2001

# Physical, environmental, and chemical methods of disease prevention and control

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Lavilla, C. R. & Erazo-Pagador, G. E. (2001). Physical, environmental, and chemical methods of disease prevention and control. In G. D. Lio-Po, C. R. Lavilla, & E. R. Cruz-Lacierda (Eds.), Health management in aquaculture (pp. 97-110). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

http://hdl.handle.net/10862/731

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CHAPTER EIGHT

# Physical, environmental, and chemical methods of disease prevention and control

*Erlinda R. Cruz-Lacierda and Gregoria E. Erazo-Pagador*

The environment determines the balance between the host and the disease agent. Microorganisms are always present in the water and some of them cause disease only when the host have been weakened through exposure to stressful environmental conditions. Thus, the fish culturist must be able to maximize the environment and make it favorable to the cultured species.

This chapter deals with the general principles involved in the physical, environmental and chemical aspects of disease prevention and control that are applicable in the hatchery and the grow-out stages of shrimps and fishes. Specific chemical treatments against a particular disease are discussed in their respective chapters.

## PHYSICAL METHODS

Physical methods of disease prevention and control are based on the physiological tolerance of disease agents to adverse conditions such as increased or low temperature, absence of moisture, presence of deleterious irradiation; and the removal of pathogen sources or presence of physical barriers to prevent contact between the disease agent and the host.

Potential pathogens can be removed by ultraviolet radiation and through the process of microfiltration. Chemical pollutants can be eliminated by carbon filtration, biofiltration and water dilution. Exposure of tank or pond to heated water and sun-drying can also eliminate some microbial flora. Infected fish must be removed quickly and destroyed.

Such health classification scheme has been proposed by Ghittino and de Kinkelin (1975):

- Fish free of specific pathogenic organisms (SPF) – refer to fish free of all species-specific pathogens. The water supply must be completely sterile and exchanges of fish is possible only between SPF classified establishments.

- Fish free of coded pathogenic organisms (CPF) – include fish free of all diseases appearing in a list drawn up by an international agreement. For Southeast Asian countries, such a list has yet to be drafted. Water supply would have to be pretreated. CPF classified farms can receive SPF or CPF fish but cannot dispatch fish to SPF farms.
- Fish free of specified diseases (SDF) – relate to fish reared in water supplies in which pathogens could exist, multiply or be disseminated by wild fish. Disease could occur but readily controlled by therapy. Certification for freedom from certain diseases can be issued but guarantees only for the diseases listed in the document. Such a farm can receive fish from SPF or SDF farms as well as enterprises of similar sanitary level.
- Uncontrolled fish consist of fish not checked for the presence of disease or pathogens. Fish exchange is possible only with farms of similar category but can receive fish from the three foregoing ones.

This sanitary classification of fish farms can be used as basis for issuance of permits for fish import, export, exchange or restocking.

The International Council for the Exploration of the Seas (ICES) have recommended policy measures dealing with the introduction of aquatic species and guidelines for implementation, including methods to minimize the possibility of disease transfers. Such recommendation is the Revised Code of Practice to Reduce the Risks of Adverse Effects Arising from the Introduction and Transfers of Marine Species (Sinderman and Lightner, 1988). The ICES Code of Practice is as follows:

- A recommended procedure for all species prior to reaching a decision regarding new introductions;
- Recommended action if the decision is taken to proceed with the introduction;
- A suggestion that regulatory agencies use the strongest possible measures to prevent unauthorized introductions;
- A recommended procedure for introduced or transferred species which are part of current commercial practice; and
- A note recognizing that countries will have different attitudes toward the selection of the place of inspection and control of the consignment.

## ENVIRONMENTAL METHODS

Monitoring of the environment is extremely important for success in fish culture. The primary objective of the environmental method is to protect the host by intercepting the pathogen or cutting its pathway to the host. The sections that follow will attempt to describe some of the ways this is carried out.

## Proper Hatchery/Pond Design

Trained personnel and well-designed hatcheries or ponds are important requirements in ensuring that fish health management practices can be incorporated in the routine operations of an aquaculture system. The hatchery or farm should have access to a good water supply free from any type of pollution (Fig. 8-1). Pond development, wherever possible, should be adjacent to mangrove areas for protection from erosion, and to provide natural filter for farm effluent. Provision of independent water supply and drainage canals (Fig. 8-2) to each individual part of an aquaculture grow-out facility will ensure that water emerging from one pond compartment does not enter the other compartments. Fishponds should be kept free of wild fish and other potential carriers of infectious agents such as invertebrates, pests and predators. The farm/hatchery should be accessible by road to avoid excessively long transport time of the larvae or fish.

## Good Water Quality

Good water quality is crucial in the hatchery or pond; it can spell the difference between success and failure of the aquaculture enterprise. The lower the water quality, the fewer fish/shrimp it will support; the higher the water quality, the higher the production potential will be. Aside from being pathogen-free, the

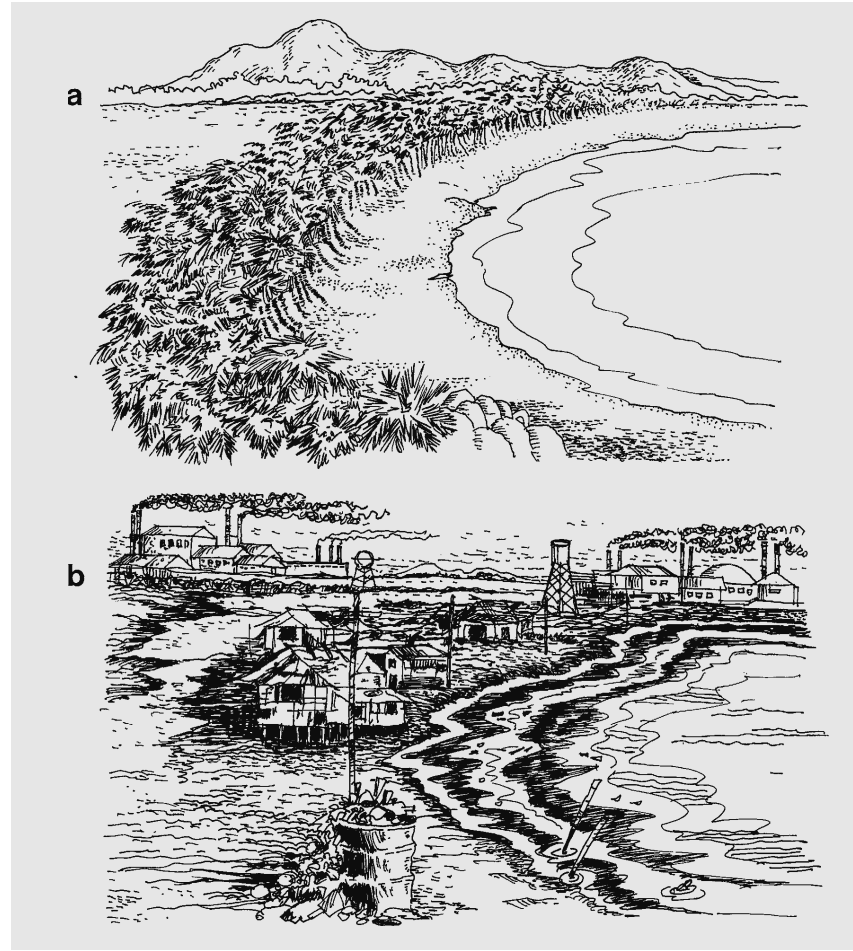


**Figure 8-1.** Ideal (a) and poor (b) hatchery sites; the latter is polluted with industrial and domestic wastes

water must meet the specific quality requirements of the cultured species. Monitor regularly the rearing water quality parameters such as salinity, pH, dissolved oxygen, ammonia and temperature. Ultraviolet radiation and filtration systems eliminate potential pathogens. Sand filters or filter bags will remove most of the debris.

Filter water with fine net (Fig. 8-3), cloth or cartridge filter before stocking in tanks. Clean filters regularly. Aerate and change rearing water regularly. Siphon off bottom sediments regularly to remove feces, organic debris and unused feed. Provide paddle wheels as aeration in ponds and a large settling reservoir to reduce the organic and particulate load before it is directed into ponds.

## Sanitary Practices

Cleanliness improves the general standard of health. It also prevents or retards the development of disease agents. Drain and dry the tank (Fig. 8-4) and pond bottom in between culture periods. Backwash or clean filters regularly (Fig. 8-5). Day-to-day hygiene measures should include siphoning out of organic material that accumulate in tank bottom, immediate removal of any dead fish, and careful control of aquatic vegetation in ponds. Provide properly labeled gear like scoop nets, buckets and pails for exclusive use in individual facilities. Use PVC or non-toxic plastic pipes, pails and other equipment parts. Workers should disinfect their hands with soap and water before preparing and administering feed, and before performing other jobs.

## Stress Avoidance

Stress plays a major role in the susceptibility of fish to disease. Most diseases are stress-related. Poor water quality, inadequate food, overstocking, handling, grading, and transfer and transport of animals are stress inducing factors. Regular monitoring of the health status of the stock can detect early signs or onset of diseases before they become uncontrollable.

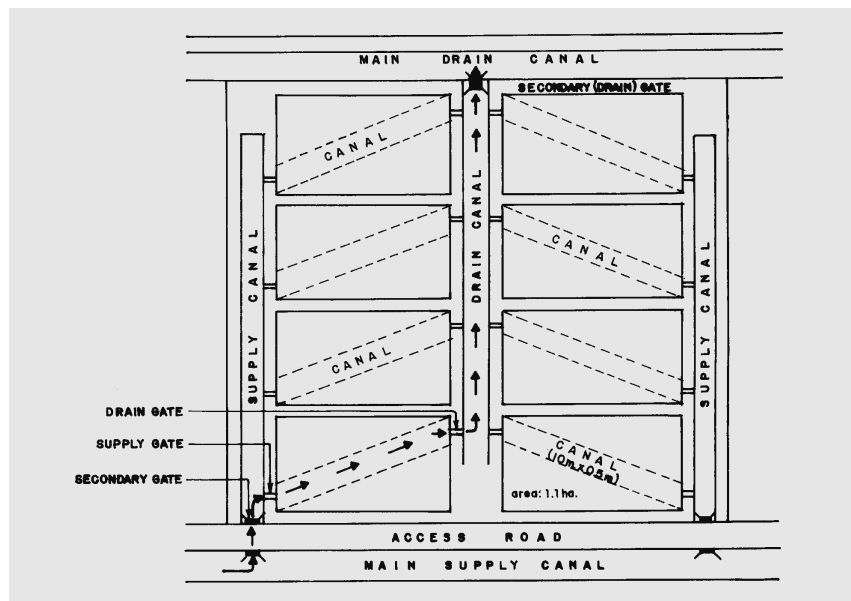


**Figure 8-2.** Lay-out of a 10-ha semi-intensive shrimp pond system

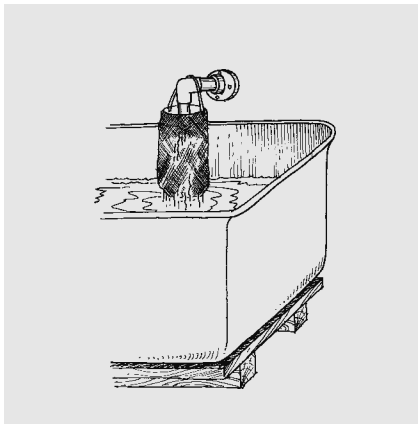

**Figure 8-3.** Filtration of water using fine net

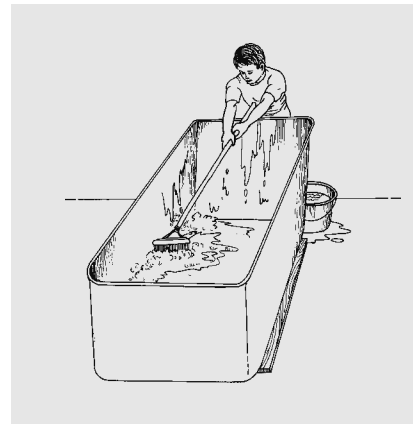

**Figure 8-4.** Thorough cleaning of rearing tank with stiff brush

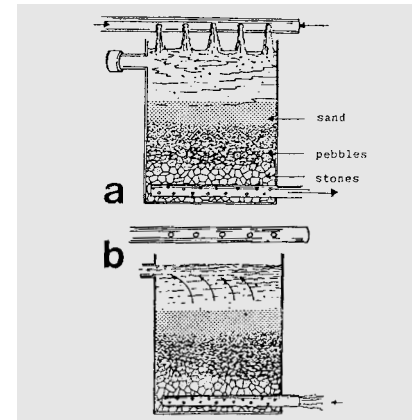


**Figure 8-5.** Sand filter system showing operationa inlet flow (a) and reverse flow or backwashing (b)

## Quarantine Procedures/ Legislation

Quarantine measures are very important for the prevention of the international spread of diseases of aquatic organisms. Legislations to impose quarantine procedures on fish imported and exported requiring health certification of incoming fish into countries will minimize worldwide spread of fish pathogens. Sanitary classification of farms can be instituted such that exchanges of fish occur only among farms of similar fish health status.

Quarantine should be practiced to minimize risk of disease among local species. Fish imported from abroad, or fish moved from one place to another within a country, should be placed in quarantine on arrival and should remain there until all danger has passed. The quarantine period should exceed the length of the longest latent period of the pathogens. Fish markets can become centers for the dispersal of pathogens. To avoid this danger, fish should be disinfected upon arrival at the market. The quarantine period for incoming stock must be observed for at least 2-3 weeks. Legislation of quarantine requirements should be imposed on all imported and exported fish to minimize the spread of disease, both within a country and outside. Quarantine ponds must be safely isolated and must be located downstream from all other ponds on the farms to minimize the danger of pathogen penetration.

## Termination Procedures

Termination procedures may also be used to control fish diseases. These include destruction of infected individuals, by burning, cooking or burying in limed pits. Disposal of infected individuals should be to areas that will not affect the culture system. Avoid contact between diseased and normal individuals. Disinfect the water supply system that may have carried the pathogen by draining and drying the affected tanks and ponds. Disinfect paraphernalia used on infected individuals.

## CHEMICAL METHODS

### Prophylactic Methods

Prophylactic treatment methods are protective or defensive measures designed to prevent a disease from occurring. They are used to combat external parasites and stress-mediated bacterial diseases.

#### Disinfecting culture facilities

*Tanks* – Rearing tanks should be disinfected in between rearing periods. Drain and scrub tank bottom and sidewalls using powdered detergent and plastic brush to remove debris. Rinse thoroughly to remove soap suds and loosened contaminants. Disinfect with 200-ppm chlorine for 1 h or with 100-ppm chlorine for several hours. Scrub tank bottom and sidewalls again. Rinse several times with clean freshwater and dry under the sun.

*Earthen ponds* – Drain the pond and then dry. Apply lime (0.5-1 ton/ha $CaCO_3$ or agricultural lime) and 20-ppm tea seed cake, or any of 600-ppm Roccal (benzalkonium chloride), Hyamine 1622 and Hyamine 3500 (quaternary compounds).

#### Disinfecting rearing water

*Chlorination method* — Chlorine is the cheapest disinfectant. One of the best and commonly used is calcium hypochlorite (powder form) or ordinary household bleach (Purex, Chlorox). Filter the water first. Chlorine loses its strength when exposed to air. It is reduced by organic matter (mud, slime, plant matter) and must be covered. Use 5 to 20-ppm chlorine for 12-24 h, then neutralize with sodium thiosulfate until residual chlorine becomes zero. Chlorinated, neutralized water must be used within 6 h as bacterial load increases after 12 h.

*Ozonation method* – Ozone ($O_3$-triatomic oxygen) is a more powerful oxidizing agent than hypochlorite. It can de-activate or destroy viruses and bacteria that might be transmitted through the water supply system. At 90-mg/L concentration and exposure for 20 min, ozone can control bacterial and viral fish pathogens in water supplies, although this level may not eliminate 100% of pathogens. Like chlorine, ozone is toxic to aquatic organisms. Oxygen ($O_2$) is a breakdown product of ozone, and oxidizing action may result in oxygen supersaturation or gas-bubble disease. The concentration of 0.005-ppm $O_3$ is the upper limit for continuous exposure. Ozonated water must be re-aerated before it is used.

#### Disinfecting materials

Materials like pails, brushes, scoop nets, secchi disk, glasswares, hose, etc. may be disinfected in between use in different culture facilities. Dip the materials in 400-ppm chlorine solution for a few seconds, and rinse thoroughly with clean water.

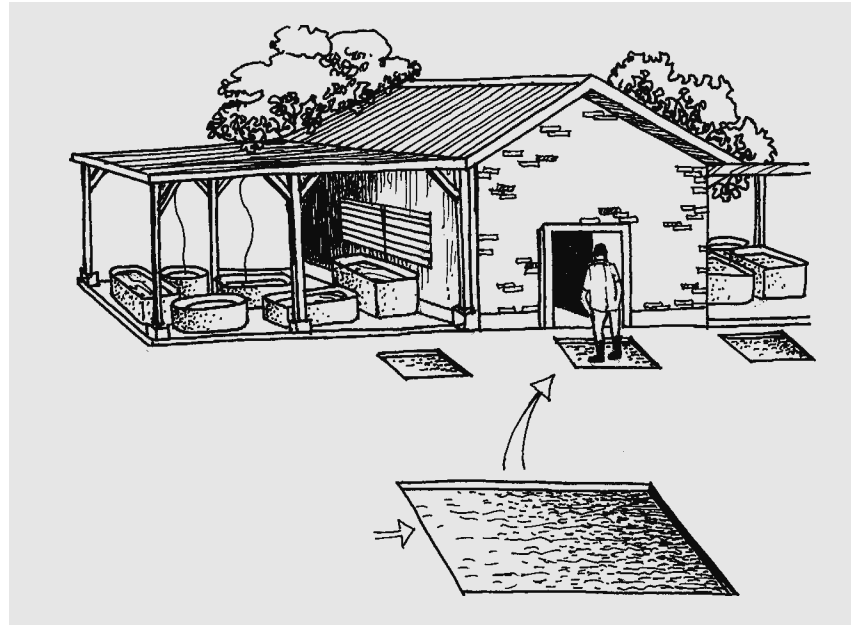

**Figure 8-6.** Disinfection rugs and trays at entrances of hatchery facilities

Disinfect footwear by placing 200-ppm chlorine or 3% Lysol solution in disinfecting rugs/trays at the entrance of aquaculture facility (Fig. 8-6). Wash rugs and change disinfectant regularly.

**Disinfecting feeds**

*Artemia* cysts – Cysts may be decapsulated in chlorine solution. Use 30 ppm chlorine or 10 ppm formalin, 1 h before hatching.

**Disinfecting the hosts (especially *Penaeus monodon*)**

Spawners – Disinfect with 5 ppm Treflan for 1 h or 50-100 ppm formalin for 30-60 min. Rinse spawners thoroughly in clean water.

Eggs – Disinfect with 20 ppm detergent for 2-4 h. Disinfection should be done at least 6 h before hatching. Rinse thoroughly and completely change water in hatching tank.

Larvae – Disinfect with 0.1 ppm Treflan (trifluralin) once every other day.

## Chemotherapy

Chemotherapy involves the use of drugs or chemicals for treating infectious diseases. It is considered as the method of "last resort" in any disease control program.

Factors to consider before using chemicals:

- Tolerance of the host to the chemical – Tolerance of fish varies with age, size, species, and health condition. Younger or smaller fish are more sensitive than bigger or older ones. Some species are better able to tolerate chemicals than others are. Fish weakened by disease become less tolerant to stress and environmental fluctuations.

- Efficiency of the chemical – The choice of what chemical to use is based on differential toxicity, that is, the chemical must be lethal to the target micro-organism but harmless to the host. It is essential to know the properties of the chemical such as the active ingredient, solubility and application method. The chemical must not harm the environment.
- Restrictions on the use of chemicals to treat food fish – Use only chemicals that break down rapidly and are eliminated quickly from all fish tissues to avoid tissue residue problems. The chemical must not form toxic or carcinogenic products during cooking of the contaminated flesh.
- Consequences of drug resistance – The indiscriminate use of antibiotics may lead to the development of drug-resistant strains.
- Economics – Chemicals are expensive, and one should know the value of the stock and the cost of treatment to determine the benefits that may be derived from their use.

The methods of chemical treatment are as follows:

**1. External methods** – These are used to control ectoparasites and other micro-organisms outside the fish. They are employed to reduce or eliminate potential pathogens from tanks, ponds, and from other materials. The external method may either be topical or by immersion.

Topical

This is the direct and simplest method for treating wounds, skin ulcers and other localized infection. Immobilize the fish before taking it out of the water for treatment. Apply the drug directly on the infected area. The method is labor-intensive and should be used only for high-value fish.

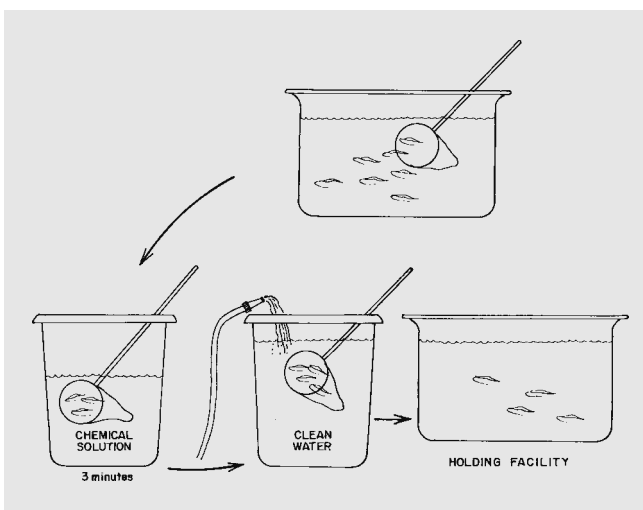


**Figure 8-7.** Dip method – fish are scooped out and suspended in the chemical solution for a few seconds to 3 min; rinse well with clean water and return to the clean/disinfected holding facility

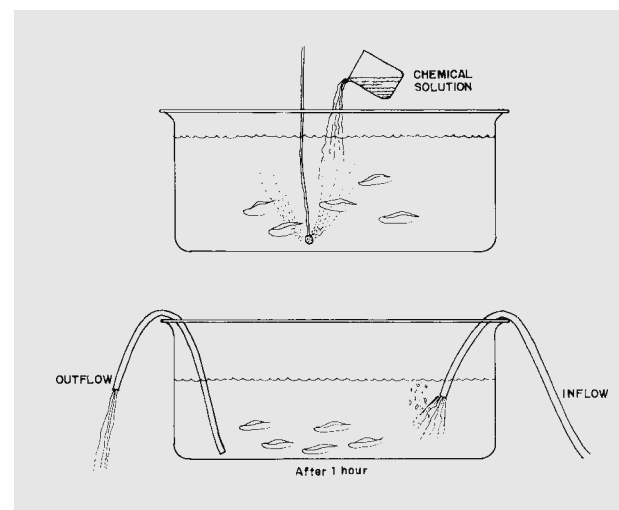


**Figure 8-8.** Short bath method – the chemical solution is added to the holding tank with fish, allowing the chemical/water mixture to remain with the fish for several minutes to 1 h; the solution is removed right after treatment and replaced with new, clean water

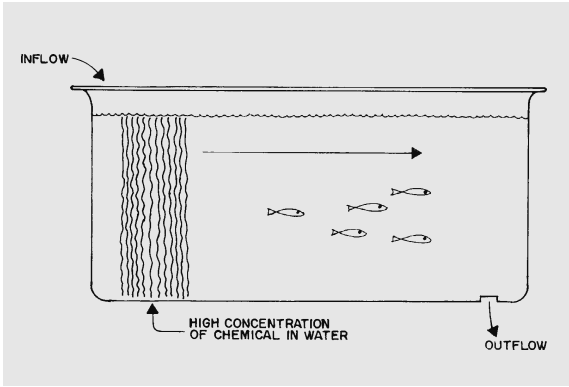


**Figure 8-9.** Flush method – the chemical solution is added in concentrated form at the inlet and allowed to pass through the water flow system and out the effluent pipe

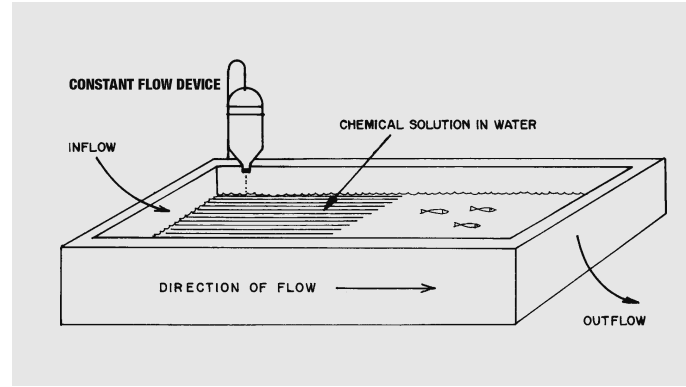


**Figure 8-10.** Flow through method – the chemical is added at a constant rate by a constant flow device; the chemical moves through and out of the container to be replaced by new clean water

Immersion

*Dip*. Place the fish in a scoop net and immerse in a high concentration of chemical solution for a specified time, usually from a few seconds to a few minutes. Rinse the fish immediately in clean water after treatment and return the fish to the clean/disinfected holding facility (Fig. 8-7).

*Short bath*. Add the chemical solution to the holding facility where the fish are to be treated, allowing the fish to remain in the chemical and water mixture for a designated time, usually a few hours or less (Fig. 8-8). After treatment, remove treated water immediately and replace with clean water.

*Flush*. Add a highly concentrated chemical solution at the water inlet and allow this to pass through the water flow system and out of the effluent pipe (Fig. 8-9).

*Long bath*. Treat the fish for a longer time, usually 12 h or more, in a chemical solution of low concentration.

*Flow-through*. Add the chemical at a constant rate through a metering device to give a consistent low concentration for the desired treatment time. The treated water moves through and out of the holding facility, and is replaced by new clean water (Fig. 8-10).

**2. Systemic treatment** – This is employed for treatment of systemic infections. Chemicals are added into the feed. The advantages of this method are that fewer chemicals are needed, environmental pollution is lessened and labor input is low. The disadvantages are the non-feeding of sick fish and that, since some drugs are not stable in moist diets, this would require introduction of more palatable components.

**3. Parenteral treatment** – This is the direct and most effective route of drug administration. Advantages are that accurate dosage can be administered and pollution of the environment is avoided. The disadvantages are that it is labor-intensive, it contributes to handling stress and it is good only for big and valuable stocks.

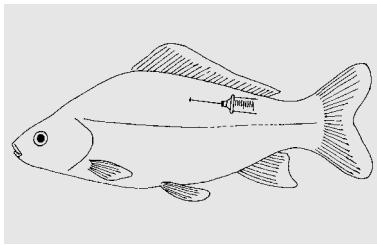

**Figure 8-11.** Intramuscular injection – needle is inserted into space posterior to the dorsal fin above the midline of the body

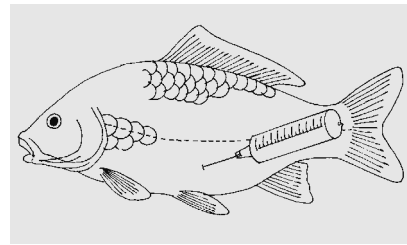

**Figure 8-12.** Intraperitoneal injection – needle is inserted into the visceral cavity or belly of the fish

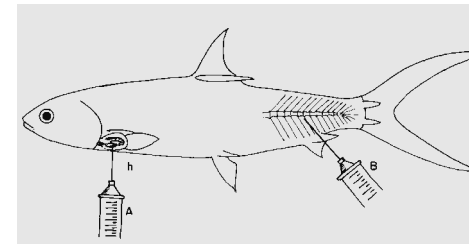


**Figure 8-13.** Intravenous injection – needle is inserted by (A) direct cardiac puncture or (B) through the caudal artery

*Intramuscular.* Insert the needle posterior to the dorsal fin above the midline of the body (Fig. 8-11). Absorption is slow (not very effective) or, sometimes, does not take place at all.

*Intraperitoneal*. This is the most common method of injection. Insert the needle into the visceral cavity or belly of the fish (Fig. 8-12). The drug must be highly absorbable and should be able to pass through either the intestinal wall or some other membrane to be absorbed into the fish system.

*Intravenous.* Insert needle by direct cardiac puncture, or through the caudal artery (Fig. 8-13). This results in rapid dispersal and is the most effective route for administering antibiotics. The only drawback is that this can be used only on large fish.

## Disadvantages of Chemical Treatment

- If used in closed recirculating systems, chemicals may cause adverse effects or destroy the nitrification processes in biofilters.
- Treatment may have adverse effect on natural food. It may induce oxygen depletion during their degradation, or may destroy algal blooms whose decay then depletes the dissolved oxygen in the water. It may also inhibit photosynthetic production of oxygen.
- Chemicals may leave harmful residues in the host.
- Diseased animals do not eat medicated feeds.
- Baths usually do not result in therapeutic tissue level and may be ineffective against systemic infection.
- Antibiotic-resistant strains may develop.
- Some drugs have immunosuppresive effects.

## Prevention of Drug Resistance

- Ensure correct diagnosis of the case.
- Use the prescribed dosage for a given period.
- Restrict use of drugs.
- Simultaneously administer two drugs that will not result in cross-resistance.
- Strictly observe implementation of the clearance/withdrawal period before the fish/shrimp can be harvested/consumed. In the tropics, this usually takes 2-3 weeks.

## Principles of Bioassay

A biological assay is a procedure involving use of the responses of aquatic organisms to detect or measure the presence or effect of one or more substances, wastes, or environmental factors, alone or in combination.

### Types of bioassay

*Short-term*. This type of bioassay reveals in relatively less time (usually 8 days or less) the relative toxicity of different toxicants to a selected test organism. It shows the relative sensitivity of various organisms to different conditions or variables like temperature and pH. It also determines the median lethal concentration (LC50), or the effective concentration values.

*Intermediate*. This bioassay is used when LC50 determination requires additional time (usually 8-90 days) for studies of the life stages of organisms with long life cycles, and to indicate toxicant concentrations for life cycle tests.

*Long-term*. This bioassay procedure is almost always a flow-through test. It determines the maximum allowable toxicant concentration, or safe concentration, for indicating water quality standards;

### Methods of adding test solutions

*Static.* The test animals remain in the same test concentration for the duration of the test.

*Renewal.* This is a static test where the test animals are transferred to a fresh test solution of the same composition at periodic intervals, usually every 24 h.

*Re-circulation*. This static test involves the circulation of test solution through test chambers. The test solution may be treated by aeration, filtration, sterilization or other means to maintain water quality.

*Flow-through*. Measured quantities of dilution water and the stock toxicant solution are mixed and delivered periodically to the test containers to provide continuous flow-through of the test toxicant.

### Test procedures

*Criteria for selecting and preparing test animals:*

Sensitivity to the materials under consideration;
Availability and abundance;
Recreational, economic and ecological importance;
Adaptability to laboratory conditions;
Suitability for bioassay tests;
Originating from a single common source;
Uniform in size and of the same stage of maturity;
Healthy, free from disease and parasites;
No previous exposure to heavy metals, pesticides, and other substances;
Acclimation to laboratory conditions for at least 10 days;
During acclimation, provide for daily feeding. Mortality should be less than 10% of the total population.

*Experimental water*. The water should not be polluted or contaminated with wastes from any source.

*Experimental design*. There should be a minimum of 5 test concentrations and control in at least duplicate containers.

*Test concentrations*. Express liquid waste concentrations as percent on a volume to volume basis. Express concentrations of non-aqueous wastes and of individual chemical composition by weight. Indicate whether the LC value is based on concentration of total material or active ingredient.

*Test containers*. Glass aquaria should be clean and of uniform size and shape. They should measure 15-30 cm deep and should be arranged at random in the testing area. If replicates are used, the series of test containers should be randomized separately.

*Number of test animals or biomass*. (1) There should be at least 10 animals per concentration. (2) Less than 10 animals may be used for the range finding test. (3) There should be a maximum of 1 g fish/ liter of water. (4) Distribute animals at random by adding one at a time to each container

*Preparing test solutions*. Test solutions must be freshly prepared. Also avoid unnecessary exposure to air and light.

*Feeding*. Do not feed for at least 2 days before the test. Do not feed during the entire experimental period for short-term tests (96 h or less).

*Biological data and observations*. Observe and record mortality at periodic intervals. The usual indicator for death is no movement, especially no gill movement in fish, and no reaction to gentle prodding. Remove dead organisms as soon as observed.

Report effects using terms like erratic swimming, loss of reflex, discoloration, changes in behavior, excessive mucus production, hyperventilation, opaque eyes, curved spine, and hemorrhaging.

*Physical and chemical water quality*. Measure temperature, salinity, hardness, alkalinity, pH, dissolved oxygen, ammonia and nitrite at the beginning of the test, and daily thereafter.

*Calculation, analysis, and reporting of results*. The recommended measures or indices of relative toxicity are 48 and 96 h LC50. Always compute for 95% confidence limits for LC50 and EC50 values.

The most widely used methods for calculating an LC50 and confidence limit are probit, logit, moving average and Lithcfield-Wilcoxon (1949). Other methods are straight line interpolation and the Reed-Muench method (1938).

Report the LC50 with specified exposure time and the confidence limits of LC50.

Provide descriptions of test organisms (species, source, size, weight), procedures for acclimation to test conditions and observations on behavior during the test). Describe also the source of experimental water and its characteristics, source and properties of tested material and concentrations of the test solutions. Indicate also the experimental temperature, test method, test conditions (type of container with volume and depth of solution, number of organisms), and the criterion of response.

## SUMMARY

Disease prevention is a primary and cost-effective method in fish health management. It is more effective and economical than attempting to stop a disease that has already set in. The recommendations given above will greatly reduce the possibility of disease outbreaks.

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