

The Use of Chemotherapeutic Agents in Shrimp Hatcheries in Sri Lanka

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ABSTRACT

In Sri Lanka, the active promotion of chemical products to prevent disease in shrimp hatcheries has led to an increase in the use of drugs and chemicals without much emphasis on understanding their efficacies. A survey was carried out to evaluate trends in the use of drugs and chemicals as therapeutic treatments for shrimp-hatchery diseases. A wide range of chemicals and drugs are being used, both for prophylactic treatment and to prevent or control parasitic, fungal and bacterial diseases in hatcheries. Without proper scientific investigation into treatment regimes, there has been a tendency for individual hatcheries to select their own treatment regimes and to do their own experimentation. Little knowledge exists among hatchery operators as to the hazardous effects of the chemicals in use. Lack of legislation on the use of chemotherapeutants in aquaculture has led to the uncontrolled use and improper selection of chemicals for use in shrimp hatcheries.

INTRODUCTION

Within the past decade, shrimp culture has progressed at a very rapid pace in Sri Lanka. At present, shrimp farming is done mainly in the northwestern coastal areas; however, it continues to expand into other regions, viz, the eastern and southern coastal areas. Around 3000 ha of land area have been allocated for the industry in the northwestern coastal areas, and the production of shrimp in 1995 was around 3,500 mt live weight. Shrimp farming as practiced in Sri Lanka is monostock monoculture of the black tiger prawn (*Penaeus monodon*). The entire industry is dependent on hatchery-bred postlarvae for the seed supply. With the rapid expansion of shrimp grow-out, the hatchery industry has progressed rapidly during the last few years. The shrimp hatchery industry has developed on different levels of economic and management scale. One of the areas in which a technical base is lacking is the therapeutic treatment of shrimp-hatchery diseases. The active promotion of chemical products has partly led to an increase in use of drugs and chemicals in shrimp hatcheries without much emphasis on determining their efficacies.

This paper summarizes general trends in the use of drugs and chemotherapeutic agents for treatment and prevention of diseases in the shrimp hatcheries of Sri Lanka. It reviews the diseases occurring in shrimp hatcheries, the clinical signs of diseases treated with chemicals, the chemotherapeutic agents used, problems and constraints associated with their use, legislation related to their use, and provides some recommendations.

METHODS

This survey was conducted during August 1995 to May, 1996. Of the 45 operating hatcheries, 36 (80%) were surveyed. Information pertaining to the use of drugs and chemicals to treat shrimp-

hatchery diseases and on the clinical signs of disease was collected based on a questionnaire. The questionnaire was designed to collect information on the use of drugs and chemotherapeutic agents for broodstock maintenance and larval and nursery rearing, and on the technical capabilities of hatchery personnel. This information is presented in tabular form and is compared with that found in the literature.

RESULTS

Diseases Recorded and Clinical Signs Observed

Monodon baculovirus (MBV) has been recorded from these hatcheries. According to unconfirmed reports, there have been viral infections due to viruses other than MBV. Larval mycosis was commonly found in zoeal and mysis stages, causing mortalities of up to 100%. Gross signs were pale yellowish-green colored tissues in the larval body. The phycomycete fungus *Lagenidium* sp. is believed to be the pathogenic agent.

Commonly seen pathogenic bacteria include *Vibrio* spp., the species suspected to be involved being *V. vulnificus*, *V. parahaemolyticus*, and *V. anguillarum*. The clinical signs of vibriosis are the necrosis of appendages with melanized tips, resulting in dark oral regions. Clear red lesions could be seen on the cuticle of *Vibrio*-affected shrimp at the mysis stage. Luminous bacteria causing up to 100% larval mortality were recorded in most of the surveyed hatcheries. *Vibrio harveyi* is believed to be the pathogenic agent involved. Filamentous bacteria were also reported from most of the surveyed hatcheries. Heavily infected larvae show discoloration of the body and gills. The main filamentous bacteria is *Leucothrix mucor*. It frequently occurs with other genera such as *Flexibacter*, *Cytophaga*, and *Flavobacter*, which contribute as fouling organisms.

Zoothamnium, *Epistylis* and *Vorticella* are the main protozoan genera that were involved as external fouling organisms. The suctorians *Acineta* and *Ephelota* were also seen. These types of infection cause problems in respiration, locomotion, feeding and molting. These organisms are responsible for black and brown discoloration of gills. Heavy infections may show a "fungus-like" appearance on the body surface and may lead to mortality.

Drugs and Chemotherapeutants used in Hatcheries

Chemicals widely used to control parasites in Sri Lankan shrimp hatcheries include formalin, malachite green, and formalin and malachite green in combination. Sixty percent of the hatcheries surveyed used formalin and 40% used malachite green to control parasites in broodstock holding facilities. Twenty seven percent of the hatcheries used malachite green and formalin in combination, in addition to using malachite green and formalin alone (Table 1). Of the hatcheries using formalin and malachite green in larval rearing facilities, 90% and 50%, respectively, used these chemicals to control parasitic infections (Table 2).

A wide range of drugs and chemotherapeutic agents are used to control bacteria in the shrimp hatcheries surveyed. Furans, oxytetracycline, erythromycin and Treflan were widely used with varying success to control all types of bacteria (Table 2). Twenty percent of the hatcheries used the first three drugs in broodstock maintenance to prevent possible bacterial infections after eye stalk ablation (Table 1). Eleven percent of the hatcheries used copper compounds, and of the formalin users, 10% used formalin to control filamentous bacteria, while 16% of the malachite green users used this dye to control luminous bacteria (Table 2).

Treflan, malachite green and furans have been used as antifungal agents. Of the hatcheries using Treflan and malachite green, 63.6% of the Treflan users and 33% of the malachite green users used these drugs as antifungal agents in larval rearing (Table 2). For broodstock, the commonly used

Table 1. Prophylactic treatment used for broodstock maintenance in *Penaeus monodon* hatcheries in Sri Lanka.

% Hatcheries	Drug	Dosage range (ppm)	Pathogen
40%	Malachite green	NA	Fungi, epibionts
20%	Furazan, furazolidone Oxytetracycline Erythromycin	1.1-5 (2 d) 10 0.4	After eye ablation for possible bacterial infections
60%	Formalin	200 (10 min) 10-25 (few h) 0.25 (indefinite bath)	Ectoparasites Epibionts Ciliates
27%	Malachite green + formalin	1.4-2 malachite green + formalin	Ectoparasites, epibionts, fungi, and ciliates
15%	None	-	-

Table 2. Chemotherapeutants and their usage for treating larvae and postlarvae in shrimp hatcheries in Sri Lanka (percentage of user hatcheries using a given chemical to treat against each pathogen is given in parentheses).

Chemical	% Users	Dose (ppm)	Pathogen
Chloramphenicol	11%	10-15 3-4	Luminous bacteria (50%) <i>Vibrio</i> spp. (50%)
Copper control	11%	0.0004-2 ² 0.24 (prolonged) ²	Filamentous bacteria (11%)
Erythromycin	17%	0.1%(daily) ¹ 0.5-0.6 ¹ 2 ² NA ³ 2 ²	Filamentous bacteria, <i>Vibrio</i> spp. (33%) Filamentous bacteria (17%) <i>Pseudomonas</i> spp. (17%) <i>Vibrio</i> spp. (17%) <i>Vibrio</i> spp./ <i>Pseudomonas</i> spp. (17%)
Formalin	49%	27 (0.5 h), 5-20 (1h) 0.05 (daily)	Ciliates (90%)
Formalin + malachite green		0.01 + 0.01	Filamentous bacteria (10%)
Furans	44%	2.0-2.5 (d) ² 2-4 (prolonged) 3-10 (12 h) 3-4 d, 2-5 (daily) ² 1 ² NA (daily) 2-5	All types of bacteria (31%) Luminous bacteria (19%) Fungi (25%) Filamentous bacteria (6%) Ciliates (6%) <i>Vibrio</i> spp. (13%)
Malachite green	17%	1.0 NA NA	Ciliates (50%) Fungi (33%) Luminous bacteria (17%)

Table 2. Continued. . .

Chemical	% Users	Dose (ppm)	Pathogen
Oxytetracycline	64%	2-4 ²	Filamentous bacteria, <i>Vibrio</i> spp., <i>Pseudomonas</i> spp. (46%)
		2.5-4	<i>Pseudomonas</i> / <i>Vibrio</i> spp. (23%)
		1-3 ¹	<i>Vibrio</i> spp. (15%)
		4-5 ²	
		4	<i>Pseudomonas</i> spp. (8%)
		2	Filamentous bacteria (8%)
		2 ²	All bacteria (8%)
		0.5-1 (daily)	Quarantine (8%)
Treflan	92%	0.003-0.006 ¹	Fungi (64%)
		0.02-0.12 (daily)	
		0.002-0.01 (daily)	Bacteria (21%)
		NA	Other purposes (7%)

¹Prophylactic treatment.

²Treatment on detection.

³NA = not available.

antifungal agent is malachite green, used either alone or in combination with formalin (Table 1).

Treflan, malachite green and formalin have been widely used by many hatcheries as prophylactic treatments for fungi and bacteria (Tables 1 and 2). Some hatcheries used furans (of the furan users, 6.2%), erythromycin (of the erythromycin users, 16.7%), oxytetracycline (of the oxytetracycline users, 15%) or Treflan (of the Treflan users, 21.2%) for prophylactic treatment in larval rearing (Table 2).

Other than the prophylactic treatment strategy, it is not very clear whether the treatment strategies adopted by the hatcheries surveyed are metaphylactic or therapeutic treatment strategies.

DISCUSSION

The actual strategy used in administration of a chemotherapeutant is an essential component of proper management. There are three basic types of strategy: prophylactic, metaphylactic and therapeutic (Bell 1992). Prophylactic treatment involves the routine use of a chemotherapeutant prior to clinical signs of disease being noted. In the present study, malachite green, Treflan and furans were found to be used as prophylactic treatment even without an established history of disease. Prophylactic treatment is best used when a long history of disease allows for accurate prediction (Bell 1992). A metaphylactic strategy calls for treatment to be administered only after a predetermined disease prevalence has been reached within the shrimp population and a full-scale outbreak is therefore probable. Therapeutic treatment relies upon the accurate diagnosis of the disease, immediately followed by proper treatment. Most of the drugs and chemotherapeutants used in the hatcheries surveyed fall into neither the metaphylactic nor the therapeutic method of administration. Other than as prophylactic treatment, they were used based on observation of the clinical signs of disease or after the occurrence of disease. The determination of actual disease prevalences and accurate diagnoses based on proper monitoring have not been done. Such use of drugs and chemotherapeutants without proper diagnosis leads to higher operating costs and the production of poor quality postlarvae, and increases the risk of chemicals in the environment.

Most of the chemicals that have been tested to control luminous vibriosis, such as chloramphenicol, Furacin, and oxytetracycline, cause mortalities, incomplete molting, or morphological deformities, such as spread carapace, bent rostrum, and curled setae in shrimp larvae when applied at effective concentrations (Baticados *et al.* 1990b). The present study reveals that the concentrations of furans used to control luminous bacteria in Sri Lanka are lower than those reported in the literature (Tables 2 and 3) (see Baticados and Paclibare 1992), and that oxytetracycline has not been used for this purpose. The concentrations of chloramphenicol used in Sri Lankan hatcheries to control luminous vibrios are three to four times higher than those reported elsewhere (Ruangpan 1987, Baticados and Paclibare 1992) and thereby increase the risk of morphological aberrations and mortality. Moreover, the use of chloramphenicol poses a danger to public health. Chloramphenicol has erythrocyte-destroying effects in humans (Fakas *et al.* 1982) and may be harmful to users who come in contact with it (Baticados and Paclibare 1992).

Of the malachite green users, 50% used it as an antifungal and antibacterial agent and were unaware of the concentrations used. Similarly, 6% of the furan users and 17% of the erythromycin users used these chemotherapeutants as antibacterial agents, and were unaware of the concentrations used. This indicates that some users are selecting arbitrary dosages of chemotherapeutants for use in shrimp hatcheries, a practice which, if continued on a routine basis, may lead to the development of resistant strains of pathogens and to associated economic losses. Sixty-seven percent and 17% of hatchery operators used malachite green in broodstock maintenance and in larval rearing, respectively. Malachite green has been used at levels of 0.002 to 0.01 ppm with varying success as an antifungal agent in larval rearing (Bell and Lightner 1992). Malachite green is reported to have potential carcinogenic and teratogenic properties (Baily 1983). As an alternate to malachite green, trifluralin has been used at levels of 0.001 to 0.002 ppm with even more success, and with apparently few, if any, side effects (Baticados *et al.* 1990a, Bell and Lightner 1992).

Sixty-four percent and 44% of the hatcheries, respectively, used oxytetracycline and furans to control all types of bacteria. The present survey also revealed that oxytetracycline and furans have been used routinely on a daily basis. Oxytetracycline is known to enhance the production of plasmid-mediated resistance in aquatic bacteria (Shotts *et al.* 1976). Nitrofurans (e.g., pefuran) may possibly cause rapid formation of bacterial resistance because of their persistence in water (Beladi *et al.* 1978). The prolonged, repeated or widespread use of antibiotics more significantly leads to the development of resistance in bacterial populations (Watanabe *et al.* 1971; Aoki 1974; Aoki *et al.* 1981, 1987). Furans (furozolidone) have been implicated as potential carcinogens (Schnick and Meyer 1978).

Chemotherapy is based on the principle of differential toxicity, i.e., the drug or chemical must kill or eliminate the pathogen at concentrations that are not harmful to the host (Baticados and Paclibare 1992). The effective concentration of 30 ppm formalin against protozoan infections of *P. monodon* larvae and postlarvae was reported to be the 12 h LC_{50} value for larval *P. monodon* (Vicente *et al.* 1979). Thus, the toxicity of drugs and chemicals limits their use as a method to control shrimp-hatchery diseases. The present survey revealed the use of 20 to 27 ppm formalin with .05-1.0 h exposure periods. Even though these concentrations are below the reported LC_{50} value, frequent use of formalin at such concentrations may cause sublethal effects that may be seen in later larval stages.

CONCLUSIONS

It is clear that without proper scientific investigations into treatment regimes, there has been a tendency for individual hatcheries to select their own treatment regimes and to do their own experimentation. Little knowledge exists among hatchery operators as to the potential hazardous effects of the chemicals in use. It is also evident that knowledge of alternate chemotherapeutants with fewer or no side effects is lacking. Since the chemotherapeutants used in aquaculture may result in adverse environmental impacts such as quantitative and qualitative changes in bacterial flora, toxic effects on wild living organisms, development of drug resistance in bacterial pathogens

Table 3. Chemotherapeutants and their usage in shrimp hatcheries as reported in the literature.

Chemical/Drug	Dosage (ppm)	Duration	Pathogen	Stage	References
Chloramphenicol	2-3	5 d	<i>Vibrio harveyi</i>	PL 6-7	Ruangpan 1987
	2.7-7	Prolonged	Bacteria	PL 2-15	Rattanavinijkul <i>et al.</i> 1988
	2-4 ¹	Every other d	Luminous bacteria	PL	Baticados and Paclibare 1992
	1.0 ¹	Every 3 d	Bacteria	PL	Sunaryanto 1986
Erythromycin	2-6 ¹	Every 2 d	Bacteria	PL	Aquacop 1983
	2-10		Bacteria	PL	Aquacop 1983
	2-4	3-5 d bath	Bacteria	Larvae	Baticados and Paclibare 1992
Formalin	25-75	Prolonged	Ectoparasites	Juveniles	Limsuwan 1987
	25-30	24 h	<i>Epistylis</i>	Juveniles	Chen 1978
	50-100	30 min	<i>Epistylis</i>	Juveniles	Ruangpan 1982
	25	10-15 min	Disinfectant	Spawners	Platon 1978
	2-5	Routine bath	Bacteria, parasites	Larvae	Baticados and Paclibare 1992
Furans (furazolidone, Furacin)	1.0	Prolonged	<i>Vibrio</i>	Larvae	Limsuwan 1987
	2.0, 0.5 ¹	Prolonged	Bacteria	Larvae	Primpol 1990
	10-20	24 h	<i>Vibrio harveyi</i>	Larvae	Baticados and Paclibare 1992
	0.5-1.0	Routine	Bacteria	Larvae	
	50	24 h	Bacteria	Larvae	Baticados <i>et al.</i> 1990b
	0.1	Every other d	Bacteria	PL	Aquacop 1977
	3.0	Few h	Disinfectant	Spawners	Platon 1978
Malachite green	5-10	NA	<i>Lagenidium</i> spp.	Larvae	Lio-Po <i>et al.</i> 1982
	0.01		Fungi	Larvae	SCSP 1982
	0.006		Fungi	Mysis	Ruangpan 1982
	0.5	10 min	Disinfectant	Eggs	Kungvankij <i>et al.</i> 1986
	0.007		Bacteria, parasites	PL	Baticados and Paclibare 1992
Oxytetracycline	2.68-5	Prolonged	Bacteria	PL	Rattanavinijkul <i>et al.</i> 1988
	1.5	Every other d	<i>Vibrio harveyi</i>	PL	Baticados and Paclibare 1992
Trifluralin (Treflan)	100	24 h	<i>Vibrio harveyi</i>	Nauplius	
	0.01	Few h	Fungi	Eggs	Ruangpanich 1988
	0.2	Prolonged	<i>Lagenidium</i> spp.	Larvae	Limsuwan 1987
	0.1	Every 2-3 d	<i>Lagenidium</i> spp.	Eggs	Baticados <i>et al.</i> 1990a
	0.2	24 h	<i>Lagenidium</i> spp.	PL	Baticados <i>et al.</i> 1990a
	0.1	Every 2-3 d for 24 h	<i>Lagenidium</i> spp.	Larvae	Baticados <i>et al.</i> 1990a
	0.01-0.02	Every 2-3 d for 24 h	<i>Lagenidium</i> spp.	Larvae	Bell and Lightner 1992

¹Prophylactic treatment.

of fish and shellfish, and transfer of resistance to human pathogens (Braaten and Hektoen 1991), knowledge on the controlled use and careful selection of chemotherapeutants is imperative.

Although the Cosmetics, Devices and Drugs Act No. 27 of 1980 controls the importation, manufacture and sale of chemotherapeutic agents including antibiotics and disinfectants, the implementation of such legislation does not appear to be very effective (Subasinghe 1992). Since chemotherapeutants are readily available through pharmaceutical outlets and as there exists no legislation on the use of chemotherapeutants in aquaculture in Sri Lanka, legislation to regulate the use of chemotherapeutic agents in aquaculture is essential. Moreover, a program should be initiated to disseminate knowledge on the potential health hazards and known efficacies of chemotherapeutants used in aquaculture. Research is needed to determine the efficacies of the wide range of chemotherapeutants used in aquaculture and their residual patterns in cultured shrimp and wild fish and shellfish.

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