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Control of Freshwater Fish Parasites: a Southeast Asian Perspective

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Abstract—Tonguthai K. 1997. Control of freshwater fish parasites: a Southeast Asian perspective. *International Journal for Parasitology* 27: 1193–1201. Parasites commonly found in freshwater fishes and other aquatic animals primarily belong to Protozoa, Platyhelminthes, Acanthocephala, Nematoda, Hirudinea and Crustacea. Most of these parasites are external parasites of the skin, fins or gills, while few are internal parasites living in the epidermal intralamellar gills, intestine or intramuscular tissues. Possible control measures involving manual removal, cleaning by topical cleaners, management practices, nutritional improvement, vaccination, chemoprophylaxis, chemotherapy, and quarantine and certification, etc., are discussed. © 1997 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: Control; freshwater; parasite; prophylaxis; quarantine; certification.

INTRODUCTION

The process of disease control is quite complex and depends upon the interplay of three factors: diagnosis, preventive measures and treatment. Correct diagnosis of the disease is a critical step in any disease control programme. No progress is possible in controlling the disease unless the cause is clearly and correctly identified. For effective control of parasitic diseases it is also necessary to understand the life-cycle and ecology of pathogenic parasites.

Diseases of aquatic animals are extremely difficult to control or cure. Once the diseases occur it is often too late to do anything to prevent losses. Thus preventive measures to alleviate diseases are fundamental. Prevention is generally related to the management practices of aquaculture systems, including the following major aspects:

- desirable water qualities;
- adequate nutrition;
- appropriate stocking density;
- adherence of proper preventive measures, such as
  - —development of disease-resistant stocks,
  - —environmental manipulation,
  - —reduction of other forms of environmental stress;
  - —vaccine development for immunological protection;
  - —chemical prophylaxis;
  - —law and regulation to prevent disease transfer.

In the case of certain diseases, however, treatment may also become a useful measure to reduce losses if preventive measures have failed.

There is a large body of literature on the control of freshwater fish parasites (Kimura, 1960; Sano & Ushiyama, 1970; Molnar, 1970; Takahashi & Egusa, 1976). However, a review of these works is not within the scope of this paper. Only the most practical and effective preventive and curative treatment measures are discussed herein, and the information is restricted mainly to the problems occurring in Asia.

Parasitic problems

External parasites are the most common parasites encountered in aquatic animals raised in both ponds and aquaria. The major groups of parasites include protozoans, monogeneans and crustaceans. Among

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protozoan parasites, the most common species belong to the ciliated protozoa, such as Trichodina, Ichthyophthirius, Epistylis, Apiosoma, Scyphidia, Oodinium and Chilodonella. As these parasites live on the skin and gills of hosts, they are quite susceptible to chemotherapeutic treatment.

There are also many parasitic protozoans found in the internal organs and tissues, such as the alimentary canal, kidney, gall bladder and muscles. In general, internal parasites are able to cause much greater damage to the hosts than the external ones. However, the severity of the damage depends upon the organ of the host and the number of parasites.

Among crustaceans, the copepods which parasitize fish, are the most commonly known pathogenic parasites of farmed freshwater fish in many Asian countries. Their injurious effects on the host are believed to be direct or indirect as their infection causes formation of lesions and inflammation at the site of attachment, which often leads to secondary infections by opportunistic bacterial invaders. Monogeneans, such as Dactylogyrus and Gyrodactylus, are gill and skin parasites which may occur in large numbers causing damage to gill tissues and resulting in respiratory problems.

Internal parasites, such as digeneans, cestodes, nematodes and acanthocephalans do not cause severe damage to fish unless they are present in large numbers. However, certain species of trematode (metacercaria) that live in fish gills may disrupt the respiratory system leading to high mortality.

Parasitism by large numbers of tapeworms can result in growth retardation. Abe (1973) reported that a species of acanthocephalan induced chronic catarrhal inflammation accompanied by deformation and loss of mucosal epithelium caused by penetration of the proboscis into the submucosal tissue of the intestine.

CONTROL OF FRESHWATER FISH PARASITES

Control of fish parasites is generally achieved by application of chemotherapeutic agents to infested fishes. However, in view of the potential hazards of using chemicals in water for the control of freshwater fish parasites, other methods of parasite control have been included in this review.

Manual removal of parasites

For aquarium fish, parasites can be removed manually from infected fish. Although this method is quite effective, it is labor intensive and impractical when there are large numbers of fish or parasites. Furthermore, prolonged handling of fish to remove parasites can cause physical and physiological stress and may be impractical with small fish. The job becomes more difficult when the organisms are small; the parasites must be large enough to be seen by the naked eye. This method is probably more applicable for fish infected by crustacean parasites which are relatively large enough to be removed manually. However, extreme care and skill are needed as some of these parasites, such as Lernaea and Ergasilus, are strongly anchored to the host. Incomplete extraction of the attached animal or rupture of host muscle tissues can cause damage, leading to secondary microbial infections.

Topical cleaners

There have been several attemps to remove parasites by using topical cleaner fish. Cowell *et al.* (1993) demonstrated that juvenile bluehead wrasse, neon goby and cleaning goby were able to remove ectoparasitic monogeneans, e.g., *Neobenedenia melleni*, from saltwater, cultured Florida red tilapia and the cleaners' guts were found to contain three species of monogenean parasites. A similar method was applied to remove Lernaea from infected arowana fish (*Scleropages* sp.), one of the most valuable aquarium fish, by using *Tilapia nilotica* fry of 3–5-cm long as cleaner fish. The result showed that *Tilapia nilotica* were effective cleaner fish for Lernaea infestation (Menakarn, personal communication, 1996).

Management practices

The best procedure to control cestodes in culture ponds is through adherence to good management practices. Draining the ponds and subsequently allowing them to completely dry out eliminates infected intermediate hosts and cestode eggs. Ponds that cannot be drained completely can be treated with chlorine or lime to kill intermediate copepod hosts and cestode eggs. Crustacean intermediate hosts may be eliminated by a single application of Dipterex applied at the rate of 0.25 p.p.m.

The most effective control measure for Acanthocephala is to eliminate water-borne crustaceans by using organo-phosphate pesticides such as Dipterex, but such compounds also cause environmental safety concerns.

Prophylactic measures such as limiting the first intermediate host and the definitive hosts are the best approach to control digenetic trematodes. The bottoms of ponds should be treated with quicklime at 250–300 kg ha^{-1} or tea-seed cake at 110 kg ha^{-1}. The treatment is more effective when tea-seed cake is presoaked in water for 24 h.

Life-cycles of nematode and cyclopoid copepods can be interrupted by Dipterex treatment at 0.5–1.0 p.p.m. in ponds a few days prior to stocking.

The free-swimming stages of many species of parasite can survive for only a short time if they do not find their host. Ponds or aquaria should be left empty with no host fish for at least 3–7 days in order to facilitate destruction of this group of parasites in their larval stages. Care is also needed while draining the pond. There is possibility of parasites gaining entry to the nearby water system if infected pond water is drained immediately after harvesting.

Nutritional control measures

The influence that dietary factors may have on disease outbreaks in cultured fishes has been recognized for many years. Early investigators concentrated their studies on the effects of various diets on the incidence and severity of common infectious diseases. Later investigators attempted to understand the mechanism for some of the observed nutritional effects. However, studies on the effects of dietary quality on parasitic infestation appear to be very limited. Wahli *et al.* (1986) demonstrated the effect of dietary ascorbic acid on the disease resistance of rainbow trout infested with the parasite *Ichthyophthirius multifiliis*. Fish were fed with ascorbic acid at 0 or 5000 mg kg^{-1} of diet for 5 days before exposure to the parasite. After 50 days of exposure, no parasites could be detected on fish that survived infestation. They concluded that the results definitely indicated the presence of an immune response against the parasite and this response might have been enhanced by dietary ascorbic acid.

Vaccination

Attempts to vaccinate to protect fish have mostly been against bacterial diseases. In the history of vaccination, the first commercial product licence for a vaccine of fish was granted in the U.S.A. in 1976 for use against enteric redmouth disease. Since then, product licences have been granted for *Vibrio* vaccines. These two vaccines have proved to be of great commercial value. Since the beginning of the last decade, in Eastern Europe commercial scale vaccination has also been used extensively against the viral disease of carp (the Spring Viremia of Carp) However, vaccines against parasitic diseases have not been developed commercially. One reason for this is that knowledge of the immunological relationships between fish and their parasites is quite scanty in comparison to that of bacteria and viruses.

Vaccination against some protozoa and helminth parasites has received increased attention in recent years. Attention has centered around the prospects of preventive immunization as a means to control parasitic infestation. This is not only because of the obvious limitations of chemotherapeutic and other methods, but also because of the recognition that a protective immunity develops in previously infected fish. Vaccination against parasitic infestation, therefore, appears promising.

The possibility of developing a vaccine against *I. multifiliis* became feasible when it was discovered that channel catfish injected with a closely related ciliate *Tetrahymena pyriformis*, were protected against *I. multifiliis* infection (Goven *et al.*, 1980, 1981). Wolf & Markiw (1982) have also found that immunization against *I. multifiliis* provides protection against *Ichthyobodo necator*. Wahli *et al.* (1986) investigated the properties of serum and mucus of infested rainbow trout infested with *I. multifiliis* and found that during a heavy infestation the mucus immobilized the parasite.

The practical application of vaccines against ichthyophthiriasis appears promising, but no comprehensive trial has been conducted to assess the real field value of a vaccine. The mode of administration is also equally important. Bath application would be desirable for a vaccine which stimulates a secretory immune response for protection against ectoparasites.

Vaccination for controlling *Trypanosoma* may be possible. Woo (1981) has demonstrated that goldfish survived inoculation with *T. danilewskyi*. Some work has suggested that vaccination of fish using an antigenic preparation of metacercarial antigens might be possible. Bortz *et al.* (1984) were able to detect the presence of circulating antibody to metacercarial antigens in naturally infested fish and following experimental immunization of rainbow trout with sonicated metacercariae.

In fish, the development of acquired immunity to various parasites is now known to exist. Therefore, vaccination should be developed seriously and exploited to protect fish against parasitic diseases.

CHEMOTHERAPEUTIC TREATMENT

A wide range of chemicals is used in the aquaculture industry worldwide. However, there has been little work on the toxicities, pharmacology and pharmacokinetics of these chemicals in fish. Before chemotherapeutic treatment is applied, it is necessary to make a diagnosis of the problem. In the case of a parasitic problem, it may not be possible to make a complete identification of the parasite. However, at least the group of the parasite must be known.

If the treatment is new to the facilities, it is always wise to consider the use of a test dose on a few fish in a separate container. Whenever possible, treat fish in a reduced volume of water. This offers the combined benefits of being able to quickly raise the water level in order to dilute the treatment in the event of an adverse reaction and to reduce the amount and thus the cost of the chemical used. It is also essential to watch the fish through the treatment and to be ready to take any necessary emergency action.

Treatment in different holding systems

Aquarium. It is a simple procedure to treat the fish in an aquarium, as it is a highly controlled system. The dose used will be more accurate than that in the pond. Application is easier and it is possible to make a close observation of the fish throughout the treatment period.

Pond. It can be difficult to treat fish in large area of water. Accurate calculation of the volume of water may not be possible due to an uneven pond bottom or pond bank. In a small pond a common compromise is to lower the water level and add the chemotherapeutant to the inlet. After the desired amount of chemotherapeutant has been added, the pond is refilled and normal flow is resumed. In a large pond it is necessary to spray the chemical over the pond surface.

Cage. There is a bath technique which involves raising the cage bottom to a known depth. A tarpaulin is placed around and under the net to totally enclose it. To minimize the amount of chemical required for treatment the chemical is then applied, taking care to ensure that it is properly mixed.

ADVANTAGES AND DISADVANTAGES OF CHEMICAL USE

The use of chemicals in aquaculture has various advantages:

- Chemicals are effective against multiple diseases or multiple pathogens.
- Their applications are versatile.
- They give quick results.
- A wide variety are available.
- Chemicals can be used as prophylactic agents and can be applied quickly when a disease condition occurs.
- The desired chemicals can be applied in various ways, such as bath, injected or as feed additives.

On the other hand, these chemicals also have certain disadvantages, including:

- The chemical may be toxic to fish.
- The chemicals might be toxic to non-target organisms, such as the natural fish food present in the culture system.
- Their application may lead to the development of drug resistance because of overuse or misuse.
- They may accumulate harmful residues in the fish flesh and the environment.
- The cost may be prohibitive.

A number of chemicals are under use in aquaculture worldwide. With the present culture practice, chemicals are more likely to be involved not only to treat diseased animals but also to improve water quality in culture facilities. The chemicals use in aquaculture have specific effects. In general they are applied singularly or in combination.

Chemotherapeutic treatment for ectoparasites

Formalin. With respect to the treatment of ectoparasitic infestation, formalin is considered to be the most effective chemical not only for its efficacy, but also its availability and low cost. The first recorded use of formalin in the treatment of fish disease was in 1909 (Alderman & Michel, 1992) and it has, more recently, been approved by the USFDA for use in food fish treatment.

Formalin is commonly used to treat ciliated protozoan infestations. The recommended dosage rates, however, are 150 p.p.m. for 1-h bath, and 25–30 p.p.m. for long-term bath treatment. Formalin degrades very rapidly at 25, 50 and 75 p.p.m. and completely degrades within 26, 48 and 54 h, respectively. If no aeration is provided degradation takes longer, i.e. 36, 54 and 60 h, respectively (Chinabut *et al.*, 1988). Formalin also causes oxygen depletion if applied in a pond with high plankton density. It is, therefore, recommended that formalin is applied in ponds with low plankton density and that the treatment should be conducted in the afternoon when the ponds have relatively high dissolved oxygen levels.

The most successful treatment for the free-living form of *Ichthyophthirius* sp. is bathing in formalin solution of approximately 200 p.p.m. for 1 h, or 25–30 p.p.m. for long-term treatment. The mixture of formalin and malachite green at 25:0.1 p.p.m. appeared to be a very effective treatment for *Ichthyophthirius*, particularly for aquarium fish. This concentration is also applied to *Chilodonella* infestation. However, due to the teratological effect of malachite green (Meyer & Jorgensen, 1983) and the high price, formalin alone is now recommended for pond treatment. Formalin at 25–30 p.p.m. is also effective to treat *Trichodina* in aquaria and ponds. For other ciliate protozoans such as Apiosoma, Ambiphrya and Scyphidia, formalin is also recommended as an effective therapeutic. For flagellated parasites also, formalin is quite effective when applied at 25–30 p.p.m., and this concentration also appears to be very effective in treating monogenetic trematodes.

Nakajima & Egusa (1974) have observed that *Myxobolus* spores die after 1 day in 0.1% formalin. It is therefore possible to use formalin to treat spores in a pond without fish.

Salt. In small water tanks, bathing fish in 3% salt is recommended for treatment of *Ichthyobodo* spp.

Acetic acid. Treatment with 1:1000 acetic acid solution is also recommended for *Ichthyobodo* infestation.

Dipterex. The organophosphate trichlorphon (the principal ingredient of Dipterex) is most effective in eradicating anchor worm larvae. Kasahara (1962) has clarified that the nauplii were destroyed in 2 days at 0.2 p.p.m. and in 1 day at 0.5 p.p.m.. Furthermore, 0.2 p.p.m. of Dipterex is believed not to injure fish and virtually no adverse effects were seen on phytoplankton. For effective treatment of *Lernaea* larvae, two to three applications at 1-week intervals for 3 weeks should be made in order to eliminate larvae, since there is a wide range of spawning periods of the first generation. Subsequently, application should be conducted periodically in anticipation of the development of a second generation. After application, care should be taken to ensure that the concentration of effective ingredients in the pond is 0.2 p.p.m. Kimura (1960) indicated that the use of Dipterex is effective in eradication of argulids. Specifically, a concentration of 0.2–0.3 p.p.m. is effective, and adults and larvae fall off the parasitized fish and die within 12–24 h. The agent is applied by sprinkling on the pond surface so that the concentration is uniform. The chemical requirement should be calculated at the rate of 0.3 p.p.m.; the desired amount should be dissolved in enough water and sprayed over the entire pond surface. However, the eggs of argulids are not affected by this treatment and they remain capable of hatching. Accordingly, two or three treatments must be conducted at intervals of 20–25 days over which period the eggs hatch.

Bormex. Sarig (1968) reported that application of 0.3 p.p.m. of the organophosphate Bromex in ponds is effective in eradicating ergasilids. In addition, application of 0.15 p.p.m. of Bromex and Dipterex has subsequently been reported to be effective (Sarig, 1971). However, this dose is effective only on larvae. The concentration required for killing the adult reached 800 p.p.m. within 3 days (Kabata, 1970) which, however, was found completly impractical considering its toxicity on fish.

Chemotherapeutic treatment for endoparasites

Chemical compounds. Kamala, the extract of glands and hairs found on the fruit of *Mallotus phillippinensis* has been recommended for the treatment of cestodes. In the former Soviet Union, Kamala is used extensively and is reported to be effective against *Bothriocephalus* in *Cyprinus carpio* (Bauer *et al.*, 1973). To treat bothriocephalosis, Molnar (1970) attempted to use Devermin at 0.1 mg kg^{-1} fish weight. Niclosamide piperazine salt when applied at 7.5–9 mg kg^{-1} fish weight and blended two or three times with feed in a 2-week period achieved good results (Korting, 1974). Di-*n*-butyl tin oxide is recommended for cestode treatment but does not seem to be available, particularly in Southeast Asia.

Since there is no suitable chemical known to eradicate nematode infestation, the intermediate host copepods can be eradicated by sprinkling Trichlorfon in culture ponds from which the disease outbreak is reported. Nakajima *et al.* (1975) have reported that bithionol, pyruvinium pamoate and dithiazanine iodide destroy parasites such as *Acanthocephalus opsariichthydis* within 24 h at a concentration of 10 p.p.m. However, the most effective measure would be the elimination of water-borne crustaceans using organophosphate to break the life-cycle.

Antibiotics. When sulfamonomethoxydine is administered to treat *Hexamita* infestation at 0.02% of fish weight for 13 consecutive days, a decrease in the number of parasitized fish can be noticed after 10 days (Sano & Ushiyama, 1970). Furazolidone is also an effective drug for hexamitiosis. It has been reported that daily administration of 20–40 mg kg^{-1} of fish for 4–5 days is effective, but 9 consecutive days is actually preferable (Sano, 1970).

Fumagillin is a type of antibiotic which is used effectively against microsporidian disease of the honeybee and the silkworm. More recently it has been used to treat microsporidians in fish. Molnar *et al.* (1987) reported that a concentration of 0.1% fumagillin DCH when applied with the feed immediately after experimental injection with early developmental stages of *Sphaerospora renicola* prevented spore formation. In a farm pond experiment, they also reported that renal sphaerosporiosis disappeared earlier in the summer in medicated fish than in the control fish and that fumagillin significantly reduced the number of fish affected. However, they suggested that fumagillin was not effective against the early stages of *S. renicola* infestation.

Medicinal plants. It is interesting to note that the first therapeutic substances to be used against crustacean parasites were natural compounds, accidentally discovered to have parasiticidal properties. In China, a local plant, *Pirasma quassioides*, is used to control *Trichodina*. The twigs and leaves of *P. quassioides* are applied at 50 kg ha^{-1}. These are dumped in the pond at an interval of 7–10 days (Kabata, 1985).

Chinese workers have used a mixture of powdered betel palm (*Areca catechu*) and the powdered seeds of a melon (*Curcurbita moschata* var. *melonaeformis*) in the ratio 1:2 and it added to food containing peanut cake, bean powder and animal blood. Some component of the melon seeds appears to act by destrobilating the parasite, but they are unable to expel the scoleces, whereas betel has a strong anaesthetic influence on the scolex (Kabata, 1985). However, studies on the effects of medicinal diets on parasitic infestation appear to be very limited.

QUARANTINE AND HEALTH CERTIFICATION

An effective quarantine and certification system is the key for preventing and controlling exotic aquatic parasites and diseases. Quarantine refers to a period of isolation of newly transported fishes until the possibility of introducing any pathogens they may carry can be eliminated. Certification may involve either certification of the source of origin or certification of individual lots. Certification at origin ensures that the facilities from which fish or other aquatic organisms originate have been inspected, either by the exporting or the importing country, and found to be free from specific pathogens. Both quarantine and certification should be based on recognized examination techniques for specified pathogens and should require examination of sample sizes sufficient to detect a minimum predetermined carrier prevalence with a predetermined degree of statistical confidence.

Fish quarantine and certification regulations are formulated to prevent potentially dangerous pathogens from entering and leaving the country. No amount of success can be achieved, no matter how well these regulations are formulated, unless they are supported by sound implementation guidelines and stringent enforcement.

Many countries, especially in Southeast Asia, have no specific and clear-cut regulations for fish disease control through quarantine and certification. In practice, quarantine is not applied. Health certificates are not usually issued unless required by the importing country. However, because quarantine and health certificates for export and import of live fish are likely to be the most effective measure in preventing disease transmission, regulations pertaining to the importation and exportation of live aquatic animals and plants should be reviewed and developed. The Department of Fisheries should give top priority to reviewing and updating its fish quarantine and certification policies in order to safeguard the aquaculture industry and native fish stocks from exotic diseases. It also demands high level of co-operation among trading nations for the effective implementation of quarantine and certification practices.

Regarding the control of fish parasites, it must be emphasized that the introduction and movement of fish should be subject to strict quarantine measures and a reliable programme for inspecting and certifying health certificates.

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