Ventral	Dorsal	
No. of worms observed (Mean ± SD)	49.3 ± 33.6	37.4 ± 26.2	33.7 ± 19.4	
Significant differences	a	b	b	
Udawalawa reservoir				
Arch	2	1	3	4
No. of worms observed (Mean ± SD)	124.5 ± 98.6	123.1 ± 96.4	108.3 ± 88.1	92.2 ± 78.5
Significant differences	a	a	b	c
Segments of arch	Poximal	Distal		
No. of worms observed (Mean ± SD)	78.3 ± 55.7	35.6 ± 28.3		
Significant differences	a	b		
Hemibranch	Middle	Ventral	Dorsal	
No. of worms observed (Mean ± SD)	48.1 ± 35.4	33.4 ± 25.0	32.4 ± 23.7	
Significant differences	a	b	b	

Table 2.27B : The spatial distributuion of *C. colombensis* over gill apparatus. The sites carrying a significantly different number of parasites at 95 % level are assigned with similar letters.

Koggala lagoon				
Arch	1	2	3	4
No. of worms observed (Mean ± SD)	4.0 ± 4.6	3.8 ± 3.4	3.3 ± 3.8	1.8 ± 1.9
Significant differences	a	a	a	b
Segments of arch	Poximal	Distal		
No. of worms observed (Mean ± SD)	2.8 ± 1.6	0.8 ± 0.7		
Significant differences	a	b		
Hemibranch	Ventral	Middle	Dorsal	
No. of worms observed (Mean ± SD)	2.1 ± 1.2	1.4 ± 1.1	0.1 ± 0.3	
Significant differences	a	a	b	
Udawalawa reservoir				
Arch	2	1	3	4
No. of worms observed (Mean ± SD)	3.7 ± 3.0	3.5 ± 2.3	2.2 ± 1.7	0.9 ± 1.1
Significant differences	a	a	b	c
Segments of arch	Poximal	Distal		
No. of worms observed (Mean ± SD)	2.8 ± 1.6	0.8 ± 1.0		
Significant differences	a	b		
Hemibranch	Ventral	Middle	Dorsal	
No. of worms observed (Mean ± SD)	1.9 ± 1.5	1.8 ± 1.2	0.1 ± 0.2	
Significant differences	a	b	b	

Table 2.27C: The spatial distributuion of *D. amplectens* over gill apparatus in Koggala laggon fish. The sites carrying a significantly different number of parasites at 95 % level are assigned with similar letters.

Arch	2	1	3	4
No. of copepods observed (Mean ± SD)	12.3 ± 8.9	10.8 ± 7.1	8.7 ± 8.1	5.6 ± 4.3
Significant differences	a	ab	b	c
Segments of arch	Internal	External		
No. of copepods observed (Mean ± SD)	19.5 ± 13.6	17.9 ± 12.7		
Significant differences	a	a		
Hemibranch	Dorsal	Ventral	Middle	
No. of copepods observed (Mean ± SD)	18.6 ± 11.6	11.8 ± 8.3	7.1 ± 8.6	
Significant differences	a	b	c	

(4) The host response to the parasites

Ectoparasites on gills

1. *Ancyrocephalus etroplus*

The gills were infected with a few to a large number of *A. etroplus* worms. Gross observations showed that the gills were not unhealthy in general appearance.

The parasites were mostly concentrated around the proximal portions of primary lamellae, rather than at the distal portions. They were settled in the space between two secondary lamellae, sinking the two pairs of hooks into the base of the lamellae (Figure 2.38). The tips of the hooks either penetrated the epithelium or pierced the blood vessels. The immediate reaction was slight. The only response to attachment observed was hyperplasia which was initiated near the attachment point. The epithelial cells, as well as goblet cells, seemed to be proliferating. The growing hyperplastic tissue seemed to gradually push the worms away from the bases of secondary filaments eventually dislodging them from their attachment. Some worms seemed capable of grasping a hold even when they were pushed all the way along the length of the secondary filaments (Figure 2.39).

The hyperplastic response was most frequently seen in tissue sections half way along the secondary filaments without any worms attached (Figure 2.40 & 2.41). It is possible that the developing tissue may have disrupted the attachment of the worms and they may have dropped off or moved to another site. Other than the chronic hyperplastic reaction, no inflammatory response seemed to be involved.

The intensity of infection with *C. colombensis* was very low (generally 1-2 per gill arch). Therefore, the attachments with *C. colombensis* were not easily seen in histological sections and the gill pathology observed was almost all due to *A. etroplus*. A comparison between the pathology of the two gill monogenean species could not be made from this material.

2. *Dermoergasilus amplectens*

These copepods attach themselves to the distal ends of the primary filaments,

encircling them in a permanent grip with their specialized second antennae. Owing to the mode of attachment, the body of the copepod lies adjacent to the outside of the primary filaments.

The mechanical irritation seemed to responsible for the pathology observed. The most evident feature was the tissue damage caused by the constriction of tissue by the pair of antennae (Figure 2.42). The gill structure was completely destroyed underneath the antennae; the secondary lamellae were broken down and not visible. The cells in the primary lamellae around the cartilage and blood vessels were flattened. The goblet cells were absent from the "grasped" area. However, despite the severe changes due to attachment, it did not seem that the compression was such that the blood vessels were compressed on to the cartilaginous bar, and no blockage was evident (Figure 2.42).

Hyperplastic epithelium was evident on the either side along the filament. The whole length of the primary lamella towards the tip from the attachment point was completely covered by hyperplastic epithelium (Figure 2.43). On the other hand, only a short distance towards the base of the filament was hyperplastic. The Goblet cells were also proliferating, and migrated to the side edges with the hyperplastic epithelium (Figure 2.43). The irritation caused by the body of the copepod may lead to the proliferation along the length of primary lamellae from the attachment towards the tip. The primary filaments on either side of the filament to which the parasite was attached were also affected by hyperplasia to varying degrees, probably due to the irritation caused by direct contact or the lateral movements of copepod (Figure 2.43). No inflammatory reaction seemed to be involved in the tissue reaction. A few hypertrophic cells were seen in the areas newly affected, but this was probably masked later by the hyperplasia.

Endoparasites

1. Renicolid metacercaria

The low infestation levels of this parasite made it particularly difficult to sample liver as it was difficult to judge if the tissues were infected from its external

appearance. Therefore only a few cysts could be observed for histopathology.

The parasite was enclosed in a cyst of parasite origin but a capsule of host origin was absent (Figure 2.44 & 2.45). It would appear that the metacercariae were not provoking any host inflammatory response which would lead to host capsule formation. Cellular activities or the products of inflammatory reaction were absent around all cysts.

The liver tissue in the region of the parasite was damaged. The cytoplasm of the damaged cells and pyknotic nuclei were evident at the margin of the parasite cyst wall (Figure 2.44 & 2.45). Eosinophilic granular cells were found near the cysts, aggregated in the regions where much of the damaged cell cytoplasm occurred (Figure 2.45).

2. Cyathocotylid metacercaria

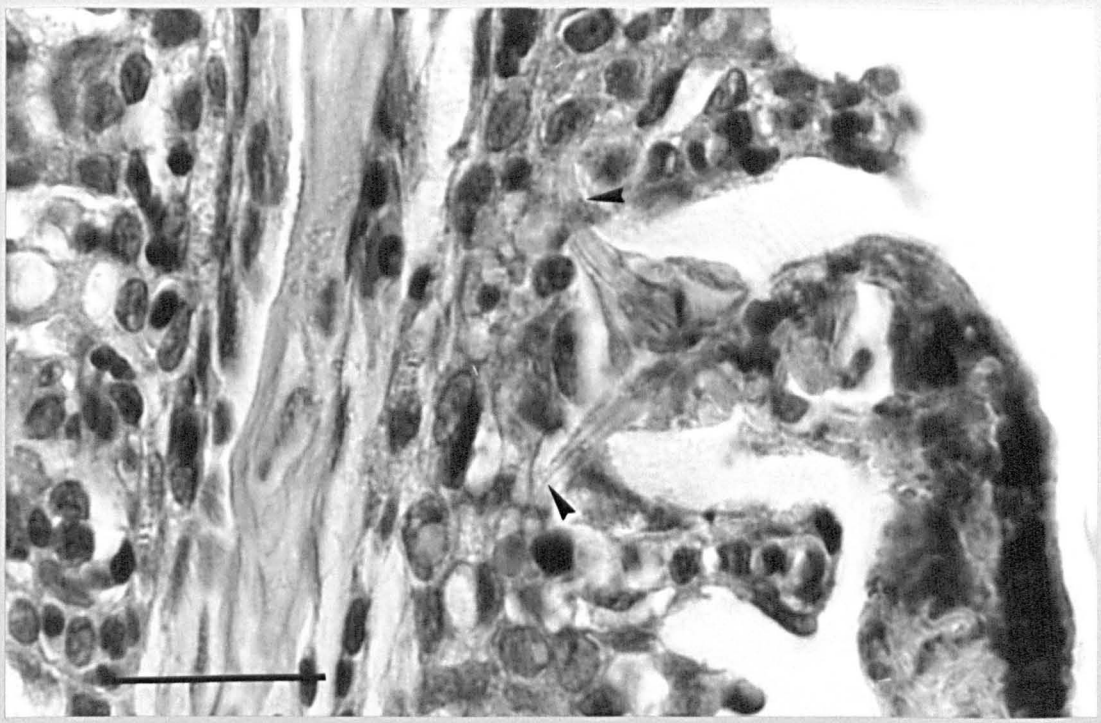
All of the cysts observed in the muscle were walled off by a fibrous tissue capsule of host origin. The capsule was very thin and seemed to be made up of fibroblasts (Figure 2.46). As the capsule was very thin it could not be divided into regions, nor could it be said whether there was any macrophage response which might lead to the formation of an epithelioid cell layer making up the innermost layer of a host capsule.

Outwith the capsule, and very near to it, large macrophage cells were seen with brownish to black particles in the cytoplasm. The cysts with macrophages near to the host capsule mostly had metacercariae which seemed to be dead with no live tissue, or cuticle and no cyst wall (Figure 2.47).

The damaged myo-fibrils of the muscles in the migratory path of the cercaria had been repaired and replaced by fibrocytes (Figure 2.46). Along these tracks, macrophages containing phagocytosed melanin in the cytoplasm were common. Melanocytes were also concentrated along these repaired areas.

Figure 2.38: Initial attachment of *Ancyrocephalus etropli* between the secondary lamellae sinking the two pairs of hooks (arrows) into the base of lamellae. Scale bar= 20 μm. (H & E).

Figure 2.39: An *Ancyrocephalus etropli* worm pushed all the way along the length of the secondary lamellae by hyperplasia. Scale bar= 20 μm. (H & E).



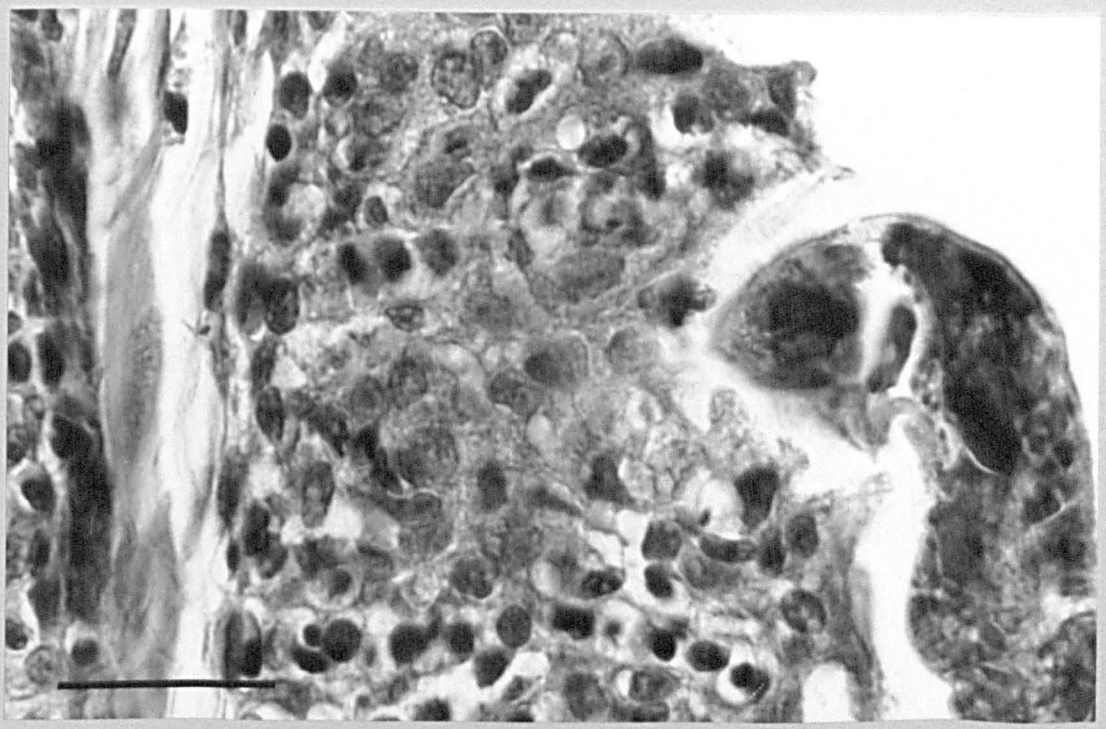
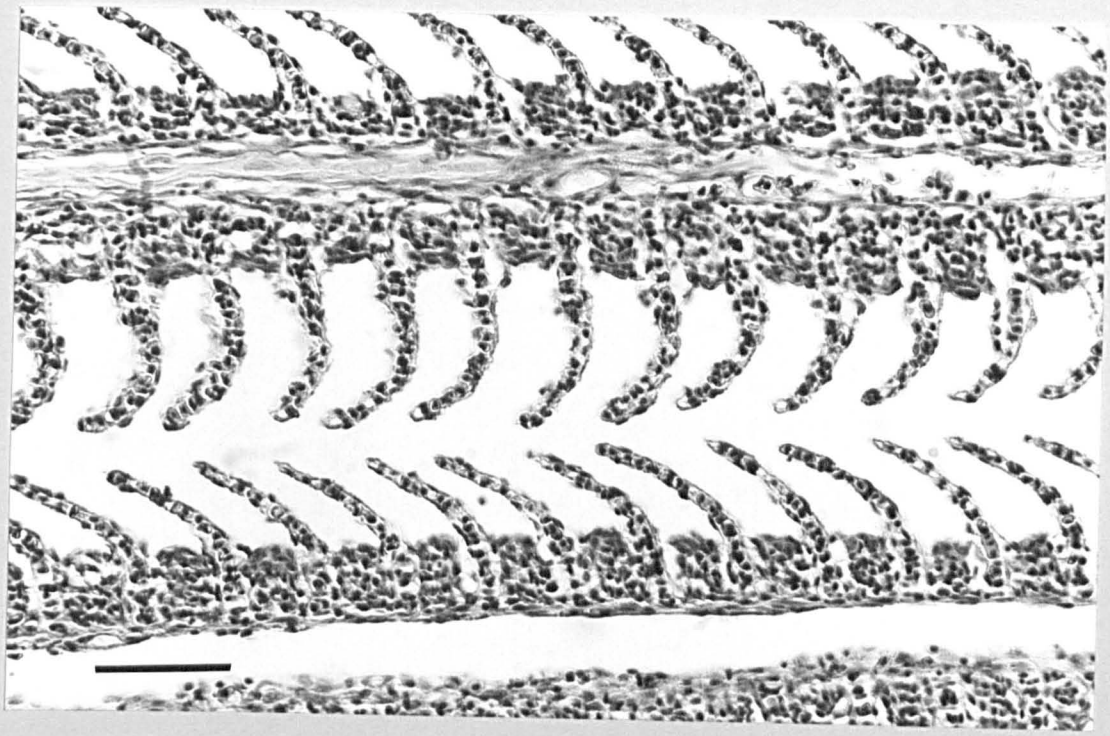


Figure 2.40: Normal gill structure of *Etroplus suratensis*. Scale bar= 50 µm. (H & E).

Figure 2.41: Focal hyperplastic response (arrows) to the presence of *Ancyrocephalus etroplus*. Compare with the normal gill structure. Scale bar= 10µm. (H & E).



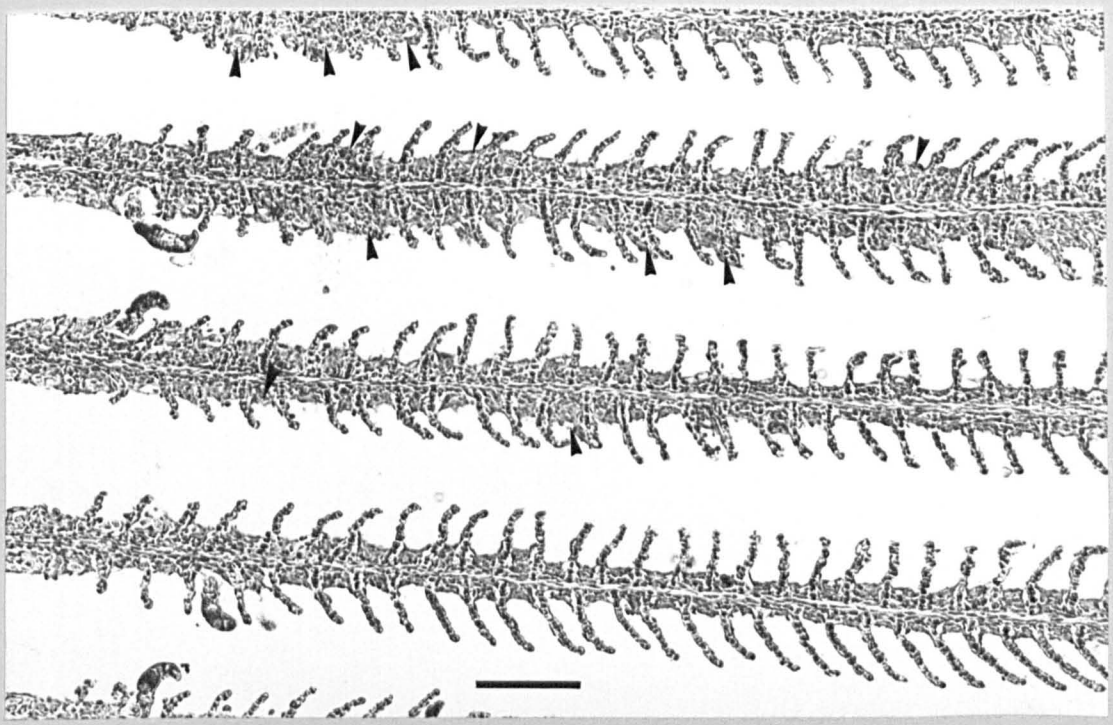
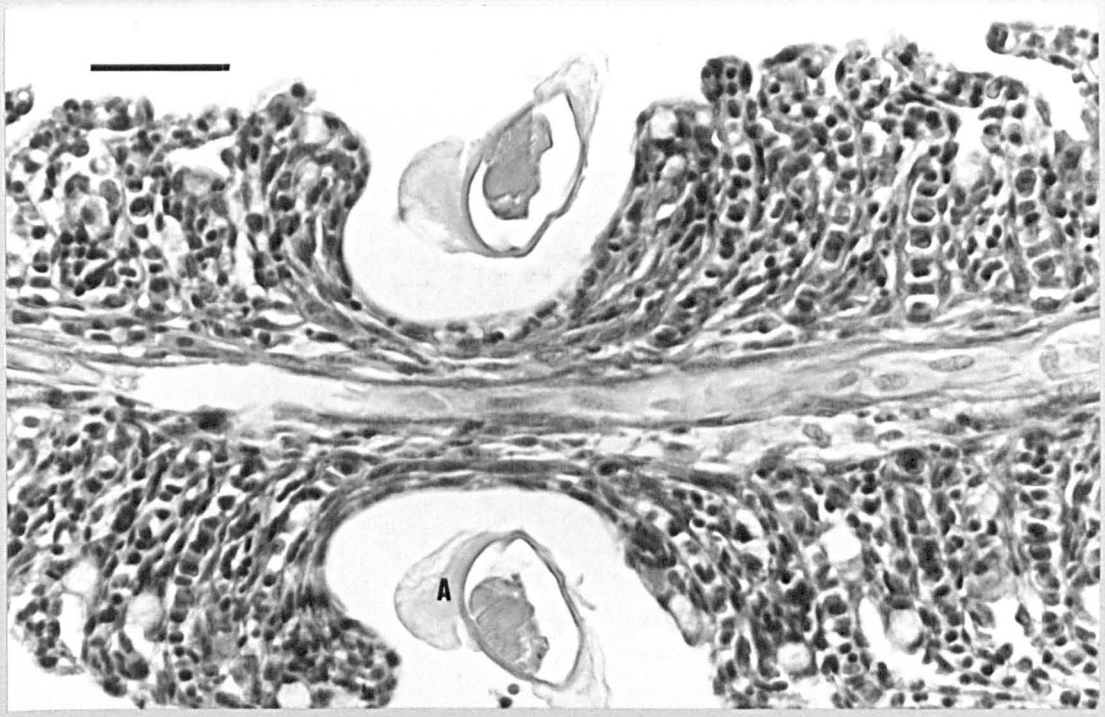


Figure 2.42: The damage caused by *Dermoergasilus amplectans* due to attachment. Note the damage around the attachment and no blockage of blood circulation. A = Antenna. Scale bar= 50 μm. (H & E).

Figure 2.43: The damage caused by *Dermoergasilus amplectans* due to mechanical irritation. Note the hyperplasia of gill epithelium and goblet cell proliferation (arrow). The neighbouring primary filaments without attached copepods are also affected. Scale bar= 100 μm. (H & E).


A

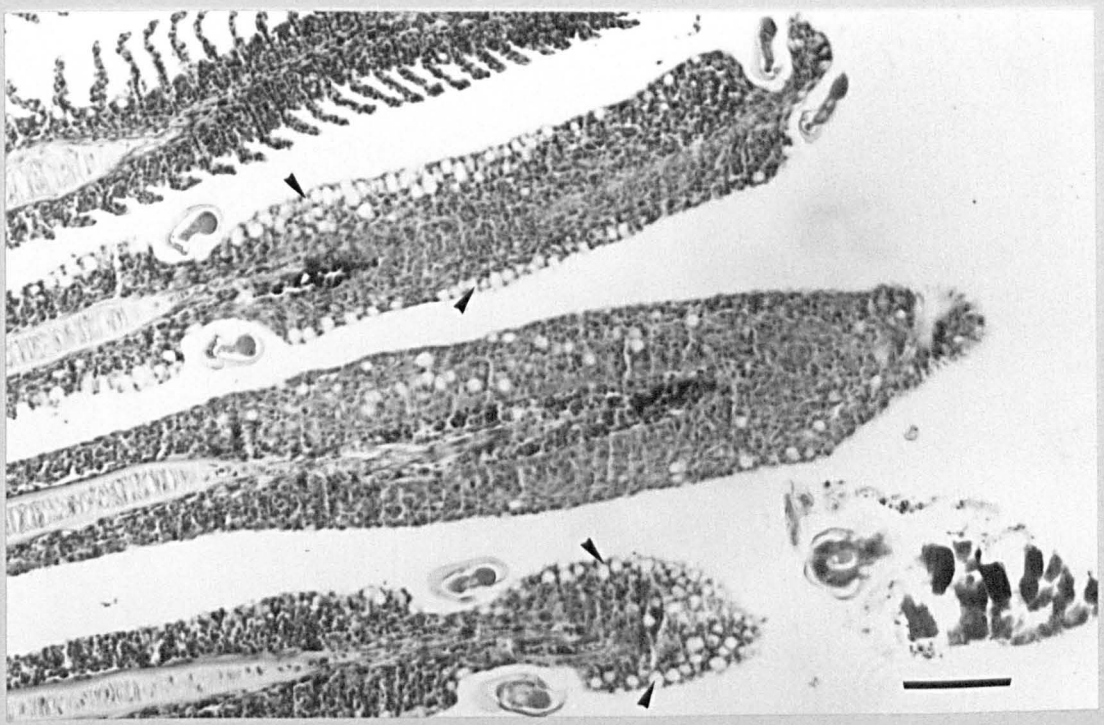
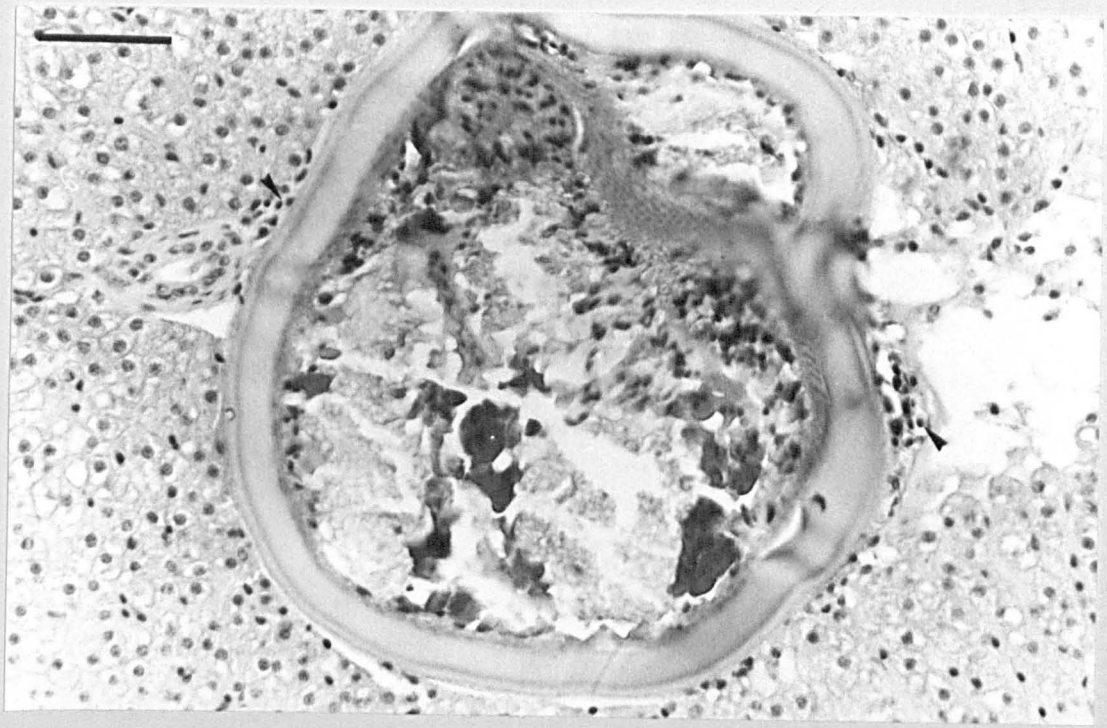


Figure 2.44: The cyst of the renicolid metacercaria in liver and its damage. A capsule of host origin and the inflammatory cells (macrophages or fibroblasts) are absent. Note the pycnotic nuclei of the destroyed liver tissue (arrows). Scale bar= 50 μm. (H & E).

Figure 2.45: The cyst of the renicolid metacercaria in liver with the presence of eosinophilic granular cells (arrows) near damaged cell cytoplasm. Scale bar= 50μm. (H & E).



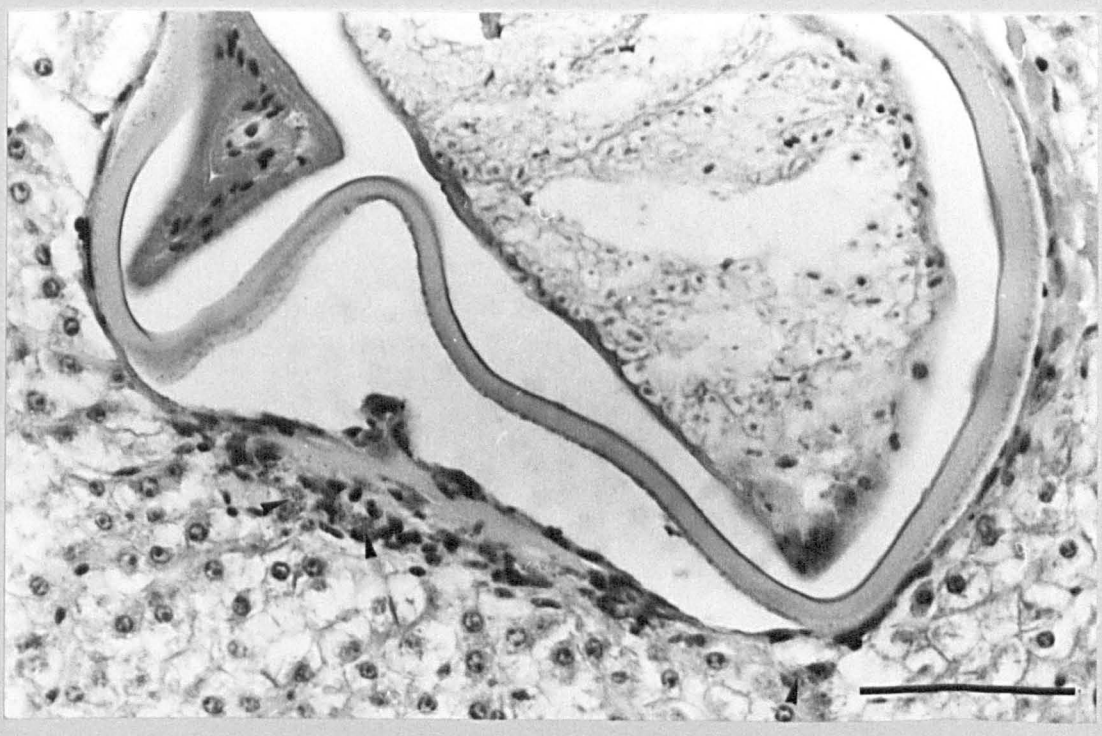
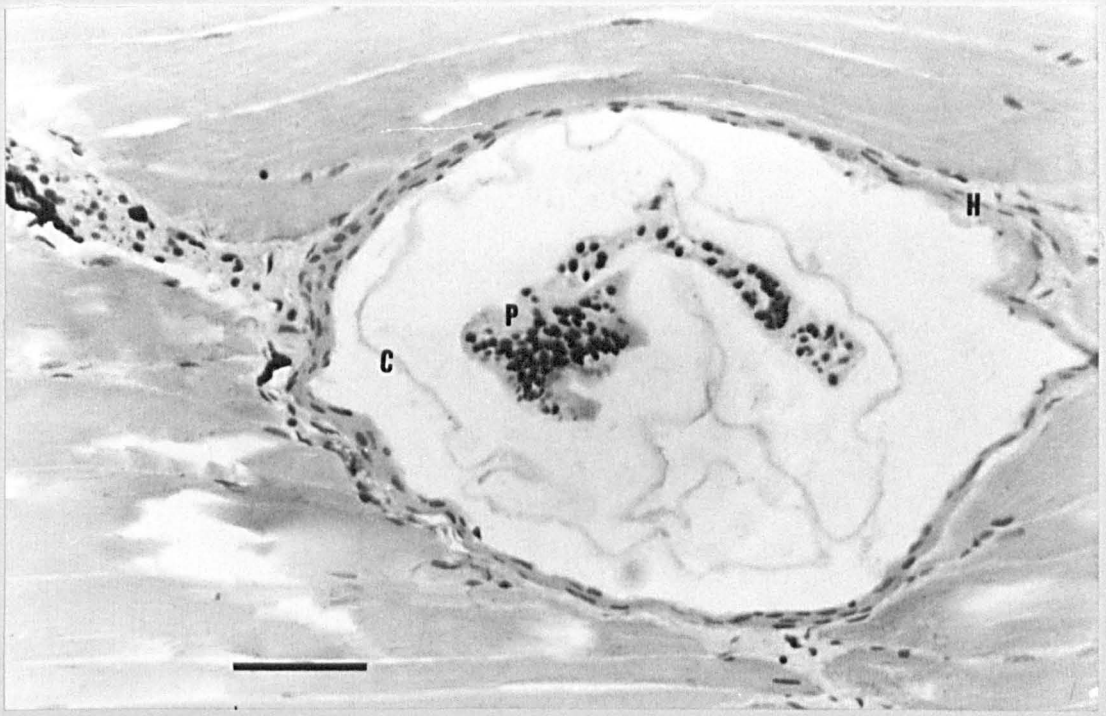


Figure 2.46: The cyst of the cyathocotylid metacercaria and the replacement of damaged myo-fibrils of the muscles in the migratory pathway of cercaria by fibrocytes. Melanocytes are attracted. H = host capsule, C = Cyst of parasite, P = Parasite. Scale bar= 50 μm. (H & E).

Figure 2.47: The cyst of a dead cyathocotylid metacercaria with macrophages (arrow) in vicinity. Live tissue, cuticle and cyst of parasite are absent. Scale bar= 50 μm. (H & E).


H
P
C

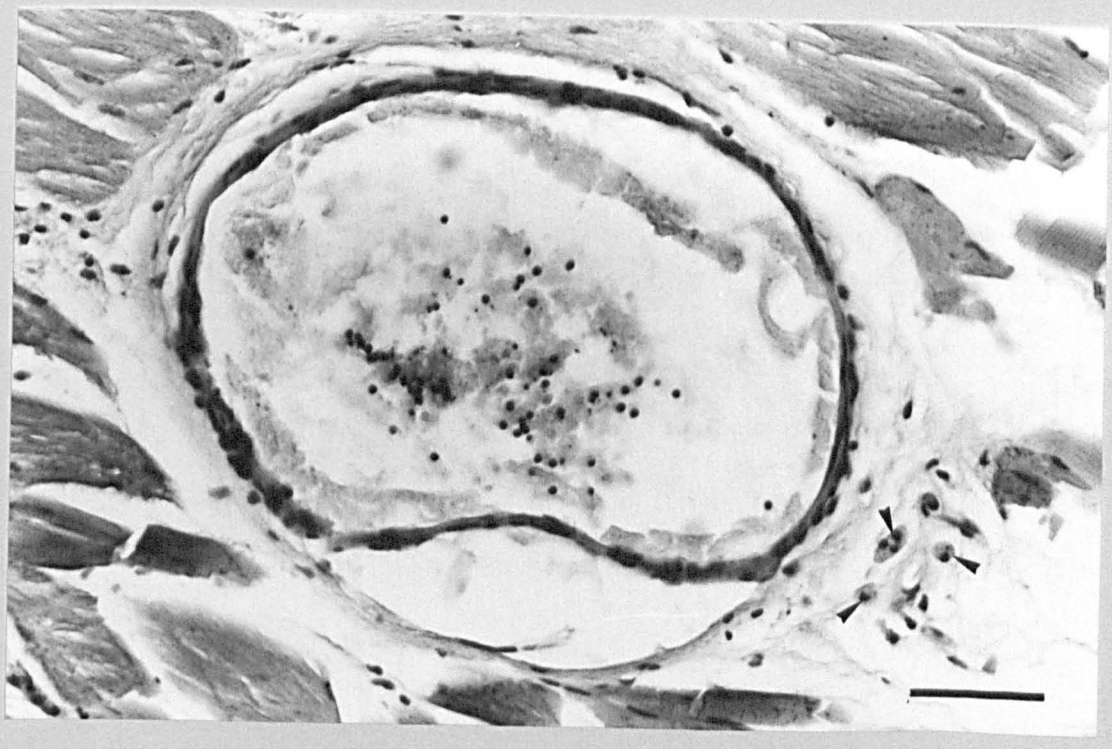
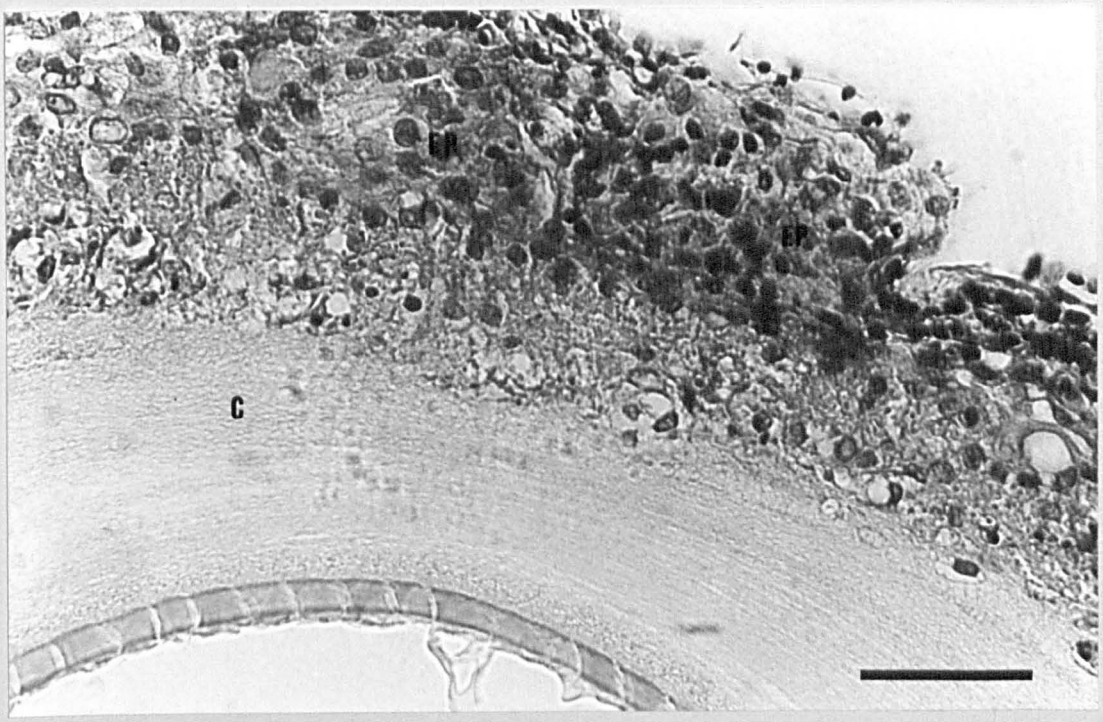


Figure 2.48: The early inflammatory response to strigeid metacercarial cyst. The periphery of the cyst of parasite was attacked by macrophages. Most of these macrophages are now in the form of epithelioid cells. C = cyst of parasite, EP = epithelioid cells. Scale bar= 40 μm. (H & E).

Figure 2.49: The late response to strigeid metacercarial cyst and the replacement of epithelioid cells by fibrocytes (arrows). C = cyst of parasite, P = parasite. Scale bar= 40 μm. (H & E).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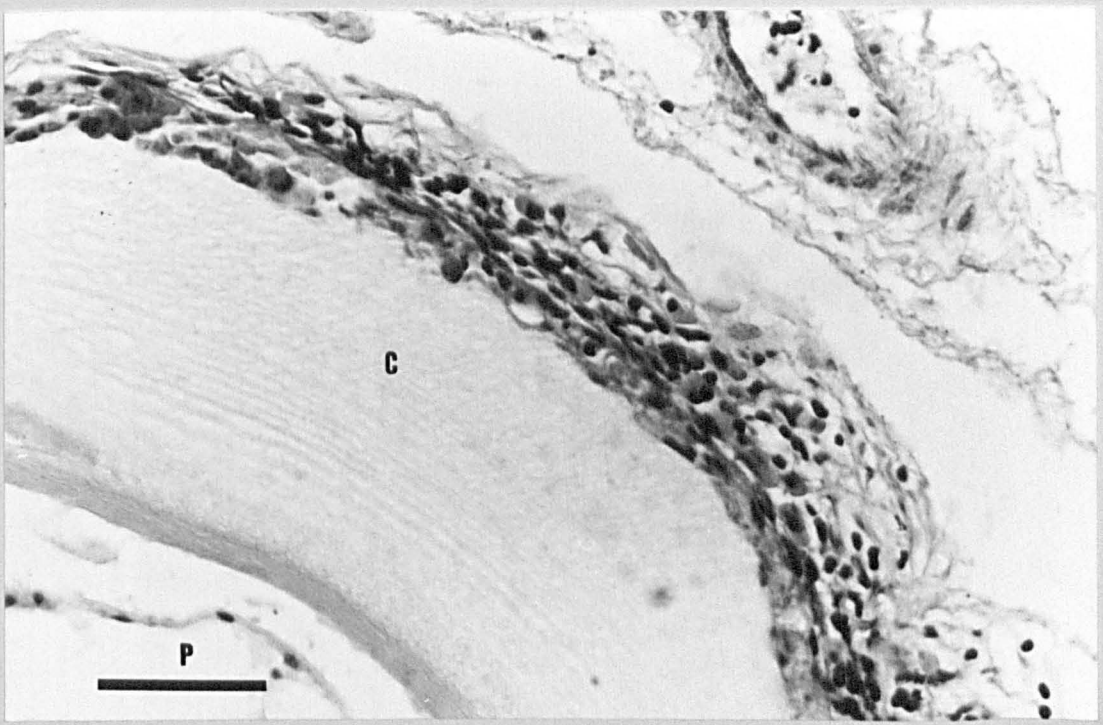
C
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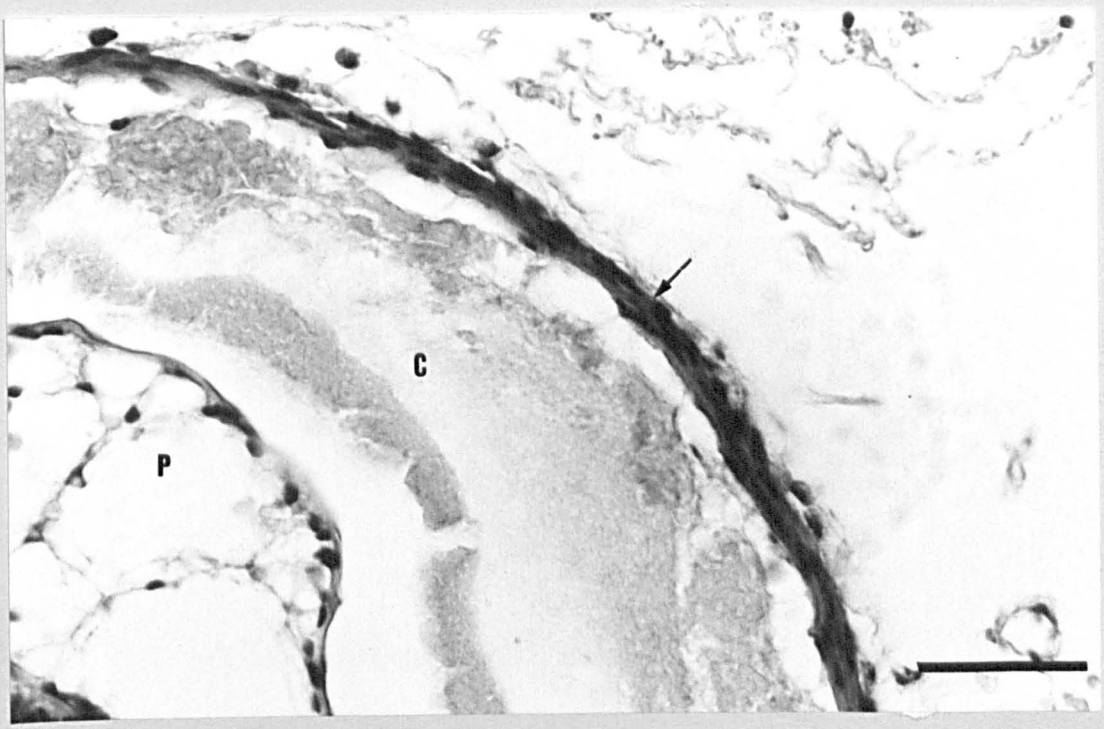
Figure 2.50: The fibrocytic host capsule formed around the cyst of a strigeid metacercaria at a later stage (arrow). C = cyst of parasite, P = parasite. Scale bar= 40 μm. (H & E).

Figure 2.51: The presence of eosinophilic granular cells (arrows) around the host capsule of a strigeid metacercarial cyst. Scale bar= 40 μm. (H & E).


C
P

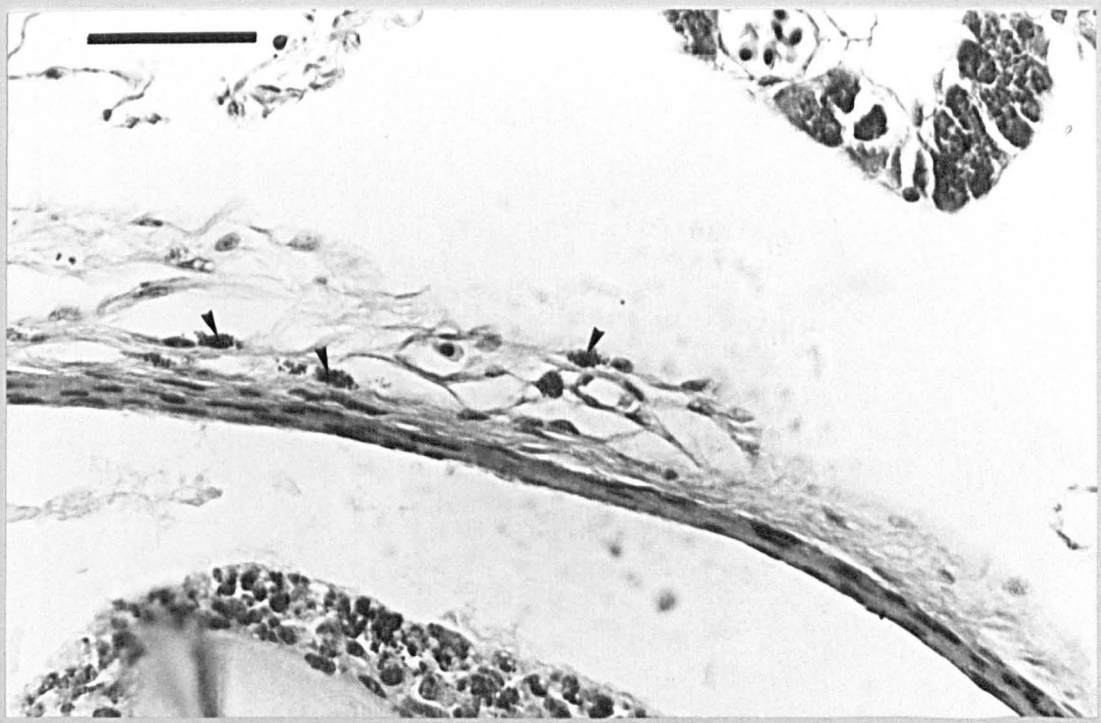
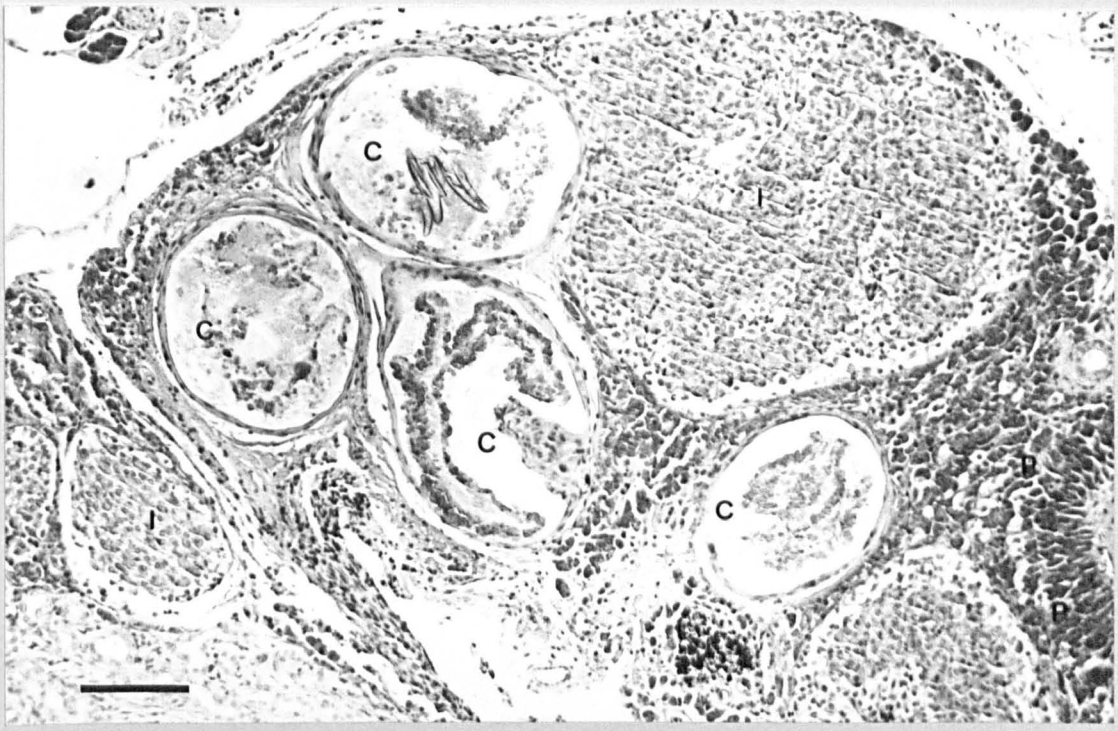


Figure 2.52: The cysticerci of *Paradilepis scolecina* destroyed by the host response. C = cysticerci, I = islet of Langerhan, P = pancreatic tissue. Scale bar= 100 μm. (H & E).

Figure 2.53: Rodlet cells (arrows) associated with the endothelium of a blood vessel in the region of cysticerci *Paradilepis scolecina* infection. Scale bar= 50 μm. (H & E).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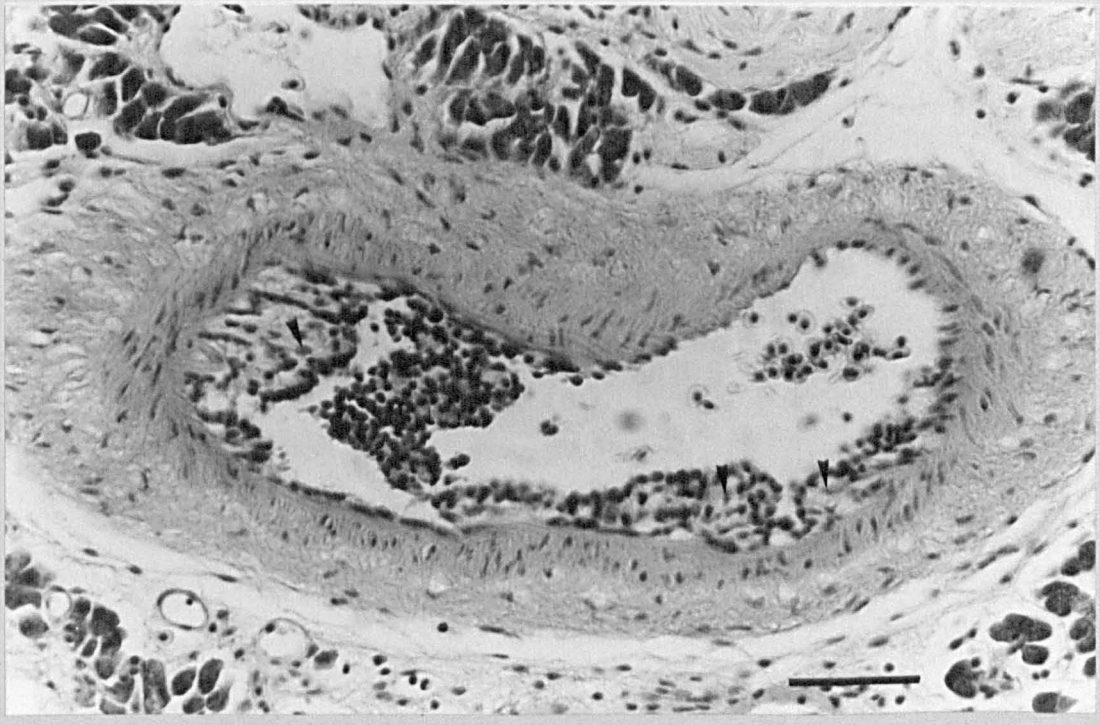
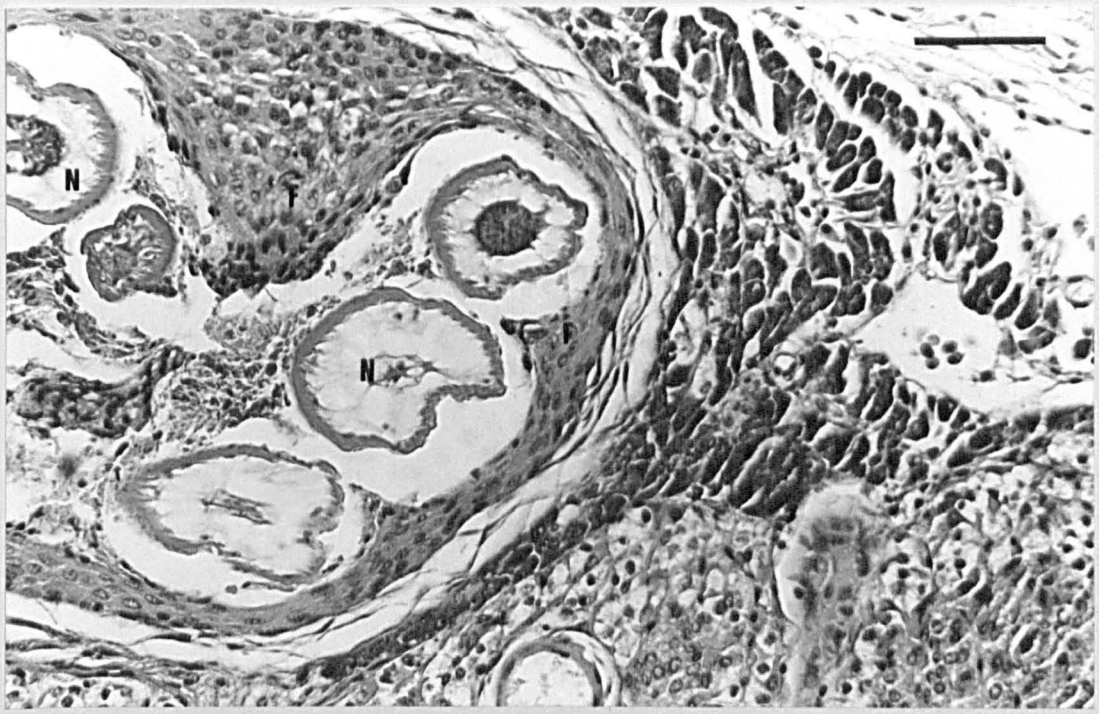


Figure 2.54: Encysted *Contracaecum* larva in the region of the pancreatic tissue. Note the massive number of fibroblasts and fibrocytes surrounding them. L = nematode larva. Scale bar= 40 μm. (H & E).

Figure 2.55: A nematode larva loosely attached to the mesentery. Note the thin fibrocyte capsule (arrows) surrounding them. L = nematode larva. Scale bar= 100 μm.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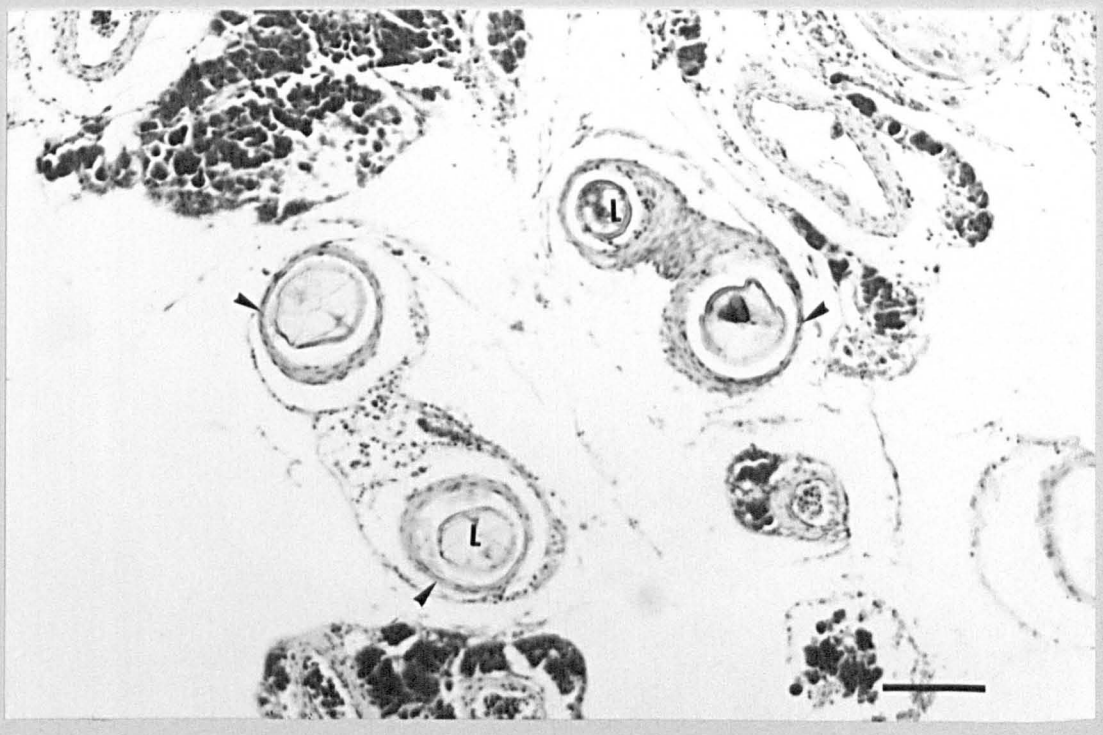

L
L

3. Strigeid metacercaria

These cysts were found in the abdominal cavity attached to the mesentery. The secreted parasitic cyst layer was thick. As some of the infections in the fish were recent, the process of the host reaction could be traced as follows.

The periphery of the secreted parasite cyst was seem to be under attack and phagocytosed by macrophages (Figure 2.48). This area of activity stained more eosinophilic than the inner part of the parasite cyst. The macrophage cells were very enlarged, possibly due to the accumulation of phagocytosed material. Most of these macrophages were in the form of epithelioid cells with elongated pale nuclei and cloudy eosinophilic cytoplasm whose outline merged with that of its neighbours. Thus it appeared that they were in the process of forming a capsule covering the parasite cyst. The epithelioid cells in the periphery still had darkly staining nuclei. There were free macrophage cells near to the periphery. No fibrocytes or fibroblasts were seen to be present.

In some other cysts, probably cysts a little older, the epithelioid cell layer was tightly packed or absent. Fibrocytes were intruding to and covering the periphery of the cyst. The parasite cyst under attack was more eosinophilic and a very few macrophages were present (Figure 2.49).

In the oldest cysts found, the host capsule has become much thinner maybe due to the compression of the cells in the capsule, as well as the destruction of the innermost epithelioid cell layers (Figure 2.50 & 2.51). Macrophages were absent too. A large number of eosinophilic granular cells and melanocytes were present around the host capsule (Figure 2.51) and melanomachrophage aggregations had formed near some cysts.

4. Cysticercus *Paradilepis scolecina*

The cysts containing these larvae were found attached to the mesentery in the region of the bile duct. In histological section, the cysticerci were surrounded by a host capsule of fibrous appearance. In all the cysts the cysticerci inside seemed to be

dead, showing no live tissue or cuticle or cyst wall. The material inside was armophous and sometimes highly eosinophilic (Figure 2.52). This confirmed the fact that only a few cysts with live worms (transparent worms exhibiting body movements and with excretory corpuscles) were found in the parasitic survey as a whole. The absence of cysts with live worms suggested that these cysts were missed by the sampling. The cysts carrying dead and live worms could not be distinguished by the external appearance. No evidence could be gathered to explain how the host reaction could be responsible for the killing of the parasite. It may be that the material observed histologically was at a later stage, ie. following the death of the parasite and the actual reaction responsible missed.

Of the 8 infected fish, 2 showed a massive presence of rodlet cells in a large blood vessel very closely situated to the bile duct. This was probably the hepatic artery with thick walls (Figure 2.53). However, in some small blood vessels in the region, they were absent. In all other infected fish material, the rodlet cells were present only in small numbers. Their presence cannot be attributed only to a host reaction against parasite, as the cells were also found in uninfected fish material. However, in these, the intensity was low and, in some, very few rodlet cells were observed.

5. Third stage *Contracaecum* larva

Only the encysted larvae in the mesentery could be studied and the larvae in the liver and stomach were missed by sampling. The larvae were surrounded by a fibrous host capsule. Sometimes the capsule was spherical, encircling the whole larva in one entity (Figure 2.54) and in the other times it was adjacent to the body wall (Figure 2.55). This difference might be due to the degree that the larva was coiled.

The cellular response which led to the formation of the capsule was not evident. In some instances, the pancreatic tissue near the encysted site seemed to be destroyed due to the occupation of the site by the worm. However, the worms which were loosely attached to the mesentery thus causing less damage (Figure 2.55). The effect of the damage in the liver would be expected to be greater than in the

mesentery.

In the histological sections, any second stage cuticle retained with the third stage larva could not be observed. The absence of the second stage cuticle is contradictory with the findings of life-cycle studies on *Contracaecum* by Huizinga (1966, 1967). This may be completely shed before they encyst or may be removed by the host reaction.

2.3.4. Experimental environment manipulation

The results of the experiment carried out to find the effect of salinity on the external parasite fauna by leaving the fish brought from freshwater reservoir in different salinities are given in the Table 2.28.

According to these results, it is evident that the external parasitic protozoans are affected considerably by the salinity of the surroundings, whilst the monogeneans show no effect between 0-24 ‰. Of the protozoans, *Ichthyobodo* sp. is very salinity tolerant and was able to survive in the experimental tank with a salinity of 16 ‰. Other species, Trichodina, *Glossatella* sp. and *I. multifiliis*, survived only in the tank with fresh water.

The monogeneans were able to survive in all salinities tested (0 - 24 ‰), with no change in their population levels. The proportion of *A. etropli* to *C. colombensis* was similar indicating that salinity has no effect on their biology and therefore on their life-cycles.

Table 2.28: The development of initial parasite infection levels on *Etroplus* in different constant salinities maintained for 3 weeks (fish from Udawalawa reservoir).

Salinity	0 ‰	8 ‰	16 ‰	24 ‰
No. of fish in tanks	8	8	8	8
Trichodina sp.	1.1 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Apiosoma sp.	1.4 ± 2.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Ichthyobodo sp.	50.4 ± 27.1	27.8 ± 18.3	3.8 ± 4.5	0.0 ± 0.0
I. multifiliis	111.4 ± 70.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
A. etropli	75.8 ± 30.7	77.5 ± 32.7	64.9 ± 27.8	71.4 ± 26.0
C. colombensis	5.75 ± 3.5	5.4 ± 3.6	7.5 ± 4.8	5.3 ± 2.3

2.4 Discussion

2.4.1 Parasites found in the study

Parasites of a wide variety of groupings, protozoans, monogeneans, digeneans, cestodes, nematodes and crustaceans were recorded from *E. suratensis* during the survey. Some of them have previously been described (Table 2.29). With the exception of the four monogeneans and the leech *Placobdella undulata* (Mendis and Fernando, 1962) none of the other parasites have been previously recorded on *E. suratensis* in Sri Lanka. The adult trematode, *M. indica* and the ergasilid, *E. parvitergum* have been described in India but this is their first record in Sri Lanka. The only monogenean recorded on *E. suratensis* in India was *E. globidiscus*; a few specimens of this stomach inhabiting monogenean were recorded from specimens found on gills (Kulkarni, 1969).

In this study, five protozoan parasites, seven species of metacercariae, one adult digenean, one larval cestode, one nematode larva, one adult nematode and two crustaceans are newly recorded for *E. suratensis* (Table 2.29C). Unfortunately, many of the protozoans and larval stages of the metazoan could not be identified to the species level. Further detailed morphological studies for protozoans and life cycle studies for the larval stages, will be necessary in the future to determine their identity. Of these new records on *E. suratensis*, some are already described on other fish in Sri Lanka. The common protozoan parasite, *I. multifiliis* was reported by Balasuriya (1983) and the adult trematode *T. patialense* by Crusz & Sathananthan (1960). The adults of the larval cestode *P. scolecina* was reported by Burt (1940). Three species of adult of *Contracaecum* sp., *C. rudolphi* (= *C. spiculigerum*), *C. haliaeti* Baylis & Daubney 1925 and *C. microcephalum* (Rudolphi, 1809) were reported (Baylis, 1936) in birds, and may well be the adults of the *Contracaecum* found in *E. suratensis*. The presence of the protozoan genera *Trichodina* sp. and *Ichthyobodo* sp. are already described from Sri Lanka by Balasuriya (1983, 1987) on carps though he did not identify them to the species level.

Table 2.29: The status of parasite records of *Etroplus suratensis*.

A. Parasites of *E. suratensis* recorded in Sri Lanka

Parasite	Site	Habitat	Reference
Ancyrocephalus etropli	on gills	?	Gussev, 1963
Celonotrema colombensis	on gills	?	Gussev, 1963
Enterogyurus globidiscus	in stomach	fresh water	Gussev & Fernando 1973
Enterogyrus papernai	in stomach	fresh water	Gussev & Fernando 1973

B. Parasites of *E. suratensis* recorded in India

Parasite	Site	Habitat	Reference
Enterogyrus globidiscus	on gills	?	Kulkarni, 1969
Malabarotrema indica	intestine	?	Zhukov, 1972
Ergasilus parvitergum	on gills	brackish	Ho, Jayarajan & Ramakrishnan (per.comm.)

C. Parasites of *E. suratensis* newly recorded in the present study

Parasite	Site	Habitat
Protozoans		
Trichodina sp.	skin & gills	fresh/brackish
Apiosoma sp.	skin	fresh/brackish
Ichtyobodo sp.	skin & gills	fresh/brackish
Ichthyophthirius multifiliis	skin & gills	fresh water
Trypanosoma sp.	blood	fresh/brackish
Digeneans		
Acanthostomidae gen. sp.	fin ray & gill filament cartilage	brackish water
Exorchis sp.	skin & gills	brackish water
Renicolidae gen. sp.	liver	freshwater
Centrocestus sp.	gills	brackish water
Cyathocotylid metacercaria	muscle, liver mesentery	fresh/brackish
Diplostomid metacercaria	muscle	freshwater
Strigeid metacercaria	abdominal cavity on mesentery	freshwater
Transversotrema patialense	under scales	fresh/brackish
Cestodes		
Paradilepis scolecina	mesentery	freshwater
Nematodes		
Contracaecum sp.	mesentery	fresh/brackish
Rhabdochona sp.	duodenal wall	brackish
Crustaceans		
Dermoergasilus amplectens	gills	brackish
Ergasilus parvitergum	gills	brackish
Argulus sp.	skin	brackish

Although some of the protozoan genera found were already recorded (Balasuriya, 1983, 1987), this is the first time the two protozoan genera *Apiosoma* (=*Glossatella*) and *Trypanosoma* are recorded in Sri Lanka. The parasite species of the already recorded genus *Trichodina* from carps may vary from the species found on *Etroplus*. Further, the two genera *Trichodina* and *Apiosoma* found in both fresh and brackish water localities, may be represented by more than one species. The occurrence of families of digeneans other than Echinostomatidae, Heterophyidae and Transversotrematidae, is reported in Sri Lanka for the first time in this study, either from the definitive or the intermediate host. In the family Heterophydae, the genus *Centrocestus* has not been previously reported. Although the adult forms were reported earlier, the cysticerci of *Paradilepis scolecina* and the third stage larva of a *Contracaecum* sp are reported for the first time. This is the first record of a *Rhabdochona* sp. too. Although *Argulus foliaceus* was reported previously from common carp by Mendis & Fernando (1962), the *Argulus* found in the present investigation is clearly different from *A. foliaceus*. The two ergasilids, *Dermoergasilus amplectens* and *Ergasilus parvitergum* are also new records for Sri Lanka. The record of *Ergasilus ceylonensis* in the Negombo lagoon by Wijeyarathne & Gunawardena (1988) seems a misidentification if it is not euryhaline, as this species is previously recorded by Fernando & Hanek (1972) in freshwater reservoirs. No such form was found in the Koggala lagoon during the period of survey in this study. In addition, the parasites *Cichlodogyrus sclerosus* and *Gryporhyncus pusillum* are described for the first time in Sri Lanka. Although these parasites were not found on *E. suratensis* but are described from *Oreochromis niloticus* from Udawalawa reservoir. *Lernaea* sp. was reported to occur on *E. suratensis* inhabiting Udawalawa reservoir by Tennakoon (pers. comm., cited by Balasuriya, 1987) but was not observed in this study. The three leech specimens collected from Koggala lagoon were not identified, thus it is impossible to comment on whether they belong to the species *Placobdella undulata* as previously reported on *E suratensis* by Mendis and Fernando (1962) or to any other species.

2.4.2 The life cycle studies

Of the metacercariae discovered, the molluscan intermediate hosts of three

were found. *Melanoides tuberculata* was found to be the host for the Acanthostomid metacercaria as well as for *Transversotrema patialense*. *Thiara* sp. was the host for the renicolid metacercaria. Both these species are euryhaline but, *Thiara* sp. was found to be much more abundant in the reservoir, whilst *M. tuberculata* was common in the lagoon. The presence of the molluscan intermediate host in both localities shows that the absence of the intermediate host is not the limiting factor where the parasite is found in only one of these localities eg. the absence of Acanthostomid metacercaria in the reservoir and renicolid metacercaria in the lagoon. The difference in the abundance of the two species of molluscs in these two localities, however, may have a slight influence on the locality preference. The absence of the definitive host in these localities and/or the inability of larval stages to survive in low or high saline conditions may also be the reasons responsible.

Whenever a good collection of metacercariae could be obtained, they were force fed to chicks and rats, the two kinds of laboratory host available. This was carried out with very fresh material prior to the identification of metacercariae, thus without knowing the possible group, ie. fish, amphibian etc., of final host. A subsequent assessment of earlier studies by other authors reveals that Cyathocotylids do not generally appear to develop in laboratory hosts. For example, Anderson and Cable (1950) were not able to obtain adults of *Linstowiella szidati* (Anderson, 1944) in white rats, 2-day old chicks and mallard ducks. Later, they were obtained in newly-hatched chicks that had been unfed. Mishra and Chubb (1969) were not able to obtain adults of a metacercaria of a *Prohemistomulum* species in chicks and ducklings. Vernberg (1952) reported low intensities of cyathocotylids even in natural infections.

The inability of Cyathocotylid metacercaria and *Contracaecum* sp. to establish in chicks was demonstrated by their presence in faeces of chicks, two hours after force feeding. The retention of the parasite cyst around the Cyathocotylid metacercaria passing out with faeces suggested the inability of chicks to provide the physiological requirements for excystment, possibly due to the low proteolytic enzyme concentration which may be present in them compared to fish-eating birds and the high rate of food digestion. The inability of the cysticercus *P. scolecina* to develop in the experimental

birds might be due to the same reason. In addition, the actual dose had been relatively low due to the difficulty of differentiating cysts bearing live and dead cysticerci. The adults of the family Renicolidae develop within and inhabit the kidneys of birds (Yamaguti, 1975), a site which was, unfortunately, not checked, as this is not a common site of infection in birds. The bird and mammal final hosts used for Acanthostomid metacercaria were undoubtedly not suitable for their development as they are physiologically very different from their natural definite host, fish.

There are practical difficulties in carrying out life-cycle studies in the laboratory. The laboratory hosts are physiologically incompatible with the natural host and therefore the parasites find it difficult to establish in them. Even though the parasites establish, they may be expelled easily within few days with the development of host immunity. Therefore, the use of wild natural host is more reliable for successful parasite establishment. Sometimes, it is impracticable to find the host in the wild. Since wild hosts are naturally infected, only the newly hatched or born host can be used; otherwise the parasites infection have to be removed by drugs. The other problem of carrying out final host infection studies is the difficulty in getting considerable numbers of metacercariae, as the metacercarial infection levels in wild fish are very low.

Life-cycle studies of *D. amplectens* revealed the presence of six naupliar stages, similar to the studies on *E. sieboldi* (Abdelhalim *et al.*, 1991) and *Neoergasilus japonicus* (Urawa *et al.*, 1980a). The copepodid stages were also very similar to these other species, suggesting the similarity of ontogenic development and therefore the life-cycles of ergasilids.

Although the structure of the nauplius stages were basically similar, *D. amplectens* was smaller in size compared to both the already described species. The minor morphological differences observed were; (i) presence of fewer numbers of setae on the apical segment of the antennule; ie. one less than in *E. sieboldi* and two less than in *N. japonicus* at naupliar stage 6, (ii) appearance of rudiments of swimming legs in sixth nauplii stage instead of the third stage in *E. sieboldi* (iii)

having a lesser number of caudal rami appearing at stages compared to *E. seiboldi*. *D. amplectens* was similar to *N. japonicus* in the last two points. Medial spines on the maxillule could not be observed on *D. amplectens* whereas two have been described for *N. japonicus* and *E. sieboldi*.

The morphology of the copepodid stages were shown to have basically similar development with the two species already described. The minor differences were (i) ability to differentiate the sex at the copepodid stage 3 in the case of *N. japonicus* while it could be done at the stage 4 in *D. amplectens* and *E. sieboldi*, (ii) the difference in the number of segments of the antennule appearing at the stages and the number of setae carried by them; in *E. sieboldi*, the antennule become 6 segmented in the third copepodid stage, while it occurs at the adult stage of *N. japonicus* and in *D. amplectens* adult males have 5 segments and parasitic females have 6, (iii) the absence of a vestigial exopod on antennae of *D. amplectens* while it is present in the other two and (iv) the difference of armature in swimming legs, although the development is similar; however, the fourth pair of swimming legs is comparatively vestigial in *N. japonicus* and the fifth pair is completely absent.

Therefore, the study of the life-cycle stages of *D. amplectens* strengthens the view that the parasitic copepods have six naupliar stages and show similarity in the development of copepodid stages. The discrepancies in the number of naupliar stages identified in past studies, may be due to the categorisation of a fewer number of stages due to the little differences which exist between some stages, and also to the missing of some stages due to low frequency of sampling. In addition, the rate of growth of *D. amplectens* is approximately similar to the rate of growth of *N. japonicus* studied by Urawa *et al.* (1991), at the same temperature. The development time from egg to nauplii stage 6 was around 4 days and to adult was (estimated) 7 days at 30° for *N. japonicus*. An interesting finding from the aquaculture point of view is to know that different species of ergasilids have similar growth rates at the same temperatures. This type of information is useful when developing control strategies.

2.4.3 Ecological aspects

2.4.3.1 The macro-habitat effect on the parasitic species composition and parasite infection levels

(1) Comparison of the fauna between water bodies

The nature of the difference in the parasite fauna of the *E. suratensis* in the lagoon and those in the reservoir appeared to largely depend on the mode of life-cycle of parasite. The monogeneans, having monoxenous life-cycles, were the main group with species common to both habitats. Although the digenean and nematode groups were found in both the habitats, only a few species were common to both. The group totally absent in the freshwater was the crustaceans. The main environmental parameter which characterizes these two localities ie. the salinity, seemed to have a direct effect on presence or absence, or was involved in enhancing or restricting the presence of intermediate hosts required for the completion of life cycles, thus being the factor creating the difference. Some parasites common to both localities showed a higher preference for one locality whilst others showed an equal preference for both localities.

Only one species is known for the genera *Ichthyobodo* and *Ichthyophthirius*. Experimentally it was found that the *Ichthyobodo* spp. from the reservoir could tolerate a wide range of salinity whereas *I. multifiliis* had a low range, being unable to survive in 8‰. Both the species show little host specificity, thus they are species which are common in any type of locality which can provide conditions within their tolerance limit. Two genera of protozoans, *Trichodina* and *Apiosoma* unfortunately could not be identified to the species level and therefore it is not known whether the parasites in the different habitats belong to different species. Due to the experimental finding that the species present in Udawalawa did not survive in the salinity 8‰ or above, it is more likely that these two genera are represented by different species in these two different habitats However, their infection levels were low and similar in both habitats.

The dioxenous protozoan *Trypanosoma* sp., was also found in the two different localities. If the intermediate host is euryhaline, it is possible that a single species is

involved in both habitats. The other possibility is that the parasite in the reservoir is able to survive with an alternate intermediate host species. However, the prevalence and intensity of *Trypanosoma* in the freshwater habitat was higher than in brackish water. If the parasite is the same species introduced from lagoon to reservoir, this poses the question "why do they have higher performance in freshwater than in the habitat they evolved in together with the host?". The environmental parameters may be more amenable to their population growth in the freshwater habitat than in the lagoon or two species, one freshwater and one brackish water, may be involved in the two different habitats. Usually not more than one species of trypanosome is recorded from one species of fish (Becker, 1977). However, the situation here is different as the fish were from physically and chemically two different localities. On the other hand, duration of the time in the freshwater habitat would be too short for such a development.

It was found that the monogeneans are the parasites which are most likely to survive with introductions to new habitats (Izyumova, 1988). Monogeneans have a direct life-cycle and, since no intermediate host is necessary which may be absent in a new water-body into which the fish are introduced, establishment of the parasites is easier, providing they can tolerate the water characteristics. All the monogeneans found in this study were ancyrocephalins. The Ancyrocephalinae is a marine and freshwater subfamily (Yamaguti, 1963; Malmberg, 1990). Thus, the parasites may have evolved with a wide range of salinity tolerance, even though they are found in one habitat. They may therefore be able to survive in new habitats by tolerating the salinity change. The tolerance ranges of oncomiracidia of *Enterogyrus* support this hypothesis.

Of the monogeneans, *A. etropli* were at similar abundance levels in both the habitats. *C. colombensis* was found at higher levels in the freshwater reservoir. *Enterogyrus* spp. were more abundant in the lagoon compared to the reservoir. It is possible that the similar levels shown by *A. etropli* may reflect their rapid multiplication in the holding tanks, thus masking the situation in the two different habitats. Although the abundance levels were higher, the preference of *C. colombensis*

for freshwater was not greatly different. However, the prevalence and intensity levels of *Enterogyrus* in the lagoon habitat was much greater than the reservoir. The Koggala lagoon being shallow and small may provide a high chance for host finding rather than the comparatively four times larger and six times deeper freshwater reservoir. Otherwise, the parasites may be propagating better in brackish water conditions rather than in freshwater, which is unlikely as shown by the study of their biology in chapter 3.

The digenean fauna of the two localities varied considerably. In contrast to monogeneans, they showed the effect of having intermediate and final hosts. However, the cyathocotylid metacercaria and *T. patialense* were found in both localities indicating that the hosts required for life-cycle propagation are available in both habitats and also that these parasites are euryhaline. Digenean metacercariae, as well as most adult stages, are generally loosely host specific, thus, these parasites may not have been introduced to freshwater with the fish, but possibly existed independently in both localities before their introduction. The higher abundance of cyathocotylid metacercariae in freshwater and *T. patialense* (zero for *E. suratensis*) in brackish water suggests that the reason for a diminuation in the numbers of parasites might be a lower density of intermediate host and/or the presence of a more preferable fish host in the other habitat. Further, the lagoon environment can be relatively harsh owing to the wide range of salinities and the free living stages may be destroyed at higher salinities.

Liver metacercaria and strigeid metacercaria were found only in the freshwater reservoir. Thus, these two were the digeneans picked up by the fish from their new habitat as a result of the introduction. It seems the first intermediate and/or the final hosts are restricted to this freshwater habitat. As the first intermediate host of the liver metacercaria is the euryhaline *Thiara* sp., their absence in Koggala lagoon indicates the absence of final host in the vicinity, or physiological inadaptability of the free living stages to brackish water conditions.

The absence of the Acanthostomid metacercaria *Exorchis* sp. and *M. indica* in

the freshwater indicates the absence of at least a single host required for life-cycle continuation or their physiological inability to tolerate the freshwater conditions. Since these parasites are exposed to very low salinity levels even in the lagoon, it seems unlikely that they are intolerant of freshwater.

Only a few species of the genus *Exorchis* have been described and the adults were found in freshwater catfishes (Yamaguti, 1971). However, the metacercarial stages infecting *Etroplus* were found only in lagoon fish. Therefore the final host may be a fish species dwelling in the lower part of rivers and brackish water areas and may not ascend to higher areas to reach into reservoirs. In the family Cryptogonimidae, to which the *Exorchis* belongs, freshwater as well as marine species occur. Since, only one genus of the subfamily Exorchinae and a few species of the genus *Exorchis* have been recorded, there is a possibility that there are unrecorded representatives in the marine environment.

The family Acanthostomidae includes parasites inhabiting marine fish as final host (Yamaguti, 1971). The inability of this parasite to survive in freshwater despite having the euryhaline *M. tuberculata* as an intermediate host, strongly suggests that the final host is a brackish water or marine species.

P. scolecina is a freshwater form which appears to have been picked by *Etroplus* after its introduction into the reservoir. The intermediate copepod hosts and/or the final host may well be restricted to the reservoirs. Even though numerous cysticerci were found in *E. suratensis* most of them were dead, with only few live specimens; the live larvae may have been recent infections. This strongly suggests that the parasite has its own freshwater fish host/s, and *E. suratensis* is either a paratenic host or an abnormal host in which the parasite fails to survive.

The *Contracaecum* sp. was very common in the reservoir and very few specimens were found in the lagoon. It is not clear whether they belong to one or two species. The very low levels of worms in the lagoon suggests the intolerance of the existing salinities in the lagoon by the larval stages and/or the copepod intermediate

host of a freshwater or a marine species of *Contracaecum*. The lagoon species could be a freshwater or a marine form, since the genus *Contracaecum* is present in both freshwater and marine fish. The mouth of the river or the mouth of the lagoon seems to be the edge of the normal distribution range of the larvae or intermediate host. Outside of this range, ie. in the lagoon, their density must be very low with a correspondingly low possibility of infecting fish. Even though the Bolgoda lagoon had slightly higher infection values, the low values, nevertheless indicate the same possibility. Since *Contracaecum* L_3 stages are not very host specific, the introduction of fish to freshwater habitats may have enabled them to acquire the freshwater species.

Rhabdochona is considered to be a freshwater genus though there are some *Rhabdochona* spp. recorded in brackish and marine waters (Dogiel, 1964). In this study this nematode was present only in Koggala lagoon. The absence in the Bolgoda lagoon indicates a geographical barrier or a non-representative, too-small sample. Alternatively, the absence of the parasite may be regional within the lagoon which is much more elongated than its breadth, giving greater zonation with the salinity gradient. As with the other brackish water parasites, the non-existence of them in Udawalawa may be due to the absence of intermediate hosts or the physiological intolerance.

Ergasilids are freshwater as well as marine forms (Kabata, 1988). The ergasilid, *D. amplectens* was reported on *Mugil cephalus*, the marine and brackish water inhabiting fish. This indicates that the parasite is salinity tolerant at least within the range found in the lagoon. Since this parasite has a direct life cycle it suggests that they are not able to adapt physiologically to the freshwater condition.

Ergasilus parvitergum and the branchiuran *Argulus* sp. were found in the lagoon with very low numbers. Neither was present in the freshwater reservoir. The very low prevalence and intensities may indicate that the lagoon is not within their normal distribution range and the parasite may prefer freshwater areas or high saline areas adjacent to lagoon. Since the ergasilids and argulids are reported from both fresh water and marine habitats there is an equal chance for them to be belong to any one

of these. On the other hand the host, *Etroplus*, may be an accidental host, thus the infection was very low even though they prefer brackish water and are present on a brackish water host.

(2) Seasonal variations

Care must be taken when interpreting the variations in the parasitic burden as these may not be purely due to the effect of the seasonal variations. The small sample sizes may not represent the real picture of the water body. The patchy occurrence of infective stages in the water-body, may mask or create false seasonal fluctuations especially when the burdens are low. In this study, most of the parasites showed low burdens. Because of this, only generalized and obvious variations in the burdens can be taken into consideration. Even though it was considered that the fish size variation had a minimal effect on monthly data, this may be another factor.

The behavioural changes of different stages of host, the aggregations or dispersals, can change the host-finding ability of infective stages, thus varying the burdens. In addition, the life-cycle pattern of parasites may lead to the recruitment of parasites in a particular period and their disappearance after a particular period. This may or may not have any influence on seasonal changes.

Of the protozoans, only *Ichthyophthirius* showed significant differences in abundance in tanks in the aquarium. Some samples showed higher numbers coinciding with lower temperatures in December, whilst other samples showing an increased abundance of *Ichthyophthirius*, coincided with high numbers of *Ichthyobodo* (June & August). Since fish are able to produce a very effective immune response to *I. multifiliis* it seems likely that the increase in intensity in aquarium during the months June, August & December is due to increased susceptibility. Both low temperatures and stress can depress the host's immune system and thus the defence against the parasite. It seems that both of these parasites may be a threat in culture systems as they rapidly multiplied in the aquarium. *Ichthyophthirius* may be a problem in the colder seasons and also in drier seasons where fish can become stressed and a concentration of parasites occur due to less flushing/dilution of infective stages.

Ichthyophthirius was completely absent in the brackish water. Although *Ichthyobodo* was present at low levels in the Koggala samples, the infection did not build up in stocking tanks. It seems that the environmental conditions may not have been suitable for their population growth even though they were able to survive.

There was an increase in abundance of all the monogeneans occurring during or just after the rainy seasons; the first peak of abundance was from May to July and the second from December to March. A behavioral change leading to aggregation of the fish hosts, due to rain, flooding or onset of some other activity due to rain, may be responsible for the peaks found in the monogenean burdens. It is difficult to separate the exact cause due to the lack of studies on the normal behavioral pattern of fish in these water bodies and their response to environmental stimuli. The decrease of salinity during the rainy season may be a major feature in lagoons. The preference for inundated macrophytes by *Etroplus* as their food, and the aggregation of fish in these areas was observed by Schiemer & Hofer (1983), and Chandrasoma *et al.* (1986), suggesting that the increase in parasite abundance is due to increased host density in a particular area, thus increasing the host finding ability of the parasite.

The peaks of *Enterogyrus* occurred a little later than the peaks of *A. etroplus*, ie. at the end of the rainy periods. The peaks coincided with breeding season of *Etroplus* which occurs in July and February in the lagoons and in February in the reservoirs (Costa, 1983). The increase in abundance of the parasite can be due therefore to aggregation behaviour in the breeding season.

Any two parasites with similar host finding requirements must be similarly and simultaneously influenced by the aggregation of the host. It was found that the monogeneans with distant affinities but on the same host showed similar types of host finding behaviour (Whittington, 1987). Any aggregation which enhances the population growth of one species should therefore enhance that of the other. However, this cannot occur when the water quality parameters which are favourable for one species are not favourable for the other. It is evident from the water quality data that there was not any considerable variation occurring within the rainy period or at the

end of it. Therefore, the reason for one species to exhibit low population densities whilst the other showed high densities is not clear. A possible explanation can be provided by considering the life cycle duration and generation time of the two species. *A. etropli* has a very short generation time which probably led it to multiply rapidly in the holding tanks whilst this time is comparatively longer for *Enterogyrus*. The increase in population due to increased host finding ability occurred during the rainy season may become evident much more quickly in *A. etropli* i.e. within the rainy season and later in *Enterogyrus* i.e. at the end of the rainy period.

The peak times for metacercarial prevalence and abundances are in December to January. Only the cyathocotylid metacercaria exhibited two peaks, one at the same time as for other metacercariae, and the other peak in July. For strigeid metacercaria no peaks could be traced. The molluscan hosts of the metacercariae therefore seem to be influenced, not by rainfall, but directly or indirectly by the colder climate prevailing during this period, probably in combination with rain. Unlike the others, the molluscan host of the cyathocotylid metacercariae seems to be influenced by rainfall as the peaks occur in rainy or just after the rainy season.

Since the life cycle duration of *T. patialense* is shorter and about 3 months in length, for the prevalence and abundance to be the same cercarial release must be continuous throughout the year. The prevalence of infection of *Melanoides tuberculata* with the cercaria of *T. patialense* was similar throughout the year supporting this suggestion.

It is very difficult to explain the presence or absence of seasonal variation in the helminth adults with complex life-cycles without background knowledge of the seasonality of the intermediate hosts involved and the biology of the larval stages. However, it is possible to speculate on the possibilities which will mask or create seasonality in these parasites.

As with other members of the Family Waratrematidae, *M. indica* metacercariae must encyst in open water or on vegetation (Yamaguti, 1975) and therefore depend

on the feeding of fish to gain entry. Since there was no significant difference in the abundance values throughout the year, the cercarial release may be uniform throughout the year or otherwise the seasonal cercarial release may be masked by the excess production of cercariae to make the metacercariae to be available throughout the year (if the encysted metacercariae are infective for long periods). Any seasonal variation of adults can also be masked by accumulation of the worms in the fish intestines. It was observed that the adults produce eggs throughout the year. Therefore, if there is a seasonal variation it is most probably due to the variation of cercarial release. A detailed survey of the annual cycle of length-frequency distribution of adults in intestines would give information on high recruitment periods and will show whether accumulation exerts any effect.

The prevalence and abundance values of the cestode and nematode parasites, propagating through the planktonic crustacean intermediate host, did not show any seasonal variation. There may be no seasonal variation in egg production by adults and therefore the infective stages within crustacean intermediate hosts are readily available to fish hosts in the environment throughout the year. Since the infected crustacean intermediate hosts are not long lived, the uniformity of abundance values within fish hosts suggests the non-existence of seasonality. The year round presence of live cysticerci and L_2 *Contracaecum* larva also supports this view. Accumulation of worms in the fish host was not evident as shown by the investigation into host age preference, where the number of worms in the three size host groups was the same.

The nematode *Rhabdochona* which is transmitted via benthic crustaceans, amphipods, isopods or dragonfly larvae, showed a marked seasonal variation. They were present in the fish host only from May to August. It seems that the fish become infected at the beginning of this period and within a period of 3-4 months the nematodes produce eggs and die off. At the other times of the year, they were not completely absent, but the numbers were very low. The reason for the high burden in a particular period may be due to a change in the feeding habits of the host, whereby they feed more on the intermediate hosts in this period and thus get infected by the worm. The intermediate host may be most prevalent in this period and increase the

host finding probability of the larvae emerging from the eggs. The increased abundance may influence the fish to change their diet to feed on a more available food item.

Of the crustaceans only the ergasilid *D. amplectens* showed variations in abundance declining in abundance just after the rainy season and increasing in abundance before the onset of the next. This increase is also in accord with the salinity increase. This parasite very clearly shows the influence of salinity on the intensity. The low salinity might kill the free living larval stages (not the adult stages), and/or they may be carried away from the lagoon with the flush rates of floods. Both explanations are equally possible.

E. sieboldi and *Argulus* abundances do not show any variation. The low densities of these parasites in the lagoon may be insufficient to show the effects of environmental parameters. On the other hand, since these two parasites have free living copepodid stages which can be carried away by flush rate and possibly show salinity effects, variations should be visible.

2.4.3.2 The micro-habitat effects on the parasites

(1) Host preference

The host specificity of the parasites of *Etroplus* was investigated by comparing the parasite fauna of *E. suratensis* with that of the closely related cichlid, *Oreochromis* spp. which inhabit the same water body. There is a considerable overlapping of feeding habits of *E. suratensis* and *Oreochromis* spp. as both feed on the bottom. However, *Oreochromis* spp. depends relatively more on plankton whilst *Etroplus* utilise macrophytes as well as plankton (Costa, 1983; De Silva *et al.*, 1984). Due to this overlapping of food habits, there is a high probability of their ingesting the same type of food as well as inhabiting the same areas in the water-bodies, thus providing for similar exposure to the same parasites. It is worth noting that the *Oreochromis* spp. have been recently introduced into Sri Lanka, thus this is a new host for the parasite fauna of *Etroplus*.

Though the ecto-parasitic protozoans are not very host specific, only Trichodinids were found on *Oreochromis* spp. Trichodinids seem to display varying degrees of host specificity, with some species infecting a wide range of hosts where others are restricted to certain genera or even to a single host species (Van As and Basson, 1987). The host specificity, or lack of it, is not well understood (Hoffman, 1978) in sessile peritrichs. Therefore, *Apiosoma* probably have similar host specificity to trichodinids. The two genera *Ichthyophthirius* and *Ichthyobodo* are each suggested to comprise one species, and are not host specific. The apparent absence of these two non-specific protozoans in *Oreochromis* spp. may be due to their extremely low prevalence levels.

According to Becker (1977) trypanosomes are specific to particular species or genus and mostly to members of one family. Therefore the species found in the two hosts may be different or the same. However, since these two hosts have only been sharing the same habitat for a short time, there is a possibility that the species in *Etroplus* is specific to them and the species in *Oreochromis* sp. is different.

According to Rohde (1979) most monogeneans show a distinct 'phylogenetic host specificity'. According to data collected from various sources, Rohde (1978) shows that only a few species of monogeneans have replaced their phylogenetic specificity by an 'ecological host specificity'. The monogenetic parasites found on *Etroplus* therefore display their extreme specificity to *Etroplus*. Though *Oreochromis* spp. and *Etroplus* spp. are closely related taxonomically, the introduced species has been geographically and taxonomically separated from *Etroplus* for a long time. Thus the monogeneans may have developed their specificity to this isolated genus *Etroplus*.

All the digeneans found in *Etroplus* in Koggala lagoon were found in *O. mossambicus* sampled from there. However, in Udawalawa strigeid metacercaria and cyathocotylid metacercaria were absent in the *O. niloticus* sample surveyed. As the abundance of strigeid metacercariae was very low this may be due to the small sample size studied. The cyathocotylid metacercaria was present in *Etroplus* as well as in *O. mossambicus* in Koggala lagoon; with a lower burden in *Etroplus from Koggala*

compared to the reservoir. Therefore, it is surprising that they were completely absent in *O. niloticus* in the habitat where the parasite is most abundant, even though the number of fish surveyed was less. Possibly *O. niloticus* is more resistant to the parasite. Since there is a high probability for *Etroplus* and *O. niloticus* to occupy similar habitats, habitat difference is unlikely to be responsible.

Cestodes were found only in Udawalawa and the genera of cestodes found in the two cichlids were different, one species in each host. The cysticerci of *G. pusillum*, which was present in *O. niloticus* were surviving well in their host. However, *P. scolecina* in *Etroplus* succumbed, possibly due to a host response, or due to a failure to adapt to physiological conditions provided by the host. These cysticerci are described from a variety of host species by Bykhovskaya-Pavlovskaya (1962), thus they cannot be host specific. The absence of these in one or other fish therefore shows their inability to establish. Though *P. scolecina* was able to establish in *Etroplus*, it was not generally able to survive for prolonged periods. The nematodes found were not host specific.

According to Kabata (1988) most species of *Argulus* are loosely host specific. Their non-representation in *O. mossambicus* must be due to very low prevalence. *D. amplectens* was already described from mullets by Ho & Do (1982). Only one specimen was found attached to an *Oreochromis* specimen observed, thus proving the low preference for *O. mossambicus*. Possibly it can resist their attachment or may have an immunological resistance acting against their feeding. *E. parvitergum* was found to occur at a very low prevalence and intensity on *Etroplus*, and could possibly have been missed in a small sample.

It seems that, in general, the monoxenous parasites, except for a few non-specific genera and crustaceans, are host specific; this was seen mostly in the gill parasites. Most of the dioxenous parasites were not specific. A few dioxenous parasites in the encysted stage, the stage which usually exhibits a looser specificity to improve the chances of infection (Shulman, 1958), were found to be restricted to one of the two host investigated. This was evident in the Udawalawa reservoir, where both

the hosts investigated were introduced and therefore the parasites had not evolved together with them.

(2) Age of host

In tropical fish, living under relatively low climatic variations, the growth rings in the otolith and in the scales are not a useful guide to determine the age of fish (Batra, 1984). In addition, since the growth rate of cichlids is high and the fishing effort in the reservoirs and lagoons is high, it is difficult to expect that the fish live for more than 1-2 years. With the estimation of the growth by De Silva *et al.* (1984), that it takes about 2-3 months for them to reach a size of 6 cm, it was assumed that approximately not more than one year is taken for the fish to attain about 12 cm. The majority of the fish investigated were under one year of age, therefore it was no use separating them into year classes. Assuming that the size of the fish is proportional to their age fish were divided into three size groups according to their lengths. It is worth noting that this study includes fish only above 6 cm, since this was the minimum size obtained in samples, thus it does not includes the whole spectrum.

The most common pattern found was the increase in parasite burden with the increasing size. The trichodinids, monogeneans, cyathocotylid metacercaria and *M. indica* all showed this tendency. As found for most other monogeneans (Frankland, 1955; Paling, 1965; Hanek & Fernando, 1978d,1978e; Paperna, Diamant & Overstreet, 1984; Buchmann, 1989 and Khidr, 1990) the size of the surface area available for settling, the larger body surface area for the attachment of the invasive stage or the increased volume of water passing over the gills in larger fish have been responsible. In the case of the cyathocotylid metacercaria, the accumulation of parasites contributed to the size factor of availability of more space and surface area for infection. The fact that adult fish ingest more food (Pennycuick, 1971; Furtado & Tan, 1973) was most likely to be responsible in the case of *M. indica*, whilst the other factors are unimportant.

The smallest size group of fish was most heavily infected by the trypanosomes. This is more likely to be due to the development of an acquired immunity against

them with the increase in the age of the fish. Lom (1973) demonstrated the development of the immunity and loss of infection in a group of survivors after an experimental infection. Further, the vector may prefer to feed on smaller fish rather than larger fish. Indeed, the habitat of the small fish may in the areas favoured by the vector.

Contracaecum L_3 worms as well as *P. scolecina* (live and dead worms included) cysticerci did not show any difference between different size groups of the fish. The number of second stage migrating larvae of *Contracaecum* in size group 3 and the number of live cysticerci of *P. scolecina* in size groups 1 & 2, though showing significantly higher levels were still in low numbers, and are thus not considered to be worthy of comment. More data are required to get a better picture of the real situation. Usually the burden of the larval stages of cestodes and nematodes which propagate via a copepod intermediate host is highest in smaller fish due to their zooplankton feeding habit of (Pennycuick, 1971; Zaman & Leong, 1987). The similarity in levels shown for all size groups of fish may be due to the survival of the parasites for a long time in the fish.

Even though there was clearly more space availability in older fish for the attachment of *D. amplectens*, only size 1 and 2 were significantly more commonly and heavily parasitised. One explanation for this observation might be a host response in older fish successfully avoiding the attachment of the parasite. A decrease in abundance of *Ergasilus centrarchidarum* in the eldest fish group (4-years old) was attributed to increased physiological resistant because the evidence for the elicitation of an immune response was lacking (Cloutman & Becker, 1977). Leong (1986) attributed the decrease in intensity of *Lamproglena minuta* in larger fish to the increase in size of gill filaments, thus making them less vulnerable. On the other hand an increase in intensity of *Ergasilus* sp. on *Wallago attu* in an Indian reservoir was considered to be due to increased surface area (Sommerville and Wootten pers. comm.).

(3) Site preference

There were one or two possible factors leading to the site preference shown by the parasites; avoidance of the water current and/or increase of intraspecific contact for mating. The gill arch preference was the same for both the monogeneans and the copepods i.e. decreasing in the order of second, first, third and fourth arch. In most cases, an equal preference was found for the first and the second. This correlates with the gill area; the second being the largest, the first being the next and the fourth being the smallest. Since most water flows over the middle two arches (Paling, 1968; Wootten, 1974), the equal preference for the first two arches in most cases neither indicates the avoidance of water current, nor agrees with the fact that the greater amount of water flowing through the gills brings more larvae to settle. It is not known whether the anatomy of cichlids is such that more water is passed through the larger first two arches than the posterior two.

Wootten (1974) experimentally observed the distribution of glochidia on the gill of *Gymnocephalus cernua* (L.) and suggested that their concentration on the ventral segment and distal region of the primary filaments was a result of the greater water flow over this area i.e. they had not selected this site for attachment but that this was the area of earliest contact.

The distribution of copepods over the segments of gill arches of *E. suratensis* indicated the preference of *D. amplectens* for the ventral and dorsal gill segments. This was similar to the findings of Hanek & Fernando (1978a) on *E. centrachidarum* living on *Lepomis gibbosus*. It was difficult to explain this distribution by considering the differences in water volumes passing over the gill sites. However, they mostly preferred either end of the gill arches where flow rate was mostly minimal, due to the protection given by the branchial chamber. High water currents may detach the copepods in the early stages of attachment before it becomes permanent.

Of the segments of the second gill arch, the middle segment was preferred by the monogenean, *A. etropli*. This was similar to the distribution of the monogenean *Urocleidus ferox* Mueller 1934, and glochidia of the mollusc *Lampsilis radiata*

(Gmelin 1792) infecting *Lepomis gibbosus* (Hanek & Fernando, 1978a). *A. etropli* preferred the proximal part of the gill filaments as did the monogenean *Dactylogyrus amphibothrium* Wagener, 1857 on the gills of *Gymnocephalus cernua* (L.) (Wootten, 1974). The high volume of water passing over the distal parts of the primary lamellae may force the *A. etropli* worms to be restricted to the proximal part. The preference for the medial segment is difficult to explain. This may possibly be due to the arbitrary separation of gill areas, which would have resulted in a higher overall area being assigned to the middle segment.

The site preference of *C. colombensis* was very different to that of the *A. etropli*. They seemed to concentrate in the proximal ventral and proximal dorsal areas. Due to the very small population numbers, this aggregation would help them to maintain intra-specific contacts.

(4) Pathology

Taken overall, the parasites encountered were not found to be causing severe damage to the host in the natural environments, the lagoons and reservoirs.

Ectoparasites

A. etropli was found to cause a moderate tissue hyperplasia in slight and moderate burdens. However, when the parasite burdens were heavy, potentially serious hyperplasia was found.

The mobility of the parasite may however reduce its' harmfulness due to the migration from damaged hyperplastic areas to new sites, allowing regeneration to occur in disturbed sites (Cone & Burt, 1982; Cone and Odense, 1984). The parasite mobility and the regenerative ability of the epithelium which enables the fish to cope with the damage resulting from feeding or attachment (Kearn, 1963a, 1967a; Smyth and Halton, 1983) may help to reduce the effect of high parasite intensities. However, very heavy infections may cause damage exceeding this regenerative ability (Lester, 1972; Lester & Adams, 1974a). The hyperplastic gills may become a serious disadvantage for fish in low-oxygen or polluted conditions by reducing respiratory

efficiency.

As Paperna (1964a) suggested, the hyperplastic response may result in the reduction of mechanical irritation experienced by the fish by keeping the parasite at a distance. However, the worms thus pushed away may seek another site for a more stable attachment. As seen in histology, some worms were attached to the same site even though the hyperplasia had developed along the entire length of the secondary filaments. Therefore, it is difficult to understand whether they move to another site following the hyperplasia, or remain in the same site and drop off. Since the worms appear to move along the primary filaments, both of these are possibilities. Paperna (1964a) pointed out that when the majority of the gill area is affected, having no suitable areas to move to, the parasites drop off and die. Thus, hyperplasia is a mechanism for 'self cure'.

A. etropli was found on the gills of fish of all ages. Therefore the hyperplastic areas may repair by the sloughing of the excess epithelium. There was no evidence for the development of immunity, possibly due to the little damage they cause.

The antennae of the ergasilid *D. amplectens* which encircle the gill filament probably causes less damage than ergasilids which pierce the filament. The damage seemed due to the mechanical irritation of attachment and feeding. The filaments on either side seemed to be irritated in the same way, thus a similar sort of reaction was evident whenever the gill was in contact with the parasite. In this way a large area of hyperplasia results from individual parasites.

D. amplectens seemed to induce neither inflammatory reaction nor any attraction of lymphocytes, unlike the pathology of *Dermoergasilus acanthopagari* Byrens, 1985 which usually caused hyperplastic reaction, inflammation which involved leucocytes including lymphocytes (Roubal, 1989). It was interesting to consider the mild reaction in relation to the low infection level in adult fish. If there is no immune response and only a mild cellular response developed what could be the reason for the lower infection levels? It may be that the gills become unsuitable for

attachment due to the earlier damage and repair, or due to the large size of the gills compared to that of small fish. An ecological factor like habitat difference of young and adult fish may also be responsible, which may lead to a difficulty in finding adult fish as a host.

Endoparasites

The encysting larval forms with the exception of the renicolid metacercaria seemed to elicit inflammatory reactions leading to host capsule formation. Even though its onset was not evident in some parasites the ultimate product, the fibrous capsule, was attained. In the case of the strigeid metacercaria, owing to the recent infection, the stages of the inflammatory response leading to fibrous capsule formation were evident. The macrophage involvement, the formation of epithelioid tissue and the laying down of fibrous cyst were found in these stages. This was in accord with the sequential study of the involvement of inflammatory cells in the host response described by Sommerville (1981) on the encystment of the metacercaria of *Stephanochasmus baccatus* (Nicoll, 1907) infecting flat fish.

The renicolid metacercaria did not elicit the host inflammatory reaction and even the fibrous capsule of host origin was absent. Some metacercariae *Stictodora lari* Yamaguti, 1939 in *Gambusia affinis* (Baird & Girard)(Howell, 1973) and *Haplorchis pumilio* (Looss, 1896) on tilapia (Sommerville, 1982), seems to resist the inflammatory response and the renicolid metacercaria appears to behave in the same way as evidenced by the absence of a host response.

The reason for the death of the cysticerci of *P. scolecina* is not clear. *E. suratensis* is an introduced fish species into reservoirs and thus seems to be an abnormal host for this parasite. The host response may be aggressive enough to kill them. According to Wakelin (1976), in the natural host, inflammation does occur but without deleterious effects, whereas in an unnatural host, inflammation frequently leads to elimination and/or death of a parasite. There is also a possibility of an involvement of immunologically mediated inflammatory reaction, but no evidence could be gathered on this phenomenon in the material studied. However, this could

be clarified by a histological study of recent infections, and will be an important area for further investigation regarding host immunity.

There is no agreement as to the function of eosinophilic granular cells in fish. In higher animals, true eosinophils are often associated with parasitic infections. Eosinophilic granulocytes are reported in normal fish tissues such as epithelium (Roberts, Young & Milne, 1971), gills and normal gut epithelium (Chaicharn & Bullock, 1967; Ezeasor & Stokoe, 1980; Ellis, 1985). These eosinophils are not equivalent to mammalian eosinophils (Roberts *et al.*, 1971; Lester & Daniels, 1976).

The presence of eosinophilic granular cells in the inflammatory areas are recorded for some parasites. Lester & Daniels (1976) found a large number of eosinophilic granulocytes in the inflammatory response of the white sucker, to the parasites *Actinobdella inequiannulata* and *Lernaea cyprinacea* L. (Shariff & Roberts, 1989) and it seems likely that these are eosinophilic granular cells. Noga (1986) also found eosinophilic granular cells close to *Lernaea* in the scale pocket area of large mouth bass, *Micropterus salmoides* (Lacépède). In *Etroplus*, eosinophilic granular cells were found with the infections of strigeid and renicolid metacercariae and around the attachment of *Enterogyrus papernai* (see Chapter 3, Section 3.3.1.4). It was very clear in the tissue reaction to strigeid metacercariae that the eosinophilic granular cells appeared later, when the fibroblast capsule was laid. The presence of eosinophilic granular cells with a few attached *E. papernai* also suggests that these attachments could be much older thus the eosinophilic granular cells were attracted at only this later stage.

In general, rodlet cells are seen in many epithelia of different teleost groups, both freshwater and marine, but not always in the same epithelia or in constant numbers in individual fish within a species. Although the nature of these cells remains controversial, recent studies on fine structure supported the view that rodlet cells are of teleost origin (Barber & Westermann, 1985, 1986a, 1986b). The work of Desser & Lester (1975), Morrison & Odense (1978) showed that the rodlet cells have the morphology of secretory cells. Light and electron microscopic cytochemical studies

by Leino (1982) suggested that the secretory function of rodlet cells differs from that of mucous and granular cells in the skin and intestine. The proteinaceous nature of their granules led to the proposal that the granules contain enzymes (Morrison and Odense, 1978) or toxins which might have an "antibiotic" function and could be released at the endothelial surface, into the vascular system, or into the body cavity.

The presence of the rodlet cells in the blood vessels near the infected sites by *P. scolecina* and the death of most of the parasites seems to have been related. This might therefore support Leino's (1982) view on rodlet cells, that the cells secrete enzymes or toxins into the blood vessels in the region, which can help in killing the parasite. Leknes (1986) investigated the rodlet cells in the bulbous arteriosus of cichlids in close connection to endothelial cells. As the rodlet cells occurred in small numbers in most of the infected fish and uninfected fish it can be argued that this is a common occurrence as they usually appear to a varying degree in individual fish of same species. Otherwise, if these are related to the response to the parasite infection, the low occurrence of rodlet cells in some infected fish may be explained by the distribution within the fish and the limited selection of areas for histological studies. Thus, further investigations are necessary to examine the correlation of occurrence of rodlet cells and infection in fish. Such a study might also throw light on the function of rodlet cells.

2.4.4 Summary

From the ecological point of view, the overall study provides information on the relationships of the parasites of *Etroplus* to their macro and micro environments. The potential for existence of particular parasites in freshwater and brackish water is also disclosed by reference to potential salinity tolerance and the presence of their intermediate or final hosts. The extent to which the seasonality of environmental factors effect the parasite population levels were also investigated. The possibility of finding the parasites amongst other fish, the infection levels of fish of different ages and the attachment site selection of parasites were added.

From the aquaculture point of view this reveals the parasite fauna of *Etroplus*

in brackish and freshwater habitats. Their prevalence and intensity levels provide information on the extent of the infection and make it possible to estimate the extent to which the levels can be build up in an aquaculture impoundment. The life-cycle studies (molluscan host) and estimation of life-cycle durations of monoxenous parasites provide information for developing methods of prevention of infection. Finally, the histopathological studies assess the potential pathogenicity of the parasites.

Chapter 3

The study on *Enterogyrus* spp.

3.1 Introduction

The usual site of infection of monogeneans is the external surface of fish, where they mainly inhabit the skin and gills. However, the polyopisthocotylean family Polystomatidae is well-known for parasitizing the mouth, pharynx, oesophagus or urinary bladder of amphibians and reptiles. A few other polyopisthocotyleans also inhabit internal sites: eg. *Merizocotyle* sp. lives in the nasal fossae of rays, mainly *Raja undulata* Lacépède (Kearn, 1968b), *Dictyocotyle coeliaca* Nybelin, 1941 in the coelom of *Raja naevus* Fowler, 1941 (Kearn, 1970) and *Calicotyle kröyeri* Diesing, 1850 in rectal glands and cloaca of species of *Raja* (Kearn, 1987). Some dactylogyrids also show an unusual localization: eg. *Diplectanotrema* Johnston and Tiegs, 1922 was found in the oesophagus of *Balistes* and *Trachurus* species, *Acolpenteron* Fischthal & Allison, 1940 in the urinary bladder and uterus of *Lepomis* and *Micropterus* species and *Dactylogyrus nasalis* Strelkov & Ha Ki, 1964 in the nasal cavity of roach (Pariselle, Lambert & Euzet, 1991). Llewellyn (1965) found the eggs of a dactylogyrid monogenean which habitually passed through the intestine of the fish *Sebastes madurensis* Doderlein, 1891 and discovered the adults inhabiting the oesophagus of the host. Considering the large number of species of Monogenea these examples represent but a few which are not ectoparasitic. One genus of monogeneans of subfamily Ancyrocephalinae has become adapted to one of the most hostile of microhabitats, this is the genus *Enterogyrus* Paperna, 1963 which is found in the stomachs of cichlids (Paperna, 1963b).

Since Paperna's first description of the genus *Enterogyrus* from the fore-gut (stomach) of cichlids in 1963, nine *Enterogyrus* spp. have been described and these are listed in Table 3.1. Pariselle *et al.* (1991) suggest that the *Enterogyrus* sp. reported in *Pomacanthus paru* (Bloch, 1787), originating in the Caribbean Sea and raised in an aquarium, is not an *Enterogyrus* spp. but more closely related to the genus *Diplectanotrema* Johnston & Tiegs, 1922 or *Pseudempleurosoma* Yamaguti, 1965, which have previously been described in the pharynx of carangids.

Table 3.1: *Enterogyrus* species described from Cichlids.

	Species	Host	Reference
1.	*E. cichlidarum* Paperna, 1963	*Tilapia zilli* Gervais, 1848 in Israel *Oreochromis niloticus* (L.) (syn. *Tilapia nilotica*) in Israel	Paperna, 1963b
2.	*E. globidiscus* (Kulkarni, 1969) Gussev & Fernando, 1973 (syn. *Haplocleidus globidiscus*)	*Etroplus suratensis* Bloch, 1790 in India & Sri Lanka	Kulkarni, 1969 Gussev & Fernando, 1973
3.	*E. papernai* Gussev & Fernando, 1973	*Etroplus suratensis* Bloch, 1790 in Sri Lanka	Gussev & Fernando, 1973
4.	*E. hemihaplochromii* Bender, 1979	*Hemihaplochromis multicolor* Hilgendrof, 1903 (Cichlidae, originating in East Africa) in an aquarium in Germany	an unpublished thesis quoted in Pariselle, Lambert & Euzet, 1991
5.	*E. niloticus* Eid & Negm, 1987	*Oreochromis niloticus* in Egypt	Eid & Negm, 1987
6.	*E. malambergi* Bilong Bilong, 1988	*Oreochromis niloticus* in Cameroon	Bilong Bilong, 1988
7.	*E. melanensis* Bilong Bilong, Birgi & Lambert, 1989	*Hemichromis fasciatus* in Cameroon	Bilong Bilong, Birgi & Lambert, 1989
8.	*E. foratus* Pariselle, Lambert & Euzet, 1991	*Sarotherodon melanotheron melanotheron* (Rüppel, 1853) and *S. m. heudelotii* (Duméril, 1859) in Ivory Coast	Pariselle, Lambert & Euzet, 1991
9.	*E. coronatus* Pariselle, Lambert & Euzet, 1991	*Tilapia guineensis* Bleeker, 1862 in Ivory Coast	Pariselle, Lambert & Euzet, 1991

Most of the reports on *Enterogyrus* describe the morphology of the species. Pariselle *et al.* (1991) identify a novel shape of the haptors of the two described species; *E. foratus* and *E. coronatus*. The haptor comprises two segments; an elongated posterior peduncular segment and a bulbous anterior segment. The posterior segment bearing the two pairs of hamuli and marginal hook pairs I and II, was reported to deeply penetrate the stomach wall.

In an unpublished thesis Bender (1979) studied the life-cycle of *E. hemihaplochromii* (cited by Cone, Gratzek, & Hoffman, 1987). Khidr (1990) describes the population dynamics and the site selection of *E. cichlidarum*. Even though it cannot be regarded as an *Enterogyrus* species (Pariselle *et al.*, 1991), the study on the pathology of the dactylogyrid in the host *Pomocanthus paru* by Cone *et al.* (1987) is an important contribution to the understanding of the attachment mechanisms of the endo-parasitic monogeneans to the host tissue.

Two species of *Enterogyrus* were found in *Etroplus suratensis* in the survey carried out during this study, *E. globidiscus* and *E. papernai*. These had previously been reported by Gussev & Fernando (1973) on the same fish stomach. In this study, these two *Enterogyrus* species were found to share the same stomachs of *E. suratensis* in both the freshwater and in brackish water habitats. Since much of their biology is unknown and they were found in two ecologically different habitats, these organisms were selected as an ideal tool for studying the biology of parasites in relation to their ecology.

Literature review of the biology of monogeneans

3.1.1 Population studies

Length frequency distribution studies on a population help understanding its structure ie. an overall picture of the numbers representing the length groups, and the numbers of one length group comparative to another. When considering the growth phase of parasite populations, length-frequency distributions are helpful in roughly estimating the age (or lengths) at which the parasite is invasive. When length

frequency studies are carried out on a seasonal basis, the length frequency analysis may provide information on the inherent and/or external factors influencing the biology of the parasite.

Although there are many studies concerning the variation in population size of monogeneans with time, especially in experimentally infected hosts (Lester & Adams, 1974b; Scott, 1982, 1987; Scott & Anderson, 1984) only a few studies are available on length-frequency distributions. Kearn (1967a) used length-frequency graphs to identify the population structure of monogeneans on the dorsal and ventral sides of the host. With the seasonal monitoring of length-frequency distributions of parasites of different age, Cone & Burt (1985) were able to understand the invasion periods of the parasite as well as the spring growth of overwintering worms. Even though there are some differences in individual graphs, in all these cases the overall picture was a bell-shaped graph. The length-frequency graphs obtained for the infections of *Entobdella soleae* (van Beneden & Hesse, 1863) Johnston, 1929 collected from its host *Solea solea*, showed a situation where the greatest number of observed parasites occurred on host at the smallest length range (Kearn, 1967b). It appears that there is a gradual fall of frequencies with increasing size. This situation probably indicates the possibility of monogenean populations to have smallest individuals in the highest proportion similarly as in the cases of other stable animal populations.

It is known that the growth of some poikilotherms is continuous after they attain sexual maturity. However, Prost (1963) stated that the haptor, copulatory apparatus and the size of the parasite body attained their final size at the moment of sexual maturity in the case of *Dactylogyrus extensus* Mueller & Van Cleave, 1932, but not in *Dactylogyrus anchoratus* (Duj., 1845) Wagener, 1857 in which the sexual maturity appears before parasite has reached its' maximum size. Kearn (1963c) found the growth continuous in *E. soleae,* especially in the body size, haptor size, anterior hamulus and the accessory sclerite. Owing to these variations in the growth phenomenon it is not reliable to depend on a general rule such as; the worms with highest length of sclerites are mature, to separate sexually mature worms. The finding of individuals with a complete set of reproductive organs seems a little unreliable too,

as Paperna (1963a) showed that even though the complete set of organs was present on the third day of the life of *Dactylogyrus vastator* Nybelin, 1924, the egg laying did not commence until the fourth day at 28-29°C. The delay in time from maturation to the appearance of the first egg, may be explained in that this intervening period allows for mating to occur.

The occurrence of several species of monogeneans in a single host species on similar sites is not uncommon (Prost, 1963; Paperna, 1964b; Suydam, 1971; Shaharom-Harrison, 1984; Dzika, 1987; Pojmańska & Dzika, 1987). Of these studies, Paperna (1964b) reports on competitive exclusion of one species by the other. Rohde (1976, 1978), report clearly defined micro-settlement sites, resulting in separation of species even when coexisting. The work by Dzika & Szymański (1989) suggests similar distribution patterns for four species of *Dactylogyrus* on the same host, so that they occur numerously in the same micro sites.

Khidr (1990), working on the site preference of *E. cichlidarum* inside the stomach of *Oreochromis niloticus* and *Tilapia zillii* Gervais, 1848 found no noticeable difference in the distribution in either host. The different stages of the life cycle, however, seemed to prefer different sites, immature enterogyrids being more abundant in the posterior sector of the stomach, whilst the adults occurred towards the anterior sector.

There have been even fewer studies of the pathology of *Enterogyrus*. The study of the pathology caused by "Enterogyrus" (= *Diplectanotrema*?) species on *Pomacanthus paru* by Cone *et al.* (1987) describes the piercing of fore-gut wall by hamuli. The main content of their report, however, relates the adherence of worms between adjacent folds in the stomach, to the descriptions of the attachment of the gill parasitic ancyrocephalines by Kearn (1971) and Cone & Burt (1982). The latter worms position themselves between adjacent secondary lamellae, clinging to the host tissue by the blades of the dorsal and ventral hamuli with little role being played by the marginal hooks. No comments were made on the effect on host tissue.

3.1.2. Reproductive biology

Observations of the mode of egg formation are available on some capsalid monogeneans. Jahn & Kuhn (1932) and Kearn (1985) described the egg formation of the capsalid monogeneans *E. soleae* and *Epibdella (=Benedenia) melleni* (MacCallum, 1927). The egg assembly, function of the oötype in egg formation and the egg shell formation was described in detail by Kearn (1985). Shaharom-Harrison (1984) described the egg formation and the egg laying behaviour of the ancyrocephaline monogenean, *Cichlidogyrus sclerosus* Paperna & Thurston, 1969 and commented on its similarity to the descriptions of *Microcotyle spinicirrus* MacCallum, 1918 by Ramley (1942). Tinsley (1983) identified the role of the uterus of egg laying monogeneans as not just a tube carrying eggs to the exterior, but as a site where shell-hardening proceeds without impeding the assembly of successive eggs in the oötype.

Measurement of the rate of oviposition has been attempted by several authors. However, a significant difference in the rate of oviposition of monogeneans removed from the gills, and those allowed to remain *in situ* was found for *D. vastator* by Ljaiman (1951) and Izyumova (1953) (cited by Paperna, 1963a). The *in situ* rate being lower than the rate of the detached worms. Prost, (1963) made the same observation for *D. anchoratus*. Her observation on the diminishing of the egg size in the detached worms during the course of the egg laying period, led to the suggestion that production of a larger number of smaller eggs by the dying worms contributed to the increased egg output. Thus conclusions of such observations *in vitro* should be treated with caution.

Different oviposition rates (fecundities) have been demonstrated for different monogenean species. A high variation in egg laying rates is found even within the same genus at the same temperature. Izyumova (1953) cited by Paperna (1963a) recorded 9.3 eggs per worm per a day at 18°C for *D. vastator*. Paperna (1963a), Prost (1963) and Molnár (1971) recorded 29, 2.13 and 15 eggs per worm per a day at 28°C for *D. vastator*, *D. anchoratus* and *Dactylogyrus lamellatus* Achmerow, 1952 respectively. Therefore, even the egg laying rates of closely related worms differ markedly at the same temperatures.

Izyumova (1956 cited by Kearn 1986), did not find any significant difference between the number of eggs laid by *D. vastator* during the day and during the night. However, Thurston (1968), and Macdonald & Jones (1978) found day and night differences in egg laying rates and related this difference to the behaviour of the host fish. In *Diplozoon homoion gracile* Reichenbach-Klinke, 1961 the difference was due to the difference in egg production rates (Macdonald & Jones, 1978) but in *Diclidophora luscae* (Van Beneden & Hesse, 1864) (Macdonald, 1975) and *Oculotrema hippopotami* Stunkard, 1924 (Thurston, 1968), it was due to the accumulation of eggs in the uterus.

As might be expected, there is a tendency in monogeneans to produce more eggs as the temperature increases. However, at temperatures at the upper end of the range, egg output may fall. Bauer (1954) and Ljaiman (1951) cited by Paperna (1963a), Prost (1963) and Imada & Muroga (1978) found this relationship, with the optimal temperatures occurring around 20-25°C. Paperna working in the tropical environment found that the oviposition rates at temperatures 12°C and 37°C were comparatively very low compared to the rate at 24°C and 28°C. Even though there was no marked difference in oviposition rates at 24°C and 28°, at 28°C the rate was higher.

The only other aspect of the egg laying rate studied is the effect of the salinity. Anderson (1981) studied the effect of salinity on the oviposition rates of the monogenean parasites of the grey mullet *Chelon labrosus* (Risso, 1826). In *Ligophorus angustus* Euzet & Suriano, 1977 infecting mullet living in the open sea from the age of 2 years, the egg laying rates were proportional to salinity, with highest at 100% sea water while *Ergenstrema labrosi* Anderson, 1981 infecting young mullet in tidal pools, have their highest oviposition rates at 50% sea water with reduced rates at either side of this value. Thus it seems the worms have adapted suitably to live in the ambient salinities they experience most, and that they produce more eggs at these salinities. This may however make very little contribution to a stomach parasitizing monogenean as the osmolarity of water ingested by fish is changed in the stomach to an average value almost similar to the osmolarity of their body (Möller, 1978). Even though a

small difference can exist it might not be strong enough to cause a change in oviposition rate.

The effect of other physical factors such as pH have not been studied in relation to egg production, nor have factors such as host species or food substrate been addressed. Llewellyn (1954) suggested that *Leptocotyle minor* (Monticelli, 1888) Gallien, 1937 and *Acanthocotyle* sp. may feed on eroded host tissue or on mucus or may merely be commensals. Kearn (1963a) showed *E. soleae* and *Acanthocotyle* sp. erode the epidermis of their host by means of proteolytic secretions produced by conspicuous gland cells in the feeding organ. He suggested that the majority of other monopisthocotyleans living on the skin and gills are also epidermal feeders, although there are some, including *Amphibdella torpedinis* (Chatin, 1874) MacCallum, 1916 which may feed on the blood of their host. Since there is no variation in food availability, any effect of this on egg laying rates cannot be expected.

It is unlikely that the monogeneans which first evolved as ecto-parasites, have developed an ability to absorb the food through the tegument. The endo-parasitic monogeneans living in food rich environments probably evolved this ability. Even if they do not, they may be able to feed on digested food material in the stomach, in addition to the intestinal epithelium. Therefore a variation of egg laying rates may be expected to be influenced by the feeding condition or food type of the host.

3.1.3. Life-cycle studies

The eggs produced are the link to the next generation and the source for transmission. Auto-infection by vivipary is the method adopted by gyrodactylids for their successful transmission. All other monogeneans are oviparous. There is a great deal of evidence suggesting that the eggs of dactylogyrids and ancyrocephalines drop off the host and sediment on the bottom (Prost, 1963; Paperna, 1963a; Molńár, 1971; Cone, 1979a; Shaharom-Harrison, 1984, 1986). Almost all the endoparasites, whatever the method adopted, release their eggs to the external environment for the purpose of dispersion of offspring to new hosts. In this respect endoparasitic monogeneans can be no exception.

The eggs of endoparasitic monogeneans have two options, the first is to develop at the site of the adults and the second, is to develop externally, as in the other endoparasitic helminths. All ectoparasitic and other known endoparasitic monogeneans pass their eggs to the exterior passively with some action of host origin, eg. *Polystoma* spp., *Merizocotyle* sp. from the nasal fossae, *Dictyocotyle coeliaca* and *Calicotyle kröyeri* in the body cavity and cloaca of rays respectively. Llewellyn (1965) found that the eggs of a monogenean inhabiting the oesophagus of *Sebastes madurensis* were passed out habitually with the faeces of the host, suggesting that the egg movement was passive with the food ingestion and the parasite played no active role in directing the eggs towards the branchial chamber. Bender (1979) cited by Cone *et al.* (1987) also reported that the eggs of *E. hemihaplochromii* inhabiting stomachs of the cichlid *Hemihaplochromis multicolour* Hilegendrof, 1903 pass via the digestive tract to the outside environment.

Kearn, in his review on eggs of monogeneans (1986) describes the remarkable diversity in the shapes and sizes of the eggs of monogeneans. A wide range of sizes exists, and closely related monogeneans may produce eggs of different shapes. As redrawn by Kearn (1986) the eggs of *D. vastator* are spheroidal (Nybelin, 1924) in shape but eggs of *Dactylogyrus chranilovi* Bychowsky, 1931 are usually tetrahedral (Izyumova, 1969). In contrast with most digenean and cestode eggs, many monogenean eggs have appendages. In some monogenean eggs, especially those laid by parasites of fresh water vertebrates, appendages are either absent, as in *Discocotyle sagittata* (Leuckart, 1842) Dies., 1850 (Owen, 1970), or very short as in *D. vastator* (redrawn from Nybelin, 1924 by Kearn, 1986). In the eggs of *E. soleae*, *Entobdella australis* Kearn, 1978 and *Acanthocotyle lobianchi* Monticelli, 1888 the appendage carries sticky droplets to anchor eggs on to the sand grains (Kearn, 1963b, 1978, 1967a respectively). Some eggs, eg. those of *Calicotyle kröyeri* Diesing, 1850, have sticky substances on the surface of the egg shell itself (Kearn, 1970).

There have been no studies of the permeability of the eggshell of monogeneans comparable to that of Wilson (1967) on *Fasciola hepatica* L. Kearn and Macdonald (1976) pointed out that the size of the molecules such as urea, which induce hatching

are at the ultra microscopic level, such that specialized pores or channels in the shell would be unnecessary to facilitate their passage. Paperna's (1963a) finding that the development of *D. vastator* eggs at a wide range of salinities was possible but the oncomiracidia died soon after hatching at higher salinities, indicates that the egg shell serves to protect the developing embryo and unhatched larva from detrimental osmotic effects.

Kearn (1975a) demonstrated that digestive enzymes applied internally to the egg shell (into partly opened eggs without damaging the operculum) can open the operculum, but not if applied externally. The passage of monogenean eggs unharmed through the alimentary canal of microphagous animals (Llewellyn, 1965; Kearn, 1975a) together with the observation of Llewellyn (1965) on the habitual passing of eggs of an oesophagus inhabiting dactylogyrid through the intestine of fish, proves the protection provided by the egg shell or the opercular cement against the digestive enzymes.

The embryonic development of dactylogyrid and ancyrocephaline monogenean eggs was described by Paperna (1963a), Cone (1979a) and Shaharom-Harrison (1984, 1986). The procedure appears to be common to all the species studied. The freshly laid eggs are filled with the ovum and vitelline cells, which then pass through stages such as the change to a translucent appearance, the formation of larvae, the reduction of vitelline material and confinement of vitelline material in two sacs. The eggs of *Urocleidus adspectus* Mueller, 1936 remained constant in dimensions during its development according to Cone (1979a), while *C. sclerosus* and *Dactylogyrus nobilis* Long & Yu, 1939 eggs increased in size (Shaharom-Harrison, 1984, 1986). Paperna (1963a) too, reported that *D. vastator* undergoes swelling during its development.

The only report of the effect of salinity on egg development is by Paperna (1963a). He found that the eggs of *D. vastator* left in water salinities 255-4000 mg Cl/L (0.26-4.00 ‰) have similar hatching rates (72 - 97 %), but there was a slight delay in larval development with increasing water salinity. The larvae however, were killed immediately upon hatching in higher salinities.

The duration of development of most monogenean eggs is shortened at higher incubation temperatures up to the optimal temperature for development, but at temperatures higher than the optimal, the development of embryos was delayed and eggs failed to hatch (Paperna, 1963a; Prost, 1963; Imada and Muroga, 1978). Paperna's (1963a) findings on *D. anchoratus* and *D. vastator* showed that, although development may proceed more rapidly at higher temperatures, a smaller proportion of these eggs may complete their development and hatch.

The hatching process in monogenea involves the detachment of the operculum prior to the emergence of the oncomiracidium. Detachment of the operculum in the digenean *F. hepatica* is known to be brought about by an increase in internal pressure caused by the swelling of a "viscous cushion" lying beneath the operculum (Wilson, 1968). A viscous cushion has not been reported in any monogenean eggs (Kearn, 1986) other than eggs of *C. sclerosus* (Shaharom-Harrison, 1984). Kearn (1975a) found evidence suggesting that head glands secrete a proteolytic hatching fluid that dissolves the opercular cement. Some mechanical pressure then applied is necessary to dislodge the operculum. Kearn (1982) observing the rapid hatching of *Entobdella diadema* (Monticelli, 1902) Johnston, 1929 within a few seconds in response to the stimulus, suggested that the opercular cement was already weakened in readiness for rapid escape from the egg. This observation also shows the possibility of weakening the opercular cement by hatching enzymes.

Many workers have expressed the opinion that muscular exertion by the larva plays a part in dislodging the operculum (Frankland, 1955; Prost, 1963; Owen, 1970; Macdonald, 1974; Tinsely and Owen, 1975; Cone, 1979a; Shaharom-Harrison, 1984). There has been no agreement on the extent to which this is involved. Prost (1963) and Cone (1979a) described repeated thrusts by the larva of *Urocleidus adspectus* usually directed, but not always, on the opercular pole. Cone (1979a) found that the larva of *U. adspectus* is accompanied by two fluid filled sacs within the egg. These sacs gradually become enlarged after the operculum becomes detached and as the larva is emerging their swelling helps in ejecting the larva. There is a further possibility that the swelling prior to hatching may exert sufficient force to dislodge the operculum.

An increase in internal fluid pressure appeared to round the ridges and the corners of well-developed eggs of *Diplectanum aequans* (Wagener, 1857) Dies., 1858 and the egg resumed its tetrahedral shape after hatching (Oliver, 1969 cited by Kearn, 1986).

In some monogeneans, hatching is spontaneous and arhythmic (Kearn, 1975b; Cone, 1979a). Though different hatching rhythms have been worked out for some others. *E. soleae* larvae hatch during the first 4 h of the period of illumination. Even the eggs previously exposed to LD 12:12 until hatching begins and left in total darkness or in constant light, hatched rhythmically suggesting a strong endogenous rhythm (Kearn, 1973). In *Entobdella hippoglossi* (Müller, 1776) Johnston, 1856 hatching occurred during the first few hours of darkness (Kearn, 1974a). Hatching shortly before and after dusk was reported by Macdonald and Jones (1978) in *Diplozoon homoion gracile*. Even though monogenean eggs become sclerotized they appear to be sufficiently translucent to transmit light. This is clearly important in order to be stimulated to hatch by a change in light intensity or in those eggs which have hatching rhythms that are controlled by the photoperiod. Some monogenean eggs, eg. *E. diadema* were stimulated to hatch by a sudden reduction in light intensity within 3-5 s. This would occur naturally through shadow by the host (Kearn, 1982).

The hatching of some oncomiracidia is known to be stimulated by chemical substances from the host. Host skin mucus was found to induce hatching in larval *Acanthocotyle lobianchi* Monticelli, 1888 (Macdonald, 1974), *E. soleae* (Kearn, 1974b), *C. sclerosus* and *D. nobilis* (Shaharom-Harrison, 1984, 1986). Urea is another stimulant for *A. lobianchi* (Kearn and Macdonald, 1976). Kearn (1986) showed that even the mechanical transfer of eggs during the experiments initiated hatching.

Oncomiracidia vary in length between about 0.1 and 0.3 mm and, at least in artificial cultures, have a life-span no longer than 24 h. Bychowsky (1957) described two phases of activity of oncomiracidia: first, a free-swimming phase in search of a host, and secondly a "creeping" or "gliding" phase, the locomotion of which over the surface of its host resembles that of a turbellarian. In the first phase, locomotion is purely by means of epidermal cilia, but in the second, muscular movements take place

and may include a leech-like progression. Izyumova (1956) cited by Kearn (1967a), found that the larvae of *D. vastator* swam freely for 6-17 h but were infective for only 4-8 h at 10-14°C. Prost (1963) reported that the longevity of a larva which fails to meet its host is short, and the period in which the larvae are distinctly viable and make quick movements, is usually a little less than their whole life.

The only published work on the effect of salinity on oncomiracidia is by Paperna (1963a) who found that *D. vastator* oncomiracidia die soon after hatching at salinities higher than 1500-2000 mg Cl/L (1.50-2.00 ‰).

Izyumova (1956) cited by Paperna (1963a), found that the longevity of the hatched larva of *D. vastator* is reduced at low temperatures and extended at moderate temperatures. Paperna's (1963a) findings show a decrease in the active life of the larva with both decreasing and increasing temperatures. According to his findings, at the optimal temperatures, 24°C and 28°C, the active life period was 12 hours and the total life span, including the later inactive phase, was 24-36 hours. In contrast to the above investigation, Kearn (1967a) found that the duration of free swimming activity increases at the low temperatures for *Entobdella soleae* oncomiracidia. Investigating the active life period of *E. soleae* he found that the time period at 7°C was 20-30 hours whilst at 17°C it was 9-14 hours. However, this is a parasite of colder northern waters and the temperature range been 9-14°C on the sea at Plymouth, where the investigation took place.

In monogeneans other than gyrodactylids, invasion of a new host is by the larva. The larvae, with ciliated epidermis, find their host actively. Prost (1963) found that there were two routes for *D. anchoratus* invading young carp. With young fish up to a month old (10 mm long) the active skin route was the choice. In older fishes, passive entry into the buccal cavity was the most frequent route of invasion. The passive entry route has also been reported by Paperna (1963a) for *D. vastator* invading young carp of 8 mm long. Bovet (1967) observed with a stereo-microscope that the larval *Diplozoon paradoxum* Nordman, 1832 were drawn into the mouth with the gill ventilating current and they never crept over the fish's skin. Kearn (1968a)

investigating the youngest parasites of *Neodactylogyrus crucifer* (Wagener, 1857) Price, 1938, *Tetraonchus monenteron* (Wagner, 1857) Dies., 1858 (freshwater species) and *Diplectanum aequans* (marine species) in the skin and buccal cavity scrapings, concluded that the invasion route could be by attachment to, and subsequent migration over, the general body skin, or by passive ingress with the gill ventilation current and attachment to the buccal skin.

A considerable amount of literature suggests the restriction of monogenean species to single host species. Hargis (1953) found a high degree of host specificity amongst freshwater monogeneans. Llewellyn (1956) collected over 900 gill monogeneans belonging to 18 species from 17 species of marine fishes and found that all species, except 2, were strictly specific to their hosts. In a series of experiments Kearn (1967a) showed that larval *E. soleae* display a narrowly specific chemotactic response to the skin mucus of *Solea solea* (L.). In addition to these, Price & McMahon (1967), Rhode (1978) and Shaharom-Harrison (1984) dealt with the findings of host specificity of monogeneans and found that they were highly host specific.

Following attachment to the host by the larval hooks, an adult form of haptor, mainly the hamuli, develop in dactylogyrid monogeneans. The only existing published work on the haptor development of an ancyrocephaline monogenean is by Cone (1979c) on *U. adspectus*. The ventral pair of hamuli were absent in oncomiracidia, but by day 1 post infection, the rudiments developed in parenchymal tissue immediately anterior to the haptor in the worms attached to skin. The ventral hamuli developed faster than the dorsal hamuli. According to Llewellyn (1963), in some oncomiracidia the ventral pair of hooks are present in a relatively advanced stage of development and this occurrence is widespread through the various groups of monogeneans. The post-oncomiracidial haptor development of *Diplectanum aequans*, *Tetraonchus monenteron* and *Neodactylogyrus crucifer* (Kearn, 1968a), *D. anchoratus* and *D. extensus* (Prost, 1963), and *Pseudodactylogyrus microrchis* Ogawa & Egusa, 1976 (Imada & Muroga, 1978) have been well described.

There is little information on the longevity of the dactylogyrid monogeneans according to existing literature. The only detailed studies are of Prost (1963) and Paperna (1963a). According to their investigations, the longevity is also temperature dependent. The survival time of *D. anchoratus* was 42 days at the temperature 20-23°C (Prost, 1963). The whole development of *D. vastator* from egg to sexual maturity and oviposition lasted 9 days at 24-25°C and 4-5 days at 28-29°C, longevity of the mature worm was 3-5 days (Paperna, 1963a).

3.1.4. Objectives of the present study

In this study, the biology of the stomach inhabiting monogeneans was studied in relation to the environmental parameters of the two different habitats in order to understand their adaptability to the two environments.

The length-frequency distribution of the two *Enterogyrus* species were first studied to identify their species composition, size composition, the stages of the life-cycle living in stomach as well as the length at which they attain maturity. The site preference of the two species was investigated to see whether there was any site segregation between the two parasites. The stomach tissue was viewed histologically as well as with scanning electron microscopy to examine any damage the parasite might cause on the host as well as to understand their method of attachment.

The rate of reproduction of worms was investigated by *in vitro* and *in vivo* methods and these were compared for suitability and an attempt was made to take into consideration all the factors which might influence the rate of reproduction. The effects of external factors like host feeding, ambient water temperature, pH and digested food in the environment on the oviposition rates were also investigated. The path the eggs take to reach the outside was also studied.

The durations of the external life-cycle stages, eggs and the oncomiracidia, as well as the effect of the environmental parameters salinity, temperature and pH on the duration were investigated. The attachment sites of oncomiracidia were also studied.

All the *Etroplus* fish encountered in this study were infected by the gill monogeneans and stomach monogeneans. Therefore, the possibility of using a substitute host for experimental infection studies was examined using the other two common cichlids species occurring in the same habitats but available from aquarium bred populations i.e. *O. mossambicus* and *O. niloticus*. As these trials failed, the infected fish had to be treated to make them devoid of parasites. Using these cleaned fish, the life cycle durations of the pre-stomach stages (skin and gill inhabiting) and stomach inhabiting stages were able to be investigated.

3.2. Materials and Methods

3.2.1. Population studies.

3.2.1.1. The description of *E. papernai* and *E. globodiscus*

This is given in the section, the preservation and description of parasites (2.2.1.3).

3.2.1.2. The populations in stomach

(1) The population structure

A group of 23 fish brought from Koggala lagoon in mid March 1991, of standard lengths between 9 - 12 cm were left in a separate holding tank and surveyed for a period of 26 days. The stomachs were removed from decapitated fish, split open and the contents removed by washing in physiological saline. The interior of the stomach wall was scraped on to glass slides until all the monogenean worms were removed. The worms were then fixed using a few drops of glacial acetic acid, followed by a few drops of alcohol. When these added solutions were about to dry a few drops of glycerin were added and a cover-slip applied. This preparation was used for counting and measuring each species. As some worms were not lying in a flattened position, the lengths could not be measured directly using an eye-piece graticule. Therefore the outer edge of worms were drawn on a piece of paper using a drawing tube and the lengths were measured along the middle line of the body using a bendable ruler. These measurements were converted into micrometer values to obtain the actual lengths of the worms. About a fourth of worms measured in this way.

The fish were left in a separate tank and surveyed through out a period of 26 days. As changes of length frequency distribution of parasites within this period may reflect

* the effect of the change of external environment due to confinement of fish into a small area in the aquarium and
* the absence of oncomiracidia in the first few days in aquarium water until they hatch from the eggs laid in the aquarium,

the data within 26 days were divided into five stages to investigate any changes.

(2) Measurements of some morphological characters with the age

Considering the minimum and maximum lengths found under the Section 3.2.1.2(1), the length range in stomachs were established for the two species. These ranges were divided into 20 μm length sub-ranges and 10 worms from each range were measured (for some ranges only a few worms could be found) for the lengths of dorsal and ventral hamuli (external hamuli length), dorsal bar, marginal hooks, and the copulatory tube.

(3) The differentiation of young and mature worms

While the experiment under Section 3.2.1.2(1) was being carried out the structure of the hard parts and the reproductive organs were observed to see whether there were any morphological differences which would indicate maturity.

Since the stomachs were heavily infected with *Enterogyrus* and consisted with worms of different ages, it was difficult to differenciate young adults and even to differenciate species. Therefore, stomachs with very low infections were found for this purpose. Such stomachs were available from fish experimentally infected with *Enterogyrus* and these stomachs also carried worms of the same age. Therefore experimentally infected fish prepared for the Sections 3.2.3.6(3) and 3.2.3.6(4) were also used for this experiment.

The opened stomachs were left in saline for 6 hours in an attempt to find the smallest size of egg laying worms of each species, so as to determine the size at which these worms attain maturity. The egg laying worms and number of eggs laid were noted, worms were separated, and the lengths of both categories, ie. egg laying and non-egg laying worms, were measured.

3.2.1.3 The site preference of *Enterogyrus* spp.

A group of 50 fish brought from Koggala lagoon, on 3 occasions within three months, and with standard lengths between 8 - 12 cm were left in a stock tank and

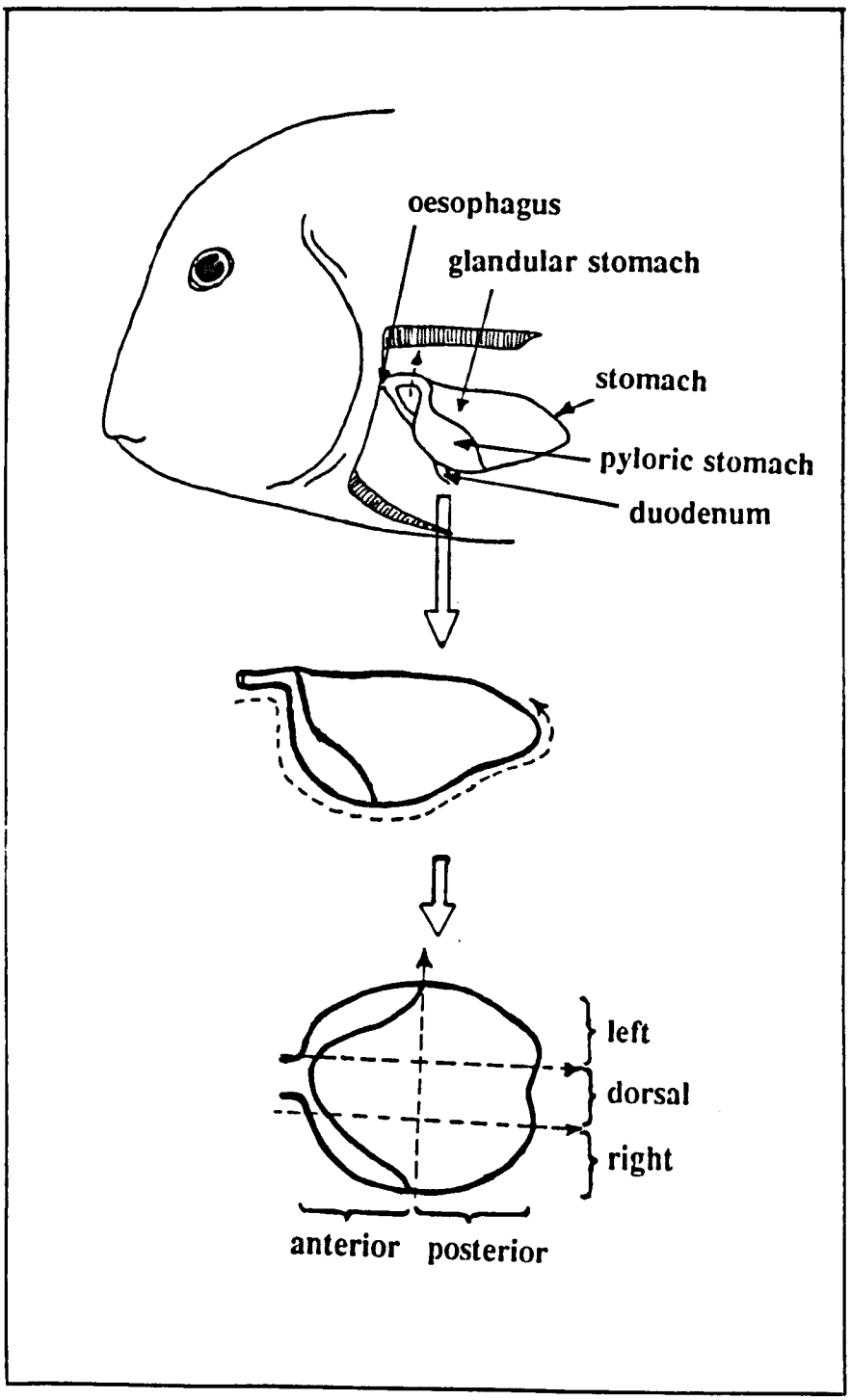


Figure 3.1: Diagram of *Etroplus* stomach showing the divisions used in finding the distribution of stomach monogeneans.

used during this period for the experiment. The compressed sac like stomachs were removed from the decapitated fish and opened with an incision along the more convexed ventral fold, partitioning at its middle, the patch like pyloric portion situated anteroventrally (Figure 3.1). The opened stomachs were washed in physiological saline, and each was divided into six approximately equal portions with a scalpel blade. First, a transverse cut separating stomachs into anterior and posterior portions, then each of these portions longitudinally into 3 portions namely right, dorsal and left according to their relative positions in the fish (Figure 3.1). Each of these pieces was scraped onto separate glass slides, and the slides prepared as described under section 3.2.1.2(1) The number of worms present was counted according to the species.

This was repeated using 33 fish of the same size range from Udawalawa reservoir brought within a period of 3 months.

The six sites were from the same stomach, the sites were not independent from each other. Therefore, the Analysis of Variance test cannnot be applied to find whether there is any significant variation among the populations of the sites. The sign test (Daniel, 1990) which removes the effect of dependance was used to compare each site with the other. This test can compare only two levels at a time, and there are 15 combinations to check. Therefore the probability value which should be used for the whole test was divided by 15 to compare by testing the statistic value. The probability values obtained were compared with the value 3.33E-3 (0.05/15) for the probability 95% level and 6.66E-4 (0.01/15) for 99 %. level. The site of preference for both species was checked separately. Then the correlations for the numbers of worms of the two species at each site and the number of worms in the each stomach was found out.

3.2.1.4 Pathology

(1) Histology

The procedure was given under Section 2.2.3.3(4).

(2) Tissue preparation for Scanning Electron Microscopy

Stomachs were excised from freshly killed fish, slit open, and the contents and mucus removed by washing thoroughly in phosphate buffer several times. The tissues were then fixed in 10 % buffered formalin with phosphate buffer according to the method used by Cone *et al.* (1987). They were then stored, refrigerated, until the tissues were processed at Institute of Aquaculture, University of Stirling.

On return to Stirling, the tissues were transferred to 3 % glutaraldehyde with phosphate buffer. They were then dehydrated with acetone and subjected to critical point drying. The dried tissues were mounted on aluminium stubs and sputter coated with gold. The coated specimens were viewed and photographed using a Scanning Electron Microscope model Phillips 500.

3.2.2. Reproductive Biology

3.2.2.1. Egg laying behaviour

E. globidiscus worms from infected stomachs were used for this study, as it was helpful to observe a selected worm without interferences from other worms so as to avoid any confusion.

The stomachs were split open, washed well and the tissue was immersed in physiological saline in a small petri-dish, keeping the inside of the stomach facing down-wards and just touching the bottom of the petri-dish. Then a worm was selected under the magnification 400 of the Olympus IMT2 inverted microscope. The behaviour of the worm was observed until it produced and laid an egg. In the same manner, egg laying of a total of 10 worms was observed.

3.2.2.2. In vitro egg laying rate

Rates at pH 7 and pH 4.5

The egg laying rates of both *Enterogyrus* spp. were determined from the worms in vitro, after removal from the stomach wall. Since the worms could not be

differentiated into species whilst they were attached to the stomach wall, this was the only method whereby the egg counts could be made for the two species separately and was thus the method of choice.

Experiment 1

The stomachs were obtained from freshly killed fish around 9-10 am, and were thoroughly washed to remove any eggs already laid. The worms were scraped out with the edge of the narrow side of a glass slide into small petri-dishes (5 cm diameter) with physiological saline adjusted to either pH 4.5 or 7 with the buffer HEPES Sodium (N-[2-Hydroxyethylpioerazine N'-ethanesulfonic acid). The biological buffer was useful at the pH range 6.8-8.2. The buffer was used to maintain the saline solution at pH 7, otherwise the addition of gastic acid changed the pH drastically.

Twenty worms from each species, were removed with a pulled out Pasteur pipette and placed singly on glass slides with drops of saline adjusted to the required pH. Another set up was made in the same way with saline of the other pH value. Care was taken not to select any inactive worms as these may have been damaged during the scraping. The slides were left in a wetted container, not protected from day light, and the number of eggs laid in each hour were counted for six hours.

Experiment 2

The worms from five stomachs were scraped into each of five petri-dishes containing saline of one pH, thus there were 10 petri-dishes for both pH values tested. The fish were killed at approximately 9-10 am and the number of eggs laid by both species (cumulatively since the eggs of the two species could not be differentiated) in each hour were counted for six hours. At the end of this period, the total number of worms, (both species) present in the petri-dishes were counted after killing them by adding Glacial Acetic Acid. The petri-dishes were left unprotected by day light during the egg laying.

3.2.2.3.In vivo egg laying rates

(1) With live fish

Egg laying rates were also determined by assessing the output of *Enterogyrus* eggs with faeces over a known time period. The number of worms present in stomachs were counted after killing the fish at the end of the experiment. As the eggs of the gill monogeneans were indistinguishable from those of the stomach monogeneans, fish were chemically treated in order to get rid of the gill infection prior to the study, as follows:

A group of 18 fish brought from Koggala lagoon, in the size range of 8.0-10.0 cm, were left in five tanks in the density of three fish per five litres of declorinated tap water for 2 weeks until they were accustomed to the environment. During this time they were fed *ad libitum* with the plant *Hydrilla* and the water was changed every other day. Earlier, it was found out that these fish behaved normally when kept in groups but not when placed individually. They were then treated with 250 mg l^{-1} solution of formalin (2.5 ml for 10 litres) bath for one hour after which they were transferred to a new set of clean tanks with aeration for 12 hours. This treatment had previously been tried and found to completely kill all the mature worms.

Immediately after the 12 hour period, fish were transferred into a set of five experimental tanks in the density of 3 fish per 5 litres of water in each, filtered through a mesh of 30 μm to reduce the amount of suspended matter in the water. After 24 hours, the fish were transferred to another set of tanks keeping the same three fish together. They remained in this tank till the next day, until they were killed for counting the number of monogeneans present.

The eggs of the stomach monogeneans which were released to the outside with the faeces of host, were collected from the bottom of the tanks and counted. Fish were killed and the number of stomach monogeneans responsible for laying the respective number of eggs determined. Opened stomachs were washed in saline to remove the contents and were then pressed between two slides so that the number of monogeneans could be counted by focusing on the inner stomach wall. Whilst counting, the immature *E. papernai* worms without copulatory tubes were excluded, and, as all the *E. globidiscus* had complete sets of reproductive organs, no differentiation was made

for them. The experiment was repeated later in the same manner with another set of fish.

Egg collection

A net with a mesh size of 30 μm fixed on to a Buckner funnel with rubber bands was used for filtering the water containing the eggs and faeces. The egg collection method using filter papers (Shaharom-Harrison, 1984) was unsuccessful, as it took a long time to filter the water even with the suction pressure of a motor and needed 3-4 filter papers due to the clogging, thus requiring a lot of time for counting. In addition, faeces did not get separated out to release the eggs trapped within.

The entire contents of the tanks was sucked out and passed through the net with the help of a siphon formed from a new plastic tube and gravitational pressure. The pressure was used to suck up the eggs adhering to the walls at the same time. The suspended matter from the water, the faeces and the food particles etc. on the net masked the visibility of eggs and hindered the counting of the eggs whilst they were on the net. Therefore, the net was washed into a series of petri-dishes (3-5) consecutively to remove all eggs to the petri-dishes. By this method almost all eggs were found to be well separated and sunk to the bottom with only the exceptional few adhering to the suspended matter. The eggs lying on the bottom of the petri-dishes were counted under the total magnification x 200 using an IMT2 inverted microscope. This magnification was necessary to distinguish some particles with the same shape and size as eggs. The eggs of each species were too similar to be distinguished, and thus egg counts represent the product of both species.

(2) Egg laying rates of *Enterogyrus* spp. on excised stomachs

Influence of tissue degradation on egg laying

As the excised stomach tissues may cause adverse effects on the egg production of stomach monogeneans due to its post-mortem degradation and its inability to supply the necessary food for them, it was decided to find out the longest period that the tissue could be left in physiological saline without affecting the egg laying rates of worms.

Five fish in the size range of 10.0 - 11.0 cm were killed from 9.00 - 10.00 am with a time gap of 10 minutes to allow time inbetween for egg counting, after exactly one hour periods. Stomachs were excised, slit opened and thoroughly washed to remove eggs already laid. These stomachs were kept in 5 cm diameter petri-dishes in physiological saline. The stomachs were laid down with the inside of stomachs uppermost. After each 60 minutes, the number of laid eggs were counted. This was carried out for eight consecutive hours. The number of the worms (excluding the immature *E. papernai*) on the stomachs were also counted by mounting stomachs between two slides. The egg laying rates at each hour were calculated. The same experiment was repeated for another 2 sets of fish in the next 2 days.

The egg laying rates for the hour were not independent from each other as the same stomachs were used for the experiment. The Analysis of Variance test cannnot be applied to find whether there is any significant variation amongst the hours. Therefore, the sign test which removes the effect of dependance was used. This test can compare only two levels at a time; there are 28 combinations to check. Therefore the probability value which should be used for the whole test was divided by 28 to compare with testing statistic value. The probability values obtained were compared with the value 1.79E-3 (0.05/28) for the probability 95% level and 3.57E-4 (0.01/28) for 99 %. level.

Egg laying rate

N.B. The following series of experiments were carried out on *Enterogyrus* spp. eggs for both species combined.

(i) Fish treated as same as the fish of Section 3.2.2.3(1) and killed at the same time

This experiment was planned in order to find out whether the two methods, determination of rates with live fish and the determination with the worms on the excised stomachs, give the same results. For the purpose of comparing the rates obtained for two experiments, one with a duration of a day and the other of 4 hours, it had to be assumed that there is no difference of egg laying rate at any time of the day.

A group of 12 fish, collected and treated in the same way as the fish treated under Section 3.2.2.3(1), were used for this experiment. They were killed, the same day that the fish used in the experiment of Section 3.2.2.3(1) were left for egg laying. Fish were killed between 9.00-10.00 am with a time gap of 10 minutes. The excised stomachs were opened and thoroughly washed to remove food and eggs already laid. Then they were placed in petri-dishes, one in each, keeping the inside facing upwards. At the end of four hours the number of eggs laid was counted according to species whilst they were on the tissue. The number of worms present were counted (both species together) under the magnification 100 by mounting the stomachs in between two glass slides and focusing on to the inner wall. The egg laying rates per worm per hour was determined.

(ii) Fish brought directly from the natural environment

Since the leaving of fish in the aquarium may cause behavioural, as well as physiological changes, in the fish thus affecting the physiology of the worms, it was decided to determine the egg laying rates in the fish just after bringing them from the lagoon.

A group of 10 fish within the size range of 8.0-10.0 cm were selected from a group of fish just brought from the lagoon. The egg laying rates per worm per hour were determined after leaving thoroughly washed stomachs in saline for four hours starting from 9.00 am in the morning.

3.2.2.4 The factors effecting the egg laying rates

N.B. The following series of experiments were carried out on *Enterogyrus* spp. eggs for both species combined.

(1) Effect of leaving fish in the aquarium

As the change of the habitat, the physical and chemical environment, the natural food sources etc., can affect the normal physiology of fish thus affecting the physiology of parasites, it was decided to investigate the egg laying rates whilst the fish were living in the aquarium with routine maintenance. The density of fish was 20 or less fish per 200 litre water. Water quality was maintained by renewal of 1/3 of

water every day. Feeding with *Hydrilla* was carried out *ad libitum.*

It was observed that the adult fish of a size greater than 10 cm, behave very agressively in the aquarium conditions. They stopped feeding for about a week after they were brought into the aquarium and resumed feeding later later than smaller fish. The smaller fish acclimated very quickly and the feeding was considered to be normal. Therefore it was decided to compare the two size groups of standard lengths (i) above 10 and (ii) between 8.0-10.0 cm. The size range 8.0-10.0 cm was especially selected as this was the size range which it was decided to use for all the experiments on reproductive biology. Therefore these fish were used to determine a suitable time period over which the fish should be allowed to adjust to aquarium conditions (if the oviposition varies with the time the fish leave in the aquarium).

Fish were selected immediately after they were brought from the lagoon. The fish of one length group were left in two tanks, initially in the density of 18 fish per 200 litres, four tanks altogether for two size groups. These fish were fed *ad libitum* with *Hydrilla.* Six groups of fish (three from each tank) were sampled from each group of tanks in the days 1, 4, 7, 10, 13 and 16, taking the day they were brought from the lagoon as day one. Fish were killed at 9.00 - 10.00 hours on these days and the egg laying rates were assessed using the method described under the Section 3.2.2.3(2).

(2) Diurnal variation

A group of 30 fish having standard lengths between 8-10 cm were selected from a group of fish left in the aquarium for 2 weeks. A day was divided into six time intervals having four hours in each. At the beginning of each time period 5 fish were killed and the egg laying rate of the stomach monogeneans within this period were determined as described under the Section 3.2.2.3(2).

The same experiment was carried out with a sample of freshly collected fish from the lagoon in the same manner.

(3) Effect of temperature

Three groups of 4 fish left in the aquarium for 12 days and having standard lengths between 8-10 cm were acclimatized over 6 hours and left in temperatures 23±1.8, 29±0.5 and 35±1.2°C for one day. As the temperature change may cause stress in the fish, they were not fed for this one day. At the end of this period fish were decapitated at 9.00-10.00 am. The excised tissues were prepared for determination of egg laying rates as described under the Section 3.2.2.3(2), but the petri-dishes containing the tissues were floated for four hours in the tank water at the respective temperatures. The next day, the same experiment was repeated in the same manner.

(4) Effect of pH

The effect of pH on egg laying rates was investigated using Acetate buffers of pH 3.6, 4.6 and 5.6. The buffer solutions were made as described by Golterman, Clymo & Ohnstad (1978). A 1.6 % NaCl solution was made and the buffer solutions were added to this solution in 1:1 proportion to gain the concentration of physiological saline (0.8 %). The pH of the buffered solutions was then checked to ensure that they had not changed due to the dilution.

A group of 16 fish in the size range 8-10 cm and left in the aquarium for 2 weeks with *ad libitum* feeding with *Hydrilla* were used for the experiment. Fish were killed between 9.00 - 10.00 hours and sets of 4 stomachs were kept in each of the three buffer solutions and one set was kept in normal saline as the control. Before placing the stomachs in the solutions they were thoroughly washed with the respective solutions in which they were to be kept in order to bring the pH of the stomachs to the appropriate pH value. Each of the stomachs was divided longitudinally into two. One portion was to be used for determination of egg laying rates and the other was used to measure the actual pH existing on the tissue half way through the duration of the experimental period; the two pieces of each stomach were left in the same petri-dish.

The pH of the stomach wall was measured by making contact between the

inner wall (always with a thin layer of mucus) with the tiny apperture on the electrode wall which allows for the passage of the solution to be measured towards the electrodes. Before carrying out this experiment, the pH meter reading was checked for accuracy of the method by using a buffer solution and a tissue paper moistened with the same buffer, and contact was made with the apperture. This provided assurance that the correct pH values were being measured, even when measuring a thin layer of liquid on a surface.

After 4 hours, the egg laying rates were determined as described in the Section 3.2.2.3(2). The same experiment was repeated the next day with another set of 16 fish.

(5) Effect of host feeding

A. Effect of fish feeding rate

The only food which was readily accepted by the fish was the plant *Hydrilla*, in its natural form as a plant. Therefore, it was decided to use the same for this experiment. Fish grazed only on the leaves leaving the stems. This therefore, made it difficult to calculate the weights for feeding rations. The weight proportions of stem to leaf varied widely when measured. By taking the whole range possible, and weighing the wet stems and leaves separately, the ratio was determined, in order to calculate the feeding rations. The wet weight ratio of stem to leaves was approximately 8:1.

For the experiment, a group of 30 fish, having the standard length range of 8-10 cm was selected and placed in the aquarium with *ad libitum* feeding of *Hydrilla* for 2 weeks. Subsequently, they were divided into groups of 5 fish and placed in separate tanks of 20 litres. All the fish in each tank were weighed to obtain their total body weight. These fish were fed with the following rations for 5 days.

Group (a) 10 % feeding ration; 90 % *Hydrilla* of the total body weight of fish in the tank.

Group (b) 5 % feeding ration; 45 % *Hydrilla* of the total body weight of fish in the tank.

Group (c) 2.5 % feeding ration; 22.5 % *Hydrilla* of the total body weight of fish

in the tank.

Group (d) 1 % feeding ration; 9 % *Hydrilla* of the total body weight of fish in the tank.

Group (e) without any food.

After the period of five days, the fish were killed between 9.00-10.00 hours and the egg laying rates of the parasites in the five groups of fish were determined as described under the Section 3.2.2.3(2).

B. Effect of Glucose in the surrounding medium

A group of 15 fish in the standard length range of 8-10 cm, held in the aquarium for 2 weeks with *ad libitum* feeding with *Hydrilla* were used for this experiment. The fish were decapitated between 9.00-10.00 hours and the stomachs removed. Stomachs of three fish were held in petri-dishes with each of the Glucose solutions made with physiological saline (0.8% NaCl) to the concentrations of 0.10 M, 0.05 M, 0.01 M, 0.005 M and one without glucose. After 4 and 8 hours the number of eggs laid was counted. The egg laying rates for first four hours, the next four hours and the whole 8 hour period were calculated. The same procedure was repeated with another group of 15 fish the next day.

C. Effect of Egg albumen in the surrounding medium

The concentrations of 10, 5, 1 and 0 % of egg albumen were made up in physiological saline (0.8%) considering the concentration of egg albumen obtained from the chick eggs as 100 %. The effect of egg albumen on the egg laying rates was determined in the same way as with the determination of the effect of glucose.

3.2.2.5 Location of egg embryonation and development; determination of egg viability

N.B. The following series of experiments were carried out on *Enterogyrus* spp. eggs for both species combined.

(1) Potential for egg development in the stomach

In this experiment the diurnal pH range of the stomach was investigated, in

order to check whether the range will overlap with the pH range in which the eggs can develop (Section 3.3.3.2(3).

A group of 30 fish, having the standard length range 8-10 cm, and held in the aquarium for 2 weeks with *ad libitum* feeding with *Hydrilla* were used for this experiment. Six fish were killed after every four hours starting from 8.00 hour. The ingested material in the excised stomachs were removed with forceps and the inner wall of the stomach was kept in contact with the apperture on the electrode wall as the pH of the stomachs was measured.

In addition to this, all the eggs in the stomachs were observed throughout the whole experimental period to check whether there were any eggs developing beyond the non-transparent stage. The various stages of development are given under the Section 3.2.3.1.(2).

(2) Method of egg release into the environment

If the eggs cannot develop into adults inside the stomach, they must pass out into the external environment for further development to take place. There are two possible ways, (i) passing along the alimentary canal with faeces or (ii) passing out through oesophagus. The latter would seem to be unlikely to occur passively. However it would be possible by way of activity of host origin or parasite origin to deposite eggs in the oral cavity or on to the gill tissue of host, so that they could be passed out with the respiratory current.

As there was no way to collect eggs passing out of the oral or branchial cavity from those passing via the anus, a direct determination of the number of eggs passing via the oral or branchial cavity was impossible. Therefore, an indirect method was used which required direct observations in combination with the counting of number of eggs along the alimentary canal. This included (i) finding the eggs in the mouth cavity and gill chambers (ii) finding any movement of egg laying parasites towards the oesophagus and mouth cavity which would facilitate egg passage to mouth cavity and (iii) checking the gut contents through out a day to find whether there was a

continous passing of eggs along the alimentary canal and whether this was supported by gut content movements.

The procedure was as follows:

1. A group of 10 fish treated with 250 mg l^{-1} Formalin for 1 hour to kill the gill parasitizing monogeneans were decapitateded around 10.00 am. All four gill arches were excised and the number of eggs present in the buccal cavity and on gills were counted. In addition a check was made to determine whether there were any egg bearing worms in these regions.
2. The same fish were used and the stomachs with the oesophagus were excised when they were freshly killed. They were opened instantly, each stomach was divided into two regions along the length of the sac and the number of egg bearing stomach monogeneans in these two regions were counted.
3. A group of 30 fish of the standard length size range 8-10 cm held in the aquarium for 2 weeks with *ad libitum* feeding of *Hydrilla* were selected. Five of these fish were killed at four hour time intervals. The number of monogeneans in the stomach and the number of eggs in the following intestinal regions were counted:- stomach, fore-intestine, mid-intestine, hind-intestine and rectum. The fullness of these intestinal portions with food contents were roughly estimated by eye as a percentage of the volume of the lumen.

(3) The viable egg percentage

Stomachs from five fish left in the aquarium for 2 weeks with *ad libitum* feeding with *Hydrilla* were used for this experiment. They were slit opened, thoroughly washed and left in saline for four hours for eggs to be laid. The laid eggs were then washed into the same petri-dishes. Then, the eggs in each Petri-dish were washed twice by pipetting all the eggs into new Petri-dishes with sterilized pond+sea water of salinity 8‰. The number of developed eggs and the number of hatched eggs as a percentage of the total number of eggs present was determined on the third and fifth days post-excision.

3.2.3. Life cycle studies

3.2.3.1. Egg development and its stages

N.B. The following series of experiments were carried out on *Enterogyrus* spp. eggs for both species combined.

Excised stomachs from freshly killed fish were slit opened and thoroughly washed to remove eggs already laid. These were placed in Petri-dishes containing physiological saline (0.8% NaCl) and left for two hours for fresh eggs to be laid. The laid eggs were washed into the same Petri-dish. They were then picked up one by one using a pulled out Pasteur pipette and placed in another Petri-dish containing the medium in which the eggs were allowed to develop. This egg transferance to a clean medium was repeated once. This additional changing was necessary to reduce tissue debris, mucus etc., and consequent bacterial growth. In addition, it reduced the changing of the quality of the experimental media, in the experiments carried out to find out the effect of salinity and pH.

(1) The differentiation of eggs of the two *Enterogyrus* species

Egg bearing worms of the two species were separated and left in cavity slides. After they had laid eggs, a cover slip was applied and a total of 30 eggs from each species was measured.

(2) Egg development under natural condition

Eggs obtained as described under the section 3.2.3.1 were placed in a petri-dish containing filtered, sterilized Koggala lagoon water (salinity 6.8‰). Sterilization was achieved by autoclaving.

Fifteen of these eggs were placed in cavity slides, one in each, together with a few drops of sterilized lagoon water. The cavity slides were kept in a closed plastic container floating on water to prevent drying. When necessary a few more drops of lagoon water were added to prevent drying of the eggs.

These cavity slides were studied every four hours under the microscope to

observe egg development. The development was categorized into four main stages;

1. non-transparent stage
2. transparent stage
3. developing stage
4. active stage

and the durations taken for these stages were noted.

The remaining eggs in the petri-dish were removed after every 24 hours, mounted in a drop of water, under a cover slip and examined microscopically. Drawings were made from these specimens.

Every 24 hours the length and width of the eggs in the cavity slides were measured.

When the larvae were about to hatch, they were observed more frequently, ie. once an hour to record the hatching time to the nearest hour.

The experiment was conducted at room temperature, which varied between 27.5-30.8°C. This is somewhat similar to the lagoon temperature considering the diurnal variation.

3.2.3.2. Effect of environmental factors on egg development

N.B. The following series of experiments were carried out on *Enterogyrus* spp. eggs for both species combined.

The three most important parameters which can vary in the natural environment were tested. With each environmental parameter tested, a control as similar to the natural condition as possible was used.

An appropriate medium for all the experiments was worked out to keep uniformity. Pond water was mixed with sea water to obtain salinity 8 ‰ unless

otherwise specified. Pond water was used instead of lagoon water because 0‰ salinity could not be prepared with lagoon water. The medium was then sterilized and filtered. As a buffer was required to maintain pH values around neutral pH (see Section 3.2.3.1(2)) HEPES Sodium (N-[2-Hydroxyethyl]pioerazine N'-ethanesulfonic acid), the biological buffer useful at the pH range 6.8-8.2, was added in the concentration of 5 mM l^{-1}. Unless otherwise specified, the pH was adjusted to 7 using dilute solutions of HCl and/or NaOH made using 0.8% NaCl instead of water. The medium in the controls therefore differed from the natural medium (lagoon water) by having pond+sea water mixture and HEPES. The salinity, pH and temperature were however maintained to approximately similar values. All the experiments were carried out at room temperature, unless otherwise specified. This varied between 27.5-31.0°C. The light regime was 12:12 day and night.

In all these tests, in addition to measuring the duration of development of the whole egg period, the durations for each developmental stage was noted to see whether effects were acting on a particular stage.

(1) Effect of salinity on egg development

Eggs were obtained as described under the Section 2.3.1 and the eggs were washed into small petri-dishes containing medium made up to salinities 0, 8, 16, 24 and 32 ‰. The eggs were then transferred into a plastic container with wells of half an inch in diameter carrying 5 ml water of the selected salinities at a density of one well carrying approximately five eggs. There were four wells for each salinity each containing approximately 5 eggs. The eggs were observed under Olympus IMT2 inverted microscope and the developmental stage of eggs were noted at every 4 hours. When the eggs were at the moving stage they were placed individually into single wells having 2 ml water with appropriate salinities and observed every hour to note the time of hatching. This transferring of eggs to single wells was carried out as it was important to keep hatched oncomiracidia separately in a small amount of water so that they could be used to study the effect of salinity on oncomiracidial longevity (Section 3.2.3.2.).

(2) Effect of temperature on egg development

Eggs were obtained as described under the Section 3.2.3.1. They were then placed in six multiwell plastic containers, with approximately 10 eggs per container and 1 egg per well together with 2 ml of medium. These containers were covered and floated in water baths. Three bath temperatures, 23±1.5, 29±1.0 and 35±1.0°C were used. One well per container was filled only with egg developing medium and was used to measure the temperature in the container. Every four hours the temperature of the water baths was adjusted to maintain minimum variation in temperature. The developmental stages over time were recorded and the hatching time was noted to the nearest hour. Care was taken not to leave containers for long periods out of the baths for microscopic observations.

(3) Effect of pH on egg development

A preliminary trial was carried out to see how the pH of the media could be maintained within set values for 3-4 days. The pH value around the neutral pH was difficult to maintain or adjust without a buffer. It was impracticable to add the very small volumes of diluted acid/base required for adjustments. Therefore, the use of HEPES was tested. This facilitated the maintainance and adjustments of pH values around neutral pH. The extreme pH values were then difficult to maintain, but these values were comparatively easy to adjust. Therefore, it was decided to use HEPES and carry out the experiment while correcting the extreme pH values twice a day with dilute solutions of HCl and NaOH (made with 0.8% NaCl instead of water).

Eggs were obtained as described under the section 3.2.3.1. The eggs were washed into small petri-dishes containing media made to pH values 3, 4, 5, 6, 7, 8, 9 and 10. Then, 32 wells of the multiwell plastic containers were filled with 2 ml of media with different pH values; four wells having the same pH. Eggs from the petri-dishes with the respective pH values were picked up using a pulled out Pasteur pipette and placed in wells in the density of approximately 5 per well. Observations on the egg developmental stages were made as previously stated and, when at the moving stage they were placed individually in single wells with medium containing the appropriate pH value where they were observed in every hour to obtain the hatching

time.

3.2.3.3. Oncomiracidium stage

N.B. The following series of experiments were carried out on *Enterogyrus* spp. oncomiracidia obtained from eggs of both species combined.

(1) The description of the oncomiracidium

Eggs used for measurements (separated according to species) were allowed to develop on the same cavity slides and were then transfered to glass slides before hatching. They were allowed to hatch in a very small amount of water on glass slides as this made it easy to locate them. When the eggs were hatched, the oncomiracidia were studied and measured under slight coverslip pressure.

(2) Behaviour of oncomiracidia

A set of eggs were left in individual small plastic Petri-dishes (diameter of 3.5 cm) containing 5 ml of filtered and autoclaved Koggala lagoon water. Eight oncomiracidia hatched out and were observed continuously for 2 minute every hour under a stereo microscope to examine their behaviour.

The remaining hatched larvae were killed using glacial acetic acid prior to making diagrams as they were moving very actively.

3.2.3.4. Effect of experimental environmental factors on the duration of the oncomiracidium stage

N.B. The following series of experiments were carried out on *Enterogyrus* spp. oncomiracidia obtained from eggs of both species combined.

The oncomiracidia hatched out from the experiments under the Section 3.2.3.2 were used to evaluate the effect of these parameters on the oncomiracidium stage. After the hatching of the eggs, the oncomiracidia in the wells were continually observed in every hour. It was not possible to focus on to the swimming oncomiracidia all the time, but whether they were moving actively or lying stationary

with sluggish movements was noted. Finally, the time at which they became motionless ie. dead was noted.

Since the same experimental set-up was used for the testing of the egg development, the controls were the same.

3.2.3.5. Mode of entry to host

N.B. The following series of experiments were carried out on *Enterogyrus* spp. oncomiracidia obtained from eggs of both species combined.

A group of eggs collected as explained in Section 3.2.3.1 were left to develop and, when they were about to hatch, were transferred to five 5 cm diameter Petri-dishes containing 8 ‰ filtered and autoclaved pond+sea water, at a density of 10 per Petri-dish. When almost all eggs were hatched, equal size pieces of fish tissue were placed in the Petri-dishes. In three of the seven Petri-dishes, the larvae were provided with a choice of tissue as follows.

Petri-dish number	Tissues placed
1	skin
2	gills
3	oesophagus
4	stomach
5	skin & gills
6	gills & oesophagus
7	gills & stomach

NB. When two pieces of tissue were placed in the same Petri-dish, they were placed at the maximum distance from each other, minimum 4 cm.

Small pieces of tissues were used as large pieces might conceal some of the oncomiracidia from view. After 6 hours the number of oncomiracidia attracted or not attracted to the tissues was counted.

As this type of behavioural experiment results in large variation, the experiment was repeated once in the same manner.

3.3.3.6. Life cycle duration of worms

(1) Infection of *Oreochromis* spp. (*O. niloticus* and *O. mossambicus*) with *Enterogyrus* spp.

Etroplus suratensis, infected with *Enterogyrus*, were brought from Udawalawa reservoir and left in close proximity with *Oreochromis* spp. (not a natural host fish) brought from same locality in 3 sets as given below;

A. *Etroplus : Oreochromis* ratio of 2:1 during the whole experimental period of four weeks in 200 l tanks.

B. Same as A but with the ratio of 1:1.

C. Same as A but *Etroplus* were removed after one week and *Oreochromis* were left alone for the rest of the period.

In all the tanks, the number of *Oreochromis* was 8 and the two species of *Oreochromis*, *O. niloticus* and *O. mossambicus* (identified by external features) were held in equal numbers. Everyday 1/3 of water was exchanged to maintain water quality but without disturbing the sediments. Once a week the sediments were roughly, ie. not thoroughly, removed. Half of the *Oreochromis* were sampled after two weeks (equal numbers from both spp.) and the rest were sampled after another two weeks (week 4). All the gills and the stomachs of the *Oreochromis* spp. were examined thoroughly to check for the infection of *Enterogyrus* spp.

(2) Removal of *Enterogyrus* with administration of Droncit

The estimation of life cycle duration can only be done by infecting clean fish and estimating the duration they live on these fish. As almost all *E. suratensis* were infected with *Enterogyrus* and no other cichlids were suitable as a host, a suitable clean host was not available. Therefore, it was decided to treat the infected fish and remove the infection. The antihelminthic drug Droncit was used mainly due to the availability, and its known low toxicity to the fish. This drug has been found to be a useful anthelmintic against Cestoda and Digenea (C. Sommerville, per. comm.)

The following trials, each with four fish, were made consequently to find out the reliable Droncit dose rate to remove *Enterogyrus* from the stomachs.

Method

1. 20-30 mg/kg, one treatment and stomachs were examined after 3 hours.
2. 20-30 mg/kg, 3 times at 24 hour intervals and stomachs were examined after 3½ days.
3. 20-30 mg/kg, 3 times at 6 hour intervals and stomachs were examined after 28 hours.
4. 200 mg/kg, one treatment and stomachs were examined after 3 hours.
5. 200 mg/kg, 2 times at 6 hourly intervals and stomachs were examined after 24 hours.
6. 300 mg/kg, 3 times at 2 day intervals and examined after 7 days.
7. 300 mg/kg, 3 treatments, the second treatment on the third day, then a 250 ppm formalin 1 hour bath on fifth day and the third Droncit treatment on the sixth day.
8. 300 mg/kg, 3 treatments at 72 hour intervals with a 250 ppm formalin 1 hour bath treatment 24 hours before Droncit treatments.

Powdered Droncit was made into a suspension with physiological saline and administrated directly into the stomach of anaesthetized fish with the help of a very flexible plastic tube. A 50 ppm solution of Benzocaine was used for anaesthetic (Benzocaine was dissolved in a 95% solution of ethanol before adding to water). The tanks were continuously supplied with aeration and, during and after treatments, heavy aeration was used.

(3) Life cycle duration of adults

Forty *E. suratensis* in the size range 8-12 cm standard length, brought from Koggala lagoon, were treated with the treatment method 8 which killed almost all the *Enterogyrus* in the previous Section. The 8-12 cm size range was selected because of the ease of handling and the requirement for smaller quantities of Droncit to achieve the appropriate dose rate. At the end of the treatment period, five fish were killed and skin, gills and stomach were examined to check the absence of any type of monogeneans. After a further two weeks, another 5 fish were killed and thoroughly

examined to ensure the absence of worms.

A group of thirty freshly collected, untreated fish were left in a tank for 24 hours (8.00 am-8.00 am) to collect the eggs (both species combined) of *Enterogyrus* released with faeces of fish. The 8.00 pm was considered as the zero time of egg laying. Since the eggs hatch in the fourth day, the fish devoid of *Enterogyrus* were introduced for infection by oncomiracidia at the beginning of the fourth day at 8.00 am. Very light aeration was supplied by several small aerator bulbs for minimal disturbance of the movements of oncomiracidia. The tanks were cleaned and treated as normal stock fish tanks from the sixth day. Two fish were killed every other day, the stomachs were examined, the number of worms present were counted and their lengths were measured as described under the Section 3.2.1.2(1).

The stomachs with light infections were required for the experiment on differentiation of young and mature worms (Section 3.2.1.2(3), especially because in those it was easier to keep an eye on the moving worms and separate the egg laying worms from non egg laying. Therefore, lighly infected stomachs in this experiment were left in saline for 6 hours prior to killing of parasites, the egg laying worms were noted and separated from non egg laying and their lengths measured.

(4) Pre-stomach stages of *Enterogyrus*

The fish used in the previous section were infected by gill monogeneans in addition to *Enterogyrus*, and the eggs laid by the gill monogeneans were also retained in the tanks. It was found that the pre-stomach *Enterogyrus* stages on skin and gills amongst the larvae of gill monogeneans were difficult to differentiate and separate. Therefore this experiment was designed and carried out to infect the skin and gills of fish exclusively by the stomach monogeneans.

Twenty fish of the size range 8-12 cm were treated, 300 mg/kg three treatments at 72 hour intervals with a 250 ppm formalin 1 hour bath treatment 24 hours before Droncit treatment, to remove all the gill and stomach monogeneans. Two weeks after the end of the treatment period, a group of six fish were killed and the

skin, gills and stomach were examined to check the absence of monogeneans.

The remaining 14 fish were infected only with *Enterogyrus* eggs according to the previous experiment (Section 3.2.3.6(3)) They were infected with approximately 160 eggs (both species combined). The hatching percentage was considered to be 87 (see Section 3.3.2.4(3)), thus a burden of 10 oncomiracidia could be expected. Fish were introduced to the tank containing eggs in the third day of egg development and observed. Two fish were killed and examined for *Enterogyrus* on days 4, 5, 7, 9, 11, 13 and 15 taking the day of egg laying as the starting point, day 1.

3.3. Results

3.3.1. Population studies.

3.3.1.1. The description of two species.

The two species of *Enterogyrus* are described in Part 1, under the Section 2.3.1.1, description of the parasites found on *Etroplus suratensis.*

3.1.1.2. The population in stomach.

(1) The population structure.

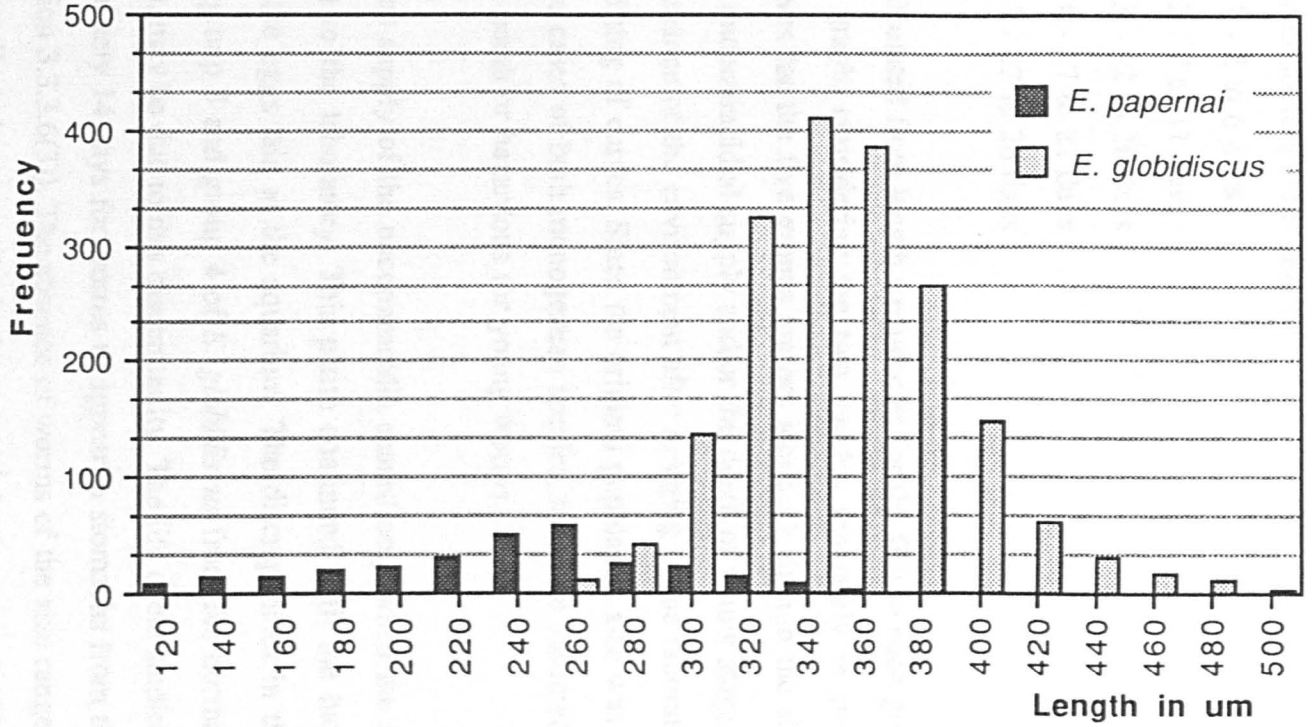
The length-frequency of the population of each species obtained from all the 23 fish examined is illustrated in the Figure 3.2.

There were 1843 *E. globidiscus* and 327 *E. papernai* in total in all 23 fish, thus 80.13 ± 21.88 *E. globidiscus* and 13.70 ± 2.79 *E. papernai* per fish. Therefore the population size of the two species showed a great difference. This is proportional to the area under the two length-frequency curves, the population size of *E. papernai* being 17.74 % of that of *E. globidiscus.*

The length range found for *E. globidiscus* was 260-500 µm, whilst the range for *E. papernai* was 120-360 µm. The highest frequencies of worms occurred at the lengths of 260 and 340 µm in *E. papernai* and *E. globidiscus* respectively. Therefore in general the size of the *E. papernai* worms are smaller than *E. globidiscus* worms. The observations of internal structures revealed that *E. papernai* has a more heterogenous population probably reflecting the life stages. Whereas *E. globidiscus* has a homogenous population representing mature worms only.

The low frequencies of the smaller worms of both populations indicate that the immature worms migrate into the stomach from an outside site. The reduction in the number of worms of greater size than the peak indicates the death of the mature worms or their loss with the out going food materials. Several times during the parasitic survey, *Enterogyrus* worms were found in the duodenum wall. Thus it is

Figure 3.2: Length-frequency distribution of *E. globidiscus* and *E. papernai;* totals of the frequencies obtained from 23 fish.



reasonable to believe that some worms are lost with the out-going food material.

Since the population structure of the stomach monogeneans was surveyed over a period of 26 days, the data were categorized into five stages, each consisting of five days, to divide the period and to incorporate proximately surveyed fish together. The stages were as follows, the day 1 was the day the fish were brought into the laboratory and they were examined starting from next day.

Stage 1 - 2 to 6 days
Stage 2 - 7 to 11 days
Stage 3 - 12 to 16 days
Stage 4 - 17 to 21 days
Stage 5 - 22 to 26 days

The means calculated from length frequencies for the fish in each group were plotted in the same graph; considering the two species separately in two graphs (Figure 3.3). All curves for the five groups are not identical; though the shapes are similar. A change in oncomiracidial supply and/or the death of young stages on skin and gills due to the change of the environment after bringing to the laboratory, may be responsible for shifting of curves. Since the original population size was regained by the group 5, in the cases of both monogenean species, it can be assumed that the environment was not harsh or hazardous for young worms.

The continuous supply of the oncomiracidia ceased only when the fish were brought from lagoon to the laboratory. This pause continued until the hatching of oncomiracidia from the eggs laid at the aquarium. The discrepancies in the length frequency curves of group 3 and group 4 of *E. globidiscus* from the normal pattern (in some size ranges), may be due to this discontinuity. The life cycle studies showed that it takes approximately 14 days for worms to appear in stomachs from the day of oviposition (see Section 3.3.3.6(3)). The absence of worms of the size range 260-280 μm representing the smallest size occurring in the stomach in the group 3 (13-16 days) could be due to the absence of the supply of oncomiracidia about 14 days previously. It takes another 6-7 days for the worms to attain the size of 380 μm after reaching the

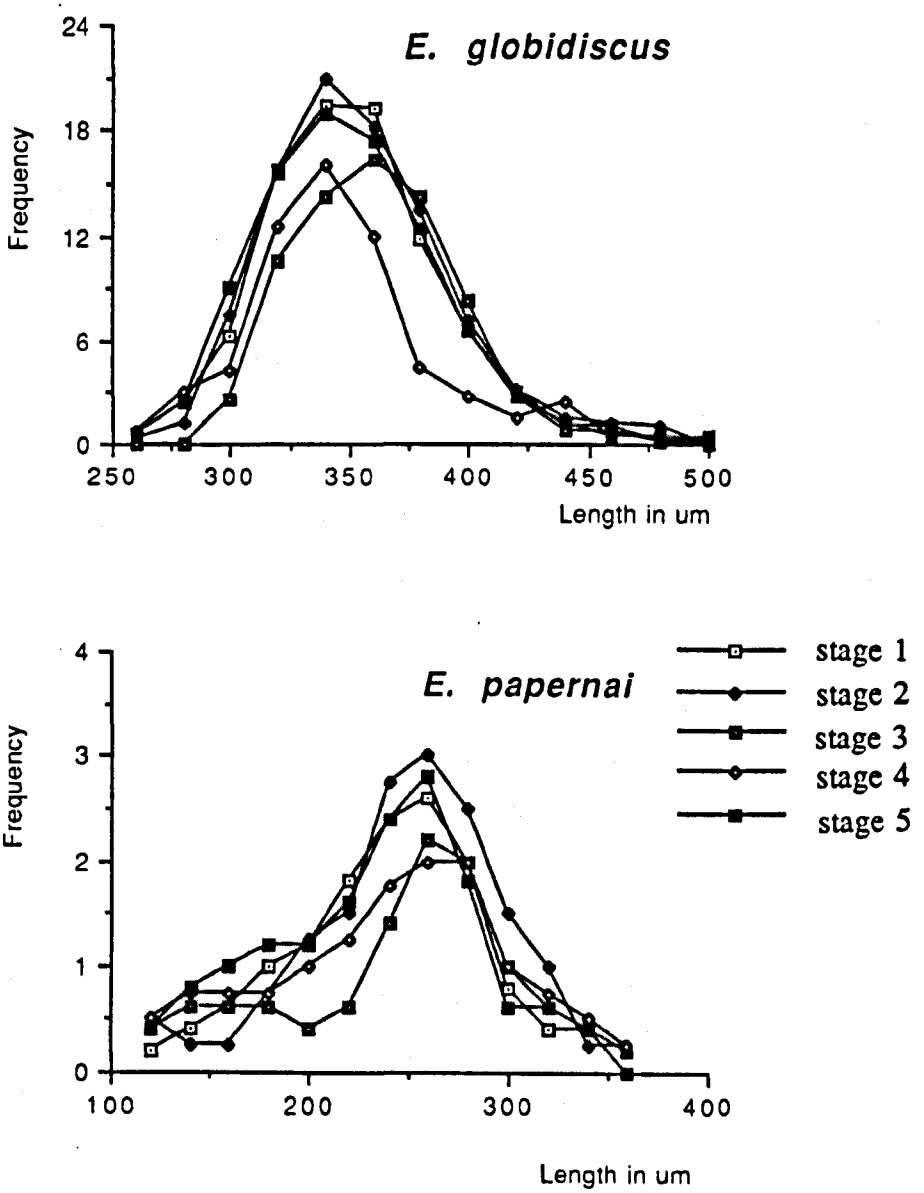


Figure 3.2: Mean length frequency distribution for the five groupes of fish (categorised dividing the whole period of observation with the time) for stages 1,3 &4 n=5; 2 & 5 n=4.

stomach. The marked low number of worms of the length range 360-400 μm in the group 4 (19-21 days) length frequency curve shows the shift of the discrepancy which occurred in the group 3 after about 6 days. Since the differences in the curves between the groups correlate with the life cycle information generated from the life cycle studies (Section 3.3.3.6(3)) this reinforces the hypothesis that the differences are due to the temporary discontinuity in the supply of oncomiracidia.

In the case of *E. papernai*, a similar discrepancy is evident in group 2 and 3. Again, this can be explained using the data from the life-cycle study. It takes 9 days for the worms to appear in stomach from the egg stage. So, the deficiency of 140 - 180 μm worms in group 2 (7-11 days) indicates the cessation of oncomiracidiae supply about 9 days previously. The deficiency of 180 - 240 μm worms in the group 3 (13-16 days) was created by the same cause where the shift needs about 5 days according to life-cycle studies.

The low peaks obtained in the groups 3 and 4 in the both species of stomach monogeneans, is difficult to explain, but may be due to low egg production rates and therefore the low recruitment of young worms.

(2) The measurements of sclerites of worms of different lengths.

Table 3.2 gives the data of the measurements of sclerites of the worms of different sizes; representing worms of different ages. As can be seen from the table the sclerites do not show any development during the life-time of worms once they have been formed.

(3) The differentiation of young and mature worms.

The observation of hard parts and the reproductive organs in the experiment under the Section 3.3.1.2(1) revealed that all the *E. globidiscus* worms found in the stomach had a complete set of reproductive organs including the copulatory tube. In addition, the haptorial armature was alike in all worms observed. The case with *E. papernai* was different. The worms having lengths around 180 μm and below did not

Table 3.2 : The measurements of some morphometric characters in relation to length of monogeneans, *Enterogyrus* spp.

Length of worms	Number measured	Length of dorsal hamulus	Length of ventral hamulus	Length of dorsal bar	Length of copulatory tube	Length of marginal hook pair 5
oncomiracidia						
110-129	4	absent	17 - 18	absent	absent	13 - 14
E. papernai						
110-129	2	36 - 40	16	16	-	13
130-149	6	36 - 40	16 - 17	14 - 17	-	13
150-169	10	38 - 42	16	15 - 16	-	13 - 14
170-189	10	37 - 44	16 - 17	16 - 18	-	13
190-209	10	38 - 42	15 - 18	17 - 18	78 - 86	13 - 14
210-229	10	36 - 42	16 - 18	15 - 18	76 - 86	13 - 15
230-249	10	40 - 44	15 - 17	15 - 18	84 - 96	13 - 14
250-269	10	39 - 43	16 - 18	15 - 17	76 - 93	13 - 15
270-289	7	40 - 44	16 - 17	15 - 18	84 - 98	13 - 14
290-309	3	40 - 42	16	15 - 16	78 - 92	13 - 14
310-329	1	39	17	16	88	13
330-349	0	-	-	-	-	-
350-369	3	40 - 43	16 - 18	16 - 17	84 - 102	14
370-389	0	-	-	-	-	-
390-409	1	40	17	16	104	14
E. globidiscus						
250-269	4	36 - 38	16 - 18	24 - 27	62 - 68	13 - 14
270-289	4	37 - 38	16 - 18	25 - 28	66 - 74	13 - 14
290-309	10	35 - 38	18 - 19	24 - 28	68 - 72	13 - 15
310-329	10	36 - 38	18 - 20	23 - 27	68 - 76	14 - 15
330-349	10	36 - 40	17 - 20	23 - 27	64 - 74	13 - 14
350-369	10	36 - 38	16 - 20	22 - 28	68 - 78	13 - 14
370-389	10	37 - 40	16 - 20	22 - 26	68 -.76	13 - 14
390-409	10	36 - 40	16 - 19	24 - 27	68 - 74	13 - 15
410-429	8	38 - 39	17 - 20	22 - 27	64 - 78	13 - 15
430-449	6	37 - 40	18 - 20	24 - 27	70 - 74	13 - 14
450-469	4	36 - 38	16 - 19	26 - 27	66 - 76	14 - 15
470-489	5	38 - 40	17 - 20	25 - 27	68 - 74	13 - 15

Table 3.3 : Differentiation of young and mature worms of *Enterogyrus* spp.

Day of the life-cycle	Lengths of the worms found (μm) with indications of the egg laying (⊙) and/or presence of the copulatory tube (†).	
	Fish 1	Fish 2
Experiment under the Section 3.3.3.3(3)		
E. papernai		
8	136, 160	146, 164, 174
10	186, 194†, 200†	148, 166, 176†, 180†, 210†•
12	220†, 228†•, 240†•, 256†•, 260†•, 282†••	234†•, 242†•, 264†•
14	263†•, 268†•, 277†•	244†•, 246†•, 270†••
E. globidiscus		
14	264†, 268†•, 274†•, 278†••, 278†•	262†, 265†••, 275†•, 278†•, 280†••
Experiment under the Section 3.3.3.3(4)		
E. papernai		
15	236†	248†•
E. globidiscus		
15	272†•	268†, 285†•

• worms produced 2 eggs within the period of 6 hours.

have copulatory tubes and the reproductive organs were absent. Some worms around the length 180 μm had developing reproductive organs faintly visible. But, in all the *E. papernai* worms, immature or mature, the haptorial armature was similar in size and shape.

As the worms laid eggs only while they were on the stomach tissue, it was very difficult to find and separate non-egg laying worms from the egg laying worms in naturally infected stomachs, especially due to the confusion made by high densities. Lightly infected stomachs were available with experimental infections carried out in the Sections 3.3.3.3(3) and 3.3.3.3(4) with the advantage of each stomach having worms of a particular age. Therefore the lengths of the egg laying and non-egg laying worms at the age of 9-15 days of the life-cycle were measured in these experiments and are given in the Table 3.3. *E. papernai* worms without copulatory tubes and smaller worms with copulatory tube did not lay eggs. In these worms the copulatory tubes might not have been functionally ready by then. Most of the *E. globidiscus* worms laid eggs in 1-2 days after they appear in the stomach, but a few of the smaller worms did not, even when they were morphologically ready. Probably they had not copulated or there is a latent period between copulation and laying eggs.

3.3.1.3 The site preference of *Enterogyrus* spp.

The number of worms of the two species of monogeneans present in the stomach sites of roughly equal sizes are given in Table 3.4, separately for the two different localities Koggala lagoon (brackish) and Udawalawa reservoir (freshwater). The results of the sign test applied to find the sites with different population numbers at 95 % significance level are indicated.

Each species of worm showed a significantly different population size for the anterior and posterior portions of the stomachs. The three sites within these anterior or posterior portions showed a little or no difference with the dorsal site having a marginally higher population number. Thus the results show that both species of worm have a similar site preference, where the posterior portion was greatly preferred by

Table 3.4 : The results of the sign test applied to compare the population sizes in the six sites of the stomachs. The Mean ± SD of population for each site are given.

E. globidiscus of Koggala lagoon (n = 50)

Anterior right	Anterior dorsal	Anterior left	Posterior right	Posterior dorsal	Posterior left
3.12 ± 2.38 ^{a}	4.86 ± 3.52 ^{b}	3.74 ± 2.66 ^{ab}	16.34 ± 7.81 ^{c}	18.36 ± 10.02 ^{c}	17.06 ± 8.35 ^{c}

E. papernai of Koggala lagoon (n = 50)

Anterior right	Anterior dorsal	Anterior left	Posterior right	Posterior dorsal	Posterior left
0.64 ± 0.94 ^{a}	1.20 ± 1.16 ^{a}	0.94 ± 1.20 ^{a}	3.36 ± 2.48 ^{bc}	3.94 ± 2.57 ^{c}	2.20 ± 1.71 ^{b}

E. globidiscus of Udawalawa reservoir (n = 33)

Anterior right	Anterior dorsal	Anterior left	Posterior right	Posterior dorsal	Posterior left
1.88 ± 1.32 ^{a}	2.85 ± 1.54 ^{a}	1.94 ± 1.69 ^{a}	13.27 ± 4.54 ^{b}	18.36 ± 5.73 ^{c}	14.03 ± 4.79 ^{b}

E. papernai of Udawalawa reservoir (n = 33)

Anterior right	Anterior dorsal	Anterior left	Posterior right	Posterior dorsal	Posterior left
0.64 ± 0.74 ^{a}	0.82 ± 0.98 ^{a}	0.61 ± 0.83 ^{a}	2.46 ± 1.12 ^{b}	3.12 ± 1.11 ^{b}	3.24 ± 1.42 ^{b}

The sites with similar sizes of populations at 95 % significance level are marked with similar superscripts.

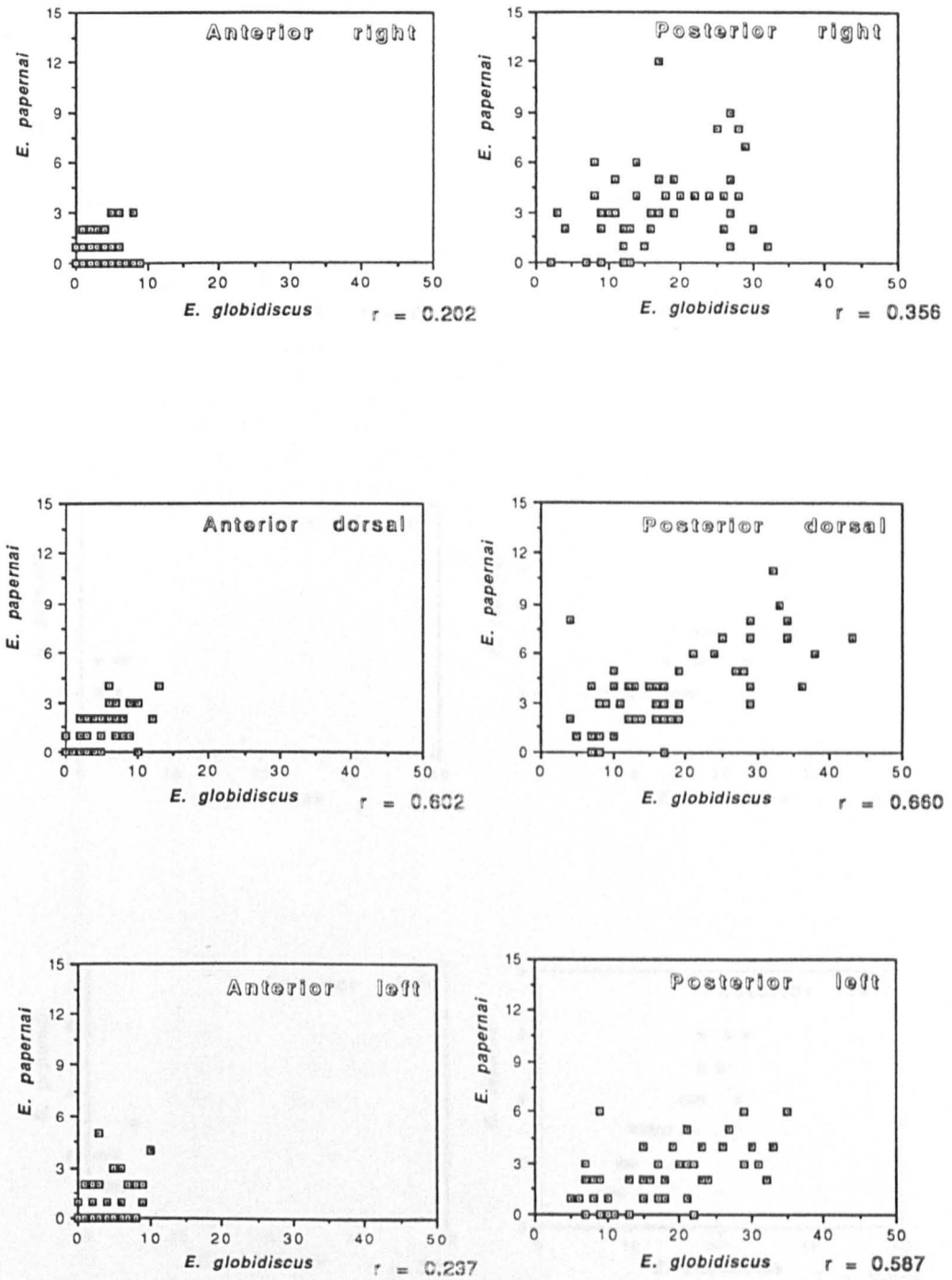


Figure 3.4: The scatter plots of population numbers of *E. globidiscus* against *E. papernai*, for the six sites of the stomachs of the fish from Koggala Lagoon (n=50). r=correlation coefficient, $r_{0.05(2),48}$= 0.279.

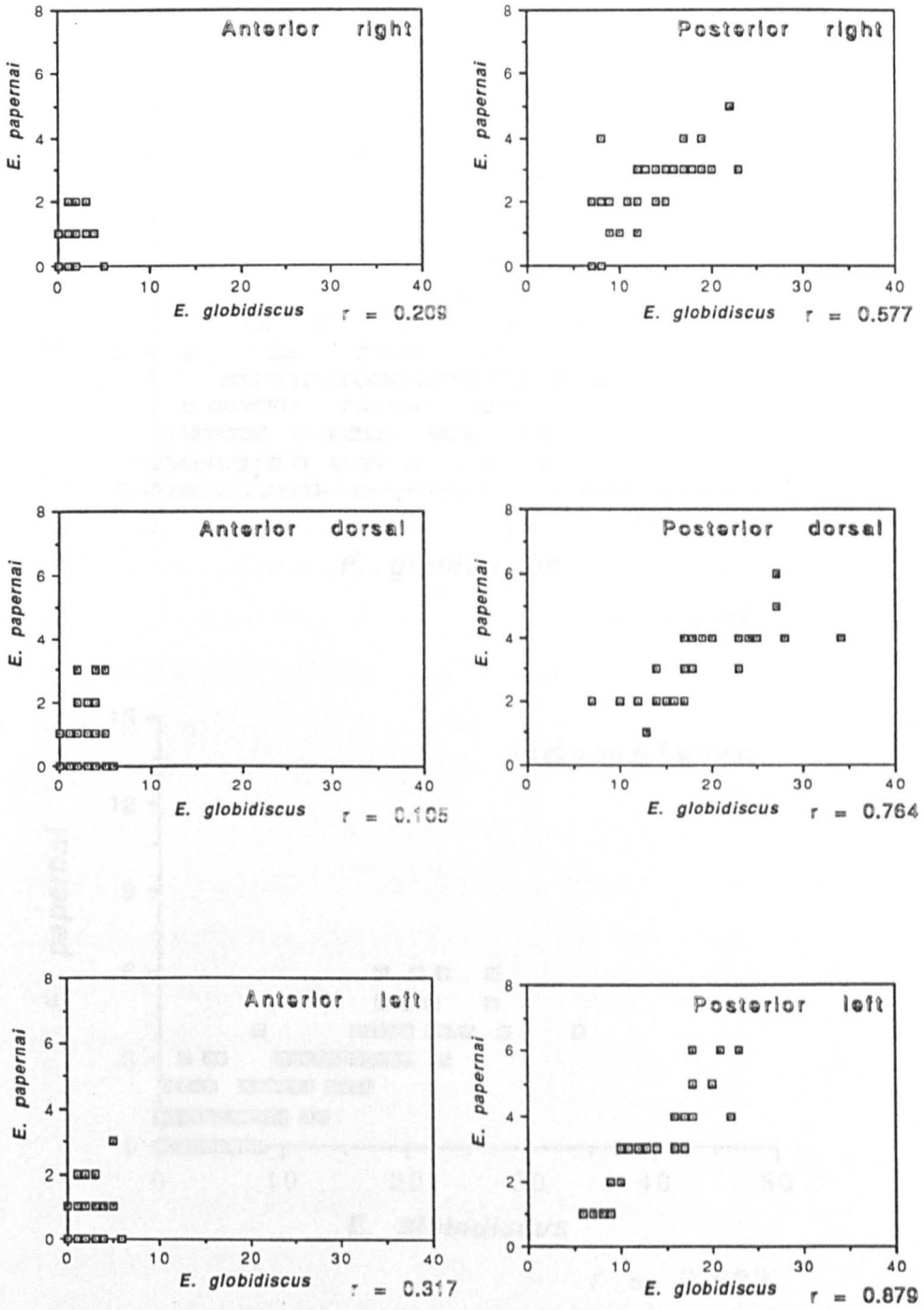


Figure 35: The scatter plots of population numbers of *E. globidiscus* against *E. papernai*, for the six sites of the stomachs of the fish from Udawalawa reservoir (n=33). r=correlation coefficient, $r_{0.05(2),31}$= 0.344.

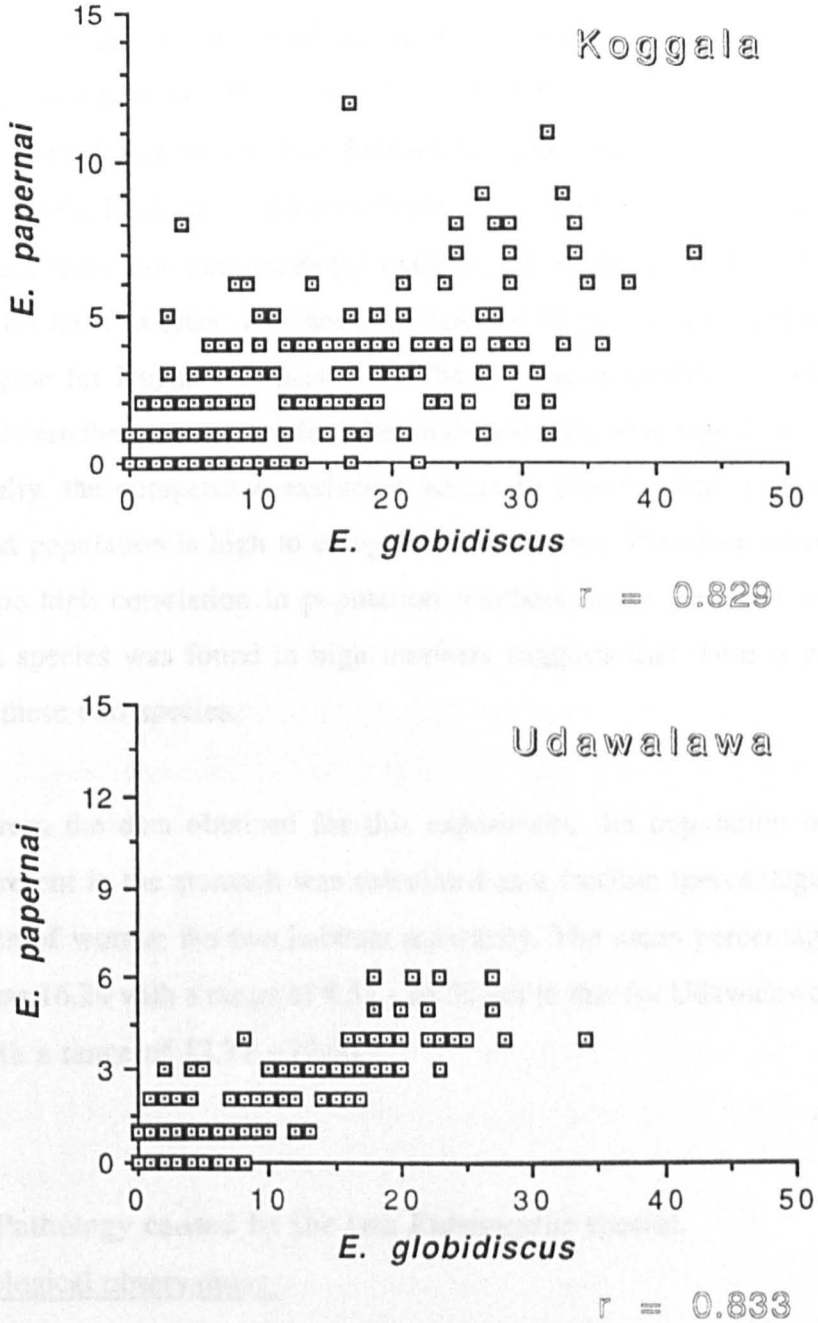


Figure 3. : The scatter plots of population numbers of *E. globidiscus* against *E. papernai*, all sites of stomachs on one plot, for fish from Koggala Lagoon and Udawalawa reservoir. r=correlation coefficient. For Koggala Lagoon; n=300, $r_{0.05(2),298}$= 0.113. For Udawalawa reservoir; $r_{0.05(2),196}$= 0.138.

both species and the dorsal site of both anterior and posterior portions, was preferred to the vertical sites.

The similar preference of worms for the same sites are also shown by the correlation coefficients. The correlation coefficients for the two species were calculated considering the numbers for each site and overall numbers for the stomachs (Figure 3.4-3.6). In all cases, the correlation coefficients were positive, indicating that one species does not competitively exclude the other. However, the coefficients obtained for anterior sites were not significant at 95 % level, except for the anterior dorsal region for Koggala Lagoon fish. The correlation coefficients for all posterior portions where the worms were found in high numbers, were significant at 95 % level. In generally, the competitive exclusion occurs in places where the numbers of the dominated population is high to compete for resources. Therefore according to these results, the high correlation in population numbers in the posterior sites where the dominant species was found in high numbers suggests that there is no competition between these two species.

From the data obtained for this experiment, the population of *E. papernai* worms present in the stomach was calculated as a fraction (percentages) of the total population of worms; the two habitats separately. The mean percentage for Koggala lagoon was 16.24 with a range of 8.51 - 28.85 while that for Udawalawa reservoir was 17.17 with a range of 12.77 - 22.00.

3.3.1.4. Pathology caused by the two *Enterogyrus* species.

(1) Histological observations.

The inner surface of the blind, sac-shaped stomach exhibited two different textures; a less convoluted smaller portion situated anteroventrally near to the one opening of the stomach to the oesophagus as well as to the duodenum. The rest exhibited deep infolding of the mucosa. Histologically the former carried numerous mucous secretory cells but no gastric glands whilst the latter was abundant with gastric glands as well as mucous secretory cells. Both these portions had columnar

epithelial cells. Due to the absence of gastric glands, the presence of columnar epithelial cells and its position in the stomach, the smaller area was identified as the pyloric portion of the stomach. An area which could be considered as the cardiac portion was not found. In the histological study, the worms of both species were only observed attaching to the deeply infolding mucosa of the glandular region.

On these folds, the morphologically smaller species, *E. papernai*, were mostly on the vertical walls of the folds, with the body axis aligned approximately perpendicular to the longitudinal axes of the folds (Figure 3.8). *E. globidiscus* seemed to prefer crypts. Lying in the crypts, the bodies were extended between the folds towards the lumen of the stomach (Figure 3.7). Occasionally, *E. globidiscus* was found at the apices of the folds and on the vertical walls. This may however have occurred during the course of movement from one location to another. *E. papernai* seemed to be a more sedentary species.

In all cases, at the site of attachment to the host tissue, the haptor was sunken into host tissue damaging the host cells. In the case of *E. papernai* the entire opisthaptor and about 1/3 of body was embedded in the host stomach wall (Figure 3.8 & 3.9), whilst only about 1/2 to 2/3 of the opisthaptor was embedded in the case of *E. globidiscus* (Figure 3.7). In both cases, the columnar epithelium, the tubular part of the gastric glands and the secretory part of the gastric glands lying just beneath the epithelium at the site of attachment were damaged due to compression. The damage caused by *E. papernai* penetrated more deeply into the gastric glands than did that of *E. globidiscus*.

The opisthaptor of *E. papernai* was more closely applied to the host tissue than in the case of *E. globidiscus* (Figure 3.10 & 3.11). In both species, the degree of dorsal hamuli penetration into the host tissue was similar. The posterior most part of the opisthaptor of *E. papernai* which contained the ventral hamuli and two pairs of marginal hooks, was penetrated deeper into the host tissue and curved ventrally holding host tissue (Figure 3.11), thus making a more firm attachment. The penetration of hamuli was always accompanied by the presence of a few pycnotic nuclei in the

adjacent cells.

In some regions of the mucosa and sub-mucosa, patchy infiltrations of inflammatory cells were observed. In a few cases, these patches were evident directly under the attachments of worms (Figure 3.9), mostly in *E. papernai*. The infiltrations contained macrophages and a few leucocytes. Even though scattered red blood cells were found near a few of the pits this was not common to all. The presence of eosinophilic granular cells varied and these were to be found mostly under the attachment of *E. papernai*. A few attachment sites of *E. papernai* were seen with a considerable number of eosinophilic granular cells.

The damage due to feeding of the morphologically smaller *E. papernai* was usually evident near the attachment (Figure 3.9). Here, the cytoplasmic portion towards the surface of the columnar epithelial cells was damaged. The feeding damage caused by *E. globidiscus* was not apparent. As this species is more elongated, it can feed over a wider area and thus focal damage may be minimized.

On some sections, depressions without evidence of the worms were seen. The worms may have been detached during processing, or these may represent the older, abandoned attachment sites. In same sections, damage similar to the feeding damage of *E. papernai* was often evident in the absence of a worm in the vicinity. These may represent feeding damage of worms of either of the two species or the regeneration of abandoned attachment sites. The regeneration process may be quite fast; thus making these areas normally indistinguishable.

(2) Scanning Electron Microscopic (SEM) observations.

The three dimensional SEM observations confirmed the two dimensional histological observations. Mainly, the varying degree to which the two species of worms were embedded in the host tissue was clearly evident (Figure 3.12 & 3.13). The depression made by the *E. globidiscus* was more superficial with just the haptor embedded, but *E. papernai* seemed to be attached more permanently and were feeding

from the surrounding area. Their body movements had raised the edges of the pits in which they were lying.

A large number of depressions or pits were found on the inner surface of the stomachs. The bottom of these pits were punctured with the evidence of sharp cuts or holes (Figure 3.14). This is an evidence for the puncturing of the bases of the depressions mainly by the dorsal pair of hamuli. On the bases of some pits more than two holes were seen (Figure 3.15). The additional punctures may be the damage cause by ventral hamuli or the posterior most tip of the haptor of *E. papernai.*

The number of *E. globidiscus* worms seen on stomach under SEM were much fewer than the number of *E. papernai* seen. The possible explanation for this is the greater loss of the more lightly embedded *E. globidiscus* during the storage, transportation and processing for SEM. The folds near the cut edge of the stomachs were unfolded and straightened, therefore the *E. papernai* worms attached onto the side walls the folds were easily seen in these straightened areas, compared to the more convoluted middle region, as if they were concentrated to the edges.

Figure 3.7: An *Enterogyrus globidiscus* worm in the stomach, between the folds and with no host response (H & E). Scale bar = 50 μm.

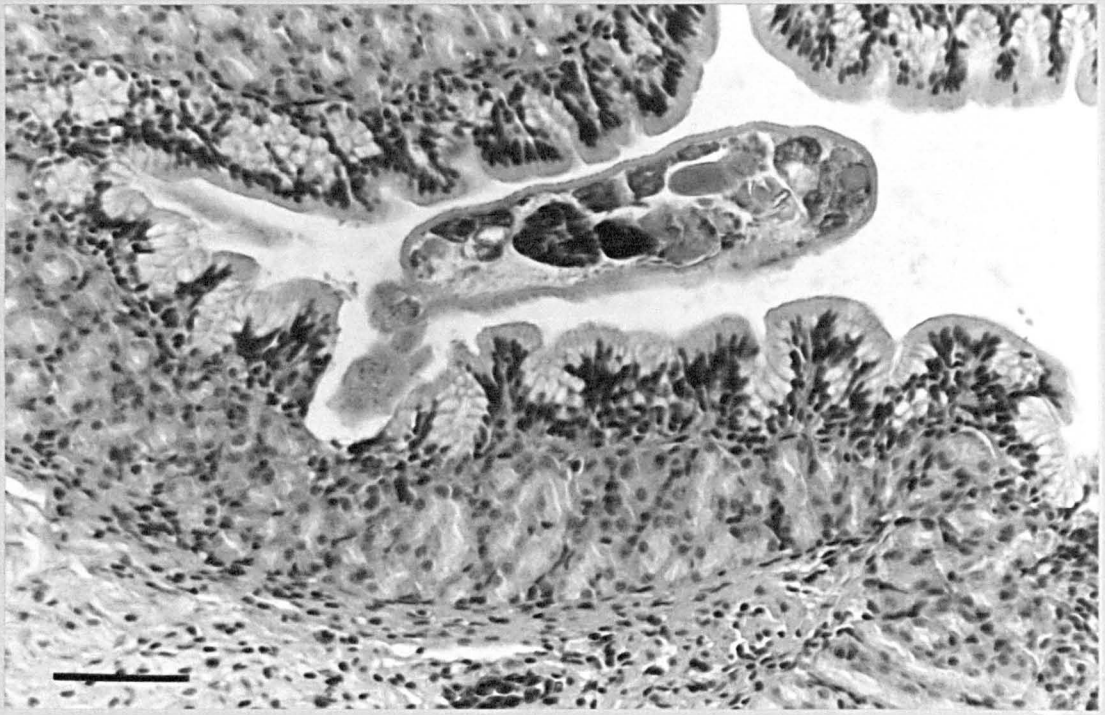


Figure 3.8: An *Enterogyrus papernai* worm probably recently attached to the site, thus showing less host response (H & E). Scale bar = 50 μm.

Figure 3.9: An *Enterogyrus papernai* worm probably staying in the attached site for a long period, thus showing massive host response (area bounded with dotted lines; note the feeding damage (arrow) (H & E). Scale bar = 50 μm.

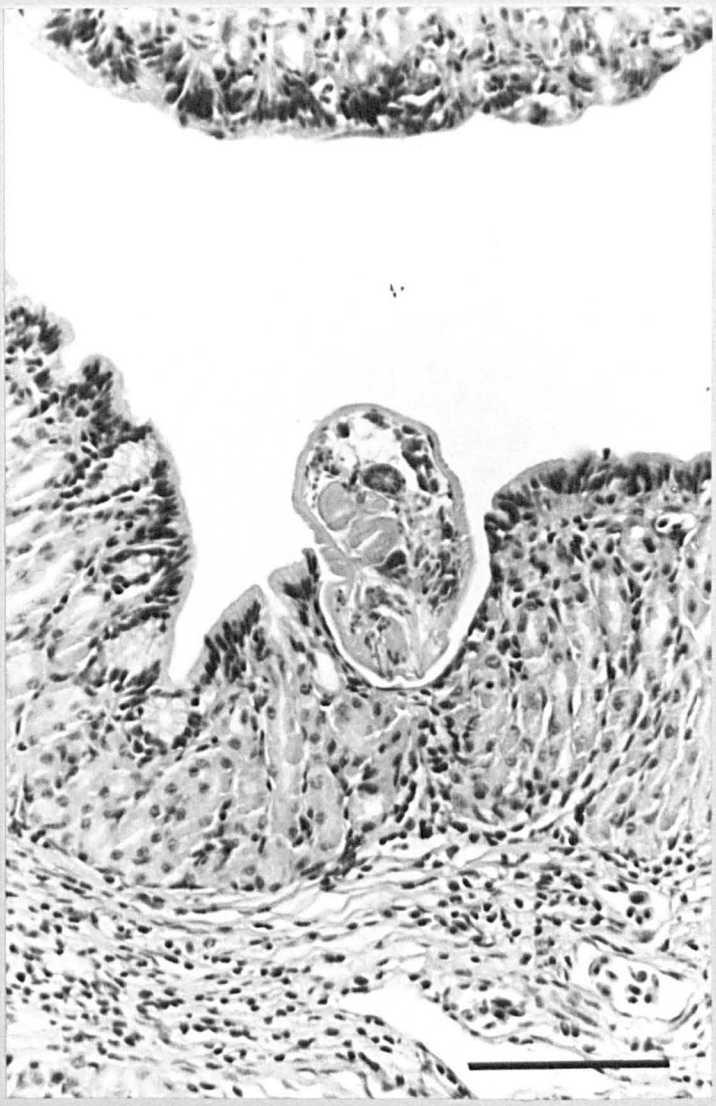
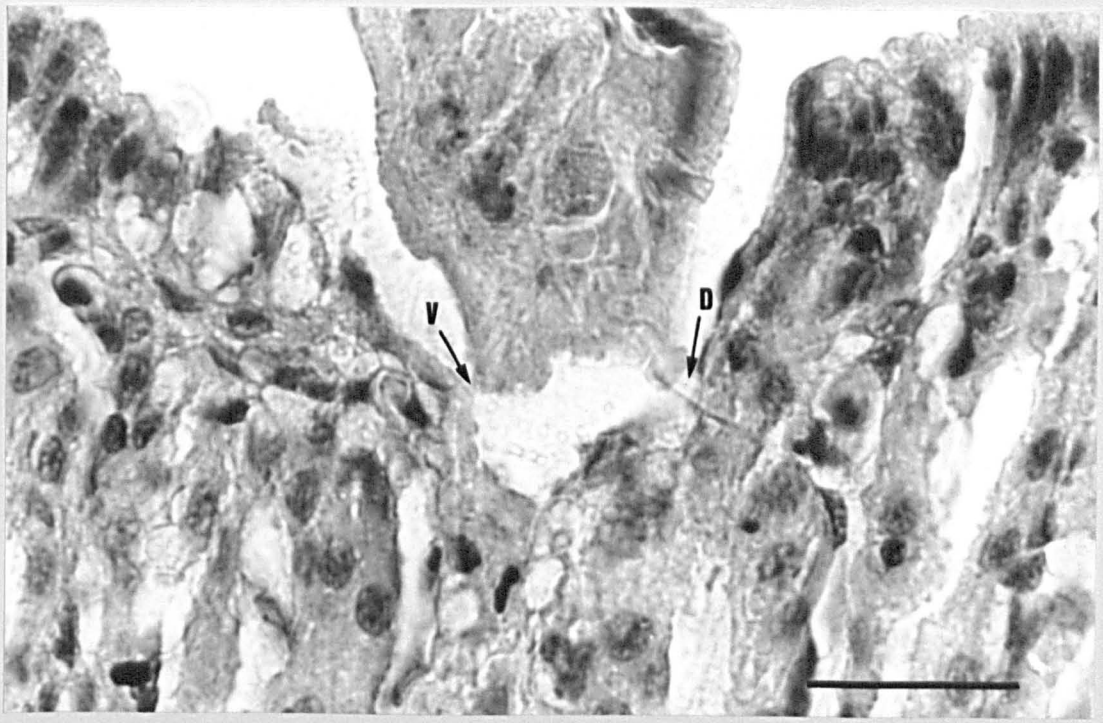


Figure 3.10: The attachment of *Enterogyrus globidiscus* (H & E). Scale bar = 20 µm. D = dorsal hamulus V = ventral hamulus.

Figure 3.11: The attachment of *Enterogyrus papernai*; note the posterior portion of the opisthaptor bearing ventral hamuli holding the host tissue (H & E). Scale bar = 20 µm.


V
D

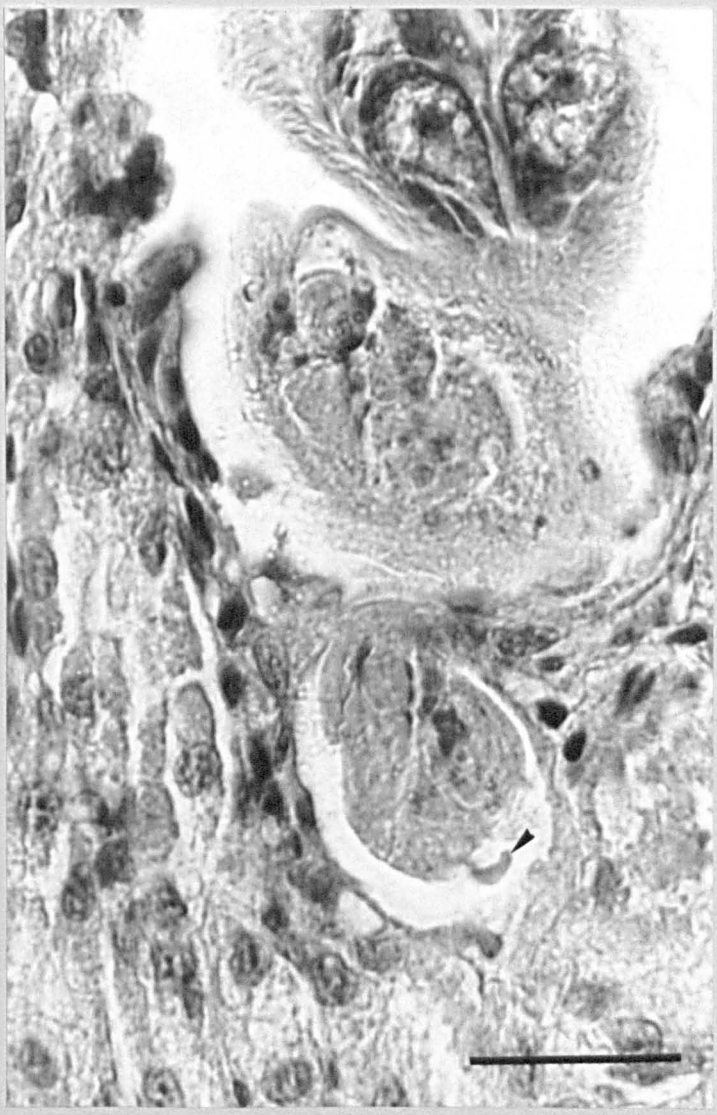


Figure 3.12: SEM view of an *Enterogyrus globidiscus* worm; note the extent of the haptor penetration into the host tissue. Scale bar = 10 µm.

Figure 3.13: SEM view of an *Enterogyrus papernai* worm. A considerable part of the body of the worm is embedded. Scale bar = 10 µm.

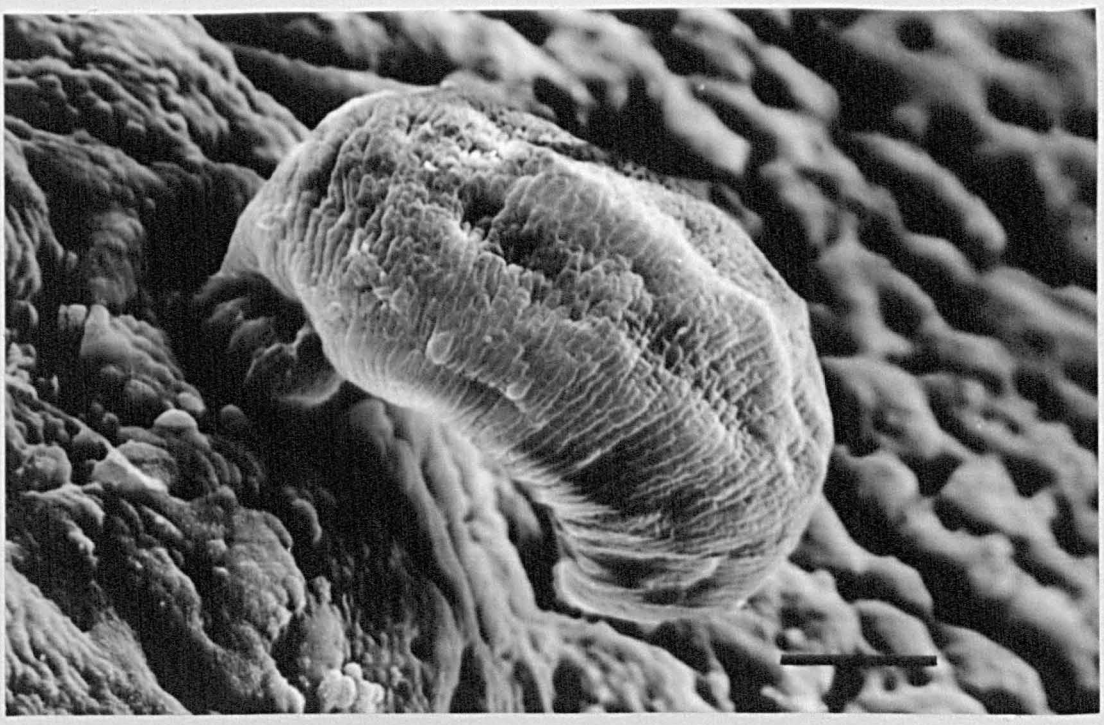
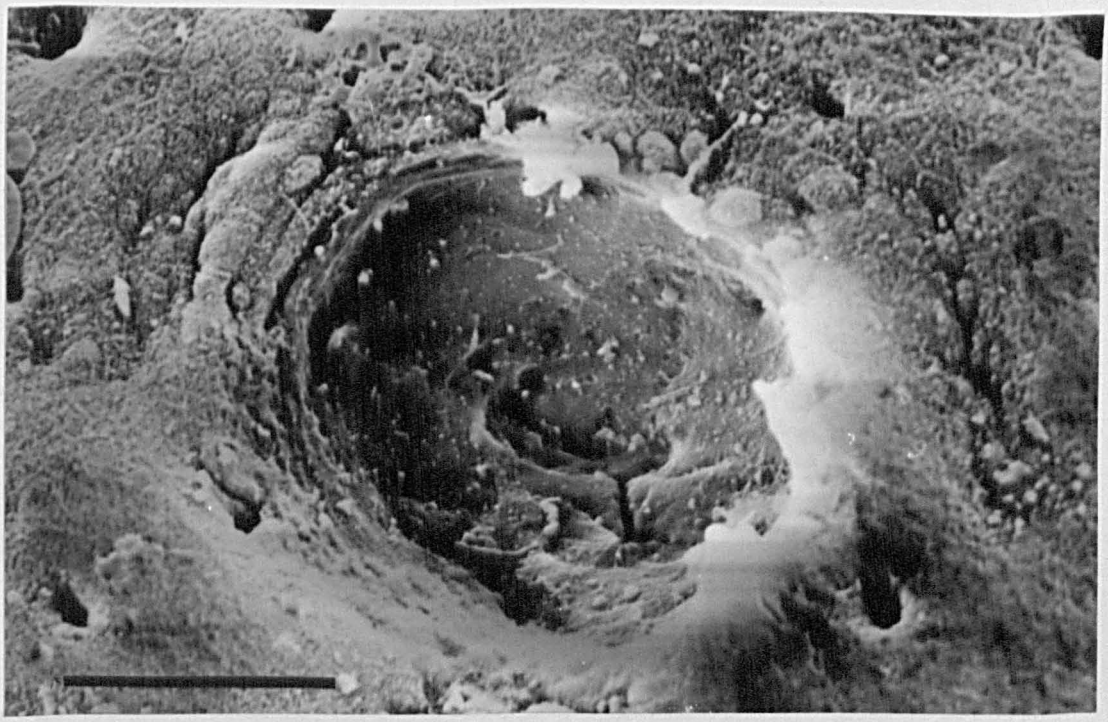
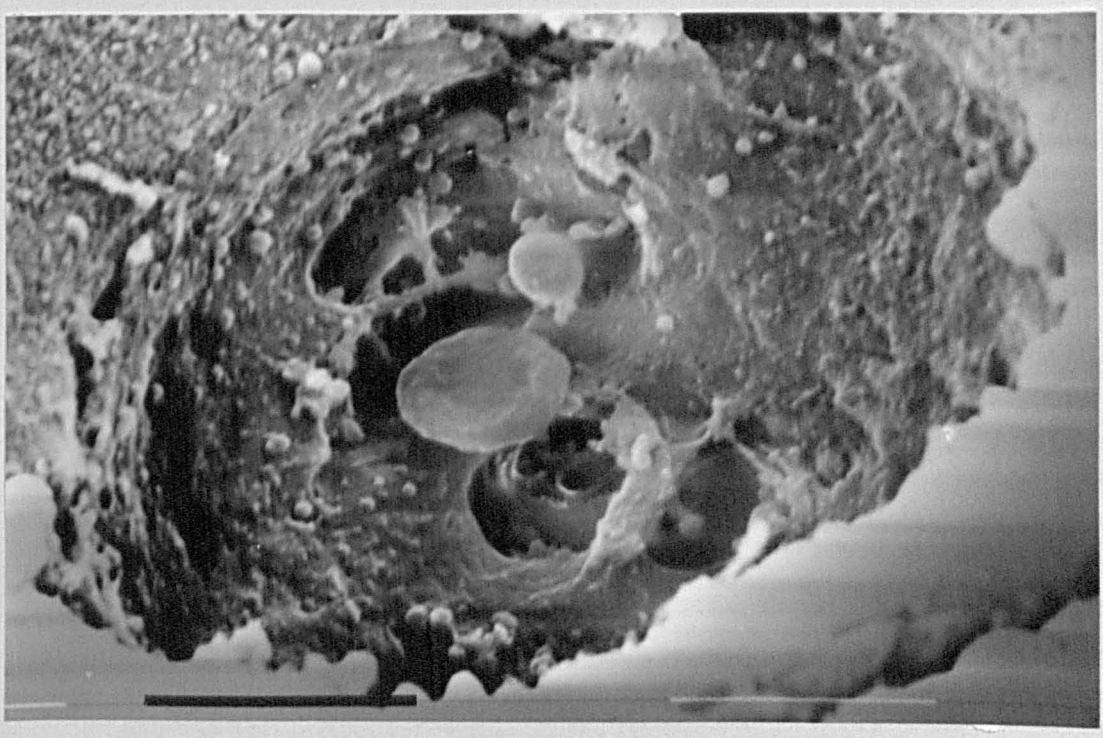


Figure 3.14: A depression in the host tissue with two sharp cuts in the base. Scale bar = 10 µm.

Figure 3.15: A depression in the host tissue with four holes in the base. Scale bar = 10 µm.





3.3.2. Reproductive Biology.

3.3.2.1. The egg formation and oviposition behaviour.

The summary of the behaviour of the 10 *E. globidiscus* worms observed is given below. The oviposition behaviour of *E. papernai* worms was difficult to observe as they were comparatively smaller and due to the low population numbers it was difficult to locate egg laying worms.

In general, *E. globidiscus* worms lay stationary, displaying only searching behaviour for food location. Occasionally under microscopic observation they changed the site of attachment and moved to another site. The worms became stationary at least 4-5 minutes before the ovum became lightly visible in the ovo-vitelline duct with vitelline cells. The ovum was in this position for about 1-2 minutes. Then it started to move forward along the duct with vitelline cells as a shapeless mass. When the mass moved the first 1/3 of its way, spending 4-5 minutes moving, the movement stopped. The mass seemed to be shaken around this point for about 3-4 minutes. After a lag phase of about 2 minutes the mass appeared to be a little more rigid with a lightly tanned shell giving the shape of an egg. Due to this function, this location was considered as the ootype.

With this more rigid and lightly tanned appearance, it started to move along the uterus. When it reached about 1/2 of the length of the uterus, after about 5-7 minutes, it was well tanned with a brown colour and had acquired the rigid constant shape. When the egg became gradually hardened, the worm, which had shown random searching and resting behaviour earlier, started to assist the egg movement by pushing movements. This movements occurred as peristaltic movements, starting 2/3 away from anterior region, posterior to the anterior direction. Around 5-11 minutes were required for the movement along the final 1/2 of the length of the uterus. The longer duration was required as, sometimes, the worm rested in the middle of this pushing behaviour for short periods of 2-4 minutes.

When the egg was at the genital pore, the worm started bending movements which lasted for about 1-2 minutes. When the egg was released, the worm returned

to the straightened position and rested for about 3-4 minutes. Then, again the normal behaviour pattern, the mixture of searching and resting behaviour started. Some worms moved to other sites a little later. The shortest time the worms stayed in an egg laying site after laying an egg was 4 minutes.

Altogether it took about 20-30 minutes for an egg to be laid from the time of its appearance as an ovum in the ootype.

3.3.2.2. In vitro oviposition rate.

Rates at pH 7 and pH 4.5.

Experiment 1

None of the 20 worms from each species, left in saline of pH 4.5 and 7 laid eggs. Active and non egg bearing worms were selected for the experiment, no eggs were formed within worms during the six hour period of observation. Due to the failure of this experiment, the only method which would allow the calculation of the rate separately for the two species had to be abandoned.

Experiment 2

The findings of the second experiment on in vitro oviposition rates are given in Table 3.5. In this experiment, as the worms could not be separated, the rates were calculated considering two species together. The oviposition rates were 0.069 eggs $worm^{-1}hour^{-1}$ at the pH 7.0 and 0.072 eggs $worm^{-1}hour^{-1}$ at pH 4.5, indicating that a worm lays an egg 14.5 hours after the previous one at pH 7.0 and 13.9 hours at pH 4.5. When compared the overall oviposition rates for 6 hours for the two pH values, there was no difference with a probability of 0.84 suggesting that pH values in the medium have not affected the oviposition rates.

Constant oviposition rates could not be maintained by the worms throughout the experiment and the hourly rates for six hours were very low. During the first hour they laid the eggs formed before they were removed from the stomach. Most of the eggs laid later, were also formed within the first hour after removal from the stomach. These eggs were formed in worms which were attached to the pieces of broken tissue

Table 3.5 : In vitro egg laying rates of the two *Enterogyrus* species together for 6 hours at the pH values 7.0 and 4.5.

pH = 7.0

Egg laying rates at each hour No worm^{-1}hour^{-1}

Hour	Petri-dish number					Mean ± SD
	1	2	3	4	5	
1	0.049	0.013	0.220	0.204	0.177	0.133 ± 0.095
2	0.138	0.106	0.136	0.144	0.159	0.137 ± 0.019
3	0.008	0.013	0.017	0.028	0.009	0.015 ± 0.008
4	0.000	0.000	0.000	0.000	0.009	0.002 ± 0.004
5	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.000
6	0.000	0.000	0.000	0.006	0.000	0.000 ± 0.000
Rate for the whole period	0.054	0.046	0.082	0.083	0.080	0.069 ± 0.018
No. of worms	123	160	59	181	113	

pH = 4.5

Egg laying rates at each hour No worm^{-1}hour^{-1}

Hour	Petri-dish number					Mean ± SD
	1	2	3	4	5	
1	0.106	0.129	0.031	0.190	0.216	0.135 ± 0.073
2	0.141	0.089	0.134	0.135	0.284	0.157 ± 0.074
3	0.000	0.008	0.021	0.024	0.014	0.013 ± 0.010
4	0.000	0.008	0.000	0.000	0.000	0.002 ± 0.004
5	0.000	0.000	0.010	0.000	0.000	0.002 ± 0.005
6	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.00
Rate for the whole period	0.059	0.065	0.043	0.078	0.115	0.072 ± 0.027
No. of worms	85	124	97	126	74	

which therefore continued feeding. The reduction in the oviposition rates in later hours and the fact that eggs were formed in the first hour only, strongly suggested that the unavailability of food in the medium may be the reason.

In contrast to the observations in the Section 3.3.2.2 (Experiment 1) where the worms remained attached to the stomach tissue, the time period taken by some worms in this experiment for an egg to be laid, once it was formed, was very long. Therefore it seems that they require an attachment to perform the necessary movements to lay eggs. In addition to the unavailability of food, the absence of the substratum may have affected the oviposition rates.

During the time period of six hours, the worms became gradually inactive, the worms unattached to debris earlier than the attached worms. Because of the shortcomings, especially the absence of food and substratum, it was decided that the oviposition rates are not reliable. Even the conclusion that the pH values in the medium have no affect on the oviposition rates was doubtful and thus oviposition was studied in vivo.

3.3.2.3. In vivo oviposition rates.

(1) With live fish.

The in vivo oviposition rates of *Enterogyrus* spp. were 0.122 eggs worm^{-1}hour^{-1} (2.928 eggs worm^{-1}day^{-1}, ≈3 eggs worm^{-1}day^{-1}) showing that the time gap between laying the two consecutive eggs was approximately 8 hours (Table 3.6A). This shows that the in vitro egg laying rates obtained, approximately 0.070 eggs worm^{-1}hour^{-1} at the pH 4.5 and 7.0 were generally low and confirmed that there were shortcomings in the in vitro method.

(2) With *Enterogyrus* spp. on excised stomachs.

Influence of tissue degradation on egg laying.

The results of the sign test applied to the differences in oviposition rates at each hour for 8 hours are given in the Table 3.7A. The results reveal that the oviposition rates for the first four hours were not significantly different from each

Table 3.6 : The comparison of the egg laying rates of *Enterogyrus* spp. when in live fish and when in excised stomachs.

Table 3.6A : In vivo egg laying rates of the two *Enterogyrus* species together, when in live fish.

Tank number	Population of 3 fish	Number of eggs laid within 24 hours	Rate of egg laying Eggs worm^{-1}hour^{-1}
1	64+ 52+ 38 = 154	408	0.110
2	80+ 62+ 70 = 212	548	0.108
3	62+ 86+ 42 = 190	608	0.133
4	52+ 62+ 92 = 206	638	0.129
5	108+ 66+ 54 = 228	494	0.090
6	82+ 52+ 68 = 202	478	0.099
7	74+ 65+142 = 281	804	0.119
8	54+ 75+ 45 = 174	664	0.159
9	32+ 73+ 65 = 171	546	0.133
10	109+ 84 +37 = 230	782	0.142
Mean rate ± SD			0.122 ± 0.021

Table 3.6B : The egg laying rates of *Enterogyrus* species on excised stomachs.

(i) Fish treated the same as the live fish used to find out the egg laying rates and killed at the same time.

(ii) Fish just brought from the natural environment.

Fish serial No.	Experiment (i)			Experiment (ii)		
	No. of worms	No. of eggs laid (4 hours)	Rate Eggs worm^{-1} h^{-1}	No. of worms	No. of egg laid (4 hours)	Rate Eggs worm^{-1} h^{-1}
1	48	41	0.214	137	151	0.276
2	70	54	0.193	96	130	0.339
3	59	68	0.288	54	83	0.384
4	84	78	0.232	182	264	0.363
5	53	49	0.321	117	165	0.353
6	76	104	0.342	92	106	0.288
7	63	48	0.190	66	100	0.379
8	47	37	0.197	89	87	0.244
9	56	74	0.330	110	131	0.298
10	36	45	0.313	64	89	0.348
Mean ± SD			0.253 ± 0.059			0.327 ± 0.047

Table 3.7 : Influence of tissue degradation on egg laying.

Table 3.7A : The number of eggs laid by the stomach monogeneans on excised stomachs for 8 successive hours.

Stomach	Number of monogeneans present on excised stomachs	Number of eggs laid in each hour							
		1	2	3	4	5	6	7	8
1.	99	14	9	7	2	6	5	7	3
2.	102	11	8	6	3	2	7	3	9
3.	110	17	10	15	3	5	1	5	1
4.	79	15	18	13	17	9	12	4	5
5.	287	30	14	25	33	4	9	4	3
6.	202	52	21	27	39	3	3	8	7
7.	60	14	9	8	5	1	1	2	0
8.	90	14	25	16	1	0	2	1	10
9.	162	14	69	16	22	4	2	4	3
10.	160	21	31	20	8	9	5	4	7
11.	122	28	36	16	9	4	3	1	0
12.	183	45	39	22	12	8	2	3	2
13.	92	26	38	20	15	9	3	5	6
14.	108	47	36	25	14	15	8	3	5
15.	58	16	24	13	8	2	5	4	0

Table 3.7B : The results of the sign test on the comparison of egg laying rates during 8 hours to find out which hours have the same egg laying rates. The probability value for 95 % level was 0.0018 and for 99 % level was 0.0004.

Hour	1	2	3	4	5	6	7
2	1.0000 ns						
3	0.0074 ns	0.0352 ns					
4	0.0352 ns	0.0074 ns	0.1185 ns				
5	0.0001 * **	0.0001 * **	0.0001 * **	0.1185 ns			
6	0.0001 * **	0.0001 * **	0.0010 *	0.0352 ns	0.5811 ns		
7	0.0001 * **	0.0001 * **	0.0001 * **	0.0225 ns	1.0000 ns	1.0000 ns	
8	0.0001 * **	0.0010 *	0.0010 *	0.0352 ns	0.0352 ns	1.0000 ns	0.6072 ns

ns - non significant at 95 % level, * - significantly different at 95 % level, ** - significantly different at 99 % level.

other at 95% level and those of 1-3 hours were different from 5-8 hours. Only the rate of the fourth hour was similar with that of fifth and sixth at 95% level (Table 3.7B).

The oviposition rates of the stomach monogeneans on excised tissue lessened with the time. Monogeneans present on exised stomachs were almost certainly affected by post-mortem changes in the tissues leading to the gradual disappearance of the latter through conversion of non-assimilable products. According to the results it was decided to leave the stomachs for not more than 4 hours to find out the oviposition rates.

Oviposition rate on excised stomach tissue.

(i) Fish treated the same as the fish used in the Section 2.3.2.3.(1) (rate with live fish) and killed at the same time.

With this experiment, the oviposition rate obtained within the time period 10.00-14.00 hours (at 12.00 hour) was a mean of 0.253 eggs worm^{-1} hour^{-1} (Table 3.6B(i)) indicating that it takes only about 4 hours for the second egg to be laid after the first.

This showed a greater difference between the two methods. The method applied in this experiment gave a twice as high rate as the method of finding oviposition rates using parasites living inside the live fish (Section 3.3.2.3(1)). There is a diurnal variation of egg laying (the rate at 12.00 noon is about 158.37 % higher than the normal daily rate; see Table 3.6B(i). When the correction is made, the value should be 0.160 eggs worm^{-1}hour^{-1} (3.834 eggs worm^{-1}day^{-1}, 6 1/4 hours in between laying two eggs), which is still higher than the rate obtained with parasites in live fish.

(ii) Fish just brought from the natural environment.

The oviposition rate obtained within the time period 10.00-14.00 hours (at 12.00 hour) with the parasites freshly brought from the lagoon was highest with the rate of 0.327 eggs worm^{-1}hour^{-1} (Table 3.6B(ii)) indicating that the time gap between laying two consecutive eggs was approximately 3 hours. The difference suggests a possibility that the aquarium conditions creates an unfavourable situation for the

parasites, possibly through the physiological changes occurring in host, thus lowering the rate of egg production.

The correction of the result at 12.00 hour for the diurnal variation gave the rate 0.206 eggs worm^{-1}hour^{-1} or 4.955 eggs worm^{-1}day^{-1} and 5 4/5 hours in between laying two eggs.

3.3.2.4. Factors affecting the oviposition rate.

(1) Effect of leaving fish in the aquarium.

The oviposition rates of *Enterogyrus* spp. obtained while the fish of the two size groups were kept in the aquarium are given in the Table 3.8 and Figure 3.16. The graph clearly shows that the oviposition rates vary very much between the two size groups of fish (probability 0.002). The comparison of the oviposition rates on the same days by t-test revealed that the rates were mostly similar in the first two days but deviated later. The rates obtained for small fish were mostly similar throughout the experimental period (probability 0.019) with the exception of the third day. But the drop in the rates of the large group of fish was clearly evident from the sixth day to the final day, day 15 (probability 0.000). But, towards the end of the experiment the rates seemed to be recovering.

The major difference observed between the two groups of fish was the difference in feeding, where the small fish took food from the beginning, the large group of fish took food later and very small quantities. The effect of the feeding ration of fish on the oviposition rate of the parasite is discussed under the Section 3.3.2.4(5)A. The feeding may not be the sole factor responsible for this variation. The other possibilities are the physiological and behavioral changes of fish which effect the environment of the parasite thus affecting the oviposition rates.

(2) Diurnal variation.

The diurnal variations of the oviposition rates of parasites in (i) fish freshly brought from the lagoon and (ii) fish left for 2 weeks in the aquarium are given in the Table 3.9. A similar rhythm of diurnal variation was evident in both the cases (Figure

Table 3.8 : The effect of leaving fish in the aquarium on the egg laying rates of the *Enterogyrus* spp. in two size ranges of fish.

The day of examination	Egg laying rates Eggs/worm/hour (Mean ± SD)		Probabilities of the comparison of days by t-test
	Fish size equal to or higher than 10 cm (n = 6)	Fish size equal to or higher than 8 cm and less than 10 cm (n = 6)	
0	0.300 ± 0.065 [b]	0.259 ± 0.018 [b]	0.200 ns
3	0.236 ± 0.032 [b]	0.228 ± 0.025 [ab]	0.660 ns
6	0.134 ± 0.040 [a]	0.191 ± 0.010 [a]	0.020 *
9	0.110 ± 0.032 [a]	0.211 ± 0.038 [ab]	0.001 * **
12	0.095 ± 0.008 [a]	0.215 ± 0.056 [ab]	0.004 * **
15	0.153 ± 0.013 [a]	0.223 ± 0.010 [ab]	0.000 * **
Overall	0.171 ± 0.082	0.221 ± 0.036	0.002 * **
Probability-comparison of different days	0.000 * **	0.019 *	

Figure 3.16 : Effect of leaving fish in the aquarium on oviposition

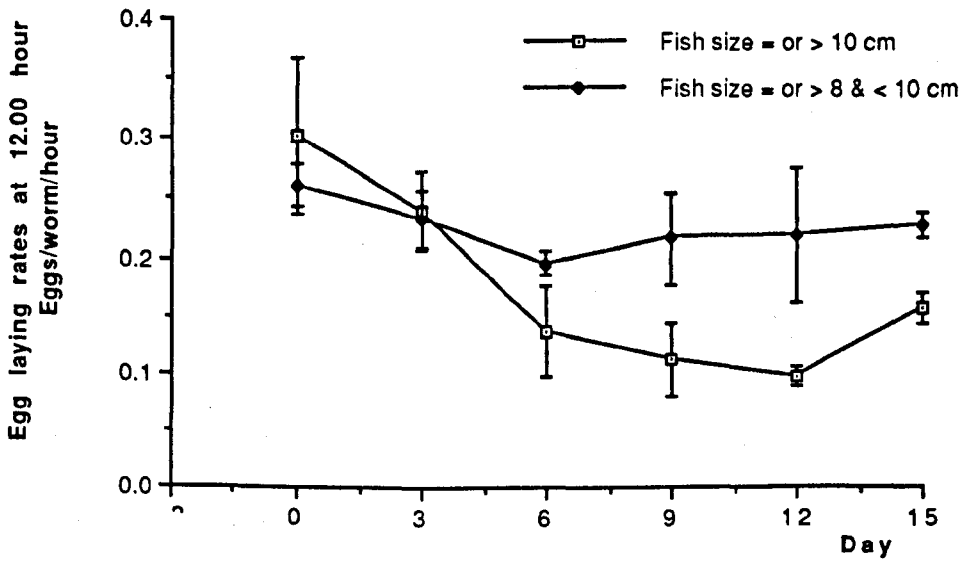


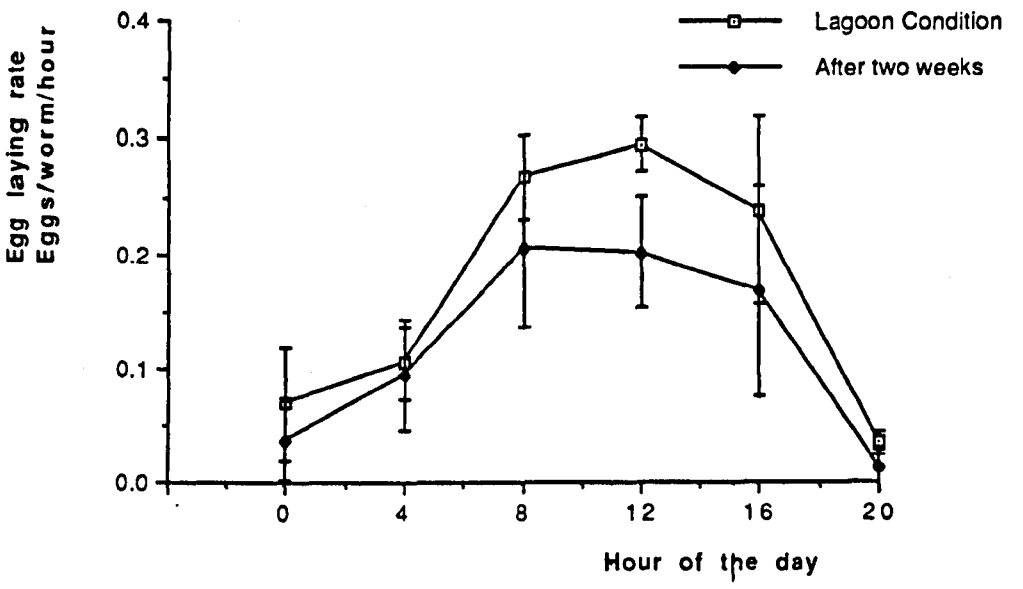
Table 3.9 : The diurnal variation of the egg laying rates of the *Enterogyrus* spp., in fish just brought from the lagoon and the fish left for 2 weeks in the aquarium.

Hour of the day	Egg laying rates, eggs/worm/hour (Mean ± SD)		Means of the two experiments	Probabilities of the comparison of hours by t-test
	Fish just brought from lagoon (n = 6)	Fish left in aquarium for 2 weeks (n = 5)		
0	0.069 ± 0.050 ^{a}	0.038 ± 0.034 ^{a}		0.250 ns
4	0.104 ± 0.032 ^{a}	0.094 ± 0.048 ^{ab}		0.710 ns
8	0.264 ± 0.036 ^{b}	0.203 ± 0.067 ^{b}		0.130 ns
12	0.291 ± 0.023 ^{b}	0.199 ± 0.047 ^{b}	0.245	0.010 * **
16	0.235 ± 0.081 ^{b}	0.165 ± 0.091 ^{b}		0.220 ns
20	0.034 ± 0.010 ^{a}	0.012 ± 0.017 ^{a}		0.051 ns
Overall	0.166 ± 0.110	0.119 ± 0.092	0.143	0.060 ns
Probability - comparison of different hours	0.000 * **	0.000 * **		

ns- non signisicant; * - significantly different at 95% level; ** - Significantly different at 99% level.

The egg laying rate at 12.00 hours is 158.37 % higher than the overall rate calculated per the day considering diurnal variation.

Figure 3.17 : The diurnal variation of the egg laying rates.



3.17). The rates in the day time (8, 12 and 16 hours) were higher and significantly different from the rates of the night time. The only disagreement is the statistically insignificant rate at hour 16 with the hour 20, in the case of fish left in the aquarium for 2 weeks.

The diurnal rates of the two group of fish were similar with a probability of 0.060 when the rates at same hours were compared with the t-test. Only the rate at 12 hour showed a difference when the rates at the same hours were compared. This shows that the natural oviposition rate is regained by the fish left for 2 weeks in the aquarium. This confirms the results of the previous Section (3.3.2.4(1)) which showed that the oviposition rates of the parasites were regained after leaving the fish for 2 weeks in the aquarium. When considering the two groups of fish together, the oviposition rate at 12.00 hour is 158.37 % higher than the overall daily rate.

(3) Effect of temperature.

The worms were able to lay eggs at the same rate in the temperature of 23 and 29°C, but at a significantly lower rate (probability 0.001) at the temperature 35°C (Table 3.10 and Figure 3.18). This suggests clearly that the temperature of 35°C, which is higher than the natural environmental temperature, (the range is 27.5-31.0°C) affects the rate of reproduction, whilst lower temperatures such as 23°C have no effect. Probably the worms are not adapted to higher temperatures and spend more energy on general metabolic reaction rather than on reproduction.

(4) Effect of pH.

The results in the Figure 3.19 illustrate that there is an effect of pH on the oviposition rate. The graph was plotted using the mean pH values of the stomach walls as measured half way through the experimental period. The Table 3.11 shows that the mean pH values of the stomach walls were different from the pH values in the saline medium. Therefore, it was assumed that the worms were experiencing the pH on the stomach wall.

The rates of oviposition were not significantly different at pH values 3.62 and

Table 3.10 : The effect of temperature on the egg laying rates of *Enterogyrus* spp.

Temperature in °C	Temperature variation	Egg laying rates at 12 hour Eggs/worm/hour (Mean ± SD) n = 8
23	22.4 - 24.2	0.226 ± 0.066[b]
29	28.5 - 29.5	0.239 ± 0.071[b]
35	34.6 - 36.0	0.077 ± 0.040[a]
Probability		0.001 * **

Figure 3.18: Effect of temperature on the egg laying rate.

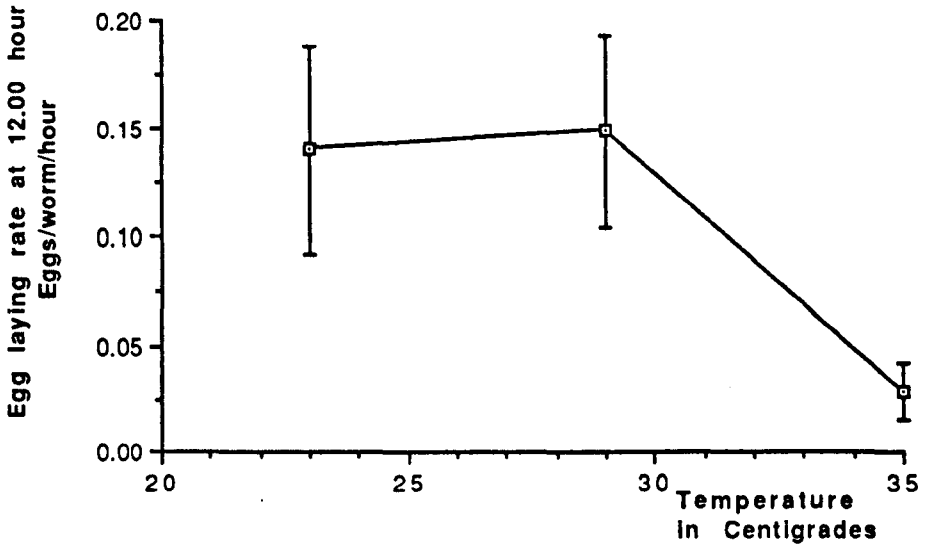
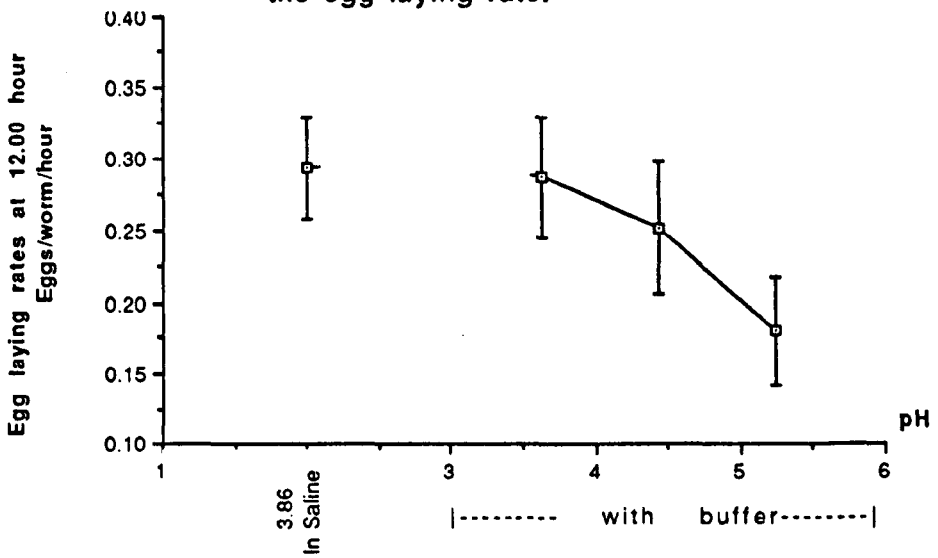


Table 3.11 : The effect of pH on the egg laying rates of *Enterogyrus* spp.

pH	The mean pH of the inner wall of the stomachs at the half time of the experiment duration	Egg laying rates at 12 hour Eggs/worm/hour (Mean ± SD) n = 8
Natural†	3.86	0.293 ± 0.036[b]
3.6	3.62	0.287 ± 0.041[b]
4.6	4.43	0.252 ± 0.046[b]
5.6	5.24	0.180 ± 0.037[a]
Probability		0.000 * **

† stomachs in saline without any buffer

Figure 3.19 : Effect of pH of the surrounding medium on the egg laying rate.



4.43 obtained with buffer and saline, and the pH value 3.86 which existed on the stomach walls which were left in saline without buffer. It was only the pH value 5.24 which gave a significantly (probability 0.000) lower rate. It seemed that there was no particular effect exerted by the acetate buffer on the oviposition rate, as the stomachs left only in saline and with similar pH to the stomachs left in the buffer gave similar rates.

(5) Effect of feeding.

A. Effect of feeding rate.

The results of the oviposition rates of parasites obtained by feeding the host with different rations of feed are given in the Table 3.12. The graph in Figure 3.20 shows a visible reduction of the oviposition rates when the feeding ration was reduced. However the relationship was not significant, having a probability of 0.082 probably due to the very high variation. The oviposition rate was not zero for the worms in unfed fish, suggesting that the worms are capable of reproducing, even when the outside food source is absent and may utilise another food source within the stomach, if it is required.

B. Effect of Glucose in the surrounding medium.

This experiment examine whether the worm could utilise an external source of energy. The effect of different concentrations of Glucose in the surrounding medium in vitro (Table 3.13) is illustrated in the Figure 3.21. The glucose concentrations between 0.005-0.050 M gave similar oviposition rates which did not differ from the control, whilst the concentration 0.100 M drastically decreased the rate during first 4 hours. During the next four hours the egg producing rate at all glucose concentrations and control were lower and were similar.

Therefore, according to the results, it was evident that the glucose present in the medium up to 0.050 M could not exert any enhanced effect on the egg laying rates and the higher concentration of 0.100 M caused a clear adverse effect, reducing the oviposition rates drastically. This suggests that glucose in the surrounding medium was not helpful to maintain the egg producing rates.

Table 3.12 : The effect of feeding ration of fish on the egg laying rate of *Enterogyrus* spp.

Feeding ration as a percentage of the body weight of fish	Egg laying rates at 12 hour Eggs/worm/hour (Mean ± SD) n = 6
10.0	0.194 ± 0.048
5.0	0.179 ± 0.046
2.5	0.159 ± 0.046
1.0	0.146 ± 0.058
0.0	0.114 ± 0.047
Probability	0.082 non significant

n= the number of fish used for each treatment

Figure 3.20 : Effect of feeding rate of fish on the egg laying rates of the parasite

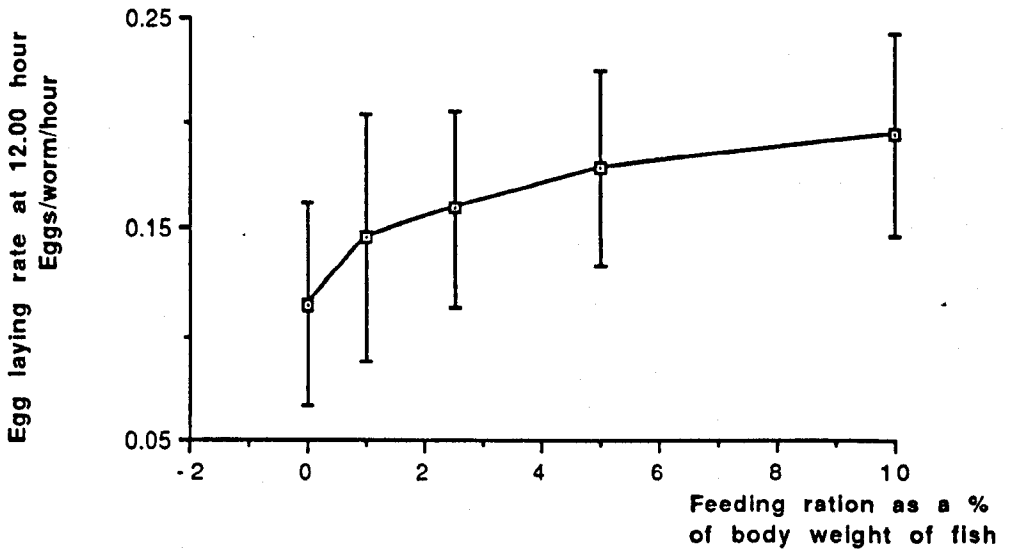


Table 3.13 : The effect of glucose concentration in the surrounding medium on the egg laying rates of *Enterogyrus* spp.

Glucose concentration in moles	Egg laying rates at 12 hours Eggs/worm/hour (Mean ± SD) n = 6		
	Between 0-4 hours	Between 4-8 hours	For 0-8 hours
0.000	0.216 ± 0.038[b]	0.065 ± 0.017	0.141 ± 0.017[b]
0.005	0.240 ± 0.046[b]	0.069 ± 0.033	0.155 ± 0.039[b]
0.010	0.233 ± 0.040[b]	0.066 ± 0.013	0.150 ± 0.019[b]
0.050	0.223 ± 0.047[b]	0.051 ± 0.014	0.137 ± 0.025[b]
0.100	0.041 ± 0.019[a]	0.035 ± 0.024	0.038 ± 0.011[a]
Probability	0.000 * **	0.053	0.000 * **

Figure 3.21 : Effect of glucose concentration in the surrounding medium on the egg laying rate

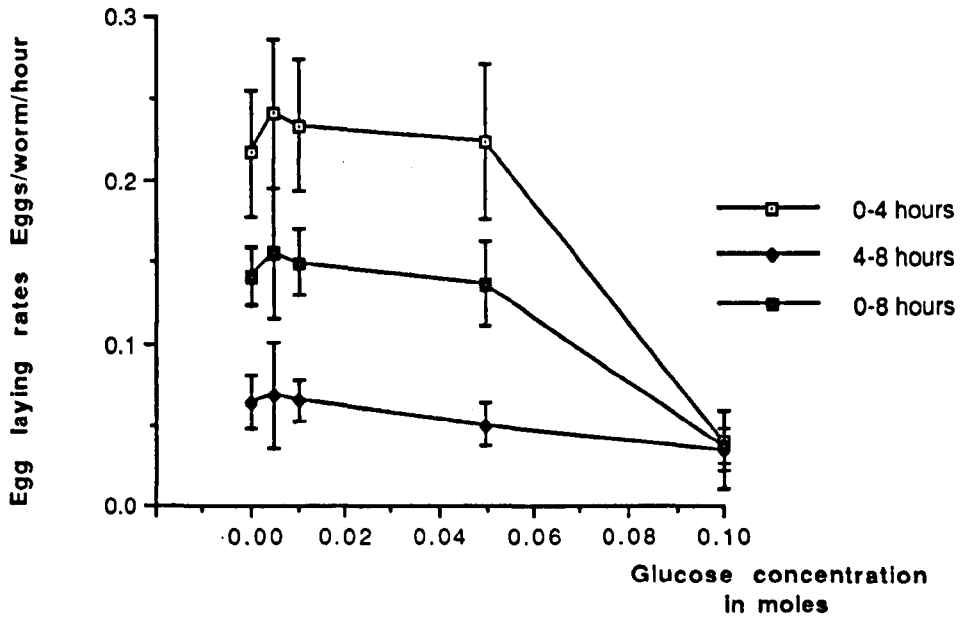
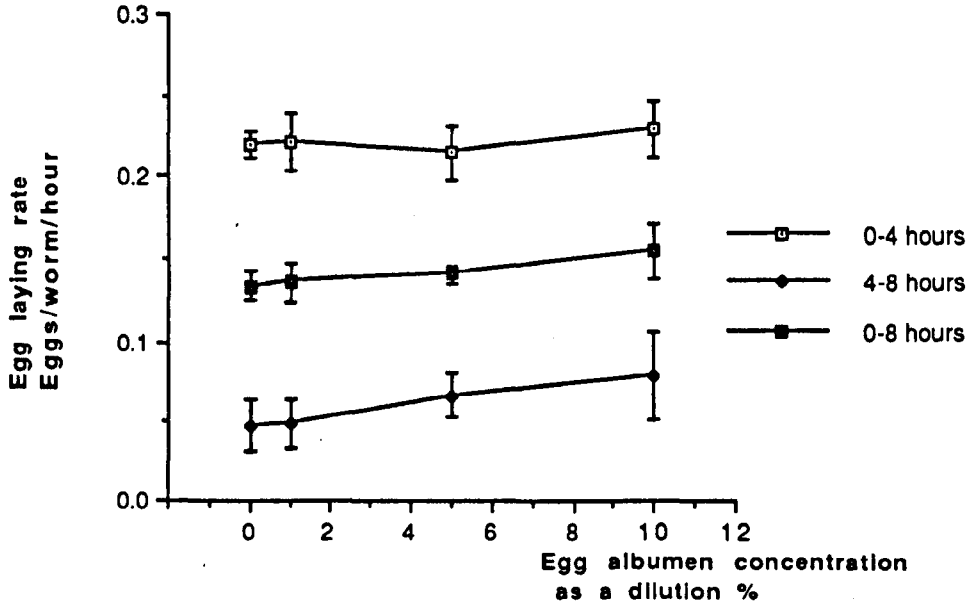


Table 3.14 : The effect of egg albumen concentration in the surrounding medium on the egg laying rate of *Enterogyrus* spp.

Fresh chick egg albumen concentreation as a dilution %	Egg laying rates at 12 hours Eggs/worm/hour (Mean ± SD) n = 6		
	Between 0-4 hours	Between 4-8 hours	For 0-8 hours
0	0.219 ± 0.008	0.048 ± 0.016[a]	0.133 ± 0.009[a]
1	0.221 ± 0.018	0.049 ± 0.016[a]	0.135 ± 0.012[a]
5	0.214 ± 0.017	0.067 ± 0.013[ab]	0.140 ± 0.006[ab]
10	0.229 ± 0.017	0.079 ± 0.027[b]	0.154 ± 0.016[b]
Probability	0.413 ns	0.022 *	0.022 *

ns - non significant, * - significantly different at 95% level, ** - significantly different at 99% level.

Figure 3.22 : Effect of egg albumen concentration in the surrounding medium on the egg laying rate



C. Effect of egg albumen in the surrounding medium.

As an alternative to a carbohydrate source of food, a protein source in the external medium was tested. The effect of the tested egg albumen concentrations, 0-10 % dilutions of the concentration existing in chick eggs, on the oviposition rates for 8 hours is presented in the Table 3.14 and the Figure 3.22.

The oviposition rates gained during the first four hours were similar with all the concentrations tested. During the next four hours the rates of all the concentrations dropped considerably. But, within this time period the rates obtained for different concentrations differed significantly at 95 % level (see Table 3.14). The effect of the concentration 0 & 1 % were similar and low compared to that of 10 % and the effect of 5 % was intermediate. When the whole 8 hour period considered together, the same effect was evident.

Therefore, it seems that egg albumen in the medium, in the dilutions up to 10 %, can not enhance the oviposition rates considerably. However, a small but significant enhancement was shown at 10 %. Since this was the highest concentration tested, it would be worthwhile testing concentrations above this.

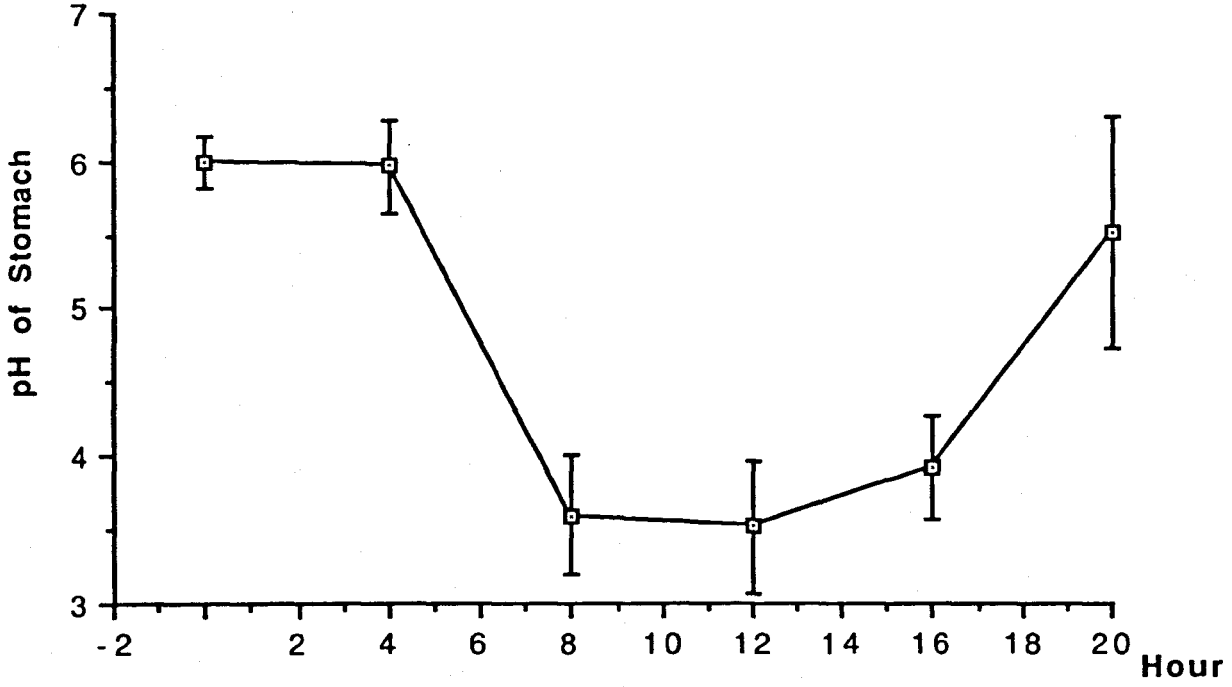
3.3.2.5. Egg viability and location of development.

(1) The ability of the eggs to develop in the stomach.

The diurnal variation of pH in the stomachs was found to be very high (Figure 3.23). During the day, its range lay between pH 3-4, whilst the range at night was between pH 5 and 6. The results of the experiment to determine the effect of pH on egg development (Section 3.3.3.1(3)c) showed that egg development is arrested at pH values below 6. Thus, for most of the day, approximately 14 hours (Figure 3.23) the prevailing pH of the stomachs were not suitable for egg development.

When the development stages of the eggs in stomachs were assessed all the eggs were in the non-transparent stage. Only two eggs in the later developmental stages were found during the whole experimental period on stomach monogeneans.

Figure 3.23 : The diurnal variation of the stomach pH.



(2) Route of egg release into the environment.

The observation of gills (devoid of gill monogeneans) revealed that there is no *Enterogyrus* egg retention on gills.

When the number of egg bearing worms in the stomachs and oesophagus were counted as soon as the stomachs were opened the counts revealed that the numbers in the anterior region was significantly lower than the numbers in posterior region with a probability of 0.02 (Table 3.15). No worms were found in the oesophagus. This suggests that the worms lay eggs in their normal attachment sites and do not move anteriorly towards the opening of the stomach nearest to gills to lay eggs.

The number of egg present in different gut regions at four hour intervals of the day and the gut fullness of these regions as a percentage of the volume of the lumen are given in the Figure 3.24. If the rate at which the gut contents were passing through had been known (the rate may not be similar at different times of the day and through different regions of the gut), with the help of the oviposition rates, rough estimations could have been made to see whether all the eggs laid were passed through the gut. However, from these results it is clear that the egg number correlates very well with the passage of food through the gut and it can be concluded that almost all the eggs laid are passed out through the intestine with the help of the passage of digested food.

(3) The viable egg percentage.

The results of this experiment is presented in the Table 3.16. According to these results it is evident that only a mean of 87.4 % of the eggs laid produce oncomiracidia (range 85-90). Of the rest a mean of 7.7 % do not develop at all and 5.7 % develop, but the larvae get trapped inside the egg shell without being able to open the lid of the shell.

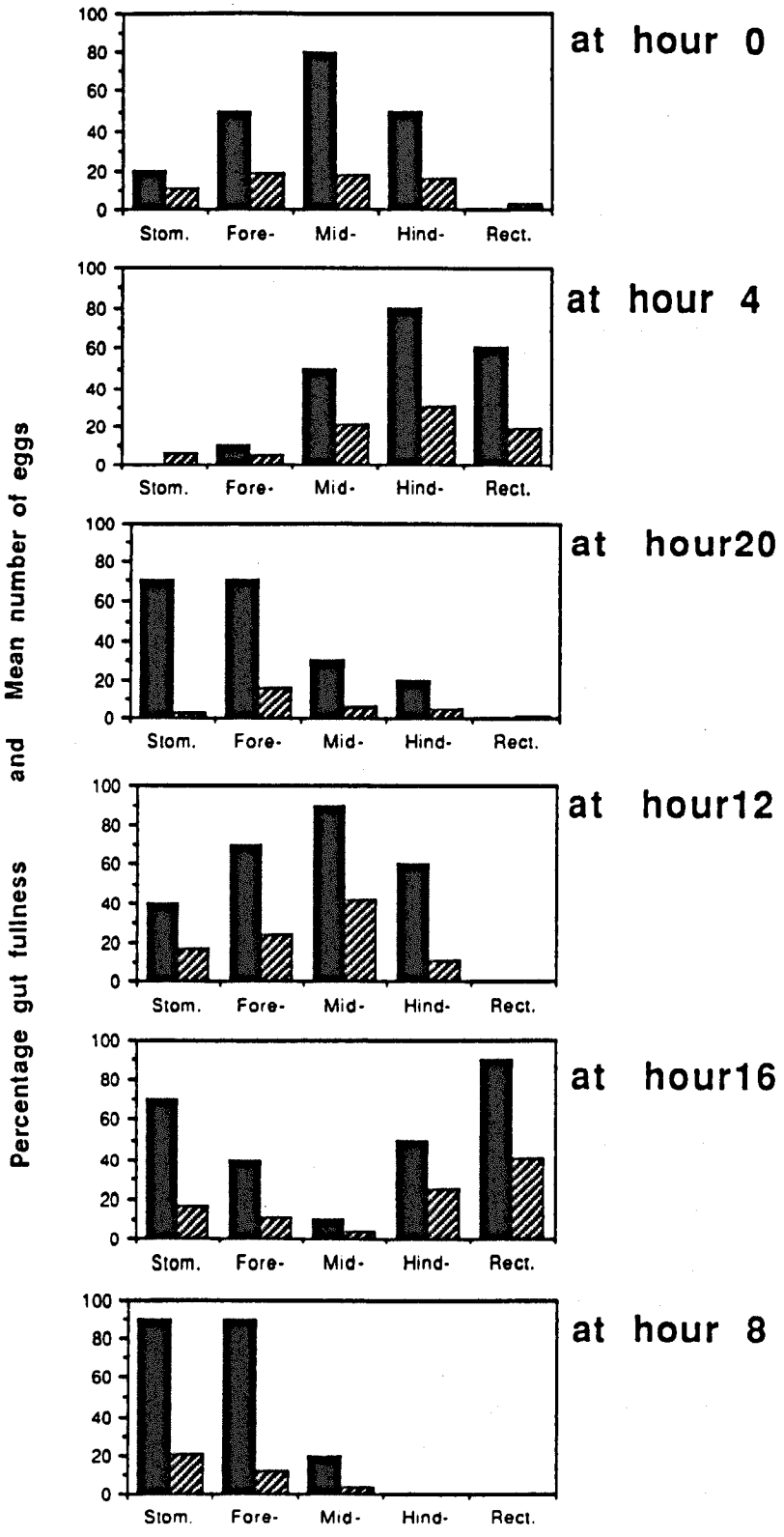


Figure 3.24: Percentage gut fullness (■) and the mean number of eggs present (▨) (if 50 worms are in stomachs) in different regions of digestive tract at 4 hour intervals of a day. In all cases n=5.

Table 3.15 : The number of egg bearing stomach monogeneans present in the anterior region and posterior region of the stomach.

Stomach	Number of egg bearing monogeneans in the anterior region	Number of egg bearing monogeneans in the posterior region
1	3	14
2	2	15
3	4	26
4	2	13
5	0	9
6	2	18
7	3	22
8	1	14
9	1	8
10	2	17

The probability given when the two regions were compared by sign test (0.02) is less than the probability level 0.05 (95 % level) indicating the significant difference.

Table 3.16 : The non developing, trapped and hatched percentages of stomach monogenean eggs.

Total number of eggs in the petri-dishes	Percentages of eggs which did not develop	Percentages of eggs with trapped larvae inside	Percentages of hatched eggs
1. 49	8.2	6.1	85.7
2. 73	6.8	2.7	90.4
3. 54	7.4	3.7	88.9
4. 87	9.2	10.6	87.4
5. 72	6.9	5.6	84.7
Mean Percentages	7.7 ± 1.0	5.7 ± 3.0	87.4 ± 2.3

3.3.3. Life-cycle studies.

3.3.3.1. Egg development and its stages.

In all experiments under this section, the eggs tested were of mixed species. As the adults did not lay eggs when they were separated from host tissue and the eggs were not morphologically different, there was no way to separate the eggs of two species.

(1) The differentiation of the eggs of the two species

The size of the eggs of the two species of *Enterogyrus* were as follows;

E. globidiscus	Range	48-60 x 35-52	
	Mean± SD	54.03±3.78 x 45.47±5.24	n=30
E. papernai	Range	32-54 x 26-42	
	Mean±	44.23±6.55 x 35.68±5.37	n=30

Therefore it was evident that the ranges of the size of the eggs of the two species overlap with each other.

(2) Egg development under natural condition.

A. Egg development.

The laid eggs were brown in colour with vitelline cells filling the inside. Sometimes the developing ovum could be seen (Figure 3.25A), but the tiny spine, which is clearly evident on the cast egg shells, was not visible. After about 12-24 hours following oviposition, a translucent patch appeared on eggs mainly in the mid-region (Figure 3.25B). During the next 20-32 hours, this translucent patch turned into a transparent area which gradually enlarged. In the latter half of the second day, about 36-48 hours after oviposition, two dark spots appeared on this transparent area (Figure 3.25C).

In the early hours of the third day, 48-72 hours, four eye spots were apparent and the developing embryo could be recognised (Figure 3.25D). In the late half of the third day the eye spots became larger and were well developed; the outline of the embryo became distinct. The vesicles lying external to the embryo always occupied the concave space made by the curving of the embryo and were expanded laterally

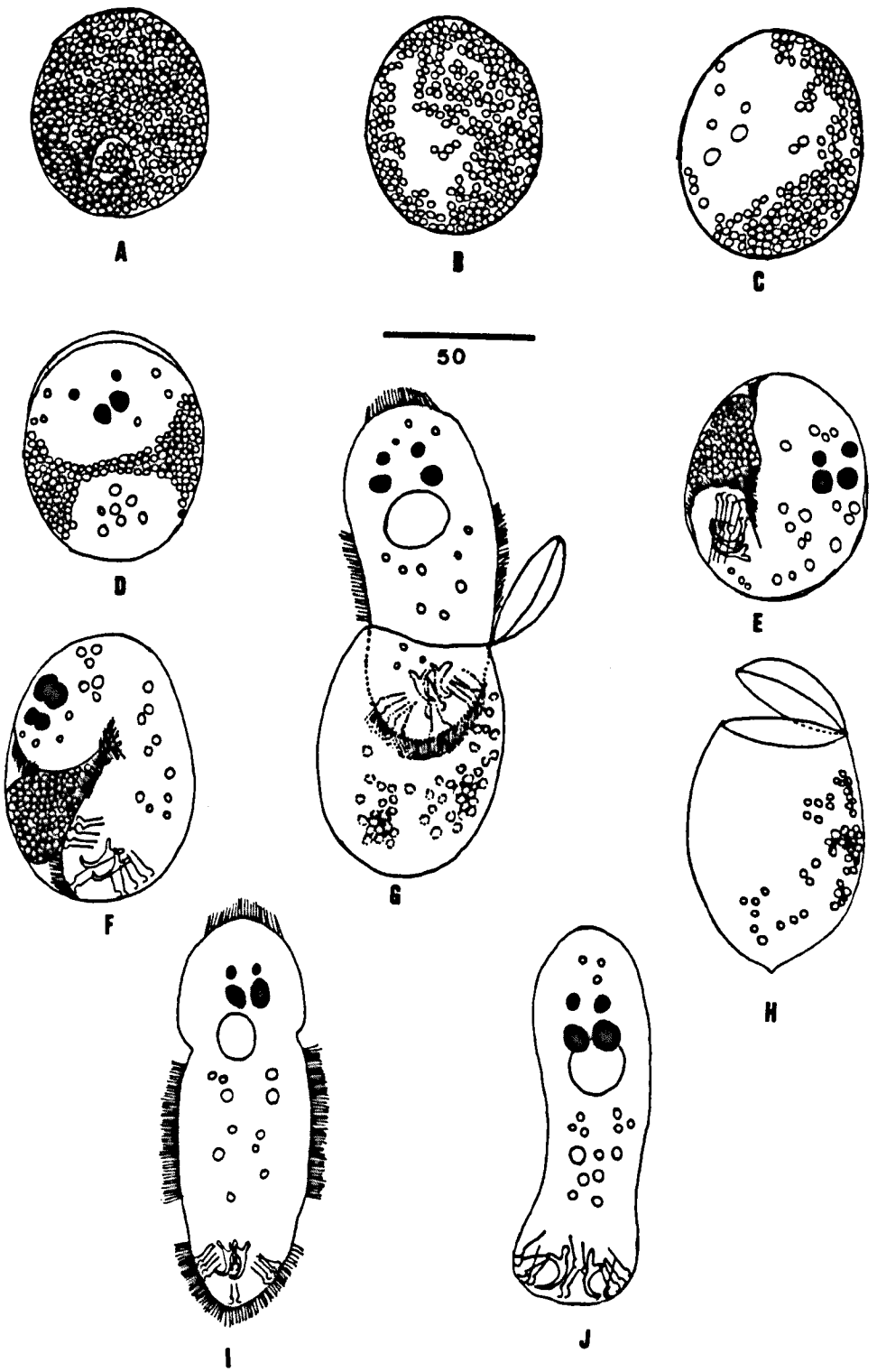


Figure 3.25: The egg development and oncomiracidia. (A) Just after laying. (B) After 24 hours. (C) After 36 hours. (D) After 48 hours. (E) After 72 hours. (F) The exertion of pressure against the egg wall. (G) The hatching. (H) Cast egg shell. (I) Oncomiracidia with ciliated epidermis. (J) Oncomiracidia after shedding the epidermis.

giving an appearance of a sac. Even though the wall of the sac was not distinct, the way the vesicles were arranged and the way the sac was positioned, (ventrally between the opisthaptor and the anterior end) it was possible to speculate that this was either a type of bladder which contained excretory granules or a yolk sac containing yolk cells.

By 72 hours, cilia were present and the embryo started to move occasionally by muscular movements, probably assisted by the cilia. Later this day the frequency of movements and their duration was extended. By this time (72-96 hours), the opisthaptor armature was well developed (Figure 3.25E).

It was observed that, prior to hatching, the oncomiracidium appeared to thrust its head region, dorsal side of the neck region and/or the mid body region, against the opercular pole (Figure 3.25F), and occasionally against the opposite pole, of the egg shell. The cilia of the oncomiracidium moved rapidly and continuously whilst its body was contracting and elongating. Gradually the operculum was opened and the oncomiracidium squeezed its body through the aperture. At the moment of hatching, the sac enlarged and burst. The occurrence of this phenomenon was hard to catch so that it was observed only three times and missed several times, despite hours of waiting. The tiny spine on the egg shell was only apparent on the cast shells and was directly opposite to the opercular pole.

Occasionally, even with powerful thrusts some oncomiracidia could not succeed in opening the operculum and continued to try to open it for more than half a day. In these cases, the strength of the activity gradually diminished and continued for one more day before the larva died, trapped inside the shell.

B. The developmental stages

According to the observations, the process of development was divided into four major categories, the non-transparent stage, the transparent stage, the developing stage and the active stage. The starting point of the developing stage was considered to be the time when the developing embryo could be identified.

The time durations required for these stages are as follows;

non-transparent stage	25.85 ± 4.79 hours
transparent stage	16.00 ± 2.83 hours
developing stage	18.77 ± 1.92 hours
active stage	25.73 ± 2.98 hours

C. The dimensions of the eggs during development

The measurements of the eggs after each 24 hours are as follow.

Time in hours	Length X Width in µm Range (Mean) n=14
2-4	52-60 X 40-54 (55.92 x 45.43)
24	62-72 X 48-64 (66.36 X 54.21)
48	68-76 X 56-66 (72.07 X 58.43)
72	72-80 X 57-69 (76.36 X 61.86)
88 (about to hatch)	82-92 X 58-69 (86.64 X 62.00)

These measurements show that the size of the eggs increases with the development of eggs.

D. Time taken for egg development

Under the natural conditions which were provided in the laboratory, it took a mean of 86 hours and 21 minutes (3 3/5 days) for the completion of the egg stage (Table 3.17). The range was 81 to 95.5 hours.

According to the Table 3.16, the hatching success is 87.4 %. A 7.7 % eggs did not develop and the larvae were trapped inside in 5.7 %.

(3) The effect of environmental factors on egg development.

The control tests carried out with each environmental testing are marked in the Table 3.17. The egg development durations of these controls were similar to those under the natural condition and showed no statistically significant difference at 95 % level (probability 0.504). This indicates that similar conditions were supplied for each set of experiments, even though the sets were carried out independently.

The results of the effect of the environmental factors studied on the duration of egg development are given in the Table 3.17. Figure 3.26 illustrates the effects graphically and also shows the effect of these parameters on the different egg developmental stages. Figure 3.27 elucidates the specific effects of these parameters on the duration taken for each developmental stage.

A. Effect of salinity on egg development.

Table 3.17 shows the effect of salinity on egg development, the results are significantly different with a probability of <0.01. A salinity of 16 ‰ seemed to be the most favourable for egg development. The time taken was approximately 2 2/3 days, the shortest when compared with other salinities. The next favourable salinity was 0 ‰, taking 3 1/4 days. Salinity 8 ‰ required 3 1/2 days. Salinity 24 and 32 ‰ needed much longer periods 4, 5 1/2 days respectively.

Figure 3.26 shows that the salinities affect different developmental stages differently, the most vulnerable stages being the non-transparent stage and the active stage, to a lesser degree, where the hatching ultimately occurs. Therefore it seems that the initial developmental stages and the process of hatching is delayed at unfavourable salinities. Further, the transparent stage in salinity 16 ‰ took less time compared to the other salinities which showed similar durations.

B. Effect of temperature on egg development.

The effect of temperature showed significant differences at 95 % level (probability <0.01) and this agrees with the general rule of higher temperatures giving higher egg developmental rates (Table 3.17). The highest experimental temperature applied, 35°C, brought the eggs to hatch in 2 2/3 days. Under the temperature 29°C, which is the closest to the natural conditions, egg development required approximately 3 1/2 days. The temperature of 23°C delayed the development rate such that it required 6 1/3 days to hatching (Figure 3.26).

Temperature affected all the developmental stages in a similar manner. The lower temperatures required more time for all the stages whilst shorter times were

Table 3.17 : The sole effect of salinity, temperature and pH on the egg development rate of *Enterogyrus* spp.

Experimental Environmental parameters			No. of eggs / oncomiracidia observed (No. undeveloped, No. trapped)	Time taken for egg development in hours	Life time of oncomiracidia in hours
Salinity ‰	Temperature °C	pH			
6.8[1]	28.0 - 30.8	7.68	13 (1, 1)	86.35 ± 4.38	11.27 ± 1.33
0			19 (2, 2)	77.58 ± 4.39[b]	11.21 ± 1.00
8[1]			22 (2, 1)	84.11 ± 7.30[c]	11.32 ± 1.05
16	28.5 - 31.3	7 (6.84 - 7.63)	20 (1, 1)	64.70 ± 6.49[a]	11.50 ± 1.24
24			17 (2, 3)	96.38 ± 8.46[d]	10.71 ± 1.47
32			18 (0, 2)	132.44 ± 7.30[e]	10.50 ± 1.13
	23.0 (22.5 - 24.0)		19 (1, 2)	153.26 ± 4.90[c]	12.05 ± 1.37[c]
8	29.0 (28.2 - 30.0)[1]	7.47	17 (2, 1)	82.79 ± 5.56[b]	10.77 ± 1.02[b]
	35.0 (34.8 - 35.5)		18 (0, 1)	64.67 ± 3.99[a]	2.94 ± 1.06[a]
		3 (2.95 - 3.16)	0 (23, 0)	-	-
		4 (3.96 - 4.14)*	2 (13, 2)	88.00 ± 5.66	1.25 ± 0.35
		5 (4.93 - 5.13)	14 (1, 3)	90.04 ± 5.29[c]	1.79 ± 1.05[a]
8	28.8 - 31.0	6 (5.95 - 6.11)	16 (1, 1)	79.16 ± 8.28[a]	6.19 ± 1.30[b]
		7 (7.00 - 7.04)[1]	14 (0, 3)	84.25 ± 6.82[b]	10.93 ± 1.36[d]
		8 (7.83 - 8.04)	17 (0, 3)	94.91 ± 6.62[c]	9.12 ± 1.39[c]
		9 (8.82 - 9.08)*	2 (11, 3)	106.00 ± 19.80	2.25 ± 1.06
		10 (9.74 -10.10)	0 (19, 0)	-	-

[1] the control tests carried out. * due to the small sample size results were not subjected to statistical analysis.

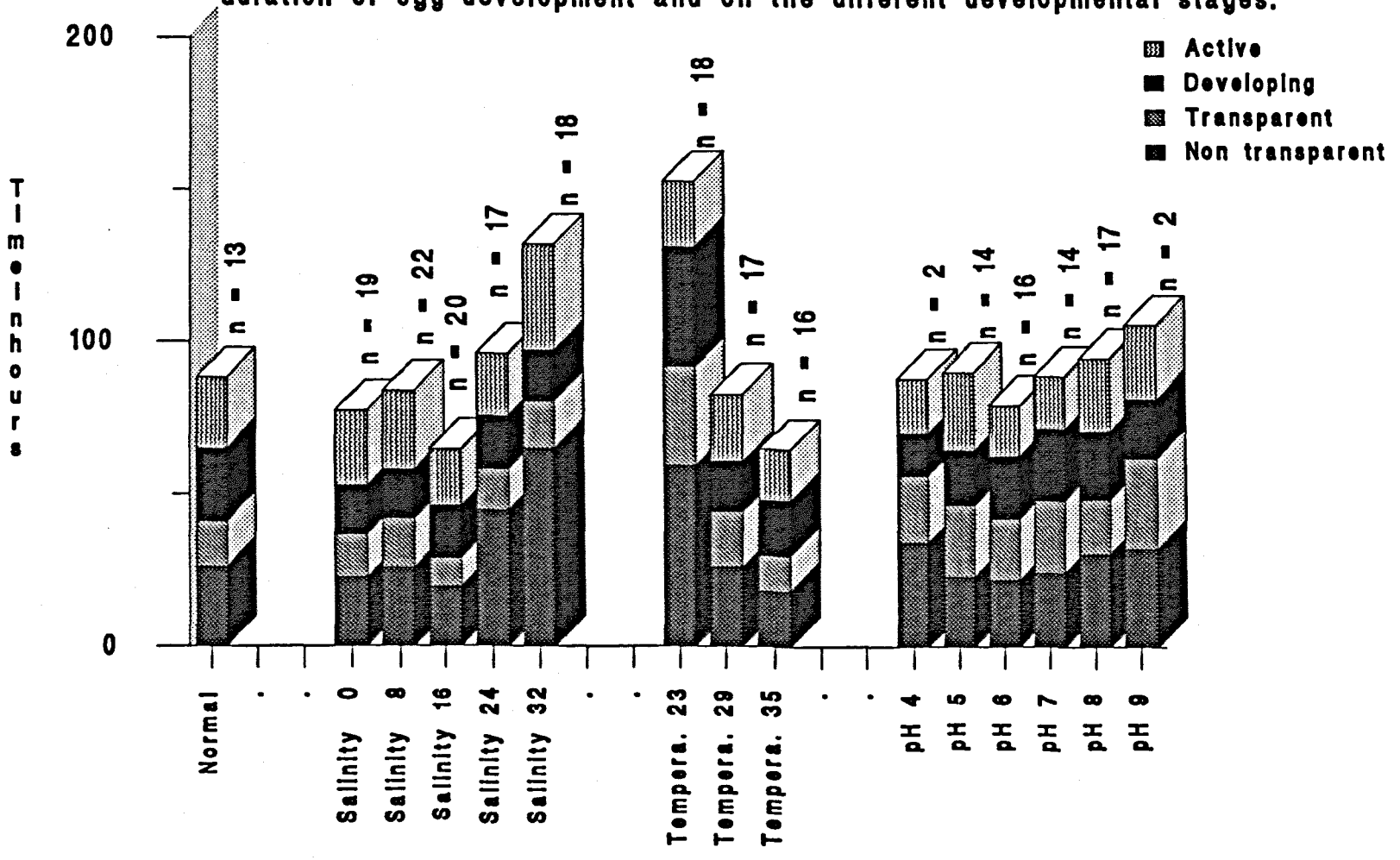


Figure 3.26: The independent effect of environmental parameters on the duration of egg development and on the different developmental stages.
Active
Developing
Transparent
Non transparent
200
100
0
n = 13
n = 19
n = 22
n = 20
n = 17
n = 18
n = 18
n = 17
n = 16
n = 2
n = 14
n = 16
n = 14
n = 17
n = 2
Normal
Salinity 0
Salinity 8
Salinity 16
Salinity 24
Salinity 32
Tempera. 23
Tempera. 29
Tempera. 35
pH 4
pH 5
pH 6
pH 7
pH 8
pH 9

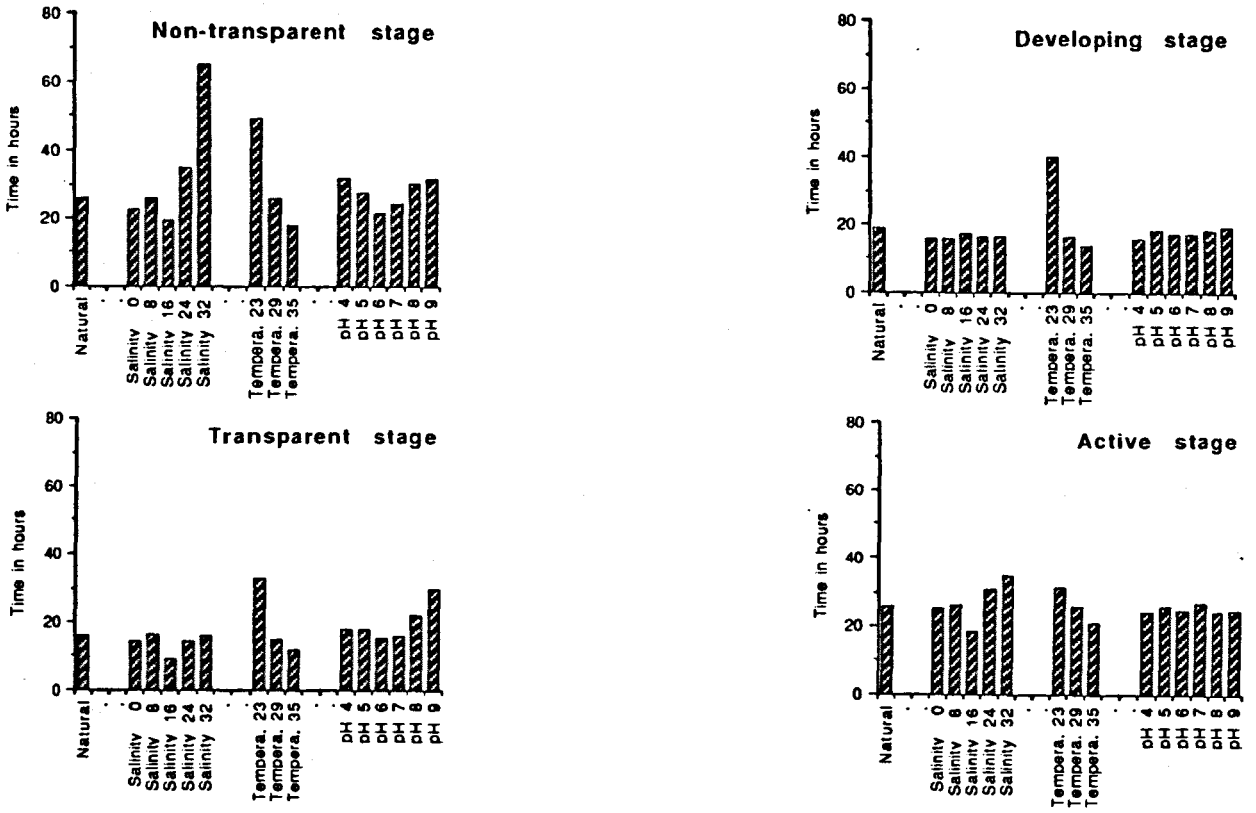


Figure 3.27: The independant effect of environmental parameters on the duration of egg development; the time taken for different developmental stages are given in separate graphs.

recorded for the higher temperatures (Figure 3.27).

C. Effect of pH on egg development.

Of the pH values tested pH 3 and 10 did not allow any eggs to develop. The pH values 4 and 9, allowed the development to some extent but were harmful and only 12 % hatching occurred. The pH values 5, 6, 7 and 8 allowed the normal process of developing and hatching, but at significantly different rates (probability <0.01) (Table 3.17). The most suitable pH for egg development was pH 6, requiring approximately 3 1/3 days. There was little difference between this and pH 7 taking 3 1/2 days for the development. The pH values 5 and 8 showed a similar effect requiring 3 3/4 and 4 days respectively (Figure 3.26).

The non-transparent stage of egg development and the transparent stage, to a lesser extent were the stages most affected by the tested pH values. The preferred pH values reduced the duration of these stages and the unfavourable pH values increased the durations on these stages.The egg development durations at developing and active stages were not affected (Figure 3.27).

3.3.3.2. Oncomiracidium stage (hatched larval stage).

(1) The description of the oncomiracidia.

The shape and the structure of the two species of oncomiracidiae were the same (Figure 3.25I). They had the oblong body shape with the blunt anterior and posterior ends. The haptor was not separated from the body. The two pairs of eyes were clearly evident. The cilia were in four zones over the body, anteriorly, posteriorly and both lateral regions. The seven pairs of marginal hooks and the pair of ventral hamuli were on the haptor. The last two pairs were centrally placed between the pair of ventral hamuli. The other five pairs were on the lateral margins of the haptor. The large pharynx was evident posterior to the two pairs of eyes. Other internal structures were unclear and were difficult to observe.

The size ranges of the oncomiracidia (in micrometers) of the two species were

as follows;

E. globidiscus	Range	108-126 x 44-66	
	Mean±SD	116.64±7.54 x 53.74±4.85	n=18
E. papernai	Range	95-117 x 36-59	
	Mean±SD	105.73±8.45 x 46.48±6.34	n=14

Therefore the size ranges of the oncomiracidia of the two species overlap with each other.

(2) The behaviour of oncomiracidia and their longevity under natural conditions.

The study of the behaviour of oncomiracidia showed that two phases could be identified; the active phase, which pertained for most of their life-time and the short, final, inactive phase terminating in death.

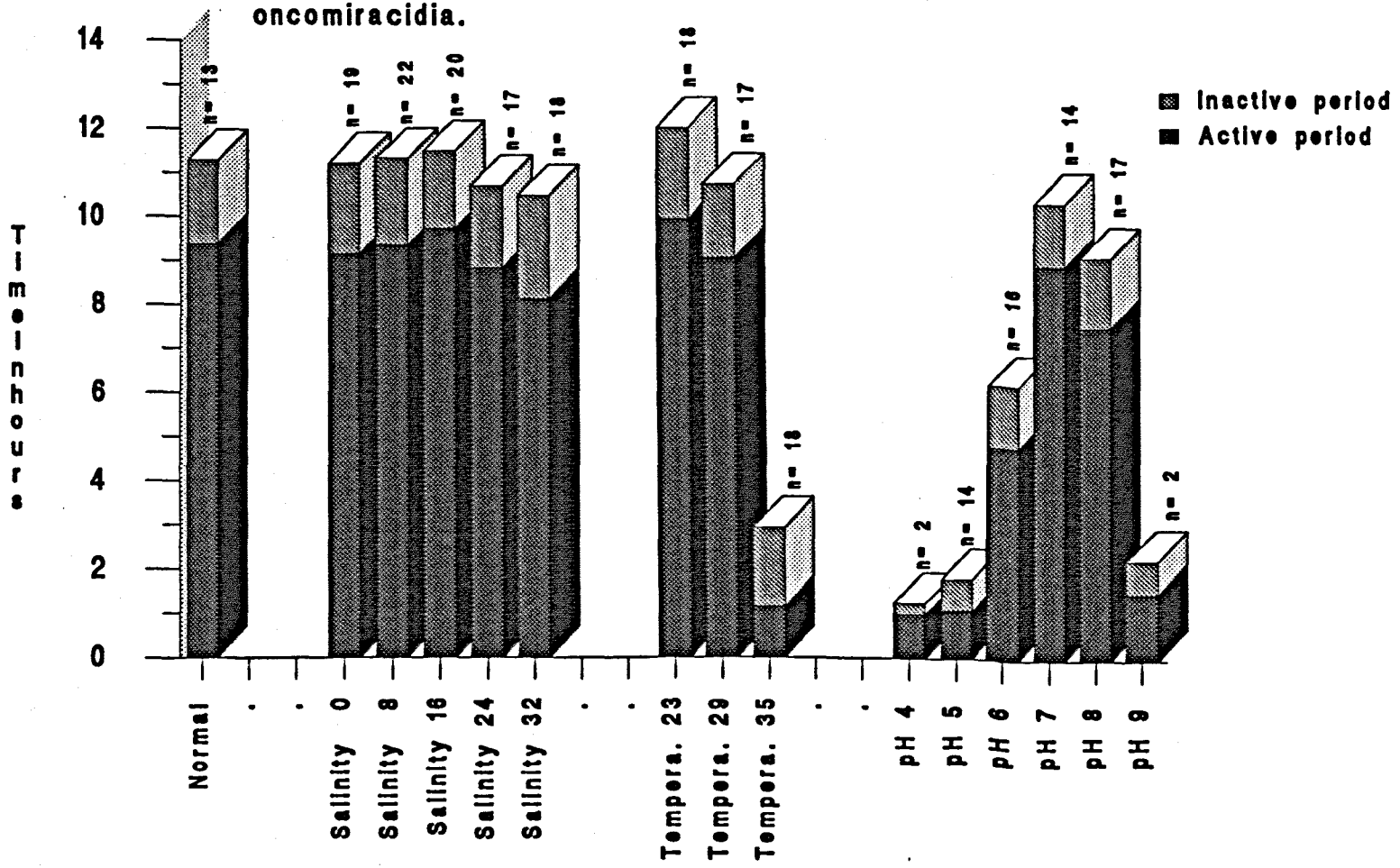
From the time of hatching, the oncomiracidia were very active. They swam actively with ciliary movements, in straight lines. Probably the muscular movements were important for changing directions. Occasionally they reached the bottom of the petri-dish and with the help of cilia, moved on the bottom a little slower than at their swimming rate. If they come across any object such as detritus, they moved about it showing apparent investigatory activity. Under the natural conditions provided, this active stage proceeded for approximately 9-10 hours.

Towards the end of the life they became stationary on the bottom or near the objects to which they had been attracted. While stationary they showed the contraction and relaxation movements of body. Rarely, they swam small distances. After approximately 2 hours of this inactive stage they ceased all the body movements and died.

(3) Effect of experimental environmental factors on the duration of oncomiracidium stage.

The duration of the life of the oncomiracidia with respect to the experimental environmental factors provided are given in the Table 3.17 and illustrated in the Figure 3.28. In these experiments the controls gave similar results to those under

Figure 3.28 : The independent effect of environmental factors on the life duration of oncomiracidia.



natural condition (probability level 0.415), confirming the similarities of the conditions provided.

A. Salinity

None of the experimental salinities tested showed any statistically significant effect at 95 % level on the longevity of the oncomiracidia (probability 0.053). The duration of active and inactive periods were not affected (Figure 3.28).

B. Temperature

The highest experimental temperature used (35°C) markedly decreased the active life-time duration to about 1 hour, but the inactive period was not affected. The duration of the whole life period was 3 hours. The low temperature tested (23°C) significantly increased the life-time of oncomiracidia, the active phase as well as the inactive phase (Figure 3.28).

C. pH

The effect of pH was similar to that found for the egg development, the extreme pH values tested were not favourable for the oncomiracidia. The pH values 3 and 10 did not allow the eggs to develop, so that no oncomiracidia could not be obtained. With pH 4 and 9 only two oncomiracidia for each pH was tested since only these small number were hatched. The pH values 4, 5 and 9 were very harmful to oncomiracidia, killing them within 1 to 2 hours (Figure 3.28). The active phase as well as inactive phase were affected. Even though pH 6 accelerated the egg development, it was not suitable for oncomiracidia thus shortening its life-time. The most favourable pH was 7 and the next favourable was pH 8. In all tested pH values, both the phases active and inactive, were affected similarly.

(4) Mode of entry to host.

According to the results of the experiment designed to find out which host tissue the oncomiracidia preferred (Table 3.18), it appeared that the skin and gill tissues were similarly preferred, whether placed singly or together. Oesophagus was the less preferred and the most rejected tissue was the stomach. The presence of gill

Table 3.18 : The host tissue preference by oncomiracidia.

Set number	Tissues placed	Expt. number	No. of oncomiracidia (No. unhatched eggs)	Number of worms attracted				
				Skin	Gills	Oesophagus	Stomach	Swimming
1	skin	1	10 (0)†	9	—	—	—	0
		2	8 (2)	8				0
2	gills	1	9 (1)	—	9	—	—	0
		2	10 (0)‡		9			0
3	oesophagus	1	8 (2)	—	—	4	—	4
		2	9 (1)			5		4
4	stomach	1	7 (3)	—	—	—	1+1*	5
		2	8 (2)				1*	7
5	skin & gills	1	10 (0)‡	6	3	—	—	0
		2	8 (2)	4	4			0
6	gills & oesophagus	1	9 (1)	—	8	1	—	0
		2	7 (3)		6	0		1
7	gills & stomach	1	8 (2)	—	6	—	0	2
		2	10 (0)‡		9		0	0

† the balance number of worm was attracted to the side of the skin with muscle; ‡ the balance number of worm was attracted to pieces of detritus; * number of worms attracted to the outer wall of stomach.

tissue with either the oesophagus or stomach tissues resulted in the oncomiracidia being attracted to the gill tissue, suggesting that they first attach to skin and/or gills before entering into stomach via oesophagus.

Six hours after the introduction of the tissue pieces, the oncomiracidial attraction to the skin and gill tissues was maintained. They did not leave the tissues even when the tissue was disturbed eg. as created by tapping on to the petri-dish. Therefore, it was concluded that they had shed their ciliary epithelia by this time.

3.3.3.3. Life-cycle duration of worms.

(1) Infection of *Oreochromis* spp. (*O. niloticus* and *O. mossambicus*) with *Enterogyrus* spp.

None of the *Oreochromis* spp. from all 3 of the experimental systems used were infected by *Enterogyrus* spp. thus demonstrating their likely specificity to *Etroplus*. Even in the system 3 where the host fish species, *Etroplus*, was removed after the first week, the parasites did not infect the *Oreochromis*.

(2) Removal of *Enterogyrus* with the administration of Droncit.

The trials were carried out serially with the aim of finding a suitable dose rate and administration frequency to produce *Enterogyrus* free fish. The results are given in the Table 3.19.

The observation of the *Enterogyrus* from stomachs after trial 1 showed that the worms were not affected by the drug and behaved as normal. Even when the high administration frequencies were used the results showed the inadequacy of the dose rate 20-30 mg/kg of fish at any frequency. The observation of stomachs after trial 4 revealed that the worms became inactive with the dose rate 200 mg/kg and some were detached from the wall but the others were attached. Trials 5 and 6 showed a marked reduction of the worm numbers, and the remaining worms were mostly young indicating the possibility of migration of worms present on the gills into the stomach post treatment. Thus, it was decided to treat external monogeneans in between the

Table 3.19 : Results of the testing of the efficacy of different dose administration and frequencies, with the aid of other treatments to remove the *Enterogyrus* infections.

	Droncit dose rate (mg/kg fish weight)	Treatment frequency	No. of fish treated	Observe d after	Mean No. of parasites in stomach per fish (individual numbers for fish)
1.	20-30	one treatment	3	3 hours	79.66 (69,78,92)
2.	20-30	3 times at every 24 hours	3	3½ days	67.67 (52,64,87)
3.	20-30	3 times at every 6 hours	2 (one died)	28 hours	83.00 (92,74,)
4.	200	one treatment	3	3 hours	50.00(42,38,70)
5.	200	2 times, 6 hour intervals	3	24 hours	15.33 (18,7,22)
6.	300	3 times after every second day	3	7 days	11.00 (9,14,10)
7.	300	2 treatments the second one after two days, then 250 ppm formalin 1 hour bath on the fifth day and the third Droncit treatment on sixth day	3	7 days	2.00 (2,0,4)
8.	300	3 treatments at every 72 hours and 250 ppm formalin 1 hour bath treatments 24 hours before each Droncit treatment.	3	9 days	absent

Droncit treatments. Finally, trial 7 gave an acceptable result and in trial 8, the fish were devoid of stomach monogeneans.

Due to the unreliability of the treatment and therefore the possibility of parasites remaining in a treated population, it was decided to hold the treated fish for a considerable time (2-3 weeks) and to check a sample before carrying out experimental infections.

(3) Life-cycle duration of adult worms.

The number of worms (two species separately) found in the stomachs and their lengths are given in the Table 3.20 at 2 day intervals post infection, considering the time of oviposition as the starting point.

The gills of source fish were infected with monogeneans normally inhabiting the gills. The eggs from these were present with the eggs of stomach monogeneans in the eggs collected from the tanks, thus the gill monogeneans infections occurred. Therefore it was difficult to identify the young forms of *Enterogyrus* amongst them on the fish. Thus, only the worms present in the stomachs were studied here.

E. papernai was the first species to appear in the stomach on the eighth day, indicating that they inhabit a pre-stomach site after attaching to fish and before reaching the stomach. This constitutes an approximately 3-4 day gap taking into account the time for egg development. The other species, *E. globidiscus* reached the stomach on the fourteenth day, staying 9-10 days in the pre-stomach site.

At the time of reaching the stomach *E. papernai* worms were not mature and were lacking the copulatory tube and they did not lay any eggs until day 10. In contrast, *E. globidiscus* worms laid eggs from the first two days of entering the stomach indicating that they were already mature on entering to the stomach (Table 3.20).

At the beginning of the experiment, when there were only *E. papernai* worms,

Table 3.20 : The size measurements (lengths) of *Enterogyrus* worms in the stomach with time, after introducing them to clean fish as eggs.

Day of the life cycle	No. of *E. papernai* worms measured (fish 1, fish 2)	Mean lengths of *E. papernai* collected from both fish ± SE (in µm)	No. of *E. globidiscus* worms measured (fish 1, fish 2)	Mean lengths of *E. globidiscus* collected from both fish ± SE (in µm)
4	0,0	—	0,0	—
6	0,0	—	0,0	—
8	2,3	156.0 ± 15.0	0,0	—
10	3,5	182.5 ± 19.7	0,0	—
12	6,3	247.3 ± 19.7	0,0	—
14	3,3	261.3 ± 13.4	5,5	272.2 ± 6.8
16	2,6	250.0 ± 10.7	49,38	294.0 ± 14.0
18	4,7	274.5 ± 12.9	39,39	335.1 ± 18.3
20	4,4	292.5 ± 33.7	34,25	368.0 ± 22.3
22	2,3 1,0†	320.0 ± 0.0 120.0	8,14	414.0 ± 25.3
24	1,0 3,2†	360.0 140.0 ± 14.1	4,5	420.0 ± 31.6

† worms of the second generation.

the population of this species in the stomachs were low. The population of *E. papernai* remained low for the duration of the experiment. The population of *E. globidiscus* was low at the first day of their appearance in stomach but increased considerably afterwards (Table 3.20). The relative proportions of the two populations at this time approximately reflected the natural population proportions (14.4 % *E. papernai* of the total number of worms in the stomach). This may have been due to the presence of eggs of the two species in the same proportions in the infecting tanks, thus suggesting that the rate of egg production in the two species is similar.

On the 22nd day the next generation of *E. papernai* appeared in the stomachs. The second generation of *E. globidiscus* had not appeared when the experiment was terminated on the 24th day as all the fish had been sacrificed by this time. As this worm stays longer in the pre-stomach site the time period was not sufficient to allow the next generation to enter into stomachs.

According to these results, the generation time of *E. papernai* is approximately 22 days. Since *E. globidiscus* laid eggs on the fourteenth day of their development from the egg laying, their generation time is approximately 28 days. Since the experiment could not be continued due to the lack of fish this time could not be determine exactly for this species.

The life time of the worms could not be calculated directly from the experiment. However, the number of worms surviving on the stomachs towards the end of the experiment decreased and the lengths of the remaining worms reached the highest values obtained for the natural infections (Section 3.3.1.2.(1)). Thus, even though the experiment could not be continued until the stomachs were cleared of the first generation, it was likely that the worms were close to the end of their life time.

(4) The pre-stomach stages of *Enterogyrus*.

The length of the worms found for each location after the infection are given in the Table 3.21 and the diagrams of the worms found are given in Figure 3.29. The results clearly show that the existence of pre-stomach stages on the skin and mainly

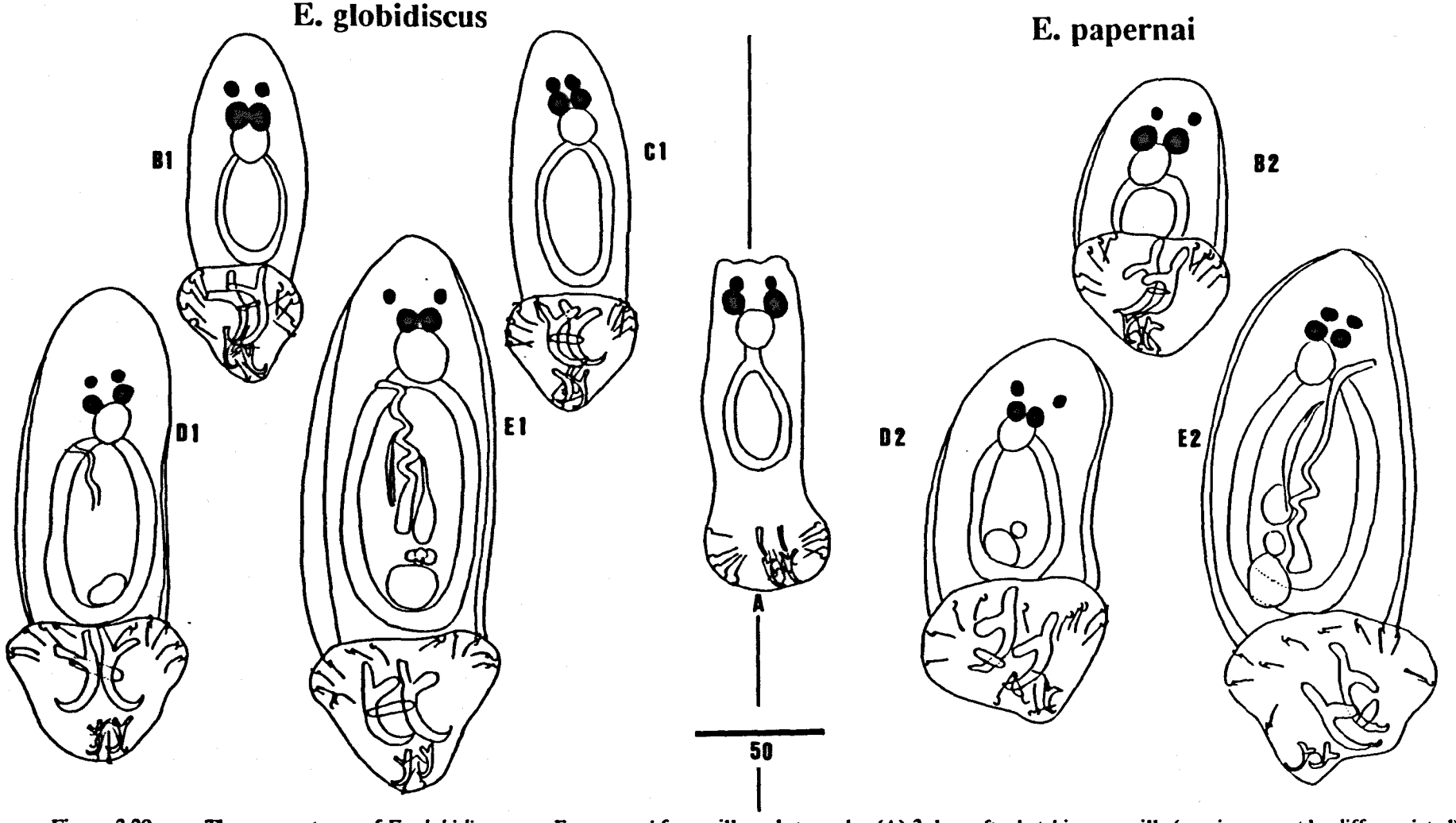


Figure 3.29: The young stages of *E. globidiscus* ans *E. papernai* from gills and stomachs. (A) 3-days after hatching, on gills (species cannot be differenciated). *E. globidiscus*: B1) 6-days after hatching, on gills; C1) 8-days after hatching, on gills; D1) 10-days after hatching, on gills; E1) 12-days after hatching, in stomach.
E. papernai: B1) 6-days after hatching, in stomach; D1) 10-days after hatching, in stomach. E1) 12-days after hatching, in stomach.

Table 3.21 : The lengths of the young *Enterogyrus* worms found in each site (Mean ± SE). The data collected from two fish on each day were pooled.

Days of the life cycle (day post-infection)	Skin	Entire gill surface			Stomach		
	Uncertain	*E. globidiscus*	*E. papernai*	Uncertain	*E. globidiscus*	*E. papernai*	Uncertain
4 (1)	124,128,134 (128.7±5.0)	___	___	108	___	___	Absent
5 (2)	122	___	___	120,126,142 (129.3±11.4)	___	___	Absent
7 (4)	Absent	___	___	146,138,148 (144.0±5.3)	___	___	Absent
9 (6)	Absent	148,152 (150.0±2.8)	Absent	___	Absent	136	___
11 (8)	Absent	162,174,164, 158,182 (168.0±9.8)	146	___	Absent	Absent	___
13 (10)	Absent	186,195,202 (194.3±8.0)	Absent	___	Absent	224	___
15 (12)	Absent	224,235,240, 266,242 (241.4±15.4)	Absent	___	272,268,285 (275.0±8.9)	236,248 (242.0±8.5)	___

___ used when the catagory was of no use.

Table 3.22 : Egg laying activity and the presence of copulatory tube to differentiate young and mature worms of *Enterogyrus* spp.

Day of the life-cycle	Lengths of the worms found (μm) with indications of the egg laying (o) and/or presence of the copulatory tube (†).	
	Fish 1	Fish 2
Experiment under the Section 3.3.3.3(3)		
E. papernai		
8	136, 160	146, 164, 174
10	186, 194†, 200†	148, 166, 176†, 180†, 210†•
12	220†, 228†•, 240†•, 256†•, 260†•, 282†•*	234†•, 242†•, 264†•
14	263†•, 268†•, 277†•	244†•, 246†•, 270†•*
E. globidiscus		
14	264†, 268†•, 274†•, 278†•*, 278†•	262†, 265†•*, 275†•, 278†•, 280†•*
Experiment under the Section 3.3.3.3(4)		
E. papernai		
15	236†	248†•
E. globidiscus		
15	272†•	268†, 285†•

* worms produced 2 eggs within the period of 6 hours.

on the gills.

The early young stages of the stomach monogeneans were found on the skin on the day of exposure to oncomiracidia and for a day post-exposure. As the fish were exposed to eggs just ready to hatch and not to oncomiracidia, an exact time of infection cannot be given.

The young stages were also found on the gills from the day of exposure. The clarification of the species of these worms could not be made properly until the 4th day post-infection (7th day of life cycle), mainly due to the lack of dorsal hamulus. *E. papernai* was found on the gills until the 8th day post-infection. *E. globidiscus* was found on the gills until the completion of the experiment due to the lack of experimental fish, on the 12th day post-infection.

According to these results it seems that *E. papernai* starts migrating to stomach before *E. globidiscus*, around the 6th day post-infection. *E. globidiscus* started migration on the 12th day post-infection. The copulatory tube appeared on both species around the post-infection day 11. This suggests that the copulation of *E. papernai* takes place in the stomach after the migration when the copulatory tube then develops. Although the copulatory tube was formed around the day 11 post-infection, the egg laying of *E. globidiscus* was observed on day 14, the first day they appeared in the stomach. Whether they start to lay eggs even before this was not investigated, but probably the entering into the stomach may act as a triggering factor for oviposition. Two specimens of *E. globidiscus* with the copulatory tube were found on gills and all the worms found on the stomach had this organ. Considering that *E. globidiscus* can lay eggs on the first day after migration to the stomach (Table 3.22), this implies the possibility of their copulating before reaching the stomach. This may be the main factor determining the segregation of the two species.

The results gained under this Section agree with the experiment of the Section 3.3.3.3(3), where the *E. papernai* worms appeared in the stomach around day 8 of the life cycle and *E. globidiscus* worms around day 14.

3.4 Discussion

3.4.1 The populations in stomach

Two *Enterogyrus* populations, *E. papernai* and *E. globidiscus*, were found in similar proportions in the stomachs of fish in both fresh and brackish water. This suggests that the salinity did not affect either species.

There was a wide range of sizes within each infrapopulation, suggesting that there were differences in age.

The major differences observed between the populations of the two species are

1. the markedly different length ranges of the two species, 260-500 µm for *E. globidiscus* and 120-360 µm for *E. papernai*, and
2. the small population size of *E. papernai*; 50 fish from Koggala lagoon sampled over four months gave a mean percentage of 16 % (range 8.5 - 29) *E. papernai* of the total number of worms found in the stomach, while a sample of 33 fish collected over a period of three months from Udawalawa gave a mean of 17 % (range 13 -22).

The morphometry of the worms in the stomach compared with that of the oncomiracidia showed that there is a size range of worms in between the size of the oncomiracidium and the smallest size found to be present in the stomach.

E. globidiscus worms position their body in between the folds of the stomach and place the haptor in the depression between the folds. This would seem to be a more protected position than the *E. papernai* worms which attached to the side walls of the folds. The firmer attachment and the smaller size of the body seems to be advantageous to *E. papernai* in reducing the instability, probably, in such a position, therefore, would have helped them to adapt to their niche.

The low number of *E. papernai* compared to *E. globidiscus* suggests that this may be a result of competition between the two populations. Though there may be other factors which regulate the population levels, competition being solely, or partially, the cause. It may be, otherwise, due to the factors intrinsic to the species, e.g. reproductive capacity, or to macro- or micro-environmental effects acting differently on the two species. All the factors may have contributed to the population size to different degrees.

Usually a bell-shape length-frequency graph represents an unstable population as the population of the younger generation is smaller than the existing mature generation. Here, the low numbers in the younger generation probably reflects the immigration of young worms from another site. It is not that the worms of a specific size enter into stomachs, but the range below the highest frequencies are entering.

The shape of the graphs obtained by Keam (1967a) for *A. lobianchi* on the ventral surface of *Raja clavata* L. and Cone & Burt (1985) for the Ancyrocephaline *U. adspectus* on the gills of *Perca flavescens*, are of similar shape to that obtained for *Enterogyrus* probably suggesting a similar situation that they immigrate to the site in a range of lengths which is below the length at highest frequency. Where as the situation found by Keam (1967b) for *E. soleae*, the highest frequency of smallest size worms in the population, represents the host invasion by larvae of same size.

The changes in the length frequency distributions of *U. adspectus* in consecutive samples collected were attributed to seasonal changes by Cone & Burt (1985). The overwintering of adult worms, the cessation of oviposition during cold months and the completion of higher number of generations, about 8, during summer were responsible. As significant temperature changes are absent in the tropics, much variation in the generation time cannot be expected, but the changes in rainfall and probably salinity may cause a significant contribution to the

population structure possibly by changing the host finding ability. Therefore it would be interesting if the length frequency distributions had been studied seasonally for both localities. As minor variations, such as the absence of oncomiracidium supply for three days, could be detected in the present study there is a possibility of detecting the effect of minor environmental changes too.

Though the worms varied highly in their size, the sclerite size did not varied with the increase in the size of the worms, in either *Enterogyrus* species. Larger hooks may be not a necessity as the size of the dorsal hamuli developed at the beginning may be long enough to support the relative small size of the body.

A morphological character which would be useful to separate mature worms from young, was not found. Only the lengths they attain at maturity were found by directly observing the egg laying young adults. *E. papernai* worms attained maturity, copulated and laid eggs only after reaching stomachs. The status of *E. globidiscus* is not clear. Since they lay eggs within 1-2 days after reaching the stomach and the worms arriving in the stomachs are already morphologically mature, they may copulate when they are on the gills and then proceed to the stomach.

The work of Khidr (1990) on the distribution of *E. cichlidarum* in the stomach was unable to show a difference in colonization in the stomachs of *T. nilotica* (= *O. niloticus*) and *T. zilli* comparing the anterior, middle and posterior regions divided along the length of the stomach. These results are contrary to the present findings of *E. globidiscus* and *E. papernai*, both of which showed a greater preference towards the posterior part. Further, discrimination was shown by the worms in this study as a preference towards the dorsal side in either the anterior or posterior section.

In the same study, Khidr (1990) showed a decline in the percentage of adult specimens from the anterior to the posterior sector, while young worms showed the

reverse trend. He suggested that the reasons for this were the strain on the haptor of the young is less in the posterior region because less food enters to this sac-like posterior sector and also an increase in mating ability of the adults in the anterior sector due to their concentration in this region. If the mating occurs on the gills as in the case of *E. globidiscus*, the presence of only mature worms in the stomach waives the necessity of finding a mate there, thus no aggregation is required. The situation of *E. papernai*, where the immature worms are capable of moving and the adults incapable (they attach more or less permanently) is not clear. An investigation of site preference, taking into account the size of the *E.papernai* monogeneans is therefore a necessity.

Taking into account the number of each species in the sites of the posterior region, there was a high positive correlation, showing the same preference for the same sites agreeing with the findings of Dzika & Szymńaski (1989) where positive correlations occurred between different species of monogenean gill worms in the same sites on gills. The data for both the localities were also the same. In addition, according to incidental observations, these two species appeared to coexisted throughout the year almost in the same proportions in the stomachs. This ability to co-exist in the same site suggests the preference of these sites by both species rather than the others and the low importance of competition to control the two levels of the populations.

E. globidiscus worms were positioned between adjacent folds. The attachment was mainly by piercing the host tissue with hamuli. There were no evidence of the attachment by the marginal hooks, but these may be assisting. The pathology described by Cone *et al.* (1987) on an 'Enterogyrus' species inhabiting the fore gut of the French Angelfish *Pomocanthus paru* is very similar to the attachment of *E. globidiscus.*

On the other hand, the positioning and the anchorage of *E. papernai* worms living on the folds were very different from *E. globidiscus* living between the folds.

E. papernai were firmly attached on to the side walls of the folds and, in addition to the dorsal hamuli, the elongate posterior peduncle of the haptor was aiding in holding it to the host tissue. The inability to move or the restricted movements of *E. papernai* seemed to be due to this additional holding. Although it restricted the movement, this may have enabled the worms to live in a different micro-environment from *E. globidiscus*, which may reduce the competition with them. In addition, they need a very permanent attachment as they are attached to a relatively unstable position and the higher pressure on attachment by the moving food compared to *E. globidiscus*. This almost permanent attachment seemed to be responsible for provoking greater inflammatory response. The localized feeding of *E. papernai* may also be a contributing factor to the inflammation. The damage caused by both species by the hamuli and the feeding seemed to be resolved rapidly.

3.4.2 Reproductive biology

Oviposition behaviour

E. globidiscus worms were very small (0.26-0.50 mm in length) and had to be attached on to the stomach tissue for the initiation of the process of egg formation. This therefore, hindered the observations of details of the process of oviposition. But, the outline of the process is most similar to the descriptions of oviposition in the capsalid monogeneans *Epibdella* (=*Benedenia*) *melleni* by Jahn & Kuhn (1932) and *E. soleae* by Kearn (1985). Although an ootype is not clearly evident in the specimens mounted in Malmberg's fluid, the place where the ovum-vitelline complex is shaken, about 1/3 distance along the egg moving path from the ovary, can be regarded as the ootype. Unlike *E. soleae*, all the eggs expelled from the ootype were less rigid and only lightly tanned but all the laid eggs were fully tanned. The tanning process seemed to occur with the movement along uterus.

The laying of fully tanned eggs could be an adaptation to the stomach habitat so as to protect the ovum from the digestive enzymes of the alimentary

canal. The uterus seems important in this context for the hardening of egg shell and has no function of storing eggs for allowing subsequent eggs to be assembled in ootype. According to Tinsley (1983) the uterus provides a site where shell-hardening can proceed without impeding the assembly of successive eggs in the ootype. In *E. globidiscus* the shell hardening seem to be the only important function of the uterus as a second egg assembled in ootype was never seen whilst the first one was in the uterus. The whole process, up to the laying of the egg, took 20-30 minutes and for a further 3-4 hours no eggs appeared. The time taken by *C. sclerosus* was approximately 45 minutes according to Shaharom-Harrison (1984). Jahn & Kuhn (1932) reported an average of 5 minutes for *E. melleni*. It seems the time taken varies a great deal depending on the species.

The oviposition behaviour of *E. papernai* could not be observed mainly because of the difficulty of differentiating them when they were among the *E. globidiscus* worms on unpressed stomachs between glass slides. Of the few differentiated worms most were not ready for oviposition at the moments they were located and a long time had to be spent on waiting until the process started. Even in the few worms where the process was observed, the exact process was not clear.

Oviposition rate

The oviposition rates for the two species could not be investigated separately. The only possible way to separate the worms of the two species and therefore the eggs they laid, was by the in vitro investigation. Since these trials were not successful for the finding of oviposition rates, a method in which the worms were attached to the host tissue had to be employed. The main difficulty incurred in these methods was the inability to separate eggs of the two species. The size range of the eggs of the two species overlapped and morphologically they were similar. Therefore all the rates found were combined rates for both the species. Since the *E. papernai* worms represented a 17.79 % of all the worms in stomach, the oviposition rates mostly reflect the rate of *E. globidiscus*. In the

experiment where the egg laying worms were separated from non egg laying worms (Section 3.1.2.2), and when the worms were left to lay eggs for six hours, few *E. papernai* worms layed two eggs within this period as did some *E.globidiscus* worms. Though this indicates a possibility for similar egg laying rates the experiment was not giving strong evidence.

In the literature, high oviposition rates were reported for gill monogeneans removed from gills compared to worms in situ; eg. *D. vastator* (Ljaiman, 1951 and Izyumova, 1953 as cited by Paperna, 1963a) and *D. anchoratus* (Prost, 1963). In contrast to these observations the rate of egg production of *Enterogyrus* spp. removed from the stomach was very low (0.072 eggs $worm^{-1}hour^{-1}$) when compared with the in situ rate on excised stomachs (0.327 eggs $worm^{-1}hour^{-1}$) left 4 hours for egg laying for fish freshly collected from the lagoon in both cases. When the worms were removed from the stomach, egg formation initiated only within the first hour. An absence of a triggering factor, lack of food and the abnormal environment could all have been responsible. Due to the absence of the substratum some worms were unable to lay the formed eggs and others demanded longer times.

The oviposition rate calculated using live fish was lower than the in situ rate on excised stomachs for fish treated the same. There are 4 major reasons which can contribute to this difference:

(a) the ineffective egg collection method which would lead to the loss of eggs by adhering on to the vessels and filtering net together with the difficulty of differentiating eggs from the large amount of detrital material could contribute to under estimation,

(b) the loss of diurnal rhythm of passing out of faeces due to less food ingestion thus masking the actual number of eggs laid in a fixed time

(c) oxygen tension in situ stomachs of live fish may result in low rates compared to the excised open stomachs and

(d) the overestimation of the rate on excised stomach due to finding the rate

between 10-14 hours, when the diurnal oviposition rate is high and extrapolating this value for the whole day rate. Even if the diurnal variation is taken into consideration, the rate is still low, showing that (a) and/or (b) are possible reasons for this variation.

All the experiments on investigating oviposition rates on excised stomachs (other than diurnal rhythm experiments) were carried out leaving the tissue for 4 hours, from 10-14.00 hours. Therefore, the rates were termed as the 'rate at 12.00'. When the rate at 12.00 was considered for the fish freshly brought from the lagoon the oviposition rate for *Enterogyrus* spp. was 8 eggs worm^{-1}day^{-1} (0.327 eggs worm^{-1}hour^{-1}). But, when the diurnal rhythm of oviposition was taken into account only 4 eggs worm^{-1}day^{-1} were produced (0.166 eggs worm^{-1}hour^{-1}).

The oviposition rate of *Enterogyrus*, 4 eggs worm^{-1}day^{-1} is very low when compared with the rates of other monogeneans studied at temperatures between 24-28°C, eg. for some dactylogyrids the rates were 29, 15, 13.95, 13.27, for *D. extensus* (Papema, 1963a), *D. lamellatus* (Molńár, 1971), *C. sclerosus* (Shaharom-Harrison 1984) and *D. nobilis* (Shaharom-Harrison, 1986) respectively. The rate for *Enterogyrus* is higher than the oviposition rate of *D. anchoratus* which was found to be 2.13 (Prost, 1963). This low rate of oviposition may be due to the intrinsically low capacity for egg production by *Enterogyrus*. Houlihan and Macdonald (1979) reported that the reduction of ambient partial pressure of oxygen lowered, or sometimes ceased the egg production. By being evolved in this oxygen deficient environment, *Enterogyrus* may have adapted to survive with low oviposition rates. If this is the case, then the opening up of stomachs to find out the oviposition rates would over estimate the oviposition rates. Then, the actual oviposition rates may be even less than those found here.

An increase in egg production with increasing age is recorded for some monogeneans. As cited by Kearn (1986), Izyumova (1956) has shown that, in general, egg output increased with the age of the parasite over a period of about

10 days. Kearn (1985) found the same relationship for *E. soleae*. The experimental infections of the fish under Section 3.3.3.4 provided worms of same age on stomachs, which could be used for this investigation. However, due to the small number of fish available, the investigation of oviposition rate by the worms of different ages was not attempted.

Effect of external factors on oviposition rate

The holding of fish in the aquarium affected the oviposition rate of the parasites. The affect was most pronounced in the large size group of fish, where the feeding was observed to be minimal or ceased for about 7-10 days after bringing them from the lagoon, but was resumed later. In the small group of fish who were feeding normally, the rate was not affected much, although there was a significant drop around the sixth day after collection. If a factor other than the feeding, such as the physiological and behavioral changes of the fish due to the habitat change which could have changed the environment of parasite is responsible, the rate should be affected in both sizes in the same manner. This may have made at least a little contribution to the reduction of overall egg production rate in both cases and it must have affected both sizes in the same manner.

A daily rhythm of oviposition as shown by the significant difference between the number of eggs laid between day and night was obtained for *Enterogyrus* spp. Izyumova (1956) as cited by Kearn (1986), did not find any significant difference between the number of eggs laid by *D. anchoratus* for day and night. It is known that some monogeneans, *O. hippopotami* (Thurston, 1968), *Diclidophora luscae* (Macdonald, 1975) and *Diplozoon homoion gracile* (Macdonald and Jones, 1978), restrict their egg laying to a particular period of daylight or darkness or to a specific time, and they lay large number of eggs at this time. Their hosts seem to use the same site repeatedly for resting and disperse at other times of the day. They release large numbers of eggs at these times by temporarily retaining the produced eggs in uterus. *Enterogyrus* laid eggs as they were formed and did not store in the uterus. Therefore, the diurnal variation is due

to a difference in egg production, as in *Diplozoon homoion gracile* (Macdonald & Jones, 1978) and is not due to temporary retention.

The results of the effect of temperature on oviposition rates agrees with the observations of Paperna (1963a) in the tropical environment; the rate at 23°C and 29°C being significantly similar, with a somewhat greater number of eggs at 29°C and comparatively a lower rate at 35°C. As can be expected, in the temperate regions the optimal temperatures are in a lower range and a temperature of 28°C has been found to be negatively affecting the process (Ljaiman, 1951 & Bauer, 1954 as cited by Paperna, 1963a; Prost, 1963; Imada & Muroga, 1978).

The effect of pH on oviposition rate is an area to which no attention has previously been paid. This may be due to the more uniform nature of the pH of the surrounding aquatic environment of these external parasites. However, the pH of the environment of *Enterogyrus* spp. was found to vary between 3-6.5. According to the results of this study, it seems that pH around 3-4 is optimal for the egg production with a gradual reduction in the rates up to around pH 5. Therefore, the optimal pH for egg production determined experimentally correlates well with that of its natural habitat. The quality of the results of the experiment would have been improved, if the effect of higher pH values were tested. The difficulty of attaining the desired pH values on the stomach walls (pH on the stomach walls differed considerably from the pH of the buffered saline solutions in which they were placed) made this impossible.

From the histological picture, the feeding of *Enterogyrus* spp on the epidermis of stomach was evident. They may feed on the epithelium of the stomach as their ectoparasitic counterparts feed on the skins and gills of their hosts (Kearn, 1963a). In addition, this highly specialized monogenean, living in a food rich environment, may be adapted to feed on readily available source, probably in a partly digested form.

Although there was a visible increase in oviposition rates with the increase in feeding rates of the host, the relationship was not statistically significant. This casts doubt the possibility that the parasite takes an advantage of the food of the host. However, the experiment on the effect of duration of time the fish were held in the aquarium on the oviposition of these parasites, clearly shows a difference in oviposition rates between the feeding and non-feeding fish, even though the feeding rates were not monitored and only the anorexia was observed. It was thus worthy of further investigation to see if supplementary food in the relatively simple form of glucose and albumen protein would enhance egg production rates.

Glucose in the surrounding medium did not change the oviposition rates. High concentrations (0.10 M) inhibited egg production totally possibly due to osmoregulatory stress. The egg albumen at a dilution percentage of 10, showed a little enhancing effect. This visible effect was statistically significant during 4-8 time interval. Therefore, they may be displaying the effect of albumen when the generally preferred food reserve has been lost. Thus, when food in an appropriate form is present in the surrounding medium, the parasite may be able to consume it. This is worthy of further investigation.

The partial loss of natural feed available for fish and/or the complete cessation of feeding in the aquarium may result in inadequate nutrition of the parasite and, as a result they may produce eggs at lower rates when they were left in the aquarium. The natural feed of *Etroplus* is shown to consist of a variety of food material (Costa, 1983; De Silva *et al.*, 1984). Therefore, the feed used in the aquarium, *Hydrilla*, may not have been satisfactory to demonstrate a significant effect of diet on egg production, in the experiment on the effect of feeding ration.

Location of egg embryonation and development and determination of egg viability.

Developing eggs were not observed in the stomach or anywhere in the alimentary canal with only one or two exceptions. The eggs passing out with faeces

were always undeveloped. Clearly stomachs do not provide a favourable environment for egg development as the pH for 12 hours per a day varies between 3-4.5, the range which was unsuitable experimentally. The rare presence of developing eggs may be due to the accidental ingestion when fish were feeding on detritus from the bottom of the holding tanks.

The eggs laid in the stomach may be able to pass through the intestine without any damage caused by digestive enzymes. Llewellyn (1965) and Kearn (1975a) found that dactylogyrid eggs passed unharmed through crustacean guts. The inability of digestive enzymes, applied from out-side, to remove the opercular cement, in contrast to enzymes applied internally, made Kearn (1975a) suggest that the egg shell is resistant to digestive enzymes.

According to the counts of the eggs in the intestine at different time of the day, it can be said confidently that the only way out for the eggs is via the intestine. No evidence was found to prove that the eggs were pushed against the food flow towards the oesophagus. Llewellyn (1965) reported the habitual passing of the eggs of the oesophagus inhabiting dactylogyrid monogenean along the intestinal canal of the host *Sebastes madurensis*. Since the eggs of oesophagus inhabiting monogeneans are passing through the gut, there is a high possibility that the stomach monogenean eggs also are being passed through in the same way. Bender (1979, cited by Cone *et al.*, 1987) suggested the passing of the eggs of *E. hemihaplochromii* from the stomach of *Hemihaplochromis multicolor* via the digestive tract.

In summary, the rate of egg production by the parasite is optimal in the range of environmental parameters they encounter in stomachs most of the time; temperature 27-31°C and the pH range 3-4. The rate of oviposition was higher during day time when the host is feeding. During this time the existing pH of the stomachs are favourable for egg laying and there is an extra source of food for worms. But at night, the pH is highest, between 4.0-6.5 and the extra source of

food is absent. It is difficult to say which factor is most responsible. As the major food source of the parasite, the stomach epithelium, is present in plenty at all the times, the pH may have an inhibitory response during night time.

4.3 Life-cycle studies

Egg development, hatching and its stages

The eggs of *Enterogyrus* are small and oval and, therefore, may be adapted to obtain enough oxygen lying on the bottom of the water body where oxygen is lacking. All the eggs sank to the bottom, even in seawater. Thus, even if the eggs are carried away by the water turbulence, there is a high possibility for re-sedimentation as shown for the eggs of *D. sagittata* (Paling, 1965). The bottom substrate of the habitats inhabited by *Etroplus* are have low gradients or are almost flat and therefore may not be much affected by the current. Thus the eggs may not be carried far away from the areas they are laid.

The appendage on the abopercular end is a tiny spine which is barely visible, until the hatching. There are some monogenean eggs, especially those laid by parasites in freshwater habitats, in which the appendages are either absent, as in *Discocotyle sagittata* (Owen, 1970), or very short, as in *Dactylogyrus vastator* (redrawn from Nybelin, 1924 by Kearn, 1986). Cone (1979a) reports that the eggs of the ancyrocephaline, *U. adspectus* having a short but longer appendage than that of *Enterogyrus*, sink in water and become entangled in fibrous debris or rest with their sides on the sand particles. Eggs of *Enterogyrus* always rested on their sides and the appendage was always out of the reach of the bottom. Therefore it seems that the appendage is not useful for attaching the eggs onto any substratum. The egg shell did not show any adhesive property on to glass, but a very little on to plastic. This non-adhesive nature may be helpful in preventing attachment to faecal matter as it passes along the intestine and to depart from the faeces in the outside environment so as to obtain oxygen etc. for development.

Irrespective of the time taken for development, the embryonic development

of the eggs of *Enterogyrus* is similar to the development of eggs of *U. adspectus* described by Cone (1979a) and *C. sclerosus* by Shaharom-Harrison (1984). In *U. adspectus* incubated at 20°C the embryo was seen as a translucent clump of cells midway between the poles after two days. After four days, the larva was clearly distinguishable as an oncomiracidium and after 5 days, the fluid filled sac was evident. After 5-6 days, the eggs hatched. In *C. sclerosus* the time taken for these processes at 25°C incubation were 1, 2½, 3 and 4 days respectively. The major differences in the development of *Enterogyrus* eggs with those of *C. sclerosus* are the prerequisite of host mucus for the development of *C. sclerosus* eggs and the presence of the "viscous cushion". Shaharom-Harrison (1986) also reported the same requirement of host mucus for the development of eggs of *Dactylogyrus nobilis*, when placed in distilled water. Sterilized aquarium water was found to be little less effective than the host mucus added to distilled water.

The eggs of *Enterogyrus* increased in size during its development as in the case of *D. vastator*, *C. sclerosus* and *D. nobilis* (Paperna, 1963a; Shaharom-Harrison, 1984, 1986 respectively) but was not the case in *U. adspectus* (Cone, 1979a).

No evident external stimuli were required for the hatching of the oncomiracidia of *Enterogyrus* and approximately 80-90 % hatching occurred without any difficulty. Although the eggs were subjected to alternative periods of light and darkness 12:12 hours for 3 days in all the experiments, they hatched arhythmically. It is hard to believe that the mesopelagic host of *Enterogyrus* can apply a considerably strong shading, or mechanical or chemical stimulus on the eggs lying on the bottom. The only reasonable stimuli, the photoperiod, does not seem to influence egg hatching. No specific stimuli were required before the hatching of the eggs of *U. adspectus* according to Cone (1979a).

Although the requirement of a proteolytic head gland secretion for the weakening of the opercular cement has been reported by Kearn (1975a) for the

hatching of *E. soleae*. No secretions were observed with the dactylogyrids, *U. adspectus* (Cone, 1979a) and *C. sclerosus* (Shaharom-Harrison, 1984). This seems to be the case with *Enterogyrus* too. The mechanical dislodgment of the operculum seemed to be the main process involved in the hatching of *Enterogyrus*. The thrusts exerted by the oncomiracidia were very strong, even to temporarily protrude the egg shell. Even though some workers expressed the opinion that muscular exertion of larva plays a part in dislodging the operculum, only the work by Cone (1979a) suggests its full involvement. The role of the enlargement of egg size during its development may be due to osmosis and this may be useful to stretch the walls due to the pressure and thereby aid in mechanical dislodgment. However, it is doubtful that an osmotic hatching mechanism can be involved in hatching in salinities above 8 ‰, where the osmotic pressure inside the eggs is lower than the outside.

The eggs left in water salinities throughout the range 0-32‰ exhibited similar developmental stages and hatching percentages. But, a delay in larval development was observed with the increasing salinity. The delay was mainly for the duration of the first and the last stages of development, ie. the non-transparent and the actively moving stages were affected. Therefore, it may be the initiation of development and the hatching processes which are affected at high salinities. Paperna (1963a) found the same sort of relationship for *D. vastator* within the small salinity range 0.26 - 4 ‰. This parasite may be considerably stenohaline when compared with *Enterogyrus* and thus show the same effect within the small salinity range. The rapid hatching at 16 ‰ is difficult to explain. This may be due to an experimental error or it may represent the optimal salinity for egg development. As the genus *Enterogyrus* has evolved in a freshwater habitat, (Cichlids being fish originated in freshwater, and most of the other *Enterogyrus* spp. being freshwater) it is difficult to accept this as an explanation. The delay in hatching in higher salinities probably may be due to the inability of the involvement of osmotic pressure. The measurements of the developing eggs in different salinities through out the developing process are required to see how the osmolarity of the developing medium effect the size of eggs.

The rate of development of *Enterogyrus* eggs increased with the increase in temperature from 23°C up to 35°C, showing the effect similarly on each stage of egg development. Paperna (1963a) found the same relationship between 12° and 28°C for *D. vastator*. But, at the temperature 37°C, the development of the embryo was delayed and hatching failed. Prost (1963) also demonstrated that the development time of *D. anchoratus* and *D. extensus* shortens at higher incubation temperatures, but a smaller proportion completed their development and hatched at these temperatures. The results for *Enterogyrus* do not show any reduction of hatching percentages at the highest temperature tested, 35°C. Therefore, for *Enterogyrus*, the incubation temperature 35°C may still be within its limits of tolerance for egg development, even though the ambient temperatures they encounter in the natural habitat are between 27-31°C.

The ambient pH range 5-8 was tolerated by the eggs of *Enterogyrus* during their development and pH 4 and 9 showed their unsuitability by allowing for only a very low percentage to be developed and hatched. Of the range, pH 6 was optimal, 7 being the next, and 5 and 8 the least reliable for development. This preferred range supports the finding that the eggs do not develop inside the stomach, where the range is 3-5 for about 14 hours per day. The adaptability for pH 5-6 may be helpful because the eggs laid during non-feeding time of the host, ie. when the stomach pH is around 5-6, are probably retained within the stomach for a longer time due to the unavailability of digested food material for the eggs to pass out with. Therefore the adaptability of *Enterogyrus* eggs for the less acidic as well as less alkaline conditions may be advantageous as the eggs face acidic conditions in the stomachs and less alkaline conditions in the external environment.

The only work which has paid attention on the effect of the ambient water pH is that by Shaharom-Harrison (1984). According to her findings, the egg development was favoured by the alkaline environment. Even though the findings for the egg development of *Enterogyrus* do not agree with this, the oncomiracidia seem to prefer alkaline conditions.

The oncomiracidia

The general body plan of the oncomiracidium of *Enterogyrus* is similar to the descriptions of Ancyrocephaline oncomiracidia (Kingston, Dillong & Hargis, 1969; Prost, 1963; Cone, 1979b) having ciliated locomotory cells in four zones, four pigmented eyes, a prominent pharynx, and 10 lateral, 2 posterior and 2 central marginal hooks. In addition, the ventral pair of hamuli were fully formed so that further development did not take place.

The duration of the life of the oncomiracidium was around 12 hours and for the last 2 hours they were inactive. Prost (1963) reported that the period in which the larvae are viable and make rapid movements is usually little less than their whole life. The behaviour of the oncomiracidium was in two phases as described by Bychowsky (1957) a free swimming phase and "creeping" and a "gliding" phase. As cited by Kearn (1967a), Izyumova (1956) reports that the infective period of *D. vastator* was 4-8 hours of a 6-17 hours active period at 10-14°C. Therefore, the oncomiracidium of *Enterogyrus* may not be infective during the whole period of activity. This could be tested in future studies.

The findings for *Enterogyrus* suggest that the oncomiracidia withstand the salinity range 0-32 ‰ without any effect of life-cycle duration, or the active period. This is different from the findings of Paperna (1963a) on the oncomiracidia of *D. vastator* which seems to be a stenohaline species as they succumbed when exposed to salinities higher than 1.5-2.0 ‰, compared to *Enterogyrus*.

The results of the effect of temperature on oncomiracidia of *Enterogyrus* agree with the pattern found by Kearn (1967a), the lengthening of free swimming activity as well as the inactive periods by lower temperatures. At higher temperatures than those experienced in the natural habitat of *Enterogyrus*, a marked shortening of longevity occurred as found by earlier workers (Izyumova, 1956 as cited by Paperna 1963a; Paperna 1963a).

Of the pH range tested the optimal pH was 7 with a reduced longevity at 8, the range occurring in the natural habitat. Even though the eggs were able to develop in a ambient pH 5 and 6, the oncomiracidia could not survive normally even in a slightly acidic environment. This supports the fact that the oncomiracidia live outside the stomach and do not enter it.

As soon as the oncomiracidia hatched, they swam very actively using cilia, and sometimes they reached the bottom and moved on the bottom of the container by the ciliary movements little slower than the swimming rate . Inactive sinking of larvae was not observed. The attraction to the pieces of detritus and the searching behaviour performed, show that it possibly investigates any obstacle it meets in its pathway. The horizontal swimming of oncomiracidia may increase the chances of finding the meso-pelagic host.

The oncomiracidia of *Enterogyrus* hatched arhythmically. Since the active period is around 10 hours, the oncomiracidia which hatch during the early dark hours will face a difficulty in host finding if they depend on the photo-positive and negative behaviour described for *E. soleae* (Kearn, 1980). The ability of host finding in darkness is reported for the oncomiracidia of *Entobdella hippoglossi*, which hatch during early dark hours by Kearn (1974a), who suggested that the resting of the host *E. hippoglossi*, the halibut, which occurs during night on the sea bottom provides for a greater opportunity for parasites of finding a host. This close proximity does not exist between the host *Etroplus* and *Enterogyrus*. In addition, if the parasite can find the host in the dark, the value of the two pairs of eye spots, even for orientation of larva, is doubtful. Therefore, a method which allows the larva to hatch during day time may be operating.

Llewellyn (1965, 1972) considered that the monogenean eggs do not undergo development within the alimentary canal of host and embryonic development begins as soon as the eggs are exposed to water. He suggested that the simultaneous oviposition, followed by synchronous embryonic development

lead to simultaneous hatching. The eggs of *Enterogyrus* are exposed to the outside at two times in a day, when the faeces are laid by the host. Therefore, they may be undergoing synchronous development to hatch at the same time.

Etroplus suratensis passes faeces just before commencement of feeding in the aquarium, therefore, probably in the foraging sites in the natural habitat. If these eggs develop in the same sites and do not get carried away by currants, it provide an ample opportunity for finding a host at the time of their foraging, whilst slow moving as they feed on vegetation and detritus. Therefore, it may be that the eggs hatch just before the foraging time of host, when the 3 3/4 days development time is considered.

In the infection experiments, the larvae of *Enterogyrus* were found attached to the skin and the gills of the host. Even when the oesophagus and stomach tissues were presented, skin and gill tissues were more attractive than these. This suggested that the main route of infection may be by attachment to the skin, and the other alternative may be the passive ingress with the gill ventilation current with subsequent attachment to the buccal skin or gills. The non-preference for the stomach tissue clearly indicates the non adaptability of oncomiracidia for the stomach environment, and therefore, the attachment has to be somewhere outside it. Bender (1979, cited by Cone *et al.*, 1987), reported the attachment of the larvae of *E. hemihaplochromii*, first onto the skin of the head region and the migration on to gills before entering into the stomach. The most abundant gill monogenean of *Etroplus*, *Ancyrocephalus etropli*, first attach to skin or buccal cavity and then migrate to gills (personal observations). Therefore the same method may be used by *Enterogyrus* due to the similar kind of pressure in host finding faced by the oncomiracidia of both species. Whittington (1987) showed a similar type of host finding exhibited by two unrelated monogenean species *Hexabothrium appendiculatum* Kuhn, 1829 Nordmann, 1832 (gill parasitic) and *Leptocotyle minor* (Monticelli, 1888) Gallien, 1937 (skin parasitic) on the common dogfish, *Scyliorhinus canicula* L.

Host-attached *Enterogyrus* spp.

After attachment to the skin or gill, the oncomiracidia lose the ciliated epidermis. Some larvae attach to the skin whilst others possibly attach directly to the gills. The species identification could not be done until the 5th day post infection, when the dorsal hamuli formation was complete. *E. papernai* migrated in the immature form into the stomach about 5 days earlier than *E. globidiscus* on the 9th day post infection. It is only the mature forms of *E. globidiscus* which entered into the stomach. This shows that *E. papernai* has acquired adaptations to life in the stomach at an earlier stage in its life, so that it must mature in the stomachs, thus increasing its chances of finding a mate. Since they occur only as a small population, this may help in their survival, as they do not have to compete with the high populations of gill monogeneans on the gills. The oviposition of *E. papernai* also commences about 3 days before that of *E. globidiscus*, around 8-9 days post-infection. When considering both the species, the time taken to attain maturity of *Enterogyrus* is longer than the records in literature for other species such as *D. vastator*, *D.anchoratus* , *Pseudodactylogyrus metorchis* and *U. adspectus* (Paperna, 1963a; Prost, 1963; Imada and Muroga, 1978; Cone, 1979b respectively).

The oncomiracidium haptor of *Enterogyrus* is similar to the ancyrocephaline oncomiracidial haptors described (Cone, 1979c). The only difference being the presence of well-developed ventral hamuli between the marginal hook pairs 2 and 3 (marked from posterior to anterior). Llewellyn (1963) stated the occasional occurrence of ventral hamuli of relative advanced stage of development. From the second day of infection, the primordia of the dorsal hamuli were seen, and by day four development was completed, with similar shapes and sizes to the adult hamuli.

The longevity of the adult worms could not be determined properly. The first oviposition of *E. papernai* commences around 8-9 days post infection, while it occurred 11-12 days post-infection in *E. globidiscus*. When considering the whole life cycle, the longevity of both species is approximately 30 days. The

generation time of *E. papernai* is around 26 days. This duration found out at 28-30°C is much higher than the 8-10 days recorded for *D. vastator* by Paperna (1963a), and lower than the 42 days recorded for *D. anchoratus* at 20-23°C by Prost (1963).

The oviposition rate of *D. anchoratus* is comparatively lower than the other dactylogyrids and its life span is comparatively longer. The data obtained for *Enterogyrus* are almost similar to *D. anchoratus*. Therefore, there may be a possibility for monogeneans having low egg rates to have long life periods.

Overall, it was seen that the life-cycle stages of *Enterogyrus* are well adapted to the environments in which they occur.

Adults live in a more constant iso-osmotic environment with pH range 3.0-6.5 and experiencing the temperature of the host's environment. They are adapted to lay eggs at the time the fish is feeding, when the environment is much more acidic. Therefore they have high oviposition rates in the range of pH values 3.0-4.0. The temperature range 23-29°C is the optimal for egg laying. Therefore it overlaps with the range 27-31°C, which is the normal ambient temperature range experienced. During the hours of fish feeding, parasites probably use the digested food material of host as an extra source of energy for egg production.

The eggs and oncomiracidia could withstand the maximum range of salinities ie. 0-32‰, they are likely to experience. But, the egg development was faster in the actual range of salinities they experience in the lagoon, 0-16‰. The egg development period decreased with increasing temperature. However, the range 24-29°C was the best for the performance of oncomiracidia. Therefore, when the both factors, faster egg development and longer oncomiracidium performance are considered 29°C is the best temperature, the temperature they experience naturally. Eggs developed in the pH range 5-8, but the range 6-7 was better. Oncomiracidia survived well in the pH range 7 & 8. Therefore, the pH 7, which is the commonly

occurring pH in the natural environment, is the best for external stages. Therefore, even though there are acceptable wide ranges for some stages, when both the external stages are considered together the most suitable are the ranges they naturally experience.

The oncomiracidia cannot infect the stomachs with high pH as they are not adapted for this condition. Therefore, they attach to an external site, the skin and then to gills, until they gain this ability and until the dorsal pair of hamuli are formed.

There is no difficulty for *Enterogyrus* to inhabit the two localities under study as the salinity is the only factor different in them. The external stages survive well in the range 0-16‰ where the salinities of the two localities lie.

Chapter 4

Summary and Conclusions

Summary and Conclusions

A considerable number of parasites are newly recorded for *E. suratensis*. Of these, two parasite species, ie. one nematode, *Rhabdochona* sp., and one crustacean *Argulus* species appear to be previously unrecorded. Several larval intermediate stages were found, which could not be identified to the species level. There may be other unrecorded species amongst these. The monogenea seem to be the only group which was fully reported previously on *E. suratensis*. Most of these records, however, are new to Sri Lanka., and this reflects the paucity of studies on the fish parasite fauna of Sri Lanka. Of the parasites found, most seem to be loosely host specific, thus it is possible that they also occur in other species of fish, especially amongst its close relatives the *Oreochromis* spp., as was shown in this investigation. The monogenean fauna showing 'phylogenetic specificity', however, are highly specific to the genus *Etroplus*.

With the introduction to freshwater, the only group which has survived successfully are the monogeneans. The digeneans, cyathocotylid metacercaria and *Transversotrema patialens* are the species with the widest distribution and salinity tolerance. However, the low preference of cyathocotylid metacercaria for lagoons is indicated by their low intensity levels. The very low prevalence of *T. patialens* in the reservoir was possibly due to the low abundance of the intermediate host and/or the dilution of parasites in the large water body. All the other parasites which were newly acquired in the freshwater habitat were mainly encysting larval stages of helminths. These parasites must have evolved with non-cichlids since all the cichlids in the reservoir are introduced species. The infectivity of these parasites to cichlids therefore denotes their loose specificity to fish hosts. The death of cysticerci of *Paradilepis scolecina* in this new host indicated the poor suitability of the host.

The absence of the requirement for an intermediate host, together with a wide range of salinity tolerance are the most likely factors contributing to the success of the monogeneans in the freshwater habitat. In contrast, the copepods, despite having direct

life-cycles, have not been able to thrive at all in freshwater probably due to the inadequacy of their salinity tolerance.

It is not certain whether parasites with indirect life-cycles did not transfer with *E. suratensis* to freshwater due to the absence of the host/s required for the completion of the life-cycle or to the inability of the parasites to tolerate freshwater conditions. The background information on host/s or the locality range of these first time records of parasites is lacking and it is therefore difficult to determine the reason. Further experimental work on the tolerance limits of the life-cycle stages of the parasites is required in order to determine the reasons for the distribution observed.

In general, it would appear that the equable environmental conditions seem to be responsible for the lack of any drastic changes in the parasite infection levels over a period of one year. A situation very different from the temperate condition with its demarcated seasonal changes. The rainfall, however, does seem to have some influence on the infection levels, directly or indirectly. For example, it influenced cercarial release in cyathocotylid metacercaria, and the copepodid intermediate stages of *D. amplectans* may have been washed away by floods or affected by a decrease in the salinity level in the lagoon. The rain indirectly caused a rise in the level of monogeneans, possibly by creating behavioural changes such as aggregation of fish due to flooding. Though the temperature changes were minimal, the small drop which occurred in December-January influenced the cercarial release of most of the species of metacercaria found. Therefore, these two changes in the climate seem to be the most important factors influencing parasite population changes. The similarity in the abundance through the year of the adults of parasites with indirect life-cycles suggest that the climatic factors affecting the larval stages are masked by other factors.

The number of fish in the samples used for the seasonal survey may, in some cases have been too low and not representative of the real situation. It would be interesting if this work could be followed up by sampling for two years, with one sample from each locality for each month. A minimum number of 30 fish per sample would be preferable.

The larger fish appeared to carry a higher load of parasites possibly due to their larger size or to the accumulation of parasites with age (assuming that the larger fish are indeed older). The smaller fish seemed to be more vulnerable to trypanosomiasis and the medium sized fish were preferred by *D. amplectans*. It would have been interesting to find out if the parasites using copepods as intermediate host are acquired during the earliest stage of the fish where zooplanktivory predominates. The lack of very small fish as a result of the sampling method meant that this could not be effectively investigated in the present study. However, the presence of infective stages in the larger fish indicated the possibility that they can enter at any time of the life and that the fish continue to feed on zooplankton to some extent throughout life.

The protozoans which multiply rapidly under optimal conditions are already causing problems in aquaculture systems. *I. multifiliis* and *Ichthyobodo* are common pathogens of cultured fish in many parts of the world and would cause problems in any stressful culture situation.

The gill inhabiting metazoan parasites were restricted to different regions of the gills. It may be that at high infection levels the parasites will spread to the other, less preferred sites. However, if their site preference is governed by the rate of water flow through the gill, it is unlikely that the parasites will survive in these sites. The hyperplasia which develops may be able to dislodge the worms in a self-curing process and may explain why the infection of *A. etropli* decreased spontaneously following peaks in the aquarium. However in severe conditions, the hyperplastic response can interfere with respiration. Though *D. amplectans* infects only the tips of the primary filaments, it may exacerbate the situation.

None of the common internal parasites were highly pathogenic. The fish were able to respond to their presence in the tissue, and wall them off in a fibrocytic capsule with the exception of the renicolid metacercaria which appeared to evade the host response. The response to *P. scolecina* appeared to be very intense and was effective in destroying most of the parasites. The destruction of cyathocotylid metacercaria was also seen, and could also be due to the host response. Though they

appeared to be non-pathogenic, high burdens of these parasites can weaken the fish making them vulnerable to other infections and may result in poor growth rates or food conversion efficiency. *Etroplus* seemed to be more vulnerable to many of the parasitic infections compared to *Oreochromis* species and did not seem to be very hardy in the aquarium conditions.

The study of the two *Enterogyrus* species revealed many of their adaptations to the unusual and hostile habitat in the fish.

The relative proportions of the two *Enterogyrus* populations were found to be stable and similar in the fresh as well as brackish water environments. Therefore, it seems that these two species were equally well adapted to the two environments they encounter, suggesting that the salinity changes experienced in these habitats did not interfere with their overall biology. It would be interesting to extend the investigation for at least a period of a year to provide a better picture of the influence of the salinity and the other climatic changes occurring on the two *Enterogyrus* populations with the picture of the population structure in the stomach. These findings would help to uncover more differences in the biology of the two species such as in host finding ability and recruitment periods.

The population size difference of the two species of *Enterogyrus* was apparent and, superficially, indicated a competition between the two *Enterogyrus* spp. Other evidence however, showed that this was not necessarily the case. The two species were aggregated mostly in the same site, the posterior dorsal side of the stomach. This appeared to be the most favoured area for both the species as shown experimentally. However, within this site, the two species showed a distinct niche segregation; the *E. papernai* worms showing a greater affinity for the depressions between the folds in the stomach and *E. globidiscus* worms showing a greater preference to the side walls of the folds in the stomachs. If there was a space limitation, it would most probably operate amongst the individuals of the species *E. globidiscus*, rather than between the two species, as they occur in high numbers and their niche area is comparatively smaller.

The lower number of *E. papernai* worms in the stomach may therefore be attributable to some difference in their biology or the host reaction compared to *E. globidiscus*. The more sedentary *E. papernai* was relatively more pathogenic than the mobile *E. globidiscus*. Its mode of attachment and its sedentary nature could reasonably be involved in this increased pathogenicity. The stronger host reaction against *E. papernai* may be responsible for less success in establishing, resulting in a limiting of the population size. However, since the relative proportions of the two populations appeared to be similar throughout, this constant level suggests that *E. papernai* is well able to maintain its established level.

The oviposition rates and the factors involved in the changes of oviposition rates were determined for the combined species. The values obtained would be much closer to the real values for *E. globidiscus*, since these worms were predominant. The egg laying rate of *Enterogyrus* for fish directly brought from the lagoon was 4 eggs $worm^{-1}day^{-1}$ and the rate differed depending on the time of the day; the rate at 12.00 hour being 8 eggs $worm^{-1}day^{-1}$. There is little recorded information on the variations in egg production of monogeneans or other helminths in response to diurnal changes.

When the fish were left in the aquarium the oviposition rates dropped and then later resumed. This apparently resulted from the changes in the feeding of the fish following removal from the natural environment and its subsequent influence on the micro-habitat of the parasite. Factors such as the change in the type of food presented to the fish host may also be important. This calls into question whether the parasite consumes the partly digested food of the host or digestive products as well as host tissue. The experiments indicated a possibility that *Enterogyrus* feeds on simple proteinaceous compounds, though this needs further clarification. At the temperature 35°C the rate of egg production drastically dropped whilst at 23°C it was the same as at 29°C, the ambient temperature of the parasites' natural environment. The pH 5.6 was found to be unfavourable whilst at 3.6-4.6 the rate was not affected. This reflects the range that the worms experience during the times of the highest oviposition.

It seems, therefore, that the physiology of the worms living in this hostile

environment is adjusted to optimise egg production. Thus, this modification in physiology could have been a part of their process of adaptation to the environment. There were two possible factors influencing the rate of egg production, the availability of the external food source and the gradual rise of acidity starting around 6.00 a.m. However, other related factors appearing at this time, such as the presence of digestive enzymes, may have been the stimulating factor. This study did not determine which of these was responsible but this will be an interesting subject for further investigation in the future.

The eggs produced seemed to passively move out with the food contents of the host, and the egg development process was delayed until the eggs reached the external environment. Clearly pH values less than 4 inhibited the egg development. However, the eggs appeared to tolerate high acidity for short times until they passed out of the stomach with the digested food material. The intolerance of low pH values may require the eggs to pass out as soon as possible.

The higher the temperature, the more rapid the egg development with eggs developing fastest at 35°C. The most important adaptation which helped the parasites to thrive in any of the habitats was the salinity tolerance of their eggs, though the development was a little slower in the higher salinities.

The oncomiracidia were only capable of surviving at the pH values around neutral i.e. the normal value existing in the water body. The highest duration of life of the oncomiracidia was at the lowest temperature tested, 23°C. The highest temperature tested, 35°C, caused them to die faster. Though the eggs developed fastest at this temperature, the oncomiracidia were incapable of surviving. The ambient temperature of their natural habitat, 29°C, gave a considerably higher survival time, though it was slightly less than the value at 23°C. The oncomiracidia were capable of surviving equally well in all the salinities tested (0-32 ‰). This is a considerable advantage as a long survival time for the oncomiracidia is vital for host location.

The oncomiracidia attached to both the skin and the gills of the host. The skin

is likely to be the most common site of attachment, as there is a higher probability of contacting with it. The youngest worms of the two species found in the stomach were larger and more developed than the oncomiracidia suggesting that they live for a period in a site other than the stomach, eg. the gills, before they finally enter the stomach. An interesting finding was that *E. papernai* worms entered the stomach in a relatively immature state whilst all the *E. globidiscus* worms found in the stomachs had a complete set of reproductive organs. This suggests that their sites of mating were probably separated; *E. papernai* mating in the stomach and *E. globidiscus* in the gills or a particular site on their way to the stomach. This maybe one of the key factors in the separation of the two species.

E. papernai started egg laying around the eleventh day of life (including egg stage) whilst it started around the fourteenth day for *E. globidiscus* showing a difference in generation time. The life span for both of the species was more than 24 days. These time periods are long compared with other dactylogyrid monogeneans. Possibly the longer generation time and longevity of adult worms have evolved as a requirement for the adaptation to the stomach environment.

The major problem encountered in this study on *Enterogyrus* was the difficulty in differentiating the eggs of the two species. Thus, the egg laying rates and the life-cycle duration data for the larvae were for the combined species. The results of the two experiments carried out with very low infection levels of worms in the stomachs showed that most of the worms of both species produced 1-2 eggs, 12.5 % of *E. papernai* and 20 % of *E. globidiscus* worms produced 2 eggs within a period of six hours, indicating that there is a high probability that the oviposition rates for both species are similar. Furthermore, the data from the egg and oncomiracidia studies did not show any considerable variability as indicated by the standard deviations of the means. This shows either that the eggs and oncomiracidia of only one species were tested or that there is similarity of the data for both species.

Finally, this study has shown a high degree of deviation in the biology of the stomach inhabiting ancyrocephaline worms from their gill inhabiting counterparts.

Each stage of their life-cycle was highly adapted for the environment they inhabited. Furthermore, it elucidated the differences between the two species of *Enterogyrus* wherever possible. There are a few interesting points which need further clarification; the stimulation of high oviposition rates during the daytime and the possibility that the monogeneans utilize host food. The reason for the low population number of *E. papernai* and the extent of the stability of this proportion with seasonal fluctuations is another interesting field of research. Finding hosts harbouring only one species of *Enterogyrus* or finding methods of single species infection experimentally would be a step forward in such a study.

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