

IMPORTANCE OF ECTOPARASITIC CILIATE INFESTATIONS IN THE
FARMING OF SNAKEHEAD FISHES (CHANNA SPP.)
(TELEOSTEI:CHANNIDAE) IN SRI LANKA

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ABSTRACT

Three snakehead species of Sri Lanka, namely, Channa striatus, C. punctatus and C. marulius are suitable for farming. The fry and fingerlings for snakehead farming are usually collected from the wild. Three ciliate ectoparasites, namely, Ichthyophthirius multifiliis, Chilodonella sp. and Trichodina sp. were found to infect snakehead fry and fingerlings collected from the wild in Sri Lanka causing high mortalities. Although the fry and fingerlings in the natural habitat did not show signs of infestations, the parasites developed rapidly under culture conditions. I. multifiliis, which was the most important, showed up in large numbers within five days of the capture of fry and led to 90-100% mortalities unless treated immediately. A continuous bath for 3-4 days in 0.1 ppm malachite green + 25 ppm formalin (10 ppm formaldehyde) removed the parasite completely without affecting the fry. Chilodonella sp. and Trichodina sp. were successfully treated with a three-day bath in either 2,500 ppm NaCl or 50-100 ppm formalin. Wild fingerlings of 50 mm usually did not develop epizootics under the same culture conditions, and even those fry that were infected and recovered showed some degree of resistance when they reached the 50 mm fingerling stage. Therefore, collection of fingerlings from the wild rather than fry is recommended (this will also shorten the culture period). On the other hand, if fry were collected, it is advisable to give them a 24-hour bath in malachite green and formalin to remove any low level of infection present in them.

INTRODUCTION

Snakeheads are very tasty and popular food fishes in most southern and south-eastern Asian countries. They occur mainly at low altitudes in shallow areas of lakes, irrigation reservoirs, swamps and streams. Five species of snakeheads occur in Sri Lanka, and other species occur in both tropical Asia and Africa. Only one species, channa orientalis is endemic to Sri Lanka. As in other Asian countries such as Cambodia, Hong Kong, Thailand and Taiwan, the economically most important snakehead species in Sri Lanka is C. striatus, although C. marulius (which is rare)

and C. punctatus (which is smaller) are also consumed. C. gachua and C. orientalis are very small (not more than 15 cm in length) and are not usually consumed, and are therefore not suitable for farming.

Snakehead farming is a relatively recent development, and even today, most of the snakehead fish supply comes from natural sources. Successful farming of snakeheads is carried out in south eastern Asian countries such as Thailand. As snakeheads are carnivorous, the production cost is high, and until cheap sources of feed are found their farming will remain rather limited. Most of the seed for farming is still collected from the wild, although there have been reports of successful artificial spawning of snakeheads (Ling, 1977).

Fry and fingerlings of snakeheads are easily subject to parasitic infestations under culture conditions, unless precautionary measures are taken. Although these infestations could cause very high mortalities, they are relatively easy to deal with. Three ectoparasitic ciliates are known to occur in snakeheads (Table 1). These are, in their order of importance for farming, Ichthyophthirius multifiliis, Chilodonella sp. and Trichodina sp.

The high percentage of mortalities observed in Thailand in fry and fingerlings of snakeheads soon after collection from the wild for culturing could mainly be due to stress and ciliate ectoparasites (Wee, 1983). Since snakeheads, particularly C. striatus, could provide a suitable alternative to Chinese carps, Indian carps, and tilapias, presently used in aquaculture in Sri Lanka, special attention must be paid to any cause of high mortalities of fry and fingerlings of snakeheads. The present study reports observations on infestations of I. multifiliis, Chilodonella sp. and Trichodina sp. in fry and fingerlings of snakeheads in laboratory culture.

These three ciliate parasites have also been reported from many other commercially important tropical as well as temperate fish species including carps, catfishes, eels, salmon, trout and ornamental fishes (Bauer, 1956; Paperna, 1972; Hines & Spira, 1973; Nigrelli, Pokorny & Ruggieri, 1976; Goven, Dawe & Gratzek, 1980). I. multifiliis has been reported in Sri Lanka in carps in Colombo (Mendis & Fernando, 1962) and Uda-Walawe (U.B. Edirisinghe, personal communication). However, Chilodonella sp. and Trichodina sp. do not seem to have hitherto been recorded from Sri Lanka. The paucity of information on these parasites in Sri Lanka is probably due to the scanty attention that has so far been paid to fish pathology in this country.

METHODS AND OBSERVATIONS

Fry and fingerlings of C. striatus and C. punctatus were routinely collected from the irrigation reservoirs in Anuradhapura, Kurunegala and Polonnaruwa districts from December to March following the rainy season. Fry of C. marulius were very difficult to find and only a few were collected. The first batch of about 50 was collected from the Mahakandarawa reservoir near Anuradhapura, and the second batch of only 10 was collected from a stream at Mahiyangana. The fry of the first batch died within a week of collection owing to I. multifiliis infection. From the second batch only two survived; however, the mortalities were not due to parasitic infestation. Despite a fairly intensive search for the fry of C. marulius in all three districts mentioned earlier, which were the most promising for the purpose because of the occurrence of the adults, for two years no other fry were located. The following observations were made only on the fry and fingerlings of C. striatus and C. punctatus.

Fry and fingerlings in their natural habitats appear to be quite healthy and free from the parasites. Routine examination of the fry under a dissection microscope, as well as the examination of smears of skin and gills did not reveal any parasites. However, the infestations often developed under culture conditions when fry were reared in either cement tanks or fibreglass tanks. Some batches from all three districts developed infestations. Paperna (1972), working on fish species other than snakeheads, was also unable to detect I. multifiliis infestations in the wild fish, but the infestations developed in several fish species under culture conditions.

In order to eliminate the possibility of the infestation coming through the water supply, chlorinated pipe-borne water, stored for about 24 hours, and tanks which were previously cleaned and dried, were used for fry rearing. Even with these precautions, some batches developed the infestations. It is unlikely that the infestations came with the tubicid oligochaetes with which the fry were fed, because only some batches developed the disease.

The fry that were starved for more than 24 hours during the processes of collection, transport and acclimation to culture conditions developed the diseases more easily than those that were not starved. The diseases also developed more easily in those fry that were kept in tanks in stationary water (even with aeration) than in those that were kept in tanks in which a substantial flow rate was maintained. These observations indicate that stress in fry was of prime importance in allowing the epizootics to develop.

Fingerlings (50-60 mm) collected from the wild seldom developed the infestations under similar culture conditions. However, if their cultures were contaminated by diseased fish, and when the water quality deteriorated through accumulation of left over food etc., the infestations appeared.

Ichthyophthirius multifiliis

Signs of I. multifiliis often appeared (to the naked eye) 2-3 days after collection, and mortalities started within 48 hours of the appearance of the infestation, and reached a peak in 5-6 days after the first appearance. After about 10 days of the first appearance of the disease, some fry recovered on their own, but often 90-100% mortalities occurred unless treatment was given.

The recommended treatments of malachite green and formalin (Sommerville, 1983; Wee, 1983) were tried individually in various concentrations and in mixtures of different proportions, varying the duration of treatment. The best results were obtained with a mixture of 0.1 ppm malachite green + 25 ppm formalin. More than 80% of the fry of highly infected batches recovered completely in a continuous bath of this mixture for 3-4 days. With moderately infected fry, the recovery rate was almost 100%. Aeration of the bath, and limiting the treatment to only several hours per day, as have been recommended (Sommerville, 1983; Wee, 1983), were found to be unnecessary. The high tolerance of the snakeheads to malachite-green and formalin mixture is probably due to their air breathing habit. The fry were given their usual feed of tubicid oligochaetes while under treatment. Most of the infection disappeared within two days in the bath, but three and four days were required respectively for the total disappearance of the parasite from moderately and highly infected fry. Infected fingerlings also showed a similar response to malachite green formalin mixture. The colour of the bath changes from green to colourless with time, and fish may be transferred to a fresh bath every 24 hours or so. 5-10 fry (15-30 mm) or 2-4 fingerlings (50-60 mm) were kept per one litre of the bath.

Some batches of C. striatus, recovered after treatment at fry stage, were reinfected due to contamination during the fingerling stage (50-60 mm). However, the mortalities of the untreated fingerlings were only about 25%, with 75% recovering on their own, provided the cultures were kept clean (in flowing water). The recovery rate of those that were treated with malachite-green formalin mixture was 100%. A few individuals of these were reinfected again when they were 125-150 mm (only 19 from a batch of 72 were thus reinfected). Malachite green and formalin treatment completely removed the infection from 17 individuals, but two heavily infected individuals did not feed, lost weight, and died during treatment. A post mortem examination of these revealed a very severe infection, with epidermis and gills covered with white spots and large amounts of mucus.

In order to assess the benefit of malachite green formalin treatment as a prophylactic measure, an experiment was carried out in the following manner. Five batches of fry were collected from a reservoir on the same day and brought to the laboratory and left overnight in separate tanks for acclimation. Next morning, 50 fry were randomly selected from each batch and each selected group was divided into two sub-groups of 25 each. From each group, one sub-group was given a bath of malachite green formalin for 24 hours, and the other sub-group was kept untreated as the control. Of the treated five sub-groups only one developed I. multifiliis infection, whereas four of the five control sub-groups (including the one corresponding to the sub-group that developed the infection after treatment) developed infection. Although these results are not conclusive, they do indicate that pre-treatment of the fry with malachite green formalin mixture is helpful in reducing the chance of I. multifiliis infection.

Chilodonella sp.

Chilodonella sp. infection appeared in the fry and fingerlings of both C. striatus and C. punctatus when the water quality deteriorated owing to decay of left over food etc. The infective species appeared to be C. cyprini. The infection was not observed in fish larger than 120 mm. It caused mortalities as high as 80% in both fry and fingerlings. The infection was successfully treated with a 3-day continuous bath of either 2,500 ppm NaCl or 50-100 ppm formalin. More than 70% of the infected fry and fingerlings were recovered by this treatment. Reinfection occurred at fingerling stage of the fish that recovered after treatment at fry stage. This second infection was successfully treated with NaCl or formalin with more than 90% recovery.

Trichodina sp.

Trichodina sp. was also observed in fry and fingerlings of C. striatus and C. punctatus. It was often seen in fry and fingerlings infected with Chilodonella sp., and about 50% of the fry infected with Chilodonella sp. also had Trichodina sp. It occurred in relatively small numbers in comparison with Chilodonella sp. In a random count of parasites in smear preparations only about 8% was Trichodina sp. and the rest Chilodonella sp. The parasite involved is probably T. mutabilis, but the occurrence of more than one species of Trichodina cannot be ruled out. Because of its presence together with Chilodonella sp., its effect on the host could not be assessed. However, in a single batch in which Trichodina sp. occurred alone, but in small numbers, no mortalities were observed. The treatment with NaCl or formalin, which was given for Chilodonella sp., was also effective in eliminating Trichodina sp. completely.

DISCUSSION

Ichthyophthirius multifiliis occurs in most, if not all, snakehead species (Table 1) causing very high mortalities in fry and fingerlings, as has also been reported by Wee (1983). Attempts have been made to control the parasite with chemotherapy, electrotherapy, UV therapy and immunotherapy. Initial studies with silver nitrate (Farley & Heckmann, 1980) and UV treatment (Gratzek, Gilbert, Lohr, Shotts & Brown, 1983) were promising, but electrotherapy was ineffective (Farley & Heckmann, 1980) in controlling the parasite. Although the usual treatment for I. multifiliis is a mixture of malachite green and formalin (Sommerville, 1983), Wee's (1983) recommendation of 25 ppm malachite green + 3,500 ppm (14 g in 4 l) formalin for snakeheads was found to be too strong and caused high mortalities in the present study. A continuous bath for 3-4 days, which was found to be effective in the present study, is much easier to administer, specially if large numbers of individuals are involved, than the two-hour bath every other day for one week recommended by Wee (1983) for snakeheads, or the three 6-hour baths every other day recommended for tilapia by Sommerville (1983). It is also much easier and shorter than the 3-week continuous bath in 3 ppm methylene blue, with regular changes of fresh solutions recommended by Wee (1983) for snakeheads. Furthermore, in Wee's study, even a 15 ppm methylene blue solution failed to control the parasite in C. maculatus in one instance. In the present study, 5 ppm methylene blue solution was found to be quite effective (with about 75% recovery) when the infection was moderate, but was found to be less effective (with only about 40% recovery) when the infection was heavy. Chemical treatments recommended by various authors to control I. multifiliis are summarised in Table 2.

The development of the disease in some hatches of fry, a few days after collection, is probably due to the presence of a few parasites in at least some fry, undetected by the screening procedure. These would have developed into an epizootic because of the stress induced in fry as a result of capture, transportation, new conditions in culture etc. Development of epizootics in only some batches but not in others, which were collected on the same day from the same reservoir (but from different locations) and kept in culture under similar conditions, tends to support the view that a low level of infection was present in some batches prior to capture.

The apparent absence of infection in the wild fry is probably because the chances of survival of such infected fry is minimal under the conditions prevailing in the natural habitat. For instance, if a heavy infection develops in the wild it will probably cause 100% mortalities in the nest of fry, as fry are gregarious and tend to remain close together favouring transfer of parasite from one individual to another. Furthermore, even

before the parasite could cause mortalities, the fry may easily become prey to various predators because the diseased fry are sluggish and fail to move in a co-ordinated manner to avoid predators.

The observed higher resistance of the wild fingerlings of 50-60 mm (in comparison to that of fry) to I. multifiliis infection may be due to three factors. 1. They may have been exposed to the parasite during early stages and developed some degree of immunity. However, this is rather unlikely as the chances of survival of diseased fry in the wild is minimal. 2. As the fish grows it may become more resistant to diseases in general, and may recover more easily even if a disease does occur. 3. As the fish grows it may not become as easily stressed as the earlier stages during capture, transport, acclimation to new conditions etc., thus giving less chance for the epizootics to develop. The data in the present study are not sufficient to determine which one of the latter two factors is important. However, it may well be that the two factors are related and operate together.

Reinfection by I. multifiliis, observed in the present study, has also been reported by Paperna (1972) and others. It is also known that some degree of immunity may develop as a result of previous exposure to the parasite and that the degree of immunity to subsequent infections depends on the initial infective dose (Hines & Spira, 1973; Goven, Dave & Gratzek, 1980). Failure of the subsequent infections to establish in some individuals and the much lower mortality rates among those that developed subsequent infections, observed in the present study, may indicate the development of some degree of immunity to I. multifiliis in snakeheads through initial exposure. On the other hand, it may also be that the fish as it grows becomes more resistant to parasitic attacks and becomes better adapted to recover on its own from such attacks, if they do occur. Recent studies have shown that even ciliary preparations of I. multifiliis as well as those of the closely related Tetrahymena pyriformis could confer a high degree of immunity to subsequent infections by I. multifiliis (Goven, Dawe & Gratzek, 1981).

The possibility of the occurrence of several physiological races of I. multifiliis and even of several species of Ichthyophthirius has been discussed by Nigrelli, Pokorny & Ruggieri (1976). One of the main lines of evidence for the suggestion is the occurrence of I. multifiliis in fishes with different ranges of temperature tolerance and the observation that the parasite in different geographical areas shows different optimal temperature ranges for reproduction and development. For instance, the optimal temperature ranges of I. multifiliis in Japan, Uganda and the Soviet Union are 14-17.5°C (Suzuki, 1930), 25-27°C (Paperna, 1972) and 25-27°C (Bauer, 1956) respectively. I. multifiliis in Sri Lanka appears to have an optimal temperature range of 25-27°C. Wee (1983) noted 100% mortalities due to I. multifiliis at 28 + 1°C in snakeheads from Hong Kong and Thailand.

Chilodonella cyprini, that causes high mortalities in carp in the Soviet Union, has an optimal temperature range of 9-10°C and its fission stops completely and the parasite starts dying at 20°C (Bauer, 1956). If the species occurring in Sri Lanka is also the same, as appears to be the case, then the high degree of infection observed at 24°C (room temperature at which the cultures were kept) in Sri Lanka raises the possibility of different physiological races in C. cyprini also.

van As, Basson & Theron (1984) showed that there is some host specificity at least among some species of Trichodina. For instance, T. mutabilis, which infects the common carp Cyprinus carpio, does not appear to infect the tilapia Oreochromis mossambicus, and T. minuta, which infects O. mossambicus, does not appear to infect C. carpio. Both O. mossambicus and C. carpio are exotic species in Sri Lanka, and while the former species is very wide spread the latter species also occurs in many water bodies. If the species of Trichodina that infects Channa spp. is T. mutabilis, as appears to be the case, then the possibility that it was introduced to Sri Lanka with C. carpio cannot be ruled out. van As, Basson & Theron (1984) are also of the opinion that the distribution of T. mutabilis is linked to the distribution of C. carpio. Because of the wide occurrence of O. mossambicus in Sri Lanka, it is interesting to see whether snakeheads are also infected with T. minuta (and also with other species of Trichodina).

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REFERENCES

- Bauer, O.N. (1956). Parasitic diseases of cultured fishes and methods of their prevention and treatment. In: Parasitology of Fishes. eds. Dogiel, V.A., Petrushevski, G.K. & Polyanski, Y.I. Translated to English by Kabata, Z. pp 265-298. Oliver & Boyd, Edinburgh. 1961.
- Farley, D.G. & Heckmann, R. (1980). Attempts to control Ichthyophthirius multifiliis Fouquet (Ciliophora: Ophryoglenidae) by chemotherapy and electrotherapy. J. Fish. Dis., 3:203-212.

- Goven, B.A., Dawe, D.L. & Gratzek, J.B. (1980). Protection of channel catfish, Ictalurus punctatus Rafinesque, against Ichthyophthirius multifiliis Fouquet by immunization. J. Fish Biol. 17:311-316.
- Goven, B.A., Dawe, D.L. & Gratzek, J.B. (1981). Protection of channel catfish (Ictalurus punctatus) against Ichthyophthirius multifiliis (Fourquet) by immunization with varying doses of Tetrahymena pyriformis (Lwoff) cilia. Aquaculture, 23:269-273.
- Gratzek, J.B., Gilbert, J.P., Lohr, A.L., Shotts, F.B. & Brown, J. (1983). Ultraviolet light control of Ichthyophthirius multifiliis Fouquet in a closed fish culture recirculation system. J. Fish. Dis. 6:145-153.
- Hines, R.S. & Spira, D.T. (1973). Acquired immunity of the Mirror Carp (Cyprinus carpio L.) to Ichthyophthiriasis. Refuah. Vet. 30:17-19.
- Ling, S-W. (1977). Aquaculture in Southeast Asia- A Historical Overview. Univ. Washington Press, Seattle, 107 pp.
- Mendis, A.S. & Fernando, C.H. (1962). A guide to freshwater fauna of Ceylon. Bulletin No.12, Fisheries Research Station, Dept. of Fisheries, ceylon, 21 p.
- Nigrelli, R.F., Pokorny, K.S. & Ruggieri, G.D. (1976). Notes on Ichthyophthirius multifiliis, a ciliate parasitic on freshwater fishes, with some remarks on possible physiological races and species. Trans. Amer. Micro. Soc. 95:607-613.
- Paperna, I. (1972). Infection by Ichthyophthirius multifiliis of fishes in Uganda, Prog. Fish. Cult. 34:162-164.
- Sommerville, C. (1983). Control and treatment of parasitic diseases. In: Notes to the workshop on hatchery techniques for freshwater fish culture in S.E. Asia. December 4-17, Colombo, 8 pp (mimeographed).
- van As, J.G., Basson, L. & Theron, J. (1984). An experimental evaluation of the use of formalin to control trichodiniasis and other ectoparasitic protozoans on fry of Cyprinus carpio L. and Oreochromis mossambicus (Peters). S. Afr. J. Wildl. Res. 14(2):42-49.
- Wee, K.L. (1983). Studies on intensive snakehead (Channa spp.) culture with special reference to their nutrition. Ph.D. Thesis, University of Stirling, 209-229.

Table 1

Ectoparasitic species recorded from various snakehead species

Parasite	Host	Country	Source
Mastigophora-Kinetoplastida <u>Ichthyobodo</u> sp.	<u>C. micropeltis</u>	Thailand	1
Ciliophora-Holotricha <u>Chilodonella</u> sp.	<u>C. maculatus</u>	Hong Kong	1
	<u>C. micropeltis</u>	Thailand	1
	<u>C. punctatus</u>	Sri Lanka	2
	<u>C. striatus</u>	Sri Lanka	2
<u>Ichthyophthirius</u> <u>multifiliis</u>	<u>C. maculatus</u>	Hong Kong	1
	<u>C. micropeltis</u>	Thailand	1
	<u>C. qachua</u>	Sri Lanka	2
	<u>C. marulius</u>	Sri Lanka	2
	<u>C. punctatus</u>	Sri Lanka	2
	<u>C. striatus</u>	Sri Lanka	2
		Thailand	1
Chilophora-Peritricha <u>Trichodina</u> sp.	<u>C. maculatus</u>	Hong Kong	1
	<u>C. micropeltis</u>	Thailand	1
	<u>C. punctatus</u>	Sri Lanka	2
	<u>C. striatus</u>	Sri Lanka	2
		Thailand	1
Trematoda-Monogenea <u>Gyrodactylus</u> sp.	<u>C. maculatus</u>	Hong Kong	1
	<u>C. qachua</u>	Sri Lanka	1

1- Wee (1983); 2 - K.H.G.M. de Silva (present study)

Table 2

Treatments recommended for ectoparasites recorded from snakeheads.

Parasite	Host	Treatment	Source
<u>Ichthyobodo</u> sp.	snakehead	125 ppm formalin- 30 min. bath on alternate days for one week	2
	tilapia, carp	15-25 ppm formalin- indefinite bath	1
		150-166 ppm formalin 1 hour bath	1
		1000-2000 ppm NaCl- indefinite bath 3000 ppm NaCl- 20-30 min. bath	1
<u>Chilodonella</u> spp. <u>Trichodina</u> sp.	snakehead	same as for <u>Ichthyobodo</u> sp.	2
	snakehead	50-100 ppm formalin- continuous bath for three days	3
		2500 ppm NaCl- continuous bath for three days	3
	tilapia, carp	same as for <u>Ichthyobodo</u> sp.	1
<u>Ichthyophthirius</u> <u>multifiliis</u>	snakehead	25 ppm malachite green + 3500 ppm (14 g in 4dm ³) formalin- 2 hour bath on alternate days for one week	2
		3 ppm methylene blue- continuous bath for three weeks	2
	snakehead	0.1 ppm malachite green + 25 ppm formalin- continuous bath for 3-4 days	2
	tilapia, carp	0.1 ppm malachite green + 25 ppm formalin- 3 treatments of 6-hour baths on alternate days for one week	1
<u>Gyrodactylus</u> sp.	snakehead	150 ppm formalin - 30 min. bath on alternate days for one week	2
	tilapia, carp	same as for <u>Ichthyobodo</u> sp. or <u>Ichthyophthirius</u> <u>multifiliis</u>	1

1 - Sommerville (1983); 2-Wee (1983); 3- K.H.G.M. de Silva
(present study)