

# Parasitic infections in freshwater ornamental fish in Sri Lanka

I. D. S. I. P. Thilakaratne<sup>1,\*</sup>, G. Rajapaksha<sup>2</sup>, A. Hewakopara<sup>3</sup>,  
R. P. V. J. Rajapakse<sup>4</sup>, A. C. M. Faizal<sup>1</sup>

<sup>1</sup>Veterinary Research Institute, Gannoruwa, Peradeniya, Sri Lanka

<sup>2</sup>Veterinary Investigation Centre, Welisera, Sri Lanka

<sup>3</sup>Animal Quarantine Station, Colombo, Sri Lanka

<sup>4</sup>Department of Para Clinical Studies, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka

**ABSTRACT:** A total of 1520 ornamental fish of 13 species from 26 export farms in Sri Lanka were collected between October 1999 and March 2000 and examined for parasites. Fish species examined were guppy *Poecilia reticulata*, goldfish *Carassius auratus*, platy *Xiphophorus maculatus*, molly *Poecilia sphenops*, angel *Pterophyllum scalare*, swordtail *Xiphophorus helleri*, tetras *Hyphessobrycon* species, barbs *Capeota* and *Puntius* spp., gourami *Colisa* sp., carp *Cyprinus carpio*, fighters *Betta splendens* and others (*Brachydanio* and *Astronotus* spp.). Nine species of monogenean trematodes (*Dactylogyrus extensus*, *Dactylogyrus* cf. *extensus*, *D. vastator*, *Dactylogyrus* cf. *vastator* *Dactylogyrus* spp., *Gyrodactylus turnbulli*, *G. katherineri*, *Gyrodactylus* cf. *katherineri*, *Gyrodactylus* spp.), 7 protozoan species (*Trichodina nigra*, *Trichodina* spp., *Tetrahymena corlissi*, *T. pyriformis*, *Ichthyophthirius multifiliis*, *Ichthyobodo necator*, *Piscinoodinium* spp.), 3 species of copepod arthropods (*Lernaea cyprinacea*, *Ergasilus ceylonensis*, *Argulus foliaceus*), 1 metacercarial stage of a digenean trematode (*Centrocestus* spp.) and 1 nematode (*Capillaria* spp.) were identified. Parasites were found in fish from 23 of the 26 farms with an overall prevalence of parasitism in 45.3% of fish. The variation in farm prevalence among different parasites was significant ( $p < 0.01$ ). Fish infection rates with monogenean trematodes, protozoans, copepod crustaceans, digenean trematodes and nematodes were 28.3, 18.4, 4.8, 0.8 and 0.4%, respectively. In all, 50 out of 590 (50/590) guppies were infected with *Tetrahymena*, compared with 13/930 for all other species, which is a statistically significant result ( $p < 0.01$ ). Similarly, 13/44 and 18/44 carp were infected with *Argulus foliaceus* and *Lernaea cyprinacea*, compared with 7/1476 and 15/1476, respectively, for all other species combined ( $p < 0.01$ ). *Capillaria* spp. was found only in guppies (4/590) and angel fish (3/92) while *Centrocestus* spp. was found in goldfish (12/153) only.

**KEY WORDS:** Parasite · Prevalence · Ornamental fish · Tropics

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## INTRODUCTION

During the last 2 decades, ornamental fish export has emerged as an important activity generating foreign exchange in Sri Lanka, with over 300 million Sri Lankan Rupees (~US\$35 000) earned annually (Weerakoon 1998). Sri Lanka has a reputation of exporting high quality marine ornamental fish. As importing countries have imposed regulations to prevent import of ornamental fish caught from the wild, in view of

resulting depletion and extinction of natural stocks (Andrews 1990), demand for cultured freshwater ornamental fish is increasing. Although the freshwater ornamental fish industry in Sri Lanka has tremendous potential for development, one of the weaknesses observed is the lack of understanding of the disease situation (Mee 1993).

Parasitism is one of the most impacting problems for cultured fish (Scholz 1999). Fish in intensive culture are continuously affected by environmental fluctua-

tions and management practices such as handling, crowding, transport, drug treatment, undernourishment, fluctuating temperatures, and poor water quality. All of these factors can impose considerable stress on the homeostatic mechanisms of fish, rendering them susceptible to a wide variety of parasites (Subasinghe 1997).

Besides direct losses caused by mortality, parasites may have a considerable impact on the growth and behavior of fish (Scholz 1999), and consequently reduce farm efficiency and production, which in turn increases costs, and reduces profit and impacts on foreign exchange earnings.

The present study was undertaken to estimate the prevalence of selected important parasites associated with the production of ornamental fish for export in Sri Lanka.

## MATERIALS AND METHODS

**Study area.** The study was carried out in the Western and Northwestern Provinces of the country, where almost all of the ornamental fish export farms are located. Some of these exporters partly depend on small-scale grower farms situated in nearby rural areas for a continuous supply of ornamental fish, which helps to keep costs down. In 1999, there were 30 registered ornamental fish exporters in the country. The area of freshwater ornamental fish farms varied from 0.5 to 10 ha in size, and monthly production varied from 40 000 to >500 000 fish.

**Ornamental fish management.** Ornamental fish are raised in cement, glass or fiberglass tanks and mud ponds, with export producers usually maintaining their own breeding stock. More than 75% of the exports are varieties of guppy. Guppies are viviparous and 1 d old larvae are collected and managed in separate tanks. After ca. 3 wk, the juveniles are sexed and separated in growing units. At 2 to 3 mo, depending on the length of the fish, guppies are ready to be marketed and are quarantined for a period of 1 wk prior to export. Other fish species are grown for longer periods before export. Feeding regimens vary with stage of growth. Feed includes brine shrimp *Artemia salina* L., tubifex worms and formulated fish feed. Most of the farmers use methylene blue (2 ppm) and salt before transferring the fish to the growing units and at the beginning of the pre-export quarantine period as a routine hygiene measure. Before being exported, representative samples of fish are examined by an Animal Quarantine Officer of the Department of Animal Production and Health; only healthy fish are recommended for export.

**Study design.** Twenty-six registered ornamental fish export farms in the Western and Northwestern

provinces of Sri Lanka were visited during the period from October 1999 to March 2000. These farms represented 87% of registered exporters who accounted for >95% of total ornamental fish exports in 1999. At each farm, tanks/ponds were numbered and random sampling was used to choose tanks/ponds (Fowler & Cohen 1994) from which to select fish for examination. A total of 1520 fish of 13 species (Table 1) were selected in this manner. Numbers of fish selected varied from 16 to 195 per farm, with species and growth stage dependent on their availability on the day of collection. Guppies were the smallest fish collected (1 to 3 cm long) whereas carp were the largest (3 to 15 cm). Live ornamental fish were taken to the laboratory in polythene bags filled with oxygenated pond water. Each farm was visited once only and information on farm management was also recorded.

**Parasitological techniques.** At the laboratory, fish were initially examined for the presence of any parasites or lesions visible to the naked eye. Next, wet mounts of scrapings (of body surface mucus from behind the pectoral fin adjacent to the dorsal fin and operculum, excised gills, lesions, and intestines) of freshly killed fish were examined for parasites using a compound light microscope at  $\times 10$  and  $\times 40$  magnification. Use of fresh specimens facilitates the visualization of motile parasites (Post 1987, Southgate 1994, Wildgoose 1998). Specimens were then preserved in 10% buffered formalin and 70% ethanol for storage prior to further identification, using keys described by Yamaguti (1963), Kirtisinghe (1964), Fernando & Hanek (1971, 1973), Elliot (1973), Cheng (1986), Liguó et al. (1991), Cone (1995), Dickerson & Dawe (1995), Lom (1995) and Hoffman (1999). Representative specimens were lodged in the Zoology Department of the National Museum, Colombo, Sri Lanka.

**Data analysis.** Prevalence rates were calculated for each recovered genus of the parasite. Where there were sufficient numbers, chi-square tests were used to compare prevalence using the computerized statistical package, Minitab release 10.1 (Minitab).

## RESULTS

### Identification of parasites

A list of parasites identified from different body locations in different fish species is shown in Table 2. Eighteen parasite species were found comprising 9 species of monogenean parasites, 7 species of protozoans, 3 species of copepod arthropods, 1 round worm and 1 metacercarial stage of a digenean trematode. Of these, 15 were identified to species level with the balance identified to genus level only (Table 2).

Table 1. Species and number of ornamental fish examined, number of different parasites recovered in different species of fish and the prevalence of parasites and number of farms infected

Species of ornamental fish	No. examined	No. infected	Parasite species											
			<i>Dactylogyrus</i>	<i>Gyrodactylus</i>	<i>Trichodina</i>	<i>Tetrahymena</i>	<i>Ichthyophthirius</i>	<i>Ichthyobodo</i>	<i>Piscinoodinium</i>	<i>Lernaea</i>	<i>Ergasilus</i>	<i>Argulus</i>	<i>Capillaria</i>	<i>Centrocestus</i>
Guppy <i>Poecilia reticulata</i>	590	262	91	63	42	50	0	13	0	0	14	0	4	0
Goldfish <i>Carassius auratus</i>	153	94	47	35	7	3	0	0	9	7	2	4	0	12
Platy <i>Xiphophorus maculatus</i>	143	35	15	7	11	0	5	0	0	0	2	3	0	0
Molly <i>Poecilia sphenops</i>	106	65	33	9	22	5	0	5	0	6	0	0	0	0
Barbs <i>Capeota</i> and <i>Puntius</i> spp.	95	36	11	7	12	1	7	3	0	0	0	0	0	0
Angel <i>Pterophyllum scalare</i>	92	71	36	2	26	1	5	0	0	0	0	0	3	0
Fighters <i>Betta splendens</i>	84	30	15	4	6	0	6	0	4	0	0	0	0	0
Tetras <i>Hyphessobrycon</i> sp.	75	28	12	8	10	0	0	0	0	0	0	0	0	0
Swordtail <i>Xiphophorus helleri</i>	66	11	5	2	2	0	0	0	0	0	2	0	0	0
Gourami <i>Colisa</i> sp.	64	28	9	10	3	0	0	0	4	2	0	0	0	0
Carp <i>Cyprinus carpio</i>	44	21	6	4	0	3	0	5	2	18	0	13	0	0
Other ( <i>Brachydanio</i> and <i>Astronotus</i> spp.)	08	0	0	0	0	0	0	0	0	0	0	0	0	0
Total fish	1520	689	280	151	141	63	30	26	19	33	20	20	7	12
Prevalence (%) of infected fish		45.3	18.4	9.9	9.3	4.1	2.0	1.7	1.3	2.2	1.3	1.3	0.5	0.8
Number of infected farms	26	23	23	21	13	9	4	3	5	10	5	3	4	3

The identification of *Dactylogyrus extensus* recovered from goldfish and carp was confirmed by its large size, with a body length varying from 1120 to 1280  $\mu\text{m}$  and width varying from 150 to 160  $\mu\text{m}$ . The hamuli were relatively long (75 to 85  $\mu\text{m}$ ) (Museum Accession No. PS-2002-6-1). Similar specimens recovered from angel fish were identified as *Dactylogyrus* cf. *extensus*. The identification of *Dactylogyrus vastator* recovered from platy and goldfish was confirmed by its relatively small body size with a length varying from 320 to 380  $\mu\text{m}$  and an approximate width of 80  $\mu\text{m}$ . The hamuli of these were relatively small (38 to 40  $\mu\text{m}$ ). (Museum Accession No. PS-2002-6-2). The specimens recovered from molly and guppy fish with similar morphology were identified as *Dactylogyrus* cf. *vastator*.

Specimens of *Gyrodactylus turnbulli* recovered from guppy and molly fish were identified by their small length, varying from 275 to 310  $\mu\text{m}$ , and the length of the hamuli (56 to 59  $\mu\text{m}$ ) (Museum Accession No. PS-2002-6-3).

Specimens of *Gyrodactylus katherineri* collected from goldfish were identified by their large body length, varying from 800 to 1100  $\mu\text{m}$ , and the length of the hamuli (80 to 108  $\mu\text{m}$ ). (Museum Accession No. PS-2002-6-4). Similar specimens recovered from angel and platy fish were identified as *Gyrodactylus* cf. *katherineri*.

Specimens of *Trichodina nigra* collected from guppy, barbs, platy and goldfish were identified by the dia-

meter of the adhesive disc (38 to 60  $\mu\text{m}$ ) with a dark center and denticulate ring (20 to 35  $\mu\text{m}$ ) (Museum Accession No. PS-2002-6-5).

The identification of *Tetrahymena corlissi* recovered from guppy fish was confirmed by its ovoid-shaped body (40  $\times$  60  $\mu\text{m}$ ) and the presence of caudal cilium (Museum Accession No. PS-2002-6-6), whereas the identification of *T. pyriformis* recovered from most of the fish was confirmed by its pyriform body (50  $\times$  30  $\mu\text{m}$ ), and the absence of caudal cilium (Museum Accession No. PS-2002-6-7).

Specimens of *Ichthyophthirius multifiliis* were identified by their oval to round body, with a diameter of 500 to 800  $\mu\text{m}$ , and a horseshoe shaped macro nucleus of the trophonts (Museum Accession No. PS-2002-6-8).

Specimens of free-swimming forms of *Ichthyobodo necator* were identified by their oval body (10  $\times$  5  $\mu\text{m}$ ), which contained 2 free unequal flagella ventrally, and a centrally located nucleus (Museum Accession No. PS-2002-6-9).

Specimens of *Lernaea cyprinacea* were identified by their length (6.5 to 6.8 mm, with a width of ca. 0.6 mm), and the length of their egg sacs (ranging from 1.4 to 1.5 mm, with a width of ca. 0.25 mm) (Museum Accession No. PS-2002-6-10).

The identification of *Ergasilus ceylonensis* was confirmed by its size (1.1 to 1.3 mm), the barrel-shaped genital segment and the characteristic random

Table 2. Parasites recovered from different body locations of ornamental fish

Parasite	Host fish species	Body location
<i>Dactylogyrus extensus</i>	Goldfish, carp	Gill lamellae
<i>Dactylogyrus</i> cf. <i>extensus</i>	Angel	
<i>Dactylogyrus vastator</i>	Goldfish, platy	Gill lamellae
<i>Dactylogyrus</i> cf. <i>vastator</i>	Guppy, molly	
<i>Dactylogyrus</i> spp.	Tetras, gourami, swordtail, molly, goldfish, barbs, fighters	Gill lamellae
<i>Gyrodactylus turnbulli</i>	Guppy, molly	Body surface, fins
<i>Gyrodactylus katherineri</i>	Goldfish	Body surface, fins
<i>Gyrodactylus</i> cf. <i>katherineri</i>	Angel, platy	
<i>Gyrodactylus</i> spp.	Tetras, gourami, swordtail, carp, goldfish, barbs, fighters	Body surface, fins
<i>Trichodina nigra</i>	Goldfish, guppy, barbs, platy	Body surface
<i>Trichodina</i> spp.	Angel, tetras, gourami, swordtail, molly, fighters	Body surface, gills
<i>Tetrahymena corlissi</i>	Guppy	Body surface, gills, muscles
<i>Tetrahymena pyriformis</i>	Guppy, goldfish, molly, carp, angel, barbs	Body surface, gills
<i>Ichthyophthirius multifiliis</i>	Angel, platy, barbs, fighters	Body surface
<i>Ichthyobodo necator</i>	Guppy, molly, barbs, carp	Body surface
<i>Piscinoodinium</i> spp.	Goldfish, fighters, Carp, Gurami	Body surface
<i>Lernaea cyprinacea</i>	Carp, gourami, molly, goldfish	Skin close to caudal fin
<i>Ergasilus ceylonensis</i>	Guppy, goldfish, platy, swordtail	Gills
<i>Argulus foliaceus</i>	Goldfish, platy, carp	Body surface
<i>Capillaria</i> spp.	Guppy, angel	Gut
<i>Centrocestus</i> spp.	Goldfish	Gills

arrangement of large numbers of eggs in the egg sacs (Museum Accession No. PS-2002-6-11).

Specimens of *Argulus foliaceus* were identified by the size of their cephalothorax (length 9 mm), with distinct thoracic areas, and posterior lobes that do not reach the base of the abdomen, which has distinct lobes separated by a clear cleft (Museum Accession No. PS-2002-6-12).

The overall prevalence of parasitism in the 1520 fish was 45.3% (95% CI of 42.8 to 47.9%). The genus *Dactylogyrus* (18.4% of fish infected) was the most common, and *Capillaria* was least common (0.4%).

#### Farm infection rates

Parasitized fish were found from 23 of the 26 fish farms visited. The variation in farm prevalence among parasites was significant ( $p < 0.01$ ). The majority of farms (23/26) had fish infected with dactylogyrids; gyrodactylid infected farms were also common (21/26). The lowest farm prevalence (3/26) was recorded for *Ichthyobodo necator*, *Argulus foliaceus* and *Cetrocestus* spp. (Table 1).

#### Fish infection rates

For those parasite genera for which there were sufficient data, infection rates varied significantly with fish species ( $p < 0.01$ ). Fifty out of 590 guppies were infected with *Tetrahymena* spp., compared with

13/930 for all other species ( $p < 0.01$ ). Similarly 13/44 and 18/44 carp were infected with *Argulus foliaceus* and *Lernaea cyprinacea*, compared with 7/1476 and 15/1476, respectively, for all other fish species combined ( $p < 0.01$ ). Dactylogyrids, gyrodactylids and trichodinids were present in 11/12, 11/12 and 10/12 of the sampled fish species, respectively, suggesting that they were not specific for any particular ornamental fish species.

#### DISCUSSION

With Sri Lanka being a tropical country, one would expect a wider spectrum of parasites in ornamental fish than that found in this study. Although complete parasitological examinations were made for all collected fish, the failure to find certain groups of parasites may be due to a number of reasons. Firstly, the majority of farms had an independent water supply for each pond or tank to prevent cross contamination, and ponds and tanks were drained and cleaned after each production cycle. Secondly, the ponds and tanks in the study farms were covered with wire mesh to prevent the entry of birds and other animals which can act as definitive hosts for digenean trematodes. Finally, the fish were collected from export farms where there is regular use of antiparasitic compounds such as formalin, malachite green, acriflavine and methylene blue. Nevertheless, the overall prevalence of parasitized fish in these farms was high (45.3%), although most infections were mono-specific (37.9% of fish). However, the

presence of detectable parasites at any level is significant since the study fish came from populations destined for export, and any rejections at quarantine certification may negatively influence the generation of foreign exchange.

The dactylogyrids (*Dactylogyrus* cf. *extensus*) isolated from angel fish were morphologically similar to *D. extensus* recovered from carp and goldfish, while the dactylogyrids (*Dactylogyrus* cf. *vastator*) recovered from guppy and molly fish were morphologically similar to *D. vastator*. The gyrodactylids (*Gyrodactylus* cf. *katherineri*) recovered from angel and platy fish were morphologically similar to *G. katherineri* recovered from goldfish.

The monogenean trematodes (*Dactylogyrus extensus*, *Dactylogyrus* cf. *extensus*, *Dactylogyrus vastator*, *Dactylogyrus* cf. *vastator*, *Dactylogyrus* spp., *Gyrodactylus turnbulli*, *G. katherineri*, *Gyrodactylus* cf. *katherineri*, *Gyrodactylus* spp.) were found in 23 out of 26 study farms. They also had the highest fish infection rate, suggesting that they were the most common parasites in ornamental fish species prepared for export from Sri Lanka. The high prevalence of these infections could be explained, firstly, by the fact that monogenean trematodes have high reproductive rates, and effective transmission is increased under poor management conditions (Soulsby 1982, Cone 1995). Secondly, viviparous *Gyrodactylus* and oviparous *Dactylogyrus* have very short direct life cycles that require no intermediate host, which allows them to quickly multiply to dangerous levels under the management conditions prevailing in ornamental fish farms (Citino 1996).

Apart from monogenean trematodes, protozoan parasites, particularly *Trichodina nigra* and *Trichodina* spp., were commonly encountered in this study. These obligatory ectoparasites can survive without fish for hours, perhaps days, and can temporarily be supported by several species of hosts other than fish (Lom 1995). This may explain their widespread occurrence and comparatively higher prevalence.

Most of the farmers involved in this study were familiar with the risk of mortality from *Tetrahymena* infection. In Sri Lanka, when lesions appear on fish in a tank or pond, control by destroying the fish in the affected tank or pond is a common practice. This may explain the observed low prevalence (4.1%) of *T. corlissi* and *T. pyriformis* infection in the present study, which is much lower than expected.

Parasitic copepods *Lernaea cyprinacea*, *Ergasilus ceylonensis* and *Argulus foliaceus* were identified in the present study and all 3 species have been recorded previously from freshwater fish (Fernando & Hanek 1973, Fernando 1990). Prevalence of these parasites was also low (2.2, 1.3 and 1.3% respectively). The length of the life cycle of copepod parasites may be

extended up to 3 mo depending on the temperature (Soulsby 1982). Most of the common export fish species are grown for only 2 to 3 mo before being marketed. Hence, there is less opportunity for such fish to be exposed to parasitic copepod infestation if the ponds and tanks are drained and cleaned properly after each production cycle. As the copepods, particularly *A. foliaceus* and *L. cyprinacea*, are visible to the naked eye, farmers may also implement early control measures, which may be another reason for the observed low prevalence.

The life cycle of *Centrocestus* spp. requires an intermediate snail host; therefore, with good management, infestations can be controlled (Citino 1996). This could explain the very low observed prevalence of the metacercarial stage of *Centrocestus* spp., and the absence of other metacercarial stages during the study.

The lowest fish infection rates were found for nematodes. This was expected, as nematodes are a lesser problem in fish when compared with terrestrial vertebrates (Anderson 1996).

Twenty-three of 26 farms were infected with one or more parasites. Parasitism in fish occurs as a result of interactions among the parasite, the fish and the environment (Wildgoose 1998). Fish in intensive culture are continuously affected by environmental fluctuations and management practices such as handling, crowding, transport, drug treatments, undernourishment, fluctuating temperatures, and poor water quality (Subasinghe 1997, Wildgoose 1998). A slight change in management, which is common under any standard of management, results in considerable stress on the homeostatic mechanisms of fish, rendering them susceptible to infection with a wide variety of parasites (Ling et al. 1996, Scholz 1999). However, the proportion of parasitized fish found in this study varied among farms. This may be a true difference or may be explained by the variation in the number of fish that were selected from each farm (16 to 195), resulting in different chances of detection.

Fifty out of 590 guppies were infected with *Tetrahymena*, compared with 13/930 for all other fish species combined, which is a statistically significant result ( $p < 0.01$ ) and indicates that this parasite is more common in guppies. This observation may also be explained by the lack of disease resistance in guppy varieties due to development of newer varieties for improved appearance and color, with little emphasis on other factors. Breeding for new varieties is commonly practiced in guppies, which constitute the major share of Sri Lanka's export market. Except for *Ergasilus ceylonensis*, other copepods were observed mainly on carp and goldfish. Unlike other commonly exported fish species, these fish need a growing period of 4 to 12 mo before being marketed, which therefore

leads to a higher possibility of infection with copepods, which have a relatively long life cycle.

In conclusion, of the 12 genera (21 species) of parasites recovered, *Gyrodactylus*, *Dactylogyrus*, and *Trichodina* were the most common, and the occurrence of parasites in ornamental fish export farms was widespread. Parasite genera such as *Tetrahymena* were more prevalent in guppies, whereas *Argulus* and *Lernaea* were more common in carp, suggesting that they were specific for such fish species.

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