Sri Lanka J. Aquat. Sci. **10** (2005): 85-96

Evaluation of the effects of infestation by trematodes and *Lernaea* on *Catla catla*, an Indian carp cultured in Sri Lanka

N.D. WIMALAWICKRAMA AND A. PATHIRATNE*

Department of Zoology, University of Kelaniya, Kelaniya, Sri Lanka

*Corresponding author (E-mail: asoka@kln.ac.lk)

Abstract

Juvenile stages of *Catla catla* collected from Udawalawa Fish Breeding Station of the National Aquaculture Development Authority of Sri Lanka were found to be infested mainly with *Gyrodactylus* sp., digenean metacercarial cysts and *Lernaea cyprinacea*. In the present study, the intensities of parasite infections, preference sites of the parasites on fish, oxygen consumption rate and histological structure of the infested sites of the host fish were examined in comparison to the control fish to evaluate the effects of parasitic infestation on the host fish. *Gyrodactylus* sp. and digenean metacercarial cysts were recovered from the gills, whereas adult females of *L. cyprinacea* were found attached to the skin and fins of the fish. The intensities of *Gyrodactylus* sp. and the metacercarial cysts were 1-2368 individuals and 1-80 cysts per infected fish respectively. The intensity of *L. cyprinacea* ranged from 1 to 5 individuals per infected fish. *Gyrodactylus* sp. and metacercarial cysts had no specific preference sites on the gills whereas caudal peduncle region of the fish was preferred by *L. cyprinacea*. Histopathological study revealed that infection of fish with *Gyrodactylus*, and digenean metacercarial cysts had induced moderate hyperplasia of the infected gill lamellae and deformation of part of some gill filaments of the host fish. Attachment of *L. cyprinacea* had triggered inflammatory tissue reaction at the infested site of the fish. In spite of these histopathological changes induced by the parasites on the infected sites, the oxygen consumption rates of the fish were not affected significantly by the presence of parasites at the current infestation level.

Introduction

An Indian carp, *Catla catla* is a freshwater fish species cultured in several Asian countries mainly as a human food source (Pillay 1990; Mohan and Bhatta 2002). It is one of the exotic fish species currently used in extensive culture in inland reservoirs of Sri Lanka. As Indian carps are not

bred naturally in Sri Lankan conditions, these fish are maintained in freshwater fish breeding stations of the National Aquaculture Development Authority (NAQDA) of Sri Lanka for continuous seed production for various culture systems. However, ectoparasitic infections of *C. catla* especially with trematodes and copepods have become a major problem in the fish breeding stations (Balasuriya 1987; Subasinghe 1992; OIC of the Udawalawe Fisheries Station, Pers. Comm.). The present study was carried out to assess the intensity of infestation of the trematode and copepod parasites, preference sites of these parasites on *C. catla*, the effects of infestation of the parasites on the histological structure of the infested sites and the oxygen consumption rate of the host fish.

Materials and Methods

Fish

Samples of *C. catla* (1.1–38.2 g in body weight) were obtained from Udawalawa Fish Breeding Station, NAQDA. The fish were brought to the laboratory in polythene bags with oxygen-saturated water. They were kept in separate aquaria filled with water obtained from their previous habitat for about two days and were used for respirometry, parasitological survey and histopathological studies.

Determination of oxygen consumption rates

Prior to sacrificing the fish for parasitological survey, oxygen consumption rates of the fish were measured using static respirometers as described by Cech (1990). Oxygen concentrations in water of the reference and experimental respirometers were determined by the Winkler method (Taras et al. 1971).

Parasitological survey

Fish were killed by pithing and parasitological survey was conducted following the procedure described by Kabata (1985). The skin, fins and buccal cavity were examined for ectoparasites and the attachment of parasites on different areas of the body surface and fins was recorded. The operculum was cut and all the gills were removed and examined under the light microscope for parasites. The distribution of parasites on upper and lower surfaces of each gill was recorded. The parasites were identified to the genus/species level using their morphological characters (Kabata 1985; Paperna 1996). The fish free of parasites were considered as control fish.

Histology

Gill, skin and muscle tissues of the fish infested with the parasites were preserved in 10% neutral buffered formalin and embedded in paraffin wax following standard procedures (Bucke 1989). Sections of the gill and

liver tissues were cut at 5-7 µm thickness and stained with haematoxylin and eosin. Gill, skin and muscle tissues of the fish free of parasites were also processed concurrently for comparison of histological structures.

Data analysis

According to the presence or absence of trematodes and *L. cyprinacea*, the fish were divided into four groups: control (apparently healthy and no parasitic infestations), Fish infected only with trematodes, fish infected only with *L. cyprinacea*, fish infected only with *L. cyprinacea* and trematodes. Fish infested with other taxonomic groups of parasites (protozoans) were eliminated from the analysis. The intensities of the parasitic infestation and preference sites of the parasites were analysed statistically using Kruskal-Wallis test followed by Nemenyi test where ever necessary (Zar 1999).

The experimental data on oxygen consumption rates were processed statistically. The least squares method was used to calculate the power equation for the control and infected fish in relation to oxygen consumption rates and body weights. The power equation was expressed as $Q = aW^k$, where Q is the oxygen consumption rate of individual fish (mg of oxygen $hour^{-1}$); W is the body weight of the fish in grams; a is a coefficient equal to metabolism at W=1; and k is a coefficient indicating the rate of change in metabolism with increasing body weight. The log transformed respiratory rate equation was expressed as $\log Q = \log a + k \log W$ (Withers, 1992). The regression analysis between log Q and log W was carried out and the slopes of the regression lines were compared using Student's t-test (Zar 1999).

Results

Prevalence of the parasites

The *Gyrodactylus* sp. recovered from the gills of the fish ranged from 0.5 to 1mm in length. The size of the metacercarial cysts seen in the gills of the fish was around 0.15 mm along the long axis and around 0.12 mm along the short axis. By the dimensions of the cysts, they were presumptively identified as *Centrocestus* metacercarial cysts. *L. cyprinacea* recovered from the fish were adult females and they had attached to various locations on the surface of the fish.

Of the total of 65 *C. catla* examined, 12 fish (19%) were infected only with trematodes (*Gyrodactylus* sp. and metacercarial cysts) and 10 fish (15%) were infected only with *L. cyprinacea*. A mixed infection of both *L. cyprinacea* and trematodes was found in 13 fish (20%) and a mixed infection of *L. cyprinacea*/trematodes and protozoan parasites (*Trichodina* sp. and *Piscinoodinium* sp.) was found in 10 fish (15%). In addition, 31% of the total number of fish were free of parasites (apparently healthy or controls). The fish infected with protozoan parasites were not used in the analyses as the

present study focused mainly on the effects of trematode and copepod parasites.

Intensity of infestation of the parasites

The intensity of infestation of the parasites on three groups of *C. catla* is presented in Table 1. In the fish which were infected only with trematodes, the intensities of *Gyrodactylus* sp. and metacercarial cysts infections ranged from 1-134 individuals and 2-10 cysts respectively per infected fish. The intensity of infection in fish which were infected only with *L. cyprinacea* was 1-3 individuals per infected fish. In the fish which were infected only with trematodes and *L. cyprinacea*, intensities of infection were 1-2,368 individuals of *Gyrodactylus* sp., 1-80 metacercarial cysts and 1-5 individuals of *L. cyprinacea* per fish.

Table 1. Intensity of infection by trematodes and *L. cyprinacea* on *Catla catla*

Fish	Intensity of infection*		
	Gyrodactylus sp.	Metacercarial cysts	*L. cyprinacea*
Apparently healthy fish (n = 20)	-	-	-
Fish infected with only trematodes (n = 12)	22.2±11.8 (1-134)	4.8±0.6 (2-10)	-
Fish infected with only *L. cyprinacea* (n = 10)	-	-	2.0±0.3 (1-3)
Fish infected with only trematodes and *L. cyprinacea* (n = 13)	45.2±237.8 (1-2368)	9.1±6.1 (1-80)	3.1±0.3 (1-5)

*Data are presented as mean ± SEM and the ranges, n = number of fish examined.

The distribution of trematodes on the four gills is presented in Table 2. Majority of the *Gyrodactylus* sp. was found attached to the 3rd gill (33%). The lowest number (13%) of *Gyrodactylus* sp. was recorded in the 1st gill. The major sites of attachment of metacercarial cysts on the fish were 3rd and 4th gills representing 31% and 30% of the total cyst attachments respectively. However, no significant difference was found with respect to the preference sites of attachment of *Gyrodactylus* sp. or metacercarial cysts.

Total attachment of *L. cyprinacea* at various locations on fish is presented in Table 3. Of the total of 60 adult females of *L. cyprinacea* recovered in the fish examined, nearly 72% of the individuals were found attached to the skin and 28% of the parasites were attached to the fins.

Distribution of adult *L. cyprinacea* on different parts of the body of the fish was significantly different. Further analysis revealed that the attachment of *L. cyprinacea* at the caudal peduncle was significantly higher than that of pelvic and anal fins.

Table 2 Distribution of trematodes on the gills of *Catla catla*.

Location	Intensity of infection*	
	Gyrodactylus sp.	Metacercarial cysts
1st gill	32.1 ± 15.8 (1-353)	1.4 ± 0.1 (1-31)
2nd gill	48.5 ± 28.6 (1-804)	1.2 ± 0.6 (1-16)
3rd gill	81.6 ± 42.8 (1-866)	2.2 ± 0.6 (1-13)
4th gill	66.5 ± 37.4 (1-761)	2.1 ± 0.8 (1-20)

*Data are presented as mean ± SEM and the range. Number of fish examined = 25, Intensities of infection among the four gill arches were not significantly different from each other (Kruskal-Wallis test, $P > 0.05$).

Table 3. Distribution of adult female *L. cyprinacea* on *Catla catla*

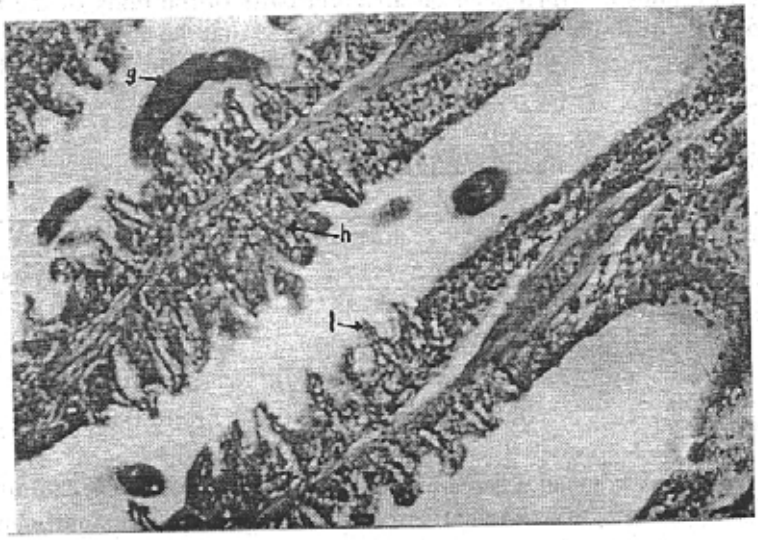
Location	Number (percentage)*
Head	12 (20%)
Dorsal skin	5 (8%)
Ventral skin	10 (17%)
Caudal peduncle	16 (27%)
Caudal fin	4 (7%)
Dorsal fin	7 (12%)
Pelvic fin	3 (5%)
Anal fin	3 (5%)

*Number of fish examined = 23. Number of copepods attached to the caudal peduncle was significantly different from the numbers attached to the pelvic fin or anal fin (Kruskal–Wallis test, Nemenyi test, $P < 0.05$).

Histological changes induced in fish by parasitic infections

Some gill filaments of *C. catla* were affected by the presence of both *Gyrodactylus* sp. and metacercarial cysts (Plate 1). The gills of the fish which harbored a low intensity of trematodes (1-134 individuals per fish) had minor pathological changes or no tissue response. Areas of lamellar hyperplasia, and necrosis at the attachment site were observed in the gill filaments of several fish infected with *Gyrodactylus* sp. at higher infestation levels (1,370-2,368 individuals per fish). Metacercarial cysts have developed in the cartilaginous tissue of the gill filaments or between the cartilage and

(a)



(b)

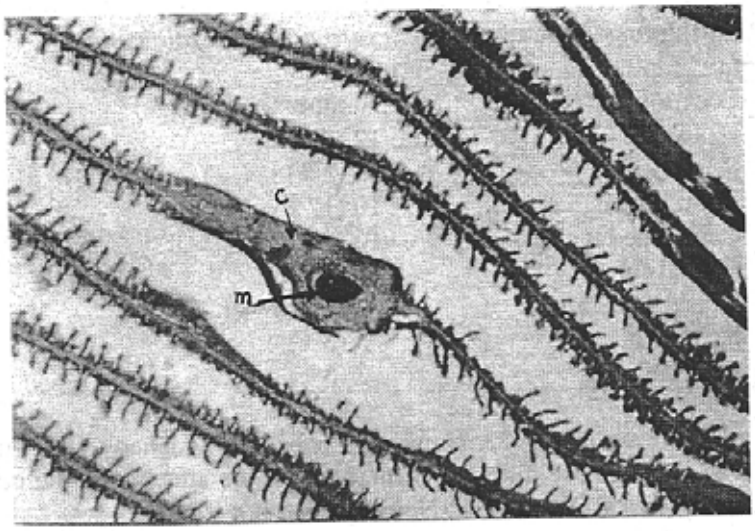


Plate 1. (a) Gill filaments of *Catla catla* infected with *Gyrodactylus* (X 200) (l - gill lamellae, g – *Gyrodactylus*, h - hyperplasia) (b) Gill filaments of *Catla catla* infected with metacercarial cysts showing deformation of part of the gill filament (X 100) (c - cartilage, m - metacercarial cyst).

epidermis. Some gill filaments were found to contain 2-3 cysts. Due to the growth of metacercaria and formation of its cyst wall, the cartilage and the blood vessels were pushed and strained leading to deformation of the part of the gill filament structure. Skin and muscle tissues of the fish infected with

L. cyprinacea showed barring effects of penetration and inflammatory tissue reactions (Plate 2). Penetrating females of *L. cyprinacea* caused disruption of the epidermis and dermis, tissue necrosis and hemorrhages.

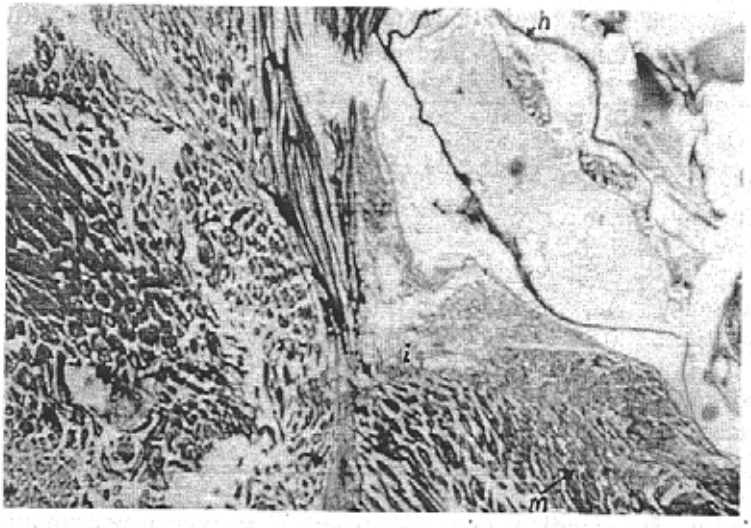


Plate 2. Damaged skin area in *Catla catla* due to attachment of *Lernaea cyprinacea* (X 400) (h - holdfast organ of the parasite, i - inflamed area due to the attachment, m - muscle tissue of the fish).

Effect of parasitic infestations on oxygen consumption

The correlation coefficients (r) between log oxygen consumption rates (Q) and log values of body weights (W) of the control fish and three groups of parasite infected fish were considerably high ($r = 0.733$-0.916) and statistically significant ($p<0.05$). Parameters of respiratory rate equation of controls and three groups of infected fish are presented in Table 4. The absolute value of the coefficient k for the control fish was 0.396. The values for the fish infected with trematodes alone and trematodes and *L. cyprinacea* alone were not significantly different from the value obtained for the control fish.

Discussion

In the present study, *C. catla* which were collected from Udawalawe Fish Breeding Station was found to be infected with *Gyrodactylus* sp., digenean metacercarial cysts and *L. cyprinacea*. Generally most *Gyrodactylus* species parasitize primarily on the skin and fins, but several species which parasitize only the gills of fish have been recorded (Kabata 1985; Paperna 1996). All the *Gyrodactylus* sp. recovered from the fish in this study were found attached to the gills of the fish. *Gyrodactylus* sp. have also been

recovered from the gills of the fingerlings of two other Indian carps, namely rohu and mrigal collected from Dambulla Fish Breeding Station (Balasuriya 1987).

Table 4. Parameters of respiration rate equations of apparently healthy *Catla catla* and the fish infected with parasites.

Fish	a ($mg\ hr^{-1}\ g^{-1}$)	k	r	Equation
Apparently healthy (control)	1.200	0.396	0.916	$Q = 1.200W^{0.396}$
Fish infected with				
Trematodes only	1.435	0.311**	0.806	$Q = 1.435W^{0.311}$
L. cyprinacea only	0.346	0.919	0.878	$Q = 0.346W^{0.919}$
Trematodes and *L. cyprinacea* only	1.423	0.313**	0.734	$Q = 1.423W^{0.313}$

*Respiration rate equation, $Q = aW^k$ where Q = oxygen consumption rate (mg of Oxygen $hour^{-1}$ $fish^{-1}$), W = body weight of fish (g), a = coefficient equal to metabolism at W = 1 g and k = coefficient indicating the rate of change in metabolism with increasing body weight, r = correlation coefficient.
**Significantly not different from the control (Student's t-test, $P > 0.05$).

The matacercaria of *Centrocestus* sp. have been identified as the most prevalent parasite of carp fry in Sri Lanka in addition to the *Trichodina* sp. (Subasinghe 1992). There are several records on effects of *Centrocestus* sp. on the health status of several species of freshwater fish cultured in Sri Lanka (Balasuriya 1988; Sajeevanee and Hettiarachchi 1998; Hettiarachchi 2002). The dimensions of the cysts recorded in the present study (around 0.15 mm along the long axis and around 0.12 mm along the short axis) were in the range of the size of *Centrocestus* metacercarial cysts recorded by Balasuriya (1988) and Sajeevanee and Hettiarachchi (1998). Therefore, the metacercarial cysts present in the gills of *C. catla* examined in the present study were presumptively identified as metacercarial cysts of *Centrocestus* sp. It was found that the first gill harbored fewer individuals of *Gyrodactylus* sp. and *Centrocestus* cysts. The third gill harbored the highest number. This may be due to the provision of a stable and safer attachment area by the third gill as it is far away from the respiratory water currents compared with the first gill. However, no statistical difference was found with respect to the preferred site of attachment of trematodes.

Learnea species parasitized on *C. catla* examined in the present study was identified as *L. cyprinacea*. Copepodids of *L. cyprinacea* are generally found attached to gills of the fishes, especially cyprinids (Kabata 1985; Paperna 1996). However, copepodid stages of *L. cyprinacea* were not found on the gills of *C. catla* examined in the present study. The results show that the major sites of adult *L. cyprinacea* attachment on *C. catla* are the caudal peduncle, head and ventral skin representing 26.7%, 20% and 16.6% of the total parasitic attachments respectively. The lowest percentage was recorded in pelvic fins and anal fins. In view of the much smaller total surface area of the fins in comparison to the skin, the results suggest that the skin regions may be preferred by adult *L. cyprinacea* to maintain their larger bodies in a stable position.

Prominent histopathological alterations have been recorded in the gills of sparid infected with monogeneans and copepods (Roubal 1986) and in the gills of rainbow trout infected with copepods (Sutherland and Wittrock 1985). In the present study, histopathology of the gills of trematode infested *C. catla* indicates the host tissue responses ranging from no reaction to moderate hyperplasia depending on the intensity of infestation. Some gill lamellae of the fish, which harbored relatively high numbers of *Gyrodactylus* sp., exhibited areas of necrosis at the attachment sites in addition to lamellar hyperplasia. *Gyrodactylus* sp. feeds on the epithelial cells and it also engages in active crawling using the adhesive process at the anterior tip of the body and the posterior adhesive sucker (Kabata 1985; Post 1987). The observed pathological changes in the gill tissue of *C. catla* could be attributed to the reaction of the host response to the feeding and crawling process of the parasite. It was observed that *Centrocestus* cysts were in close association with blood vessels and the cartilage of the gill filaments of *C. catla* making part of the gill filament distorted. Similar observations in the histological structure of the gills of gold fish infected with metacercarial cysts have been made earlier by Sajeevanee and Hettiarachchi (1998) and Hettiarachchi (2002). *Lernaea* species injures its host fish as the result of attachment, producing disruption of the host's attached site. Most serious consequence of intensive attack results if the cephalic horn of *Lernaea* sp. is embedded in the vital organs such as brain, liver and heart (Post 1987). In the present study, histological sections of infected skin showed lesions around the holdfast and ulcers at the surface. Penetrating females of *L. cyprinacea* caused disruption of the epidermis and dermis, necrosis and hemorrhages. Inflamed muscle tissues were also observed in the attachment area.

In the present study, weight specific oxygen consumption rates of *C. catla* infected with parasites were not significantly different from that of the control fish. Similar situation has been reported earlier by Pathiratne (1992) for the Asian cichlid *Etroplus suratensis* infected with copepod *Ergasilus ceylonensis*. In the present study, intensity of infestation of *C. catla* by parasites were 1 - 2,368 individuals of *Gyrodactylus* sp., 1 - 80 metacercarial

cysts and 1 - 5 individuals of *L. cyprinacea* per fish. Even though some gill filaments could not be properly functional due to histopathological changes induced by the attachment of the parasites, the changes were not sufficient to cause a significant effect on the exchange of gases through the respiratory surfaces of *C. catla* as most of the remaining gill filaments were functional. However, these infestation levels may cause an additional stress to the fish especially if the water quality parameters in the culture facility are at sub-optimal levels. In conclusion, the results show that the respiratory functions of the host fish, *C. catla* were not affected by the presence of *Gyrodactylus* sp., metacercarial cysts of *Centrocestus* sp. and *L. cyprinacea* at the current infestation levels in spite of moderate changes in histological structure induced by the parasites on the infested sites.

Acknowledgements

Thanks are due to Mr. Upali Wanigasekara of the Department of Zoology, University of Kelaniya for the assistance with the histological preparations and photomicrographs.

References

Balasuriya, L.K.S.W. 1987.
Current fish disease problems in Sri Lanka. In: Fish Quarantine and Fish Diseases in South and South East Asia: 1986 update (J.R. Arthur ed.), pp. 36-40. Asian Fisheries Society Special Publication No.1, Manila. 86 p.

Balasuriya, L.K.S.W. 1988.
A study on the metacercarial cysts of a *Centrocestus* sp. (Digenia: Heterophylidae) occurring on the gills of cultured fishes in Sri Lanka. Journal of Inland Fisheries 4: 3-10.

Bucke, D. 1989.
Histology. In: Methods for the Microbial Examination of Fish and Shellfish. (B. Austin & D. A. Austin eds), pp. 69-97. Ellis Horwood Limited. West Sussex.

Cech, J.J.1990.
Respirometry. In: Methods for Fish Biology. (C.B. Schreck & P.B. Moyle eds), pp. 335-362. American Fisheries Society, Maryland.

Hettiarachchi, M. 2002
Effects of praziquantel on metacercarial cysts of *Centrocestus* sp. on the gills of gold fish *Carassius auratus*. In: Diseases in Asian Aquaculture IV (C. R. Lavilla-Pitogo & E. R. Cruz-Lacierda eds), pp. 285-292. Fish Health Section, Asian Fisheries Society, Manila/

Kabata, Z. 1985.
Parasites and Diseases of Fish Cultured in the Tropics. Taylor & Francis, London, 318p.

Mohan, C.V. & R. Bhatta 2002.
Social and economic impacts of aquatic animal health problems on aquaculture in India. In: Primary Aquatic Animal Health Care in Rural, Small Scale, Aquaculture Development. FAO Technical Paper No. 406. pp. 63-75. FAO, Rome.

Paperna, I. 1996.
Parasites, Infections and Diseases of Fishes in Africa, An update. CIFA Technical Paper 31, FAO, Rome. 220 p.

Pathiratne, A. 1992.
The effects of infection by the copepod *Ergasilus ceylonensis* on the oxygen consumption of the Asian cichlid *Etroplus suratensis*. In: Diseases in Asian Aquaculture I (M. Shariff, R.P. Subasinghe & J.R. Arthur eds), pp. 361-369. Fish Health Section, Asian Fisheries Society, Manila.

Pillay, T. V. R. 1990.
Aquaculture, Principles and Practices, Blackwell Science Ltd., London. 575 p.

Post, G. 1987.
Text book of Fish Health. TFH Publications Inc. New Jersey. 288 p.

Roubal, F. R. 1986.
Studies on monogeneans and copepods parasitizing the gills of a sparid, *Acanthopagarus australis* (Gunther) in Northern New South Wales. Canadian Journal of Zoology 64: 841-849.

Sajeewani, H. K. C. & M. Hettiarachchi 1998.
Prevalence, intensity and distribution of the metacercarial cysts of a *Centrocestus* sp. (Digenia: Heterophylidae) on the gills of goldfish, *Carassius auratus*. Proceedings of Fourth Annual Sessions of Sri Lanka Association of Fisheries and Aquatic Resources. 12 (Abstract only).

Subasinghe, R.P. 1992.
Hatchery diseases of fresh water fish in Sri Lanka. In: Diseases in Asian aquaculture I (M. Shariff, R.P. Subasinghe and J.R. Arthur eds), pp. 209-214. Fish Health Section, Asian Fisheries Society, Manila.

Sutherland, D. R. & D. D. Wittrock, 1985.
The effects of *Salminicola californiensis* (Copepoda: Lernaeopodidae) on the gills of farm-raised rainbow trout, *Salmo gairdneri*. Canadian Journal of Zoology 63: 2893-2901.

Taras, M.J., A.E. Greenberg, R. D. Hoak, & M. C. Rand, 1971.
Standard Methods for the Examination of Water and Waste Water. American Public Health Association, Washington D.C. 874 p

Withers, P. C. 1992.
Comparative Animal Physiology, Saunders College Publications, San Diego. 949p.
Zar, J. H 1999.
Biostatistical Analysis. Prentice Hall, New Jersey. 663 p.