STUDIES ON PARASITES OF ORNAMENTAL FISH FROM SOUTH AMERICA WITH PARTICULAR REFERENCE TO THEIR PATHOGENICITY AND POTENTIAL FOR TRANSFAUNATION.

A thesis submitted to the University of Stirling
for the degree of Doctor of Philosophy

by

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August 1995

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DECLARATION

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

Abstract

This study, by a variety of approaches, investigated the health of wild ornamental fish species prior to export and on their arrival at the importers. It was conducted in collaboration with British importers in the UK, exporters from Venezuela, and fishermen and exporters from Brazil.

The study in the UK involved a series of interviews with British importers and hobbyists and a long-term period of sampling of shipments of fish from the 4 major exporting countries of the Amazon Basin. A total of 735 specimens of tropical ornamental fish, comprising 9 families, 22 genera and 33 species, were sampled. Although around 100 species of ornamental fish are being exported from South America, the majority of the species selected for study belonged to the family Callichthyidae, predominantly the genus *Corydoras* Lacépède, 1803.

In the 28 shipments of ornamental fish examined in the UK, 8 species of protozoans, 7 species of monogeneans, 1 crustacean and 13 species of digeneans (of which 6 were adults and 7 metacercariae), 13 species of nematodes (of which 10 were adult stages), 1 pentastomid nymph and 1 acanthocephalan species were found. With the exception of the protozoan *Piscinoodinium* sp., and the mesocercariae of Strigeoidea, all major groups of parasites were generally found at low prevalence and intensity of infection. These results may be related to the exporters' procedures and/or a series of prophylactic treatments that the fish were exposed to prior to export or because heavily infected fish die prior to export.

Very few importers in the UK deal with the direct import of fish from South America. Importers of wild freshwater fish often prefer to buy wild quarantined fish from the United States, Germany or the Netherlands rather than buy directly from South America because they believe that these quarantined fish carry less parasites and present fewer disease problems.

The study in South America was conducted in Brazil and Venezuela during the beginning of the fishing season for ornamental species. Routine techniques for the detection of common fish diseases are not used by the ornamental fish trade in Venezuela and quarantine procedures appear to be related more to the demand of the market rather than to a concern for the health of the fish. In Brazil, 11 exporters were dealing with ornamental fish from the Amazon basin and the study was conducted in collaboration with the three major exporters from the region and selected fishermen working under their supervision. In general, no fish health programmes are reported by Brazilian exporters.

A total of 456 specimens of fish, comprising 5 families, 6 genera and 15 species, were sampled at the exporters' holding facilities. The parasite fauna was composed of 9 species of protozoans, 10 species of monogeneans, 6 digeneans, (of which 5 were metacercarial stages), 6 nematodes, (of which 1 was a larval stage) and 1 acanthocephalan. Only minor differences were observed between the results obtained in Brazil and in the UK and these appeared to be related more to the exporters' procedures than to differences in the natural composition of the parasite fauna of the fish.

Fifteen monogeneans were found in the species of fish studied. In the UK samples, 7 species were found in single infections on the skin, gills, and excretory system. Five species belonged to the genus *Gyrodactylus* Nordmann, 1832 of which 4 were parasites of the callichthyids *Corydoras* spp., *Brochis splendens* (Castelnau,1855) and *Callichthys callichthys* (Linnaeus,1758) and one a parasite of a curimatid, *Semaprochilodus taeniurus* (Steindachner). One of these species, *G. gemini* Ferraz, Shinn and Sommerville, 1994 has been fully described and published. Amongst the dactylogyrids, an interesting finding was the presence of one species parasitising the excretory system of *Mylossoma aureum* (Spix), the silver dollar, from Colombia and Peru. This species is fully described and placed in the genus *Kritskyia*

Kohn, 1990. This appears to constitute only the second species to be described for this genus.

In the fish sampled in Brazil, 10 monogeneans, all gyrodactylids, were found, of which 8 occurred either in single or in mixed infections in *Corydoras* spp. and two occurred in mixed infections in *B. splendens*. Overall, single infections of gyrodactylids were more common than mixed infections. It is possible that some of the mixed infections were transient infections as these fish are commonly captured in large schools and kept under overcrowded conditions. These *Gyrodactylus* spp. were examined using recently developed SEM techniques and 8 species are fully described.

Many of the problems associated with wild ornamental fish species from South America were found to be related to the stressful holding and transport conditions, and to opportunistic parasites, which take advantage of the favourable conditions to multiply and cause mortalities. Only a limited number of parasite species were associated with pathological conditions on arrival of the shipments in the UK. Among these species the most common were the protozoans *Piscinoodinium* sp., *Chilodonella hexasticha* (Kiernik, 1909) and *Ichthyophthirius multifiliis* (Fouquet, 1876), the mesocercariae of Strigeoidea, and the monogenean *Kritskyia* sp. The pathology of *Piscinoodinium* sp., the mesocercariae of Strigeoidea and the monogenean *Kritskyia* sp. have previously been given little attention and were therefore studied in detail. A unique feature of *Piscinoodinium* was described where individual groups were enclosed in crypts within the epithelium. The mesocercariae were also unusual in that 1 to 15 individuals were commonly found enclosed by the same host cyst. Such cysts were widespread in the body of callicthyids. Specimens not enclosed by a host cyst were only found within atresic oocytes. Minimal pathology was associated with the monogenean *Kritskyia* sp. in light infections. However, heavily infected fish presented an intense infiltrative cellular response. The epithelium of the

urinary bladder was metaplastic and separating from the basal layer with the formation of vesicles.

Several species of parasites with direct or indirect life cycles with the potential for transfaunation were found in the UK samples. Experimental studies were conducted utilising nematodes of the genus *Spirocamallanus* Olsen, 1952 and native and commercial species of copepods commonly used as live food for ornamental species, to evaluate their potential for transfaunation. Only early L_3 were obtained from the copepods that became infected with the larvae of *Spirocamallanus* after 17 d.p.i. The low temperature (19-23°C) appeared to be responsible for the slow development of the larvae and its thermophilic character appears to limit the range of distribution of these nematodes to warm waters. In the UK, these stages were developed in the copepod, *Cyclops viridis* (Jurine), which commonly occurs in British waters and is also commonly supplied commercially as live food for ornamental species.

The life cycle of one species of *Spirocamallanus* was also investigated in Brazil, utilising two native species occurring naturally in the holding tanks at the exporters' holding facilities where the fish were kept. One of these species of copepods, *Thermocyclops dicipiens* (Kiefer, 1929) exhibits a wide distribution in South and Central America and the tropical regions of Asia. The complete development of the larvae to the infective stage was obtained in both species in 10 days, contrasting with the results obtained in the UK. The development of this *Spirocamallanus* in copepods collected from ponds where the fish were held supports the hypothesis that parasites with indirect life cycles can be acquired by fish during the holding period. The development of these nematodes in *T. dicipiens* also illustrates the vulnerability of those countries with a large aquaculture production of freshwater aquarium fish to the introduction of parasites from other tropical regions.

Acknowledgements

A special thank you to Dr Christina Sommerville, my supervisor, for her valuable guidance, interest and friendship throughout the course of this study.

I would like to thank all the collaborating British importers, and Brazilian and Venezuelan exporters for their help during the sampling periods, in England, Scotland, Venezuela and Brazil. My special thanks are also extended to Mr. Cliff Murray for all his help and guidance, particularly at the beginning of this study.

A very special thank you must go to Maureen Menzies, for her guidance in the laboratory and encouragement in the most difficult moments and to my dear friends James Bron, Tom Turnbull and Andy Shinn for their support, long-term discussions on my work, and friendship.

I am extremely grateful to "my driver", Dave Gibson, for all the many hours he gave up to act as "my personal fish transporter".

I would also like to thank all other staff and students with whom I have had the pleasure of working especially Dr. Rod Wootten, Keith Ranson, Marguerite, Sheila, Obdulio, Andy Bell, Bill, Helen, MacGeorge, Vino and Nayana.

I am greatly indebted to the following experts who willingly gave up their time to confirm the identity of various fish, parasites and intermediate hosts encountered during the course of this study. They were: Dr. Sven Kullander from the Swedish Museum; Michel Jegu from

ORSTON/INPA; Dr. J.C. Malta, Roserval Leite, Jansen Zuanon, Nelson da Silva from INPA; Dr. A. Batista from IBAMA; Dr. L. Chao from Universidade do Amazonas; Dr. G. George from the Institute of Freshwater Ecology, Windermere Laboratory; Dr. Delane Kritsky from Idaho State University, USA, and Dr. David Gibson from The Natural History Museum.

I must to extend my thanks to my family, especially to my mother for all her support and encouragement in the most difficult moments, and to Val and Chris, who first encouraged me to study for a PhD in Britain.

I would like to thank the British Council and The Brazilian Research Council, CNPq, for provision of a research studentship, and my employers, The Instituto Nacional de Pesquisas da Amazonia, INPA, for allowing me to conduct my studies in Scotland. I am extremely grateful to my friends and colleagues from the Parasitology Laboratory, INPA, for assuming my responsibilities during my absences.

Finally I would like to say a big thank you to Clive, my husband, for all his hard work in translating my "Portangles" into English and for his support, patience and love.

Table of Contents

List of Figures

List of Tables

Chapter 1

General Introduction

General Introduction

1.1 The size of the international market

The aquarium fish industry is an important element in the economy of several exporting and importing countries. In the exporting countries the industry has been contributing not only to the entrance of foreign currency but also in the creation of jobs in very remote areas where people have little opportunity to earn money. In the importing countries beyond the sales of their primary products, the fish and associated accessories, the aquarium fish industry has also brought pleasure to millions of hobbyists.

Although the most recent statistical figures are not available the global sales of fish, plants and associated accessories for the ornamental fish industry have increased steadily from U.S.$ $4x10^9$ in 1971 to U.S. $ $7.2x10^9$ in 1986 (Axelrodi, 1971, Bruton and Impson, 1986, Andrews, 1990; Anon. 1992). For the UK, more recent figures show that the annual total retail sales associated with the ornamental fish trade rose from 115 x 10^6 pounds in 1987 to 178 x 10^6 in 1990 (MINTEL, 1991). In the UK, fish are the third largest item in the hobbyist trade sales having risen in value from £ 19 million in 1987 to £ 27 million in 1990, as illustrated in the Table 1.1.

Tropical aquarium fish have been exported from several countries in Asia, Africa and South America as well as from industrialised countries such as the United States and

Germany. On a regional basis the origin of exported tropical aquarium fish is as follows: Asia, 60%, South America 30%, Africa, the Caribbean and other areas, 10% (Anon., 1992). Although Africa has vast natural resources of aquatic life, its exports of fish are estimated at around 2% of the global total, and the potential market for West and Central African species is only just beginning to be exploited (Anon. 1992). The same is true for other exporting countries which have only recently started to explore new aquatic resources.

Table 1.1

Retail sales of water garden and fishkeeping products (at current prices) 1987-1990 in the United Kingdom.

	1987 £m	1990 £m
Aquarium hardware and accessories	42	66
Ponds and fountains	25	44
Fish	19	27
Fish food	12	16
Pumps, filters, other accessories	10	14
Aquatic plants	7	11
Total	115	178

Source: MINTEL (1991)

In this context, some of the South American countries such as Brazil present good opportunities in this growing market, mainly due to the richness of its fish fauna, which is

only partially explored.

From Southeast Asian countries most of the freshwater species exported are tank bred in captivity and include both the native species and varieties raised from breeding stock imported from other parts of the world. In contrast, the majority of the freshwater species exported from South American and African countries are usually caught wild.

Currently, the major markets for ornamental fish are the USA, Western European countries and Japan, which have become internationally recognised according to their market size and the type of fish commercialized. The United States for example, has been supplying mostly South American wild fish shipped from Miami or tank raised in Florida. Among the Western European countries, The Netherlands, and Germany have been supplying wild caught fish from different parts of the world which have been quarantined and tank bred species. The Netherlands has become the largest supplier in the European market (Bassleer, 1995) importing fish from small and major exporting countries around the world (Fig. 1.1). The most recent import figures available are for Germany and are presented in the Table 1.2.

Table 1.2

Import value (DM) of ornamental fish in Germany for the period between 1991-1993.

Origin	1991	1992	1993
Singapore	10,122,000	10,161,000	9,760,000
Europe	7,796,000	8,305,000	7,785,000
Brazil	2,819,000	2,996,000	3,627,000
Colombia	1,583,000	1,750,000	1,872,000
Thailand	871,000	1,311,000	2,024,000
USA	1,193,000	1,027,000	787,000
Israel	992,000	774,000	1,000,000
Indonesia	569,000	641,000	562,000
Hong Kong	766,000	453,000	262,000
Nigeria	338,000	481,000	376,000
Japan	305,000	424,000	595,000
China	96,000	310,000	516,000
Peru	275,000	225,000	411,000
Zaire	456,000	268,000	138,000
Zambia	94,000	236,000	271,000
Tanzania	104,000	166,000	309,000
Malaysia	160,000	205,000	214,000
Malawi	293,000	154,000	107,000
Burundi	175,000	128,000	171,000

Source: Bassleer (1995).

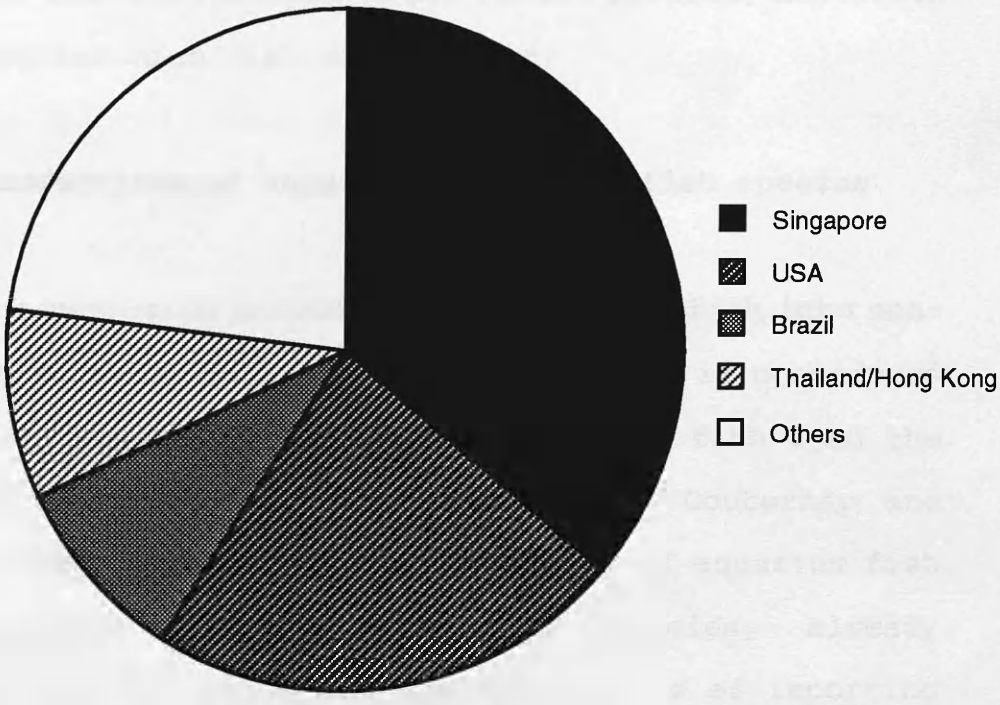


Fig. 1.1 Country of origin (as % market share) of ornamental fish imported into the Netherlands in 1987.

Source: Shenoy (1987)

1.2 Problems faced by the international ornamental fish industry.

At the same time that the growing market in ornamental fish has brought an increase in business for importing and exporting countries, it has also created a series of problems relating to the introduction of non-native species, depletion of wild stocks, high fish mortality etc.

1.2.1 Introductions of aquarium freshwater fish species.

The un-regulated introduction of aquarium fish into non-native habitats, by both industry and hobbyist is considered to be one of the major sources of introduced fish into the waters of many nations (Couternay, 1989; Couternay and Stauffer, 1990). However, the actual number of aquarium fish species, mainly tropical freshwater species, already introduced and established in the open waters of importing countries is not well known. Since the introduction of *Cyprinus carpio* Linnaeus, 1758 into Europe for aquaculture purposes during the Roman Empire (Welcomme, 1981, 1984, 1988) at least 237 species of fish have been introduced worldwide (Welcomme, 1988) of which a substantial number are commonly found in the world aquarium fish trade. Although collection of one or several individuals of a particular species of aquarium fish from open waters does not provide evidence of establishment, clearly fish are being released from the aquarium fish

culture facilities and by hobbyists creating the potential for adaptation of imported species to a new environment (Couternay and Stauffer, 1990). Introductions of aquarium species is a world-wide problem and the situation seems to be most critical in countries where environmental conditions (e.g. Australia and Singapore) and large-scale aquacultural operations (e.g. United States and Singapore), favour escape and establishment of non-native species (Andrews, 1990).

The problems relating to the introduction of aquarium fish and other exotic species into Australia have been reported by McKay (1977; 1984; 1989). According to this author, Australia has an impoverished freshwater fish fauna, and a "permissive" freshwater habitat which, according to Couternay *et al.*, (1974) is the type of habitat particularly susceptible to the establishment of non-indigenous exotic species. So far, the establishment of aquarium fish in Australian waters has been limited to a few species, *Poecilia reticulata,* Petters, guppy, *Xiphophorus hellieri* (Heckel), swordtail, *X. maculatus* (Guenther), southern platy, *X. variatus* (Meek), variegated platy, and *Poecilia sphenops* Valenciennes, black mollies. These species have become successfully established as feral populations and yet are still included in the list of "approved" species by the government despite their capacity to harm native freshwater fish. (Mckay, 1984; Datodi, 1993). Although instances of the introduction of *Geophagus brasiliensis* (Quoy and Gaimard, 1824) have been recorded (McKay, 1984) in Australia, there are no records of the successful establishment of this South

American species in Australia.

Several factors have contributed to the rapid introduction of aquarium fish species into the United States and Singapore over the years, including the high volume of live fish imported, the large number of aquarium fish farms, and their locations at the centre of major air and shipping routes to and from different parts of the world. Couternay and Stauffer (1990) reported that "the first exotic fish to establish a reproducing population in open waters of North America was an aquarium fish species, the goldfish, *Carassius auratus* (Linnaeus), introduced three centuries ago. Since then, around 30 species of aquarium fish escaped or were released into waters of the United States and became established as feral population". Out of these 30 species, five species, *Hypostomus* spp., suckermouth catfish, *Pterygoplichthys multiradiatus* (Hancock), radiated ptero, *Astronotus ocellatus* (Cuvier), Oscar, *Cichlasoma bimaculatum* (Linnaeus), black acara, and *Geophagus surinamensis* Bloch, red striped earth-eater, are species native to South America. A list of all exotic species of freshwater fish (aquarium species, food fish species etc.) collected from open waters of the United States, without evidence of establishment, was provided by Couternay, Hensley, Taylor and McCanan (1984) and Couternay and Stauffer (1990).

In Singapore, considered the centre of the trade, 37 species of aquarium fish have already become established, of which one species, the catfish, *Pterygoplichthys* sp., suckermouth, is native to South America (Ng and Lim, 1989;

Ng, Chou and Lam 1993). Additionally, this species has also been reported to have established in other parts of Southeast Asia (Malaysia Peninsular and Java) and adapted to a wide variety of habitats (Ng *et al.*, 1993).

Most of the introduced species in Singapore are lacustrine (e.g. cichlids) or hard water (e.g. poeciliids) which, in part explains why the native fauna has apparently not suffered greatly at the expense of the introductions (Fernando, 1990). However, the recent establishment of species able to invade the acid water habitats of native species is a matter of serious concern (e.g. the catfish, *Pterygoplichthys* sp. and the tiger barb, *Puntius tetrazona* (Bleeker)). Although large numbers of South American/ Amazonian species of characins, tetras and catfish which also originate from acid waters, are imported and sold in Singapore, however, it appears that none of them have yet become established. (Ng *et al.*, 1993).

In the United Kingdom, the steady growth in imports of ornamental fish species over the last decade has contributed to increased concern about the introduction of non-native species via the trade. The freshwater fish fauna of the British Isles is scarce, around 50 species (Maitland, 1972). Out of these, 12 species have been successfully introduced and are established as part of the fauna (Wheeler and Maitland, 1973). *Cyprinus carpio* and *Carassius auratus*, popular species in the aquarium industry, are among the introduced and established species in the UK. The status of other species, such as *Poecilia reticulata*, the guppy, is

uncertain (Wheeler and Maitland, 1973).

Notwithstanding the large number of tropical fish species that have been imported into the UK, no species is known to have been introduced and established in this country, although there have been reports of the release of some species into artificially heated localities in England (Wheeler and Maitland, 1973).

Unfortunately, the problem of introductions of aquarium species by the ornamental fish industry has only been seriously considered by the major importing countries. Their consequences in the exporting countries, where large number of species are constantly been moved from different geographical regions and stocked at the main exporters' holding facilities prior to export, are so far unknown.

1.2.2. Depletion of wild stocks

There is growing concern among environmentalists that the large-scale capture of tropical fauna for commercial purposes will lead to gradual extinction of such fauna and consequent damage to the environment (Anon., 1992). It is also becoming clear that the lack of studies (at least on the most commercialized species) on their native ecosystem to find out what is happening to the fish population, makes it very difficult to evaluate what is the real situation.

South American and African countries are major world suppliers of wild freshwater ornamental species, but the impact of the trade appears to have been reported only for

a few species, such as *Paracheirodon axelrodi* (Schultz), *Symphysodon* sp. from the Rio Negro, Amazon region (Bayley and Petrere, 1990) and several species from Sri Lanka and Malaysia (Banister, 1989) as a result of over-collection for the ornamental fish trade. Andrews (1990) based on the 1988 *Red List* (IUCN, 1988) reported that "596 species of fish are considered to be threatened, but only a very small number of these are of interest to the ornamental fish trade". According to this author, some of these species are of interest only for a minority of hobbyists and others such as *Barbus* species, *Belontia signata* (Günther) and *Scleropages formosus* (Müller and Schlegel), are already being produced for the trade on fish farms .

Unfortunately, problems relating to the depletion of wild stock, have only recently started to be addressed in the Amazon region, (Chao, 1993; Barlleta, 1994; Prada-Pedreiros, 1992). Consequently, the damage caused to the wild stocks by the trade is still unknown. To date, it appears that these types of study have not yet been conducted in African countries.

1.2.3 High mortality rates

High mortality rate is one of the most critical problems still faced by the ornamental fish industry. High losses in their natural environment following capture and in transit to importing countries have been commonly reported (Conroy, 1975; Conroy *et al.*,, 1981; Anon., 1992; Chao,

1992a). However, as there is very little reliable export and import data available no confidence can be placed in the overall accuracy of these figures.

In the early 1980's, the mortality rate for the Amazonian's exporters were estimated to be between 50 and 70%, of which 40-50% died before export (Conroy *et al.*, 1981). Comparisons between old figures and the most recent data, suggest that, although some improvements have been achieved by the trade, the number of losses is still high, at around 50%, between the fish's capture and their arrival in the European markets (Anon., 1992). These figures become more alarming when compared with the losses suffered by the Southeast Asian countries, where an average mortality rate of around 2-3% may be experienced by Singapore's exporters (Lim and Chua, 1993).

In response to the need to conserve wild populations, and to improve the post shipment survival rates of wild caught species, the ornamental fish industry has been undergoing a series of changes in the last few years, which, in part have contributed to a general improvement within the trade. In this regard, the ornamental fish industry and those related to it, started to develop close working links with government policy-forming bodies, which has already resulted in the creation of the closed season for the Amazon region. Another important change is related to the appearance of new sectors in the market, involved mainly with re-exporting of quarantined wild fish, which have been contributing to the

post-shipment survival of these species. Germany and The Netherlands have become recognised as the major suppliers of quarantined wild fish due to the good quality of the fish that they have been re-exporting. The importance of Germany in this respect is indicated by the imports value of freshwater ornamental fish for the last three years which are presented in the Table 1.2.

The brighter colours presented by the wild caught species in relation to the tank-bred species, the greater number of varieties, the relatively low costs of collection, in combination with the decrease in domestic tank-bred supplies of tropical ornamental fish in the industrialized countries due to the drastic increases in costs, especially heating, are some of the factors contributing to new market opportunities for the developing countries to export wild species. However, according to Bassleer (1995) good quality is dominant over cheaper price. At the same time that new opportunities have arisen, the large majority of the exporting countries, with the exception of some Southeast Asian countries, still need to make considerable improvement to their aquarium fish trade. Fish are still perishing as a consequence of a lack of adequate collection methods and the poor transport and sanitation technique of the fishermen and exporters in the developing countries.

1.3 Background to the present study

The aim of this study was to investigate the health status of the ornamental fish directly imported from South America into the UK and their condition before export, by a range of techniques and approaches.

The study was divided into two phases, one conducted in the UK and other in two South American countries, Brazil and Venezuela. Studies in the UK included direct approaches to importers to investigate the main problems they experience, combined with long term sampling programmes of shipments of ornamental fish, in order to detect and identify the diseases that are entering the UK with their hosts and their pathogenic potential. Experimental infections were also conducted to investigate the possibility of transfaunation of a parasites from imported fish to native fish utilizing native copepods and those commonly commercialized as live food as potential intermediate hosts.

In South America, studies included direct approaches to exporters in Venezuela and exporters and fishermen in Brazil, to investigate the main problems experienced by the ornamental fish trade prior to export, combined with a short term sampling programme of the shipments at different stages before export to investigate the main diseases present. Experimental infections were also conducted with the same group of parasites investigated in the UK, to evaluate the possibility of acquiring the infection at the exporters' holding facilities via potential native intermediate hosts

commonly found in the holding tanks.

Chapter 2

General Materials and Methods

Chapter 2

General Materials and Methods

2.1 Materials and methods

The methods commonly employed throughout the period of study are described in this chapter. The methodology utilised for the preparation of specific materials has been described in the appropriate chapters.

2.1.1 Fish

2.1.1.1.Source of Fish

Samples of shipments of species of tropical ornamental fish imported from South America were taken on their arrival in Britain. Importers collaborating with the project provided information in advance of the species of fish ordered, their origin, and the date and expected arrival time of the shipments.

Shipments from South America arrived at London, Manchester or Glasgow airport either directly, or indirectly via Miami, USA.

Shipments arriving in Glasgow, or re-routed to Glasgow from Manchester were sampled on their arrival at the importer's holding facilities in Glasgow.

Shipments arriving in London were either re-routed to Glasgow and sampled on their arrival at the importer's or sampled by English importers at their holding facilities and sent by courier to the University of Stirling. Samples taken in London were re-oxygenated and forwarded in their original

shipping water. Apart from re-oxygenation of the water, the collaborating importers were asked not to treat in any manner the samples of the shipments to be analyzed.

2.1.1.2 Maintenance of fish

Each lot of species of fish sampled was kept for 1-3 days in a 25l aquarium in the tropical aquarium facility at the Institute of Aquaculture. Each aquarium was provided with constant aeration and half of the volume of water was changed after the two first days in order to ensure good water quality. During the holding period, the fish were not fed until parasitological examination of the sub samples was complete when they were fed with a commercial tilapia formulation.

2.1.1.3 Establishment of parasite free stock

On the arrival moribund fish and those showing clear evidence of ectoparasitic infections were examined immediately. For this the fish were anaesthetized in a solution of 130 ppm benzocaine and examined under the dissecting microscope for the presence of *Gyrodactylus* and the cysts of protozoans. Fish which were free of ectoparasites were placed in a separate aquarium for subsequent use in experimental infections. The aquarium was provided with constant aeration and a EHEIM external filter (EHEIM GmbH and Co.KG, Germany). During the holding period,

the fish were fed twice per day with commercial trout pellets (EWOS Bakers No.3, EWOS Ltd., UK) .

2.1.1.4 Identification of the species of fish

The first identification of each species of fish was provided on their arrival by the importers. Subsequently, one specimen from each species sampled was fixed in 10% phosphate-buffered formalin for confirmation of identity by a specialist in the group of fish. The common names of the species of fish presented in this study are used in accordance with Axelrod, Burguess, Pronek and Walls (1991). Species for which common names were not found are referred to only by their latin names.

2.1.2 General post-mortem techniques

Prior to examination fish were killed by insertion of a needle into the cranial cavity and the length recorded. They were then examined under a dissecting microscope for the presence of ectoparasites and cysts. Skin scrapes and squash preparations of the gills were taken prior to opening the abdominal cavity and removing the internal organs.

The internal organs were examined separately, only in the specimens with the total length greater than two inches. Squash preparations of the kidney were also prepared and examined microscopically (x100 - x400).

During the post-mortem examination, pieces of normal

tissue and all pathology observed were taken for histological processing.

2.1.3. Parasitological techniques

Parasitological examination was conducted simultaneously with the post-mortem examination. Fresh squashes showing myxosporeans were air-dried, fixed in absolute methanol and stained with Giemsa. Those showing ciliata, such as *Chilodonella*, were air-dried and stained with Klein's silver nitrate impregnation technique (Klein, 1958).

Monogenoidea collected from skin and gill scrapes of the hosts were prepared for study according to the procedures of Malmberg (1970) and Shinn, Gibson and Sommerville (1993).

The endoparasites found were placed in a physiological saline solution, separated by group, counted and fixed in Berland's fluid (Glacial acetic acid 19 parts and Formalin 1 part). Subsequently, they were transferred to 70% alcohol for storage prior to processing. They were prepared for taxonomic studies according to the procedures of Gibson (1984). The terms prevalence and intensity of infection were used in accordance with Margolis, Holmes, Kuirs and Schad (1982). The intensity of infection of the protozoans was not determinated.

Crustacean parasites of the gills were collected, counted and preserved in 70% alcohol.

All measurements were recorded in micrometres (µm) followed by the mean in parentheses.

2.1.4. Histological techniques

2.1.4.1 Tissue sampling

Samples of normal tissue and all pathology observed were fixed in 10% buffered-formalin for a minimum of 24 hours.

2.1.4.2 Tissue processing

All tissues to be processed were trimmed, wrapped in paper, cassetted, labelled and autoprocessed in a histokinette (HISTOKINETTE 2000). The tissues were positioned in suitable sized metallic moulds, blocked in molten wax from a Reichert-Jung wax embedder and cooled rapidly on a freezing plate.

2.1.4.3 Sectioning and staining

The blocks were trimmed to bring the desired tissues to the surface. When necessary, the surface of the block was decalcified in RDC (Rapid Decalcifier from Histopath) for a period of 30 minutes and then washed. Otherwise the block was placed directly in water for a period of between 30 minutes and 1 hour . Subsequently, they were dried and cooled on a cold plate before being sectioned.

The tissues were cut at 4-5 μm in a Leitz-Weitzlar microtome using Reichert-Jung disposable blades. The sections were floated on water maintained at 40°C in a water bath and

collected on pre-washed wet glass slides. The slides were then marked with diamond pen, dried on a hot plate and the wax melted at 60°C.

For routine observations the sections were stained with Haematoxylin and Eosin (HE) and Schiff's Periodic Acid (PAS). Giemsa staining was used only for routine observations of kidney.

2.1.5. Scanning electron microscopy

2.1.5.1 Processing

Samples of fish were fixed in fresh 1% glutaraldehyde for 1 hour. Subsequently, they were transferred to 3% glutaraldehyde for a period of 48 and 72 hours.

Fixed tissues were trimmed and rinsed in cacodylate phosphate buffer and post-fixed in 1% osmium tetroxide for 2-4 hours.

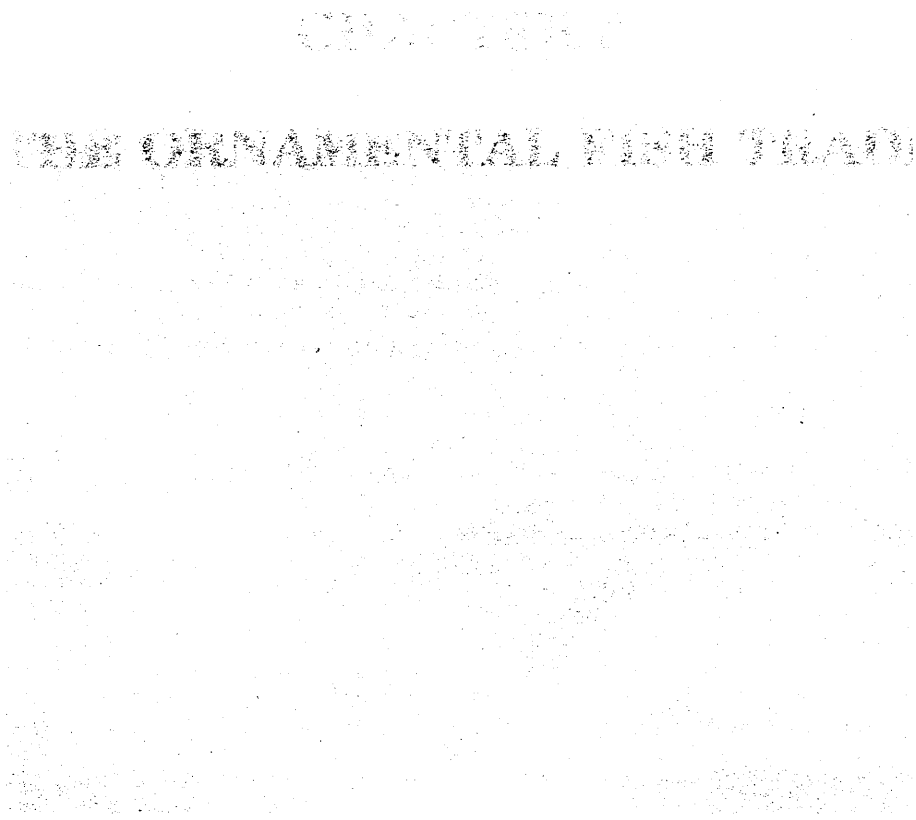
Following post-fixation, the tissues were dehydrated in a graded acetone series of 70-100%. The samples were critical point dried using an Acetone-E 300 critical point drier.

The tissues were mounted on aluminium stubs using silver paint or Araldite. Dried tissues were coated with gold palladium in an Edwards S-150 Sputter coater. A Phillips ISI-60A scanning electron microscope was used to examine the samples.

2.1.6. Drawings and photographs

Illustrations of the parasites were prepared with the aid of a drawing tube on a Olympus BH2 microscope.

Photomicrographs from the parasites and histological sections were taken on a Olympus BH2 microscope equipped with Automatic Exposure Photomicrography by PM 10AD using black and white (Ilford Pan F-135 ASA 50) or colour film (Kodak - ASA 100).



CHAPTER 3

THE ORNAMENTAL FISH TRADE

3.1 Introduction

3.1.1. The ornamental fish industry in South America and the international market

Although the majority of freshwater fish for the ornamental trade are produced on fish farms, large numbers are still removed from the wild, especially in South America and Africa where ornamental fish farming is rarely practised (Andrews, 1990).

From South America, ornamental fish are principally exported from the Amazon Basin. This region is considered to be the world's largest river basin, comprising an area of 7,000,000 km^2, and is probably the primary source for the world market of wild freshwater aquarium fish. (Fig. 3.1)

Ornamental fish from the Amazon Basin are exported by Brazil, Colombia, Peru and Venezuela (Junk, 1984). Colombia and Venezuela are exporting fish from the Amazon and Orinoco Basins whereas Brazil and Peru are exporting fish almost exclusively from a few areas of the Amazon Basin (Welcomme, Richards and Neiva, 1979). Other countries such as Argentina, Paraguay, Bolivia and Guyana also export fish but in smaller numbers.

Information relating to the catch and export of ornamental fish from South America is scarce, and in some cases contradictory, making it difficult to provide an accurate picture of the trade.

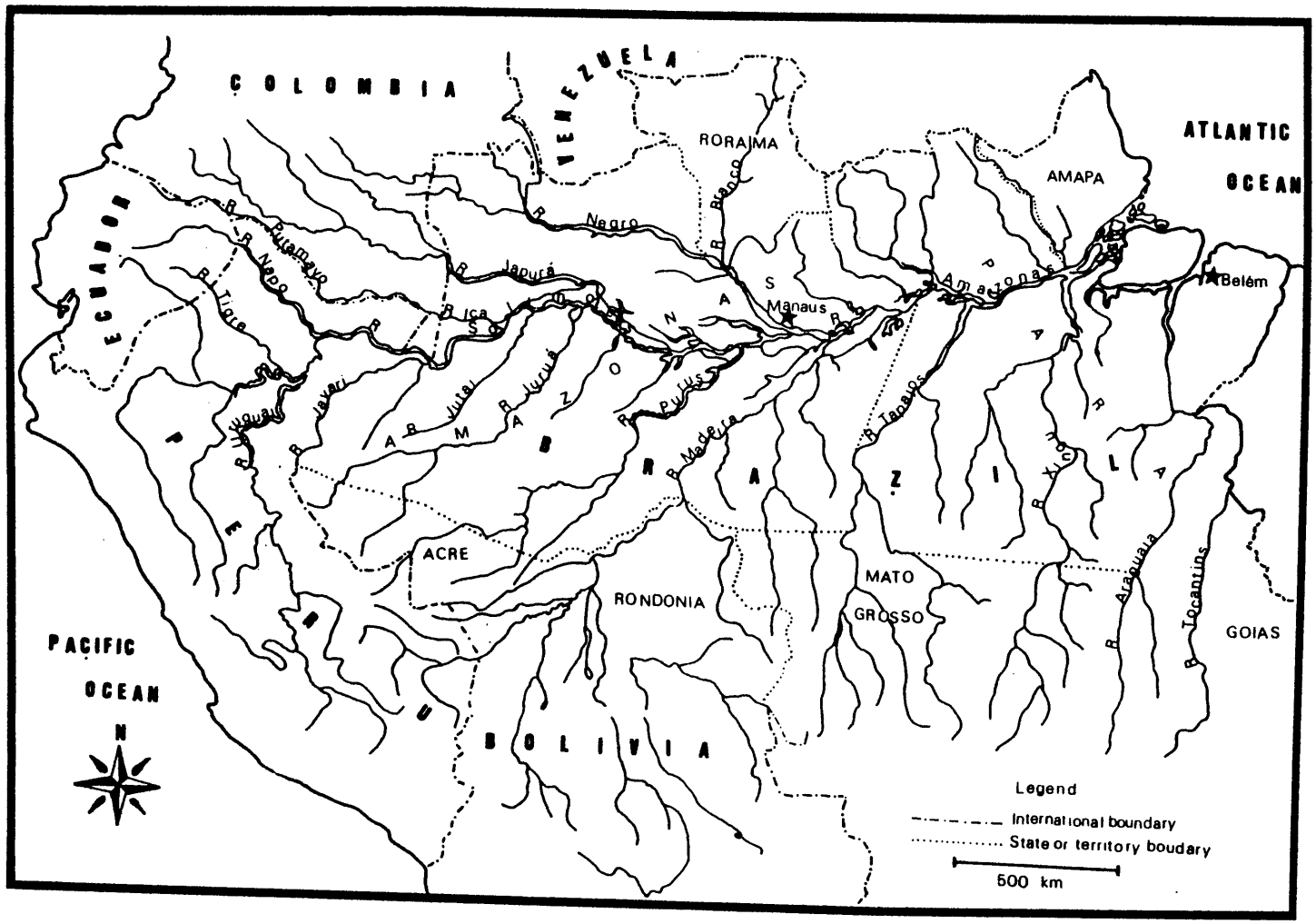


Fig.3.1 Amazon Basin with major rivers and political boundaries. Source: Bayley and Petrere, 1990

Reviews of the ornamental fish trade from South America were provided by Conroy (1975), who concentrated on the situation in Colombia, Peru and Venezuela, and by Welcomme *et al.*, (1979), Denis (1985), Falabella (1985), McGrath (1990), Eisentadt (1992) and Chao (1995) who concentrated on the situation in the Amazon state, Brazil.

According to Conroy (1975), in 1970 over 13 million fish were exported from Peru, and between 1972 and 1974 at least 13 million fish were exported from Venezuela.

Some statistics exist regarding the export of ornamental fish from Brazil, but the figures are sometimes contradictory and incomplete. Junk (1984) reported that the fishery development research programme of Brazil (Programa de Pesquisa e Desenvolvimento de Pesquisas do Brasil-PDP) stated that 11,008,899 specimens of fish were exported in 1976. For the same year, Denis (1985) reported 10,890,703, whereas independent statistics (SUDEPE/PDP) indicated 12,532,417 specimens for the state of Amazonas alone.

Based on the U.S. import data for 1985-1988, the major exporting countries in South America are Brazil (34.2%), Colombia (30.6%) and Peru (23.6%). However, Colombia exports 50% of the total value of the US imports while Brazil and Peru each account for only 20%, with Guyana accounting for most of the remaining 10% (McGrath, 1990; Eisentadt, 1992). Thus, although Brazil has traditionally been the major exporting country in South America it has been losing its position in the U.S.Market in recent years, mainly due to the increase in supply by Colombia and Peru. In 1988, for

example, Colombia accounted for 34.6% of US aquarium fish imports from South America compared to 28% and 27% for Brazil and Peru respectively (McGrath, 1990). Additionally, as Colombia accounts for a proportionately greater share of the total value of US aquarium imports than either Brazil or Peru, it appears that Colombia tends to export higher value fish than the other South American countries (McGrath, 1990). Despite the obvious importance of the US market, however, it should be noted that South America is only the third largest exporter of aquarium fish for the US market, behind Southeast and East Asian countries.

Regarding the European Market it is quite difficult to establish the relative proportions of aquarium fish imported into each European country from South America, principally due to the difficulty of collecting complete statistical data on European imports. Nonetheless, it is clear that some of the European countries, such as the Netherlands and Germany, play a very important role in the South American market. On the basis of import statistics for freshwater fish, the import value of the main European markets can be assessed according to Table 3.1.

Although accurate supporting data are lacking, it appears that around 90% of the freshwater fish available in the European aquarium market are captive-bred (Mintel, 1988).

Among the main exporting countries in South America, Brazil and Colombia were cited by Andrews (1990) as being the principal sources of wild freshwater aquarium fish for the United Kingdom. Nonetheless, the participation of the South

American aquarium trade in this market is small. For the period between 1987-1989 for example, the total landed weight of freshwater fish imported into the U.K from South America was 28,255 - 30,385 kg (Andrews, 1990) and for the year of 1990, only for Colombia Mintel (1991) reported 19,000 kg. The principal countries exporting freshwater ornamental fish into the UK and their market shares during 1990 (the most recent year for which data are available) are presented in Table 3.2. According to Mintel (1991) freshwater fish imports were recorded in 1990 as originating from 47 countries.

On the basis of the available Brazilian export statistics the number of fish exported to the United Kingdom market for periods between 1970 and 1983 are presented in Table 3.3. These data suggest that the proportion of the Brazilian market exported to the UK did not exceeded 1% during this period.

3.1.2. The Brazilian ornamental fish trade

Although Brazil has vast natural resources of aquatic life the majority of fish for export come from a single region, the middle Rio Negro basin. Brazil has recently started to export fish from other basins, such as the Rio Paraguay basin, but the number of fish exported from such areas is still very low and the vast majority of Brazil's natural aquatic resources remain unexplored.

The major exporting regions of the Brazilian ornamental fish trade are the states of Para and Amazonas, accounting

Table 3.1

Import value (US$) of freshwater fish in 1992 of each European country member and their classification.

EUROPEAN COUNTRY	**Import Value (US$)**
1. Germany	20,280,000
2. United Kingdom	19,910,000
3. France	14,270,000
4. The Netherlands	6,630,000
5. Italy	6,490,000
6. Spain	6,365,000
7. Belgium	5,960,000
8. Denmark	1,440,000
9. Portugal	785,000
10. Ireland	470,000
11. Greece	285,000
TOTAL	82,885,000

* Source: Bassler, 1995

Table 3.2

The principal exporting countries of freshwater ornamental fish into the United Kingdom and their market shares for the year 1990

COUNTRY	VALUE (£M)	MARKET SHARE (%)	VOLUME (Tonnes)	MARKET SHARE (%)
Singapore	4.45	50	526	49
Israel	1.09	12	155	15
U.S.A	0.88	10	142	13
Japan	1.03	11	45	4
Hong Kong	0.35	4	43	4
Colombia[1]	0.12	1	19	2
Germany	0.16	2	15	1
Nigeria	0.07	1	13	1
Others	0.85	10	110	10

Source: HM CUSTOMS and EXCISE MINTEL. [1]Only South American country listed.

Table 3.3

Total number of ornamental fish exported from Brazil to the United Kingdom.

YEAR	QUANTITY	%
1970	67,306	0.52
1971	62,796	0.47
1972	34,447	0.31
1973	16,438	0.20
1974	34,100	0.46
1975	49,075	0.55
1976	40,818	0.37
	-	-
1983	40,818	0.34

*Source: Data for 1970-1976 are from Denis (1985). Data for 1983 is from Falabella (1985) and corresponds only to the major exporting state of Brazil, Amazonas.

for 98% of the total exported ([1]*Cacex 1982-1988 in McGrath (1990)). The state of Amazonas alone is responsible for 90% of all exports (Leite and Zuanon, 1991).

Reviewing the available literature it is clear that the Brazilian aquarium fish trade has experienced several different phases since the initial boom in the 1960's (Falabella, 1985; Denis, 1985; McGrath, 1990; Leite and Zuanon, 1991). Exports from the state of Amazonas for example, reached their lowest number in 1974 and then peaked in 1979 (Table 3.4). More recently, Leite and Zuanon (1991) reported that the average number of fish exported has been about 15 million per year.

Table 3.4

Ornamental fish exported from the state of Amazonas, Brazil

YEAR	QUANTITY
1974	6 021 140
1975	10 373 524
1976	12 532 417
1978	17 903 485
1979	19 352 254
1980	16 301 049
1981	15 951 624
1982-1983	< 12 500 000
1987	> 18 100 000

Source: Data for 1974-1976 are from Welcomme *et al.* (1979). 1978-1981 are from Falabella (1985).1982-1983 and 1987 are from McGrath (1990).

[1] Cacex = Brazilian Customs & Excise

3.1.3. Problems faced by the South American aquarium trade

3.1.3.1 High rates of mortality

One of the most well documented problems facing the ornamental fish industry in Brazil and other South American countries is the high mortality rates (Vasquez, 1974; Welcomme et al. 1979; Conroy *et al.*, 1981; Junk, 1984; Falabella, 1985; Chao, 1992b; Eisenstadt, 1992). This study has shown that mortalities can occur at any stage between the initial capture of the fish and their arrival at the final destination, but the most critical periods are during transport to, and storage at, the exporters' local reception areas, and during transport from these areas to the exporters' principal holding facilities.

The mortality rates during these periods can vary widely, with the species of fish and the husbandry skills of the fishermen and exporters, commonly reported as factors influencing these rates. The season of the year (dry or wet) also appears to influence mortality rates reported during the process of collection and storage of fish for export, although detailed studies on this aspect are lacking. High fish mortality is known to occur when lakes start to dry out completely during the peak of extreme dry seasons (Junk, 1984). These are the periods during which large numbers of different ornamental fish species are caught by the fishermen.

No previous studies have adequately addressed the causes

of mortalities of wild ornamental fish from South America. The mortality rates reported tend to be under-estimated by the exporters (see section 3.4.2.3), and reliable information is not easy to obtain. Several authors have reported high mortality rates (Vasquez, 1974; Conroy *et al.*, 1981; Chao, 1992a), which, if allowed to rise much higher, would soon render the business unprofitable. Reported estimates of mortality rates at different stages of the export process are summarised in Table 3.5.

The lack of information regarding the cause(s) of mortalities is due, in part, to:

1) A lack of monitoring by exporters and dealers;

2) Secrecy about the number of fish caught and subsequent mortalities, to avoid competition, negative publicity and regulatory controls;

3) Few specialists in the field;

4) Lack of integration between fishermen, importers and exporters, and the scientific community at national and international level.

Table 3.5

Mortality rates of wild ornamental fish at different stages of the export process for Peru, Brazil and Colombia.

Country/Stage of the Operation	Rates of Mortalities (%)
PERU[1]	
Capture by the fishermen and transport to the exporters' holding facilities in Iquitos	30
Maintenance in the Exporters' Holding Facilites	10-20
Transport by air to the final destination (e.g. Miami)	10-20
BRAZIL[2]	
Capture by the fishermen	±60
Transport between the local reception areas and the exporters' holding facilities	5-10
Maintenance in the exporters' holding facilities	30-35
Transport by air to the final destination (e.g. Miami)	5-10
COLOMBIA[3]	
Maintenance in the exporters' holding facilities	50-60

Source: [1] Conroy *et al.*, (1981);[2]Eisenstadt (1992) and Chao (1992a); [3]Vasquez (1974)

3.1.3.2 Depletion of wild stocks

Depletion of wild stocks of ornamental fish is another problem often associated with the ornamental fish industry. Although an increasing number of ornamental fish are now farmed, large numbers are still removed from the wild in South America.

Up until recently, the only ornamental species from South America, particularly from the Amazon region, reported

in the world literature as disappearing from some areas as a consequence of intensive fishing for the ornamental fish trade, were the discus, *Symphysodon* spp., in the lower Rio Negro, and the cardinal, *Paracheirodon axelrodi*, in the middle Rio Negro (M. Goulding, cited by Bayley and Petrere, 1989;). However, no data was provided to confirm these statements.

Chao (1995) reported that it appears that the composition of fish exported from the Amazon has been changing in the last decade and the disappearance of some species from IBAMA's 1993 statistics may be a consequence of the successful farming of these species elsewhere, the decline of the population in the wild, or the hobbyists becoming interested in other fish. According to this author, over-exploitation of some species of "Pleco" is likely to occur if it has not already happened, but again, no data was provided. His contribution, although valid, it is quite difficult to interpret, because the latin name of the species was not provided and several species of catfish are commonly called "Pleco" in the Brazilian Amazon.

There have been few studies of the Amazon concerned with the natural stocks of ornamental species and they have all been conducted in the last 7 years (Prada-Pedreros, 1992; Chao, 1993; Barletta, 1994; Chao, 1995). For this reason, it is very difficult to evaluate the real impact that the trade has had on natural stocks of fish from this continent. Junk (1984) reported that ornamental fish from the Amazon basin might not be seriously endangered. According to this author,

most species colonise habitats that are subject to strong natural modifications. For example, the annual dry period provokes high natural mortality rates. Most fish compensate for this loss by high production rates and/or short reproduction cycles. This view is shared by Chao (1993; 1995) who believes that "no Rio Negro forest fish is threatened or endangered with extinction. The fishery can be managed in both environmentally and economically sound ways". Clearly, however, more studies on the biology or habitats of these fish are required so that these statements can be either substantiated or refuted.

Those connected with the trade believe that the removal of ornamental species of fish is having little or no impact on wild populations because the primitive collecting methods, and seasonal extremes in rainfall, limit the degree of exploitation (Axelrod, 1988; H. Bleher (pers. comm.) cited by Andrews, 1990). More damage to the wild stock has been caused by oil spills and gold mining, activities that are changing natural habitats and destroying many of the creeks where fish for the industry are caught (Schwartz, 1993).

Notwithstanding the views of some exporters and scientists, the damage caused by the ornamental fish industry to wild stocks in South America is still largely unknown. In response to the need to conserve wild populations, countries exporting from the Amazon basin started to regulate the ornamental fishery through the adoption of laws prohibiting fishing and trading during the spawning season (May to July) of *P. axelrodi*, and by restricting the export of food fish

species as ornamentals unless they are tank-bred.

3.1.3.3 Misidentification of the species of fish

Due to the large number of species and sub-species of wild freshwater fish available in the market, the misidentification of species is another difficulty faced by the wild ornamental fish industry.

Although a large number of studies on the taxonomy of freshwater fish have been published in scientific journals and books (Burguess, 1989; Axelrod, Burguess, Pronek and Walls 1991) the identification of aquarium species prior to export/import is generally made by non-specialised people who separate the species using a variety of external features which may be of little importance from a taxonomic viewpoint.

Commonly, different species of the same genus possessing similar external characteristics can be found in the same shipment under the same latin name or under the same common name without having been correctly identified by a specialist. Among the species of the genus *Corydoras* for example, this problem is commonly faced because of the pattern of similarity observed in the species which belong to the same species-group. The division of the genus *Corydoras* was proposed by Nijssen and Isbrücker (1980a) based on the colour pattern, snout length etc. According to these authors the genus has five species-groups: *punctatus*-group with 31 species, *barbatus*-group with 11 species, *aeneus*-group with 25 species, *elegans*-group with 8 species and *acutus*-

group with 19 species.

The species Blackband headstander, *Chilodus gracilis* Isbrücker and Nijssen, 1980 and the Narcissus cory, *Corydoras narcissus* Nijssen and Isbrücker, 1988 are among the several South American species known as aquarium fish for decades but only recently with their taxonomic status recognised. These examples amongst the several others found in the ornamental fish industry confirm the report of Leite and Zuanon (1991) that new species from the Amazon basin had often been described in the United States and Europe based on material exported only under the common name.

3.2 Study aims

The aim of this chapter was to provide basic information, commonly not available in the literature, regarding the wild ornamental fish industry in the United Kingdom, Brazil and Venezuela, obtained through the collection of primary data utilizing questionnaires and interviews with importers, exporters and fishermen.

The first part of this study was conducted with the importers in the United Kingdom to determine:

1. The most common species of wild freshwater ornamental fish that have been directly imported from South American countries;
2. The main problems caused by parasites and parasitic diseases during and after transportation to the UK;
3. The mortality rates of fish on arrival and during the

quarantine period at the importers' holding facilites;

4. The importers' quarantine procedures.

The second part of the study was conducted firstly with exporters from Venezuela and secondly with exporters and fishermen from Brazil. The main objectives were to determine:

1. The species and the numbers of wild ornamental fish that have been exported from South American countries, principally Brazil, into the UK;

2. The main problems caused by parasites and parasitic diseases of ornamental fish prior to their export

3. The mortality rates and the main factors responsible for mortalities at different stages prior to export;

4. The exporters' quarantine procedures.

3.3 Materials and methods

3.3.1 Investigations in the United Kingdom

3.3.1.1 Area of study

The study in the United Kingdom was conducted at the Institute of Aquaculture, University of Stirling, Stirling, Scotland. The species of ornamental fish imported from South America were obtained from collaborating importers in Scotland and England.

3.3.1.2 Collection of data

A survey of the ornamental fish trade was conducted with particular reference to problems associated with mortalities. The survey utilized a series of interviews and questionnaires with people associated with the ornamental fish industry in South America and the UK.

- Selection of the importers

The main criterion for the selection of the importers in the United Kingdom was that they should be importing tropical ornamental fish from South America. The initial selection was made according to the information given by each importer in the 1990 edition of the O. F.I. Year Book. Subsequently, importers were contacted by telephone or

fax and asked if they would be willing to collaborate on the project.

Those that agreed to collaborate were interviewed and were initially requested to fill out a simple questionnaire. This questionnaire was subsequently redesigned / elaborated to give one composed of 46 questions (Appendix I).

The questionnaires were principally used to determine:

1. The most common species of ornamental fish that were being imported from South America;
2. The general procedures for importation;
3. The calendar for importation of different species of fish;
4. The main problems relating to diseases that the importers were facing in Britain.

- Selection of the species of fish

The selection of the species of fish to be studied was made according to:

1. Importance of the species as an ornamental fish;
2. Price of the species;
3. Ability to obtain the species in the UK at different periods of the year;
4. Problems reported in the literature relating to the species of fish and its parasites and diseases;
5. Information obtained from the importers.

3.3.2 Investigations in Brazil

3.3.2.1 Area of study

- **MANAUS**

Manaus is the capital of the Amazon state in Brazil (Fig. 3.2). The city is situated at the left margin of the Rio Negro ±2 km from where it combines with the Rio Solimoes, to form the Rio Amazonas. Manaus is the principal centre for export for the Amazonian aquarium trade.

- **BARCELOS**

Barcelos is the trading post for ornamental fish in the upper Rio Negro. The city is situated at the right margin of the Rio Negro 430 km upstream from Manaus. Barcelos is the principal reception point for all ornamental fish species captured in the middle and upper Rio Negro (Fig. 3.2).

3.3.2.2 Collection of data

- **Selection of the exporters**

The criteria for the selection of the exporters from Brazil was that they should be exporting fish from the Amazon basin and be based in Manaus. As only one Brazilian exporter was cited in the 1990 edition of the Ornamental Fish International Year book, contact with others exporters was made through The Ornamental Fish Exporters Association of Amazonas (ACEPOAM).

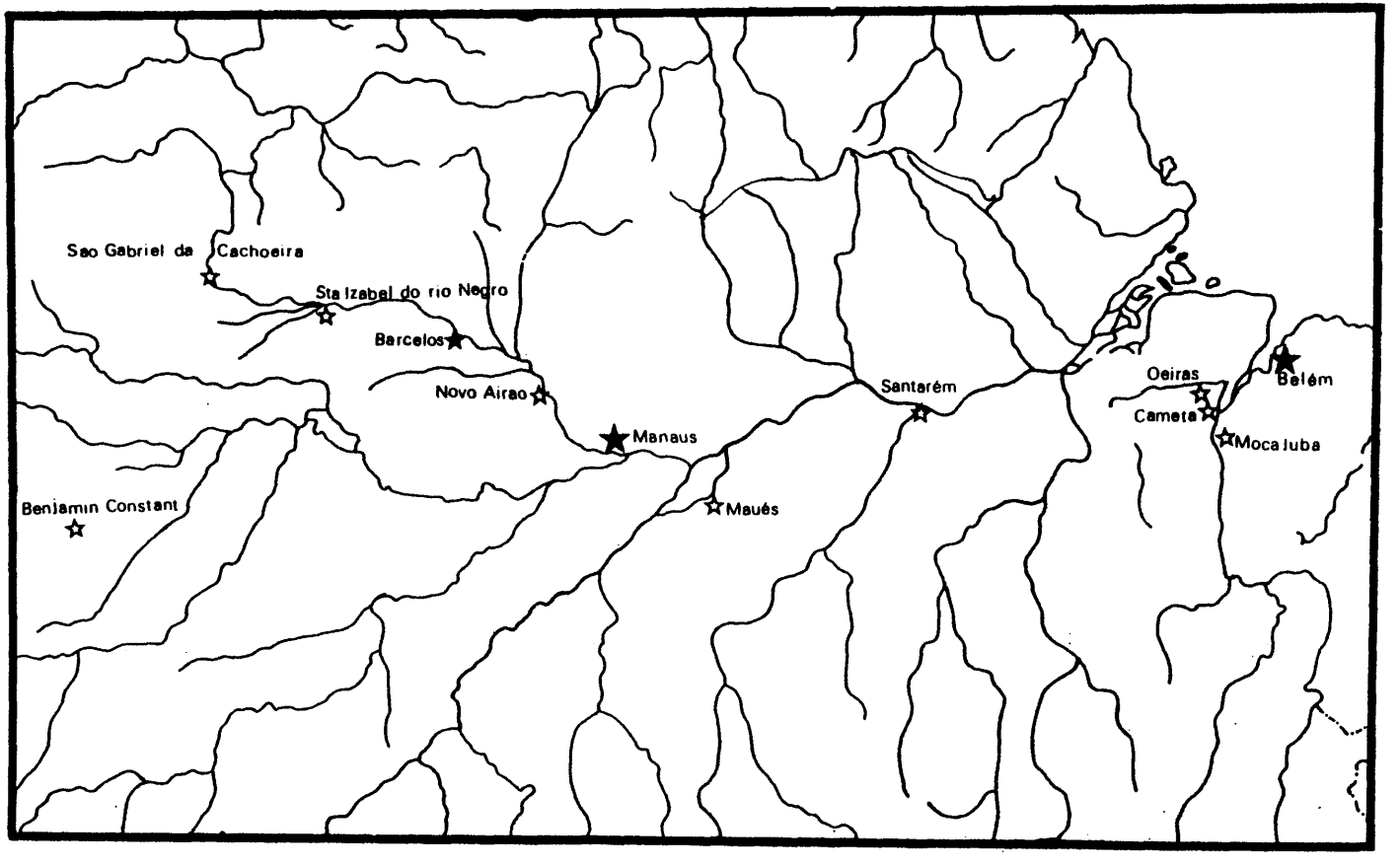


Fig.3.2 Source for Amazon aquarium fish.

The procedures for contact and interview were the same as those utilised for the importers. A similar questionnaire to that applied to the importers was applied to the exporters (Appendix II).

- Selection of the fishermen

Fishermen in Barcelos were selected randomly from those fishing for the exporters' interviewed. The fishermen selected were interviewed and asked to complete a questionnaire comprising 54 questions (Appendix III).

3.3.2.3 Collection of data during transportation of fish between Barcelos and Manaus

During the transportation of fish between the local holding facilities in Barcelos and the exporters' main holding facilities in Manaus, specific shipments were followed. The water temperatures of the tanks located at the top of the stacks, and at different locations in the boat, were taken every 4 hours. The water temperatures of the tanks located in the middle and lower positions of the stacks were taken approximately midway through the journey, just before the original water in the tanks was replaced with fresh water. At this time an estimate of the number of mortalities was also recorded.

The fishermen and the middle-men responsible for each shipment in the boat were interviewed and asked to complete

a questionnaire comprising 15 questions designed to determine the general procedures for the transport of fish by boat, the species most sensitive to deterioration in water quality, and the problems most commonly associated with high mortality rates during the transportation of ornamental fish (Appendix IV).

3.3.2.4 Collection of exportation data

The number of species exported and the number of specimens of fish exported per exporter from the Amazon basin for the year of 1993 were obtained from the government environmental agency, IBAMA (Brazilian Environmental Control Committee).

The raw data were organized according to the species, month of the year, and the country to which the fish were exported.

3.3.3 Investigations in Venezuela

3.3.3.1 Collection of Data

- Selection of the exporters

As no exporters from Venezuela were included in the 1990 edition of the Ornamental Fish Industry Year Book, the initial contact was made through an importer in United Kingdom. The procedures for contact and interview were the same as those described in sections 3.3.1.2 and 3.3.2.2.

3.3.4 Limitations of the study

Studies in the United Kingdom were constrained by the very small number of importers importing fish directly from South America, thus severely limiting the number of shipments available for study. Similarly, studies in Venezuela were reliant on the co-operation of the only exporter. In Brazil, studies were conducted in collaboration with the three largest exporters, but it was not possible to work with the eight smaller exporters during the period of the study as they were not fully operational at this time (June to September, the beginning of the season).

The ornamental fish industry is a highly sensitive industry with many legal and environmental issues to be considered. Business is usually based on trust between the importers and exporters, at the international level, and the exporters and fishermen in the country of origin, relationships which are built up over many years. Information regarding the catch, export, mortality rates and diseases is scarce in the literature and are treated as confidential by the importers and exporters.

3.4 Results

3.4.1 Investigations in the United Kingdom

According to the 1990 edition of the Ornamental Fish Industry Year Book, 30 British importers were registered in the directory of full members of the Ornamental Fish Industry. Although the large majority of these companies indicated some involvement with ornamental fish, the results presented in this study were based on interviews with five importers of which four were occasionally dealing with the direct import of wild freshwater fish from South America and one who directly imported wild fish from South America and Africa on a more regular basis.

The results of the questionnaires completed during the interviews were separated into five main topics as follows:

3.4.1.1. Species of fish commonly imported from South America

Several species of wild ornamental fish are imported directly from South America into Britain, although shipments are infrequent.

One of the most important species imported from South America is the cardinal tetra, *Paracheirodon axelrodi*. A large variety of catfish, mainly of the genus *Corydoras*, cichlids and characids are also imported. Additionally, large South American characids have been imported, but they are not in high demand due to the size that the adult specimens can reach.

No figures could be obtained in terms of the number of specimens of each species imported into the UK.

3.4.1.2 Barriers to the import of wild ornamental fish from South America.

Several reasons were given by the importers as being negative factors for the importation of wild ornamental fish.

Of those relating to the health of the fish the most common were:

1. They believe that tank-bred fish are able to adapt better to aquarium life than their wild counterparts;
2. Tank-bred species are normally healthier than wild fish;
3. Wild fish are commonly infected by a high burden of parasites;
4. Following importation, the recovery period of the wild fish is longer than that of their farmed counterparts.

Other points referred to by the importers were related to the high price of transport and some problems with packing.

3.4.1.3 Problems caused by diseases

The general opinion of the importers was that wild fish are frequently infected by a high burden of parasites. However, they believe that the main disease problems faced following the importation of fish are caused by bacteria and viruses. Therefore, it is not clear what the basis is for their particular view.

Only two importers reported that their most common problems were caused by ectoparasites, mainly protozoans (*Oodinium* sp.), sporozoans (*Pleistophora* sp.) and monogeneans. Crustacean ectoparasites, *Argulus* and *Dolops* were reported as occasional findings in shipments of *Paracheirodon axelrodi*.

Very little pathology or deformities were reported. The most common pathology observed was lesions on the fins, and occasionally on the body, that might be caused during the transport process if the packing quality was poor.

3.4.1.4 Mortality rates

Among the South American species imported, *Paracheirodon axelrodi* and *Hemmigrammus rhodostomus* Ahl, 1924 were reported as being most likely to present high mortality

rates. For the species *P. axelrodi* figures quoted ranged from 2-3%, if the specimens were apparently healthy on arrival, to about 30% or even higher when the fish appeared to be in poor condition on arrival.

The estimates of mortalities reported by four of the importers were similar, around 5% during transportation. This value could rise to 8-10% or fall to 2% depending on whether the fish were in good or bad condition on their arrival and whether the exporter was directly involved with the packing.

3.4.1.5 Quarantine

All importers interviewed reported that they placed some fish in quarantine following their arrival, but it appears that this procedure is not followed for all species. The period of quarantine appears to vary according to the species, demand of the market and the health of the fish. If the fish are in good condition they will be held on the premises for only a few days, to allow them to acclimatise to their new environment and start to feed. Otherwise, the fish will be kept in quarantine for a period necessary to be properly treated.

Among the South American species, *Paracheirodon tetra Symphysodon* spp.and *Hemmigrammus rhodostomus* were reported as the species most requiring quarantine, because they recover slowly from the transport process.

3.4.2 Investigations in Brazil

In the year that this study was conducted in Manaus, 11 exporters were involved in the export of wild ornamental fish. Of these eight are small companies not operating during the closed-season (1 May - 31 July).

Three exporters, who control around 80% of the market in Manaus, Amazonas State, Brazil, and 15 fishermen, were interviewed. The results were divided into three categories and are presented as follows:

3.4.2.1 Species of fish exported from Brazil

According to the list published by the Brazilian Environmental Agency, IBAMA, on 15th June, 1992, it is permissable to export 177 species of wild ornamental fish from Brazil (Appendix V). The results summarised below were obtained by analysis of the large volume of unpublished raw data requested from IBAMA relating to the numbers of ornamental fish exported from Brazil during 1993.

In 1993, 16,605,238 specimens of wild ornamental fish were exported from the Amazon state (Table 3.6). Of this total only 54,490 (0.32%) specimens were exported directly into the United Kingdom, corresponding to 8 shipments and around 17 species (Fig 3.4). The major countries importing wild ornamental fish from Brazil were the United States, Germany, Holland and Japan who share 93.04% of the market (Fig.3.5), of which the United States alone is responsible for 47.25%.

Paracheirodon axelrodi, H. rhodostomus, Corydoras spp., *Carnegiella* spp., *Crenicara* spp. and *H. erythrostigma* were the species most commonly exported from Brazil, accounting for 94.15% of the total exported from the state of Amazonas. The wild ornamental fish industry in Brazil is principally based on the export of one species of fish, *P. axelrodi*, which accounted for 74.78% of the total exports (Table 3.6; Fig.3.6). Although fishing was restricted for three months, the species was exported throughout the year (Fig. 3.6).

Paracheirodon axelrodi (36,500), *Corydoras* spp. (6,400) and *H. rhodostomus* (5,200) were the species most exported from Amazonas into the UK, accounting for 88.81% of the total (Table 3.6).

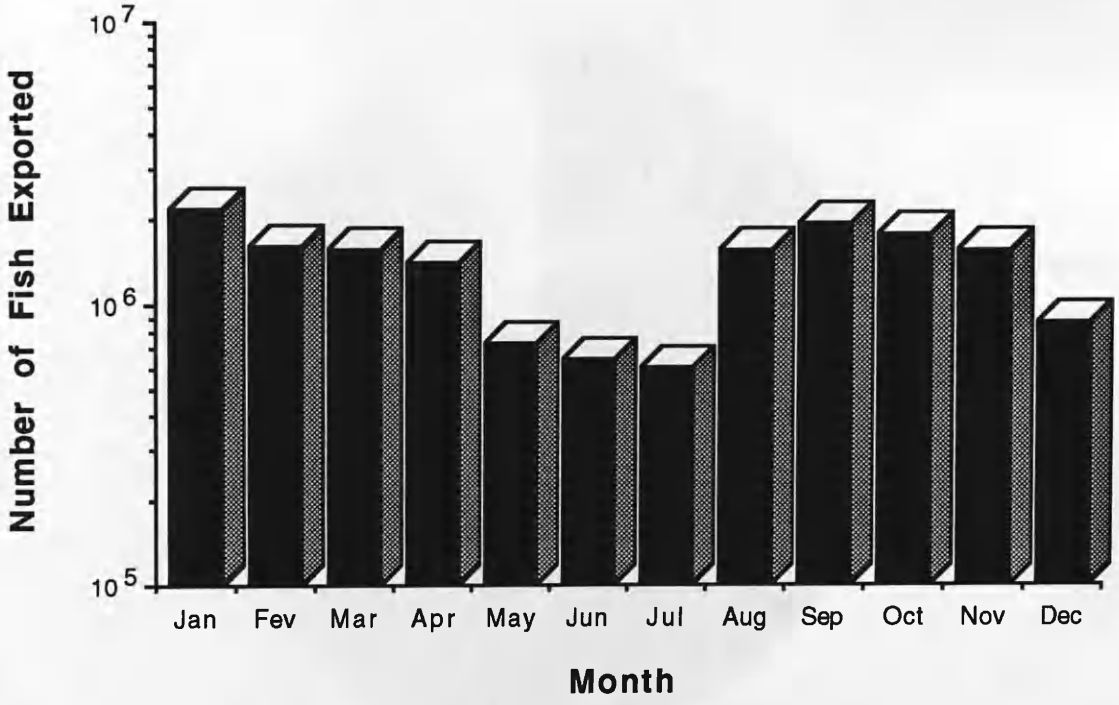


Fig. 3.3 Amazonas aquarium fish export: 1993
Source: Brazilian Environmental Control Committee - IBAMA

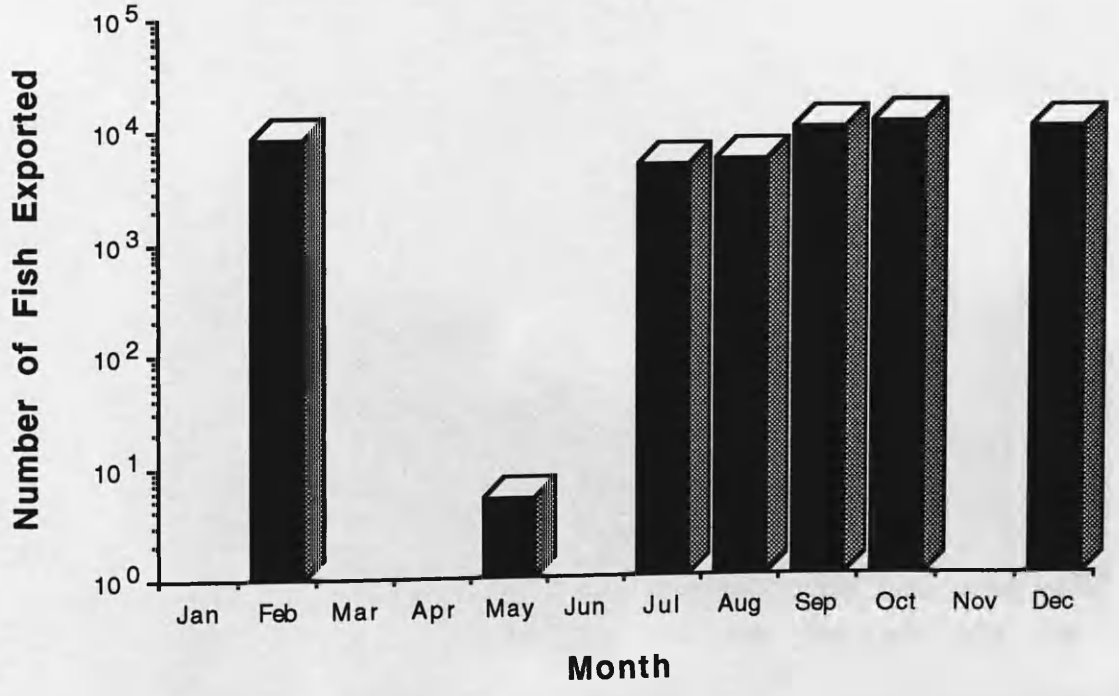


Fig.3.4 Freshwater ornamental fish exported from Amazonas, Brazil to Britain:1993. Source: Brazilian Environmental Control Committee - IBAMA

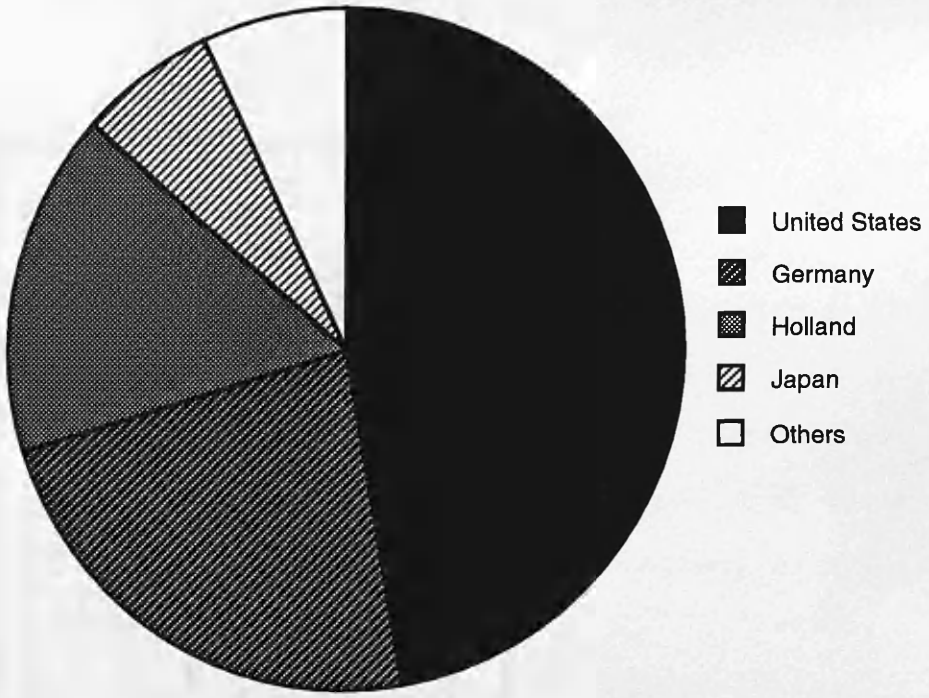


Fig. 3.5 Major importing countries of ornamental fish species from Amazonas. Source: Brazilian Environmental Control Committee - IBAMA

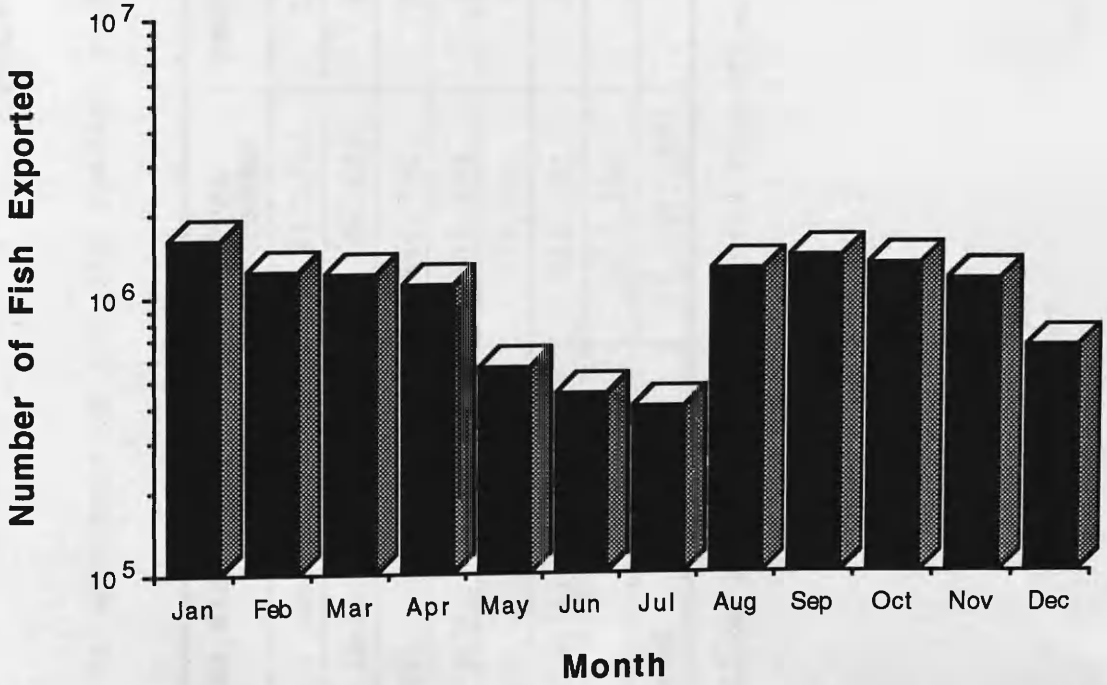


Fig. 3.6 Seasonal variation of *Paracheirodon axelrodi* exports in 1993. Source: Brazilian Environmental Control Committee - IBAMA

Table 3.6

Total number of specimens of the six species most commonly exported from Amazonas to the United Kingdom.

SPECIES EXPORTED	TOTAL EXPORTED	PERCENTAGE (%)	TOTAL EXPORTED TO UK	PERCENTAGE (%)
Paracheirodon axelrodi	12,417,510	74.78	36,500	67
Hemmigramus rhodostomus	1,384,625	8.33	5,200	9.55
Corydoras spp.	946,791	5.70	6,400	12
Carnegiella spp.	351,383	2.11	400	0.73
Crenicara spp.	272,770	1.64	1,400	2.56
Hyphessobrycon erythrostigma	264,600	1.59	1,000	1.84
Others	967,559	5.8	3,590	6.58
Total Exported	16,605,238		54,490	

Source: Brazilian Environmental Control Committee IBAMA

3.4.2.2 Problems caused by diseases

All of the exporters interviewed reported some problems with diseases, the large majority believing that the major problems are caused by bacteria rather than parasites.

Among the parasites, the only species known to be causing some mortalities was the protozoan *Ichthyophthirius multifiliis*. This species appears to be responsible for severe losses during the period between May and June, when unexpected cold fronts (*friagens*) reach the region, causing a drop in the temperature.

Four species of fish, the cardinal tetra, *P. axelrodi*, Discus, *Symphysodon* spp., Rodostomus, *Hemigrammus rhodostomus* and *Corydoras* spp. were referred to by the exporters as the fish most likely to show symptoms of diseases and to present high mortality rates after arrival at the exporters' holding facilities. However, as the diseases were not diagnosed, the cause of these losses was unknown.

Symphysodon spp., *P. axelrodi* and *H. rhodostomus* were reported not to recover easily from the stress caused by handling during capture and transportation.

Paracheirodon axelrodi was reported to exhibit black spots on the dorsal part of the body, which appeared to spread quickly and cause changes in the colour of the fish. It appears that when some of the fish were caught they were already infected and that after capture the disease spread rapidly amongst the other specimens, causing severe losses for the fishermen and exporters.

Severe losses of *Corydoras* spp. have been observed when the specimens are stocked for long periods. A velvet condition is observed and the specimens become completely lethargic at the bottom of the tanks.

3.4.2.3 Factors responsible for mortalities

The aquarium trade in the Amazon basin is very seasonal. The permitted season is between August and April, but the period of peak activity corresponds principally with the low water season, that is from August/September to November/ December.

The results obtained from the interviews were divided into three major stages of the export process: capture, transport and storage at the exporters' holding facilities.

- During the capture

The information given by the fishermen suggests that the problems faced during the capture of the fish become more important during the long period of the dry season. The main factors reported were:

1. Fish easily captured due to the concentration of fish in the lakes and bays during the low water period;
2. Fish were often already injured and debilitated when captured due to the lack of food in the lakes which have become isolated from the river.
3. Overcrowded conditions in the fishermen's holding facilities.

4. Poor water quality in the igapó. (igapó=flooded forest).

- During transport

During transport by boat between Barcelos and Manaus several factors were reported as being responsible for causing mortalities. However all respondents reported poor transport conditions as being the most important. Other factors reported were:

1. Shipments maintained for long periods at high temperatures before transport;
2. Overcrowded conditions in the boat due to the large number of shipments during the dry season;
3. A progressive decrease in water quality during transport.
4. Tanks transported in the hold and/or the top deck of the boats are exposed to low ventilation, high temperatures and pollution by diesel fumes;

The temperatures of the water during one journey between Barcelos and Manaus, recorded according to the position of the tank in the stack and in the boat are presented in Table 3.7.

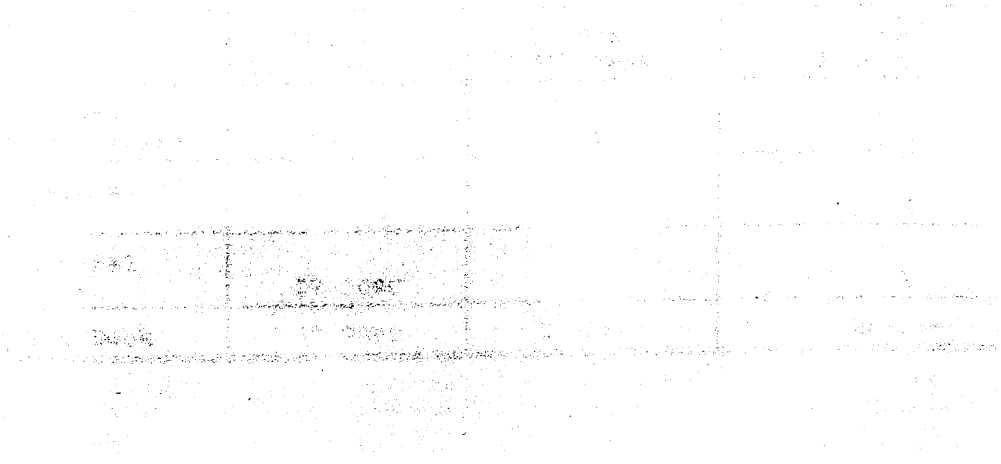


Table 3.7

Variation of the water temperature in tanks used for the transport of ornamental fish by boat between Barcelos and Manaus, Amazonas (X = unobtainable).

TIME: 6.40-8:30 AM	
LOCATION OF THE TANK	
BOAT	**STACK**
	Top - First Tank
Lateral to the engine	26-29°C
Central area	25.5-27°C
Lateral area	24-28°C
Top Deck	24-26°C

TIME: 11:00-13:00 AM			
LOCATION OF THE TANK			
BOAT	**STACK**		
	Upper First Tank	**Middle 5-6th tank**	**Lower 13th tank**
Lateral to the engine	27-29°C	29-32°C	32-38°C
Central area	26-27°C	27.5-28°C	28-28.5°C
Lateral area	26-28°C	28-30°C	28-30°C
Top Deck	27-28°C	X	X

TIME: After 16:00 PM			
LOCATION OF THE TANK			
BOAT	**STACK**		
	Upper First Tank	**Middle 5-6th tank**	**Lower 13th tank**
Lateral to the engine	28-29	X	X
Central area	27-28	X	X
Lateral area	27-30°C	X	X
Top Deck	27-28°C	27-28°C	28-30°C

3.4.2.4 Mortality rates

Before export, the wild ornamental fish pass through a number of different stages during which they can suffer severe losses. Based on the information given by fishermen, transporters and exporters, estimated mortality rates are presented according to the stage of the process leading up to export.

- Capture

No figures regarding mortalities were provided by the fishermen but the exporters quoted mortality rates of 5-10%. However, these figures can vary and may reach 30% or more depending on the species, the condition of the fish at the time of capture, and the fishermens'skills.

- Transport by boat

Exporters, transporters and middle-men quoted losses of about 5-10%. Again, these figures were variable depending mainly on the period of the season, the location of the tanks in the boat, the condition of the fish during capture, and the fishermens' skills.

The number of tanks of fish lost during one journey by boat between Barcelos and Manaus at the beginning of the season (August) was recorded in three different shipments transported in the same boat, as shown in Tables 3.8 and 3.9

Table 3.8

The percentage of tanks of fish lost during a single transportation by boat between Barcelos and Manaus.

PERCENTAGE OF TANKS LOST			
EXPORTER	No. TANKS TRANSPORTED	No. TANKS LOST	% TANKS LOST
EXPORTER I	1400	10	0.14%
EXPORTER II	200	3	1.5%
MIDDLEMAN	680	100	14.7%

Table 3.9

Estimated number of specimens of fish lost during a single transportation between Barcelos and Manaus.

ESTIMATED NUMBER OF SPECIMENS OF FISH LOST			
	No. TANKS LOST PER SPECIES	No. SPECIMENS OF FISH PER TANK	TOTAL No. OF FISH LOST
EXPORTER I	5 *P.axelrodi* 3 *Carnegiella* sp 2 catfish	1200-1500 200-250 20-30	7,500 750 60
EXPORTER II	3 *Carnegiella* sp	200-250	750
MIDDLEMAN	100 *P.axelrodi*	1200-1500	150,000
NUMBER OF SPECIMENS LOST			158,760

- Storage at the exporters' holding facilites

Estimated losses at the exporters' holding facilities were around 5-10% of the initial stock, although one exporter reported losses of only 1.4 - 2%. However, if a particular species is considered separately, such as *Symphysodon* spp. or *P. axelrodi*, mortality rates can reach up to 100% following arrival at the exporters' holding facilities.

3.4.2.5 Quarantine[2]

In general, the quarantine period for fish at the exporters' holding facilites was reported to be around one week. However, according to the information of the exporters the quarantine period may decrease depending on the period of the season, demand of the market, species, and the health of the fish.

It was reported by all exporters' interviewed that at the beginning of the season (August) fish in good condition

[2] Quarantine: Isolation imposed on persons or animals that have arrived from elsewhere or been exposed to, and might spread, infectious or contagious disease (OED).

can be exported in the five days following their arrival. However, this procedure can not be followed for all species. The species *Symphysodon* spp., *P. axelrodi* and *H. rhodostomus* are usually held over for at least ten days. The exporters claim that these species are very sensitive and will die in large numbers if precautions were not taken after reception.

The basic procedures at the exporters' holding facilites can be summarised as follows:

On their arrival the fish are separated into four main categories:

1. Fish that need special care - included in this category are those species that need to be held over for more than one week (e.g. *Symphysodon* spp., *P. axelrodi*);
2. Fish in good condition - these fish will be exported within the five days following their arrival (Fig. 3.7).
3. Fish with problems which they think can be cured - these fish will be exported only after being treated (Fig. 3.8).
4. Fish in poor condition which are unlikely to recover - these are released into small creeks.

3.4.3 Investigations in Venezuela

Only one exporter was dealing with the export of wild ornamental fish in Venezuela in the year that this study was conducted.

The close season in this country is from 15 May - 16 July. About 100 species of fish from the Orinoco and Amazon basins are listed as being exported. The main export restriction for this country relates to the export of alevins of food fish as ornamentals. According to the exporter only tank-bred species can be exported.

3.4.3.1 Problems caused by diseases.

Although the exporters reported problems with diseases no information was given on particular diseases.

No routine examinations are made on the incoming shipments.

Fig. 3.7 *Otocinclus* sp. Shipment just arrived at exporters' holding facilities.

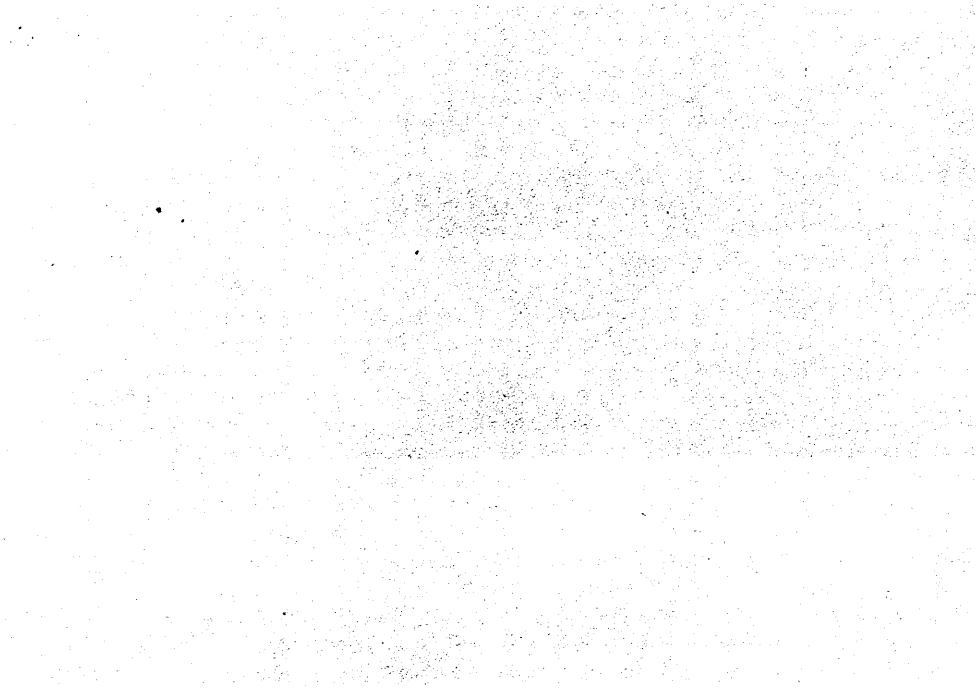
Fig. 3.8 Trimming of the caudal fin of the sucker-mouth catfish prior to administration of a cocktail of drugs on the arrival of the shipment.

Diagnosis of the diseases, when it occurs, is based on the type of lesions observed. As no microscopical examination is made the parasites and parasitic diseases that may be causing problems in the exporters' holding facilities are not recorded.

3.4.3.2 Mortality rates

As no fishermen were interviewed, the mortality rates presented are based only on information given by the exporter. Losses of 10-20% were reported between capture and transport. During storage at the exporters' holding facility losses of about 5-10% were reported.

The method of fishing and transport, the period of the year, and the site of collection, were the main factors cited as responsible for mortalities.



3.5 Discussion

Very few importers in the UK are dealing with the direct import of fish from South America. Those involved with the import of wild freshwater fish often prefer to buy wild quarantined fish from the United States or Germany rather than buying directly from South America because they believe that these quarantined fish carry less parasites and present fewer disease problems. However, it is not possible to confirm this hypothesis since there are no studies available in the literature concerning the efficacy of quarantine of ornamental fish.

Studies of ornamental fish receiving prophylactic treatment prior to export revealed that infestations of protozoans were reduced to minimal levels (Shotts and Gratzek, 1984). Clearly, if the prophylactic treatment is applied correctly the complete eradication of protozoan ectoparasites would be expected. However, if the level of infestation is reduced dramatically, but the parasite is not completely eradicated, it is very difficult to detect the presence of the parasites during routine examinations. Given the appropriate conditions this can lead to unexpected outbreaks of parasitic disease shortly after the arrival of apparently healthy fish at either the importer's holding facility or the customer's aquarium.

Although the importers were aware that parasitic diseases can cause severe losses of aquarium fish they believe that their main problems with wild fish are related

to bacterial and viral diseases. It should be noted that, in general the importers diagnose these agents because fish are sick and no parasite was detected. According to them parasites do not cause significant problems provided that good water quality is maintained and high quality equipment, such as ultra-violet water purification systems, is used for disinfection. Clearly, good husbandry and hygiene will reduce the problems caused by external parasites and other diseases. However, all current methods for the control of diseases have their limitations. For example, ultra-violet irradiation, which was referred to most frequently by the importers, apparently only destroys pathogens which are free in the water and is ineffective against stages attached to the fish (Michel, Maurand, Loubes, Chilmonczyk and Kinkelin, 1989) or substrate. Generally, this treatment is not powerful enough to kill macroparasites, such as whitespot (Burguess, 1993).

Although the importers believed that bacteria and viruses were responsible for most of their disease problems, with parasites being of much lesser significance, they did not provide any details regarding the clinical signs or causative agents of the diseases affecting their fish, even though in some cases the nature of the disease would have been diagnosed by specialists. This was not surprising as importers are often reluctant to discuss their disease-related problems with outsiders, understandably wishing to avoid negative publicity by keeping such information secret.

The absence of deformities and external pathology on arrival of wild fish in the U.K. was expected because the

fish are screened during selection and graded at the exporters' holding facilities. Fish that are not of the required quality are removed from the shipment.

Figures relating to mortalities during transport from South America, and during the quarantine period in the U.K., were difficult to assess because the mortality rate is affected by a wide array of variables, including the species of fish, the health of the fish, the quality of the exporters' packaging and the skills of the importer's. However, all interviewees reported that if the fish were in good health, and packaging was of good quality, the losses on direct shipments of the cardinal, *Paracheirodon axelrodi*, from South America were around 2-3%. However, figures of about 30% or more have also been experienced by the importers for this species.

For all South American species of fish imported into the UK mortality rates during transport were reported to be about 5% for small fish and less than 5% for large fish. Although these figures are estimates they are very close to the estimated mortality rates for SouthEast Asia, which achieves the lowest loss ratio during transport, 2-3% (Chuan and Hee, 1993). On the other hand, overall mortality rates for supplies from South America to European markets have been reported to be as high as 30%.(Anon., 1992), a figure reported by UK importers only for certain shipments of individual species. However, the low overall mortality rates reported on arrival in the UK by the importers may well be a realistic estimate because the total number of species, and the number of

specimens of each species, imported directly from South America, is relatively low. The low volume of the UK market enables importers to be selective with regard to the competence and facilities of the exporters they trade with, thus ensuring that the fish they receive are usually of good quality.

Andrews (1990) reported that the total combined weight of wild freshwater ornamental fish imported into the UK from Nigeria, the Congo, Zaire, Brazil and Colombia represents 7.3-8.5% of the total landed each year. To my knowledge, there are no detailed reports regarding the number of specimens of wild ornamental freshwater fish commonly imported from Brazil or other South American countries into the UK (e.g. the data available from Colombia reported by MINTEL (1991) only present the total imports by weight). However, according to Denis (1985), Falabella (1985), and figures supplied by the Brazilian Environmental Agency (IBAMA), Brazil has been exporting less than 1% of its total exports of wild ornamental fish to the United Kingdom (Table 3.3), with the cardinal, *Paracheirodon axelrodi* (Schultz), *Corydoras* spp., Banded Rummy-nose, *Hemigrammus rhodostomus* and Bleeding heart tetra, *Hyphessobrycon erythrostigma*, being most commonly exported to the UK in recent years.

Some of the difficulties in obtaining and analysing export data for ornamental fish were discussed by Conroy (1975), Wood (1985), and Andrews (1990). In the present study the major problem to be surmounted in analysing the raw export data provided by IBAMA was that all of the species of

fish were recorded under their common names. Such records are often difficult to interpret because more than one species can be recorded under the same common name and sometimes the common name does not appear on IBAMA's list of species that it is permissable to export (Appendix V).

The trade in wild ornamental fish is very important for many South American countries because it brings foreign exchange into the country and involves people living in remote areas, who have little opportunity to earn money in other ways. In response to the need to conserve wild populations of fish, the South American countries exporting fish from the Amazon basin have started to introduce regulatory controls and have restricted fishing for several species of fish. Almost all species can legally be exported as long as they are not regarded as food fish. Only tank-bred species of food fish can be exported. Fry of food fish have been exported from Colombia, Venezuela and Peru, and quite often can be found in the UK market, although they are less popular than the traditional ornamentals. It is not clear whether food fish are still being removed from the wild. In addition to the restrictions on export of food fish some countries, such as Brazil and Venezuela, have also established a closed-season during the main spawning period.

For Brazil, the closed-season is crucial because the Brazilian industry is principally based on the export of one species of fish, *P. axelrodi* (Table 3.6), already reported as having disappeared from some areas in the middle Rio Negro by Bayley and Petrere (1990). Despite these restrictions

IBAMA's statistics show that *P. axelrodi* was exported throughout the year in 1993, although there was a sharp decline in exports coincident with the closed-season (Fig. 3.6). McGrath (1990) stated that this species was apparently available in large numbers all year round, although the data he presented suggest that no exports of cardinals occurred in June and July 1985. However, his data do indicate that exports of cardinals occurred at the beginning of the closed season, in May. These exports of cardinals during the closed season are explained by the fact that exporters store fish for long periods in their holding facilities. This is only legal if the exporters have registered their stock numbers prior to the start of the closed season (A. Baptista, personal communication - IBAMA).

Unfortunately, the South American ornamental fish industry is mainly based on wild caught fish from the Amazon basin, and little effort has been made to modernise the trade. The techniques for fish capture and transportation are basically the same as those used in the early 1950's (Schwartz,1993). Consequently, the problems facing the industry in the past, such as disease and high mortality rates, are still present.

The exporters in Brazil and Venezuela shared the view of the UK importers that parasitic diseases were of only minor significance, believing that the principal problems at the holding facilities were related to bacterial diseases. Consequently, antibiotic therapy is the most common treatment in use.

Generally, the exporters do not conduct routine examinations or disease diagnosis on incoming shipments irrespective of whether or not the fish show signs of disease. Consequently, it was difficult to obtain detailed information regarding the most common diseases present at the exporters' holding facilities. In Brazil and Venezuela this situation is principally due to the lack of specialised people in the region and the fact that many importing countries did not request a health certificate for their fish.

The only species of parasite reported by the exporters to be common in fish stored in the holding facilities was *I. multifiliis*. This finding was similar to that reported by Welcomme *et al.*, (1979) in their evaluation of the ornamental fish trade in Brazil. Some of the Brazilian exporters admitted that this parasite often caused severe losses to their fish stocks, with peaks of infection usually coincident with unexpected decreases in water temperature. In the Amazon region this phenomenon is known as *friagem* and occurs mostly in the months of May and June, when cold fronts reach the region (Santos, 1973).

Among the species exported, *P. axelrodi*, *Symphysodon* spp., *H. rhodostomus* and *Corydoras* spp. were commonly reported as presenting symptoms of disease, and *Symphysodon* spp., *H. rhodostomus* and *P. axelrodi* as having high mortality rates after their arrival at the exporters' holding facilities. However, as the diseases are not diagnosed, the cause of these losses was not clear.

The ratio of mortalities at different stages of the process prior to export was difficult to assess, principally because the fishermen do not record numbers of mortalities during capture or local transport and the exporters are generally unwilling to allow access to written records of mortalities (where they exist) which occur during transport by boat or at their holding facilities. These problems highlight the difficulty of obtaining reliable statistics for mortalities of ornamental fish in South America (and elsewhere), and hence all figures reported to date are estimates.

Mortalities have previously been reported to occur most commonly during capture and during transport by boat (Chao, 1993; Anon., 1992; Conroy et al., 1981). These stages were confirmed by the fishermen and exporters as being the periods when severe losses can occur.

Several factors were mentioned by the fishermen as contributing to mortalities during capture. However, it appears that the strong seasonal fluctuations in water levels in the Amazon basin, which cause modifications in the habitats, behaviour and metabolism of aquatic organisms, combined with the lack of new techniques to improve husbandry and transport of fish, are the main factors responsible for the high losses faced by the ornamental fish industry in the Amazon basin.

All fishermen and exporters interviewed reported some losses during capture, but the highest mortality rates appear to occur during the long dry season, when fish may be very

weak due to the shortage of food or receive injuries due to overcrowding with the associated risks of predation and aggressive behaviour.

It should be noted that Amazonian fish exhibit high natural mortality rates during the dry season under some circumstances. The dramatic decrease in oxygen concentrations and the occurrence of hydrogen sulphide (H_2S) in the entire water-body, due to the total circulation during the *friagens*, kills many fish in the *várzea* (white water rivers and floodplain lakes) virtually every year (Santos, 1973). Similarly, a drastic decrease in O_2 concentrations can occur in shallow lakes during low water periods due to the strong stirring of the mud which results from intensive fishing activities (Junk,[X] 1984). Moreover, during the low water season, fish have to live at least partially on fat reserves stored during high water and feeding is often reduced because of the development of the gonads.(Junk, 1984; 1985).

As large numbers of fish can be collected during the dry season, overcrowded conditions are commonly observed at the fishermen's local holding facilities. When combined with the poor condition of the fish and the deterioration of the water quality of the igarapé (forest streams) and the igapó (flooded forest), it is not surprising that these conditions produce high mortalities. Since debilitated fish are more susceptible to bacterial, mycotic and parasitic diseases, more attention clearly needs to be paid to the methods of capture and stocking densities in local holding facilities, particularly during the long dry season.

As previously mentioned, the other stage presenting a high risk of mortalities is the transport of fish by boat between the exporters' local reception facility in Barcelos and the main holding facilities in Manaus. Analysis of the points referred to by the fishermen, transporters and exporters suggests that problems principally arise because of a lack of basic infrastructure. In the Brazilian Amazon there are no boats specifically designed for the transport of fish. All boats transporting fish are also utilized to carry passengers and other goods, thus decreasing the useful space in the boat. Consequently, during the peak fishing period all available space is used for fish transport. Thus, fish are not only transported on the middle deck, where they receive adequate ventilation, but also in the hold and on the top deck, where they are exposed to low ventilation, high temperatures and diesel fumes from the engine of the boat.

Temperatures of 32-38°C were recorded in all tanks located around the engine of the boat (Table 3.10). Consequently, all tanks located in this area were lost (Table 3.11 and 3.12). It should be noted that the losses reported during the present study are probably an underestimate as they were recorded only half-way through the journey when the water was exchanged, and the tanks were subsequently maintained under identical conditions.

The exporters did not overcrowd the fish during the transport by boat. Nevertheless, as the water is exchanged only about 14-16 hours after the departure of the boat, and often large numbers of tanks are transported, debilitated and

injured specimens, and species most sensitive to the progressive decrease in water quality, are the most likely to die. Additionally, during the long dry season the journey time is increased by about 6 hours, but the fish do not receive any extra attention. Clearly then, the variety of problems associated with transport by boat, coupled with the condition of the fish following capture, can potentially lead to high losses for the industry.

At the exporters' holding facilities in Venezuela and Brazil the mortality rates quoted, including all losses occurring on arrival of the shipment and during the 5-10 day quarantine period, were about 5-10%. Little information could be obtained regarding losses of fish which are held for long period due to their slow recovery from the stress caused by capture and transport, such as *P. axelrodi*, *Symphysodon* spp. and *H. rhodostomus*. Losses of around 10% were reported for *P. axelrodi* during the first 24 hours after arrival sometimes with similar losses occurring again in the next 4 to 5 days. It was also reported that even the most experienced exporters can sometimes be faced with mortality rates of 100% for these species. Similar figures were quoted by the importers in UK for the same species of fish during the quarantine period.

Although all exporters interviewed claim to be quarantining their fish, the view of the British importers was that wild freshwater ornamental fish are not being quarantined prior to export, and that they are carriers of parasites and diseases.

Comparing the information given by importers in the UK and exporters in Brazil and Venezuela it is clear that they are basically following the same quarantine procedures in terms of the quarantine period. Obviously, quarantine has its limitations, because fish may appear healthy throughout the quarantine period but still harbour infections (e.g. viruses, internal parasites etc). However, for the exporters of wild ornamental fish it is important that quarantine procedures are performed consistently, in order to ensure the health of the fish, and that quarantine is not sacrificed when the market demand is high. Exporting only healthy high quality fish will ensure that the exporters are able to compete in the international market.

In conclusion, it appears that British importers believe that wild ornamental fish are carriers of parasites and diseases. Although both importers and exporters believed that the main problems associated with wild fish were caused by bacteria and viruses no evidence was provided to support this. Ornamental fish from the Brazilian Amazon basin still have the potential to compete with fish of high quality in the international market, and to recover their market share. However, studies on the biology of the most important species of fish and their associated viral, bacterial and parasitic diseases, improvement in the basic infrastructure for capture and transport, adoption of new techniques to handle and care for the fish, and a better understanding of the ecology of the region, should be considered as important factors to improve the quality of the wild species of fish exported.

Chapter 4

Parasite Survey

4.1 Introduction

A problem commonly reported by the importers of wild freshwater ornamental fish was that such fish are not parasite-free. Wild fish, at the time of capture, usually have a great diversity of parasites. Several reasons can be given to explain this high diversity, including:

1. Fish have evolved in close association with a great variety of invertebrates for a longer period of time than any other vertebrate. Thus, these invertebrates have become integrated into the fish food web as well as providing intermediate hosts for a large variety of parasites;
2. Fish can work as intermediate or paratenic hosts for parasites of other aquatic animals or animals inter-related with the aquatic environment (e.g. mammals, birds, reptiles).

Despite the great diversity, parasites with indirect transmission will be unable to build up a large infrapopulation and cause problems after fish capture. Many will disappear due to the absence of the intermediate host necessary for the continuity of the life cycle. However, under the conditions maintained after capture of fish, some groups of parasites, particularly those with direct cycles, will be able to build up a large infrapopulation resulting in disease and other problems in the fish kept in the holding facilities.

Under natural conditions, the factors known to induce changes in the parasite population may be grouped into three major categories (Schad, 1977):

1. External environmental factors;

2. Host factors which determine the host's suitability as an environment for further development;

3. Parasite-related factors, either genetic or density dependent.

However, Chubb (1977) simply described these factors as abiotic and biotic.

Under the artificial conditions associated with the export/import of wild ornamental fish, Sommerville (1980) and Gratzek (1988) stated that successful groups of parasites will be able to increase the size of their populations, principally because of:

1. The increase in fish density in the holding facility;
2. The stress caused by handling and transportation;
3. Sub-optimal conditions of the artificial environment;
4. The mixing of fish from different incoming shipments;
5. Static water conditions;
6. Lack of hygiene.

Several publications have addressed the problems associated with parasitic diseases of freshwater ornamental fish. Some were published as general guides with the objective of helping veterinarians and hobbyists to diagnose, treat and prevent the most common diseases observed in aquarium fish (Goldstein, 1971;Reichenback-Klinke and Landolt, 1973; Richards,1977a-c;Gratzek, 1980; 1981; 1988; Sommerville, 1980; Herkner,1987a-b; Axelrod,1989; Hoffman, 1992; Biffar *et al.*, 1991). Others concentrated on specific problems and diseases in certain species including goldfish

(*Carassius* spp.), carp (*Cyprinus* spp.) and a variety of South American and African species, tank bred in Asia, the United States and Europe (Kent and Hedrick, 1985; Leibovitz 1980a-e; 1981; Shamsudin, Tajima, Kimura, Shariff and Sommerville, 1990; Molnár,1976; Dixon,Yamashita and Evelyn, 1990; Michel *et al.*,1989; Lom *et al.*, 1989; Székely and Molnár, 1992).

However, little detailed work has been conducted specifically to ascertain the species of parasites carried by wild freshwater ornamental fish, their pathogenicity, procedures for their control and prevention, and the problems they create for the ornamental fish industry, quite apart from their potential for transfaunation (discussed in more detail in Chapter 7).

Only 3 surveys of parasites present on imported fish have been reported in the scientific press. Giavenni (1978) presented the results of two years of observations on diseases caused by virus, bacteria and parasites on 63 species of ornamental fish from one importer's holding facility in Italy. Unfortunately, the author only presented a summarized description of the main problems found without presenting data relating to the prevalence of the common diseases and mortality rates. Gratzek *et al.*, (1978) examined 77 bags of ornamental fish imported to the United States from Hong Kong, Taiwan, Singapore and Bangkok for the presence of parasites, bacteria and viruses. No intermediate stages of digeneans and tapeworms were found in the fish species examined. According to the authors, these results appear to be related to the type of culture of ornamental fish in Far

East, where the fish are raised in aquaria or small ponds with little chance for them to be exposed to the infective stages of these parasites. In their study, monogeneans of gills were the commonest parasites observed, but at a low intensity of infection. Subsequently, Shotts and Gratzek (1984) conducted a comparative study of shipments of species of ornamental tank bred fish in the United States and Far East, with shipments of wild species imported from South America, for the presence of parasites and other pathogens that might cause problems on the health of humans, domestic animals and fish. Approximately 98% of the fish from South America were infected with the monogeneans and the intermediate stages of digeneans, cestodes and nematodes comprising the majority of the parasite fauna.

Very few parasitic diseases of wild ornamental fish have been studied in depth. One of the most documented parasitic diseases in wild ornamental fish, and subsequently also in tank bred fish, is the 'neon tetra disease' caused by the microsporidian *Pleistophora hyphessobryconis* Schäperclaus, 1941. This disease has been responsible for severe losses in the ornamental fish industry. So far it has been recorded in 15 species but is commonly associated with the neon tetra, *Paracheirodon innesi* (Myers) (Schäperclaus, 1941; van Dujin, 1967; Canning and Lom,1989).

The great majority of papers relating to parasites and the problems they cause in wild freshwater aquarium fish have been based on the results of material submitted for diagnostic purposes after the import of fish (Heinze, 1933;

Lucky, 1970; Lom et al.,1989; Leibovitz, 1980d; 1981; Majeed, Gopinath and Jolly,1981; Moravec,Gelnar and Rehulka, 1983; Moravec and Gut., 1982; Moravec et al., 1984; Moravec *et al.*, 1987; Mellergaard and Bloch, 1988; Cheung,Nigrelli and Ruggieri, 1986). Unfortunately, two types of problems are frequently found in these publications:

1. There is often insufficient information about the origin of the fish or other details that can help to assess how the fish became infected (Moravec *et al.*, 1987; Mellergaard and Bloch, 1988; Szekely and Bereczky, 1992; Frank et al., 1983; Frank, 1984).

2. Misidentification of the species of parasite. For example, all capillariids found in aquarium fish in Europe were originally considered to belong to the species *Capillaria pterophylli* Heinze, 1933. This South American species was reported not only from fish of the family Cichlidae, for which it seems to be host specific, but also from species of cyprinids and catfishes (Moravec *et al.*, 1984). However Moravec *et al.*, (1984, 1987) demonstrated that capillariids found in the cyprinid *C. tetrazona* belonged to *Pseudocapillaria brevispicula* (Linstow, 1873) a common parasite of free-living cyprinids in Europe. These authors also suggested that the frequent occurrence of the species *C. pterophylli* in aquarium-reared catfishes (Siluriformes) described by Frank *et al.*, (1983) and Frank (1984) may be erroneous.

There have also been very few studies on parasitic diseases, their prevention and control, prior to export. Welcomme, Richards and Neiva (1979) conducted a general

investigation of the ornamental fish industry in Brazil and they included some information on diseases and provided suggestions for the improvement of husbandry skills and control of the most common diseases they found. The other principal studies were conducted on a cooperative basis between Colombia, Peru and Venezuela by Conroy *et al.*, (1981; 1982). In the first paper the authors presented data on prevalence of various diseases encountered in incoming shipments of fish, and fish maintained in the exporters' holding facilities prior to exportation. Subsequently, on the basis of the previous study, the same authors suggested how some commercial products might be used for the prevention and control of diseases.

Other studies in this area include occasional descriptions of particular diseases (Conroy, 1963; 1964; Valdéz and Conroy, 1963; Santacana,Conroy, Mujica, and Lopez, 1982) wide-ranging reports for governmental agencies, which are often difficult to access (Conroy and Vásquez, 1975; Montadon, 1972; Vásquez, 1974) and a variety of MSc or BSc theses from South American universities, also difficult to obtain outside South America (in Colombia: Alvarez ,1974; Bermudez ,1974; Borrero,1977; in Peru: Roos, 1972; in Venezuela: Urdaneta ,1981; Mujica, 1982; Medina, 1986; and Castillo, 1991).

The great majority of South American studies relating to wild freshwater ornamental fish are taxonomic descriptions of new species of parasites involving one or two species of fish (Thatcher and Dossman, 1974; 1975; Thatcher, 1981a-c; 1984; Thatcher and Robertson, 1982; Kohn and Fernandes,

1988a; Thatcher and Padilha, 1977; Thatcher and Paredes, 1985; Ferraz and Thatcher, 1992) with few studies discussing the pathogenicity caused by parasites (Teixeira de Freitas and Lent, 1946; Thatcher, 1981a; Thatcher and Varella, 1980; Thatcher and Boeger, 1983a-b; Ferraz and Thatcher, 1990).

The scarcity of information on parasites of wild ornamental fish is probably related to the difficulties inherent in the identification of these parasites and the diagnosis of parasitic diseases. These difficulties arise because:

1. There are a great number of species of fish from different parts of the world that have been exported together with their parasite fauna;
2. Some exotic species of fish from South America and Africa are also being tank-bred in fish farms in South East Asia; These species might be acquiring a new parasite fauna and, consequently, exhibiting different diseases from their wild relatives.
3. Many parasites associated with internal tissues (e.g., microsporidians) and hence their harmful effects may not be observed externally;
4. Ornamental fish may receive many treatments before shipment, thus removing or greatly reducing the level of infection of many ectoparasites also parasite acquisition post capture and import.

4.2 Study aims

The aim of the study described in this chapter was to provide a baseline of information concerning the parasitic diseases of samples of wild ornamental freshwater fish both before and after importation into the United Kingdom from South America.

The first part of the study, conducted in collaboration with importers in the United Kingdom, aimed to determine:

1. The species of parasites carried by the most commonly imported species of wild ornamental fish following their arrival in the United Kingdom from South America;
2. The species of parasites which are potentially pathogenic and hence may be responsible for mortalities of wild ornamental fish during transportation from the country of origin and after their arrival at the importer's holding facilities.

The second part of the study, conducted in collaboration with exporters in Brazil, on the same genus/species examined in the United Kingdom, aimed to determine:

1. The most common species of parasites and parasitic diseases present in shipments of fish on their arrival at the exporters' holding facilities and in shipments of fish waiting to be exported;
2. The pathogenic species of parasites and parasitic diseases which may be responsible for mortalities of wild fish prior to export.

4.3 Materials and methods

4.3.1 Investigations in the United Kingdom

4.3.1.1 Collection of samples of fish

During a period between August 1991 to May 1993 and November 1993 to March 1994, samples of tropical ornamental fish were taken monthly from shipments originating from four countries in South America. The methods of collection transport and maintenance of fish were described in the sections 2.1.1 to 2.1.3, Chapter II.

4.3.1.2 Identification of the species of fish

The procedures for collection and identification of the species of fish were described in the section 2.1.1.4, Chapter II . The species of the genus *Corydoras* Lacépède, 1803 for which the only identification has been provided by the importers (including those for which confirmation of identification is currently being undertaken by specialists) were arranged in group-species according to the classification of Nijssen and Isbrücker (1980a). In this study, they were presented as follows: latin name (provided by the importers) followed by the name of the group-species in brackets.

4.3.1.3 Parasitological techniques

All parasitological techniques utilized in the present survey were described in detail in the Chapter 2, section 2.1.3.

4.3.2. Investigations in Brazil

4.3.2.1 Collection of the species of fish

During the period of July to September of 1993, 15 species of fish were sampled in two exporters' holding facilities in Brazil, 10 of which were previously sampled in United Kingdom.

Three types of samples were taken:

1. Samples of fish of shipments just arrived at the main exporters' holding facilities in Manaus. These samples included species of fish delivered directly by the fishermen to the exporters in Manaus as well as fish from the exporters' holding facilities situated in areas of the middle Rio Negro. The fish delivered directly by the fishermen still needed to be sorted, graded and separated.
2. Samples of fish kept in holding tanks. The fish kept in holding tanks were already sorted and were under routine prophylactic treatment with Furazolidone and Acriflavine or Acriflavine and Potassium Permanganate.
3. Samples of fish of shipments ready to be exported. These fish were already separated, counted and placed in plastic

tanks of 401. They were only waiting to be packed and exported.

4.3.2.2 Transport and maintenance of fish

Fish were examined in the Laboratory of Parasitology at the National Institute for Amazon Research, INPA, in Manaus. Samples of fish were transported separated in plastic bags containing well water or water from the exporters' holding facilities usually supplied by a small stream or river. All bags were fully oxygenated prior to being sealed. The procedures of holding and maintenance of the fish were described in the section 2.1.1.2, Chapter II.

4.3.2.3 Analyses of fish and collection of parasites

The procedures for analysing the specimens of fish, collection, fixation and staining of the parasites were the same as those described for the study conducted in United Kingdom.

4.4 Results

4.4.1 Investigations in the United Kingdom

4.4.1.1 Fish species examined

Although a large variety of wild ornamental fish species imported directly from South America can still be obtained in the UK market, direct shipments were not frequent. During a period of 26 months, only 28 shipments of the species previously selected for study from 4 exporting countries of South America, were available for sampling.

A total of 735 specimens of fish, comprising 9 families 22 genera and 33 species were analyzed. These are listed in the Tables 4.1-4.2, according to the family, together with the number of fish examined.

The family Callichthyidae comprised the greatest number of species and specimens sampled (Table 4.1). The family was represented by 4 genera *Corydoras* Lacépède, 1803, *Dianema* Cope, 1870, *Callichthys* Scopoli,1777 and *Brochis* Cope, 1871 and 13 species, 10 of which belong to the genus *Corydoras*.

Other families sampled were Characidae, Serrasalmidae and Cichlidae each with 6, 5 and 4 species respectively (Table 4.1) and the families Curimatidae, Loricariidae, Anostomidae, Pimelodidae and Hemiodontidae with 1 species each (Table 4.2).

The greatest number of individual specimens examined were *Mylossoma aureum* (70) (Serrasalmidae) *Paracheirodon axelrodi* (60) (Characidae) and *Brochis splendens* (58)

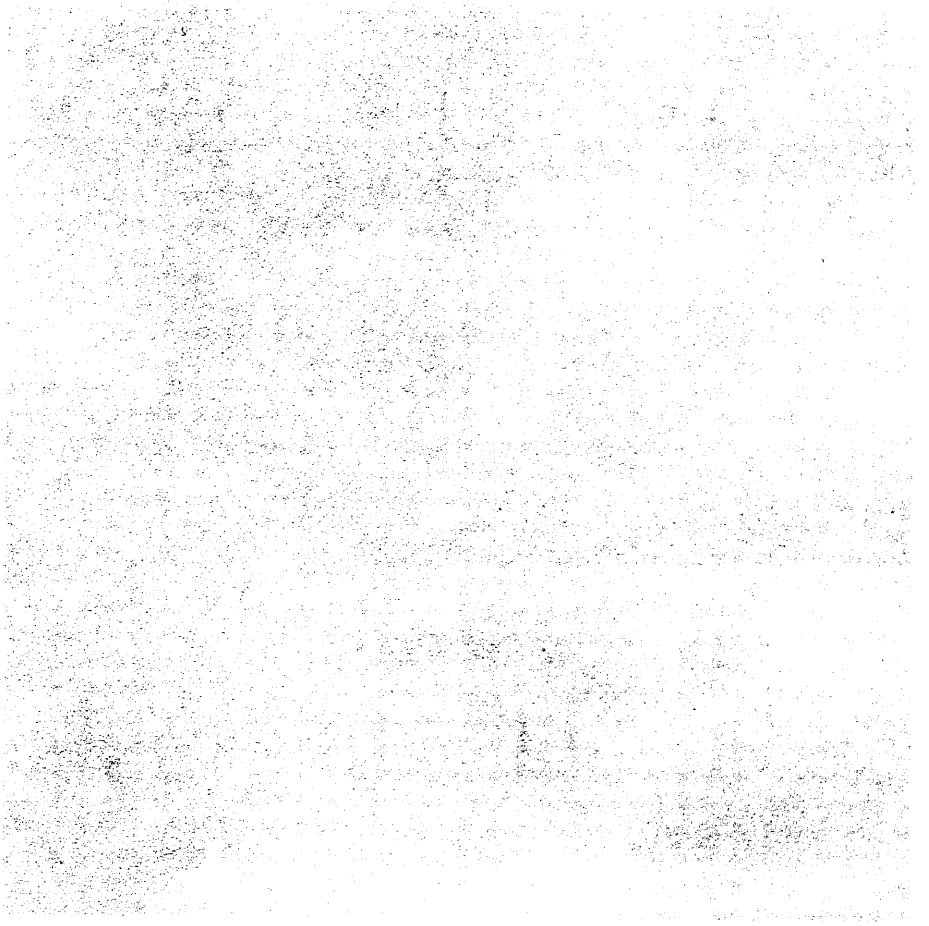
(Callichthyidae) and these are illustrated in Figure 4.1. Among the species of the genus *Corydoras*, were *Corydoras melanistius* (40), *C. punctatus* (40), *C. metae* (35), *C. schwartzi* (33) and *C. julii* (26). Alevins of 2 food fish species, classed as ornamental, were also sampled *Colossoma macropomum* and *Semaprochilodus taeniurus*.

Colombia and Brazil were the South American exporting countries with the greatest number of shipments (13, 7), species (23, 13) and specimens (383, 242) sampled. Peru and Venezuela had 4 shipments and three species each sampled. A total of 60 and 50 specimens were analyzed respectively. The number of species sampled according to the origin of the shipment is presented in the Table 4.3 and the number of shipments and the range of the number of species sampled in the Table 4.4.

Amongst the species imported from Brazil, 4 species (*C. punctatus, C. robinae, C. schwartzi* and *C. sterbai*) were not included in the published list of the Brazilian Environmental Control Committee, IBAMA as permissible to be exported from this country.

The species *B. splendens, C. arcuatus, C. julii, C. punctatus, C. schwartzi, P. axelrodi, Otocinclus* sp. and *Hyphessobrycon* sp. were commonly available for sampling in the shipments from Colombia and Brazil and the species *M. aureum* only in the shipments from Colombia and Peru. The collection data presented in the Table 4.3 suggest that amongst the South American exporters' countries, the largest number of species and specimens were sampled in shipments

from Colombia. However, it should be noted that this figure does not reflect the total participation of these countries in the UK market because, only the shipments of the species previously selected for this study were sampled.



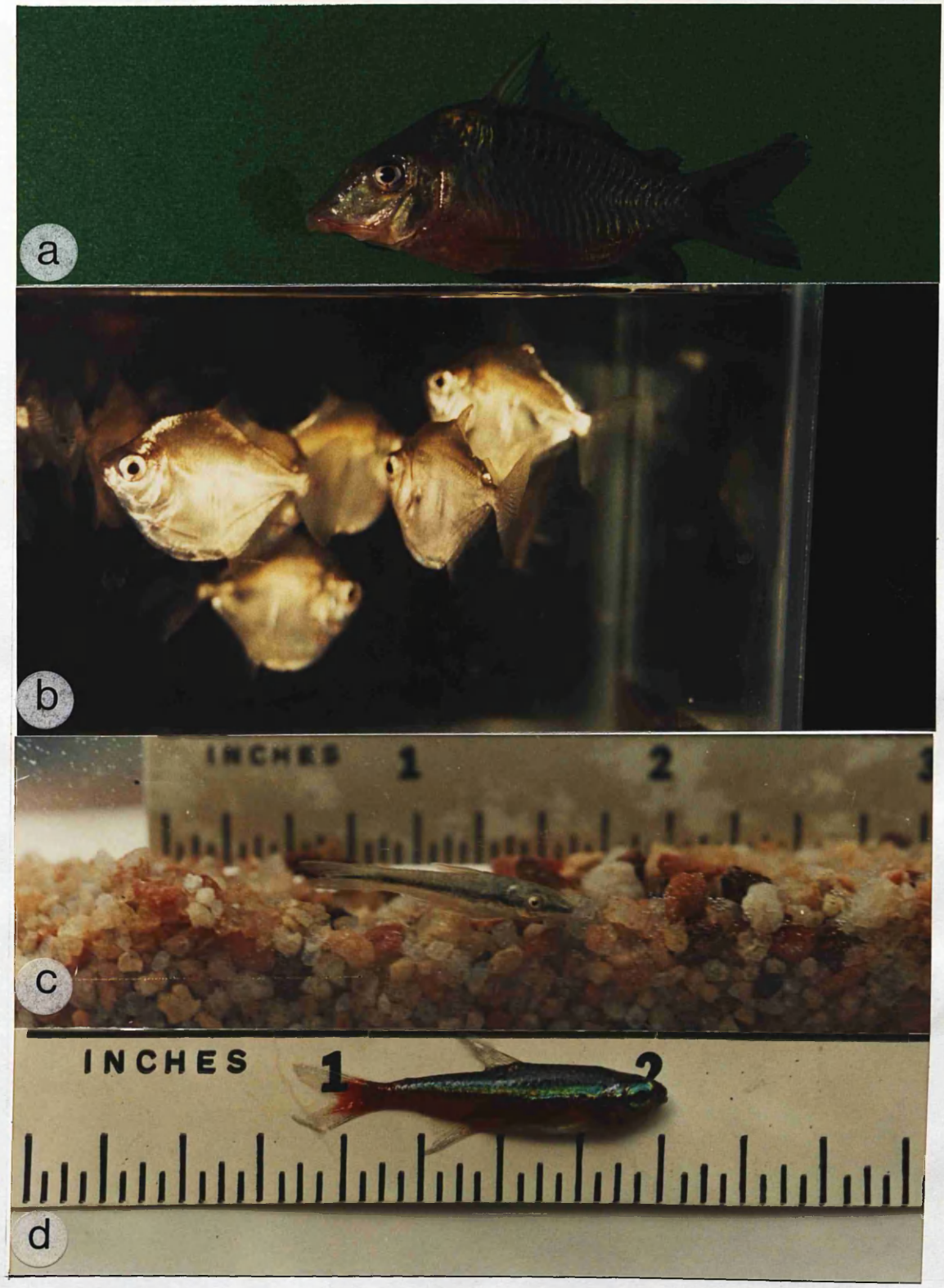


Fig. 4.1 a-d Species of ornamental fish most commonly sampled in the shipments imported from South America into the UK. a - *Brochis splendens*; b - *Mylossoma aureum*; c - Otocinclus sp.; d - *Paracheirodon axelrodi*

Table 4.1

Total number of species of ornamental fish examined from the families Callichthyidae, Characidae, Serrassalmidae, and Cichlidae imported into the U.K. from South America examined.

Family/ Species of Fish	Number Examined	Common Name
CALLICHTHYIDAE		
Dianema longibarbis Cope, 1870	5	Porthole Catfish
Callichthys (Linnaeus,1758)	6	Slender Armored Catfish
Brochis splendens (Castelnau, 1855)	58	Common Brochis
Corydoras arcuatus Elwin, 1939	30	Skunk Cory
C. julii Steindachner, 1906	26	Julii Cat
C. metae Eigenmann, 1914	35	Bandit Cory
C.robinae Burguess, 1983	15	Robina's Cory
C.schwartzi Rössel, 1939	33	Schwartz's Cory
C. sterbai Knaack, 1962	10	Sterba's Cory
C. pygmaeus (*elegans*-group)	5	Pygmy Cory
C.melanistius (*punctatus*-group)	40	Black Sail Cory
C.punctatus (*punctatus*-group)	40	-
C.reticulatus (*punctatus*-group)	15	Reticulated Cory
CHARACIDAE		
*Megalamphodus sweglesi**	7	Swegles's Tetra
Hyphessobrycon sp*.	20	Bleeding Heart Tetra
*Nematobrycon palmerai**	8	Emperor Tetra
Paracheirodon axelrodi (Schultz)	60	Cardinal Tetra
Paracheirodon innesi (Myers)	20	Neon Tetra
*Petitella georgiae**	5	Black-finned Rummy-nose
SERRASALMIDAE		
Colossoma macropomum (Cüvier, 1818)	11	Black-finned Pacu
Metynnis hypsauchen (Müller and Troschel, 1844)	10	Plain Pacu
Metynnis sp	10	-
*Myleus rubripinnis**	15	Redhook Pacu
Mylossoma aureum (Spix)	70	Silver Dollar
CICHLIDAE		
*Aequidens pulcher**	2	Blue Acara
*Cichlassoma festivum**	6	Flag Cichlid
*Geophagus jurupari**	5	Earth eater
Symphysodon aequifasciatus Pellegrin	5	Green Discus

* Species identified only by the importers or under process of identification by the specialist.

Table 4.2

Total number of species of ornamental fish from the families Curimatidae, Loricariidae, Anostomidae, Pimelodidae and Hemiodontidae imported into U.K. from South America examined.

Family / Species of Fish	Number Examined	Common Name
CURIMATIDAE		
Semaprochilodus taeniurus (Steindachner)	25	Plain-body Prochilodus
LORICARIIDAE		
*Otocinclus arnoldi**	111	"Otocinclus"
ANOSTOMIDAE		
Leporinus sp*.	2	"Leporinus"
PIMELODIDAE		
Pimelodella pictus	15	Angelicus pimelodus
HEMIODONTIDAE		
*Nannostomus trifasciatus**	10	Three-lined Pencilfish

* Species identified only by the importers or under process of identification by specialist.

Table 4.3

Ornamental fish species sampled in shipments imported from four South American countries into the U.K.

Family	Number Examined/Country Origin			
Fish Species	**Colombia**	**Brazil**	**Peru**	**Venezuela**
C. callichthys	60	-	-	-
D. longibarbis	-	50	-	-
B. splendens	280	300	-	-
C. arcuatus	200	100	-	-
C. julii	160	100	-	-
*C. punctatus**	255	150	-	-
C. schwartzi	133	200	-	-
*C. melanistius**	400	-	-	-
C. metae	355	-	-	-
*C. pygmaeus**	55	-	-	-
C. robinae	-	150	-	-
C. reticulatus	-	150	-	-
C. sterbai	-	100	-	-
M. sweglesi	70	-	-	-
Hyphessobrycon sp.	100	100	-	-
P.axelrodi	200	400	-	-
P. innesi	-	-	200	-
N. palmerai	80	-	-	-
P. georgeae	50	-	-	-
C. macropomum	110	-	-	-
M.hypsauchen	100	-	-	-
M.aureum	350	-	350	-
Metynnis sp.	-	-	-	100
Myleus sp.	-	-	-	150
C.festivum	60	-	-	-
G.jurupari	50	-	-	-
Aequidens sp.	-	20	-	-
S. aequifasciatum	-	-	50	-
N. trifasciatus	100	-	-	-
P. pictus	150	-	-	-
Otocinclus spp.	510	600	-	-
Leporinus sp.	20	-	-	-
S. taeniurus	-	-	-	250

Table 4.4

Total Number of Shipments and Species of Fish Sampled in the U.K.

COUNTRY ORIGIN	NUMBER OF SHIPMENTS	NUMBER OF SPECIES EXAMINED	RANGE OF THE NUMBER OF FISH SPECIES SAMPLED	TOTAL EXAMINED
COLOMBIA				
	13	23	1-8	383
BRAZIL				
	7	13	1-6	242
VENEZUELA				
	4	3	1	50
PERU				
	4	3	1	60
TOTAL	28	42	-	735

4.4.1.2 Parasite survey

The survey showed that all the major groups of parasites were present and they are listed in the Tables 4.5 and 4.7 to 4.11. The parasite fauna was composed of adult parasites that complete their life cycle in these hosts and intermediate stages which complete their life-cycle either in other species of fish (L_3 of camallanids), crocodiles, piscivorous snakes and fish (Acanthostomidae) birds (mesocercariae of Strigeoidea) crocodiles (nymph of pentastomids) etc.

The ectoparasite fauna comprised principally of protozoans and monogeneans of which, *Chilodonella hexasticha* (Kiernik, 1909), *Piscinoodinium* sp.and *Gyrodactylus* spp. were the most common. Parasitic crustaceans were represented only

only by one species of copepod, *Myracetyma* sp., which was found in the gills of *Semaprochilodus taeniurus*.

All of the species of protozoans found (Table 4.5) are potentially pathogenic under favourable conditions. The intensity of infection presented by *Piscinoodinium* sp., *Chilodonella hexasticha* parasites of skin and gills and *Myxobolus* sp. parasite of the excretory system on samples of fish (Table 4.11) suggest that these parasites may be responsible for high mortalities of the species at the importers holding facilities or subsequently in the domestic aquaria. *Piscinoodinium* sp. was found infecting more than one species of fish in the same shipment from Colombia (Table 4.6) and *Chilodonella hexasticha* was found in more than one species in the same shipment from Colombia. *Myxobolus* sp. was found in single or mixed infection with *Myxidium* sp. in the same host, *Mylossoma aureum* imported from Colombia or Peru. All the protozoans found are listed in the Tables 4.5.

Five *Gyrodactylus* species were found in the UK fish, four of which were parasitizing catfish of the families Callicthyidae and Loricariidae and one, *Gyrodactylus gemini* sp.n. (Ferraz *et al.*, 1994) was found on specimens of the family Curimatidae. Dactylogyrids were also found in this survey parasitizing the gills of four species of fish. These may comprise five species, however, few specimens were recovered from the gills and they were not sufficient to allow for identification. The only dactylogyrid identified was that found in the excretory system. *Kritskyia* sp. was found

parasitising the excretory system of *M. aureum*, the silver dollar, in samples of fish imported from both Colombia and Peru. All monogeneans species are listed in the Table 4.7.

The fauna of endohelminths mainly comprised nematodes (both adults and larvae) and digenea (both adults and metacercariae). Nematodes of the genus *Spirocamallanus* and *Contracaecum* and digeneans of the family Derogenidae and Strigeidae were found to be very common in the intestine and mesentery of a number of fish.

Amongst the nematodes, species of the genus *Spirocamallanus* were the most common and *Spirocamallanus inopinatus* Travassos *et al.*, 1928 was found in three species, *C. julii* from Brazil and *Metynnis hypsauchen* and *C. macropomum* from Colombia (Table 4.10). Generally, the specimens of *Spirocamallanus* found were immature stages (mainly non-gravid females). Oxyurids were represented by three species *Rondonia rondoni* Travassos, 1919, *Spectatus spectatus* Travassos, 1923 and specimens of the genus *Ichthyouris* Inglis 1962. The two first species were found in mixed infections in the intestine of *Myleus rubripinnis* and the last in the intestine of *M. hypsauchen*. Oxyurids were only found in the intestine of fish of the family Serrasalmidae. All nematodes species, site of infection and hosts are listed in the Table 4.10.

Adult stages of digeneans were represented by specimens of the family Gorgoderidae, Derogenidae and Paramphistomidae. These are listed in the Table 4.8. The families Gorgoderidae and Derogenidae were represented by species of *Phyllodistomum* sp. and *Genarchella* spp. and the family

Paramphistomidae by three species, *Dadayus* sp., *Dadaytrema* sp. and one species not identified. The latter species required further detailed studies on their genitalia which were inappropriate to this survey. The first two species of paramphistomids were found in mixed infections in the intestine of *M. hypsauchen* imported from Colombia.

Several species of metacercariae were found in the fish examined and they are summarized in the Table 4.9. Mesocercariae of Strigeoidea were commonly found in callichthyids, both from Brazil and Colombia. These larval forms were found enclosed in a host cyst in many tissues of the body (Fig.4.2a). Metacercariae of *Apatemon* sp. were found as commonly as the mesocercariae but only occurred in the mesentery. Interestingly a single metacercaria of *Apatemon* was commonly found enclosed in the same host cyst which enclosed numerous mesocercariae (between 1-15) (Fig. 4.2b). Three other types of metacercariae were recorded but could not be identified.

Cestoda, Acanthocephala and Pentastomida were the other major Taxa found and they were mainly represented by a single species from each taxon (Tables 4.8 and 4.10).

Overall, it would appear that only relatively few species found in the survey were associated with mortalities during transport between the exporting and importing countries or be responsible for significant losses of fish after the arrival of the shipment in UK.

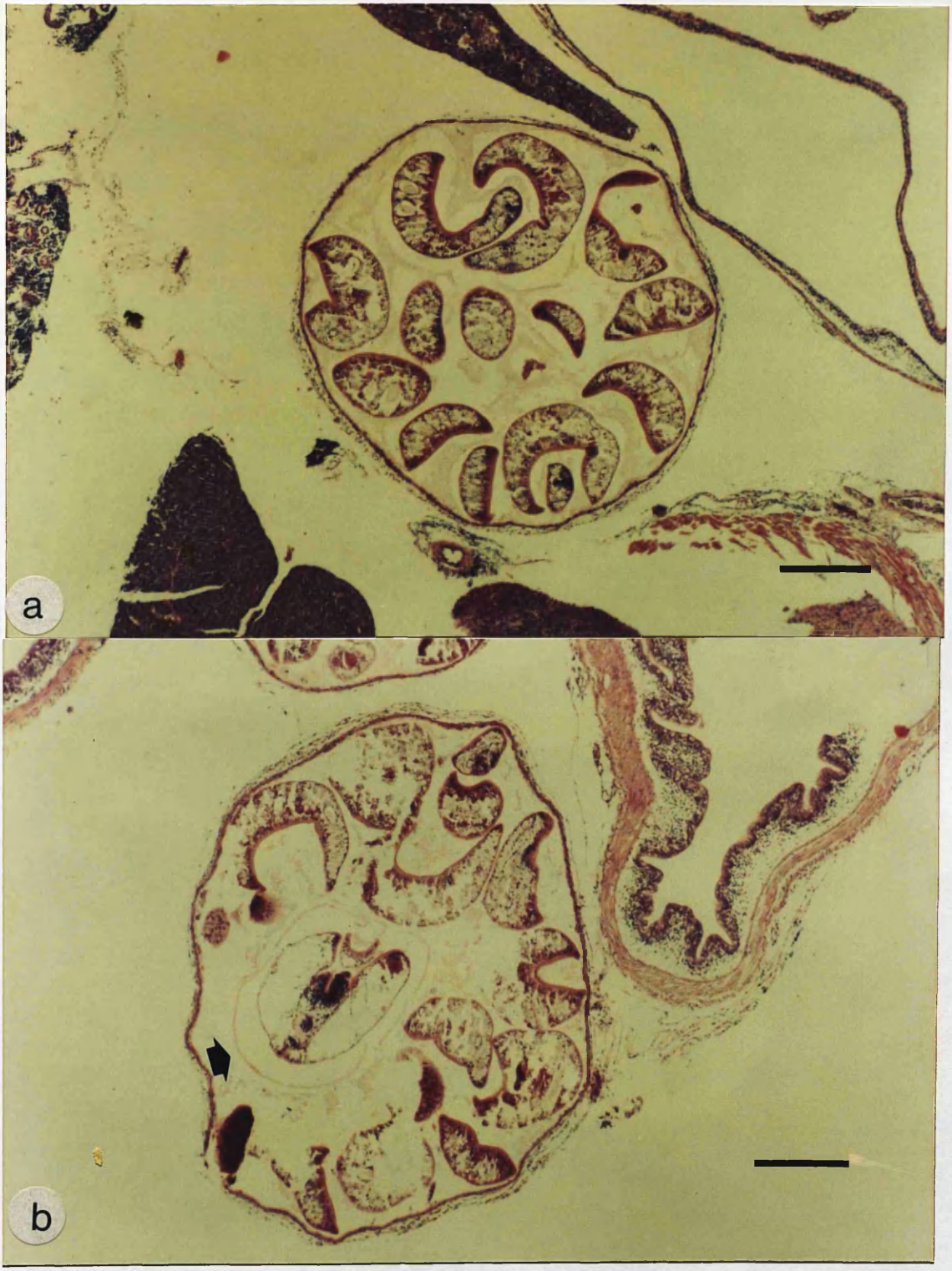


Fig 4.2 a-b Mesocercariae of Strigeoidea enclosed by the host cyst. b - Mesocercariae of Strigeoidea and *Apatemon* sp. enclosed by the same host cyst. Note the parasite cyst around the metacercaria of *Apatemon* (arrow). *Scale bar* = 100µm

Table 4.5

Protozoans found on species of ornamental fish imported into the UK from four South American countries and their site of infection.

Protozoa	Hosts	Site of Infection
Shipments from Colombia		
Myxobolus sp.	*M. aureum*	Kidney (interstitial tissue and renal tubules)
I. multifiliis (Fouquet,1876)	*P. pictus*	Body surface and gills
Ch. hexasticha (Kiernik,1909)	*C. arcuatus* *B. splendens*	Body surface and gills
Piscinoodinium cf.*pillulare* (Schaperclaüs, 1954)	*C. melanistius* *C. metae* *C. julii* *C. punctatus* *B. splendens*	Skin, gills, nasal and oral cavities
Henneguya sp.	*N. palmerai* *M. sweglesi*	Body surface and fins
Shipments from Brazil		
Ch. hexasticha	*P. axelrodi*	Gills and skin
Ch. hexasticha	*C. schwartzi*	Gills and skin
Trichodina sp.		Skin
Shipments from Venezuela		
I. multifiliis	*S. taeniurus*	Skin and Gills
Myxobolus sp.	*S. taeniurus*	Gall bladder, Intestine and oesophagus
Henneguya sp.	*S. taeniurus* *Myleus rubripinnis*	Skin and Fins Gills
Shipments from Peru		
Myxobolus sp.	*M. aureum*	Kidney (interstitial tissue and renal tubules)
Myxidium sp.	*M. aureum*	Kidney (Glomeruli)
Ch. hexasticha	*P. innesi*	Gills and Skin
Protoopalina symphysodonis Foissner *et al*, 1974	*S. aequifasciatus*	Intestine

Table 4.6

Number of fish species in each shipment infected by the same species of external protozoans

ORIGIN/ SHIPMENT	No SPECIES SAMPLED	No SPECIES INFECTED	SPECIES OF PROTOZOAN
COLOMBIA			
SHIPMENT I	8	3	*Piscinoodinium* sp.
SHIPMENT II	4	3	*Piscinoodinium* sp.
SHIPMENT III	4	2	*Chilodonella hexasticha*
BRAZIL			
SHIPMENT I	1	1	*Chilodonella hexasticha*
SHIPMENT II	3	1	*Chilodonella hexasticha* *Trichodina* sp.
PERU			
SHIPMENT I	1	1	*Chilodonella hexasticha*
VENEZUELA			
SHIPMENT I	1	1	*Ichthyophthirius multifiliis*

Table 4.7

Monogeneans and parasitic crustaceans found on ornamental fish species imported from Colombia, Brazil, Peru and Venezuela into the U.K, and their site of infection.

Species	Hosts	Site of Infection
Shipments from Colombia		
Gyrodactylus sp[1]	*Otocinclus* sp.	Body and fins
Gyrodactylus sp[2]	*C. callichthys*	Fins
Gyrodactylus sp[4]	*B. splendens*	Fins
Kritskyia sp.	*M. aureum*	Collecting ducts and urinary bladder
Dactylogyridae (unidentified)	*M. hypsauchen* *C. festivum* *G. jurupari*	Gills
Shipments from Brazil		
Gyrodactylus sp[1]	*Otocinclus* sp.	Body and fins
Gyrodactylus sp[3]	*C. robinae*	Fins
Gyrodactylus sp_4	*B. splendens*	Fins
Shipments from Peru		
Kritskyia sp.	*M. aureum*	Collecting ducts and urinary bladder
Shipments from Venezuela		
G. gemini sp.n. Ferraz, Shinn and Somerville, 1994	*S. taeniurus*	Body and fins
Dactylogyridae (unidentified)		Gills
CRUSTACEA		
Myracetyma sp.		Gills

Table 4.8

Adult stage of digeneans, and adult and larval stages of cestodes, found on species of ornamental fish sampled in the UK, and their site infection .

Species	Hosts	Site of Infection
Shipments from Colombia		
CESTODA		
plerocercoids	*C. melanistius* *B. splendens*	Mesentery
DIGENEA		
Genarchella sp.	*C. julii* *C. arcuatus*	Intestine
Dadaytrema sp.	*M. hypsauchen*	Intestine
Dadayus sp.	*M. hypsauchen*	Intestine
Shipments from Brazil		
CESTODA		
Proteocephalus sp.	*Dianema longibarbis*	Intestine
plerocercoids	*C. arcuatus* *C. robinae*	Mesentery
DIGENEA		
Phylodistomum sp.	*Dianema longibarbis*	Urinary bladder
Genarchella spp.	*Corydoras punctatus* *C. reticulatus* *C. arcuatus*	Intestine
Shipments from Venezuela		
CESTODA		
Scolex pleuronectis	*S. taeniurus*	Intestine
DIGENEA		
Paramphistomidae	*Myleus* sp.	Intestine
Digenea not identified	*S. taeniurus*	Intestine

Table 4.9

Larval stages of digeneans found on species of ornamental fish sampled in the UK and their site of infection.

Species	Hosts	Site of Infection
Shipments from Colombia		
Unidentified metacercariae[1]	*Hyphessobrycon* sp.	Mesentery
Mesocercaria of Strigeoidea	*Corydoras punctatus* (*punctatus*-group) *C. melanistius* (*punctatus*-group) *C. julii* *C. metae*	Mesentery, head, liver and Muscles
Shipments from Brazil		
Acanthosthomidae	*Dianema longibarbis* *Corydoras reticulatus*(*punctatus*-group) *Brochis splendens*	Mesentery
Mesocercaria of Strigeoidea	*Brochis splendens* *Corydoras punctatus* (*punctatus*-group) *C. schwartzi* *C. reticulatus* (*punctatus*-group) *C. arcuatus* *C. robinae*	Mesentery, head, liver and Muscles
Apatemon sp.	*Brochis splendens* *Corydoras punctatus*(*punctatus*-group) *C. reticulatus* (*punctatus*-group)	Mesentery
Unidentified metacercariae[2]	*Brochis splendens*	Mesentery
Shipments from Venezuela		
Acanthostomum sp.	*Semaprochilodus taeniurus*	Gills
Acanthostomidae	*Metynnis* sp.	Skin
Unidentified metacercariae[3]	*Myleus* sp.	Mesentery

Table 4.10

Adult and larval stages of nematodes, adults of Acanthocephala and larval stages of Pentastomida, parasitic crustaceans found in species of ornamental fish sampled in UK and their site of infection.

Species	Hosts	Site of Infection
Shipments from Colombia		
NEMATODA		
S. inopinatus	*Colossoma macropomum* *Metynnis hypsauchen*	Intestine and pyloric caeca
Spirocamallanus sp[1]	*Brochis splendens*	Intestine
Spirocamallanus sp[3]	*Corydoras metae*	Intestine
Spirocamallanus sp[4]	*Mylossoma aureum*	Intestine
Ichthyouris sp.	*M. hypsauchen*	Intestine
Contracaecum sp.	*C. punctatus* *Petitella georgeae* *B. splendens*	Encysted on the mesentery
L_3 of Camallanidae	*Hyphessobrycon* sp. *Callichthys*	Encysted on the mesentery
Larvae not identified	*C. arcuatus*	Encysted mucosa of stomach
Shipments from Brazil		
NEMATODA		
Spirocamallanus sp[1]	*B. splendens*	Intestine
Spirocamallanus sp[2]	*C. sterbai*	
S. inopinatus Travassos *et al.*, 1928	*C. julii*	Intestine and pyloric caeca
Contracaecum sp.	*Aequidens* sp. *Dianema longibarbis* *B. splendens*	Encysted on the mesentery
Larvae not identified	*C. robinae* *C. arcuatus*	Encysted on the mesentery
PENTASTOMIDA		
Nymph	*D. longibarbis*	Encysted in the bladder swim
ACANTHOCEPHALA		
Neoechinorhynchus sp	*C. reticulatus*	Intestine
Shipments from Venezuela		
NEMATODA		
Rondonia rondoni Travassos, 1919	*Myleus* sp.	Intestine
Spectatus spectatus Travassos, 1923	*Myleus* sp.	Intestine
Spinitectus sp.	*S. taeniurus*	Intestine
Philometra sp.	*S. taeniurus*	Body cavity

4.4.1.3 Prevalence and intensity of infection

In general, the prevalence and intensity of infection of all groups of parasites found on the wild ornamental fish imported into the UK from Colombia, Peru, Brazil and Venezuela were low. The data for prevalence and intensity of infection are shown in the Tables 4.11 to 4.14. Quantitatively these results obtained cannot be compared because of the way in which the samples were collected.

From 735 specimens of wild ornamental fish sampled 369 (50.2%) were infected with one or more species of parasites.

Protozoans were found in shipments of all countries examined. As can be seen in the Table 4.11, the shipments from Colombia presented a greater number of both fish infected (11) and protozoan species (5). *Piscinoodinium* sp. was the species most prevalent. Shipments from other South American countries presented two to three fish species infected and *Chilodonella hexasticha* was the species most prevalent in the shipments from Brazil and Peru.

Although 5 species of gyrodactylids were found in the UK samples, no species identified appeared to occur in more than one host species. These are presented in the Table 4.12. *Gyrodactylus* sp[1] parasite of *Otocinclus* sp. and *Gyrodactylus* sp[4] were the only species found in the same host from different origin. In both samples, it was found at low prevalence and intensity of infection. Other species were also found at low prevalence and intensity of infection, with

exception of *Gyrodactylus* sp[2] that was infecting 83% of the Porthole cat, *Callichthys callichthys* examined.

The most prevalent metazoan parasite from the fish examined was the mesocercaria or diplostumula of the superfamily Strigeoidea. The intensity of infection of this mesocercaria appeared to be very high, because in each host cyst 1 to 15 mesocercariae could be found as illustrated in the Figure 4.2a. An average of 50 host cysts could be found occurring in the mesentery alone. These mesocercariae were found in callichthyid species imported from both Brazil and Colombia.

The prevalence and intensity of the metacercariae found in this survey can be assessed in the Table 4.13. For the mesocercariae of Strigeoidea, the intensity of infection is represented by the range of the number of cysts present.

A single cyst of the metacercaria of *Apatemon* was often enclosed within the host cyst together with the mesocercariae as illustrated in the Figure 4.2b. *Apatemon* sp. was also found together in tight clusters of cysts in the mesentery of *B. splendens.* The intensity of infection of this metacercaria was also not determined because they were often found enclosed by the host cyst containing the mesocercariae. The prevalence and intensity of infection of other metacercariae found can be accessed in the table 4.13.

Adult of digenea, cestodes and acanthocephalans presented low prevalence and intensity of infection in all samples of fish examined. These figures are presented in the Tables 4.13 and 4.14.

Eleven genera of nematodes were found in the samples of fish examined, of which the genus *Spirocamallanus* presented the greatest number of species (5). *Spirocamallanus* sp[1] and *S. inopinatus* were found as adult stages in the samples of fish from both Brazil and Colombia at low prevalence and intensity of infection and *S. inopinatus* appeared to be the species most prevalent. *Contracaecum* sp. appeared to be the most prevalent larval stage and it was found in 5 host species. Strangely, no nematodes species, adult or larval stage, were found in the samples of the shipments from Peru. The prevalence and intensity of infection of the species of nematodes found are shown in Table 4.14.

Table 4.11

Prevalence of the species of protozoans found on tropical ornamental fish imported into the U.K. from four South American Countries.

Protozoa	Host	Number Examined	Prevalence (%)
Shipments from Colombia			
Myxobolus sp.	*M. aureum*	35	31.4
Ichthyophthirius multifiliis (Fouquet, 1876)	*P. pictus*	15	100
Chilodonella hexaticha (Kiernik, 1909)	*C. arcuatus*	20	10
	B. splendens	28	20
Piscinoodinium cf. *pillulare* (Schaperclaus, 1954)	*C.melanistius*	40	45
	C. metae	35	14.28
	C. julii	16	62.5
	C. punctatus	25	20
	B. splendens	28	14.3
Henneguya sp.	*N. palmerai*	8	12.5
	M. sweglesi	7	14.3
Shipments from Brazil			
Ch. hexasticha	*P. axelrodi*	40	42.5
	C. schwartzi	20	65
Trichodina sp.	*C. schwartzi*	20	9
Shipments from Venezuela			
Henneguya sp.	*S. taeniurus*	25	36
Myxobolus sp.	*S. taeniurus*	25	32
I. multifiliis	*S. taeniurus*	25	8
	Myleus sp.	13	15.38
Shipments from Peru			
Myxobolus sp.	*M. aureum*	35	54.2
Myxidium sp.	*M. aureum*	35	5.7
Ch. hexasticha	*P. innesi*	20	64
Protoopalina symphysodonis Foissner, Schubert and Wilbert, 1974.	*Symphysodon* sp.	5	60

Table 4.12

Prevalence and intensity of infection of the species of monogeneans found on species of tropical ornamental fish examined in the UK.

Species	Host	Number Examined	Intensity of Infection (Range)	Prevalence (%)
Shipments from Colombia				
Gyrodactylus sp[1]	*Otocinclus* sp.	51	1-35	25.5
Gyrodactylus sp[2]	*C. callichthys*	6	1-10	83.3
Gyrodactylus sp[4]	*B. splendens*	28	5	7
Kritskyia sp.	*M. aureum*	35	6-50	31
Dactylogyridae	*M. hypsauchen*	10	2-8	20
	C. festivum	6	3	17
	G. jurupari	5	4	20
Shipments from Brazil				
Gyrodactylus sp[1]	*Otocinclus* sp	60	1-11	7
Gyrodactylus sp[3]	*C. robinae*	15	10-20	13
Gyrodactylus sp[4]	*B. splendens*	30	4-9	7
Shipments from Venezuela				
G. gemini Ferraz, Shinn and Sommerville, 1994	*S. taeniurus*	25	1-25	12
Dactylogyridae	*S. taeniurus*	25	2-4	16
Crustacea				
Miracetyma sp.	*S. taeniurus*	25	1-15	16
Shipments from Peru				
Kritskyia sp.	*M. aureum*	35	1->50	54

NB. The intensity of infection of the monogeneans in this table is expressed by the **Range**, according to Margolis *et al.*, 1982, because the number of *Kritskyia* sp. was only counted in 5 infected specimens. Other samples were fixed for histology.

Intensity (frequently expressed as a numerical range) - number of individuals (determined directly or indirectly) of a particular parasite species in each infected host (Margolis *et al.*, 1982).

Table 4.13

Prevalence and intensity of infection of the adult and larval stages of digeneans and cestodes on species of tropical ornamental fish imported from South America.

Species	Host	Number Examined	Mean Intensity	Range	Prevalence (%)
Shipments from Colombia					
CESTODA					
plerocercoids	*C. melanistius*	40	2.5	-	10
	B. splendens	28	3	-	3.5
DIGENEA					
Genarchella sp.	*C. julii*	16	1	-	6
	C. arcuatus	20	1	-	5
Dadayus sp.	*M. hypsauchen*	10	2	-	30
Dadaytrema sp.	*M. hypsauchen*	10	4.5	-	80
Mesoc. Strigeoidea	*C. punctatus*	25	-	10-20	24
	C. melanistius	40	-	10-20	5
	C. julii	16	-	1-15	12.5
	c. metae	35	-	>50	3
Unidentified metac.	*Hyphessobrycon* sp.	10	-	1-3	30
Shipments from Brazil					
CESTODA					
Proteocephalus sp.	*D. longibarbis*	5	1	-	20
plerocercoids	*C. arcuatus*	10	3	-	10
	C. robinae	15	1.6	-	20
DIGENEA					
Phyllodistomum sp.	*D. longibarbis*	5	1	-	20
Genarchella sp.	*C. punctatus*	15	1	-	7
	C. reticulatus	15	1	-	13
	C. arcuatus	10	1	-	10
Acanthostomidae	*D. longibarbis*	5	5	-	20
(Metac.)	*C. reticulatus*	15	4.5	-	13
Dipl. Strigeoidea	*B. splendens*	30	-	5->50	60
	C. punctatus	15	-	10-20	20
	C. schwarzti	20	-	3-50	80
	C. reticulatus	15	-	20-40	40
	C. arcuatus	10	35	-	10
	C. robinae	15	20	-	10
Apatemon sp.	*B. splendens*	30	-	1-20	60
	C. punctatus	15	-	-	20
	C. reticulatus	15	-	-	40
Metac. not identified	*B. splendens*	30	2	-	3
Shipments from Venezuela					
CESTODA					
Scolex pleuronectis	*S. taeniurus*	25	3	-	12
DIGENEA					
Paramphistomidae	*Myleus* sp.	15	4	-	33
Acanthostomum sp.	*S.taeniurus*	25	-	-	8
Acanthostomidae	*Metynnis* sp.	10	-	-	80
Metac. not identified	*Myleus* sp.	15	2	-	7

Table 4.14

Prevalence and intensity of infection of nematodes, acanthocephalans and pentastomids found on species of tropical ornamental freshwater fish imported from South America.

Species	Host	Number Examined	Mean Intensity	Prevalence (%)
Shipments from Colombia				
S. inopinatus	*C. macropomum*	11	1.18	9
	M. hypsauchen	10	1.33	30
Spirocamallanus sp[1]	*B. splendens*	28	1.66	25
Spirocamallanus sp[3]	*C. metae*	35	1.5	23
Spirocamallanus sp[4]	*M. aureum*	35	1.75	23
Ichthyouris sp.	*M. hypsauchen*	10	1.5	20
Contracaecum sp.	*C.punctatus*	25	1	12
	P. georgeae	5	1	20
	B. splendens	28	3	11
L_3 of Camallanidae	*Hyphessobrycon* sp.	10	2	20
	C. callichthys			
Unidentified larvae	*C. arcuatus*	20	2	20
Shipments from Brazil				
NEMATODA				
S. inopinatus Travassos *et al.*,1928	*C. julii*	10	1	10
Spirocamallanus sp[1]	*B. splendens*	30	1.37	27
Spirocamallanus sp[2]	*C. sterbai*	10	1.33	30
Contracaecum sp.	*Aequidens* sp.	2	2	50
	B. splendens	30	1	3
	D. longibarbis	5	1	20
Larvae not identified	*C. robinae*	15	2.33	13
ACANTHOCEPHALA				
Neoechinorhynchus sp.	*C. reticulatus*	15	1	40
PENTASTOMIDA				
Nymph	*D. longibarbis*	5	1	20
Shipments from Venezuela				
Rondonia rondoni Travassos, 1919	*Myleus* sp.	15	3	46
Spectatus spectatus Travassos, 1923	*Myleus* sp.	15	5	7
Spinitectus sp.	*S. taeniurus*	25	1	4
Philometra sp.	*S. taeniurus*	25	3	4
CRUSTACEA				
Myracetyma sp.	*S. taeniurus*	25	7.5	16

4.4.2 Investigations in Brazil

4.4.2.1 Fish species examined

A total of 456 specimens of wild freshwater ornamental fish, comprising 5 families and 15 species were sampled at the exporters' holding facilities in Brazil. The species of fish, their common name and the total number sampled are presented in Table 4.15.

The family Callichthyidae comprised the greatest number of species (11) and specimens (306) sampled. It was represented by 2 genera *Brochis* with 1 species and *Corydoras* with 10 species. Other families sampled were represented by only 1 species each.

Paracheirodon axelrodi was the species with the greatest number of specimens analyzed (100). Amongst the species of the genus *Corydoras* were *C. maculifer* (51), *C. sterbai* (47) and *C. julii* (40). These are illustrated in Fig. 4.3.

Amongst the 15 species sampled at the exporters' holding facilities in Brazil, 10 species had been previously examined in the shipments from Brazil, Colombia and Peru imported into the UK. Only 5 species were sampled for the first time and these were *C. haraldschultzi* and *C. elegans* (*elegans*-group), *C. maculifer*, *C. hastatus* and *C. agassizi*.

Seven species sampled in this study do not have their names printed in the IBAMA's list of species as being allowed to be exported as ornamental fish from Brazil.

Table 4.15

Total number of species of ornamental fish of the families Callichthyidae, Serrassalmidae, Characidae and Pimelodidae examined in Manaus, Brazil.

FAMILY/ SPECIES OF FISH	NUMBER EXAMINED	COMMON NAME
CALLICHTHYIDAE		
Brochis splendens (Castelnau, 1855)	20	Common Brochis
Corydoras julii Steindachner, 1906	40	Julii Cat
C. haraldschultzi Knaack,1962	30	Harold Schultz's Cory
C. elegans (*elegans*-group)	20	Elegant Cory
C. maculifer Nijssen and Isrbrückner,1971	51	-
C. sterbai Knaack, 1962	47	Sterba's Cory
C. schwartzi Rössel, 1963	20	Schwartz's Cory
C. hastatus Eigenmann and Eigenmann ,1888	30	Spotlight mini cory
C. agassizi (*punctatus*-group)	28	-
C. punctatus (*punctatus*-group)	20	-
C. robinae Burguess, 1983	20	Robina's Cory
SERRASSALMIDAE		
Mylossoma aureum (Spix in Agassiz, 1929)	10	Silver Dollar
PIMELODIDAE		
*Pimelodella pictus**	10	Angelicus Pimelodus
CHARACIDAE		
Paracheirodon axelrodi (Schultz)	100	Cardinal tetra
CICHLIDAE		
Symphysodon spp.	10	Discus

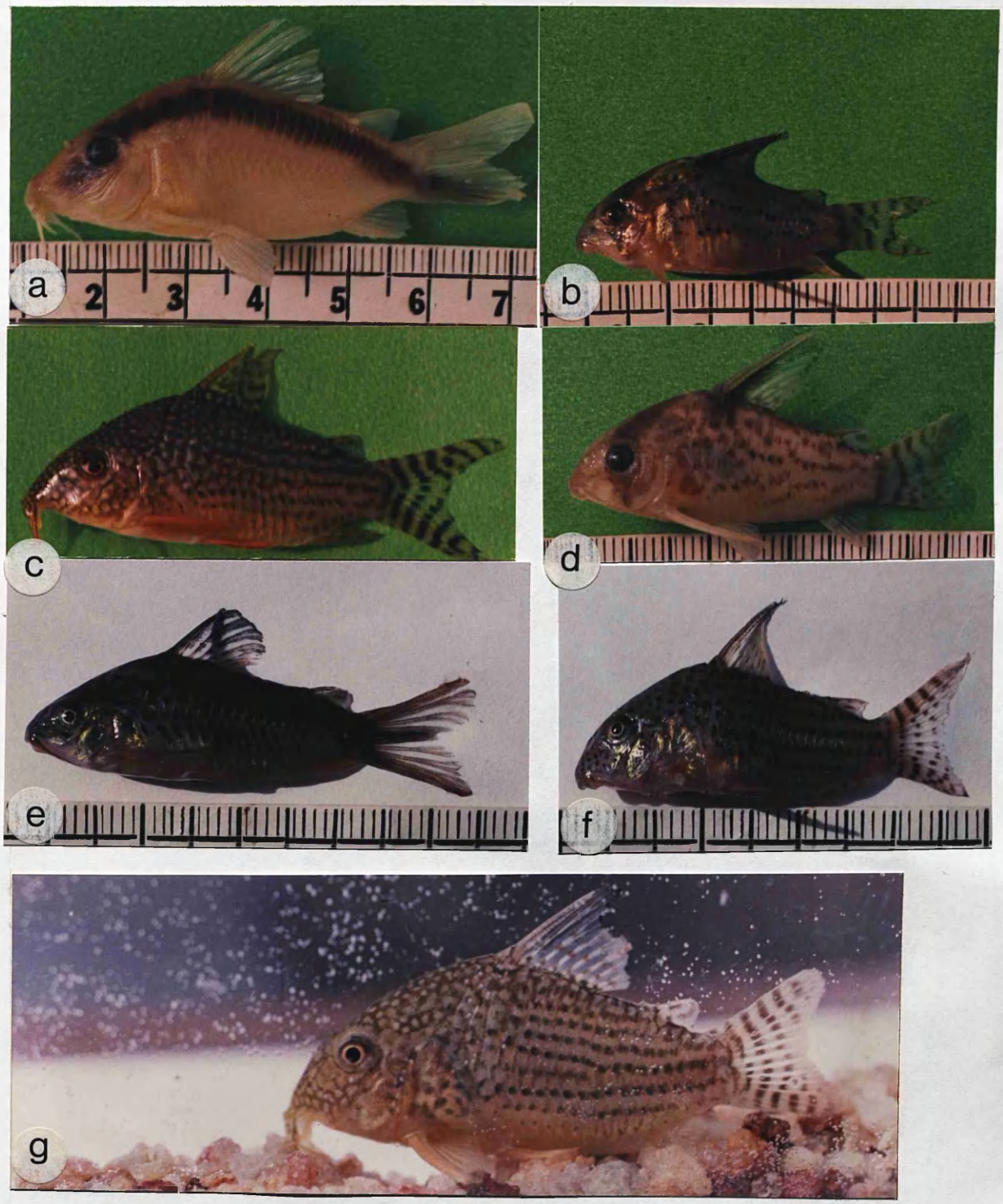


Fig. 4.3 a-g. Species of the genus *Corydoras* commonly available at the exporters' holding facilities in Brazil. a - *C. arcuatus*; b - *C. julii*; c - *C. sterbai*; d - *C. schwartzi*; e - *C. elegans*; f - *C. maculifer*; g - *C. haraldschultzi*.

4.4.2.2. Parasite survey

Thirty four species of parasites were found in 15 fish species examined at the exporters' holding facilities in Brazil compared to 47 species of parasites found in 33 fish species examined in the UK . These are shown in the Tables 4.16-4.17 and 4.19. Leeches were sporadically observed attached to species of catfish on incoming shipments and were not observed in the shipments arriving in the UK.

Monogenean parasites of the skin, were commonly observed on all types of shipments examined and should be noted that from the 7 species of callichthyids sampled on their arrival at the exporters' holding facilities, 5 were infected with 1 or 2 *Gyrodactylus* species (Table 4.16). In total 10 *Gyrodactylus* species were found, 6 of which were in mixed infections. These are presented in the Table 4.16.

Nine species of protozoans were present in the samples of fish stocks examined and 2 of them, *Piscinoodinium* sp. and *Chilodonella hexasticha* were found in the samples of *C. julii*, *C. schwartzi* and *B. splendens* ready to be exported (Table 4.17-4.18). Sessiline peritrichs of the family Epistylididae were found in a very high intensity of infection on the skin of very debilitated specimens of *Symphysodon* sp.. They were also found on specimens of *C. hastatus* kept in tanks without aeration and with high level of organic material in the bottom. The species of protozoans according to their site of infection can be assessed in the Tables 4.17.

The endoparasite fauna was composed mainly of larval stages of digeneans and nematodes and few adult specimens of

digeneans, nematodes and acanthocephalans. These are summarized in the Table 4.19.

Several species of metacercariae were found in the fish examined in Brazil (Fig. 4.4) of which the mesocercaria of Strigeoidea was the most prevalent infecting 6 host species and presenting the greatest number of parasites per fish. The mesocercariae were widespread throughout different parts of the body including the muscles of the head, abdominal cavity (Fig. 4.2) and oocytes of the females which had the ovary completely developed.

Nematodes of the genus *Spirocamallanus* were common findings among the callichthyid species and *Spirocamallanus* sp[5] appears to be infecting two species of callichtyids, *Corydoras punctatus* and *C. haraldshultzi*.

The findings presented in this survey are the results of the examination of fish from two exporters' holdings facilites in Brazil. However, as the study was carried out at the beginning of the season, and one of the collaborators was not fully operational, the large majority of the samples of fish were obtained from the major exporter in Manaus. The number of parasites species actually found in the species of callichthyids is probably greater than shown here, as relatively few individuals were examined and only at one period of the year. Species of callichthyids are mainly captured during the dry season, when the level of the water of the lakes is very low.

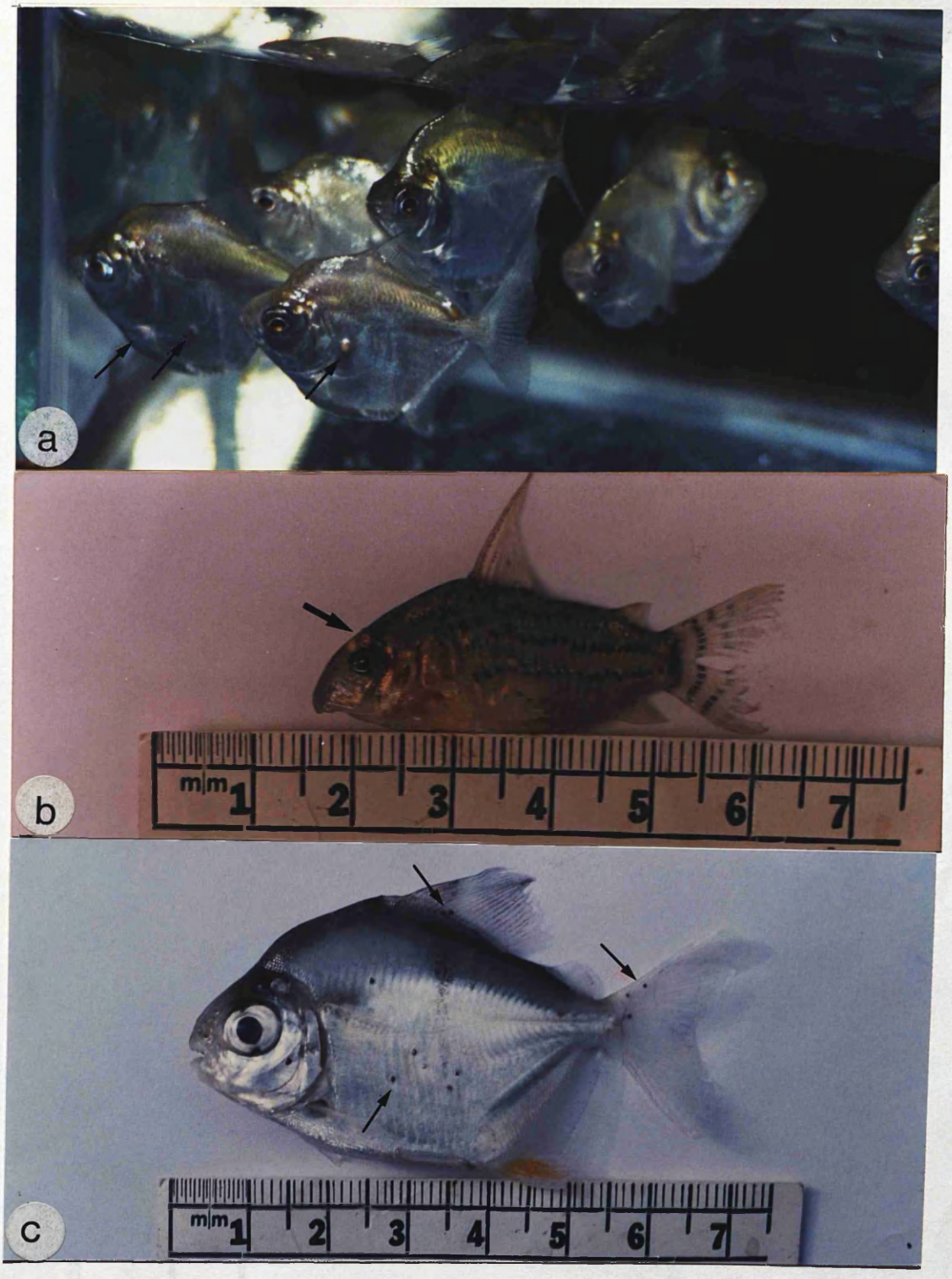


Fig 4.4 Types of metacercariae found in species of ornamental fish prior to export. a - b *Clinostomum* sp. a - Note the presence of the grubs in the base of the dorsal fin, and lateral part of the body of *Mylossoma aureum;* b - note the yellow cysts in the eye lid above the eye of *Corydoras* sp. c - Black spot diseases in *Metynnis* sp.

Table 4.16

Presence of monogeneans in the shipments of fish of the family Callichthyidae at different stages prior to the export in the exporters' holding facilities in Manàus, AM.

Host	Species	Site of Infection	Origin	Condition of the Shipment		
				Just Arrived	Holding Tank	Ready to be Exported
Corydoras julii	*Gyrodactylus* sp[6]	Fins	Amazon region	+	+	+
C.maculifer	*Gyrodactylus* sp[8]	Fins	Amazon region	+	+	+
C. hastatus	*Gyrodactylus* sp[10] *Gyrodactylus* sp[11]	Body and fins	Amazon region	Not examined	+++	Not examined
C. sterbai	*Gyrodactylus* sp[7] *Gyrodactylus* sp[12]	Body and fins	Rio Guapore	+	+	Not examined
C. robinae	*Gyrodactylus* sp[3]	Fins	Rio Negro	+	+	+
C. punctatus	*Gyrodactylus* sp[5]	Fins	Amazon region	+	Not examined	Not examined
Brochis splendens	*Gyrodactylus* sp[4] *Gyrodactylus* sp[9]	Body and fins	Amazon region	Not examined	+	+

Light Infection (+)= 1-25 Moderate Infection (++) = 25-50

Heavy Infection (+++)= >50 specimens

Table 4.17

Protozoans and their site infection found in species of ornamental fish sampled in the exporters' holding facilities in Brazil.

Species	Host	Site of Infection
Piscinoodinium sp.	*Corydoras julii* *B. splendens*	Skin, nasal and oral cavity and gills
Ch. hexasticha	*C.schwartzi* *B. splendens* *P. pictus*	Skin and gills
Myxobolus sp.	*C. hastatus* *Symphysodon* sp.	Fins
Henneguya sp.	*C. hastatus*	Gills
I. multifiliis	*P. pictus* *Symphysodon* sp.	
Epistylididae	*C. hastatus* *Symphysodon* sp.	Skin
P. symphysodonis	*Symphysodon* sp.	Intestine
Tetrahymena sp.		Intestine
Hexamitidae		Skin

Table 4.18

External protozoans found on scrapes of skin and gills of species of ornamental fish sampled at the exporters holding facilites.

Family/Species of Fish	**Parasite Species**	**Condition of the Shipment**		
CALLICHTHYIDAE		**Just Arrived**	**Holding Tank**	**Ready to be Exported**
Corydoras julii	*Piscinoodinium* sp.	-	+	+
C. schwartzi	*Ch. hexasticha*	-	+	+
C. hastatus	Epistylididae	Not Examined	+++	Not examined
Brochis splendens	*Piscinoodinium* sp. *Ch. hexasticha*	- -	+ +	- +
PIMELODIDAE				
Pimelodella pictus	*I. multifiliis* *Ch. hexasticha*	- -	+ +	- +
CICHLIDAE				
Symphysodon sp.	*Tetrahymena* sp. Hexamitidae *I. multifiliis* Epistylididae	Not examined	+ + + +++	Not examined

Light infection (+) Moderate (++) Heavy (+++)

Table 4.19

Metazoans found on species of ornamental fish sampled in the exporters holding facilities in Brazil, and their site of infection.

Species	Host	Site of Infection
DIGENEA		
Paramphistomidae	*Symphysodon sp.*	Intestine
Genarchella sp.	*C. punctatus* *C. schwartzi* *C. julii*	Intestine
Diplostomum sp.	*C. punctatus*	Eyes
Clinostomum sp.	*C. punctatus* *M. aureum*	above the eye (eye lid) Muscles
Dipl.Strigeoidea	*C. schwartzi* *B. splendens* *C. sterbai* *C.haraldshultzi* *C. julii* *C. maculifer*	Mesentery, muscles of head and ovary
Apatemon sp.	*B. splendens* *C. sterbai* *C. julii* *C. elegans*	**Mesentery**
Metac. not identified	*P. axelrodi*	Body cavity
NEMATODA		
S. inopinatus	*M. aureum*	Intestine
Spirocamallanus sp[1]	*B. splendens*	Intestine
Spirocamallanus sp[2]	*C. sterbai*	Intestine
Spirocamallanus sp[5]	*C. punctatus* *C. haraldshultzi*	Intestine
Phylometra sp.	*C. sterbai*	Swimming bladder
Contracaecum sp.	*B. splendens* *C. maculifer* *C. sterbai* *C. julii*	Mesentery
Larvae not identified	*C. sterbai*	Mucosa of stomach
ACANTHOCEPHALA		
Neoechinorhynchus sp	*C. agassizi*	Intestine

4.4.2.2.1 Prevalence and intensity of infection

The prevalence and the intensity of infection of all species of parasites found in this survey at the exporters' holding facilities in Brazil are presented in Tables 4.20 to 4.22.

From the 456 specimens of fish sampled in the 2 exporters' holding facilities, 363 (79.60%) were infected with one or more species of parasites.

Among the protozoans, *Ch. hexasticha*, *I. multifiliis*, Hexamitidae, and Epistylididae were the most prevalent species (Fig. 4.20). It should be noted that these protozoans are all common pests of aquarium fish and the high prevalence was mainly observed in very debilitated fish kept in tanks with poor water quality (e.g. *C. hastatus*) and species that do not recover fast from the stressful conditions of capture, handling and transportation (e.g. Discus, *Symphysodon* sp.).

Ten species of *Gyrodactylus* were found of which the lowest prevalence was shown by *Gyrodactylus* sp[6], a parasite of *C. julii* and the highest by the species parasitic on *C. hastatus*, *B. splendens* and *C. robinae* (Table 4.21)

Among the endoparasites the highest prevalence and intensity of infection was shown by the mesocercaria of Strigeoidea (Table 4.22), which was found in 5 host species. Each fish could be infected with more than >50 host cysts in the mesentery alone.

Table 4.20

Prevalence of protozoans on species of tropical ornamental fish sampled in the exporters' holding facilities in Brazil.

Protozoa	Host	Number Examined	Prevalence (%)
Piscinoodinium sp.	*C. julii*	40	50
	B. splendens	20	50
Ch. hexasticha	*C. schwartzi*	20	35
	P. pictus	10	100
I. multifiliis	*P. pictus*	10	100
	Symphysodon sp.		
Epystilididae	*C. hastatus*	30	100
Myxobolus sp.	*C. hastatus*	30	23
	Symphysodon sp.	10	10
Henneguya sp.	*Symphysodon* sp.	10	30
Hexamitidae			100
Protoopalina symphysodonis			70
Tetrahymena sp.			70

Table 4.21

Prevalence and intensity of infection of monogeneans on species of tropical ornamental fish sampled in the exporters' holding facilities in Brazil.

Host	Number Examined	Species	Intensity of Infection (Range)	Prevalence (%)
C. julii	40	*Gyrodactylus* sp[6]	1-25	7.5
C. maculifer	51	*Gyrodactylus* sp[8]	1-25	68.62
C. hastatus	20	*Gyrodactylus* sp[10]	>50	100
		Gyrodactylus sp[11]		
C. sterbai	47	*Gyrodactylus* sp[7]	1-25	63.82
		Gyrodactylus sp[12]		
C. robinae	20	*Gyrodactylus* sp[3]	1-25	100
C. punctatus	20	*Gyrodactylus* sp[2]	1->50	50
B. splendens	20	*Gyrodactylus* sp[4]	1-25	100
		Gyrodactylus sp[9]		

Table 4.22

Prevalence and intensity of infection of metazoans on species of tropical fish sampled in the exporters' holding facilities in Brazil.

Species	Host	Mean Intensity	Range	Prevalence (%)
DIGENEA				
Paramphistomidae	*Symphysodon sp.*	1	-	10
Genarchella sp.	*C. punctatus*	1	-	5
	C. schwartzi	3.5	-	20
	C. julii	2		5
Diplostomum sp.	*C. punctatus*	2	-	5
Clinostomum sp.	*C. punctatus*	2.66	-	15
	M. aureum	4.8	-	50
Dipl.Strigeoidea	*C.schwartzi*	65	65	5
	B. splendens	-	1->50	100
	C. sterbai	-	1->50	17
	C.haraldshultzi	-	>50	40
	C. julii	-	>50	25
		-	>50	15.68
Apatemon sp.	*B. splendens*	8	-	25
	C. sterbai	-	-	17.02
	C. julii	-	-	25
	C. elegans	-	-	25
Metac. not identified	*P. axelrodi*	-	-	100
NEMATODA				
S. inopinatus	*M. aureum*	1.5	-	40
Spirocamallanus sp[1]	*B. splendens*	1.66	-	15
Spirocamallanus sp[2]	*C. sterbai*	1.12	-	17
Spirocamallanus sp[5]	*C. punctatus*	1	-	5
	C. haraldshultzi	1		3
Phylometra sp.	*C. sterbai*	1	-	2
Contracaecum sp.	*B. splendens*	1.5	-	10
	C. maculifer	1	-	2
	C. sterbai	1.5	-	4.25
	C. julii	4	-	2.5
ACANTHOCEPHALA				
Neoechinorhynchus sp	*C. agassizi*	15	-	15

4.5 Discussion

4.5.1 Fish species examined in the UK and Brazil

Very few importers in the UK were receiving fish imported directly from South America. Consequently, shipments of wild ornamental fish were infrequent (see Chapter III - Tables 3.2 and 3.8). This situation was evident from the present investigation where only 29 shipments of the fish species selected for study were obtainable during the 25 month sampling period. Although not every shipment from South America to the UK during this period was sampled, firstly because the study was limited to certain species, and secondly because shipments of highly valuable species (e.g. Discus) were not sampled routinely due to price restrictions, the present study reflects the fact that the number of shipments from South American exporting countries into the UK are indeed very small compared with the numbers arriving in the UK from the major source of freshwater fish, Singapore (MINTEL, 1991).

Shipments were sampled from four exporting countries of the Amazon basin, Brazil, Colombia, Peru and Venezuela. Most shipments sampled were from Colombia and Brazil (Table 4.3) which were the main South American countries exporting wild freshwater fish into the UK from 1987-1989 (Andrews, 1990). Colombia was listed amongst the six major sources of freshwater fish imports into the UK for the year of 1990, behind Singapore, Israel, US, Japan and Hong Kong (MINTEL, 1991). Whereas import figures for the period of collection 1991-1994 were not obtained to support our observations,

Colombia, appeared to be supplying the UK market with a greater variety of fish species than other exporting SA countries. This situation was mainly observed through the list of the species supplied by the importers before arrival of the shipments and this reflects the greater number of shipments and species sampled from Colombia in this study.

Consignments from Peru and Venezuela were not commonly available and it appears that shipments from Venezuela were usually imported under special order.

Basically, all species of ornamental fish from the Amazon basin can be obtained in the UK market, including juveniles of food fish species. Juveniles of two species of food fish species *Colossoma macropomum* (Cuvier, 1818) and *Semaprochilodus taeniurus* (Steindachner) were sampled, but they were only found in consignments from Colombia and Venezuela, where the legislation allows the export of juveniles of tank-bred species of food fish. It is not clear whether or not they were removed from the wild. *Colossoma macropomum* is the largest species amongst the Serrasalmidae, the adult reaching around 30 kg (Saint-Paul, 1986). According to the importers this species is not popular because of the large size that the adult can reach, and hence is only available under special order.

Amongst the ornamental species, all major families were sampled, and the highest number of shipments examined, and species analyzed, were from the family Callichthyidae, genus *Corydoras* Lacépède, 1803 (Table 4.1). This was expected because information obtained from a preliminary study and discussions with the importers (see Chapter III), suggested

that this genus was one of the most commonly imported from South America into the UK.

The family Callichthyidae is a large family of relatively small, heavily armoured freshwater catfishes found in South America and Trinidad (Burguess, 1989), where the genus *Corydoras* is one of the most important genera of catfish for the aquarium trade. The genus is very prolific with approximately 94 species known (Nijssen and Isbrücker, 1980b).

The large number of species of *Corydoras* available, complicated the sampling process during the present study because different species with a similar external appearance were often exported together in the same bag under the same latin name. This occurrence can be attributed to the following factors:

1) Adults and juveniles of different species may be found in the same schools, or in aggregations of hundreds or thousands of individuals, in their natural environment (Burguess, 1989), and hence be captured at the same time;

2.) The high degree of variability in the colour pattern exhibited by species of this genus is not well defined in the juveniles (Zuanon, pers. comm - INPA, Brazil).

3) Identification prior to export is usually made by non-specialists who often separate the species using the dorsal view of the specimens rather than the lateral view, from which specific characters such as the lateral line and the colour pattern can be better observed (Zuanon, pers. comm.).

Consequently, some identifications of species from the genus *Corydoras* provided by the importers were either not confirmed as correct by the specialist or the species-group

name was added following the latin name for those species for which the specific characters were not clear (Tables 4.1 and 4.15). The division in group-species for the genus *Corydoras* was proposed by Nijssen (1970), Nijssen and Isbrücker (1980b) and was defined on the basis of colour pattern and snout length.

Similar problems of identification were also observed with specimens of *Metynnis* of the family Serrassalmidae imported from Venezuela and Colombia (Table 4.3) and specimens of *Symphysodon* of the family Cichlidae (Table 4.3) imported from Peru. Initially, these specimens were identified as *Metynnis screitmulleri* and *Symphysodon discus*, but subsequently they had their identification confirmed as *M. hypsauchen* (Müller and Troschel, 1844) by M. Jegú (ORSTON/INPA) and *S. aequifasciatus* Pelegrin by S. Kullander (Swedish Museum of Nat. Hist.) respectively. The species *M. screitmulleri* is commonly referred to by the importers but it is not clear whether this species has been properly described.

With regard to the specimens of Discus, their small size made it quite difficult to confirm whether the species was *S. discus* or *S. aequifasciatus*, but, as the scale size was more appropriate to the latter (i.e. quite large), the specimens were identified as *S. aequifasciatus* Pelegrin (Kullander, pers. comm.).

The identification of the specimens of *Pimelodella pictus*, Pimelodidae (Table 4.2) and specimens of *Otocinclus*, Loricariidae (Table 4.2), have not yet been completed by the specialist. However, there is often confusion in the identification of the species of these two genera. Species

of the genus *Pimelodella*, for example, are often confused with species of the genus *Pimelodus*, and different species of the so-called "Angelicus pimelodella" are sold in the trade under the name *Pimelodella pictus* (Burguess, 1989).

Species of the genus *Otocinclus* are also often erroneously identified (Fig. 4.1d) During the sampling period all specimens sampled in shipments from Brazil and Colombia were identified under the name *Otocinclus arnoldi* Regan, 1909 by the importers. The species that has most often been exported from Brazil according to Burguess (1989) is *Otocinclus vestitus* Cope, 1872, from Southeast Brazil. It is possible that some shipments also included this species and other species.

The misidentification of the species of fish exported, caused by a lack of adequate monitoring, is perhaps one of the most common problems faced by the South American ornamental fish industry. All South American exporting countries appear to have the same general procedure for the export of ornamental fish as that reported by Conroy (1975). However, it is difficult for the governments to provide adequate monitoring because:

1. The large number of exotic species exported.
2. The control of the species to be exported is based upon lists provided by the exporters with the name of the species, number of specimens and price of the consignments ready to be exported. Unfortunately, the species are often listed under their common names, which are often applied to more than one species of fish in the same genus, making it difficult to determine the real number of each species exported. For example, in Brazil 10 species of the genus

Corydoras are registered under the common name of "Corredora" (IBAMA's list, 1992); in Venezuela 7 species of the genus *Corydoras* are known as "Conchinita de rio" (Medina, 1986). In the present study, 4 species of the genus *Corydoras* sampled in the UK in shipments from Brazil, and 7 species sampled at the exporters' holding facilities in Brazil, do not have their name listed among the species it is permissible to export.

3. The list of the species of fish which can be legally exported published by the government is not updated regularly. Consequently, new species have been commercialized on the international market without the knowledge of the environmental control committees of the exporting countries. This is of particular importance in the Amazon basin because the ichthyofauna of the region is not well known and often species new to science and the industry have been offered in the international market (e.g. *"Corydoras correa", "Ancistrus gomesi",* "Ancistrus plane head"). It is not clear how species not listed by the environmental control committees of the exporting countries are receiving licenses for export.

4.5.2 Parasitic survey in the UK

The parasite fauna present on species of ornamental fish sampled in shipments imported from South America into the UK comprised mainly of ectoparasites (protozoans and monogeneans) and intermediate stages of the major groups of parasites which complete their life-cycle in piscivorous vertebrates (especially birds and crocodilians) other than fish (Tables 4.5, 4.7-4.10).

These findings in wild ornamental fish may provide, firstly, an indication of the conditions that these species of fish experienced prior to export, and secondly, an indication of the types of association which may be present in their natural environment.

Some differences appear to be present in the composition of the parasite fauna of the species of ornamental fish imported from the four South American countries, but the results obtained in this study were not quantitatively compared because:

1) The samples were not uniform (e.g., different sizes, different species);

2) All species of fish were submitted to a series of different prophylactic treatments prior to export, which may have influenced the results obtained.

4.5.2.1. Ectoparasite fauna

Several known pathogenic species of protozoans were observed on the skin, gills of the species of fish examined (Table 4.5), and many of these could be responsible for high mortalities before and after export.

The species, *Chilodonella hexasticha*, *Piscinoodinium* sp., *I. multifiliis* and *Trichodina* sp., found in this study, are common pests in fish aquaria which, under favourable conditions, are able to proliferate and account for significant losses. The problems caused by these species to the culture of fish has been described by several authors, and their infection of species of ornamental fish normally appears to be associated with lack of hygiene, stress,

debilitated fish, poor water quality, and overcrowding (Hoffman,Kazubskii, Mitchel and Smith,1979; Paperna and van As, 1983; Imai,Hatai and Ogawa,1985; Paperna and Ventura, 1985; Huchzermeyer, 1995).

Other species of protozoans found included three species of myxosporeans, *Henneguya* sp., *Myxobolus* sp. and *Myxidium sp.*, and one opalinid, *Protoopalina* sp. Myxosporean parasites are very common on teleost fish, and the cysts of one or two species were found dispersed in the skin, gills, kidney and other inner organs of 5 species of fish examined (Table 4.5). The source of infection of these myxosporeans species in these fish is not known. However as they were wild species, they may have acquired the infection by ingestion of the infected invertebrate host or exposure to the actinosporeans recently released.

Although the specimens of *Mylossoma aureum* presented heavy infection of myxosporeans in the kidney, no mortalities and no visible clinical signs were observed on the arrival of this fish. However, the period of survival of the infected specimens in the home aquaria may be limited, depending on the damage caused by the myxosporean in the kidney.

Future work involving importers, fish dealers and fish keepers of this species could be followed via survey form to be received with the fish from a specific shipment . They would be asked particularly for information on the survival period of the species and the possible causes of death. In parallel, some experimental infection could be developed under laboratory conditions involving infected wild species, tank bred species and oligochaetes commonly commercialized as live food for ornamental species to determine the possible

role of these oligochaetes on the life cycle of these species.

Protoopalina sp. was only found in the intestine of very debilitated specimens of Discus, *Symphysodon aequifasciata*, which arrived in one shipment from Peru. The specimens found may belong to the species *Protoopalina symphysodonis* Foissner, Schubert and Wilbert, 1979, described from the intestine of this species of cichlid. The pathogenicity of this protozoans is not well known. Only one report was found in the literature where this species was associated with mortalities of several specimens of Discus (Foissner *et al.*, 1979). According to these authors, "the infection has no influence on the light microscopical structure of the epithelium of the rectum, but most infected animal died". Untergasser (1989) reported that a moderate infection causes only minimal damage to discus and a more precise prognosis on the effect of this parasite is difficult to do. It appears that the pathogenicity of this parasite is associated with debilitating conditions caused by mixed infections with other protozoans such as *Spironucleus* and parasitic worms such as *Capillaria* sp. and stress.

Mortalities associated with infections by protozoans on arrival of the shipments were observed only in those shipments of fish infected with *Piscinoodinium* sp. from Colombia and *Chilodonella hexasticha* from Colombia, Brazil and Peru. Both protozoans were found infecting fish of different species which arrived in the U.K. in the same shipments but packed separately (Table 4.6), although few fish were actually dead on arrival.

Piscinoodinium sp. was only found infecting specimens of callichthyids (*Brochis splendens* and *Corydoras* spp.) whereas *Chilodonella hexasticha* was found infecting specimens of *Corydoras schwartzi*, *Paracheirodon axelrodi* and *P. innesi* (Table 4.5). All callichthyids infected by *Piscinoodinium* sp. exhibited similar clinical signs of which the most evident was that they swam weakly and gave no resistance during handling. No clinical signs were observed in the specimens of cardinal *P. axelrodi* and neon tetra *P. innesi*, infected by *Ch. hexasticha*, although a high mortality rate was observed in neon tetra a day after their arrival in the U.K. Details of the pathology caused by *Piscinoodinium* sp. on the skin of callichthyids are discussed in Chapter 6.

The presence of these species of protozoans infecting different species of fish in the same shipment suggest that the infections may have been acquired by:

1. The fishermen transporting different species of fish to the exporters' holding facilities in the same tank;
2. Use of the same nets to sort fish from different tanks by size and species without disinfection of the nets between tanks; or
3. Mixing specimens of fish from consignments obtained from different collection areas (which initially are maintained in separate tanks) to complete the number of the order to be exported.

Monogenea was the other group of ectoparasites commonly found on the fish species examined. In this study, they were mainly represented by species of *Gyrodactylus*, parasites of the body and fins, and Dactylogyridae, parasites of the

gills, collecting ducts, ureters and urinary bladder. (Table 4.7).

In general, the prevalence and intensity of infection of the external monogeneans was low, possibly reflecting the series of treatments used by the exporters prior to export. Unfortunately, very little information related to treatments prior to export was obtained by the British importers. However, it appears that some of the treatments applied by the some South American exporters are the same as those utilized 15-20, years ago such as salt, Malachite green, Methylene blue, Furazolidone and a cocktail of antibiotics.

In contrast, to the low intensity of infection presented by the external monogeneans, a moderate to high intensity of infection was observed among the internal monogenean parasites of the excretory system (Table 4.12). This may be related to the fact that some of the current drugs utilized for treatment of the external monogeneans are not efficacious against internal monogeneans.

Although no mortalities were associated with infection by monogeneans on arrival of the shipments of fish, these parasites play an important role as pathogens because they affect the skin, gills and other tissues and organs which are vital to the normal function of the fish.

Generally, infected gills are coated with excess mucus resulting in a reduced respiratory capacity. This is an important consideration for the ornamental fish industry because, in the long journeys to the international market, frequent delays in flight times contribute to an increased deterioration of the water quality. Consequently, heavy infections of these parasites may cause severe losses during

transport with those fish species most susceptible to oxygen deficiency being the first to perish.

4.5.2.2 Endoparasite fauna

Metacercariae were the most common parasites found in all species of fish examined with two representatives of the strigeids (mesocercariae or diplostumula of Strigeoidea and *Apatemon* sp.), one acanthostomid (*Acanthostomum* sp.), and three others species which could not be identified (Table 4.9).

As discussed in Chapter III, the ornamental fish industry in South America is very seasonal and is mainly concentrated on the low water period. During this time those species that do not migrate into the river become more susceptible to predation with the continuing drying up of the lakes. Large concentrations of piscivorous birds (final hosts for some digeneans and nematodes) are attracted to these areas due to the abundance of easily available food, and consequently, they will disperse the eggs of their parasites amongst the fish species enclosed in the lakes which will develop the intermediate stages if favourable conditions are present.

In this study, the large majority of the intermediate stages were found on species of callichthyids (*Corydoras*, *Brochis* and *Dianema*). These fish are mainly caught during the low water period and are well adapted to the low oxygen conditions present in the water during the dry season. (Krammer and Braun, 1983; Krammer and McLure, 1981; 1983).

Generally, the presence of intermediate stages of parasites in imported ornamental fish is discovered only by necropsy, because all fish presenting observable abnormalities are removed from the stock prior to export, unless the cysts are not visible or are very deep in the muscles and do not cause devaluation of the product (Fig.4.5.1 and 4.5.3).

Mesocercariae or diplostumulas of Strigeoidea and metacercariae of *Apatemon* sp. were the most abundant stage in terms of the number of species and specimens per fish infected. Mesocercariae do not become encysted and are less well developed than metacercariae (Thatcher, 1991).

In this study, all mesocercariae of Strigeoidea found were not encysted but were encapsulated by a thin connective tissue host cyst. They were commonly observed as a white bladder-like sac cyst in the mesentery, viscera and muscles of the head, containing between 1-15 mesocercariae (Fig. 4.2.a). The parasites and the type of host cysts were very similar to those described by Schaperclaüs (1992) as syncysts, from the brain of rudd, *Scardinius erythrophthalmus* (Linnaeus, 1758) but they differed from syncysts in that they were not formed by several individual cysts combined in clusters.

Several individual cysts grouped together were observed containing a metacercaria of *Apatemon* spp., but in all cases the host and parasite cyst of *Apatemon* could be very well differentiated and they were only found in the mesentery. The metacercariae of *Apatemon* were also commonly found encysted in the mesentery and enclosed by the same host cysts with the mesocercariae (Fig 4.2.b). Although not all the cysts of

Apatemon were counted due to the difficulty in visualising those enclosed by the host cysts, it appears that the intensity of infection of this metacercaria was low.

Amongst the nematodes, the larvae of *Contracaecum* spp. encysted in the mesentery and L_3 of camallanids free in the intestinal lumen were the most commonly found larval stages on the species of ornamental fish sampled (Table 4.10). However, the prevalence and intensity of infection was low (Table 4.14). Amazon fish normally present numerous larvae of nematodes encysted in the mesentery. Some of these represent species of *Contracaecum, Multicaecum, Terranova, Dujardinascaris* and *Eustrongylides* that mature in piscivorous vertebrates (Thatcher, 1991).

Other intermediate stages found in this study were represented by scolex pleuronectis free in the intestinal lumen, plerocercoids encysted in the mesentery and the nymph of Pentastomids encysted on the swim bladder (Tables 4.8 and 4.10). Travassos *et al.*, (1928) called the pentastomid larva from the fish "nymph" and identified them as *Porocephalus gracile* (Diesing, 1836). The genus *Porocephalus*, as presently defined by specialists, occurs as an adult in the lungs of snakes and as a nymph in the organs of mammals. *Porocephalus* nymphs probably do not occur in fish. The pentastomid nymphs from fish are all thought to represent species of *Sebekia* and it has been reported in several species of fish from South America and Central America, Antillas and Mexico (Thatcher, 1991; Olson and Cosgrove, 1982; Pineda-Lopez, 1991).

No significant pathology was found associated with the intermediate stages of digeneans, cestodes and nematodes encysted in the species of ornamental fish examined. High

mortalities may only be associated with heavily infected fish, mainly when they are exposed to large numbers of infective stages simultaneously due to the damage caused during the migration of the infective larvae to the target organs. In this regard Leibovitz (1981a) reported that "certain species of aquarium fish, such as *Corydoras* spp., are often heavily infected at the time of purchase and are subject to significant mortalities following introduction into the aquarium". However, no studies have been reported concerning the longer-term survival of wild freshwater fish infested with any intermediate or adult stages in domestic aquaria.

Few adult stages of digeneans were found in this study (Tables 4.13 and 4.14), and they were mainly represented by specimens of the families Gorgoderidae, *Phyllodistomum* sp., Derogenidae, *Genarchella* spp., and Paramphistomidae, *Dadayus* sp., *Dadaytrema* sp. and one adult paramphistomid of *Myleus* not identified.

All specimens of Derogenidae found in our study were tentatively placed in the genus *Genarchella* Travassos, Artigas and Pereira, 1928, based on the characters of the genus described by Kohn,Gibson and Froes,(1990). Currently, two species were reported by Kohn *et al.*, (1990) parasitizing South American freshwater fish species, *G. genarchella* Travassos *et al.*, 1928 from *Salminus maxillosus* Valenciennes, 1894 and *G. parva* Travassos, Artigas and Pereira, 1928 from *Astyanax fasciatus fasciatus* Cuvier and *Salminus maxillosus* Valenciennes. *Corydoras* species were not reported previously as hosts for these digeneans, therefore, it will be necessary to study the genitalia of these specimens in detail to

separate the individual species and confirm if they belong to one of the species described or not.

Three species of paramphistomids appeared to be occurring amongst the species examined and *Dadayus* sp. and *Dadaytrema* sp. were found in mixed infections in the intestine of *M. hypsauchen*. Paramphistomids are common findings in the intestine of South American "pacus", (Travassos, Freitas and Kohn, 1969; Ostrowsky de Nuñez, 1979; Hamann, 1982; Thatcher, 1991) however no pathology has been described associated with these parasites.

Amongst the nematodes, *Spirocamallanus* species were the most common finding. They were found in the intestine of five species and the species *S. inopinatus* Travassos, Artigas and Pereira, 1928 was found in one species of callichthyd *C. julii* from Brazil and two species of serrasalmids, *C. macropomum* and *M. hypsauchen* from Colombia (Table 4.10). This species has been recorded from a wide range of hosts from several South American countries (see chapter 7, tables 7.1-7.2) but not from Colombia (Travassos *et al.* 1928; Pinto and Noronha, 1972; Petter and Dloughy, 1985; Thatcher, 1991).

Generally, the specimens of *Spirocamallanus* found in this study were immature stages (mainly non-gravid females). This suggests that the infections were acquired in their natural environment only shortly before capture or at the exporters' holding facilities during the stocking period.

In general, the camallanids are one of the few nematodes recognised by aquarists because of their size and the reddish colour characteristic of the gravid females. Although some species attach to, or bury their heads in, the mucosa of the intestine, there are only a few records in the literature

relating to their pathogenicity (Petter *et al.*, 1974; Ferraz and Thatcher, 1990). Thatcher (1991) reported that there is always a localised inflammatory reaction at the attachment site and that the worms can probably cause primary anaemia from blood loss.

Regarding the species found in this study, *S. inopinatus* may be considered more pathogenic for small fish, causing intestinal blockage due to the large size that the adults can reach. Males were reported to reach between 5-8.7mm and females 16-28mm (Travassos et al., 1928;Petter and Thatcher,1988). In specimens of *C. julii* the species was found with the anterior extremity attached on the inside of the pyloric caeca which may cause perforation.

Oxyurids were represented by three species, *Rondonia rondoni* Travassos, 1919, *Spectatus spectatus* Travassos, 1923 and *Icthyouris* sp. (Table 4.14).*Rondonia rondoni* is commonly found in the intestine of wild pacus, Serrasalmidae, forming large infrapopulations which can be observed as a large white mass which sometimes completely fills the intestine. Infestations of up to 120,000 nematodes per fish have been reported (Moravec, Kohn and Fernandes, 1992, Hamann, 1982; Kohn, Fernandes, Macedo and Abramson, 1985). Generally, adult and fourth stage larvae are found in the intestine of the infected fish, because the females are live-bearing and give birth to fourth stage larvae or sub-adults inside the intestine. Eggs are not found in these nematodes and the embryos and larval stages obtain nourishment from the uterine wall (Thatcher, 1991).The life cycle appears to be direct and no intermediate hosts are required. The low prevalence and moderate intensity of infection found in our studies may be associated with the

small size of the specimens of *Myleus rubripinnis* (5.5 - 6.0 cm) and *Metynnis hypsauchen* (4.5-5.0 cm) examined.

No pathology associated with these nematodes was observed in the present study, and to my knowledge there are no reports in the literature relating to this topic, even for those species of fish presenting a high intensity of infection with *R. rondoni*. As they do not attach to the wall of the intestine the relationship of these nematodes to their hosts seems to be of a more commensal nature (Thatcher, 1991), although studies are required to assess the effect of the large burden of *R. rondoni* on the growth rate of fish. For the wild ornamental fish industry, oxyurids may play an important restricting factor for the import of "wild pacus". Generally, prior to export, some importers have been suggesting the application of anti-helminthic drugs for treatment against these nematodes.

Other species of nematodes found in this study were represented by three specimens of *Philometra* sp., and one specimen of *Spinitectus* sp., which possesses cuticular spines and may cause severe damage to the intestinal mucosa.

The results of this study suggest that the protozoans were the most pathogenic group of parasites present. However, some pathogenic species were difficult to find because the intensity of infection was very low (e.g. *Trichodina* sp. and *I. multifiliis)*. Consequently, the number of species which are potentially pathogenic may be higher than presented here.

Prior to export, ornamental fish, both wild and tank-bred, are exposed to a series of treatments which remove or greatly reduce the level of infection of many ectoparasites.

As evaluation of the treatments used does not seem to be routine, it appears that species of ornamental fish are often exported while infested with potentially harmful species of parasites at a low intensity of infection. Consequently, following importation, when conditions predisposing to outbreaks are present, these parasites will multiply intensively causing losses for the importers and hobbyists.

The endoparasites, mainly digeneans, cestodes and acanthocephalans, do not appear to present a problem for the wild ornamental fish held in captivity, because they tend to disappear due to the interruption of the life cycle. This may also be true for the nematodes, although it is recommended that this group of parasites be kept under observation. Some species may be able to complete their life-cycle either because they do not need an intermediate host (e.g. oxyurids) or because their intermediate host is often introduced into the aquaria as live food.

4.5.3 Parasitic survey in Brazil

The parasite fauna present in the species of ornamental fish sampled at the exporters' holding facilities in Brazil was very similar to that described for the species of ornamental fish sampled in the U.K. Nevertheless, it appears that some species of parasites may have been acquired, or had their infrapopulation increased, during the period spent at the exporters' holding facilities.

No protozoans were found in the routine scrapes of skin and gills of callichthyids from the incoming consignments

(Table 4.18). In part, this finding may reflect the results of the treatments that the species of fish were exposed to after capture. Generally, when the fish are transferred from the floating cages to the plastic tanks they are kept permanently in a bath of antibiotic, acriflavine and salt which, if used correctly, is considered one of the possible combinations for the treatment of external protozoans and bacterial infections (Richards, 1977c; Gratzek, 1980; etc).

Generally, protozoan infections were found at low intensity on stocked fish and on shipments ready to be exported.

High intensity of infection was mainly observed in fish that did not recover fast from the stressful conditions caused by handling and transportation (e.g. Discus) or fish kept in tanks with poor water quality, without aeration and with a high content of organic matter (e.g. *Corydoras hastatus*) (Table 4.18). In this survey this condition was mainly caused by sessiline peritrichs, Epistylididae, outbreaks of which have been reported in the literature especially in reservoirs with high level of organic matter, thermal pollution and in combination with the presence of bacterial pathogens, such as *Aeromonas hydrophila* (Lom and Dyková, 1992). Unfortunately, some of these conditions were commonly observed at exporters' holding facilities in Brazil and Venezuela, and the losses caused by these protozoans may be higher than suspected.

Piscinoodinium sp., *Chilodonella hexasticha* and *I. multifiliis* were the most common species of protozoans found, and generally, *I. multifiliis* is the only protozoan recognized by the exporters due to the readily visible white

spots. This protozoan appears to be present at low intensity of infection in the two exporters' holding facilities examined and it appears to be responsible for severe losses mainly during the period of the "friagens" (see chapter 3).

Piscinoodinium sp. was seen to be one of the most common protozoans affecting stocked callichthyids at the exporters' holding facilities. This protozoan is mainly recognized through scrapes of the skin and gills. No clinical signs were commonly observed other than the dusty colour which becomes visible only when large number of trophonts are already attached the skin.

Species of *Corydoras* and *Brochis* are bottom feeders. Typically, they alternate periods of inactivity, in which they rest on the substrate, with periods of foraging, in which they move slowly over the substrate. Periodically, they can be observed to move up towards the surface of the water, taking air, and quickly returning to the bottom (Gee and Graham, 1978; Krammer and McLure, 1980; Burguess, 1989). Although infected callichthyids generally become very lethargic at the bottom of the ponds, this behaviour is not considered to be a reliable indicator of illness because of their aforementioned natural tendency to rest on the substrate.

Infections by protozoans may be easily acquired at the exporters' holding facilities because:

1. During the open season, large numbers of fish are arriving and the tanks, nets, pipes etc. may not be disinfected properly;
2. Fish are often held in overcrowded conditions and;
3. Protozoans primarily affect fish kept under less than optimal rearing conditions, which have become generally

weakened or subjected to additional stressors such as handling, which have further reduced their ability to resist pathogenic organisms (Wedemeyer, 1974; Pickering, 1993). Unfortunately, wild ornamental fish are subjected to a series of stress factors from their capture until their arrival at the final destination.

Monogeneans of the genus *Gyrodactylus* were commonly found in the incoming consignments of five species. In general, the intensity of infection was not high, but severe infection appears to be associated with the dry season (Brazilian Exporter - personal communication). A severe infection was only observed in one species, *Corydoras hastatus*, but it is not clear whether the fish were severely infected when they arrived or if the intensity of infection increased during the holding period. Infested fish were observed clustered in the corners of the tanks near the air stone and/or swimming at the surface of the water.

Generally, the collaborating exporters of this study do not conduct routine examinations on incoming shipments and all fish on their arrival are treated with a cocktail of drugs, which normally does not affect the monogeneans present and may lead to an increase in resistance to the drugs used. Monogenoidosis is more prevalent in conditions of artificial fish culture than in natural waters (Bauer, 1988), and the density of the fish plays a very important role in the increase in size of the infrapopulation of these parasites.

Amongst the endoparasites, larval stages of digeneans and nematodes of the family Camallanidae were the most common finding in the species of fish examined in Brazil. Metacercariae of strigeids were the most prevalent group of

parasite and their pathogenicity appears to be related to the localisation of the cysts. Infected callichthyids presented high concentrations of whitish cysts with a thin capsule in the mesentery. Free mesocercariae were also observed inside the lens of one specimen of *Corydoras punctatus* and inside the oocytes of four specimens of *Corydoras maculifer*, which was the only species examined which exhibited a developed ovary.

Metacercariae of *Clinostomum* sp. were not found in fish imported from Brazil but were found in the species examined in Brazil. These metacercariae are large and present a yellow coloration and are commonly called " yellow grubs" (Fig.4.4a) Generally, heavily infected fish are not exported but sometimes the metacercariae are deeply embedded in the musculature and hence are not easily detectable during the inspection prior to export.

In specimens of *Corydoras*, all *Clinostomum* sp. were localised in the eye lid (Fig.4.4.b) and not deeply embedded in the musculature as was commonly observed in the specimens of *Mylossoma aureum* (Fig.4.4a). This difference may be associated with the presence of scutes on the dermis of the species of *Corydoras* which may limit the penetration of the cercariae. Scutes are plates localised in the dermis which are more mineralised than bone. The body of the adult *Corydoras* is covered by two rows of thick scutes, dorsal and ventral, that overlap antero-posteriorly within both rows and also partially between rows at the level of the horizontal septum (Sire, 1993) . The pathogenicity caused by the metacercariae of *Clinostomum* sp. on *Mylossoma aureum* is discussed in Chapter 6.

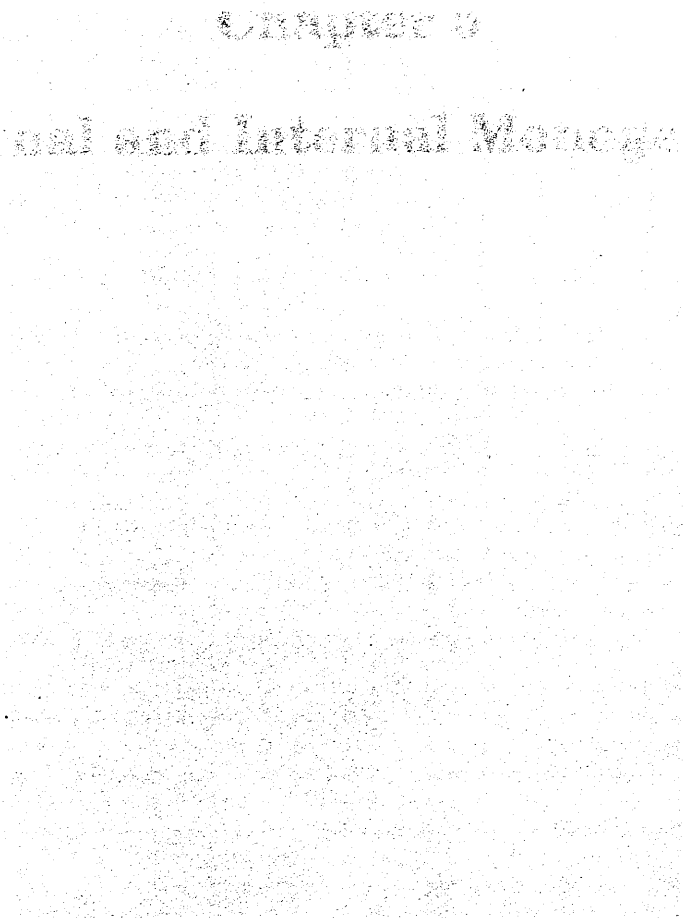
Other types of metacercariae found were those responsible for the "black spot diseases", which in this survey were caused by specimens of the family Acanthostomidae. The family Acanthostomidae and Heterophyidae were reported by Thatcher (1991) as responsible for the black spot diseases in the tropics. The black colour is caused by the presence of melanophores surrounding the encysted metacercariae which do not appear to cause problems for the export of the fish (Fig.4.4c). Gratzek (1988) stated that " the black spots are not particularly offensive and many mistake them for the normal colouring or genetic variation".

Three species of camallanids appeared to be present in the ornamental fish examined and the possibility of these parasites also being acquired at the exporters' holding facilities cannot be discounted. Generally, species of callichthyids were kept in earth ponds which were supplied with unfiltered water pumped directly from nearby creeks. It is highly probable that free copepods, the intermediate hosts, were able to enter the ponds with the unfiltered water (See Chapter 7).

In conclusion, the results of this study show that parasitic diseases are one of the major problems currently faced by the exporters of wild ornamental fish from South America. This was clearly demonstrated by the results of the analyses of fish sampled in the UK and information obtained from the exporters regarding the most common diseases present, their treatment procedures, and the results of the analyses of fish sampled in Brazil.

Wild fish would, in general, be expected to present a low level of parasitic infection which they acquired in their

natural environment. However, the majority of the parasitic diseases found in this survey with the potential to cause severe losses in the different stages before and after export, appear to be those associated with a lack of husbandry skills (e.g. sanitation, water quality control) and debilitating conditions.



Chapter 5

External and Internal Monogeneans

5.1 Introduction

Several reports on monogenean parasites of freshwater fish from the neotropical region have been published in the last decade (Harris, 1983; Kritsky, Boeger and Thatcher, 1985; 1986; 1988; Boeger and Kritsky, 1988; Jara and Cone, 1989; An,Jara and Cone, 1991; Kritsky and Boeger, 1991). However, the fauna of gyrodactylids and those dactylogyrids occurring in natural cavities of the body and internal organs, such as the nasal cavity and urinary bladder, are practically unknown. Only recently, have greater numbers of species started to be described (Kritsky *et al.*, 1988; Jara and Cone, 1989; Kohn, 1990; An *et al.*, 1991; Boeger,Kristsky and Belmont-Jegu, 1994; Ferraz and Thatcher, 1994; Kritsky, van Every and Boeger, in press).

Currently, 9 genera of gyrodactylid parasites of skin and gills are known from South America. These are: *Gyrodactylus* Nordmann, 1832, *Anacanthocotyle* Kritsky and Fritts, 1970, *Paragyrodactyloides* Szidat, 1973, *Phanerothecium* Kritsky and Thatcher, 1977, *Ooegyrodactylus* Harris, 1983, *Acessorius* Jara and Cone, 1989, *Scleroductus* Jara and Cone, 1989, *Nothogyrodactylus* Kritsky and Boeger, 1991, and *Hyperopletes* Boeger *et al.*, 1994.

From the natural cavities and internal organs of neotropical freshwater fish 4 genera of dactylogyrids are known. These are: *Rhynoxenus* Kritsky *et al.*, 1988 (with 4 species), *Rhinonastes* Kritsky *et al.*, 1988 (monotypic) and *Telethecium* Kritsky *et al.*, in press (with 2 species), all parasites of the nasal cavity, and *Kritskyia* Kohn, 1990 (monotypic), parasites of the excretory system.

5.1.1 Gyrodactylid parasites of skin and gills of South American freshwater fish.

Among the 18 genera described for the family Gyrodactylidae van Beneden and Hesse, 1863 (see *inter alia* Spencer-Jones and Gibson, 1990), only 4 genera and 10 species are known in the neotropics.

The first report of gyrodactylids from neotropical freshwater fish was by Kritsky and Fritts (1970), from Costa Rica, Central America. Prior to the late 1980's only 3 further descriptions were published, Szidat, (1973), Kritsky and Thatcher (1977) and Harris (1983).

The large majority of the known species of the genus *Gyrodactylus* Nordmann, 1832 from South America were described from freshwater fish from Peru and these are: *Gyrodactylus bimaculatus* An, Jara and Cone, 1991, *G. slendrus* An, Jara and Cone, 1991, *G. lebiasinus* An, Jara and Cone, 1991, and *Acessorius peruensis* Jara, An and Cone, 1991, all from *Lebiasina bimaculata* Cuvier and Valenciennes, Characidae; *G. pimelodellus* An, Jara and Cone, 1991 and *Scleroductus yuncensis* Jara and Cone, 1989 from *Pimelodella yuncensis* Steindachner, Pimelodidae; and *G. turnbulli* Harris, 1986 from *Poecilia reticulata* Peters, Poeciliidae. *Gyrodactylus turnbulli* has been recorded once from feral guppies in Peru (An *et al.*, 1991) but in this case, the fish had been introduced from elsewhere, as the natural range of the guppy is restricted to the Caribbean basin of northeastern South America and the Lesser Antilles (Jacobs, 1971,in Harris and Lyles, 1992). For marine fish, the only available literature

was the record of *Gyrodactylus* sp. by Jara (1986) in *Mugil cephalus* Linnaeus, 1758 from Peru.

From Venezuela two species are known: *G. gemini* Ferraz, Shinn and Sommerville, 1994, a parasite of *Semaprochilodus taeniurus* (Steindachner) and *Gyrodactylus curemae* Conroy and Conroy, 1985 of *Mugil curemae* Valenciennes, 1836, Mugilidae.

Gyrodactylus gemini was described based on material collected from an ornamental fish imported into the UK from Venezuela. However, the host, *S. taeniurus* as well as other species of the genus *Semaprochilodus* occur in the Amazon region which is shared by Venezuela, Brazil, Peru and Colombia (Saint-Paul, 1986).

Paragyrodactyloides superbus (Szidat, 1973) Ostrawski de Nuñez, 1975 appears to be the only species described in fish from Argentina. This gyrodactylid was described in an ornamental fish, *Corydoras paleatus* (Jennys), Callichthyidae by Szidat (1973), who believed it to be responsible for mortalities of specimens of *C. paleatus* kept in captivity in a public aquarium.

For Central America, Thatcher (1991) reported the occurrence of 2 genera and 4 species: *Gyrodactylus bullatarudis* Turnbull, 1956 and *G. costaricensis* Kritsky and Fritts, 1970 from *Poecilia sphenops* Valenciennes, Poeciliidae; *G. neotropicalis* Kritsky and Fritts, 1970 and *Anacanthocotyle anacanthocotyle* Kritsky and Fritts, 1970, from *Astyanax fasciatus* (Cüvier).

Other species of the superfamily Gyrodactyloidea described from Neotropical freshwater fish belong to the family Ooegyrodactylidae Harris, 1983. Four genera and 8 species are known all of which were described in freshwater

fish from South America. The species, hosts and the type-locality are summarised in the Table 5.1.

Table 5.1

Species of Gyrodactyloidea of the family Ooegyrodactylidae Harris, 1983 described from freshwater fish of the Neotropics.

Family/Species of Parasites	Family/Fish Species	Origin/Country
Ooegyrodactylidae	**Loricariidae**	
Ooegyrodactylus farlowellae Harris, 1983	*Farlowella amazonum* (Günther,1864)	Peru
Nothogyrodactylus clavatus Kritsky and Boeger, 1991	*Ancistrus* sp.	Brazil
N. amazonicus Kritsky and Boeger, 1991	*Ancistrus* sp.	Brazil
N. plaesiophallus Kritsky and Boeger, 1991	*Ancistrus* sp.	Brazil
Phanerothecium caballeroi Kritsky and Thatcher, 1977	*Cephalosilurus zungaro* (Humbollt)	Colombia
P. harrisi Kritsky and Boeger, 1991	*Plecostomus plecostomus* (Linnaeus)	Brazil
P. spinatus Boeger *et al.*, 1994	*Hypostomus punctatus* (Valenciennes)	Brazil
Hyperopletes malmbergi Boeger *et al.*, 1994	*Rhineloricaria* sp.	Brazil

5.1.2 Monogenean parasites of the urinary bladder

In contrast to the gyrodactylids, of which there are over 400 species known around the world (Harris, 1993), there are few species of monogeneans which are known to parasitise the excretory system (urinary bladder, ureter and collecting ducts) of fish. This situation may reflect an under estimation of this system as a possible site of infection for monogeneans or may be related to the difficulties in removing it during routine examinations, especially in small fish.

Currently, only 2 genera are known to parasitise the urinary bladder of fish: *Acolpenteron* Fischthal and Allison, 1940 (Calceostomatidae) and *Kritskyia* Kohn, 1990 (Dactylogyridae).

The genus *Acolpenteron* has been described from several species of fish in the United States, South Africa and Soviet Union and five species are known: *A. ureteroecetes* Fischthal and Allison, 1940, *A. catostomi* Fischthal and Allison, 1942; *A. ignotum* Gussev, 1955; *A. nephriticum* Gvozdev, 1945 and *A. pavlowskii* Bychowsky and Gussev, 1955.

For the genus *Kritskyia* only one species is known, *K. moraveci* Kohn, 1990, which parasitises the urinary bladder of the South American freshwater catfish *Rhamdia quelen* (Quoy and Gaimard, 1824). The genus was recently reviewed and the species *K. moraveci* re-described based on three voucher specimens (Kritsky *et al.*, in press).

Unfortunately, little is known of the biology and pathogenicity of these monogeneans. The only paper found in the literature regarding this matter, was published as long ago as 1948 by du Plessis where the author presented a summarised description of the life-cycle and discussed the pathology caused by *A. ureterocoetes* in the kidney and ureters of fingerlings of largemouth bass, *Micropterus salmoides* (Lacépède) at the Jonkershoek Inland Fish Hatchery, Stellenbosch, South Africa.

It appears from du Plessis's work that the monogenean parasites of the urinary bladder follow the same development pattern in their life-cycle as ectoparasitic dactylogyrids, where the oncomiracidium, after having been liberated from the egg, is ready to attach to the same or a different host.

However, as du Plessis (1948) found the oncomiracidium inside the urinary bladder, he suggested that the cycle may be completed within of the urinary bladder.

For the intensive culture and/or import of ornamental fish these internal monogeneans may represent a serious problem because they can be easily introduced to the fish farm facilities with the introduction of wild or tank-bred infected broodstock without detection, due to their localisation in internal organs. In this respect Hoffman (1970) reported that the species *A. ureterocoetes* was introduced into South Africa with *M. salmoides* imported from the United States.

In the survey conducted in the present study it was noted that the species of fish of the families Callichthyidae, Loricariidae, and Serrasalmidae were commonly carrying light infections of gyrodactylid and dactylogyrid monogeneans. Many of these species of fish are very popular ornamental species (e.g. *Corydoras* spp.) which have been widely disseminated throughout the world together with their parasite fauna. More recently breeding populations have been established in centres of the ornamental fish industry such as Singapore and Florida, where they may be susceptible to the indigenous parasite fauna. Few studies have been conducted on their monogenean fauna and it is important to determine the range of parasite species exported/imported. This study was conducted in two stages involving collection of material from samples of ornamental fish species imported from South America into the UK and samples from tropical fish at the exporters' holding facilities in Brazil.

5.2 Study aims

The main aim of the study carried out in the UK was:

To identify the species of Monogenea which have been commonly entering the UK with species of ornamental fish of the families Callichthyidae, Loricariidae and Serrasalmidae, imported from South America.

The main aims of the study carried out in Brazil were:

1. To identify the species of Monogenea infecting the fish of the family Callichthyidae, sampled at the exporters' holding facilities.

2. To determine the stages of the process prior to export that would contribute to an increase in the intensity of infection or transmission of these parasites.

5.3. Materials and Methods

5.3.1 Collection of the specimens

The general post-mortem techniques utilised in this study were described in Chapter 2, Section 2.1.2.

The specimens of monogeneans from the skin were collected from skin scrapes of the body, fins and gills. The parasites of the urinary bladder and collecting ducts were collected after removal and washing of the kidney and urinary bladder. Specimens were examined either fresh, mounted in 0.97% buffered saline under light cover glass pressure or fixed in 70% alcohol. Those specimens which were fixed in 70% alcohol were prepared for study according to the procedures of Malmberg (1970) and Shinn *et al.*, (1993) .

5.3.2 Preparation of the specimens

5.3.2.1. Processing

For general purposes, the specimens of *Gyrodactylus* (Gyrodactylidae) and *Kritskyia* (Dactylogyridae) were directly mounted in a drop of ammonium picrate-glycerine and prepared according to Malmberg (1970) covered with a cover slip, and sealed with Pertex.

Some specimens were also stained in Mayer's Carmalum, dehydrated gradually by serial passage through alcohol, cleared in Beechwood creosote, and mounted directly in Canada Balsam (Gibson, 1984).

For the study of the excretory system, fresh specimens were used. They were each placed in a drop of water and covered with a cover slip for subsequent studies.

5.3.3 Scanning Electron Microscopy

5.3.3.1 Digestion technique

The digestion technique followed was that described by Shinn *et al.*, (1993). Fixed specimens were washed twice, centrifuged at 5,000 rpm for 5 min and the supernatant decanted. The pellet was resuspended in the digestion fluid and kept at 37 °C for 15 to 30 days. Every 5 days, the material was re-washed with distilled water and replaced with fresh digestion fluid. Subsequently, the specimens were processed for the "post-sclerite release procedures" in preparation for scanning electron microscopy.

5.3.3.2 Processing

The digestion fluid was washed with distilled water and centrifuged (Shinn *et al.*, 1993). Following centrifugation one or two drops of the concentrate were placed on the top of each of 20 to 30 round cover slips of 13 mm diameter and left to dry inside a petri dish.

When dried, each cover slip was scanned microscopically under phase contrast microscope and the structures present (marginal hooks, hamuli, cirrus and accessory piece) were marked with an adhesive paper arrow to facilitate their

location during scanning with the electron microscope (Shinn, 1993).

The cover slips were then mounted on aluminium stubs using Araldite and left to dry for 24 hours. Dried stubs were coated with gold palladium in an Edwards S-150 sputter coater. A Philips ISI-60A scanning electron microscope was used to examine the samples.

Specimens of gyrodactylids which were found on the cover slips to be only partially digested, i.e. the sclerites were not completely separated, were mounted face down in Malmberg's solution and sealed with Pertex.

5.3.4 Description of the specimens, drawings and photographs

All measurements are in micrometres (μm) and in the descriptions the range is followed by the mean in parentheses.

Measurements were made on specimens fixed in 70% alcohol under light cover glass pressure. In the descriptions, the abbreviations before the prevalence indicate:

UK-Br: prevalence of the parasite in the species of fish examined in the UK following import from Brazil.

UK-C: prevalence of the parasite in the species of fish examined in the UK following import from Colombia.

UK-P: prevalence of the parasite in the species of fish examined in the UK following import from Peru.

Br: prevalence of the parasite in the species of fish species examined in Brazil.

In the tables the range is followed by the mean and the number of specimens measured in parentheses. The prevalence

has been given as a percentage. The number of infected specimens/ number of specimens of fish examined are provided, in parentheses. The mean intensity of infection is followed by the range in parentheses.

The methodology for measurements of the angle of the hamuli was the same as that utilised by Shinn (1993) where micrographs of the hamuli were analyzed and processed by means of a Kontron image analysis package (Vidas 2.1) running on an IMCO 10 80386DX computer. A schematic illustration of the procedure for morphometric measurements of the haptoral sclerites using scanning electron microscopy and light microscopy are presented in Fig. 5.1.

The terminology utilised for description of the species of *Gyrodactylus* in this study was that described by Malmberg (1970). The haptoral terminology for Dactylogyridae was that described by Mizelle, Kritsky and Crane, (1968).

The techniques for drawing and photomicrography of the specimens in permanent slides were described in Section 2.1.6.

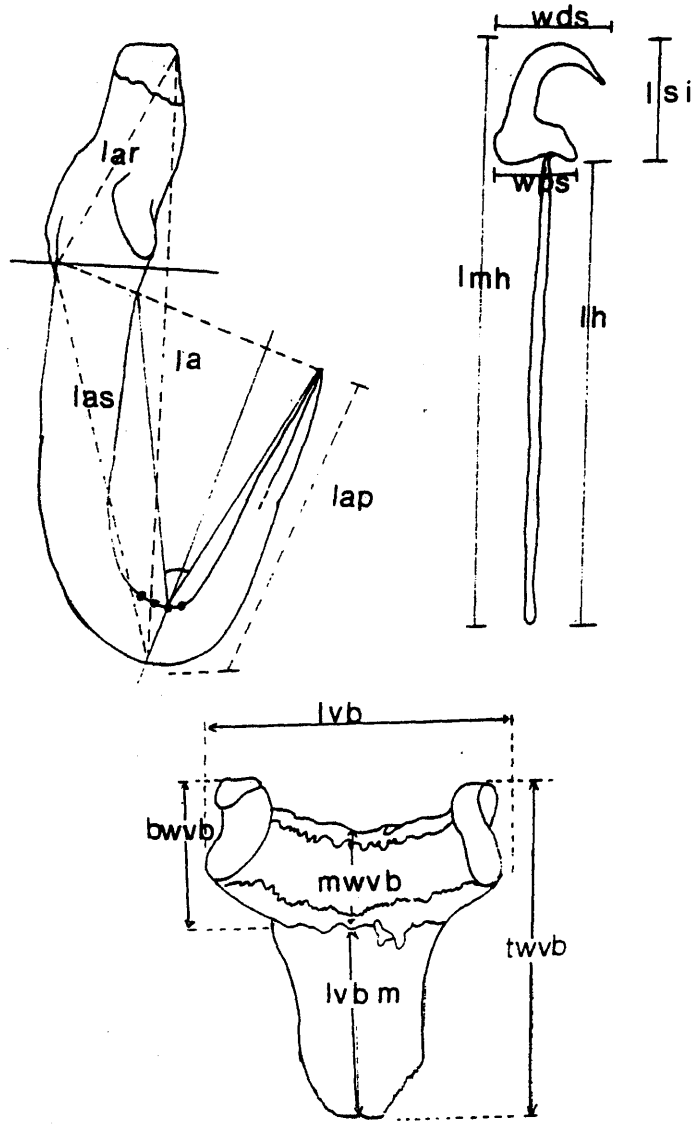


Fig. 5.1. Schematic illustration of the morphometric measurements of the haptoral sclerites. Source: Drawings of hamuli and marginal hook from Shinn (1993). lar = length anchor root; las= length shaft; la= total length anchor; lap= length point; lvb= total length ventral bar; twvb= total width; bwvb= basal width ventral bar; mwvb= median width ventral bar; lvbm= length ventral bar menbrane; lmh= total lenght hook; lsi= length sickle; lh= length handle; wds= width sickle distally; wps= width sickle proximally.

5.4 Results

Monogeneans were common findings in the South American ornamental fish species examined in the UK and in Brazil.

In the survey conducted in the UK, although their prevalence and intensity of infection were not high, 5 species of *Gyrodactylus* parasites of skin and fins and 4 species of dactylogyrids were found parasitizing the gills and urinary bladder of the fish species examined.

Amongst the gyrodactylids, one species was already published, *G. gemini* sp.n. (Ferraz, Shinn and Sommerville, 1994) and a species parasitizing *Callichthys callichthys* has not been completely identified because it presents some structural complexities in the haptor which require further study beyond the scope of this work. For this reason, its taxonomic description is not presented.

Although 5 dactylogyrids were found, only the species parasitic in the urinary bladder was identified. The other species were identified only to family level as too few specimens were available for study. All monogeneans found in the samples examined in the UK are shown in the Chapter 4, Table 4.7 and 4.12.

Ten species of gyrodactylids were found in the callychtids examined in Brazil (see Chapter 4, Table 4.16 and 4.21), which 7 species were identified. Out of the 7, 2 species parasites of *Corydoras robinae* and *Brochis splendens*, were also found in the samples analyzed in the UK.

Gyrodactylus mixed-infections were found in 3 species of callichthyids sampled, *C. hastatus*, *C. sterbai* and *B. splendens*. The gyrodactylids from *C. hastatus* and one species

from *C. sterbai* were separated and they are listed in the tables 4.16 and 4.21, however, their taxonomic descriptions are not presented in this study because they require further analysis in order to confidently separate the species.

The fauna of gyrodactylids from South America is poorly studied, and it appear that some of the specimens of gyrodactylids identified in this survey may constitute new species for the genus. For the purpose of this chapter, each species of monogenean separated was identified by number and their taxonomic description is presented in numerical order. Those missing in the series, correspond to those species for which the taxonomic description is not included.

5.4.1 Monogeneans parasites of skin and gills

The gyrodactylid species were classified according to the system of Malmberg (1970), which sub-divided the genus *Gyrodactylus* into subgenera and species group. The taxonomic description of eight species of gyrodactylids are presented.

Family Gyrodactylidae Cobbold, 1832

Gyrodactylus Nordmann, 1832

Generic diagnosis: Body elongated. Two terminal cephalic lobes present (head organs). Postero-lateral glands present or absent. Pharynx with short or long pharyngeal processes. Gut bifurcate into two unbranched caeca. Opisthaptor sub-ovate, with 16 marginal hooks, 2 ventral hamuli linked by ventral and dorsal bars. Cirrus armed with small spines in one or more arched row. Testis median. Genital pore

submedian. Ovary median, post-testicular. Uterus contains embryo with next generation inside. Parasites of body surface and gills of freshwater and marine fish, cephalopods, crustaceans and amphibians.

Gyrodactylus sp[1]

(Figs. 5.2a-e; Table 5.2)

Host: *Otocinclus* sp.; Loricariidae.
Site of infection: Surface of body and fins
Type-locality: Brazil (Br) and Colombia (C), Amazon Region.
Prevalence: UK - Br = 7% (4/60)
UK - C = 25.5% (13/51)

Description (based on 23 specimens): Body 296-416 (348) long by 66-110 (89) wide. Pharynx 34-37 (35.5) long by 20-34 (27) wide, with small pharyngeal process. Cirrus ventral, 7-9 (8.5) in diameter, armed with 4 - 5 small spines in one arched row (Fig.5.2c). Excretory system with small excretory bladders present. Hamuli 51.5-66 (58) long with a small elevation localized near the point marking the junction between the regions of shaft and point. This elevation is observed in both hamuli (Fig. 5.2a). Angle shaft/point 49.74°. Dorsal bar 6-20 (12.5) long by 1-2 (1.5) wide, without the central notch (Fig. 5.2a). Ventral bar ridged longitudinally, 18-21 (19) long by 20-30 (23) wide (Fig. 5.2b). Ventral bar process small but prominent (Fig. 5.2b). Marginal hooks 26-38 (33) long (Fig. 5.2d-e). The measurements of the sclerites according to Malmberg (1970)

are given in the Table 5.2. Testis not observed. Uterus with one or two generations at different stages of development.

Remarks: *Gyrodactylus* sp[1] is readily separated from the other gyrodactylids described from South American freshwater fish by its unique haptoral sclerites that include: 1) a thin hamulus with the anchor root relatively long and the shaft with a prominent elevation localized near the point marking the junction between the regions of the shaft and point (Fig.5.2a). 2) the marginal hook sickle with the proximal part broad and a thick marginal hook shaft (Fig. 5.2d-e). 3) a ventral bar strongly ridged longitudinally with small but prominent ventral bar processes and a triangular-shaped ventral bar membrane (Fig. 5.2b).

The morphology of the haptoral sclerites of *Gyrodactylus* sp[1] resemble those of *G. lucii* Kulakovskaya, 1952, sub-genus *G. (Limnonephrotus)*, parasites of skin and fins of *Esox lucius* (L.), according to the description presented by Malmberg (1970). However, it can be differentiated by having a prominent elevation near the point marking the junction regions between shaft and point (Fig. 5.2a) and the marginal hook shaft is not so long as that observed in the specimens of *G. lucii*.

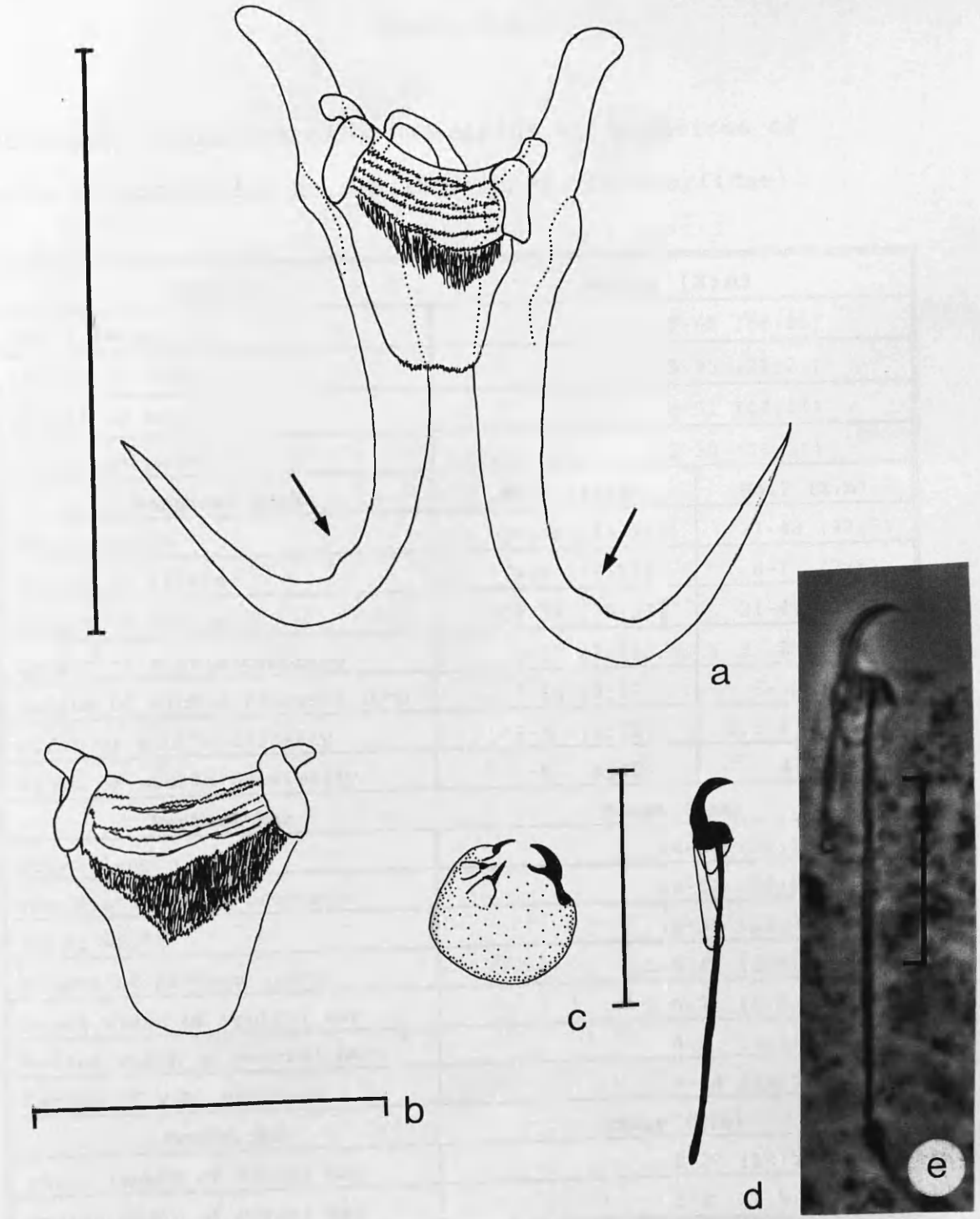


Fig. 5.2 a-e. *Gyrodactylus* sp[1]. a - Hamuli/bar complex. b. ventral bar. c. cirrus. d - e. marginal hook. *Scale-bars*: a 50μm; b 30 μm; c-e 20 μm.

Table 5.2

Sclerite dimensions of *Gyrodactylus* sp[1] parasites of skin of *Otocinclus* sp. (Siluriformes, Loricariidae).

Hamuli	**Range (X;n)**	
Total length	51.5-66 (58;21)	
Length of root	15.5-25 (21;21)	
Length of shaft	38-51 (44;21)	
Length of point	22-30 (26;21)	
Marginal Hook	**No.1 (X;n)**	**No.7 (X;n)**
Total length	26-38 (33;13)	27-48 (33;5)
Length of sickle	6-9 (7;13)	6-7 (7;5)
Length of handle	20-32 (25;13)	21-41 (27;5)
Length of sickle membrane	1-2 (2;5)	2 (2)
Length of sickle filament loop	7-14 (9;13)	6-12 (9;5)
Width of sickle distally	2-6 (4;12)	2.5-4 (3;4)
Width of sickle proximally	3-5 (4;12)	4 (4)
Ventral Bar	**Range (X;n)**	
Total length	18-21 (19;10)	
Max.dist.between processes	14-23 (20;8)	
Total width	18-30 (22;7)	
Length of process	2.5-4 (3;8)	
Basal width of ventral bar	5.5-7 (6.5;10)	
Median width of ventral bar	4-9 (6;10)	
Length of V.B. membrane	8-14 (10.5;6)	
Dorsal Bar	**Range (X;n)**	
Total length of dorsal bar	6-20 (12.5;14)	
Median width of dorsal bar	1-2 (1.5;16)	

Gyrodactylus sp[3]

(Fig. 5.3a-e;Table 5.3)

Host: *Corydoras robinae* Burguess, 1983, Callichthyidae

Site of Infection: Mainly caudal and dorsal fins.

Type-locality: Rio Negro, Amazon Region, Brazil

Prevalence: UK - Br = 13% (2/15)

Br = 100% (20/20)

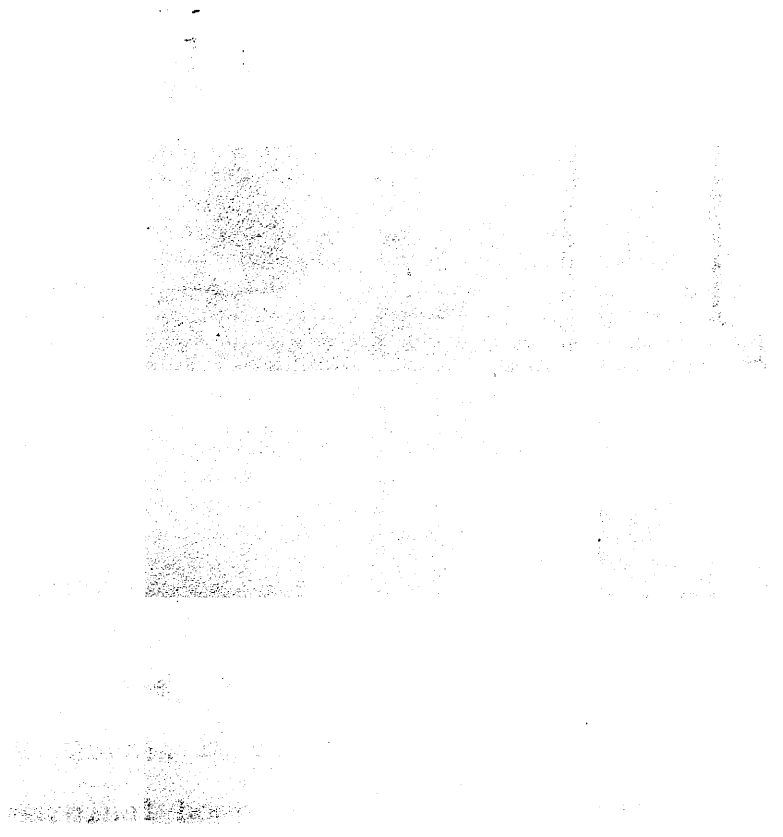
Description (based on 12 specimens): Body 605-825 (718) long by 53-98 (75.5) wide. Pharynx 37.2 long by 23 wide. Pharyngeal process not observed. Cirrus ventral, 14-15.5 (15) in diameter, armed with one row of small spines (Fig.5.3e). Excretory system lacking excretory bladders. Flame bulbs IIf5a,IIf5,IIf5d (Malmberg, 1970) in the opisthaptor are situated between marginal hooks 2-3, 5-6 and 7-8 respectively. Hamuli 56-68 (63) long (Fig.5.3a). Angle shaft/point 60.65°. Dorsal bar 15-22 (18) long by 2-3 (2) wide. Ventral bar 20-26 (22) long by 20.5-30 (24) wide (Fig.5.3c). Ventral bar processes small. Marginal hooks 28-35 (32) long (Fig.5.3b;d). Detailed measurements of sclerites presented in Table 5.3. Testis not observed. Uterus with one or two generations at different stages of development.

Remarks: The haptoral sclerites of *Gyrodactylus* sp[3] present similarities with those found on specimens of *C. punctatus* (see *Gyrodactylus* sp[5]). The hamulus is long and slender with a continuous curve from the point of the shaft to the distal tip, giving a convex form to it (Fig. 5.3a). However, the length of the hamulus of the specimens found in

C. robinae is bigger than that described for *C. punctatus* (Table 5.3).

The morphology of the marginal hook is similar, however these specimens present a bigger marginal hook (Table 5.3). The shape of the marginal hook (Fig. 5.3b;d) and the distribution of the flame cells in the main canals suggest that this species may belong to the subgenus *G. (Gyrodactylus)* Malmberg, 1964, *G. phoxini*-group. However, the sickle filament loop is not as long as that observed in *G. phoxini* Malmberg, 1957 and *G. linnaeus* Malmberg, 1964.

Gyrodactylus sp[3] was found in the specimens of *C. robinae* both at the exporters' holding facilities in Brazil and also in those imported the UK from South America.



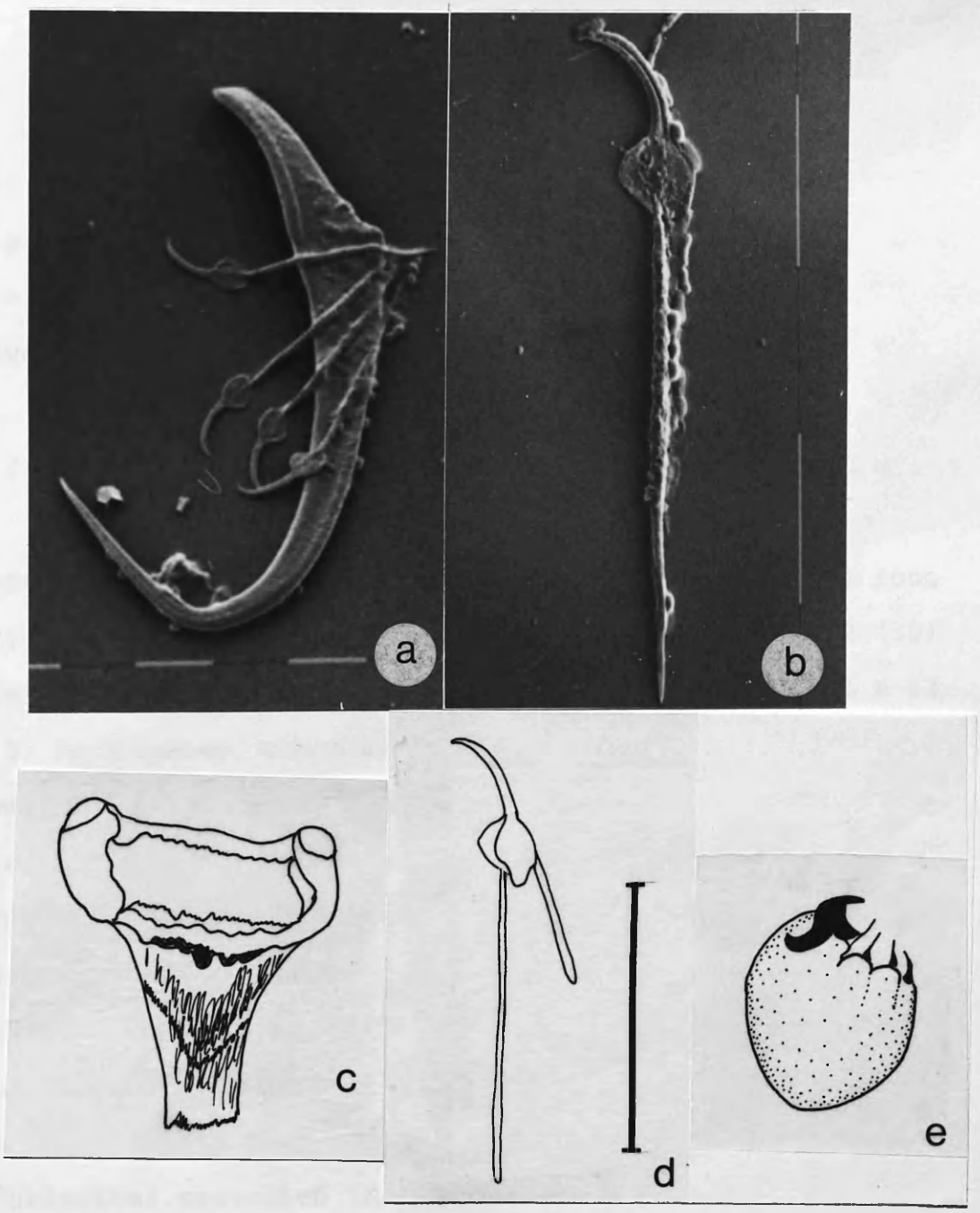


Fig. 5.3 a-e. *Gyrodactylus* sp[3]. a. Hamulus and marginal hooks (SEM 1250X). b. marginal hook (SEM 2500X). c. ventral bar. d. Marginal hook. e. cirrus. *Scale bar:* c-e 20µm.

Gyrodactylus sp[4]

(Figs. 5.4a-d; Table 5.4)

Host: *Brochis splendens* (Castelnau, 1855).

Site of infection: Mainly dorsal and caudal fins.

Type-locality: Brazil, Amazon Region

Prevalence: UK - Br= 7% (2/30)

Uk - C= 3.5% (1/28)

Br= 100% (20/20)

Description (based on 13 specimens): Body 413-585 (458) long by 104-126 (115) wide. Pharynx 30-37 (36) long by 28-32 (30) wide. Pharyngeal processes not observed. Cirrus ventral 8-12 (9.5) in diameter, armed with small spines distributed in two rows. Excretory system lacking excretory bladders. Flame bulbs IIF5a, IIf and II5d in opisthaptor are situated between marginal hooks 2-3, 5-6 and 7-8. Hamuli 63-72 (67) long (Fig. 5.4a;c). Angle shaft/point 58°45. Dorsal bar 20 long by 1-2 (2) wide. Ventral bar 20-23 (21.5) long by 22-26 (24) wide. (Fig. 5.4c). Ventral bar processes small (Fig.5.4c). Marginal hooks 22-25 (24) long (Fig. 5.4b;d). Detailed measurements of sclerites presented in Table 5.4 Testis not observed. Uterus with one or two generations at different stages of development.

Remarks: Amongst the gyrodactylids found in the callychthids examined in this survey, the haptoral sclerites of these specimens appear to be most closely related to those from *Gyrodactylus* sp[3], parasites of *C. robinae*. Both species present the thin hamuli, with curved shaft (Fig. 5.4a,c; 5.3

b-c) however, *Gyrodactylus* sp[4] can be differentiated from those specimens found in *C. robinae* by having: 1) the cirrus with small spines distributed in one arched row. 2) the hamuli not so convex, bigger and with the angle shaft/point less open (Fig. 5.4a; Table 5.3-5.4). 3) the marginal hook small and the marginal hook shaft appears to be longer and slightly broader (Fig. 5.4b; d).

These specimens of *Gyrodactylus* sp[4] were found in samples of *B. splendens* examined in the UK and Brazil. However, in the specimens of *B. splendens* sampled at the exporters' holding facilities in Brazil, these gyrodactylids had a higher intensity of infection and were found in mixed infections with other species of *Gyrodactylus*. The haptoral sclerites of these gyrodactylids suggest that these specimens may belong the sub-genus *G.* (*Gyrodactylus*).

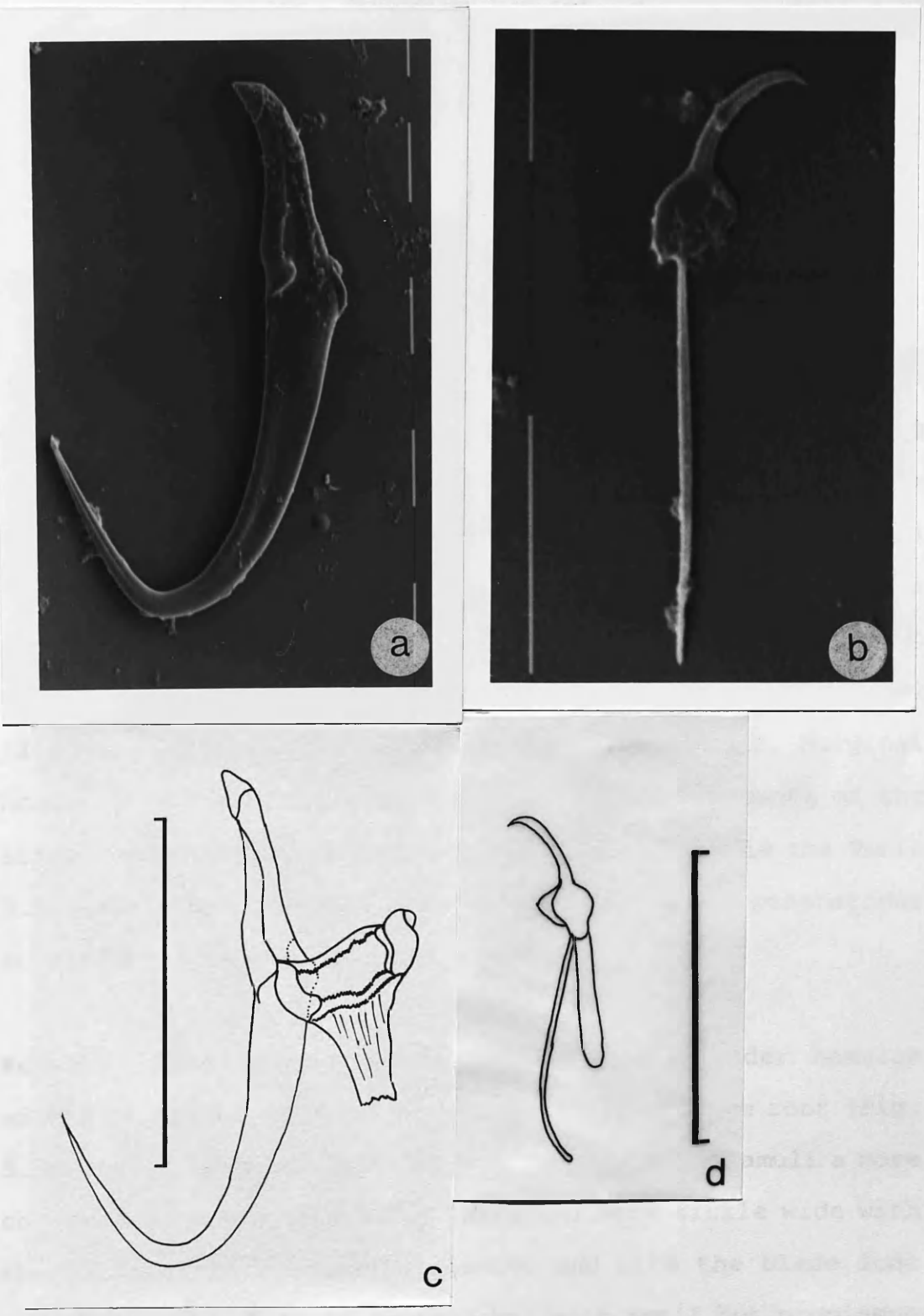


Fig. 5.4 a-d. *Gyrodactylus* sp[4]. a - Hamuli (SEM 1250X). b - marginal hook (SEM 1250X). c - Hamuli and ventral bar. d - marginal hook. *Scale-bar*: c = 50µm; d = 20 µm.

Gyrodactylus sp[5]

(Figs.5.5a-c;Table 5.3).

Host: *Corydoras punctatus* (*punctatus*-group)

Site of infection: Mainly caudal and dorsal fins

Type-locality: Brazil, Amazon Region

Prevalence: Br = 50% (10/20)

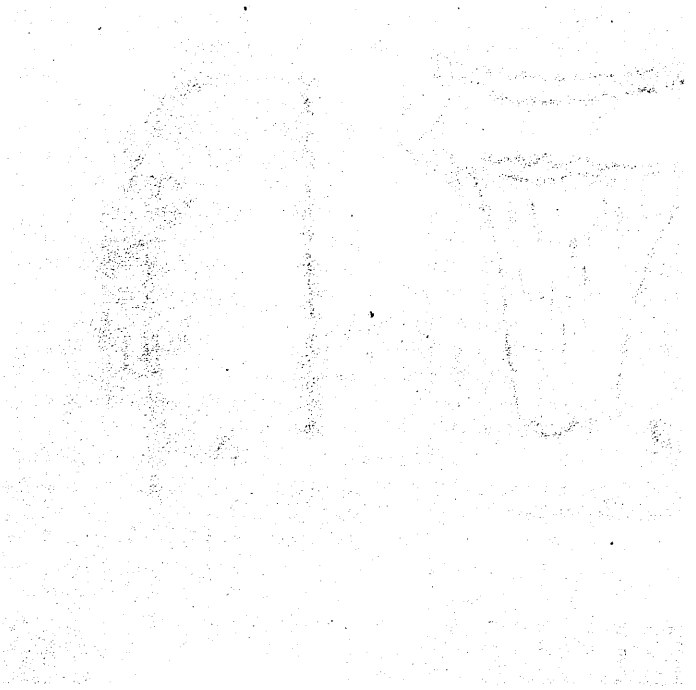
Description (based on 10 specimens) Body 258-508 (354) long by 72.5-135.5 (97) wide. Pharynx 28.5-69 (40) long by 28.5-81 (42) wide with small pharyngeal process. Cirrus ventral 6-9 (7) in diameter. Hamuli thin 52-62 (57) long (Fig. 5.5a). Angle shaft/point 58.44°. Dorsal bar 12-16 (14) long without medial notch (Fig.5.5a). Ventral bar 18-22 (20) long by 19-22 (21) wide. Ventral bar process short (Fig. 5.5c). Marginal hooks 21-30 (24) long (Fig. 5.5a-b). The measurements of the sclerites according to Malmberg (1970) are given in the Table 5.3. Testis not observed. Uterus with one or two generations at different stages of development.

Remarks: *Gyrodactylus* sp[5] presents a long slender hamulus with a prominent notch in the proximal part of the root (Fig. 5.5a-arrow). Hamulus root divergent, giving the hamuli a more convex appearance (Fig.5.5a). Marginal hook sickle wide with the proximal part dorsally concave and with the blade long and thin. (Fig. 5.5a-b) Ventral bar with small but prominent anterolateral process (Fig. 5.5c).

Amongst the gyrodactylids described in fish from South America, this species found in *Corydoras punctatus* (*punctatus*-group) presents some similarities with *G. slendrus*

An *et al.*, 1991 parasite of *L. bimaculata*. It differs from *G. slendrus* by having: 1) small ventral bar processes (*G. slendrus* = 6.5 long and *Gyrodactylus* sp[5] = 1-2.5 (1.5) long). 2) the hamuli appearing to be more convex than those described from *G. slendrus*. 3) the sickle not angled and the blade not so long and curved.

Gyrodactylus sp[5] appears to be similar to members of *G. rarus* species group in having thin hamuli with curved blades, small ventral bar processes and a marginal hook sickle with a long and slender shaft.



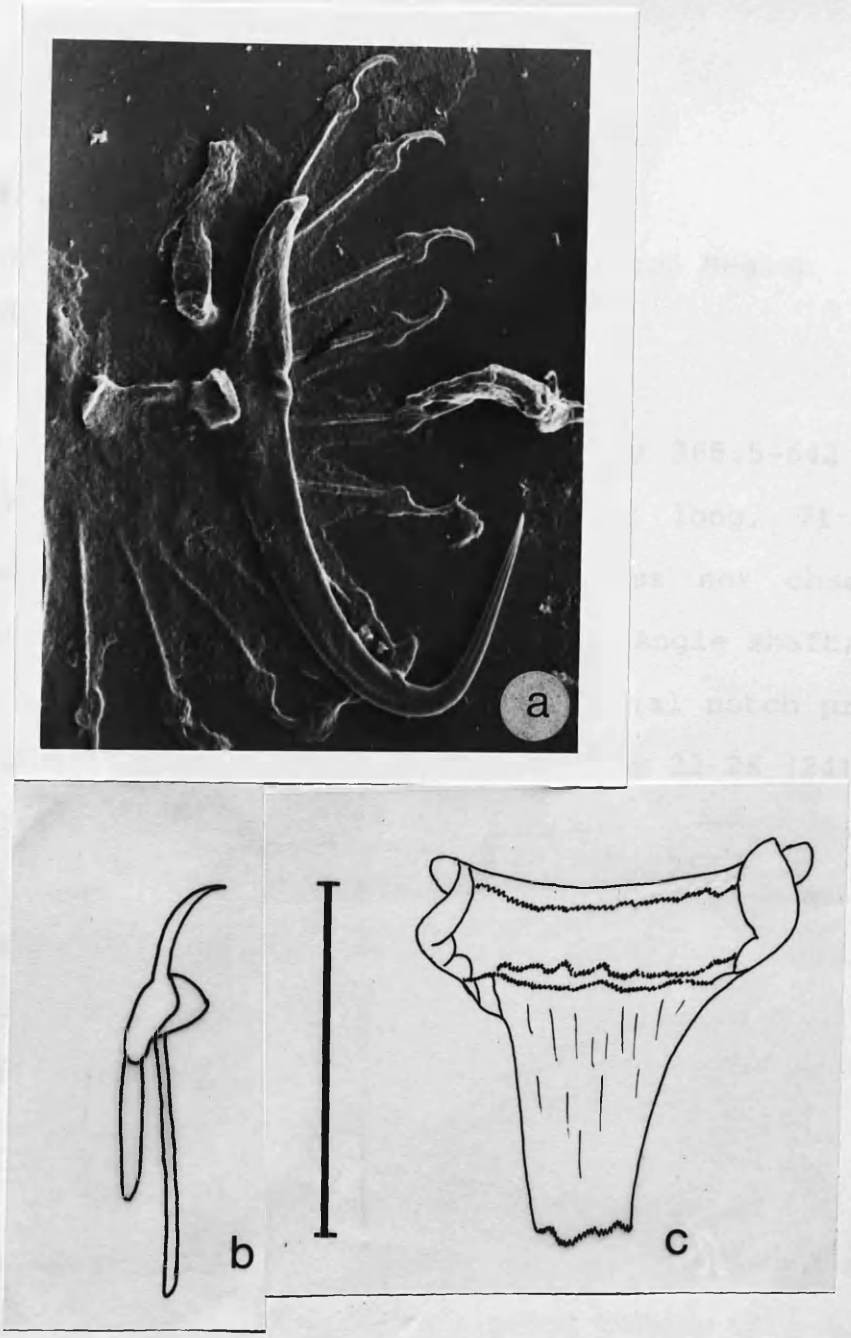


Fig. 5.5a-c *Gyrodactylus* sp[5]. a- Hamuli and marginal hook (SEM 1250X). Note the prominent notch in the proximal part of the root (arrow) b - marginal hook. c - ventral bar. *Scale bar:* b-c 20 µm.

Gyrodactylus sp[6]

(Figs. 5.6a-e; Table 5.3)

Host: *Corydoras julii* Steindachner, 1906.

Site: Mainly caudal and dorsal fins.

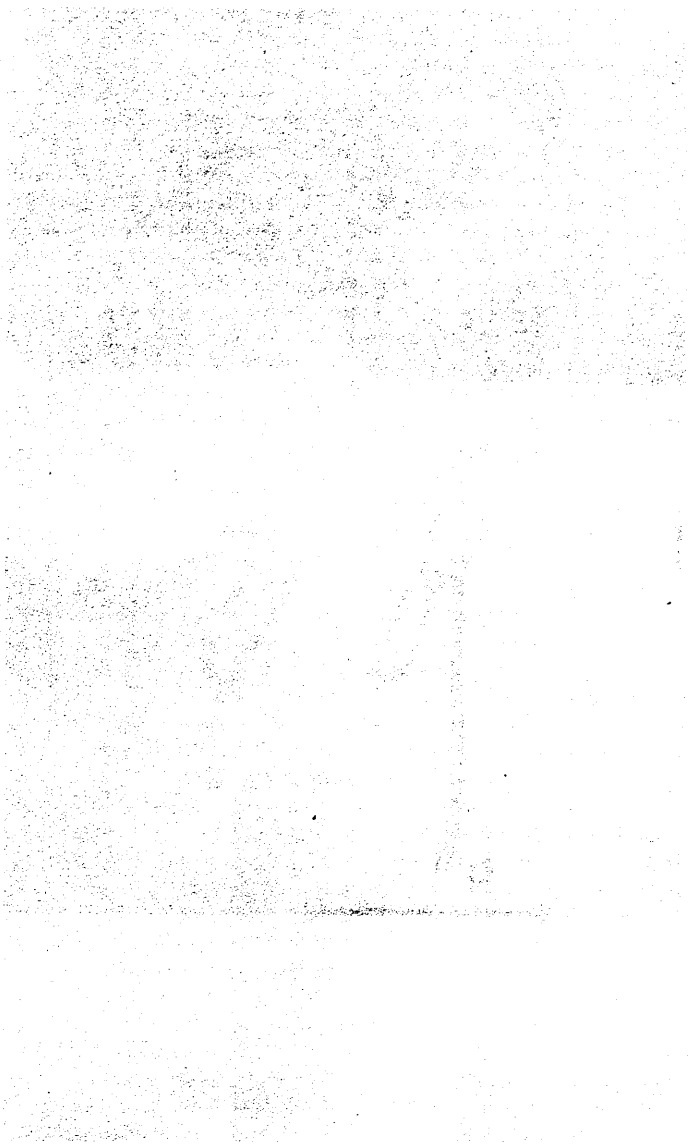
Type-locality: Rio Tocantins, Brazil, Amazon Region

Prevalence: Br = 7.5% (3/40)

Description (based on 14 specimens): Body 305.5-642 (452) long, 47-25-134.5 (92) wide. Pharynx 82 long, 71 wide. Pharyngeal processes not observed. Cirrus not observed. Hamuli stout 48-66 (62) long. (Fig.5.6a). Angle shaft/point 40°88. Dorsal bar 9-20 (16) long with medial notch present (Fig. 5.6c). Ventral bar 17-23 (21) long by 22-26 (24) wide (Fig. 5.6e). Ventral bar processes small (Fig. 5.6e). Marginal hook 25-29 (27) long (Fig. 5.6b,d). Detailed measurements of sclerites presented in Table 5.3. Testis not observed. Uterus with one or two generations at different stages of development.

Remarks: From all gyrodactylid species found in the *Corydoras* species examined in this survey, *Gyrodactylus* sp[6] can be readily separated by having stout hamuli (Fig.5.6a), the dorsal bar with a medial notch (Fig. 5.6c), the marginal hook with the proximal part of the sickle dorsally convex and the marginal hook shaft slightly straightened with the point protruding in an angle of approximately 90° (Fig. 5.6b;d). This species appears to belong to the subgenus *G. (Gyrodactylus)* and, therefore, similar to the other species found in this survey and placed in this subgenus,

Gyrodactylus sp[6] does not present the sickle filament as long as those observed in the members of the two species-group described for the sub genus *G. (Gyrodactylus)*.



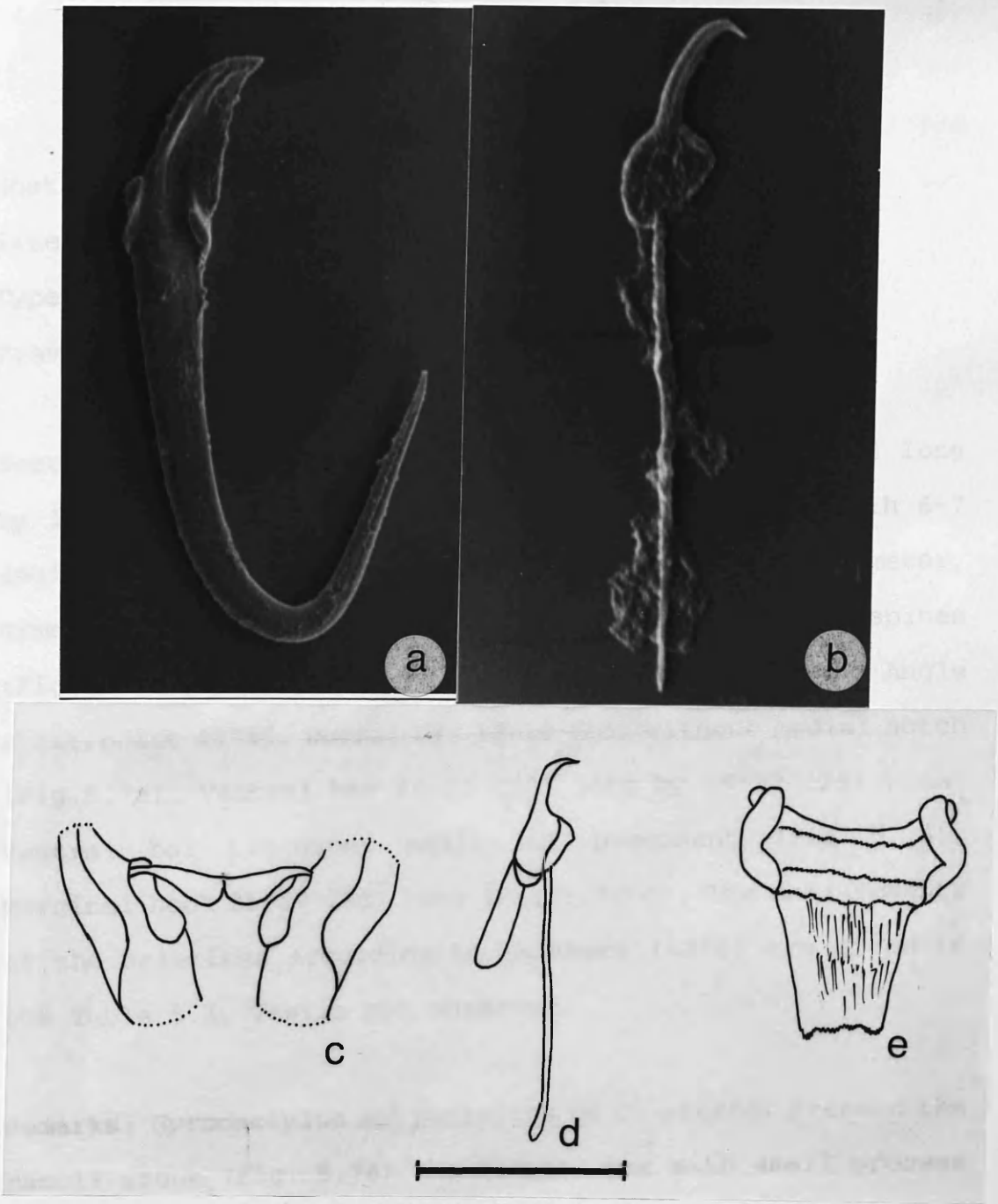


Fig 5.6 a-e *Gyrodactylus* sp[6]. a. Hamulus (SEM 1250X). b. marginal hook (SEM 2500X). c. dorsal bar. d. marginal hook. e. ventral bar. *Scale bar:* c-e 20µm.

Gyrodactylus sp[7]

(Fig.5.7a-d ; Table 5.3)

Host: *Corydoras sterbai* Knaack, 1962.

Site: Mainly dorsal and caudal fins

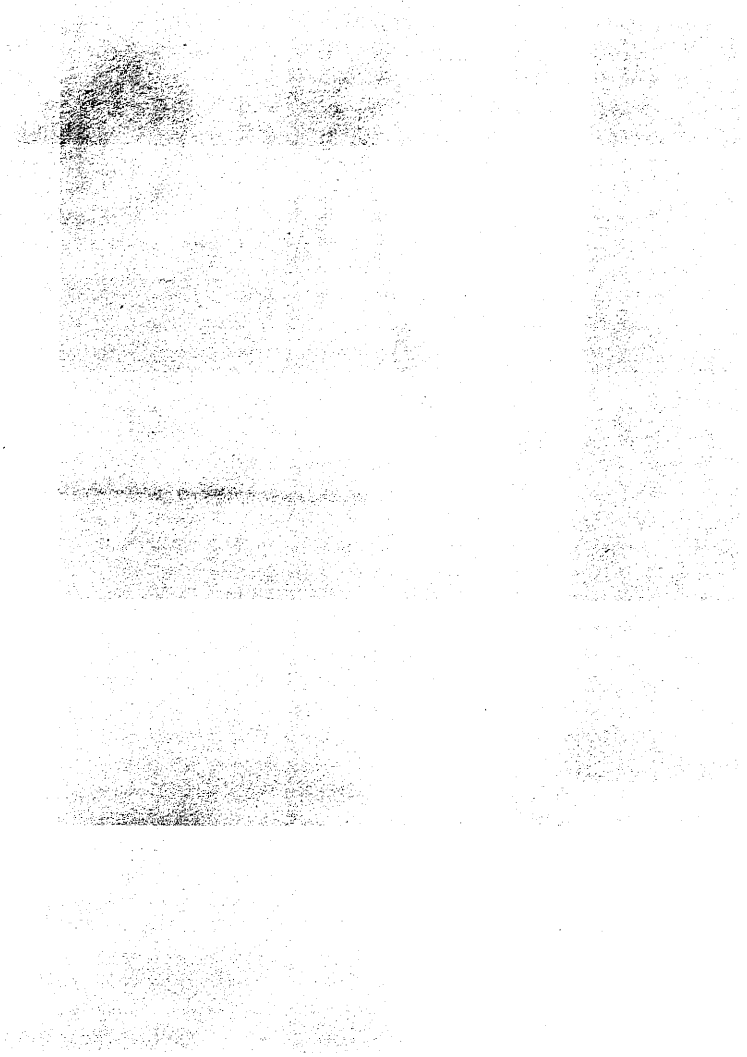
Type-Locality: Rio Guapore, Brazil

Prevalence: Br = 63.82% (30/47)

Description (based on 10 specimens): Body 585-636 (608) long by 140-197 (163) wide. Pharynx 61 long by 65 wide with 6-7 small pharyngeal processes. Cirrus ventral 9 in diameter, armed with one large spine and one row of 5-6 small spines (Fig. 5.7b). Hamuli stout 77-84 (82) long (Fig. 5.7a). Angle shaft/point 40°45. Dorsal bar 12-18 (15) without medial notch (Fig.5.7a). Ventral bar 26-30 (27) long by 25-32 (29) wide. Ventral bar processes small but prominent (Fig 5.7a). Marginal hook 25-27 (26) long (Fig.5.7c-d). The measurements of the sclerites according to Malmberg (1970) are given in the Table 5.3. Testis not observed.

Remarks: *Gyrodactylus* sp[7] parasites of *C. sterbai* present the hamuli stout (Fig. 5.7a) the ventral bar with small process and the ventral bar membrane slightly triangular (Fig.5.7a). The marginal hook of this species presents a very peculiar morphology with the proximal part, dorsal side of the sickle with small protrusion and the shaft running obliquely to the anterior side of the sickle. The marginal hook shaft is broad in the proximal part of the sickle, with the point turning sharply away and being somewhat elongated (Fig.5.7c-d). The haptoral sclerites of *Gyrodactylus* sp[7] appear to be similar

to those found in species of the subgenus *G.*(*Limnonephrotus*), *G.* (*Wageneri*) species-group. However, the morphology of the haptoral sclerites and the excretory system of more specimens should be better studied to confirm the position of these specimens in this subgenus..



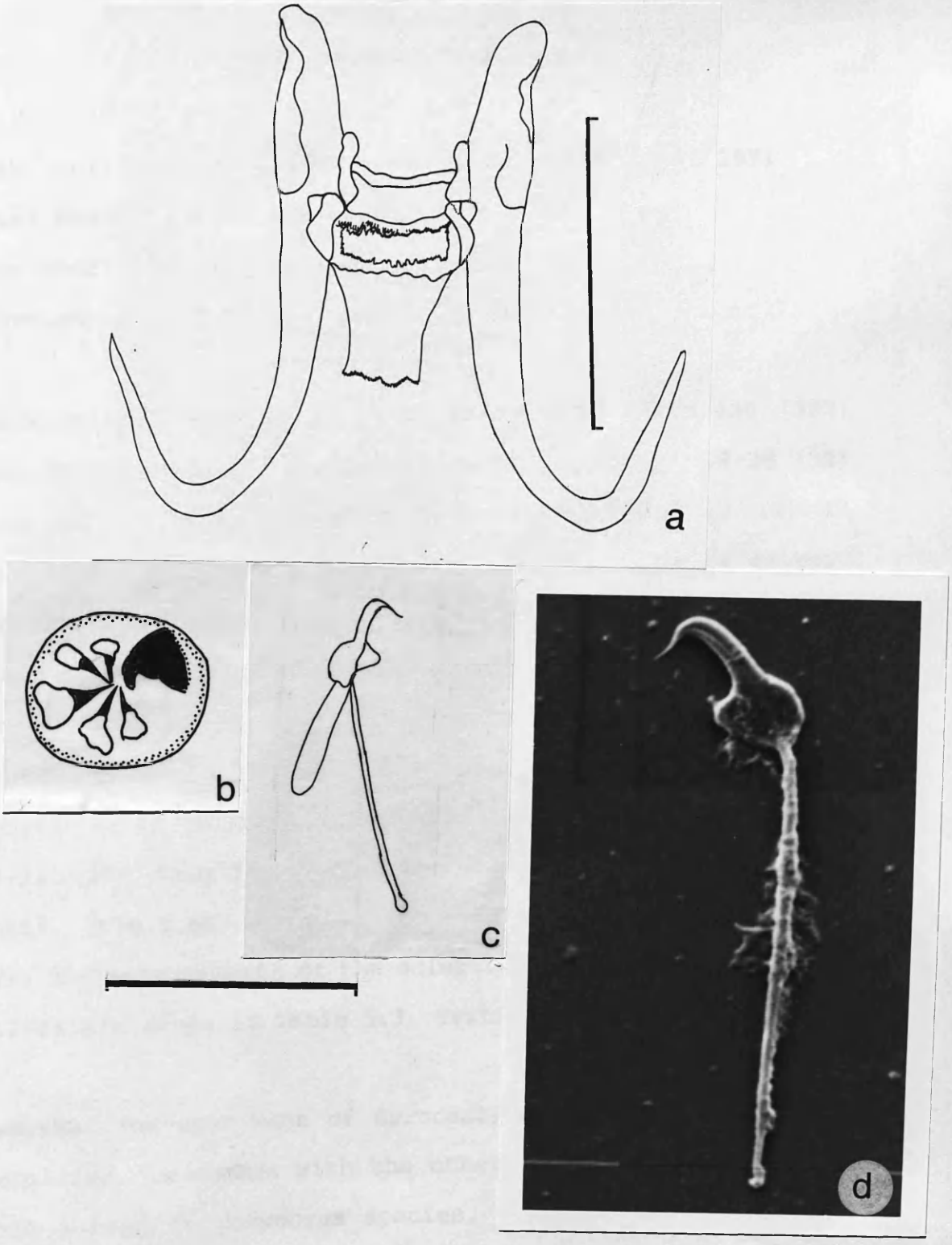


Fig.5.7 a-d. *Gyrodactylus* sp7 a - Hamuli/bar complex. b - cirrus. c - marginal hooks. d - marginal hook (SEM 2500X). *Scale bar:* a. 50µm; b-c 20µm.

Gyrodactylus sp[8]

(Figs. 5.8a-d;Table 5.3)

Host: *Corydoras maculifer* Nijseen and Isbrückner, 1971

Site: Mainly dorsal and caudal fins

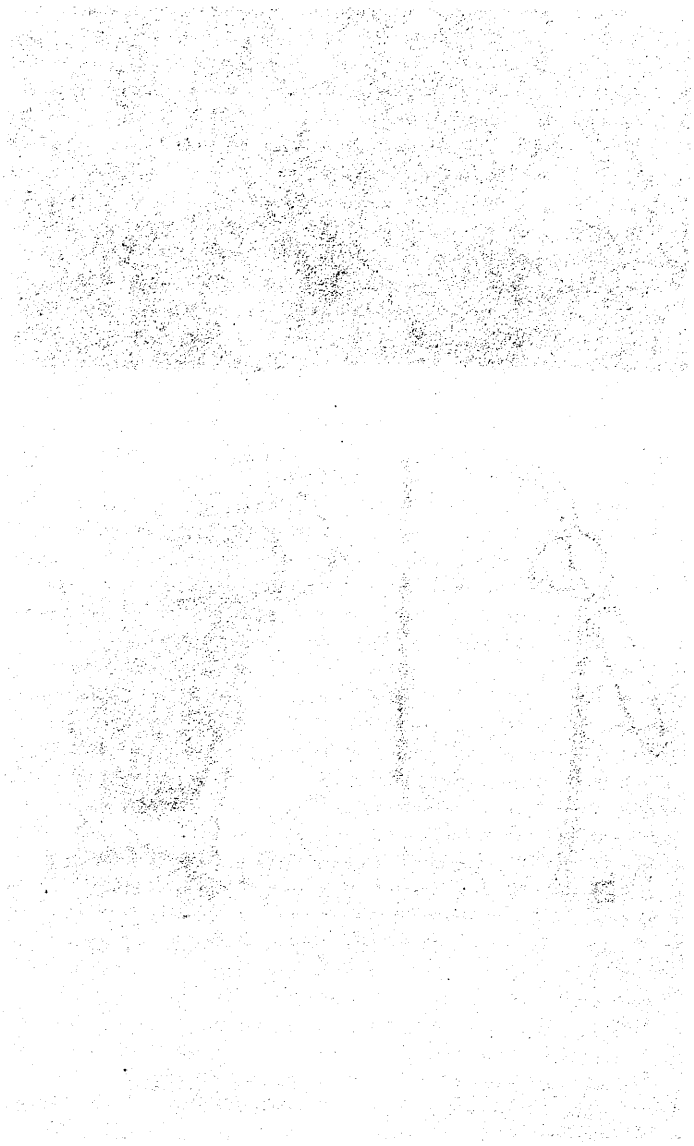
Type-locality: Brazil, Amazon region.

Prevalence: Br = 68.62% (35/51).

Description (based on 11 specimens): Body 362.5-420 (387) long by 64-129 (96). Pharynx 32-38 (35) long by 28-38 (32) with 4-5 small pharyngeal processes. Cirrus 6-13 (9) in diameter with one large spine and two rows of small spines. Excretory system with excretory bladders apparently absent. Flame bulbs IIf5a, IIf5 and IIf5d in opisthaptor are situated between marginal hooks 2-3, 5-6 and 7-8 respectively. Hamuli 58.5-64 (60) long (Fig 5.6a). Angle shaft/point 44.50°. Dorsal bar 12-18 (15) long without medial notch. Ventral bar 18-22 (20) long by 18-23 (22) wide. Ventral bar process small. (Fig.5.6c). Marginal hook 22-27 (24) long (Fig.5.8b ;d). The measurements of the sclerites according to Malmberg (1970) are given in Table 5.3. Testis not observed.

Remarks: The specimens of *Gyrodactylus* sp[8] parasites of *C. maculifer*, in common with the other gyrodactylids found in this survey in *Corydoras* species, present the ventral bar with small anterolateral processes (Fig. 5.8c). However the morphology of the hamuli (Fig. 5.8a) and the marginal hook (Fig. 5.8a;b;d) are different. The hamulus is stout with a straight shaft (Fig. 5.8a) and the marginal hook sickle is wide, dorsally concave and the sickle shaft thin and curved

(Fig. 5.8b; d). The haptoral sclerites of these specimens resemble those of the subgenus *G. (Gyrodactylus)*, *G. phoxini* species-group.



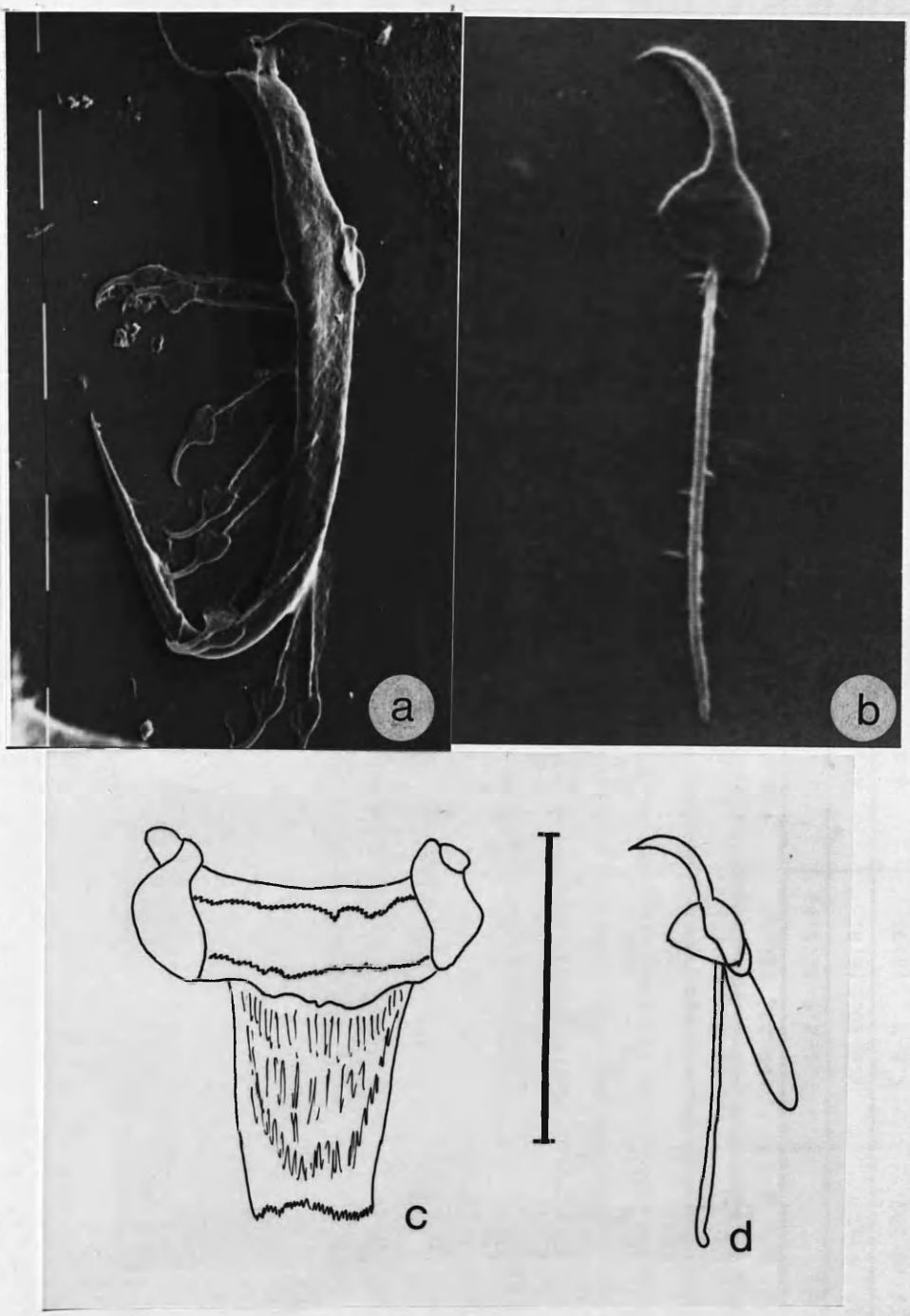


Fig.5.8 a-d. *Gyrodactylus* sp[8]. a - Hamulus and marginal hooks (SEM 1250X). b - marginal hook (SEM 2500X). c - ventral bar. d - marginal hook. *Scale bar:* 20μm.

Table 5.3

Sclerite dimensions of the species of *Gyrodactylus* parasites of body and fins of five species of ***Corydoras***.

Haptoral Sclerites	*Gyrodactylus* sp[3]	*Gyrodactylus* sp[5]	*Gyrodactylus* sp[6]	*Gyrodactylus* sp[7]	*Gyrodactylus* sp[8]
Hamuli	**Range (X; n)**	**Range (X; n)**	**Range (X; n)**	**Range (X; n)**	**Range (X; n)**
Total length anchor	56-68 (63;12)	52-62 (57;9)	48-66 (62;14)	77-84 (82;9)	58.5-64 (60;8)
Length of anchor root	16-29 (24;12)	15.5-22 (20;9)	12-24 (20;14)	23.5-30 (26;9)	14-23 (21;8)
L. of anchor shaft	44-51 (48;12)	41-50 (44;9)	40-59.5 (48;14)	59.5-64 (62;9)	42-50 (47;8)
L. of anchor point	20.5-28 (25;12)	20-32 (30)	25-34 (31;14)	32-34 (32;9)	28-32 (29;8)
Angle shaft/point	60°65	58°44	40°88	40°45	44°49
Marginal Hook	No.1 (X;n)	No.1 (X;n)	No.1 (X;n)	No.1 (X;n)	No.1 (X;n)
Total length M.H.	28-35 (32;10)	21-30 (24;9)	25-29 (27;11)	25-27 (26;8)	22-27 (24;11)
Length of sickle	7-9 (8;10)	6-7 (7;10)	5.5-9 (8;11)	5.5-7 (6;8)	7-8 (8;11)
L. of handle	22-30 (23;10)	14-22 (16;9)	15.5-22 (19;11)	19-21 (20;8)	15-19 (16.5;11)
L. sickle membrane	1-2.5 (1;9)	1-2 (1.5;9)	1-1.5 (1;6)	1-2 (1.5;8)	1-2 (1;11)
L. sickle filament loop	7-12 (9;10)	6-11 (9;10)	8-9 (9;6)	9-12 (10;7)	7-10 (8.5;11)
Width of sickle distally	2.5-4 (3;8)	3-5 (5;9)	4-5 (4.5;8)	4-5.5 (4.5;3)	3-4 (4;7)
W. proximal part sickle	2.5-5 (4;8)	3-5 (10)	4-6 (5;10)	4-5 (4;7)	4-5 (4;10)
Length of Ventral Bar	20-26 (22;11)	18-22 (20;8)	17-23 (21;7)	26-30 (27;10)	18-22 (20;8)
Total width V.B.	20.5-30 (24;11)	19-22 (21;8)	22-26 (24;6)	25-32 (29;10)	18-23 (22;8)
Length of process V.B.	1 (4)	1-2.5 (1.5;6)	1-2.5 (2;2)	2-3 (2.5;7)	1-2 (1.5;8)
Basal width of V.B	6-12 (9;11)	6-10.5(8;8)	8-11 (9.5;7)	12-15 (12.5;10)	6-10 (8;8)
Median width of V.B	6-12 (9;11)	5.5-7 (6;9)	6-9 (7.5;7)	8-11 (9;10)	6-8 (7;8)
Length of V.B. memb.	12-16 (13;11)	9-14 (12;9)	12-14 (13;6)	13-19 (15;10)	11-14 (13;7)
Total length Dorsal Bar	15-22 (18;10)	12-16 (14;9)	9-20 (16;8)	12-18 (15;5)	12-18 (15;7)
Median width of D.B.	2-3 (2;11)	1-3 (9;2)	2-2.5 (2;8)	2-2.5 (2;7)	1-2.5 (2;8)

Gyrodactylus sp^{9}

(Figs. 5.9a-e; Table 5.4)

Host: *Brochis splendens* (Castelnau, 1855).

Site: Body surface and fins.

Type-locality: Brazil, Amazon Region

Prevalence: Br = 100% (20/20)

Description (based on 13 specimens): Body 613-1216 (834) long by 153 wide. Pharynx 93 long by 80 wide. Pharyngeal processes not observed. Cirrus ventral, 12-18(14) in diameter with 6-8 (7) small spines, in more than one arched row (Fig.5.9e). Hamuli stout 76-82.5 (79) long (Fig. 5.9a). Angle shaft/point 44°37. Dorsal bar 22-23.5 (22) long by 2-2.5 (2) wide without medial notch. Ventral bar 19-25 (23) long by 22-24 (23) wide (Fig.5.9c). Ventral bar processes small but prominent. Marginal hooks 25-28 (26) long (Fig.5.9b; d). The measurements of the haptoral sclerites according to Malmberg (1970) are presented in the Table 5.4. Testis not observed. Uterus with one or two generations at different stages of development.

Remarks: *Gyrodactylus* sp^{9} was only found in the specimens of *B. splendens* sampled in Brazil and was only found in mixed infections with *Gyrodactylus* sp^{4} also parasitizing the body surface and fins. The two species can be easily differentiated mainly based on the size (Table 5.4) and the morphology of the haptoral sclerites (Fig. 5.4a-d; 5.9a-e).

The haptoral sclerites of *Gyrodactylus* sp[9] resemble those found in *Gyrodactylus* sp[7], parasites of *C. sterbai*. Both species present the large and stout hamuli (Fig.5.7a; 5.9a; Table 5.3-5.4) and similar marginal hook (Fig. 5.7d ;5.9b). However the marginal hook of these specimens appear to present a shaft which is less oblique anteriorly and ending in a long, thin and concave tip (Fig. 5.9b). *Gyrodactylus* sp[9] appears to be a member of the subgenus *G. (Limnonephrotus)*, a lineage that occurs on freshwater fish from Eurasia and North America.

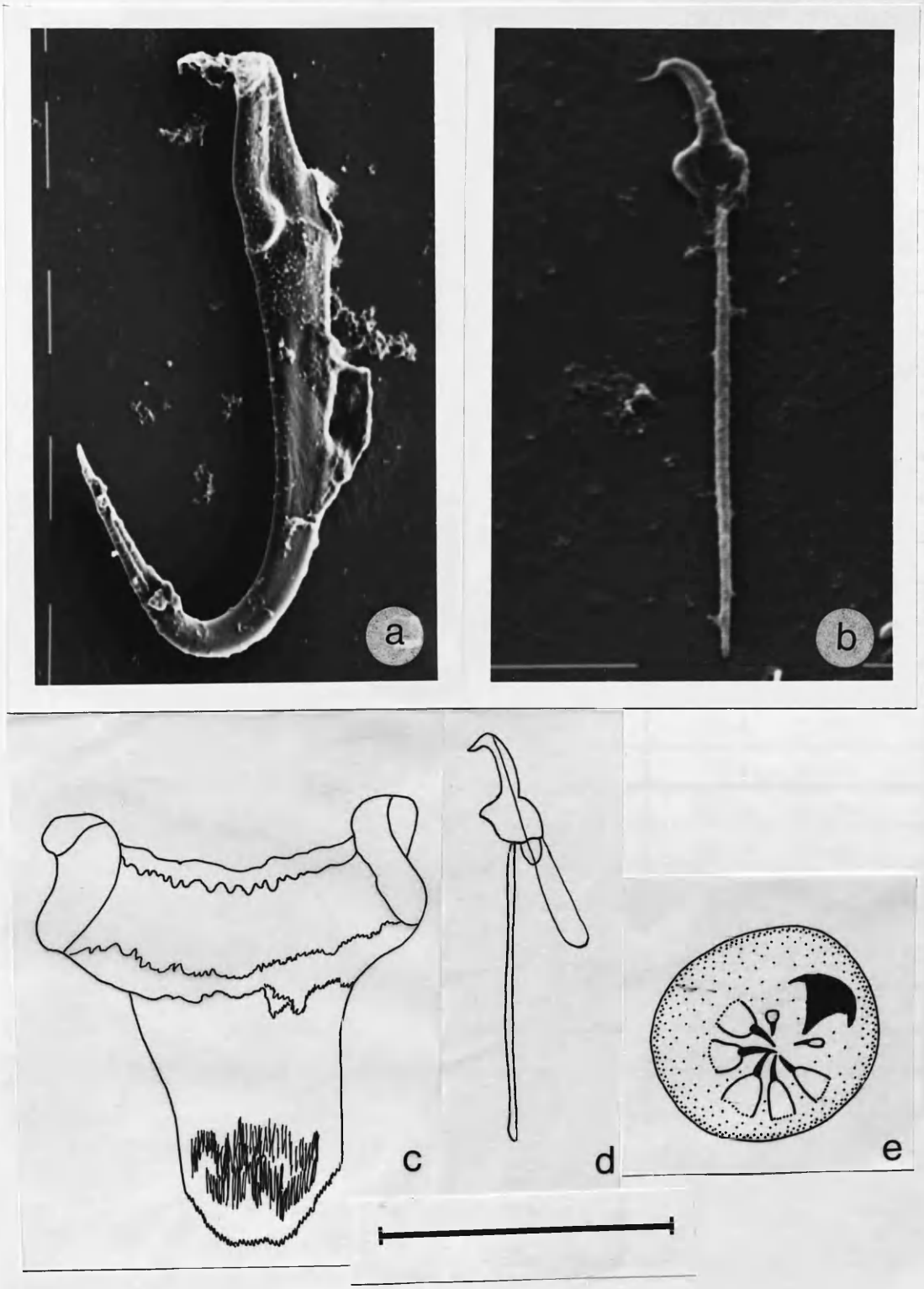


Fig. 5.9 a-e. *Gyrodactylus* sp[9]. a. Hamulus (SEM 1250X). b. marginal hook (SEM 2500X). c. ventral bar. d. marginal hook. e. cirrus. *Scale-bar*: c-e 20 µm.

Table 5.4

Sclerite dimensions of the species of *Gyrodactylus* parasites of body and fins of ***Brochis splendens***

Haptoral Sclerites	*Gyrodactylus* sp[4]	*Gyrodactylus* sp[9]
Hamuli	**Range (X;n)**	**Range (X;n)**
Total length anchor	63-72 (67;11)	76-86.5 (79;9)
Length of anchor root	23-30 (27;11)	22-28 (26;9)
L. of anchor shaft	45-52 (48;11)	58-62 (60;9)
L. of anchor point	26-30 (27.5;10)	31-33.5 (32.5;9)
Angle shaft/point	58°45	44°37
Marginal Hook	No.1	No.1
Total length M.H.	22-25 (24;7)	25-28 (26;7)
Length of sickle	6-9 (7;7)	5.5-7 (7;6)
L. of handle	16-19 (17;7)	17-22 (19.5;7)
L. sickle membrane	0.6-1 (1;7)	0.6-1 (1;6)
L. sickle filament loop	8-10 (9;7)	9-10.5(9.5;6)
Width of sickle distally	4-6 (5;4)	2.5-4 (3;4)
W. of sickle proximally	3-4 (3;7)	3-4 (4;7)
Length of Ventral Bar	20-23 (21.5;11)	19-25 (23;8)
Total width	22-26 (24;11)	28-33 (29;8)
Length of process	1.24	2
Basal width	8-10 (9;11)	12-15.5(13.5;8)
Median width	5.5-9 (7;11)	9-12 (10;8)
Length of V.B. memb.	12-16 (14;11)	15-19 (16.5;8)
Total length Dorsal Bar	20	22-23.5(22;4)
Median width	1-2 (2;4)	2-2.5 (2;5)

5.3.2 Monogenean parasites of the urinary bladder.

Seventy specimens of *Mylossoma aureum* (Spix), the silver dollar, were sampled in shipments from Colombia and Peru, after their import to the UK. Thirty specimens (42.85%) were infected with monogeneans in the urinary bladder (Fig. 5.10a-d), collecting ducts and ureters. These specimens were identified as members of the family Dactylogyridae, genus *Kritskyia* Kohn, 1990. Recently, Kritsky *et al.*, (in press) proposed an emended diagnosis for the genus based on three voucher specimens. In order to accommodate the specimens from this study in this genus, the diagnosis presented by Kritsky *et al.* (*op.cit*) had to be further emended. The emended diagnosis and the taxonomic description of this dactylogyrid is presented.

Family Dactylogyridae sensu Gussev, 1976

Subfamily Ancyrocephalinae sensu Kritsky and Boeger, 1989

Genus *Kritskyia* Kohn, 1990

Kritskyia Kohn, 1990 emended by Kritsky *et al.*, (**in press**) (**amended diagnosis**).

Generic Diagnosis: Dactylogyridae: Ancyrocephalinae. Body elongate, fusiform, divisible into cephalic region, trunk, peduncle, haptor. Cephalic lobes undifferentiated. Head organs inconspicuous. Cephalic glands indistinct. Eyes present or represented by 4-6 accumulations of elongate-ovate granules. Mouth mid-ventral at level of anterior margin of

pharynx; pharynx muscular, glandular; oesophagus present; intestinal caeca lacking diverticula, confluent in posterior trunk. Genital pore mid-ventral. Gonads intercaecal. Testis single, post-ovarian. Seminal vesicle large, C-shaped; One or two prostatic reservoirs present. Male copulatory complex comprising coiled cirrus with counter-clockwise rings (Kritsky *et al.*, 1985) ; accessory piece articulated or not. Vagina sinistral may or may not be sclerotized. Seminal receptacle present. Uterus indistinct. Vitelline follicles well developed, coextensive with caeca. Haptor cup-shaped, lacking anterior rim, armed with 14 marginal hooks. Anchors, bars and 4A hooks lacking. Parasites of urinary bladder, collecting tubules and ureters of Neotropical fish.

Kritskyia sp.

(Figs 5.10a-d; 5.11a-c; Table 5.5)

Host: *Mylossoma aureum* (Spix), Serrasalmidae, "silver dollar."

Site of Infection: Ureter, collecting tubules and urinary bladder.

Type-locality: Colombia and Peru, South America

Prevalence: UK - C = 31 (11/35)

UK - P = 54 (19/35)

Description: (based on 6 adult specimens) Body small, elongate, 579-998.5 (785) long by 159-189 (173) wide.

Cephalic lobes not differentiated. Head organs inconspicuous. Cephalic glands postero-lateral to the pharynx. Two pairs of eyes approximately equidistant present; posterior pair larger. Haptor lacking an anterior rim, antero-ventrally concave, 53- 79 (64.5) long by 84-145 (116) wide, with 14 marginal hooks. Anchors, bars and 4A hooks absent (Fig 5.10a). Marginal hooks 23-24 (23.5) long, each with a delicate point, thumb erected and shaft uniform along its length. FH loop 7-9 (9) long (Fig. 5.10d). Pharynx spherical 22-36.5 (25) long, 26-36 (26) wide. Oesophagus short. Crura united posteriorly. Gonads in the posterior half of body (Fig. 5.10a). Testis single, post-ovarian, 28-42 (34.5) long, 30-37 (31) wide (Fig.5.10a). Vas deferens enlarged anteriorly. Seminal vesicle saccular and elongate. One prostatic reservoir present which is rounded in shape and opening into the base of the cirrus. Prostatic reservoir round-shaped 25-65 (44) long by 26-48 (36) wide. Cirrus coiled, strongly sclerotized, with anti-clockwise spiral and inflated base (Fig5.10b; 5.11c). Accessory piece very sclerotized, divided in two parts. Part one, the accessory piece cirrus guide, is elongated, tapering anteriorly, with a broad base and a groove in the dorsal part through which the cirrus is conducted to the outside 53-86 (74). Part two with a small bifurcation at the anterior end, broad base with two long lateral projections that articulate to the part one of the accessory piece, 51-64 (56) long (Fig. 5.10b; 5.11c). Vitellogenic reservoir ovalate, 25-46.5 (37.2) long by 26-35 (32) wide. Mehlis'gland not observed.

Vitelloduct transverse anterior to ovary. Vitellogenic follicles dense co-extensive with caeca (Fig. 5.10a). Vagina 49-162 (90) long, clarinet-shape, strongly sclerotized, overlooping posteriorly and with a small inverse forked structure in the distal extremity (Fig.5.10a; 5.10c). Ovary elongated, strap-shape, 82-148 (110) long, with the anterior end wide and tapering distally. Eggs not observed. The Table 5.5 summarizes all measurements of *Kritskyia* sp. and together with *Kritskyia moraveci* Kohn,1990 for comparison.

Description: (based on 14 immature specimens) Body 413-642 (503) long by 85-223 (160) wide. Haptor 47-85 (67) long by 98-144 (124) wide. Marginal hooks 22-25 (24) long, FH loop 7-10 (8) long. Pharynx 22-42 (29) long, 22-40 (27) wide. Accessory piece, part one 64-86 (73) long. Part two 43-59 (52) long. Vitellogenic reservoir not observed. Vagina 66-162 (84) long. Testis not observed. Ovary 86-30 long.

Remarks: The majority of the generic characters described by Kohn (1990) and Kritsky *et al.*, (in press) were observed in the specimens studied with the main differences concentrated in the composition of the eyes, morphology of the accessory piece, number of prostatic reservoirs and localization and type of vagina.

In *Kritskyia* sp. two pair of eyes, a bipartite accessory piece, one prostatic reservoir and a strongly sclerotized clarinet-shape vagina, localized in the middle region of the body were observed (Fig. 5.10a; 5.10c). In *K. moraveci*

although there were differences in the descriptions of Kohn (1990) and Kritsky *et al.*, (*in press*) they both agreed on the presence of two prostatic reservoirs and the localization of the vagina which is sinistral in the anterior trunk. However, Kritsky *et al.* (*op.cit*) described the vagina of *K. moraveci* as being lightly sclerotized and opening into a fusiform seminal receptacle.

The eyes, according to the original description of Kohn are represented by scattered pigment granules, variable in shape and size. However, according to Kritsky *et al.*, *K. moraveci* has eyes sub-equal, lying dorsal to the anterior margin of the pharynx and the accessory granules are common in the cephalic, anterior trunk region. Other important differences described by these authors were related to the morphology of the accessory piece which was re-described as being sheath-like with a longitudinal groove and a recurved distal end.

Kritskyia sp. is the second species described for this genus and for this reason, its generic diagnosis had to be expanded.

Table 5.5

Measurements of *Kritskyia* sp. and *Kritskyia moraveci* Kohn, 1990, parasites of the excretory system of freshwater fish from South America.

	Kritskyia sp.(Adult specimens)	*Kritskyia* sp. (immature specimens)	*K. moraveci* Kohn, 1990	Kritsky *et al.*(**in press**)
	Range (X;n)	Range (X;n)	Range (X;n)	Range (X;n)
Body Length	579-998 (785;6)	413-642 (503;11)	360-740 (545;13)	409-466 (438;2)
Body Width	159-189 (173;6)	85-223 (160;11)	130-220 (157;14)	141-159 (150;2)
Haptor Length	53-79 (64.5;4)	47-85 (67;7)	42-72 (53;13)	56-59 (58;2)
Haptor Width	84-142 (116;4)	98-145 (124;8)	63-108 (88;16)	-
No. Marginal Hooks	14	14	14	14
Total Length	23-24 (23.5;6)	22-25 (24;14)	21-24 (23;16)	25-26 (9)
Filament Loop Length	7-9 (9;4)	7-10 (8;11)	-	-
Pharynx Length	22-36 (25;6)	22-42 (29;14)	48-83 (58;13)	48-50 (49;2)
Pharynx Width	26-36 (26;6)	22-40 (27;14)	40-68 (52;13)	84-94 (89;2)
Testis Length	28-42 (34.5;3)	-	37-38 (37.5;2)	-
Testis Width	30-37 (31;3)	-	23-28 (25.5;2)	-
Ovary Length	82-148 (110;3)	86	70-140 (102;12)	68-79 (73;2)
Ovary Width	18-20 (19;3)	30	30-65 (44;12)	27-33 (30;2)
Vagina	68-134 (102;4)	66-162 (84;5)	-	-
Eggs Length	-	-	75 (1)	-
Eggs Width	-	-	57 (1)	-

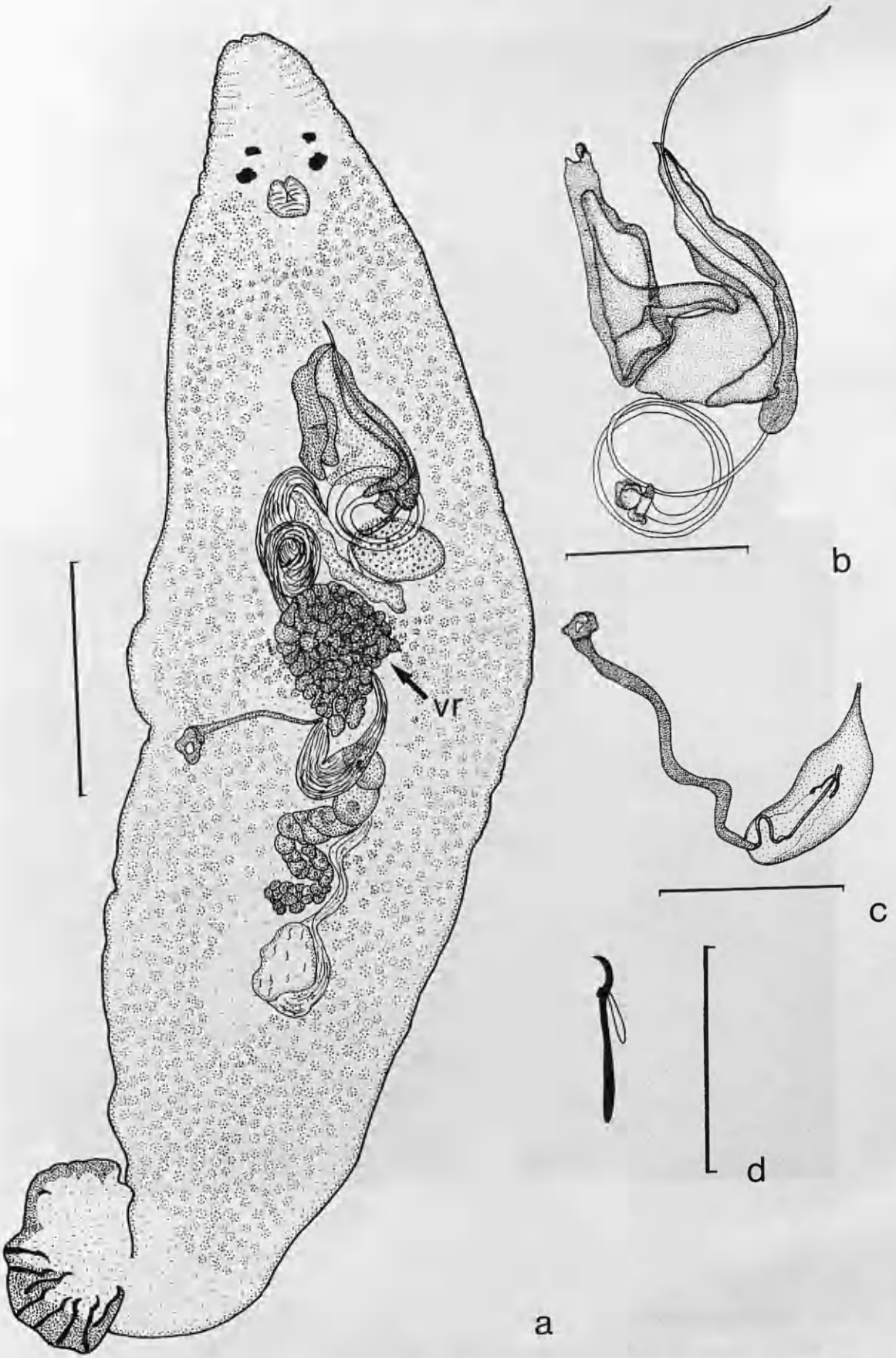


Fig.5.10 a-d *Kritskyia* sp. a - Holotype, ventral view. b - accessory piece and cirrus. c - vagina. d. marginal hook. Scale - bars: a, 100 µm; b-c, 50 µm; d, 30 µm. *Abbreviation*: vr, vitellogenic reservoir.

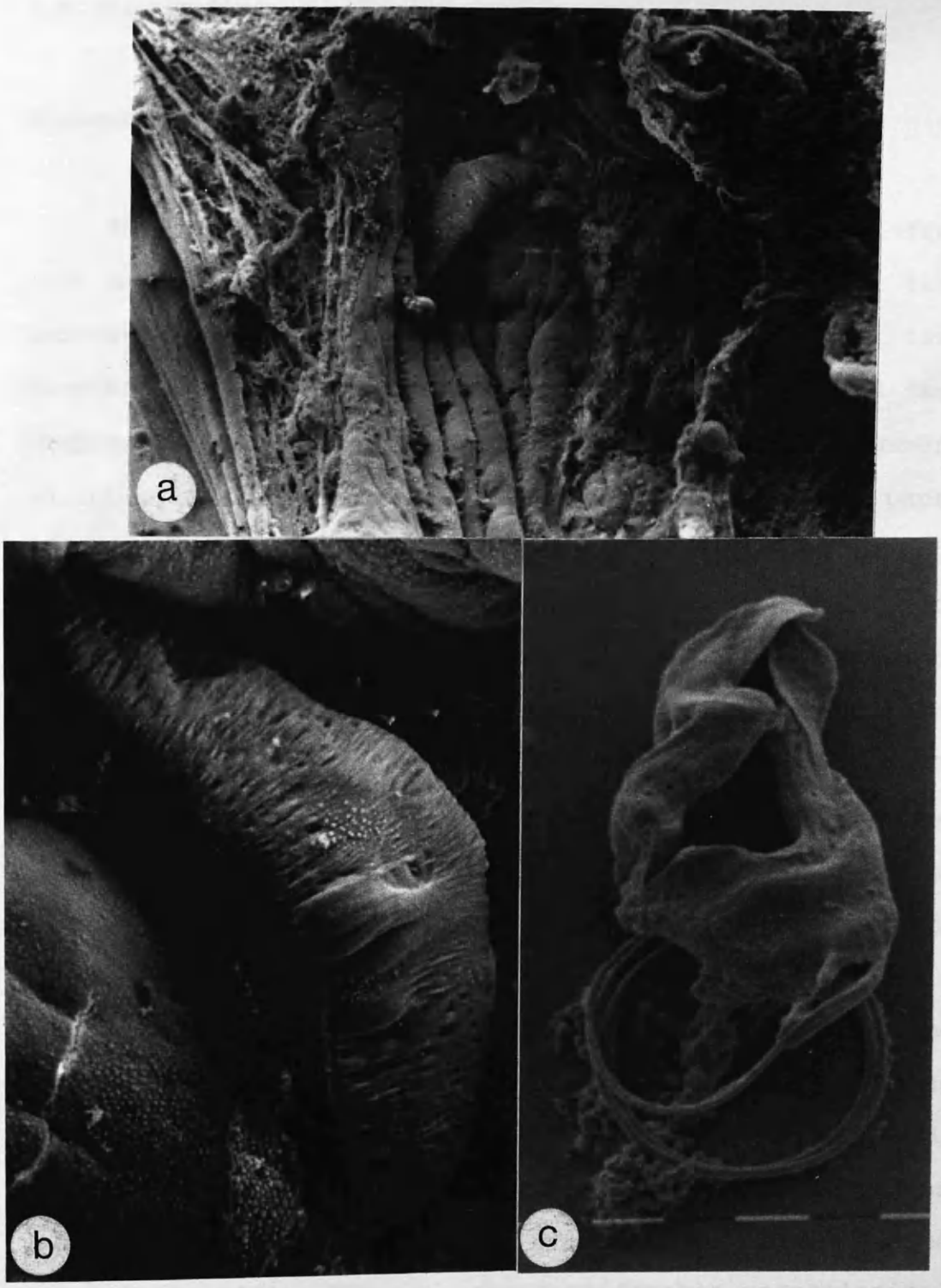


Fig. 5.11 a-c *Kritskyia* sp. a - Urinary bladder opened to show specimens of *Kritskyia* sp. attached to the mucosa (SEM - 80X). b. dorsal view (SEM -320X). Note the haptor attached to the mucosa of the urinary bladder. c - Accessory piece and cirrus (SEM - 640X). Note the anti-clockwise coiled cirrus.

5.5 Discussion

Monogenean parasites of the body and fins.

Thirteen species of *Gyrodactylus* were collected from five host taxa examined in the samples of ornamental fish imported into the UK from South America and seven host taxa examined at the exporters' holding facilities in Brazil (see chapter 4, Tables 4.12 and 4.33). In addition to *G. gemini* sp. nov., already published (Ferraz *et al.*, 1994; see paper enclosed), the species of *Gyrodactylus* from *Otocinclus* sp., *C. punctatus*, *C. robinae*, *B. splendens*, *C. julii*, *C. maculifer* and *C. sterbai*, described here also appear to represent new species for the genus. With the exception of the species parasitising *S. taeniurus*, *Otocinclus* sp., *C. hastatus*, *C. sterbai* and *B.splendens* which were collected from both the body surface and fins (see chapter 4, Tables 4.12 and 4.33), all other species were collected mainly from the fins.

Several authors have suggested that over-emphasis of the importance of host preference and site of infection in the descriptions of *Gyrodactylus* taxa might have led, erroneously, to the multiplicity of species within this genus (Ergens, 1965; Prudhoe and Bray, 1982; Bakke, Harris, Jansen and Hansen, 1992). However, the considerable morphological differences found between the parasites of the loricariids, curimatiids and callichthyids, and between the forms from different callichthyid species (*Corydoras* and *Brochis*) suggest that each new parasite described does represent a distinct species.

The number of gyrodactylids found in the small samples of South American wild ornamental fish species examined is far greater than expected, suggesting that the number of gyrodactylids that have been transported with their hosts around the world might be higher than suspected. However, these results are not surprising considering that the majority of the wild ornamental fish species exported from South America come from the Amazon basin, a region well known for the diversity of its fauna but which has been little studied.

Gyrodactylus species have often been reported in shipments of tropical ornamental fish imported from South America and the Far East (Gratzek *et al.*, 1978; Conroy *et al.*, 1981; Leibovitz, 1980d) but very little effort has been made to identify these parasites to species level, at least in the most popular tropical fish species. The few taxonomic studies found in the literature regarding this matter were conducted by Harris (1983,1986, 1989) on guppies, *Poecilia reticulata* Peters, on swordtails, hybrids of *Xiphophorus helleri* and *X. maculatus*, imported from Singapore, on *Farlowella amazonum* Günther, 1864 imported from Peru and on *S. taeniurus* imported from Venezuela into the UK by Ferraz *et al.* (1994), as part of the present study.

Among the species examined in this survey, the genus *Corydoras* is considered to be one of the two most important genera of tropical freshwater catfish as far as aquarists are concerned, with around 100 species described (Burguess, 1989). However, only one species of gyrodactylid, *P. superbus* has been described parasitising one species of this genus, *C. paleatus* (Szidat, 1973). This lack of studies may be a

consequence of the taxonomic complexity of both parasite and host, and the difficulties experienced in obtaining basic information relating to the host (e.g. site of collection, methods of handling fish prior to export and/or after import, country of origin of the fish samples), from those involved with the trade.

In general, the species of fish examined in the UK were found to be presenting single infections of different species of *Gyrodactylus* (see chapter 4; Table 4.12). In contrast with these results, out of the seven species presenting *Gyrodactylus* infections in the samples examined in Brazil, three species, *C. hastatus, C. sterbai* and *B. splendens*, exhibited mixed infections of *Gyrodactylus* (see chapter 4; Table 4.33).

Mixed infections of *Gyrodactylus* species are not commonly reported in the literature, although, up to three species have been described coexisting on the skin or the gills of some species of fish (Harris, 1985). Where mixed infections are observed in samples of wild ornamental fish obtained via trade, it should be considered that they may be transient infections resulting from the conditions to which the fish were exposed in the different stages prior to export (e.g. overcrowding, mixed stocks of different fish species, lack of disinfection of nets, lack of removal of dead infected fish from the tanks). These conditions, commonly present in the ornamental fish trade, appear to fall into the categories considered by Bakke *et al.*, (1992) as possible routes of transmission of gyrodactylids when describing transmission of *G. salaris* Malmberg, 1957 in Norway.

In general, where there were mixed infections, at least one species of *Gyrodactylus* was identified, except for those from *C. hastatus* which were not identified. Two of the species found in mixed infections in *B. splendens* and *C.sterbai*, *Gyrodactylus* sp[9] (Fig.5.9a-e) and *Gyrodactylus* sp[7] (Fig.5.7a-d) presented haptoral sclerites with some morphological similarities. Nevertheless, it appears that they may represent distinct species. Both species were provisionally placed in the sub-genus *G.* (*Limnonephrotus*), *G. wagneri* species-group, which includes several pathogenic gyrodactylid species. *Gyrodactylus* sp[1], parasitic of *Otocinclus* sp. (Fig. 5.2a-e), was also placed in this sub-genus, members of which occur throughout the Northern hemisphere.

Gyrodactylus sp[5] (Fig. 5.5 a-c) was the only member of the sub-genus *G. rarus* found in this study. Members of this sub-genus have been reported on the gills of coastal and freshwater fishes throughout North America and Eurasia (Malmberg, 1970). In South America, this sub-genus was previously reported from the body surface of *L. bimaculata* by An *et al.*, (1991) and in the present study on the fins of *C. punctatus*. Other species of *Gyrodactylus* identified were placed in the sub-genus *G.*(*Gyrodactylus*), which was represented in this study by five species, of which four were found mainly on the fins of *Corydoras* species and one, *G. gemini* was found on the body surface and fins of a curimatid.

In conclusion, the considerable number of gyrodactylids found in the samples of fish, both before and after their import from South America into the UK, suggests that they constitute one of the commonest group of parasites

transported with their hosts and present a high risk of establishing in intensive fish culture around the world, when favourable conditions are present.

Monogeneans parasites of the urinary bladder

The genus *Kritskyia* was recently erected by Kohn (1990) to accommodate the specimens of monogeneans found in the urinary bladder of South American catfish *Rhamdia quelen*. Only the type-species, *K. moraveci* is known. According to Kohn (1990) the genus *Kritskyia* is closely related to the genus *Acolpenteron* Fischthall and Allison, 1940, also found in the urinary bladder of fish but from which it differs mainly by the shape of the haptor, the absence of the 4A hooks, a sinistral vagina and by the male copulatory complex comprising of a coiled cirrus and bipartite accessory piece.

Although the specimens found in *M. aureum* presented strong morphological differences from the type-species *K. moraveci* they were also placed in the genus *Kritskyia*.

At the generic level, the main differences were observed in the number of prostatic reservoirs, the type and position of the vagina (Fig 5.10a; c) and the shape of the accessory piece (Fig.5.10b; 5.11c). At the specific level, the main differences observed were in the shape and size of the pharynx, which in *Kritskyia* sp. are small and appear to be situated further to the posterior (Table 5.5), and in the presence of the vitellogenic reservoir, which was not described in the specimens of *K. moraveci* (Fig. 5.10a). Unfortunately, this structure could only be observed in a

fully developed state in four specimens and it appears that it only becomes visible in mature specimens.

In the specimens of *Kritskyia* sp. studied only one prostatic reservoir was found in the base of the cirrus and the vagina was localized in the middle region of the body (Fig 5.10a; c). It is strongly sclerotized and has a small inverse fork structure at the end which was not always visible (Fig.5.10c). Therefore, these differences observed are not of sufficient magnitude to warrant the creation of a new genus. Consequently, an amended diagnosis has been proposed to the generic diagnosis proposed by Kritsky *et al.*, (in press) to accommodate these specimens in the genus *Kritskyia* Kohn, 1990.

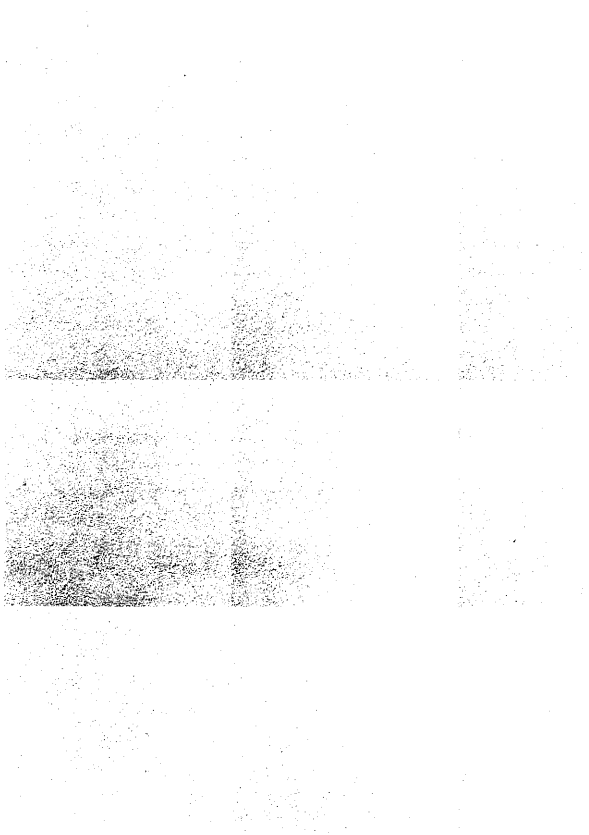
Although the specimens of *Kritskyia* sp. were found in the ureters, collecting ducts, urethra and urinary bladder, in the heavy infections they appear to concentrate in the urinary bladder where they cause severe damage in the mucosa.

As no eggs or larval stages were found in the urinary bladder of the infected fish, it was not possible to assess the development of this parasite and evaluate the hypothesis of du Plessis (1948). This author found empty egg shells, free-living larvae, and young parasites, inside the ureters, which lead him to suggest that the whole life-cycle may be completed within the ureters. Young parasites were also observed inside the urinary bladder and urethra in the present study, but it was not possible to determine if they were recently acquired by the host or if they were a result of auto-infection.

The silver dollar is a very popular ornamental fish and wild and tank-bred species have been imported into the UK

from South America, the United States and Asian countries. As no tank-bred silver dollars were examined it is not known if *Kritskyia* sp. has already been introduced in intensive cultures into the United States and Far East, a strong possibility since wild broodstock have often been exported from South America to these countries for breeding purposes.

In conclusion, the morphological differences observed between the species of *Kritskyia* sp. parasite of *M. aureum* and *K. moraveci* parasite of *R. quelen* indicate that *Kritskyia* sp. does constitute a new species for the genus. Its life-cycle may be completed inside the urinary bladder, but, more studies should be conducted to investigate its complete development.



Chapter 6

Pathology

6.1 Introduction

Protozoans and helminths are commonly found on wild and tank bred fish and some are responsible for severe mortalities of fish under both natural and artificial conditions. In addition to mortalities these parasites can cause a number of deleterious effects which may result in the rejection and/or devaluation of fish destined for the ornamental fish market.

The pathogenicity of the most common species of protozoans and helminths has been studied through several different approaches in the last 25-30 years and can be found in several compilations, e.g. Hoffman (1967) Ribellin and Migaki (1975), Reichenbach-Klinke and Landolt (1973), Roberts (1978), Thatcher (1991) and Ferguson (1992). However, the pathology of many species is still poorly understood. In this study, although several species of parasites were found (see chapter 4), only 4 species were seen to be causing some pathogenicity to their hosts, of which one was responsible for mortalities both before and after export. The most serious pathogens were the protozoan *Piscinoodinium* sp., the monogenean parasite of the urinary bladder, *Kritskyia* sp., the larval stages of two digeneans, *Clinostomum* sp., and the mesocercariae of Strigeoidea. Consequently, their pathology was studied in more detail.

6.1.1. The pathology associated with *Piscinoodinium* sp.

The genus *Piscinoodinium* (Protozoa, Dinoflagellida) was proposed by Lom (1981) to contain the species, *Oodinium*

pillulare (Schaperclaüs, 1954). This parasite of freshwater tropical fish is responsible for the disease commonly known as "rust disease" or "velvet disease" due to the appearance of the fish caused by the attached trophont. The trophont, after a period of growth of approximately 6 days at 25 °C, releases from the fish, sinks to the bottom, rounds off and becomes a tomont. The tomont divides successively into small cells, tomites, which differentiate into gymnospores. After having found a convenient host, the gymnospore adheres to its surface by the sulcal flagellum, and in the bottom sulcal region a protuberance appears to develop into the holdfast, the attachment disc (Lom and Dyková, 1992).

The trophont of *Piscinoodinium* has a sac-like or pyriform shape, a short peduncle and a small disc of attachment as shown in Fig 6.1. (Lom, 1977; Lom and Schubert, 1983; Lom and Dyková, 1992). This protozoan appears to be non-specific and has been found attached to the skin, gills, fins, epithelium of the oesophagus, and intestine of several species of fish from tropical and temperate regions (Lom and Dyková, 1992). Although considered to be one of the major pests of freshwater aquarium and cultured fish, and commonly reported in the literature (Laird, 1960; Ghittino, 1970; Schubert, 1978; Needham and Wootten, 1978; Reichenbach-Klinke, 1980; Rogers and Gaines, 1975), only a few studies have been conducted on their pathogenicity (Lucky, 1970; Shaharom-Harrison, Anderson, Siti, Noor, Shazili and Azmi, 1990). According to the most recent studies of Lom and Schubert (1983), their pathogenicity may be caused by the rhyzocysts, microfilamentous structures present on the disc of attachment of the trophont that penetrate into the

cytoplasm of the epithelial cells (Lom and Schubert,1983; Lom and Dyková, 1992; Fig. 6.2). Alternatively, the effects of this protozoan may be similar to those of the marine species *Amyloodinium ocellatum* (Brown, 1931) which was suggested by Paperna (1980) to produce toxic substances or irritants which could be responsible for the extensive epithelial changes observed.

Piscinoodinium pillulare was considered by Becker (1977) to be primarily a skin parasite which may spread to the gills of fish. However, whereas some studies reported both skin and gill pathology (Schaperclaüs, 1951;1954; Lucky, 1970), Shaharom-Harrison *et al.*, (1990) reported that this protozoan mainly induced gill pathology.

Heavily infected fish have been reported to present signs of "discomfort", spreading opercula, clamped fins and emaciation (Shaharom-Harrison *et al.*, 1990). The parasite was observed in numerous colonies on the skin and gills where they caused a thickening of the epithelium in which a large number of "pouchy" (Schaperclaüs, 1951) mucous cells appeared (Schaperclaüs, 1951; Lucky, 1970). On the gills, Shaharom-Harrison *et al.*, (1990) reported a range of responses varying from acute (the oedematous separation of the respiratory epithelium) to chronic (acute epithelial hyperplasia involving entire gill filaments), leading eventually to epithelial cell degeneration and necrosis which can result in the death of fish less than one week after exposure.

As *Corydoras* species are mainly captured during the dry season, they are subjected to a long holding period quite often under overcrowded conditions. Consequently, *Piscinoodinium* infections were commonly found in the holding

tanks at the exporters' holding facilities and in several species from the same shipments. This parasitic disease appears to be responsible for severe losses prior to export. Because *Piscinoodinium* sp. was found to be such a common and serious parasite, the pathogenicity of which is group of fish is little understood, it was subjected to further studies.

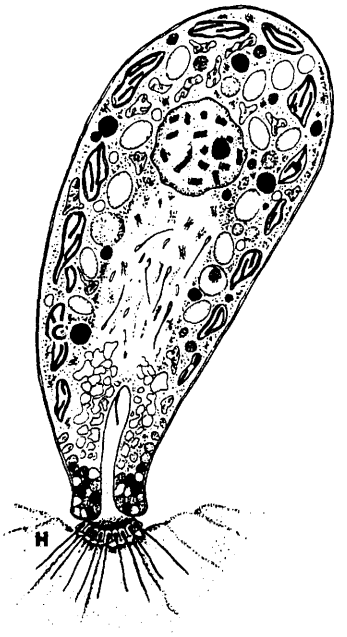


Fig. 6.1 *Piscinoodinium pillulare*: diagram of a trophont attached to the host cell (H). (Source: Lom and Schubert, 1983)

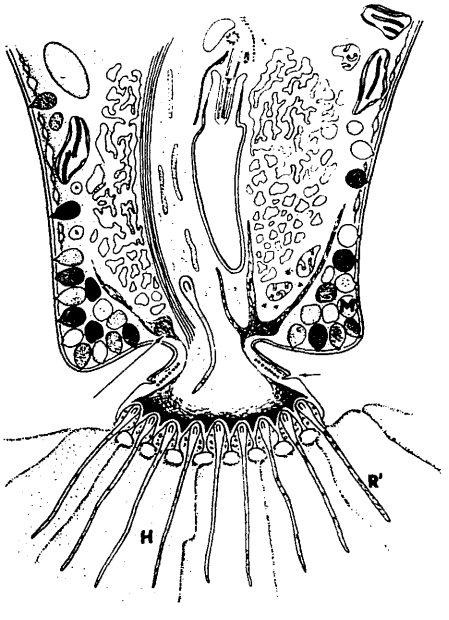


Fig. 6.2 *Piscinoodinium pillulare*: diagram of the basal part and attachment disc. (Source: Lom and Schubert, 1983) R - rhyzocysts "in position" within rhyzotheca. H - Host cell.

6.1.2 The pathology associated with monogenean parasites of the urinary bladder.

The deleterious effects of the monogenean parasites of the natural cavities and internal organs of fish are virtually unknown. In part, this lack of information may be related to the fact that very few species are known and the majority of them have been described from material collected from wild hosts, where these parasites appear to occur at a low intensity of infection. Currently, all of the species known to parasitise the excretory system of fish do not present hamuli in the haptor (Fischthal and Allison, 1941; du Plessis, 1948; Kohn, 1990) and the pathology, if present, appears to be mainly caused by mechanical effects due to obstruction of the ureters in heavy infections (du Plessis, 1948), feeding, and/or irritant substances secreted by the cephalic glands, which are not so obvious in fish exhibiting a low intensity of infection .

Among the species so far described, only the pathogenicity of one species, *Acolpenteron ureteroecetes* Fischthal and Allison, 1940, has been reported (du Plessis, 1948). However all the pathology described by this author was based on the gross appearance of the kidney; no histopathology was performed. The external signs observed were mainly related to the intensity of infection of the parasite, with the infected fish generally having a cloaca with a reddish and swollen border, a condition which became extended to the region immediately anterior to the cloaca in the acute infections. Heavily infected fish were reported to

have an obstructed ureter and the deaths were generally thought to be associated with the failure of the kidney.

For the other genus of parasite of the urinary bladder, *Kritskyia* Kohn, 1990, no pathology has yet been described.

A large number of studies have been conducted on the monogenean parasites of the skin and gills. However the presence of monogeneans in the urinary bladder is unusual, and hence the damage caused by these parasites in the excretory system is largely unknown. Due to its unusual location, and because *Kritskyia* sp. may represent a serious problem for fish in intensive culture and/or home aquaria, this parasite was subject to further studies.

6.1.3. The pathology associated with metacercarial stages

Several species of metacercariae were found in the fish species examined (see Chapter 4; Tables 4.9 and 4.19), but only two of these species were associated with pathologies in the samples of ornamental fish examined. These were the metacercariae of *Clinostomum* sp. and the mesocercariae of Strigeoidea.

Metacercariae of *Clinostomum* sp., commonly known as yellow-grubs, may be found in all tissues throughout the body of freshwater fish. These metacercariae have been responsible for severe losses of fish (mainly cichlids) in culture and in managed fisheries, where they have also been reported to be responsible for the rejection of these fish for human consumption due to the high level of infection (Paperna, 1964; Kabunda and Sommerville, 1984). Their pathology has been quite extensively studied in different fish species and

the damage to hosts has been mainly related to their large size, the number of metacercariae present, the localisation of the cysts, and the excystment of the metacercariae. Large cysts localised near the surface of the body cause erosion of the skin and may ulcerate (Sommerville, 1980). Histologically, degenerative changes in the muscle tissues around the cysts, pressure atrophy, degeneration of the hepatic cords, and cellular infiltrations in the kidney, have been reported Kalantan, Arfin and Nizami (1987) and Lo, Wang and Khou (1992).

Four types of metacercarial forms have been described among the strigeids, of which tetracotyle, neascus and diplostomulum appear to be the most common. The fourth type, known as the mesocercaria, is interposed between the cercaria and metacercaria, so these larval stages require four successive hosts. (Olsen, 1974; Chandler and Read, 1971).

The larval stages of strigeids appear to be a world-wide problem and are well known by the pathology that they cause in the eyes, heart, skin, brain and other organs of fish (Hoffman, 1975; Shariff, Richards and Sommerville, 1980; Watson, Pike and Priede, 1992). According to Szidat (1969), these larval forms appear to constitute the predominant group among the metacercariae found in freshwater fish, particularly in tropical and sub-tropical regions.

The pathologies caused by some of these larvae, such as those from the genera *Diplostomum, Apatemon* and *Posthodiplostomum,* are quite well understood (Kozicka, 1958; Hoffman, 1975; Shariff *et al.*, 1980; Watson *et al.*, 1992), but others such as those caused by the mesocercariae, appear to have been little studied. Thatcher

(1991) reported that these mesocercariae are quite often found spread throughout the body of fish from the Amazon region, but no pathology was described.

Metacercarial forms were one of the most common findings on the ornamental fish species examined, and they may be responsible for some losses for the wild ornamental fish industry, principally during the dry season. The pathology caused by these forms from tropical regions is not well known. Consequently, further investigations were conducted with those species found to be in the present study associated with pathological conditions.

6.2. Study aims

Protozoans and adult and larval stages of helminths were commonly found in the species of ornamental fish sampled in the UK and Brazil. However, the protozoan *Piscinoodinium* sp., the monogenean *Kritskyia* sp., and the metacercariae discussed above, were consistently observed to induce pathology in their hosts. The aim of this study was to describe the main pathology caused by these parasites in the ornamental fish examined in order to understand the pathological processes involved.

6.3 Materials and methods

6.3.1 Histological techniques

The methods for sampling tissue were described in Chapter II, section 2.1.4. The main modification to the

general procedure was performed with the samples of skin of *Corydoras* and *Brochis* species. After fixation, all samples were placed in a solution of EDTA di-sodium (for decalcification of the scutes) for a period of 4 to 5 days, during which time the solution was changed twice. Subsequently, they were processed using routine histological methods.

6.3.2 Scanning electron microscopy (SEM)

The procedures for the SEM studies of the skin of *Corydoras* and *Brochis* species were described in chapter 2, section 2.1.5.

For the studies of the monogeneans of the urinary bladder *in situ*, the urinary bladder and kidney were removed intact from the host and fixed. Subsequently, they were dissected and processed as described in chapter 2, section 2.1.5.

6.4 Results

6.4.1 Pathology caused by *Piscinoodinium* sp. on *Corydoras* and *Brochis* species.

6.4.1.2 External signs

Hosts: *C. melanistius; C. metae; C. julii; C. punctatus;* and *Brochis splendens*

All infected fish, *Corydoras* spp. and *B. splendens*, showed similar clinical signs of which the most evident was that they swam weakly to the surface of water and gave no resistance during handling. The rust colour was observed mainly in fish which were heavily infected. Petechia and slight inflammation were often observed on the surface of the body, although these can also be caused by perforation of the skin by the spines of fins from other specimens of fish in overcrowded conditions.

6.4.1.3. Pathology observed in the skin.

Infected fish were found with large numbers of trophonts of different sizes (9x8 to 70x39 μm) on the surface of the body, surrounding the eyes, nasal and buccal cavities and on the gills.

A heavy infection was also evident from the SEM studies of the skin of the *Corydoras* species infected. Large numbers of trophonts were found deeply embedded in the crypts formed by their penetration of the epidermis (Figs. 6.3 and 6.4).

Each attached trophont appeared to be damaging 3 to 4 epithelial cells on the surface of the epidermis (Fig.6.4) and the cavities so formed were seen enclosing one or more trophonts or were empty. Unfortunately, due to their deep penetration in the epidermis, the disc of attachment with the rhizocysts in rhizothecas were not observed. For this reason, the identification of this species was only to generic level.

In histological sections of the skin, trophonts of varied size were seen to be attached deep within depressions of different depths (Fig. 6.5a-b). The trophonts were oval or round with a basophilic macronucleus, filled with large achromic and refractile granules, and with a short peduncle and its disc of attachment (Fig.6.6).

The crypts so formed were lined by flattened epithelial cells with nuclei compressed into an elongated shape and pale eosinophilic cells into which the parasite rhyzocytes were inserted (Fig.6.5b; 6.6).

An extensive epidermal erosion was caused by the attached trophonts in the epidermis of *B. splendens* and *Corydoras julii* (Fig.6.7 a-b) and an intensive cellular infiltration was commonly present in the basal layer where the trophonts were attached (Fig.6.6). Cloudy swelling, pycnotic and karyorhectic nuclei, were also observed in the cells surrounding the point of attachment of the parasite, suggesting that these cells were undergoing a degenerative process (Fig. 6.8). Trophonts were never found in the dermis of infected specimens of *B. splendens* and *Corydoras* spp., but were exclusively found in the epidermis above the basement membrane.

Trophonts were also commonly found completely enclosed by hyperplastic skin cells some of which were apparently dead (Figs 6.9 and 6.10). Large vesicles containing granular material, probably the debris of dead club cells or dead trophonts, were commonly found in the area around the enclosed trophont.

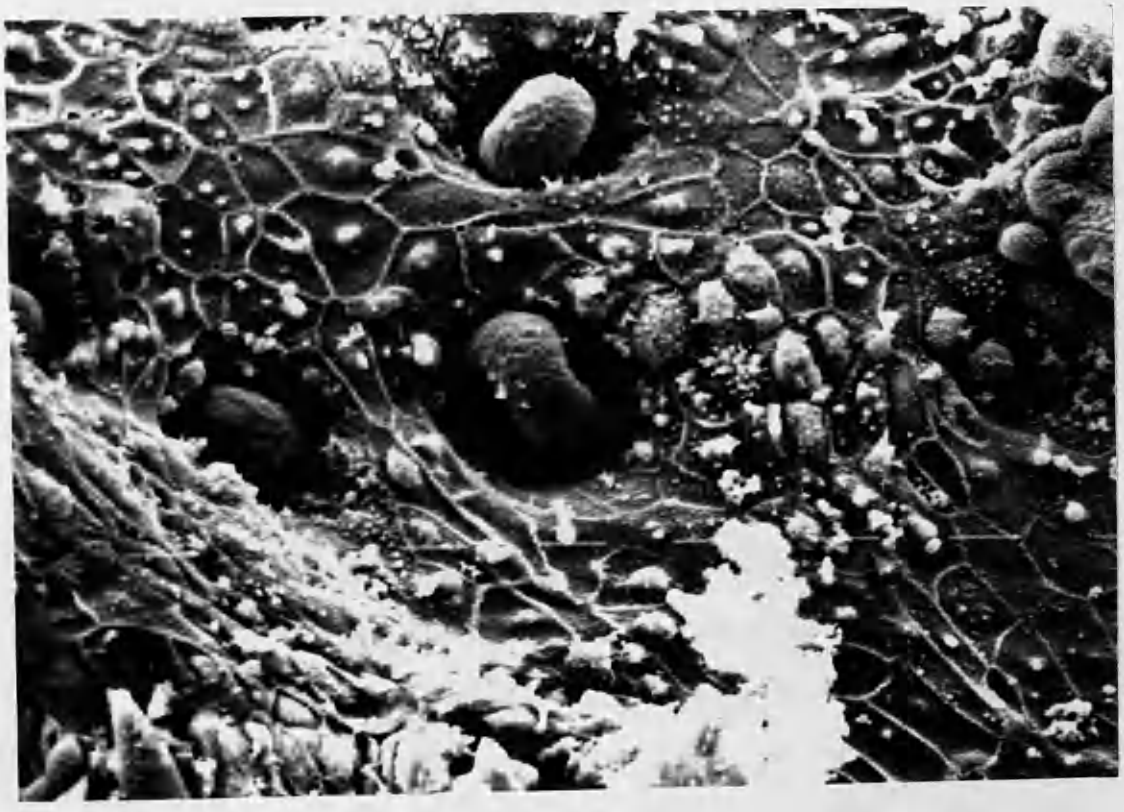
In general, the pathology caused by *Piscinoodinium* sp. on the skin of *Corydoras* spp. and *B. splendens* was very similar, with the main difference appearing to be related to the different level of intensity of infection present.

6.4.1.4 Pathology found in the gills.

In all of the gills, trophonts were attached to the epithelium between filaments where they appeared to be located in a slight depression of the epithelium or in crypts formed by the hyperplastic epithelial cells or fusions of the secondary lamellae (Fig. 6.11). Only a few specimens were found presenting severe infection in the gills. In these specimens, a generalised hyperplasia was present in all of the filaments causing a complete fusion of the filaments of the secondary lamellae. On the gills the *Piscinoodinum* infection was commonly associated with hypertrophy, focal and diffuse hyperplasia, oedema of the respiratory epithelium, and lamellar fusion, resulting in a reduced respiratory surface and efficiency. Although this protozoan was seen to cause severe damage to the gills, some pathology, associated with the water quality of the holding tanks where the fish were kept prior to export was also evident in these fish.

Fig. 6.3 SEM lower power view of the skin of *Corydoras* spp. showing the presence of empty cavities left in the skin after the trophonts have dropped from the host (arrow) (SEM-1250X).

Fig.6.4 Trophonts of *Piscinoodinium* sp. inside the crypts formed in the areas of attachment of the protozoan. Note that each cavity formed appear to be covering 3 to 4 epithelial cell on the surface of the epidermis. (SEM - 2500x)



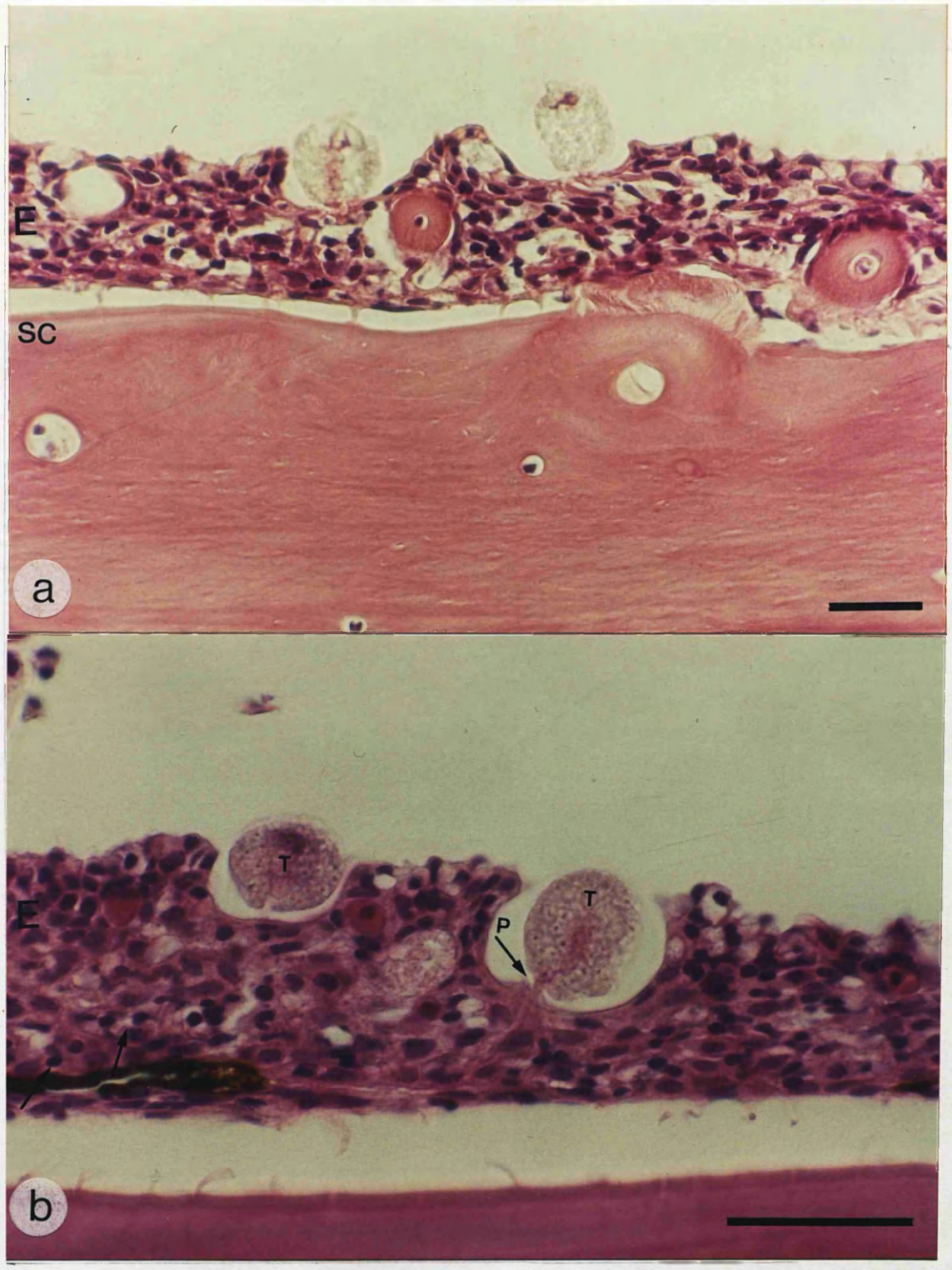


Fig. 6.5 a-b Cross section of infected skin of *B. splendens*. Note the flattened cells and pale eosinophilic cells surrounding the point of attachment of the parasite. T= trophont; P= peduncle (arrow); E= epidermis; Sc= scutes (H+E). *Scale bar* - a-b 50 µm.

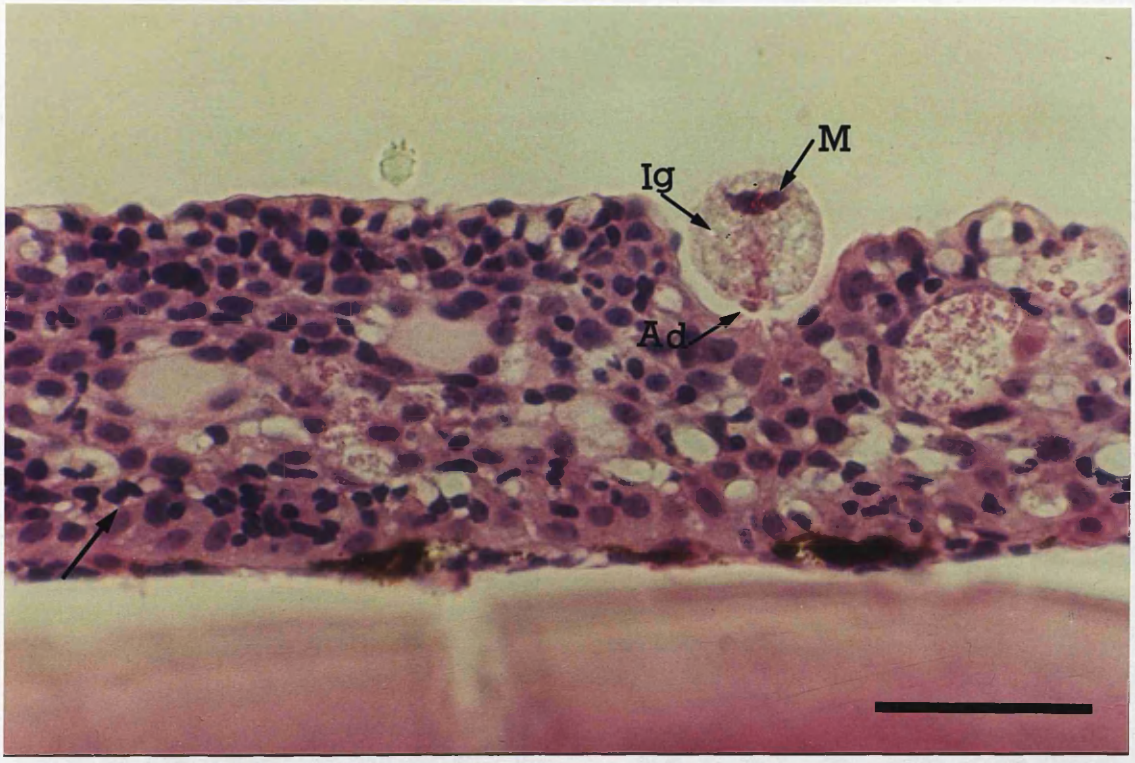


Fig.6.6 Trophonts attached to the epidermis of *B. splendens*, note the intracytoplasmic granules (arrowed Ig), macronucleus (M) and attachment disc (Ad) and cellular infiltration in the basal layer (arrowed) (H+E). *Scale bar* - 50µm.

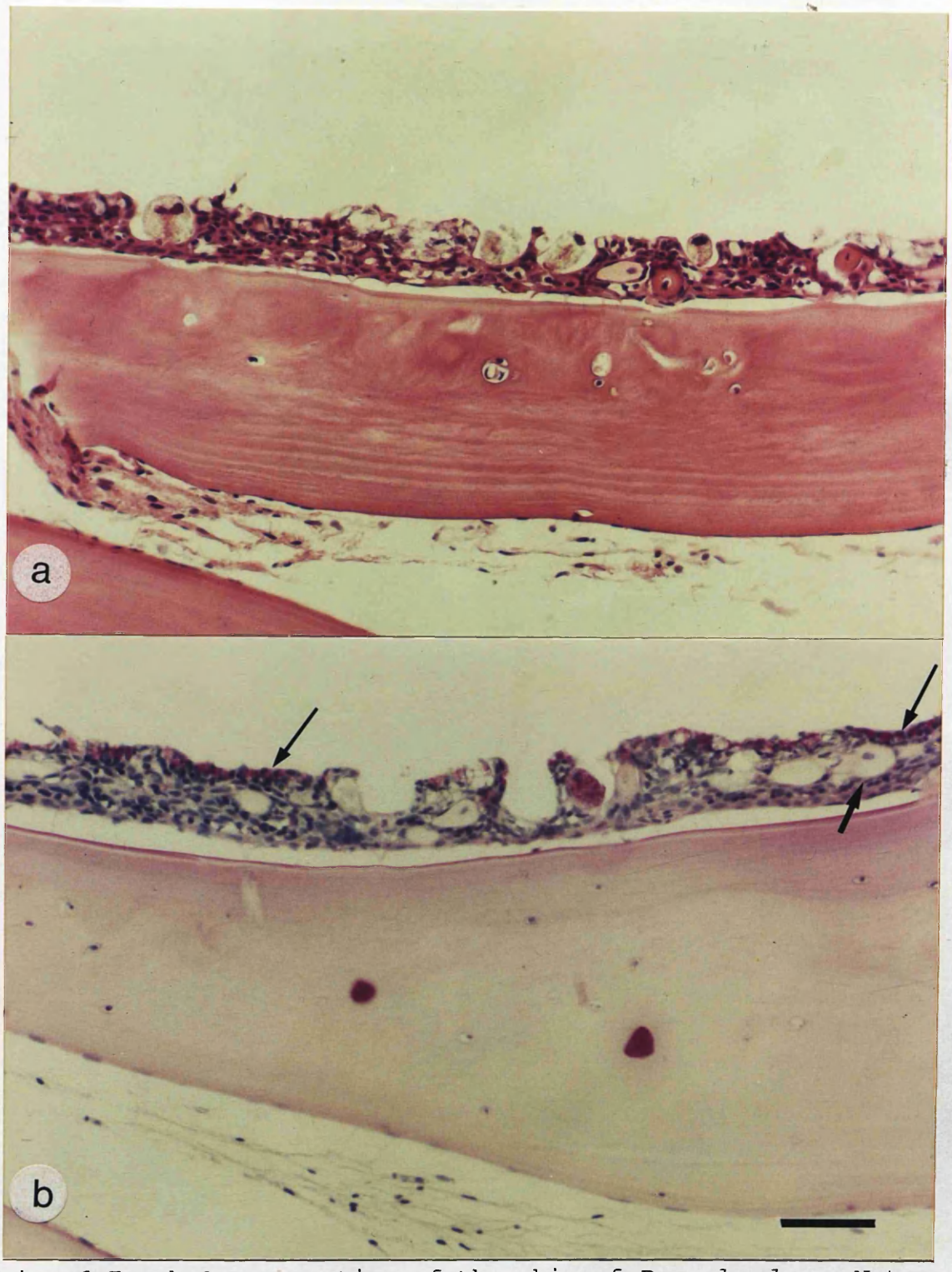


Fig. 6.7 a-b Cross section of the skin of *B. splendens*. Note the extensive epidermal erosion caused by the trophonts of *Piscinoodinium* sp. a. Epidermis with the trophonts attached (H+E); b. Epidermis showing a layer of mucous cells (long arrow), club cells (short arrow), and ex-point of attachment of the trophonts (PAS). *Scale bar* - a-b 50µm.

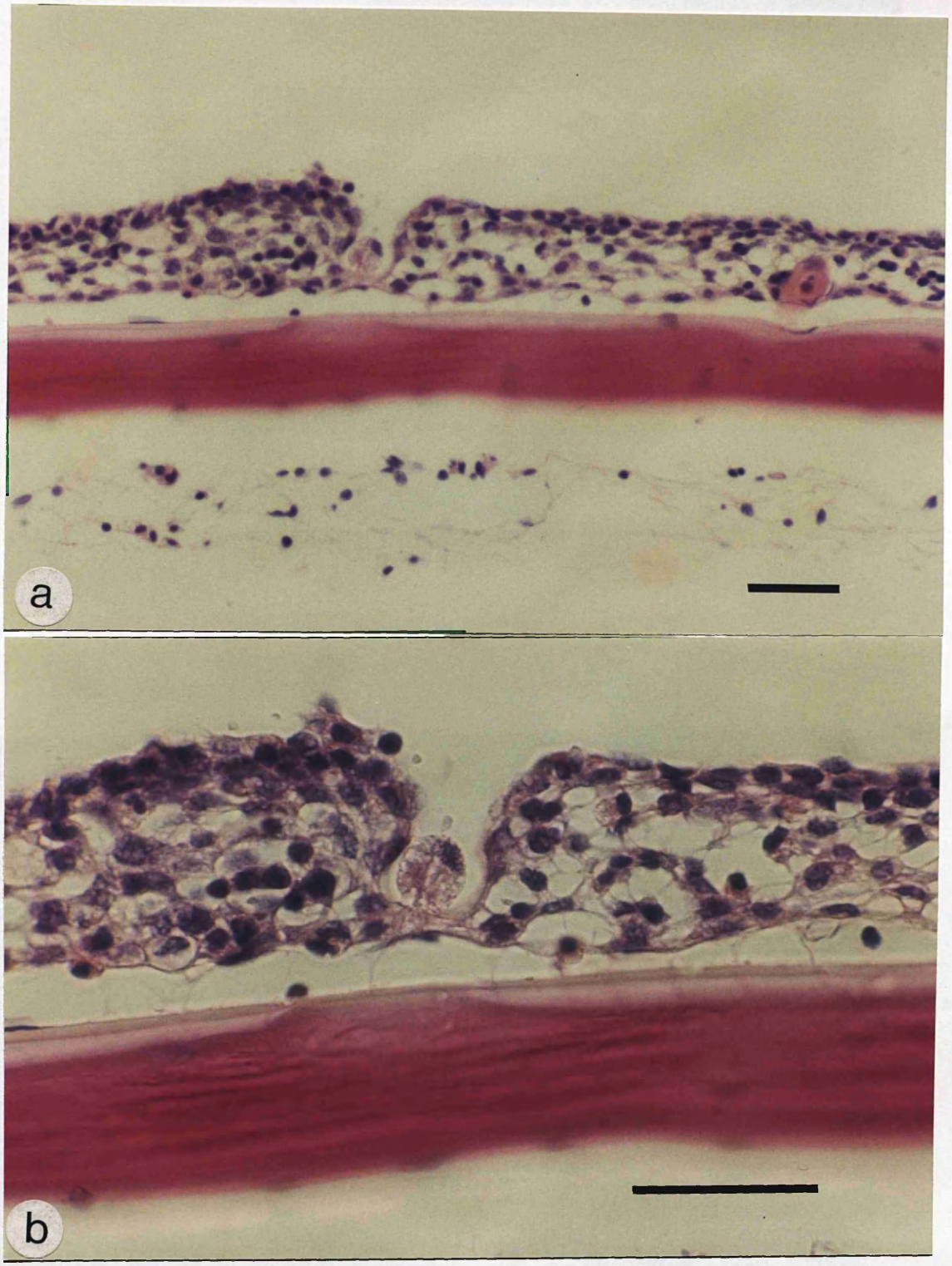


Fig.6.8 Cross section of the skin of *Corydoras julii*. Note that the cells are undergoing degenerative processes and the presence of pycnotic debris in the area surrounding the attached trophont (H+E). *Scale bar* - a-b 50µm

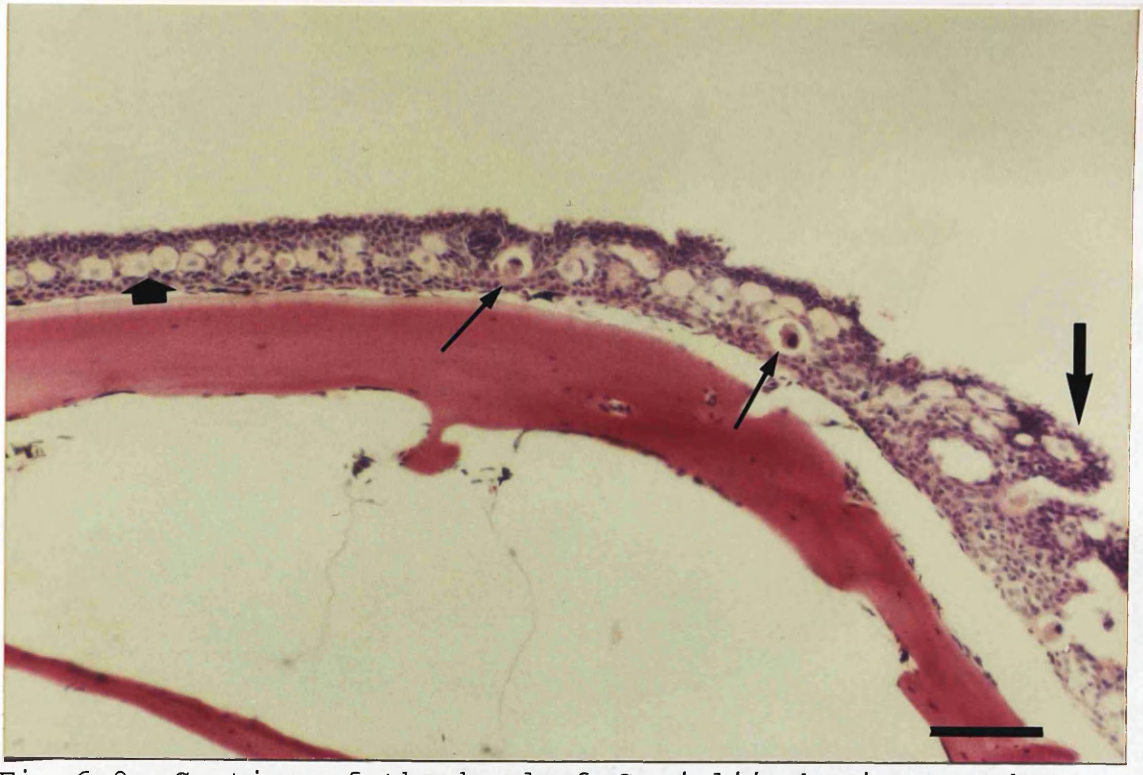


Fig.6.9 Section of the head of *C. julii* showing trophonts enclosed by the epithelial cells (long thin arrow). Note the row of club cells present (short thick arrow) and an area of marked hyperplasia where the trophonts are attached (long thick arrow) (H+E). *Scale bar* - 100µm.

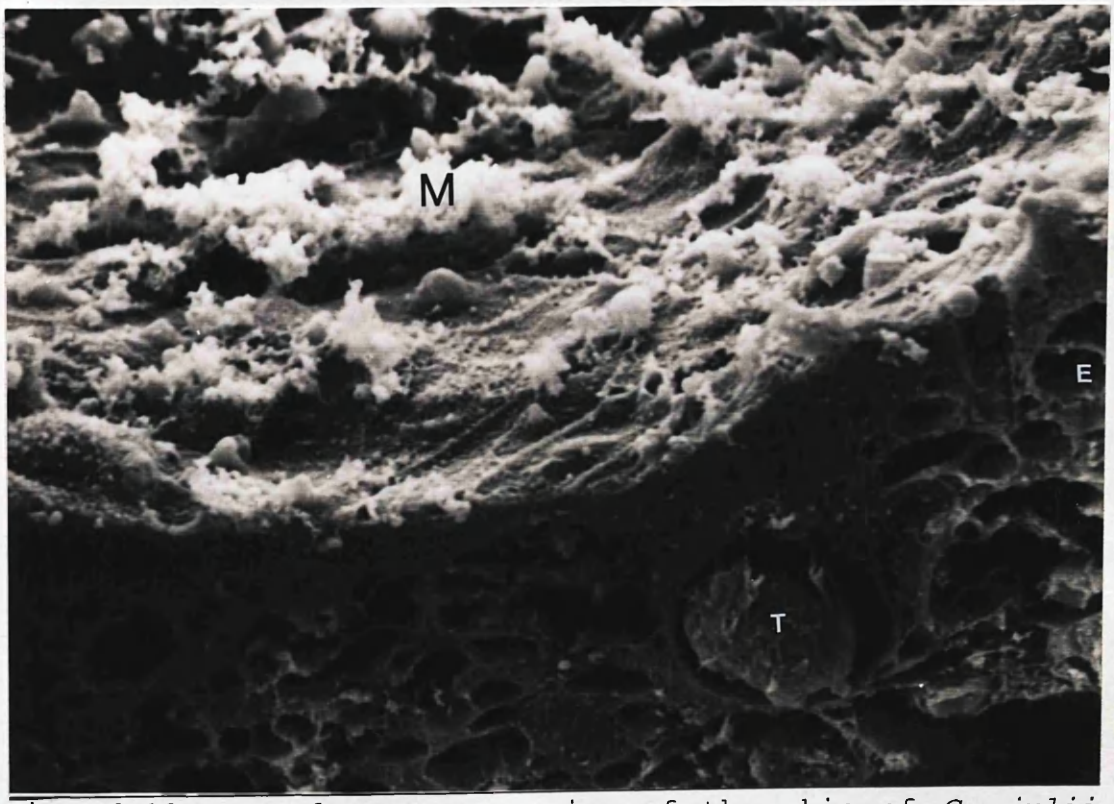


Fig. 6.10 SEM lower power view of the skin of *C. julii* showing a trophont completely enclosed within the epidermis (SEM). M = Mucous on skin surface; E= epidermis; T= trophont enclosed in epidermis (SEM - 1250X).

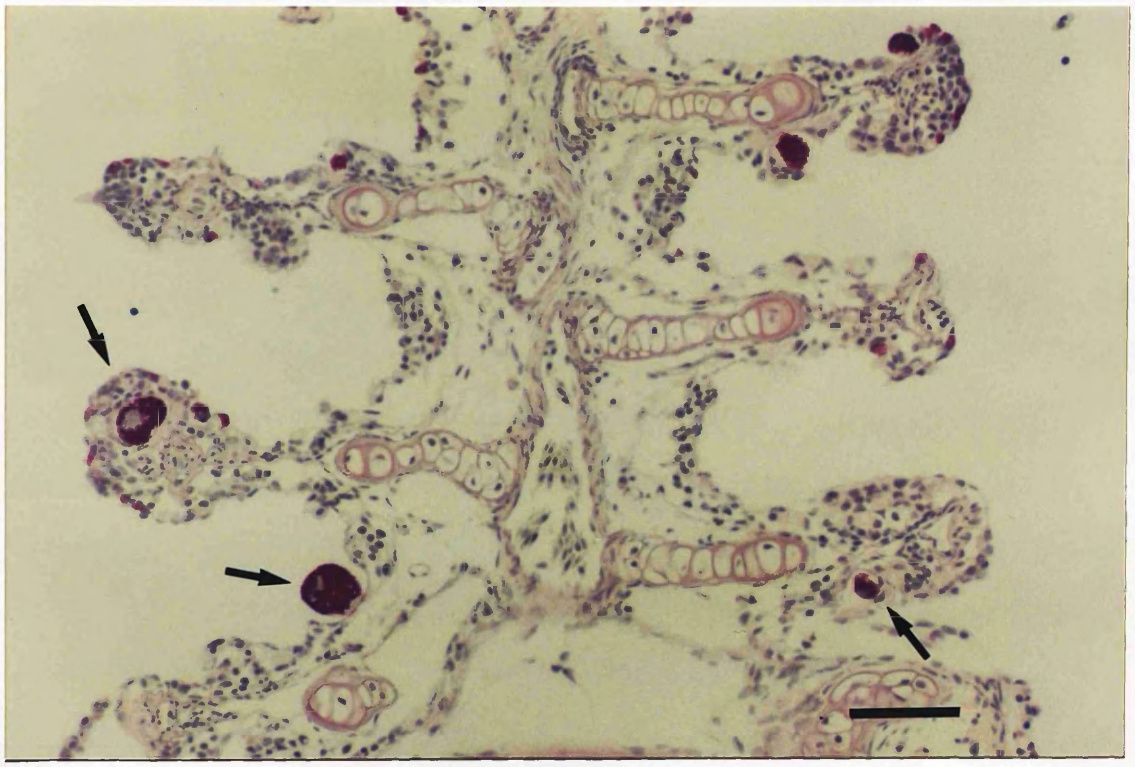


Fig. 6.11 Section of the gill filaments showing attached trophonts between filaments of the secondary lamellae and trophonts enclosed by the hyperplastic cells (arrowed). Note also the presence of oedema of the epithelium of the primary lamellae (PAS) *Scale bar* - 50µm.

6.4.2 Histopathology of *Kritskyia* sp. in the urinary bladder.

6.4.2.1 General description of the urinary bladder.

Host: *Mylossoma aureum* (Spix)

The urinary bladder is a thin elongated sac-like structure located in the posterior region of the ureter. Two distinct layers were distinguished in the urinary bladder: the inner layer consisting of one or two layers of columnar epithelial cells lining the inner lumen, and the external layer consisting of a layer of connective and smooth tissue.

6.4.2.2 Pathology caused by *Kritskyia* sp.

Generally, no external signs were observed in the infected fish and the monogeneans were found only on necropsy.

Monogeneans were found attached to the epithelium of the collecting ducts, ureters, urinary bladder and urethra or loose in these areas. However, all infected specimens were also infected by myxosporideans, with spores of the genus *Myxobolus* found in the epithelium of the urinary bladder and all the way up to the proximal part of the renal tubules. For this reason, in some parts of this description, some pathologies which may be associated with the presence of the myxosporideans are also presented.

In light infections the monogeneans were found distributed only in the ureter and in the urinary bladder.

They were associated with minimal pathology. Generally, some focal hyperplasia was present in the epithelium of the urinary bladder at the point of attachment of the parasite (Fig. 6.12). Hyperplastic areas were also present in the epithelium of the collecting ducts and ureter where the parasite was not attached, possibly indicating previous points of attachment of the parasite. The cells of the inner layer of the epithelium were often swollen and the cytoplasm appeared faintly granular suggesting that the epithelium was undergoing a degenerative process (Fig. 6.12). Consequently, the epithelium of the urinary bladder, independent of the level of infection by the monogenean, was seen to be completely lifted away (Fig.6.13) or more frequently, the cell junction between the epithelium and the basal layer were separated, resulting in the formation of vesicles (Fig. 6.12 and 6.18).

Areas of disruption of the epithelium were occasionally present which may have been caused by feeding of the parasite and/or release of the spores of *Myxobolus* sp. (Fig.6.12). No inflammatory cell response was found in the surrounding urinary bladder of these specimens.

Specimens exhibiting moderate infections were found with a localised inflammatory cell response in the tissue surrounding the urinary bladder. As granulocyte cells appear to be distributed in different parts of the basal layer, and small granulomas were also present (Fig 6.14), it is not clear if this response was due to the mechanical effect of the parasite and/or the presence of sporogonic stages of the myxosporideans in the epithelial cells. In these specimens the epithelium of the urinary bladder and ureter were

extremely hyperplastic and were producing papillary ingrowths of epithelial cells of different sizes into the lumen. Occasionally degenerated spores and necrotic cells were found in these areas.

Heavily infected specimens were found with the urinary bladder slightly distended and surrounded by an intense inflammatory infiltrative cell response (Fig.6.15). The monogeneans were mainly concentrated inside the urinary bladder, but young and mature specimens were also found in the collecting ducts, ureters and urethra.

In the urinary bladder, the epithelium was exhibited severe changes which may have been caused by the presence of large numbers of the monogeneans. The normal epithelium of the urinary bladder of *M. aureum* appears to be composed of columnar cells. In the infected specimens the epithelium showed localised hyperplastic areas which were seen surrounding the small granulomas present and/or as small papillary ingrowths of epithelial cells into the lumen of the urinary bladder (Fig. 6.15), and areas where the cells became flattened giving the appearance of an epithelium composed of squamous cells (Fig.6.16). It should be noted that in these metaplastic areas the cell-to-cell contact in the basal layer appeared to be breaking down (Fig.6.17 and 6.18). Cell debris and disrupted epithelium were also present inside the urinary bladder (Fig.6.16), probably caused by the attachment of the parasite, feeding, or release of the spores of *Myxobolus* sp. present in other parts of the excretory system.

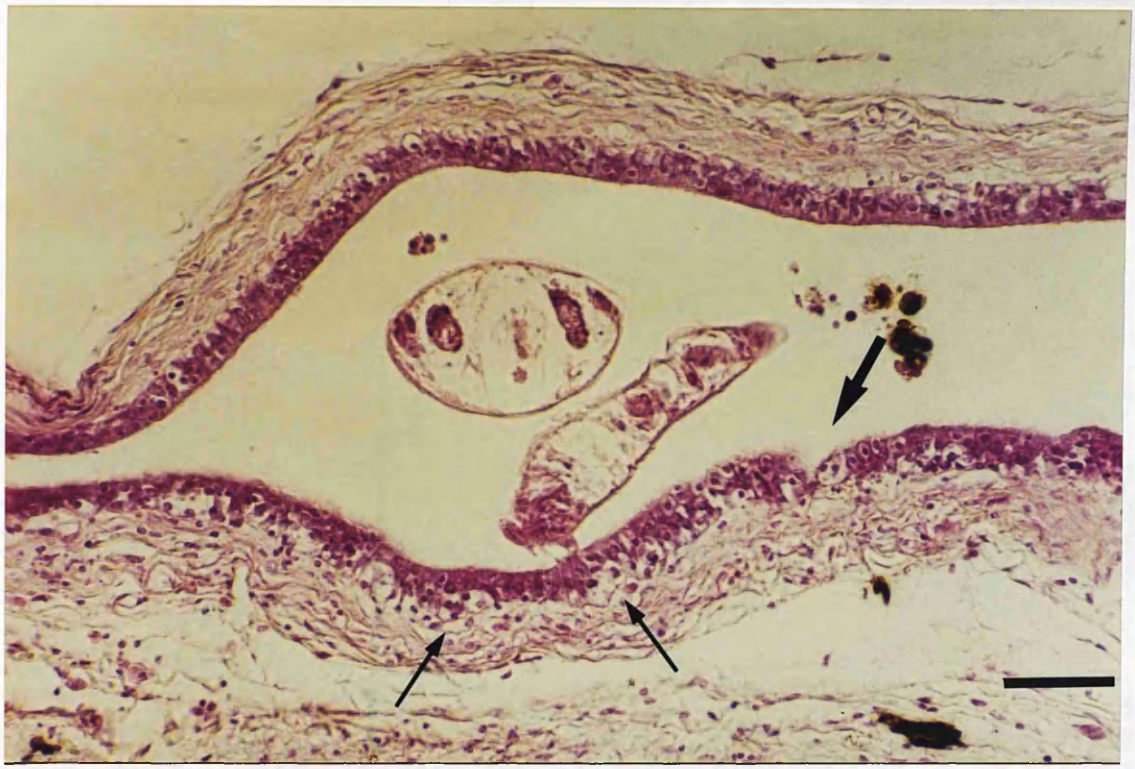


Fig. 6.12 *Kritskyia* sp. attached to the epithelium of the urinary bladder. Note the cells of the basal layer undergoing a degenerative process (thin arrow) and the disruption of the epithelium caused by feeding or release of spores of *Myxobolus* sp (thick arrow). (H+E) *Scale bar* = 50µm.

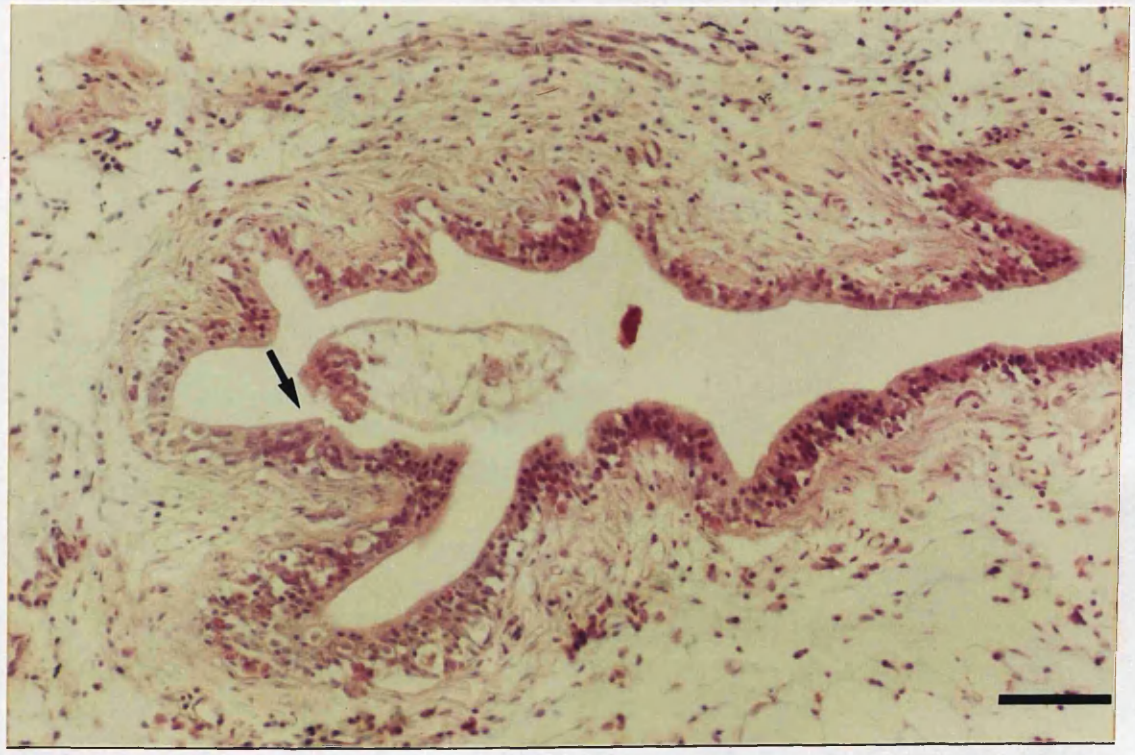


Fig. 6.13 *Kritskyia* sp. - Note at the point of attachment the epithelium is lifted out (arrow) (H+E). *Scale bar* = 50µm.

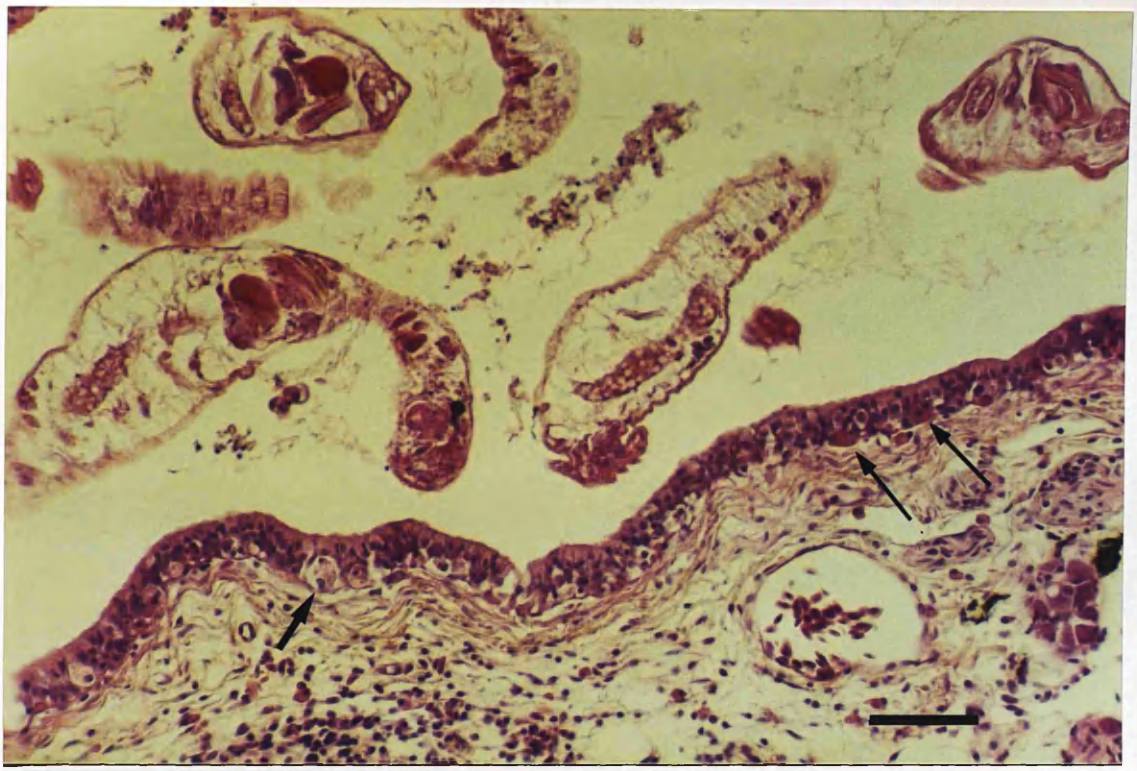


Fig 6.14 Section of the urinary bladder of a specimen presenting moderate infection. Note the presence of the granulocyte cells (thin long arrow) and small granulomas in the basal layer (short arrow) (H+E). *Scale bar* = 50µm

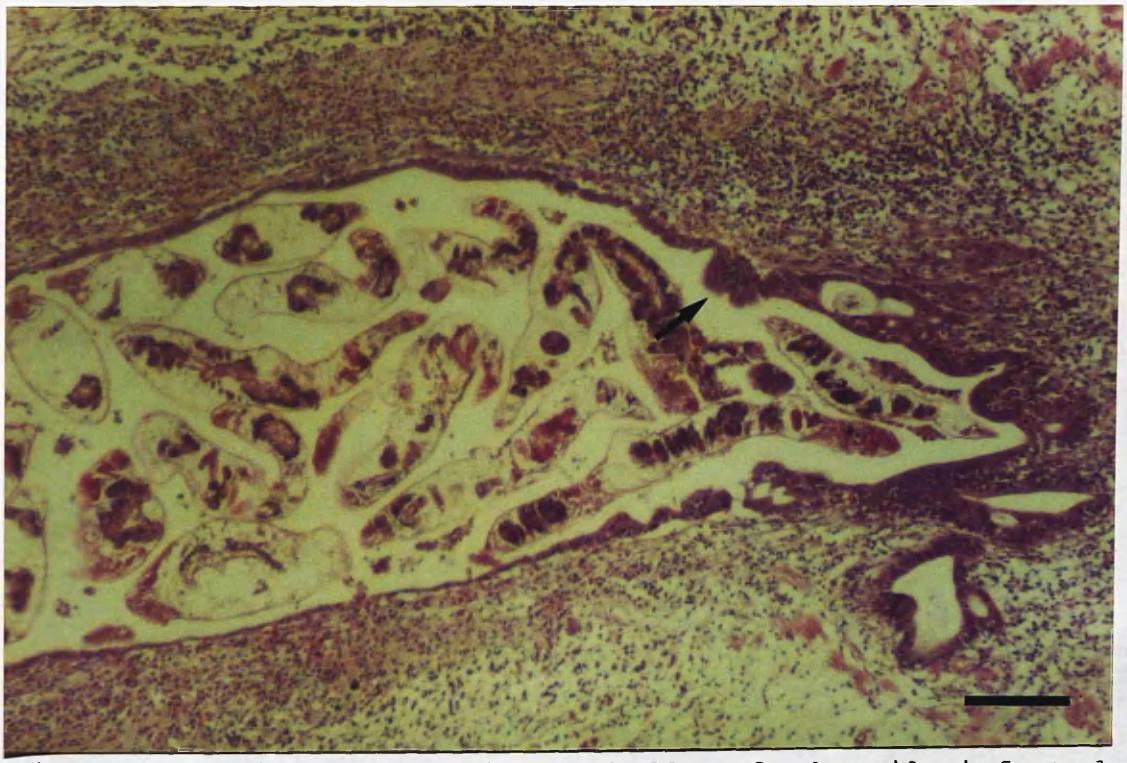


Fig.6.15 Section of the urinary bladder of a heavily infected specimen. Note the intense inflammatory cell response surrounding the urinary bladder, the large number of monogeneans and the papillary ingrowth of epithelial cells into the lumen (arrow) (H+E). *Scale bar* - 100µm

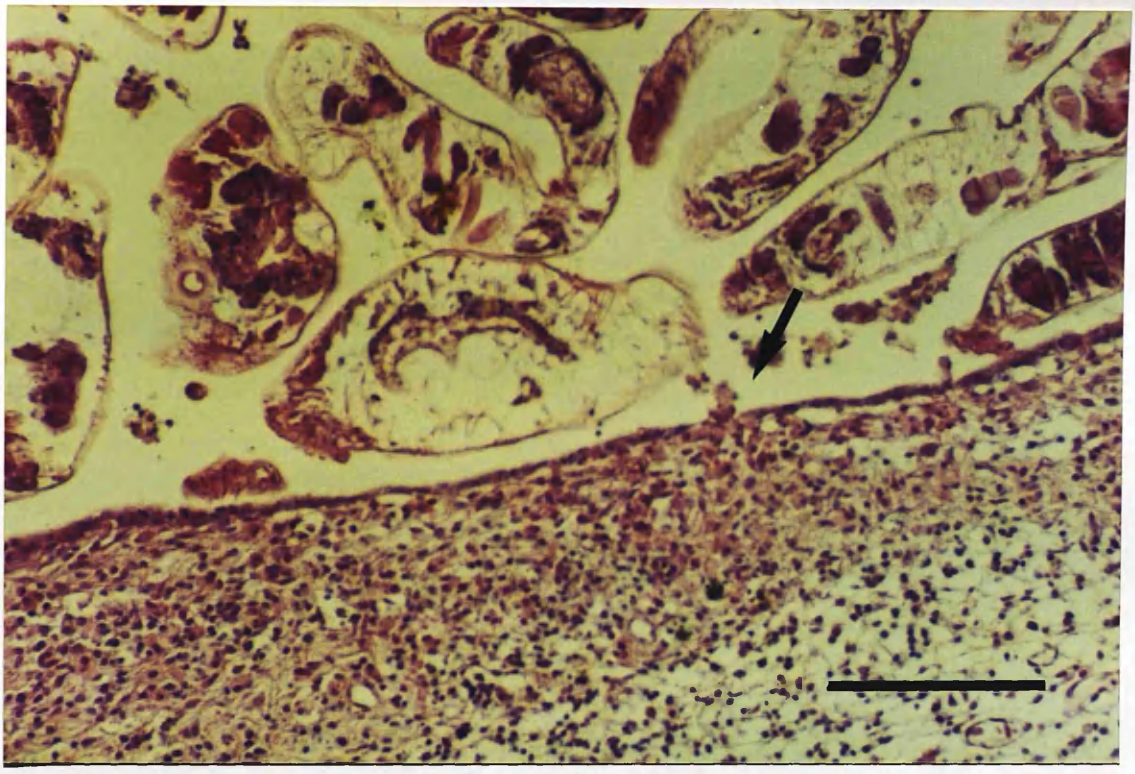


Fig.6.16 High magnification of the metaplasic area of the epithelium of the urinary bladder showing the layer of flattened cells, an intense inflammatory cell response and disruption of the epithelium (arrow) probably caused by feeding. (H+E). *Scale bar* = 100µm.

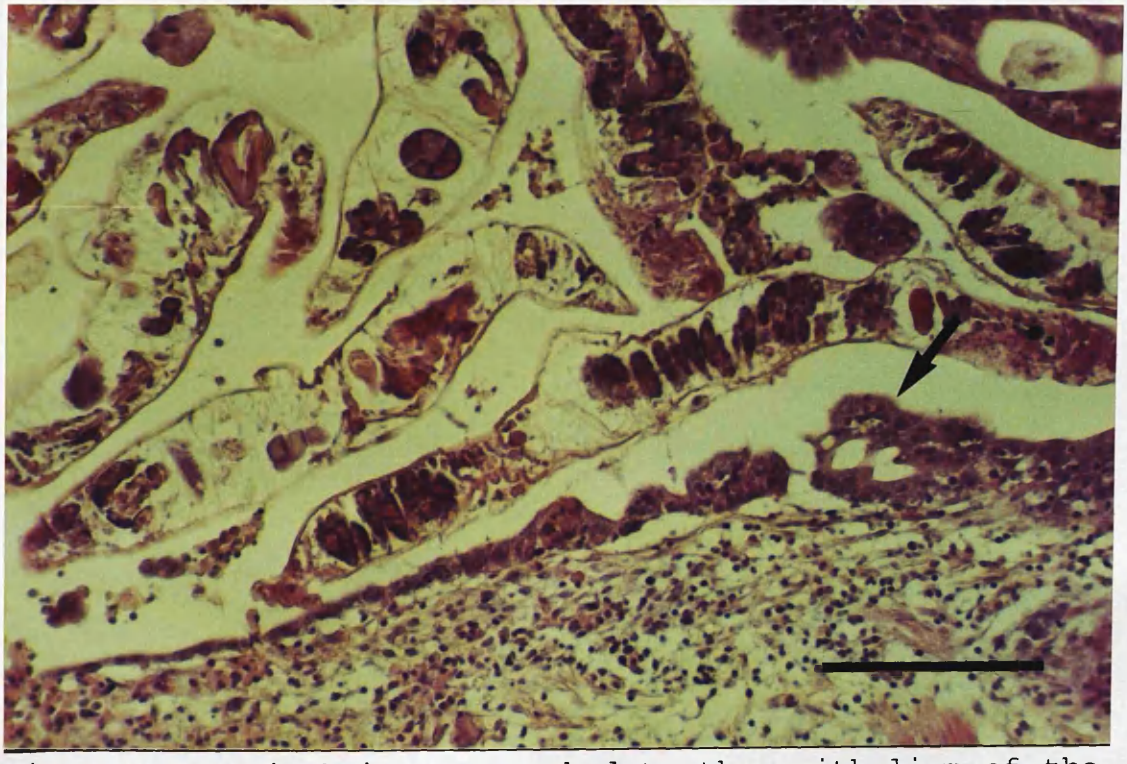


Fig. 6.17 *Kritskyia* sp attached to the epithelium of the urinary bladder. Note the papillary ingrowth of epithelial cells into the lumen (arrow). (H+E). *Scale bar* = 100µm.

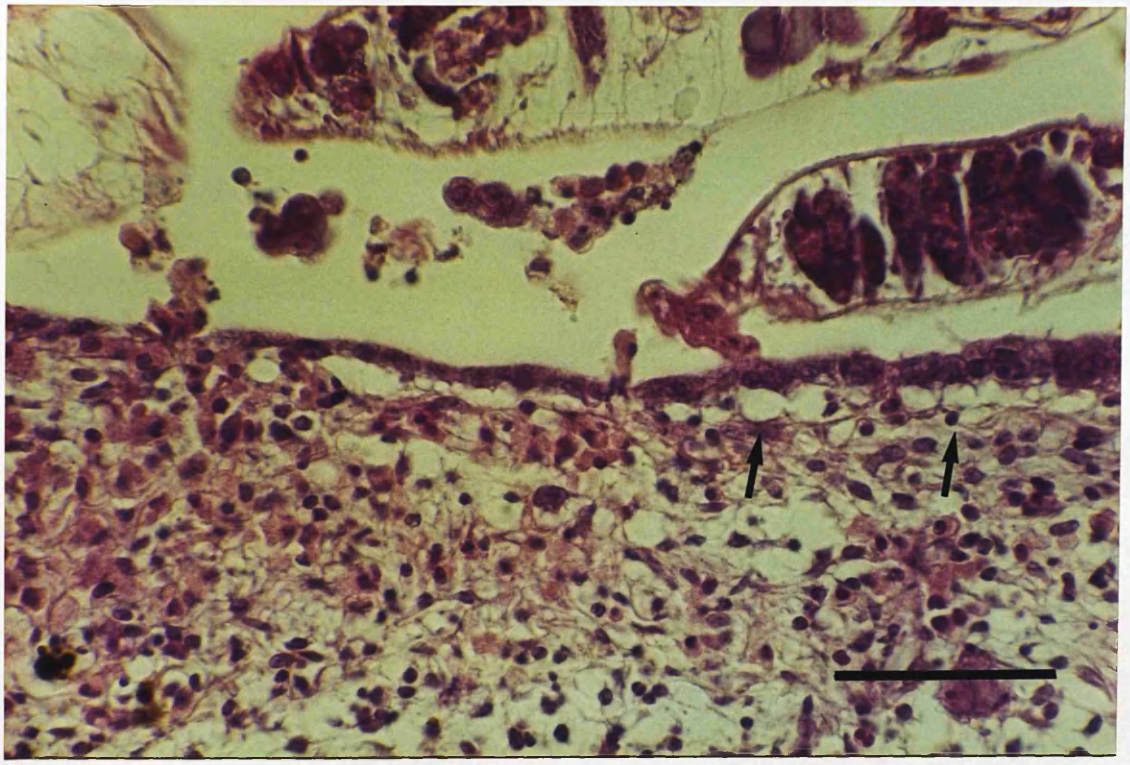


Fig.6.18 The attachment of *Kritskyia* sp. Note the single layer of the epithelium and the basal layer near the point of attachment with the cell-to-cell contact breaking down and the formation of vesicles (arrow) (H+E). *Scale bar* - 50µm

6.4.3. Histopathology Caused by Metacercariae

6.4.3.1 *Clinostomum* sp.

6.4.3.1.1 External Signs

Hosts: *Mylossoma aureum* (Spix)

Infected fish could be separated easily in the shipments, mainly due to the presence of nodules of different sizes localised in the muscles of the trunk, underneath the mouth and in the operculum and fin bases (Fig.6.19). The host response was variable according to the size of the metacercariae and their site of infection.

6.4.3.1.2 Pathology

All metacercariae present in the histological sections were found in a cyst composed of a membrane of parasite origin and an outer capsule of host cells. The main pathology associated with them was muscle atrophy, mainly caused by the development of the larvae and their unusual size (4-5 mm long by 1.5-2 mm wide). The lateral somatic muscle was almost completely replaced by the metacercariae (Fig.6.20).

Intense cellular infiltration was present partially surrounding the parasite cyst and adjacent muscles in the vicinity of the acetabulum. It was only present in those specimens in which the acetabulum of the metacercaria was attached to the cyst wall. This area showed a marked basophilia (Fig. 6.21). The remaining host capsule was stained intensively eosinophilic.

Fig.6.19 Specimens of *M. aureum* infected with metacercariae of *Clinostomum*. Note the presence of the nodules (arrow) at the base of the dorsal fin, near the opercula and underneath the mouth.

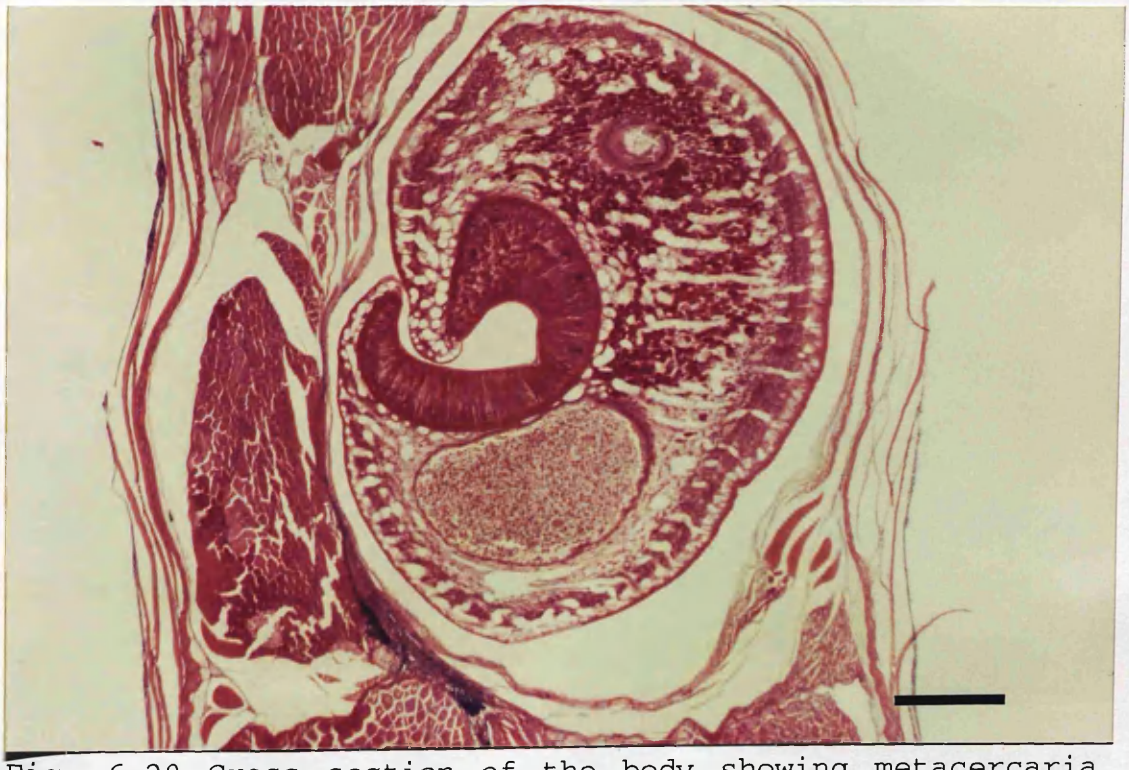


Fig. 6.20 Cross section of the body showing metacercaria encysted. Note the marked atrophy of the lateral somatic muscle adjacent to the encysted metacercaria (H+E).

Scale bar - 50µm.

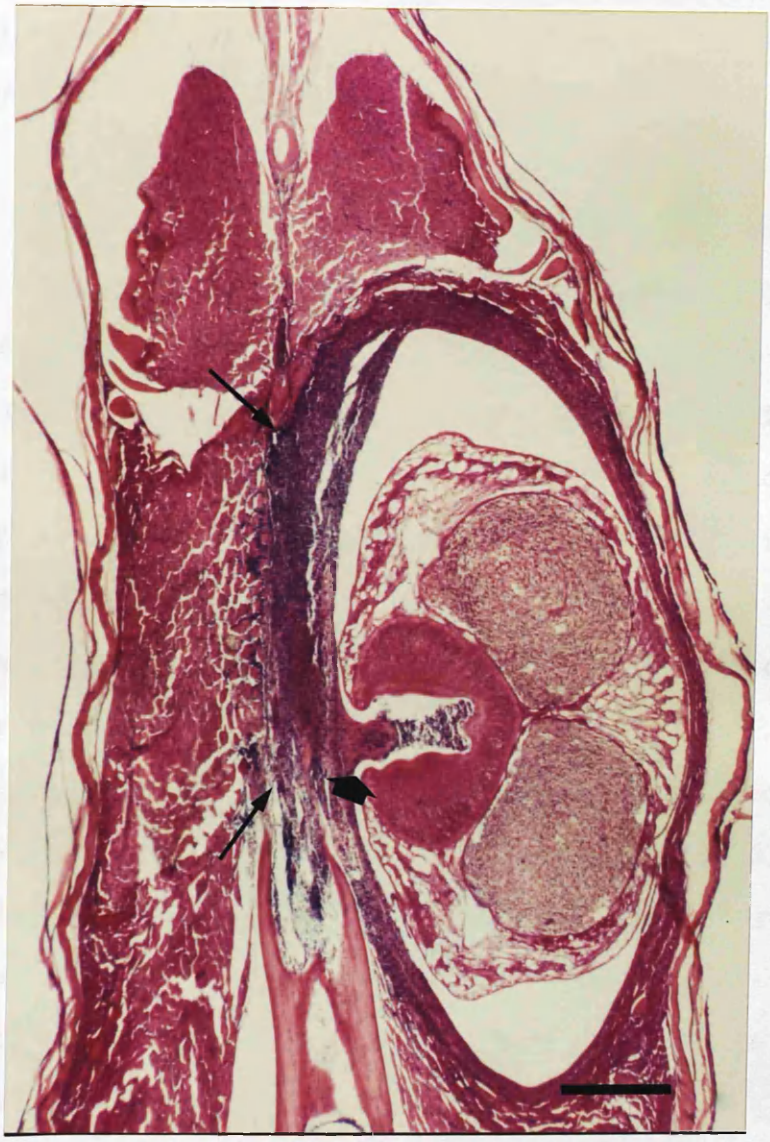


Fig. 6.21 Cross section of the body of *M. aureum*. Note the intense cellular infiltration partially surrounding the encysted metacercariae (long arrow) and the acetabulum attached to the cyst wall (short arrow) (H+E).

Scale bar - 50µm.

6.4.3.2 Mesocercariae of Strigeoidea

Hosts: *Brochis splendens; Corydoras punctatus; C. schwartzi; C. reticulatus; C. arcuatus; C.robinae; C. sterbai; C. haraldshultzi; C. julii* and *C. elegans.*

No clinical signs were observed in infected specimens and the mesocercariae were only found on necropsy.

The mesocercariae did not become encysted, i.e. there was no cyst of parasite origin. In the infected fish they were mainly found enclosed by the host cyst, which was seen as a capsule formed from a thin layer of connective tissue. The size of the cysts was variable according to the number of the mesocercariae enclosed, varying from 300 to 636 µm in diameter. Generally, 1 to 15 mesocercariae, at apparently similar stages of development, (464-742 (598) µm long by 146-242 (202) µm wide), were found enclosed in each host cyst (Fig.6.22). Frequently, a single tetracotyle of *Apatemon* sp. was found enclosed by the same host cyst (Fig. 6.23).

Such cysts were found spread throughout the body, but mainly in the mesentery and muscles of the head. They were also found in the submucosa of the mouth, barbels and ovary. Generally, no inflammatory host response or melanisation was present in the areas surrounding the parasite cyst and the damage caused appeared to be principally related to the location of the cyst. Those localised in the submucosa of the head appeared to cause pressure atrophy of the dermis and epidermis by virtue of their space occupying nature (Fig. 6.24).

In some cases, one or two mesocercariae were present in the ovary, situated within the oocyte (Figs. 6.25 and 6.26). The type of ovary observed in the fish, *Corydoras maculifer*, suggests that this species is a batch spawner, in which the presence of large numbers of atretic oocytes appears to be quite common. Histologically, the atretic oocytes were superficially identified by the occasional presence of dark pigments and also by their irregular shape (Fig.6.26 - short arrow)

All mesocercariae found in the ovary in this study were within atretic oocytes, which were seen to be surrounded by a layer of cuboidal cells and partially filled with a mass of loosely-bound cells and light yolk granules (Fig. 6.26). It was not possible to determine if the atresia (or degeneration) observed in the infected oocytes was caused by the parasites or if they had simply invaded oocytes which had already undergone atresia.

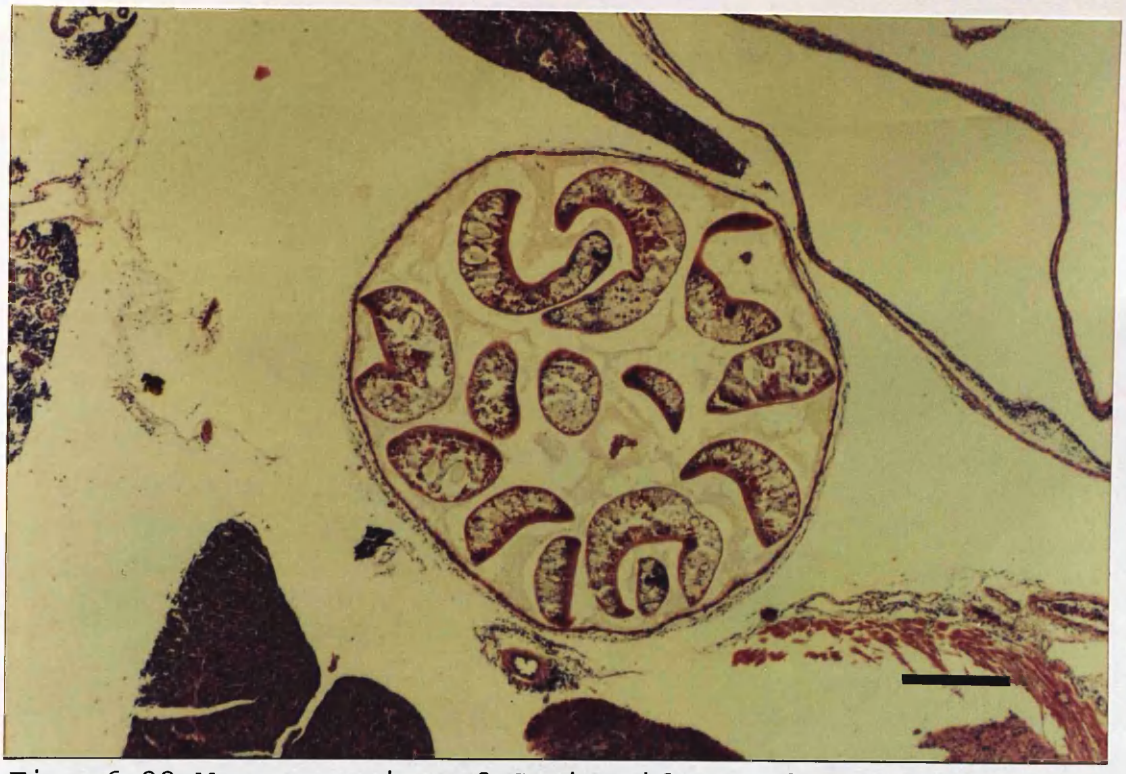


Fig. 6.22 Mesocercariae of Strigeoidea enclosed by the host cyst in the mesentery (H+E). *Scale bar* - 100µm.

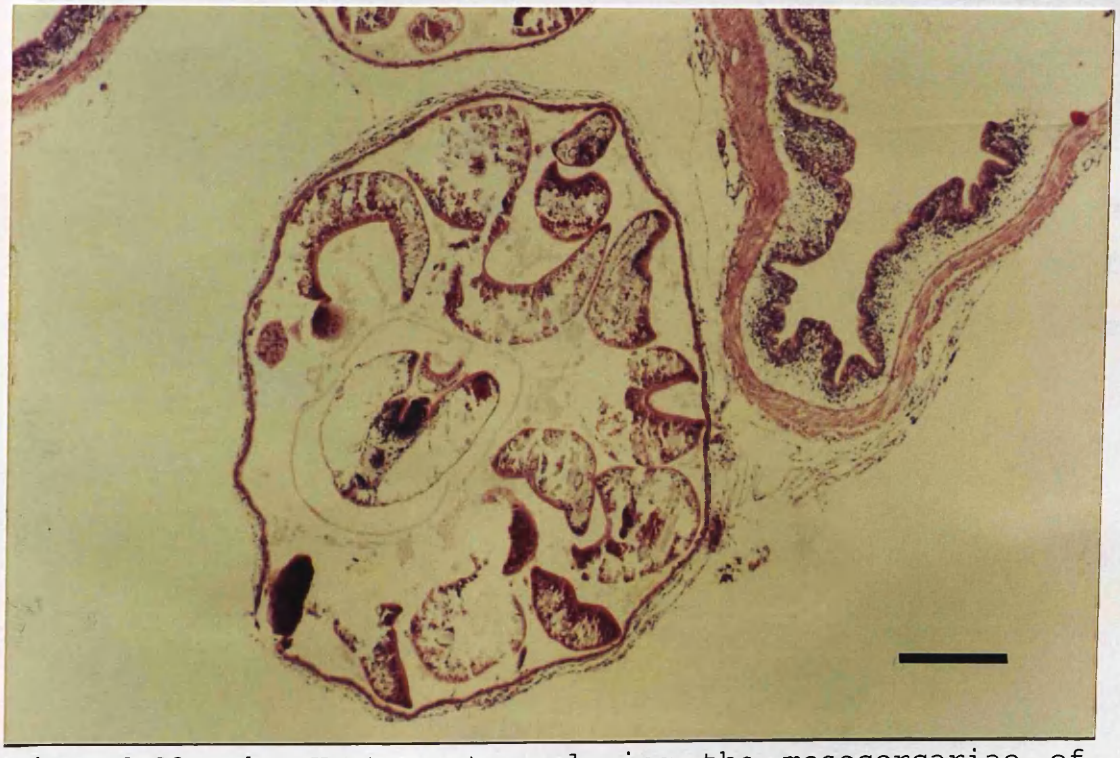


Fig. 6.23 b. Host cyst enclosing the mesocercariae of Strigeoidea and tetracotyle of *Apatemon* sp. (H+E) *Scale bar* - 100µm.

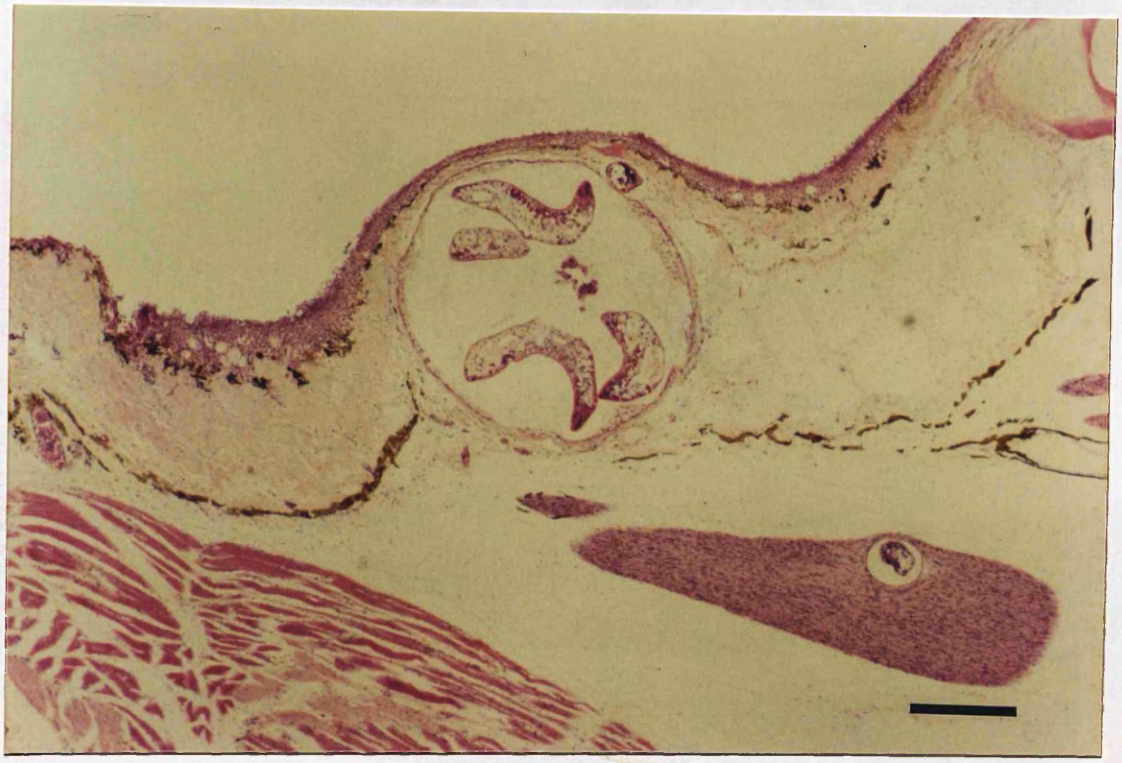


Fig.6.24 Section of the head of *Corydoras* sp showing host cyst enclosing mesocercariae and cyst of other metacercariae not identified localised in the dermis. Note the atrophy of the epidermis and dermis caused by compression of the host cyst and disruption of the pigment layer. (H+E).

Scale bar - 100µm.

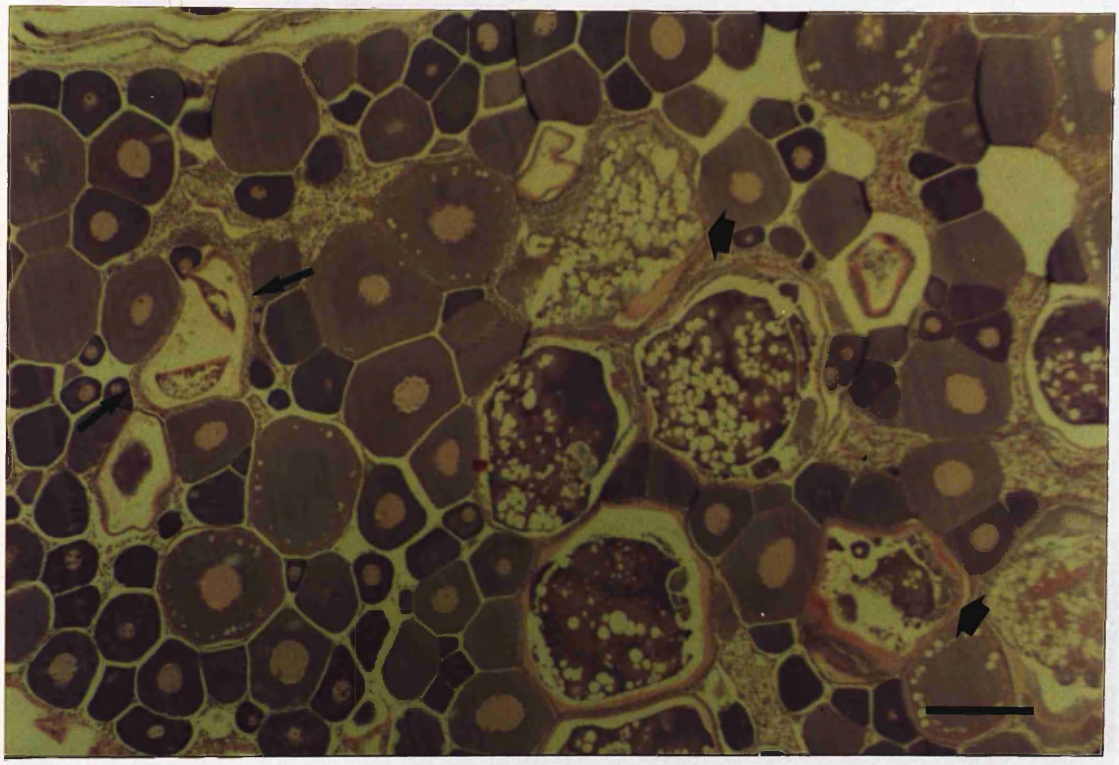


Fig. 6.25 Ovary of *C. maculifer* infected with two mesocercariae of Strigeoidea (arrow). Note the presence of large numbers of atretic oocytes (large arrow). *Scale bar* = 50µm.

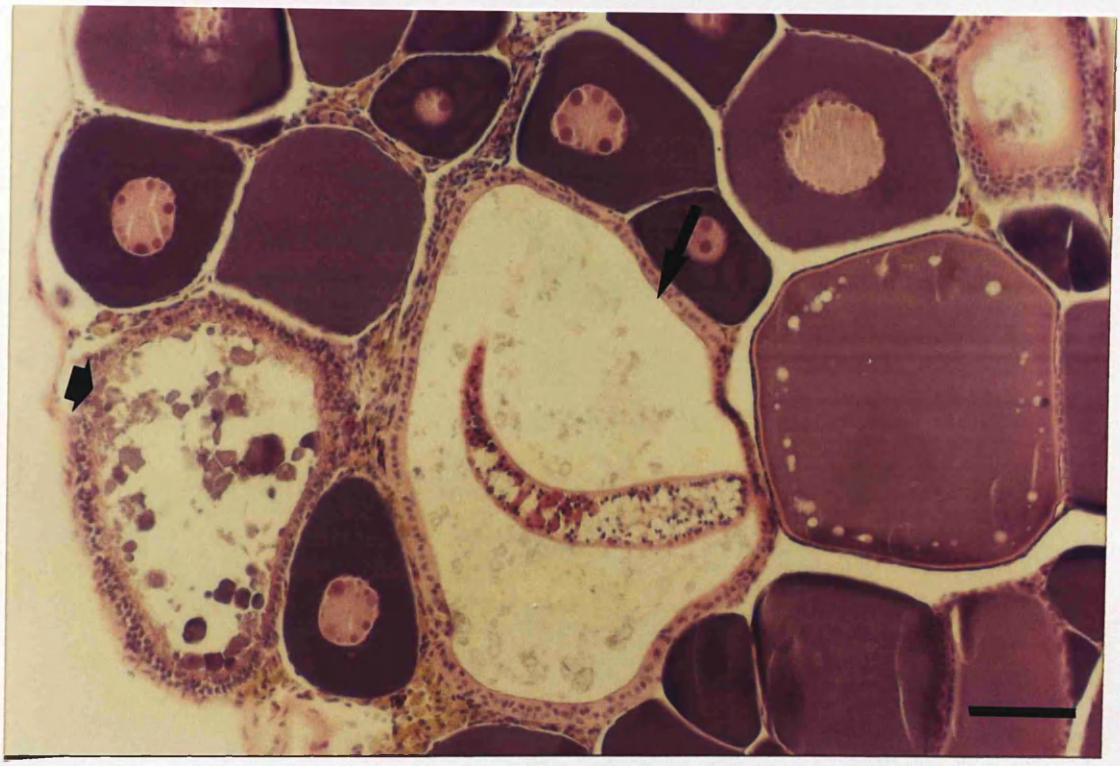


Fig.6.26 Ovary of *C. maculifer* - Note the different stages of atresia present between the infected (long arrow) and uninfected oocyte (short arrow). Infected oocyte shows a layer of cuboid cells of the granulosa, the presence of light yolk granules and one mesocercaria (H+E). *Scale bar* - 100µm.

6.5. Discussion

6.5.1 Pathology associated with *Piscinoodinium* sp.

Large numbers of trophonts of *Piscinoodinium* sp. were mainly concentrated in the epidermis of *Corydoras* spp. and *Brochis splendens* where they appeared to be responsible for the extensive epidermal erosion found on the skin of these fish. Although they were also found on the gills, the number of trophonts found was not as high as in the skin. These findings in callichthyids contrast with those described by Shaharom-Harrison *et al.* (1990) who reported that in cultured tropical pond fish in Malaysia a related *Piscinoodinium* mainly induced gill pathology.

Trophonts of different sizes were found on the epidermis, but it was not possible to differentiate the pathology caused by trophonts of different sizes/ages, because quite often they were present in areas previously damaged by other trophonts that had already fallen off the hosts. The varied sizes of the trophonts may be indicative of different periods of attachment to the hosts or a very active infection.

In all infected fish the trophonts were found attached within crypts of different depths in the epidermis, sometimes reaching the basal layer, but no trophonts were found in the dermis (Figs. 6.6 and 6.8). These crypts in the skin were seen in the SEM studies as large crypts either empty or containing one or more trophonts (Figs. 6.3 and 6.4). The presence of these crypts in the epidermis was previously reported by Schaperclaüs (1954), Reichenbach-Klinke (1954)

and Lucky (1970). The first two authors described them as hollows caused by a thickening of the epithelium in which a large number of "pouchy", mucous cells appeared. However, histological sections of the skin of *Corydoras* spp. and *B. splendens* suggest that these crypts are formed as a consequence of the deep penetration of the rhyzocyts in the epidermal cells and their subsequent destruction (Fig. 6.6). Large empty spaces were occasionally found, but were mainly related to the degeneration of club cells, which in these fish are found in one or more rows according to the area of the body.

An interesting finding in this study was the presence of trophonts enclosed by hyperplastic cells in some infected areas. These trophonts appeared to be dead, possibly indicating that the movement of live trophonts and/or the secretion of substances from them may have prevented the host cells from enclosing them, so that the attached live trophont was protected from host effects; once the trophonts die for whatever reason and therefore do not detach from the host, the hyperplastic cells are able to enclose such trophonts. Paperna (1980) suggested that epithelial changes in *Amyloodinium ocellatum* (Brown, 1931) infection may be related to toxic substances secreted by this parasite. It is possible that *Piscinoodinium* sp. also excrete irritants but further studies should be conducted to better assess the cause of the pathology induced by this protozoan.

Trophonts enclosed in crypts were also found in the filaments of the gills (Fig. 6.11). A similar condition was also reported by Shaharom-Harrison *et al.*, (1990), although according to these authors the crypts appeared to be open at

the ends which would have allowed continuous contact with the external environment.

In this study, trophonts were not found in large numbers on the gills of callichthyids. However, for this particular group of fish, *Piscinoodinium* infections may be responsible for severe losses prior to export, because callichthyids are often kept under overcrowded conditions in tanks with minimal or no aeration at the exporters' holding facilities due to their ability to remove oxygen from the atmospheric air (see chapters 3 and 4). Consequently, callicthyids which are lethargic and thus unable to swim to the surface for air, and are also suffering from a reduction of their respiratory surface area appear to be the first to perish. For the same reasons, *Piscinoodinium* infections in the gills can also be responsible for severe losses during transit of the ornamental species between the exporting and importing countries as a consequence of the deterioration in water quality.

6.5.2 Pathology associated with *Kritskyia* sp. in the urinary bladder.

Very little pathology was associated with light infections of *Kritskyia* sp. on the specimens of *M. aureum*. This might be due to the non-permanent nature of the attachment of the parasite, which means it is not always attached to the same place, and the absence of hamuli in the haptor, as hamuli are generally associated with irritation and mechanical damage of the host tissue. Consistent with this hypothesis, no inflammatory cell response was observed

surrounding the points of attachment to the hosts, although the epithelium appeared to be lifted up in some of these areas (Fig.6.13).

The ability of *Kritskyia* specimens to move about was clearly observed when removing the kidney and urinary bladder of the infected fish, as the urinary bladder was generally perforated by the forceps revealing the monogeneans actively moving about the surrounding tissues. The worm was observed to extend its body forward, attaching firstly by means of the anterior margin of the head and secondly by the haptor.

No pathology has been reported for those ancyrocephalids attaching superficially to their host. However, for those presenting a more permanent attachment, such as *Enterogyrus papernai* Gusev and Fernando, 1973, and *E. globidiscus* (Kulkarni, 1969), a severe inflammatory response in the tissue surrounding the point of attachment, independent of the intensity of infection, has been reported (Cone and Burt, 1982; Nilakarawasan, 1993). This condition was not observed in the places where the *Kritskyia* specimens were attached.

A localised inflammatory cell response became evident in those specimens presenting moderate infections (Fig. 6.14). However, as the epithelium also exhibited degenerative changes, possibly associated with the presence of the spores of *Myxobolus*, it is not clear if the type of host response observed was entirely due to the presence of the monogenea and/or sporogonic stages present in the epithelial cells.

Although little pathology was associated with the specimens of *M. aureum* presenting light to moderate infection, the inter-cellular break down observed in the basal layer appeared to cause weakness of the epithelium

which could then become easily detached by the stresses exerted by the attachment of the monogenean. The etiological agent of the weakening of the junctions of the basal layer was not determined, but dermo-epidermal separation has been reported in salmonids when they enter freshwater on their migration prior to spawning. The early lesions included break downs of the intercellular junctions which became secondarily infected with *Saprolegnia* sp. (Ferguson, 1992).

Severe damage to the urinary bladder was mainly observed in heavily infected specimens (Fig.6.15). In these specimens, the monogeneans were mainly concentrated in the urinary bladder, and probably contributed to the distension observed. The epithelium seemed to be metaplastic (Fig.6.16), a condition which appears to be a consequence of the combined effects of various irritants, such as the attachment and secretions of the cephalic glands, and feeding of a large number of monogeneans. The continuous irritation can also lead to a chronic inflammatory response as was observed surrounding the urinary bladder. Subsequently, this chronic inflammatory cell response may also be stimulated by the destruction of the inflammatory cells producing substances which further stimulate this type of response.

In contrast to the infections caused by *A. ureterocoetes* in largemouth bass, in which the ureters were blocked (du Plessis, 1948), no blockage of the ureters was observed associated with the presence of the monogenean in specimens of *M. aureum* heavily infected with *Kritskyia* sp. However, it should be noted that the large number of monogeneans observed in the urinary bladder of some specimens may cause some

urinary stasis and heavier infections may be lethal so that they did not survive the transportation.

The pathology of the urinary bladder caused by monogeneans has not previously been reported. However, more studies should be conducted on the pathology of *Kritskyia* sp. because all of the specimens of fish examined were also infected by the myxosporean, *Myxobolus* sp., in the kidney and urinary bladder, making it difficult to relate some of the pathology found to specific parasite effects.

6.5.3 Pathology associated with metacercarial forms

Among the metacercarial forms found in the fish examined, only two types were associated with pathology in their hosts and these were the metacercariae of *Clinostomum* sp. and the mesocercaria of Strigeoidea.

Clinostomum sp. was mainly found in specimens of *M. aureum*, where the pathology associated with them was principally related to the size of the metacercariae and their localisation.

All metacercariae studied were encysted. Consequently, the pathology found was mainly related to the atrophy caused by prolonged pressure in the muscles due to the development of the metacercariae (Fig.6.20). This condition has been associated with an inability to move the mouth and gill operculum in heavily infected fish (Lo *et al.*, 1992). Degenerative changes were also found partially surrounding the cyst and adjacent muscles and it appeared that these were mainly caused by the traumatic effect of the acetabulum attached to the cyst wall (Fig.6.21). A prolonged irritation

and consequent weakness of the cyst wall could contribute to the encystment of the metacercaria with fatal consequences for the host.

Another metacercarial form found in this study was the mesocercaria of Strigeoidea. This larval form was found spread throughout the body of callichthyid species, usually enclosed by the host cyst (Figs.6.22 and 6.24). Very little obvious harm was associated with them, the most obvious being the pressure atrophy in the muscles caused by the size of the cysts (Fig.6.24). Although host cysts were also found in the head, no free mesocercariae or host cysts were found inside the cranial cavity. Szidat (1969) reported that the presence of very young metacercariae in the brain were responsible for severe mortalities of "pejerreyes" from the Rio Negro, Amazon basin. Although these larval forms were placed in the genus *Diplostomum* (*Tylodelphys*) the diagram of these stages presented by the author suggested they were very similar to the mesocercariae found in this study. Unfortunately, this author did not provide the latin name for the host, making it difficult to identify the host since the common name varies with the region and country.

The only place where the mesocercariae were not found enclosed by the host cyst was in the ovary, where one or two mesocercariae were commonly found inside the oocytes (Figs.6.25 and 6.26). Although they appear to be occurring at a low intensity in the ovary, they may induce reduction of fertility and/or castration of the host. This condition may be brought about by the destruction of the follicular cell layers caused by the migration of the larvae following the degeneration of the oocyte where they were previously

enclosed. The follicular cell layer, according to Bromage and Cumaranatunga, (1988), is responsible for the formation of the different layers and components of the follicle.

Chapter 7

Transformation

Chapter 7

Transfaunation

7.1 Introduction

7.1.1 Introductions of parasitic diseases associated with aquarium fish species.

With the world-wide growth and spread of aquaculture several species of fish have been transferred to countries or continents in which they were previously absent (Couternay, 1979). Consequently, there has been an increasingly widespread concern about introductions of parasites and the diseases associated with them, principally in those countries where there have been increases in fish farming, fisheries management and stocking (Hoffman, 1970; Bauer and Hoffman, 1976; Shotts and Gratzek, 1984; Molnar, 1984; Bauer, 1991; Körting, 1993; Kennedy 1993, 1994).

In this context, the ornamental fish industry may play a very important role because of the large number of fish species involved (cold, temperate and tropical species), the large variety of parasites that have been transported together with their natural hosts, the expertise in packing technology for live ornamental fish transport, and the increased speed with which these species of fish have been transported around the world.

The control of diseases at the level of the airport presents serious problems because of the large number of species and volume of fish arriving from different countries at any time (Wootten, 1991). To check all of the consignments

would only cause delay and the number of losses would be much higher. Ornamental species and their transport waters have been associated both with the spread of diseases of fish between different continents (Hoffman, 1970; Hoffman and Schubert, 1984) and the transmission of pathogens of potential danger to public health (Trust and Bartlett, 1974; Shotts and Gratzek, 1984; Vandelpitte, 1983).

Vandelpitte (1983) reported that the bacterium, *Edwardsiella tarda*, was the causative agent of diarrhoea in 2 month old Belgian infants. This organism was isolated from a tropical aquarium fish in the home of the patient. Subsequently, *Salmonella arizona* was reported as an important primary agent with potential danger to public health present in shipping waters (Shotts and Gratzek, 1984). Recently, one case of diarrhoea in a 5 year old Scottish infant was associated with *Salmonella java*. This bacterium was isolated from the ornamental fish, the Tiger barb, *Punthius partipentazona*, in the home of the patient, apparently imported from Singapore (British importer, pers. comm.).

Information on the spread of fish diseases associated with aquarium fish is scarce and import restrictions associated with these criteria have currently been applied to only a few species of fish (e.g. in Australia, all goldfish, *Carassius auratus*, must be certified to be from farms known to be free from goldfish ulcerative diseases - Datodi, 1993).

There is no standardisation of import requirements between countries although many seek to minimise the risk of introducing pathogens by requiring a health certificate for imports of some species, limiting the number of species to be imported, only allowing the import of fish from registered exporters and adopting severe quarantine procedures such as those observed in Australia (14 days) and New Zealand (42 days) (Wootten, 1991; Datodi, 1993). In the UK, although the legislation is quite strict for the import of wild animals, it distinguishes food fish from ornamental fish. Unfortunately, ornamental and food fish species may carry the same infectious agents. Recently, with the creation of the single European market, there has been an increased concern with the trade of aquatic organisms and their products because of the risk of spreading new parasites to new regions, especially if the presence of these parasites is not notifiable.

Although large numbers of parasites have been transported with aquarium fish species around the world, relatively few species of parasites have been recorded as introduced and established in new environments associated with the importation of ornamental fish species, this being especially true for tropical species.

The trade in goldfish, *C. auratus*, carp, *Cyprinus carpio*, and their ornamental varieties, is perhaps the most spectacular example of the importation of diseases and pathogens associated with the fish trade; both generalist and

specialist parasites have been able to colonise new environments with the dispersal of these hosts (Kulakovskya and Krotas, 1961; Edward and Hines, 1974; Riley, 1978; Meier and Pfister, 1981; Lucky, 1984; Molnar, 1984; Hoffman and Schubert, 1984; Grabda-Kazabska, Baturo-Warzawaska and Pojmanka 1987; Gelnar and Lux, 1991; Bauer, 1991; Pojmanska and Cabros, 1993; Kennedy, 1993; 1994). In the British Isles, for example, the introduction of three monogeneans (*Dactylogyrus anchoratus*, (Dujardin, 1845) *D. extensus* Mueller and Van Cleave, 1932, and *D. vastator*, Nybelin, 1924), one digenean (*Sanguinicola inermis* Plehn, 1905), and one cestode (*Bothriocephalus acheilognathi*, Yamaguti, 1934), were associated with successful introductions of these species of fish, (Kennedy,1993).

In the few lists of internationally transferred parasites found in the literature, it is mainly specialist parasites of tropical species of fish which appear to be recorded. Consequently, their distribution is limited to the distribution of their hosts which may be restricted to the ornamental fish culture facilities (e.g. the South American nematode, *Capillaria pterophylli* Heinze, 1933, a parasite of the angelfish, *Pterophyllum scalare* Curs and Valens, 1831 was transferred to Germany and other ornamental fish culture facilities in Europe (Hoffman, 1970; Bauer, and Hoffmam, 1976; Hoffman and Schubert, 1984; Moravec *et al.*, 1987). Another important feature of these lists relates to the presence of cosmopolitan parasites, such as *Ichthyophthirius multifiliis*, *Chilodonella hexasticha*, and *Ichthyobodo necator*

(Henneguy, 1883), which have a broad temperature tolerance and host range and have commonly been transhipped from the Far East, South America, United States and other parts of the world with infected aquarium fish species (Robertson, 1985; Hoffman, Kazubskii, Mitchel and Smith, 1979; Matthews, 1994).

The number of species of parasites of tropical aquarium fish transferred internationally is not known and it would be very difficult to provide a list with all species for several reasons. One of these is related to the fact that the parasite fauna of the large majority of the aquarium species available in the ornamental fish market is poorly known and sometimes neither the range of distribution of the hosts nor the identity of the parasites are known. Other reasons are related to the literature available involving parasites and other pathogens of tropical ornamental fish from South America, the USA and Asia. Generally, these papers do not provide the species names of the parasites found, only higher groups, which makes it difficult to assess the species present in their native environment and how far they have become distributed with their hosts (Gratzek *et al.*, 1978; Conroy *et al.*, 1981; Shotts and Gratzek, 1984; Gratzek, 1988). Consequently, the role played by species of aquarium fish in the introduction, spread and colonisation of parasites is so far unknown. A broad ecological view of the introductions of parasites and diseases associated with aquatic organisms was recently discussed by Bauer (1991) and Kennedy (1993; 1994). Clearly a series of factors relating

to the species of aquarium fish involved in the trade, associated with the obstacles to be overcome by the parasites, will determine the success of these invasions.

This chapter will investigate the potential for transfaunation of several species of nematodes of the genus *Spirocamallanus*, family Camallanidae, commonly found in the intestine of tropical ornamental fish species imported from South America into Britain.

7.1.2 Camallanid parasites of ornamental fish species from South America

According to Petter (1979) the Family Camallanidae Railliet and Henry, 1915, is composed of two sub-families: Camallaninae Yeh, 1960 and Procamallaninae Railliet and Henry, 1915. These nematodes are primarily parasites of the intestine of fish, frogs and turtles and have so far been reported from all continents except for Antarctica (Stromberg and Crites, 1974).

Although they have often been reported from Brazil, the parasite fauna of camallanids from this continent is generally not very well known (Travassos *et al.*, 1928; Pinto, FFabio, Noronha and Rolas,1974; 1975; Petter and Thatcher, 1988; Ferraz and Thatcher, 1990, 1992; Petter and Dlouhy, 1985). Of the 9 genera described for the Family, only 5 genera were described as occurring in South America. Four of these are parasites of fish; *Camallanus* Railliet and Henry, 1915, *Paracamallanus*

Yeh, 1960, *Procamallanus* Baylis, 1923, *Spirocamallanus* Olsen, 1952, and one is a parasite of reptiles, *Serpinema* Yeh, 1960.

Only one species of *Paracamallanus* has been described from South America, *P. amazonensis* Ferraz and Thatcher, 1992, which was found in the intestine of *Hyppostomus affinis* (Steindachner, 1876) in the North of Brazil. Subsequently, the species has been recorded in the South of Brazil from the intestine of *H. edentatus* Spix and *Pterodoras granulosus* (Valenciennes) by Ferraz and Thatcher (1992) and Moravec, Kohn and Fernandes, (1993).

The genera *Camallanus* and *Procamallanus* are each represented by two species which have been recorded in the Amazon region in the North of Brazil. These are: *Camallanus tridentatus* (Drasche, 1884), a parasite of the pirarucu, *Arapaima gigas* (Cuvier), *C. acaudatus* Ferraz and Thatcher, 1991, a parasite of the arowana, *Osteoglossum bicirrhosum* Vandelli, *Procamallanus peraccuratus* Pinto, Fabio, Noronha and Rolas, 1976, a parasite of fish of the Family Cichlidae including *Geophagus brasiliensis* (Quoy and Gaimard, 1824) and *Cichlasoma facetum* (Jennys, 1842), and *P. petterae* Kohn and Fernandes, 1988, a parasite of a siluriform fish, *Hypostomus albopunctatus* Regan, 1908.

Among the camallanids described from South America, the genus *Spirocamallanus* contains the most described species of which 16 are parasites of freshwater fish and one is a parasite of marine fish. So far this genus has been described from Brazil, Paraguay, Argentina, French Guyana, Peru, Chile

and Colombia (Thatcher, 1991). The list of these species and their geographical distribution in South America is summarised in Tables 7.1 and 7.2.

The level of specificity and the range of distribution of the large majority of these species is not known, mainly because some species have not been recorded subsequent to their description or because they have only recently been described. Others such as *Spirocamallanus inopinatus* Travassos *et al.*, 1928 appear to be generalists with a broad range of hosts (Table 7.1).

Nematodes of the family Camallanidae appear to be a common finding in the intestine of tropical ornamental species. They have been commonly reported by hobbyists and exporters mainly because they are often seen hanging out of the vent of fish and can be recognised by the reddish colour and large size of the females of some species.

One species of Camallanidae, *Camallanus cotti* (Fujita, 1927) has been reported as transfaunated from Japan and established in ornamental fish cultures in Malaysia, Europe, the United States and Australia (Stumpp, 1975; Hoffman and Schubert, 1984). The species was recorded by Stumpp (1975) from the intestine of guppies, *Poecilia reticulata*, platy, *Xiphophorus* sp., and from black and check mollies, *Mollienisia* sp. imported from Singapore. Previously, the species was recorded from the intestine of *Symphysodon* sp., *Pterophyllum* sp. and *Corydoras* sp. (Schubert, 1971; cited by

Stumpp, 1975). It was not clear whether these South American species of fish were wild or tank bred.

The life cycle of *C. cotti* was studied by Stumpp (1975) who stated, "in tank kept fish the infection is possible without an intermediate host for two generations". Unfortunately, there is no evidence in the literature supporting this statement. The life cycle was not followed completely and it appears that finding larval stages in the detritus of the aquarium and in the mucosa of the intestine of infected fish, lead the author to suggest development without an intermediate host.

Several species of camallanids have completed their life cycle under experimental conditions. For the Sub-Family Camallaninae the following life-cycles have been studied: *Camallanus sweeti* (Moorthy, 1937) in India by Moorthy (1938); *Camallanus lacustris* (Zoega, 1776) by Mecznikow (1866), Leuckart (1876), Linstow (1909), Leiper (1910), Kuprjanova (1954), Campana-Rouget (1961) and Moravec (1969,1971). *Paracamallanus cyathopharynx* (Baylis, 1923) by Moravec (1974) in Europe; *Camallanus oxycephalus* Ward and Magath, 1916 in the United States by Stromberg and Crites (1974).

The life-cycles of nematodes of the family Camallanidae appear to be very simple and generally involve one copepod, as intermediate host. Non-cyclopoid invertebrates such as *Asellus aquaticus* and *Agrion* sp. larvae have also been suggested as intermediate hosts (Leuckart, 1876; Linstow, 1909) although Moravec (1969), in his study involving

infection of different species of aquatic invertebrates, including *Asellus aquaticus* and *Agrion* sp., observed that no parasites developed in these specimens. Similar results were reported by Stromberg and Crites (1974) who reported that the arthropods *Gamarus, Hyalella, Asellus, Chiromus* and *Cricoptus* fed upon the first larvae when exposed to them, but no infections were subsequently observed. In part, these results may be related to the way in which these arthropods eat their food. It is well known that the copepod intermediate hosts of these nematodes swallow the larvae whole whereas those invertebrates used by Stromberg and Crites (1974) might have crushed or macerated their food. The species of aquatic invertebrates utilised experimentally as intermediate hosts for the different species of camallanids are presented in Table 7.3. It is important to note that the successful development of the larvae of the camallanid parasites of freshwater fish has only been achieved in free-living copepods (Moravec, 1974; Bashirullah and Ahmed, 1976; Strombeg and Crites, 1974; Nie and Kennedy, 1991).

For the Sub-Family Procamallaninae the development and life cycle of four species of *Spirocamallanus* and one species of *Procamallanus* have been investigated: *S. fulvidraconis* (Li,1935) by Li (1935); *S. cearensis* (Pereira, Dias and Azevedo, 1936) (= *S. hilarii* (Vaz and Pereira, 1934)) by Pereira et al., (1936); *S. xenopodis* (Baylis, 1929) by Thurston (1970); *S. intestinecolas* Bashirullah, 1973 by Bashirullah and Ahmed (1976); *S. cricotus* Fusco and

Overstreet, 1978 by Fusco (1980); *P. laeviconchus* (Wedl, 1862) by Moravec (1974).

Information relating to the life cycles of the camallanids in fish from South America are limited to studies on *S. cearensis* (= *S. hilarii*) by Pereira, Dias and Azevedo, (1936) and the description of the L_4 stage of *Camallanus acaudatus* by Ferraz and Thatcher (1990).

Under natural conditions, the life cycle of camallanids can be summarised as follows: after the release of the first stage larvae by the gravid females they are eaten by cyclopoid copepods. Larvae penetrate the gut wall and migrate to the haemocoel. Two moults occur and the larvae become third stage. Infected copepods are eaten by the final hosts or other species of fish which can act as paratenic hosts. Normal development continues in some species but the final moult is inhibited in others (paratenic hosts). When the paratenic hosts are eaten by larger predatory fish species the larvae are transferred and develop to maturity (Moorthy, 1938; Pereira *et al.*, 1936; Stromberg and Crites, 1974).

Under experimental conditions, the life cycle has only been successful through infection of copepods. The time necessary for the development of the larvae in the copepods is greatly dependent on the temperature. Moravec (1975) stated that "the temperature is one of the most important factors influencing the rate of development of members of the family Camallanidae and, evidently, is an important limiting

factor in the distribution and occurrence of the individual species".

There appears to be little or no difference in the type of life cycle presented by members of the Family Camallanidae so far studied, although a few contradictory opinions were found in the earliest papers, mainly concerning the number of moults in the intermediate host and the morphology of the buccal capsule of the larvae (Li, 1935; Perreira *et al.*, 1936).

With regard to the morphology of the individual larval stages, Moravec (1975) did not find any difference between the first and second stage larvae of *Procamallanus, Spirocamallanus and Camallanus*. Differences, however, were found in the structure of the buccal capsule and the number of the processes at the end of the tail of the third stage larvae. Three conical processes at the end of the third-stage larvae of the camallanids have been described (Moravec 1967, 1969, 1974; Bashirullah and Ahmed 1976; Campana-Rouget 1961; Pereira *et al.*, 1936; Stromberg and Crites 1974; Fusco 1980). The only exceptions were found in *Procamallanus laeviconchus, Spirocamallanus cearensis* (=*S. hilarii*) and *S. fulvidraconis*, which possess four small conical processes (Moravec 1975).

Fish, the final host of these nematodes, become infected through the ingestion of a copepod infected with the third-stage larva. Generally, copepods are one of the different types of microcrustaceans utilised as a source of live food for the species of ornamental fish kept under artificial

conditions and the potential exists, therefore, for transmission to occur in ornamental aquaria and for the continuation of the life cycle within the enclosed system.

The survey detected several parasite species which have the potential to transfaunate, such as the parasites with direct life cycles, notably protozoans and monogeneans. The occurrence of *I. multifiliis* for example, is widespread in UK waters and it is now established on indigenous fish species (Sommerville, pers. comm.). It seems likely that it was originally introduced with tropical ornamental fish since it is well known to be a warmwater parasite. The establishment of parasites with indirect life cycles would be less likely but has nevertheless occurred (Gibson, 1993).

The release of ornamental fish into natural waters is a problem that has already been encountered by several countries (see Chapter 1). However, the impact of their release associated with their parasites is so far unknown.

This study investigates the life cycles of parasite species with indirect cycles which are commonly found infecting tropical ornamental fish. It was conducted with several species of nematode of the genus *Spirocamallanus* Olson, 1952, including *S. inopinatus*, a parasite with a widespread distribution in South America which has been recorded from 27 species of fish. The development of their larval stages in native copepods, and in species of copepods commonly supplied commercially as live food for ornamental species, is described.

Table 7.1

Species of nematodes of the Family Camallanidae described from freshwater fish from South America - Brazil

Genus/ Species of Parasites	Fish Host Species	Common Name
Camallanus tridentatus (Drasche, 1884)	*Arapaima gigas*	Arapaima
C. acaudatus Ferraz *et al.*, 1990	*Osteoglossum bicirrhosum*	Arowana
Paracamallanus edentatus Ferraz *et al.*, 1992	*Hypophthalmus affinis* *H. edentatus* *Pterodoras granulosus*	- - Sandpaper bacu
Spirocamallanus amarali (Vaz and Pereira, 1934)	*Leporinus* sp.	-
S. barroslimai (Pereira, 1935)	*Triportheus* sp.	-
S. hilarii (Vaz and Pereira, 1935)	*Salminus hilarii* *Astyanax bimaculatus*	- Two-spot Astyanax
S. inopinatus (Travassos *et al* 1928)	*Astyanax b. lacustris* *Brycon hilarii* *B. erythropterum* *B. brevicaudatus* *Brycon* sp. *Hoplias malabaricus* *Leporinus fasciatus* *L. copelandii* *L. friderici* *Leporinus* sp. *Schizodon nasutus* *Serrasalmus natterreri* *S. marginatus* *S. spilopleura* *Pterodoras granulosus* *Trachydoras paraguayensis* *Pygocentrus* sp. *Astronotus ocellatus* *Cichla ocellaris* *Crenicichla haroldoi* *Potamotrygon motoro*	- - - Short-tailed Trout Tetra - Common Trahira Banded Leporinus - Frederici's L. - - Red Piranha Fire-mouth Piranha Sandpaper Bacu Paraguay Doradid - Oscar Peacock Cichlid - Ocellated Stingray
S. iheringi (Travassos et al., 1928)	*Salminus hilarii* *Astyanax fasciatus* *Leporinus* sp. *Hoplias malabaricus* *Schizodon fasciatus*	- Silvery Tetra - Common Trahira -
S. intermedius (Pinto *et.al.*, 1974)	*Pimelodus clarias*	
S. paraensis (Pinto and Noronha, 1976)	possibly Erythriniidae	
S. pexatus (Pinto *et al.*, 1974)	*Pygidium brasiliensis*	
S. pimelodus (Pinto *et al.*1975)[1]	*Pimelodus clarias* *Pimelodella lateristriga*	
S. pintoi (Kohn and Fernandes, 1988)	*Corydoras pintoi*	
S. solani (Pinto *et al.* 1975)	unidentified catfish	

[1] *S. pimelodus* was considered junior synonymous of *S. intermedius* (Moravec *et al.*, 1993)

Table 7.1 (Cont.)

Species of nematodes of the Family Camallanidae described from freshwater fish from South America - Brazil.

Genus/Species of Parasites	Fish Host Species	Common Name
S. rarus Travassos, 1929	*Pimelodella lateristriga* *Rhinodoras d'orbignyi*	- Speckled Growler
S. freitasi Moreira *et al.*, 1991	*Pimelodus maculatus* *Pimelodus* sp.	- Triangle-fin Pimelodus
Procamallanus peracccuratus Pinto *et al.*, 1976	*Geophagus brasiliensis* *Cichlasoma facetum* *Hoplias malabaricus*	Pearl Cichlid - Common Trahira
P. annipetterae (Kohn and Fernandes, 1988)	*Hyppostomus affinis*	-
Procamallanus sp.	*Apistogramma ramirezi* *Aequidens pallidus* *Pterophyllum scalare*	Ramirezi Pale Flag Cichlid Angelfish

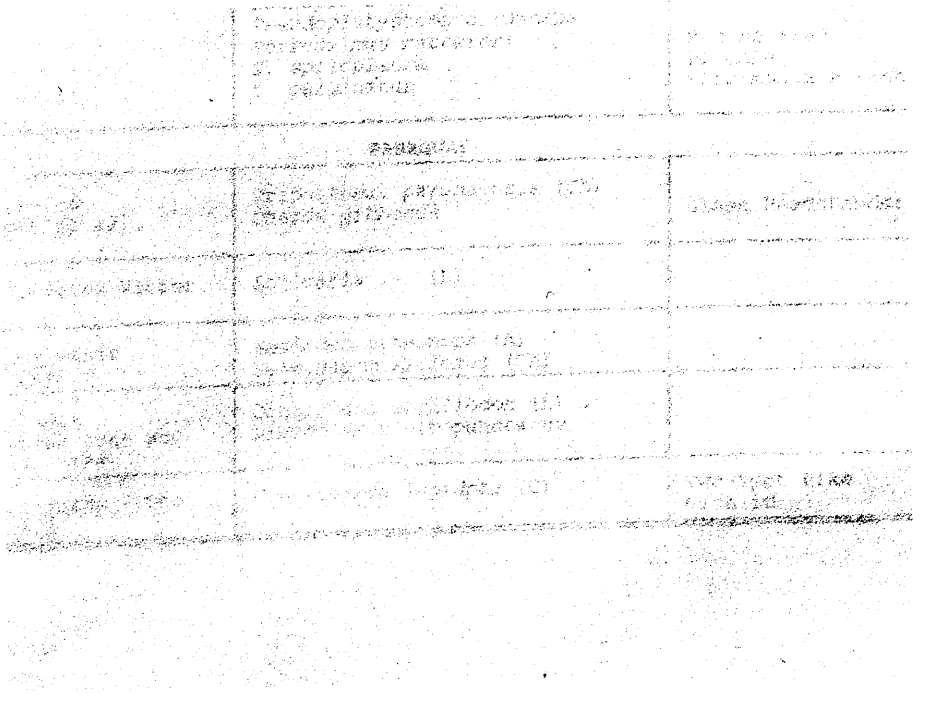


Table 7.2

Species of nematodes of the Family Camallanidae described from freshwater fish from South America - Peru, French Guyana, Argentina and Paraguay.

Genus/Species of Parasites	**Fish Host Species**	**Common Name**
	PERU	
Spirocamallanus hilarii (Vaz and Pereira, 1935)	*Pygidium punctulatum* (L)	-
S. chimusensis Teixeira de Freitas and Ibanez, 1968	*Pygidium punctulatum* (L)	-
	FRENCH GUYANA	
S. krameri Petter, 1974	*Hoplerythrinus unitaeniatus* (E)	Golden Trahira
	ARGENTINA	
S. inopinatus	*Brycon orbygnianus* *Ephyppicharax orbicularis paraguayensis* *Leporinus obtusidens* *L. maculatus* *Luciopimelodus pati* *Pseudoplatystoma coruscans* *Serrasalmus nattereri* *S. spilopleura* *S. marginatus*	- - - - - - Red Bellied Piranha Fire-mouth Piranha -
	PARAGUAY	
S. inopinatus (Travassos *et al.*, 1928)	*Triportheus paranaensis* (Ch) *Charax gibbosus*	- Glass Headstander
S. cervicalatus Petter, 1990	*Loricaria* sp. (L)	-
S. paraguayensis Petter, 1990	*Hemiodus orthonops* (A) *Salminus maxillosus* (Ch)	- -
Procamallanus annipetterae Kohn and Fernandes, 1988	*Cochliodon cochliodon* (L) *Plecostomus albopunctatus*	- -
P. peraccuratus Pinto *et al.*,1976	*Crenicichla lepidota* (C)	Two-Spot Pike Cichlid

Table 7.3

Species of water invertebrates utilised experimentally as intermediate hosts for nematodes of the Family Camallanidae

Aquatic Invertebrates	Parasite Species	Positive Hosts	Source
Acanthocyclops viridis **C** *Macrocyclops* sp.**C**	*Camallanus lacustris* (Zoega, 1776)	+ +	Campana-Rouget, 1961
Megacyclops viridis **C** *M. albidus* **C** *Acanthocyclops vernalis* **C** *M. leuckarti* **C** *Eucyclops serrulatus* **C** *Cyclops strenurus* **C** Harpacticidae (Copepoda) *Diaptomus* sp. **C** *Agrion* sp. (**O**) *Asselus aquaticus* **I**	*Camallanus lacustris*	+ + + - + - - - - -	Moravec, 1969
Asellus aquaticus **I**		-	Leuckart , 1876
Agrion sp. (**O**)		-	Linstow, 1909
Cyclops vernalis **C** *C. bicuspidatus* **C**	*Camallanus oxycephalus* Ward and Magath, 1916	+ +	Stromberg and Crites, 1974
Mesocyclops leuckarti **C**	*Paracamallanus cyathopharynx* (Baylis, 1923)	+	Moravec, 1974
Mesocyclops leuckarti **C** *Cyclops* sp. **C**	*Procamallanus laeviconchus* (Wedl., 1862)	+ +	Moravec, 1975
M. leuckarti **C** *Thermocyclops crassus* **C**	*Spirocamallanus intestinecolas* (Bashirullah, 1973)	+ +	Bashirullah and Ahmed, 1976
Diaptomus cearensis **C****	*Spirocamallanus cearensis*	+	Pereira *et al.*, 1936
Mesochra sp.**C** *Tigriopus californicus* **C** *Penaeus setifurus* **P**	*Spirocamallanus cricotus* Fusco and Overstreet, 1978.	+ + +	Fusco, 1980

****N.B.** The identification of the intermediate hosts might be erroneous. This study was conducted in Brazil and it appears that this genus does not occur in Brazil. C= Copepoda I= Isopoda O= Odonata P= Peneidae.

7.2 Study aims

The transfaunation studies were conducted in the UK and Brazil. The main aims of these studies were:

- UNITED KINGDOM

1. To establish the infection of larval stages of species of the nematode *Spirocamallanus* in free-living copepods commonly found in the lakes of Scotland and in other species of copepods commonly supplied as live food for ornamental fish species.
2. To infect native fish species and natural hosts in order to evaluate the development of the adult stages of nematodes of *Spirocamallanus* in these species of fish.

- BRAZIL

To develop the infective stages of *Spirocamallanus* spp. in species of copepods commonly found in the earth ponds of the exporters' holding facilities, with special emphasis on the selection of species of copepods with a broad distribution in the neotropical regions.

7.3 Materials and methods

7.3.1. Development of the life cycle in the United Kingdom.

7.3.1.1 Collection of the gravid females of nematodes

Gravid adult females were obtained from the intestine of *Corydoras metae* Eigenman, 1914, *Mylossoma aureum* (Cuvier, 1818) and *Brochis splendens* (Castelnau, 1855). They were placed in petri dishes containing physiological saline solution and kept in a refrigerator at 4°C for a period of between 24 and 48 hours. The physiological saline solution was changed every 12 hours.

7.3.1.2 First stage larvae

Generally, the first stage larvae were obtained by placing the gravid females containing active larvae in the uterus into fresh saline solution and bursting their body with small needles. Gravid females were also placed in petri dishes containing filtered pond water or spring water and allowed to burst naturally.

7.3.1.3 Period of survival of the first stage larvae

To evaluate the period of survival of the larvae outside the uterus, samples of 10-20 active larvae were placed in small petri dishes containing one of the three different

types of water utilised in these experiments (see below), and kept at room temperature. In the first 12 hours, the petri dishes were examined at intervals of 1 hour after which they were examined at intervals of 8 hours.

7.3.1.4 Types of freshwater

Three different types of freshwater were utilised in these experiments:

- Filtered pond water from Airthrey Loch, University of Stirling
- Peaty water from Dunblane Reservoir;
- Artificial spring water (Macinnis and Voge, 1970)

Filtered pond water

Samples of water from Airthrey Loch were collected regularly, filtered through a mesh of 50µm and kept in 40 litre, constantly aerated, containers. The containers were left at room temperature between 20-22 °C.

Filtered water from Dunblane reservoir

Two samples of peaty water were collected from Dunblane reservoir. The samples were filtered as above and kept constantly aerated. The containers were left at room temperature between 20-22°C.

Artificial spring water

Artificial spring water is an artificial medium utilised for the culture of zooplankton or molluscs with a pH of around 7.2. It is composed of different concentrations of ferric chloride, anhydrous calcium chloride, magnesium sulphate and phosphate buffer, diluted in distilled water previously kept under constant aeration. The methodology for the preparation of the stock solutions was as recommended by Macinnis and Voge (1970), as described in Appendix VI.

7.3.1.5 Water quality.

For the determination of the physical and chemical water parameters of the water from Airthrey Loch and Dunblane reservoir samples were collected in 300 ml plastic bottles and refrigerated prior to determination of the alkalinity, calcium and magnesium. The alkalinity was determined by the method of Total Alkalinity and Phenolphthalein Alkalinity described in Golterman, Clymo and Ohnstad (1978). Calcium and magnesium were determined by atomic absorption spectrophotometry, Perkin-Elmer 2280.

The hardness, mg equivalent $CaCO_3$/L was calculated according to the following formula:

$$2.497\ [Ca,mg/L] + 4.118\ [Mg,\ mg/L]$$

The temperature was measured with a -1 to 105°C glass thermometer, the pH with a Digital pH meter PW 9409 and the conductivity with a conductivity-meter pHOX 52. Measurements

were taken during the collection of the water samples and/or during the experimental periods.

7.3.1.6 Free-living copepods

Samples of free-living copepods were collected with a 20 µm plankton net from Airthrey Loch, University of Stirling, Scotland. Initially, the copepods were separated by size and species where possible. Subsequently, the females containing egg-sacs were placed in a small aquarium containing filtered pond water and plants for laboratory culture. Five to 10 females containing egg-sacs from the specimens separated for culture were fixed in 70% Alcohol for subsequent identification.

Pure cultures of *Cyclops viridis* (Jurine), a species of copepod commonly sold commercially as live food for ornamental fish, were obtained through Sciento Ltd. On arrival of the cultures all females containing egg-sacs were separated from the main culture and placed in a small aquarium to be cultured under laboratory conditions according to the instructions supplied .

7.3.1.7 *In vitro* culture of copepods

Female copepods collected from Airthrey Loch were placed in a small 2 l aquarium containing filtered pond water and plants. They were fed with fine particles of oats once per week and occasionally with egg yolk.

The procedures for culture of *Cyclops viridis* were those recommended by Sciento Ltd. and the copepods were fed with *Paramecium* spp. and *Chlorella* spp. 3 times per week. All cultures of copepods were kept at room temperature and under low aeration.

7.3.1.8 Identification of the intermediate host

The species of free-living copepods collected in Airthrey Loch, were sent to the Institute of Freshwater Ecology, Windermere Laboratory, for confirmation of identification.

7.3.1.9 Infection of intermediate hosts

Twenty-four to 48 hours prior to infection between 25 and 100 copepods were taken with a small plankton net from the cultures of free-living copepods kept under laboratory conditions.

The copepods were separated by species and size and divided into groups of 5, 10 or 20 specimens each. Subsequently, each group was placed in a 5 cm diameter petri dish containing 1 of the 3 types of water utilised in the experiment. A small quantity of detritus (aquatic plants) was occasionally added to the petri dishes because this material can be utilised by the L_1 for attachment (Moravec, 1969, 1974). Individual specimens of copepods were also placed in 2-3 ml capacity Multiwell plates.

All copepods were starved for a period of 24 to 72 hours prior to infection. However, in those cases where no feeding of the larvae had been observed during the previous experiments, the period of starvation was increased to 120 hours.

Five to 20 very active larvae were placed in each petri dish together with the copepods. Those copepods placed individually in the Multiwell plates were each exposed to 2 to 3 larvae.

In the first 12 hours after the larvae had been placed together with the copepods each plate was examined under the stereoscopic microscope at intervals of one hour. Subsequently, they were examined at intervals of 8 hours.

Those copepods that became infected and those observed swallowing the larvae were removed with a pipette and placed in another petri dish containing only spring water or filtered pond water. Infected copepods, and all copepods which had been exposed to the L_1 utilised in each experiment, were kept under observation for up to 21 days post-infection (d.p.i). Assessment of the developmental stage of the larvae was conducted according to Moravec (1969, 1975).

7.3.1.10 Infection of final hosts

The final hosts of the species of *Spirocamallanus* sp., *Brochis splendens* and *Corydoras sterbai* utilised in the experiment, had been held in a tropical aquarium for at least

four months. In the experimental fish hosts, food was withheld for 24 hours prior to the infection.

Each potential host fish was placed in a 300 ml small glass vessel containing filtered pond water and kept under low aeration. One or more specimens of copepod infected with L_3 of *Spirocamallanus* sp. were placed in the glass vessel to allow the fish to swallow the copepods spontaneously. When the copepods were not swallowed, they were introduced into the stomach of the fish through a small pipette.

Infected fish were killed between 45 and 60 days after infection. The procedures for maintenance of fish and the general post-mortem and parasitological techniques were described in Chapter 2.

7.3.1.11 Descriptions, drawings and photographs of the larval stages

In the descriptions and in the tables, all measurements are in micrometers (μm), unless otherwise indicated. The methodology for measurements was the same as described in Chapter 5, section 5.3.4.

7.3.2 Development of the life cycle in Brazil.

7.3.2.1 Collection of the gravid female nematodes

Female adults containing first stage larvae in the uterus were collected from the intestine of specimens of *Corydoras*

sterbai Knaack, 1962, sampled at the exporters' holding facilities in Manaus, Brazil. The procedures for collection and maintenance of the females and the first stage larvae were the same as those described in sections 7.3.1.1 and 7.3.1.2.

7.3.2.2 Samples of freshwater from the earth ponds

In the experiments conducted in Brazil samples of water were collected regularly from the earth ponds where specimens of *Corydoras* were held in the exporters' holding facilities. The water supply of these ponds comes from a small stream, which also supplies all the water for the exporters' holding facility.

The samples were collected in 40 litre containers, filtered and kept under constant aeration at room temperature.

7.3.2.3 Water quality.

The same physical and chemical parameters measured in the UK (section 7.3.1.5) were measured in the samples of water collected at the exporters' holding facilites in Brazil. The physico-chemical analyses were conducted in the water quality laboratory at the National Institute for Research in the Amazon (INPA), Manaus, Amazonas, Brazil.

7.3.2.4 Collection of free-living copepods in Brazil

Samples of free-living copepods were collected with a 20 µm plankton net from the earth ponds at the exporters' holding facilities (see section 7.3.2.2). Generally, in these ponds specimens of *Corydoras* spp. are stocked for a long period (between 4-6 months).

Five to 10 female copepods containing egg-sacs were fixed in 10% formalin for subsequent identification.

7.3.2.5 Culture of free-living copepods

The samples of copepods were separated by size and species and the females of each species with egg-sacs were removed from the samples. They were placed in 1 litre beakers containing filtered pond water and plants for the development of the cultures *in vitro*.

Other specimens sampled were individually placed in a small petri dish with a drop of water and examined under a stereoscopic microscope for the presence of larvae of nematodes in the haemocoel. Uninfected copepods were kept in 1 litre beakers containing filtered pond water and plants for subsequent utilisation in the life-cycle experiments.

The procedures followed for the maintenance of the copepods were those described for the cultures of zooplankton from Airthrey Loch (section 7.3.1.7).

7.3.2.6 Identification of the intermediate host

The species of free-living copepods collected in the earth ponds at the exporters' holding facilities were identified in the Zooplankton Section of INPA.

7.3.2.7 Infection of the intermediate Host.

Two hundred specimens of *Thermocyclops dicipiens* (Kiefer, 1929) and 50 specimens of *Mesocyclops brasilianus* Kiefer, 1933, of different sizes were utilised in 2 experimental infections.

In the first experiment, 100 specimens of *T. dicipiens* and 50 of *M. brasilianus* were separated into 5 groups of 20 and 10 respectively. In the second experiment, 100 specimens of *T.dicipiens* were separated into 5 groups of 20 specimens each. The groups were placed in small petri dishes containing filtered pond water, detritus and first stage larvae recently released from the uterus. One group of each species was placed in a small petri dish containing only water and detritus (control group).

The period of starvation prior to the infection was from 24 to 48 hours and the infected copepods were kept under observation for up to 38 days post-infection (d.p.i.). Other procedures were the same as those described in section 7.3.1.9

7.3.2.8 Infection of the final hosts.

Specimens of *Brochis splendens*, *Corydoras sterbai*, *C. julii* and *C. haroldschultzi* were exposed to copepods experimentally infected with L_3 of *Spirocamallanus* spp. The procedures for infection of the fish were basically the same as these described in section 7.2.1.10. Due to the limited time available for studies in Brazil the experiments could not be continued beyond 30 d.p.i of the final hosts.

7.4 Results

7.4.1 Experiments in the United Kingdom

7.4.1.1 Species of *Spirocamallanus*

Among the 5 species of *Spirocamallanus* found in the ornamental fish examined in the UK, only one species has been previously described, *S. inopinatus* Travassos, Artigas and Pereira, 1928 (Figs. 7.1a,b and 7.2c). Two species *Spirocamallanus* sp[1] (Fig.7.2a) and *Spirocamallanus* sp[2] (Fig.7.2b), parasites of *Brochis splendens* and *Corydoras sterbai* respectively, although presenting similarities with other South American species, appear to be new species. The other two species, *Spirocamallanus* sp[3] and *Spirocamallanus* sp[4], parasites of *Corydoras metae* and *Mylossoma aureum*, could not be identified to a specific level because only immature specimens or only females were found.

The measurements of the specimens of the 5 species of *Spirocamallanus* are presented in Table 7.4.

7.4.1.2. Prevalence and intensity of infection of *Spirocamallanus* spp.

The five species of *Spirocamallanus* were found in 6 species of fish but the large majority of the specimens found were immature adult stages (non-gravid females or females with only small numbers of larvae in the uterus). The

proportions of each are shown in Table 7.5. Larval stages representing recent infections of camallanids were found in the intestine of *Hyphessobrycon* sp.. In the species *C. callichthys* larval stages were found encysted in the mesentery. Since this is an unusual finding in camallanids, and because the host is a predator, it appears that this fish was acting as paratenic host. *Spirocamallanus* sp[1] and *S. inopinatus* presented the highest prevalence, 36 and 100% respectively, and *S. inopinatus* was found in as many as 3 host species, *Corydoras julii*, *Colossoma macropomum* and *Metynnis hypsauchen*. The prevalence and intensity of infection of the species of *Spirocamallanus* spp. are presented in Table 7.6.

7.4.1.3 Period of survival of first stage larvae.

The period of survival of the first stage larvae of *Spirocamallanus* spp. depended on:

1. the stage of development of the L_1 in the uterus of the gravid female;
2. the type of medium to which they were exposed after release;
3. the temperature of the medium.

Around 50-60% of the larvae released from gravid females containing only larvae in the uterus, and 90-95% (sometimes 100%) of the larvae released from females containing both completely formed larvae and embryos at different stages of

development in the uterus, died in the first hour after release from the uterus in all types of medium used.

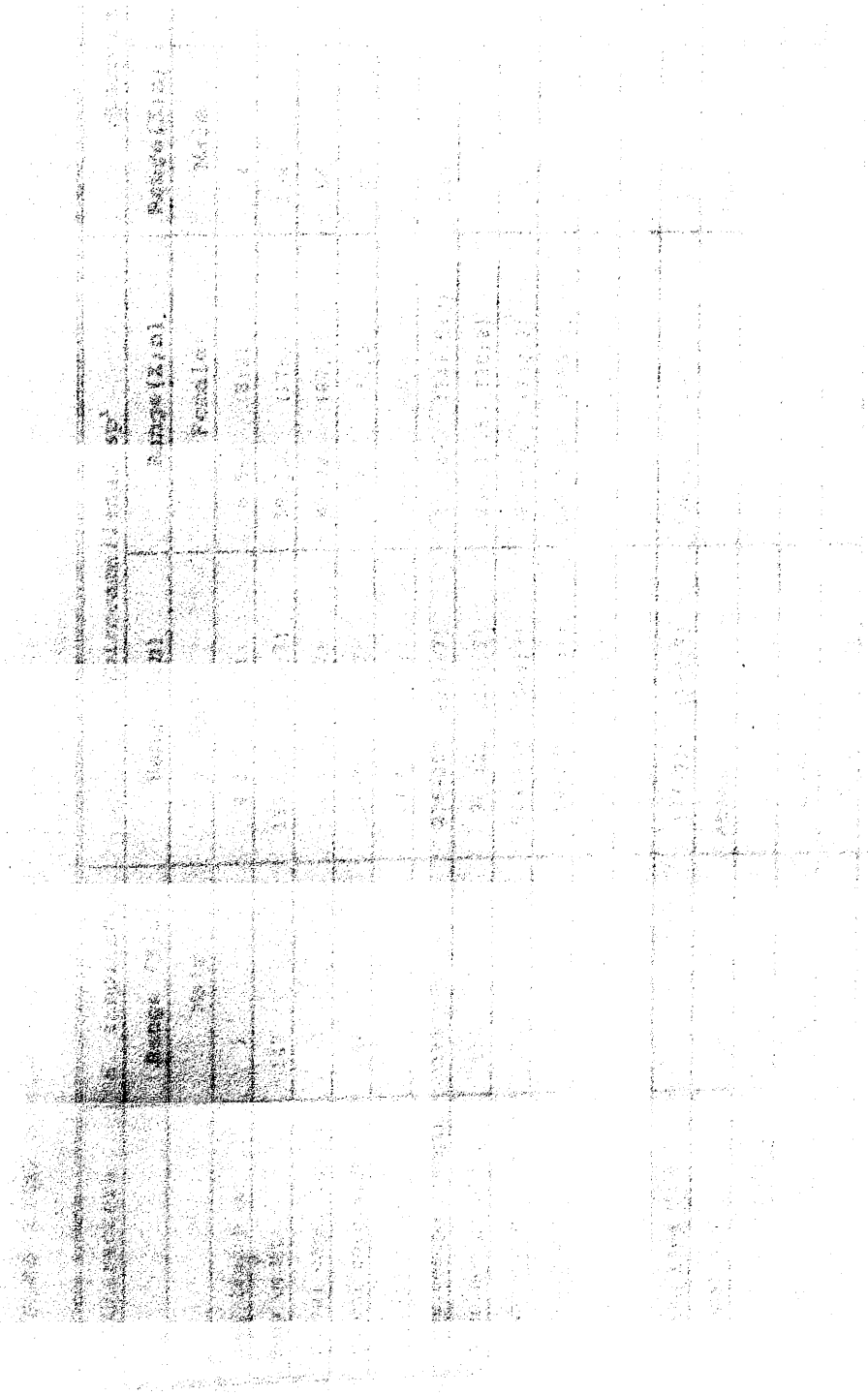


Table 7.4

Measurements of the species of *Spirocamallanus* found in the intestine of ornamental fish imported from South America.

Characters	*S. inopinatus*	*Spirocamallanus* sp[1]		*Spirocamallanus* sp[2]	
	Range (X;n)	**Range(X;n)**	**Range(X;n)**	**Range(X;n)**	**Range(X;n)**
	Male	Male	Female	Male	Female
Body length mm	2	4.5-5 (5;7)	6.5-9 (8;2)	2	6-14 (9;3)
Body width	147	139-201 (166;7)	98-252 (175;2)	108	211-549 (326;3)
Buccal caps. length	58	76-84.5 (79;7)	85-88 (87;2)	56	56-63 (58.5;3)
Buccal caps.width	44	51-72 (58;7)	68-73 (71;2)	43	43-58.5 (51;3)
No. spires b.c.	-	4-6 (6;7)	6-7 (6;2)	3	4-7
Musc.oesoph. length	172	275-307 (287;7)	334-349 (341.5;2)	250	255-295 (278;3)
Musc.oesoph.width	116	94-123 (108;7)	123-137.5 (130;2)	78.5	69-127 (100;3)
Gland.oesoph.length	357	548-655 (584;7)	697-766 (732;2)	442	481-746 (583;3)
Gland.oesoph.width	75	94-145 (124;7)	157-220 (189;2)	147	93-118 (101.5;3)
Total oesoph.length	529	731-962 (848;7)	1-2 (1;2)	692	736-805 (782;3)
Excretory pore	-	194	-	-	207
Nerve ring	-	177-210 (194;7)	196-206 (201;2)	134	161-134 (147;3)
Caudal alae	-	absent	-	Absent	-
Total No. papillae	10	6	-	6	-
Right spicule	60	47-91 (63;7)	-	54	-
Left spicule	66	48-85 (69;7)		54	-
Vulva mm	-	-	5-6 (6;2)	-	4-5 (5;3)
Tail	93	200-238 (218;7)	260-471 (365.5;2)	117	201-304 (257;3)

Table 7.4 (Cont.)

Measurements of the species of *Spirocamallanus* found in the intestine of ornamental fish imported from South America.

Characters	*Spirocamallanus* sp[3]		*Spirocamallanus* sp[4]
	Range (X;n)	Range (X;n)	Range(X;n)
	Male	Female	Female
Body length mm	3 (3;1)	10.5-11 (11;2)	6-11 (8.5;2)
Body width	88 (88;1)	523-612 (567.5;2)	252-365 (308.5;2)
Buccal caps. length	47 (47;1)	56-59.5 (58;2)	56-66 (61;2)
Buccal caps.width	33 (33;1)	44-47 (45.5;2)	47-59.5 (53;2)
No. spires b.c.	5 (5;1)	5-8 (6.5;2)	6-8 (7;2)
Musc.oesoph. length	222 (222;1)	266-288 (277;2)	257-297 (138.5;2)
Musc.oesoph.width	59.5 (59.5;1)	-	94-128 (111;2)
Gland.oesoph.length	353 (353;1)	-	541-659 (600;2)
Gland.oesoph.width	37.5 (37.5;1)	-	94-122 (108;2)
Total oesoph.length	575 (575;2)	-	-
Excretory pore	-	-	88-241 (164.5;2)
Nervous ring	94 (94;1)	141-188 (164.5;2)	141-165.5 (153;2)
Caudal alae	-	-	-
Total No. papillae	-	-	-
Right spicule	39 (39;1)	-	-
Left spicule	45 (45;1)	-	-
Vulva mm	-	2.5 (2.5;1)	6
Tail	113 (113;1)	195 (195;1)	244

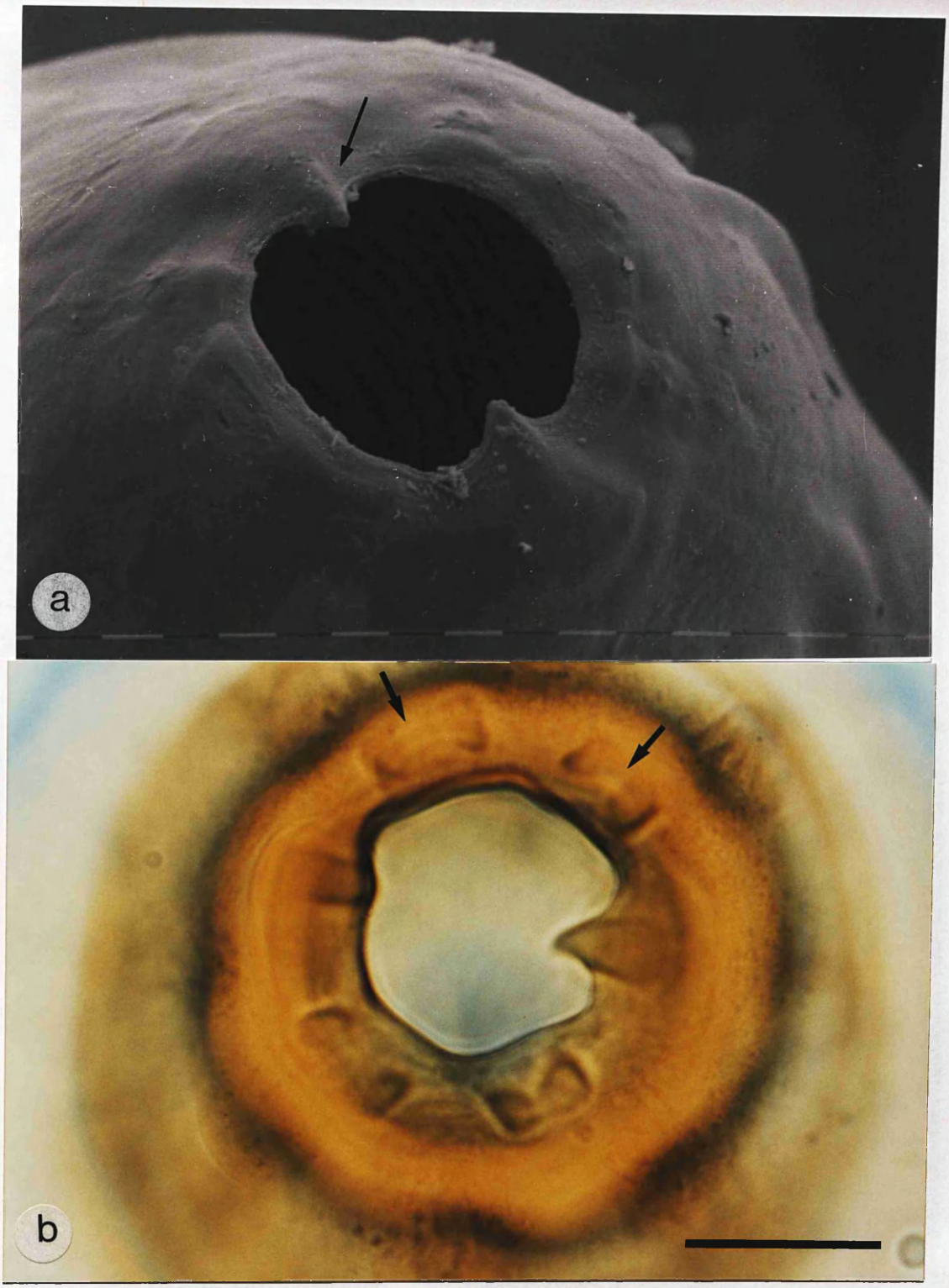


Fig. 7.1a-b. *Spirocamallanus inopinatus* Travassos *et al.*, 1928. a - en face showing the two teeth (arrow) localised in the aperture of the buccal capsule (SEM x640). b - en face showing the two teeth and 4 cutting plates (arrow). *Scale bar:* b 20µm.

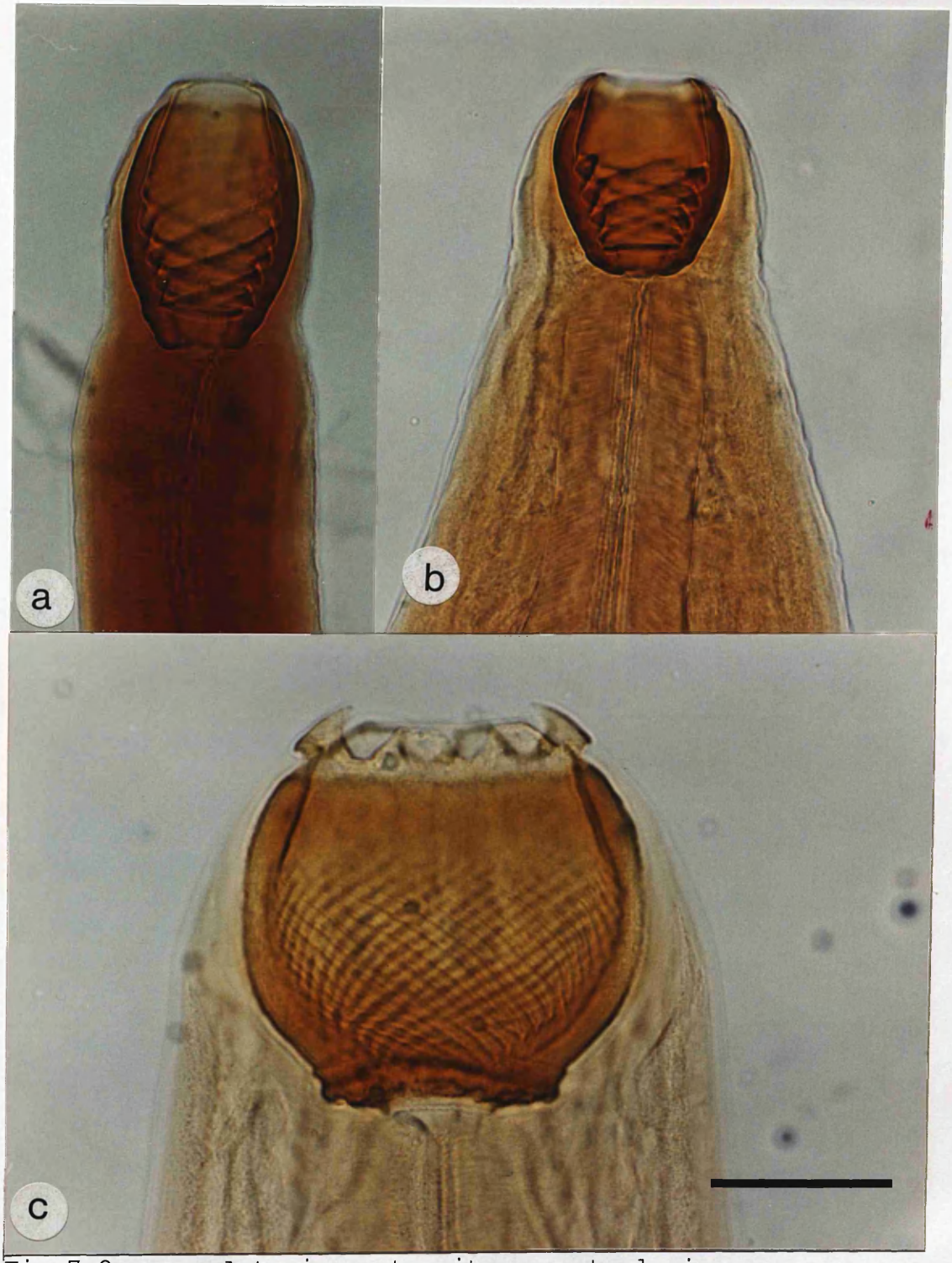


Fig.7.2 a-c. Anterior extremity - ventral view

a - *Spirocamallanus* sp[1] b - *Spirocamallanus* sp[2]

c - *S. inopinatus*. *Scale bar:* a-c 50µm. Note the differences in the thickness of the spiral bridges and their restriction to the proximal part of the buccal capsule.

Table 7.5

Total number of adult males and females and larval stages of species of *Spirocamallanus* spp. found in the species of ornamental fish sampled in the UK.

Family/Species of Fish	Number of Males	Females		Number of Larvae
CALLICHTHYIDAE		**Immature**	**Gravid**	
Brochis splendens	8	16	5	-
Corydoras sterbai	-	3	-	-
Corydoras julii	1	-	-	-
Corydoras metae	3*	7	3	-
Callichthys callichthys	-	-	-	2
CHARACIDAE				-
Colossoma macropomum	5*	8	2	-
Metynnis hypsauchen	2	1	-	-
Mylossoma aureum	-	12	1	-
Hyphessobrycon sp.	-	-	-	2
TOTAL	19	47	11	4

* Immature

Table 7.6

Prevalence and intensity of infection of nematodes of the family Camallanidae in ornamental fish sampled in the UK.

Family/Species of Fish	Family/Species of Nematode	Prevalence (%)	Intensity of Infection (Range)
CALLICHTHYIDAE	**CAMALLANIDAE**		
Brochis splendens	*Spirocamallanus* sp[1]	36	1-3
Corydoras sterbai	*Spirocamallanus* sp[2]	30	1-2
Corydoras julii	*S. inopinatus*	4	1
Corydoras metae	*Spirocamallanus* sp[3]	23	1-3
Callichthys callichthys	L_3	33	1-2
SERRASALMIDAE			
Colossoma macropomum	*S. inopinatus*	100	1-3
Metynnis hypsauchen	*S. inopinatus*	30	1-2
Mylossoma aureum	*Spirocamallanus* sp[4]	13	1-3
CHARACIDAE			
Hyphessobrycon sp.	L_3	20	1-3

No larvae survived longer than 10 minutes when the females of *Spirocamallanus* sp[1] were forced to burst directly into filtered pond water at 15-18°C which had just been recently collected from Airthrey Loch.

However, larvae which were obtained from females by tearing with a fine needle and kept in physiological saline solution survived up to 3 days after subsequent transfer to petri dishes containing filtered pond water at room temperature (19-27°C). Those placed in artificial spring water survived up to 7 days under the same conditions.

Larvae kept in physiological saline solution survived up to 10 days at room temperature and up to 1 month under refrigeration. The saline solution was exchanged every day.

7.4.1.4 Water Quality

The physical and chemical characteristics of the samples of water from Airthrey loch, Dunblane reservoir and the artificial spring water are presented in Table 7.7. As can be seen from this table, the characteristics of the two natural waters, Dunblane reservoir and Airthrey loch were very different. The first presented high values for pH, conductivity range, and magnesium, and the second high values for calcium (19.76mg/1) and hardness (64.16). According to the ASTM (1980) the samples from Dunblane reservoir would be classified as very soft water (hardness between 0-30 mg/L $CaCO_3$) and the samples from Airthrey loch as moderately soft (hardness between 60-120 mg/L $CaCO_3$).

Table 7.7

UK Freshwater Parameters: Physical and Chemical

Parameters	Dunblane Reservoir	Spring Water	Airthrey Loch
PH	7.02-8.0	5.93-7.10	6.40 -7.44
Conductivity (μS)	60-133.80	15.53	117.40
Alkalinity	0.29	0.26	0.984
Calcium (mg/l)	10	8.42	19.76
Magnesium (mg/l)	9	2.08	3.60
Hardness (mg/l)	19	29.59	64.16

7.4.1.5 Infection of the Intermediate Host

A total of 10 experiments were conducted under laboratory conditions for the infection of the intermediate hosts. Overall, the success of infection of the intermediate hosts was very low, with the prevalence varying between 3.3 and 15.6%.

Five species of free-living copepods collected from Airthrey loch were utilised as potential intermediate hosts. These were: *Cyclops strenuus abyssorum*, Sars, *C. agilis*, Koch, *C. viridis* (Jurine), *C. bicuspidatus*, Claus, and *C. macruroides* s.str., Lilljeborg.

Only specimens of *Cyclops strenuus abyssorum* and *C. viridis* were observed swallowing and developing larvae in the haemocoel. No specimens of *C. agilis*, *C. bicuspidatus*, and *C. macruroides* were found to be infected even though they

were starved for a total of 120 hours compared to the 24-36 hours starvation of *C. strenuus* and *C. viridis*.

The results of the experiments are summarised according to the species of nematode and fish host and are presented below:

EXPERIMENT 1

Host: *Corydoras metae* Eigenmann, 1914.
Species of Parasites: *Spirocamallanus* sp[3]
Number of Females = 3 specimens

Condition of the female of nematodes: All females utilised in these experiments were immature. They presented a light pink colour and had both completely formed larvae and embryos at different stages of development in the uterus.

Type of water: Filtered pond water
Artificial spring water

Results: Three experiments were conducted utilising filtered water from Airthrey loch, Dunblane reservoir and artificial spring water. The water temperature of the samples was between 23 and 24 °C. No larvae survived more than 10 minutes when the body of the female was forced to burst in these media.

EXPERIMENT 2

Host: *Mylossoma aureum* (Cuvier, 1818)

Species of Parasite: *Spirocamallanus* sp[4]

Number of Females = 2

Condition of the female of nematodes: All females utilised in these experiments presented a red colour and the uterus was completely full of very active larvae.

Type of Water: Filtered pond water

Artificial spring water

Species of Copepod: 14 *Cyclops strenuus abyssorum*, Sars.

14 *C. viridis* (Jurine).

10 *C. agilis*, Koch,

10 *C. bicuspidatus*, Claus.,

Results: Four experiments were conducted. In the first two experiments, specimens of *C. strenuus abyssorum* and *C. viridis* were placed with first stage larvae in separate petri dishes containing filtered water from Airthey loch with first stage larvae and kept at room temperature between 19 and 23°C. Five copepods, 3 *C. strenuus* and 2 *C. viridis*, became infected. The 3 infected specimens of *C. strenuus* died between the 1st and 3rd day. They were infected with 5,2 and 1 larvae in the haemocoel and/or intestine. The specimens of

C.viridis survived longer, between 11 and 22 d.p.i. and they were each infected with 1 larva.

In the other 2 experiments, specimens of *C. agilis* and *C. bicuspidatus* were placed in a petri dish containing artificial spring water and L_1 larvae. The room temperature was between 19 and 23°C. No infection was found and the larvae were observed to remain alive in the petri dish up to 7 days.

EXPERIMENT 3

Host: *Brochis splendens* (Castelneau, 1855)
Species of parasites: *Spirocamallanus* sp[1]
Number of females = 5 specimens

Condition of the female of nematodes: All females utilised in these experiments presented a red colour and the uterus was completely full of very active larvae.
Type of water: Filtered water from Airthrey loch
Artificial spring water
Species of copepods: 40 *Cyclops strenuus abyssorum*
140 *C. viridis*
Results: Three experiments were conducted. The first was conducted with the *C. strenuus abyssorum*. Copepods and larvae were placed in filtered water from Airthrey loch and kept at room temperature between 20 and 24°C. No infections were found.

The other two experiments were conducted with *C. viridis*. Copepods and larvae were placed in artificial spring water and kept at room temperature between 19 and 23 (21.3)°C.

Nine copepods became infected with 1 to 2 larvae of *Spirocamallanus* sp[1] of which 5 died between the 1st and 2nd d.p.i., 1 died 17 d.p.i. and 3 survived up to 22 d.p.i.. Out of the 3 survivors to 22 days, 2 copepods had 1 larva and 1 copepod had 2 larvae. The larvae started to present a yellowish colour in the intestine between 5 and 6 d.p.i. and started to coil in the haemocoel of the copepod after 15 d.p.i. (Fig. 7.3).

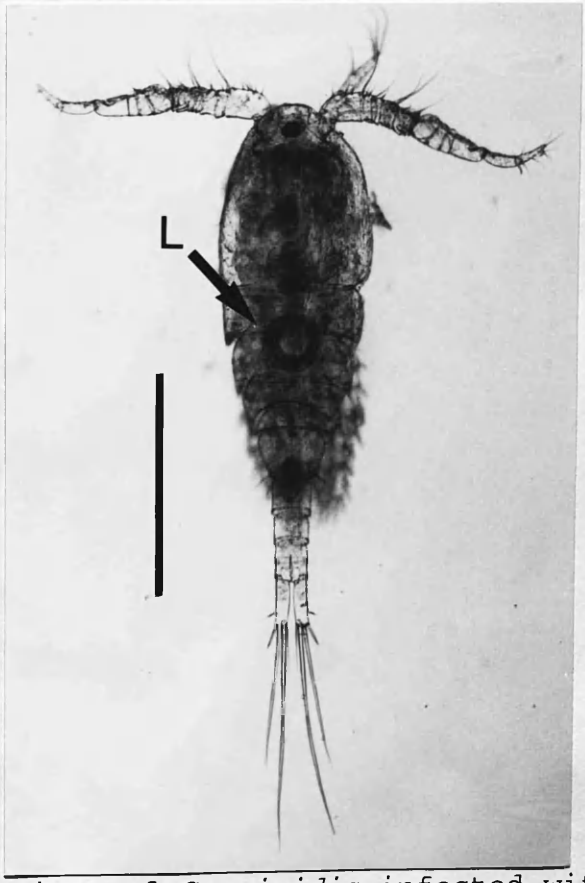


Fig. 7.3. - Specimen of *C. viridis* infected with one larva 22 d.p.i. L=larva. *Scale bar:* 1mm.

7.4.1.6 Description of the larval stages of *Spirocamallanus* sp[1].

First stage larvae (based on 4 larvae found in the haemocoel): The cuticle was very thin with dense cuticular striations mainly concentrated in the anterior extremity. The body was very slender with a long, transparent and sharply pointed tail. Cephalic papillae were not observed. Cephalic end with dorsal tooth present. The whole body of the larva had a granular appearance which made it difficult to define internal structures in detail.

In the larva from the copepod which died between 24 and 36 hours p.i. a fine buccal tube connected to the anterior extremity of the oesophagus became visible (Fig. 7.4 a-b). The first moult appeared to start on the 3rd d.p.i. The measurements of the larvae are presented in Table 7.8.

Third stage larvae

11 days p.i. based on 1 larva: Body ensheathed, colourless, slender and tapering towards extremities. Cuticle very thin with fine cuticular striations. Cephalic papillae 4 in number. Buccal capsule slightly sclerotized divided into two cavities with first cavity finely striated. Arcade cells present. Nerve ring visible. Excretory pore posterior to the nerve ring. Division of oesophagus into muscular and glandular not very distinct. Intestine with fine colourless granules. Tail with three small mucrones. The measurements

are presented in Table 7.8. For the later third stage larvae found, only the main differences are presented below:

17 days p.i - based on 1 larva: Cuticle thin with fine cuticular striations. Buccal capsule divided into 2 cavities, first cavity finely striated and with the walls thicker and more sclerotized than those from the second cavity. Second cavity with a funnel shape and walls thin and weakly sclerotized (Fig.7.5a). Division of oesophagus into muscular and glandular distinct. Glandular oesophagus formed but not yet completely separated from the intestine. Oesophagus-intestine valves not observed. Intestine with fine yellowish granules. Tail with three small mucrones. The measurements are presented in Table 7.8.

20 d.p.i - based on 1 larva: Cuticle slightly thicker with fine cuticular striations. Buccal capsule divided into 2 cavities and with the walls more sclerotized (Fig.7.5b). Division of oesophagus into muscular and glandular distinct. Glandular oesophagus completely separated from the intestine. Oesophagus-intestinal valves present. Intestine with fine dark yellow granules. Genital primordium oval. Tail with three small mucrones (Fig.7.5c). These larvae did not completely undergo the second moult. The measurements are presented in Table 7.8.

7.4.1.7 Infection of the Final Host

Three specimens of *Brochis splendens* were experimentally exposed to 3 infected copepods. In the first experiment one specimen of *B. splendens* was exposed to one copepod infected with two larvae 22 d.p.i.. The copepods were not observed after 2 hours and were assumed to have been ingested. No fourth stage larvae were recovered from the intestine of the fish 45 d.p.i..

In the second experiment, 2 specimens of *B. splendens* were exposed to 2 copepods which had been infected for 22 days with one larvae each. No larvae were recovered 2 months after infection.

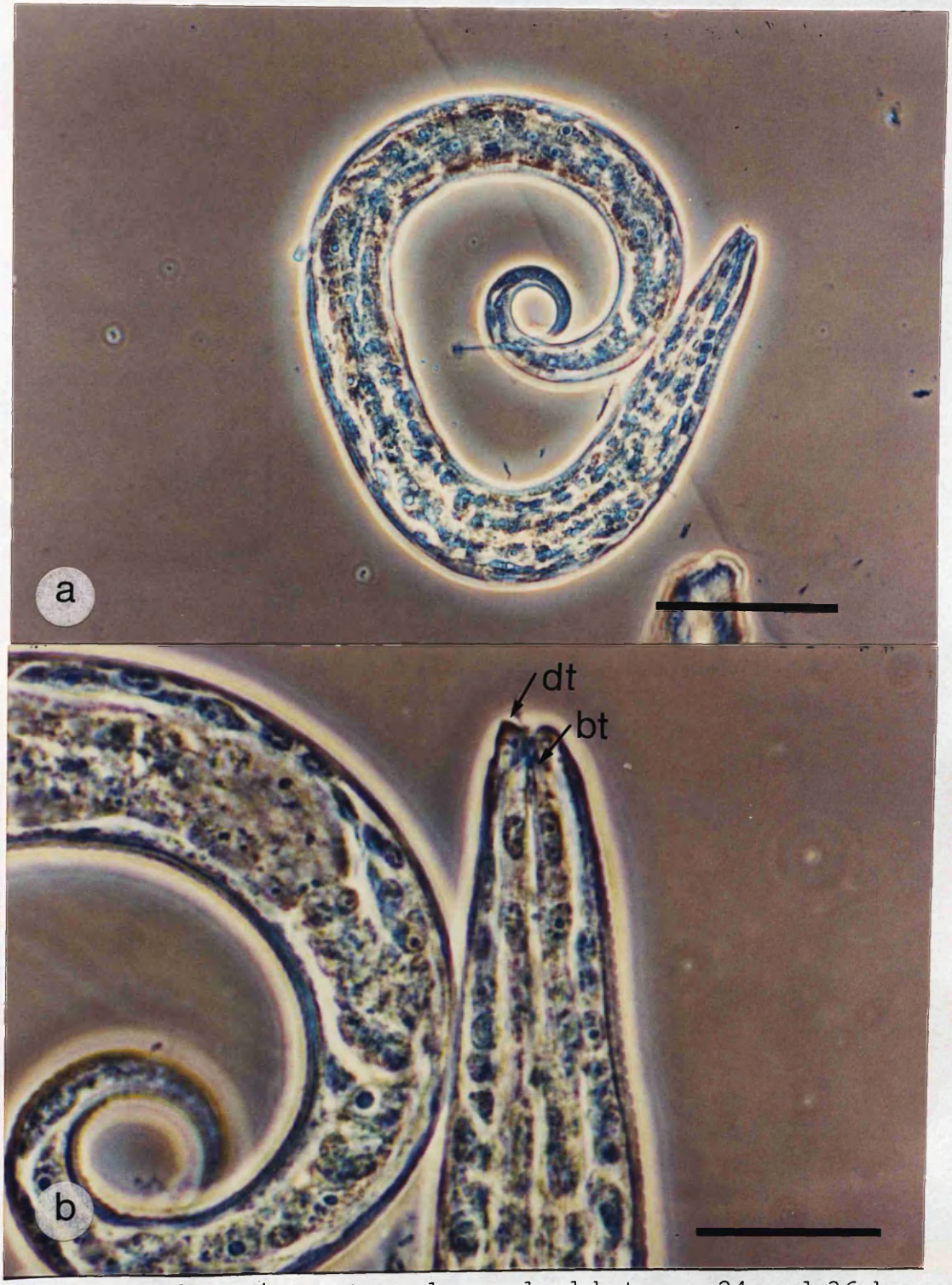


Fig. 7.4 a-b. First stage larva dead between 24 and 36 hrs p.i. a - total view. b - anterior extremity. dt= dorsal tooth bt= buccal tube. *Scale bar:* a 50µm b 20µm.

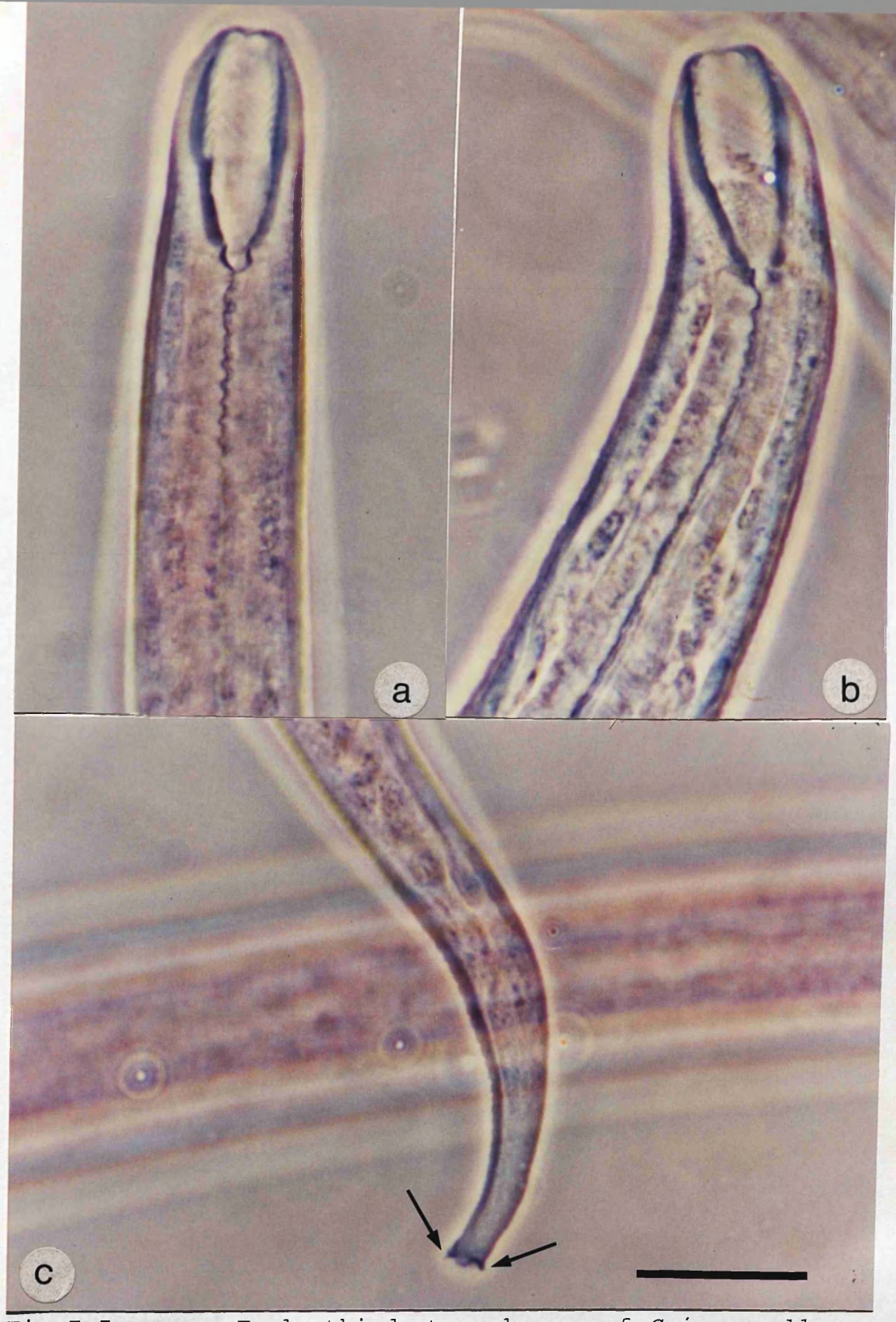


Fig 7.5 a - c Early third stage larvae of *Spirocamallanus* sp[1]: a - L_3 17 d.p.i, anterior extremity. b - L_3 20 d.p.i., anterior extremity. Note the difference in the level of sclerotization of the buccal capsule. c - L_3 posterior extremity showing the mucrones (arrow) *Scale bar:* a-c 20µm.

Table 7.8

Measurements of the larval stages of *Spirocamallanus* sp[1] in free-living copepods infected experimentally in the U.K.

	First Stage Larvae	Second Stage Larvae		
Character	**12-24 h. p.i Range (X;n)**	**11 d.p.i (n=1)**	**17 d.p.i (n=1)**	**20 d.p.i (n=1)**
Length of body	396-441 (418.5;2)	498	661.5	586
Width of body	20-27 (23.5;2)	20	31.5	22
Buccal capsule	-	First Cavity		
Length	-	14	16	14
Width	-	11	9.5	11
		Second Cavity		
Length	-	13	12.5	11
Width	-	6	9	8
Musc. oesophagus				
Length	-	113	110	111.5
Width	-	11	17	14
Gland.oesophagus				
Length	-	81	91	94
Width	-	15.5	19	16
Nerve ring*	-		66	_
Excretory pore*	-		67	_
Length of Tail	78.5		82	74

* Distance from the anterior extremity

7.4.2 Experiments conducted in Brazil

7.4.2.1 Prevalence and intensity of infection of *Spirocamallanus* spp.

Four species of nematodes of the genus *Spirocamallanus* were found in ornamental fish examined at the exporters'

holding facilities in Brazil of which 3 species, *S. inopinatus*, *Spirocamallanus* sp[1] and *Spirocamallanus* sp[2], were also recorded in the species of ornamental fish examined in the UK. The large majority of the specimens found were represented by immature stages or were single specimens, in similar proportions to those found in the UK. *Corydoras sterbai* Knaack, 1962, was the only species examined containing gravid females with fully developed larvae in the uterus. The prevalence and intensity of infection of the species are summarised in Table 7.9.

Table 7.9

Prevalence and intensity of infection of nematodes of the family Camallanidae in species of ornamental fish sampled in the exporters' holding facilities in Brazil

Family/Species of Fish	**Family/Species of Nematode**	**Prevalence (%)**	**Intensity of Infection (Range)**
CALLICHTHYIDAE	**CAMALLANIDAE**		
Brochis splendens	*Spirocamallanus* sp[1]	15	1-2
Corydoras sterbai	*Spirocamallanus* sp[2]	17	1-2
C. haraldschultzi	*Spirocamallanus* sp[5]	3	1
SERRASALMIDAE			
Mylossoma aureum	*S. inopinatus*	10	1-2

7.4.2.2 Period of survival of first stage larvae

When females of *Spirocamallanus* collected from *C. sterbai* were forced to burst in petri dishes containing either pond water or physiological saline, the immature L_1

larvae could be distinguished from the mature larvae. Generally, 50-60% of the immature first stage larvae died in the first hour after release from the uterus. Fully developed, mature first stage larvae, survived up to 15 days in petri dishes containing filtered pond water exchanged once per day at room temperature between 23 and 28°C.

7.4.2.3 Water quality

The physical and chemical parameters of the samples of water from the earth ponds in the exporters' holding facilities are presented in Table 7.10. As can be seen in this table, the samples exhibit the characteristics of acid waters, which may be due to the low pH of the soils through which the pond water drains and the high input of organic matter.

Table 7.10

Physical and chemical characteristics of freshwater from the earth ponds of the exporters' holding facilities in Brazil.

Water from Earth Ponds	
pH	4.36-4.43
Conductivity (µS)	11-13
Alkalinity	0.00
Calcium (mg/l)	0.071-0.106
Magnesium (mg/l)	0.26-0.28
Hardness (mg/l)	1.34
KCl (mg/l)	0.808-0.836
Temperature (°C)	25-27 °C

7.4.2.4 Infection of the Intermediate Host

Five species of copepods were sampled in the earth ponds at the exporters' holding facilites: *Mesocyclops brasilianus* Kiefer, 1933, *M. longisectus* (Thiebaud, 1914), *Thermocyclops dicipiens* (Kiefer, 1929), *Microcyclops* cf. *anceps* (Richard, 1987), *Metacyclops* cf. *brauni* Herbst, 1962 and *Tropocyclops* sp.. Two of these species were chosen for experimental studies, *Mesocyclops brasilianus* and *Thermocyclops dicipiens*, because they were the most abundant species in the earth ponds where the *Corydoras* species were held.

Three experiments were conducted utilising 250 specimens of copepods and 4 gravid females of *Spirocamallanus* sp[2]. The prevalence of infection of the intermediate host varied between 74 and 100%. The maximum number of larvae found inside single *M. brasilianus* and *T. dicipiens* copepods varied between 1-15 and 1-10 respectively. The results of the experiments, summarised according to the species of nematode and species of fish, are presented as below:

Experiment 1

Host: *Corydoras sterbai* Knaack, 1962
Species of Parasites: *Spirocamallanus* sp[2]
Number of Females = 4 specimens

Condition of the female of nematodes: All females utilised in these experiments presented a red colour and a uterus completely full of very active larvae.

Type of Water: Filtered pond water

Species of Copepods: 100 *Mesocyclops brasilianus*
70 *Thermocyclops dicipiens*

Results: Two experiments were conducted. The first experiment was conducted with specimens of *M. brasilianus*. The copepods and larvae were placed in a petri dish containing filtered pond water and left at room temperature between 25 and 28°C. Eighty-one copepods, corresponding to 74% of the total exposed to the L_1 of *Spirocamallanus* sp[2], were infected. Out of these, 66 specimens survived more than 21 d.p.i.. Severe mortalities were observed after 21 d.p.i., a result which may be associated with the sudden drop in temperature to 21°C, "friagem"[3], for 2 days, from 20 to 22 d.p.i..

The second experiment was conducted with *T. dicipiens*. Seventy specimens were utilised and 100% infection was obtained. Generally, between 1 and 10 larvae were found in each copepod. However, one specimen was found with 20 larvae.

[3] Friagem - a cold front from the Andes which occur occasionally in July.

7.4.3 Behaviour of the copepod

Generally, the copepods were attracted by the movement of the larvae, which wiggle their bodies up and down vigorously. The copepods were observed swallowing the larvae after the first hour of exposure. No alterations of the behaviour were observed in those infected with 1 to 5 larvae. Heavily infected copepods (up to 15 larvae) were observed mainly on the bottom and walls of the petri dish and appeared to have difficulty in swimming.

7.4.4 Infection of the final hosts with *Spirocamallanus* sp[2]

Two specimens of *Brochis splendens*, 2 of *Corydoras sterbai*, 2 of *C. julii* and 2 of *C. haraldschultzi* were utilised. The specimens of *B. splendens* were exposed to 3 copepods each containing two L_3, 22d.p.i. The specimens of *Corydoras* spp. were exposed to 2 copepods containing 1-3 L_3, 22 d.p.i.

One fourth-stage larva was recovered from the intestine of *C. sterbai* 15 d.p.i.. No fourth-stage larvae or young specimens of nematode were recovered in the other fish examined 30 d.p.i..

7.4.5 Description of the larval stages of *Spirocamallanus* sp² in the copepods.

First stage larvae (based on 8 specimens): Body slender, colourless, with dense transverse cuticular striations and tapering toward extremities. Cephalic papillae not observed. Cephalic end with dorsal tooth present in the larvae until 24 hours p.i. (Fig.7.6a). Muscular part of the oesophagus developed. Glandular part of the oesophagus poorly defined. Intestine containing granules of different size. Rectum indistinct, narrow short tube visible in the larvae undergoing the first moult. Tail very long and thin (Fig.7.7a).

The first moult was observed at the end of the second day and the beginning of the third day p.i. One of the most important external characters observed was the changing of the colouration of the intestine of the larvae to a dark orange. This process was followed by the separation of the old cuticle which is better observed in the anterior extremity and tail (Fig.7.7b). At this stage, the anterior extremity of the glandular oesophagus started to be visible and a large concentration of granules could still be observed inside the body.

As a consequence of the change in colour of the intestine of the larvae, the infected copepods could easily be differentiated from those not infected. They appear to present an orange colour in the cephalothorax which becomes more visible with the development of the larval stage. The

process was better observed in copepods with a transparent cephalothorax (e.g. *Thermocyclops*).

The measurements of the first stage larvae recovered from the infected copepods are presented in Table 7.11.

Second stage larvae (based on 8 specimens): The early stage was similar to the previous stage except for the absence of the dorsal tooth and the shape and size of the tail. The cuticle had fine striations and the mouth was a narrow weakly sclerotized tube.

The second moult started between the end of the fourth and beginning of the fifth day p.i.. The complete development of the buccal capsule occurred between the 5th and 7th d.p.i. and can be summarised as follows:

The buccal capsule started to be formed with the appearance of a hyaline, translucent bell-shaped, poorly sclerotized formation (Fig.7.6c). Subsequently, the anterior extremity starts to dilate and the anterior extremity of the muscular oesophagus becomes localised inside this expansion (Fig.7.6d). A lightly sclerotized buccal tube, connected between the anterior extremity of the oesophagus and the buccal capsule, was observed (Fig.7.8a). With the development of the larvae, the walls of the buccal capsule became more sclerotized and the anterior extremity of the oesophagus started to move posteriorly to form the second cavity (Fig.7.8b).

Between the 5th and 6th d.p.i. the cephalic papillae and the first spiral bridges of the buccal capsule were

completely visible and a second cavity with weakly sclerotized walls could be clearly differentiated (Fig.7.8c). The second cavity will form a kind of basal ring in the buccal capsule of the adult.

The division between the glandular and muscular oesophagus was completely distinct at the end of the 7th d.p.i. and the oesophagus-intestinal valves started to be formed in the late L_2 stage. The excretory pore and nerve ring were visible. The tail was shorter than the previous stage with three mucrones (Fig.7.9a-b).

The morphology of the late second stage larva is similar to the third stage except for the presence of the arcade cells posterior to the buccal capsule and the shape of the tail and rectal glands. The measurements of the second stage larvae recovered from infected copepods are presented in Table 7.12

Third stage larvae (based on 13 specimens): Third stage larvae were first observed in the free-living copepods 9 days p.i.. The larvae appeared to be very inactive and were always found coiled or coiling up inside the haemocoel of the copepods (Fig.7.10a-b). The body was fairly plump with a reddish colour and thick cuticle (Fig. 7.8d). After the second moult the cuticle was smooth and subsequently began to show a dense transverse striation in the middle part of the body. The buccal capsule was divided into two cavities and strongly sclerotized (Fig. 7.8d-e). The first cavity had thick lateral walls with the spiral bridges very well

defined. Cephalic papillae were present. The second cavity was more narrow than the first cavity. Arcade cells were not observed. Muscular and glandular oesophagus well defined. Oesophago-intestinal valves developed. Nerve ring and excretory pore visible. Genital primordium oval and localized in the third posterior part of the body (Fig. 7.11a). Intestine with large reddish granules. Rectal glands, 8 in number. Tail blunt, rounded, with small spines at the posterior extremity (Fig.7.11b). The measurements of the L_3 larvae are presented in Table 7.12.

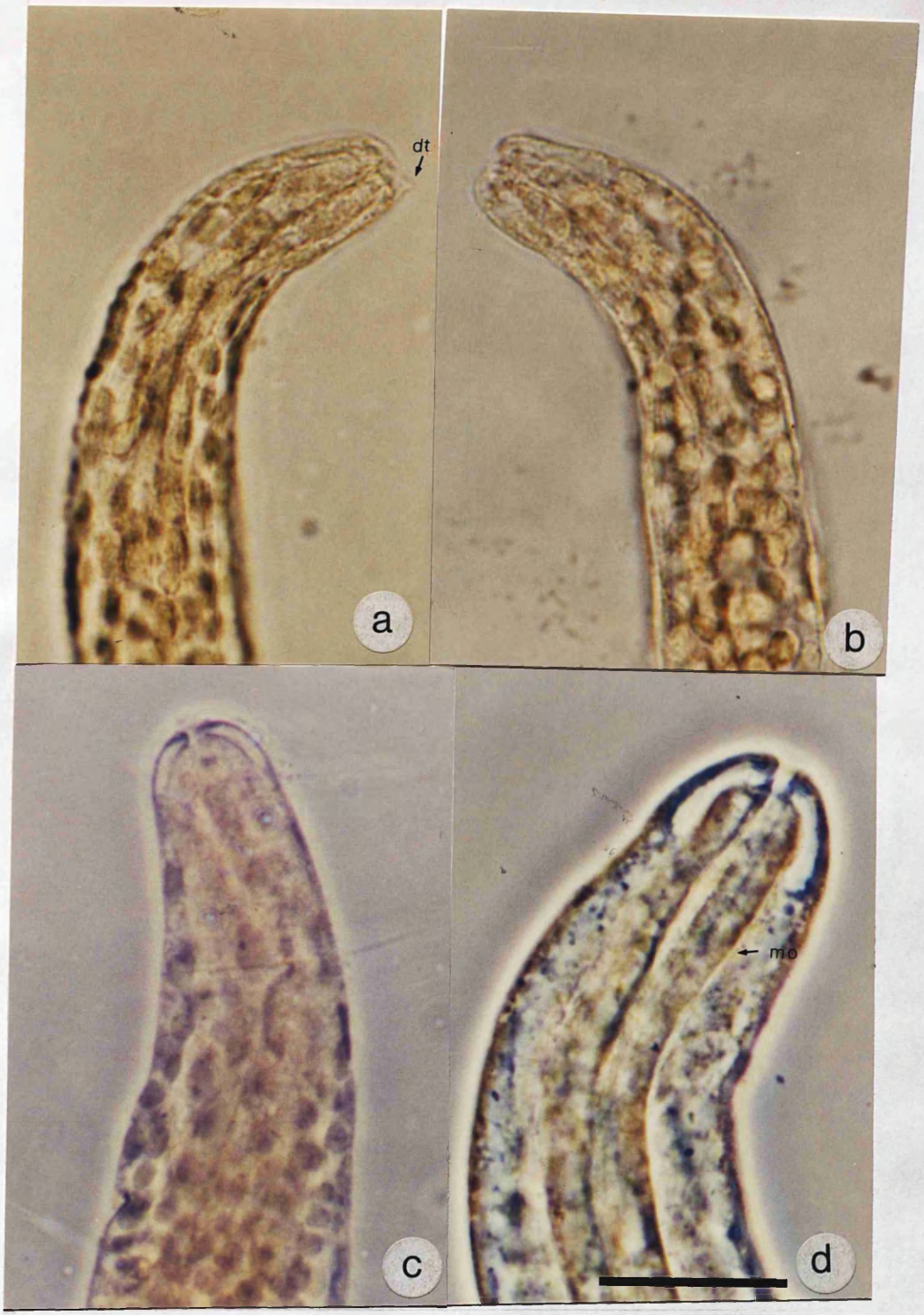


Fig. 7.6 - Larval stages of *Spirocamallanus* sp[2]. Note the decrease in granular appearance with development.

a - First stage larva - anterior extremity; b - d. Second stage larvae - development of the buccal capsule. dt= dorsal tooth mo= muscular oesophagus. *Scale bar:* 20µm.

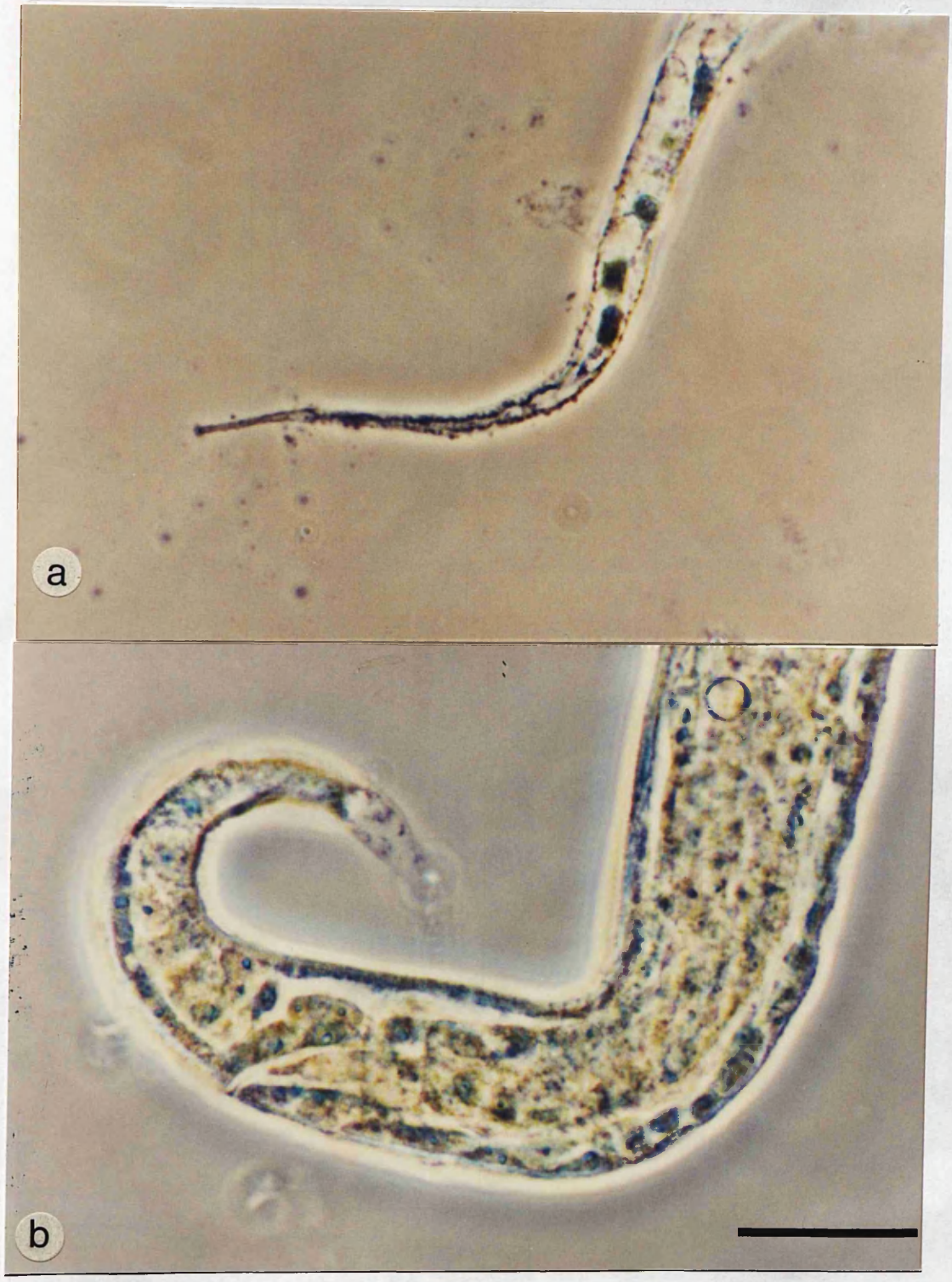


Fig. 7.7 - First stage larva. a - posterior extremity. b - Second stage larva - posterior extremity with the old cuticle. oc= old cuticle. *Scale bar*: 20µm.

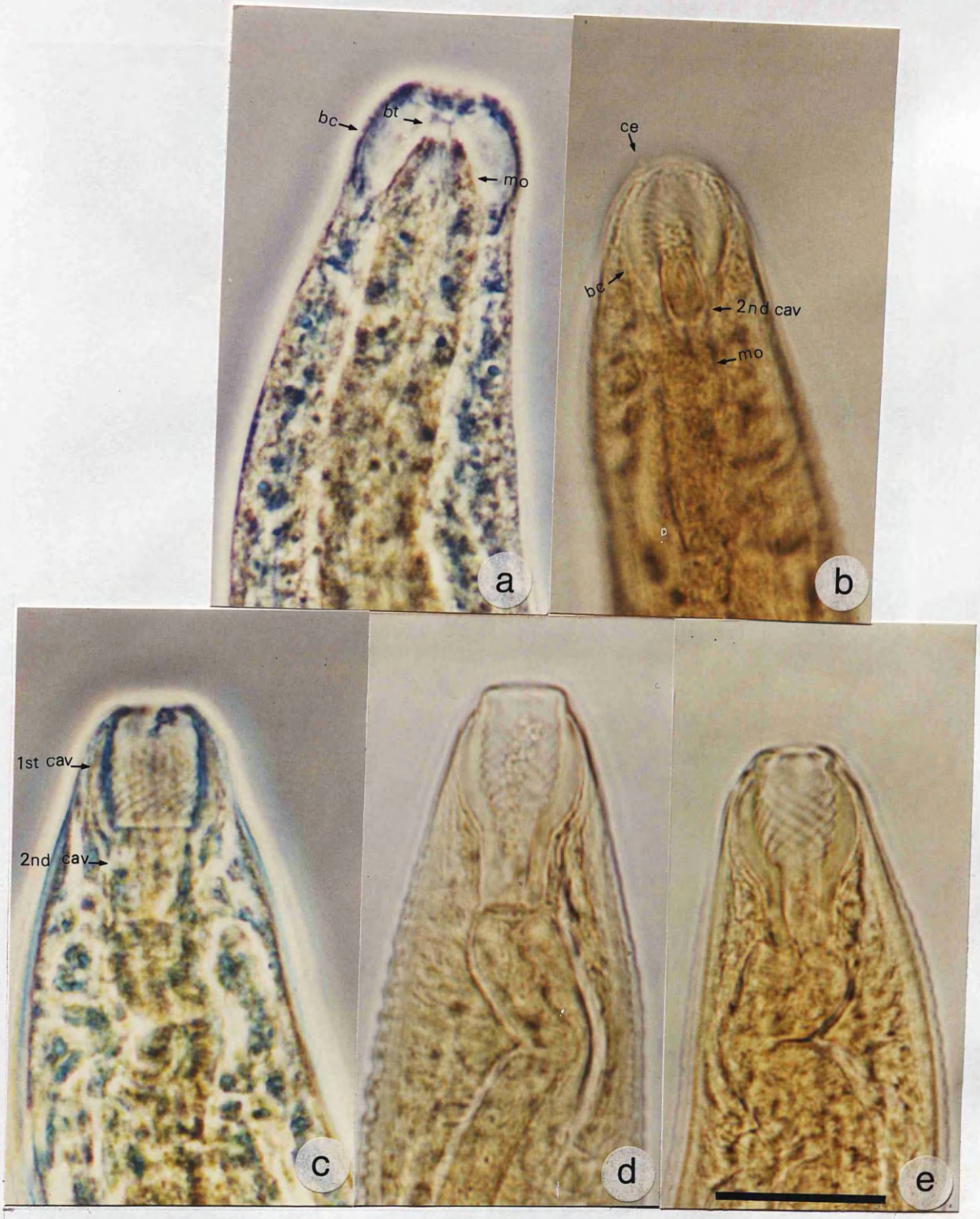


Fig. 7.8 - Developmental stages of *Spirocamallanus* sp[2]: anterior extremity. a - b second stage larva: 5 to 6 d.p.i; c - e third stage larva. c - early L_3, 7d.p.i.; d - L_3, 10 d.p.i; e - 38 d.p.i. 1st= first cavity; 2nd cav = second cavity; ce = cephalic papillae; bc = buccal capsule; bt= buccal tube; mo =muscular oesophagus. *Scale bar* = 20µm.

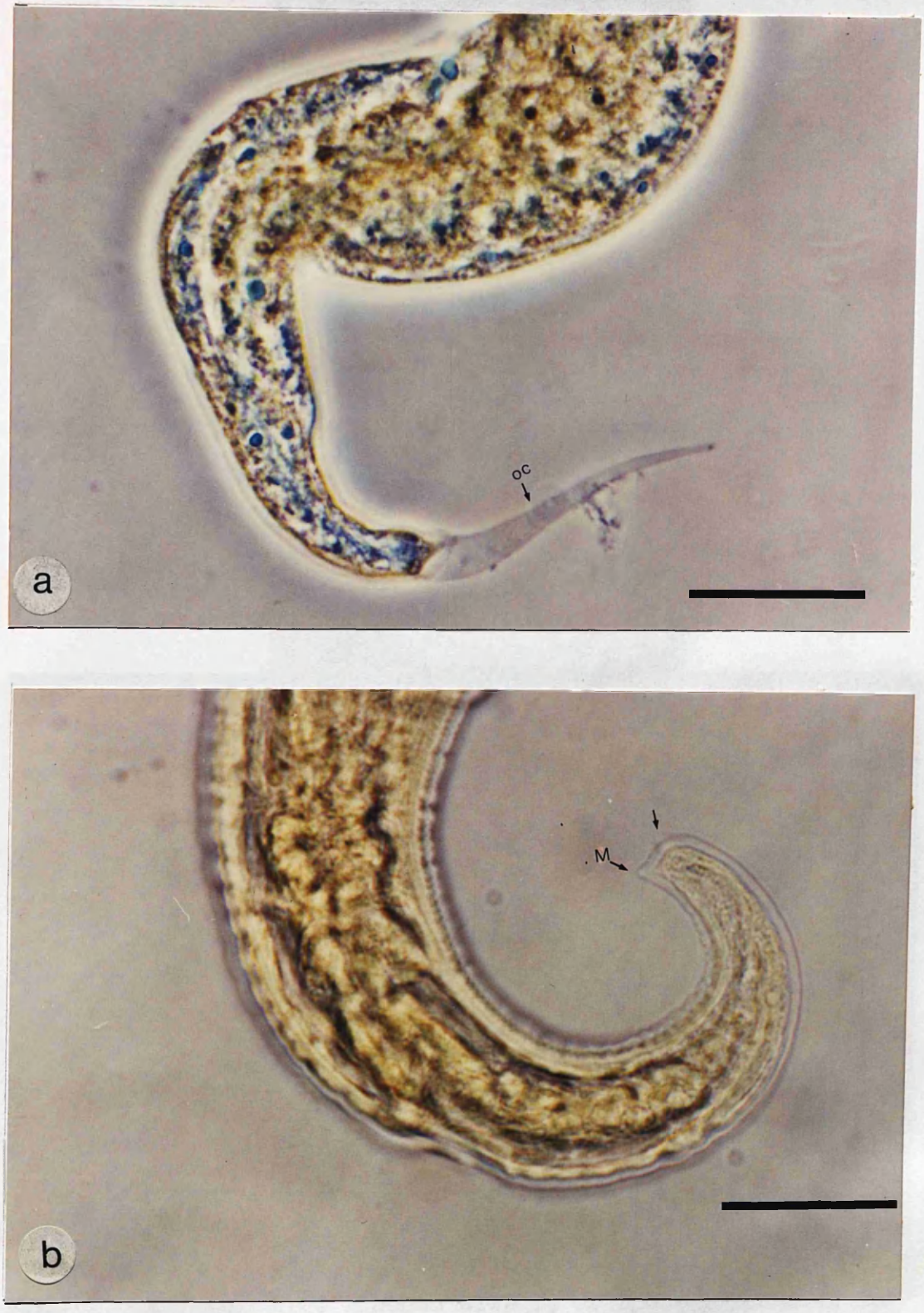


Fig. 7.9 a-b. Second stage larvae. a - posterior extremity with the old cuticle. b - posterior extremity with mucrones visible. oc= old cuticle m= mucrones. *Scale bar:* 20µm.

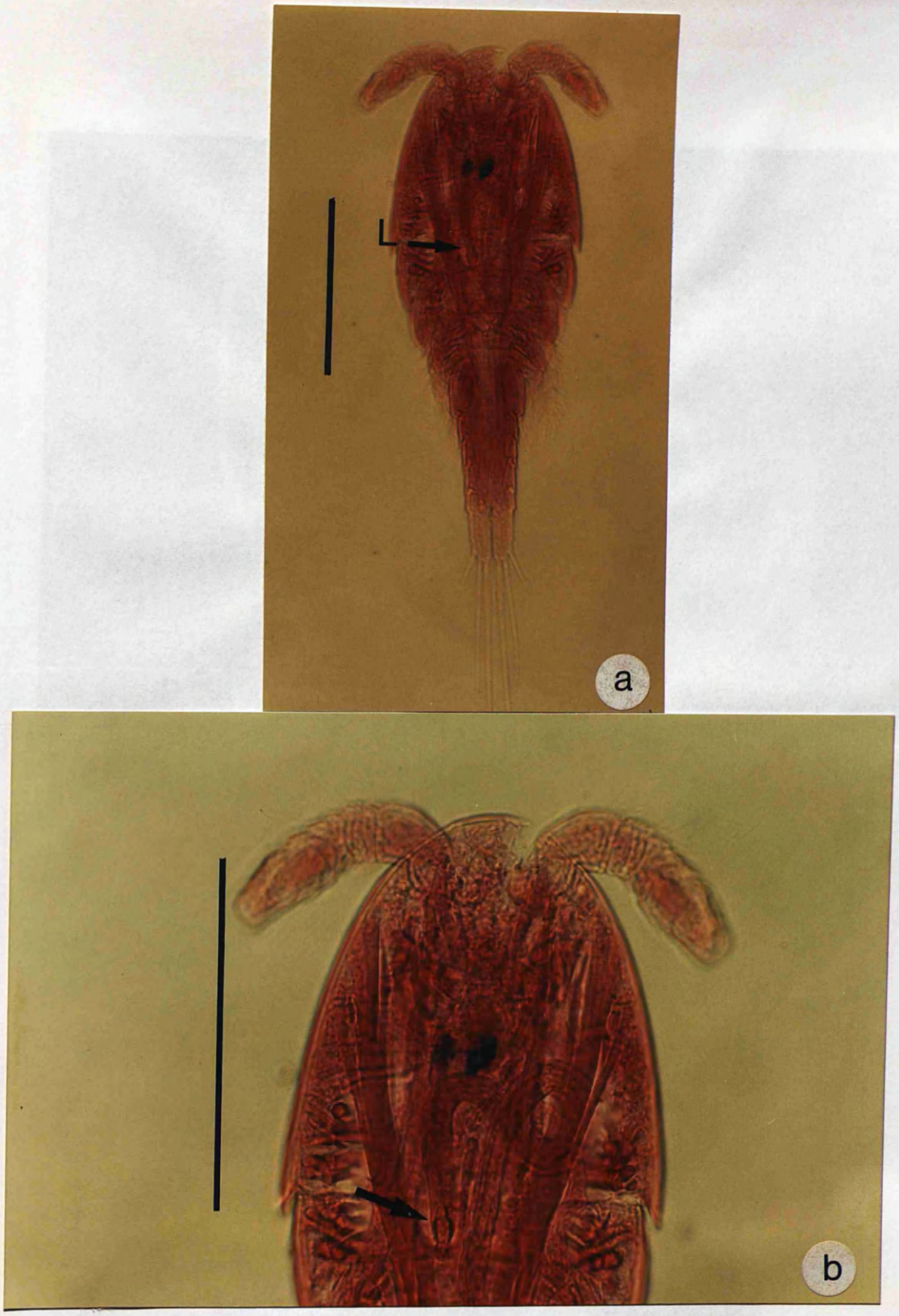


Fig. 7.10. - Infected copepod with L_3 of *Spirocamallanus* sp[2] in the haemocoel. a - total view b - note the division of the buccal capsule (arrow). L= larva bc= buccal capsule a-b 1mm.

Fig 7.11. - Third stage larva. a - total view. b - posterior extremity. Note the absence of mucrones in the tail. gp = genital primordium (arrow). *Scale bar:* a 50µm. b 20µm.

Table 7. 11

Measurements of the first stage larvae of *Spirocamallanus* sp[2] in free-living copepods.

First Stage Larvae		
Character	**1 - 2 d.p.i**	**2 - 3 d.p.i**
Length of body	419-461.5(440)	450-472.5(461.5)
Width of body	17	20-24(22)
L. musc. oesoph.	55-58(56.5)	86-97(92)
L. gland. oesoph.	47	45-63(54)
Dist. nerve ring	31	34.5-61(48)
Distance exc. pore	39	66-78(72)
Rectum	8	8
Length of tail	-	89-91(90)

Table 7.12
Measurements of the second and third stage of *Spirocamallanus* from the intermediate host

	Second stage larvae		**Third stage larvae**		
	4 - 6 days p.i.	**7 - 9 days p.i.**	**10 - 15 days p.i.**	**16 - 21 days p.i.**	**21 -38 days p.i.**
Length of body	394-586 (504.5)	435-542 (481)	466-633 (518)	505-697 (583)	540-769 (643)
Width of body	19-88 (34)	34.5-42(37)	23.5-34.5(29)	23.5-30(27)	28-39(31)
Buccal Capsule	First Cavity				
Length	12.5-15 (14)	14-16(15)	14-17(16)	15-16(16)	14-16(15.5)
Width	9-15 (11)	11-12.5(12)	11-14(12)	11-22(14)	11-12.5(11)
Second Cavity					
Length	8-10 (8.5)	8-11(9)	9-12.5(9)	7-11(9)	8-11(9)
Width	5-10 (7)	3-6(5)	6-5(6)	6-9(7)	5-8(6)
Muscular oesophagus					
Length	75-124(103)	94-124(113)	89-129(110)	97-119(107)	97-129(116)
Width	12.5-16(14.5)	12.5-16(15)	14-16(15)	13-18(15)	16-23.5(18)
Glandular oesoph.					
Length	47-88 (75)	82-88(85.5)	69-102(89)	78-113(87)	78.5-116(101.5)
Width	14-16 (15)	11-19(16)	16-20(18)	15-19(18)	17-19(18)
Nerve Ring*	47-63(55)	55-71(65)	63	53-75(65)	60-63(61)
Excretory pore*	74	50-63(59)	55-78.5(67)	58-75.5(67)	-
Rectum	9	-	19	10-16(13)	19-23.5(19.5)
Length of tail	63-81 (73)	60-88(72)	67.5-82(50)	60-78(68)	71-94(81)

* Distance from the anterior extremity.

7.5 Discussion

In this study adult and larval stages of nematodes of the family Camallanidae were found in nine species of fish (Table 7.6). The large majority of the nematodes were immature stages (females without larvae or with few larvae in the uterus) (Table 7.5). These results may reflect recent recruitment of these parasites in their natural environment or at the exporters' holding facilities during the holding period.

Among the 4 species of nematodes of the genus *Spirocamallanus* found it appears that only one species was previously described, *S. inopinatus* Travassos, Artigas and Pereira, 1928 (Figs. 7.1a-c and 7.2c). The specimens found in *Brochis splendens*, *Spirocamallanus* sp[1], and those species found in *C. sterbai*, *Spirocamallanus* sp[2], present similarities with 4 South American species, *S. krameri* Petter, 1974, *S. incaracorai* Freitas and Ibanez, 1970, *C. paraguayensis* Petter, 1990, and *S. inopinatus* Travassos, Artigas and Pereira, 1928, from which they mainly differ by the morphology of the buccal capsule and distribution and/or number of caudal papillae.

Spirocamallanus inopinatus has been described from several different species of freshwater fish and seems to be wide-spread in Brazil (Table 7.1) (Petter and Thatcher, 1988; Travassos *et al.*, 1928; Moravec *et al.*, 1993; Pinto and Noronha, 1972; Pinto *et al.*, 1974; Petter and Thatcher, 1988; Thatcher, 1991). So far this species has been described in 27 species of freshwater fish from two other South American countries, Argentina and Paraguay (Table 7.2) (Petter, 1990;

Hamman, 1978), and it would not be surprising if its geographical distribution also extended to other South American countries, mainly those that share part of the Amazon region and consequently its fauna. Other species of *Spirocamallanus* found in this study were not identified to a specific level because only one specimen or only females were found (Table 7.6).

For the transfaunation experiments to be carried out in the UK all mature females of *Spirocamallanus* containing large numbers of larvae in the uterus were utilised. *S. inopinatus* would have been the most suitable species for study in this way because of its well known wide host specificity (Tables 7.1 and 7.2) and wide ranging distribution (Hamman, 1978; Pinto *et al.*, 1974, 1975; Petter and Thatcher, 1988; Thatcher, 1991; Moravec *et al.*, 1993). Although these are not all the attributes necessary for a successful invader and good coloniser (Kennedy, 1994; MacArthur and Wilson, 1967) they will clearly assist a successful establishment (Kennedy, Bates and Brwon, 1989; Kennedy, 1994). Unfortunately, the experiments with this species were limited by the stage of development of the specimens found in the intestine of the infected fish, which were mainly immature stages.

7.5.1. Experiments conducted in the UK.

Survival period of the first stage larvae.

One of the major problems faced during the development of the life cycle of nematodes of the genus *Spirocamallanus* was related to the survival period of the first stage larvae.

In the experiments conducted, around 50-60% of the larvae from gravid females only containing fully developed larvae in the uterus, died in the first half an hour following their release. These figures sometimes reached 100% when gravid females having few fully developed larvae and a large quantity of embryos at different stages of development in the uterus were utilised.

As the high mortality rates were observed in all experiments conducted, independent of the type of water utilised (Table 7.7), it appears that all larvae present in the uterus of the gravid females do not mature at the same time and only the fully developed first stage larvae are able to survive different conditions from those present in the uterus. Under natural conditions the number of surviving larvae naturally released by the female into the environment are not known. However, as the larvae are released in batches by the gravid females inside the lumen of the fish's intestine, it is possible that some physiological adaptation take place in the intestine prior to release into the environment.

The first stage larvae are very active and after their release they fix themselves to the bottom of the petri dish or to the substrate with their tail continuously contracting and relaxing their body. They appear to be able to survive under different physico-chemical water conditions, but the temperature appears to be a limiting factor for them.

The results of the experiments conducted in the UK show that the first stage larvae of these tropical parasites were able to survive for a short time in all types of water tested experimentally since the water temperature was not below 19°C

(Table 7.7). The longest survival period, 7 days, was observed in the larvae placed in petri dishes containing artificial spring water of pH 5.5-5.9, and kept at room temperature between 19-26°C. In the other types of water utilised, larvae were observed to live for up to 3 days after their release from the uterus. It should be noted that when the larvae were released at a water temperature of about 19°C their movements became very slow until eventually they became completely paralysed.

This ability to survive in different types of water may in part be explained by their ability to survive in the different types of water conditions found in the Amazon region. In general the larvae of South American species of *Spirocamallanus* appear to be able to survive in a wide range of water conditions in which the pH varies from about 3.6 to 5.8, as occurs in the Rio Negro, and from 6.5 to 7.3, as found in the Rio Solimoes-Amazonas. However, the water temperature appears to play a very important role in their survival outside the uterus mainly because, in their natural environment, the water temperature ranges from about 28 to 31°C in the Rio Negro and from 26.6 to 29.5°C in the Rio Solimoes-Amazonas (Goulding, *et al.*, 1988; Gessner,1962).

The decrease in the survival period of the larvae and the reduction in their movements exhibited at low temperatures is likely to severely restrict the ability of these parasites to attract and therefore infect free living copepods under natural conditions in the UK.

The active movements of the first stage larvae appear to be one of the strategies utilised by the parasite to attract the intermediate host, free living copepods (Moravec,

1975). However, this ability to attract the intermediate host does not often result in ingestion of the larvae. When a predator encounters a prey organism a complex series of behavioural interactions may follow, which can be broken down into several sequential components including encounter, attack, capture and ingestion (Williamson and Gilbert, 1980).

Under experimental conditions, when the available prey consist of only a single species, prey body size seems to be the most important factor regulating predator selectivity. Reduced ingestion rates may result from problems in handling prey at different levels of the encounter, attack, capture and ingestion sequence (Williamson, 1983).

These points may explain the absence of ingestion observed in some species of copepods utilised in this study. The small size of *Cyclops agilis* (female 0.8-1.45 mm), *C. bicuspidatus* (female around 1mm) and *C. macruroides* (female 1.04-1.25 mm) in relation to the size of the larvae (419-450 µm) was probably the major factor inhibiting their infection with larvae of these tropical parasites.

In all experiments conducted, ingestion of the larvae was only observed in the large copepods, *Cyclops strenuus abyssorum* (female 1.20-1.47 mm) and *C. viridis* (Jurine) (female 1.5-3.0mm), but the rates of ingestion and the prevalence of infection were very low (3-15.6%), possibly reflecting the decline in the survival period of the larvae or a decline in their infectivity.

In the majority of the studies on the life cycle of camallanids reported in the literature, the range of infection of the copepods was 70-100% (Stromberg and Crites, 1974; Bashirullah and Ahmed, 1976; Fusco, 1980). Clearly,

however, the results obtained in the present study cannot be compared with those found in previous studies because they were carried out mainly with species of copepods that are normally utilised by the nematodes as their intermediate hosts under natural conditions.

Another important point that should be noted in the above experiments is related to the fact that the natural predators were starved and the prey were abundant. These conditions cause maximal predator response and relatively high encounter rates. In the experiments carried out in the UK, although the copepods were also starved for the entire period that larvae were still observed alive in the petri dishes, these copepods were not their natural intermediate hosts. Consequently, no ingestion or a very low rate of infection of the copepods was observed.

Development of the first stage larvae in the intermediate host.

After ingestion of the first stage larvae by the copepod, the larvae penetrate into the haemocoel where two moults will occur. In the studies carried out in the UK the L_1 was first observed in the haemocoel at 12 hr p.i. at a room temperature of 20°C. This is within the range of time reported in the literature which is quite broad, 2-18 h.p.i., a variation which is probably related to the temperature, species of camallanid studied, and the suitability of the intermediate hosts. (Stromberg and Crites, 1974, Bashirullah and Ahmed, 1976; Fusco, 1980).

Unfortunately, due to the limitations of the material, samples of infected copepods could not be used to study the development of the larval stages. In the copepods infected with larvae of *Spirocamallanus* from *Mylossoma aureum* and *Brochis splendens* that died in the first 12 hr.p.i virtually no morphological changes were observed. These larvae were almost identical to those obtained from the uterus of the females. The most significant change observed in the larvae from the copepods which died between 24 and 36 hours was the development of a fine tube connecting the anterior extremity of the body and the anterior extremity of the oesophagus (Fig. 7.4b). No other differences were observed between the larvae from the uterus and the first stage larvae recovered from the copepod.

A marked morphological change was observed in the larva recovered from the copepod which died 11 d.p.i. The larva was undergoing the beginning of the second moult. The buccal capsule was narrow, very weakly sclerotized, separated into two cavities and with very thin spiral bridges in the first cavity. The division of the oesophagus was distinct and the intestine was straight, granulated and without colouration.

Although the number of larvae examined were very small, it appears that the first moult started to occur between 24 and 36 hr.p.i.. This moult was quite difficult to observe and it has been reported that it becomes more distinct at the end of the anterior extremity, when the old cuticle starts to separate (Moravec, 1969; 1974; 1975).

Few copepods infected with first stage larvae of *Spirocamallanus* sp[1] from *Brochis splendens* survived up to 22 days p.i. The larvae that were recovered 22 d.p.i. were early

third stage and were characterised by a yellowish colour in the intestine (5 and 6 days p.i.) and their coiling in the haemocoel of the copepod after 15 days p.i.. (Fig.7.3). This change in colour in the intestine of the larvae has been reported to appear in the larvae of camallanids after they start to undergo the first moult. With the development of the larvae this colour becomes strong and easily distinguishable in the body of free living copepods examined at low magnification under a dissecting microscope (Moravec, 1974; 1975).

The coiling up of the larvae has been normally observed after the late second stage. The third-stage larvae have a tendency to coil up and remain in this position for some time. According to Moravec (1969) this procedure appears to be "connected with the fact that larvae of this type are invasive larvae, infecting passively their definitive hosts".

The period for the complete development of the larvae to the infective stage in the intermediate hosts is variable and appears to be mainly related to the temperature, which has been reported as one of the most important factors influencing the rate of development of members of this family (Stromberg and Crites, 1974; Moravec, 1975). In general, the range reported has been between 6 and 12 days (Moravec, 1969; 1974; 1975; Stromberg and Crites, 1974; Bashirullah and Ahmed, 1976; Fusco, 1980). For the species of the genera *Procamallanus* and *Spirocamallanus*, sub-family Procamallaninae, infective larvae were just obtained between 6 and 11 d.p.i.. The shortest periods, 5-6 days and 10-11 days at a water temperature of 23 - 24°C was reported for *P.*

laeviconchus and *Spirocamallanus cricotus* respectively (Stromberg and Crites, 1974; Moravec, 1974).

Based on the age and development of the larvae obtained in the experiments conducted in the UK, it is estimated that the infective stage would be obtained at about 30 days at an average room temperature of 22°C. The slow development observed appears to be related to the thermophilic character of the larvae of camallanids, although in this study, the suitability of the intermediate hosts should also be considered. This slow development of the larvae may also explain the negative results found in the specimens of *Brochis splendens* exposed to copepods infected with larvae of 22 d.p.i..

The results obtained in these studies strongly support the views of Kennedy (1993) who suggested that the species introduced from the tropics "may be unable to tolerate the lower temperatures of the British lakes or rivers, or a species may be very sensitive to water pH or enhanced ionic content in its free living stages". The larvae of the species of *Spirocamallanus* appear to be able to survive a quite wide range of pH, but they seem to be quite sensitive to temperatures below 19°C. The thermophilic character of the larvae, together with the apparent unsuitability of the intermediate hosts utilised experimentally, were some of the barriers that these species of parasites of tropical fish did not overcome. Consequently, the results suggest that it is unlikely that these parasites could be successfully introduced into the UK. However, the potential of these species to establish in tropical fish culture facilities in the UK should be considered since, depending on the species

of fish, the temperature in these facilites is kept at around 23-25°C, and different tropical species of copepods, which may be utilised as intermediate hosts, are commonly introduced as live food for these species of fish.

7.5.2 Experiments conducted in Brazil

In the samples of ornamental fish examined at the exporters' holding facilities in Brazil, 4 species of *Spirocamallanus* were found. Three of these had previously been found in the UK, two, *Spirocamallanus* sp[1] and *Spirocamallanus* sp[2], in the same species of fish.

Similar to the results obtained in the studies conducted in the UK, high mortality rates of the first stage larvae were also observed in all experiments utilising water from the main water supply to the earth ponds (very soft acid water - Table 7.10) at the exporters' holding facilities where the species of *Corydoras* were normally held.

The survival period of the first stage larvae was longer than in the UK experiments, 15 days at a room temperature of 23-28°C, although high activity of the larvae was mainly restricted to the first 10 days. After 10 days the larvae started to move less actively and the mortality rates started to increase. It should be noted that these larvae normally appear to survive quite well in very soft acid water, which is commonly found in the Rio Negro and some of its main tributaries (Goulding *et al.*, 1988). The survival period was close to the period reported for the first stage larvae of *Spirocamallanus intestinecolas* (Bashirullah, 1976), 17 days at 26°C (Bashirullah and Ahmed, 1976).

Infection of the intermediate host and behaviour of the infected copepod.

Although six species of copepods were sampled in the earth ponds at the exporters' holding facilities, only two species, *Thermocyclops dicipiens* (Kiefer, 1929) and *Mesocyclops brasilianus* Kiefer, 1933, were utilised as intermediate hosts. These species were principally selected because of their abundance in the ponds where the fish were held, and their large size.

Another reason for selecting *T. dicipiens* relates to its widespread geographical distribution. This species is broadly distributed in South and Central America, and has also been recorded in Africa south of the Sahara, Egypt, Australia and tropical and sub-tropical Asia (Reid, 1989).

The rates of infection observed in both species were high, 74-100% for *M. brasilianus* and 100% for *T. dicipiens*. These rates were close to those described for *Spirocamallanus intestinecolas*, *S. cricotus* Fusco and Overstreet, 1978 and *Camallanus oxycephalus* Ward and Magath, 1916, in studies in which the life cycle was examined under similar conditions with regard to the intermediate host (Stromberg and Crites, 1974; Bashirullah and Ahmed, 1976; Fusco, 1980).

In general, the critical period occurred in the first 24 to 48 hr.p.i., when mortalities were mainly associated with the number of larvae ingested. During this time, infected copepods were not observed taking food and the deaths appeared to be related to the damage caused by the

larvae during their migration from the intestine to the haemocoel, where the moults occur. A similar observation was reported by Moravec (1969) who suggested that the deaths are mainly caused by the perforation of the intestine and damage to other internal organs.

The behaviour of the copepods also appeared to be affected by the presence of the larvae in the haemocoel. However, there is little information available in the literature to support this observation. Although several studies have been conducted utilising copepods as intermediate hosts of camallanids, only one paper reported that infected copepods experienced difficulty in swimming (Moravec, 1969).

Little alteration in behaviour was observed in large copepods infected with 1 to 5 larvae. Difficulties with swimming and/or swimming in circular movements, long periods of resting at the bottom of the plate or long periods of floating in the surface of the water, were commonly observed among the small infected specimens of *M. brasilianus* and *T. dicipiens* and in the large copepods heavily infected with up to 15 larvae. These alterations in behaviour appear to be associated with the presence of the parasite because, generally, the swimming behaviour of *Mesocyclops*, for example, consists of short rests between prolonged hop and sink sequences (Williamson and Gilbert, 1980).

Development of the first stage larvae in the intermediate host.

The progress of the first stage larvae to the infective stage was followed in specimens of both species of copepods infected experimentally but no differences in the development of the larvae or in the length of the developmental period were found.

In the infected copepod, the first moult appeared to occur just after the intestine of the larvae became a dark yellow. This colour became so intense that infected copepods could easily be separated from those not infected. The changing in colour of the intestine appears to be associated with the moulting process, and has been observed in some larvae prior to the first or the second moult (Moravec, 1969; 1975; Bashirullah and Ahmed, 1976), but sometimes has only been reported in late third stage larvae (Moravec, 1969).

The L_1 first appeared in the haemocoel of the copepod after the first hour. The first moult was observed at the end of the second day and beginning of the third day p.i. (between 24 and 36 hr.), when the larvae exhibited dark yellow granules in the intestine. The anterior extremity of the oesophagus was visible and the body started to show separation of the old cuticle. (Fig.7.6a). The larva obtained in the UK from copepods which died between 24 and 36 hours p.i., appeared to be less developed. The muscular oesophagus was not yet well differentiated and the granules of the intestine were colourless (Fig. 7.4a). A yellowish colour in the intestine was observed between 5 and 6 d.p.i.

In general, the development of the first and second stage larvae of *Spirocamallanus* sp^2 was similar to other members of the family Camallanidae previously described. No second stage larvae of *Spirocamallanus* sp^1 were examined in the copepods infected in the UK.

The development of the third larva of *Spirocamallanus* sp^2 was also very similar to those described in the literature (Figs. 7.6a-d and 7.8a-e). The main differences found appear to be related to the formation of the buccal capsule and the number of mucrones in the posterior extremity of the body.

The L_3 of *Spirocamallanus* sp^2 was first observed in the haemocoel 9 d.p.i.. The buccal capsule of these larvae was very similar to the buccal capsule of the adult except for the presence of the second cavity (Figs. 7.8d-e and 7.2b) and the distribution of the spiral bridge in the buccal capsule, which in the larva is distributed throughout the entire first cavity whereas in the adult the spiral bridge is restricted to the proximal half of the buccal capsule (Figs.7.8d-e and 7.2b).

No fully developed L_3 of *Spirocamallanus* sp^1 was obtained in the copepods infected in the UK. However, the morphology of these larvae obtained between 11 and 22 d.p.i. shows clearly that they represent young L_3 stages. The buccal capsule was exhibiting light to moderate sclerotization and the spirals were not so clearly visible (Fig. 7.5a-b).

The life cycle of 3 species of *Spirocamallanus* have so far been described: *S. intestinecolas* (Bashirullah, 1973), *S. cearensis* (Vaz and Pereira, 1936) and *S. cricotus* Fusco and Overstreet, 1978, and it appears that there are quite

strong morphological differences in the development of the buccal capsule which may be related to different lines of evolution among the Procamallaninae (Petter, 1979). One of these differences relates to the absence of the spiral bridges in the buccal capsule of the L_3 of *S. intestinecolas*. In this species, it appears that the spiral bridges do not show in the L_3 (Bashirullah and Ahmed,1976). Another is related to the presence of the remainder of the second cavity which has been reported occurring in the L_3 of *S. cearensis* by Pereira *et al.*, (1936) and *S.cricotus*, and as absent in the L_3 of *S. intestinecolas* by Bashirullah and Ahmed (1976).

The other main difference observed in the development of the larval stages of *Spirocamallanus* sp[2] was related to the posterior extremity of the L_3 which has generally been reported with 3 mucrones by Fusco (1980) in *S. cricotus* or 4 mucrones by Pereira *et al.*, (1936) for *S. cearensis*. In the specimens studied in Brazil no mucrones were observed in the posterior extremity of the L_3. The tail is blunt and appears to have several very small spines (Fig. 7.11b). This type of morphology of the tail has not previously been reported for any of the species of the family Camallanidae studied.

The development of *Spirocamallanus* sp[2], a parasite of *Corydoras sterbai*, in the specimens of *M. brasiliensis* and *T. dicipiens*, confirm these two species of copepods as intermediate hosts for this species of nematode and possibly for other South American species of camallanids as well. The development of the larvae in specimens of *T. dicipiens* particularly constitutes an important finding, because this species has a broad geographical distribution among the

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neotropical regions and could play an important role in the development of these nematodes under appropriate conditions.

A further conclusive finding relates to the possibility of infection of the ornamental fish species with species of camallanids at the exporters' holding facilities during the holding period. The abundance of *M. brasilianus* and *T. dicipiens* in the earth ponds at the exporters' holding facilities suggests that species of *Corydoras* held for long periods have a high risk of becoming infested by nematodes of this family or other parasites that utilise micro-crustaceans as intermediate hosts.

Chapter 8

General Discussion

8.1 General Discussion

This is the first study that has attempted, by a variety of approaches, to investigate the health of wild ornamental fish species at the level of both importing and exporting countries. It was conducted in collaboration with British importers in the UK, exporters from Venezuela and fishermen and exporters from Brazil. The latter is considered one of the major exporting countries of wild species from South America. The study has accumulated information on the general health of the fish species examined, their parasite fauna, aspects of their life cycle and some of the management procedures of the collaborating importers and exporters.

A period of two months, when interviews with British importers were conducted, followed by a long term sampling programme of shipments arriving from South America, provided the information relating to the main problems routinely faced by the importers of wild freshwater fish and the main diseases carried by the fish species examined. In South America, a similar approach was adopted for a three month period, which comprised mainly the beginning of the fishing season.

Although accurate statistical figures are not available, Colombia and Brazil appear to be the main South American countries exporting to the UK, Colombia having exported 19 tonnes to the UK in 1990, according to the latest figures available (Mintel, 1991). Unfortunately, this figure only reflects the total landed weight of freshwater fish imports from Colombia without specifying the number of specimens per

species imported. No figures were obtained in the UK, relating to imports of ornamental fish from Brazil. For the period of 1993, according to the Brazilian Environmental Control Committee, IBAMA, the state of Amazonas alone had exported to the UK a total of 54,490 specimens of ornamental fish, which corresponded to a total of 16 species. These figures however, should be viewed with caution because in Brazil the records of the species to be exported were made under the common name. Out of a total of 177 ornamental species permitted for export, at least 86 species can be found in IBAMA's list under more than one common name (See Appendix V). These difficulties in obtaining and interpreting the import and export figures from the UK and Brazil, demonstrate some of the problems experienced when trying to obtain reliable data on ornamental fish, and the difficulties faced by government agencies with respect to controlling the exports of ornamental fish. As a consequence of the difficulties in assessing the information relating to the species most commonly imported into the UK directly from South America, this study has utilised a large number of species, of which the genus *Corydoras* was the most commonly sampled.

One of the approaches used to assess the problems faced by importers in the UK, involved with the direct import of fish from South America, was the questionnaires. This was composed of a series of questions relating to transport, post-shipment procedures, diseases and treatment, and quarantine. Unfortunately, the majority of the questions were not answered clearly, or were answered in a very

contradictory way, making it difficult to obtain a complete picture of both the health of the ornamental fish directly imported from South America into the UK and the problems faced by the importers. However, it was clear from the answers of the interviewees that they preferred to buy South American quarantined fish from Germany, the Netherlands or United States rather than to be dealing with direct imports.

Diseases, treatment, mortality rates and quarantine are very sensitive issues in the ornamental fish industry and hence were very difficult to assess among the British importers. Only limited information was obtained with no specific details given by any importer. Quarantine was perhaps the most difficult to assess and although its purpose is the control of the transmission of infectious diseases it appears that, the so called "quarantine period" can be, for all practical purposes, defined as "the period necessary to acclimatize the fish to the new environment and for them to start to feed". This period is normally well below the period necessary for the control of disease.

In contrast to the sparse information obtained from the British importers, the exporters in Brazil and Venezuela, were more willing to discuss the diseases problem they faced. In general, light microscopes are not available to them, and none of the most common techniques of disease screening, such as gill and skin biopsy, are routinely used to check the fish's health on and subsequent to their arrival at the exporters' holding facilities. Consequently, the most common diseases, such as the parasitic diseases, are not diagnosed.

The only disease that appeared to be recognised by the exporters was "white spot" or "Ich", due to the easy observation of the large white spots formed by the parasites, which are visible with the naked eye. Similar information was obtained by Welcomme *et al.*, (1979) from the Brazilian exporters'. The repetition of the information 16 years later, by some exporters, clearly shows the little progress achieved in this time by some of the exporters from South America.

Among the problems faced by the exporters, the most difficult to assess, as with the British importers, were those related to quarantine. This is due principally to the need to move large numbers of fish quickly during the beginning of the season to meet the demand of the international market. However, there was general consensus on the necessity to "quarantine" some species, such as *P. axelrodi*, *Symphysodon* spp. and *H. rhodostomus*, principally because of their slow recovery after transport down river to the exporters' holding facilities.

The parasitological study of the species sampled in the UK revealed the presence of all major groups of parasites in the fish species sampled. With the exception of the mesocercariae of Strigeoidea and the protozoan *Piscinoodinium* sp., in general all major groups of parasites were found at low prevalence and intensity of infection. These results appeared to be related to the series of prophylactic treatment that the fish were exposed to prior to export and/or the mortality of heavily infected fish.

The parasite fauna was mainly composed of larval stages, but only one of them was readily visible in the fish. This

was metacercaria found in the skin which is responsible for a condition known as "black spot disease" and has often been confused by hobbyists as normal pigmentation of the fish.

Among the larval stages found in this study, the mesocercariae of Strigeoidea were perhaps the most interesting finding. These mesocercariae were found in several species of callichthyids examined and in all of them they were spread throughout the body but always enclosed by the host cysts. Generally, 1 to 15 mesocercariae were found enclosed by the same host cyst. Such cysts have not been previously reported and the mechanism that attracts all the mesocercariae to the same place to be enclosed by the host cyst is unknown.

These mesocercariae are very young stages for which the adults are not known, but it is possible that they belong to one of the genera of Strigeoidea that parasitises crocodilians, turtles or other aquatic vertebrates that occur in the same environment.

In the samples of fish examined for parasites at the exporters' holding facilities in Brazil, more or less the same parasites found in the UK samples were present. The differences that were observed appeared to be related more to the exporters' procedures (e.g. lack of sanitation, poor water quality) than to differences in the natural composition of the parasite fauna of the fish. The main differences were:
1. the presence of large colonies of epibionts, Epistylididae, on the skin and gills of *C. hastatus* and *Symphysodon* sp. in the holding tanks. The heavy

growth on the surface of the gills and skin may indicate that the fish had been predisposed to some debilitating environmental and/or infectious factor and the repellent ability of their surface has decreased. Other contributing factors could be the presence of large amount of organic material in the bottom of the holding tanks (e.g.excess food and dead fish) and the lack of aeration. 2. the presence of metacercarial stages which can affect the aesthetically pleasing features of the species. Generally, these specimens are removed from the stock prior to export in those that are easily visible. 3. high prevalence and intensity of infection of gyrodactylid species, mainly on the species of callichthyids examined. These results may be related to the overcrowding conditions of the tanks and the lack of necessity for an intermediate host a vector in gyrodactylids.

A considerable number of species of the genus *Gyrodactylus* were found in this survey. In the samples examined in the UK they were represented by five species of which four were parasites of callichthyid species (three species in *Corydoras* spp. and one in *Brochis splendens)* and one was a parasite of a curimatid species, *Semaprochilodus taeniurus*. In Brazil, 10 species were present in the fish examined of which eight were found in *Corydoras* spp and two in *B. splendens*.

It is interesting to note that all of the gyrodactylid infections found in the UK samples were represented by single infections occurring at low prevalence and intensity of infection contrasting with some of the results obtained in the samples examined at the exporters' holding facilities in

Brazil. In these samples, three species of fish, *B. splendens*, *C. hastatus* and *C. sterbai*, were found presenting mixed infections and only one species *C. julii* was found presenting low prevalence. For other species a moderate to high prevalence and intensity was found, with the species *C. hastatus* and *C. punctatus*, presenting the highest figures.

The fauna of gyrodactylids from South America is poorly studied and it appears that the majority of these species of parasites of callichthyids found may represent new species. It would be of interest to carry out experiments involving host specificity in order to clarify if some of these infections were transient infections resulting from the close contact of the different species of fish during transportation and the holding period prior to export.

Gyrodactylids have been reported in shipments of freshwater fish from Singapore, the United States and South America (Gratzek *et al.*, 1978; Shotts and Gratzek, 1984). However, it is not known if the parasites reported mainly on South American aquarium fish tank bred in Singapore and the United States, are native or introduced with their hosts subsequently become established in these culture facilities. A follow up study to compare these fauna would elucidate some of this points raised in this study.

An important finding among the monogenean species found in this study was the presence of one species parasitising the excretory system of the ornamental fish, *Mylossoma aureum*. This monogenean was identified as a member of the genus *Kritskyia* Kohn, 1990 and appears to constitute the second species so far known for the genus. *Kritskyia* sp.

appears to be a pathogenic species but due to its location in the excretory system fish which are severely infected, but do not present clinical signs, are normally exported without detection. Its host, *M. aureum* is farmed in Asian countries and the United States, but it is not clear if this parasites is already established in these culture facilities.

Kritskyia sp. may be responsible for mortalities prior to and after export, mainly because of their location in the excretory system. In heavily infected fish they may cause urinary stasis with severe consequences for the hosts. This monogenean appears to be difficult to control because the majority of the current treatments available are for the control of external monogeneans and hence are not for appropriate control of this species of internal monogenean.

All major groups of parasites were found in the samples of ornamental fish examined in the UK and several of them may present potential for transfaunation (e.g. *Gyrodactylus* spp., *Spirocamallanus inopinatus* sp.; *Piscinoodinium* sp.) or establishment in ornamental fish culture (e.g. *Gyrodactylus* spp., *Philometra* sp., *Spirocamallanus* spp.; *Piscinoodinium* sp. and others). Experimental studies on transfaunation were conducted with *Spirocamallanus* spp because: 1. these nematodes were the most commonly reported by the importers and hobbyists in the UK, as occurring among the tropical species; 2. one of the species, *S. inopinatus*, presents a wide host specificity, and has been reported in more than 27 different host species from South America; 3. several species of copepods, including native species, which have the

potential to be first intermediate hosts, are commonly introduced as live food for ornamental species.

In this study, five species of *Spirocamallanus* were identified, of which three were found in the shipments from Brazil and two in the shipments from Colombia. Of these five, two species, *S. inopinatus* and *Spirocamallanus* sp[1] were present in the fish sampled in shipments from Brazil and Colombia. The species *S. inopinatus* was found in three fish species, *C. julii, Colossoma macropomum* and *M. hypsauchen,* which had not previously been recorded as host for this parasite.

Although several species of the genus *Spirocamallanus* were found, the majority of them were represented by immature specimens, and possibly reflecting recent infection of these parasites in their natural environment or at the exporters' holding facilities. Gravid adult stages containing larvae in the uterus were found mainly parasitising specimens of *C. metae, M. aureum* and *B. splendens,* which have not previously been reported as hosts for any Camallanidae species.

Five species of copepods were utilised as potential intermediate hosts, but only two species, *C. strenuruus abyssorum* and *Cyclops viridis,* were found swallowing and developing the larval stage which subsequently showed development. It should be noted that although both species are commonly commercialized among the importers, they also occur naturally in British waters.

The larvae of the *Spirocamallanus* species appeared to be able to survive in a quite wide range of pH, but they seemed to be quite sensitive to temperatures below 19°C. Low temperatures appeared to influence their ability to attract

the intermediate host possibly through a reduction in their motility, thus decreasing their infectivity. Low temperatures may also slow down their development in the intermediate host.

The early L_3 stage was first found in the haemocoel 22 d.p.i., but it was not possible, to determine whether the slow development was related to the thermophilic character of the larvae, the unsuitability of the intermediate host, or both.

The results obtained in this study, suggested that *Spirocamallanus* sp[1], a tropical species, may not be able to become an established parasite in British waters. The potential to establish in tropical fish culture facilities in the UK may also be doubtful because: 1. the range of water temperature, in which the fish are kept may not be suitable for the survival of some species of copepods commonly utilised as live fish food in this country. At temperatures above their normal range, the copepods may not survive long enough for the parasite to develop to the infective stage. 2. generally, closed systems are utilised for culture of tropical species and the methods for water sterilization, such as, UV filters and other chemical methods, may destroy these larvae or interfere with their motility etc. Experimental studies principally involving eurythermic copepods would clarify some of the questions raised in this study, not only relating to *Spirocamallanus* species but also to other species of parasites that require microcrustaceans as intermediate hosts.

The development of one species of *Spirocamallanus* was also studied in Brazil, utilising the most abundant species of copepods *Thermocyclops dicipiens* and *Mesocyclops brasilianus*, present in the earth tanks, where the species of *Corydoras* spp. were being held. The rates of infection in both intermediate hosts were high and the first L_3 was first found in the haemocoel 9 d.p.i. The results of this study support the hypothesis, that aquarium species can become infected with these nematodes at the exporters' holding facilities.

Another important point arising from this study was related to one of the species of intermediate host utilised in the experiment conducted in Brazil, *Thermocyclops dicipiens*. This species, according to Reid (1989), exhibits a widespread geographical distribution in South and Central America but is also reported to occur in tropical and neotropical regions of Asia. The wide ranging distribution of this species, associated with the recent reports of Ng *et al.*, (1993) of the establishment of South American fish species in the acid habitats of native species in Singapore and Malaysia, illustrates the vulnerability of those places with a large aquaculture production of freshwater aquarium fish to introductions not only of fish species from other tropical regions, but also to some species of parasites carried by them, since their natural intermediate hosts may also occur naturally in these regions.

The pathology studies indicates that although several species of parasites were found, only a limited number were associated with pathological problems in the hosts at the

point of export or import. The protozoans *Piscinoodinium* sp. *Ch. hexasticha, I. multifiliis* and *Trichodina* sp. which are common pest of aquarium fish, and some exotic species such as the mesocercariae of strigeoidea and the monogenea *Kritskyia* sp., were part of the spectrum of species able to cause significant pathology. The contribution of other species to the morbidity and mortality of fish prior to arrival at the exporters holding facilities in not known and deserves further studies.

Many of the problems faced by wild ornamental fish species appear to be related to the stressful holding and transport conditions and opportunistic parasites, such as protozoans and bacteria, which take advantage of the favourable conditions to multiply and cause mortalities. Other groups, such as nematodes, digeneans and acanthocephalans etc, may also act as debilitating agents if present in large number, but in general very few of them are associated with pathological problems. However, their presence in the ornamental fish species is obviously undesirable and not aesthetically pleasing. Currently, some of them, such the nematodes, are being removed by anti-helminthic treatments after to export. This practice appears to be becoming very common in those importing countries which specialise in quarantined fish (British importer, pers.comm.).

Unfortunately, this is not yet a common practice and informed practices are carried out among the South American exporters and, sometimes, due to the long holding period at the exporters' holding facilities, new parasites can be

acquired. The development of the life cycle of *Spirocamallanus* sp. utilising copepods present in the holding tanks at the exporters' holding facilities in Brazil, clearly demonstrated this possibility. Simple measures such as placing nets in the main water influx would help to minimize fish infections and reduce the ability of parasites to complete their life-cycle within the ponds.

Inadequate sanitation was another aspect reflected in the samples of fish on their arrival in the UK. More than one fish species from the same shipment was found to be infected with the same protozoan, *Piscinoodinium* sp. This protozoan, and others which were at low intensity of infection in this study, are principally associated with stressful conditions and inadequate sanitation. Pathological studies of the infected specimens revealed the presence of a chronic condition in the majority of the specimens examined which may suggest long term infection in the tanks prior to export.

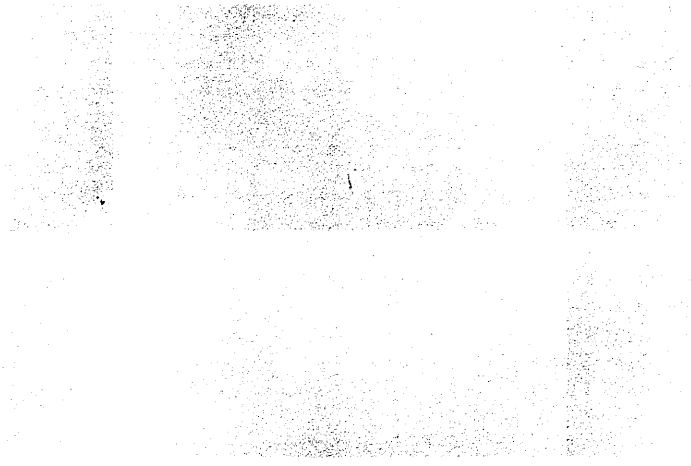
In Brazil, this situation was also found in the species examined at the exporters' holding facilities, in samples from different tanks, suggesting a wide distribution of the disease in the holding facility, probably arising from the mixing stocks or inadequate disinfection of the nets, pipes etc.

As a consequence of the series of problems faced by the South American exporters, some of them discussed in this study, drugs are indiscriminately utilised, as "preventive medicine" in order to minimise the fish health problems faced. Although an evaluation of the efficacy of these treatments was beyond the scope of this study, some of the

results in this study clearly indicated that some of treatments were unsuccessful.

By concentrating on preventive methods, such as good sanitation, water quality control, feeding and quarantine, the problems with diseases could be minimised. Consequently, the problems associated with the indiscriminate use of drugs for fish treatment, such as antibiotic resistance, risk of toxicity, and high costs, would be avoided.

New opportunities have arisen for the South American ornamental fish industry, but, the exporting countries still have to overcome basic problems in management to offer fish at competitive prices in the international market. In this respect, the rapid increasing competition from European countries specialising in "quarantined fish" may increase the pressure on the exporting countries to change their procedures.



References

References

ALDERTON, D. (1993). An introduction to Cites. OFI Journal. Issue Number **3**, May 1993

ALVAREZ, D. B. (1974) Aporte al conocimiento de la ictiofauna ornamental de Colombia y algunos aspectos sobre sus enfermedades y control. Fundaciom Universidad de Bogota Jorge Tadeo Lozano, Facultad de Ciencias del Mar. B.Sc Thesis, 137 pp.

AN, J., JARA, C.A. and CONE, D.K. (1991) Five species of *Gyrodactylus* Nordmann, 1832 (Monogenea) from freshwater fishes of Peru. *Canadian Journal of Zoology*, **69:** 1199-1201.

ANDREWS, C. (1990) The ornamental fish trade and fish conservation. *Journal Fish Biology* **37**: 53-59.

ANDREWS, C. (1984).The economic importance and control of the infectious diseases of freshwater fish, with special reference to fish farming, angling and ornamental fish-keeping. 4. European Multicolloquium of Parasitology, Izmir (Turkey), EMOP 4. THE FOURTH EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY, IZMIR, TURKEY, ABSTRACTS. (Tumbay, E.; Yasarol, S.;Ozcel, M. A. eds.) 252-253.

ANDREWS, C., CHUBB,J.C., COLES, T. and T. DEARSELEY (1981) The occurrence of *Bothriocephalus acheilognatti* Yamaguti,1934 (*B. gowkongensis*) (Cestoda: Pseudophylidea) in the British Isles. *Journal of Fish Diseases* **4**: 89-93.

ANG, K.J., GOPINATH, R. and CHUA, T. E. (1988) The status of introduced fish species in Malaysia. Exotic Aquatic Organisms in Asia. Proceedings of a workshop on Introduction of Exotic Organisms in Asia. (Silva, S.S. ed.) **3:**71-82.

ANONYMOUS (1992) Study of Technical and Economic Feasibility of a European Hatchery and Production Unit Serving the Ornamental Fish Trade. Internal Report. Stirling Aquaculture. Institute of Aquaculture. University of Stirling.

ANONYMOUS (1992) Ornamental Fish - World Trade. *Aqua Farm News* **10**: 2-4.

ASTM (1980) American Standards for Testing and Materials. Philadelphia, P.A.

AXELROD, H.R. (1989). Handbook of Fish Diseases. T.F.H. Publications Inc., 160pp.

AXELROD, H.R. (1988) Editorally speaking....conservation? *Tropical Fish Hobbyst* **36**: 6.

AXELROD, H.R. (1971) The aquarium fish industry. New Jersey. T.F.H. Publications

AXELROD, H.R., BURGUESS, W.E., PRONEK, N. and WALLS,J.G. (1991) Dr. Axerold's atlas of freshwater aquarium fishes.T.F.H. Publications Inc., 1150pp.

BAKKE, T.A., HARRIS, P.D., JANSEN, P.A. and HANSEN, P.L. 1992. Host specificity and dispersal strategy in gyrodatcylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* **13**, 63-74.

BANISTER, K. (1989) Shoals of fish. *Naturopa* **62**, 20-21.

BARLETTA,M. (1994) Estudo da comunidade de peixes bentonicos em tres areas do canal principal, proximas a confluencia dos rios Negro e Solimoes-Amazonas (Amazonia Central, Brasil). MS thesis. Universidade do Amazonas

BASHIRULLAH, A. K. M. and AHMED, B. 1976. Larval development of *Spirocamallanus intestinecolas* (Bashirullah, 1973) Bashirullah, 1974 in copepods. *Rivista di Parassitologia* **37**: 303-311.

BASSLEER, G. (1995) Exploring new markets & market opportunities Aquarama'95 - The 4th International Aquarium Fish Conference - 25-28 May, Singapore, 92-101

BAUER, O. N. (1991) Spread of parasites and diseases of aquatic organisms by acclimatization: a short review. *Journal of Fish Biology* **39**: 679-686.

BAUER, O.N. (1988) Epizootiological Significance of Monogeneans. *In*: Monogeneans as pathogens of fish diseases.(SKARUATO, O.A ed.) Translations of Monogeneans in the USSR. Rurrisan Translation series 62 (1988). A.A. BALKEMA, Vseseyuznae Simpozium po Monogeneyam (1976, Leningrad).

BAUER, O.N. and HOFFMAN,G.L. (1976) Helminth range extension by translocation of fish. In Wildlife Diseases (Page, L.A., ed.), pp. 163-172. New York: PLenum.

BAYLEY, P.N. & PETRERE, M. (1990) Amazon fisheries: assessment methods, current status and managment options. *In*: *Special Publication of the Canadian Journal of Fisheries and Aquatic Sciences*, 385-398.

BECKER, C.D. (1977) Flagellate parasites of fish. In: Parasitic Protozoa. I. Taxonomy, Kninetoplastids and Flagellates of Fish. (ed. by Kreier, J.P.). Academic Press, New York, NY, pp 360-370.

BERKA, R. (1986) The transport of live fish. A review. *EIFAC Technical Paper* 48.

BERMUDEZ, D. (1974) Aporte al conocimiento de la ictiofauna ornamental de Colombia, y algunos aspectos de sus enfermedades. *Thesis in Biological Sciences*, Facultad de Ciencias del Mar, Universidad de Bogota, 138pp.

BIFFAR, M., GROSSHEIDER, G., KRUSE, P. and STEINHAGEN, D. (1991) Improved clinical examination of ornamental fish. *Der Praktische Tierarzt* **72**: 37.

BLASIOLA, G.C. (1987) Ophthalmic disease of fishes. *Freshwater Marine Aquar.* **10**: 40-50.

BOEGER, W.A. & KRITSKY,D.C. (1988) Neotropical Monogenea. 12. Dactylogyridae from *Serrassalmus nattereri* (Cypriniformes:Serrasalmidae) and aspects of their morphologic variation and distribution in the Brazilian Amazon. *Proccedings of the Helminthological Society of Washington*, **55**: 188-213.

BOEGER, W.A., KRITSKY,D.C. and BELMONT-JEGU,E. (1994) Neotropical Monogenoidea.20. Two new species of oviparous Gyrodactyloidea (Polyonchoinea) from loricariid catfishes (Siluriformes) in Brazil and the phylogenetic status of Ooegyrodactylidae Harris, 1983. *Journal of the Helminthological Society of Washington* **61:** 34-44.

BORRERO, I. (1977) Inventario patologico de la ictiofauna ornamental Colombiana. Facultad de Ciencias del Mar, Universidad de Bogota, B.Sc. Thesis, 140pp.

BROMAGE, N. and CUMARANATUNGA,R. 1988. Egg production in the rainbow trout. *In*: Recent Advances in Aquaculture. Volume 3, 420pp.

BRUTON, M.N. and IMPSON, N.D. (1986) Report on the survey of the South African ornamental fsih trade. Grahamstonwn, Republic of South Africa: J.L.B.Smith Institute of Icthyology (unpublished)

BURGUESS, P. (1993) Fish Vaccines - An alternative to chemical treatments? Aquarama'93 - The 3rd International Aquarium Fish Conference - 24-27 June, Singapore, 13pp.

BURGESS, W.E. (1989) An atlas of freshwater and marine cat fishes. T.F.H. Publications, Inc, 784pp.

CAMPANA-ROUGET, Y. 1961. Remarques sur le cycle evotulif de *Camallanus lacustris* (Zoega, 1776) et la phylogenie des Camallanidae. *Annales de Parasitologie Humaine et Comparée* **36**: 425-434.

CANNING, E.U. and LOM, J. (1989) The Microsporida of Vertebrates. Academic Press, New York, 136-138p.

CASTILLO, D.T. (1991) Estudios de la utilizacion de ciertos productuos quimicos para el tratamiento de determinadas enfermedades de peces dulciaquicolas. Universidad Central de Venezuela. Facultad de Agronomia. Instituto de Producion Animal, Departamento de Zootecnia. B.Sc., Thesis, 120pp.

CHANDLER, A.C. and READ,C.P. 1971 Introduction to Parasitology. Toppan Company, Ltd., Tokyo, Japan, 822pp.

CHAO, N.L. (1995) Ornamental fish resources of Amazonia and aquatic conservation.Aquarama'95 - The 4th International Aquarium Fish Conference - 25-28 May, Singapore, 33-40.

CHAO, N.L. (1993) Conservation of Rio Negro Ornamental Fishes *Tropical Fish Hobbyst*, **41**: 99-114.

CHAO, N.L. (1992a) Ornamental fishes and fisheries of the Rio Negro. *Tropical Fish Hobbyst*, **40**: 84-102.

CHAO, N.L. (1992b) Diversity and conservation of ornamental fishes - the gems from flooded forests in Amazonia. *Canadian Biodiversity*, **2**: 2-7.

CHEUNG,P.J., NIGRELLI, R.F. and RUGGIERI, D (1986) *Calyptospora serrasalmi* sp.npv. (Coccidia: Calyptosporidae) from liver of the black piranha, *Serrasalmus niger* Schomburgk. *Journal of Aquariculture Aquatic Science* **4**: 54-57.

CHOU, L.M. and LAM, T.J. (1989) Introduction of exotic aquatic species in Singapore. *In:* Exotic Aquatic Organisms in Asia, S.S.De SILVA (ed.). Proceedings of the Workshop on Introduction of Exotic Aquatic Organisms in Asia. *Asian Fishery Society Special Publication* **3**: 91-97.

CHUAN, L.L. and HEE, L.C. (1993) Transportation of Ornamental Fish for Export - The Singapore Experience. Aquarama'93 The 3rd International Aquarium Fish Conference - 24-27 June, Singapore, 20pp.

CHUBB, J.C. (1977) Seasonal occurrence of helminths in freshwater fishes. Part I. Monogenea. *Advances in Parasitology*, **15**: 133-199.

CONE, D.K. and BURT, M.D.B. (1982) The behaviour of *Urocleidus adspectus* Mueller, 1936 (Monogenea) on the gills of *Perca flavescens*.*Canadian Journal of Zoology* **60**:3237-3240.

CONROY, D. A. (1975) An evaluation of the present state of world trade in ornamental fish. FAO *Fishery Technical Paper*, **146**: 128pp.

CONROY, D. A. (1964) Nocardiosis as a diseases of tropical fish. *Veterinary Record* 76:676.

CONROY, D. A. (1963) The study of tuberculosis-like condition in neon-tetras (*hyphessobrycon innesi*). I. Symptoms of the diseases of the organisms isolated. *Microbiol.espan.* **16**:47-54.

CONROY, D.A. and VASQUEZ, C. (1975) Informe del trabajo del proyecto (Col/552) en el campo de la ictiopatolgia durante el ano de 1974. Publicacion del Proyecto Desarrollo y Pesca Continental, INDERENA-FAO, 46pp

CONROY,D.A., MORALES, J., PERDOMO, C., RUIZ, R.A., SANTACANA, J.A. (1981) Preliminary observations on ornamental fish diseases in Northern South America. *Rivista Italiana di Piscicultura i Ittiopatologia* **16**: 86-98.

CONROY,D.A., MORALES, J., PERDOMO, C., RUIZ, R.A., SANTACANA, J.A. (1982) The prevention and control of diseased conditions in South American ornamental fish. *Rivista Italiana di Piscicultura i Ittiopatologia* **17**: 127-132.

COUTERNAY, R. W.,Jr., (1989) Dispersal of exotic species for aquaculture purposes: freshwater species. Abstracts of the Annual Meeting of the National Shellfisheries Association, Los Angeles, California, 553.

COUTERNAY, R. W.,Jr., (1982) Introductions - country and lake Kinneret case. Report of the Symposium on stck enhancement in the management os freshwater fisheries. EIFAC Technical Paper **42**: 24-25.

COUTERNAY, R. W.,Jr., (1979) The introduction of exotic organisms. *In*: Wildlife and America (BROKAW,H.P. ed.), 237-251.

COUTERNAY, R. W., Jr., and STAUFFER, J.R.,Jr. (1990) The introduced fish problems and the aquarium fish industry.*Journal of the World Aquaculture Society* **21**: 145-159.

COUTERNAY, R. W., Jr., HENSLEY,D.A, TAYLOR,J.N and McCANN, J.A. (1984) Distribution of Exotic Fishes in the Continental United States.In: Distribution, Biology and Managment of Exotic Fishes. (Courtenay, W.R.,Jr.; Stauffer, J.R.,Jr. eds.) pp. 41-77.

COUTERNAY, R.W., Jr., SAHLMAN, H.F., MILEY II, W.W. and HERRENA, D.J. (1974) Exotic fishes in freshwater and brackish waters of Florida. *Biological Conservation* **6**: 292-302.

DATODI, R. (1993) The effects of goverment controls and 14 days quarantine on the aquarium industry in Australia. Aquarama'93 - The 3rd International Aquarium Fish Conference 24-27 June, Singapore, 23pp.

DENIS, I.R.M. (1985) Exportacao Brasileira de Peixes Ornamentais. Serie Documentos Tecnicos **32**: 121-158. Ministerio da Agricultura - MA. Superintendencia do Desenvolvimento de Pesca - SUDEPE.

DIXON, B.A., YAMASHITA, J., EVELYN, F. (1990) Antibiotic resistance of Aeromonas spp. isolated from tropical fish imported from Singapore. *Journal of Aquatic Animal Health* **2**: 295-297.

van DUJIN, C. (1967) Diseases of Fishes. Illife Books, London.

DYKOVA, I. (1984) The role of the melanomacrophage centres in the tissue reaction to myxosporean infections of fishes. *Bulletin European Association of Fish Pathology* **4**:65

DYKOVA, I. and LOM, J. (1982). *Sphaerophora renicola* n.sp., a myxosporean from carp kidney, and its pathogenicity. *Zeitschrift fur Parazitekunde* **68**: 259-268.

DYKOVA, I., LOM, J. and GRUPCHEVA, G. (1987) Pathogenicity and some structural features of *Myxidium rhodei* (Myxozoa: Myxosporea) from the kidney of the roach *Rutilius rutilus*. Diseases of Aquatic Organisms **2**: 109-115.

EDWARDS, D.J. and HINE, P.M. (1974) Introduction, preliminary handling, and diseases of Grass Carp in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **8**: 441- 454.

EISENSTADT, T.A. (1992) The Rio Negro Basin's Aquarium Fish Trade: Harbinger of Sustainable Development, or the "One that Got Away". Report to Fundacao Vitoria Amazonica Report, 14pp.

ERGENS, R. 1965. Die morphogenese der chitinoidenteile des haptors bei *Gyrodactylus decorus* Malmberg, 1965 (Monogenoidea) und ihre morphologisch-metrische variabilität. *Zeitschrift für Parazitenkunde* **25**: 359-370.

ESCH, G.W., HAZEN, T.C. and AHO,J.M. (1977) Parasitism r and k-selection. *In:* Regulation of Parasites Populations. (Esch, G.W. ed.) New York Academic Press.

ESCH, G.W., KENNEDY,C. R., BUSH, A.O. and AHO,J. M. (1988) Pattern in helminth communities in freshwater fish in Great Britain: alternative strategies for colonization. *Parasitology* **96**: 1-14.

FAIN, A. and LAMBRECHTS, L. (1985) A new anoetid mite parasitic in the swim-bladder of the aquarium fish *Pangasius sutchi*. *Bulletin Annual Socieded Royal Belge Entomologie* **121**:4-6.

FAIN,A. LAMBRECHTS, L. and DELPAIRE, C. (1986) Acariasis, a new parasitic disease of aquarium fish. *Rivista Italiana di Piscicultura Ittiopatologia*. **21**: 33.

FALABELLA, P.G.R. (1985). A pesca no Amazonas: Soluçao e problemas. Impresso do Brasil, 156pp.

FERGUSON, H.W. (1992) SYSTEMIC PATHOLOGY of FISH. A Text and Atlas of Comparative Tissue Responses in Diseases of Teleosts. Iowa State University Press/Ames, 263pp.

FERNANDO, C.H. (1990) Freshwater biology in Southeast Asia and the role of Singapore-based research. *In*: Essays in Zoology, L.M.CHOU & P.K.L.NG Department of Zoology, National University of Singapore, Singapore, 211-219.

FERRAZ, E and THATCHER, V.E. (1990) *Camallanus acaudatus* sp.n. (Nematoda:Camallanidae) and a description of the male of *Camallanus tridentatus* (Drasche, 1884) parasites of fish of the Brazilian Amazon. *Amazoniana* **11**: 135-145.

FERRAZ, E. and THATCHER, V.E. (1992) *Paracamallanus amazonensis* sp.n. (Nematoda:Camallanidae) um parasita de bagre, *Hypophthalmus edentatus* (Pisces: Hypophthalmidae) da Amazonia Brasileira. *Amazoniana* **12:** 1-6.

FERRAZ, E., SHINN, A.P. and SOMMERVILLE, C. (1994) *Gyrodactylus gemini* n.sp. (Monogenea: Gyrodactylidae), a parasite of *Semaprochilodus taeniurus* (Steindachner) from the Venezuelan Amazon. *Systematic Parasitology*, **29**: 217- 222.

FISCHTHAL, J.H. and ALLISON, L.N. (1941) *Acolpenteron ureteroecets* Fischthal and Allison, 1940, a monogenetic trematode from the ureters of the black basses, with a revision of the family Calceotomatidae (Gyrodactyloidea).*Journal of Parasitology* **27**: 517-524.

FOISSNER, W., SCHUBBERT, G. and WILBERT, N. (1979) Morphologie, infracilitur and Silberliniensytem von *Protoopalina symphysodonis* nov.spec. (Protozoa: Opalinata), einer Opalinidae aus dem intestinum von *Symphysodon aequifasciata* Pellegrin (Percoidei: Cichlidae). *Zoologiche Anzeiger*, **202:** 71-85

FRANK, S.(1984) Aquaristics. Publications House Práce, Praha 365pp.

FRANK, S., RATAJ,K. and ZUKAL, R. (1983) Times how and why. Publications House Práce Svépomoc, Praha, 374 pp.

FUSCO, A.C. 1980. Larval development of *Spirocamallanus cricotus* (Nematoda:Camallanidae). *Procedings of the Helminthological Society of Washington* **47**: 63-71.

GEE, J.H. and Graham, H. (1978) Respiratory and hydrostatic functions of the intestine of the catfishes *Hoplosternum thoracatum* and *Brochis splendens* (Callichthyidae). *The Journal of Experimental Biology* **74**: 1-16.

GELNAR, M. and LUX, E. (1991) On the dissemination of two Far East monogenean parasites (*Dactylogyrus achmerovi* Gussev, 1955 and *Gyrodactylus kherulensis* Ergens, 1974, of carp *(Cyprinus carpio)* in Czechoslovakia. *Folia Parasitologica* **38:** 131-132.

GESSNER, F. (1962). O regime do oxigenio do rio Amazonas. *Boletim do Museum Paraense Emilio Goeldi* **1**: 43-71.

GHITTINO, P. (1970) Piscicultura e Ittiopatologia, Vol. 2, *Rivista di Zootecnia*, Stampa, Strada, Milan.

GHITINO, P. (1965) Segnalazione in Italia di una verrminosi del Persico-trota Nord-Americano d' importazione. *Atti della Socied Italiana delle Scienze Veterinarie* **19**: 448 -449.

GIAVENNI, R. (1981) Considerazioni sulle piu diffuse forme morbose riscontrabili a carico del pesci ornamentali. *Rivista Ittaliana di Piscicultura i Ittiopatologia* **16**: 130-133.

GIBSON, D.E. (1993) *Monobothrium wagneri*: another imported tapeworm established in wild British freshwater fishes? *Journal of Fish Biology* **43**: 281-285.

GIBSON, D.E. (1984) Technology as applied to museum collections: The collection, fixation and conservation of helminths. *Systematic Parasitology* **6**: 241-255.

GIORGETTI, G. (1988) The importation of fish and relative biological risks. *IL PESCE* **5**: 49-52.

GOLDSTEIN, R. (1971) Diseases of Aquarium Fishes. T.F.H. Publications. 126pp.

GOLTERMAN, H.L., CLYMO, R.S. and OHNSTAD,M.A.M (1978) IBP Handbook No.8. Methods for Physiscal and Chemical Analysis of Freshwaters. 100pp.

GOULDING, M, CARVALHO, M.L. and FERREIRA, E.G. (1988) Rio Negro - Rich Life in Poor Water. SPB Acad. Publ., The Hague, The Netherlands, 200 pp.

GRABDA-KAZABSKA, B. BATURO-WARZAWSKA, B and POJMANSKA, T. (1987) Dynamics of parasite infestation of fish in lakes Dgal Wielki and Warniak in connection with introduction of phytophagous species. *Acta Parasitologica Polonica* **32**:1-28

GRATZEK, J.B. (1988) Parasites associated with ornamental fish. *Veterimary Clinics of North America Small Animal Practice* **18**: 375-399.

GRATZEK, J.B. (1981). An overview of ornamental fish diseases and therapy. *Journal of Small Animal Pract.* **22**: 345-366.

GRATZEK, J. B. (1980) An overview of the diseases of ornamental fishes. *Proceedings of the 4th Kal Kan Symposium*, 25-39.

GRATZEK, J.B., SHOTTS, E.B. and BLUE, J.L (1978) Ornamental fish: diseases and problems. *Marine Fisheries Review* **40**:58-60.

GUSEV, A.V. (1976) Freshwater Indian Monogenoidea. Principles of Systematics, analysis of the world fauna and their evolution. *Indian Journal of Helminthology*, **25-26**:1-241.

HAMANN, M.I. (1982). Parasitos del pacu (*Colossoma mitrei*) del rio Parana medio, Republica Argentina (Pisces, Serrassalmidae). *Historia Natural* **2**: 153-160.

HAMANN, M.I. (1978) *Procamallanus inopinatus* Travassos, Artigas y Pereira, 1928 (Nematoda, Camallanoidea) en peces de ambientes loticos and lenticos de la Provincia de Corrientes, Republica Argentina. 103. *Historia Natural*, 103.

HARRIS, P.D. (1993) Interactions between reproduction and population biology in gyrodactylid monogeneans - a review. *Bulletin Français de la Peche et de la Pisciculture*, **328**: 47-65.

HARRIS, P.D. (1989) Interactions between population growth and sexual reproduction in the viviparous monogenean *Gyrodactylus turnbulli* Harris, 1986 from the guppy *Poecilia reticulata* Peters. *Parasitology* **98**: 245-251

HARRIS, P.D. (1986) Species of *Gyrodactylus* von Nordman, 1832 (Monogenea: Gyrodactylidae) from poeciliid fishes, with a description of *G. turnbulli* sp.nov. from the guppy, *Poecilia reticulata* Peters. *Journal of Natural History* **20**: 183-191.

HARRIS, P.D. (1985) Species of *Gyrodactylus* von Nordman, 1832 (Monogenea: Gyrodactylidae) from freshwater fishes in southern England, with a description of *Gyrodactylus rogatensis* sp.nov. from the bulhead *Cottus gobio* L. *Journal of Natural History* **19**: 791-809.

HARRIS, P.D. (1983) The morphology and the life-cycle of the oviparous *Oogyrodactylus farlowella* gen. et sp.nov. (Monogenea, Gyrodactylidea). *Parasitology*, **87**: 405-420.

HARRIS, P.D. and LYLES, A.M. (1992) Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on Guppies (*Poecilia reticulata*) in Trinidad. *Journal of Parasitology*, **78**: 912-914.

HASTEIN, T. (1993) Consequences of more open borders in Western Europe from a parasitological point of view. Parasites causing diseases in aquatic Animals. *Bulletin of the Scandinavian Society for Parasitology* **3**: 6-9.

HEINZE, K. (1933) Die gattung Capillaria Zeder (1800) als fischparasit. *Zeitschrift Parasitenkunde* 5:393-406.

HERKNER, V.H. (1987a) The most frequent parasitic disorders of oranmental fish *Der Praktische Tierartz* **68**: 34-39.

HERKNER, V.H (1987b) Protozoal parasites of ornamental fish. *Praktische Tierartz* **68**: 40-41.

HOFFMAN, G. L. (1975) Lesions due to Internal Helminths of Freshwater Fishes. In: The Pathology of Fishes (ed. by Ribelin, W.E and Migaki, G.). The University of Wiscossin Press. 151-186pp.

HOFFMAN, G. L. (1970) Intercontinemtal and transcontinemtal dissemination and transfaunation of fish parasites with emphasis on whirling diseases (*Myxosoma cerebralis*). *In A Symposyum of Diseases of Fishes and Shelfishes* (Snieszko, S.F., ed.), *American Fishery Society Special Publications* **5**: 69-81.

HOFFMAN, G. L. (1967) Parasites of North American Freshwater Fishes. University of California Press, 486pp.

HOFFMAN, G.L., KAZUBSKII,S.L., MITCHELL, A.J. and SMITH, C.E. (1979) *Chilodonella hexasticha* (Kiernik, 1909) (Protozoa, Ciliata) from North American warmwater fish. *Journal of Fish Diseases* **2**, 153-157.

HOFFMAN, G.L., SCHUBERT, G. (1984) Some parasites of exotic fishes.In Distribution, Biology and Managment of Exotic Fishes. (Courtenay, W.R.,Jr.;Stauffer, J.R.,Jr. eds.) pp. 233-261.

HOFFMAN, R.W. (1992) Diseases of ornamental fish and their treatment. *Der Praktische Tierarzt* **73**: 1057-1059.

HUCHZERMEYER, D. (1995) Establishing, monitoring and maintaining heath on fish farms. Aquarama'95 - The 4th International Aquarium Fish Conference - 25-28 May, Singapore, 196-207.

IMAI, S., HATAI,K. and OGAWA,M. (1985) *Chilodonella hexasticha* (Kiernik,1909) found from gills of a Discus, *Symphisodon discus* Heckel, 1940. *Japanese Journal of Veterinary and Science* **47**: 305-308.

INGLIS, W. G. (1962) *Icthyouris ro* gen. et sp. nov. (Nematoda): an oxyurid from a freshwater fish. *Journal of Helminthology* **36**: 45-50.

IUCN (1988) 1988 IUCN Red List of Thretened Animals. Gland, Switzerland and Cambridge, UK: International Union for the Conservation of Nature and Natural Resources.

JARA, C.A. (1986) Finding of *Gyrodactylus* sp. and *Anacanthocotyle* sp. (Monogenea, Gyrodactylidae) in fishes from the Moche river, Trujillo, Peru. *Hydrobios*, **10**: 8-13.

JARA, C.A. and CONE, D.K. (1989) *Scleroductus yuncensis* gen et sp.n. (Monogenea) from *Pimelodella yuncensis* (Siluriformes: Pimelodidae) in Peru. *Proccedings of the Helminthological Society of Washington*, **56**: 125-127.

JUNK,J.W. (1985) Temporary fat storage, an adaptation of some fish species waterlevel fluctuations and related environmental changes for the Amazon river. *Amazoniana* **3**: 315-351.

JUNK,J.W. (1984) Ecology of the várzea floodplain of Amazonian whitewater rivers. *In*: SIOLI, H. (ed.): The Amazon - limnology and landscape ecology of a mighty tropical river and its basin. Junk, The Hague: 216-243.

KABUNDA, M.Y and SOMMERVILLE, C. (1984) Parasitic worms causing the rejection of tilapia (*Oreochromis species*) in Zaire. *British Veterinary Journal* **140:** 263-268.

KALANTAN, A.M., ARFIN, M. and NIZAMI, W.A. (1987) Seasonal incidence and pathogenicity of the metacercariae of *Clinostomum complanatum* in *Aphanius dispar*. *Japanese Journal of Parasitology* **36:** 17-23.

KENNEDY, C.R. (1994) The ecology of introductions. *In*: Parasitic Diseases of Fish (ed. A.W. PIKE and J.W.Lewis) 189-208.

KENNEDY, C.R. (1993) Introductions, spread and colonization of new localities by fish helminth and crustacean parasites in the British Isles: a perspective and appraisal. *Journal Fish Biology* **43**: 287-301.

KENNEDY, C.R. (1976) Reproduction and dispersal. In: Ecological Aspects of Parasitism (Kennedy, C.R.,ed.) pp. 143-160. Amsterdam: North-Holland Publishing Company.

KENNEDY, C.R. (1975) The distribution of some crustacean fish parasites in Britain in relation to the introduction and movement of freshwater fish. *Journal of the Institute of Fishery Management* **6**: 36-41.

KENNEDY, C. R. (1974) A checklist of British and Irish freshwater fish parasites with notes on their distribution. *Journal Fish Biology* **6**: 613-644.

KENNEDY, C.R. and FITCH, D.J (1990) Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *Journal Fish Biology* **36**: 117-131.

KENNEDY, C.R., BATES,R. M. and BROWN,A.F. (1989) Discontinuous distributions of the fish acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in Britain and Ireland: an hypothesis. *Journal Fish Biology* **34**: 607-619.

KENNEDY, C.R., R. HARTVIGESN and O. HALVORSEN (1991) The importance of fish stocking in the dissemination of parasites throughout a gorup of reservoirs. *Journal Fish Biology* **38**: 541-552.

KENT, M.L. and HEDRICK, R.P. (1985) The biology and associated pathology of *Goussia carpelli* (Léger and Stankovitch) in goldfish *Carassius auratus* (Linnaeus). *Fish Pathology* 20:485-494.

KLEIN, B.M. (1958) The "dry" silver method and its proper use. *The journal of Protozoology* **5**:99-103.

KOHN, A. (1990) *Kritskyia moraveci* n.g.,n.sp. (Monogenea: Dactylogyridae) from the urinary bladder and ureters of *Rhamdia quelen* (Quoy & Gaimard, 1824) (Pisces: Pimelodidae) in Brazil. *Systematic Parasitology* **17:** 81-85.

KOHN, A. and FERNANDES,B.M.M. (1988a) Helminth parasites of fishes from the hydroeletric power station of Eletrosul (Brazil). I. *Procamallanus petterae* n.sp. and *Spirocamallanus pintoi* n.sp. (Nematoda:Camallanidae) from the reservoir of "Salto Osorio". *Memórias do Instituto Oswaldo Cruz*, **83**: 293-298.

KOHN, A. and FERNANDES,B.M.M. (1988b) Revision of the Brazilian species of the genus *Halipegus* Loos, 1899 (Trematoda: Derogenidae). *Systematyc Parasitology* **11**: 129-137.

KOHN, A., FERNANDES,B.M.M., GIBSON, I. and FROES, O.M. (1990) On the Brazilian species of halipegine genera (Trematoda: Derogenidae) from fishes, with new morphological data, hosts and synonyms. *Systematic Parasitology* **16**: 201-211.

KOHN,A., FERNANDES, B.M.M., MACEDO, B. and ABRAMSON, B. (1985) Helminth parasites of freshwatr fishes from Pirassununga, SP, Brazil. *Memorias do Instituto Oswaldo Cruz*, **80:** 327-336.

KÖRTING, W. (1993). Aquarium fish diseases and their control. Second Symposium on Diseases in Asian Aquaculture. *Aquatic Animal Health and the Environment*, 25-29 October, 1993. Phuket, Thailand.

KOZICKA, J. (1958) Diseases of fishes of Druzno lake. *Acta Parasitologica Polonica* **6**: 393-436.

KOZICKA, J. and NIEWIADOMSKA, K. (1960) Studies on the biology and taxonomy of trematodes of the genus *Thylodelphis* Diesing, 1850 (Diplostomatidae). *Acta Parasitologica Polonica* **8**: 379-401.

KRAMER, D.L. and BRAUN,E.A. (1983) Short-term of food avaibility on air-breathing frequency in the fish *Corydoras aeneus*. *Canadian Journal Zoology* **61**: 1964-1967

KRAMER, D.L. and McLURE, M. (1981) The Transit Cost of Aerial Respiration in the Catfish *Corydoral aeneus* (Callichthyidae). *Physiological Zoology* **54**: 189-194.

KRAMER, D. L. and McLURE, M. (1980). Aerial respiration in the catfish, *Corydoras aeneus* (Callichthyidae). *Canadian Journal Zoology* **58**: 1984-1991.

KRITSKY, D.C. and BOEGER, W.A. (1991) Neotropical Monogenea. 16. New species of oviparous Gyrodactylidea with proposal of *Nothogyrodactylus* gen.n. (Oogyrodactylidae). *Journal of the Helminthological Society of Washington*, **58**: 7-15.

KRITSKY, D.C. and FRITTS, T.H. (1970) Monogenetic Trematodes from Costa Rica with the proposal of *Anacanthocotyle* gen.n. (Gyrodactylidae: Isancistrinae). *Proceedings of the Helminthological Society of Washington*, **37**: 63-68.

KRITSKY, D.C. and THATCHER, V.E. (1977) *Phanerothecium* gen. nov. and *Fundulotrema* gen.nov. two genera of viviparous Monogenoidea (Gyrodactylidae), with a description of *P. caballeroi* sp.nov. and a key to the subfamilies and genera of the family. *Excerta Parasitologica en memória del doctor Eduardo Caballero y Caballero. Instituto de Biologia, Mexico, Publicaciones Especiales*, **4**:53-60.

KRITSKY, D.C., BOEGER, W.A. and THATCHER, V.E. (1988) Neotropical Monogenea. 11. *Rhinoxenus*, new genus (Dactylogyridae: Ancyrocephalinae) with description of three new species from the nasal cavities of Amazonian Characoidea. *Proceedings of the Biology Society of Washington*, **101**: 87-94.

KRITSKY, D.C., BOEGER, W.A. and THATCHER, V.E. (1986) Neotropical Monogenea. 9. Status of *Trinigyrus* HANEK, MOLNAR & FERNANDO, 1974 (Dactylogyridae) with descriptions of two new species from loricariid catfishes from the Brazilian Amazon. *Proceedings of the Biology Society of Washington*, **99**: 392-398.

KRITSKY, D.C., BOEGER, W.A. and THATCHER, V.E. (1985) Neotropical Monogenea. 7. Parasites of the pirarucu, *Arapaima gigas* (CUVIER), with descriptions of two new species and redescription of *Dawestrema cycloancistrium* PRICE & NOWLIN, 1967 (Dactylogyriae, Ancyrocephalinae). *Proceedings of the Helminthological Society of Washington*, **97**: 321-333.

KRITSKY, D.C., VAN EVERY,L.R. and BOEGER, W.A. (**in press**) Neotropical Monogenea. 27. Two new species of *Thelethecium* gen.n. from the nasal cavities of Central Amazonian fishes and a redescription of *Kritskyia moraveci* Kohn, 1990 (Dactylogyridae, Ancyrocephalinae). *Journal of the Helminthological Society of Washington.*

KRITSKY, D.C., MIZELLE, J.D. and BILQUES, F.M. (1978) Studies on Monogenea of Pakistan. III. Status of the Calceostomitidae (Parona and Perugia, 1890) with a redescription of *Neocalceostoma elongatum* Tripathi, 1957 and the proposal of *Neocalceostomoides* gen.n. *Proceedings of the Helominthological Society of Washington* **45**:149-154.

KULAKOVSKAYA,O.P. and KROTAS, R.A. (1961) A parasite introduced into carp hatcheries of the Western USSR from the far East. *Doklady Akademii Nauk* SSSR **137**: 1253-1255.

KUPRJANOVA, R.A. (1954) K biologii nematod ryb *Camallanus lacustris* (Zoega, 1776) i *Camallanus truncatus* (Rudolphi, 1814) (Nematodes: Spirurida). Dokl. AN SSSR, *nov.ser.*, **97**: 373-376.

LAIRD, M. (1960) Aspects of fish parasitology. *Proceedings of the 2nd Joint Symposium of the Scientific Society of Malaysia and Malayan Mathematical Society*, 46-54.

LANSDELL, W., DIXON, B., SMITH, N. and BENJAMIN, L. (1993) Isolation of several *Mycobacterium* species from fish *Journal Aquatic Animal Health* **5:** 73-76.

LEIBOVITZ, L.(1981) Digenetic Trematode Infection. *Journal American Veterinary Medicine Association*, **177**: 40-42.

LEIBOVITZ, L.(1980a) Ichthyophthiriasis. *Journal American Veterinary Medicine Association* **176**: 30-31.

LEIBOVITZ, L. (1980b) Lymphocystis Disease. *Journal American Veterinary Medicine Association* **176**: 202-203.

LEIBOVITZ, L.(1980c) Chilodonelliasis.*Journal American Veterinary Medicine Association* **176**: 222-223.

LEIBOVITZ, L. (1980d) Monogenetic trematodes Infections. *Journal American Veterinary Medicine Association*, **176**: 608-609.

LEITE, R.G. and ZUANON, J.A. 1991. Peixes ornamentais - Aspectos de commercializacao, ecologia, legislacao e propostas de acoes para um melhor aproveitamento. In Basis Cientificas para Estrategias de Presevacao e Desenvolvimento da Amazonia: Fatos e Perspectivas vol.1 (eds. Val, Figliuolo and Feldeberg) 327-331 pp.

LEIPER, T.R. (1910) Exhibition of a specimen of *Cyclops* containing living embryon of *Cucullanus elegans*. *Proceedings of Zoology Society of London*, **2**: 387.

LEUCKART, R. (1876) Die menschlichen parasiten un die von ihnen herrührenden Krankheiten, II. *Leipizig*.

LI, H. C. 1935. The taxonomy and early development of *Procamallanus fulvidacronis n.sp. Journal of Parasitology* **21**: 103-113.

LIM, L.C. and CHUA, L.H. (1993) Transportation of ornamental fish for export-The Singapore experience. Aquarama'93 - The 3rd International Aquarium Fish Conference - 24-27 June, Singapore, 24pp.

LINSTOW, O. (1909) Parasitische Nematoden. *In*: Brauer's: Die Süsswasserfauna Deutchlands, H. 15. Jena.

LO, CHU F., WANG, CH. H. and KOU, G.H. (1992) The pathology of loach (*Misgurnus anguillicaudatus*) infected with *Clinostomum complanatum* (Rudolphi, 1814). *Acta Zoologica of Taiwan* **3**: 145-154.

LODGE, D.M. (1993) Biological Invasions: Lessons for Ecology. *Tree* **8**: 133-137.

LOM, J. (1981) Fish invading dinoflagellates: a synopsis of existing and newly proposed genera. *Folia Parasitologica (Praha)* **28**: 3-11.

LOM, J. (1977) Mode of attachment to the host in a dinoflagellate parasite of freshwater fishes. *Journal of Protozzology* (Supplement) **24**, 16A.

LOM, J. and CORLISS, J.O. (1967) Ultrastructural observations on the development of the microsporidian protozoan *Pleistophora hyphessobryconis* Schäperclaus. *Journal of Protozoolology* **14**: 141-152.

LOM, J. and DYKOVA, I. (1992) Protozoans Parasites of Fishes. Development in Aquaculture and Fisheries Science **26**: 30-31.

LOM, J. and DYKOVA, I. (1984) Pathogenicity of some protozoan parasites of cyprinid fishes. *Symposia Biologica Hungarica* **23**: 99-118.

LOM, J. and SCHUBERT,G (1983) Ultrastructural study of *Piscinoodinium pillulare* (Schaeperclaus, 1954) Lom, 1981 with special emphasis on its attachment to the fish host. *Journal Fish Diseases* **6**: 411-428.

LOM, J., DYKOVA, I., KÖRTING, W and KLINGER, H. (1989) *Heterosporis schuberti* n.sp., a new microsporidian parasite of aquarium fish. *European Journal of Protistology* **25**: 129-135.

LUCKY, Z. (1984) Investigation on invasive diseases of the herbivorous fish fry and their treatment.In: Fish, Pathogens and Environment in European Polyculture (Olah,J.ed.) **23**: 173-180.

LUCKY, Z. (1970) Pathological changes with oodinosis of aquarium fish. *Acta Veterinaria BRNO*, Supplement **1**: 70-73.

MACINNIS, A.J. and VOGE, M. (1970) Experiments and Techniques in Parasitology. W.H. Freeman and Company, 232pp.

MacARTHUR,R.H. and WILSON, E. O. (1967) The Theory of Island Biogeography. Princenton, NJ: Princenton Unversity Press.

MAITLAND, P.S. (1972) A key of the freshwater fishes of the British Isles with notes on their distribution and ecology. *Scientific Publications of the Freshwater Biological Association* **27**, 1-139.

MAITLAND, P.S. and PRICE, C.E. (1969) *Urocleidus principalis* (Mizelle, 1936) a North American monogenetic trematode new to the British Isles, probably introduced with the largemouth bass *Micropterus salmonoides* (Lacepede, 1802). *Journal of Fish Biology* **1**: 17-18.

MAJEED, S.K., GOPINATH, C. and JOLLY, D.W. (1981) Pathology of Spontaneous Tuberculosis and Pseudotuberculosis in Fish. *Journal of Fish Diseases* **4**: 507-512.

MALMBERG, G. (1970) The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea) *Arkiv för Zoologi*, **23:** 1-235.

MALMBERG, G. (1957) [On the occurrence of *Gyrodactylus* on Swedish fish] (In Swedish, with description of the species and summary in English). *Skr. sod. Sver. FiskFor. Arsskr.* 1956, 19-76.

MARGOLIS, L., ESCH, G.W., HOLMES, J.C., KUIRS, A.M. and SCHAD,G,A. (1982) The use of ecological terms in parasitology. *Journal of Parasitology* **68**, 131-133.

MATTHEWS, R.A. (1994) *Ichthyophthirius multifilis* Fouquet, 1876: infection and protective response within the fish host. *In:* Parasitic Diseases of Fish (Pike, A.W & Lewis, J.W. eds.) 17-42.

McGRATH, D.G. (1990) Preliminary investigation of the trade in extractive products in the Brazilian Amazon. World Wildlife Fund-US Report, 30pp.

McKAY, R.J. (1989) Exotic and translocated freshwater fishes in Australia. *In:* Exotic Aquatic Organisms in Asia. (S.S.de Silva, ed.) *Proceedings of the Workshop on Introduction of Exotic Organisms in Asia. Asian Fishery Society Special Publications* **3**: 21-34.

McKAY, R.J. (1984) Introductions of exotic fishes in Australia. *In*: Distribution, Biology and Managment of Exotic Fishes. (Courtenay, W.R.,Jr.;Stauffer, J.R.,Jr. eds.) pp. 177-199.

McKAY, R.J. (1977). The Australian Aquarium Fish Industry. Fisheries Paper No. 25, Fisheries Division Department Industry, 36pp.

MECZNIKOW, I.I. (1866) Entgegen auf die Erwiderung des Herrn Prof. Leuckart in Gissen, in Betreff der Frage über die Nematodenentwicklung. Berlin.

MEDINA,J.H. (1986) Peces ornamentales de Venezuela. *NATURA*, Caracas, **80**: 21-27.

MEIER, W. and PFISTER,K. (1981) Bothriocephalosis in grasscarps (*Ctenopharyngodon idella*) in Switzerland. *Schweizer Archiv fur Tierheilkunde.*,**123**: 51-53.

MELLEGAARD, S. and BLOCH, B (1988) Herps-virus particles in angelfish *Pterophyllum altum*. *Diseases of Aquatic organisms*, **5**: 151-155

MICHEL, C., MAURAND,J., LOUBES,C, CHILMONCZYK and KINKELIN, P. (1989) *Heterosporis finki*, a microsporidian parasite of the angel fish *Pterophyllum scalare*: pathology and ultrastrucutre. *Diseases of Aquatic Organisms* **7**: 103-109.

MINTEL (1988) Water gardens and aquaria. *Leisure Intelligence* **1**, 1-31.

MINTEL (1991) Water gardens and aquaria. *Leisure Intelligence* **2**, 1-24.

MIZELLE, J.D., KRITSKY, D.C. and CRANE, J.W. (1968) Studies on monogenetic trematodes. XXXVIII. Ancyrocephalinae from South America with the proposal of *Jainus* gen.n. *The American Midland Naturalist* **80**: 186-198.

MOLNAR, K. (1984) Parasite range extension by introduction of fish to Hungry. EIFAC - Technical Paper **42**: 534-540.

MOLNAR, K. (1976) Histological study of coccididosis caused in silver carp and the bighead by *Eimeria sinensis* Chen, 1956. *Acta Veterinary of the Academy of Science of Hungary* **26:** 303-312.

MONTADON, J. (1972) Producción y comercialización de peces de acuario en Colombia y la región de Amazonas, y estudio de las possibilidades de exportación a los Estados Unidos de America. International Trade Centre, Geneve. ITC/TPAS 53pp.

MOORTHY, V.N. (1938) Observation on the life history of *Camallanus sweety*. *Journal of Parasitology* 24:323-342.

MORAVEC, F. (1983) Some remarks on the biology of *Capillaria pterophylli* Heinze, 1933. *Folia Parasitologica* **30**: 129-130.

MORAVEC, F. (1975). The development of *Procamallanus laeviconchus* (Wedl, 1862) (Nematoda: Camallanidae). *Vestnik Ceskoslovenske Spolecnosti Zoologicke* **39**: 23-38.

MORAVEC, F. (1974) The development of *Paracamallanus cyathopharynx* (Baylis, 1923) (Nematoda: Camallanidae). *Folia Parasitologica* **21**: 333-343.

MORAVEC, F. (1971) On the problem of host specificity, reservoir parasitism and secondary invasions of *Camallanus lacustris* (Nematoda; Camallanidae). *Helminthologia* **10**:107-114.

MORAVEC, F. (1969) Observations on the development of *Camallanus lacustris* (Zoega, 1776) (Nematoda: Camallanidae). *Vestnik Ceskoslovenske Spolecnosti Zoologicke* **1**: 15-33.

MORAVEC, F. (1967) Observations on the development of *Camallanus lacustris* (Zoega, 1776) (Nenatoda, Camallanidae) *Vestnik Czeslovenske Spolecnoti Zoologiche. Acta Societatis Zoologicae Bohemoslovacae* **1**:15-33.

MORAVEC, F. and GUT, J. (1982) Morphology of the nematode *Capillaria pterophylli* Heinze, 1933, a pathogenic parasite of some aquarium fishes. *Folia Parasitologica* 29: 227-231.

MORAVEC, F., ERGENS,R and REPOVA, R. (1984) First record of the nematode *Pseudocapillaria brevispicula* (Linstow, 1873) from aquarium fish. *Folia Parasitologica* 31: 241-245.

MORAVEC, F., GELNAR, M. and REHULKA, J. (1987) *Capillostrongyloides ancistri* sp. n. (Nematoda: Capillariidae) a new pathogenic parasite of aquarium fishes in Europe. *Folia Parasitologica*. **34**: 157-161.

MORAVEC, F., KOHN, A. and FERNANDES, B. M. M. (1993). Nematode parasites of fishes of the Parana river, Brazil. Part 3. Camallanoidea and Dracunculoidea. *Folia Parasitologica* **40**: 211-229.

MORAVEC, F., KOHN, A. and FERNADES, B.M.M. (1992a). Nematode parasites of fishes of the Parana River, Brazil. Part I.Trichuroidea, Oxyuroidea and Cosmocercoidea. *Folia Parasitologica* **39**: 327-353.

MORAVEC, F., KOHN, A. and FERNANDES, B. M. M. (1992b). Three new species of oxyuroid nematodes, including two new genera, from freshwater catfishes in Brazil. *Systematic Parasitology* **21**: 189-201.

MUJICA, A. E. M. (1982) Estudios preliminares sobre enfermedades que afectam a peces continentales de aguas calidas aptos para el cultivo, en la Estacion Hidrobiologica de Guanapito, Estado Guarico, Venezuela. Universidad Central de Venezuela, Facultad de Ciencias, Escuela de Biologia, B.Sc.Thesis, 100pp.

NEEDHAM, T. and WOOTTEN, R. (1978) The Parasitology of Teleosts. *In*: Fish Pathology (ed. by R.J.Roberts), pp. 144-182. Bailliere Tindall, London.

NG, P.K.L. and LIM, K.K.P. (1989) A preliminary report of a South American loricariid catfish, *Pterygoplichthys* sp.,in Singapore freshwaters. *The Pangolin* **2**:69-71.

NG, P.K.L., CHOU,L.M. and LAM, T.J. (1993) The status and impact of introduced freshwater animals in Singapore. *Biological Conservation* **64**: 19-24.

NIE, P. and KENNEDY, R. (1991) Occurrence and seasonal dynamics of Pseudogyrodactylus anguillae (Yin & Sproston)(monogenea in eel, Anguilla anguilla (L.), in England. *Journal Fish Biology* **39**: 897-900.

NIJSSEN, H. (1970) Revision of the Surinam catfishes of the genus *Corydoras* Lacépède 1803 (Pisces, Siluriformes, Callichthyidae). *Beuafortia* **230**: 1-75.

NIJSSEN, H. and ISBRUCKER, I.J.H. (1979) Chronological enumeration of nominal species and subspecies of *Corydoras* (Pisces, Siluriformes, Callichthyidae). *Bull. Zool. Mus.*,Univ. Amsterdan, **6**: 129-135.

NIJSSEN, H. and ISBRUCKER,I.J.H. (1980a). On the identity of *Corydoras nattereri* Steindachner, 1877 with the description of a new species, *Corydoras prionotos* (Pisces, Siluriformes, Callichthyidae). *Beaufortia* **30**: 1-9.

NIJSSEN, H. and ISBRUCKER,I.J.H. (1980b) A review of the genus *Corydoras* Lacepede, 1803 (Pisces, Siluriformes, Callichthyidae). *Bijdragen tot de Dierkunde,* **50**: 190-220.

NIJSSEN, H. and ISBRUCKER, I.J.H. (1980c) Three new *Corydoras* species from French Guiana and Brazil (Pisces, Siluriformes, Callichthyidae). *Netherlands Journal Zoology* **30**: 494-503.

NILAKARAWASAN, N. (1993) Ecological studies on the parasites of *Etropolus swatensis* (Bloch) (Pisces:Cichlidae) with special reference to *Enterogyrus* spp. (Monogenea: Ancyrocephalinae). Institute of Aquaculture (University of Stirling). Ph.D thesis 467pp.

OLSEN, W.O. (1974) Animal Parasites. Their Life Cycles and Ecology. University Park Press, 562 pp.

OLSON, A.Jr. and COSGROVE, G.E. 1982 Pentastomida. *In*: Aquatic Biota of Mexico, Central America and the West Indies. (eds. Hubert, S. and Villalobos, A.). University of San Diego Press, USA, 172-174.

OSTROWSKY de NUÑEZ, M. (1979) Fauna de agua dulce de la Republica Argentina. IX. Sobrre representantes de la familia Paramphistomidae (Trematoda). *Physis* **38**: 55-62.

PAPERNA, H.I. (1980) *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida) infestations in cultured marine fish at Eilat, Red Sea: epizootiology and pathology. *Journal of Fish Diseases* **3:** 363-372.

PAPERNA, H.I. (1964) The metazoan parasite fauna of Israel inland water fishes. *Bamidgeh* **16**: 3-66.

PAPERNA, I. and van AS, J.G. (1983) The pathology of *Chilodonella hexasticha* (Kiernick). Infections in cichlid fishes. *Journal of Fish Biology* **23**, 441-450.

PAPERNA, I. and VENTURA, T.M. (1985) Histopathology of *Ichthyophthirius multifilis* infections in fishes. *Journal of Fish Biology* **27**, 185-203.

PAPERNA, I, VENTURA, T.M. and MATOS, A.P de (1987) Lymphocystis infection in sneakskin gourami (Regan), Anabantidae. *Journal Fish Diseases* **10:** 11-19.

PEREIRA, C., VIANA DIAS, M and AZEVEDO, P. 1936. Biologia do nematoide *Procamallanus cearensis* n.sp. *Archivos do Instituto de Biologia* **5**: 209-226.

PETTER, A.J. (1990) Nématodes de poissons du Paraguay. VI. Description de deux nouvelles especes du genre *Spirocamallanus* et complements a la description de *Procamallanus annipetterae* Kohn & Fernandes, 1988. *Revue Suisse Zoologie* **97**: 327-338.

PETTER, A.J. (1979) Essai de classification de la sous -famille des Camallaninae (Nematoda: Camallanidae). *Bulletin Muséum National d'Histoire Naturelle* Paris, 4 serie, section A, **4**: 991-1008.

PETTER, A.J. and DLOUHY, C. (1985) Nématodes de Poissons du Paraguay. III. Camallanina. Description d'une espèce et d'une sous-espèce nouvelles de la famille des Guyanemidae. *Revue Suisse Zoologie* **92**: 165-175.

PETTER, A.J. and THATCHER, V.E. (1988) Observation sur la capsule buccale de *Spirocamallanus inopinatus* (Nematoda), parasite de poissons brésiliens. *Bulletin Muséum National d'Histoire Naturelle* **4**: 685-692.

PETTER, A.J., CASSONE, J. & FRANCE, M. (1974). Un nouveau nématode *Camallanus* pathogène dans de élevages poissons exotiques. *Annales de Parasitologie Hummaine et Comparee* **49:** 991-1008.

PICKERING, A.D. (1993) Stress and Adaptation: b.Stress and Disease resistance. *Recent Adavances in Aquaculture* IV, 170-175.

PINEDA-LOPEZ,R. 1991 Hallazgo de nimfas de pentastomidos en *Ictalurus meridionalis* (Pisces: Ictaluridae) en el Rio San Pedro, Balancan, Tabasco. Nota de Investigacion. *Universidad y Ciencia* **8**:97-100.

PINTO, M.R. and NORONHA, D. (1972) Redescriçao de *Procamallanus inopinatus* Travassos, Aritgas & Pereira, 1928, (Nematoda, Camallanoidea). *Atas da Sociedade de Biologia do Rio de Janeiro* **15,** 105-108.

PINTO, M. R., Fabio, S. P. de, Noronha, D. Rolas, T. 1975. Novas contribuicoes ao conhecimento do genero *Procamallanus* (Nematoda: Camallanoidea). *Memorias do Instuto Oswaldo Cruz* **73**: 183-191.

PINTO, M.R., Fabio, S.P. de, Noronha, D. & Rolas,T. 1974. Procamallanus brasileiros - Parte 1 (Nematoda: Camallanidae). *Memorias do Instuto Oswaldo Cruz* **72**:205 -211, pls.I-V.

du PLESSIS, S.S. (1948) A gyrodactyloid parasite from the ureters of largemouth bass at the Jonkershoek inland fish hatchery, South Africa. *Transactions of the American Fisheries Society*, **75**: 105-109.

POJMANSKA, T. and CHABROS, M. (1993) Parasites of common carp. *Acta Parasitologica* **38**: 101-108.

PRADA-PEDREIROS, S. (1992) Abundancia e distribuiçao do cardinal tetra, *Paracheirodon axelrodi*, e diversidade dos peixes nas planicies inundaveis de tributarios do rio Negro, Amazonas, Brasil. MSc thesis, Universidade do Amazonas.

PRUDHOE, S. and BRAY, A. (1982) Platyhelminth parasites of the Amphibia. British Museum (N.H.), Oxford University Press.

RALPHS, J.R. and BENJAMIM, M. (1992) Chondroitin and keratan sulphate in the epidermal club cells of teleosts. *Journal Fish Biology* **40**: 473-475.

REICHENBACH-KLINKE, H.H. (1980) Krankheiten und Schädigungen der Fisch. G. Fischer Verlag, Stuttgart, New York.

REICHENBACH-KLINKE, H.H. (1954) Untersuchungen über die bie Fischen durch Parasiten hervogerufenen Zysten und deren Wirkung auf den Wirtskörper. *Zeitschrift für Fischerei und deren Hilfswissenschaften*, 565-636.

REICHENBACH-KLINKE, H.H. & LANDOLT, M. (1973) Fish Pathology. T.F.H. Press, Neptune City, N.J. 521pp.

REID, J.W. (1989) The distribution of species of the genus *Thermocyclops* (Copepoda, Cyclpoida) in the Western Hemisphere, with description of *T. parvus*, new species. *Hydrobiologia* 175: 149-174.

RIBELIN, E.W and MIGAKI, G. (1975). The Pathology of Fishes. The University of Wisconsin Press 420 pp.

RICHARDS, R. (1977a) Diseases of aquarium fish. 2. Skin diseases. *The Veterinary Record* **101**: 132-135.

RICHARDS, R. (1977b) Diseases of aquarium fish. 3. Disease of the internal organs. *The Veterinary Record* **101**: 149-150.

RICHARDS, R. (1977c) Diseases of aquarium fish. 4. Treatment. *The Veterinary Record* **101**: 166-167.

RILEY, D.M. (1978) Parasites of grass carp and native fish in Florida. *Transactions of the American Fishery Society* **107**:207-212.

ROBERTS, R.J (1978) Fish Pathology.Bailliere Tindall, London, 318pp.

ROBERTSON, D. (1985) A review of *Ichthyobodo necator* (Henneguy,1883) an important and damaging fish parasite. *Recent Advances in Aquaculture* **2**: 1-30.

ROGERS, W.A. and GAINES, J.L.Jr., (1975). Lesions of Protozoans Diseases in Fish. *In*: The Pathology of Fishes (ed. by Ribelin, W.E. and Migaki, G.) 117-136 pp.

ROOS, J.W. (1972) Onderzoek naar parasitaire ziekten bij siervis in het Peruaanse Amazonegebied. Rijksuniversiteit te Utrecht, B.Sc. Thesis, 19pp.

SAIDAPUR, S.K. (1978). Folicular atresia in the ovaries of nonmammalian vertebrates. *International Review of Cytology* **54**: 225-244.

SAINT-PAUL, U. (1986) Potential for Aquaculture of South American Freshwater fishes: a review. *Aquaculture*, **54**: 205-240.

SANTACANA, J.A., CONROY,D.A., MUJICA, M.E. and LOPEZ, N. (1982) Acid-fast bacterial infection and its control in three-spot gouramies, *Trichogaster trichopterus* Pallas. *Journal of Fish Diseases* 5: 549-552.

SANTOS, M.de U. (1973) Beobachtungen uber Wasser-bewegungen, chemische Schichtung und Fisswanderungen in Varzea-Seen am mittleren Solimoes (Amazonas). *Oecologia* **13:** 239-246.

SCHAD, W. (1977) The role of arrested development in the regulation of nematode parasites. *In*: The Regulation of Fish Parasite Populations (ed. G.W. ESCH). Academic Press, New York and London.

SCHÄPERCLAUS, W. (1992) Fish Diseases **2,** 1275pp.

SCHÄPERCLAUS, W. (1954) Fischkrankheinten, Berlim, 708 pp.

SCHÄPERCLAUS, W. (1951) Der *Colisa*-Parasit - ein neuer krankheitserreger bei Aquarien fishen. *Die Aqu Terr. Zeitschrift*, 169-171.

SCHÄPERCLAUS, W. (1941) Eiine neue Mikrosporidienkrankheit beim neonfisch und seinen Verwandten. *Wochenschr. Aqua. Terr.* **38:** 381-384.

SCHWARTZ, A. (1993) The ornamental aquatic Industry in South America. Aquarama'93 - The 3rd International Aquarium Fish Conference - 24-27 June, Singapore, 14pp.

SEAGRAVE, C. (1993). Ornamental Fish production in UK. Present and Future. *Trout News* **16:** 12-17.

SHAMSUDIN, M.N., TAJIMA, K., KIMURA,T., SHARIFF, M. and ANDERSON, I.G. (1990) Characterization of the causative organism of ornamental fish mycobacteriosis in Malasyia. *Fish Pathology*, Tokyo, **25:** 1-6.

SHAHAROM-HARRISON, F.M., ANDERSON, I.G., SITI,A.Z., NOOR, A.M., SHAZILI,K.J. and AZMI, T.I. (1990) Epizootics of Malaysian cultured freshwater pond fishes by *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981. *Aquaculture* **86: 127-138.**

SHARIFF, M., RICHARDS, R.H. and SOMMERVILLE, C. (1980). The histophatology of acute and chronic infections of rainbow trout *Salmo gairdneri* Richardson with eyeflukes, *Diplostomum* spp. *Journal of Fish Diseases* **3,** 455-465.

SHENOY, A.S. (1987). Netherlands - a good market for tropical aquarium fish. *Seafoood Export Journal*, **19.**

SHINN, A.P. (1983) The application of new byosystematic techniques in the discrimination of the genus *Gyrodactylus* (Monogenea) on salmonid fish. Ph.D. thesis, Institute of Aquaculture, University of Stirling.

SHINN, A.P., GIBSON, D.I. and SOMMERVILLE, C. (1993). An SEM study of the haptoral sclerite of the genus *Gyrodactylus* Nordmann, 1832 (Monogenea) following extraction by digestion and sonication techniques. *Systematic Parasitology* **25**: 135-144.

SHOTTS, E.B. and GRATZEK, J.B. (1984) Bacteria, parasites, and viruses of aquarium fish and their shipping waters. *In*: Distribution, Biology and Managment of Exotic Fishes (Courtenay,-W.R.,Jr.;Stauffer,-J.R.,Jr) pp. 215-232

SHOTTS, E. B. Jr, KLECKNER, A.L., GRATZEK,J. B. and BLUE, J.L. (1976) Bacterial flora of aquarium fishes and their shipping waters imported from Southeast Asia. *Journal Fisheries Research Board Canadian* **33**: 732-735.

SCHUBERT, G. (1978) Krankheiten der Fische. Kosmos, Francksche Verlagshandlung, Stuttgart, 68 pp.

SIRE, J.Y. (1993) Development and fine structure of the bony scutes in *Corydoras arcuatus* (Siluriformes, Callichthyidae). *Journal of Morphology* **215**: 225-244.

SOMMERVILLE, C. (1980). Parasites of Ornamental Fish. *Journal of Small Animal Practice* **22**: 367-376.

SPENCER-JONES, M.E. and GIBSON, D.I. (1990) A list of old and recently erected monogenean genus-group names not included in Yamaguti's *System helminthum*. *Systematic Parasitology*, **16**: 213-226.

STROMBERG, P.C. and CRITES, J.L. (1974). The life cycle and development of *Camallanus oxycephalus* Ward & Magath, 1916 (Nematoda: Camallanidae). *The Journal of Parasitology* **60**: 117-124.

STUMPP, M. (1975) Unterschungen zur morphologie und biologie von *Camallanus cotti* (Fujita, 1927). *Zeit Parasitenkunde* **46**: 277-290.

SZEKELY, C. and BERECZKY, M.Cs. (1992a) An unusual case of disease in pet fish stocks caused by *Coleps* sp. (Protozoa: Kinetoflagminophorea). *Diseases of Aquatic Organisms* **13**: 143-145.

SZEKELY, C. and MOLNAR, K. (1992b) *Goussia trichogasteri* n. sp. (Apicomplexa: Eimeriidae) infecting the aquarium-cultured golden gourami *Trichogaster trichopterus trichopterus*. *Diseases of Aquatic Organisms* **13**: 79-81.

SZIDAT, L. (1973) Morphologie und Verhalten von *Paragyrodactylus superbus* n.g.,n.sp, Erreger eines Fischsterbens in Argentinien. *Angewandte Parasitologie* **14**: 1-10.

SZIDAT, L. (1969) Structure, development, and behaviour of new strigeatoid metacercariae from Subtropical fishes of South America. *Journal Fisheries Research Board of Canada* **26**: 753-786.

TEIXEIRA DE FREITAS, J.F. and LENT, H. (1946) Infestaçao de apaiaris "*Astronotus ocellatus*" (Agassiz) pelo nematódeo *Goezia spinulosa* (Diesing, 1839). *Revista Brasileira de Biologia* **6**: 215-222

TEO, L.H. and CHEN, T.W. (1989). Closed system packing of tropical aquarium fish for air transport. *EAS Special Publication* **10**. European Aquaculture Society.

THATCHER, V.E. (1991) Amazon fish parasites. *Amazoniana* **11**:263-572.

THATCHER, V.E. (1984) The parasitic crustaceans of fish from the Brazilian Amazon. 7. *Acusicola tucunarense* n.sp. (Copepoda:Cyclopoidea) from *Cichla ocellaris*, with emmendation of the genus and the proposal of Acusicolinae subfam.nov. *Revista Brasileira de Biologia* 44: 181-189.

THATCHER, V.E. (1981a) Patologia de Peixes da Amazônia Brasileira. 1. Aspectos Gerais. *Acta Amazônica*, **11**:125 -140.

THATCHER, V.E. (1981b) Os crustáceos parasitos de peixes da Amazônia Brasileira, 1. *Ergasilus bryconis* n.sp. (Copepoda: Cyclopoidea) da matrincha (*Brycon melanopterus* (Cope). *Acta Amazônica*, **11**: 437-442.

THATCHER, V.E. (1981c) Os crustáceos parasitos de peixes da Amazônia Brasileira, 1. *Ergasilus leporinidis* n.sp. (Copepoda: Cyclopoidea) das brânquias de (*Leporinus fasciatus* (Bloch)). *Acta Amazônica*, **11**:723-727.

THATCHER,V.E. and BOEGER,W.A. (1983a) The parasitic crustaceans of fish from Brazilian Amazon 4. *Ergasilus colomesus* n.sp. (Copepoda, Cyclopoida) from an ornamental fish and aspects of its pathogenicity. *Transactions of the American Microscopical Society* **102**: 371-379.

THATCHER,V.E. and BOEGER,W.A. (1983b) Patologia de peixes da Amazônia Brasileira 3. Alteraçoes histológicas em brânquias provocadas por *Ergasilus, Brasergasilus* e *Acusicola* (Crustacea: Cyclopoidea,Ergasilidae). *Acta Amazônica* **13**, 441-451.

THATCHER, V.E and DOSSMAN, M.D. (1975) *Univoelium prochilodorum* gen et sp.n (Trematoda:Haploporidae) from a freshwater fish (*Prochilodus reticulatus*) in Colombia. *Proceedings ohf the Helminthological Society of Washington* 42: 28-30.

THATCHER, V.E and DOSSMAN, M.D. (1974) *Lecithobothrioides mediacanoensis* n.g.,n.sp. (Trematoda: Haploporidae) from a freshwater fish (Prochilodus reticulatus) in Colombia. *Transactions American Microscopy Society* 93: 261-264.

THATCHER, V.E. and PADILHA, T.N. (1977) *Spinitectus jamundensis* sp.n. (Nematoda: Spiruroidea) from Colombian freshwater fish, Prochilodus reticulatus Steind. *Revista Brasileira de Biologia* **37**: 799-801.

THATCHER, V.E. and PAREDES, V. (1985) A parasitic copepod, *Perulernea gamitanae* gen.et sp. nov. (Cyclopoida: Lerneidae), from the nasal fossae of a Peruvian Amazon food fish. *Amazoniana* **9**: 169-175.

THATCHER, V.E. and ROBERTSON, B.A. (1982) The parasitic crustaceans of fish from the Brazilian Amazon, 3. *Ergasilus jaraquensis* n.sp. (Copepoda:Cyclopoidea) from the gills of *Semaprochilodus insignis* (Schomburgk).42: 503-513.

THATCHER, V.E. and VARELLA,A.B. (1980) Patologia de peixes da Amazônia Brasileira. 2. Um tumor maligno das brânquias relacionado com as metacercárias de um trematódeo. *Acta Amazônica* **10**: 651-656.

THURSTON, J.P. (1970) Studies on some protozoa and helminth parasites of *Xenopus*, the African clawed toad. *Revue de Zoologie et de Botanique Africaines* **82**: 349-364.

TRAVASSOS, L., ARTIGAS, P. and PEREIRA, C. (1928). Fauna helmintologica dos peixes de agua doce do Brasil. *Archivos do Instituto Biologico* **1**: 5-68.

TRAVASSOS, L., TEIXEIRA de FREITAS, J.F. and KOHN, A. (1969) Trematódeos do Brasil. *Memorias do Instituto Oswaldo Cruz* **67**: 1-886.

TRUST, T.J. and BARTLETT, K.H. (1974) Occurrence of potential pathogens in water containing ornamental fishes. *Applied Microbiology* **28**: 35-40.

UNTERGASSER, D. (1989) Handbook of Fish DiseasesT.F.H. Publications, 160.

URDANETA, Ch.H. (1981) Enfermedades que afectam peces comestibles y ornamentales en el Edo Zulia. Universidad el Zulia. Facultad de Humanidades, B.Sc. Thesis, 116pp.

VALDÉZ, I.E. and CONROY, D.A. (1963) The study of a tuberculosis-like conditon in neon tetras (*Hyphessobrycon innesi*). II. Characteristics of the bacterium isolated. *Microbiologia Espanola*, **16**: 249-253.

VANDELPITE, J., LEMMENS, P. and SWERT, L.D. (1983). Human edwardsiellosis traced to ornmanental fish. *Journal*

VASQUEZ, C. (1974) Algunos aspectos de la ictiopatologia in Colombia. **Symposium de la Aquaculture en Latin America**, FAO/CARPAS.

WATSON, J.J., PIKE, A.W. and PRIEDE, I.G. (1992). Cardiac pathology associated with the infection of *Oncorhynchus mykiss* Walbaum with *Apatemon gracilis* Rud. 1819. *Journal of Fish Biology* **41:** 163-167.

WEDEMEYER, G.A. (1974) Stress as a predisposing factor in fish disease. U.S. Department International, Fish Wildlife Service Publications FDL-38, Washington, DC.

WEDEMEYER, G.A. (1970) The role of stress in the disease resistance of fish. 30-35.

WELCOMME, R.L. (1988) International introductions of inland aquatic species. FAO Fisheries Technical Paper No. 294.

WELCOMME, R.L. (1984) International Transfers of Inland Fish Species. In: Distribution, Biology and Managment of Exotic Fishes. (Courtenay, W.R.,Jr.;Stauffer, J.R.,Jr. eds.), 22 -40.

WELCOMME, R.L. (1981) Register of international transfers of inland fish species. Food and Agriculture Organization Fisheries **Technical Paper** No. 213, 120pp.

WELCOMME, R.L., RICHARDS, R. and NEIVA, S.G. (1979) Relatorio da missao sobre peixes ornamentais. **Serie Documentos Traduzidos** No.9, 41pp. Ministerio da Agricultura. Superintendencia de Desenvolvimento da Pesca. Programa de Pesquisa e Desenvolvimento Pesqueiro do Brasil.

WHEELER, A and MAITLAND, P.S. (1973) The scarcer freshwater fishes of the British Isles I. Introduced species. *Journal of Fish Biology* **4**, 49-68.

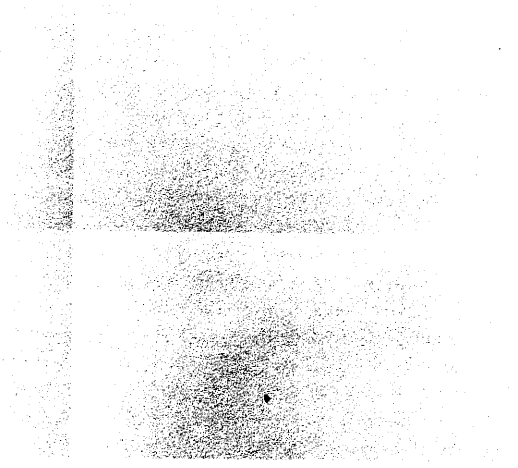
WILLIAMSON, C.E. (1983) Behavioral interactions between a cyclopoid copepod predator and its prey. *Journal of Plankton Research,* **5:** 701-711.

WILLIAMSON, C.E. and GILBERT,J.J. (1980) Variation among Zooplankton Predators: The potential of *Asplanchna, Mesocyclops* and *Cyclops* to attack, capture, and eat various rotifer prey. American Society of Limnology Oceanography. SPC. Symposium **3**:509-517.

WOOD, E. (1985) Exploitation of Coral reef fishes for the aquarium trade. Report to the Marine Conservation Society 119pp.

WOOTTEN, R. (1991) Legislation and the control of fish diseases. Fish Health Management in Asia Pacific. Report on a Regional Study and Workshop on Fish Disesase and Fish Health Management.Asian Development Bank, Manila Philippines; Network of Aquaculture Cent.in Asia, Bangkok Thailand MANILA PHILIPPINES ADB 1991. no. 1 pp. 543-548

WYATT,R.J. and KENNEDY,C.R. (1989) Host-constrained epidemiology of the fish tapeworm Ligula intestinalis. *Journal Fish Biology* **35**: 215-227.



Appendices

Appendix I

IMPORTERS' QUESTIONNAIRES

NAME OF IMPORTER

ADDRRESS

CITY POST CODE

COUNTRY

TELEPHONE NUMBER FAX NUMBER

General Aspects of Fish

1. What species are you importing from South America?

2. What species are you importing from Brazil, Colombia, Venezuela and Peru?

3. How many species of:

CHARACINS CICHLIDS

SILURIFORMS

are you importing?

4. Are you receiving different species of fish at the same time or do you receive particular species of fish only at certain time?

5. In your oppinion, which species are the most important?

6. Do you receive these fish:

weekly monthly

7. When are you receiving the next stock of fish? Date:

8. Do you have a specific calendar to receive these fish or not?

9. Do you know of *Colossoma macropomun*?

10. Are you importing this species of fish?

Transportation

12. What is the percentage of mortality during transportation?

13. What is the percentage of mortality after the transportation?

14. What is the percentage of mortality in your holding facility?

15. Have you observed different levels of mortality, at different periods of the year?

16. How are you transporting these fish?

17. How many fish are keeping/receiving in each plastic bag?

18. What are the common types of problems that you are facing during the transportation?

Water Quality

19. Which types of water quality analysis are you doing?

20. Do you send water samples for analyses in specialized laboratory or are you analysing them yourself with kits?

21. Which water quality parameters are you checking regularly?

22. How are you getting your water?

23. Are you using any type of treatments in the water before using the water for the fish?

Importer's Holding Facilities

24. Where are you keeping these fish? What type of tanks?
25. How long are these fish retained in these tanks before distribution to pet shops or fish dealers?
26. Do you have any type of steriliser (filters) in your holding facility?

Feeding

27. What are the dietary requirements of these fish?
28. Are you producing any types of fish food or are you buying this externally?
29. Do you have different procedures for feeding different species of fish? Or do you have a basic diet?

Diseases

30. What are the most common problems that you have had?
31. What type of problems or diseases are you having sporadically?
32. Do you have any species of fish that you consider to be problematic? Which species?
33. What type of problems are you having with this species?

34. Have you observed any parasites or any pathology in these fish?

35. If yes, do you know what type of parasite? or what part of body have you seen these parasite? Is it frequent or is it related with a period of year? Could you please describe?

Treatment

36. Are you treating any of these fish?

37. If yes, what kind of treatment? When ?

38. What do you believe the drugs treat?

39. What types of drugs are you using for:

a) organophosphates

b) antibiotics

c) sulphur

d) others

40. How are you doing the evaluation the efficacy of your treatment?

41. What routine technique are you using for diagnosis of the diseases?

42. Do you request any particular procedure (treatment) from your export suppliers to be applied to the fish prior to import? If you do, which type?

43. During the holding period of fish, what are the most common treatments applied? Why?

Quarantine

43. What is the nature of your quarantine procedures?

a) water quality

b) temperature

c) period of quarantine

d) Techniques for disease screening

How long are you keeping these fish after import in your holding facilities?

44. As you are doing quarantine, what are the most common problems faced?

Desinfection

45. How are you doing the disinfection of the nets, aquaria etc?

46. What type of disinfection are you doing?

Appendix II

EXPORTERS' QUESTIONNAIRES

NAME OF EXPORTER

ADDRRESS

CITY POST CODE

COUNTRY

TELEPHONE NUMBER FAX NUMBER

General Aspects of Fish

1. Which species of fish are you exporting from the Amazon region?
2. Are you importing fish from other regions of Brazil? Which regions?
3. What are the most important species of fish from this region for exportation?
4. What is the most important species for exportation from this Amazon region?
5. Do you have a specific timetable for receiving these fish or not?

Transportation

First Stage: From the Fishermen to the Exporter

6. How do you transport your fish?

7. How long do you keep your shipment in Barcelos's holding facility before trans-shipment to Manaus?

8. What is the percentage mortality during the travel between Barcelos and Manaus?

9. What factors do you consider to be responsible for these mortality rates?

10. What is the percentage mortality between arrival at your holding facilities and the time of export?

11. What is the percentage mortality in your holding facility?

12. Have you observed different levels of mortality, at different periods of the year?

13. What are the most common problems observed during tranportation by boat?

14. What is the percentage mortality :

a. Between the fishermens' holding facilities and exporters' holding facilities in Barcelos.

b. In the exporters' holding facilities in Barcelos

c. During travel by boat from Barcelos to Manaus

d. Between the arrival in the exporters' holding facilites in Manaus and exportation

15. Have you observed a different percentage of mortalities at different periods of the year?

16. What are the species that you consider most susceptible to diseases / or to have problems during and after transportation?

17. What are the most commom problems observed during transportation?

18. After arrival to the exporters' holding facilities, what are your procedures?

19. How long do you keep the fish prior to export?

20. Are you using a quarantine procedure ? For how many days/weeks?

Water Quality

21. Where does the water at the exporters' holding facilities come from ?

22. What type of water quality analyses are you doing?

23. Have you had any problem with the water? What type? Could you please describe?

24. Do you transport the fish in the river water or do you transport them in water from the holding facility?

25. Are you using any type of prophylactic treatment for the fish during transportation? What are you doing?

26. Do you know if the species of fish from the Rio Negro or the Rio Solimoes have different requirements for transportation?

27. Does the type of water (white or black) contribute towards mortalities during tranportation of fish ?

EXPORTERS' HOLDING FACILITIES

28. What are the types of ponds/ tanks that you have? (Size, earth or concrete tanks, etc.)?

29. Do you have any type of protection in these tanks against birds or others predators (nets)?

FEEDING

30. What types of food are you using ?

31. Are you preparing the food yourself or are you buying it comercially ? What type of food do you use ?

32. Do you feed the fish during transport by boat?

33. Do you feed all species of fish with the same type of food?

34. Do you have any special food requirements for any particular species of fish?

35. How many days prior to exportation do you stop feeding the species of fish to be exported?

DISEASES

36. What are the most common diseases that you have encountered ?

37. What are the types of disease problems that you are observing during the transportation by boat?

38. Have you observed any particular problem associated with a particular period of the year?

39. Which species of fish do you consider most susceptible to different types of diseases/problems?

40. Have you observed any type of parasite? Could you describe it ?

41. Among the different species of Corydoras, which one do you consider most susceptible to diseases? Which type of diseases?

42. Are you having disease problems with the species of Corydoras? Could you describe them ?

TREATMENT

43. Are you treating the species of fish in any way before transportation by boat?

44. What are your procedures regarding treatment of the fish on their arrival in Barcelos and Manaus?

45. What type of drugs are you administering to the species of fish?

46. How are you evaluating the efficacy of your treatments?

47. Are you using any type of organophosphates? Which ones?

DISINFECTION

48. What are your procedures for disinfection at the exporters' holding facilities?

49. What type of disifectant are you using for general disinfection ?

50. How do you disinfect the nets, pipes, aquaria, etc?

51. What type of disinfectant are you using for these items?

Appendix III

FISHERMEN'S QUESTIONNAIRE

NAME OF FISHERMEN

EMPLOYED BY - NAME OF THE EXPORTER

ADDRRESS

CITY POST CODE

COUNTRY

TELEPHONE NUMBER FAX NUMBER

General Aspects of Fish

1. What species of ornamental fish are you capturing ?
2. What type of river are you fishing? (black water or white water)
3. What are the most important species ? or for which species are you getting better prices?
4. Do you have a specific timetable for catching different species of fish?

FISHERMENS' HOLDING FACILITIES

1. What type of holding facilities do have?
2. What are the size of yours cages/tanks?
3. How do you transport the fish to your holding facilities?
4. For how long do you keep the fish in your holding facilities?

5. Do you have any idea about how many fish you are losing, during the period that are you holding them?

6. Do you have any idea about possible causes for these losses?

7. Have you noticed whether there is any particular period of the year where the fish are dying more easily?

8. What type of food are you giving to your fish?

9. How many times per day do you feed these fish?

10. How many specimens of fish do you keep per cage/tank?

11. Do you mix different species of fish in the same cage?

12. If yes, which species do you keep together?

13. In this case, are you transporting these species together to the exporters' holding facilities?

14. Are you doing any type of treatment in your holding facilities? What are you doing?

15. What type of treatment are you doing?

16. What doses are you giving to the fish? (1 soup spoon, tea or coffee or other type of measurement?

17. What do you believe you are treating?

18. Are you giving the same type of treatment for all species of fish? or do you have different types of treatment?

19. What are the species more susceptible to disease/ or what are the species the are dying more easily?

20. What are the types of problems that you face most often in your holding facilities?

21. Have you observed any particular type of disease associated with different periods of the year?

22. Which type of diseases ? Could you describe?

23. Do you have problems in your holding facilities with birds, snakes or other types of predators?

TRANSPORT

24. How do you transport the fish from the river to your holding facilities? Could you describe?

25. How do you transport the fish to the exporters' holding facilities? Could you describe the type of packing?

26. How long is the trip from your facilities to Barcelos?

27. Do you have high percentage of mortality during the transport to Barcelos?

28. Are you observing different percentage of mortalities between different species of fish during the transport?

29. Which factors do you believe are responsible for these mortalities?

30. Which type of tank do you use to transport the fish?

31. How many specimens of fish are you transporting in these tanks?

32. What is the maximum capacity for this type of tank?

33. How many specimens of fish are you transporting to save space and time?

34. Are you doing any type of treatment during the transport?

35. Which type of drug are you using? What is the dose?

36. Are you using the same type of drug or are you using different types of drugs for particular species of fish?

37. Do you believe that the treatment is making a difference or not?

38. Who advised you about this type of treatment?

39. How are you getting this medication? Are you buying or is the exporter giving it to you? Are you receiving this drug from the exporter free or do you have to pay later?

40. What type of water are you transporting the fish in ?

41. How many times during the transportation are you changing the water?

42. After you change the water are you adding more drug in the water?

43. Do you believe that the type of river water you use (ie black / white) can cause any problem?

44. Do you have any idea of how many fish you are losing between capture and arrival of fish in the exporters' holding facilities in Barcelos?

45. Do you have any problem with Corydoras? Could you describe it?

46. During the process of fish capture and transport, do you have any idea, where the largest mortalities occur?

47. Which period of the year do you have more problems in keeping your fish alive?

48. Do you deliver your fish in Barcelos or in Manaus?

49. When you deliver the fish in Manaus, are you having a high percentage of mortalities?

50. How do you count your fish?

51. When do you count your fish?

52. How are you calculating your losses?

53. Do you use a spoon or a small net to count your fish?

54. When you treat the fish, what type of measurement are you using: soup spoon, cup.

Appendix IV

FISHERMAN OR MIDDLE-MAN RESPONSIBLE FOR THE TRANSPORT OF THE FISH FROM BARCELOS TO MANAUS - QUESTIONNAIRES

NAME OF THE FISHERMAN/MIDDLE-MAN

DESTINATION OF THE BOAT:

DEPARTURE TIME FROM BARCELOS:

DEPARTURE TIME IN MANAUS:

CAPACITY OF THE BOAT TRANSPORT FISH:

1. How many tanks are you responsible for each trip?
2. How are the tanks organized in the boat? Are the exporters booking for any particular space in the boat or not?
3. Are all the species transported in the same conditions in the boat?
4. How many times do you change the water from the tanks?
5. Are you changing the water of all species, or there are some species where the water is not changed?
6. Do you have to have any special care with any particular species?
7. During the travel, when do you start to change the water?
8. Are there any particular area, that you believe the water of the river has better quality? Why do think this way?
9. What is the percentage of mortality during the transport from Barcelos to Manaus?
10. Which factors do you believe are responsible for these mortalities?

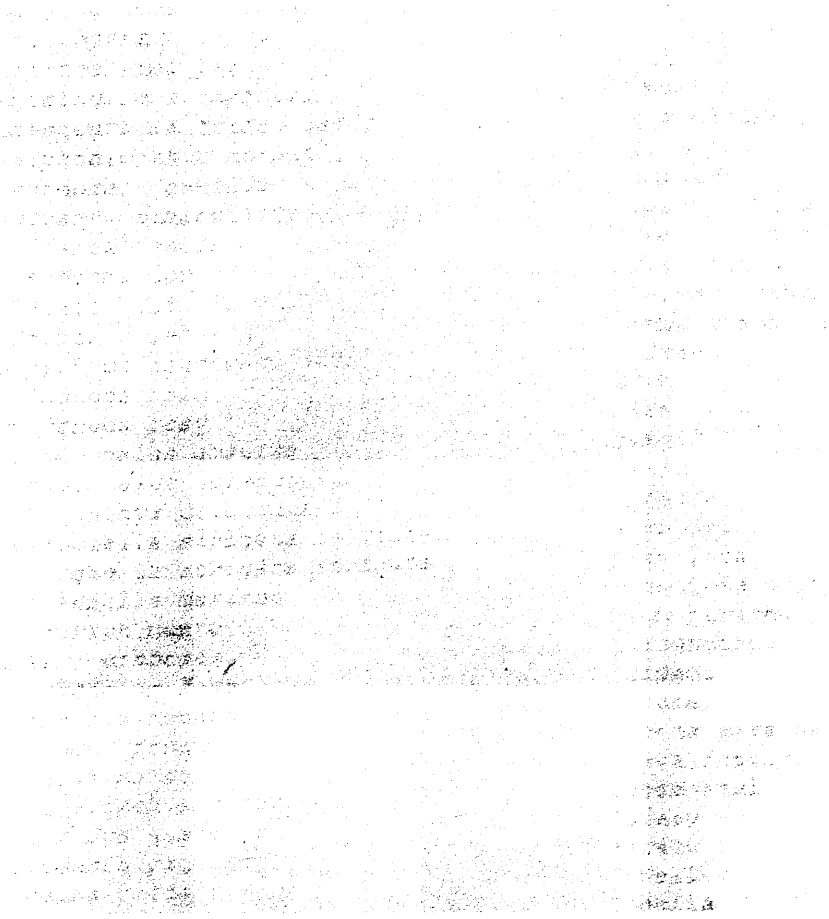
11. Which species do you consider more susceptible to die during transportation?

12. Do you do any type of treatment during the travel?

13. Which type of drug, do you normally use?

14. After change the water, do you add more drug or not?

15. After arrival of the boat in Manaus, how long do you have to wait for the truck of the exporter?



Appendix V

List of ornamental fish that can be captured, commercialised and exported legally from Brazil. Source: IBAMA Of. no. 531/92.

Scientific Names	Local Names
01. *Abramites hypselonotus*	Abramites
02. *Acanthodoras spinosissimus*	Ronca ronca
03. *Acarichthys heckeli*	Acará amarelo
04. *Achirus lineatus*	Soia
05. *Achirus errans*	Soia
06. *Aequidens curviceps*	Acarazinho
07. *Aequidens dorsigerus*	Acará-bobo
08. *Aequidens mariae*	----------
09. *Aequidens portalegrensis*	Cará-moita
10. *Amblydoras hancocki*	Cascudo mole
11. *Ancistrus* sp.	Cascudo, bodó seda
12. *Ancistrus dolichopterus*	Tigre
13. *Ancistrus lineolatus*	Ancistrus
14. *Anostomus anostomus*	Anostomus
15. *Anostomus gracilis*	Anostomus
16. *Anostomus taeniatus*	Lápis
17. *Anostomus ternetzi*	Anostomus
18. *Anostomus trimaculatus*	Anostomus
19. *Aphyocharax anisitsi*	Enfermeirinha
20. *Apistogramma agassizi*	Agassizi
21. *Apistogramma borelli*	Apistograma
22. *Apistogramma corumbae*	Apistograma
23. *Apistogramma ortmanni*	Apistograma
24. *Apistogramma pertence*	Pertence
25. *Apistogramma ramirezi*	Ramirezi
26. *Apistogramma trifasciatum*	Apistograma
27. *Asiphonichthys condei*	Peixe vidro
28. *Aspidoras poecilus*	Aspidora
29. *Astyanax bimaculatus*	Piaba do rabo amarelo
30. *Astyanax fasciatus*	Piaba do rabo vermelho
31. *Biotodoma cupido*	Acará cupido
32. *Brochis britskii*	Coridora gigante
33. *Brochis splendens*	Limpa fundo verde
34. *Bryconops caudomaculatus*	Brincon
35. *Bryconops gold*	Gold
36. *Bryconops rosy*	Rose
37. *Bunocephalus amaurus*	Banjo
38. *Bunocephalus coracoideus*	Banjo
39. *Callichthys callichthys*	Taboatá
40. *Carnegiella strigata fasciata*	Borboleta
41. *Carnegiella strigata strigata*	Borboleta
42. *Carnegiella marthae*	Borboleta branca
43. *Catoprion mento*	Pacu piranha
44. *Charax gibbosus*	Corcundinha
45. *Characidium fasciatum*	Torpedo
46. *Cheirodon notomelas*	Caramelo
47. *Chilodus punctatus*	Cabeça para baixo
48. *Cichlasoma festivum*	Acara festivo
49. *Coelurichthys microlepis*	Tetra azul
50. *Colomesus asellus*	Baiacu
51. *Colomesus psittacus*	Baiacú
52. *Copeina guttata*	Copella
53. *Copella arnoldi*	Copella
54. *Copella metae*	Copella
55. *Copella nattereri*	Copella
56. *Copella nigrofasciata*	Copella
57. *Corydoras acutus*	Coridora

58.	*Corydoras aeneus*	Coridora
59.	*Corydoras adolfoi*	Coridora
60.	*Corydoras agassizi*	Coridora
61.	*Corydoras arcuatus*	Coridora
62.	*Corydoras barbatus*	Coridora.
63.	*Corydoras caudimaculatus*	Coridora.
64.	*Corydoras elegans*	Coridora
65.	*Corydoras griseus*	Coridora
66.	*Corydoras haraldschultzi*	Coridora
67.	*Corydoras hastatus*	Coridora mini
68.	*Coyidoras julii*	Coridora leopardo
69.	*Corydoras myersi*	Coridora
70.	*Corydoras nattereri*	Coridora
71.	*Corydoras paleatus*	Coridora
72.	*Corydoras reticulatus*	Coridora
73.	*Crenicara maculata*	Kadrez
74.	*Crenicara filamentosa*	Kadrez
75.	*Crenicara punctulata*	Kadrez
76.	*Crenuchus spirulus*	Crenucho
77.	*Cynolebias adloffi*	Cinolébia
78.	*Cynolebias nigripinnis*	Cinolébia
79.	*Dianema urostriata*	Dianema
80.	*Dianema longibarbis*	-------
81.	*Exodon paradoxus*	Miguelzinho
82.	*Farowella acus*	Farowella
83.	*Farowella sp.*	Totoxi
84.	*Gasteropelecus sternicla*	Borboleta falsa, Sapopema
85.	*Gasteropelecus levis*	Peixe galo
86.	*Gymnocorymbus ternetzi*	Tetra preto
87.	*Hemigrammus erythrozonus*	--------
88.	*Hemigrammus marginatus*	Torpedinho
89.	*Hemigrammus ocellifer*	Torpedinho
90.	*Hemigrammus pulcher*	Olho de fogo
91.	*Hemigrammus rhodostomus*	Rodostomus
92.	*Hemigrammus ulreyi*	Ulreyi verdadeiro
93.	*Hemigrammus unilineatus*	Piquira
94.	*Hemiodopsis gracilis*	Cruzeiro do sul
95.	*Hemiodopsis goeldii*	Cruzeiro
96.	*Hemiodopsis sterni*	--------
97.	*Hypnessobrycon bifasciatus*	Tetra amarelo
98.	*Hypnessobrycon bentosi*	Rosa-ceu
99.	*Hypnessobrycon callistus*	Mato grosso
100.	*Hypnessobrycon erythrostigma*	Rosa-ceu
101.	*Hypnessobrycon flammeus*	Engraçadinho
102.	*Hypnessobrycon georgettae*	Rosa-céu
103.	*Hypnessobrycon griemi*	--------
104.	*Hypnessobrycon herbertaxelrodi*	Neon negro
105.	*Hypnessobrycon heterornabdus*	Falso ulreyi
106.	*Hypnessobrycon serpae*	Mato grosso
107.	*Hypnessobrycon socolofi*	Rosa-ceu
108.	*Hypnessobrycon sp.*	Platinado
109.	*Hypnessobrycon vilmae*	Falso neon negro
110.	*Iguanodectes spilurus*	Iguanodectes
111.	*Leporellus vittatus*	Aracú, anostinha
112.	*Leporinus agassizi*	Aracú
113.	*Loricaria parva*	Cascudo comprido
114.	*Megalamphodus megalopterus*	Tetra fantasma negro
115.	*Moenkhausia affinis*	Piaba
116.	*Moenkhausia barbouri*	Piaba
117.	*Moenkhausia collettii*	Piaba

118.*Moenkhausia dichroura*	Piaba bota fogo
119.*Moenkhausia gracilima*	Piaba
120.*Moenkhausia hasemani*	Piaba
121.*Moenkhausia intermedia*	Piaba
122.*Moenkhausia jamesi*	Piaba
123.*Moenkhausia lepidura*	Piaba
124.*Moenkhausia megalops*	Piaba
125.*Moenkhausia oligolepis*	Piaba rabo de ouro
126.*Moenkhausia sanctaefilomenae*	Piaba
127.*Monocirrhus polyacanthus*	Peixe folha
128.*Myleus rubripinnis*	Pacuzinho vermelho
129.*Nannostomus beckfordi*	Lápis
130.*Nannostomus digrammus*	Lápis
131.*Nannostomus eques*	Lápis
132.*Nannostomus espei*	Lápis
133.*Nannostomus marginatus*	Lápis
134.*Nannostomus trifasciatus*	Lápis
135.*Nannostomus unifasciatus*	Lápis
136.*Otocinclus affinis*	Limpa vidro
137.*Otocinclus arnoldi*	Cascudinho
138.*Otocinclus vittatus*	Limpa vidro
139.*Paracheirodon axelrodi*	Cardinal
140.*Paracheirodon innesi*	Neon tetra
141.*Paracheirodon pulcher*	Neon tetra
142.*Parodon affinis*	Mariposa
143.*Parodon suborbitale*	Mariposa
144.*Parotocinclus maculicauda*	Otocinclus pintado
145.*Peckoltia pulcher*	--------
146.*Peckoltia vittata*	--------
147.*Petitella georgiae*	Rodostomo
148.*Poecilia reticulata*	Guppy
149.*Poecilocharax weitzmani*	Brilhante
150.*Polycentrus schomburgki*	Marajó
151.*Prionobrama filigera*	Prionobrama
152.*Pseudacanthicus leopardus*	Assacú pintado
153.*Pterophyllum dumerili*	Acará bandeira
154.*Pterophyllum scalare*	Acará bandeira
155.*Pterolebias longipinnis*	Rivulo
156.*Pyrrhulina brevis*	Pyrrhulina pintada
157.*Pyrrhulina laeta*	Pyrrhulina
158.*Pyrrhulina vittata*	Pyrrhulina
159.*Pyrrhulina rachowiana*	Pyrrhulina
160.*Rineloricaria fallax*	Rabo de chicote
161.*Rineloricaria lima*	Rabo de chicote
162.*Rineloricaria lanceolata*	Rabo de chicote
163.*Rivulus punctatus*	Rivulo
164.*Rivulus uropthalmus*	Pacu
165.*Serrasalmus calmoni*	Piranha
166.*Serrasalmus hollandi*	Piranha
167.*Serrasalmus nattereri*	Piranha
168.*Serrasalmus rhombeus*	Piranha
169.*Sturisoma barbatum*	Cascudinho disco
170.*Symphysodon aequifasciata aequifasciata*	Disco
171.*Symphysodon aequifasciata axelrodi*	Disco
172.*Symphysodon aequifasciata haraldi*	Disco
173.*Symphysodon discus*	Disco
174.*Tatia aulopygia*	Tatia
175.*Thayeria obliqua*	Taéria
176.*Thoracocharax stellatus*	Borboleta·
177.*Trygonectes strigapunctis*	Trigonectes

Appendix VI

Recommended Compositon for Artificial Spring Water

Stock Solution	Composition	Quantity g/L
A	$FeCl_3.6H_2O$	0.25
B	$CaCl_2$ anhydrous	11.0
C	$MgSO_4.7H_2O$	10.0
D	Phosphate buffer*	
Solutions[1]		Quantity (ml)
A		0.5 ml
B		2.5
C		2.5
D		1.25

Source: Macinnis & Voge, 1970. * Phosphate buffer - dissolve 34g of KH_2PO_4 in 500 ml of distilled water. Add aproximately 175 ml 1N NaOH until a pH 7.2 is reached. Subsequently add 1.5g $(NH_4)_2SO_4$ and dilute to one liter.

[1] Make up to 1 litre with distilled water.

Systematic Parasitology **29**: 217–222, 1994.

Gyrodactylus gemini n. sp. (Monogenea: Gyrodactylidae), a parasite of *Semaprochilodus taeniurus* (Steindachner) from the Venezuelan Amazon

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Accepted for publication 6th October, 1993

Abstract

Gyrodactylus gemini n. sp. (Monogenea, Gyrodactylidae) is described from the surface of the body and fins of the fish *Semaprochilodus taeniurus* (Steindachner) imported into Britain from the Venezuelan Amazon. The new species differs from other species of the genus, including those described from South and Central America, by having: (i) stout hamuli with straight shafts and diverging roots; (ii) marginal hooks with the sickle length larger than the width; (iii) a dorsal bar without a medial constriction; (iv) a rectangular ventral bar with short processes; (v) a triangular ventral bar membrane; and, most obviously, (vi) at least two generations which can develop two embryos simultaneously. This is the first known species of the genus *Gyrodactylus* from the Venezuelan Amazon and the first record of the subgenus *Gyrodactylus* (*Gyrodactylus*) from South America.

Introduction

South America possesses the richest ichthyofauna of all the continents. In the Amazon region alone, it is estimated that over 2,000 species of fish occur (Bohlke *et al.*, 1978). Despite this great diversity, the gyrodactylid fauna of this continent is not well known.

Amongst the genera described for the family Gyrodactylidae van Beneden & Hesse, 1863 (see *inter alia* Spencer-Jones & Gibson, 1990), only four genera and nine species are known in South America. These are *Paragyrodactyloides superbus* (Szidat, 1973) Ostrowski de Nuñes, 1975 from *Corydoras paleatus* (Jennys), Callichthyidae, in Argentina; *Scleroductus yuncensi* Jara & Cone, 1989 from *Pimelodella yuncensis* Steindachner, Pimelodidae, in Peru; *Acessorius peruensis* Jara, An & Cone, 1991, *Gyrodactylus bimaculatus* An, Jara & Cone, 1991, *G. slendrus* An, Jara & Cone, 1991 and *G. lebiasinus* An, Jara & Cone, 1991 all from *Lebiasina bimaculata* Cuv. & Val., Characidae, in Peru; *G. pimellodus* An, Jara & Cone, 1991 from *Pimelodella yuncensis*, in Peru; *G. turnbulli* Harris, 1986 from *Poecilia reticulata* Peters, Poeciliidae, in Peru; and *G. curemae* Conroy & Conroy, 1985 from *Mugil curemae* Valenciennes, Mugilidae, in Venezuela. *Gyrodactylus turnbulli* has been recorded once from feral guppies in Peru (An *et al.*, 1991), but in this case the fishes had been introduced from elsewhere, as the natural range of the guppy is restricted to the Caribbean basin of northeastern South America and the Lesser Antilles (Jacobs, 1971, in Harris & Lyles, 1992).

Other species of the superfamily Gyrodactyloidea described from South American freshwater fish belong to the family Ooegyrodactylidae

Harris, 1983. There are three genera and six species described: *Phanerothecium caballeroi* Kritsky & Thatcher, 1977, from *Cephalosilurus zungaro* (Humboldt), Pimelodidae, in Colombia; *P. harrisi* Kritsky & Boeger, 1991, from *Plecostomus plecostomus* (L.), Loricariidae, in Brazil; *Nothogyrodactylus amazonicus* Kritsky & Boeger, 1991, *N. clavatus* Kritsky & Boeger, 1991, and *N. plaesiophallus* Kritsky & Boeger, 1991, all from *Ancistrus* sp., Loricariidae, in Brazil; and *Ooegyrodactylus farlowellae* Harris, 1983, from *Farlowella amazonum* Günther, in Peru.

For Central America, Thatcher (1991) reported the occurrence of two genera and four species: *Gyrodactylus bullatarudis* Turnbull, 1956, by Kritsky & Fritts (1970) and *G. costaricensis* Kritsky & Fritts, 1970, from *Poecilia sphenops* Val., Poeciliidae; *G. neotropicalis* Kritsky & Fritts, 1970 and *Anacanthocotyle anacanthocotyle* Kritsky & Fritts, 1970, from *Astyanax fasciatus* (Cuvier) in Costa Rica.

The present study describes a new species, *Gyrodactylus gemini* n. sp., a parasite of *Semaprochilodus taeniurus* (Steindachner) from the Venezuelan Amazon.

Materials and methods

Twenty-five specimens of *Semaprochilodus taeniurus* were obtained from a local importer on their arrival into Britain after their importation direct from Venezuela. Helminths were collected from skin scrapes of the hosts and prepared for study according to the procedures of Malmberg (1970) and Shinn, Gibson & Sommerville (1993).

Illustrations were prepared with the aid of a camera lucida. All measurements in the diagnosis are in micrometres followed by the mean in parentheses and were made according to the procedures of Malmberg (1970).

***Gyrodactylus gemini* n. sp.**

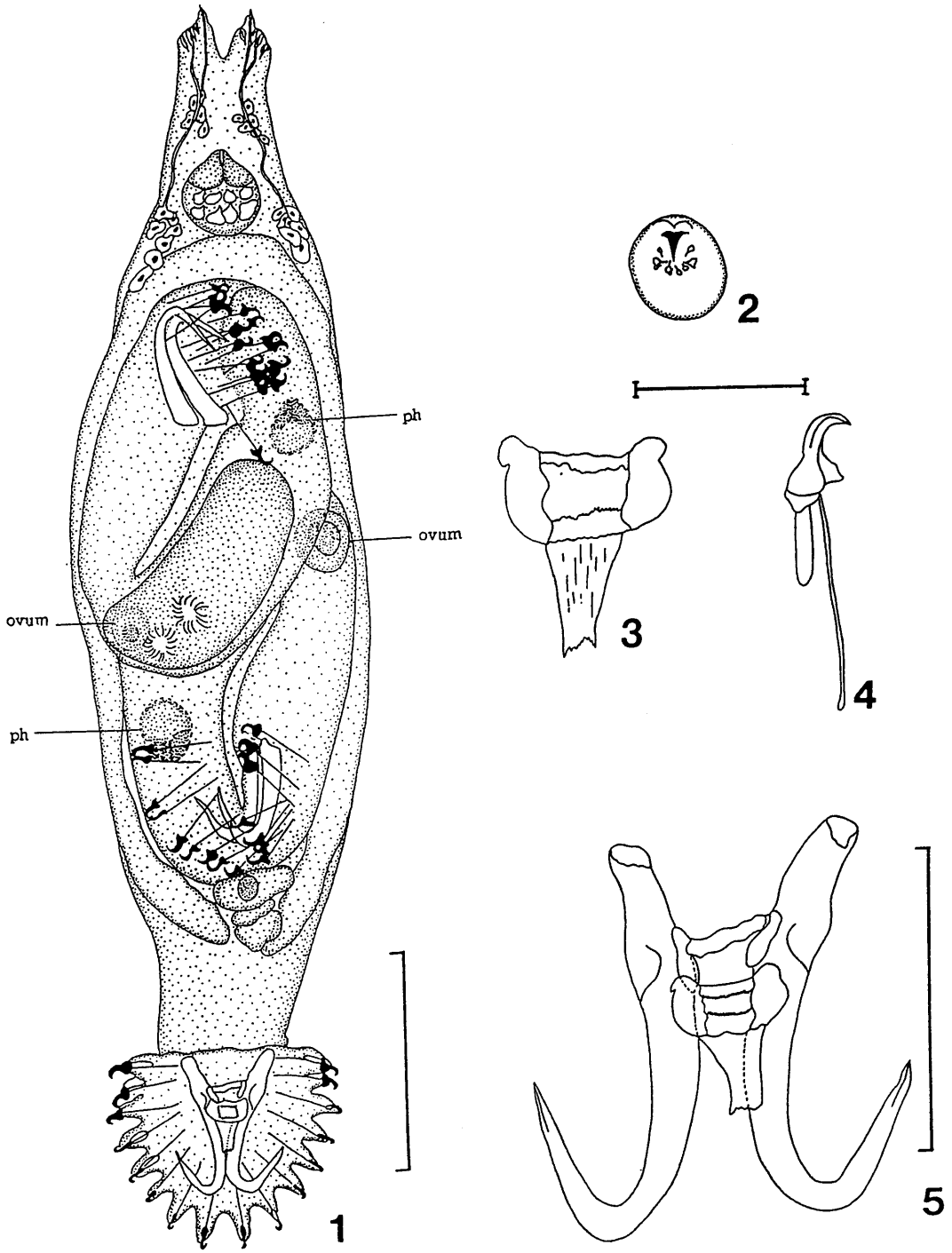
Type-host: *Semaprochilodus taeniurus* (Steindachner), Curimatidae, Prochilodinae; "red prochilodus".
Site: Surface of body and fins.
Type-locality: Venezuelan Amazon.
Type-material: Fourteen flattened specimens were studied. Holotype (Reg. no. 1993.11.25.1) and paratypes in The Natural History Museum, London, (Reg. no. 1993.11.25.2–14).
Prevalence: Three of 25 fish examined.
Etymology: The specific name relates to the simultaneous development of two embryos in the uterus.

Description (Figs 1–10; Table I)

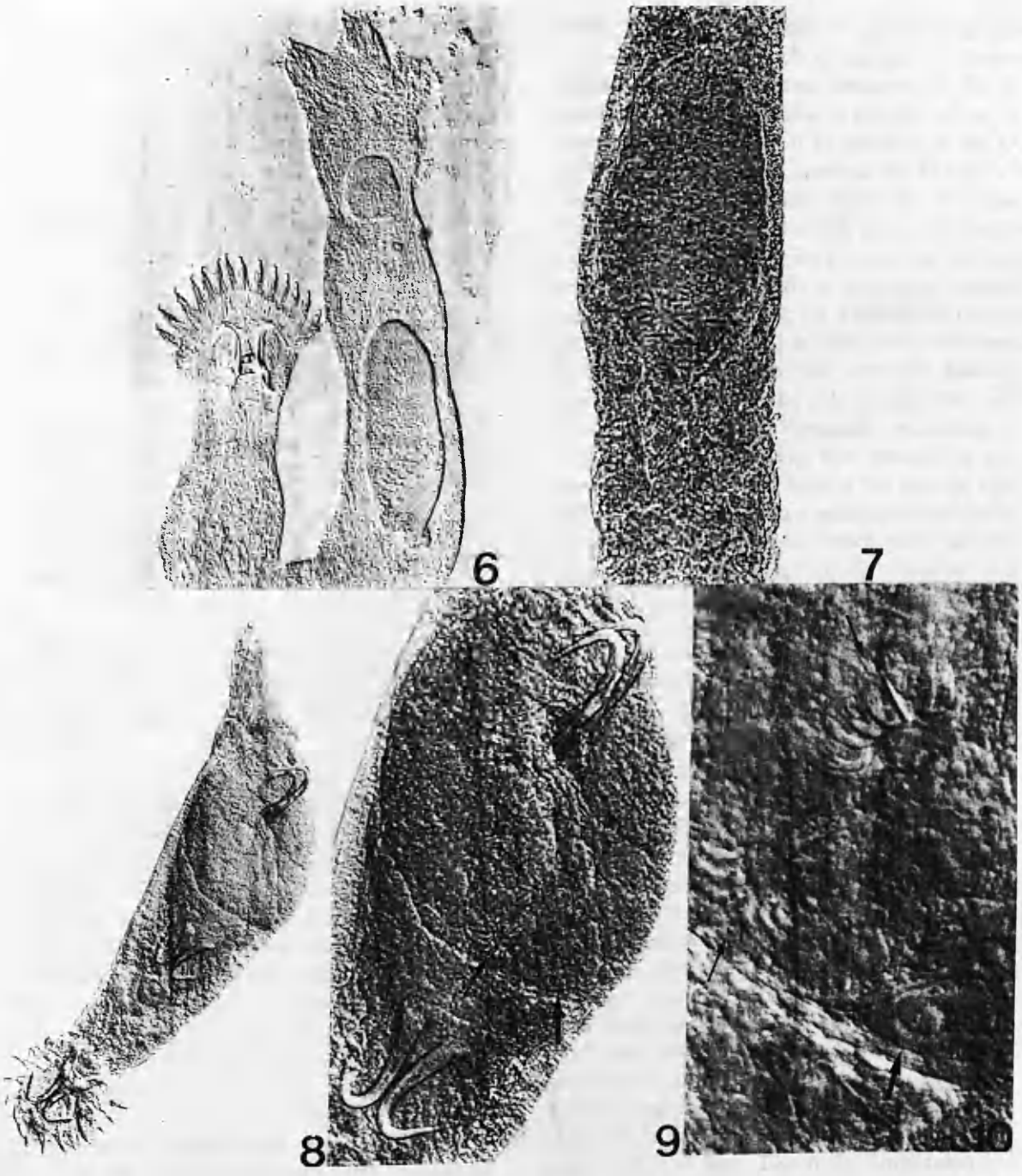
Gyrodactylidae; with the characters of the genus *Gyrodactylus* Nordmann, 1832. Body fusiform. Two terminal cephalic lobes, well developed, each with spike (sensillum). Flattened specimens 346–1,089 (573) × 69–141 (110). Postero-lateral cephalic glands well developed. Pharynx 31–59 (43) × 28–53 (39), with 8 small pharyngeal processes. Gut bifurcates into 2 unbranched caeca which do not extend beyond level of testis. Opisthaptor sub-ovate, 85–98 (90) × 88–110 (99), with 16 marginal hooks, 2 ventral hamuli linked by ventral and dorsal bars (Fig. 1). Cirrus ventral to posterior pharyngeal bulb, 12–15 (13) in diameter, armed with 5–7 (6) small spines, in more than one arched row (Fig. 2). Vas deferens may dilate into sac-like structure prior to approaching basal bulb of cirrus. Excretory system of *G.* (*Gyrodactylus*)-type, lacking excretory bladders. Flame bulbs IIf5a, IIf5 and IIf5d (Malmberg, 1970) in opisthaptor are situated between marginal hooks 2–3, 5–6 and 7–8, respectively (Fig. 6).

Detailed measurements of sclerites presented in Table I.

Hamuli 56–66.5 (62) long (Fig. 5). Dorsal bar 11–20 (15) × 2–2.5 (2.3) (Fig. 5). Ventral bar rectangular 17–21 (18.5) × 19–24.5 (22) (Fig. 3). Ventral bar processes short. Basal width of ventral bar 8.5–10.5 (9.5). Ventral bar membrane (=



Figs 1–4. Gyrodactylus gemini n. sp. 1. Holotype, ventral view; 2. Cirrus; 3. Ventral bar; 4. Marginal hook; 5. Hamuli/bar complex. *Scale-bars*: 1, 100 μm; 2–4, 20 μm; 5, 50 μm. *Abbreviation*: ph. pharynx.



Figs 6–10 *Gyrodactylus gemini* n. sp. Developmental stages. 6–7. Specimens with two embryonal marginal hook corollas; 6. Specimen with cirrus developed (×40); 7. Specimen without cirrus developed (×40); 8. Specimen with more mature embryos, ventral view (×16); 9. Anterior-most embryo with two developing marginal hook corollas (small arrow) and ovum (large arrow) (×40); 10. Two embryonal marginal hook corollas (small arrow) and ovum (large arrow) (×100).

Table I. Sclerite dimensions of *Gyrodactylus gemini* n. sp. from *Semaprochilodus taeniurus*.

Hamuli		
Total length	56–66.5 (62)	
L. of root	19–27.5 (22.5)	
L. of shaft	43–49 (46)	
L. of point	27–30 (28)	
Marginal hooks	No. 1 (n = 14)	No. 8 (n = 6)
Total length	31–37 (34)	33–36 (35)
L. of sickle	8–10 (9)	8–10 (9.5)
L. of handle	22–28 (25)	24–27 (25)
L. of sickle membrane	0.6–2(1)	1
L. of sickle filament loop	7–12 (10)	8–12 (10)
W. of sickle distally	4–5 (4)	3–4 (3.5)
W. of sickle proximally	5–6 (5.5)	5–6 (5.5)
Ventral bar		
Length	17–21 (19)	
Max. dist. between processes	16–20 (19)	
Total width	19–24 (22)	
L. of processes	1–2 (1.5)	
Basal width	8–10 (9.5)	
Median width	6–10 (8)	
L. of membrane	9–12 (11)	
Dorsal bar		
Total length	11–20 (15)	
Median width	2–2.5 (2.3)	

L: length; W, width.

"shield" of Conroy & Conroy, 1985) small, triangular and weakly sclerotised (Fig. 3). Marginal hooks with sickle length greater than width; total length 31–37 (34.5) (Fig. 4; Table I).

Uterus with 2 embryos developing simultaneously (Fig. 1; 6–10). One large oöcyte may be observed within oötype posterior to embryos. One large testis present posterior to oötype.

Discussion

The presence and positions of three flame bulbs in each half of the opisthaptor, the absence of excretory bladders, a cirrus with small spines in more than one arched row, and the type of marginal hooks indicate that *Gyrodactylus gemini* n. sp. is a member of the subgenus *Gyrodactylus* (*Gyrodactylus*), as defined by Malmberg (1970).

Amongst the six species of gyrodactylids described from South America, *G. gemini* n. sp. can be distinguished from *G. lebiasinus*, *G. bimaculatus* and *G. pimelodellus*, (members of the *G. arcuatus*-group), *G. slendrus* (a member of the *G. rarus*-group), *G. turnbulli* (a member of the *G. eucaliae*-group) and *G. curemae* by having: (i) stout hamuli with straight shafts and diverging roots; (ii) marginal hooks with the sickle length greater than the width; (iii) a dorsal bar without a medial constriction; (iv) a rectangular ventral bar with short processes; (v) a triangular ventral bar membrane; and (vi) at least two generations present which develop two embryos simultaneously. *G. curemae* is the only species of *Gyrodactylus* described from Venezuela. According to Conroy & Conroy (1985), this species has one articulation between the ventral bar and the ventral bar membrane and two spines and four spinelets in the cirrus, features which were not observed in *G. gemini* n. sp. *G. curemae* was described as a parasite of *Mugil curemae*, a euryhaline fish.

From the three species described for Central America, *G. costaricensis*, *G. neotropicalis* and *G. bullatarudis*, the new species can be distinguished mainly by the morphology of the haptoral elements and by the type of embryonic development.

The process of development of the embryos exhibited by *G. gemini* n. sp. appears to differ from that of other members of the Gyrodactylidae. Generally, two to three embryonic generations occur in the uterus of the gyrodactylids (e.g. *G. bullatarudis* Turnbull, 1956, *Macrogyrodactylus polypteri* Malmberg, 1957). However, they are always at different stages of development. In the specimens of *G. gemini* n. sp. examined, some specimens had at least two embryos at the same stage of development in the uterus. The two embryonic marginal hook corollas can be observed in tandem or slightly obliquely in relation to each other (Figs 6–7). Unfortunately, there was insufficient material to ascertain the sequence of development of this gyrodactylid. However, in nine specimens in which the embryos were more well developed, the haptoral elements of the two embryos and a large ovum were observed in the anterior-most embryo, whereas the post-

erior-most embryo contained only a single large ovum (Figs 1, 8–10). The cirrus was visible in only three of the nine specimens (Fig 6).

Despite its similarities with the sub-genus *G.* (*Gyrodactylus*), the embryology of *G. gemini* is clearly different from that of other members of the Gyrodactylidae. Since the embryology of this parasite is of great interest, future studies should aim to clarify the process of embryonic development of the species. It is possible that such investigations will demonstrate that this species represents a new genus.

Acknowledgements

The authors wish to thank Dr Goran Malmberg, University of Stockholm, for his helpful comments, the British Council and CNPq-Brazil for the financial support of this project and Mrs Marguerite Mason for the translation of German papers.

References

An, L., Jara, C. A. & Cone, D. K. (1991) Five species of *Gyrodactylus* Nordmann, 1832 (Monogenea) from freshwater fishes of Peru. *Canadian Journal of Zoology*, **69**, 1,199–1,201.

Bohlke, J.E., Weitzman, S.H. & Menezes, N.A. (1978) Estado actual da sistematica dos peixes de agua doce da America do Sul. *Acta Amazonica*, **8**, 657–677.

Conroy, G. & Conroy, D.A. (1985) Gyrodactylosis in silver mullet (*Mugil curemae* Val.) from Venezuelan coastal waters, and a description of *Gyrodactylus curemae* n. sp. *Rivista Italiana Piscicultura e Ittiopatologia*, **4**, 140–147.

Harris, P.D. & Lyles, A.M. (1992) Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) in Trinidad. *Journal of Parasitology*, **78**, 912–914.

Malmberg, G. (1970) The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv för Zoologi*, **23**, 1–235.

Spencer-Jones M.E. & Gibson, D.I. (1990) A list of old and recently erected monogenean genus-group names not included in Yamaguti's *Systema helminthum*. *Systematic Parasitology*, **16**, 213–226.

Shinn, A.P., Gibson, D.I. & Sommerville, C. (1993) An SEM study of the haptoral sclerites of the genus *Gyrodactylus* Nordmann, 1832 (Monogenea) following extraction by digestion and sonication techniques. *Systematic Parasitology*, **25**, 135–144.

Thatcher, V.E. (1991) Amazon fish parasites. *Amazoniana*, **11**, 1–571.