
Use of Chemicals in Aquaculture in Asia

Proceedings of the Meeting on the Use of Chemicals In Aquaculture in Asia

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FOREWORD

The use of chemicals in aquaculture systems for various purposes is widely recognized. While aquaculturists acknowledge that some operations are reliant on chemical usage, they also realize the potential danger associated with chemical misuse. A meeting in the use of chemicals in aquaculture in Asia was conceived by Dr. Uwe Barg of FAO and Dr. Jurgenne Primavera of SEAFDEC Aquaculture Department during a GESAMP working group meeting in Victoria, Canada in late 1994. A Local Organizing Committee was created at SEAFDEC to coordinate closely with FAO on the choice of reviewers and Asian experts to put together the papers for presentation and discussion in the meeting.

The Expert Meeting on the Use of Chemicals in Aquaculture in Asia was convened at the Aquaculture Department of SEAFDEC last May 20 –22, 1996. More than a hundred participants and observers composed of scientists and aquaculturists, both from the private and government sectors, from 20 countries – Australia, Bangladesh, Cambodia, the People’s Republic of China, Denmark, India, French Polynesia, Indonesia, Japan, Malaysia, New Caledonia, Panama, Singapore, Sri Lanka, Taiwan, Thailand, United Kingdom, the USA, Viet Nam, and the Philippines – attended. The meeting synthesized all information on the use of chemicals in aquaculture Asia with emphasis on the various aquaculture systems and species to which they were applied, and the country regulations regarding their distribution and usage. This was achieved through the various country and area papers presented by known experts. Special review papers covering topics on the effects of chemicals on human health and the environment, problems with drug resistant fish pathogens, as well as their delivery through feeds and water were presented by scientists only from Asia but also from various parts of the world. The discussions and workshops came up with recommendations on how to mitigate the impact of chemical use on the environment and consumers. Experts estimated that there may be no less than 50 veterinary drug products in each country that have found their way to fish farms. The meeting was opportune because sustainability of the aquaculture industry has been increasingly linked to the integrity of the environment.

We believe that the meeting was a success, its objectives having been met as documented in this volume. However, the meeting brought a realization that mitigating the impact of chemical use could be a drawn out and expensive process. Governments need to impose restrictions or institute policies to regulate chemical use; the private sector needs to be educated on disease development, prevention and control, and the proper use of chemicals; and the research-and-development sector needs to conduct more studies and find more environment-friendly alternatives to chemicals. But we are hopeful that we have taken the first step.

The recommendations made in this volume were discussed by the Working Group on Environmental Impacts on Coastal Aquaculture of GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environment Protection) during its meeting from 24 to 28 May 1996 also held at the SEAFDEC Aquaculture Department. The proceedings of that meeting are contained in GESAMP Reports at Studies No. 65 entitled “Towards safe and effective use of chemicals in coastal aquaculture” (GESAMP, 1997).

We thank our co-organizer, the Inland Water Resources and Aquaculture Service of the Food and Agriculture Organization (FAO) of the United Nations; and our cooperators, the Network of Aquaculture Centres in Asia and the Pacific (NACA), Japan International Research Center for Agricultural Sciences (JIRCAS), Taiwan Fisheries Research Institute, and the Canadian International Development Agency (CIDA) through its ASEAN Canada Fund. The effort of my predecessor, Dr. Efren Ed. Flores, in organizing this meeting is very much appreciated.



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REVIEW PAPERS

Chemicals in Asian Aquaculture: Need, Usage, Issues and Challenges

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ABSTRACT

This paper outlines the opening introductory presentation made at the “Expert Meeting on the Use of Chemicals in Aquaculture in Asia,” which was held 20-22 May 1996 at the SEAFDEC facilities in Tigbauan, Iloilo, the Philippines. Its purpose is to provide a balanced and realistic perspective on the needs, issues and challenges with respect to the use of chemicals in Asian aquaculture. We hope to assist participants in identifying development opportunities and in differentiating real hazards from hypothetical threats to cultured organisms, end-users and the environment as a consequence of chemical use. We do not attempt to provide answers to issues related to chemicals in Asian aquaculture, but rather offer some basic directives and opportunities to the workshop participants to assist them in their discussions and in the compilation of realistic recommendations.

INTRODUCTION

During the past decade, world aquaculture production has grown tremendously, averaging an annual growth rate of 9.4% during the period 1984-1994. Total world aquaculture production is now on the order of 25.5 million mt, valued at \$US 39.8 billion, and accounts for some 21.7% of the total world fishery landings. China remains the largest producer, accounting for 60.4% of total world production.

Although the culture of high-priced species such as shrimp and salmon often receives the lion's share of attention, it is important to note that low-value inland finfish (e.g., Indian major and Chinese carps, tilapia, etc.) produced in extensive or semi-intensive culture systems comprise the bulk of world aquaculture production. Crustaceans, by comparison, represent only 4.2% of total aquaculture production by weight, and only 18.1% by value. Developing countries contribute more than 86% of total world production, with LIFDCs (Low Income Food Deficient Countries) accounting for more than 75% of the total. The LIFDCs contribute more than 80% of the world finfish production, of which more than 95% is derived from inland freshwater fish culture. Production from the LIFDCs continues to grow at an above average rate of some 13% annually, indicating aquaculture's real and potential contribution to providing low cost protein to those among the world's most impoverished sectors.

In aquaculture, as in all food production sectors, one of the external inputs required for successful crop production is chemicals. In the most simple, extensive systems, this may be limited to fertilizers (most often manure), while in more complex semi-intensive and intensive systems a wide range of natural and synthetic compounds may be used. It is safe to say that, as in agriculture, chemicals

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are an essential “ingredient” to successful aquaculture, one which has been used in various forms for centuries.

The purpose of this introductory presentation is to provide a balanced and realistic perspective on the needs, issues and challenges with respect to the use of chemicals in Asian aquaculture. We hope to assist in identifying development opportunities and in differentiating real hazards from hypothetical threats to cultured organisms, end-users and the environment as a consequence of chemical use. We will not attempt to provide answers to issues related to chemicals in Asian aquaculture, but intend to offer some basic directives and opportunities to the workshop participants to assist them in their discussions and in the compilation of realistic recommendations.

CHEMICALS IN AQUACULTURE

What are Chemicals?

There are many different classifications and working definitions of “chemicals” (see Van Houtte, this volume). These include classification of “drug groups” (see Alderman and Michel 1992), the classification provided by the International Council for the Exploration of the Sea (ICES 1994), a classification developed specifically for prawn culture (see Primavera *et al.* 1993), as well as various working definitions for scientific and legal purposes. In aquaculture, chemicals can be classified by the purpose of use, the type of organisms under culture, the life cycle stage for which they are used, the culture system and intensity of culture, and by the type of people who use them.

Why are Chemicals Used in Aquaculture?

Chemicals have many uses in aquaculture, the types of chemicals used depending of the nature of the culture system and the species being cultured. They are essential components in:

- pond and tank construction,
- soil and water management,
- enhancement of natural aquatic productivity,
- transportation of live organisms,
- feed formulation,
- manipulation and enhancement of reproduction,
- growth promotion,
- health management, and
- processing and value enhancement of the final product.

The benefits of chemical usage are many. Chemicals increase production efficiency and reduce the waste of other resources. They assist in increasing hatchery production and feeding efficiency, and improve survival of fry and fingerlings to marketable size. They are used to reduce transport stress and to control pathogens, among many other applications.

Concerns Regarding Chemical Usage

There are several important concerns with regard to the use of chemicals in aquaculture. These include:

- Human health concerns related to the use of feed additives, therapeutants, hormones, disinfectants and vaccines.
- Product quality concerns related to such issues as the occurrence of chemical residues in

aquaculture products, their use in the enhancement of product quality and in the preparation of value-added products, the need for consumer protection from hazardous usage, and issues surrounding consumer acceptance of the use of chemicals in the production of fish and shellfish destined for human consumption.

- Environmental concerns, such as the effects of aquaculture chemicals on water and sediment quality (nutrient enrichment, loading with organic matter, etc.), natural aquatic communities (toxicity, disturbance of community structure and resultant impacts on biodiversity), and effects on microorganisms (alteration of microbial communities and the generation of drug-resistant strains of bacteria).
- The general lack of knowledge concerning the effects and fates of chemicals and their residues in cultured organisms and within the aquaculture system itself. Similarly, information is lacking on the actions and fate of chemicals used in aquaculture in the aquatic environment in general (impacts on non-cultured organisms, sediments and the water column).
- The lack of alternative means for chemical application. Development of highly specific targeted chemicals that have reduced side effects and environmental implicates is needed. The availability of affordable treatments suitable for aquaculture systems raising low-value species needs to be improved.

Human health and environmental concerns regarding the use of chemicals in aquaculture are reflected in the FAO Code of Conduct for Responsible Fisheries (FAO 1995). In fact, the Code calls upon States to:

- Promote effective farm and fish health management practices favouring hygienic measures and vaccines. Safe, effective and minimal use of therapeutants, hormones and drugs, antibiotics and other disease control chemicals should be ensured. (Article 9.4.4).
- Regulate the use of chemical inputs in aquaculture which are hazardous to human health and the environment. (Article 9.4.5)

ISSUES AND OPPORTUNITIES

Future Issues

There a number of currents trends in global aquaculture which will continue to make the use of chemicals a subject for future discussion and debate.

Increased market demands which create pressure for the production of high-value species such as shrimp and salmon may, in turn, lead to increased intensification, more highly sophisticated culture systems, and a corresponding increase in both responsible and irresponsible chemical usage.

Recent measures such as the Agreement on the Application of Sanitary and Phytosanitary Measures (GATT 1994) have a major impact on the conditions of international trade in aquaculture products, both increasing the freedom of movement of products and requiring that exporting countries meet uniform standards with regard to quality, production procedures, etc. Various organizations have put forth real and suggested standards (via legislation, agreements, codes of conduct, guidelines, etc.) in such areas as production procedures and ethics, minimal residue levels (MRLs), allowable daily intakes (ADIs), withdrawal periods for chemicals used in treatment and prophylaxis, and for standards for aquatic animal health.

For many of these issues, policy and legislation is rapidly advancing, outstripping the advances in

technical knowledge through applied research which are necessary to make informed decisions and to support implementation of policy and law. This is particularly true when standards are set for chemical usage in aquaculture. An example is the need for research data related to chemical residues in aquaculture products, where information is needed on MRLs and withdrawal periods, and for chemical registration and approval.

It is clear that chemicals are an important component in aquaculture systems, and that further advancement of the aquaculture industry, particularly in systems undergoing intensification, may, in some cases, continue to be tied to increased chemical usage. However, development of vaccines, for example, may also lead to reduction in the use of therapeutants, and the use of synthetic chemicals is not required in all systems.

Major Challenges and Opportunities

There are three broad groups of people who deal directly with aquaculture chemicals: manufacturers and traders, farmers, and consumers.

Manufacturers and traders should work towards manufacturing and supplying “appropriate” species- and systems-specific chemicals. They should facilitate availability through ensuring an adequate supply of such chemicals; should provide accurate and adequate information to farmers, and avoid illegal trade. The private sector should also conduct more research and development towards reducing the harmful impacts of chemicals in aquaculture systems, and should work to improve public awareness of the pros and cons of chemical use.

Farmers should work to understand the on-farm management of chemical use in order to increase effectiveness and minimize adverse impacts. They should also inform themselves of the advantages and disadvantages of chemical use in each specific situation. Aquaculturists need to increase their awareness of the short-, medium- and long-term implications of the use of a chosen chemical.

Consumers should be aware of the health consequences of chemical misuse. They should inform themselves of the benefits, as well of the hazards, arising from chemical use, and should guard against undue influence by criticisms against aquaculture based mainly on emotional arguments that have little basis in scientific fact. However, where evidence strongly indicates the need for constructive change within the aquaculture industry, consumers should support advocacy groups working towards this goal.

Policy makers, researchers, and scientists should work together in addressing the issues of chemical use, with the view to reduce the adverse impacts. More research is needed, and they should focus on providing answers to the problems related to the use of chemicals. More research efforts should be made towards finding non-chemotherapeutic solutions to health management and disease control. There is a need to distinguish between perceived problems (i.e., subjective views) and potential hazards (which can be pre-determined and evaluated scientifically).

REFERENCES

- Alderman DJ, Michel C. 1992. Chemotherapy in aquaculture today. p. 3-24. In: Chemotherapy in Aquaculture: from Theory to Reality. OIE, Paris.
- GATT Secretariat. 1994. Agreement on the application of sanitary and phytosanitary measures, p. 69-84. In: The results of the Uruguay Round of multilateral trade negotiations. The legal texts. Secretariat of the General Agreement on Tariffs and Trade, Geneva, 558 p.

- ICES 1994. Report on the Working Group on Environmental Interaction of Mariculture. Cork, Ireland, 25-31 March 1994. Copenhagen, ICES. CM 1994/F:3. Mariculture Committee. Ref. E, Marine Environmental Quality Committee, ACME, 159 p.
- Primavera JH, Lavilla-Pitogo CR, Ladja JM and dela Peña MR. 1993. A survey of chemical and biological products used in intensive prawn farms in the Philippines. Mar. Pollut. Bull 26: 35-40.

Antibacterial Chemotherapy in Aquaculture: Review of Practice, Associated Risks and Need for Action

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ABSTRACT

This paper briefly reviews the use of chemicals to prevent and treat bacterial diseases in aquaculture, and provides a detailed summary of the current state of knowledge on the development of bacterial resistance to antimicrobial agents in fish and shellfish. The topics covered include mechanisms of resistance, resistance of bacterial fish pathogens, resistance to antibacterial agents associated with use in aquaculture, and factors causing selection of resistant variants. Emphasis is placed on avoiding and solving problems related to bacterial resistance in aquaculture, and recommendations on antibiotic usage in aquaculture are made.

INTRODUCTION

Interactions leading to bacterial disease in fish depend on the availability of the pathogen, the quality of the environment and the general health status of the fish. Balance of these conditions can ensure fish health without the use of antibacterial agents.

Health is promoted by ensuring good water quality, optimizing stocking densities and providing balanced nutrition. It is very important to eliminate highly specific pathogens from the stock and system (e.g., *Renibacterium salmoninarum*) or, in the case of opportunistic pathogens such as vibrios or motile aeromonads, to reduce the bacterial load. Resistance to disease can be enhanced by use of specific vaccines, where they are available, or more generally, by non-specific stimulation of the innate defenses.

However, aquaculture is driven by commercial forces, and stocking densities and rearing conditions are adjusted to maximize returns within the limits of acceptable risk. Within this scheme antibacterial agents are used widely. They are used both prophylactically, at times of heightened risk of disease, and therapeutically, when an outbreak of disease occurs in the system.

ANTIBACTERIAL CHEMOTHERAPY IN AQUACULTURE

History of Use

Antibacterial chemotherapy has been applied in aquaculture for over 50 years, with early attempts to use sulphonamides in the treatment of furunculosis in trout and the tetracyclines against a range of Gram-negative pathogens. However, they didn't come into general use until the 1970s when the sulphonamides were used, potentiated with trimethoprim. Since then, their use has grown, both in numbers and quantity, as the problem of bacterial disease has increased.

The potential of most veterinary antibacterials for use in aquaculture has been considered, and now countries vary widely in the drugs they use in their aquaculture systems. More details of their use throughout Asian countries are given by other contributors to this volume.

Methods of Application

Antibiotic usage in aquaculture is predominantly by three methods of administration, namely:

- a) oral therapy (in feed)
- b) immersion therapy (bath, dip, flow or flush) or
- c) injection.

Topical therapy, by ointments, sprays or brush, is also used for valuable individual fish or broodstock, but the most commonly used methods are those given above. Combination therapy i.e., oral and bath, is used in some situations.

Medicated feed is usually prepared on site by mixing the drug with pelleted feed and surface coating with an agent such as oil, gelatin or whole egg, or simply mixing with trash fish. Alternatively, the drug may be incorporated by the feed mill where commercial diets are prepared. The main advantage of oral therapy is that it does not stress the fish. The disadvantages are that this route is unavailable when fish are anorexic, a clinical sign often present when fish are sick and, moreover, leaching of the drug from the feed may occur prior to ingestion.

Immersion therapy, commonly used for problems involving ectoparasites, is used less often to treat bacterial disease; however, land-based hatcheries and tank systems, especially marine finfish hatcheries, do use antibiotic baths. These usually last 1-2 h, but more prolonged baths are not uncommon. This method is employed where the biomass is small, such as with fry, and when adequate oral therapy is impractical, as with larvae. Tank water volume is usually reduced, and consequently, the amount of drug required is reduced. The discharge of such treated water, however, poses an environmental threat that should not be dismissed.

Injection of antibiotics, usually by interperitoneal or intramuscular routes, has been utilized historically for individual fish or valuable broodstock. Recently, there has been increased interest in this method as an effective means of clearing bacterial infections from carrier fish or in conjunction with vaccination to confer protection before the immune response is mounted (Inglis *et al.* 1996).

Concerns About the Use of Antibacterials in Aquaculture

Antibacterial chemotherapy has been a cornerstone upon which the aquaculture industry has been built. In the growing industry there were many outbreaks of disease, as wild species were first kept in captivity and before the full significance of environmental aspects of health control was appreciated. Initially, field developments outstripped the rate at which the body of scientific knowledge underpinning applied chemotherapy was being gathered, and the use of antibiotics was essential in preventing the commercial collapse of many aquaculture enterprises. At first antibacterial chemotherapy was highly successful, possibly to the extent that drugs were relied upon to increase yields and obviate more costly disease control strategies. Unfortunately, this has led to problems, and concern is now centred on treatment failures, environmental impacts and risks to human health.

Antibacterials may disturb the balance of the environmental microflora, and this is the subject of a later paper (see Weston, this volume). The risk posed to human health by disturbance of the gastrointestinal flora, selection of resistant strains and allergies is also addressed elsewhere (see Sinhaseni *et al.*, this volume).

Treatments may fail for several reasons, but probably the most consistent and fundamental cause of their failure is the emergence of resistant bacteria. This paper will deal with antibacterial chemotherapy and associated resistance.

RESISTANCE

Mechanisms of Resistance

An improved understanding of how resistance emerges and is selected for among bacteria is essential in evaluating the impact of aquacultural use of antibacterial agents, identifying the high risk procedures and designing ways to reduce these effects. Bacteria acquire resistance by acquisition of foreign DNA or by modification of the chromosomal DNA. Examples of both are found among bacterial fish pathogens, and these are well illustrated in relation to the tetracyclines and the quinolones. A brief consideration of these compounds is helpful in elucidating the causal relationship of drug use and emergence of resistance and in planning intervention strategies to reduce negative effects.

Tetracycline resistance. Firstly, there is evidence that in evolutionary terms, the origins of tetracycline resistance is remote. Tetracyclines are produced by species of *Streptomyces* (which produce numerous other groups of antibiotics) possessing tetracycline resistance determinants. A popular theory is that resistance determinants originate in such organisms and were then disseminated by interspecies transfer by a variety of routes (Chopra 1985). DNA encoding resistance may be transferred by plasmids, conjugative transposons, and bacteriophages, as well as free DNA. Plasmid-mediated resistance can occur with a high transfer frequency. It may be expressed by extrachromosomal replication of the plasmid with subsequent opportunities for spread within the species or to other genera; or it may be transferred to the chromosome where it becomes integrated. Particularly in the latter case, when the selection pressure is removed, the potential for expressing resistance remains.

It has been suggested (Levy 1989) that tetracycline resistance has been evolving for a very long time (millions of years), perhaps in response to competition with organisms producing tetracycline-like substances. The use of tetracyclines in human and veterinary medicine has been relatively recent, yet resistance factors were found before use was widespread and from remote locations.

Quinolone resistance. A major group of antimicrobial agents used in aquaculture, but which has been synthetically produced, is the quinolones. Transferable resistance of the type described has not yet been recorded (Courvalin 1990). Nevertheless, resistance to these agents does arise and increases rapidly under pressure of their use. Quinolones kill bacteria by interrupting the DNA supercoiling (Hooper and Wolfson 1989). The DNA repair mechanisms may then cause mutations coding for resistance (Lewin *et al.* 1990). Laboratory evidence suggests that these mutations are stable. The mutants survive well and may grow to produce a dominant sub-group. In this case, use of the drug has been the cause of resistance developing where it had not been before. The long-term effect on the environmental microflora is not known, and the full implications of this are not yet realized.

Expression of resistance. Laboratory studies have shown that expression of resistance is selected against in the absence of the drug (Lee and Edlin 1985, Modi *et al.* 1991). The term "persistence" has been used (Bryan 1989) to describe the form of resistance only manifested in the presence of the antibiotic. "Persistent" strains are detected only during and shortly after therapy. They then recede but remain in the environment until they emerge under positive selection pressure. This is of particular importance when sensitivity is determined at the outset of an epizootic and therapy introduced to which the infecting strain rapidly becomes resistant. This mechanism may also affect findings with laboratory collections that have been cultured for some time *in vitro* before

minimum inhibitory concentrations are determined (Smith *et al.* 1994).

Tetracycline resistance has been found to survive in the microbial flora of farm animals years after tetracycline has stopped being used in the feed (Smith 1975). Such persistence within an ecosystem suggests either the continuing presence of the tetracycline or else that the intrinsic deleterious effect of encoding the resistance has been attenuated.

The use of antibacterial agents may result in mutations to resistance among bacteria as well as the selection of resistant variants that are already present in the environment. Under positive selection pressure of drug use, they will increase in relative proportion. When the drug is withdrawn they may recede but are unlikely to disappear.

Resistance of Bacterial Fish Pathogens

Interpretation of results. Resistance is a relative term allowing comparison of variants within a strain or between species. It is determined for fish pathogens, as more generally, *in vitro*, and the numerical value of a zone size in a disk diffusion test or end-point in a serial dilution test translated into resistant, moderately resistant or sensitive. Many other methods are available (see Piddock 1990) and include measurement of a range of bacterial activities such as pH change, bioluminescence, electrical conductivity or impedance. Results are affected by between-laboratory variation in techniques, but more importantly, by variation in interpretations of results. With some drugs (e.g., oxytetracycline), many groups of bacteria display a clear bimodal distribution of sensitivity, and classification into sensitive or resistant is easy. Problems arise with strains designated of intermediate sensitivity, as happens when resistance is increased in small steps (Inglis and Richards 1991). The problem of differences in the interpretation of results has been well exemplified in a between-laboratory study involving six countries in Europe. However, while it should be possible to overcome this source of error within a laboratory or groups of co-ordinated workers by use of standardized techniques, and to achieve comparable results and classification of the same group of bacteria, this alone would be insufficient to predict clinical efficacy. Culture conditions also must be considered.

Cultural conditions in determining sensitivity. The environment of a pathogen in artificial culture conditions and in clinical use differs, and as a consequence, the concentrations required to inhibit or kill in the two situations may differ. Laboratory media, especially those designed for antimicrobial sensitivity testing, do not simulate *in vivo* conditions. The biological activity of oxolinic acid and oxytetracycline is reduced in the presence of Mg^{2+} and Ca^{2+} so that the efficacy of these agents in fish in sea water is much lower than in fresh water (Barnes *et al.* 1995). The presence of buffers, availability of iron and incubation temperature may all be different from the *in vivo* situation, and have an effect on the outcome (Inglis *et al.* 1991, Martinsen *et al.* 1992). The condition of the bacterial inoculum, which has been grown in the laboratory on artificial media, subjected to centrifugation etc., is also different from that *in vivo*.

Clinical relevance. Determination of *in vitro* sensitivity is required to be reliable, not only to allow detection of resistance changes, but also to be a good predictor of clinical efficacy. In medicine, prediction of efficacy is based on the minimum inhibitory concentration (MIC), pharmacokinetics and clinical experience. If the lowest MICs of sensitive strains are low, the prediction of a good clinical outcome can be made with considerable confidence. With intermediate resistance, the laboratory prediction of the outcome of a clinical efficacy is less reliable.

A major factor affecting clinical outcome is the concentration of the antibacterial agent, in its active form, that is achieved at the site of infection. This is further influenced by the terminal half-life of the agent and the total amount present during the dosing period. While many sites within the animal can become infected, in the case of fish the window of opportunity for treatment may be restricted to the pre-clinical stage, when it is still possible to deliver an effective dose by feeding.

Resistance to Antibacterial Agents Associated with Use in Aquaculture

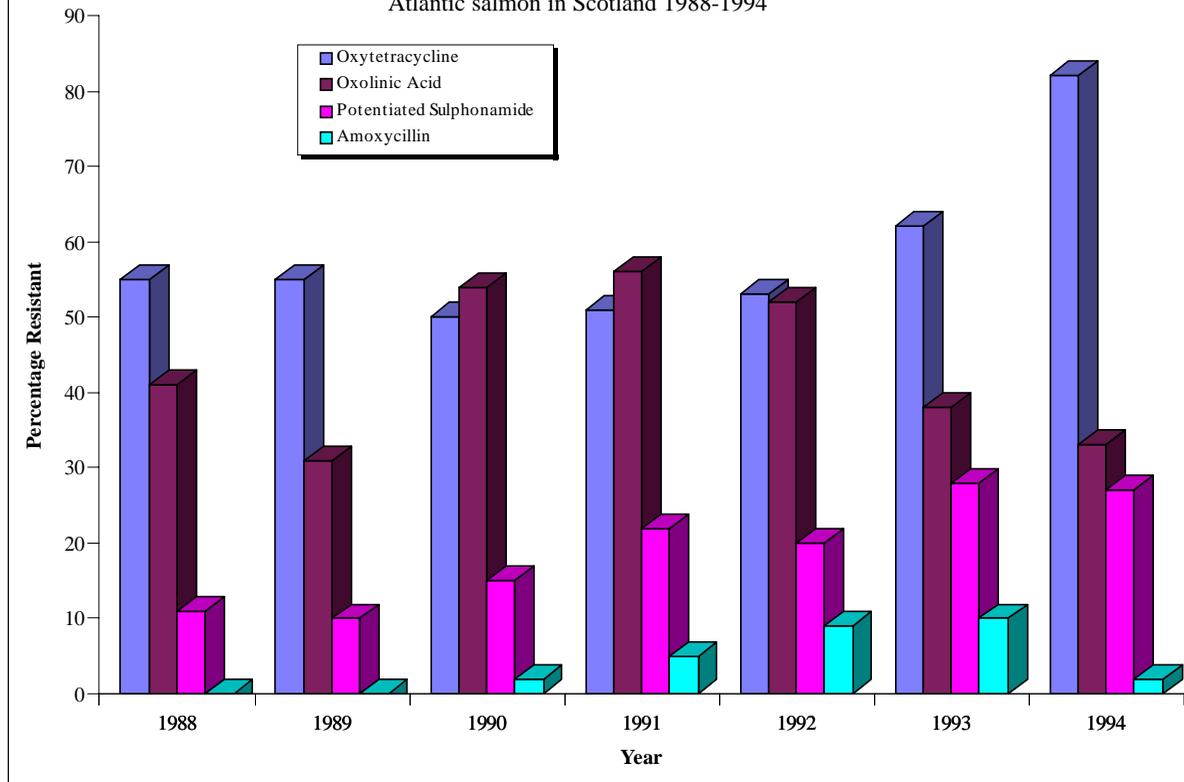
Frequency of drug use and emergence of resistance. There is widespread concern that the use of antibacterial agents in aquaculture has led to the emergence and selection of resistant bacteria. In general terms, it is agreed that antimicrobial resistance is associated with frequency of use in an environment, and there are several studies that illustrate this (Hamilton-Miller 1990, Kruse 1994). In food-producing animals kept under intensive conditions, common pathogens emerged with resistance against commonly used drugs. Increased frequencies of resistance to penicillin of *Staphylococcus aureus* causing mastitis on dairy farms (Prescott and Baggot 1988) and resistance of *Escherichia coli* from pigs to sulphonamides, streptomycin and tetracycline has been reported. The response in these industries has been to move from one drug to another as resistance catches up. In aquaculture, it is reasonable to assume that a similar thing has happened: that the increased use of antibacterial agents has led to an increase in the incidence of resistance among relevant pathogens.

Emergence of resistance to new antibiotics. A causal relationship between use of drugs and selection of resistant mutants can be inferred from first reports of resistance to drugs newly introduced to aquaculture. The history of the use of quinoline, oxolinic acid, in Europe is well documented. It was identified as being very useful in the control of furunculosis in salmonids in Europe in 1983 (Austin *et al.* 1983), although it had been used earlier in Japan. Initially it was very effective in treating furunculosis in Scotland, but in 1987 the first outbreaks occurred which failed to respond to therapy, and resistant strains were isolated (Hastings and McKay 1987). By the 1990s, 40-50% of isolates of *Aeromonas salmonicida* in Scotland were resistant (Inglis *et al.* 1991). Similarly, amoxycillin was not used in aquaculture in the UK before 1990. It had been used earlier in Japan, where initially the treatment of pasteurellosis in yellowtail was very successful; but resistance started to emerge in 1982 and is now widespread. In the UK, however, isolates of *A. salmonicida* taken between 1988-90 were all sensitive (Inglis *et al.* 1991, Barnes *et al.* 1994). However, three years after the introduction of the drug in 1990, Inglis *et al.* (1993a) reported a furunculosis outbreak from which resistant variants were isolated.

Surveys of resistance. Survey data upon which to assess the extent of the problem or upon which to evaluate intervention strategies are poor. At present, it is not possible to make direct comparisons between published information on resistance of bacterial fish pathogens because of the lack of standardization of procedures and systems to interpret results. Moreover, the composition of the sample sets of bacteria tested is often ill-defined and subject to numerous biases. Ideally, they should be statistically representative of a defined aqua-system, reflecting the geographical spread of aquaculture sites and species of fish cultured and collected with information on local drug use. More often, the set is a collection from a diagnostic laboratory where pathogens subjected to frequent antimicrobial treatments are likely to be over-represented and repeat isolates from the same site or same outbreak may be included. Awareness of these sources of error can improve, but not eliminate, bias. Other sample sets reported appear to be little more than random collections, sometimes assembled initially for some other reason, such as antigen analysis or phenotyping. With these reservations in mind, the records of established diagnostic laboratories provide useful information which gives some insight into antibiotic usage and antibiotic resistance patterns over a longer time span e.g., for Switzerland (Meier *et al.* 1992) and Germany (Schlotfeldt 1992).

A survey of the resistance of *Aeromonas salmonicida*, isolated in Scotland from Atlantic salmon with furunculosis, has been conducted, along with monitoring of the antibiotics in use in that country. Bacteria in this study came from 36 geographically separate seawater or freshwater sites distributed throughout the Scottish salmon farming industry. Between 1988 and 1992, oxtetracycline resistance was 50-55%, but more recently has risen to greater than 80%. Resistance to oxolinic acid was 50% between 1990 and 1992 but has fallen in recent years (Richards *et al.* 1992, and see also Fig. 1). There has been a slow gradual increase in resistance to potentiated sulphonamide, and some resistance to amoxycillin, but with a very low incidence.

Figure 1: Antibiotic resistance of *Aeromonas salmonicida* isolated from Atlantic salmon in Scotland 1988-1994



Since 1991, management practices in Atlantic salmon farming have greatly improved; effective furunculosis vaccines have been introduced for the first time and disease outbreaks and drug usage have been much reduced. Reduction in the amounts of drugs used in Norway preceded that in Scotland. By 1993, a decline in the resistance of *A. salmonicida* to oxytetracycline had already been recorded in Norway (Høie *et al.* 1992). Decline in resistance of *Vibrio anguillarum* associated with reduced drug use had previously been seen in Japan (Aoki *et al.* 1985). This appears to provide only temporary respite, however, because levels of resistance have been found to rise again when the drugs were re-introduced to the system (M. Endo pers. comm.).

Some efforts have been made to relate frequency of resistance to exposure to antibacterial treatments, but available data are not satisfactory. It has often been observed that patterns of resistance reflect patterns of use, but the choice of agents tested usually is a reflection of the agents available for use in the area the samples came from (Aoki *et al.* 1981, Takashima *et al.* 1985).

To evaluate the relationship between drug use in aquaculture and the development of resistance, it is essential that the sets of bacteria used are representative collections and that the surveys are repeated to analyze trends. In an attempt to measure the present situation in South East Asia, a project was set up with participants from five countries in the region. The aim was to assemble a representative collection of aquatic bacterial pathogens from each country. Initially, this was restricted to *Vibrio* and *Aeromonas* species. Samples were drawn from a wide range of aquaculture facilities in each country and the antibiograms of each isolate determined. Information on environmental conditions and drug use was collected simultaneously. The bacterial collection and records are

held by the Aquatic Animal Health Research Institute, Bangkok (Inglis *et al.* 1997). This provides a baseline against which changes can be measured, either on a regional basis, in which case repeat surveys will be needed each 5 or 10 years, or in local studies to evaluate the effectiveness of controlled drug withdrawals.

Although there is an insufficient database to prove a causal effect or evaluate interventions, it is generally felt among the community of scientific aquaculturists that there is a need to modify practice. While there can be no justification for delaying action while epidemiological data are being collected, it is of value to review the information available and identify what more is needed to demonstrate and quantify the causal effect, identify high risk practices and design modifications, and allow efficacy of interventions to be analyzed.

Selection of Resistant Variants in Aquaculture

Predisposing conditions. Resistance is either caused or selected by the presence of antibacterial agents in concentrations insufficient to kill the bacteria treated, and evidence is beginning to accumulate to allow identification of the high risk practices in aquaculture.

Whether by chromosomal mutation, by DNA transfer or by selection, outgrowth of resistant variants is strongly favored by prolonged exposure to sub-inhibitory concentrations of antibacterial drug. This opportunity may arise within the fish, in the water or in the sediments in fish ponds, or below fish cages. Antibacterials may reach the aquatic environment and be deposited in sediments as a result of leaching from feed, inappetence in the fish and excretion of active metabolites. This is particularly important in relation to ubiquitous opportunistic pathogens. Sub-inhibitory levels may also occur in fish, due to insufficient dose delivery and during the elimination period. Efficacy is heavily dependent upon a sufficient concentration of the drug reaching the relevant site within the host. Little work of this kind has been done on fish. The general assumption is that a tissue concentration of 3-4 times the MIC is required to eradicate the pathogen (Stamm 1989). Since blood is easy to collect, most reported studies measure serum concentrations of drug. This may be a good indicator in generalized septicemias, but is less useful in localized infections. In effect, most treatments are prophylactic. Treatment is usually started at the first signs of disease in the population but while the majority are still unaffected and, therefore, still feeding actively. At this stage the infection may not yet be systemic, and in many diseases (e.g., furunculosis in salmonids), it is unclear where the initial site of infection is and, therefore, what is the relevant tissue for an inhibitory drug concentration.

The duration for which effective concentrations are maintained is critical in determining outcome. Little is known about drug levels attained in key tissues throughout therapy as a basis for planning the most effective dosing strategy. Two approaches have been used: pharmacokinetic studies, either following single dose administration by the intravenous, intramuscular, intraperitoneal or oral routes, or during and after a course of medicated feed. Pronounced species differences have been found and a marked effect of temperature on drug elimination and metabolism. These studies have shown that bioavailability of oxytetracycline is very low, being 0.38% for carp and 1.25% for trout after a single oral dose of 60 mg/kg, while plasma concentrations of 0.65 and 0.37 µg/mL were achieved in trout at 10 °C and 19 °C and 0.15 and 0.81 µg/mL in carp at 8 °C and 20 °C, respectively (Nouws *et al.* 1992). Very little has been done to measure drug levels during oral therapy. In a study on serum and liver concentrations of oxolinic acid in Atlantic salmon during and after a 10-d treatment with oxolinic acid at 10 mg/kg (see Tables 1 and 2), up to four fold variation was found between individuals taken at the same sampling point. Drug concentrations were highest immediately on completion of the 10-d course; the mid-course levels were similar to those after 3-d withdrawal. The effect of temperature was clearly demonstrated, in that fish treated at 15 °C achieved higher concentrations than those treated at 8 °C, but the residues persisted longer at lower temperatures (unpublished results). The effects of temperature are discussed further

Table 1. Oxolinic acid concentration in serum of Atlantic salmon following oral therapy at 10 mg/kg in fresh water at 8 °C.

Concentration of Oxolinic Acid in µg/mL Serum							
During Treatment (2.5 d)	Days after treatment						
	0.8	2.8	4.7	7.7	10.7	14.8	21.8
3.2	3.3	2.9	1.6	1.4	1.0	0.2	ND ¹
3.0	5.4	2.4	3.3	1.1	1.5	0.2	0.1
3.1	3.1	3.6	2.1	1.4	0.8	ND	ND
3.1	6.1	2.4	2.0	1.7	0.6	0.2	ND
2.7	4.8	3.7	1.2	1.8	0.6	ND	ND
	4.5	3.7	3.1	1.5	1.2	ND	ND
	4.8	3.5	3.7	1.7	0.3	0.3	ND
	6.5	3.8	3.2	0.6	1.3	0.3	ND
	11.5	3.8	2.5	1.1	0.7	0.2	ND
	7.6	4.3	4.2	1.2	0.2	0.3	ND
Mean: 3.0	5.7	3.4	2.7	1.3	0.8	0.2	
SD: 0.2	2.3	0.6	0.9	0.4	0.4	0.1	

¹ND = not detected.**Table 2.** Oxolinic acid concentrations in the liver of Atlantic salmon following oral therapy at 10 mg/kg in fresh water at 8°C.

Concentrations of Oxolinic Acid in µg/gm Liver							
During treatment (2.5 d)	Days after treatment						
	0.8	2.8	4.7	7.7	10.7	14.8	21.8
7.1	6.0	4.5	2.4	1.5	0.7	ND ¹	ND
5.1	6.1	4.9	4.6	2.1	1.6	ND	ND
5.7	7.5	7.2	3.3	1.8	1.2	ND	ND
5.7	5.5	4.6	3.1	3.0	1.3	ND	ND
7.1	9.5	5.0	1.8	3.2	0.6	ND	ND
	9.9	8.0	5.9	3.6	1.4	ND	ND
	9.9	6.2	6.8	3.4	0.5	0.6	ND
	6.0	10.7	5.4	1.1	1.5	1.0	ND
	11.1	7.2	4.4	1.5	0.7	ND	ND
	9.7	6.6	4.9	1.4	0	0.3	ND
Mean: 6.1	6.1	8.1	6.5	4.3	2.2	0.9	
SD: 0.8	0.8	2.0	1.8	1.5	0.9	0.5	

¹ND = not detected.

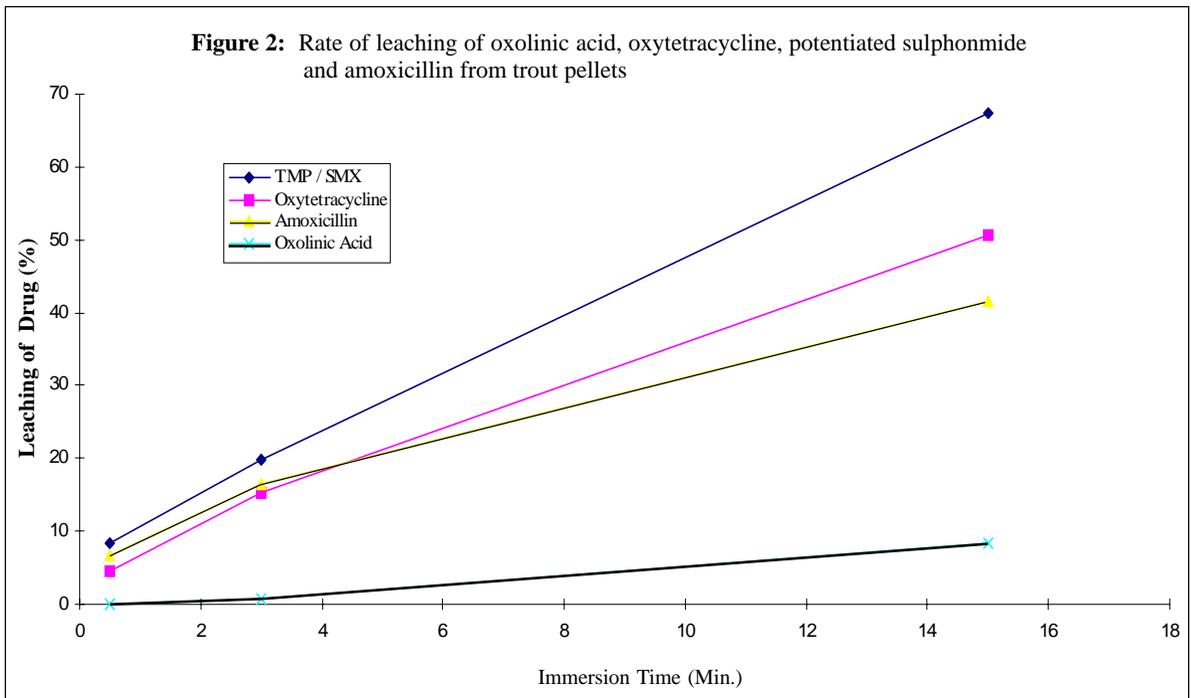
later, however, there is a shortage of information on tissue concentrations achieved in different species and the relationship between the dose delivered, regimens and variations within fish populations.

Clinical experience, added to pharmacokinetics studies and MIC data, together provide a powerful predictor of outcome. In most areas of aquaculture, information from all three sources is unavailable.

Dose delivery. Delivering an effective dose depends on selection of a drug to which the pathogen is sensitive and a medication system that is capable of achieving levels in the fish, for a prolonged period, in excess of the MIC. With increasingly good diagnostic laboratory services supporting aquaculture, the choice of an appropriate drug is unlikely to be a major problem provided that the national regulations allow an adequate range to choose from. With ever tightening regulations for drug registration and strict residue testing requirements in some countries, this may become difficult.

Delivery systems are, at present, a greater source of difficulty. Although dips, flushes and injections are all possible (Austin and Austin 1993), overwhelmingly, the method of choice is *per os* with drug attached to the feed (Rae 1992). Usually this is achieved by simply mixing the drug with the feed, often with a coating, such as oil, also applied. Compared with bath treatment, the amounts of drug required are much smaller and the impact on the environment much reduced. Nevertheless, using this method, drug may be lost by leaching from the surface-coated pellet as it passes through the water column. This is particularly important in species such as shrimp, which feed slowly (Goldblatt *et al.* 1980), but it is significant for all feed which remains in the water for even a few minutes before it is eaten (Fribourgh *et al.* 1969, Duis *et al.* 1995a). The leaching rate is dependent on many variables, including size of food particle, water temperature and turbulence. It varies also with the solubility of the drug. Rates of leaching of oxolinic acid, oxytetracycline, potentiated sulphonamide and amoxicillin from trout pellets are shown in Figure 2. The effect of pellet size is shown in Figure 3, where the greater surface area to volume ratio in smaller pellets results in relatively more drug loss by leaching.

More sophisticated presentation systems have been suggested to reduce these losses. The incorporation of antibiotics and chemotherapeutants into live food organisms has been suggested as an economic and environmentally friendly way of delivering drugs in larviculture (Cherel and Nin 1992, Verpraet *et al.* 1992). Bio-encapsulation in *Artemia* has been achieved with potentiated



sulphonamides and chloramphenicol (Mohney *et al.* 1990) and with quinolones (Duis *et al.* 1995b). Although there was little loss of drug from the *Artemia* before it was taken up by the fish, there was a very high level of wastage in preparing the medicated *Artemia*. This method has potential only in specialized situations, such as treatment of valuable fry. Alternative binding agents to replace oil have also been considered, for example, the efficacy of an algininate, commonly used as a thickening and gelling agent in the food industry, to surface coat antibiotics to fish food pellets was investigated (Duis *et al.* 1995a). This was effective in reducing leaching losses by up to 50% depending on the drug, in an immersion time of 15 min (see Fig. 4).

Another effective modification which reduced losses from medicated shrimp feed was a simple change to using an aqueous solution of oxytetracycline instead of a drug in the powder form to apply to the feed (Pearson and Chanratchakool 1993). While the additional steps in preparing medicated feed are often rejected *prima facie* because they involve cost and extra effort, they may well be highly cost effective. Tightly controlled field trials are necessary to develop this aspect.

Feeding behavior and medication. Another factor in effective eradication of a pathogen is the quantity of medicated feed taken by individual fish. It is well accepted that feeding rates differ greatly between individual fish in a population, even when these are initially matched for size (McCarthy *et al.* 1993). A social hierarchy develops, with aggressive individuals affecting the behavior of others. This has an important bearing on the receipt of medicated feed. In studies on drug palatability, we have often observed dominant fish which restricted the feeding behavior of others in the group and, thereby, the dose of drug reaching the individual.

In the tightly controlled trial referred to earlier, there was wide variation in intake of oxolinic acid between healthy individuals. Unevenness of uptake of drug is a major factor in field treatments. In studies on amoxycillin treatment of furunculosis in Atlantic salmon, there were great differences between drug serum levels in populations of fish nominally receiving the same treatment. There were also big differences between members of the same population (Inglis *et al.* 1993b). An important factor contributing to this is the health status of the population. Inappetence is one of the first clinical signs of disease, and if treatment is not started very early in an epizootic, uptake of drug may be very low.

Drug excretion. Temperature has a marked influence on the elimination of drugs from fish, excretion being more rapid at higher rather than at lower temperature, within the range of tolerance. A widely held convention describes elimination time in degree days, this being the product of temperature in degrees Celsius and the withdrawal period in days since the cessation of treatment. Within this range, it is proposed that a 10% increase in metabolic rate should be allowed for every 1 °C rise in temperature. However, adequate data sets are unavailable for many drugs and fish species, and some studies simply give elimination rates for specified temperature ranges (e.g., Jacobsen 1989). There are distinct differences in the elimination periods required by different species; oxytetracycline residues were cleared from rainbow trout (*Oncorhynchus mykiss*) in 348 degree days (at 12 °C) and from African catfish (*Clarias gariepinus*) in 775 degree days (at 25 °C). Elimination of various antibiotics from fish has been reviewed by Ellis (1991). In conclusion, available data indicate that elimination is faster at higher temperatures, but the relationship is not always linear and moreover, differs between species. However, excretion can be very slow at lower temperatures and in Figure 5 it can be seen that, in the oxolinic acid study, excretion was slower at the lower temperature, and detectable residues of drug were present in blood and liver for 10 d. Slow excretion provides an opportunity for selection of resistant bacteria among the normal gut flora and in pathogens persisting in the host. This is a potential hazard in cold water temperature. Even at 15 °C, excretion was much faster, suggesting that this risk may be only minimal in warm-water systems.

Antibacterial agents are widely used in aquaculture, and this is likely to remain so in the foreseeable

Figure 3: Effect of pellet size on rate of leaching of oxolinic acid

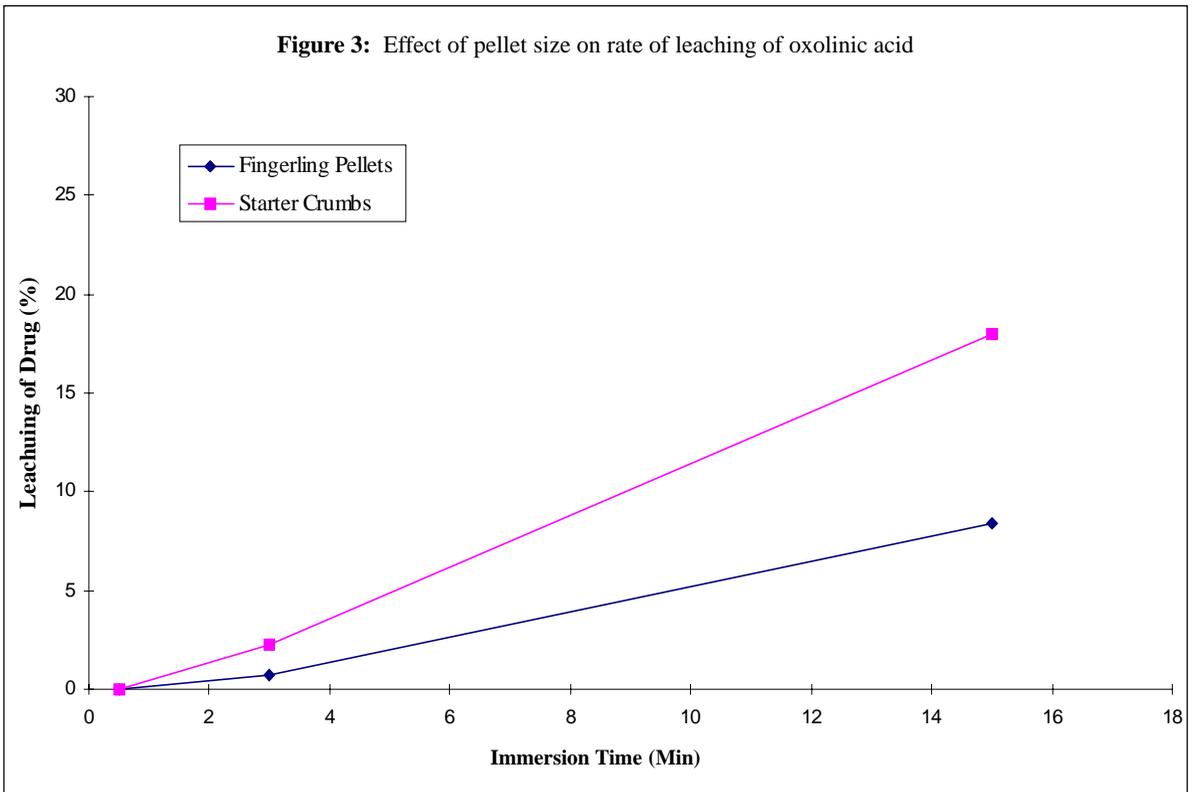


Figure 4: Effect of coating agent on rate of leaching of oxolinic acid

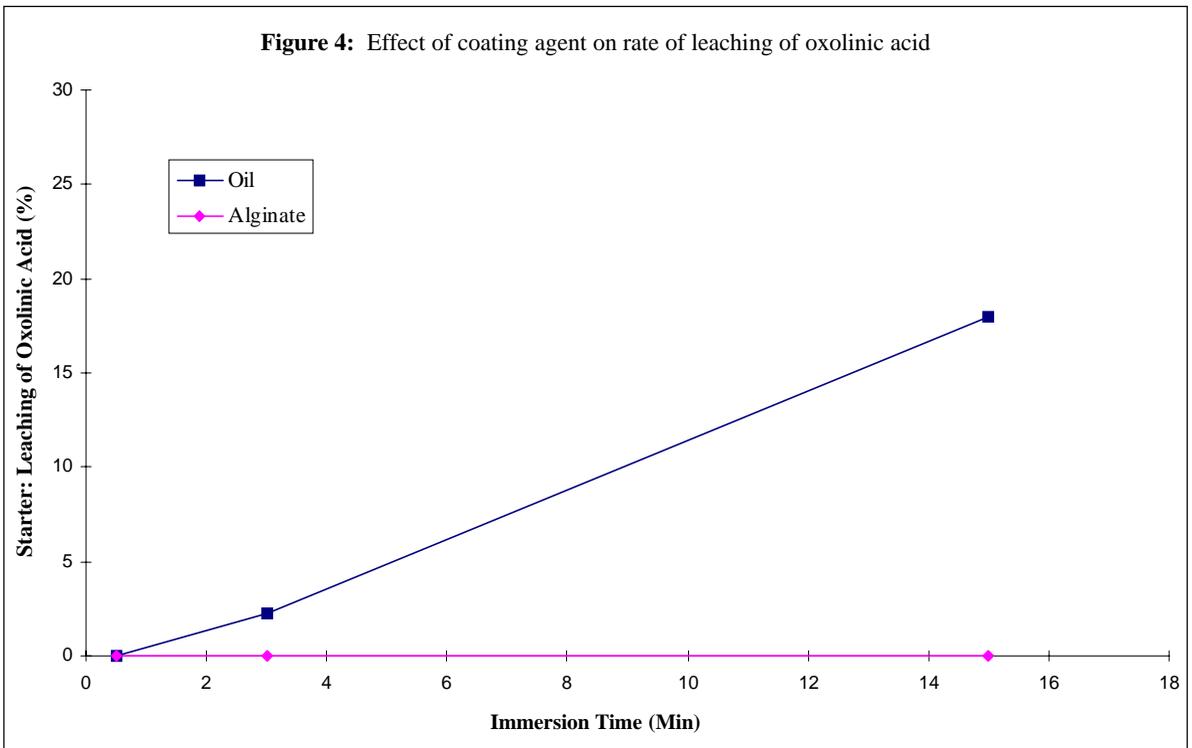
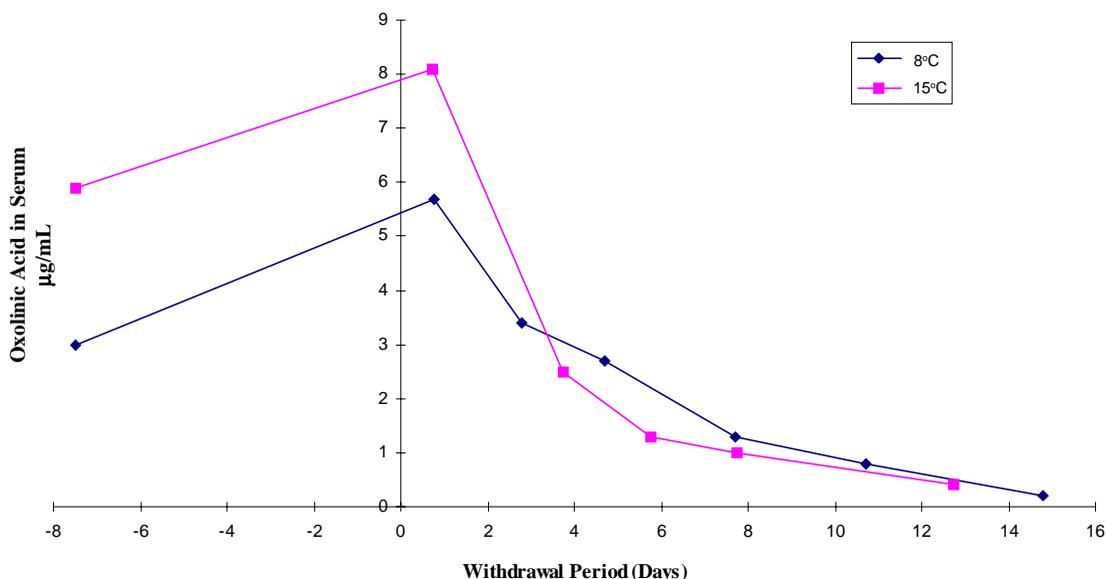


Figure 5: Oxolinic acid residues in the sera of Atlantic salmon in fresh water, after oral treatment with 10 µg/kg for 10 d at 8 °C and 15 °C



future. The task facing aquacultural scientists is to develop and support implementation of a code of practice to ensure the greatest benefits in efficacy and commercial terms with minimal environmental impact and damage to health.

Actual use of antibacterial agents is influenced strongly by commercial forces of cost and supply, services of veterinarians and diagnostic laboratories, available guidance on good procedure and local practice. Agreement and adoption of a scheme for antibacterial chemotherapy in aquaculture must be reached following wide-ranging discussion and negotiation for each country within the Asian Region. The following highlights the points that must be included in this discussion.

RECOMMENDATIONS FOR ANTIBIOTIC USAGE IN AQUACULTURE

1. Establish the cause of the disease condition.

Can the disease be treated with antibiotics, and if it can, should they be used? The clinician responsible must decide if therapy will be worthwhile and consider what, if any, impact may result on the local environment.

2. Establish an antibiogram or sensitivity pattern for the pathogen.

This will ensure that an effective antibiotic is utilized. In the absence of such, the clinician must decide on the drug of choice based on previous applications, history of antibiograms in any previous isolations, drugs available and economics.

3. Use the correct dosage for the recommended duration.

Suboptimal dosage or duration of therapy can result in rapidly developing antibiotic resistance.

Even if a positive result is obtained before the full course of treatment is complete, the total duration of therapy should be undertaken.

4. Adhere to careful storage of antibiotics.

Antibiotics are subject to degradation and should be stored in a cool, dark, secure, rodent-free facility. The quality of the chemicals should be ensured by utilizing only licensed products obtained from reputable wholesalers. A high standard of hygiene should be maintained to prevent cross contamination. All antibiotics have expiry dates and out-of-date drugs should not be used.

5. Use as narrow a spectrum of antibiotic as possible and avoid indiscriminate use of drugs, especially with live feeds (rotifers and *Artemia*).

6. Avoid oral therapy if fish are inappetent.

Treatment should, however, be instituted as soon as possible.

7. Avoid repeated use of the same antibiotic and blanket treatment for prophylactic use.

Rotation of available antibiotics should reduce the chances of resistant organisms being selected.

8. Antibiotic resistance patterns should be monitored as a routine.

9. Avoid polypharmacy.

The only exclusion to this is if synergism is likely, as with trimethoprim and the sulphonamides.

10. Whenever possible use products licensed for the species.

If no licensed product is available, then use that licensed for another food-producing species. In cross-species prescription, an application of a minimal withdrawal period of at least 500 °C d is recommended.

11. For all treatments, the prescribing clinician should record the date of examination, the clients, the number of fish treated, the diagnosis, product prescribed, dosage, duration of treatment and withdrawal period recommended.

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REFERENCES

- Aoki T, Kanazawa T, Kitao T. 1985. Epidemiological surveillance of drug resistant *Vibrio anguillarum* strains. Fish Pathol. 20:199-208.
- Aoki T, Kitao T, Kawano K. 1981. Changes in drug resistance of *Vibrio anguillarum* in cultured ayu, *Plecoglossus altivelis* Temminck and Schegel, in Japan. J. Fish Dis. 4:223-230.

- Austin B, Austin DA. 1993. Bacterial Fish Pathogens. 2nd ed. Ellis Horwood, Chichester.
- Austin B, Rayment J, Alderman DJ. 1983. Control of furunculosis by oxolinic acid. *Aquaculture*, 31:101-108.
- Barnes AC, Hastings TS, Amyes SGB. 1994. Amoxicillin resistance in Scottish isolates of *Aeromonas salmonicida*. *J. Fish Dis.* 17:357-364.
- Barnes AC, Hastings TS, Amyes SGB. 1995. Aquaculture antibacterials are antagonized by seawater cations. *J. Fish Dis.* 18:463-466.
- Bryan LE. 1989. Two forms of antimicrobial resistance: bacterial persistence and positive function resistance. *J. Antimicrob. Chemother.* 23:817-819.
- Cherel P, Nin F. 1992. Antibiotherapy using biocarriers (*Artemia salina*) in hatcheries. In: Michel C, Alderman DJ. eds. *Chemotherapy in Aquaculture*. Office International des Epizooties, Paris, p. 389-393.
- Chopra I. 1985. Mode of action of the tetracyclines and the nature of bacterial resistance to them. In: Hlavka JJ, Boothe JH. eds. *Tetracycline*. p. 317-392. Springer-Verlag. Berlin.
- Courvalin P. 1990. Plasmid-mediated 4-quinolone resistance: a real or apparent absence? *Antimicrob. Agents Chemother.* 34:681-684.
- Duis K, Inglis V, Beveridge MCM, Hammer C. 1995a. Leaching of four different antibacterials from oil- and alginate-coated fish-feed pellets. *Aquacult. Res.* 26:549-556.
- Duis K, Hammer C, Beveridge MCM, Inglis V, Braum E. 1995b. Delivery of quinolone antibacterial to turbot, *Scophthalmus maximus* (L.), via bioencapsulation: quantification and efficacy trial. *J. Fish Dis.* 18:229-238.
- Ellis AE. 1991. Tissue residues of chemotherapeutants in fish. *Bull. Eur. Assoc. Fish Pathol.* 11:22-29.
- Fribourgh JH, Meyer FP, Robinson JA. 1969. Oxytetracycline leaching from medicated fish feeds. *US Bur. Sport Fish. Wildl. Techn. Pap.* 40, p. 3-7.
- Goldblatt MJ, Conclin DE, Brown WD. 1980. Nutrient leaching from coated crustacean rations. *Aquaculture*, 19:383-388.
- Hamilton-Miller JMI. 1990. The emergence of antibiotic resistance: myths and facts in clinical practice. *Intens. Care Med.* 16 (Suppl. 13):206-211.
- Hastings TS, McKay A. 1987. Resistance of *Aeromonas salmonicida* to oxolinic acid. *Aquaculture*, 61:165-171.
- Høie S, Martinsen B, Sohlberg S, Horsberg TE. 1992. Sensitivity patterns of Norwegian clinical isolates of *Aeromonas salmonicida* subsp. *salmonicida* to oxolinic acid, flumequine, oxytetracycline and sulphadiazine/trimethoprim. *Bull. Eur. Assoc. Fish Pathol.* 12:142-144.
- Hooper DC, Wolfson JS. 1989. Mode of action of the quinolone antimicrobial agents - review of recent information. *Rev. Infect. Dis.* 11 (Suppl. 5):S902-S916.
- Inglis V, Abdullah SZ, Angka SL, Chinabut S, Chowdhury MBR, Leano EM, MacRae IH, Sasongko A, Somsiri T, Yambot AV. 1997. Survey of resistance to antibacterial agents used in aquaculture in five South East Asian countries. In: Flegel TW, MacRae IH. eds. *Diseases in Asian Aquaculture III*. p. 331-337. Fish Health Sect., Asian Fish. Soc., Manila, Philippines.
- Inglis V, Frerichs GN, Millar SD, Richards RH. 1991. Antibiotic resistance of *Aeromonas salmonicida* isolated from Atlantic salmon, *Salmo salar* L., in Scotland. *J. Fish Dis.* 14:353-358.
- Inglis V, Millar SD, Richards RH. 1993a. Resistance of *Aeromonas salmonicida* to amoxicillin. *J. Fish Dis.* 10:389-395.
- Inglis V, Palmer R, Shatwell JP, Branson EJ, Richards RH. 1993b. Amoxicillin concentrations in the serum of Atlantic salmon (*Salmo salar* L.) during furunculosis therapy. *Vet. Rec.* 133 (25/26):617-621.
- Inglis V, Richards RH. 1991. The *in vitro* susceptibility of *Aeromonas salmonicida* and other fish-pathogenic bacteria to 29 antimicrobial agents. *J. Fish Dis.* 14:641-650.

- Inglis V, Robertson D, Miller K, Thompson KD, Richards RH. 1996. Antibiotic protection against recrudescence of latent infection during vaccination against furunculosis. *J. Fish Dis.* 19:341-348.
- Jacobsen MD. 1989. Withdrawal times of freshwater rainbow trout, *Salmo gairdneri* Richardson, after treatment with oxolinic acid, oxytetracycline and trimetoprim. *J. Fish Dis.* 12:29-36.
- Kruse H. 1994. Antimicrobial resistance - epidemiological aspects. Thesis for degree of Doctor Scientiarum. Norwegian College of Veterinary Medicine, Oslo.
- Lee SW, Edlin G. 1985. Expression of tetracycline resistance in PBR 322 derivatives reduces the reproductive fitness of plasmid-containing *Escherichia coli*. *Gene*, 39:173-180.
- Levy SB. 1989. Evolution and spread of tetracycline resistance determinants. *J. Antimicrob. Chemother.* 24:1-3.
- Lewin CS, Allen RA, Amyes SGB. 1990. Potential mechanisms of resistance to the modern fluorinated 4-quinolones. *J. Med. Microbiol.* 31:153-161.
- Martinsen B, Oppegaard H, Wichstrøm R, Myhr E. 1992. Temperature-dependent *in-vitro* antimicrobial activity of four 4-quinolones and oxytetracycline against bacteria pathogenic to fish. *Antimicrob. Agents Chemother.* 30:1738-1743.
- McCarthy ID, Houlihan DF, Carter CG, Moutou K. 1993. Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. *Proc. Nutr. Soc.* 52:427-436.
- Meier W, Schmitt M, Wahli T. 1992. Resistance of antibiotics used in the treatment of freshwater fish during a ten year period (1979-1988) in Switzerland. In: Michel C, Alderman DJ. eds. *Chemotherapy in Aquaculture*. Office International des Epizooties, Paris, p. 263-275.
- Modi RI, Wilke CM, Rosenzweig RF, Adams J. 1991. Plasmid macro-evolution - selection of deletions during adaptation in a nutrient-limited environment. *Genetica*, 84:195-202.
- Mohney LL, Lightner DV, Williams RR, Brauerlein M. 1990. Bioencapsulation of therapeutic quantities of the antibacterial Romet-30 in nauplii of the brine shrimp *Artemia* and in the nematode *Panagrellus redivivus*. *J. World Aquacult. Soc.* 21:186-191.
- Nouws JFM, Grondel JL, Boon JH, Van Ginneken VJT. 1992. Pharmacokinetics of antimicrobials in some fresh water fish species. In: Michel C, Alderman DJ. eds. *Chemotherapy in Aquaculture*. Office International des Epizooties, Paris, p. 437-447.
- Pearson MD, Chanratchakool P. 1993. Leaching of oxytetracycline from surface-coated shrimp feed. Second Symposium on Diseases in Asian Aquaculture. Aquatic Animal Health and the Environment. 25-29 October, 1993, Phuket, Thailand. Scientific Sessions Abstracts, Abstract PP 30, p. 101. *Fish Health Sect., Asian Fish. Soc.*
- Piddock LJV. 1990. A review: techniques used for the determination of antimicrobial resistance and sensitivity in bacteria. *J. Appl. Bacteriol.* 68:307-318.
- Prescott JJ, Baggot JD. 1988. *Antimicrobial Therapy in Veterinary Medicine*. Blackwell Scientific Publications, Boston.
- Rae GH. 1992. Constraints on chemotherapy: the fish farming industry view. In: Michel C, Alderman DJ. eds. *Chemotherapy in Aquaculture*. Office International des Epizooties, Paris. p. 95-102.
- Richards RH, Inglis V, Frerichs GN, Millar SD. 1992. Variation in antibiotic resistance patterns of *Aeromonas salmonicida* isolated from Atlantic salmon *Salmo salar* L in Scotland. In: Michel C, Alderman DJ. eds. *Chemotherapy in Aquaculture*. Office International des Epizooties, Paris, p. 276-287.
- Schlottfeldt HJ. 1992. Current practices of chemotherapy in fish culture. In: Michel C, Alderman DJ. eds. *Chemotherapy in Aquaculture*. Office International des Epizooties, Paris, p. 25-38.
- Smith HW. 1975. Persistence of tetracycline resistance in pig *E. coli*. *Nature*, 258:628-630.
- Smith P, Hiney MP, Samuelson OB. 1994. Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. *Ann. Rev. Fish Dis.* 4:273-313.

- Stamm JM. 1989. *In vitro* resistance by fish pathogen to aquaculture antibacterials, including the quinolones difloxacin (A-56619) and sarafloxacin (A-56620). J. Aquat. Anim. Health 1:135-141.
- Takashima N, Aoki T, Kitao T. 1985. Epidemiological surveillance of drug-resistant strains of *Pasteurella piscicida*. Fish Pathol. 20:209-217.
- Verpraet R, Chair M, Leger P, Helis HJ, Sorgeloos P, de Leenheer A. 1992. Live-food-mediated drug delivery as a tool for disease treatment in larviculture: the enrichment of therapeutic in rotifers and *Artemia* nauplii. Aquacult. Eng. 11:133-139.

Ecological Effects of the Use of Chemicals in Aquaculture

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ABSTRACT

Many aquaculture chemicals are, by their very nature, biocidal, and may be released to the surrounding environment at toxic concentrations either through misuse, or in some cases, even by following generally accepted procedures for use. Thus, there is a potential for mortality of non-target organisms. Illustrations are provided of three classes of aquaculture chemicals and their effects on non-target biota: 1) use of a carbaryl pesticide and mortality of non-target invertebrates; 2) use of an organophosphate parasiticide and suspected effects on nearby biota; and 3) effects of antibacterial residues in aquatic sediments on the associated microbial community. Efforts to assess the risks posed by aquaculture chemicals are often frustrated by a lack of information on environmental fate and effects, and data needs to resolve this situation are identified.

INTRODUCTION

Many aquaculture chemicals are, by their very nature, biocidal, and achieve their intended purpose by killing or slowing the population growth of aquatic organisms. Chemicals used in this manner include:

- antifoulants
- disinfectants
- algicides
- herbicides
- pesticides
- parasiticides
- antibacterials

For the purpose of this assessment, mortality of the target organism is accepted as a given, and, from the perspective of the aquaculturist, a desirable outcome. Mortality of a pest species is in itself an ecological effect with potential implications to the surrounding ecosystem, but the implicit assumption is that the commercial value of elimination of the pest outweighs the ecological value of its presence. The emphasis in this discussion is on effects on non-target species.

In order to illustrate the potential range of ecological effects, three general classes of aquaculture chemicals are discussed below: 1) pesticides, 2) parasiticides, and 3) antibacterials. This assessment does not consider human health aspects or stimulation of antibacterial resistance in natural microbial communities, as both these topics are discussed elsewhere in this volume.

DISCUSSION

Pesticides

Perhaps the greatest potential for ecological effects arises from the use of aquaculture chemicals to remove pest species from the surrounding environment. An example of such an approach is the use of the carbaryl pesticide Sevin to control ghost shrimp (*Callinassa californiensis*) and mud shrimp (*Upogebia pugettensis*) in oyster culture areas of Washington State, U.S.A. Oysters (*Crassostrea gigas*) are produced by bottom culture on intertidal mudflats in Willapa Bay and Grays Harbor, Washington. Dense infestations of burrowing shrimp reduce production, either by creating suspended sediments which smother oyster seed, or by softening the substrate to the point where it cannot support the adult oysters (WDF/WDOE 1985). Since 1963, the pesticide Sevin has been used to control burrowing shrimp, with application on exposed mudflats during low tide, either by hand or by aerial spraying from helicopters. Application requires approval of a state fisheries biologist, and is given only when shrimp burrow entrances exceed a density of 10 burrows m². Application rate is 8.5 kg active ingredient/ha, with no more than 20 ha sprayed in a single treatment, and no more than 320 ha sprayed statewide each year. A single oyster bed may be treated in two successive years, but on average, treatment of any given bed is required once every six years.

Immediately after spraying, maximum observed concentrations of Sevin in overlying water have been reported to range from 1-20 ppm (WDF/WDOE 1985, 1989 and references therein). Sevin concentrations in water are reduced to 0.1 ppm within hours, either by hydrolysis to 1-naphthol or adsorption to sediments. In sediments, however, residues may persist for a few weeks (WDF/WDOE 1985). Sevin is highly toxic not only to burrowing shrimp, but to non-target organisms. In plots sprayed at a dosage of 11.3 kg/ha, the densities of gaper clams (*Tresus capax*) and bent-nosed clams (*Macoma baltica*) were reduced 69% and 28%, respectively (Armstrong and Millemann 1974). The 24 hr LD₅₀ for many arthropods, including the Dungeness crab (*Cancer magister*) is approximately 0.1 ppm (Table 1), suggesting that these species may experience mortality at the concentrations observed after application.

Effects of Sevin spraying on Dungeness crab are of particular concern because of the commercial importance of the Dungeness crab fishery. Crabs, particularly juveniles, are at risk not only because aqueous concentrations of Sevin may exceed lethal levels, but because of their behavior of ingesting the carcasses of burrowing shrimp and other invertebrates that have been killed by Sevin treatment. Invertebrates collected from treated areas contain 5-75 ppm carbaryl (Table 2). Ingestion of food containing 43-167 ppm carbaryl has been shown to result in 7-80% mortality within 24 h in Dungeness crabs (Tufts 1988). Dungeness crabs have also been shown to develop irreversible paralysis after ingesting bivalves that had been exposed to 1 ppm Sevin (Buchanan *et al.* 1970).

It has been estimated that during the period 1984-1988 an average of 9,300 juvenile and 73 adult crabs were killed per hectare treated with Sevin. With an estimated 0.4% of juveniles surviving to a harvestable size, the mortality rate in adult crab equivalents is 110 individuals/ha, or 35,100 individuals over the entire 320 ha treated each year (WDF/WDOE 1989). This mortality represents an annual loss of \$47,000 to the Dungeness crab fishery, but this cost is overshadowed by the value of the \$7 million/yr oyster industry, and the loss of a large proportion of the beds if Sevin treatment were not done. This comparison, however, fails to take into account the ecological value of the Dungeness crabs and the many non-commercial species that are adversely affected by carbaryl treatment. Lacking any mechanism to place a monetary value on these species, the ecological impacts of aquaculture chemicals are often ignored, and regulatory decisions based largely on commercial considerations.

Table 1. Carbaryl LC₅₀ values for selected invertebrate species (modified from WDF/WDOE 1989).

Species	Exposure Time (h)	LC ₅₀ (ppm carbaryl)
Arthropoda		
Amphipoda (<i>Gammarus</i> spp.)	24	0.04
Mud shrimp larva	24	0.03-0.16
Ghost shrimp larva	48	0.17-0.47
Ghost shrimp adult	24	0.13
Dungeness crab larva	24	0.08
Dungeness crab juvenile	24	0.08
Dungeness crab adult	24	0.49
Fiddler crab larva	24	0.1
Mollusca		
Bay mussel larva	48	1.4-2.9
Pacific oyster larva	48	1.5-2.7
Adult cockle clams	24	7.3

Table 2. Average carbaryl residues (mg/kg wet weight) found in the tissues of invertebrates after pesticide application (from Tufts 1988).

Rate of Application (kg/ha)	Residues in Burrowing Shrimp	Residues in Annelids
11.3	13.8	75.7
8.5	8.7	57.0
5.6	5.3	58.6

Parasiticides

Organophosphate compounds are occasionally used in aquaculture for a wide variety of applications, including control of ectoparasitic crustaceans, treatment of trematode or ciliate infections in shrimp hatcheries, or removal of mysids from shrimp ponds. They are sold under a variety of trade names including Nuvan®, Neguvon®, Aquaguard®, Dipterex®, Dursban®, Demerin®, and Malathion®. Neguvon® (trichlorphon) and its degradation product Nuvar® (dichlorvos) are used for treatment of ectoparasitic crustaceans such as salmon lice, *Lepeophtheirus salmonis*, on marine fishes, or *Argulus* sp. and *Lernaea* sp. on freshwater fishes. Their use has generated considerable controversy in the UK regarding toxicity to non-target species (Ross 1989). Egidius and Møster (1987) provide one of the few pieces of evidence, albeit anecdotal, of non-target organism toxicity associated with the use of these parasiticides. In Norwegian salmonid cage culture, growers surround the cages with tarpaulins, and add Neguvon® to achieve a concentration ranging from 10 up to 300 ppm. After completion of treatment, the tarpaulins are removed, allowing the solution to disperse into surrounding waters. As a result of unexplained mortality of lobsters (*Homarus gammarus*) held near a salmon farm, subsequent investigation showed the lobsters were extremely sensitive to these parasiticides, with mortality occurring after 24 h exposure to 0.5 ppm Neguvon or 0.1 ppm Nuvar (Egidius and Møster 1987).

Antibacterials

Antibacterials are commonly administered as a bath or as a feed supplement. As a bath, there is an obvious route of release of unabsorbed antibacterials to the surrounding environment via the effluent. Even as a feed supplement, however, this loss can occur either via uningested waste feed or through elimination in the feces or urine. Oxytetracycline, one of the most widely used antibacterials in aquaculture worldwide, is notorious in this regard. The vast majority of oxytetracycline supplied in medicated feed can be found in hatchery effluent at concentrations that account for nearly all of the drug supplied (Smith *et al.* 1994). It has been estimated that only 7-9% of the oxytetracycline ingested is absorbed during gut passage in freshwater rainbow trout (Cravedi *et al.* 1987). Thus, even if all medicated feed were ingested, >90% of the drug could leave the facility via fecal matter or dissolved in the effluent. Fecal sources are also important for oxolinic acid, where 62-86% of the ingested drug has been shown to remain in the feces of rainbow trout (Cravedi *et al.* 1987). Conversely, chloramphenicol is absorbed very efficiently in the gut, and <1% of drug ingested is released via the feces (Cravedi *et al.* 1987).

Antibacterial residues in the surrounding water are not a major concern, given the rapid dilution and susceptibility of many drugs to photodegradation. However, the sediments serve as a long-term reservoir for residues of many drugs (Table 3). Of the antibacterials examined, oxytetracycline is among the most persistent, and is lost from the sediment only by dissolution and diffusion into the overlying water (Samuelsen 1989). Under conditions of rapid sedimentation, as would be expected near many aquaculture facilities, sediment containing oxytetracycline residues may be quickly buried, and the drug may persist indefinitely.

The quinolones oxolinic acid and flumequine are also very persistent in sediments, and detectable residues may remain for 6 mo or more after drug treatment. Sulfadiazine and sulfadimethoxine have been shown to persist for many months in a sealed vial (Samuelsen *et al.* 1994), but are far less persistent in an open system with flowing water overlying the sediments (Capone *et al.* 1996).

Residues of trimethoprim, ormetoprim and furazolidone are all relatively short-lived in aquatic sediments, with concentrations becoming immeasurable within 2 mo or less. Furazolidone, in particular, is rapidly degraded microbially, and has a half-life of less than 1 d in aquatic sediments (Samuelsen *et al.* 1991).

The organic-rich sediments typical in the vicinity of aquaculture operations are characterized by intense microbial activity including high rates of oxygen consumption and sulfate reduction. Since antibacterial residues can be found in sediments surrounding aquaculture operations and persist there for a year or more in the case of some antibacterials, the question arises as to what affect these residues may have on natural microbial communities in the sediments. Despite the potential environmental effects, little work has been done on changes in sedimentary microbial abundance or biogeochemical processes following aquacultural use of antibacterials. The most extensive data on biogeochemical effects of antibacterial treatment come from work recently completed at a salmon net-cage farm in the United States and in laboratory microcosms (Capone *et al.* 1994, Herwig and Gray 1997, Herwig *et al.* 1997). Use of 186 kg oxytetracycline at a farm and the presence of residues of the drug in the sediments (1-4 mg/kg) had no measurable affect on total microbial density in the farm sediments, the flux of ammonium from the sediments, sulfate concentrations in pore water, or sediment oxygen consumption. Dosing of microcosms with oxytetracycline and Romet® 30 (sulfadimethoxine and ormetoprim) at a rate intended to mimic field conditions also had no affect on the same parameters. The lack of an effect on microbial density or activity was due to an observed increase in antibacterial resistance that compensated for the loss of more susceptible microorganisms, complexation of the oxytetracycline with divalent cations, and a rapid disappearance of the sulfadimethoxine.

Table 3. Persistence of antibacterial residues in sediments. Bold text indicates data from sediments beneath a farm; all other data from laboratory microcosms. (modified from Weston 1996).

Antibacterial	Half-life (d)	Persistence ¹ (d)	Farm or Lab. Data	Reference
Flumequine	155	>185	L	Hansen <i>et al.</i> 1992
		>180	L	Samuelsen <i>et al.</i> 1994
Furazolidone	0.75	9	L	Samuelsen <i>et al.</i> 1991
Ormetoprim		>2-<23	L	Capone <i>et al.</i> 1996
		<30	L	Samuelsen <i>et al.</i> 1994
Oxolinic acid	165 48	6	F	Björklund <i>et al.</i> 1991
		>77	L	Björklund <i>et al.</i> 1991
		>185	L	Hansen <i>et al.</i> 1992
		>210	L	Samuelsen 1992
		>180	L	Samuelsen <i>et al.</i> 1994
Oxytetracycline	9-419 36 16 125 70 32 30-64 55 87-144	>7-> 308	F	Björklund <i>et al.</i> 1990
		> 12	F	Björklund <i>et al.</i> 1991
		>77	L	Björklund <i>et al.</i> 1991
		> 300	F	Capone <i>et al.</i> 1996
		>60	L	Capone <i>et al.</i> 1996
		> 33-<71	F	Coyne <i>et al.</i> 1994
		>185	L	Hansen <i>et al.</i> 1992
		> 84	F	Jacobsen & Berglind 1988
		>84	L	Jacobsen & Berglind 1988
		> 39	F	Samuelsen 1989
		>220	L	Samuelsen 1989
		>210	L	Samuelsen 1992
		> 550	F	Samuelsen <i>et al.</i> 1992
>180	L	Samuelsen <i>et al.</i> 1994		
Sulfadiazine		>180	L	Samuelsen <i>et al.</i> 1994
Sulfadimethoxine		>2-<22	L	Capone <i>et al.</i> 1996
		>180	L	Samuelsen <i>et al.</i> 1994
Trimethoprim		>30-<60	L	Samuelsen <i>et al.</i> 1994

¹Length of time after medication during which detectable residues were present in the sediment.

In the only other study on the topic, Hansen *et al.* (1992) reported a 40-50% reduction in microbial density and a >90% decrease in the rate of sulfate reduction in sediments dosed with either oxytetracycline, oxolinic acid or flumequine. The disparity between these results and the lack of microbial response observed in Capone *et al.* (1994) is probably due to differences in the concentrations of antibacterials used. Hansen *et al.* (1992) dosed the sediments with 400 mg/kg oxytetracycline and 100 mg/kg of the other drugs. However, Capone *et al.* (1994) used 5-15 mg/kg oxytetracycline in the microcosms and reported <4 mg/kg in the farm sediments.

The reported failure of antibacterial residues in sediment to alter microbial densities or biogeochemical processes, except under extraordinarily high dosing rates, should not be considered justification to eliminate this possibility. These results are very likely a function of the particular drugs used and the marine environments examined. All three of the drugs that have been examined (oxytetracycline, oxolinic acid and flumequine) form complexes with divalent cations, principally calcium and magnesium (Lunestad and Goksøyr 1990). In the marine systems studied (Hansen *et al.* 1992, Capone *et al.* 1994, Herwig *et al.* 1997, Herwig and Grey 1997), approximately 95% of the drug residues were in the complexed form, which has no antimicrobial activity (Lunestad and Goksøyr 1990). Much more of the drug is likely to be in the free, bioavailable form in fresh waters. The calcium and magnesium content of sea water is 400 and 1,272 mg/L, respectively (Sverdrup *et al.* 1942). In fresh water, the calcium and magnesium concentrations are about 2-60 mg/L and 2-50 mg/L, respectively, depending upon water hardness (EPA 1989). With one to two orders-of-magnitude less complexing cations in freshwater systems, it is very likely that more of these drugs will be in the microbially-active free form, and effects on the microbial community more evident. No work has been published, however, on the environmental effects of these drugs in freshwater systems, nor is any information available on other antibacterials which do not form cation complexes.

If antibacterial residues were to reduce bacterial numbers and slow the rate at which they degrade organic matter, the effects are likely to be of limited spatial extent since, in net-cage culture at least, antibacterial residues are only elevated within tens of meters of farm sites (Coynne *et al.* 1994, Capone *et al.* 1996). There could, however, be adverse consequences to the farm aside from the potential stimulation of antibacterial resistance. Aerobic degradation of organic matter results in the production of non-toxic products such as carbon dioxide and nitrates, while anaerobic degradation yields toxic products such as sulfides and ammonium. If the presence of antibacterial residues reduced the extent of aerobic degradation of organic matter, more labile organic carbon would be incorporated into the anaerobic portion of the sediment column. Subsequent anaerobic degradation could result in an increased production of toxic end products. Such a scenario is entirely speculative, but illustrates the need for more information on antibacterial effects on sediment biogeochemistry.

CONCLUSIONS

There is remarkably little information available on the effects of aquaculture chemicals on non-target organisms. Emphasis has been on the efficacy of the chemical on the target species, and there has been little consideration of the environmental effects of any chemical residues remaining in wastewaters from the culture facility. There are rarely data available on the concentration of aquaculture chemicals in effluents. For most chemicals, there is little information on environmental persistence, obviously a key consideration in predicting ecological effects. Toxicity data are often lacking or minimal, and in any event, are difficult to relate to field conditions because of unknown dilution rates. Surveys are rarely conducted to document population or community-level effects due to release of aquaculture chemicals to receiving waters. Consequently, this evaluation is based on very limited data, and is biased towards temperate latitudes from which the majority of the data are available.

Ideally, information such as that listed in Table 4 is available to determine the likelihood that use of an aquaculture chemical will have unintended and adverse ecological effects. All the information listed may not be necessary for each compound; for example, rapid degradation would mitigate the need for chronic toxicity data. Nevertheless, for many aquaculture chemicals, we have very little of the data needs identified. In most developed countries, such information is now required before new aquaculture chemicals are approved for use. However, no comparable data are available for many chemicals already in widespread commercial use, and there is a particular lack of data for tropical environments where aquaculture chemical use is less strictly regulated.

Table 4. Primary data needed to assess ecological effects of aquaculture chemicals.

USE PATTERNS

- Dosage
- Frequency of use
- Wastewater management
- Solid waste management
- Dilution prior to release
- Rate of dilution in receiving waters

ENVIRONMENTAL FATE

- Absorption efficiency (if a feed supplement)
- Metabolism
- Chemical reactivity
- Microbial degradation rates
- Photodegradation rates
- Temperature/pH dependence of environmental fate
- Vapor pressure
- Aqueous solubility
- Degradation products (with same environmental fate and toxicology needs for all)

TOXICOLOGY

- Bioaccumulation potential (K_{ow} , BCF)
- Acute toxicity (multiple species, multiple life stages)
- Chronic toxicity (multiple species, multiple life stages)
- Stimulation of resistance (for antibacterials)
- Effects on sediment biogeochemistry

REFERENCES

- Armstrong DA, Millemann RE. 1974. Effects of the insecticide carbaryl on clams and some other intertidal mud flat animals. *J. Fish. Res. Board Can.* 31:466-469.
- Björklund H, Bondestam J, Bylund G. 1990. Residues of oxytetracycline in wild fish and sediments from fish farms. *Aquaculture*, 86:359-367.
- Björklund HV, Råbergh CMI, Bylund G. 1991. Residues of oxolinic acid and oxytetracycline in fish and sediments from fish farms. *Aquaculture*, 97:85-96.
- Buchanan DV, Milleman RE, Stewart NE. 1970. Effects of the insecticide Sevin on various stages of the Dungeness crab, *Cancer magister*. *J. Fish. Res. Board Can.* 27:93-104.
- Capone DG, Miller V, Love J, Shoemaker C. 1994. Effect of aquacultural antibacterials on biogeochemical processes in sediments: field and microcosm observations. In: Weston DP, Capone DG, Herwig RP, Staley JT. eds. *Environmental Fate and Effects of Aquacultural Antibacterials in Puget Sound*. Appendix 3. Report from the University of California at Berkeley to the National Oceanic and Atmospheric Administration.
- Capone DG, Weston DP, Miller V, Shoemaker C. 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. *Aquaculture*, 145:55-75.
- Coyne R, Hiney M, O'Connor B, Kerry J, Cazabon D, Smith P. 1994. Concentration and persistence of oxytetracycline in sediments under a marine salmon farm. *Aquaculture*, 123:31-42.

- Cravedi J-P, Chouber G, Delous G. 1987. Digestibility of chloramphenicol, oxolinic acid and oxytetracycline in rainbow trout and influence of these antibiotics on lipid digestibility. *Aquaculture*, 60:133-141.
- Egidius E, Møster B. 1987. Effect of Neguvon and Nuvan treatment on crabs (*Cancer pagurus*, *C. maenas*), lobster (*Homarus gammarus*) and blue mussel (*Mytilus edulis*). *Aquaculture*, 60:165-168.
- EPA. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd edn. U.S. Environmental Protection Agency, EPA/600/4-89-001, Cincinnati, OH.
- Hansen PK, Lunestad BT, Samuelsen, OB. 1992. Effects of oxytetracycline, oxolinic acid, and flumequine on bacteria in an artificial marine fish farm sediment. *Can. J. Microbiol.* 38:1307-1312.
- Herwig RP, Gray JP. 1997. Microbial response to antibacterial treatment in marine microcosms. *Aquaculture*, 152:139-154.
- Herwig, RP, Gray JP, Weston DP. 1997. Antibacterial resistant bacteria in surficial sediments near salmon net-cages farms in Puget Sound, Washington. *Aquaculture*, 149:263-283.
- Jacobsen P, Berglund L. 1988. Persistence of oxytetracycline in sediments from fish farms. *Aquaculture*, 70:365-370.
- Lunestad BT, Goksøyr J. 1990. Reduction in the antibacterial effect of oxytetracycline in sea water by complex formation with magnesium and chloride. *Dis. Aquat. Org.* 9:67-72.
- Ross A. 1989. Nuvan use in salmon farming: the antithesis of the precautionary principle. *Mar. Pollut. Bull.* 20:372-374.
- Samuelsen OB. 1989. Degradation of oxytetracycline in seawater at two different temperatures and light intensities, and the persistence of oxytetracycline in the sediment from a fish farm. *Aquaculture*, 83:7-16.
- Samuelsen OB. 1992. The fate of antibiotics/chemotherapeutics in marine aquaculture sediments. In: Michel CM, Alderman DJ. eds. *Problems of Chemotherapy in Aquaculture: from Theory to Reality*. p. 87-95. Office International des Epizooties, Paris.
- Samuelson OB, Lunestad BT, Ervik A, Fjelde S. 1994. Stability of antibacterial agents in an artificial marine aquaculture sediment studied under laboratory conditions. *Aquaculture*, 126:283-290.
- Samuelsen OB, Solheim E, Lunestad BT. 1991. Fate and microbiological effects of furazolidone in a marine aquaculture sediment. *Sci. Total Environ.* 108:275-283.
- Samuelsen, OB, Torsvik V, Ervik A. 1992. Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *Sci. Total Environ.* 114:25-36.
- Smith P, Donlon J, Coyne R, Cazabon DJ. 1994. Fate of oxytetracycline in a fresh water fish farm: influence of effluent treatment systems. *Aquaculture*, 120:319-325.
- Sverdrup HU, Johnson MW, Fleming RH. 1942. *The Oceans: their Physics, Chemistry and General Biology*. Prentice-Hall, Englewood Cliffs, NJ.
- Tufts DF. 1988. The use of the insecticide Sevin to control burrowing shrimp in Willapa Bay and Grays Harbor in 1987. *Wash. Dep. Fish. Spec. Shellfish Rep. No. 4.*, Olympia, WA.
- WDF/WDOE. 1985. Use of the insecticide Sevin to control ghost and mud shrimp in oyster beds of Willapa Bay and Grays Harbor. *Final Environmental Impact Statement*. Wash. Dep. Fish., Wash. Dep. Ecol., Olympia, WA.
- WDF/WDOE. 1989. Use of the insecticide carbaryl to control ghost and mud shrimp in oyster beds of Willapa Bay and Grays Harbor. *Supplemental Environmental Impact Statement*. Wash. Dep. Fish., Wash. Dep. Ecol. Olympia, WA.
- Weston DP. 1996. Environmental considerations in the use of antibacterial drugs in aquaculture. . In: Baird D, Beveridge M, Kelly L, Muir J. eds. *Aquaculture and Water Resources Management*. p. 140-165. Blackwell Science.

Transferable Drug Resistance Plasmids in Fish-Pathogenic Bacteria

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ABSTRACT

Chemotherapeutic agents have been developed for treating bacterial infections and have been widely used for cultured fish for the last 30 years in Japan. The extensive use of chemotherapeutants has resulted in an increase in the occurrence of drug resistance in fish-pathogenic bacteria and also in the bacterial flora of the intestinal tract of cultured fish. The kinds of chemotherapeutants used are correlated with the occurrence of the corresponding drug-resistant genes in fish-pathogenic bacteria. Almost all multiple-drug resistant strains are carried on the transferable R plasmid, although resistance in fish pathogens to nitrofurans and pyridonecarboxylic acids is associated with a chromosomal gene. The DNA sequences of R plasmids generally differ depending on the species of fish pathogen. Exceptions are the R plasmids of *Aeromonas hydrophila* and *A. salmonicida*, which have the same resistance markers as chloramphenicol, streptomycin, and sulfonamides (SA); and the R plasmids of *A. hydrophila* and *Edwardsiella tarda*, which have the same resistance markers as SA and tetracycline. The fish pathogens *A. hydrophila*, *A. salmonicida*, *E. tarda*, *Enterococcus seriolicida*, *Pasteurella piscicida*, and *Vibrio anguillarum* are all widely distributed in fish farms in various areas, and within each species the R plasmid has an identical DNA sequence. The chloramphenicol resistance (*cat*) gene of the R plasmid from Gram-negative bacteria was classified into CAT I, II, III, and IV according to the DNA sequence. The *cat* gene of *P. piscicida* was classified as CAT I, those of *A. salmonicida* and *E. tarda* were classified as CAT II, and that of *V. anguillarum* was classified as CAT II or IV, depending on the time the strains were isolated. The tetracycline-resistance determinants (Tet), which occur in six classes (Tet A through Tet G), were class D in the R plasmids obtained from strains of *V. anguillarum* that were isolated from 1989 to 1991. The Tet for strains of *V. anguillarum* isolated from 1973 to 1977 was classified as Tet B, while for strains isolated from 1980 to 1983 it was classified as Tet G.

INTRODUCTION

Intensive fish culture has resulted in the massive use of chemotherapeutic agents for the treatment of bacterial infectious diseases. The most common chemotherapeutants in use in fish farms in Japan are amoxicillin, ampicillin, bicozamycin, florfenicol, lincomycin, macrolide antibiotics, novobiocin, pyridonecarboxylic acids, sodium nifurstyrenate, sulfonamides, the sulfonamide:ormetoprim complex, tetracycline derivatives, and thiamphenicol (Aoki 1992a, Kitao *et al.* 1992).

Drug-resistant Gram-negative bacteria carrying the transferable R plasmid were isolated with high frequency from the intestinal tracts of cultured eel (*Anguilla* spp.) (Aoki and Watanabe 1973a), carp (*Cyprinus carpio*) (Aoki 1974), amago salmon (*Oncorhynchus rhodurus*) (Aoki *et al.* 1972), ayu (*Plecoglossus altivelis*) (Aoki 1975a, Aoki *et al.* 1980), and yellowtail (*Seriola quinqueradiata*) (Aoki *et al.* 1973) in which chemotherapeutants had often been used for treatment. On the other hand, drug-resistant bacteria were isolated at low frequencies from the intestinal tracts of wild ayu,

as well as from cultured ayu that were not administered chemotherapeutants (Aoki 1975a, Aoki *et al.* 1980).

Various drug-resistant bacteria were isolated with varying frequency from the water of ponds used for culturing eel (Aoki and Watanabe 1973a), carp (Aoki 1974), ayu (Aoki 1975a), and rainbow trout (Aoki and Watanabe 1973b).

The use of chemotherapeutants in aquaculture has been associated with an increased occurrence of drug-resistant fish-pathogenic bacteria, including *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *Enterococcus seriolicida*, *Pasteurella piscicida*, and *Vibrio anguillarum* (Aoki 1988, 1992a, b; Aoki *et al.* 1990).

When donor and recipient cells were cultured together in a test tube, the R plasmid could be easily transferred from donor cells of drug-resistant strains of fish pathogens to recipient cells of a strain of *Escherichia coli*. Transfer of R plasmids was also observed in sterilized sea water (Goodman *et al.* 1993), sediments (Sandaa and Enger 1994), and pond water used for fish culture (Aoki 1974); however, *in vitro* transfer of R plasmids was rare in the intestinal tracts of carp (Aoki 1975b) and mouse (Wiedemann 1972).

Multiple drug-resistant strains of fish pathogens that carry the transferable R plasmid were widely distributed in fish farms in various areas (Aoki 1992a, b). The detected R plasmids from Gram-negative fish pathogens were encoded with resistance to ampicillin, chloramphenicol, florfenicol, kanamycin, ormetoprim, sulfonamides, and/or tetracycline. Drug-resistant markers of R plasmids depend on the origin of each fish pathogen. The kind of chemotherapeutant used was correlated with the appearance of the corresponding drug-resistant marker, except for kanamycin (KM) and streptomycin (SM) of *P. piscicida* and *V. anguillarum*. KM and SM have never been used to treat any bacterial infectious disease in Japanese fish farms (Aoki 1988, 1992a, b). Gram-negative pathogenic bacteria of fish that also have chromosomal genes that provide resistance to pyridonecarboxylic acids and nitrofurans were isolated frequently in fish farms (Aoki 1988, 1992a, b).

Drug-resistant strains of the Gram-positive *Enterococcus seriolicida* have resistance to various combinations of CP, erythromycin (Mls), lincomycin (LIM), and TC. These resistant strains were classified as having either intermediate- or high-level resistance to CP, LIM, Mls, or TC. Transferable R plasmids were detected in high-level resistant strains with resistance to CP, LIM, Mls, and/or TC. Intermediate-level resistance determinants were not transferred (Aoki *et al.* 1990).

The DNA sequences of R plasmids from *A. hydrophila*, *A. salmonicida*, *E. tarda*, *E. seriolicida*, *P. piscicida*, and *V. anguillarum* differed from each other (Aoki 1988, 1992b). R plasmids of *A. hydrophila* and *A. salmonicida* have the same markers as CP, SA and SM, and the R plasmids of *A. hydrophila* and *E. tarda* also have the same resistance markers as SA and TC (Akashi and Aoki 1986, Aoki *et al.* 1986). The R plasmids detected from each fish pathogen, which were isolated in different areas but in the same year, had identical DNA sequences.

The chloramphenicol resistance (*cat*) gene of the R plasmid from Gram-negative bacteria was classified into CAT I, II, III and IV according to the DNA sequence. The *cat* gene of the R plasmid detected in *P. piscicida* was classified as CAT I and those from *A. salmonicida* and *E. tarda* were classified as CAT II. The *cat* gene of the R plasmid from *V. anguillarum* was classified as CAT II for strains isolated between 1973 and 1977 and as CAT IV for strains isolated between 1989 to 1991 (Aoki 1988, Kim and Aoki 1993).

Six classes of tetracycline resistance determinant (Tet) of the R plasmids from Gram-negative bacteria (Tet A through G) were recognized. The Tet in R plasmids from *A. hydrophila*, *E. tarda*,

P. piscicida and *V. anguillarum* (isolated from 1989 to 1991) was classified as Tet D (Aoki and Takahashi 1987), which is very common in fish pathogens. The Tet of R plasmids from *V. anguillarum* isolated from 1973 to 1977 was Tet B, while that from those isolated from 1980 to 1991 was Tet G (Aoki 1988).

REFERENCES

- Akashi A, Aoki T. 1986. Characterization of transferable R plasmids from *Aeromonas hydrophila*. Bull. Jpn. Soc. Sci. Fish. 52:649-655.
- Aoki T. 1974. Studies of drug-resistant bacteria isolated from water of carp-ponds and intestinal tracts of carp. Bull. Jpn. Soc. Sci. Fish. 40:247-254. (in Japanese).
- Aoki T. 1975a. Effects of chemotherapeutics on bacterial ecology in the water of ponds and the intestinal tracts of cultured fish, ayu (*Plecoglossus altivelis*). Jpn. J. Microbiol. 19:7-12.
- Aoki T. 1975b. Transfer of R factor in the intestinal tracts of fish and sterilized water of cultured pond. Fish Pathol. 9:167-173. (in Japanese).
- Aoki T. 1988. Drug-resistant plasmids from fish pathogens. Microbiol. Sci. 5:219-223.
- Aoki T. 1992a. Chemotherapy and drug resistance in fish farms in Japan. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 519-529. Fish Health Section, Asian Fisheries Society, Manila.
- Aoki T. 1992b. Present and future problems concerning the development of resistance in aquaculture. In: Chemotherapy in Aquaculture: from Theory to Reality. p. 254-262. Office International des Epizooties, Paris.
- Aoki T, Akashi A, Sakaguchi T. 1986. Phylogenetic relationships of transferable R plasmids from *Edwardsiella tarda*. Bull. Jpn. Soc. Sci. Fish. 52:1173-1179.
- Aoki T, Egusa S, Watanabe T. 1973. Detection of R+ bacteria in cultured marine fish, yellowtails (*Seriola quinqueradiata*). Jpn. J. Microbiol. 17:7-12.
- Aoki T, Egusa S, Yada C, Watanabe T. 1972. Studies of drug resistance and R factors in bacteria from pond-cultured salmonids. I. Amago (*Oncorhynchus rhodurus macrostomus*) and yamame (*Oncorhynchus masou ishikawae*) Jpn. J. Microbiol. 16:233-238.
- Aoki T, Jo Y, Egusa S. 1980. Frequent occurrence of drug resistant bacteria in ayu (*Plecoglossus altivelis*) culture. Fish Pathol. 15:1-6. (in Japanese).
- Aoki T, Takahashi A. 1987. Class D tetracycline resistance determinants of R plasmids in fish pathogens *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Pasteurella piscicida*. Agents Chemother. 31:1278-1280.
- Aoki T, Takami K, Kitao T. 1990. Drug resistance in a non-haemolytic *Streptococcus* sp. isolated from cultured yellowtail *Seriola quinqueradiata*. Dis. Aquat. Org. 8:171-177.
- Aoki T, Watanabe T. 1973a. Studies of drug-resistant bacteria isolated from eel-pond-water and intestinal tracts of the eel (*Anguilla japonica* and *Anguilla anguilla*). Bull. Jpn. Soc. Sci. Fish. 39:121-130. (in Japanese).
- Aoki T, Watanabe T. 1973b. Studies of drug resistance and R factors in bacteria from pond-cultured salmonids. II. Rainbow trout (*Salmo gairdneri* f. *irideus*). Fish Pathol. 8:83-90.
- Goodman AE, Hild E, Marshall KC, Hermansson M. 1993. Conjugative plasmid transfer between bacteria under simulated marine oligotrophic conditions. Appl. Environ. Microbiol. 59:1035-1040.
- Kim EH, Aoki T. 1993. The structure of the chloramphenicol resistance gene on a transferable R plasmid from the fish pathogen, *Pasteurella piscicida*. Microbiol. Immunol. 37:705-712.
- Kitao T, Yoshida T, Kusuda R, Matsuoka T, Nakano S, Okada M, Oshima T. 1992. *In vitro* antibacterial activity of bicozamycin against *Pasteurella piscicida*. Fish. Pathol. 27:109-113.
- Sandaa RA, Enger O. 1994. Transfer in marine sediments of the naturally occurring plasmid pRAS1 encoding multiple antibiotic resistance. Appl. Environ. Microbiol. 60:4234-4238.
- Wiedemann B. 1972. Resistance transfer *in vivo* and its inhibition. In: Bacterial Plasmids and Antibiotic Resistance. p. 75-90. Springer-Verlag, Berlin.

The Use of Chemicals in Aquafeed

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ABSTRACT

Various chemicals and additives used in fish and shrimp feeds may have impacts on animal health, product quality and the environment. This paper reviews the use and effects of vitamins (vitamins C and E), essential fatty acids, carotenoids, immunostimulants, hormones and attractants added to feeds for cultured fish and shellfish.

INTRODUCTION

The rapid growth of the fish and shrimp culture industries, especially in Asia, has been accompanied by the negative impacts of disease. Although parasitic (e.g., *Zootamnium*) and bacterial diseases (e.g., *Vibrio* spp.) sometimes result in heavy losses in hatcheries, viral diseases are the most serious problem in grow-out facilities. Chemotherapy is now recognized as an increasing source of problems in aquaculture. The presence of residual antibiotics in sediments and in the tissues of shrimp may have public health implications, and these substances are regarded as dangerous for humans. Effective vaccines for shrimp are not available; such vaccines may not be practical because of the non-self-recognition immune system of these animals. Several appropriate measures could be employed to reduce losses from disease. One preventive measure is to ensure optimal diet quality. In mammals, one of the first signs of malnutrition is immunosuppression. This review paper emphasizes chemicals such as vitamins, fatty acids, carotenoids, immunostimulants, hormones and attractants used in feed which impact on health, product quality and the environment.

VITAMINS

So far, four fat-soluble and 11 or 12 water-soluble vitamins are known to be required by fish and shrimp, respectively. Vitamin deficiencies lead to aberrant biochemical functions and consequent cellular and organal disfunctions which gradually manifest as clinical deficiencies. The weak integrity of skin and epithelial tissues consequently predisposes fish to infections. Moreover, cells involved in the generation of both specific and nonspecific immune responses are metabolically active and are also likely to be affected by vitamin deficiency. Most studies on the positive correlation between vitamin and immune response of fish are confined to the antioxidant vitamins C and E.

Vitamin C

The clinical signs of ascorbic acid (AA) deficiency in fish were described by Halver *et al.* (1975). They include lordosis, scoliosis, distortion of support cartilage, hyperplasia of gill tissue, shortened opercles and petechial hemorrhages. External signs of AA deficiency have been described in rainbow trout (*Oncorhynchus mykiss*) (Kitamura *et al.* 1965; Halver *et al.* 1969; Hilton *et al.* 1977, 1978), brook trout (*Salvelinus fontinalis*) (Poston 1967), coho salmon (*O. kisutch*) (Halver *et al.* 1969), cherry salmon (*O. mason*) (Halver *et al.* 1975), mrigal (*Cirrhina mrigala*) (Mahajan and Agrawal

1980), common carp (*Cyprinus carpio*) (Dabrowski *et al.* 1988), guppy (*Poecilia reticulata*) (Halver *et al.* 1975), yellowtail (*Seriola quinqueradiata*) (Sakaguchi *et al.* 1969), channel catfish (*Ictalurus punctatus*) (Lovell 1973, Lim and Lovell 1978, Miyazaki *et al.* 1985), Japanese eel (*Anguilla japonica*) (Arai *et al.* 1972), lake whitefish (*Coregonus clupeaformis*) (Zitzov and Millard 1988), plaice (*Pleuronectes platessa*) (Rosenlund *et al.* 1990), Zill's tilapia (*Tilapia zilli*) (Anadu *et al.* 1990), Mayan chichlid (*Cichlasoma urophthalmus*) (Chavez de Martinez 1990), Atlantic salmon (*Salmo salar*) (Sandnes 1991), seabass (*Lates calcarifer*) (Boonyaratpalin *et al.* 1989) and tiger shrimp (*Penaeus monodon*) (Boonyaratpalin and Phongmaneerat 1995). It is generally agreed that the clinical signs of deficiency seen in fish are caused by impaired collagen and support cartilage formation (Wilson and Poe 1973, Halver *et al.* 1975, Lim and Lovell 1978). In *P. monodon*, the signs of deficiency include soft shelling, reduced activity, opaque whitish muscle, big head with flipped gill cover, incomplete molting and mortality (Boonyaratpalin and Phongmaneerat 1995).

The role of vitamin C in disease resistance has been studied in several fish. Durve and Lovell (1982) found that a dietary supplementation of 30 mg vitamin C/kg supported normal growth and prevented deficiency signs in channel catfish. Increased resistance against infection by *Edwardsiella tarda* was observed at a supplementation level of 150 mg/kg at water temperature of 21 °C; however, at 33 °C, increasing the supplemented level of vitamin C had no significant effect on resistance against infection. Feeding channel catfish a megadose (3000 ppm) of vitamin C enhanced disease resistance against *E. ictaluri* significantly.

Fish fed a vitamin C-deficient diet showed suppressed specific antibody production against *E. ictaluri* serum complement activity and non-specific phagocytic index (Li and Lovell 1985). Liu *et al.* (1989) reported that when pond-reared catfish were immunized with *E. ictaluri* and challenged 1 mo later with virulent *E. ictaluri*, a dietary AA level of 1,000 mg/kg of diet significantly decreased mortality. Higher supplementation of vitamin C (2,000 and 4,000 mg/kg diet) did not further decrease mortality. This lack of a positive effect of megadose of vitamin C on complement activity does not agree with the findings of Li and Lovell (1985). In contrast to Li and Lovell (1985), Li *et al.* (1993) observed that high vitamin C diets appear to be ineffective as a prophylactic treatment to enhance disease resistance against *E. ictaluri* in channel catfish.

Yano *et al.* (1990) found that megadose levels of L-ascorbic acid and L-ascorbyl-2 phosphate Mg (10,000 mg/kg diet) increased the phagocytic index of pronephros cells of red seabream but did not influence the complement activity.

In salmonids, the effects of AA on immune response vary widely, and results are less conclusive. Blazer (1982) showed that rainbow trout fed a diet with 120 mg/kg AA had significantly lower phagocytic index compared to fish fed a diet containing 1200 mg/kg AA. Blazer and Wolke (1984a) challenged rainbow trout with sheep red blood cells or *Yersinia ruckeri*. Those fed diets containing higher than standard levels of AA and vitamin E had increased thymus-dependent cells (T cells) and bursa-dependent cells (B cells), as well as enhanced specific antibody titers.

Wahli *et al.* (1986) studied the effect of dietary AA on the disease resistance of rainbow trout infected with the holotrichous ciliate *Ichthyophthirius multifiliis*. Trout fed 0 and 5,000 mg AA/kg diet for 5 d were infected with 8,800 and 11,500 tomites per fish. Fifty days after infection no parasites could be detected on fish that survived the infection. At the lower infectious dose, survival rate increased from 48% (0 mg AA/kg) to 98% (5,000 mg AA/kg), while the respective values were 0% and 82% in trout infected with the highest number of ciliates. Serum from experimentally infected fish was examined for tomite-immobilizing activity, IgM and specific antibodies against *I. multifiliis* (Wahli *et al.* 1986). The authors concluded that the results definitely indicated the presence of an immune response against this parasite, and that this response may have been enhanced by dietary AA.

Navarre and Halver (1989) studied the effects of high levels of dietary AA on disease resistance and humoral antibody production against *Vibrio anguillarum* in rainbow trout. Young fish were fed purified experimental diets supplemented with 0, 100, 500, 1000 and 2000 mg AA/kg for 28 wk. Resistance to bacterial infection was related to dietary AA, and was reflected in improved survival of challenged fish fed diets with 500, 1000 and 2000 mg AA; higher antibody production was observed. Navarre and Halver (1989) concluded that rainbow trout may survive bacterial challenge and improve antibody production when the diet contains 500-1000 mg AA/kg. Verlhac *et al.* (1991) found impaired phagocytosis and antibody response in vitamin C-deficient rainbow trout. The variation in phagocytosis and antibody production of fish fed 1000 ppm calcium L-ascorbate-2 monophosphate was not significantly different when compared to those fed normal requirement level (100 ppm). Verlhac *et al.* (1996) showed that high dietary level of ascorbate polyphosphate (1000 ppm) enhanced the phagocytic index of rainbow trout but had no antibody response effect.

Lall *et al.* (1989) found no conclusive evidence that vitamin C affects the susceptibility of Atlantic salmon to vibriosis or furunculosis, the production of antibodies, or the bactericidal activity of serum from normal or immunized fish.

Olivier *et al.* (1989) fed a practical diet supplemented with 0, 50, 100, 200, 500, 1000 and 2000 mg AA/kg to Atlantic salmon for 22 wk. For all diets, no effects on non-specific resistance factors were found. Groups of fish fed 0, 100, 500 and 2000 mg AA/kg were immunized against *Aeromonas salmonicida* and *Vibrio anguillarum*. One month after vaccination, analysis for complement activity and humoral antibody response revealed no effects of dietary AA.

In Atlantic salmon parr, Sandnes *et al.* (1990) studied the production of antibodies against a soluble artificial antigen (NIP₁₁-LPH) following a feeding period of 6 wk when fish were fed different equivalent level of AA and AS in the diets (0, 500 and 5000 mg AA/kg). The antibody response was somewhat reduced in fish deprived of vitamin C, but there were no differences between fish fed 500 and 5000 mg/kg, irrespective of chemical form. The results indicated that antibody production is not dependent on a high physiological status of AA (Sandnes *et al.* 1990).

Hardie *et al.* (1991) found that serum complement activity of Atlantic salmon was significantly reduced in AA-depleted fish. On bath challenge with a virulent strain of *A. salmonicida*, a significant increase in mortality was seen in AA-depleted fish. However, specific and certain non-specific cellular defense-related responses including respiratory burst (superoxide anion) production and erythrophagocytosis were unaffected by dietary vitamin C.

Erdal *et al.* (1991) fed Atlantic salmon three diets with varying contents of AA and AS and measured general resistance after challenge with *V. salmonicida*, development of specific immune response after vaccination against *Yersinia ruckeri*, and survival after challenge. No significant differences in general or specific resistance were found; however, the experimental design renders interpretation of the results difficult. Thus, the experimental diets were supplemented with either 90 mg AA/218 mg As/kg feed (diet 1), 2980 mg AA/218 mg AS (diet 2) or 90 mg AA/4470 mg AS (diet 3).

In Pacific salmon, higher levels of dietary vitamin C showed no positive effect on immune response or disease resistance. Bell *et al.* (1984) reported that dietary ascorbate had no effect on the development of bacterial kidney disease (*Reinbacterium salmoninarum*) in sockeye salmon (*Oncorhynchus nerka*).

In summary, (1) there is evidence that high dietary dose of vitamin C protects against infectious diseases in channel catfish; (2) no clear conclusion can be drawn with regards to salmonids; and (3) there is general agreement that vitamin C-depleted fish are more susceptible to disease.

The role of vitamin C in reducing the negative impacts of environmental stress on health and on disease resistance has also been studied. Stress increases susceptibility to infection (Maule *et al.* 1989) and is increased by handling, vaccination and transport of fish. Often, acute and chronic stress causes hypersecretion of corticosteroids, particularly cortisol (Robertson *et al.* 1987), which may induce some secondary effects on the immune system (Bennett and Wolke 1987, Ellsaesser and Clem 1987). Prolonged elevation of cortisol significantly increased disease susceptibility in brown trout (*Salmo trutta*) (Pickering and Pottinger 1985). Stress-induced ascorbic acid depletion from the anterior kidney in coho salmon and rainbow trout has been observed (Wedemeyer 1969). Channel catfish fed vitamin C-free diets are more sensitive to physiological stressors such as ammonia and low oxygen levels than when fed diets with vitamin C (Mazik *et al.* 1987). Ascorbic acid reduces the effects of toxicity of waterborne copper (Hilton 1989) and nitrite (Wise *et al.* 1988), and also protects against the adverse effects of intakes of the organochlorine pesticide Aldrin (Agarwal *et al.* 1978) and the insecticide Toxophene (Mayer *et al.* 1978).

Ascorbic acid has antioxidant properties in feed (Cort 1982, Takahashi *et al.* 1986). The antioxidant effect of ascorbic acid and ascorbyl palmitate is more effective in combination with vitamin E, as vitamin E reacts with the free radicals first. Ascorbic acid and ascorbyl palmitate regenerate vitamin E until all ascorbate is consumed (Lambelet *et al.* 1985).

Vitamin E

Reduced growth, muscular dystrophy and anemia (red blood cell lysis) caused by oxidative damage of components in the cell membranes were observed in vitamin E-deficient fish (Moccia *et al.* 1984, Cowey 1986, Furones *et al.* 1992, Mcloughlin *et al.* 1992). Studies on the protective effect of vitamin E are limited.

Wise *et al.* (1993a) fed channel catfish fingerlings with purified diets either unsupplemented and deficient in both selenium and vitamin E, deficient in either selenium or vitamin E, adequate in both selenium (0.2 mg/kg) and vitamin E (60 mg/kg) or excessive in both nutrients (four times the recommended levels) for 120 d. The results indicated that higher than recommended levels of one or both nutrients may enhance macrophage function; selenium and vitamin E did not complement each other. Wise *et al.* (1993b) fed channel catfish fingerlings with purified diets containing α -tocopherol acetate to provide 0, 60 and 2500 mg vitamin E/kg for 180 d. The susceptibility of red blood cells to oxidative hemolysis decreased with increasing levels of dietary vitamin E, and the ability of macrophages to phagocytize virulent *E. ictaluri* was enhanced by vitamin E, but agglutinating antibody titers were not effected by dietary vitamin E levels.

Blazer and Wolke (1984b) reported that α -tocopherol deficiency suppressed all aspects of humoral and cellular immunity as well as the phagocytic index of rainbow trout. Macrophages from fish fed a deficient diet showed significantly reduced ability to engulf latex beads as compared to those from fish on a control diet.

Ndoye *et al.* (1989) observed a gradual increase in antibody production in trout with an increase in dietary vitamin from 23 mg to 60 and 600 mg/kg diet. In trout fed diets supplemented with 0, 45 and 450 mg vitamin E/kg for 23 wk, Verlhac *et al.* (1991) observed that antibody titre against redmouth disease at 40 d after vaccination was higher for fish fed 450 mg vitamin E/kg as compared to those fed at 45 mg/kg. However, the beneficial effect on stimulation was time dependent, and after 120 d of feeding no difference was observed. Verlhac *et al.* (1991) also concluded that vitamin E deficiency impaired antibody response and lymphoproliferation.

Rainbow trout fed diets containing 7, 86 or 806 mg vitamin E/kg for 22 wk were exposed to virulent *Yersinia ruckeri* by bath or injection. Mortalities were always least among those fed the highest concentration of vitamin E, but serum antibody production was not affected by vitamin E

levels (Furones *et al.* 1992).

Studies conducted on Atlantic and chinook salmon (*Oncorhynchus tshawytscha*) have not confirmed these observations. Lall *et al.* (1988) found that vitamin E had no effect on the non-specific resistance of Atlantic salmon challenged with *A. salmonicida*. Neither humoral response nor the complement system was affected 6 wk after vaccination with formalin-killed *A. salmonicida* cells. Hardie *et al.* (1990) reported that secretion of superoxide anion, serum lysozyme, lymphokine production and humoral immune response in Atlantic salmon were not affected by dietary vitamin intake (7, 86, 326 and 800 mg vitamin E/kg), but the complement system was compromised in fish fed a low level of vitamin E. Waagbø *et al.* (1993a) found that the interaction between dietary lipid, vitamin E and water temperature was apparent for disease resistance of Atlantic salmon challenged with *Vibrio salmonicida* by injection. Fish fed sardine oil supplemented with vitamin E showed best survival at low water temperature (8 °C), while the capelin oil diet with vitamin E was superior to the soybean oil and sardine oil diets at 13 °C. Data from all treatments showed higher total antibody level in fish fed vitamin E-supplemented diets, an opposite effect to the differences found as regards to specific antibodies.

In coho salmon, Forster *et al.* (1988) showed that disease resistance as determined by the rate of mortality induced by exposure of nonvaccinated fish to challenge with *Vibrio anguillarum* or *V. ordalii* was likewise unaffected by dietary vitamin E level (30 and 1030 IU/kg).

Blazer (1991) found that increasing the concentration of vitamin E to 2500 mg/kg of diet depressed the killing ability of macrophages and suggested that very high levels of vitamin E may have depressed other killing mechanisms such as superoxide anion and peroxide production. Lall and Olivier (1991) recommended that higher dose of vitamin E (>2000 mg/kg) should not be used in studies on immune response of fish because of problems due to hypovitaminosis.

These results indicate that the effects of vitamin E on the immune function of fish are not clear. The discrepancies between studies are difficult to explain, but they could be due to different methodologies, environmental conditions or feed compositions used and experience in determining the non-specific and specific immuno-response parameters. Since the science of fish immunology is still at the developmental stage, more research is needed to determine proper doses and durations of application to enhance the health of fish and shrimp.

As to the role of vitamin E on product quality, increasing supplements of vitamin E in the diet of channel catfish may provide additional protection against lipid oxidation in fillet tissue (Gatlin *et al.* 1992). This would mean improved quality of fillet and shelf life. This information will probably be most useful in countries where raw fish is a preferred food and freshness brings a premium price.

ESSENTIAL FATTY ACIDS

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contained in fish and crustaceans are essential fatty acids for marine animals, and these highly unsaturated fatty acids are also required by humans. Omega-3 highly unsaturated fatty acids (Ω -3 HUFA) are quantitatively the dominant fatty acids in marine fish. Fish cannot synthesize essential Ω -3 fatty acids (EFA), and therefore, these fatty acids must be provided in the feed. Requirements of salmonids for Ω -3 fatty acids are estimated to be approximately 1% of the diet in low fat diets (Castell *et al.* 1972, Yu and Sinnhuber 1979). Two highly unsaturated fatty acids (HUFA) of the Ω -3 family, namely eicosapentaenoic acid (20:5 n-3) (EPA) and docosahexaenoic acid (22:6 n-3) (DHA), are of particular importance (Gunstone *et al.* 1978, Ackman and Takeuchi 1986). Omega-3 HUFA may have a higher EFA value, and requirements for rainbow trout are satisfied by a level of 0.5% of the diet or 10% of total dietary lipids (Watanabe 1982).

The significance of Ω -3 fatty acids in various tissues, other than on growth-promoting effect, has not been determined in detail. Membrane-bound lipids are important for temperature adaptation in cold-blooded animals (Hazel 1979). At low temperatures, a selective increased incorporation of Ω -3 fatty acids into the cell membrane takes place (Hazel 1979). It is assumed that this enhances the fluidity of the cell membrane. Further, Ω -3 fatty acids are important precursors in the synthesis of eicosanoids (Bakhle 1983), which are, in turn, important mediators in inflammatory reactions and, partly, in the regulation of the immune response. The bactericidal activity of macrophage cells *in vitro* was reduced when the diet was EFA deficient. A maximum number of *Aeromonas salmonicida* survived in this group of salmon. Macrophages of fish receiving lenolenic acid (LNA) or n-3 HUFA were more effective in killing the bacteria *in vitro* (Kiron *et al.* 1995).

The effectiveness of n-3 series fatty acids in intracellular killing has been demonstrated in a study by Sheldon and Blazer (1991) on channel catfish. Using purified diets containing either menhaden oil, soybean oil or beef tallow at 7%, they correlated enhanced bactericidal activity to increasing levels of HUFAs, results that were independent of the rearing temperature. Waagbø *et al.* (1993a) observed in Atlantic salmon that the bacterial killing activity of macrophages at 12 °C was reduced in fish fed on sardine oil which contained more n-3 polyunsaturated fatty acid (n-3 PUFA) as compared to fish receiving capelin oil which had lower n-3 PUFA content. However, there was no difference in the macrophage activity at a higher temperature (18 °C). Kiron *et al.* (1995) reported that in rainbow trout, the macrophage activity *in vitro* was superior in all the groups receiving EFA (LA, LNA and n-3 HUFA) as compared with the deficient group receiving palmitic acid. In this study, the macrophages of rainbow trout receiving the PUFAs responded better against the bacteria. The immunomodulation by dietary lipid is effected by changes in the plasma membrane lipid structure of the lymphocyte subpopulation. However, caution has to be exercised in such dietary manipulations, as it has been shown that high fat concentration in the diet, particularly PUFA, can suppress lymphocyte functions when EFA requirements are met.

Erdal *et al.* (1991) illustrated the relationship between n-3 fatty acids and the immune response in Atlantic salmon. Fish fed diets with various types of oils containing increasing amounts of n-3 fatty acids (from 2.9 to 3.6-5.8) exhibited an immune suppressive effect. Waagbø *et al.* (1993a) showed that increasing the amount of n-3 PUFA from 3.5 to 6.0% in diets of Atlantic salmon was again the reason for a reduction in specific antibody production against *Vibrio salmonicida*.

Henderson *et al.* (1992) reported that antibody production was not affected by increased dietary n-3 PUFA in rainbow trout vaccinated with *Y. ruckeri*. However, Kiron *et al.* (1995) suggest that optimal use of EFA (0.5% according to Watanabe (1982) and 1.0% according to Castell *et al.* (1972) enhances antibody activity. This indicated that EFA above the required level might have impaired the defense mechanisms. Waagbø *et al.* (1993a) showed an interaction between dietary lipid, vitamin E and water temperature for disease resistance of Atlantic salmon challenged with *V. salmonicida* by injection. Dietary vitamin E requirement increases as dietary n-3 PUFA increase. Dietary n-3 PUFA requirement level decreases as water temperature increases.

The increase in clotting time observed with increasing level of dietary n-3 PUFA and vitamin E (Salte *et al.* 1987, 1988; Waagbø *et al.* 1993b) resembles the classical effects of marine lipids reported in humans and experimental animals, where n-3 PUFA-rich diets prevent the development of cardiovascular diseases by decreasing the incidence of thrombosis (Herold and Kinsella 1986, Weber 1989). An awareness of the influence DHA, contained in the mammalian brain, on intelligence and the protective role of n-3 PUFA against the development of cardiovascular disease has prompted the promotion of fish consumption. Fish rich in n-3 PUFA would seem to give the most benefit. In general, marine fish will have higher n-3 PUFA than freshwater fish; however, lipid contents, nutritional status, total lipid and phosphoglyceride DHA levels of gillhead seabream were directly correlated with dietary DHA levels (Mourete *et al.* 1993). For most fish, body lipid composition is affected by dietary lipid composition. In this connection, fish nutrition, though

feed manipulation, would play an important role in the production of fish that are rich in n-3 HUFA for the benefit of human health.

CAROTENOIDS

Coloration

One of the most obvious functions of carotenoid in feed is in coloration of aquatic animals. Carotenoid use in fish culture is mainly associated with astaxanthin and canthaxanthin pigmentation of the flesh of salmonids, or astaxanthin in the shells and flesh of shrimp and lobsters. Next to freshness, the pigmentation of the flesh of Atlantic salmon and rainbow trout is regarded as the most important quality criteria. The market demand for Atlantic salmon is for a flesh concentration of astaxanthin above 6-8 mg/kg. Because shell and flesh color of shrimp and fish whet the appetite and enhance enjoyment, food color acts as an optical seasoning. Ornamental fishes change their hues in response to background coloration to avoid being preyed upon, and also display color response during excitement and courtship (Moyle and Cech 1982).

Fish and shrimp are not able to synthesize carotenoids “*de novo*” and depend completely on the presence of necessary carotenoids in their feed. Salmonids absorb and deposit astaxanthin and canthaxanthin in the muscle during the grow-out period. At the time of sexual maturation, they mobilize stored carotenoids to the ovaries and finally, on to the progeny. This active transfer of carotenoids from the mother to the eggs has led to the hypothesis that carotenoids are vital for egg and larval development (Negre-Sadargues *et al.* 1993).

Shrimp (*Penaeus monodon*) absorb canthaxanthin, which is transformed into astaxanthin before being deposited in flesh and shell. Therefore, astaxanthin is 2-3 times more efficiently utilized by *P. monodon* than is canthaxanthin because no transformation is needed. Citranaxanthin had no effect in coloration of *P. monodon* (Boonyaratpalin *et al.* 1994). It appears that astaxanthin is more efficiently used in pigmentation of *P. monodon* than is β -carotene (Chien and Jeng 1992). This result supports the work done by Katayama *et al.* (1972) on the metabolic pathway of β -carotene to astaxanthin in shrimp. Foss *et al.* (1984) and Torrissen (1986) also found that astaxanthin was more efficacious than canthaxanthin in pigmenting the flesh of rainbow trout, but astaxanthin and canthaxanthin were not interconverted.

Growth and Survival

Torrissen (1984) found a positive effect of 30 mg/kg canthaxanthin or astaxanthin supplementation on growth during the start of the feeding period, and no significant differences were found between fish fed the astaxanthin and canthaxanthin-supplemented diets. Goswami (1993) showed that a supplement of β -carotene and canthaxanthin in the diets of Indian major carps resulted in better survival and growth as compared to conventional diets without carotenoids. Improved survival rates were also reported for kuruma shrimp (*Penaeus japonicus*) (Chien and Jeng 1992, Negre-Sadargues *et al.* 1993) by astaxanthin supplementation or supplementation with a combination of astaxanthin and canthaxanthin (1:1), but no differences were observed in growth or molting.

A study on the quantitative requirement for astaxanthin in Atlantic salmon showed increased growth and survival, and protein utilization in the range of 0.7 to 5.3 mg/kg with a constant, high value for diets supplemented above 5.3 mg/kg (Torrissen and Christiansen 1994). Thomson *et al.* (1995) found no marked differences in food conversion efficiency or growth of rainbow trout fed diets with and without astaxanthin.

Reproduction

Hartmann *et al.* (1947) reported that astaxanthin had a function as a fertilization hormone by stimulating and attracting spermatozoa, and Deufel (1965) found that fertilization increased in trout given a canthaxanthin-supplemented diet. This is supported by Longinova (1977), who reported higher survival of highly pigmented rainbow trout eggs as compared to pale ones.

In red seabream (*Chrysophrys major*), the percentage of buoyant eggs was found to be improved by a diet fortified with carotenoids (β -carotene, canthaxanthin or astaxanthin). The hatching rate was not influenced, but abnormality in number and position of the oil globules was reduced. Consequently, the total number of normal larvae was higher when the broodstock was supplied carotenoids (Watanabe *et al.* 1984). β -carotene was confirmed to be non-transferable, resulting in poor egg quality (Watanabe and Miki 1991). The freshwater fish *Heteropneustes fossilis* fed a carotenoid-free diet showed atrophied gonads with damaged germinal epithelium (Goswami 1988).

In contrast, Torrissen and Chistiansen (1994) produced Atlantic salmon eggs with astaxanthin concentrations varying from 0.1 to 20 mg/kg by keeping salmon in filtered sea water on a carotenoid-free diet from smolt stage to spawning. Comparison of the progeny with brood from fish given identical treatment but a diet fortified with 100 mg/kg astaxanthin showed no significant differences in rate of fertilization, survival during the green egg stage, from eyed egg until hatching or of the yolk-sac fry. No differences in size, deformities, general performance or tolerance to oxygen depletion could be detected. Harris (1984) and Tveranger (1986) found no effect of dietary astaxanthin or canthaxanthin supplementation on fecundity, fertilization rate or hatching rate of rainbow trout and Atlantic salmon. Torrissen (1986) found no effect of egg pigmentation on survival during the embryonic stages.

Health and Immunology

Except for the consensus that carotenoids enhance the performance of fish and their brood, there is little information on the direct effects of carotenoids on fish health. Thompson *et al.* (1995) found that astaxanthin does not appear to have any marked effect on innate or specific immunity, and therefore has little potential as immunostimulant for cultured trout.

Conclusions

As coloration is a positive selection criterion for the consumer, synthetic astaxanthin is routinely added to the diets of farmed salmon. Available data show that the amount of dietary astaxanthin required for growth is 5.3 mg/kg; for survival and egg quality, it is 20 mg/kg. However, normal dosage in commercial salmon feed is between 30 to 70 mg/kg, starting with a high dose and decreasing as fish grow. Astaxanthin feed was used through the whole production cycle (OJ Torrissen pers. comm.). Astaxanthin's effects on immune response and disease resistance need further investigation.

IMMUNOSTIMULANTS

As a number of fish and shrimp diseases are associated with intensive aquaculture, control of disease has become an urgent need. Efforts to protect fish by vaccination have found that immunity is short-lived and that there are difficulties with administration (Plumb 1988a, b). Arthropods have a simple nonspecific defensive mechanism, such as non-self recognition of foreign particles. These mechanisms involve the phagocytosis or encapsulation of large particles (Gotz 1986), blood clotting, nodule formation, the prophenaloxidase activity system and release of opsonizing factor (Perrson *et al.* 1987).

Many chemicals have been used as immunostimulators for fish and shrimp. Intraperitoneal injections of channel catfish with β -1, 3 glucan (derived from baker's yeast) greatly reduces mortality from experimental infection with *Edwardsiella ictaluri*. The results indicate that β -1,3 glucan potentially could be utilized prophylactically as an immunomodulator in channel catfish (Chen and Aingworth 1992). Verlhac *et al.* (1996) showed that the antibody response of trout can be enhanced by feeding glucan for 2 wk, and that some effects lasted 2 wk after fish has been switched back to a control diet. β -glucan, β -1,3 and 1,6 linkage polysaccharide are structural elements of fungal cell walls. *Saccharomyces cerevisiae* can enhance nonspecific disease resistance and growth in *Penaeus monodon* post-larvae via immersion, however, the protective effect of glucan treatment lasted only 14 d (Sung *et al.* 1994). Boonyaratpalin *et al.* (1993) reported that *P. monodon* fed a diet supplemented with peptidoglycan (a chemical substance from the cell walls of a bacterium, *Brevibacterium lactofermentum*) at 0.01% showed better growth, survival, disease resistance, salinity stress resistance and hemocyte phagocytic activity, whereas a higher concentration of peptidoglycan (0.1%) in feed showed an adverse effect. Sung *et al.* (1994) found similar results for high concentration of glucan, and concluded that the adverse effects were caused by gill tissue change. The mechanism of better growth probably relates to the disease resistance of shrimp. Itami *et al.* (1993) have also reported that peptidoglycan can enhance disease resistance of *P. japonicus* and increase the phagocytic activity of prawn hemocytes. Oral administration of a β -1,3-glucan derived from *Schizophyllum commune* (SPG) has produced enhanced disease resistance against vibriosis in kuruma prawn. SPG was administered orally at a level of 50 mg or 100 mg/kg body weight/d in the feed. High phagocytic activities were observed in the hemocytes of prawn fed 100 mg SPG for 3 d or 50 mg SPG for 10 d (Itami *et al.* 1994).

Based on this information, β -glucan and peptidoglycan apparently have the potential to be used prophylactically as a short-term immunostimulant for fish and shrimp. However, success of administration depended on dose and timing. Therefore, further studies are needed to find out the most practical and efficacious way of administration. The results confirm the important role of β -glucan in fish health.

HORMONES

17 α methyltestosterone

The ability to control the sex of fish populations would be advantageous to producers of economically important species, and is especially suited for prolific species such as tilapias. Androgens, 17 α methyltestosterone (MT) induced masculinization when fed to tilapia (Guerrero 1975, Nakamura 1981, Shelton *et al.* 1981); rainbow trout (Johnstone *et al.* 1978, Okada *et al.* 1979) and salmon (Fagerlund and McBride 1978). Culture of mixed sex tilapia may result in limited growth, 30-40% of the harvested fish being under marketable size. However, the use of sex steroids has sometimes yielded inconsistent results (Schreck 1974, Hunter and Donaldson 1983), presumably due to differences in duration, dose, temperature, timing of treatment, availability of natural food and species studied.

The culture of monosex tilapia has been widely practiced in Thailand, the Philippines, Indonesia and North America. Oral administration of 17 α methyltestosterone at 40-60 mg/kg feed from first feeding for a duration of 3-4 wk will produce about 98-100% male tilapia. If the fry are cultured in green water where plenty of natural food is available, then 60 mg MT/kg diet is required. This concentration can be reduced to 40 ppm when fry are cultured in clear water. The duration of treatment increases as water temperature decreases. The optimal duration treatment is 3 wk at temperature above 28 °C and 4 wk when water temperature is below 28 °C.

Concerns exist over residues of the androgen remaining in fish destined for human consumption.

Radioactivity in the carcass and viscera was evaluated in juvenile blue tilapia (*Oreochromis aureus*) (Goudie *et al.* 1986), Mossambique tilapia (*O. mossambicus*), rainbow trout (Johnstone *et al.* 1983) and coho salmon (Fagerlund and McBride 1978) fed steroid-incorporated diet (tritium and carbon¹⁴-labelled MT). Radioactivity was detected in the carcass within 1 h after initial feeding and reached the highest level by 6 h. Most of the radioactivity (>90%) was in the viscera during the period when the radiolabelled diet was being fed. Radioactivity was eliminated, exponentially decreasing by 90% within 24 h after the last feeding. By 72 h, only 4% of the original radioactivity remained, and was distributed evenly between carcass and viscera. Although less than 1% of the original radioactivity remained in *O. mossambicus* 100 h after withdrawal of radiolabelled diet (Johnstone *et al.* 1983), this level was not reached in *O. aureus* until 21 d after withdrawal (Goudie *et al.* 1986). This persistence may reflect differences in experimental protocols (continued radioisotope exposure for 21 d in *O. aureus* versus a single radioisotope exposure in *O. mossambicus*).

The short-term (about 4 d) elimination patterns for both tilapia and rainbow trout were similar. The observed low levels of residual radioactivity at the conclusion of the sex reversal period, as well as anticipated dilution through growth during the culture of fish to marketable size (250-300 gm), support the conclusion that no potential health hazard exists for people who eat fish that have been fed 17 α methyltestosterone as juveniles (<1gm). At the end of the study (21 d after last feeding of radioisotope diet), only 5 ng MT/gm remained in tissue. If all the residue persisted until the fish reached marketable size, tissue levels would be only 20 pg/gm. Therefore, the use of MT for sex-reversal was recently approved by the US FDA.

ATTRACTANTS

Attractants are mainly used to enhance feed intake and growth. In farmed fish, encouraging feed consumption can increase survival and shorten production intervals, while reducing wastage of feeds that also fouls the water. Also, effective attractants would encourage the use of bland ingredients that normally would go unused. Attractants are needed in semipurified diets in vitamin, mineral or fatty acid requirement studies. Attractants will have a greater effect on feed intake and survival of young fish at the "start feed" stage. Attractants added to feed may also help offset reduction in feed intake during times of disease or stress.

It has been demonstrated that amino acids, nucleic acid-related compounds, lipids, and nitrogenous-base and sulfur-containing organic compounds are potential attractants for abalone, fish and shrimp. The effective compounds have a wide difference among the test animals. It was ascertained for all test animals that the attraction activities were present in L-amino acids but not in D-types. When effective compounds were combined, the activities for all the test animals became higher in the order two, three and four combinations. Results showed that animals were strongly attracted by these combinations rather than by individual compounds (Harada 1985a, b, 1986; Harada and Akishima 1985; Nakajima *et al.* 1989). Nakajima *et al.* (1989) found that the sulfur-containing organic compound dimethyl- β -propiothetin (DMPT) strongly prompted the sticking behavior in goldfish and common carp when included in a synthetic diet (cellulose alone). The high-potential attractants for different test animals are compiled in Tables 1-4.

Conclusions

Feed acceptance generally is not a problem in cultured fish and shrimp except:

- fry feed
- high soybean meal feed
- semipurified diet for marine carnivorous fish
- medicated feed

Table 1. L-amino acids having high attraction activity in abalone, fish and shrimp (from Harada 1985a, b, 1986; Kanazawa 1991).

Species	Chemical	Activity
Abalone (<i>Haliotis discus</i>)	Hydroxyproline	high
	Ornithine	moderate
	Lysine	low
	Glycine	moderate
	Cystine	moderate
	Tyrosine	moderate
	Hydroxylysine	moderate
Weather fish (<i>Misgurnus anguillicaudatus</i>)	Histidine	high
	Arginine	high
	Lysine	high
	Ornithine	high
	Glycine	moderate
	L-alanine	moderate
	Homocysteine	moderate
Yellowtail (<i>Seriola quinqueradiata</i>)	Histidine	high
	Arginine	moderate
	Lysine	moderate
	Ornithine	moderate
	Glycine	high
	Valine	moderate
	Threonine	high
Kuruma shrimp (<i>Penaeus japonicus</i>)	Proline 0.7%	high
	Alanine+proline	high
Atlantic salmon (<i>Salmo salar</i>)	L-proline	
	L-alanine	
	L-lysine	
Rainbow trout (<i>Oncorhynchus mykiss</i>)	L-alanine	
	L-leucine	
	L-tryptophan	
	L-phenylamine	
Brown trout (<i>S. trutta</i>)	L-proline	
	L-methionine,taurine	
	L-cystine	
	L-glutamic acid	
	L-glycine Glutathione	

Table 2. Nucleic acid-related compounds recorded as potential attractants in abalone, fish and shrimp (from Harada 1986, Carr and Thompson 1983, Carr *et al.* 1984).

Species	Chemical	Activity
Abalone (<i>Haliotis discus</i>)	AMP+xanthine+cytosine	highest
	AMP+xanthine	higher
	AMP	high
	Cytosine	moderate
	Xanthin	low
Japanese eel (<i>Anguilla japonica</i>)	AMP	none
	IMP, UMP	low
	GMP	high
Yellowtail (<i>Seriola quinqueradiata</i>)	Cytosine	high
	GMP	high
	Guanine	moderate
	Adenine	moderate
	AMP	high
	Cytosine + AMP	higher
	Cytosine + AMP - guanine	highest
Cytosine + AMP + dGMP	highest	
Weather fish (<i>Misgurnus anguillicaudatus</i>)	Adenine	high
	Guanosine	moderate
	GMP	low
	Adenine + cytosine + GMP	higher
	Adenine + cytosine + GMP + guanosine	highest
Marine shrimp (<i>Palaemonetes pugio</i>)	AMP	high
	ADP, XMP, GMP, CMP, UMP	moderate

Attractants might be used in such feeds to improve taste or mask adverse taste. Two attractant formulations have been recommended for marine fish and shrimp in Japan (Tables 5 and 6). Betaine is one of the more commonly used chemo-attractants because it is commercially available. Finnsugar researchers have shown betaine to be an especially effective attractant for Atlantic salmon and rainbow trout (Gill 1989). Finnsugar has developed a betaine-based attractant called "Finnstim" which is a mixture of betaine and amino acids that is claimed to be very effective. The recommended dose is 1.5-2.0%.

Table 3. Nitrogenous bases which were potential attractants for fish and shrimp (from Harada 1985b, Harada and Akishima 1985, Pierce and Laws 1986).

Species	Chemical	Activity
Abalone (<i>Haliotis discus</i>)	Monomethylamine	highest
	Monoethanolamine	moderate
	Pyrrrolidine	moderate
	R-aminobutyric acid	highest
	Choline	moderate
	Ammonium acetate	moderate
Weather fish (<i>Misgurnus anguillicaudatus</i>)	Monomethylamine	high
	Dimethylamine	high
	Trimethylamine	high
	R-aminobutyric acid	high
	Trimethylamine oxide	moderate
Yellowtail (<i>Seriola quinqueradiata</i>)	Ammonia	high
	Trimethylamine	moderate
	Pyrrrolidine	low
Sole (<i>Solea solea</i>), Japanese eel (<i>Anguilla japonica</i>)	Betaine	high
Giant prawn (<i>Macrobrachium rosenbergii</i>)	Trimethylammonium hydrochloride (TMAH)	high

Table 4. Lipid as attractants for abalone (from Harada and Akishima 1985).

Species	Chemical	Activity
Abalone (<i>Haliotis discus</i>)	Phosphatidyl inositol	high
	Tristearin	moderate
	Lecithin (bean)	low

Table 5. Composition of amino acid mixture as an attractant for marine fish (from Yone 1976).

Amino Acid	gm/100 gm diet
L-Phenylalanine	0.6
L-Arginine-HCl	1.3
L-Cystine	0.7
L-Tryptophan	0.2
L-Histidine HCl H ₂ O	0.2
DL-Alanine	1.3
L-Aspartic acid Na	1.0
L-Valine	0.7
L-Lysine HCl	0.6
Glycine	0.4

Table 6. Composition of attractant for marine shrimp (from Kanazawa 1991).

Chemicals	gm/100 gm diet
Inosine 5-monophosphate	0.1
Taurine	0.3
Betaine	0.3
L-Glutathione	0.1
Glycine	0.2

REFERENCES

- Ackman RG, Takeuchi T. 1986. Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon *Salmo salar*. *Lipids*, 21:117-120.
- Agarwal NK, Juneja CJ, Mahajan CJ. 1978. Protective role of ascorbic acid in fishes exposed to organochlorine pollution. *Toxicology*, 11:369-375.
- Anadu, DI, Anozie OC, Anthony AD. 1990. Growth responses of *Tilapia zillii* fed diets containing various levels of ascorbic acid and cobalt chloride. *Aquaculture*, 88:329-336.
- Arai S, Nose T, Hashimoto Y. 1972. Qualitative requirements of young eels *Anguilla japonica* for water soluble vitamins and their deficiency symptoms. *Bull. Freshw. Fish. Res. Lab. (Tokyo)*, 22:69-83.
- Bakhle YS. 1983. Synthesis and catabolism of cyclo-oxygenase products. *Br. Med. Bull.* 39:214-218.
- Bell GR, Higgs DA, Traxler GS. 1984. The effect of dietary ascorbate, zinc and manganese on the development of experimentally induced bacterial kidney disease in sockeye salmon (*Oncorhynchus nerka*). *Aquaculture*, 36:293-311.
- Bennett RO, Wolke RE. 1987. The effect of sublethal endrin exposure on rainbow trout, *Salmo gairdneri* Richardson. I. Evaluation of serum cortisol concentrations and immune responsiveness. *J. Fish Biol.* 31:375-385.
- Blazer VS. 1982. The effects of marginal deficiencies of ascorbic acid and α -tocopherol on the natural resistance and immune response of rainbow trout (*Salmo gairdneri*). Ph.D. Thesis, University of Rhode Island, 121 p.
- Blazer VS. 1991. Piscine macrophage function and nutritional influences: a review. *J. Aquat. Anim. Health*, 3:77-86.
- Blazer VS, Wolke RE. 1984a. Effect of diet on the immune response of rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* 44:1244-1247.
- Blazer VS, Wolke RE. 1984b. The effects of alpha-tocopherol on the immune response and non-specific resistance factor of rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture*, 37:1-9.
- Boonyaratpalin M, Pongmaneerat J. 1995. Ascorbic acid derivative requirement of *Penaeus monodon*. *Phuket Mar. Biol. Cent. Res. Bull.* 60:65-73.
- Boonyaratpalin M, Unprasert N, Buranapanidgit J. 1989. Optimal supplementary vitamin C level in seabass fingerling diet. In: Takeda M, Watanbe T. eds. *The Current Status of Fish Nutrition in Aquaculture*. p.149-157. Tokyo University of Fisheries, Tokyo.
- Boonyaratpalin M, Wannagawat J, Borisut C, Boonchuy S. 1994. Effect of various dietary canthaxanthin and astaxanthin levels on pigmentation of giant tiger shrimp. *Natl. Inst. Coast. Aquacult. Techn. Pap. No. 18*, 11 p.
- Boonyaratpalin S, Boonyaratpalin M, Supamattaya K, Toride Y. 1993. Effects of peptidoglycan (PG) on growth, survival, immune response and tolerance to the stressor in black tiger prawn (*Penaeus monodon*). *Second Symposium on Diseases in Asian Aquaculture, Abstracts*, p. 5.

- Carr WES, Netherton JC III, Milstead ML. 1984. Chemoattractants of the shrimp, *Palaemonetes pugio*: variability in responsiveness and the stimulatory capacity of mixtures containing amino acids, quaternary ammonium compounds, purines and other substances. *Comp. Biochem. Physiol.* 77A:469-474.
- Carr WES, Thompson HW. 1983. Adenosine 5-monophosphate, an internal regulatory agent, is a potent chemoattractant for marine shrimp. *J. Comp. Physiol.* 153A:47-53.
- Castell JO, Sinnhuber RO, Wales JH, Lee DJ. 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*). *J. Nutr.* 102:77-86.
- Chavez de Martinez MG. 1990. Vitamin C requirement of the Mexican native cichlid, *Cichlasoma urophthalmus* (Gunther). *Aquaculture*, 86:409-416.
- Chen D, Aingworth AJ. 1992. Glucan administration potentiates immune defence mechanisms of channel catfish, *Ictalurus punctatus* Rafinesque. *J. Fish Dis.* 15:295-304.
- Chien YM, Jeng SC. 1992. Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources, levels and feeding regimes. *Aquaculture*, 102:333-346.
- Clem LW, Sizemore RC, Ellsaesser, CF, Miller NW. 1985. Monocytes as accessory cells in fish immune response. *Develop. Comp. Immunol.* 9:803-809.
- Cort WM. 1982. Antioxidant properties of ascorbic acid in foods. In: Seib PA, Tolbert BM. eds. *Ascorbic Acid: Chemistry, Metabolism and Uses.* p. 533-550. *Adv. Chem. Ser. No. 200*, Am. Chem. Soc., Washington D.C.
- Cowey CB. 1986. The role of nutritional factors in the prevention of peroxidative damage to tissues. *Fish Physiol. Biochem.* 2:179-193.
- Dabrowski K, Hinterleitner S, Sturmhuber C, El-Fiky N, Wieser W. 1988. Do carp larvae require vitamin C? *Aquaculture*, 72:295-306.
- Deufel J. 1965. Pigmentierungsversuche mit Canthaxanthin bei Regenbogenforellen. *Arch. Fish. Wis.* 16:125-132.
- Durve VS, Lovell RT. 1982. Vitamin C and disease resistance in channel catfish (*Ictalurus punctatus*). *Can. J. Fish. Aquat. Sci.* 39:948-951.
- Ellsaesser CF, Clem LW. 1987. Cortisol-induced hematological and immunological changes in channel catfish (*Ictalurus punctatus*). *Comp. Biochem. Physiol.* 87A:405-408.
- Erdal JI, Evenson Ø, Kaurstad OK, Lillehaud A, Solbakken R, Thorud K. 1991. Relationship between diet and immune response in Atlantic salmon (*Salmo salar* L.) after feeding various levels of ascorbic acid and omega-3 fatty acids. *Aquaculture*, 98: 363-379.
- Fagerlund UHM, McBride JR. 1978. Distribution and disappearance of radioactivity in blood and tissues of coho salmon (*Oncorhynchus kisutch*) after oral administration of ³H-testosterone. *J. Fish. Res. Board Can.* 35:893-900.
- Forster I, Higgs DA, Bell GR, Dosanjh BS, March BE. 1988. Effect of diets containing herring oil oxidized to different degrees on growth and immunocompetence of juvenile coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* 45:2187-2194.
- Foss P, Strocbacken T, Schiedt K, Liaaen-Jensen S, Austreng B, Streiff K. 1984. Carotenoids in diets for salmonids I. Pigmentation of rainbow trout with the individual optical isomers of astaxanthin in comparison with canthaxanthin. *Aquaculture*, 41:213-226.
- Furones MD, Alderman DJ, Bucke D, Fletcher TC, Knox D, White A. 1992. Dietary vitamin E and the response of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to infection with *Yersinia ruckeri*. *J. Fish Biol.* 41:1037-1041.
- Gatlin DM III, Bai SC, Erickson MC. 1992. Effect of dietary vitamin E and synthetic antioxidants on composition and storage quality of channel catfish *Ictalurus punctatus*. *Aquaculture*, 106:323-332.
- Gill C. 1989. Diet design: feeding stimulants. *Feed Intern.* 10(2):12.
- Goswami UC. 1988. Carotenoid-free diet and its role in reproduction of fresh water fish. Abstracts of the 8th International Symposium on Carotenoids, Boston, 27-31 July, 1988, p. 32.
- Goswami UC. 1993. Metabolism of carotenoids in freshwater fish: (i) biogenesis of 3-4 dehydroretinol, (ii) supplementation of carotenoids with fish food for better survival and growth. 10th International Symposium on Carotenoids, Trondheim, 20-25 June, 1993, Book of Abstracts, CL10-7.

- Gotz P. 1986. Encapsulation in arthropods. In: Brehelin M. ed. Immunity in Invertebrates. p. 153-170. Springer-Verlag, Berlin.
- Goudie CA, Shelton WL, Parker NC. 1986. Tissue distribution and elimination of radiolabelled methyltestosterone fed to sexually undifferentiated blue tilapia. *Aquaculture*, 58:215-226.
- Guerrero RD III. 1975. Use of androgens for the production of all-male *Tilapia aurea* (Steindachner). *Trans. Am. Fish. Soc.* 104:342-348.
- Gunstone FD, Wijesundera RC, Scrimgeour CM. 1978. The component of acids with special reference to furan-containing acids. *J. Sci. Food Agricult.* 29:569-580.
- Halver JE, Ashley LM, Smith RR. 1969. Ascorbic acid requirements of coho salmon and rainbow trout. *Trans. Am. Fish. Soc.* 98:762-771.
- Halver JE, Smith RR, Tolbert BM, Baker EM. 1975. Utilization of ascorbic acid in fish. *Ann. NY Acad. Sci.* 258:81-102.
- Harada K. 1985a. Feeding attraction activities of amino acids and lipids for juvenile yellowtail. *Bull. Jpn. Soc. Sci. Fish.* 51:453-459.
- Harada K. 1985b. Feeding attraction activities of amino acids and nitrogenous bases for oriental weatherfish. *Bull. Jpn. Soc. Sci. Fish.* 51:461-466.
- Harada K. 1986. Feeding attraction activities of nucleic acid-related compounds for abalone, oriental weatherfish and yellowtail. *Bull. Jpn. Soc. Sci. Fish.* 52:1961-1968.
- Harada K, Akishima Y. 1985. Feeding attraction activities of proteins, amino acids, lipids and nitrogenous bases for abalone. *Bull. Jpn. Soc. Sci. Fish.* 51:2051-2058.
- Hardie LJ, Fletcher TC, Secombes DJ. 1990. The effect of vitamin E on the immune response of the Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 87:1-13.
- Hardie LJ, Fletcher TC, Secombes CJ. 1991. The effect of dietary vitamin C on the immune response of the Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 95:201-214.
- Harris LE. 1984. Effect of a broodfish diet fortified with canthaxanthin on female fecundity and egg color. *Aquaculture*, 43:179-183.
- Hartmann M, Medem FG, Kuhn R, Bielig H. 1947. Untersuchungen uber die Befruchtungsstoffe der Regenbogenforelle. *Z. Naturforsch.* 2:230-249.
- Hazel JR. 1979. Influence of thermal acclimation on membrane lipid composition of rainbow trout liver. *Am. J. Physiol.* 236:R91-R101.
- Henderson RJ, Tatner MF, Lin W. 1992. Antibody production in relation to lipid composition in rainbow trout fed diets of different (n-3) polyunsaturated fatty acid content. *Aquaculture*, 100:232. (Abstract).
- Herold PM, Kinsella, JK. 1986. Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. *Am. J. Clin. Nutr.* 43:566-598.
- Hilton JW. 1989. The interaction of vitamins, minerals and composition in the diet of fish. *Aquaculture*, 79:223-244.
- Hilton JW, Cho CY, Slinger SJ. 1977. Evaluation of the ascorbic acid status of rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.* 34:2207-2210.
- Hilton JW, Cho CY, Slinger SJ. 1978. Effect of graded levels of supplemental ascorbic acid in practical diets fed to rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.* 35:431-436.
- Hunter GA, Donaldson EM. 1983. Hormonal sex control and its application to fish culture. In: Hoar WS, Randall DJ, Donaldson EM. eds. *Fish Physiology*. Vol. IXB, p. 223-303. Academic Press, New York, NY.
- Itami T, Takahashi Y, Tsuchihira E, Igusa H, Kondo M. 1993. Enhancement of disease resistance of kuruma prawn, *Penaeus japonicus* after oral administration of peptidoglycan. Second Symposium on Diseases in Asian Aquaculture, Abstracts, p. 6.
- Itami T, Takahashi Y, Tsuchihira E, Igusa H, Kondo M. 1994. Enhancement of disease resistance of kuruma prawn, *Penaeus japonicus* after oral administration of β -1-3-glucan (Schizophyllan) In: Chou LM, Munro AD, Lam TJ, Chen TW, Cheong LKK, Ding JK, Hooi KK, Khoo HW, Phang VPE, Shim KF, Tan CH. eds. *The Third Asian Fisheries*

- Forum. p. 375-378. Asian Fisheries Society, Manila.
- Johnstone R, Macintosh DJ, Wright, RS. 1983. Elimination of orally administered 17 α -methyltestosterone by *Oreochromis mossambicus* (tilapia) and *Salmo gairdneri* (rainbow trout) juveniles. *Aquaculture*, 35:249-257.
- Johnstone R, Simpson TH, Youngson AF. 1978. Sex reversal in salmonid culture. *Aquaculture*, 13:115-134.
- Kanazawa A. 1991. Recent advances in penaeid nutrition in Japan. In: Allan GL, Dall W. eds. Proceedings of the Aquaculture Nutrition Workshop, Salamander Bay, 15-17 April, 1991, p. 64-70. NSW Fisheries Australia.
- Katayama T, Kamata T, Shimaya M, Deshimaru O, Chichester CO. 1972. The biosynthesis of astaxanthin VIII. The conversion of labelled β -carotene-15.15¹-3H₂ into astaxanthin in prawn, *Penaeus japonicus* Bate. *Bull. Jpn. Soc. Sci. Fish.* 38:1171-1175.
- Kiron V, Fukuda H, Takeuchi T, Watanabe T. 1995. Essential fatty acid nutrition and defence mechanisms in rainbow trout *Oncorhynchus mykiss*. *Comp. Biochem. Physiol.* 111A:361-367.
- Kitamura S, Ohara S, Suwa T, Nakagawa K. 1965. Studies on vitamin requirements of rainbow trout, *Salmo gairdneri* - I. On the ascorbic acid. *Bull. Jpn. Soc. Sci. Fish.* 31:818-825.
- Lall SP, Olivier G. 1991. Role of micronutrients in immune response and disease resistance of fish. In: Kaushik SJ, Luquet P. eds. Fish Nutrition in Practice. IV International Symposium on Fish Nutrition and Feeding, 24-27 June, 1991, Biarritz, France, p. 101-118. INRA, Paris.
- Lall SP, Olivier GO, Hines JA, Ferguson HF. 1988. The role of vitamin E in nutrition and immune response of Atlantic salmon (*Salmo salar*). *Aquacult. Can. Bull.* 88-1:76-78.
- Lall SP, Olivier G, Weerakoon DEM, Hines JA. 1989. The effects of vitamin C deficiency and excess on immune response in Atlantic salmon (*Salmo salar* L). In: Takeda M, Watanabe, T. eds. The Current Status of Fish Nutrition in Aquaculture. Proceedings of the Third International Symposium on Feeding and Nutrition in Fish, August 28-September 1, 1989. Toba, Japan. p. 427-441. Tokyo University of Fisheries, Tokyo.
- Lambelet P, Saucy F, Loliger J. 1985. Chemical evidence for interactions between vitamins E and C. *Experientia*, 41:1384-1388.
- Li MH, Johnson MR, Robinson EH. 1993. Elevated dietary vitamin C concentrations did not improve resistance of channel catfish, *Ictalurus punctatus*, against *Edwardsiella ictaluri* infection. *Aquaculture*, 117:303-312.
- Li Y, Lovell RT. 1985. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. *J. Nutr.* 115:123-131.
- Lim C, Lovell RT. 1978. Pathology of the vitamin C deficiency syndrome in channel catfish (*Ictalurus punctatus*). *J. Nutr.* 108:1137-1146.
- Liu PR, Plumb JA, Guerin M, Lovell RT. 1989. Effect of megadose levels of dietary vitamin C on the immune response of channel catfish, *Ictalurus punctatus* in ponds. *Dis. Aquat. Org.* 7:191-194.
- Longinova TA. 1977. Carotenoids of rainbow trout during the growth of gonads and spawns. In: Karzinkin, GS. ed. Metabolism and Biochemistry of Fishes. p. 530-536 Indian Natl. Sci. Doc. Cent., New Delhi.
- Lovell RT. 1973. Essentiality of vitamin C in feeds for intensively fed caged channel catfish. *J. Nutr.* 103:134-138.
- Mahajan CL, Agrawal NK. 1980. Nutritional requirement of ascorbic acid by Indian major carp, *Cirrhina mrigala*, during early growth. *Aquaculture*, 19:37-48.
- Maule AG, Tripp RA, Kaattari, SL, Schreck C. 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). *J. Endocrinol.* 102:135-142.
- Mayer FL, Mehrle PM, Crutcher PL. 1978. Interactions of toxaphene and vitamin C in channel catfish. *Trans. Am. Fish. Soc.* 107:326-333.
- Mazik PM, Brandt TM, Tomasso JR. 1987. Effects of dietary vitamin C on growth, caudal fin development, and tolerance of aquaculture-related stressors in channel catfish. *Prog. Fish-Cult.* 49:13-16.

- Mclouglin MF, Kennedy S, Kennedy DG. 1992. Vitamin E-responsive myopathy in rainbow trout fry (*Oncorhynchus mykiss*). Vet. Rec. 130:224-226.
- Miyazaki T, Plumb JA, Li YP, Lovell RT. 1985. Histopathology of the broken-back syndrome in channel catfish. J. Fish Biol. 26:647-655.
- Moccia RD, Hung SSO, Slinger SJ, Ferguson HW. 1984. Effect of oxidized fish oil, vitamin E and ethoxyquin on the histopathology and haematology of rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 7:269-282.
- Mourente G, Rodriguez A, Tocher DR, Sargent JR. 1993. The effects of dietary docosahexanoic acid (DHA; 22:6n-3) on lipid and fatty acid compositions and growth in gilthead sea bream (*Sparus aurata* L.) larvae during first feeding. Aquaculture, 112:79-98.
- Moyle DB, Cech JJ Jr. 1982. Fishes: an Introduction to Ichthyology. Prentice-Hall Inc., Englewood Cliffs, NJ, p. 162-168.
- Nakajima K, Uchida A, Ishida Y. 1989. Effect of supplemental dietary feeding attractant, dimethyl-b-propiothetin, on growth of goldfish. Nippon Suisan Gakkaishi, 55: 1291.
- Nakamura, M. 1981. Effects of 11-ketotestosterone on gonadal sex differentiation in *Tilapia mossambica*. Bull. Jpn. Soc. Sci. Fish. 47:1323-1327.
- Navarre O, Halver JE. 1989. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. Aquaculture, 79:207-221.
- Ndoye A, Ghanmi Z, Koenig J, Deschaux, P. 1989. Effects de la vitamine E sur la production d'anticorps anti *Yersinia ruckeri* chez la truite arc-en-ciel (*Salmo gairdneri*). Ichthyophysiol. Acta 13:17-23.
- Negre-Sadargues G, Custillo R, Petit H, Sance S, Martinez RG, Milicua JCG, Choubert G, Trilles J-P. 1993. Utilization of synthetic carotenoids by the prawn *Penaeus japonicus* reared under laboratory condition. Aquaculture, 11:151-159.
- Okada H, Matumoto H, Yamazaki F. 1979. Functional masculinization of genetic females in rainbow trout. Bull. Jpn. Soc. Sci. Fish. 45:413-419.
- Olivier G, Lall SP, Weerakoon DEM, Hines JA. 1989. The effect of vitamin C deficiency and excess on immune responses in Atlantic salmon (*Salmo salar*). Abstract. International Conference on Phylogeny of Immunity, CNRS, France.
- Perrson M, Vey A, Soderhall K. 1987. Encapsulation of foreign particles *in vitro* by separated blood cell from crayfish, *Astacus leptodactylus*. Cell Tissue Res. 247:409-415.
- Pickering AD, Pottinger TG. 1985. Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. J. Fish Biol. 27:611-619.
- Pierce BA, Laws EA. 1986. Chemically-active feed additive for prawns (*Macrobrachium rosenbergii*). Prog. Fish-Cult. 47:59-61.
- Plumb JA. 1988a. Vaccination against *Edwardsiella ictaluri*. In: Ellis AQE. ed. Fish Vaccination. p. 152-161. Academic Press, New York, NY.
- Plumb JA. 1988b. Vaccination against Channel Catfish Virus. In: Ellis AQE. ed. Fish Vaccination. p. 216-223. Academic Press, New York, NY.
- Poston AH. 1967. Effect of dietary L-ascorbic acid on immature brook trout. Fish. Res. Bull. 30:46-51.
- Robertson L, Thomas P, Arnold CR, Trant JM. 1987. Plasma cortisol and secondary stress responses of red drum handling, transport, rearing density, and a disease outbreak. Prog. Fish-Cult. 49:1-12.
- Rosenlund G, Jørgensen L, Waagbø R, Sandnes K. 1990. Effects of different dietary levels of ascorbic acid in plaice (*Pleuronectes platessa* L.). Comp. Biochem. Physiol. 96A:395-398.
- Sakaguchi H, Takeda F, Tance K. 1969. Studies on vitamin requirements by yellowtail. I. Vitamin B, and vitamin C deficiency symptoms. Bull. Jpn. Soc. Sci. Fish. 35:1201-1206.
- Salte R, Nafstad P, Asgard T, 1987. Disseminated intravascular coagulation in "hitra disease" (hemorrhagic syndrome) in farmed Atlantic salmon. Vet. Pathol. 24:378-385.
- Salte R, Thomassen MS, Wold K. 1988. Do high level of dietary polyunsaturated fatty acids (EPA/DHA) prevent diseases associated with membrane degeneration in farmed Atlantic salmon at lower water temperatures? Bull. Eur. Assoc. Fish Pathol. 8:63-66.

- Sandnes K. 1991. Vitamin C in fish nutrition - a review. Fiskidir. Skr. Ser. Ernaer. 4:3-32.
- Sandnes K, Hansen T, Killie J-EA, Waagbø R. 1990. Ascorbate-2-sulfate as a dietary vitamin C source for Atlantic salmon (*Salmo salar*): I. Growth, bioactivity, haematology and humoral immune response. Fish Physiol. Biochem. 8:419-427.
- Schreck CB. 1974. Hormonal treatment and sex manipulation in fishes. In: Schreck CB. ed. Control of Sex in Fishes. p. 84-106. Virginia Polytechnic Institute and State University, Blacksburg, VA.
- Sheldon WD Jr, Blazer VS. 1991. Influence of dietary lipid and temperature on bactericidal activity of channel catfish macrophages. J. Aquat. Anim. Health, 3:87-93.
- Shelton WL, Rodriguez-Guerrero D, Lopez-Macias J. 1981. Factors affecting androgen sex reversal of *Tilapia aurea*. Aquaculture, 25:59-65.
- Sung HH, Kou GH, Song YL. 1994. Vibriosis resistance induced by glucan treatment in tiger shrimp (*Penaeus monodon*). Fish Pathol. 29:11-17.
- Takahashi M, Niki E, Kawakami A, Kumasaka A, Yamamoto Y, Kamiya Y, Tanaka K. 1986. Oxidation of lipids. XIV. Inhibition of oxidation of methyl linoleate by fatty acid esters of L-ascorbic acid. Bull. Chem. Soc. Jpn. 59:3179-3183.
- Thompson G, Choubert G, Houlihan DF, Secombes CJ. 1995. The effect of dietary vitamin A and astaxanthin on the immunocompetence of rainbow trout. Aquaculture, 133:91-102.
- Torrissen OJ. 1984. Pigmentation of salmonids. Effect of carotenoids in eggs and start-feeding diet on survival and growth rate. Aquaculture, 43:185-193.
- Torrissen OJ. 1986. Pigmentation of salmonids; a comparison of astaxanthin and canthaxanthin as pigment sources for rainbow trout. Aquaculture, 53:271-278.
- Torrissen OJ, Christiansen R. 1994. Carotenoids and their function in fish. The 1st Roche Aquaculture Centre Conference on Nutritional Prophylaxis. 23 March, 1994, The Hilton Hotel, Bangkok, 17 p.
- Tveranger B. 1986. Effect of pigment content in broodstock diet on subsequent fertilization rate, survival and growth rate of rainbow trout (*Salmo gairdneri*) offspring. Aquaculture, 53:85-93.
- Verlhac V, Doye AN, Gabaudan J, Troutaud D, Deschaux P. 1991. Vitamin nutrition and fish immunity: influence of antioxidant vitamins (C and E) on immune response of rainbow trout. In: Kaushik SJ, Luquet P. eds. Fish Nutrition in Practice. IV International Symposium on Fish Nutrition and Feeding, 24-27 June, 1991, Biarritz, France, p. 167-177. INRA, Paris.
- Verlhac V, Gabaudan J, Obach A, Schuep W, Hole R. 1996. Influence of dietary glucan and vitamin C on non-specific and specific immune responses of rainbow trout (*Oncorhynchus mykiss*). World Aquaculture Society Meeting, 29 January - 2 February, 1996, Bangkok, Abstracts, p. 425.
- Waagbø R, Sandnes K, Jørgensen J, Engatad R, Glette J, Lie Ø. 1993b. Health aspects of dietary lipid sources and vitamin E in Atlantic salmon (*Salmo salar*) II. Spleen and erythrocyte phospholipid fatty acid composition, nonspecific immunity and disease resistance. Fiskidir. Skr. Ser. Ernaer. 6:63-80.
- Waagbø R, Sandnes K, Lie Ø, Nilsen ER. 1993a. Health aspects of dietary lipid sources and vitamin E in Atlantic salmon (*Salmo salar*). I. Erythrocyte total lipid fatty acid composition, haematology and humoral immune response. Fiskidir. Skr. Ser. Ernaer. 6:47-61.
- Wahli T, Meier W, Pfister K. 1986. Ascorbic acid induced immune-mediated decrease in mortality in *Ichthyophthirius multifiliis* infected rainbow trout (*Salmo gairdneri*). Acta Trop. 43:287-289.
- Watanabe T. 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. 73B:3-15.
- Watanabe T, Itoh A, Murakami A, Tsukashima Y, Kitajima C, Fujita S. 1984. Effect of nutritional quality of diets on reproduction of red sea bream. Bull. Jpn. Soc. Sci. Fish. 50:1023-1028.
- Watanabe T, Miki W. 1991. Astaxanthin: an effective dietary component for red seabream broodstock. In: Kaushik SJ, Luquet, P. eds. Fish Nutrition in Practice. IV International Symposium on Fish Nutrition and Feeding, 24-27 June, 1991, Biarritz, France, p. 27-36. INRA, Paris.

- Weber PC. 1989. Are we what we eat? Fatty acids in nutrition and in cell membranes: cell function and disorders induced by dietary conditions. In: Proceedings of the International Conference on Fish Lipids and their Influence on Human Health. p. 9-18. Svanøy Foundation, Norway.
- Wedemyer G. 1969. Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes. *Comp. Biochem. Physiol.* 29:1247-1251.
- Wilson RP, Poe W. 1973. Impaired collagen formation in the scorbutic channel catfish. *J. Nutr.* 103:1359-1364.
- Wise DJ, Tomasso JR, Brandt TM. 1988. Ascorbic acid inhibition of nitrite-induced methemoglobinaemia in channel catfish. *Prog. Fish-Cult.* 50:77-80.
- Wise DJ, Tomasso JR, Gatlin DM II, Bia SC, Blazer VS. 1993a. Effects of dietary selenium and vitamin E on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide anion production in channel catfish. *J. Aquat. Anim. Health*, 5:177-182.
- Wise DJ, Tomasso JR, Schwedler TE, Blazer VS, Gatlin DM II. 1993b. Effect of vitamin E on the immune response of channel catfish to *Edwardsiella ictaluri*. *J. Aquat. Anim. Health*, 5:183-188.
- Yano T, Furuichi M, Nakao M, Ito S. 1990. Effects of L-ascorbyl-2-phosphate Mg on the growth and nonspecific immune system of red sea bream *Pagrus major*. World Aquaculture Society Meeting, 10-14 June, 1990, Halifax, Canada, Abstract T29.12.
- Yone Y. 1976. Nutrition studies of red sea bream. Proceeding of the First International Conference on Aquaculture Nutrition, 14-15 October, 1975. p. 39-63. Lewes/Rehoborth, DE.
- Yu TC, Sinnhuber RO. 1979. Effects of dietary n-3 and n-6 fatty acids on growth and feed conversion efficiency of coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 16:31-38.
- Zitzov RE, Millard JL. 1988. Survival and growth of lake whitefish (*Coregonus clupeaformis*) larvae fed only formulated dry diets. *Aquaculture*, 69:105-113.

Human Health Aspects of the Use of Chemicals in Aquaculture, with Special Emphasis on Food Safety and Regulations¹

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ABSTRACT

Safe and wholesome food is essential for good health. Therefore, when one considers health issues related to unsafe foods, recorded morbidity and mortality as well as economic losses in a population must be included. Due to their presence in unsafe food, micro-organisms are generally considered to pose a major risk to human health. In aquaculture, chemicals are used mainly in the treatment and prophylaxis of disease problems, which constitute the largest single cause of economic losses. However, the increasing use of chemicals in aquaculture has led to wide-spread public concern. The concerns related to human health due to chemical use in aquaculture are repeatedly found in the published literature. They include allergic reactions in previously sensitized persons triggered by chemical residues, and the potential impacts on human health resulting from the emergence of drug-resistant bacteria caused by the use of sub-therapeutic levels of antibiotics and by antibiotic residues persisting in the sediments of aquaculture environments. This paper discusses the risk evaluation principles, data requirements and the concept of maximum residue limit. The uncertainties inherent in the process include, but are not limited to, the state-of-the-art of toxicological evaluation, the level of understanding of the environmental transport process of chemicals, the exposure data available, and any assumptions and extrapolations.

¹ This paper expresses the views of the authors and does not represent those of any organization mentioned in the text.

INTRODUCTION

A major objective of risk evaluation/assessment of chemicals is to provide a reliable basis for sound management of toxic chemicals. The Joint FAO/WHO Expert Consultation on Application of Risk Analysis to Food Standards Issues which was held in Geneva, Switzerland from 13-17 March, 1995 supports the use of a science-based risk assessment process (FAO/WHO 1995). The concept of maximum residue limits of veterinary drugs adopted in 1989 by the Commission of the Codex Alimentarius was discussed as an approach aimed at guaranteeing the absence of chemical residues that might present a risk to consumer health (Boisseau 1993).

DECISION PROCESS FOR ESTABLISHING RECOMMENDED MAXIMUM RESIDUE LIMITS

In recommending a Maximum Residue Limit (MRL) for a specific compound, several factors are taken into account by the Committee. Among these are the results of toxicological and radio-labelling residue studies, the bioavailability of bound residues, the identification of target tissue(s), the existence of a residue marker for determining compliance with safe residue limits, residue data from use of the veterinary drug according to good practice in the use of veterinary drugs, withdrawal periods for adequate residue depletion, and practical analytical methods for residue analysis.

The first step in establishing a recommended MRL is the determination of an Acceptable Daily Intake (ADI) based on the available toxicological data. If the use of a veterinary drug according to good practice in the use of veterinary drugs yields concentrations of residues lower than those corresponding to the ADI, the MRL will be reduced accordingly. However, if the residues cannot be measured using a practical analytical method under these conditions of use, the MRL will be raised so that compliance with the MRL may be checked analytically. In no instance, however, will an MRL be recommended at concentrations that significantly exceed the MRL based on toxicological considerations.

An important factor to be considered in the establishment of MRLs in various edible tissues and other products of animal origin is the amount of the food item consumed. In order to protect all segments of the population, it is reasonable to use intake data at the upper limit of the range for individual edible tissues and animal products. The Committee based its recommendations on the following daily intake values: 300 gm of meat (as muscle tissue), 100 gm of liver, 50 gm of kidney, 50 gm of tissue fat, 100 gm of egg and 1.5 L of milk (WHO 1990), figures that are believed to protect the vast majority of the population of the world (WHO 1989). "The human consumption of farmed fish and prawns, for example, seems to vary considerably, and accurate food intake data are difficult to obtain at the international level. In order to protect all segments of the population, MRLs for these food commodities should be based on the food intake values noted in the 34th report of the committee" (WHO 1995b). It is recommended that governments consider whether local diets may result in intakes that exceed the ADI (WHO 1995a).

RELEVANT DATA FOR ASSESSING THE HUMAN FOOD SAFETY OF RESIDUES OF VETERINARY DRUGS

When investigating the safety of the consumption of residues of veterinary drugs in food, the Committee requires detailed reports (including individual animal data) of the following types of studies relevant to the toxicological evaluation:

- Pharmacokinetic, metabolic and pharmacodynamic studies in experimental and food-

producing animals, and in humans, when available.

- Short-term and long-term carcinogenicity, reproduction and developmental studies in experimental animals, and genotoxicity studies.
- Special studies designed to investigate specific effects, such as those on mechanisms of toxicity, non-hormonal-effect levels, immune responses and macro-molecular binding.
- For compounds with antimicrobial activity, studies by the manufacturer designed to evaluate the possibility that the compound might have an adverse effect on the microbial ecology of the human intestinal tract.
- Studies providing relevant data on the use of, and exposure to, the drug in humans, including studies of effects observed after occupational exposure and epidemiological data following clinical use in humans.

Detailed reports of studies relevant to the evaluation of drug residues in food-producing animals that are required for evaluation include information on the chemical identity and properties of the drug, its use and dose range.

As for the toxicological evaluation, pharmacokinetic and metabolic studies in experimental animals, target animals and humans, are needed. These include:

- Residue-depletion studies with radio-labelled drug in target animals from zero withdrawal time to periods extending beyond the recommended withdrawal time. These studies should provide information on total residues, including free and bound residues, and major residue components to permit selection of a marker residue and target tissue.
- Residue-depletion studies with unlabelled drug for the analysis of marker residue in target animals and in eggs, milk and honey. These should include studies with appropriate formulations, routes of application, and species, at doses up to the maximum recommended.

Also required are:

- A review of routine analytical methods that may be used by regulatory authorities for the detection of residues in target tissue.
- A description of the analytical procedures used by the sponsor for the detection and determination of parent drug residues. The sponsor is also required to describe a method that may be used by regulatory authorities for the specific determination of the marker residue with a sensitivity equal to or less than the MRL (WHO 1995a).

SOME ISSUES OF PUBLIC CONCERN AND THE SAFETY EVALUATION PROCESS

Issue of Risk Due to Allergic Reactions Due to Chemical Residues in Foods

Although allergic reactions caused by antibiotic residues in food are of great public concern, it is found that, in general, the incidence of allergic reaction following ingestion of antibiotic residues in food animals is very low (Adkinson 1980, Black 1984). Many drugs, such as penicillins, tetracyclines, sulfonamides and some aminoglycosides, are considered to have a high potential for sensitizing susceptible individuals. Penicillins have the greatest potential and cause allergic response characterized by dermatitis in most reports. It is implied that the amount of penicillin required to induce the primary sensitization may be greater than the quantities eliciting an allergic response (about 1-10 units or less) (Adkinson 1980), and that dairy products that test negative by microbiological assay may contain sufficient penicillin or its metabolites to maintain urticaria (Boonk and Van Ketel 1982). According to these studies, it is indicated that very minute levels of penicillin in food can cause allergic reactions.

WHO (1990) stated that “Although there is no evidence from which threshold doses for such effects can be determined, the Committee concluded that hypersensitivity reactions due to the ingestion of food of animal origin containing allergic drug residues were unlikely to be of major health significance. This view was supported by the small numbers of reports in the published literature. Nevertheless, the Committee recognized that reactions could occur in highly sensitized individuals and therefore recommended that residues of drugs with known or suspected allergenic properties be kept as low as practicable, particularly penicillin and other β -lactam antibiotics such as the cephalosporins.”

Difficulty still exists concerning the strategy and approach to be used for evaluating this risk, especially when a chemical residue may trigger an allergic reaction in a previously sensitized person or may undermine the immune system.

Issue of Microbial Resistance Due to the Use of Sub-therapeutic Levels of Antibiotic

The predominant public concerns on microbial resistance due to the use of sub-therapeutic levels of antibiotics are the possible impacts on human health resulting from the emergence of drug-resistant bacteria in animals caused by the prolonged use of low-level antibiotics in animal feed (Gersema and Helling 1986, Sorum *et al.* 1992), and that antibiotic residues (i.e., residues of oxytetracycline and oxolinic acid) may persist in sediment for a long time (Pedersen *et al.* 1995). These situations actually bring humans to new medical dilemma.

Recently published studies have implied that the transmission of antibiotic-resistant pathogenic bacteria of animal origin to man may be possible, and is apparently related to the sub-therapeutic use of these drugs in animal production. Furthermore, the resistant strains in the environment may also transfer R plasmid to human intestinal flora (Levy *et al.* 1976a, b; Holmberg *et al.* 1984a, b). Therefore, such antibiotic-resistant bacteria may be direct or indirect causes of human illness, and the induction a significant loss of antibiotic efficacy in bacterial infection treatments, as, for example, in the case of the *Salmonella* outbreak in the United States in 1971-1983 (Cohen and Tauxe 1986). Nevertheless, this topic is still controversial, and there is no direct evidence by reason of the difficulties in tracing the resistant strains from animal to man and the complexity of antibiotic use in humans (Black 1984).

In assessing the microbiological risk due to residues of antimicrobial drugs in food, WHO (1990) stated that “In evaluating the safety of residues of antimicrobial drugs, the specific risks associated with their antimicrobial activity should be considered in addition to their pharmacological properties. The anti-microbial activity could become the determining factor of this safety evaluation if the toxicity of the substance to be considered is such that higher levels of residues could be tolerated in food on a toxicological basis.”

In this respect, the Codex Committee on Residues of Veterinary Drugs in Foods has adopted a definition of Maximum Residue Limit for Veterinary Drugs taking into account “other relevant public health risks (that may refer to allergic and microbiological risks) as well as food technological aspects.”

In assessing the microbiological risk, two biological systems need to be considered, the intestinal flora of the consumer, and the bacteria used in the food processing industry. The risk being considered by the Committee does not include potential health effects associated with ingesting food of animal origin that contains resistant bacteria selected under the pressure of antimicrobial therapy, because the Committee’s terms of reference include only the safety assessment of drug residues (WHO 1990).

INHERENT UNCERTAINTIES IN THE SAFETY EVALUATION PROCESS

The uncertainties inherent in the risk assessment process include, but are not limited to, the state-of-the-art of toxicological evaluation, the level of understanding of the environmental transport process of chemicals, the exposure data available, and any assumptions and extrapolations. Pharmacokinetic studies also become more complicated when there is the possibility of repeated exposure due to environmental transport processes in the aquatic environment. This limited understanding of the environmental fate of chemicals will contribute to uncertainty in risk evaluation.

CONCLUSIONS

Adherence to scientific principles is the most important factor in maintaining consistency in assessments of health risk, although there are differing pharmacological and toxicological properties of chemicals used in aquaculture and widely varying amounts of information available on them.

An approach, aimed at guaranteeing the absence of chemical residues that might present a risk to consumer health, is the concept of maximum residue limits of veterinary drugs in foods, used by the Commission of the Codex Alimentarius (Boisseau 1993).

The risk evaluation/assessment process is generally accepted to provide a reliable basis for identifying and managing health risks. However, in some areas of public concern, such as the triggering of allergic reactions in previously sensitized persons by chemical residues and the issue of microbial resistance due to the use of sub-therapeutic levels of antibiotics, difficulties exist concerning the strategy and approach for risk evaluation (Black 1984).

Uncertainties exist due to a lack of knowledge of the impacts of chemical use on multiple species and the multiple routes of exposure in an ecosystem. They are also due to limited knowledge of the environmental transport of chemicals, which affects exposure of aquatic organisms to chemicals. This, in turn, affects the pharmacokinetics and metabolism of such chemicals, resulting in changing of the chemical residue profile within the organism.

The complexity of these issues, and often the lack of data as well as the lack of monitoring and surveillance systems, are the factors limiting the risk analysis process. This insufficiency of data is more pronounced in developing countries, where resources are more limited or less available. Therefore, only the policy of safe and effective use of chemicals must be developed. Appropriate strategies must be chosen, according to individual country's and region's needs. Strengthening research efforts and programs for human training and development, as well as enhancing mechanisms for information exchange, may be encouraged through international collaboration.

REFERENCES

- Adkinson FN. 1980. Immunological consequences of antimicrobials in human feeds. Committee to Study the Human Health Effects of Subtherapeutic Antibiotics Use in Animal Feeds (des). In: *The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds*. p. 301-306. National Academy of Sciences, Washington, DC.
- Black WD. 1984. The use of antimicrobial drugs in agriculture. *Can. J. Physiol. Pharmacol.* 62:1044-1048.
- Boisseau J. 1993. Basis for the evaluation of the microbiological risks due to veterinary drug residues in food. *Vet. Microbiol.* 35:187-192.

- Boonk WJ, Van Ketel WG. 1982. The role of penicillin in the pathogenesis of chronic urticaria. *Br. J. Dermatol.* 106:183-190.
- Cohen ML, Tauxe RV. 1986. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. *Science*, 234:964-969.
- FAO/WHO. 1995. Application of risk analysis to food standards issues. Report of the Joint FAO/WHO Expert Consultation. Geneva, Switzerland. 13-17 March, 1995. (WHO/FNU/FOS/95.3), Geneva.
- Gersema LM, Helling DK. 1986. The use of subtherapeutic antibiotics in animal feed and its implications on human health. *Drug Intell. Clin. Pharm.* 20:214-218.
- Holmberg SD, Osterholm MT, Senger KA, Cohen ML. 1984a. Drug-resistant *Salmonella* from animals fed antimicrobials. *New Engl. J. Med.* 311:617-622.
- Holmberg SD, Wells JG, Cohen ML. 1984b. Animal-to-man transmission of antimicrobial-resistant *Salmonella*: investigations of U.S. outbreaks, 1971-1983. *Science*, 225:833-835.
- Levy SB, FitzGerald GB, Macone AB. 1976a. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. *Nature*, 260:40-42.
- Levy SB, FitzGerald GB, Macone AB. 1976b. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *New Engl. J. Med.* 295:583-588.
- Pedersen K, Tiainen T, Larsen JL. 1995. Antibiotic resistance of *Vibrio anguillarum*, in relation to serovar and plasmid contents. *Acta Vet. Scand.* 36:55-64.
- Sorum H, Roberts MC, Crosa JH. 1992. Identification and cloning of a tetracycline resistance gene from the fish pathogen *Vibrio salmonicida*. *Antimicrob. Agents Chemother.* 36:611-615.
- World Health Organization. 1989. Evaluation of certain veterinary drug residues in food. WHO Techn. Rep. Ser. No. 789. (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). Geneva.
- World Health Organization. 1990. Evaluation of certain veterinary drug residues in food. WHO Techn. Rep. Ser. No. 799. (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). Geneva.
- World Health Organization. 1995a. Evaluation of certain veterinary drug residues in food. WHO Techn. Rep. Ser. No. 851. (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). Geneva.
- World Health Organization. 1995b. Evaluation of certain veterinary drug residues in food. WHO Techn. Rep. Ser. No. 855. (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). Geneva.

Preliminary Review of the Legal Framework Governing the Use of Chemicals in Aquaculture in Asia

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“States should regulate the use of chemical inputs in aquaculture which are hazardous to human health and the environment” (CCRF, 9.4.5)

ABSTRACT

This preliminary review looks into legislation governing the use of chemicals in aquaculture in Asia. Brief assessments are made of the legislation relating to chemical contamination and the use of veterinary drugs and feed additives, a section is dedicated to trade in aquaculture products, and a few conclusions are then drawn. While mandatory measures of control are desirable and feasible, soft law instruments, such as codes of practice and conduct, allow an element of flexibility to be maintained while avoiding undue legislative restraints on scientific and technical progress.

INTRODUCTION

National legislation on the use of chemicals in aquaculture is not abundant, and is characteristically scattered in various legislative texts, including that of basic and subsidiary legislation, which does not permit a detailed and accurate legal analysis of the use of chemicals in aquaculture. While basic legislation laying down general principles applicable to the use of pesticides, veterinary drugs, food products, etc. as a whole could be collected by the author, it was more difficult to find the subsidiary regulations, including the “vertical measures” dealing with specific categories of products (in the present case, aquaculture products), the latter often being drafted by ministerial departments or subsidiary authorities.

The study of legislation relating to the use of chemicals in aquaculture brings with it two distinct legal issues. One relates to the problem of water pollution and the contamination of the general aquatic environment by aquaculture chemicals, and the second contemplates the controls on the lawful use of a range of chemicals for medicinal, pesticidal and other uses in aquaculture.

Like all producers of food crops, aquaculturists are driven to minimize stock loss in order to maximize profitability. Moreover, countries exporting fish and fish products destined for human consumption are concerned because importing countries may no longer accept their products without a guarantee that the products contain no chemical residues of concern.

CHEMICAL CONTAMINATION

Aquaculture operations, and the farms that often adjoin them, are usually dependant on properly used chemicals to minimize problems and maximize harvests. Unfortunately, when chemicals are improperly used, or when they are accidentally discharged into waters used by aquaculture operations, they can contaminate the water and harm the product. Serious threats to aquaculture water come from herbicides used to control aquatic vegetation in fish ponds; runoff of pesticides, herbicides, and fertilizers from fields adjoining aquaculture ponds; and aquifer contamination due to pollution of the recharge water.

Aquaculture Chemicals

To a certain extent, it may be difficult for aquaculturists to avoid the risk of chemical contamination from aquaculture products. This contamination may come from neighboring, as well as distant farms. An informed aquaculturist can, however, minimize the risks by considering these issues at the time of site selection and by avoiding the creation of dangerous situations on his property.

The best way for the careful aquaculturist to avoid the risk of chemical pollution is to follow strictly the instructions for use of fertilizers and chemical pesticides. Many countries in the region have set up regulatory procedures to control trade practices and the production and use of pesticides under a “pesticide-related legislative text.”

It should be noted that the term “pesticide” is not common in all countries: the Republic of Korea uses the term “agrochemical products;” Japan speaks of “agricultural chemical products;” India has endorsed the term “insecticides;” and Pakistan, Malaysia, Sri Lanka and Hong Kong China use the term “pesticides.”

Needless to say, these terms are defined differently in different countries. In some countries, they refer to plant health products only (e.g., Republic of Korea); in others, to plant health products and veterinary products (e.g., Malaysia, Pakistan); and in others, to substances to be used to control human disease carriers (e.g., Sri Lanka, Thailand). In the case of Sri Lanka, the term covers, in addition to the above, all products used to control all forms of plant or animal life likely to affect public health and human ecto- and endoparasites. In Malaysia, the following criteria allow to identify a pesticide: its (a) chemical name, trademark and commercial name; (b) ingredients; (c) formulation; (d) manufacturer; and (e) technical features. It is sufficient for even one of these elements to differ between two pesticides under comparison for the two pesticides to be deemed to be different substances.

In order to distinguish between products in terms of the hazards that they represent, they are usually classified on the basis of the risks to which they give rise. Along with the classification, control systems on their use and/or import may be more or less severe i.e., pesticides may be either authorized, restricted or banned.

An analysis of the pesticide laws in several countries in the region (see Annex I) shows that most pesticide control systems involve authorization schemes: (i) registration procedures and appeal, including treatment of proprietary information and post-registration monitoring; (ii) licensing procedures and appeals; (iii) charges and taxes related to registration and licensing schemes, including those for pesticide analyses; and (iv) special aspects concerning manufacturing and trade, including importation (licenses, border inspections, certificates, rejection or destruction and compensation thereof), exportation and re-exportation. Provisions are also made with regard to labeling requirements and, of course, for offences and administrative or penal sanctions, including powers of the designated authority and the liability of enforcing officers, where applicable.

Several countries distinguish between the commercial activities relating to pesticides and the registration of pesticide products (e.g., Japan, Republic of Korea, Pakistan, Malaysia, Myanmar, the Philippines, Thailand). The purpose of registration is to ensure that pesticides, when used according to registered label directions, will be effective for the purposes claimed, and safe (see for example, FAO's "International Code of Conduct on the Distribution and Use of Pesticides" (FAO 1990). This enables authorities to exercise control over quality, use levels, claims, labeling, packaging and advertising. The purpose of licensing is to strengthen the control on traders in these products and the facilities and infrastructure which they use.

Registration may also occur in stages. To this effect, some countries provide for an experimental authorization, either for a limited period (e.g., Myanmar - two years; Indonesia - one year; Malaysia - six months) or for an unspecified period (e.g., Sri Lanka). Other countries have set up a system of Provisional Authorization for Sale (PAS) (e.g., India, Indonesia, Myanmar, the Philippines, Sri Lanka). As a consequence, the product can be sold, but only in limited quantities and over a limited period of time. Registration may also be subject to conditions (e.g., Malaysia). A PAS is often granted for a poorly known product, whereas a conditional registration is granted for a well known product.

The registration or licensing of a pesticide implies the existence of pesticide control institutions and the conduct of examinations or tests. The relevant authorizing authority may be an official or a committee (e.g., Malaysia) or an official upon recommendation of a committee (e.g., Pakistan, Ministry of Agriculture and the Agriculture Pesticide Technical Advisory Committee; Sri Lanka, Registrar and Pesticide Committee). It is to be noted that an authorizing officer may be subject to powerful pressure on the part of the applicant(s) which might prevent him from contemplating the application in an objective manner. On the contrary, it may be more difficult for an applicant to exercise pressure on a group. Furthermore, a joint decision implies a joint responsibility.

The duration of the registration may be limited to a certain period (e.g., India - two years; Malaysia - three years; Myanmar - 10 years) or remain unspecified in the law (e.g., Sri Lanka, Republic of Korea).

The registration procedure is subject to a fee to cover administrative costs incurred by the authority when assessing the product. The fee is usually paid when the application is filed. It is to be noted that in the Republic of Korea, the law provides for the establishment of a Pesticides Management Fund to provide the resources needed to conduct all the tests to evaluate pesticides. The fund has its own financial resources. Every manufacturer or importer is required to pay 2 % of their total sales revenues into the fund's reserves every year. Nevertheless, revenues from the sale of products tested at the expense of the manufacturer or importer may be completely or partially excluded from this requirement. The fund is used to cover, amongst others, the following: (a) the cost of training and safety campaigns regarding the use, handling and management of pesticides and (b) the management costs.

As highlighted above, many countries have a twin system: registration and license/permit. Two types of permits can be identified: (1) permits for import/sale and (2) those for manufacture/packaging. Import of pesticide may be forbidden without a permit (e.g., Sri Lanka, Malaysia). The manufacture/packaging permit may relate to a control of the technical know-how in possession of the applicant.

Labeling is important and, in particular, clear and accurate labels are needed, as they often constitute the only contact between the manufacturer/supplier and the user of the product. In practically all of the legislation studied, there are provisions concerning the requirement for labels to be perfectly affixed to (or integrated into) the package of the pesticide, and for their text to be clearly visible and legible by ordinary people under normal conditions. In most cases, there are detailed rules as

to size requirements (e.g., Malaysia, Hong Kong China). Mandatory information to be reproduced on the label relates to (i) product identification, (ii) instructions for use, and (iii) warnings as to potential hazards (e.g., Malaysia, Thailand, Sri Lanka, Hong Kong China).

Misuse of approved pesticides and the use of unapproved pesticides are examples of offences which may lead to criminal penalties or fines. The latter vary depending on the type of infringement. Penalty for infringement can also involve the withdrawal of the registration or the license. Moreover, provisions for the seizure of products which do not conform to the statutory provisions are sometimes provided (e.g., Malaysia, Sri Lanka).

Chemical Discharge

Aquaculturists should be aware that chemical drift or runoff may lead to them being legally liable in respect of the consequences caused. This might occur either under a theory of “chemical trespass” in certain jurisdictions, or more generally, as a result of an action for private nuisance.

Growing concern over the potential adverse effects of chemicals released in aquaculture effluents is being observed. As an example, the legislation in Sri Lanka can be cited. Under the National Environment Act, several tools have been developed for the purposes of control of aquaculture activities, to prevent water pollution and the alteration of water quality. The Central Environment Authority has set standards for emissions (discharge of effluents) into inland surface, brackish and marine coastal waters, in particular, for the discharge of aquaculture waste waters as described below:

- “Standards for discharge of effluents into inland surface waters” (National Environmental (Protection and Quality) Regulations, No 1 of 1990, schedule 1)
- “Tolerance limits for aquaculture waste water discharged into irrigation waters”
- “Tolerance limits for aquaculture waste water discharged into inland surface waters”
- “Soil and water quality parameters suitable for brackish aquaculture”

Regulation of Pesticides at the International Level

For many years, FAO has been the center of efforts to establish a world-wide system of cooperation to control pesticides through the exchange of technical information and administrative decisions. By Resolution 10/85, the FAO Conference adopted in 1985 the *International Code of Conduct on the Distribution and Use of Pesticides* (FAO 1990). One of its primary objectives (see Article 1.1) is to “set forth responsibilities and establish voluntary standards of conduct for all public and private entities engaged in or affecting the distribution and use of pesticides, particularly where there is no or an inadequate national law to regulate pesticides.”

The code provides for standards on pesticide management, testing, availability, use, distribution, trade, advertising, labeling, packaging, storage and disposal. The code deals with activities for reducing health hazards, regulatory and technical requirements from governments and the industry, the principles of information exchange and the prior informed consent and their respective procedures, the monitoring of the code itself, etc. National laws should “translate” the contents of the code and allow for its implementation. Guidelines have been set up to this effect (see FAO 1985a,b, 1989).

The term “pesticide” provided for in the code¹ implies that the latter (i) not only applies to pesticides in the strict sense, but also to other categories of products (e.g., desiccation agents), (ii) contemplates in addition to plant health products, also veterinary products and substances to be used to control human disease carriers, (iii) embraces pesticides to be applied for the protection of inanimate products (foodstuffs, feedstuffs, wood, wood products), and (iv) thus aims at protecting

products intended for human or animal consumption in every phase of food production.

Also of interest for this study is the definition of label and the objective pursued by the latter. Article 2 of the code defines “label” as: “the written, printed or graphic matter on, or attached to, the pesticide; or the immediate container thereof and the outside container or wrapper of the retail package of the pesticide.” In the guidelines relating to the labeling of pesticides, a label constitutes “the means of achieving a consistently high standard of communication from supplier to purchaser” and in this regard, it is recommended what information should appear on the label.

Regulations Concerning the Use of Pesticides for the Specific Purposes of Aquaculture

Specific rules for the use of pesticides in aquaculture could not be found. What made the task difficult relates partly to the author’s lack of knowledge in identifying and evaluating when a product qualifies as a pesticide and, to a lesser extent, whether or not it is used in aquaculture. Nevertheless, the general impression is that few pesticides have been registered specifically for use in aquaculture, although they may have been registered for other uses.

VETERINARY DRUGS

Each country in the world has its own legal framework governing drugs and feed additives. In general, legislation sets controls on the sale and use of drugs of all kinds for treatment of either humans or animals, including fish. Conditions of use, dosages, and species for which use of a compound is approved vary from drug to drug among countries. Other critical parameters are withdrawal times and detection methodology. For the purposes of this paper, the term “veterinary drug” refers, in general, to any preparation produced in quantity or in series and offered for sale as a therapeutic, prophylactic, diagnostic, or growth-promoting agent to be applied to animals. It includes narcotics, drugs which induce anesthesia, antibiotics, anabolics, sera, vaccines, and herbal remedies (see Wilcke and Hill 1995).

The general legal approach involves the subjection of veterinary drugs to a requirement of product registration (e.g., Indonesia, Japan, Republic of Korea, Pakistan, Philippines) and/or licensing (e.g., Indonesia, Malaysia, Pakistan) before a drug may be sold or used for any kind of treatment of either humans or animals, including fish.

Both systems involve a license or a registration certificate being issued before the veterinary drug may be sold, supplied or manufactured in the country. Applications for the product registration or licenses are submitted to the appropriate licensing/registration authority (e.g., Indonesia - Director General of Livestock Services, Ministry of Agriculture; Japan - Animal Health Division of the Ministry of Agriculture, Forestry and Fisheries; Republic of Korea - Animal Health Division of the Ministry of Agriculture, Forestry and Fisheries; Pakistan - Registration Board; the Philippines - Bureau of Food and Drugs, Department of Health; Thailand - Drug Control Division, Food and Drug Administration) in a prescribed form along with full supporting data relevant to the

¹For the FAO Code of Conduct on the Distribution and Use of Pesticides, the term pesticide means “any substance or mixture of substances intended for preventing, destroying or controlling pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs, or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.”

determination of the application relating to the safety, efficiency and quantity of the product for the purposes for which it is to be used (e.g., Japan, Pakistan, Thailand). Specifically, in relation to the consideration of “safety,” the following elements are to be evaluated by the competent authority: capacity of the substance to cause danger to the health of the surrounding environment, the harmful residue which it may leave in the animal (e.g., Republic of Korea, Japan, Thailand), the already banned ingredients which it includes (e.g., the Philippines) and safety to the animal. “Effective” often means that the product is expected to do what it is claimed it will do consistently. To this effect, the data may have to originate from adequate and well-controlled studies (e.g., Japan, Republic of Korea, Pakistan), the “dose response” relationship may have to be defined (e.g., Thailand) or studies may have had to be performed in several locations as to evaluate any geographic or environmental impacts (e.g., Malaysia). The burden of proof of the safe and effective character lays most often with the manufacturer and importer (e.g., Thailand). To this effect, foreign studies may be accepted (e.g., Thailand, Indonesia, Republic of Korea).

It is rather uncommon in the countries examined that, by law, adverse impacts of, or reactions to, an approved veterinary drug must be reported by manufacturers and importers. Exceptions, however, exist (e.g., in Japan, Republic of Korea and Thailand). On the contrary, manufacturers and importers are most often required to report post-registration (e.g., Thailand (except for feed additives), Japan (only side effects and results in the case of new drugs), Pakistan, Republic of Korea).

FEED ADDITIVES

Substances which are added to feeds to promote growth and to maintain fish health appear to be separately regulated in some countries (e.g., Japan, Indonesia, Thailand, the Philippines, Singapore). Generally speaking, such regulations refer to substances which, by their very nature or their intended uses, may become components of animal feeds or which may affect the characteristics of animal feed.

Controls upon their use and import may include registration (e.g., the Philippines), licensing (e.g., Thailand, Singapore) or listing of authorized additives. In the latter case, the use of additives in fishfeed must occur in accordance with specific regulations defining the substances which may be added (e.g., Thailand, Singapore, Malaysia, Pakistan, Indonesia, the Philippines).

Sometimes, when feed additives are added for medicinal purposes (“medicated feedstuff”), they tend to be covered by the legislation governing drugs, and thus subject to registration (e.g., Japan, Korea, Malaysia) and withdrawal periods. The citing of a withdrawal period seems generally accepted amongst the labeling requirements. Occasionally, an indication on the withdrawal period is to be provided as supporting data during the application process.

Given the importance of assuring that the feed produced meets intended specifications and is not adulterated, it should be noted that in Pakistan, local manufacturers are required to comply with the Standards of Good Manufacturing Practices (GMP). The latter provide guidance for local feed manufacturers (in particular, medicated feed manufacturers) to ensure that their products meet the identity, strength and quality which they should possess with respect to their ingredients, in particular, their drug contents. In the case of imported products, applicants for the use and sale thereof are to provide a certificate asserting the Good Management Practice standards of the manufacturer abroad or authorization to sell under the US Food and Drug Act.

Likewise for veterinary drugs, the application for registration and licensing occurs in a prescribed form. Supporting data that must be provided include information on chemical identity, animal and human safety, intended physical effect, etc.

Enforcement activities include actions to correct and prevent violations, remove illegal products or goods from the market, and punish offenders. The types of enforcement vary with the nature of the violation. Violators found guilty may be subject to penalties and to imprisonment specified by law. Criminal prosecution is sometimes preceded by warning the firm or the individual(s) involved. Other regulatory actions may involve seizure of the product(s) to remove it from the channels of commerce.

Significantly, in relation to the above, the rapid increase of aquafeed production and the need for feed additives such as growth promoters, hormones, probiotics, etc. have led to the adoption in several countries of rules relating to the control of aquafeeds (e.g., Thailand, Agricultural and Cooperatives Ministerial Regulation, 1991 and Aquafeed Regulations; Indonesia, Decree of Minister of Industry No 37/M/SK/3/1992 on standards for the shrimpfeed industry; the Philippines, Feed Control Law and Administrative Order No 84, Series of 1990).

TRADE IN AQUACULTURE PRODUCTS

Restrictions on Commerce

In addition to restrictions on the use of certain chemicals, states also have laws that require food products to be safe if they are to travel in commerce and to be branded properly (e.g., Malaysia, Pakistan).

Often the legislation regulating the quality of aquaculture food products may prohibit contaminated or adulterated food from being marketed. As the primary purpose of food legislation is the protection of the consumer, a need exists to provide the public with legal safeguards against anything that may adversely affect its health or abuse its trust. The term “adulterated” often refers to fish products that contain chemical residues in amounts beyond a level that is considered safe, or ingredients that are not approved by the competent authorities. The term “misbranded” refers to labeling aspects that may be false or misleading. Transporters of adulterated food are subject to criminal sanctions, even if they are unaware of the violation.

In all cases where food is concerned, standardization (i.e., precise requirements against which product conformity can be checked) are defined and codes of practice are present. Undoubtedly, it is in the interest of the aquaculture industry that the quality of fish from aquaculture be acceptable in both national and international trade.

Standards as a Principle of “Food Law”

The trend towards generalized standards is reflected at the national level, and also at the international level in the Joint FAO/WHO Food Standards Programme - Codex Alimentarius Commission. The Codex Alimentarius is a “collection of international food standards adopted by the commission and presented in a uniform manner.” (see FAO/WHO 1995). As such, it contemplates, amongst others, provisions in respect to food additives, pesticides residues, veterinary drugs residue and for fish and fishery products, including last but not least, for the “products of aquaculture.”

A draft *Code of Hygienic Practice for the Products of Aquaculture* has been prepared and amended/re-drafted since 1990 by the FAO Fish Utilization and Marketing Service and discussed recently at the Codex Committee on Fish and Fishery Products in Norway. The scope of this Draft Code of Practice is limited to “finfish and crustaceans produced by commercial aquaculture and intended eventually for direct human consumption.” It contains general guidelines for setting up and conducting production under the most essential requirements of hygiene up to harvesting live fish and loading for transport to market. The slaughtering process is not considered (see FAO/WHO 1996).

It is not the purpose of this survey to enter into the merits of the draft code, but the author would like to stress that the code is intended “for information purposes and as a guideline for the elaboration of national quality standards, quality control and fish inspection regulations in countries where these, as yet, have not been developed. In addition, it could be used for training of fish farmers and employees of the aquaculture sector. In other words, the implementation of the Code of Practice will have to be adapted to national and local needs, priorities and requirements.

It is clear that in order to increase the international trade in aquaculture products, governments have interest in reaching an “agreement” on the composition of these products intended for export and thus, in harmonizing their national rules governing labeling, quality, use of pesticides, feed additives, veterinary drugs, etc. likely to be used in the production process of aquaculture products. Undoubtedly, the more the relevant rules are harmonized, the more complete and effective the eradication of “technical” (non-tariff) barriers (disguised and arbitrary restrictions on trade) will be.

Codex and GATT²

Of relevance to the Codex Alimentarius Commission and to this study are two separate agreements, namely the *Agreement on the Application of Sanitary and Phytosanitary Measures* (the “SPS Agreement”) and the *Agreement on Technical Barriers to Trade* (the “TBT Agreement”) adopted by GATT members following the Uruguay Round. The SPS Agreement deals with measures necessary to protect human life or health, while the TBT Agreement contemplates other technical measures, such as those to prevent deceptive practices. The basic objective of the two agreements is to limit the use of measures that do, or may, restrict trade to those that are justified to provide importing countries the level of protection that is necessary. Temporarily, nevertheless, Members maintain the fundamental right to protect themselves, at the level they determine necessary.

As a result of the Uruguay Round Agreements, major emphasis and greater importance is given to the work of the Codex Alimentarius Commission. Full recognition is given to the important contribution that international standards, guidelines and recommendations can make with regard to the development, adoption and enforcement of sanitary and phytosanitary measures. The use of harmonized sanitary and phytosanitary measures between Members, on the basis of international standards, guidelines and recommendations developed by relevant international organizations including the Codex Alimentarius Commission, is encouraged. Nevertheless, Members may “introduce or maintain” such measures “which result in a higher level of sanitary or phytosanitary protection than would be achieved by measures based on the relevant international standards, guidelines or recommendations, if there is a scientific justification...” or where the decision to do so is based on a risk assessment, appropriate to the circumstances (see Article 2 of the SPS Agreement, GATT 1994).

As far as the TBTs are concerned, the objective is to guarantee that they do not create unnecessary obstacles to, and to minimize their impact on, international trade. The latter, when introduced should be the least disruptive measures possible, and should be of a temporary nature. Where Codex standards exist and are relevant to the circumstances, GATT members must base their measures on them.

Both agreements dedicate a crucial role to “transparency” in the development and application of trade-restrictive (regulatory) measures. This embraces requirements for notifying an intention to introduce such a measure at an early stage, whenever an international standard, guideline or

² The text of the Final Act that embodies the results of the Uruguay Round of Multilateral Trade Negotiations was finalized and came into effect in April, 1994 with its signing at the Marrakech Ministerial Meeting by ministers representing Members in the General Agreement on Tariffs and Trade (GATT).

recommendation does not exist or the content of the proposed measure is not substantially the same as the content of an international standard, guideline or recommendation, and such measure may have a significant effect on trade of other Members (see Annex B of the SPS Agreement, GATT 1994). Undoubtedly, the notification procedures and requirements enclosed therein seem intended to perform as an incentive toward adoption of international standards, guidelines and recommendations, where they exist.

The incorporation of references to Codex standards and codes of practice in GATT agreements should substantially contribute to the development of international trade in food products and reduce problems emerging from differences in national legislation (see Ronk and Dodgen 1991). It should also increase coordination and integration between Codex and the national systems and approaches for approving food additives or for establishing tolerances for contaminants.

Aquaculture Products Producers and Processing

Quality ought to be a characteristic of all commodities, basic (agricultural), processed (industrial) and traded. As for other products, an aquaculture product should not constitute a health hazard or pose threat to consumer safety. The quality control of domestically produced or imported food tends to rely on the governments, for they are responsible to protect citizens against health hazards and commercial fraud. Controls are exercised to ensure compliance of products with relevant national legislation.

A new trend is notable whereby food quality control is left to the parties concerned i.e., to the producers, in a first instance, and to the administration, as a second-tier control. In other words, in order to detect chemical residue or chemical contamination, the aquaculturist must screen the product during the food processing stage. In the interest of producers, it is highly recommended that such controls be undertaken in accordance with an “officially” recognized procedure. Today, the Hazard Analysis Critical Control Point (HACCP) system is the most widely accepted procedure for assuring safe quality of food products. Elements of this system could be found in the legislation of Pakistan (“Good Manufacturing Practice” system), Hong Kong China and the Republic of Korea.

CONCLUSIONS

A few observations can be drawn from the foregoing preliminary analysis of selected legislation governing the use of chemicals in aquaculture in the Asian Region.

Generally speaking, aquaculturists can minimize the risks to their food products by adhering to regulations relating to the distribution and use of chemicals. But, as shown by the present preliminary study, several of the “chemicals” used in aquaculture appear to be subject to various pieces of basic and subsidiary legislation. Moreover, few regulations seem to exist which are specifically drafted or well designed for the purposes of aquaculture. This appears particularly true with regard to pesticides and veterinary drugs and, to a lesser extent, with regard to feed additives and food quality control. Typically, the relevant legal provisions are widely scattered in various legislative instruments covering subject matters like agriculture, plant protection, pesticides, animal health, veterinary drugs, consumer protection, etc. Undoubtedly, this will create confusion and regulatory burdens on the aquaculturist having to comply with all of them and, also, among administrators having to enforce them.

As with the legislation, it appears that the aquaculturist has to cope with various ministries and departments dealing respectively with agriculture, fisheries, animal health, consumer health, and the environment. Indeed several “chemicals” used appear to be hazardous substances which are

controlled under different regimes by various governmental agencies. Again confusion, overlap and conflicting situations are likely to arise which may be detrimental to both the aquaculturist and the consumer. A risk of marketing “unsafe or hazardous” aquaculture products exists.

Legal aspects are essential to the control of use of chemicals in aquaculture and to the quality control of the end product. Nevertheless, this study also shows that there is a need for more scientific and legal interaction. Knowledge of a wide range of technical items is a prerequisite for drafting legislation and setting up appropriate institutional frameworks which will establish an effective system of control on the use of chemicals in aquaculture products. Undoubtedly, the legislator will often be involved in transforming technical norms into nationally binding rules. In addition, the needs of the aquaculturists should be met.

Recent trends in international, regional and sub-regional trade show that a Code of Practice could constitute an essential tool towards eradicating non-tariff barriers. The potential benefits of a Code of Practice relating to the subject matter discussed in this forum should not be underestimated. A code might promote not only rational production, but also competitiveness among producers and consequently, boost exports and protect the interest of consumers. From a legal point of view, a code offers the advantage of grouping into one document measures which are usually scattered throughout different national legislative instruments. As such, a code would likely constitute a basis for the preparation of vertical measures dealing specifically with aquaculture and thus contribute to widely differing national legislation.

RECOMMENDATIONS

In the light of the above, the following recommendations are made:

- Mandatory measures of control are desirable and feasible, but once a government has decided to introduce them, it is absolutely vital that (i) lessons are taken from experiences in the neighboring countries; (ii) adequate care is taken to involve scientists, aquaculturists and, last but not least, legal experts and administrators in the drafting process; and (iii) where possible, harmonization is sought with relevant laws and regulations of the other countries in the region and/or world wide. Harmonization does not imply altogether identical laws and regulations, rather, it is the adoption of similar and compatible approaches and provisions in the domains which could benefit all concerned (manufacturers, producers, traders, importers and exporters).
- The preparation of a Code of Practice relating to the use of chemicals in aquaculture could be critical to boost national and international trade in aquaculture products. Such a code could be based on one of several models. One possibility is a code like the *International Code of Conduct for Responsible Fisheries* (FAO 1995), which sets out general principles which require significant further elaboration through technical guidelines before being suitable for adoption in national legislation. Another possibility is the *International Code of Conduct on the Distribution and Use of Pesticides* (FAO 1990), which has been drafted in such a way that its provisions can be easily incorporated into national legislation. Most probably, it is the latter model that will have the greatest application to the present subject matter. It is recommended, therefore, that consideration be given to the formulation of a code or codes for the use of chemicals in aquaculture.
- Meanwhile, and in view of the above, the preparation of a more in-depth study could be most useful. For an environment in which science and technology continually confront aquaculturists with existing and new regulatory challenges and changing expectations, an in-depth analysis of the current situation is not only desirable, it is essential. It should focus on

the chemicals used in aquaculture and their respective legal status and related approval process. To this effect, it would be beneficial if authorities from countries involved in this exercise would compile and provide information with respect to each product used using a standard format, an example of which is given in Annex II.

REFERENCES AND SUGGESTED READING

- Barberis G, Chiaradia-Bousquet J-P. 1995. Pesticide registration legislation. FAO Legislative Study No. 51, 88 p.
- Chiaradia-Bousquet J-P. 1995. Legislation governing food control and quality certification - authorities and producers. FAO Legislative Study No. 54, 125 p.
- Clausen E. 1985. Regulation of pesticide residues in food : addressing the critical issues. Food Drug Cosmetic Law J. 40: 494-498.
- FAO. 1985a. Guidelines for the registration and control of pesticides. FAO Rome.
- FAO. 1985b. Guidelines on good labeling practice for pesticides. FAO, Rome.
- FAO. 1989. Guidelines for legislation on the control of pesticides. FAO, Rome.
- FAO. 1990. The International Code of Conduct on the Distribution and Use of Pesticides. amended version, FAO, Rome. 34 p.
- FAO. 1995. Code of Conduct on Responsible Fisheries. FAO, Rome. 41 p.
- FAO/WHO. 1995. Codex Alimentarius. 2nd Edn.
- FAO/WHO. 1996. Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission: Proposed draft code of hygienic practice for the products of aquaculture. Codex Alimentarius Commission, CX/FFP 96/7.
- GATT. 1994. The results of the Uruguay Round of Multilateral Trade Negotiations - the legal texts. GATT Publication.
- Gonzalez Vaque L. 1987. Pesticide labelling legislation. FAO Legislative Study No. 43, 129 p.
- New M, Tacon AGJ, Csavas I. 1994. Farm-made aquafeeds. FAO Fisheries Technical Paper No. 343, 434 p.
- Ronk RJ, Dodgen D. 1991. Food standards: an overall strategy for common requirements applicable to all foods. FAO/WHO Conference on Food Standards, Chemicals in Food and Food Trade. ALICOM 91/6.
- Silva Repetto R. 1995. The role of pesticide legislation. FAO-LEGN Study. FAO, Rome.
- Wilcke JR, Hill SA. 1995. Compendium of regulations and authorities for registered veterinary products. Virginia Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksbourg, Virginia, 24061 USA.

Summary of National Legislation Related to the Use of Chemicals in Aquaculture for Selected Countries of the Asian Region

(available with FAOLEX, FAO's Legal Database, maintained by the Legal Office at
FAO Headquarters in Rome, http://faolex.fao.org/faolex_eng/faolex.html)

Hong Kong China

- L.N. 422 of 1990: Agricultural Pesticides (Amendment) Regulations 1990
- The Pesticides Ordinance, amended 79 of 1990

India

- The Insecticides Act, 1968
- The Insecticides Rules, 1971

Indonesia

- Government Decree on the Control of the Distribution, Storage and Use of Pesticides, 1973
- Ministerial Decree on the Procedure of Application for Registration and Approval of Pesticides, 1973
- Ministerial Decree on the Packaging and Labelling Requirements for Pesticides, 1973
- Decree of Minister of Industry No 37/M/SK/3/1992 on standards for the shrimpfeed industry
- The law No. 8 of 1967, concerning the principle of provision of animal husbandry, distribution and administration of animal vaccine, sera, and biological diagnostic substances
- The Government Regulation No. 15 of 1977 concerning repulsion, prevention, and eradication and treatment of animal diseases
- The Government Regulation No. 78 of 1992 concerning veterinary drugs
- The Decision of Minister of Agriculture No 432/kpts/UM/8/10/974 concerning examination and control regulations for vaccine, sera, and biological diagnostic substances
- The Decision of Minister of Agriculture No. 539/Kpts/Um/12/1977 concerning licensing regulation of veterinary drug production, provision, and distribution
- The Decision of Minister of Agriculture No. 24/Kpts/Um/DJP/Deptan/1978 concerning veterinary drug registration procedures

Korea, Republic of

- The 1957 law on the handling of agricultural chemical products as amended by Law No 33 22 of 31/12 1980; and the subsidiary legislation for its implementation
- Decree No 11372 of 29/02/1984 - the Pharmaceutical Affairs laws

Malaysia

- Pesticides Act No 149, 1974 and subsidiary legislation (in particular, the Regulations governing the registration of pesticides (1976), the instrument governing exemption from registration (1979), the Regulations relating to the import of pesticides for educational and research purposes (1981), and the Pesticides (Labelling) Regulations (1984)
- The Control of Drugs and Cosmetics Regulations, 1984
- The Sale of Food and Drugs Regulations, 1952
- Poisons Act, 1952 (Revised, 1989)
- Act 366, (covers the importation of veterinary drugs)
- Animals Ordinance, 1953: Act 17 (covers the importation of veterinary biologics)
- Veterinary Act, (proposed) (covers the importation, manufacture, sale and use of veterinary biologics and drugs)

- Animal Feeds Act, (proposed) (covers the importation, manufacture, and sale of animal feeds)

Pakistan

- The Agricultural Pesticide Ordinance, 1971
- Agricultural Pesticides (Amendment) Ordinance, No XII of 1979
- Agricultural Pesticides Rules, 1973
- The West Pakistan Foodstuffs (Control) Ordinance, 1975
- The Fish Meal (Grading and Marking) Rules, 1973
- Drugs Act, 1976
- Drugs (Licensing, Registering and Advertising) Rules, 1976

the Philippines

- Feed Control Law and Administrative Order No 84, Series of 1990
- Act No. 3720 relating to the use and distribution of veterinary drugs

Sri Lanka

- Control of pesticides Act, No 33 of 1980

Thailand

- Agricultural and Cooperatives Ministerial Regulation, 1991 and Aquafeed Regulations

Guide for the Collection of Information for a Detailed Legal Survey on the Use of Chemicals in Aquaculture

- Product classification
 - pesticide
 - veterinary drug
 - medicated feedstuff
 - feed additive
 - poison
 - other (specify under which circumstances it belongs to one or another category)
- Manufacturer's name
- Product name
 - official
 - scientific
- Registration number
- License number
- Approved uses
- Label code
- Active ingredient, common name and content
- Applicable mandatory rule:
 - basic law
 - regulation
 - ministerial decision or order
- Fee(s) (amount)
- Agencies (ministry, department, committee, etc.) involved in the approval process (registration, license or other)
 - central
 - local
 - other
- Agencies (ministry, department, committee, etc.) involved in the enforcement process
- Data to provided for the application for an authorization
 - on chemical and physical properties
 - on efficacy
 - on toxicity for assessment of human health hazards
 - on residues
 - on possible environmental impacts
- Reporting obligations post-authorization
- Additional information deemed relevant

COUNTRY /AREA PAPERS

The Use of Chemicals in Carp and Shrimp Aquaculture in Bangladesh, Cambodia, Lao PDR, Nepal, Pakistan, Sri Lanka and Viet Nam

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ABSTRACT

This paper provides an overview on the use of chemicals in seven countries in Asia (Bangladesh, Cambodia, Nepal, Laos PDR, Pakistan, Sri Lanka and Viet Nam), with an emphasis on coastal shrimp aquaculture and inland carp farming systems. The data come primarily from a recently completed survey of aquaculture farms in Asian countries conducted under the ADB/NACA Regional Study and Workshop on Aquaculture Sustainability and Environment. The issues discussed include the types and uses of chemicals in shrimp and carp culture, farm management practices and use of chemicals, hazards and adverse impacts associated with chemical use, alternative approaches to chemical use, and research recommendations. In inland carp farming, apart from lime and fertilizers, which are unlikely to give rise to any significant negative environmental impact, the overall use of chemicals is extremely low. Piscicides are used in some countries to control predators prior to stocking of ponds, but the use of antimicrobials and disease-control chemicals is limited to a small percentage (<5%) of producers. Most small-scale producers, who dominate aquaculture production in these countries, simply do not have the resources or need for such chemicals. The situation is similar in shrimp culture, with lime and fertilizers, followed by piscicides, being the most common chemicals used. The use of antimicrobials increases with intensification in shrimp culture, and these chemicals are mostly used in more intensive shrimp farming. In both shrimp and carp culture, promotion of “primary” health management practices probably offers greatest scope for prevention of aquatic animal disease outbreaks and the need for chemical use.

INTRODUCTION

This paper summarizes information on the use of chemicals in several countries in Asia, with an emphasis on coastal shrimp aquaculture and inland carp farming systems. The data come from a recently completed survey of aquaculture farms in Asian countries conducted under the ADB/NACA Regional Study and Workshop on Aquaculture Sustainability and Environment (ADB/NACA 1996, 1998).

The paper briefly deals with the following issues:

- Types and uses of chemicals in shrimp and carp culture
- Farm management practices and use of chemicals
- Hazards and adverse impacts associated with chemical use
- Alternative approaches to chemical use
- Research recommendations
- Conclusions and recommendations

The information presented in this paper comes from several sources:

- Primary data collected by questionnaire as part of the farm-level surveys conducted for the ADB/NACA Regional Study and Workshop on Aquaculture Sustainability and Environment (ADB/NACA 1996). The survey covered coastal shrimp farming systems in 13 countries and inland carp farming systems in 14 countries. The data presented come from farm crops in 1994 and 1995. The survey included only ponds and not hatcheries. Carp farming systems were chosen because of their importance as inland food producing systems in many Asian countries, and shrimp farming because of its importance as an export sector in the region.
- Secondary data collected as part of the ADB/NACA study and previous FAO/NACA study on the Environmental Assessment and Management of Aquaculture Development (FAO/NACA 1995).
- Selected secondary national and regional literature.

The following classification was used for the chemical types recorded during the farm-level survey:

- Therapeutants
- Disinfectants
- Pesticides/piscicides
- Soil and water treatments
- Feed additives

The survey generally covers chemicals used in water treatment ponds (shrimp only), for pond preparation, during grow-out operations and for control of carp or shrimp diseases.

RESULTS

Bangladesh

Inland Carp Culture

The survey included 96 extensive carp farms and 522 semi-intensive carp farms. Extensive farms were classified as low-input farms with limited pond inputs apart from stocking of fish seed and low outputs in terms of fish productivity (yields generally <0.5 MT/ha/yr).

Pond preparation: The most commonly used chemical for pond preparation in carp culture was lime (used on 7% of extensive farms and 80% of semi-intensive farms). Rotenone was used on 1% of extensive farms and 11% of semi-intensive farms as a piscicide to remove “weed fish” prior to stocking of culture fish. Various forms of organic and inorganic fertilizers (1-70%) were used for pond preparation. Use of Phostoxin (a compound used as a piscicide) was also reported by only 6% of semi-intensive carp farmers.

During grow-out operations: The only chemicals used during grow-out operations were lime, which was used on 9% of semi-intensive farms, and organic/inorganic fertilizer, used on 5% of the extensive farms and all (100%) of the semi-intensive farms.

Fish disease control: The main disease problem in freshwater carp culture in Bangladesh was epizootic ulcerative syndrome (EUS). Fish diseases affected 31% of extensive farms and 24% of semi-intensive carp farms. Most farmers made no attempt to control disease outbreaks. Where farmers did use chemicals, the most common treatments were salt, lime (between 28 and 33% of farms), potassium permanganate (15% of semi-intensive farms) and occasionally dipterex. Antimicrobials (e.g., oxytetracycline and oxolinic acid) were used on less than 5% of farms affected by disease.

Coastal Shrimp Culture

The survey covered 23 semi-intensive shrimp farms and 251 extensive farms. Extensive shrimp farming contributes the bulk of shrimp produced in Bangladesh.

Water treatment ponds: Lime was used to treat water treatment ponds on all semi-intensive farms, and chlorine was used on 25% of these farms. Extensive farms did not use water treatment ponds.

Pond preparation: The most commonly used chemical for pond preparation in shrimp culture was again lime (used on 56% of extensive farms and 96% of semi-intensive farms). Teaseed cake was also in common use to remove fish prior to stocking shrimp (11% of extensive farms and 74% of semi-intensive farms). No other chemicals were reported to be used during pond preparation.

During grow-out operations: The only chemicals reported during grow-out operations were lime (27% of semi-intensive farms and 87% of extensive farms) and organic/inorganic fertilizer, used on 9% of extensive farms and 52% of semi-intensive shrimp farms.

Shrimp disease control: Disease is an important problem in semi-intensive and extensive shrimp culture in Bangladesh, affecting 13% of extensive farms and 74% of the semi-intensive farms. Of the farmers affected by disease, most extensive farmers did not use chemicals. Six percent of farms reported using chemical treatments, with oxytetracycline (mixed in feed) being the most commonly used antimicrobial. Chemical use for disease treatment was higher on semi-intensive farms, with 38% of farms reporting some use of chemical treatments. Again, oxytetracycline was the most commonly used chemical, but with chloramphenicol, oxolinic acid, BKC and formalin also occasionally reported.

Cambodia

Inland Carp Culture

Inland carp culture in Cambodia is still in the early stages of development, with most of the country's fish production coming from capture fisheries. The survey covered 148 farms, which were all classified as semi-intensive, with extrapolated fish yields of around 952 kg/ha/yr.

Pond preparation: Farmers used only lime (50% of farms), rotenone for removing predatory fish before fish stocking (64% of farms) and inorganic or organic fertilizer (>88% of farms).

During culture operations: The only chemicals used during grow out were lime (only 5% of farms) and organic/inorganic fertilizer (all farms).

Fish disease control: Only 11% of farms reported any disease problems and oxytetracycline had been used for disease control on around half of these affected farms.

Coastal Shrimp Culture

The survey in Cambodia covered both extensive and intensive shrimp farms, the latter having developed in Koh Kong Province close to the border with Thailand. The survey covered 32 intensive farms and 39 extensive shrimp farms.

Water treatment ponds: Water treatment ponds were used on 56% of the intensive farms. Lime (used by 61% of farms) and BKC (used by 11%) were the most commonly used chemicals.

Pond preparation: No chemicals were used in extensive shrimp farms, but lime (97%) and teaseed cake (66%) were used to prepare ponds prior to stocking shrimp in intensive farms.

During culture operations: No chemicals were used by extensive shrimp farmers and only lime (97% of farms) and organic/inorganic fertilizer (84% of farms) were used during grow-out operations on intensive shrimp ponds.

Shrimp disease treatment: Shrimp disease was not reported at all by extensive shrimp farmers but was common in intensive culture, affecting 81% of the farms surveyed. Oxytetracycline (38%), unknown local herbs/medicine (6%), BKC (30%) and formalin (6%) were reportedly used on the farms affected by disease.

Lao PDR

The Lao PDR was not covered during the survey, but information available from secondary sources (FAO/NACA 1995) indicates that chemical use in freshwater aquaculture is extremely low. The major chemicals reported to be used are lime and inorganic or organic fertilizers.

Nepal

Inland Carp Culture

The survey covered 345 extensive farms and 150 semi-intensive farms culturing carp. The majority of carp farms in Nepal are low-input/low-output farms and consequently, the chemical use in most farms is very low.

Pond preparation: Pond preparation involved only lime (15% of extensive and 67% of semi-intensive farms), organic fertilizer (1% of extensive and 32% of semi-intensive farms) and inorganic fertilizer (3% of extensive and 12% of semi-intensive farms). Use of piscicides was surprisingly low.

During culture operations: Chemical use during grow-out was similarly limited only to organic fertilizer (11% of extensive and 58% of semi-intensive farms), inorganic fertilizer (5% of extensive and 47% of semi-intensive) and lime (11% of semi-intensive farms).

Fish disease control: Fish disease (mainly EUS) affected 18% of extensive carp ponds and 39% of semi-intensive carp farms. The only treatments used were potassium permanganate (16% of semi-intensive farms) and dipterex (on an uncertain number of extensive and semi-intensive farms).

Pakistan

Inland Carp Culture

Inland aquaculture is a relatively new activity in Pakistan, with only around 5,000 farms throughout the country at the time of the survey. Fifty extensive farms and 729 semi-intensive farms were covered during the survey.

Pond preparation: Lime (24% of extensive and 64% of the semi-intensive farms), organic fertilizer (21% of extensive and more than 72% of semi-intensive farms) and inorganic fertilizer (no extensive but 46% of semi-intensive farms) were the main chemicals used in pond preparation.

During culture operations: Similarly, only organic fertilizer (6% of extensive and 71% of semi-

intensive farms), inorganic fertilizer (3% of extensive and 55% of semi-intensive farms) and lime (9% of semi-intensive farms) were used during fish culture operations.

Fish disease control: Fish disease was only a minor problem at the time of the survey, with only 9% of extensive farms and 2% of semi-intensive farms reporting any losses. For those farms reporting disease outbreaks, unknown antimicrobials, malachite green, potassium permanganate and lime had been used by farmers to control diseases.

Sri Lanka

Coastal Shrimp Culture

The survey in Sri Lanka only examined coastal shrimp culture, covering 90 extensive farms, 130 semi-intensive farms and 36 intensive farms.

Water treatment ponds: Only a small number of farms used water treatment ponds (2% of semi-intensive and 6% of intensive farms). Lime was the only water treatment chemical used, on 50% of intensive farms.

Pond preparation: Chemical use during shrimp pond preparation involved only lime (100% of extensive, 98% of semi-intensive and 97% of intensive farms), teaseed cake (11% of extensive, 19% of semi-intensive and 25% of intensive farms) and fertilizer (21% of extensive, 8% of semi-intensive and 11% of intensive farms).

During culture operations: The only chemicals used were lime (68% of extensive, 71% of semi-intensive and 75% of intensive farms), organic fertilizer (5% of extensive, 7% of semi-intensive and 8% of intensive farms) and inorganic fertilizer (21% of extensive, 30% of semi-intensive and 25% of intensive farms).

Shrimp disease control: Shrimp disease was reported in all farm types (16-21-17%). Eleven percent of the affected semi-intensive farms used malachite green to treat disease, while 19% of the affected semi-intensive farms and 50% of the affected intensive farms used lime. Intensive and semi-intensive farms did not report any use of antimicrobials for shrimp disease control. Twenty-five percent of the affected extensive farms reported the use of oxtetracycline in feed.

Viet Nam

Inland Carp Culture

Viet Nam has a large number of farms producing carp, which is an important food item in rural areas. The survey covered 124 semi-intensive and 54 extensive farms culturing carp in ponds in the northern, central and southern parts of the country.

Pond preparation: Farmers reported only using lime (24% of extensive and 64% of semi-intensive farms), organic fertilizer (22% of extensive and 42% of semi-intensive farms) and inorganic fertilizer (17% of extensive and 19% of semi-intensive farms) for pond preparation.

During culture operations: The use of chemicals in carp grow-out was similarly limited to lime (4% of extensive and 1% of semi-intensive farms), organic fertilizer (52% of extensive and 61% of semi-intensive farms) and inorganic fertilizer (17% of extensive and 21% of semi-intensive farms).

Fish disease treatment: Disease was reported as a problem on 26% of extensive farms and 18% of

semi-intensive farms. The types of disease reported varied (no EUS was reported). The methods of treatment were similarly varied. They included unspecified antibiotics (only on 5% of affected semi-intensive farms), various local herbs and medicines (29% of affected extensive and 23% of affected semi-intensive farms), and copper sulphate (only used on 14% of extensive farms). The use of local herbs is particularly interesting and would warrant further investigation.

Coastal Shrimp Culture

Although coastal shrimp culture has developed rapidly in Viet Nam in recent years, most shrimp farming is still carried out in very extensive farming systems. The survey covered 400 extensive farms and 81 semi-intensive farms throughout the country.

Water treatment ponds: A small number of semi-intensive farms (only 8%) had water treatment ponds, of which 27% used lime for water treatment. No other chemicals were used.

Pond preparation: Lime was commonly used for pond preparation (37% of extensive and 80% of semi-intensive farms). Teaseed cake (13% of extensive and 13% of semi-intensive farms) and rotenone (46% of extensive and 63% of semi-intensive farms) were used as piscicides for removing shrimp predators before stocking.

Pond treatment after stocking: Lime was used on 3% of extensive farms and 17% of semi-intensive farms. Inorganic fertilizer (3% of extensive and 12% of semi-intensive farms) and organic fertilizer (5% of extensive and 15% of semi-intensive farms) were also occasionally used to increase pond fertility after stocking of shrimp.

Shrimp disease control: Shrimp disease is an important constraint in Viet Nam, even though much of the shrimp farming is extensive. Disease was reported on 57% of the extensive and 66% of the semi-intensive farms. The overall use of chemicals was low, but of those attempting chemical treatments, farmers reported use of oxytetracycline (1-7% of affected farms), "other" antibiotics (5% of extensive and 16% of semi-intensive farms) and some herbal medicine/other treatments (8% of extensive and 2% of semi-intensive farms).

FARM MANAGEMENT AND THE USE OF CHEMICALS

General Findings

The farm survey in these six countries showed that the most common chemicals in use on inland carp farms are lime and fertilizers. These chemicals are used for soil and water treatment and for generating fertility in ponds, and pose limited environmental and public health risks.

Piscicides such as rotenone and teaseed cake are the second most common class of compounds used after water and soil treatments. Some of the chemicals potentially pose some local risks to the environment and health if not used carefully.

The use of chemicals for disease control in these countries is very low, with less than 5% of farmers in all countries using any form of antimicrobial compound.

In coastal shrimp farming, the survey also showed that lime and fertilizers were the most common chemicals used in the five countries where shrimp are cultured, mainly for water and soil treatment. Piscicides were the second most common class of chemicals, followed by disease-control chemicals. Use of antimicrobials generally increased with intensity of farming, and the frequency of use of disease control chemicals was higher than in carp culture. However, results suggest that the use of antimicrobial compounds is much less widespread than is commonly perceived.

Economics of Chemical Use

The survey data allow some analyses and comparisons between countries and between farming systems based on the expenditure by farmers on different chemicals. Table 1 shows the expenditure in \$/ha/yr spent on chemicals excluding fertilizer costs for carp culture in the different countries. The amounts spent are generally very low and (except for Nepal) increase from extensive to semi-intensive producers. The amounts spent represent less than 1% of carp production costs, which averaged from \$1,000-2,000/ha/yr for extensive farms and \$6,000-13,000/ha/yr for semi-intensive farms (ADB/NACA 1998).

Table 1. Estimated expenditure (\$US/ha/yr) on chemicals (excluding fertilizers) in several carp-producing countries.

	Bangladesh	Cambodia	Nepal	Pakistan	Viet Nam
Extensive	0.7		12.6	0.7	0.1
Semi-intensive	7.3	0.95	10.9	7.8	2.9

Table 2 shows the estimated expenditure on fertilizers. The amount spent in semi-intensive farming is significantly higher than expenditure on other chemicals.

Table 2. Estimated expenditure (\$US/ha/yr) on organic and inorganic fertilizers in several carp-producing countries.

	Bangladesh	Cambodia	India	Nepal	Pakistan	Viet Nam
Extensive	0		19.2	169	0.3	27.4
Semi-intensive	69.7	158.7	300	166.9	29	399.4

Tables 3-5 show similar calculations for shrimp culture in four countries. Expenditure on chemicals is again very low in extensive farming but increases in semi-intensive and intensive farming. Expenditure on chemicals is higher in semi-intensive shrimp farming than in semi-intensive carp culture and shows an increasing trend for the more intensive farming systems. Fertilizer use is lower than in carp culture. The expenditure on chemicals ranged from less than 1% to 6.0% of operating costs in extensive (\$5-4400/ha/yr), semi-intensive (\$2,200-18,400/ha/yr) and intensive culture (\$13,100-28,400/ha/yr) and up to \$0.29/kg of shrimp.

Table 3. Estimated expenditure (\$US/ha/yr) on chemicals (excluding fertilizers) in shrimp aquaculture (figures in brackets give the percent of operating costs).

	Bangladesh	Cambodia	Sri Lanka	Viet Nam
Extensive	4.5 (0.7%)	0 (0%)	15.3 (0.3%)	2.3 (1.7%)
Semi-intensive	477.7 (3.3%)		235.7 (1.3%)	44.6 (2.0%)
Intensive		791.9 (6.0%)	276.6 (1.0%)	

Table 4. Estimated expenditure on chemicals in \$US per kg of shrimp produced.

	Bangladesh	Cambodia	Sri Lanka	Viet Nam
Extensive	0.02	0	0.01	0.03
Semi-intensive	0.29		0.05	0.07
Intensive		0.19	0.04	

The expenditure on fertilizer was very low and less than in carp culture.

Table 5. Estimated expenditure (\$US/ha/yr) on organic and inorganic fertilizers in shrimp culture.

	Bangladesh	Cambodia	Sri Lanka	Viet Nam
Extensive	18.2	0	1.8	0.5
Semi-intensive	46.3	0	26.4	15.4
Intensive		61	3.9	

Hazards and Adverse Impacts

Cultured organisms

The bulk of chemicals used are fertilizers and lime, which are expected to have no adverse environmental impacts. These chemicals are widely used in agriculture as soil conditioners. Fertilizers are an important source of fertility in inland carp farming. The use of agricultural by-products in integrated farming can contribute to efficient use of nutrients and organic matter and environmental improvement (Edwards 1993).

Of the chemicals used for fish and shrimp disease control, a notable feature of the results is that farmers have a very mixed success in controlling disease problems. This suggests that more emphasis is needed on development of effective health management strategies at the farm level.

Antibiotic resistance

There is very little information on this subject available from the survey data. However, there are reports from other sources of resistance to antibiotics occurring in shrimp hatcheries in Viet Nam, and recently some research work by the Aquatic Animal Health Research Institute (AAHRI) has found antibiotic resistance in Bangladeshi carp farms (Chowdhury and Inglis 1994). The latter is interesting given the apparent low use of antibiotics in carp culture in this country. Given the generally low level of antimicrobial use in inland carp farming, the risk of antibiotic resistance arising from such farming systems is considered low. There is clearly a need to better understand the sources and effects of antibiotic resistance where it does occur.

Effects of chemicals on farm workers and consumers

There is a lack of information on the effects of chemicals on farm workers and consumers. In general, the low use of chemicals in these countries suggests that adverse effects are unlikely. In

some isolated cases, the use of certain chemicals, such as piscicides, chloramphenicol and malachite green, may pose a risk to farmer and worker health. Care in the handling and use of such chemicals would be necessary to reduce health risks.

Marketing problems

No marketing problems associated with chemical use in aquaculture products have been reported in these countries. Indeed, the extensive systems commonly used for carp and shrimp in these countries may even offer opportunities for marketing of aquaculture products which have been raised with little, if any, use of chemicals.

Environmental aspects

The major chemicals used are fertilizers and lime, which are not expected to cause adverse environmental impacts, and no environmental problems were raised in the surveyed countries. Where certain higher toxicity compounds are being used as piscicides, then further assessment is necessary to determine the environmental impacts of these chemicals. Baird (1992) has identified the use of piscicides as a possible environmental hazard in freshwater aquaculture and recommended further research to assess impacts and develop alternatives. Concern has also been raised in Bangladesh of the impacts on indigenous fish species when piscicides are used in oxbow lakes and other large water bodies as part of large-scale fish restocking programs.

Measures employed to improve productivity

Fertilizers are the most important chemicals used in freshwater carp culture and are an important source of fish productivity within these systems. As is well known, there are a range of inorganic and organic fertilizers used in inland carp culture. One important issue is the efficiency with which fertilizers are used. Efficiency studies carried out on carp culture data collected during the survey (ADB/NACA 1998) suggest significant differences in the efficiency of fertilizer use in different countries and farming systems, an aspect which requires further investigation. In shrimp culture, fertilizers are applied in lesser amounts than in carp culture and are mainly used for the stabilization of plankton blooms in intensive shrimp aquaculture.

Examples of alternative approaches

Some work has been carried out on alternatives to piscicide use. Work in Thailand has shown that stocking of larger-sized fingerlings, previously grown in hapas, results in less loss due to predation than does stocking of smaller-sized fish (Little *et al.* 1991), thus reducing the need for predator control using piscicides. There is also interesting work on “integrated pest management” in some parts of the world, such as with the control of sealice (Deady *et al.* 1995). Prevention of shrimp disease through systems management is also now being widely promoted (AAHRI 1994). Such approaches need to be further explored for carp and shrimp aquaculture systems.

Research Recommendations

The following are the major research issues, mainly summarized from the recent ADB/NACA survey and earlier FAO/NACA (1995) study.

- Research is needed on the development of preventative “systems” approaches to health management in aquaculture. Systems approaches would help to reduce the likelihood of disease outbreaks through preventative approaches in aquatic animal health care. Such approaches are particularly relevant to the countries surveyed for this paper, as aquaculture is still relatively under-developed and extensive in these countries.

- Research is needed to provide a better understanding of the risk of chemicals in use, with an emphasis on piscicides used for pond preparation and antimicrobials.
- Research on existing chemical usage would be useful to properly assess the extent of the problems. It is also necessary to put antimicrobial use in aquaculture within the overall picture of antimicrobial use in other sectors, before the wider significance of their use in aquaculture can be determined.
- The survey revealed the use of several “traditional” chemicals and indigenous products for disease control, particularly in Viet Nam. Further research should explore the use and effectiveness of these different preparations.
- The possibility of using IPM (integrated pest management) in aquaculture should be further investigated.

In all research work, more emphasis should be given to linking research findings to farmers and other end users.

Regulatory Measures

An obvious regulatory measure is the need for proper product identification and labeling. The legal and other issues relating to chemical use should be further examined in these countries. The FAO Code of Conduct for Responsible Fisheries identifies the importance of appropriate legislation which includes aquaculture chemicals (FAO 1995). Work on development of aquaculture legislation is ongoing in Viet Nam, supported by Norway and the FAO, and would provide opportunities for exploring the need for and development of such legislation.

Data/Information Availability

There is a need to build awareness on safe and effective use of chemicals in aquaculture. This information could be in the form of technical guidelines, extension materials for farmers and through promoting the sharing of information within and between countries. The countries surveyed during this study could also benefit from learning of experiences in other countries within the Asia-Pacific Region.

International Trade Issues

It is likely that issues related to the use of chemicals in aquaculture will be given increasing attention in international trade (Howarth 1997), particularly for export products such as shrimp. It is important that governments and producers are aware of the likely implications of the use of certain chemicals for trade and take precautionary measures to minimize their use and impact on product quality.

CONCLUSIONS AND RECOMMENDATIONS

This survey of carp and shrimp farming systems has provided an overview of chemical use in six countries. In inland carp farming, apart from lime and fertilizers, which are unlikely to give rise to any significant negative environmental impact, the overall use of chemicals is extremely low. Piscicides are used in some countries to control predators prior to stocking of ponds, but the use of antimicrobials and disease-control chemicals is limited to all but a small percentage (<5%) of producers. Most small-scale producers, who dominate aquaculture production in these countries, simply do not have the resources or need for such chemicals.

The situation is similar with shrimp culture in the surveyed countries, with lime and fertilizers, followed by piscicides, being the most common chemicals used. The use of antimicrobials increases

with intensification in shrimp culture, and these chemicals are mostly used in more intensive shrimp farming. Intensive shrimp farmers in Sri Lanka did not use any antimicrobial compounds. In both shrimp and carp culture, promotion of “primary” health management practices probably offers the greatest scope for prevention of aquatic animal disease outbreaks and the need for chemical use.

The following recommendations are directed to different groups as required in the guidelines used for preparation of this paper, and are based on the findings from this review.

To Farmers:

It is the impression from the survey results and other discussions with farmers that farmers are mainly searching for solutions to the day-to-day problems they face, particularly when disease outbreaks are encountered. Farmers may be easily “tempted” by various “cures” presented to them by different marketing groups, as emphasized by Fegan (1996). It is the responsibility of the government and scientific community to ensure that farmers are properly supported with information and technical assistance which provides appropriate advice on farm management and on environmentally safe ways of using approved chemicals. The importance of basic preventative health care for cultured fish and shrimp cannot be overemphasized as one key way to minimize the need for using disease control chemicals (e.g., see the excellent manual by AAHRI 1994).

To Chemical Producers and Suppliers:

Chemical producers and marketers should be responsible for proper labeling of products, substantiation of claims and precautionary measures for reducing risks from improper use of the chemicals.

To Government Agencies and Institutions:

Government agencies have a responsibility for fair regulations on chemical use and provision of appropriate information to farmers. Researchers also have a responsibility for provision of appropriate information on chemical use and impacts and for development of farming systems and practices which promote environmentally sound aquaculture production.

To Regional and International Organizations:

Regional and international organizations can support national agencies through information exchange, capacity building and promotion of research cooperation.

To Scientists:

Scientists have an important role to play in promoting research along the lines outlined above; however, a closer linkage to farmers is essential, such that research work is relevant to farmers' needs and research results are effectively disseminated.

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REFERENCES

- ADB/NACA. 1996. Aquaculture Sustainability Action Plan. Regional Study and Workshop on Aquaculture Sustainability and the Environment (RETA 5534). Asian Development Bank and Network of Aquaculture Centres in Asia-Pacific. NACA, Bangkok. 21 p.
- ADB/NACA. 1998. Final Report on the Regional Study and Workshop on Aquaculture Sustainability and the Environment (RETA 5534). Asian Development Bank and Network of Aquaculture Centres in Asia-Pacific. NACA, Bangkok, 509 p.
- Baird DJ. 1992. Pest control in tropical aquaculture: an ecological hazard assessment of natural and synthetic control agents. *Mitt. Int. Ver. Theor. Angew. Limnol.* 24:285-292.
- Chanratchakool P, Turnbull JF, Funge-Smith S, Limsuwan C. 1995. Health Management in Shrimp Ponds. 2nd Ed. Aquatic Animal Health Research Institute, Bangkok.
- Chowdhury MBR, Inglis V. 1994. Selection of resistant aeromonads to certain anti-bacterial agents in the aquaculture sites of Bangladesh. In: Report of the ODA/SEAADCP/AAHRI Workshop on Antibiotic Resistance, 16-20 November, 1994. Preliminary reports. Report to ODA by the Southeast Asian Aquatic Animal Disease Control Project, Bangkok.
- Deady S, Varian SJA, Fives JM. 1995. The use of cleaner-fish to control sea lice on two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in salmon cages. *Aquaculture*, 131:73-90.
- Edwards P. 1993. Environmental issues in integrated agriculture-aquaculture and wastewater-fed fish culture systems. In: Pullin RSV, Rosenthal H, Maclean JL. eds. Environment and Aquaculture in Developing Countries. p. 139-170. ICLARM Conf. Proc. 33. ICLARM, Manila.
- FAO. 1995. Code of Conduct for Responsible Fisheries. Food and Agriculture Organization of the United Nations, Rome, 41 p.
- FAO/NACA. 1995. Regional Study and Workshop on the Environmental Assessment and Management of Aquaculture Development (TCP/RAS/2253). NACA Environ. Aquacult. Develop. Ser. No. 1, Network of Aquaculture Centres in Asia-Pacific, Bangkok, 492 p.
- Fegan DF. 1996. Sustainable shrimp farming in Asia: vision or pipedream? *Aquacult. Asia*, 1 (2):22-28.
- Howarth W. 1996. Trade, the environment and aquaculture. *Aquacult. Asia*, 1 (1):17-23
- Little DC, Innes-Taylor NL, Turongruang D, Komolmarl S. 1991. Large fish seed for small-scale aquaculture. ICLARM NAGA, Aquabyte, p. 2-3.

The Use of Chemicals in Aquaculture in India¹

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ABSTRACT

A review of the use of chemotherapeutants and other chemicals and drugs in Indian aquaculture is presented. A large number of products are used for various purposes such as soil and water treatments, disinfectants, piscicides, herbicides, organic and inorganic fertilizers, feed additives, therapeutants, and anesthetics. Farm management techniques for the use of chemicals are discussed, as are the hazards posed by, and impacts resulting from chemical use. Other approaches to disease prevention (crop holiday, pond preparation, regulating stocking density, effluent treatment systems) are considered, and national regulations on the use of chemicals in aquaculture and current research being conducted in India are summarized. Recommendations for the improved use of chemicals in Indian aquaculture are provided for farmers, government and aquaculture institutions, the chemical industry, regional and international agencies, and research institutions.

INTRODUCTION

Aquaculture has been practiced in India in both freshwater and coastal saline waters from time immemorial. These were characteristically low-input, low-production systems depending on natural seed collection from the wild, with stocking in natural ponds, or impounding in large water bodies without any further management measures. During the last decade, aquaculture has slowly but steadily transformed itself into a profitable business activity. In freshwater carp culture, production rates of up to 15 t/ha/yr have been attained, and in shrimp culture, yields of about 8 t/ha/crop have been achieved. In tilapia culture, production rates of over 400 t/ha/yr in three crops have been reported. With the increase in area devoted to aquaculture and also the intensification of culture practices, environmental issues have come into focus, particularly issues concerning the planning and regulation of aquaculture in coastal areas.

Brackishwater aquaculture in coastal areas primarily involves shrimp culture and is widespread on the east coast in the states of West Bengal (traditional bheries), Andhra Pradesh, Orissa and Tamil Nadu. Along the west coast, Kerala has a predominantly traditional culture system (paddy-cum-shrimp culture). This also exists on a moderate scale in Goa and Karnataka. Huge concentrations of shrimp farms are situated in Nellore, Bhimavaram and Kakinada in Andhra Pradesh, and Tuticorin in Tamil Nadu.

Compared to coastal aquaculture, freshwater carp culture is widespread in the country, particularly in the states of Andhra Pradesh, West Bengal, Madhya Pradesh, Punjab, Uttar Pradesh, Orissa and Bihar. Sea-farming of oysters and pearls is still in the experimental stage in India, but interest is now growing for establishing joint-venture cage-culture projects with sea bass and grouper as

¹The opinions expressed in this paper are those of the authors and not of the organization they represent.

candidate species.

Aquaculture production in the freshwater sector has steadily increased from a level of 7.9 lakh t in 1987 to 13.25 lakh t during 1994-95. The average has increased from 900 kg/ha in 1984-85 to 2130 kg/ha in 1994-95. Similarly, aquaculture production from coastal waters has increased from 35,500 t in 1990-91 to 74,850 t in 1994-95, with average productivity increasing from 545 kg/ha to 743 kg/ha.

Freshwater fish culture is primarily comprised of Indian major carps (catla, rohu and mrigal), with the secondary species including exotic Chinese carps. In 1993, total inland fish production of India (species-wise) was comprised of 54.7% major carps, 8.1% common carp, 6.1% other carps, 3.6% murrels, 2.2% *Hilsa* sp., and 25.3% other species (GOI 1993).

In traditional coastal shrimp farming systems, production is a mixture of several species of shrimp and fish. In the bheries of West Bengal, *Penaeus monodon* comprises about 18% of the total catch, while in the pokkali fields of Kerala, *Metapenaeus dobsoni* constitutes more than 50% of the total catch. *Penaeus monodon* is the major species cultured in new brackishwater shrimp farming systems all over the country, with *P. indicus* in second place.

Basically, aquaculture in India is largely of the extensive type and primarily related to carp farming. However, there has been an emergence of large-scale commercial, semi-intensive culture of carps in a few states, especially in Andhra Pradesh, where nearly 50,000 ha of carp culture ponds and 35,000 ha of shrimp culture ponds are under commercial operation. With the increase in productivity in semi-intensive carp culture, semi-intensive or intensive shrimp farms, and related hatchery operations, there has been increased usage of artificial inputs in the form of chemicals. Fish farmers faced with serious fish health problems have resorted to management practices involving the use of chemicals and therapeutants. This has resulted in environmental problems that were not previously encountered. Although carp culture is practiced in self-enclosed systems with limited effluent release to the environment, coastal aquaculture farms need large volumes of water daily. Thus, the hazards posed by the release of effluents containing chemicals with residual effect and high nutrient loads into receiving waters are a new dimension that has unfolded in the aquaculture scenario of the country. The sudden change in culture practices that has occurred in India has caught the nation off-guard as regard to effluents affecting the environment.

As these environmental problems are of recent origin, not enough research has been done to assess the gravity of the situation. Since the outbreak of shrimp disease in 1994, the subject has drawn the attention of research and development agencies in aquaculture, and a number of studies have since been conducted on the environmental impacts of such commercial aquaculture activities. However, most studies have concentrated on the nutrient loading of recipient waters, heavy metal pollution, and changes in water and soil characteristics of adjoining areas. Hence, the present review is largely based on findings restricted to the above impacts of aquaculture. The impact of the use of therapeutants on the environment has yet to be studied in detail. However, some isolated studies on the use of chemotherapeutants in controlling fish diseases have been undertaken in various fisheries research agencies in the country.

USE OF CHEMICALS IN AQUACULTURE

The various chemicals used in grow-out farming and hatchery operations in both freshwater and coastal aquaculture in India can be classified into the following broad categories: water/soil treatment products, disinfectants, piscicides, herbicides, organic fertilizers, inorganic fertilizers, feed additives, therapeutants and anesthetics.

Water and Soil Treatment Products

In both freshwater and coastal aquaculture, it is common practice to treat the pond waters for mineralization of organic matter, for adjusting pH, and for disinfection. The chemicals used in this regard are lime in the form of lime stone (CaCO_3), slaked lime ($\text{Ca}(\text{OH})_2$) or unslaked lime (CaO). Dried ponds are also sterilized using active iodine or potassium permanganate (KMnO_4). Soil conditioners that contain high numbers of sulfur-degrading bacteria and organic matter-decomposing bacteria are also used by some intensive shrimp culture farms. Health stone, zeolite, or porous aluminium silicate is applied along with lime to re-activate the soil for stabilizing algal growth and absorbing fouling materials.

Disinfectants

Common disinfectants used are sodium hypochlorite, benzalkonium chloride (BKC), calcium carbide, Na-EDTA, and zeolite. These are used mostly in hatcheries and, to a limited extent, in grow-out ponds.

Piscicides

In both freshwater and coastal aquaculture, eradication of unwanted predatory fishes is a common pre-stocking management practice. The common fish toxicants used are mahua oil cake, teaseed cake, other plant derivatives and anhydrous ammonium substances.

Herbicides

Aquatic weeds are of common occurrence in fishponds in the country and are undesirable, as they pose serious problems by upsetting the oxygen balance and removing nutrients from the aquatic systems. The common herbicides used for controlling aquatic weeds are 2,4-D; Dalapon; Paraquat; Diuron; ammonia; and many others.

Organic Fertilizers

Use of organic manure in fish culture is an age-old practice. The manure used comes mainly from farm animals, the commonly used manures being cow dung, pig dung, poultry droppings, etc. Cattle manure and poultry droppings contain nitrates at the 0.5 % and 1-15% levels and phosphates at the 0.2 and 0.4% levels, respectively. The application of raw cow dung slurry helps to boost diatom bloom (Sarkar 1983). In modified extensive shrimp-culture ponds, 1,000 to 3,000 kg of cow dung/ha is applied initially. It is followed by application of two dosages of 200 to 400 kg of cow dung on the 8th and 14th d, respectively. Poultry droppings contain higher quantities of soluble salts, inorganic substances and organic products than does cow dung, ensuring quick zooplankton production. In semi-intensive carp culture, large amounts of cow dung are applied to increase the fertility and consequent natural productivity of the ponds. Natural food provides 50% of the food requirement in such carp culture ponds.

Inorganic Fertilizers

Considerable quantities of nutrients are removed from the pond ecosystem through the harvested fish crop. Hence, for proper management of pond soil and water, these elements need to be replenished from external sources. The fertilization schedule is prepared on the basis of the fertility status of the soil. Soils with available nitrogen of >50, 25-50, or <25 ppm; and available phosphate content of >6, 3-6, or <3 ppm are classified as "high," "medium" and "low," respectively. The

application rate of the different nitrogenous and phosphate fertilizers varies with culture practice and the nature of the fertilizer used. The doses of fertilizers applied for both carp culture and shrimp culture are presented in Table 1.

Feed Additives

With the intensification of aquaculture practices, there is a shift from using supplementary fish feeds comprised of agricultural wastes and by-products to using complete feeds developed to meet the complete nutritional requirements of the species cultured. These feeds usually have other additives in the form of pigments, vitamins, chemo-attractants, and preservatives, like mold inhibitors and antioxidants.

Therapeutants

There is an increasing occurrence of disease caused by parasitic, fungal, bacterial, and viral infections in both hatcheries and grow-out farms. In the recent past, India has witnessed three major outbreaks of disease. The first to strike was the dreaded epizootic ulcerative syndrome (EUS) in freshwater cultured species. This was followed by yellowhead-virus disease and whitespot-virus disease in shrimp farms. The outbreak of these diseases rendered severe blows to the aquaculture industry in the country, creating the need to use therapeutants in both the freshwater carp industry and in shrimp culture.

Anesthetics

The use of anesthetics in aquaculture in India is rather limited. They are sparingly used, particularly in the long distance transport of broodstock and fish seed.

Information on the uses, methods of delivery, doses, frequency of application, and other aspects for chemicals commonly used by the Indian aquaculture industry is presented in Tables 1-4. Information is presented separately for freshwater aquaculture and coastal aquaculture. Information on the use of chemicals other than therapeutants in the freshwater sector is presented in Table 1, whereas that for therapeutants is given in Table 2. The use of chemotherapeutants in freshwater aquaculture is rather limited and has gained significance only since the outbreak of EUS a few years ago. In carp hatcheries, their use is also limited.

Table 1. Use of chemicals (other than therapeutants) in freshwater grow-out culture and hatchery systems in India.

Item	Purpose	Doses	Mode of Application	Remarks
I. Soil and water treatments				
Quick lime	Correcting pH, disinfectant	400 to 2000 kg/ha	Dissolved in water and broadcast over pond surface	Basal dose of 50%. Remaining in equal monthly installments
	Mineralization	400 kg/ha	Dissolved in water and broadcast over pond surface	Applied at the time of pond preparation
II. Disinfectants				
Bleaching powder	Disinfectant	25-30 ppm	Broadcasting/water solution	Toxicity lasts 7-8 d
III. Piscicides				
Mahua oil cake(MOC) (<i>Basia latifolia</i>)	Piscicide (4-6% saponin)	200-250 ppm	Soaked in water and spread over water surface	Toxicity lasts 15-20 d, after which it acts as fertilizer
Teaseed cake (<i>Camellia sinensis</i>)	Piscicide	75-100 ppm	Same as MOC	Toxicity lasts 10-12 d, after which it acts as fertilizer
IV. Herbicides				
2,4-D	Emergent weeds, grasses	5-10 kg/ha	Foliar spraying/ root zone treatment	Hyacinth, <i>Ipomea</i> , sedges, lillies, <i>Vallisneria</i> , etc.
Dalapan	Aquatic grasses	5-10 kg/ha	Foliar spraying	
Simazine, Diuron	Submerged weeds	4 kg/ha	Root zone treatment	Horn wort, <i>Hydrilla</i> , etc.
Ammonia	Submerged weeds	10-15 ppm	Root zone treatment/ dispersal in water column	Horn wort, <i>Hydrilla</i> , etc
	Floating weeds	1-2% aq. soln.	Foliar spraying with 0.25 % wetting agent	<i>Pistia</i> , <i>Salvinia</i>
Paraquat	Floating weeds	0.2 kg/ha	Foliar spraying	<i>Pistia</i> , <i>Salvinia</i>
V. Organic fertilizers				
Cow dung	Fertilization	15 t/ha	1. Pond bottom application, 20 % of total dose 2. Balance in equal mo doses	Initial manuring done 15 d prior to stocking

Table 1. Continued . . .

Item	Purpose	Doses	Mode of Application	Remarks
VI. Inorganic fertilizers (kg/ha)				
A. Nitrogenous fertilizers	Fertilization		Sprayed or distributed over water surface	Applied alternately with organic manure at 15-d interval
Urea		150-300		
Ammonium sulfate		300-600		
Calcium ammonium nitrate		300-600		
B. Phosphate fertilizers			Sprayed or distributed over water surface	Applied alternately with organic manure at 15-d interval
Single super phosphate		150-400		
Triple super phosphate		50-150		

Table 2. Use of chemotherapeutants in freshwater aquaculture and hatchery systems in India (from Rao *et al.* 1990).

Item	Purpose	Doses	Mode of Application	Remarks
A. Parasitic diseases				
Oxytetracycline	<i>Myxobolus</i> spp.	5 gm/ 100 kg fish	Supplemented in the feed	Prevents secondary bacterial infection
Sodium chloride	<i>Epistylis</i> spp., <i>Zoothamnium</i> spp.	20-50 kg/ha	2-3 installments at 4-d interval	
Malathion/ Dichlorvos	<i>Dactylogyrus</i> spp. <i>Gyrodactylus</i> spp. <i>Argulus</i> spp., <i>Lernaea</i> spp., <i>Ergasilus</i> spp.	0.2 ppm 0.15-0.25 ppm (Malathion) 0.05-0.1 ppm (Dichlorvos)	2-3 times at 4-d interval 2-4 installments at 4-7-d interval	Pond water application
Gammexane	As above			Immersion treatment
B. Bacterial/fungal diseases				
Sulphadiazine	Surface	5 gm/	Applied for 7 d	Water dispersible powder
+ Trimethoprim	ulcerative and systemic type (<i>Aeromonas hydrophila</i>)	100 kg		
Chloro-tetracycline		7 gm/100 kg	---do---	Supplemented in feed

Table 2. Continued. . .

Item	Purpose	Doses	Mode of Application	Remarks
Oxy-tetracycline	Columnaris disease	7-10 gm/100 kg	Applied for 10 d	Supplemented in feed
Nitrofurans	Microbial gill disease	10 gm/100 kg		Immersion treatment
Trimethoprim		5-7 gm/100 kg		
Copper sulfate	<i>Saprolegnia</i> spp., <i>Branchiomyces</i>	0.2-0.5 ppm	2-3 installments at 3-4 d interval	Immersion treatment

Table 3. Use of chemicals (other than therapeutants) in coastal aquaculture and hatchery systems in India.

Item	Purpose	Doses	Mode of Application	Remarks
I. Soil and water treatment				
Quick lime (CaO)	Correcting pH	270-1130 kg/ha	Dissolved in water and broadcast over pond surface	Basal dose of 50%; remaining dose given in equal monthly installments
Slaked lime (Ca(OH) ₂)		340-1610 kg/ha		
Lime stone (CaCO ₃)		380-1690 kg/ha		Dose depends upon pH of soil and water
Gypsum	Reduces turbidity	300 kg/ha	Applied before manuring	
II. Disinfectants				
20% active iodine	Sterilization	1-2 ppm	Applied in dried ponds	
KMnO ₄	Sterilization	1-2 ppm	Applied in dried ponds	
III. Soil reformers				
Sulfur bacter	Reduces soil pH	75-120 kg/ha	Applied in wet soil and sun dried 2-3 d	
Health stone/zeolite	Reactivates soil/promotes algal growth/absorbs fouling materials	250-1000 kg/ha		Dose depends on soil pH
IV. Piscicides				
Mahua oil cake (MOC) (<i>Basia latifolia</i>)	Piscicide (4-6% saponin)	200-250 ppm	Soaked in water and spread over water surface	Toxicity lasts 15-20 d, after which it acts as fertilizer
Teaseed cake (<i>Camellia sinensis</i>)	Piscicide (10-15% saponin)	75-100 ppm	same as MOC	Toxicity lasts 10-12 d, after which it acts as fertilizer
Derris root powder	Piscicide	5-10 ppm	Same as MOC	
Calcium hydroxide + Ammonium sulfate	Piscicide	10 ppm	Applied in 1:4 ratio	

Table 3. Continued. . .

Item	Purpose	Doses	Mode of Application	Remarks
V. a. Organic fertilizers alone				
Cow dung	Fertilization	Primary dose 1-3 t/ha Secondary dose 200-400 kg/ha	Applied when water depth is 10 cm Two doses, one at 30 cm water depth and another at 100 cm water depth	
Poultry droppings	Fertilization	Primary dose 100-500 kg /ha Secondary dose 25-100 kg/ha	Applied when water depth is 10 cm Two doses, one at 30 cm water depth and another at 100 cm water depth	
b. Organic fertilizers along with inorganic fertilizers				
(i). Cow dung	Fertilization	200-500 kg/ha	Applied initially by broadcasting	
Urea Single super phosphate	Fertilization	150 kg/ha 50-70 kg/ha	Applied in 2 doses Applied in 2 doses	Following application of cow dung
(ii). Poultry droppings	Manuring	100-250 kg/ha	Applied initially by broadcasting	
Urea Single super phosphate	Fertilization	20 kg/ha 6 kg/ha	Applied in 2 doses Applied in 2 doses	Following application of cow dung
VI. Inorganic fertilizers				
1. Nitrogenous fertilizer	Fertilization	Primary dose dose 0.95 ppm N ₂ level. Secondary dose 0.475 ppm N ₂ level	Applied initially on d 1 Applied in two subsequent doses on d 8 and 14	Fertilizers are dissolved and dispersed in dilute form
2. Phosphate fertilizer	Fertilization	Primary dose 0.11 ppm P ₂ O ₅ level. Secondary dose 0.055 ppm P ₂ O ₅ level	Applied initially on d 1 Applied in two subsequent doses on d 8 and 14	Fertilizers are dissolved and dispersed in dilute forms

Table 4. Use of feed additives and therapeutants in coastal aquaculture and hatchery systems in India.

Item	Purpose	Doses	Mode of Application	Remarks
A. Feed additives		/kg feed		
1. Mineral premix	Mineral requirement		Mixed with feed	
Calcium		10-18 gm		
Phosphorus		18 gm		
Magnesium		0.8-1 gm		
Sodium		6 gm		
Potassium		9 gm		
Zinc		50-100 mg		
Copper		25 mg		
Manganese		20 mg		
Iron		5-20 mg		
Cobalt		10 mg		
Selenium		1 mg		
2. Anti oxidants				
Ethoxyquin	Protects fatty acids	150 mg		
Butylated hydroxy toluene	Protects fatty acids	0.02% of fat content	Added to lipids or vitamin premix	
Butylated hydroxy anisole	Protects fatty acids	0.02% of fat content	Added to lipids or vitamin premix	
3. Antimicrobials	Prevents growth of microbes and fungi	0.1-0.25% of feed	Added to feed	
Sorbic acid derivatives				
Propionic acid derivatives				
Sodium benzoate				
4. Pigments				
Astaxanthin	Coloration	30-35 ppm	Added to feed	
B. Therapeutants				
1. Grow-out ponds				
Teaseed cake	Necrosis of appendages	5-10 ppm		Induces molting
Oxytetracycline	Brown spot disease, bacterial septicemia, black gill disease	1.5 gm/kg feed	Mixed with feed for 10-15 d at the rate of 2-10% of biomass	

Table 4. Continued . . .

Item	Purpose	Doses	Mode of Application	Remarks
Formalin	Protozoan fouling	15-25 ppm	Applied into ponds	
		50-100 ppm	Dip treatment for 30 min	
2. Hatcheries				
EDTA	Vibriosis	10-15 ppm		
Copper sulphate	Filamentous bacterial disease	0.25-1 ppm	Dip treatment for 4-6 h	
Treflan	Larval myosis	0.1-0.2 ppm	Bath for 1 d	
Prefuran	Bacterial necrosis <i>Vibrio</i> infection	1 ppm		

Supply of Chemicals

The various chemicals and antibiotics used in aquafarming are mostly available locally, and there is no control on their sale and usage. Most of the farmers use veterinary-grade antibiotics for application in the fishponds. Certain products are imported from companies such as Aurum Aquaculture Ltd (USA), J.V. Marine (Taiwan), Argent Chemical Laboratories (USA), and Pfizer Food Science (USA). Information on the quantitative usage of these chemicals is generally not available and no agencies mandated to regulate usage are in existence at the moment. Information regarding the nature and prices of some of the chemicals available from Indian manufacturers is presented in Table 5.

Table 5. Common pesticides and antibacterials used in aquaculture and their manufacturers.

Pesticide/chemical/antibiotic	Brand name	Manufacturer	Rate (Indian rupees (rs)/kg, as of Oct. 1990)
Dichlorvos	Nuvan	Hindustan Ciba-Geigy Ltd.	225
Malathion	Cythion	Cyanamid India Ltd.	75
Quinophos	Ecalux	Sandoz India	150
Benzene hexachloride	Gammexene	Local companies	40
Copper sulphate		Local companies	20
Sodium chloride	Common salt	Local companies	0.50
Calcium oxide	Lime	Local companies	1.25
Oxytetracycline	TM-50	Pfizer Ltd.	120
Chlortetracycline	Aurofac-20	Cyanamid India Ltd.	68
Doxycycline	AFS Forte	Vesper	90
Nitrofurazone + Furazolidone	Bifuran	Eskaylab Ltd.	120
Furazolidone	Groviron	Glaxo Laboratories	120
Sulphadiazine + Trimethoprim	Bactrisol Neochlor Forte	Alved Pharma Pvt. Ltd. Vetcare Division of	290
Chloramphenicol		Tetragen Chemie Ltd.	NA
Neomycin+ Doxycycline	Neodox Forte	Vetcare Division of Tetragen Chemie Ltd.	NA

NA = not ascertained.

FARM MANAGEMENT AND USE OF CHEMICALS

Preventive Methods

Most therapeutic agents have residual effects on the tissues of the candidate species. In addition, antibiotics may also leach into natural habitats, leading to modification of native bacterial flora and the emergence of antibiotic-resistant strains. The persistence of therapeutic agents in the aquatic environment may also cause adverse effects on the ecosystem (Anon 1988, Choo 1994). The best strategy in the management of aquaculture enterprises is to prevent the occurrence of disease. The industry in India, by and large, takes the following precautions to prevent disease in hatcheries and grow-out farms:

Disease Prevention Measures in Hatcheries:

1. Proper site selection. In choosing the location for a hatchery, due care should be taken to locate it in a place where good quality water is ensured for maintenance of broodstock, spawning, and larval rearing.
2. Water treatment. Disinfect sea water with calcium hypochlorite (20-30 ppm) or sodium hypochlorite (150 ppm) for 1-2 d. Remove excess chlorine from sea water by neutralizing with sodium thiosulfate. Filter or sterilize by UV treatment or other means, sea water used for all hatchery operations.
3. Maintenance of cleanliness of hatchery facilities. The tanks used for broodstock, spawning, and larval rearing should be kept thoroughly clean.
4. Disinfection of broodstock. For this purpose, 20 ppm formalin or other appropriate drugs can be used as short bath or dip treatment.
5. Observe appropriate care at the time of spawning by thoroughly removing the scum formed after spawning.
6. Stock only healthy nauplii at an optimal stocking density. The nauplii can be disinfected by dip treatment in 200-300 ppm formalin.
7. During larval rearing, siphon out unused feed, sediments, debris, and wastes accumulated at the bottom and sides of the tanks.
8. Feed the larvae with optimal amounts of good quality well-balanced feed.
9. Use antibiotics carefully and at the correct doses, preferably after ascertaining the *in vitro* sensitivity of the pathogens. Low doses of antibiotics lead to the development of antibiotic-resistant mutants of bacteria and higher doses may be toxic to the shrimp larvae or the other fauna and flora of the culture system.
10. Examine larvae microscopically every morning before changing water for any signs of abnormality, fouling protozoa, filamentous bacteria, fungal infections, and presence of swarming bacteria within the hemocoel. Maintain good water quality parameters at all times.
11. Disease outbreaks due to viral infection can be avoided by quarantine measures, and by destroying carriers and clinically diseased animals.

Disease Prevention Measures in Grow-out Farms:

1. Select farm sites properly, ensuring that they are far from industrial, agricultural and domestic sources of pollution.
2. Before stocking, drain and sun dry the ponds thoroughly. The black layer of soil formed during the previous crops should be removed and the pond tilled. Lime can be applied at the rate of 200-600 kg/ha depending on the pH of the soil.
3. Stock only healthy postlarvae after achieving an optimal algal bloom in the ponds. Maintain optimal density of shrimp larvae.
4. Good water quality in the pond should be maintained.

5. Feed the shrimp with a balanced diet at optimal quantity. Care should be taken to avoid overfeeding and accumulation of uneaten feed.
6. Routinely examine the health status of the shrimp. Frequent microscopic examination of gills, hepatopancreas and hemolymph for microbial infections or any disease signs should be done. Clinically diseased and infected shrimp should be destroyed by burning or burying with lime in soil away from the shrimp farm.
7. Practice adequate quarantine measures before transporting shrimp postlarvae or broodstock to different geographical locations. They may harbor pathogenic microorganisms, particularly viruses, without showing external clinical signs.
8. If available, use disease-resistant stocks for culture purposes. The resistance of shrimp to various infectious agents appears to be species specific and is probably a genetically acquired trait.
9. Vaccines may also be used for controlling specific diseases.

Therapeutic Measures

Disease control programs in aquaculture must consider various factors such as stocking density, environmental parameters, rate of water exchange, the type of feed used, and phytoplankton blooms. However, in spite of the best management practices adopted in hatcheries and grow-out systems, disease outbreaks will still occur, necessitating use of drugs for their control. Although some drugs have been advocated for treatment of diseases (see Tables 2 and 4), these should be employed only as a last option. Many aspects of the dynamics of drug use in aquaculture are yet to be studied. Various aspects of using drugs for disease control such as their dosages, intervals of administration, duration of exposure of fish, their effect and efficacy in controlling the disease, withdrawal period from the tissues, effects on non-target species, etc. remain to be clearly understood. As drugs are useful only if they are applied during the early phase of disease, correct diagnosis at an early stage is a very important aspect that will help to control the disease.

Criteria for Selection of Drugs for Disease Control:

The following criteria are used to select appropriate drugs for disease control:

1. The sensitivity of the pathogen to the drug or antibiotic based on *in-vitro* tests must be known.
2. The antibiotic or chemical should reach the pathogen and kill it without adversely affecting the host.
3. The antibiotic or drug should not adversely affect the user and the natural flora and fauna.
4. The drug should rapidly be broken down to avoid problems with tissue residues.
5. The metabolites of the drug should be harmless to the cultured animal and the consumer.
6. The drug should be stable under normal storage conditions.

Methods of Application of Drugs:

The treatment methods currently being followed include applying the therapeutic agent to the pond water or administering it along with feed. The various methods that are commonly followed are given below:

1. Oral route

The chemotherapeutant may be incorporated in the feed at the correct dose and fed to the shrimp. However, application of medicated feeds needs to be clearly understood, otherwise, the drug may diffuse into the water and create problems (Choo 1994). Drug therapy by this method should preferably start during the initial stages of the disease, since fish in the advanced stages of disease feed poorly. Compounds like antibiotics, sulfa drugs and nitrofurans are widely used along with

feed to treat bacterial diseases, both in shrimp and freshwater carp culture systems in India (Tables 7 and 9).

In carp culture systems in the State of Andhra Pradesh, the required quantity of poultry feed supplement (poultry feed supplements with various proportions of antibacterial activity are commonly available to the fish farmers) is added to the normal fish feed by mixing 4:1 mixture of de-oiled rice bran and oil cake into a dough (Rao *et al.* 1992). Unlike the common practice of broadcasting the feed over the pond surface, the feed is kept suspended in perforated bags tied to bamboo poles. Normally, 10-20 bags of size 20 x 30 inches are used per ha, the bags being tied individually to bamboo poles fixed at regular intervals in the pond. Each bag has two rows of perforations and can contain up to 12 kg of feed. Typically, the feed within a perforated bag is eaten by the fish within 2 h. This method results in minimal feed wastage and reduced antibiotic leaching as compared to broadcasting the medicated feed over the pond (Rao *et al.* 1992).

2. Immersion treatment method

This method is followed to treat ectoparasitic diseases, bacterial surface ulcerative lesions and external fungal problems. The therapeutic agent is added directly to the pond water or sprayed over the surface. Agricultural grade pesticides sold under different brands have been regularly used to treat against helminth and crustacean parasites. The current method of pesticide application in carp farms in Andhra Pradesh involves dissolving the required quantity of pesticide in 10-20 L of water and spraying the solution over the pond surface with hand-held agricultural sprayers. In large fishponds, hand-held sprayers are operated from boats. Use of this method is very popular for the application of Nuvan and Malathion to combat infection by *Argulus* spp. (Rao *et al.* 1992).

3. Dip treatment

In this method, the fish or shrimp are held in containers with a strong solution of the chemotherapeutant for short durations. This method may be useful when only a small portion of the stock is affected with non-systemic infections such as fouling, shell disease, necrosis of appendages, etc.

4. Bath treatment

This method is applicable only when a small portion of the stock is affected with disease. The fish are given bath treatment in containers for 30-60 min in a solution containing the drug.

5. One-time application

In one-time application, a low concentration of the chemical is applied to the culture tanks or ponds for an indefinite period. However, this method poses pollution problems.

6. Injection

This method is practical to use when only a small number of large and valuable fish are to be treated.

7. Topical application

This method is also applicable only for a small number of valuable broodstock suffering from non-systemic diseases such as shell disease.

Hazards and Adverse Impacts on Culture Organisms and Farm Productivity

The adverse impacts of chemical inputs on farmed species and farm productivity will be discussed separately for carp culture and shrimp culture because of the inherent differences between the two industries. The interaction between the pond environment and the external environment, and the qualitative and quantitative differences in the use of chemical inputs in the two fundamentally different aquaculture practices in the country are obvious.

Freshwater Aquaculture

Freshwater fish culture contributes the bulk of production derived from Indian aquaculture. The dominant culture system is semi-intensive polyculture of Indian major carps and Chinese carps (CIFRI 1985). Most semi-intensive culture ponds are relatively isolated from the external environment. Being largely dependent on natural productivity, augmented by the use of organic and inorganic fertilizers and a supply of supplementary feed largely consisting of agricultural by-products, Indian freshwater aquaculture is environment-friendly in nature. However, in attempting to achieve higher productivity, aquaculturists have often exceeded the carrying capacity of their ponds and the sustainable limits of production. Overloading systems with fish biomass and excessive application of fertilizers and supplementary feed results in deterioration of water quality, increased plankton bloom, stress to farmed species and consequent outbreak of disease. Accumulation of unused feed and metabolites often leads to eutrophication and an inimical pond environment. The resulting outbreaks of disease, in turn, necessitate the use of prophylactic agents and chemotherapeutants. The recent outbreak of epizootic ulcerative syndrome is an example of this cycle of events.

Under the ADB/NACA/NABARD and Government of India-sponsored Regional Study on Aquaculture Sustainability and the Environment in 1995, a national farm-level survey was conducted in four states involving 1004 carp farmers covering 620 issues. The findings of the study corroborate the above views. The environmental problems encountered are indicated in Table 6.

Table 6. Occurrence of environmental problems encountered in carp-culture ponds surveyed (from FAO/NACA 1995).

Factor	Percentage of Occurrence	
	Extensive farms	Semi-intensive farms
I Water quality deterioration		
1. Dissolved oxygen	6	5
2. Plankton blooms	3	2
II Disease outbreak	4	9
III Total loss	8	5
IV Reduced harvest	78	62
V Market rejections	5	15
VI Reduced price	5	10

The losses were attributed to bacterial disease, EUS and other unknown causes. The treatments followed in the affected farms were quite varied; only 4-7% of farms did not attempt to give any treatment (Table 7).

Compilation of information on the effects on farmed species of the various chemicals used was outside the purview of the above study. However some related studies have been conducted by fisheries scientists in India.

Table 7. Use of chemotherapeutants in controlling diseases in carp farms in India (numbers are % values of farms surveyed) (from FAO/NACA 1995).

Chemotherapeutant	Extensive	Semi-intensive
None applied	7	4
Oxytetracycline	4	12
Chloramphenicol	2	1
Other antibiotics	0	3
Local herbs or medicines	7	0
Methylene blue	0	1
Copper sulfate	2	7
Trichlorofon	0	1
Sumithion	0	1
Potassium permanganate	35	45
Formalin	0	2
Medicated feed as prophylactic	0	1
Others	43	24

The negative effects of pesticides on fish are well known. Widespread use of pesticides, besides causing mass kills, may hamper growth and reproduction in fish, produce severe lesions in the vital organs, inhibit gut enzyme activity, affect hatching, and reduce feeding and respiratory rate. Extreme pH levels enhance the toxicity of organophosphorus pesticides (Konar *et al.* 1990).

There are reports of increased use of fertilizers altering the pH of water and soil, and the alkalinity of water. Application of muriate of potash (K_2O) at 20-80 kg/ha reduced the feeding rate, maturity index and fecundity of fish. High application rates of lime (2,250 kg/ha) in fish ponds reduced survival, growth, maturity index and fecundity of fish significantly (Konar *et al.* 1990).

Shrimp Farming

Semi-intensive and intensive shrimp farming systems are characterized by high inputs of fertilizers and supplementary feeds, and increased load of nutrients, organic matter and other wastes that can affect water quality in ponds and receiving waters. Feed is the most important input contributing to the waste, although fertilizers, chemicals, antibiotics, and drugs may also contribute.

The effects of waste materials can be seen both within and outside the pond. Within the pond, dissolved nutrients and organic solids stimulate growth of plankton, microbes and benthic organisms. A considerable amount of dead organisms and wastes accumulates at the pond bottom. This accumulation is associated with decreased redox potentials and the release of harmful gases including hydrogen sulphide and methane, causing stress and health risk.

The effects of these stressors on farmed shrimp were encountered during the national survey under the ADB/NACA-sponsored Regional Study on Aquaculture Sustainability and the Environment (Pathak and Palanisamy 1995). The survey included 966 shrimp farmers spread over four states in India. The results show that problems with water and soil affected 9-14% of the farms. Turbidity and filamentous algae were also important causes of problems. Plankton bloom was also reported to affect intensive farms (Table 8).

Table 8. Occurrence of water quality and soil problems in shrimp farms surveyed.

Nature of Problem		Occurrence (%)		
		Extensive	Semi-intensive	Intensive
1	Salinity	7	7	-
2	Temperature	2	2	-
3	Low dissolved oxygen	1	1	-
4	High turbidity	1	2	-
5	Plankton bloom	-	-	14
6	Filamentous algae	3	1	-
7	Soil problems	1	2	-
8	Others	-	1	-

Water and soil problems were reported to result in prawn disease. Shrimp disease was reported from 14% of extensive farms, 22% of semi-intensive farms, and 57% of intensive farms surveyed (Table 9).

Table 9. Disease problems observed in shrimp farms.

Particulars	Extensive	Semi-intensive	Intensive
Number of farms (%)	14.0	22.0	57.0
Frequency of occurrence (times/yr)	0.9	0.7	0.7
Total loss (%)	62.0	59.0	75.0
Reduction in harvest (%)	33.0	38.0	25.0

The causes of disease were unknown to farmers, thus no information was provided by the survey regarding the types of shrimp disease encountered. There were no reports of successful control measures; however, the treatments applied in attempts to control shrimp diseases are presented in Table 10. The great diversity of chemicals used by farmers and the limited success achieved reflect a need for proper extension service to educate the farmers regarding disease identification, prophylaxis and control.

Table 10. Treatments applied for shrimp disease control (values are % of farms surveyed).

	Extensive	Semi-intensive	Intensive
None attempted	25	5	33
Oxytetracycline	31	71	67
Chloramphenicol	0	5	0
Other antibiotics	13	2	0
Local herbs or medicines	7	0	0
Benzalkonium chloride (BKC)	13	2	0
Copper sulfate	1	2	0
Malachite green	0	5	0
Potassium permanganate	2	2	0
Formalin	6	2	0
Medicated feed as prophylactic	2	2	0

Impacts on Farm Workers and Consumers

Aside from reports of workers in shrimp hatcheries and farms suffering from over-exposure to bleaching powder or chlorine, incidences where toxic substances have affected farm workers or consumers, where residues have affected product quality or where pathogens resistant to chemotherapeutants have been encountered have not been reported in India.

Environmental Impacts

There are few environmental problems encountered in freshwater carp culture, as in these inland fish farms the pond-culture units are typically closed systems. However, release of large volumes of water containing nutrients, organic matter, and wastes at the time of fish harvest was reported to have resulted in eutrophication of receiving waters and algal blooms (Pathak and Palanisamy 1995). The concentration of ponds in areas with limited water resources aggravated the situation. Development of semi-intensive pond culture for Indian major carps around Kolleru Lake in Andhra Pradesh has resulted in heavy discharge of pond effluents during harvest. This has contributed to the deterioration of lake water quality, leading to increased disease outbreaks (FAO/NACA 1995).

There has been considerable debate on the environmental impacts that shrimp pond effluents may have on the receiving waters and the ecosystem. A comparison of shrimp farm effluents with other discharges has shown that their pollution potential is considerably less than that of domestic or industrial waste water. However, effluents produced during pond cleaning have greater pollution potential, although for a much shorter period. Although shrimp farm effluents are less noxious than many other coastal effluents, water pollution problems arise because of the large volumes discharged. Pollution also results when shrimp farms are concentrated in areas with limited water supply or with poor flushing capacity. In such situations, the shrimp farms themselves are most severely affected, resulting in “self-pollution.”

Although no detailed research on potential hazards due to the presence of chemicals and therapeutants in shrimp farm effluents has been done in India, studies have been conducted by the National Environment Engineering Research Institute, Nagpur (NEERI 1995) and the Central Institute of Brackishwater Aquaculture (CIBA 1995), in areas of heavy concentration of shrimp farms. The following hazards were identified:

- Contamination of drinking water source.
- Organic contamination of creek and nearshore sea water.
- Accumulation of heavy metals in creek sediments.
- Increase in bacterial and fungal count due to organic contamination of creek, discharge canal, and sea.
- Dominance of blue-green algae in receiving waters, indicating organic pollution.
- Change in soil characteristic in agricultural lands surrounding aquaculture farms.

Studies conducted by CIBA (1995) on the water quality of Kandaluru Creek in the Nellore District of Andhra Pradesh, the hub of aquaculture activity in India, in connection with the recent outbreak of shrimp disease, brought out certain interesting observations (Table 11). This work clearly indicates that the water quality parameters changed significantly as one progresses from the mouth of the creek (Station 1) to the upper reaches (Station 3). The highest level of deterioration in water quality was observed in the middle region (Station 2), where most of the semi-intensive shrimp farms are located. Even some four months after the disease outbreak, the water quality had not shown any perceptible improvement.

In April 1995, studies were also conducted by a team of 13 scientists who inspected the shrimp

farms situated in coastal areas of Andhra Pradesh, Tamil Nadu and the Union Territory of Pondicherry (NEERI 1995).

Table 11. Water quality parameters in Kandaluru Creek, Andhra Pradesh.

Parameters	Concentrations During Different Periods at Three Survey Stations Starting from Creek Mouth (S1) in the Creek to Field (S3)								
	Pre disease outbreak			During outbreak			Post disease outbreak		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
Total suspended solids (mg/L)	30	-	140	90	-	100	28	-	-
Chemical oxygen demand (mg/L)	7.5	103.2	29.4	11	120	90	12	-	180
Biochemical oxygen demand (mg/L)	3.8	76.6	12.6	3.5	60	14.5	4	52.3	92
Free ammonia (mg/L)	0.001	-	0.01	0.002	0.04	0.027	0.002	0.096	-

Impact on Well Water

The impact of shrimp farming on the quality of well water in a number of villages adjoining shrimp farms in both the states was studied. Findings from two such villages are presented in Table 12.

Table 12. Water quality characteristics of well water in two villages in Andhra Pradesh and Tamil Nadu.

Parameter ¹	Kurru Village (A.P.)		Kuchipalam Village (T.N.)	
	100 m from farm	500 m from farm	100 m from farm	500 m from farm
pH	8.0	7.9	7.9	7.8
Total alkalinity	290	275	310	260
Total hardness	276	250	260	235
Total solids	920	850	860	830
Chlorides	464	243	490	230
Ammonia-nitrogen	0.40	0.30	0.90	0.35

¹All values are expressed in mg/L except pH.

It is evident that the water quality of the wells closest to the shrimp farms (100 m from the farms) was poorer than that of the wells which were located further away (500 m from the farms). Similar observations were recorded with respect to other villages studied by the team.

Organic Contamination of Creeks

The results of the study show organic contamination of creek water (Table 13).

Table 13. Physico-chemical characteristics of water samples from Kandaleru Creek.

Creek Water Sample Source	Dis-solved Oxygen	pH	TDS	Concentration (mg/L)			NH ₃ Nitro-gen	Sul-fide	Total Nitrogen	BOD ₅
				Sus-pended Solids	Dis-solved Phosphates					
1 M/s Swamy farms	5.5	7	25000	70	0.08	0.1	ND ¹	3.5	16	
2 Star Marine	2.9	7.46	6000	1	0.02	0.82	ND	5.36	12	
3 Siris Aqua	5.6	7.2	26000	100	0.02	0.82	ND	3.2	15	
4 Far East Marine Products	5.7	7.5	28000	100	0.05	0.80	0.75	2.5	14	
5 VKVR Raju	4.6	7.43	19200	480	0.14	0.86	0.76	2.47	-	
6 Isnar Aqua	4.5	7.32	10000	470	0.12	0.56	ND	2.53	-	

¹ND = not determined.

Accumulation of Heavy Metals in Creek Sediments

The study showed the presence of some of heavy metals in the sediments of Kandaleru Creek adjoining various aquafarms (Table 14).

Table 14. Concentration of heavy metals in creek sediments in Andhra Pradesh.

Aquaculture Farm	Concentration of Heavy Metals (mg/gm)			
	Iron	Manganese	Copper	Zinc
1 VKVR Raju	50.22	0.243	0.038	0.086
2 Isnar Aqua	48.44	0.459	0.040	0.104
3 Ch.V.Surya Rao	62.23	0.111	0.085	0.012
4 Star Marine	58.39	1.007	0.084	0.114
5 K.Raghu	50.11	0.962	0.068	0.094
6 Choudhary Farm	58.32	0.640	0.089	0.104
7 Kalyan Sea Foods	50.23	0.366	0.049	0.074
8 Jagan Mohan Rao	ND ¹	0.224	0.024	0.067

¹ND = not determined.

Bacterial and Fungal Contamination

The microbiological studies show high bacterial and fungal counts that were attributed to the contamination of creek, sea, and canal by the heavy discharge of organic effluent in the receiving water (Table 15).

Table 15. Bacterial and fungal contamination in the creek, sea, and Buckingham Canal water at Kakinada, Visakhapatnam and Nellore in Andhra Pradesh.

	Sampling Station	Total Bacterial Count	Total Fungal Count
		CFU ¹ /mL Sample	CFU/mL Sample
1	Swami Aqua Farm	1800	30
2	Visaka Aqua Farm	1300	2
3	Siris Aqua Farm	2400	20
4	Far East Marine Products	3200	6
5	Magunta Aqua Farm	1400	4
6	Rank Aqua Farm	4500	10
7	Carewell Investment Pvt Ltd.	1900	15

¹CFU = colony-forming units.

Dominance of Blue-green Algae

A study on the phytoplankton composition of water samples showed dominance by blue-green algae (Cyanophyceae) which was attributed to organic pollution (Table 16).

Table 16. Phytoplankton population in creek water samples.

Sampling Station	Total	% Organisms in Group ¹			Shannon-Weaver Index
		Count/mL	1	2	
1 Ms Swamy Farm	15	42	58	-	1.2
2 Visaka Aqua Farm	18	72	28	-	1.4
3 Siris Aqua Ltd.	45	33	67	-	1.5
4 Far East Marine Products Ltd.	11	65	35	-	1.6

¹Group 1 = Bacillariophyceae, Group 2 = Cyanophyceae, Group 3 = Chlorophyceae.

Change in Soil Characteristics

The study of soil characteristics at different locations around shrimp farms in the states of Andhra Pradesh and Tamil Nadu reveals that there is usually an increase in soil pH and electrical conductivity on account of salinity intrusion in the vicinity of fish farms (Table 17).

Table 17. Soil characteristics at different locations around prawn farms in the states of Andhra Pradesh (A.P.) and Tamil Nadu (T.N.).

	Location	Parameter pH	Electrical Conductivity (mm/cm)
1	MS Swamy Farm (Visakhapatnam, A.P.)		
	100 m away	7.8	7.6
	500 m away	7.8	6.5
2	Rank Aqua Farm (Nellore, A.P.)		
	100 m away	8.0	7.6
	500 m away	7.4	6.0
3	Aqua Development India Ltd. (Prakasam, A.P.)		
	100 m away	8.0	8.2
	500 m away	8.2	6.3
4	Mayur Aqua Farm (South Arcot, Sirkhali, T.N.)		
	100 m away	8.5	8.1
	500 m away	7.3	6.2
5	Sriram Marine Harvests (Quaid-e-Milleth, T.N.)		
	100 m away	7.8	7.3
	500 m away	8.8	5.2
6.	Mac Aqua Farm (Chidambranar, T.N.)		
	100 m away	7.6	7.7
	500 m away	8.4	5.0

OTHER APPROACHES TO DISEASE PREVENTION

The approaches to disease prevention that have evolved in India after the outbreaks of shrimp disease in September 1994 and May 1995 are given below. The guidelines developed are exhaustive and only important aspects are described herein.

Crop Holiday

The widespread disease outbreak in Andhra Pradesh necessitated the declaration of a crop holiday. The idea behind the declaration of crop holiday was to allow a period long enough for the culture system and the water source with which it is associated to recover to a degree to permit successful shrimp farming. It was also envisaged that the farmers would dry their ponds thoroughly. This method is proving to be successful, as many farmers restarted farm operation without any problem after observing a crop holiday.

Adequate Pond Preparation

Removal of the accumulated black top soil, drying until cracks develop, plowing and reploting the ponds two or three times, and sun drying after each crop are now suggested to farmers as approaches to disease prevention. The importance of application of lime at the rate of 2 t/ha for

disinfecting the soil has been emphasized. Farmers are advised to keep the application of organic fertilizers to a minimum.

Regulating Stocking Density

The ADB/NACA/GOI study conducted in 1995 found that stocking density has a strong influence on the performance of shrimp culture farms (Pathak and Palanisamy 1995). The survey results show some clear differences in the environmental problems in farms related to stocking density, as summarized in Table 6. The study also showed that both profit and production increase with increasing stocking density. However, the shrimp farmers faced increased occurrence of disease, environmental problems and conflicts with higher stocking densities (Table 18). A significant increase in problems is noted when stocking densities exceed 20 PL/m². This information appears to strongly support the government's policy of not promoting intensive shrimp culture (Government of India 1995).

Table 18. Relationships between stocking density and key performance indicators of shrimp farms.

Stocking density (PL/m ²)	No. of farms surveyed	Average stocking density (PL/m ²)	Profit (\$/ha/yr)	Disease losses (\$/ha/yr)	Environment Index	Conflict Index	Production (kg/ha/yr)
<5	333	2.9	1343	275	0.20	0	717
5-10	221	6.1	2822	326	0.20	0	1367
10-20	76	12.2	4588	466	0.26	0.05	2464
20-30	22	22.8	7453	1099	0.45	0.41	5209
30+	13	39.8	12272	3214	0.30	0.50	6627

Effluent Treatment Systems

Before the outbreaks of disease, no shrimp farm had put up an effluent treatment system, which was a great omission. It has been estimated that nearly 60% of the feed given turns into biological waste. Therefore, one can imagine the extent of organic load that is being discharged with the effluents in a given area. Such effluent treatment systems have now been made an integral part of farming activity.

NATIONAL REGULATIONS ON THE USE OF CHEMICALS IN AQUACULTURE

At present, there are no regulations to control the use of chemicals and drugs in aquaculture, mainly because the use of chemicals in aquaculture is a recent phenomenon in India and the issue was non-existent a decade ago. Only after the outbreaks of disease in shrimp culture farms has discussion on the need to introduce regulations commenced. The Central Pollution Control Board and State Pollution Control Boards have certain regulations on effluents containing hazardous substances, but they are not specific to aquaculture.

Recently, the Ministry of Agriculture issued guidelines in the form of management practices and recommended parameters for water discharges from various aquaculture systems such as hatcheries, ponds, feed mills and processing plants. The parameters are on pH, suspended solids, dissolved oxygen, free ammonia, biological oxygen demand, chemical oxygen demand, dissolved phosphate and total nitrogen. However, the guideline developed by the Government of India stipulates that "chemicals use should be avoided in shrimp culture ponds for prevention or treatment of disease as

feed additives, disinfectants, for removal of unwanted fish, or for treatment of soil or water. However, chemicals may be used in hatcheries. Therefore, the entry of such chemicals into the natural waters from the hatcheries should be carefully monitored and steps should be taken to remove such materials from the waste waters.”

The guideline from the Government of India (GOI 1995) also restricts the indiscriminate use of fertilizers, as both the organic and inorganic fertilizers that are widely used in semi-intensive culture systems contribute to the nutrient load of receiving waters. Therefore, as far as possible, only manure and other plant products should be used for such purposes.

Regarding the use of piscicides, the guideline says that “Piscicides and molluscicides are widely used for removing predators and competitors from shrimp ponds. It would be advisable for aquaculture to use only biodegradable organic plant extracts for this purpose as they are less harmful than the chemical agents. Use of chemicals in culture systems should be avoided.”

On the use of chemotherapeutants, the guidelines of GOI state that “...formalin and malachite green, which are commonly used as disinfectants, are known to be toxic and may affect adversely the pond ecosystem and the external waters. Hence their usage in the culture system should be avoided.” Similarly on the use of antibiotics and drugs, GOI regulations state that “A number of antibiotics used in shrimp culture for preventing outbreak of diseases are harmful and incorrect usage may result in development of shrimp pathogens resistant to such drugs. The transfer of these pathogens into human beings might result in the development of resistance among human pathogens. Therefore, the use of antibiotics and drugs in the culture system should be avoided.”

The GOI guideline also stipulates that any farm of 40 ha and above should obtain consent from the State Pollution Control Board under Sec. 25/26 of the Water (Prevention & Control of Pollution) Act, 1974. Farms with 10 ha or less watered area shall obtain a No Objection Certificate of the State Pollution Control Boards.

The guidelines are for compliance by the aquaculturists; however, there is no enactment of the regulation as yet.

ON-GOING RESEARCH ON CHEMICAL USE FOR AQUACULTURE

Because the use of chemicals in aquaculture is a recent development in India, research to date in this area has been very limited. The research institutions of the Indian Council of Agricultural Research (ICAR), the fisheries colleges of the state agricultural universities, and some of the traditional universities undertake research in this field. The recent outbreaks of disease in shrimp culture have led to enhanced interest in research on this topic. The agencies involved in research on chemicals are given in Table 19.

CONCLUSIONS

Although information on the quantity of chemicals and therapeutants used in aquaculture is not available at present, the tremendous upsurge in their use in the last five years is primarily due to the phenomenal growth in shrimp grow-out culture and hatchery operations and to the expansion of the carp industry. Considering the stagnation in fish harvest from nature, the future fish requirements of the country can only be met from expansion of freshwater and coastal aquaculture, and the utilization of the seaward side of the country's coastline, which is presently untapped for mariculture. This definitely would call for higher usage of chemicals in aquaculture and would require careful environmental planning and prudent usage of chemicals and therapeutants. The country at present lacks a suitable policy for the manufacture, sale and use of such chemicals. It is, therefore, absolutely necessary at this stage to frame suitable policies and legislation and to create the infrastructure for

Table 19. List of agencies undertaking research on the use of chemotherapeutants in aquaculture.

Institution	On-going Research Programs
Central Institute of Brackish Water Aquaculture (ICAR)	Shrimp diseases, their prevention and control Diagnosis and control of finfish and shellfish diseases Database on shrimp diseases Development of diagnostic tests and prophylactic vaccines Impact of brackishwater aquaculture on the environment
Central Marine Fisheries Research Institute (ICAR)	Finfish and shellfish diseases Monitoring the fishery environment
Central Institute of Freshwater Aquaculture (ICAR)	Finfish and shellfish diseases and their prevention and control
Central Institute of Fisheries Technology (ICAR)	Bacteriological studies on fish affected by EUS
Central Inland Capture Fisheries Research Institute (ICAR)	Studies on EUS
College of Fisheries, University of Agricultural Sciences, Mangalore	Use of chemotherapeutic agents in aquaculture Microbiological studies on pathogens Effects of antibiotics on immune response
University of Madras, Department of Zoology	Studies on viruses, bacteria, fungi and protozoans from cultured species
Department of Fisheries Science, Andhra Pradesh Agricultural University	Parasitology Use of chemotherapeutic agents in fish culture
Cochin University of Science and Technology	Bacteriology
Andhra University	Pathology

In addition to the above, many traditional and agricultural universities undertake research on the use of chemicals in aquaculture.

their implementation and monitoring. The indiscriminate use of such chemicals can pose serious hazards to public health and aquatic life. The most serious problem at the moment is a lack of information on the use of chemicals by the aquaculturists in India and their appropriateness. Most veterinary grade chemicals and biocides are freely available to the aquaculture sector. Often, farmers are unaware of the nature of a disease and its etiology, and easily fall prey to errant advisers. This leads to indiscriminate use of hazardous chemicals, which may prove dangerous. Hence, it is necessary to create an awareness among aquaculturists of the potential hazards of indiscriminate drug use, and to provide them necessary assistance through timely diagnosis and prescription of appropriate drugs. In order to achieve this, it is incumbent on the development agencies to provide mobile diagnostic laboratories at the district level and man them with adequately trained personnel. The diagnostic laboratories, together with pollution control agencies in the states, may be empowered

to monitor the use of chemicals by the aquaculture industry, as well as to maintain information databases on the subject.

The need for further research to standardize prophylactic and control measures in aquaculture is real, since the industry is of recent origin. Research needs to be strengthened to develop safer and biodegradable chemicals for use in aquaculture. Extension services need to be strengthened to educate aquaculturists on the safe use of therapeutants and chemicals.

In order to provide adequate services and to carry out research in these areas, it may be necessary to evaluate our expertise in the field before steps are taken to strengthen our capabilities. It may be also be useful to share information with other countries in the region that have acquired expertise in the field. These steps may enable us to grow without endangering our aquatic habitat and human health.

RECOMMENDATIONS

The following recommendations are made in the larger interest of sustainable aquafarming.

For Farmers:

- Monitor closely various water quality parameters for proper farm management and early detection of adverse environmental factors.
- Avoid prophylactic use of chemicals in aquafarming, since most of them are ineffective and can lead to development of resistance among bacteria.
- Use only non-persistent chemicals.
- Strictly adhere to the “self-regulation and cluster approach” for maintaining an environmentally friendly aquaculture industry.

For Government and Aquaculture Institutions:

- Governments should frame suitable guidelines, rules, and regulations; and enforce them so as to reduce the hazards of chemical abuse.
- Disease diagnostic laboratories should be established in strategic areas to assist farmers in the identification, prevention and cure of diseases. Supply of drugs and chemicals should be effected with proper prescription from such laboratories.
- Education and training on the use of chemicals, their dosages and side effects should be imparted to the farmers and state government extension officers.
- Standards for aquaculture-grade chemicals and their use should be set and enforced.
- Regular monitoring should be undertaken to avoid overuse of chemicals in the environment. For this purpose, separate environmental monitoring agencies should be created.

For the Chemical Industry:

- Information on the efficacy, potency and effects of chemical products should be widely circulated and written on product labels.
- Separate medicines and preventive drugs suitable for the aquaculture industry should be prepared and supplied with specific directives regarding their use.

For Regional and International Agencies:

- Regional and international organizations should play a more active role in guiding and sharing information for environmental planning of aquaculture facilities and in assisting interested countries in the developmental planning of their respective aquaculture industries.
- International agencies should also assist member countries in framing drug regulations based on the latest available information.

- International collaborative research programs should be organized in areas of mutual interest.

For Research Institutions:

Future research should be concentrated in the following areas:

- Surveys to identify major fish health problems and to evaluate the procedures for chemotherapy presently being followed.
- Studies on the residual effects of antibiotics and piscicides in fish tissues and to establish environmentally safe levels of disposal.
- Identification and evaluation of less toxic and biodegradable compounds for use in aquaculture.
- Formulation of guidelines for environmentally sound aquaculture practices which can help prevent the indiscriminate use of chemicals.

REFERENCES

- Anon. 1988. Norwegian aquaculture: controlling the antibiotic explosion. Animal Pharmacy, P.J.B. Publication Ltd., Surrey.
- Choo PS. 1994. Degradation of oxytetracycline hydrochloride in fresh and seawater. *Asian Fish. Sci.* 7:195-200.
- CIBA. 1995. Shrimp diseases, their prevention and control. CIBA Bull. No. 3. Central Institute of Brackish Water Aquaculture, Madras. 17 p.
- CIFRI. 1985. Carp culture - package of practices for increasing production. Aquaculture Extension Manual, New Series No. 2. Central Inland Fisheries Research Institute, Barrackpore, West Bengal. 30 p.
- FAO/NACA. 1995. Regional Study and Workshop on the Environmental Assessment and Management of Aquaculture Development (TCP/RAS/2253). NACA Environment and Aquaculture Development Series No. 1. Network of Aquaculture Centres in Asia-Pacific, Bangkok.
- Konar SK, Ghosh TK, Dey M. 1990. Hazards of aquatic pollution, its complexity and abatement measures. In: Proceedings of the Second Indian Fisheries Forum held on May 27-31, 1990 in Mangalore, India. p. 195-198.
- Government of India (GOI). 1993. Handbook on Fisheries Statistics. Ministry of Agriculture, Fisheries Division, Government of India, New Delhi.
- Government of India (GOI). 1995. Guidelines for Sustainable Development of Brackishwater Aquaculture. Ministry of Agriculture, Fisheries Division, Government of India, New Delhi. 14 p.
- NEERI. 1995. Impacts of aquaculture farming, and remedial measures in ecologically fragile coastal areas in the states of Andhra Pradesh and Tamil Nadu. Investigation Report for the Supreme Court of India, National Environmental Engineering Research Institute, Nagpur, India. 84 p.
- Pathak SC, Palanisamy K. 1995. Shrimp and carp aquaculture and the environment in India. Report on a Regional Study and Workshop on Aquaculture Sustainability and the Environment. ADB RETA 5534, Network of Aquaculture Centres in Asia-Pacific, Bangkok.
- Rao KG, Mohan CV, Seenappa D. 1992. The use of chemotherapeutic agents in fish culture in India. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 505-513. Fish Health Section, Asian Fisheries Society, Manila.
- Sarkar SK. 1983. Influence of cow dung and mustard oil cake on the effectiveness of fertilizers in fish production. *Environ. Ecol.* 1:30-40.

The Use of Chemicals in Aquaculture in Indonesia

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ABSTRACT

Aquaculture systems in Indonesia have developed toward intensive culture. As a result of intensification of fish culture, increased outbreaks of disease have occurred. Various chemotherapeutic agents like antibiotics and other chemicals have been widely used for treatment and prevention of infectious diseases in fish and shrimp farms. Antibiotics such as oxytetracycline, chloramphenicol, neomycin, streptomycin, erythromycin, prefuran and enrofloxacin are used in the treatment of bacterial diseases. Other chemicals such as malachite green oxalate, potassium permanganate, formalin, methylene blue, chlorine and teaseed have been used for the treatment of various diseases. Organic fertilizers, such as chicken manure, and inorganic fertilizers like urea and trisodium phosphate are often applied by shrimp farmers to improve primary productivity in ponds. Bacterial products with trade names like "Multi bacter," "Enviro star" and "Super NB" have recently been used by shrimp farmers to decompose organic matter resulting from excessive feeding. Feed additives such as vitamin C, "Protec Plus," and "Super Embak" are used for disease prevention.

INTRODUCTION

Fish is the primary source of animal protein in Indonesia. The biggest source of fish in the market is marine and freshwater capture fisheries, while aquaculture accounted for 12.7% of the total fish production in 1985 (Rahardjo 1987).

Aquaculture systems have developed toward intensive culture. However, intensification of fish culture has resulted in increased outbreaks of fish disease. Outbreaks of disease caused great damage to carp production in late 1980 in West Java, where a total of 1,250 t of carp, with an estimated value of US\$ two million, was lost (Djajadiredja *et al.* 1983, Dana 1987). Important bacterial pathogens in freshwater fish culture include *Aeromonas hydrophila*, *Pseudomonas* spp. and *Flexibacter columnaris* (Supriyadi and Rukyani 1992).

Indonesia is one of the principal shrimp producers in the world, together with Thailand, Ecuador, India and The People's Republic of China (Rosenberry 1993, Born *et al.* 1994). Until the early 1980s, shrimp were grown in extensive pond systems, but in 1984 intensive culture was introduced to East Java. Since then the production of shrimp in East Java increased. However, from 1992 onwards the production per hectare has decreased dramatically. Figures presented by Rosenberry (1991, 1993) indicate a drop in harvest from 140,000 mt in 1991 to 80,000 mt in 1993 for the whole of Indonesia. Reasons for this decline in shrimp production remain unclear, although it is suspected that both infectious and non-infectious diseases may play a major role.

The primary constraint to successful fish farming in Indonesia is poor water management, as evidenced by increased disease occurrence. Infectious diseases and other health problems are often the result of environmental disfunction. Various species of *Vibrio* are implicated in vibriosis in shrimp pond-culture and in shrimp hatcheries. Similarly, these pathogens are causing disease in cultured marine fish, especially in cages (Supriyadi and Rukyani 1992).

Some antibiotics and chemicals have been successfully used to treat fish diseases. Antibiotics became very important and were frequently used for the treatment of bacterial diseases, especially in shrimp hatcheries. Similarly, formalin has been used to eradicate protozoan and crustacean fish parasites.

This paper presents the status of chemical usage in fish and shrimp culture in Indonesia and is largely based upon information collected from freshwater fish culture and shrimp culture facilities on the islands of Java and Sumatra.

THE USE OF CHEMICALS IN AQUACULTURE

Advantages of Chemical Use

Health problems have become very common in fish and shrimp culture, especially in intensive systems. Protozoan and crustacean ectoparasites, as well as bacterial diseases caused by *Aeromonas hydrophila* and *Vibrio* spp. account for significant mortality in freshwater fish culture and brackishwater shrimp culture. *Vibrio harveyi* has been reported to cause mass mortalities in both grow-out ponds and hatchery facilities of black tiger shrimp in West, Central and East Java. To overcome these problems, various chemotherapeutic agents including antibiotics and other chemicals have been widely used for treatment and prevention of infectious diseases in fish and shrimp farms. The application of chemicals by immersion and the addition of antibiotics to fish feed are the methods that are normally practiced by the farmers.

Antibiotics and Other Chemotherapeutants Used in Indonesia

The antibiotics that are usually applied to treat bacterial fish and shrimp diseases are mostly derived from human medicine, poultry science, and other branches of animal medicine. There are no antibiotics developed specifically for the purpose of treating bacterial disease of fish. Many antibiotics can be bought from local suppliers, drug stores and poultry shops. Table 1 lists some antibiotics and their usage. Oxytetracycline or terramycin is used widely for treatment of bacterial fish and shrimp diseases. Chloramphenicol, erythromycin, streptomycin, prefuran and neomycin are also used in the treatment of bacterial disease in shrimp and ornamental fish. Enrofloxacin (a derivative of quinolone), has recently been used as an antibacterial agent in food and ornamental fish culture.

Table 1. List of antibiotics and their usage in aquaculture in Indonesia.

Name of Antibiotic	Route of Administration	Dose
Oxytetracycline	Bath	5-10 ppm
	Oral	50 mg/kg/d, 7-10 d
Chloramphenicol	Immersion	5 ppm
Erythromycin	Bath	4 ppm
Streptomycin	Long bath	4 ppm
Prefuran	Immersion	1 ppm
Enrofloxacin	Bath	5-10 ppm
Neomycin	Bath	4 ppm

Antibiotic application has resulted in some negative effects. The emergence of drug-resistant bacteria has made treatment with some antibiotics difficult and ineffective. The detection of antibiotic residues in exported fish products coming from Indonesian farms has resulted in rejection by the Japanese market.

Other chemotherapeutants being used are listed in Table 2. Formalin, malachite green oxalate, potassium permanganate, methylene blue, chlorine and teaseed preparations have been used to treat various diseases and to eliminate unwanted fish that act as competitors in the ponds. These chemicals are available from chemical companies and in agricultural chemical stores or poultry shops.

Table 2. List of chemicals used in aquaculture in Indonesia.

Name of Chemical	Route of Administration	Dose
Formalin	Immersion	25 ppm
Malachite green oxalate	Immersion	0.15 ppm
Potassium permanganate	Immersion	40 ppm
Methylene blue	Immersion	1-2 ppm
Chlorine	Immersion	60 ppm
Teaseed	Long bath	10 ppm
Brestan	Long bath	0.5-1.0 ppm
Thiodan	Long bath	10 cc/ha
Fertilizers		
Chicken manure	Spreading	500 kg/ha
Urea	Spreading	50-70 kg/ha
Trisodium phosphate	Spreading	35 kg/ha
Organic Matter Decomposers		
Multi bacter	Spreading	no data
Enviro star	Spreading	no data
Super NB	Spreading	no data
Feed Additives		
C-vitamin	Oral	150-200 mg/kg
Protec plus	Oral	3-5 gm/kg feed/d
Super embak	Oral	2-4 gm/kg feed/d

Formalin and malachite green oxalate are widely used for the control of fish and shrimp diseases caused by fungi and protozoan and crustacean parasites. Potassium permanganate is used as a disinfectant. Methylene blue is used as treatment against protozoans and as a fungicide. Teaseed preparations are frequently used during pond preparation to eradicate fish species which act as competitors during culture.

Organic fertilizers such as chicken manure, and inorganic fertilizers like urea and trisodium phosphate are often applied by shrimp farmers to enhance primary productivity in ponds. Organic matter decomposers such as bacterial products with trade names like "Multi bacter," "Enviro star" and "Super NB" have recently become available in Indonesia and are used by shrimp farmers. These bacterial products are said to contain *Bacillus*, *Pseudomonas* sp., *Nitrobacter* sp., *Nitrosomonas* sp. and *Acinetobacter* sp. Feed additives such as vitamin C, "Protec Plus" and "Super Embak" are also used in shrimp culture to enhance the nutritional value of artificial feed pellets.

Government regulations made by the Directorate General of Fisheries suggest that a withdrawal period of at least two weeks be observed prior to the harvest of treated fish. These regulations also state that treated water should not be disposed into water bodies that are normally used for human activity.

FARM MANAGEMENT PRACTICES

Antibiotics and chemicals have been applied in farms for treatment and prevention of fish diseases. Oxytetracycline or terramycin (Pfizer) is used widely for treatment of bacterial diseases of food and aquarium fishes. The antibiotics are applied either as 5-10 ppm baths for 24 h, or incorporated into feeds at 50 mg/kg body weight /d, given continuously for 7-10 d.

Chloramphenicol at 5 ppm is also used as bath treatment for bacterial diseases of shrimp and ornamental fish. Application of erythromycin at 4 ppm by bath is practiced by farmers to control bacterial disease of shrimp. Streptomycin at 4 ppm is also used, especially in shrimp hatcheries as a long bath treatment. Application of 1 ppm pefuran is practiced to control bacterial diseases in shrimp hatcheries and freshwater ornamental fish culture. Enrofloxacin at a dose of 5-10 ppm was recently used as an antibacterial agent in fish and ornamental fish culture. Neomycin at a dose of 4 ppm is used to treat bacterial disease in shrimp hatcheries.

Formalin at 25 ppm is widely used for the control of fish and shrimp diseases caused by protozoan and crustacean parasites. Formalin at 25 ppm combined with malachite green oxalate at 0.15 ppm is normally used for the treatment of white spot (*Ichthyophthirius multifiliis*) on fish. The treatment is usually repeated three times at 3-d intervals.

Malachite green oxalate at 0.15 ppm is still being used to treat mycosis in fish and shrimp hatcheries. Potassium permanganate at 40 ppm is used as a disinfectant, but at 20 ppm, it is used as a long bath treatment against bacterial disease. Methylene blue at a dose of 1-2 ppm is used as an indefinite bath against protozoans and fungi.

Chlorine at 60 ppm is used to disinfect hatchery paraphernalia and to decontaminate shrimp hatchery facilities.

During the preparation of grow-out shrimp ponds, teaseed at 10 ppm is frequently used to control competitors. The molluscicides Brestan 50 EC, applied at 0.5-1.0 ppm, and Thiodan, applied at 10 cc/ha (at a water level of 5-10 cm) have also been used during pond preparation to eradicate snails considered as pests in black tiger shrimp ponds. However, both pesticides are now banned by the government for use in grow-out ponds.

Application of lime is done by shrimp farmers to condition the soil and to eradicate pathogens. Lime is also applied in freshwater fish farms for the same reasons. Liquid "Primadin" has also been used by some shrimp farmers as a disinfectant (4.0-6.0 ppm) or for disease prevention (0.5-1.0 ppm). Rivanol is a disinfectant being used in shrimp hatcheries.

ALTERNATIVE DISEASE PREVENTION METHODS

The government and fish farmers have realized that the use of chemotherapeutic agents in aquaculture sometimes leads to environmental deterioration and contributes to the development of drug-resistant strains of bacteria. Alternative disease prevention methods have been directed toward appropriate farm management methods, such as the control of water quality. Water quality improvement by using organic matter-decomposing bacteria has been practiced, especially by shrimp farmers in Java and South Sumatra. Biofilters have also been used, but their usage is confined to freshwater fish hatcheries and ornamental fish culture systems.

Disease problems are usually associated with intensive culture systems. The higher the stocking density, the more often disease outbreaks occur. To minimize disease occurrence, the farmers, especially those on Java, have tried to reduce stocking density. This approach was largely based on the carrying capacity of the pond.

Vaccination was recently introduced to prevent bacterial diseases. *Vibrio* vaccine used for preventing vibriosis in marine and coastal aquaculture, and *Aeromonas* vaccine used against *A. hydrophila* in freshwater fish culture have been developed.

The use of vitamin C at 150-500 mg/kg feed was initiated by a government-owned shrimp pond in East Java with encouraging results.

NATIONAL REGULATIONS ON THE USE OF CHEMICALS IN AQUACULTURE

There is no government agency that regulates the use of chemicals in aquaculture in Indonesia. However, regulations have been formulated by the Committee on Drugs for Animal Husbandry, under the Directorate General of Animal Husbandry. The fisheries sector is represented on this committee by fisheries scientists, particularly those from the Research Institute for Freshwater Fisheries (RIFF). The committee was formed through the Ministry of Agriculture's Decree No. 476/Kpts/OP/7/1978, which was updated by No. 300/Kpts/OP/5/1982, concerning the environmental management and environmental impact analysis regulated by Government Act No. 4, 1982 and Government Regulation No. 29, 1986.

Pesticide usage, storage and distribution are regulated by Presidential Decree No. 7, 1980. The Pesticide Commission regulates and screens pesticides that will be distributed and marketed for agricultural purposes. A fishery sector representative to this committee also comes from RIFF.

Aquaculture feeds, as well as feed additives like growth promoters, hormones, and probiotics, are governed by Ministry of Industry Decree No. 37, 1992.

ON-GOING RESEARCH ON CHEMICAL USE FOR AQUACULTURE

As was mentioned above, scientists from RIFF sit as members of the Pesticide Committee. RIFF routinely conducts research on the toxicity of pesticides that will be marketed and used in rice paddy fields. Research on the acute and chronic toxicity of the pesticides endosulphan, chlorpiriphos and chlorfluozuron against fish, shrimp and zooplankton is also being conducted. The persistence and accumulation of these pesticides in fish, shrimp and zooplankton, and their role as immunosuppressors in fish are also being studied.

The level of the antibiotics enrofloxacin, chloramphenicol and oxytetracycline in fish blood after immersion in different concentration has been studied. Research on the use of immunostimulatory substances (glucans) in *Clarias batrachus* has also been conducted.

CONCLUSIONS

Chemicals such as antibiotics are important chemotherapeutic agents; however, their indiscriminate use will lead to environmental problems such as the development of drug-resistant strains of microorganisms. The formulation of appropriate regulations and guidelines on the use of chemotherapeutic agents is needed. The benefits and risks of chemical use in aquaculture need to be intensively studied.

The improvement of water quality, and the use of immunostimulants, vaccines, and high quality fish feeds are the best approaches to disease prevention. The use of biological filters and the application of organic matter-decomposing bacteria are advisable for the improvement of water quality.

REFERENCES

- Born AF, Verdegem MCJ, Huismana EA. 1994. Macroeconomic factors influencing world aquaculture production. *Aquacult. Fish. Manag.* 25:519-536.
- Dana D. 1987. Current fish health problems in Indonesia. In: Arthur JR. ed. *Fish Quarantine and Fish Diseases in South and Southeast Asia: 1986 Update*. p. 9-11. Asian Fish. Soc. Spec. Publ. No. 1.
- Djajadiredja R, Panjaitan TH, Rukyani A, Saroni A, Satyani D, Supriyadi H. 1983. Country report. Indonesia. In: Davy FB, Chouinard A. eds. *Fish Quarantine and Fish Diseases in Southeast Asia. Report of a Workshop held in Jakarta, Indonesia, 7-10 December 1982*. p. 19-30. Intern. Develop. Res. Centre Publ. IDRC-210e, Ottawa.
- Rahardjo K. 1987. *Fisheries Statistics of Indonesia (Statistik Perikanan Indonesia)*. Direktorat Jenderal Perikanan, Departemen Pertanian Jakarta. 136 p. (in Bahasa Indonesia).
- Rosenberry B. 1991. *World Shrimp Farming 1991*. San Diego, CA.
- Rosenberry B. 1993. *World Shrimp Farming 1993*. San Diego, CA.
- Supriyadi H, Rukyani A. 1992. The use of chemotherapeutic agents for the treatment of bacterial diseases of fish and shrimp in Indonesia. In: Shariff M, Subsinghe RP, Arthur JR. eds. *Diseases in Asian Aquaculture*. p. 515-517. Fish Health Section, Asian Fisheries Society, Manila.

Government Regulations Concerning the Use of Chemicals in Aquaculture in Japan

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ABSTRACT

In Japan, fisheries research activity is of a very diversified nature and is overseen by the national and prefectural governments. Regarding the use of chemicals in aquaculture, various regulations exist to protect the safety of cultured aquatic animals intended for human consumption. Under Japan's Drug Laws, certain materials are designated as "medical products" for use in humans and animals, and their usage is strictly regulated. This paper introduces aspects of this legislation as relevant to the aquaculture industry and discusses how they are actually applied on the level of operation. Prefectural fish disease centers and extension services engage in the actual supervision of the use of such designated chemicals. In reference to government research structure, the Ministry of Agriculture, Forestry and Fisheries maintains 29 national research institutes, nine of which are fisheries institutes directly under the Fisheries Agency. The prevention and treatment of fish disease is an important research theme, and programs are being implemented, especially at the National Research Institute of Aquaculture. An auxiliary organ of the Fisheries Agency, the Japan Fisheries Resource Conservation Association operates educational and training programs for employees of prefectural centers and extension services whereby individuals receive certification as fish health specialists.

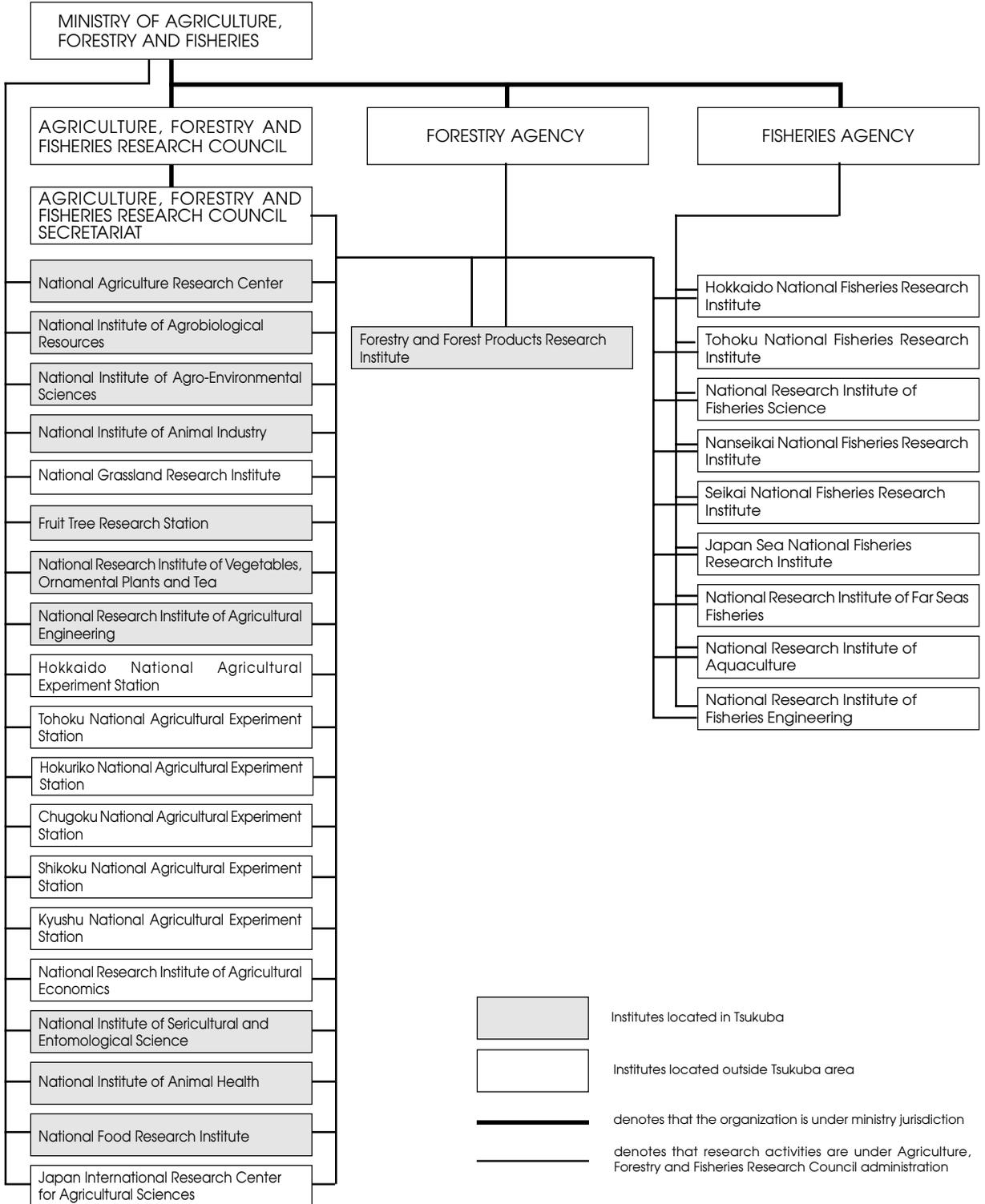
STRUCTURE OF GOVERNMENT-RELATED FISHERIES RESEARCH ACTIVITY IN JAPAN

Fisheries research in Japan is very active and wide-ranging and is carried out principally by national and prefectural government institutions, auxiliary organs, universities and the private sector. This paper will focus on government regulations concerning the use of chemicals in aquaculture in Japan with some discussion about research carried out in this context. The purpose of this section is to introduce the structure and relationships of the government and related agencies that promote and oversee fisheries and aquacultural operations and activities in Japan.

The Government of Japan comprises a number of ministries and agencies; of relevance to this discussion are the Ministry of Agriculture, Forestry and Fisheries (MAFF) and the Ministry of Health and Welfare (MHW) (which will be discussed in relation to Japan's food and drug laws). The Fisheries Agency (FA) is under the jurisdiction of the MAFF and maintains nine research institutes, such as the National Research Institute of Aquaculture (NRIA). The institute to which this author belongs, the Japan International Research Center for Agricultural Sciences (JIRCAS) contains a fisheries division but is not primarily a fisheries institute and is therefore under the direct jurisdiction of the ministry. Including the nine fisheries institutes and JIRCAS, the ministry has a total of 29 research institutes (Fig. 1).

Figure 1. General organization of the research institutes of the Ministry of Agriculture, Forestry and Fisheries

Twenty-nine research organizations are affiliated to the Ministry of Agriculture, Forestry and Fisheries: 19 are directly overseen by the AFFRC; 1 forestry and 9 fisheries institutes are under the administration of the Forestry Agency and the Fisheries Agency, respectively.



Generally, the role of these national research institutes is to conduct basic research on their respective fields in tune with the needs of the industry, consumers and agriculturists. In fisheries, dissemination of the thus-developed technologies and related extension services is the realm of auxiliary organs and prefectural institutions. Japan has three major auxiliary organs related to fisheries which carry out their operations in cooperation with the FA. These are the Japan Sea-Farming Association (JASFA), the Japan Fisheries Resource Conservation Association (JFRCA), and the Overseas Fishery Cooperation Foundation (OFCF). The scope of their activities is broad. It includes the management of coastal stocking programs and related research (JASFA), the management of fisheries resources in conjunction with the preservation of the environment (JFRCA), and the promotion of fisheries collaboration between Japan and other coastal countries (OFCF). The activities of the JFRCA as they pertain to the operation of programs related to the control of fish disease and the management of chemical usage in fisheries activities (JFRCA 1995a) will be discussed in detail later. Finally, most prefectures in Japan maintain one or more prefectural fisheries centers. Activities vary with location and needs of the region, as aquaculture species differ according to climate and habitat. However, at most centers, extension and advisory services are available for the local aquaculturists.

OVERVIEW OF JAPAN'S DRUG LAWS

Japan's Drug Laws serve to regulate the use of drugs and medical products intended for human beings or designated animal species which are the objects of human consumption. The Drug Laws contain numerous clauses specifying how products are to be tested and approved, advertised, packaged, utilized and so forth, and under what circumstances they can be prescribed. This legislation is administered by the MHW, but certain clauses contain reference to the MAFF in the case of specifications concerning the use of products or medicines in animals (Noushi 1993). Below is a brief description of the laws.

Medical products are defined as items which, although in themselves may be daily necessities, are products which constitute a group apart from ordinary necessities, in that they have an enormously large impact on the nation's public health, necessitating their regulation and proper usage. Medical products are divided into two groups, those designated for use in humans and those designated for use in animals. The latter group is further divided into items to be used in livestock administration, and those used in fisheries applications. Regarding the use of medical products in animals, legislation is geared so as to guarantee the safety for human consumption of the end product (such as eggs, beef, or cultured fish). The development of the Drug Laws has a long historical background starting from the Meiji Era in 1870. In 1979, major revisions were made to the laws including, for the first time, the inclusion of provisions relating to the use of medical products in animals.

The Drug Laws define which items are medical products (not only drugs, but also equipment and items such as syringes) and are therefore subject to regulation by them. A more detailed explanation is beyond the scope of this paper; however, it will suffice to say that a specific article, Clause 83, states that other clauses and phrases containing the words "Health and Welfare Minister" are interchangeable with "Agriculture, Forestry and Fisheries Minister" and "Ministry of Health and Welfare Promulgation" with "Ministry of Agriculture, Forestry, and Fisheries Promulgation" in interpretation of cases relating to animal-intended products (Noushi 1993). Thus, the same laws are overseen by different ministries depending on whether the product is intended for direct usage in humans or for application to livestock and aquatic species, but the overall aim is the same - to protect public welfare.

APPLICATIONS OF THE DRUG LAWS TO AQUACULTURE

The Fisheries Agency puts out a pamphlet to guide aquaculturists and fish farmers concerning the use of chemicals in aquaculture (Fisheries Agency 1995). This provides a very clear picture of how

drug use is permitted in aquaculture in Japan. Most of the following explanations are based on the contents of this pamphlet.

Medical products for fisheries use are for the treatment and prevention of disease in cultured species, the ultimate objective being to produce a product suitable for human consumption. In accordance with the Food Hygiene Law, foodstuffs may not contain traces of antibiotics or synthetic anti-bacterial agents; however, the majority of fisheries-use drugs contain such materials. Thus, regulations are in place to prevent the occurrence of residues in the final product. In Japan, it is necessary for all aquaculturists to be aware of these regulations.

Table 1 shows all of the fisheries drugs in use in Japan and for which species they may be used. An "O" symbol denotes that the item may be used in a particular species. This implies that the drug has been judged from experimental data that it is effective in treating disease in that species, and that it does not have any deleterious side-effects. It is also known what length of time is required for its clearance from the target animals; i.e., does it leave a residue for a long time or not? On the other hand, items marked with an "X" for a certain species may not be used in that species because their efficacies, side-effects and clearance times are not established.

The heavy frame in Table 1 which encloses species from yellowtail to kuruma prawn, and drugs from amoxicillin to lincomycin hydrochloride indicates "medical products" whose usage is subject to the Drug Laws. This means that the animals in which they are used, the method of administration and quantity, and the time from which administration is terminated to when the product is harvested and out on the market (withdrawal period) are strictly regulated. Failure to comply with the Drug Laws carries a term of imprisonment for up to one year, or a fine of up to 500,000 yen or a combination thereof (Fisheries Agency 1995). Drugs that fall under these restrictions are clearly labeled on their containers or wrapping with a warning that usage is subject to the regulations of the Drug Laws. Items that fall outside of the frame are outside of the regulations of the Drug Laws themselves. This does not mean that they are not regulated. If these items are not used according to the guidelines given, the final product will likely be unfit for the market under the Food Hygiene Law; and, therefore, administrative agencies must supply guidance concerning their use (JFRCA 1992).

For each of the various species targeted for aquaculture, the manner of administration, dosage level and withdrawal period is specified for each drug which can be used in that species (Fisheries Agency 1995). Table 2 presents this information representatively for yellowtail, which has the longest history of aquaculture in Japan; rainbow trout, which is also very popular; and the kuruma prawn. In most instances, the allowed method of administration is oral, by mixing the drug with the feed, although sulfamonomethoxine is given via immersion in rainbow trout. Such information is also given in detail for red seabream, coho salmon, horse mackerel, flounder, carp, eel, sweetfish, tilapia, amago salmon and crucian carp.

GUIDANCE ACTIVITIES AND DISSEMINATION OF INFORMATION

As mentioned above, the actual dissemination of information concerning drug use in aquaculture and guidance in the treatment of fish disease is dispensed by prefectural organizations. Most prefectures maintain their own research stations as well as extension service offices, with guidance and consultation services available to local aquaculturists. Here, fish health specialists advise fish farmers on the proper usage of aquaculture chemicals in conformance with the Drug Laws, give diagnoses and make recommendations. The employees of these prefectural stations and extension centers have a very strong responsibility to understand the Drug Laws, and to supervise aquaculturists on the proper use of these chemicals to ensure a safe product to the consumer and an overall healthy aquaculture industry.

Table 2. Specifics of drug usage in yellowtail, rainbow trout and kuruma prawn**Yellowtail**

Drug	Administration	Dosage	Withdrawal period
amoxicillin	oral	40 mg/kg d	5 d
bicozamycin benzoate	oral	10 mg/kg d	27 d
ampicillin	oral	20 mg/kg d	5 d
erythromycin	oral	50 mg/kg d	30 d
alkyltrimethylammonium oxytetracycline calcium	oral	50 mg/kg d	20 d
oxytetracycline hydrochloride	oral	50 mg/kg d	20 d
oxolinic acid	oral	30 mg/kg d	16 d
oxolinic acid (suspension-forming)	oral	20 mg/kg d	16 d
kitasamycin	oral	80 mg/kg d	20 d
josamycin	oral	50 mg/kg d	20 d
spiramycin	oral	40 mg/kg d	30 d
sulfamonomethoxine (or sodium salt of)	oral	200 mg/kg d	15 d
thiamphenicol	oral	50 mg/kg d	15 d
doxycycline hydrochloride	oral	50 mg/kg d	20 d
sodium nifurstyrenate	oral	50 mg/kg d	2 d
novobiocin salt	oral	50 mg/kg d	15 d
flumequine	oral	20 mg/kg d	8 d
florfenicol	oral	10 mg/kg d	5 d
lincomycin hydrochloride	oral	40 mg/kg d	10 d
sulfisozole	oral	200 mg/kg d	10 d
oleandomycin polystyrenesulfonate	oral	25 mg/kg d	30 d
phosphomycin calcium	oral	40 mg/kg d	15 d

Rainbow Trout

Drug	Administration	Dosage	Withdrawal period
oxytetracycline hydrochloride	oral	50 mg/kg d	30 d
oxolinic acid	oral	20 mg/kg d	21 d
sulfadimethoxine (or sodium salt of)	oral	100 mg/kg d	30 d
sulfamonomethoxine (or sodium salt of)	oral	150 mg/kg d	30 d
sulfamonomethoxine (for immersion bath)	immersion	10 kg/1 t saline	15 d
florfenicol	oral	10 mg/kg d	14 d
sulfisozole	oral	200 mg/kg d	15 d

Kuruma Prawn

Drug	Administration	Dosage	Withdrawal period
oxytetracycline hydrochloride	oral	50 mg/kg d	25 d
oxolinic acid	oral	50 mg/kg d	30 d

Heavy frame indicates items regulated by the Drug Laws

To accomplish these aims, JFRCA maintains several programs, including a training and certification course for fish health specialists. The program was originally initiated by the FA to provide certification to prefectural specialists. In 1977, this work was commissioned to the JFRCA. As of 1994, 425 prefectural researchers have been awarded certified status and are taking an active part in disease control management in Japan.

The JFRCA maintains the “Fish Disease Center” on its Tokyo premises where training courses and research pertaining to fish disease are carried out. Of note is JFRCA’s “specialist certification course.” After completion of this course, and provided that the applicant has at least two years of field experience, a prefectural employee can sit for the certification examination and upon passing, receive the credentials of a fish health specialist (JFRCA 1995b). Other courses are for those already possessing credentials but wishing to receive advanced training in specific areas or to undergo “re-training” to keep abreast of the latest developments. Referring back to the specialist certification course, the course is composed of three segments taught in consecutive years. The first year, various topics such as general disease theory, viral diseases, fungal diseases, and other diseases are taught by university professors. The second year, affiliated topics such as fish physiology and immunology are taught, and practical work in all areas of fish disease is conducted. In the third and final year, applicants study not only scientific areas such as pharmacology, rearing methods, and fish disease diagnostics, but also the specifics of legislation in aquaculture, learning the details of the Drug Laws and the Food Hygiene Law. At present, this system bestows a certification, not a license, as in the case of veterinary certification. Acquiring certification entails rigorous preparation, and at present, the system is serving its purpose in helping specialists to manage fish disease in this country.

STATUS OF DISEASE RESEARCH IN JAPAN

In Japan, numerous areas relating to aquatic animal disease are being investigated; research is of both pure and applied nature. Most basic research is conducted at the national institutes and universities. The National Research Institute of Aquaculture maintains a Fish Pathology Division that is composed of the following sections: Pathogen Section, Pathophysiology Section, Pharmacology Section, and Immunology Section (NRIA 1992). Basic studies are being conducted on:

- the physiology and ecology of viruses, bacteria, and parasites in the context of the establishment of disease prevention measures;
- physiology and immunology of aquatic animals leading to vaccine development and other means of health management; and
- drug metabolism to determine potential utility for disease treatment.

More information can be obtained through the headquarters of the Institute (National Research Institute of Aquaculture, Nansai, Mie 516-01, Japan).

The JFRCA has an in-house applied research program covering prevention, diagnostics, and therapies as pertains to fish disease and, in addition, carries out re-evaluation of fisheries medical products and residue analysis.

CONCLUSIONS

Japan has a well-developed legislation for the regulation of chemicals used in aquaculture that is designed to guarantee safety of the final product to the consumer. Through auxiliary organs of the government’s Fisheries Agency, prefectural fisheries experts undergo constant training in order to

supervise and guide the activities of local aquaculturists and fish farmers. Thus, Japan's system is organized from the national level down to the local level with the basic research and law-making activities of the national government being through advisory and extension services at the prefectural level.

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REFERENCES

- Fisheries Agency. 1995. A word to aquaculturists: the use of fisheries medical products in aquaculture, No. 11. (pamphlet published by the Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries). (in Japanese).
- JFRCA. 1992. Diseases of the kuruma prawn. Momoyama K, Hatai Y, Sato H, Yani I, Maeda M, Nomoto T, Okamoto A, Sakaguchi S. eds. Japan Fisheries Resource Conservation Association. 50 p. (in Japanese).
- JFRCA. 1995a. Report and outline of the 1994 Fish Health Specialist Training Courses. Japan Fisheries Resource Conservation Association. 35 p. (in Japanese).
- JFRCA. 1995b. Overview and prospectus. (pamphlet published by the Japan Fisheries Resource Conservation Association). (in Japanese).
- NRIA. 1992. Overview and prospectus. (pamphlet published by the National Research Institute of Aquaculture).
- Noushi K. 1993. The Drug Laws. Kojin Shuppan and Buffalo Press, Saitama, Japan. 42 p. (in Japanese).

The Use of Chemicals in Aquaculture in Malaysia and Singapore

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ABSTRACT

Aquaculture is an increasingly important force in both the Malaysian as well as the Singaporean economies. In recent years, Singapore has focused on the aquarium fish trade, making it one of the largest ornamental fish production and transshipment centers in the world. Similarly, the Malaysian aquaculture industry has made rapid strides in the last few years and is poised to become a major contributor to the national fish supply by the early part of the next century. A significant trend in both countries has been the growing intensification of culture systems to achieve higher production per unit area. This has led to a greater occurrence of disease, particularly among aquarium fish, shrimp and marine fish farms. To obviate and control these diseases, there has been a concurrent increase in the use of chemotherapeutants. The three major groups of commonly used chemotherapeutants are: topical disinfectants, antimicrobials and probiotics. There is a wide range of topical disinfectants used by aquafarmers. The most common of these include lime, teaseed cake, formalin, benzalkonium chloride, acriflavine, malachite green, hypochlorite and poly-vinyl pyrrolidone. Of these, lime and teaseed cake are used exclusively in ponds, and acriflavine and malachite green only in hatcheries, while the others are used in both systems. Antimicrobials being

used include sulfonamides, tetracyclines, nitrofurans, chloramphenicol, oxolinic acid and virginiamycin. A number of other chemotherapeutants are also used, albeit on a limited basis. The current concerns surrounding the use of chemotherapeutants and the legislative framework surrounding their sale and distribution are also discussed.

INTRODUCTION

The Malaysian and Singaporean aquaculture industries are of relatively recent antecedents, the first recorded attempt being the culture of Chinese carps in mining pools at the early part of the century (Tan 1998). However, by the mid-1990s, the industry in both countries had begun to acquire a strong economic profile, producing both food and ornamental fish. In 1994, total aquaculture production in Malaysia amounted to 114,114 mt of food fish valued at US\$145.8 million and 227.8 million aquarium fish valued at \$17.5 million (Department of Fisheries 1994). In the same year, the Singaporean aquaculture industry produced 2,821 mt of food fish and aquarium fish valued at \$10.7 million and \$54.5 million, respectively.

The growth of the aquaculture industry in both countries has been spurred by government support in the form of research and development, and extension and support services, as well as financial incentives. Under these circumstances, it is likely that production will continue to increase in the future. While this increase will no doubt come from an expansion of the area under culture, most of it will probably be from intensification of existing culture sites. Given the increasing cost of land and labor in both countries, it is likely that future development in the industry will gravitate toward a more comprehensive use of land and water resources, and the employment of sophisticated production technology to maximize unit output and reduce labor requirements. The implemented intensification has some consequences. In particular, the employment of high-density culture increases the chances of epizootic infections. Fish diseases, once considered only of academic importance, have acquired significant recognition in recent years, and widespread epizootic infections have been a serious obstacle in the development of aquaculture, particularly shrimp and marine fish farming. Diseases in Malaysian aquaculture have been reported as far back as the mid-1980s (Anderson 1988). Shariff and Subasinghe (1993) identified a number of parasitic, bacterial, viral and fungal pathogens common in the Malaysian aquaculture industry. Losses through disease are considered significant, although hard data are lacking. Recent disease outbreaks in Kedah are thought to have cost the industry up to \$10 million (Shariff 1995). In Singapore, diseases such as sleepy grouper disease and viral nervous necrosis in groupers have affected valuable stocks, causing significant losses in recent years (Chua *et al.* 1993).

Fish disease is usually mediated by the presence of the pathogen and unfavorable environmental conditions that predispose the animal to infection. In aquaculture, most disease problems arise out of poor sanitation and water quality. While the more obvious strategy would be to improve sanitation as a first step, the acute mortalities that characterize many epizootics do not allow a window where such sanitation measures can take effect immediately. In addition, many farmers tend to look for a "quick fix solution" for the disease problems that face them. Both these factors have encouraged the use of chemotherapeutants in disease management.

The term chemotherapeutant covers any medication, drug or chemical that is used in preventing or combating disease and which can be used either for prophylaxis or for treatment. This definition also includes those chemicals used to improve the rearing environment. However, it does not include nutritional supplements such as vitamins and immune enhancers, although these have, no doubt, an important role to play in disease management.

A wide range of chemicals and drugs is used in the aquaculture industry worldwide. Few, if any, have been specifically developed for the aquaculture industry *per se*. Most are holdovers from human and veterinary medicine or wastewater treatment. There has been little work on the toxicity,

pharmacology and pharmacokinetics of drugs and chemicals applied in fish and invertebrate culture systems. Most studies that have been undertaken were based upon temperate conditions and species. Thus, much of chemotherapeutant use is based on ease of availability and on guess-work, rather than on a systematic protocol involving identifying pathogens and prescribing the appropriate treatment.

Chemotherapeutants can be divided into four major groups, i.e., topical disinfectants, antimicrobials, probiotics and anesthetics. Topical disinfectants cover a wide range and are used mainly to eliminate external opportunistic bacteria, fungi and protozoans. Antimicrobials are basically antibacterial drugs. Although there is a great variety of such drugs, the focus has been generally on antibiotics and sulphonamides. The third group is composed of probiotics or bacterial concentrates that are used to enhance microbial degradation of organic accretions in the pond, thus reducing the biochemical oxygen demand and the possibility of anaerobiosis. The fourth group, anesthetics, includes substances that are used to sedate fish during transport and handling. The widespread use of these chemotherapeutants without control can lead to serious problems.

This paper outlines the status of use of the major chemotherapeutants used in the Malaysian and Singaporean aquaculture industries and suggests means to enhance their effectiveness.

TYPES OF CHEMOTHERAPEUTANTS USED

The main chemotherapeutants used in the local aquaculture industry are as follows:

Topical Disinfectants

Lime

Liming is considered an integral part of pond management and is undertaken for a number of reasons. These include improvement of soil chemistry like reducing soil acidity, increasing total alkalinity, neutralizing sulfides and acids, precipitating suspended or soluble organic material, decreasing biochemical oxygen demand, improving fine textured bottom soils in the presence of organic material, and improving nitrification (Kungvankij and Chua 1986). Lime is also used as a piscicide and for disinfection (Apud 1984).

There are several types of lime that are used in grow-out ponds. The most common is ground magnesium limestone (GML), which is a mix of calcium and magnesium carbonate. The extensive use of GML is related to its availability. It is used widely in the agriculture sector for soil improvement and can be found in any agriculture supply shop. However, its calcium carbonate fraction is not soluble in sea water, and is not an effective buffer (King 1973). Calcium oxide is also used, although not as frequently as GML. This is because it is caustic and must be handled with some care. Limestone products and derivatives are widely manufactured and sold within the country, although some are imported from Thailand. To increase the pH of water, 100-300 kg/ha is applied in shrimp ponds during the culture period, whereas 2-5 mt/ha is used for pond preparation. The price of lime ranges from \$40-65/mt.

Saponin

Saponin has been traditionally used by aquafarmers as a piscicide to kill predator fishes prior to stocking. It is particularly favored by shrimp farmers because it is toxic to fish but not to crustaceans. It is also used to facilitate molting in shrimp. The main source of saponin is teaseed cake, the residue from oil processing of the seeds of *Camellia* sp. that contain 10-15% saponin. As there is no teaseed cake production locally, all teaseed products are imported from China or Myanmar. However, saponin concentrates containing 100% active ingredient imported from China are now

available and are increasingly replacing teaseed cake.

Saponin is also effective against a number of pathogens. Saponin (20-30 ppm) has been recommended for the treatment of blackspot disease in shrimp. Saponin can also be used to control algal fouling. Teaseed cake at 3.5-5 kg/1000 m² is also promoted as a treatment for tail rot and is used at 5-25 ppm to treat protozoan infections (Baticados and Paclibare 1992). Locally, it is applied at 2-3 ppm for 24 h to stimulate molting in shrimp. The same application also helps prevent and get rid of protozoan and other fouling organisms on the shrimp body. The concentrated form is sold at \$6/kg, whereas teaseed cake is available at \$0.40/kg.

Formalin

Formalin has a very old history as an aquatic chemotherapeutant. The first recorded use of formalin in the treatment of fish disease was in 1909 (Alderman and Michel 1992). Formalin kills microorganisms by condensing with amino acids to form azomethines. It is active against a wide range of organisms, including fungi, bacteria and ectoparasites (Herwig 1979). However, its action is slow. At a concentration of 5,000 ppm, 6-12 h is required to kill bacteria and 2-4 d to kill spores. At 80,000 ppm (8% solution), it requires 18 h to kill spores (Harvey 1975). It is also ineffective against internal infections. Formalin has been approved by the US FDA for use in treatment of food fish.

Locally, formalin is used both in hatcheries and in ponds. In the hatchery, formalin is used for prophylaxis as well as treatment. Gravid broodstock are disinfected with formalin to prevent vertical transmission of disease. The rate used varies from 100-200 ppm for 1-2 min, although the recommended rate for this purpose is 25 ppm for 10-15 min (Platon 1978). With respect to treatment, formalin is used mainly for ciliate protozoan infestations. In addition, it is also being used to control necrotic shell and gill diseases of shrimp. The recommended dosage rates are 150 ppm for a 1 h bath and 25ppm for long-term treatment.

Formalin is also applied directly in ponds at rates varying from 10-25 ppm, especially during disease outbreaks, as a cure-all remedy. However, the effectiveness of formalin is uncertain. Formalin binds with organic matter and a large excess must be applied to overcome depletion (Harvey 1975). However, formalin also causes oxygen depletion (Plumb 1992) and this excess can be deleterious in the long run. Even if effective, the action of formalin would be too slow to be of any significant use.

On the other hand, the farmer runs the risk of destroying beneficial bacteria such as the nitrifying bacteria during formalin treatment. At the concentrations at which formalin is an effective and a rapidly acting germicide, it causes alteration of tissue proteins, causing local toxicities and promoting reactions such as eczematoid dermatitis. The chemical is also noxious to humans and can induce nausea if used for prolonged periods. Additionally, there appears some contemporary information that formalin may have some carcinogenic properties. Handling of formalin by the farmer, therefore, must be undertaken with care.

As a chemotherapeutant, formalin is inexpensive. The current cost of laboratory-grade formalin is about \$3/L. However, industrial grade formalin is available at \$16.00/25 kg/drum. Both grades are manufactured locally.

Benzaklonium Chloride

Benzaklonium chloride (BKC) is a cationic compound that, like formalin, is toxic to a wide range of bacteria, fungi, and viruses. Unlike formalin, however, it is non-irritating to tissue and has a rapid onset of action. It does not, however, kill spores. Herwig (1979) recommends its use at 2

ppm for treating bacterial, protozoan and monogenean infections. BKC has also been recommended as a bactericide and fungicide in shrimp hatcheries. Suggested dosages are 1-1.25 ppm to treat vibriosis, 0.1 ppm for 16 h for protozoa and mysis, 0.2 ppm for 24 h for early stages of postlarvae (PL), and 0.94 ppm for 24 h for PL18. (Tonguthai and Chanratchakool 1992).

Despite, its superiority over formalin, BKC is not commonly used in local hatcheries. However, an application rate of 0.8 ppm of commercial BKC (50% active ingredient) for 1-2 d is used to treat infections in shrimp ponds. The cost of the product, which is imported from Britain, is about \$4/kg. Like the use of formalin, the impact of BKC on pond conditions is uncertain. The reduction of pathogenic organisms has to be balanced by the concurrent loss of beneficial microflora that play a vital part in maintaining the integrity of the pond ecosystem. Most of the BKC available locally comes from Thailand, Taiwan or Korea.

Acriflavine

Acriflavine is a mixture of 3,6-diamino-10-methylacridinium chloride and 3,6-diaminoacridine. Known also as Trypaflavine, it has been used extensively in egg disinfection; as an antiseptic for treating wounds, ulcers, and bacterial lesions; and in protozoan and monogenean infections. Acriflavine is normally used as a long-term bath and is known to kill plants. It may also create vulnerability to bacterial disease in marine fish.

In the local context, acriflavine is used to a limited extent in shrimp and aquarium fish hatcheries as a broad-based prophylactic agent and therapeutant. The main impediment to its widespread use is cost. Although widely available, good quality acriflavine imported from Thailand costs about \$240/kg. At an application rate of about 10 ppm, this would amount to a cost of about 26 cents/100 L of water. There appears to be little information to indicate the efficacy of acriflavine on shrimp, although its use on fish has been well established (Herwig 1979). Further research into the pharmacology and pharmacokinetics of the chemical is required.

Malachite Green

Malachite green is the common name for p,p-benzylidenebis-N,N-dimethyl aniline. It was originally developed in the 1920s as a textile dye. In its original form, it contained zinc, which is toxic to fish. However, later variants came in a zinc-free form, making it more applicable as a therapeutant. Malachite green has been extensively used in controlling infections due to bacteria, fungi, protozoans and monogenetic trematodes on eggs, fry and adult fish (Herwig 1979). It is used by itself, or in combination with salt or dimethyl sulphoxide (DMSO). Next to formalin, it is, perhaps, one of the most important therapeutants in the aquaculture industry.

In recent years, however, there have been strong moves against malachite green application, especially with respect to its use in food fish. This is because the chemical has a moiety that is known to be carcinogenic. While there has been no evidence actually linking malachite green with any carcinoma, the US FDA has banned its use in food fish. Studies have also indicated that malachite green may have very long withdrawal times. Meinertz *et al.* (1995) established that residues of malachite green could be found in fry some 30 d after eggs were disinfected.

Locally, malachite green is commonly used in fish and shrimp hatcheries for the treatment of fungal and ciliated protozoan diseases. It is also extensively employed in the aquarium trade for the same purpose. It is either dissolved directly in the rearing water or combined with formalin. The cost is about \$48/kg for the dry powder, although liquid concentrates are commonly sold. Most of the malachite green used in Malaysia and Singapore is imported from Taiwan; a significant amount also comes from Germany. It is usually applied at 1-2 ppm for short exposure, and for long baths at 0.01 ppm for shrimp postlarvae and 0.1 ppm for juveniles.

Hypochlorite

Both sodium and calcium hypochlorites have a long tradition of use in the local aquaculture industry. Both have been used in hatchery as well as pond situations. Hypochlorites act by releasing hypochlorous acid, which is the primary active ingredient. Hypochlorites are potent germicidal agents. They are particularly effective in acidic conditions. For example, the bactericidal effect of hypochlorite is 10 times greater at pH 6 than at pH 9. At pH 7.0, a 0.1-0.25 ppm hypochlorite solution will kill most organisms within 15 to 20 sec. However, some acid-fast pathogens, particularly *Mycobacterium*, require concentrations 500 times higher.

Hypochlorites are too toxic to be used directly on tissues and therefore cannot be used for treatment or prophylaxis. Both products, however, are used extensively as disinfectants. In hatcheries, hypochlorites are used to disinfect tanks and equipment. They are commonly employed during the break cycle, when the entire hatchery system, including water and aeration pipes, is subject to thorough disinfection.

In ponds, hypochlorites have been traditionally used as piscicides (Apud 1984). However, a more recent use has been their application to disinfect incoming water. Water is pumped into reservoir ponds and then treated with hypochlorite (mainly calcium hypochlorite) to reduce bacterial load. This approach was particularly prevalent among farmers in the Sungai Merbok area in Kedah over the last two years to prevent their ponds from being affected by high levels of *Vibrio* in the river. Limsuwan (1993) also reported that calcium hypochlorite was being used directly on the pond bottom by Thai farmers during fallow periods to prevent yellowhead disease. Locally, farmers use 20-30 ppm of hypochlorite for pond disinfection. A dosage 0.08 ppm is used for prophylaxis in ponds with shrimp. There is no equivalent application where freshwater ponds are concerned. Hypochlorites are easily available and inexpensive. The cost of sodium hypochlorite (10%) is \$20 for a 25 kg drum. Calcium hypochlorite, which is sold in a powder form containing 65% free chlorine, costs \$120 for a 50 kg drum. Hypochlorites are manufactured locally or imported from China.

Polyvinyl Pyrrolidone Iodide

Polyvinyl pyrrolidone iodide (PVPI) is an iodophor compound sold under the name Betadine or Ovadine. An iodophor compound is basically a complex of iodine with a solubilizing agent or carrier that liberates free iodine in solution. Iodine, like chlorine, is a halogen and has a strong oxidizing capability. It is lethal to microflora and to viruses, which are killed within 15 min in a 50 ppm solution (Harvey 1975).

Like chlorine, PVPI can be used only for disinfection and is too toxic for treatment or prophylactic purposes. However, it has a much lower toxicity as compared to chlorophors (such as hypochlorite) and thus can be used to treat eggs of shrimp and fish. In addition, PVPI is not inactivated by organic matter to the extent that chlorine is. Even a solution as dilute as 10% PVPI will exert adequate bactericidal action in the presence of organic matter.

PVPI has been found to be effective against viruses on salmonid eggs (Amend and Pietsch 1972), against bacteria on trout eggs (McFadden 1969) and against fungi (Ross and Smith 1972). Shrimp eggs and nauplii can also be disinfected by immersing them in 100 ppm PVPI for 1 min or at 400 ppm for 30 sec. The eggs or nauplii are then immersed in a 1 ppm PVPI solution for 1 min before being rinsed and returned to the incubation jar.

PVPI use in local circumstances appears to be limited due to its high cost and limited availability. PVPI use in egg disinfection was only introduced in 1995, while its use as a hatchery disinfectant is still not widespread. On the other hand, PVPI is being used in ponds, especially in combatting

viral infections. Although hard data are lacking, anecdotal feedback on its use indicates that it is efficacious. PVPI is sold in 10% solution at a cost of \$7.2/L. PVPI is generally imported from Korea and the United States.

Zeolite

Zeolite is often used by fish and shrimp farmers to improve pond bottom condition. This is related to its capacity to absorb ammonia and metabolites from water. However, the efficacy of zeolite in this respect diminishes with increasing hardness. Zeolite has been found to be almost totally ineffective in absorbing ammonia when immersed in sea water. Under these circumstances, the value of using zeolite in shrimp ponds is highly questionable. Further research is needed to establish if zeolite is cost effective in shrimp ponds. Zeolite is produced locally and is also imported from Indonesia, Japan and Thailand. It is sold at \$160-400/mt. It is usually applied in shrimp ponds at 300-500 kg/ha.

Copper Sulphate

Copper sulphate is a broad-based disinfecting agent used in some shrimp and aquarium fish farms. It is effective against a wide range of organisms including blue-green algae, bacteria, fungi, protozoans, digeneans, leeches and monogeneans (Herwig 1979). Overdosing can easily kill the animals being treated, and thus caution is called for in its use. In the context of the local industry, copper sulphate is used at a rate of 0.5 ppm in freshwater aquaria and at 0.5-1 ppm in marine shrimp ponds. It is imported from China and sold at \$2.5/kg.

Organophosphates

Organophosphate pesticides are used in both freshwater fish ponds and marine shrimp hatcheries to control infections by crustaceans, and monogeneans and ciliates, respectively. The main organophosphates used are Malathion (O,O-dimethyl s-(1,2 biscalbetoxyethyl) phosphorothioate), Dipterex (also sold as Masoten) (O,O-dimethyl-2,2,2, trichloro-1-hydroxyethyl phosphate), Dichlorvos (O,O-dimethyl O-(2,2-dichlorovinyl) phosphate), and Dursban (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate). All three are effective against crustacean parasites (especially *Lernaea* and *Argulus*), and protozoans (*Ichthyophthirius*, *Trichodina*), and are used as broad-spectrum anthelmintics to control monogeneans. In Singapore, Demerin (chlorophenyl difluorobenzoyl) has been recently introduced as a treatment for *Lernaea*.

In many freshwater fish farms, these organophosphates are also used to control aquatic insects that prey upon fish fry, such as dragonfly larvae. Dosage is usually about 0.5-1 ppm administered daily for 3 to 7 d. In shrimp farms, organophosphates are used mainly to eliminate crustaceans that are vectors of whitespot virus in the rearing water. The water is treated in reservoirs at the rate of 0.5 ppm for 2 d before being channeled for use. Organophosphates are imported from Europe and sold at \$18/kg.

Antimicrobials

There is a wide variety of antimicrobials used for treating fish and shrimp diseases. In practice, however, legal restrictions prevent the use of many of them on a large-scale basis. Almost all the antimicrobials in use are generic imports from China and Thailand. Commercial preparations (non-generics) from Japan, Europe, and North America generally come with adequate labelling containing information on composition and some precaution about their application. The main antimicrobials being used are:

Sulfonamides

Sulfonamids are a group of antimicrobial agents that act by blocking the folic acid pathways of susceptible microorganisms. Sulfonamides today are popularly promoted by regulatory authorities world wide, mainly because of their rapidly declining use in human medicine (Brander and Pugh 1977). There are many sulfonamides, such as sulfamethazine, sulphamerazine and sulphathiazole. There are also potentiated sulfonamides such as Romet-30 and Tribessen, both of which are approved for use in food fish by the US FDA. Locally, the main sulphonamides used are Daimethoprim or Tribessen (which is a sulphonomide-potentiated trimethoprim) and sulphamonomethoxine and sulfadimethoxine, both of which are sold under the trade name Dimeton and cost about \$18 for a 45 gm pack.

Dimeton is used in hatcheries to control bacterial infections including *Vibrio* sp. It is, however, not a preferred antimicrobial because of its limited efficacy and high cost. Dimeton is also used in some parts of the country to treat fin rot in seabass and grouper reared in floating cages. One half gm is added to each kg of trash fish. Shrimp hatcheries use it at 20 ppm. Sulfonamides tend to induce resistance in target pathogens and thus cannot be used for any length of time. Potentiated sulfonamides are generally more effective and less amenable to inducing resistance. There are two commercially available potentiated sulfonamides on the market: Romet-30, which is sulphadimethoxine potentiated with ormetoprim, and Tribessen, a sulphadiazine potentiated with trimethoprim. The original drugs are too expensive for general use. Generic sulphadimethoxine and trimethoprim can be acquired separately at reasonable prices but must be blended by the farmers themselves. Other sulfonamides have not been extensively used in the local aquaculture industry.

Tetracyclines

Tetracyclines belong to a group of broad-spectrum antibiotics whose action is mainly bacteriostatic by causing interference with bacterial protein synthesis (Alderman and Michel 1992). They are effective against a wide range of Gram-negative and Gram-positive bacteria. The most widely used tetracycline is oxytetracycline. Developed by Pfizer at the end of World War II, it is the only antibiotic allowed for food fish use in Canada and the United States, although all use of the antibiotic must cease 21 d before fish are to be harvested for human consumption.

Locally, the use of oxytetracycline in the livestock industry, particularly swine and poultry farming, is very common. It is quite natural that aquaculture would take its cue from this situation. The drug may be administered through feeds or simply dissolved in water and absorbed through the gills. Oral administration calls for the drug to be combined with feed at a rate of 3-5 gm/kg. Dosage in a dip or bath situation is about 20 ppm. The drug is available through selected stores licensed under the Poisons Act, 1984 to retail veterinary pharmaceuticals. The cost is about \$32/kg.

The efficacy of oxytetracycline in brackishwater ponds is very limited. Oxytetracycline binds with the calcium and magnesium in sea water to form a complex that is unable to penetrate the gill lining. As much as 95% of the efficacy of the drug is inactivated this way in sea water. Oxytetracycline administered through the feed also meets a similar fate in the lumen of the shrimp's gut.

Nitrofurans

Nitrofurans, such as furoxone, nitrofurazone (Furazolidone) and nifurpirinol (Furanace) were thought to hold promise for aquaculture at one time. Furanace has since been totally banned for its carcinogenic potential, while the use of the other two has been severely limited. In Malaysia, all three are still available for use by the aquaculture industry, although they are not extensively used.

Administration is through the feed at a rate of 3-5 gm/kg. The drug is sold at licensed shops at about \$36-40/kg.

Chloramphenicol

This is another widely used antibiotic that has a broad-spectrum action against a wide range of Gram-positive and Gram-negative bacteria. Although in common use in the aquaculture industry world wide, its use has been banned in almost all developed countries because of its continued use and efficacy in human medicine. In addition, chloramphenicol can, even at very low dosages, cause irreversible aplastic anemia in humans, thus posing a risk for workers handling the products. Nonetheless, it is still available in both Singapore and Malaysia, and can be purchased from licensed dealers at about \$60/kg. It is usually administered through feed at 3-5 gm/kg.

Oxolinic Acid

This is a first generation quinoline antibiotic that is used only under exceptional circumstances. The main reason surrounding its lack of use is availability and cost. The cost of the product is \$200/kg, making it substantially more expensive than other antibiotics currently available on the market. The antibiotic is administered through feed at 3-5 gm/kg.

Virginiamycin

Virginiamycin is a peptolide antibiotic that is widely used in animal feeds as a growth promoter. It has no application in human health. The use of Virginiamycin has been shown to enhance fish growth (Padmasothy 1987), and it has been incorporated in some fish feeds.

Dimetridazole/Metronidazole

Dimetridazole and Metronidazole are potent protozoacides that are used for the treatment of internal protozoan infections in fish. Their use is largely restricted to the aquarium trade, where they are primarily employed for treatment of *Hexamita* infections, particularly in commercially valuable species such as discus. The drugs are administered through feed at a dosage of 0.15% for 3 d.

Probiotics

Probiotics are basically bacterial concentrates employed for the bioremediation of fish and shrimp ponds. They were originally developed for waste water treatment, and have since been promoted as a effective method of reducing organic accretions in aquaculture ponds. There are over 10 brands of probiotic available locally. All are imported from Australia, Europe, Japan, Thailand, and the United States. The prices range from \$20-100/kg. However, there has been little work to establish if these products really work as claimed. In addition, it is important to establish whether or not the introduction of the exotic bacteria that these products contain is deleterious to the new environment. Clearly, further research is required.

Anesthetics

Anesthetics are mainly used during handling and transport of fish to market. A significant proportion of aquaculture output in Malaysia and Singapore is sold live. Food fish and shrimp are consigned mainly to the restaurant trade, where they command prime prices (Gopinath 1990). The aquarium industry is totally reliant on live transport to move product to market. The main anesthetic used in live transport is methyl quinoline, which is sold commercially in 30 mL squeeze bottles for about \$2.50 each. Recommended dosage is at the rate of 1.0 mL of the anesthetic to 13.5 L of water,

although in practice the farmers administer it until the fish are sedated. A less commonly used anesthetic is tricaine methanosulfonate (TMS), also known as Fiquel or MS-222. Considerably more expensive at \$480/kg, TMS is used only to a limited extent. Application rates are in the range of 40-60 ppm.

Other Chemotherapeutants

A large number of other chemotherapeutants are also employed, albeit on a limited basis. These include antimicrobials such as erythromycin phosphate, Neomycin and Terramycin. Neomycin is increasingly being used in the aquarium trade as a prophylactic (10 ppm) in packing water. This is because it is colorless and thus avoids problems with regulatory authorities in importing countries, most of which actively discourage the use of antibiotics. Since analyses of packing water for antibiotics can be complicated, most of these authorities look for the tell tale yellow coloration that normally accompanies the use of antibiotics such as chloramphenicol and oxytetracycline. Other chemotherapeutants used include sodium nifurstyrenate and disinfecting chemicals, such as potassium permanganate, chloramines, chlorine dioxide and quaternary ammonium compounds. Their use, however, is limited compared with the others listed above.

FARM MANAGEMENT AND USE OF CHEMOTHERAPEUTANTS

Prophylaxis

The most common measures taken by Malaysian and Singaporean aquaculturists to obviate diseases relate to good management practices and effective environmental management. These include keeping stocking densities within prudent limits and ensuring optimal water exchange.

The most common means of chemical prophylaxis is liming. Pond liming is carried out during the pond preparation phase that precedes the beginning of a culture cycle, immediately after drying. Lime is broadcast by hand, and more often than not, it is not evenly distributed on the pond bottom. Lime is allowed to sit for varying periods of time ranging from to 1 d to 3 wk before the ponds are filled for stocking. Liming may also be carried out during the production phase, although this practice is infrequent.

Another prophylactic measure that is gaining popularity, particularly in shrimp farming, is the application of hypochlorites (chlorination) during pond preparation or immediately after harvest. This practice, however, is not common in freshwater pond culture. Many aquarium fish farms routinely disinfect their tanks with hypochlorite, formalin or PVPI prior to their use. Prophylactic treatment of aquarium fish with antiseptics and antibiotics prior to introduction into farms or during the packing process for export is also common.

Some farmers, especially those engaged in shrimp farming, administer antibiotics added to feed as a prophylactic measure. However, the relatively high cost of the drugs limits this practice to only a few farms. The use of immune enhancers such as glucans and Encap is more common. These are administered by top dressing them on the feed.

Therapeutic Measures

Therapeutic measures include administration of therapeutants in feed as well as in dips or baths. In Malaysia, both methods are common: the former is regularly used in the farming of food fish, while the latter is confined to the aquarium trade. In Singapore, administration of drugs through feed is less common. The exception to this is the use of Metronidazole-treated feed for aquarium fish.

Measures to Improve Productivity

Most farms use organic or inorganic fertilizers to produce and sustain natural productivity. There is a decided shift in the freshwater sector away from relying entirely on fertilizers to engender production, due to the potential for producing off flavors in the cultured animals. There is now a greater reliance on formulated feeds. Aquarium fish also do not rely extensively on fertilizer-generated food. For the most part, aquarium fish are reared in small ponds or tanks. Formulated feeds are the norm, with some measure of supplementation of natural food through fertilization. The same holds true for marine shrimp farming. Marine cage culture is almost totally reliant on supplied feeds.

Hazards, Impacts and National Regulations

In general, the use of chemotherapeutants has not had a negative impact on farm productivity. Occasional accidental overdose has been reported, but losses in such cases are usually limited and confined to individual farms. In 1992, a batch of Nitrofurazone from China was found to be toxic after several marine farms reported acute losses after treatment. Bioassay showed irreversible gill damage from short 10 ppm bath treatments. No further incidents were reported.

Adverse impacts of aquaculture chemicals on farm workers and consumers are also seldom encountered in Singapore. Most food-fish farmers exercise some degree of self-regulation with regard to antibiotic administration. This is because using antibiotics, particularly during the grow-out stage, is expensive in terms of both drug cost and labor. Officers from the Primary Production Department (PPD), Singapore, provide advice on withdrawal periods for food fish. Antibiotic residue surveillance is also conducted by the PPD.

In Singapore, chemicals used in aquaculture have not been implicated as causing negative environmental effects. Farms, especially those in the agrotechnology parks, have to abide by strict regulations on effluent quality set out by the Ministry of the Environment or risk being penalized. Effluents from land-based farms go through sewage treatment facilities before discharge.

In Singapore, aquaculture chemicals are regulated under the Poisons Act, which is administered by the Ministry of Health. All importers of chemicals are required to be licensed under this Act. The PPD is consulted before any new products are allowed into the local market. Only practicing veterinarians and licensed farmers may obtain drugs and chemicals from suppliers. Records of sales are monitored by the Ministry of Health.

The impact of chemotherapeutant use in Malaysia is unclear. There are, at present, no regulations or government agencies concerned with the use of chemotherapeutants in aquaculture. The Poisons Act, 1984 requires all drugs to be registered with the Drug Control Authority. Trade and distribution are also controlled by the Pharmaceutical Division of the Ministry of Health. As these regulations cover only the use of human pharmaceuticals, veterinary drugs do not require registration by the DCA and their movement and trade are thus not controlled.

The Poisons Act comes into the picture only when the drug in question has a human pharmaceutical use, i.e., in the case of antibiotics. Even then, the emphasis is more on its storage and sale rather than its use. The Animal Act, 1952 does have provisions to control the use of antibiotics in animal farming, but its provisions are rarely applied. In addition, those provisions do not apply for fish. The Fisheries Act (Amended 1993) does not have any regulations for controlling the use of drugs in the aquaculture sector. Most prescribing is done by untrained salespersons whose main concern is to move product. Farm workers have little knowledge on the specifics of the products or the manner in which they are to be handled. Often the products prescribed have little to do with the disease. For instance, the use of PVPI for systemic shrimp diseases like MBV is likely to be of

limited efficacy. As some of these chemicals and products may enter the natural environment through the discharge water, it is likely that disruptions to the natural aquatic ecosystem may occur.

ON-GOING RESEARCH ON CHEMICALS

In Malaysia, the Fisheries Research Institute in Penang has completed some work on antibiotic residues in fish and water and plans to do more work in the future. The Freshwater Fisheries Research Institute in Batu Berendam, Melaka also conducted various treatment regimes on the common freshwater diseases. At the Universiti Putra Malaysia, routine trials on treatment regimes for specific diseases are being regularly conducted, while at the Universiti Sains Malaysia, treatment trials are usually conducted on marine cage-cultured fishes. USM has also undertaken a project on the survival of grouper fry treated with chemotherapeutic agents after shipment.

In Singapore, research on chemicals is primarily undertaken by the PPD, where studies to develop treatment regimes to control specific disease agents and to enhance the post-shipment survival of fish are carried out. Such studies are sometimes conducted with academic institutions. Monitoring of chemical residues in fish destined for human consumption is undertaken by the Veterinary Public Health Laboratories of the PPD.

CONCLUSIONS AND RECOMMENDATIONS

In Singapore and Malaysia, the use of chemotherapeutants in the aquaculture industry is not as yet worrying. However, Malaysia needs more comprehensive mechanisms to monitor the types of chemotherapeutants used and the manner in which they are administered. This would be, in fact, a precursor to a legislative framework that would ultimately guide the industry. In Malaysia, this mechanism does not exist. Singapore has a much more developed administrative and legislative mechanism to manage the use of chemotherapeutants by the industry.

Several other sound recommendations were made by Shariff (1995), including the need for more studies on the efficacy of antibiotics and chemotherapeutants to specific diseases under tropical conditions. Currently, such information is limited to temperate situations. Investigations should be made on the commonly used chemotherapeutants in relation to the duration of treatment, optimal dosages, efficacy in different systems and for different fish species, and residual patterns in the host and the environment. The latter is very important in the perspective of consumer health and the impact on the environment.

Research should also be conducted to understand the pond dynamics in relation to the use of bioremediation in shrimp farming, since it is claimed that keeping the water clean reduces mortalities. However, many of the products used for bioremediation were developed in temperate countries. Investigations should be made on the possible impacts exotic microorganisms may have on the natural aquatic ecosystems. Meanwhile, researchers should also focus on the use of local microorganisms, as these might be more effective.

Overall, research should be orientated towards developing more environmentally friendly chemotherapeutants, and on the use of immunostimulants and non-specific enhancers incorporated in diets. Emphasis should also be given to the development of vaccines, which have been used successfully in the west.

A more holistic approach and a proper understanding of the environmental factors should be made, with less reliance on chemotherapeutants. Prevention will be easier by providing a better environment for the host, so that it will be able to combat a wide range of pathogens without the use of chemotherapeutants. It must be cautioned that chemotherapeutants, vaccines and immune

enhancers can only be used with success if the host is not already under stress from poor environmental conditions.

REFERENCES

- Alderman DJ, Michel C. 1992. Chemotherapy in aquaculture today. In: Chemotherapy in Aquaculture: from Theory to Reality. p. 3-24. Office International des Epizooties, Paris.
- Amend DF, Pietsch JH. 1972. Virucidal activity of two iodophors to salmonid viruses. J. Fish. Res. Board Can. 29:61-65.
- Anderson IG. 1988. Disease problems faced by marine prawn farms and hatcheries. In: Singh T, Ang KJ. eds. Marine Prawn Farming in Malaysia- Present Problems, Future Prospects. p. 67-83. Occas. Publ. No. 1, Malaysian Fisheries Society, Serdang, Selangor.
- Apud FD. 1984. Extensive and semi-intensive culture of sugpo (*Penaeus monodon*) in the Philippines. In: Prawn Industry Development in the Philippines: Proceedings of the National Prawn Industry Development Workshop; 1984 April 10-13; Iloilo City, Philippines. p. 55-73. SEAFDEC/AQD, Tigbauan, Iloilo.
- Baticados MCL, Paclibare, JO. 1992. The use of chemotherapeutic agents in aquaculture in the Philippines. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 531-546. Fish Health Section, Asian Fisheries Society, Manila.
- Brander GC, Pugh DM. 1977. Veterinary Applied Pharmacology and Therapeutics. Bailliere Tindall, London.
- Chua FHC, Loo JJ, Wee JY, Ng M. 1993. Findings from a fish disease survey: an overview of the marine fish disease situation in Singapore. Singapore J. Primary Indust. 21:26-37.
- Department of Fisheries. 1994. Annual Fisheries Statistics-1993. Department of Fisheries, Malaysia. Ministry of Agriculture, Kuala Lumpur.
- Gopinath N. 1990. Biotechnical considerations in the handling and transport of live fishery products. In: Gopinath N, Abu-Bakar A. eds. Handling and Processing: the Selling Point in Malaysia Fisheries. p. 52-64. Occas. Publ. No. 3, Malaysian Fisheries Society, Serdang, Selangor.
- Harvey SC. 1975. Antiseptics and disinfectants; fungicides; ectoparasites. In: Goodman LS, Gilam A. eds. The Pharmacological Basis of Therapeutics. p. 987-1017. Macmillan Publishing Co. Inc., New York, NY.
- Herwig N. 1979. Handbook of Drugs and Chemicals used in Treatment of Fish Diseases. Charles C. Thomas, Springfield, IL
- King JM. 1973. Recirculating system culture methods for marine organisms. S-E-A Scope 3(1):1.
- Kungvankij P, Chua TE. 1986. Shrimp hatchery design, operation and management. NACA Training Manual Ser. No.1, Tigbauan, Iloilo, 88 p.
- Limsuwan C. 1993. Shrimp diseases and health management in South East Asia. In: Subasinghe RP, Shariff M. eds. Diseases in Aquaculture: the Current Issues. p. 71-96. Malaysian Fisheries Society, Serdang, Selangor.
- McFadden TW. 1969. Effective disinfection of trout eggs to prevent egg transmission of *Aeromonas liquefaciens*. J. Fish. Res. Board Can. 26:2311-2318.
- Meinertz R., Stehly GR, Gingerich WH, Allen JL. 1995. Residues of [¹⁴C]- malachite green in eggs and fry of rainbow trout, *Oncorhynchus mykiss* (Walbaum) after treatment of eggs. J. Fish Path. 18:239-247.
- Padmasothy S. 1987. Effect of feeding Virginiamycin on the growth rate, feed conversion and carcass composition of *Pangasius sutchii*. Proceedings of the 10th Annual Conference of the Malaysian Society of Animal Production, Genting Highlands, April 2-4, 1987.
- Platon RR. 1978. Design, operation and economics of a small-scale hatchery for the larval rearing of sugpo, *Penaeus monodon* Fab. Aquaculture Extension Manual No. 1, SEAFDEC Aquaculture Department, Tigbauan, Iloilo. 30 p.
- Plumb JA. 1992. Disease control in aquaculture. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 3-17. Fish Health Section, Asian Fisheries Society, Manila.

- Ross AJ, Smith CA. 1972. Effect of two iodophors on bacterial and fungal fish pathogens. *J. Fish. Res. Board Can.* 29:1359-1361.
- Shariff M, Subasinghe RP. 1993. Diseases in Malaysian aquaculture. In: Subasinghe RP, Shariff M. eds. *Diseases in Aquaculture: the Current Issues*. p. 49-59. Malaysian Fisheries Society, Serdang, Selangor.
- Shariff M. 1995. Fish health: an odyssey through the Asia-Pacific region. Professorial Inaugural Lecture, 25 March 1995. Universiti Pertanian Malaysia, 25 p.
- Tan CK. 1998 Overview of aquaculture in Malaysia. In: Nagaraj G, Singh T. eds. *Aquaculture Practices in Malaysia*. p. 1-13. Malaysian Fisheries Society, Serdang, Selangor.
- Tonguthai K, Chanratkool P. 1992. The use of chemotherapeutic agents in Thailand. In: Shariff M, Subasinghe RP, Arthur JR. eds. *Diseases in Asian Aquaculture I*. p. 555-565. Fish Health Section, Asian Fisheries Society, Manila.

The Use of Chemicals in Aquaculture in the People's Republic of China

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ABSTRACT

Aquaculture in China has developed very rapidly in recent years. Chemicals have become important tools in the control of disease and prevention of losses in various culture systems. The occurrence of diseases stimulated the development and production of drugs for aquatic culture systems, and promoted research on chemicals and their applications. Meanwhile, there exist some problems in the application of chemicals; and there are some potential risks in their usage in aquaculture which should not be neglected.

This paper describes the use of chemicals for the prevention and control of diseases in aquaculture in China. Their production, marketing, and usage, as well as associated problems and adverse impacts are also discussed. Approaches and practices to prevent diseases are also given. National regulations, on-going research and other aspects of the use of chemicals in aquaculture in China are also highlighted.

INTRODUCTION

Aquaculture in China has experienced rapid development in recent years. In 1993, the area of freshwater culture was 4.16 million ha, including 2 million ha of ponds and 200,000 ha of industrial fish farms. The area for marine culture in 1993 was 588,000 ha. Aquatic production from freshwater culture was 6.48 million t, and 3.09 million t were produced from marine culture. In 1995, production from these areas increased to 9 million and 4 million t, respectively.

Following the increase in aquatic production, risks due to diseases became more serious. Estimates of losses due to disease in freshwater culture range from 20% to about 30% when diseases reach epizootic proportions, while in marine culture, estimated losses range from 30% to 50%. Chemical application plays an important role in the control of disease and reduction of losses. The occurrence of diseases has created an extensive market for chemicals and has stimulated the production of drugs for aquatic culture systems. This development has promoted studies on chemicals and their applications to aquaculture.

At the same time, this use of chemicals in Chinese aquaculture has led to some problems in their application, a result of immature technology and poor management of drug use. The potential risks inherent in the use of chemicals in aquaculture should not be neglected. This paper describes the use of chemicals in aquaculture for the prevention and control of diseases in China.

PRODUCTION AND MARKET SUPPLY OF CHEMICALS FOR AQUACULTURE SYSTEMS IN CHINA

Before the 1980s, there were no Chinese factories producing chemicals specifically for use in aquaculture. The chemicals used by farmers originated from chemical and pharmaceutical factories catering to other animal production industries. By 1990, however, there were already about 30 factories producing chemicals for use in aquatic systems. In 1995, there were 69 aquatic drug factories and 558 veterinary drug factories in China. Many of the factories producing veterinary drugs also produce substances that can be used in aquaculture. For example, 121 of these factories produce furazolidone, 168 produce chloramphenicol and 242 produce Terramycin for veterinary use. There are also many other factories producing these drugs for use in human medicine. Fish farmers may buy drugs from any of these factories, so that directly or indirectly, drugs from all of these factories might be used for control of aquatic diseases.

The output from these factories varies from several tons to hundreds of tons. However, due to the vastness of China, and the wide variety of aquatic species, culture practices and technical levels of culture, there still exist limitations in production capacity and transportation. Thus, it is not possible for the existing factories to meet the industry's overall need for chemicals. As a result, in recent years a number of specialized factories have been set up to produce chemicals for aquatic culture systems. The research institutes have provided prescriptions or formulas, and the factories prepared these into commodities for the market. Usually, one factory produces a series of chemicals and drugs with a large number of commercial names. For example, Guangdong Fisheries Biopharmaceutical Factory produces a series of drugs including 12 kinds of fish drugs, 20 kinds of fish hormones and 14 kinds of other veterinary drugs trade marked "Fish Happiness." Many of these drugs are produced only in small batches and used in a limited area. The output for these drugs varies continuously along with the fluctuation of market demand.

Different drugs on the local market often have the same trade name, or the same drug may have different trade names in various regions. Most locally produced drugs have no English name, making it very difficult to identify them using standard references. Besides, the factories often like to maintain secrecy as to the composition of their products. In some cases, different brands of drugs have the same or similar compositions, with only differing proportions of active ingredients. For example, there are many kinds of chlorine-based disinfectants and at least 20 kinds of anti-bacterial drugs containing various percentages of chloramphenicol.

According to estimates as of April 1995, there are 2,900 kinds of registered drugs in China, of which 134 are marketed especially for aquaculture, and 683 are for veterinary use. Both types are being used in aquaculture. In total, these include 235 antibacterials, 16 anti-parasite drugs, 43 disinfectants, 23 Chinese traditional medicines, 271 feed additives, 55 imported drugs, and 40 other drugs for various applications. Although these chemicals can be registered in national, provincial or city administrative agencies, some of them, like bleaching powder, CuSO_4 and other all-purpose disinfectants, are not listed as aquatic drugs. It is very difficult to come up with a comprehensive list of the drugs currently being used in Chinese aquaculture because some unregistered drugs are sold in the market illegally.

In China, there are no specific routes or outlets for selling chemicals for use in aquaculture. Fish farmers can purchase drugs and chemicals directly from factories or from drug sales agents without knowing whether the drugs are designated for humans, poultry, or fish. They need not obtain a prescription from a veterinarian or a license from any administrative authority before they can buy chemicals. Farmers can buy any kind of chemical if they believe that the product may relieve the clinical signs of a disease. As the chemicals used in aquaculture originate not from specialized outlets, but are available at conventional or regular sources, it is very difficult to estimate the volume of consumption per year for the entire country.

In recent years, some chemicals have been imported from such countries such as Hong Kong, Germany, the United States, Switzerland, England, Japan, Canada, France, and Norway to the coastal provinces in the southern part of China. Some foreign companies have begun to promote the sale of chemicals for aquaculture (for example, Astos, produced by Pfizer of the USA; and Flavomycin, produced by Hoechst Veterinary Company of Germany). The volume of their sales may increase in the future; however, China is a developing country with a wide aquacultural area, so for economic reasons, it is not possible to depend upon imported chemicals to meet the huge need. These imported drugs will probably not play an important role in the control of fish diseases in China.

USE OF CHEMICALS IN AQUACULTURE

The purpose of chemical use in aquaculture is to prevent and cure diseases affecting cultured commodities. Preventive measures include disinfecting water, ponds and rearing facilities, as well as eggs and other stages of fish before stocking. These measures improve the rearing environment and reduce the numbers of pathogens in the water or on the body surface of fish. The common ways of administering drugs to cure diseases are by oral application (used especially to eradicate intestinal parasites and systemic diseases), by immersion in short or long baths (to eradicate external infections), and by injection (to treat disease in broodstock and other valuable fish). Experienced fish farmers use chemicals mainly for prevention, but most farmers apply chemicals whenever fish become sick or when mortality is encountered.

Chemical application is a popular measure employed to prevent and control diseases because of the following reasons:

- Chemicals are convenient and simple to use. Most fish farmers prefer pathogen eradication by using disinfectants or medicated feed, rather than by improving the rearing environment or readjusting the culture structure to prevent disease occurrence.
- Chemicals are effective. They produce rapid results under certain conditions (e.g., arresting disease outbreaks or remedying the absence of oxygen in the environment), while other measures are unable to achieve immediate effects. For example, when bacterial disease breaks out and causes high mortality, measures such as vaccination or improvement in the environment are unable to produce instant results as compared to those achieved by chemicals.
- Chemicals are economical to use. Although building optimal culture conditions is good for reducing losses, the investment for re-structuring the culture system to conform to an ideal condition is comparatively higher than the cost of using chemicals.

Table 1 lists some common chemicals used at various stages of fish culture in China with their generic names, dosage and method of use. In practice, several kinds of drugs are often used as mixtures, such as the combination of furazolidone and rhubarb to produce a synergistic curative effect.

Table 1. The common chemicals used in aquaculture in China.

Usage: Chemical	Pond Disinfection	Fish or Egg Disinfection	Bath Treatment	Injection	Oral Administration
Quick Lime	250-1000 ppm		15-20 ppm		
Bleaching Powder	20-50 ppm	10-30 ppm	1-2 ppm		
Chlorine	0.5-1 ppm				
KMnO ₄	20-50 ppm	10-20 ppm	0.5-5 ppm		
Povidone-iodine		50-100 ppm			0.2 gm/kg
Formalin		20-40 ppm	1-10 ppm		
Dipterex		2 ppm	0.2-0.5 ppm		5-10 mg/kg
Malachite Green		70-100 ppm	0.02 ppm		
Methylene Blue			1-3 ppm		
CuSO ₄		3-10 ppm	0.5-0.7 ppm		
CuSO ₄ /FeSO ₄			0.7 ppm		
Dipterex/FeSO ₄			1.4 ppm		
K ₂ Cr ₂ O ₇			20-40 ppm		
CuCl ₂			0.7-1 ppm		
TCCA(DCCA)	5-10 ppm		0.2-0.8 ppm		
NaCl		2-5%			
EDTA			2-10 ppm		
Bromo-geramine		100 ppm			
Nystatin		20000 µg/L			
Furazolidone		3-10 ppm	0.1-1.0 ppm		20-200 mg/kg
Sulfonamide					50-100 mg/kg
Terramycin		25 ppm	2-3 ppm		50-100 mg/kg
Aureomycin		12.5 ppm			0.01-0.05 mg/kg
Penicillin			4-30 i.u./mL	10 ⁵ i.u./fish	
Streptomycin		1.5 ppm	8-30 µg/mL	10 ⁵ µg/fish	
Doxycycline					20-50 mg/kg
Erythromycin			0.05-0.1 ppm		10-30 mg/kg
Chloramphenicol			1-1.8 ppm	20 mg/kg	25-100 mg/kg
Oxolinic Acid					100-300 mg/kg

The price of drugs varies with season, location, and market supply. For example, the price of furazolidone ranges from 60 to 150 Yuan/kg, depending on whether the farmers buy it from factories or from shops.

Although a great variety of drugs are used in aquaculture, most of them are not widely applied as yet. If we calculate the amount of applied chemicals by weight, bleaching powder and quick lime account for more than half of the total used. Other commonly used chemicals are furazolidone, sulfonamide, CuSO₄, Dipterex, and iodine compounds. Antibiotics are used mainly to treat valuable broodstock, larval shrimp, and other aquatic species of high value, such as eel and softshelled turtle. Antibiotics are much less used to treat freshwater fish because they have relatively lower

economic value. For freshwater applications, furazolidone is the chemical that is most widely used.

PROBLEMS AND ADVERSE IMPACTS OF CHEMICAL USE IN AQUACULTURE

Aquaculture in lakes and reservoirs is being conducted at very low stocking density. During disease outbreaks, no chemicals are applied and the usual option is to terminate the culture because it is not economical to apply drugs to large water bodies. Therefore, very little impact of chemicals is felt in these culture systems.

In contrast, in the coastal regions of central and south China where most high-density fish culture operations are located, diseases spread very rapidly and can reach epizootic proportions. Chemicals and drugs are commonly used in these areas, and as a result, the problems and adverse impacts of chemical application to fish culture are focused there.

The main adverse impact of drug use in aquaculture in China is the development of drug-resistant strains. In some farms in southern China, for example, the dosage of drug needed to cure bacterial disease in eels is now 10 times higher than that previously required. Overcoming drug resistance not only requires an increase in dosage, but may also endanger human health, as most of the chemicals applied in aquaculture are also used to treat human diseases. Some chemicals also cause damage to the skin of fish. It has been observed that fish previously treated with formalin to kill parasites on the body surface become susceptible to secondary bacterial infection. The use of antibiotics often reduces the immunity of fish and shrimp to infectious agents.

The following problems have been recognized in connection with the use of chemicals in aquaculture systems:

- Owing to a lack of adequate knowledge on disease control, some farmers cannot apply chemicals properly. Information is lacking on the proper choice of chemicals, correct dosage, optimal time for treatment, and condition of application. For example, the action of Dipterex varies with environmental quality: it is highly poisonous at high pH and ineffective in environments with very high organic load. Because of the high cost of most drugs, farmers apply them using levels lower than the recommended effective dosage. This not only results in undesirable outcome of treatment, but also in the development of drug-resistant strains. In some cases, farmers use those chemicals readily available to them, while disregarding the nature of the disease and even lacking a prior diagnosis.
- Abuse and over-use of chemicals often occurs in farms culturing high-value aquatic species like shrimp, eel, turtle and various marine fish. Some drugs are used continuously for one month or longer. In some farms, several kinds of drugs are used at the same time or by rotation, each drug application lasting for one or two days. It is obvious that the farmers are not clear as to whether or not these drugs are actually useful and effective. This ignorance may also lead to poisoning of fish, impairment of their immune capacities, overgrowth of secondary pathogens like fungi, and eradication of beneficial bacteria.
- There are some problems related to the quality of drug products and their commercial advertisement. Some low quality drugs are sold in the market. For example, the chlorine content of bleaching powder varies from 12% to 36% due to improper transportation, packaging, storage, and the poor quality of the product itself. As a result, farmers use dosages several times higher than that recommended without getting good results. On the other hand, fish poisoning takes place when an overdose of chemicals is given. Some factories advertise their products as possessing properties for curing a wide range of diseases. These false claims lead farmers to misuse the drugs.
- A few farmers still use some prohibited chemicals like mercury-based compounds because no

better drugs can be obtained as replacements.

- In connection with the use of feed additives, some cases of illness in fish were observed to be caused by the improper usage of chemicals. For example, Olaquinox ($C_{12}H_{13}N_3O_4$), a kind of growth promoter, was used for fish and increased growth rate by 15-20% when applied at 20-50 mg/kg of food. However, some feed mills increased the amount of Olaquinox to 200 mg/kg. Common carp that received feeds with high doses of Olaquinox were prone to hemorrhage, especially during netting and transporting. The condition observed was fatal.
- In high-density culture, farmers use drugs administered through feeds and simultaneously add chemicals to the water to reduce losses. This practice leads to pollution of the environment and plankton die off. Although the condition is temporary and the environment recovers after a few days, repeated use of some heavy metal-based salts like $CuSO_4$ may pollute the pond bottom.

Despite the above problems, the direct and indirect adverse effects to the environment of chemicals used in aquaculture are relatively less than those caused by chemical wastes from industries and factories. There have been very few reports of environmental pollution by aquatic chemicals or of events of drug poisoning of fish farmers because the total amount of chemicals used in aquaculture in China is not very great. In contrast, there have been some reports of poisoning of aquatic animals due to chemical wastes from factories or run-off from agricultural lands.

None the less, potential risks associated with chemical application in aquaculture still exist. Since chemical usage is expected to increase in the future, the search for non-polluting and non-persistent drugs and chemicals specialized for use in aquaculture should be intensified.

APPROACHES AND PRACTICES TO DISEASE PREVENTION

There are a number of practices and methods to prevent and cure fish diseases without the use of chemicals. A few examples are given below:

Vaccination

There are several kinds of fish vaccines available in China. Vaccine against viral hemorrhage disease of grass carp can reduce mortality of fingerlings from 70% to 30% and has been widely applied. China produces at least 300 t of vaccine, and several billions of fish are vaccinated every year. Prof. Chen Changfu of Huazhong Agriculture University prepared vaccines against bacterial gill-rot disease caused by *Cytophaga columnaris* and bacterial hemorrhagic septicemia caused by *Aeromonas hydrophila* in Chinese perch. In 1994, 600,000 fingerlings in Zhongshan, Xinhui and Hainan counties, Guangdong Province were tested with vaccine by injection or by bath for 5-10 min. The vaccinated fish remained healthy throughout culture, while unvaccinated fish in an adjacent area became ill and suffered mortalities of more than 80%. Vaccination may be the practical way to control diseases in Chinese perch because they feed only on live fish and thus do not eat medicated pellets. In addition, vaccines against bacterial hemorrhagic septicemia of freshwater fish caused by *Aeromonas punctata* provided a 70% protective rate to silver carp after immunization by bath. The vaccine against bacterial gill-rot of grass carp, given orally and by immersion, successfully controlled the disease and is now produced commercially. As a preventive and control measure against disease, vaccines are timely, effective and clean, as no residues are left after treatment.

Application of Chinese Traditional Medicines

The use of medicinal herbs to treat fish diseases has great potential. In fact, fish farmers in various regions have their own recognized prescriptions using herbs. However, it is not easy to identify

the effective ingredients and mechanisms of action of these herbs, so that very few of them have been approved for use. Table 2 shows some common herbs applied to treat fish diseases.

Improvement of the Pond Culture System

In the traditional method of polyculture of grass carp and silver carp, the stocking ratio is one grass carp to three silver carp (or, sometimes, bighead carp). However, it has been observed that this ratio does not give the optimal yield. The pond water has to be fertilized often to meet the natural food demands of the silver carp or bighead carp, such that the water quality deteriorates rapidly and induces sickness among the grass carp. With this finding, the stocking ratio was changed from 1:3 to 1.5-2.5:1. This modification in the rearing system resulted in increased survival, improved production, and better water quality. The output in the modified ponds was three times higher as compared to traditional ponds.

Positive results have also been observed in shrimp (*Penaeus monodon*) culture systems using water with lower salinity. The environmental modification may have reduced stress among the shrimp, which prefer a brackishwater environment.

Although it is clear that chemicals can kill pathogens, the question remains as to whether they can control diseases under field situations, where various conditions come into play. This example from the field illustrates the need to consider many factors before a treatment option is chosen and successful treatment can be achieved. A case of bacterial hemorrhagic septicemia broke out in two ponds with similar conditions in the Honghu Lake area. Details about the fish and pond conditions are presented in Table 3. In pond 1, fish were fed with unmilled rice grain, and the sharp seed coats caused abrasions in their intestines, resulting in bacterial infection. At first, the disease spread

Table 2. The common medicinal herbs used in aquaculture in China.

Names of Herbs	Target Diseases
<i>Acalypha australis</i>	Bacterial enteritis and gill-rot
<i>Acorus calamus</i>	Bacterial enteritis, gill-rot, and septicemia
<i>Allium sativum</i>	Bacterial diseases
<i>Andrographis paniculata</i>	Bacterial enteritis
<i>Artemisia argyi</i>	Bacterial enteritis and gill-rot
<i>Cayratia japonica</i>	White head-mouth disease
<i>Chenopodium ambrosioides</i>	Nematode infections
<i>Cyrtomium fortunei</i>	Nematode infections
<i>Duchesnea indica</i>	Bacterial enteritis
<i>Euphorbia humifusa</i>	Bacterial enteritis and gill-rot
<i>Fructus quisoqualis</i>	Tapeworm infections
<i>Galla chinensis</i>	White head-mouth, septicemia, furunculosis
<i>Melia azedarach</i>	Trichodiniasis, Lernaeosis
<i>Perilla frutescens</i> var. <i>crispa</i>	Bacterial enteritis
<i>Pinus massoniana</i>	Bacterial enteritis, and gill-rot
<i>Piper sarmentosum</i>	Lernaeosis
<i>Polygonum hydropiper</i>	Bacterial enteritis and gill-rot
<i>Portulaca oleracea</i>	Bacterial enteritis
<i>Ricinus communis</i>	Bacterial enteritis, gill-rot, and septicemia
<i>Sambucus javanica</i>	Bacterial enteritis and gill-rot
<i>Sapium sebiferum</i>	Bacterial gill-rot
<i>Thysanospermum diffusum</i>	Bacterial enteritis, and gill-rot

among common carp and Chinese bream which ate the rice grain. Upon observing this and acknowledging the adverse effect of unmilled rice grain on the fish, the farmer stopped giving them this feed and administered furazolidone orally through artificial feeds. The fish responded well to the treatment. In pond 2, the count of pathogenic bacteria in the water was too high for the fish to resist infection, and the majority of the stock became ill. The farmer had to give medicated feed and also applied disinfectants to remedy the bad condition of the water. Thorough analysis of the culture condition is needed so that appropriate solutions can be applied.

Table 3. Comparison of curative methods applied in two fish ponds.

	Pond 1	Pond 2
Area	6.5 ha	6.8 ha
Total fish	267,000	280,000
grass carp	41,000	100,000
silver carp	161,000	50,000
bighead carp	25,000	50,000
Chinese bream	15,000	80,000
common carp	15,000	few
Inducing factor:	intestines were hurt by rice	high count of bacteria in water
Treatment:	change food oral medicine	oral medicine disinfect water

The comprehensive use of drugs is also important. Culture systems for pearl oyster are very susceptible to bacterial infection in summer, and infections usually result in high mortality. Application of 0.2 ppm furazolidone can effectively control the disease, but the sick oysters can easily become re-infected after several days because their resistance to disease becomes very low following illness and chemotherapy. It has been observed that furazolidone becomes more effective for oysters if it is given in combination with rhubarb, as rhubarb has the ability to enhance the immunity in these animals. This method has been tested in more than 300 ha of pearl farms in Ezhou County, Hubei Province.

Since the 1950s, research on fish diseases developed very rapidly in China and some practical methods on the use of chemicals have been obtained. Two popular methods of disinfection involve the use of quick lime to disinfect ponds, and the application of hanging baskets filled with bleaching powder or bags filled with CuSO_4 at the feeding sites. These methods are easy to administer and effective against fish diseases. Supplying drugs using hanging bags or baskets is an ideal and practical method, as it allows the drug or disinfectant to dissolve slowly. Toxicity due to overdose is also avoided because fish can voluntarily keep away from areas near the bags if the concentration of drugs near the site is too high.

In addition, farmers also carry out disinfection for the following purposes:

- To disinfect juveniles and fingerlings, either 3-4% salt, 10 ppm bleaching powder, 8 ppm CuSO_4 , or 20 ppm KMnO_4 is applied for 20 min.
- To disinfect feeds and feeding sites, sites can be kept clean by hanging baskets filled with bleaching powder in their vicinities or by broadcasting the disinfectant around the feeding sites.
- To disinfect manure before application in the ponds as fertilizer, 120 gm of bleaching powder

is applied to 300 kg manure. Alternatively, the bleaching powder may be applied into the water after the manure has undergone complete fermentation.

Proper feed management may also help prevent disease. The basic feed management rules such as strict adherence to good feed quality, determination of the exact feed requirements of the animals, and verification of the feeding sites and time of feeding are important measures to keep the fish healthy.

NATIONAL REGULATIONS ON THE USE OF CHEMICALS IN AQUACULTURE

The National Control Administration of Veterinary Bioproducts and Pharmaceuticals is in charge of management of production and authorization of sales of chemicals for aquaculture. Before a new drug is produced, the factory must submit an application and report the information regarding technology development, and the data from test results on safety and characteristics of the drug. After this procedure is completed, the drug is registered and approved. However, the aquaculture industry developed very rapidly in China and, as a result, chemical requirements increased very quickly. As a result, some drugs for aquaculture were produced and marketed illegally, even without licenses; and thus, some untested drugs were able to enter the market. This development aroused the attention of the government, so that at the end of 1994 an investigation of the development, production and marketing of chemicals used in aquatic systems was conducted, and all factories producing these chemicals were inspected. Unlicensed facilities were closed or their production and sales were stopped until their registration was approved. Some measures taken to strengthen the regulation of aquatic chemicals include formulating a licensing system for production, monitoring and supervising quality of products, and setting up of a specialized panel on aquatic drugs within the Veterinary Drugs Commission. Several research institutes were designated as certified clinical testing agencies for aquatic drugs. These regulations were, however, mainly embodied to control drug production and assure product quality. No regulations on the purchase and use of chemicals by consumers have been formulated.

The standards for allowable residues of chemicals in aquatic products were formulated and promulgated by the Surveillance Institute of Food Hygiene, Ministry of Public Health, and are monitored by the Food Hygiene Departments of Anti-epidemic Stations at various levels. Some processing plants and import and export corporations have their own laboratories to examine for chemical residues in exported aquatic products according to the standards prescribed by the importing country.

China has specified standards of allowable residues of hazardous materials in aquatic products (Table 4), but there are no set standards for antibiotic residues. In this case, the standards set for other food products are used as a reference. For example, the allowable terramycin residue in honey is limited to below 0.05 mg/kg.

The environmental monitoring system in China is implemented by the environmental protection agencies in the provinces and cities. They determine the types, concentrations, and origins of toxic chemicals and monitor changes in their levels. The National Bureau of Environmental Protection formulates the standards of quality for surface water (Table 5) and the standards of water quality for aquaculture in China (Table 6).

Table 4. Allowable limits for hazardous materials in aquatic products.¹

Hazardous Material	Allowable Limit (mg/kg)
Organophosphate compound	< 0.1 mg/kg
Mercury	< 0.3 mg/kg
Methyl mercury	< 0.2 mg/kg
Arsenic	< 0.5-1.0 mg/kg
BHC	< 2 mg/kg
DDT	< 1 mg/kg

¹ From Chinese national standards GB-2733-81 to GB 2745-81.

Table 5. Environmental quality standards for surface water in China.¹

Item	Class 1	Class 2	Class 3
Appearance	no visible foam, oil film and substances		
Smell	no peculiar smell	no peculiar smell	no peculiar smell
Temperature (°C)	35	35	35
pH	6.5 - 8.5	6.5 - 8.5	6.5 - 8.5
Color	< 10°	< 15°	< 25°
DO	> 90% saturation	> 6 ppm	> 4 ppm
BOD ₅	1 ppm	≤ 3 ppm	5 ppm
COD	2 ppm	4 ppm	6 ppm
Volatile phenol	< 0.001 ppm	< 0.005 ppm	< 0.01 ppm
Cyanide	< 0.01 ppm	< 0.05 ppm	< 0.1 ppm
Arsenic	< 0.01 ppm	< 0.04 ppm	< 0.08 ppm
Total Hg	< 0.0001 ppm	< 0.0005 ppm	< 0.001 ppm
Cd	< 0.001 ppm	< 0.005 ppm	< 0.01 ppm
Cr	< 0.01 ppm	< 0.02 ppm	< 0.05 ppm
Pb	< 0.01 ppm	< 0.05 ppm	< 0.1 ppm
Cu	< 0.005 ppm	< 0.01 ppm	< 0.03 ppm
Petroleum	< 0.05 ppm	< 0.3 ppm	< 0.5 ppm
<i>E. coli</i>	< 500 cfu/L	< 10000 cfu/L	< 50000 cfu/L
Total nitrogen	≤ 1.0 ppm	≤ 1.0 ppm	≤ 1.0 ppm
Total phosphorus	≤ 0.1 ppm	≤ 0.1 ppm	≤ 0.1 ppm

¹ From Chinese national standards GB 3833-88.

Table 6. Recommended standards for water quality for aquaculture in China.¹

Item	Standard
Color, smell and taste	No peculiar smell, color and taste
Surface	No obvious film and foam
Suspended particles	≤ 10 mg/L
pH value	6.5 - 8.5 in fresh water, 7.0 - 8.5 in sea water
BOD ₅	< 5 mg/L
DO	> 3 mg/L at night; 5 mg/L at daytime
Hg	≤ 0.0005 mg/L
Cd	≤ 0.005 mg/L
Pb	≤ 0.05 mg/L
Cr	≤ 0.1 mg/L
Cu	≤ 0.01 mg/L
Zn	≤ 0.05 mg/L
Ni	≤ 0.05 mg/L
As	≤ 0.1 mg/L
Cyanide	≤ 0.005 mg/L
Sulfide	≤ 0.2 mg/L
Fluoride	≤ 1.0 mg/L
Volatile phenol	≤ 0.005 mg/L
Yellow phosphorus	≤ 0.001 mg/L
Petroleum	≤ 0.05 mg/L
Acrylic aldehyde	≤ 0.02 mg/L
BHC	≤ 0.02 mg/L
DDT	≤ 0.001 mg/L

¹From Chinese national standards GB 3833-88 and GB 11607-89.

The impact of the chemicals used by aquaculture farms on the environment remains unmonitored, largely because of the perception that aquaculture is not one of the main factors causing environmental deterioration. The results of a country-wide investigation showed that the fishery sector is not only a victim of environmental pollution, but also a source of pollution. The main adverse impacts of aquaculture on the environment are believed to be eutrophication of receiving waters and the transmission of pathogens, and not pollution due to the chemicals used in the farms.

RESEARCH ON USE OF CHEMICALS IN AQUACULTURE

In the last 50 years, scientific research and education have developed rapidly in China, thus meeting the requirements of the different aspects of aquaculture. There are 52,000 technical personnel involved in fisheries in the whole country, including 2800 senior staff and 12,000 intermediate workers. There are 203 research units engaged in fishery research. The national research units, besides conducting research on pure and applied sciences, also take part in technological development and in providing technical service. The research units at the provincial level focus on applied research and on technical development, consultation and extension services. The research units at the county level deal mainly with extension. There are at present five fishery colleges and 28 universities with fisheries departments. In addition, there are 16 other fisheries schools and nine schools with attached freshwater aquaculture curricula. These entities provide basic training to prepare the staff needed to support the aquaculture industry.

Through their long-term accumulation of research results and experience, quite a number of research units are able to design medication regimes with good curative effects against fish diseases. Once a new aquatic drug is successfully formulated and tested, the technical data necessary for license application can be transferred to factories, so that batch production can commence. Most aquatic chemicals in use in Chinese aquaculture were developed in this manner.

At present, many laboratories are studying drugs, mainly on the following aspects:

- To determine if any new drugs used for humans or veterinary animals can be introduced into aquaculture systems.
- To enhance drug efficacy by establishing correct dosage, or by changing the proportions of active ingredients and additives.
- To find new methods to prevent and control diseases.

Still, the problem is that no new drugs are being synthesized solely for use on fish. Due to lack of financial support, very little basic research on residue formation or pharmacokinetics has been conducted.

CONCLUSIONS AND RECOMMENDATIONS

The production and use of chemicals for aquaculture in China has developed very rapidly in recent years. Chemicals are important tools to control disease, reduce losses, and increase fish production. The benefits derived from aquaculture are numerous; thus chemical usage will probably continue to increase.

The chemicals used in aquaculture were introduced from human and veterinary medicine. From a long-term point of view, certain types of antibiotics should be limited strictly for use in aquaculture.

The problems surrounding the use of chemicals in aquaculture (e.g., product quality, pattern of usage etc.) could be corrected or avoided by enhanced management, formulation and implementation of legislation, and training of farmers. The development of drug-resistant strains of pathogens will affect the efficacy of drugs and may have adverse impacts on human health.

As the production of chemicals for aquaculture is a recent development, not enough rules and guidelines for their management have been established. Regulations should not only control drug production and sales, but also the management of products and their handling and usage. This problem is now being recognized by the administrative agencies concerned and it is being addressed. It can be predicted that the situation will improve greatly in 5-10 years. The key point is to set up strict management of chemicals, establish standards, and license production so that unsuitable drugs can be banned from the market.

Research should focus on developing new drugs for use in aquaculture, not just introducing those already in use in human medicine. Important areas for research are the following:

- Developing antibiotics and chemicals specifically for fish and other aquaculture commodities.
- Developing drugs that will not induce resistance among microorganisms.
- Investigating the use of traditional medicinal herbs to cure fish diseases.
- Conducting studies on the pharmacology and toxicology of available chemicals to provide scientific basis for standardizing their usage.
- Finding alternative and non-medicinal control measures against fish diseases.

SUPPLEMENTARY BIBLIOGRAPHY

Although no references are cited in the text, the following books and articles are important sources of the information presented in this paper. As most are written in Chinese, we advise interested individuals to request the author's assistance in obtaining copies.

- Beijing Institute of Information for Medicine. 1992. Guidelines on the Management of Chemicals. (in Chinese).
- Chen C. 1995. A preliminary study on the immuno-prophylaxis of bacterial gill rot disease of mandarin fish (*Simiperca chuatsi* B.). J. Huazhong Agricult. Univ. 13:364-367. (in Chinese, English abstract).
- Chen Q (ed.). 1975. Handbook of the Prophylactics and Therapeutics of Fish Diseases. Shanghai Scientific Publishers, Shanghai. (in Chinese).
- Editorial Board of Yearbook of Chinese Medicine. 1993. Yearbook of Chinese Medicine: 1992. China Medical Science and Technology Publishers. (in Chinese).
- Han X. 1990. Cultivation of Freshwater Eel. Agriculture Press, Beijing. (in Chinese).
- Huang Q (ed.). 1993. Diseases of Aquatic Animals. Shanghai Scientific and Technical Publishers, Shanghai. (in Chinese).
- Liu J, He B. (eds.). 1992. Cultivation of Chinese Freshwater Fishes. 3rd edn., Science Press, Beijing. (in Chinese).
- Men Q. 1991. Handbook of the Prophylactics and Therapeutics of Shrimp Diseases. Ocean University of Qingdao Publishers, Qingdao. (in Chinese).
- Pan J-P. (ed.). 1988. Handbook of the Diagnosis, Prophylactics and Therapeutics of Fish Diseases. Shanghai Scientific and Technical Publishers, Shanghai. (in Chinese).
- Qian Z (chief ed.). 1994. The Development of the Chinese Fisheries and Manpower in Aquaculture. Aquacultural Press, Beijing. (published in Chinese and in English).
- Shen Y, Feng W, Gu M, Wang S, Wu J, Tang Y. 1994. Monitoring of River Pollution. China Architecture & Building Press, Beijing. (in Chinese, with English abstracts).
- State Medicine Administration. 1980. Collection of Medical Technology of Raw Materials in China. (in Chinese).
- Xu B, Ji W, Zhang P, Xu H. 1993. Comparison of antibacterial agents for control of pathogens in cultured shrimp, *Penaeus orientalis*. J. Ocean University of Qingdao, 23:43-51. (in Chinese, English abstract).
- Yue Y (ed.). 1992. Methods of Toxicity Tests of Fishes in Polluted Water. China Environmental Science Publishers, Beijing. (in Chinese).
- Zhu M (ed.). 1992. Toxicology of Animal Foods. Shanghai Scientific and Technical Publishers, Shanghai. (in Chinese).
- Zhu M, Li M, Yang M. (eds.) The National Control Institute of Veterinary Bioproducts and Pharmaceuticals. 1995. The Catalogue of Veterinary Drug Production in China. Aquacultural University of Beijing Publisher, Beijing. (in Chinese).
- Zhu X, Lu Q, Wang Y, Wan W. 1993. Diseases of Cultured Fishes and Prophylactics and Therapeutics. Hubei Scientific and Technical Publishers, Hubei. (in Chinese).

The Use of Chemicals in Aquaculture in the Philippines

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ABSTRACT

The intensification of aquaculture in the Philippines has made the use of chemicals and biological products inevitable. A recent survey conducted nation wide among shrimp and milkfish culture facilities revealed the use of more than 100 products for rearing, prophylaxis, and treatment purposes. The most commonly applied chemicals are disinfectants, soil and water conditioners, plankton growth promoters, organic matter decomposers, pesticides, feed supplements, and antimicrobials. All of these are readily available in the market. The dosages, purposes, patterns of use, origins, and manufacturers of these chemicals and biological products are discussed in this paper.

The indiscriminate use of chemicals has caused mortalities and morphological deformities in the host and development of antibiotic-resistant bacterial strains. The use of chemicals in aquaculture also poses dangers to public health. Government policies regulating or prohibiting the use of certain chemicals for aquaculture have helped curtail the destructive consequences of chemotherapy. Moreover, research institutions have geared their studies towards discovering environmentally safe drugs and other alternatives to disease control. However, these efforts will be futile unless a strong and aggressive campaign on the cautious and restricted use of drugs in aquaculture is conducted among shrimp and fish farmers, drug manufacturers and suppliers.

INTRODUCTION

In the Philippines, aquacultural activity is largely directed toward the production of milkfish (*Chanos chanos*) and tiger shrimp (*Penaeus monodon*). Of the 220,000 ha of brackishwater ponds, 176,000 ha are devoted to milkfish and 47,776 ha to tiger shrimp culture (ICAAE 1993, BFAR 1994). In 1994, aquaculture produced a total of 226,108 mt (Table 1) of which 135,682 mt were milkfish and 90,426 mt were shrimp (BFAR 1994). Milkfish and shrimp are mostly produced in Region VI in Western Visayas. Of the 298 commercial shrimp hatcheries operating in 1992, 59.4% are also found in this region (ICAAE 1993).

The use of chemicals has become integral to traditional and intensive aquaculture and more often than not, operations are dependent on it. Chemotherapy is widely practised in the Philippines,

especially to treat disease problems in shrimp-culture facilities. Chemicals are effective against multiple diseases or multiple pathogens and their application is versatile. Chemicals can be used as prophylactic agents and can be applied quickly when a disease condition occurs. They can be applied in various ways, such as in bath, by injection, or as feed additives.

Table 1. Milkfish and tiger shrimp production (mt) in the Philippines by region.¹

Region	Milkfish	Tiger Shrimp	Total
NCR	4,216	67	4,283
I	11,875	413	12,288
II	70	30	100
III	29,247	27,749	56,996
IV	13,162	4,161	17,323
V	1,752	925	2,677
VI	39,648	38,375	78,023
VII	10,774	2,520	13,294
VIII	1,883	204	2,087
IX	10,304	6,053	16,357
X	215	1,511	1,726
XI	7,866	5,568	13,434
XII	2,091	1,507	3,598
XIII	2,007	1,276	3,283
ARMM	572	67	639
Total	135,682	90,426	226,108

¹Source: BFAR (1994).

This paper attempts to document the general use of chemicals in aquaculture in the Philippines. It covers the chemicals and biological products used during preparation of rearing facilities, during the culture period, and during disease control, and includes notes on their patterns of use and suppliers or sources. The problems in chemical use, alternative approaches to disease prevention, the current laws and regulations on chemical use, and some recommendations are also discussed. This paper is largely based on a survey carried out throughout the Philippines with interviews conducted among fish and shrimp farmers/operators. The available published literature was also gleaned.

TYPES OF AQUACULTURE CHEMICALS USED IN THE PHILIPPINES

A survey of 18 shrimp hatcheries, 58 shrimp grow-out farms, and 26 milkfish brackishwater ponds in the Luzon, Visayas, and Mindanao areas was undertaken from July 1995 to April 1996. A questionnaire was used to obtain a comprehensive database on the types of chemicals used and their efficacies. Farm technicians, managers, and technical consultants were interviewed during these visits.

Tables 2-9 summarize the chemicals and biological products used in shrimp hatcheries and grow-out ponds, and in milkfish grow-out farms at various phases of culture. The chemical name, brand name, amount used, pattern of use, and cost and source of these chemicals are provided. More than 100 chemicals and biological products are currently being used by fish and shrimp farmers. The amounts applied vary from farm to farm and are usually based on experience, available published literature, or the supplier's recommendation. Table 10 provides a supplementary list of chemicals and biological products available in the market at the time of the study.

Table 2. Chemical and biological products used in *Penaeus monodon* hatcheries in the Philippines.

Chemical Group	Commercial Product/ % Activity	Pattern of Use	Amount Used	Price/Country of Origin/ Manufacturer/ Distributor
DISINFECTANTS: Calcium hypochlorite	Calcium hypochlorite, 70% chlorine	Disinfection of rearing tanks, few seconds to 24 h, splash or bath Disinfection of rearing water, 12-24 h Disinfection of hatchery paraphernalia, few seconds to 12 h, dip or bath <i>Artemia</i> cyst disinfectant, 5 min, short bath	200-1000 ppm 5-70 ppm 10-200 ppm	P 4,000/50 kg
Formalin Iodine	Formalin Biodin/Argentyne	Disinfection of diseased stock Spawner disinfectant, 1 min-1 h Footwear disinfectant	20 ppm 50-1000 ppm 100-500 ppm 200 ppm	P 690/2.5 L
ANTIBIOTICS: Tetracycline	Oxytetracycline	Every other day from stocking to harvest, long bath Disease control, daily until disease disappears	1-2 ppm 2-4 ppm	P 1,300/kg, Germany
Rifampicin	Rifampicin/Rimactane	Every other day from stocking to harvest, long bath Disease control, daily until disease disappears	0.1 ppm 0.2 ppm	P 28.00/600 mg, CIBA
Chloramphenicol	Bactrin Forte Chloramphenicol	Every other day from nauplii to harvest, as substitute for Rifampicin Every other day from Z ₁ to harvest, long bath	0.1 ppm 1 ppm	P 20.00/600 mg P 3,000/kg, Germany
Nitrofurantoin	Furazolidone, 98%	Disease control, 3 d, long bath Every other day from Z ₁ to harvest, long bath	2-4 ppm 0.5-1 ppm	P 1,600/kg, Taiwan
Erythromycin	Prefuran Erythromycin	Disease control, 3 d, long bath Disease control, 3 d, long bath Disease control, 3 d, long bath	2-3 ppm 1 ppm 2-3 ppm	P 2,000/100 gm
FUNGICIDES: Malachite green	Malachite green	Every other day from M ₁ to harvest, long bath	0.003-0.015 ppm	P 910/100 gm
Trifluralin	Treflan-R	Every 3-5 d from stocking to harvest, long bath For spawners, 1 h Disease control	0.1 ppm 5 ppm 1 ppm	
FEED ADDITIVES: Vitamin C	Enervon C Oderon C	M ₁ to harvest, mix with artificial feed M ₁ to harvest, mix with artificial feed	1 ppm 1 ppm	P 6.00/500 mg
Unknown	immune enhancer	Every 3-4 d from Z ₁ to harvest, long bath	0.5-1 ppm	

Table 3. Disinfectants used in *Penaeus monodon* grow-out ponds in the Philippines.

Chemical Group	Commercial Product/ % Activity	Pattern of Use	Amount Used	Price/Country of Origin/ Manufacturer/ Distributor
Benzalkonium chloride	Benzalkonium chloride	Pond prep., 10-30 cm water, broadcast	3-5 ppm	P 5,000/25 L, Clean Water
		Pond prep., 1 m water, broadcast Disease control	0.3-1 ppm 0.5-6 ppm	
	Cococide chloride	Pond prep.	0.5 ppm	P 4,400/20 L, Argent
		Rearing phase	0.5-1 ppm	
Didecyl dimethyl ammonium bromide (C ₂₂ H ₄₈ NBr)	Bromosept-50, 50%	Pond prep., 30-50 cm water, broadcast	0.5-10 ppm	P 583.45/L, Israel, Inphilco P 4,400/20 L
		Rearing phase, 1x/mo up to 1 mo before harvest	0.5-5 ppm	
		Disease control, 1x/wk for 2 wk	0.5-3 ppm	
Iodine	Biodin	Pond prep.	5 L/ha	
Alkyl dimethyl benzyl ammonium chloride	Fabcide B-50	Rearing phase	0.5-1 ppm	P 1,200/gal, Taiwan
	Aquasept	Pond prep.	5-10 ppm	P 272.90/L, Denmark, Inphilco
		Rearing phase	0.25-1 ppm	
		Disease control	0.5-1.5 ppm	
Formalin	Formalin	Rearing phase, overnight, barnacle control	25 ppm	P 690/2.5 L
Potassium permanganate	Potassium permanganate	Pond prep., spray	2 kg/ha	P 1,250/100 gm
Malachite green	Malachite green	Disease control, daily for 2 d, 1 m water	1 kg/ha	P 300/25 gm
Unknown	CDS 100	Pond prep.	0.6-1.2 ppm	P 106.60/kg, Phils., Ino-Aqua P 149.50/kg, Phils., Ino-Aqua P 173.33/kg, Phils., Ino-Aqua P 190/kg, Phils., Ino-Aqua
	PDS 100	Pond prep.	0.6-1.2 ppm	
	SHD 1000	Pond prep., 1 m water	15 kg/ha	
	SHD 2000	Pond prep., spray	13 kg/ha	

Table 4. Soil and water treatment chemicals used in *Penaeus monodon* grow-out ponds in the Philippines.

Chemical Group	Commercial Product/ % Activity	Pattern of Use	Amount Used	Price
Lime	Hydrated lime [Ca(OH) ₂]	Pond prep., broadcast Rearing phase, periodic Disease control	500-2,000 kg/ha 20-300 kg/ha 50-300 kg/ha	P 1,800/t
	Agricultural lime (CaCO ₃)	Pond prep., broadcast Rearing phase, 1x/wk-daily Disease control	200-8,000 kg/ha 10-500 kg/ha 100-300 kg/ha	P 300/t
Calcium hypochlorite	Calcium hypochlorite, 70% chlorine	Pond prep., 3-7 d, 20 cm-1.3 m	50-150 kg/ha	P 4,000/50 kg
Dolomite	Dolomite	Pond prep. Rearing phase, periodic	100 kg/ha 50-250 kg/ha	P 1,200/t
Biolite	Biolite	Pond prep. Rearing phase	100 kg/ha 100 kg/ha	
Zeolite	Zeolite	Rearing phase Disease control, daily until disease disappears	80-300 kg/ha 50 kg/ha	P 350/25 kg
	Daimetin	Rearing phase	100-150 kg/ha	
	Health lime Health stone/Wonder stone	Rearing phase, 1x/wk until harvest Disease control, plankton die-off	150 kg/ha 200-400 kg/ha	

Table 5. Plankton growth promoters used in *Penaeus monodon* grow-out ponds in the Philippines.

Chemical Group	Commercial Product	Pattern of Use	Amount Used	Price/Country of Origin/Manufacturer
Inorganic fertilizer	16-20-0 (mono-ammonium phosphate)	Pond prep., broadcast	4-100 kg/ha	P 305/50 kg
		Rearing phase, periodic, broadcast	150-300 kg/ha	
	18-46-0 (diammonium phosphate)	Pond prep.	3.2-50 kg/ha	P 405/50 kg
		Rearing phase	0.6-20 kg/ha	
	14-14-14 (NPK, complete fertilizer)	Pond prep.	7.5-15 kg/ha	P 310/50 kg
		Rearing phase	3 kg/ha	
	46-0-0 (urea)	Pond prep.	5-120 kg/ha	P 380/50 kg
		Rearing phase	3.2-5 kg/ha	
0-20-0 (solophos)	Pond prep.	3-20 kg/ha	P 220/50 kg	
	Rearing phase	5-10 kg/ha		
21-0-0 (ammonium sulfate)	Pond prep.	100-500 kg/ha	P 195/50 kg	
Organic fertilizer	Chicken manure	Pond prep., broadcast	3-50 kg/ha	P 450/50 kg
		Rearing phase, broadcast	5-10 kg/ha	
Organic fertilizer	Chicken manure	Pond prep., tea bags	100-3,000 kg/ha	P 1,000/t
		Rearing phase, tea bags	100-1,000 kg/ha	
	Cow manure	Pond prep., tea bags	100-500 kg/ha	P 75/50 kg
		Rearing phase, tea bags	100-200 kg/ha	
	Carabao manure	Pond prep., tea bags	240-300 kg/ha	P 75/50 kg
Rearing phase, tea bags	100-200 kg/ha			
VIMACA (chicken/pig manure)	Pond prep., tea bags	1,000 kg/ha		
B-4	Pond prep., substitute for manure	50 kg/ha		
Nutrients, others	Lab-me	Pond prep., 2 applications	200 mL/ha/wk	P 120/L, U.S.A., Nutri-Systems
	Algae grow	Pond prep.	0.5 ppm	P 127/kg
	Unknown growth factor	Pond prep., broadcast	30 kg/ha	Taiwan
	PA-100	Rearing phase, every 15 d up to DOC 90	15 kg/ha	P 130/L, Philippines, Ino-Aqua
Pond prep.		0.1-0.2 ppm		

Table 6. Organic matter decomposers used in *Penaeus monodon* grow-out ponds in the Philippines.

Chemical Group	Commercial Product	Pattern of Use	Amount Used	Price/Country of Origin/Manufacturer
Bacteria + enzyme Preparation	ER 49	Pond prep., broadcast	4-5 kg/ha	P 1,350/kg, U.S.A Nutri-Systems
	NS-SPO series	Pond prep.	160-320 gm/ha	P 1,100/kg, U.S.A., Nutri-Systems
		Rearing phase, every 7 d until harvest	2-3 kg/ha/culture	
	Biozyme	Disease control	1 L/ha	5 kg/ha 5 kg/ha
		Pond prep.		
	Micro aid activator	Rearing phase, every 7 d until harvest		5-20 kg/ha 10-20 kg/ha
Pond prep.				
Aquazyme	Rearing phase, 1x/wk up to harvest		0.5 kg/ha	P 370/500 gm
Twiner 19	Rearing phase, 2x/wk, every water change		2 kg/ha	5 kg/ha
	Disease control, daily for 3 d			
	Twiner 19	Rearing phase, every 7 d		

Table 7. Pesticides and algicides used in *Penaeus monodon* grow-out ponds in the Philippines.

Chemical Group	Commercial Product	Pattern of Use	Amount Used	Price
Saponin	Teaseed powder	Pond prep., broadcast, 20 cm-1 m water Rearing phase, periodic Disease control, first 30-60 d	8-30 ppm 5-25 ppm 15-35 ppm	P 1,200/50 kg
Copper compounds	Copper control	Pond prep., spray Rearing phase, until phytoplankton bloom	2 ppm 2 kg/ha/d	P 50/100 gm
Potassium permanganate	Potassium permanganate	Pond prep., spray	2 ppm	P 1,250/100 gm

Table 8. Feed additives used in *Penaeus monodon* grow-out ponds in the Philippines.

Chemical Group	Commercial Product/ % Activity	Pattern of Use	Amount Used	Price/Country of Origin/ Manufacturer	
ANTIMICROBIALS: Chloramphenicol	Chloramphenicol	DOC 1-30	3 gm/kg feed	P 3,800/kg, Germany	
		Disease control	2-2.5 gm/kg feed		
Tetracycline	Oxytetracycline	DOC 1-30	3 gm/kg feed	P 1,300/kg, Germany	
		Disease control, 3x/d for 3-7 d	1-5 gm/kg		
Oxolinic acid	Oxolinic acid	DOC 12-60, 1-3x/d Disease control, 1-3x/d for 7 d	1 gm/kg feed 0.2-4 gm/kg feed	P 18,000/kg	
Furazolidone	Furazolidone, 98%	DOC 1-100, 5x/d	1 gm/kg feed	P 1,600/kg, Taiwan	
	PE-30	Disease control DOC 1-35, alternate with vitamin/ wk, all feedings for 5-7 d	1-2.5 gm/kg feed 20 gm/kg feed	P 400/kg, Philippines	
	PE-40	Disease control, 2-3x/d for 5-7 d	20 gm/kg feed	P 800/kg, Philippines	
	PE-60	DOC 1-30, alternate with PE-30, 4-5x/d	20 gm/kg feed	P 550/kg, Philippines	
VITAMINS/LIPIDS/ MINERALS/ PROTEIN: Vitamin C	Ascorbic acid Aquamix	Rearing phase, DOC 60-120, 1x/d DOC 37 to harvest, 3x/wk Disease control, daily for 3-5 d	1-5 gm/kg feed 20 gm/kg feed 20 gm/kg feed	P 700/kg, Roche	
	Rovimix Stay C	Rearing phase, DOC 1 to harvest, 1x/d	1-20 gm/kg feed		
	Enervon C (capsule) (syrup)	Rearing phase Rearing phase	0.5-5 gm/kg feed 10 mL/kg feed	P 6.00/500 mg	
	SVT	Rearing phase	25 mL/kg feed	P 1,000/L	
	Stroner	Rearing phase, DOC 13 to harvest, 5x/d	2-3 gm/kg feed	P 370/500 gm, Taiwan	
	Hypo 66	Rearing phase, DOC 1 to harvest, 2x/d	25 gm/kg feed		
	Bactozyme	Rearing phase, DOC 13 to harvest, 5x/d	5 gm/kg feed		
	Astaxanthin + Vitamin C	Nutri Asta-C	DOC 1 to harvest, 1x/d	4-5 gm/kg feed	P 1,100/kg, U.S.A., Nutri-Systems
			Disease control, 3-4x/d	5-10 gm/kg feed	
	Vitamin A, C, E	Aquace	DOC 1 to harvest, 1x/d	1-2 gm/kg feed	P357.35/100 gm, England P 4,860.30/2 kg, PH Pharma- ceuticals

Table 8. Continued . . .

Chemical Group	Commercial Product/ % Activity	Pattern of Use	Amount Used	Price/Country of Origin/ Manufacturer
Vitamin A, D + fatty acid + protein	Nutri-Pro	Rearing phase, 5x/d	5-10 gm/kg feed	P 250/kg, U.S.A., Nutri-Systems
Enzyme/vitamin/mineral	Nutria	Rearing phase	1 gm/kg feed	P 850/kg, U.S.A., Nutri-Systems
Fatty acid	Aquatak	Coating medium	20 mL/kg feed	P 567.70/L, England, PH Pharmaceuticals
	Grow-Well	Coating medium	30 mL/kg feed	P 56/kg, Taiwan
	Nutri-Oil	Coating medium	20 mL/kg feed	P 480/gal, U.S.A., Nutri-Systems
	Fin-oil	Coating medium	2 gm/kg feed	P 157.25/L
	Cooking/squid/cod liver oil Chicken egg	Coating medium Coating medium	10-20 mL/kg feed 1-2 pc/kg feed	P 60/L, P 120/L P 2.50/pc
Calcium compound	Calcium lactate	Rearing phase, 1 wk prior to harvest, 1x/d	10 tablet/kg feed	P 30/100 tablets
	HUFA (B-meg)	Rearing phase, 5 d, 5x/d	10 mL/kg feed	
ANTIMICROBIAL/ VITAMIN/ MINERAL MIX:				
Unknown	Inoxyline	DOC 1-30, 2x/d	2 gm/kg feed	P 1,625/500 gm, Philippines, Ino-Aqua Systems
		DOC 31-1 mo before harvest, 2x/d	0.5 gm/kg feed	
	Ino-Forte	Disease control, 10 d, 5x/d, 30 d withdrawal	2 gm/kg feed	P 2,340/500 gm, Philippines, Ino-Aqua Systems
	Ino-moto	Rearing phase, 5x/d	2-3 gm/kg feed	Philippines, Ino-Aqua Systems
	Ino stress	Disease control, 5x/d	2-3 gm/kg feed	Philippines, Ino-Aqua Systems
	Terravite	Rearing phase, DOC 1-30, 5x/d, alternate with Inoxyline or PE-30	5 gm/kg feed	Pfizer
	Chronic Prevention Herbal	Disease control, 7 d, 1x/d	20 gm/kg feed	

Table 9. Chemical and biological products used in milkfish ponds in the Philippines.

Chemical Group	Commercial Product / % Activity	Pattern of Use	Amount Used	Price/Country of Origin/Manufacturer
SOIL AND WATER TREATMENT:				
Lime	Agricultural lime Hydrated lime	Pond prep., broadcast Pond prep., broadcast	300-5,000 kg/ha 150-1,000 kg/ha	P 300/t P 1,800/t
PLANKTON GROWTH PROMOTERS:				
Inorganic fertilizer	18-46-0 (diammonium phosphate) 16-20-0 (mono-ammonium phosphate) 46-0-0 (urea)	Pond prep., broadcast Pond prep., broadcast Rearing phase, every 15 d up to harvest, broadcast Pond prep., broadcast Rearing phase, every 15 d up to harvest, broadcast	50-150 kg/ha 100-300 kg/ha 3.2 kg/ha 25-200 kg/ha 12 kg/ha	P 405/50 kg P 305/50 kg P 380/50 kg
Organic fertilizer	Chicken manure Goat/pig manure Bioearth	Pond prep., broadcast Rearing phase, tea bags Pond prep., broadcast Pond prep., broadcast	500-3,000 kg/ha 200 kg/ha 500-1,000 kg/ha 500 kg/ha	P 1,000/t P 180/50 kg
PESTICIDES:				
Saponin	Teaseed powder/cake, 10%	Pond prep., broadcast	5-400 kg/ha	P 1,200/50 kg
Nicotine	Tobacco dust	Pond prep., broadcast, substitute for teaseed	400 kg/ha	
Rotenone	Derris root, 10%	Pond prep., broadcast, 25 cm water rearing phase	300-800 kg/ha	P 12/kg
Organotin	Brestan, 60% Gusathion	Pond prep., 1/yr-1/3 yr, broadcast or spray Pond prep.	300-800 kg/ha 250-600 gm/ha 0.1 ppm	P 2,500/kg, Indonesia/Malaysia, Hoescht Philippines, Planters Products
Azimphos ethyl Saponin, flavonoid, and tannin	Hostathion Protek FP (24.5%)	Pond prep. Pond prep., broadcast	1 L/3 ha 45-75 kg/ha	P 630/15 kg, Philippines, Cyanamid
Benzene hexachloride Endosulfan	Diazinon/Zumithion Thiodan	Pond prep. Pond prep.	0.1 ppm	P 600/L, Hoescht

Table 10. Supplementary list of products available in the market for use in intensive prawn farms in the Philippines. ¹

Use	Chemical Group	Commercial Product
Therapeutants and disinfectants	Erythromycin, Doxycycline Nitrofurans Oxolinic acid Sulfa drugs Assorted antibiotics Iodine Alkyl dimethyl benzyl ammonium chloride Calcium sulfide Laundry detergent Sodium hypochlorite	Y Mycin Furazan Oxalic acid Bacta-S 051 Sulfa Drug Antibiodice 106 Chiefiodo Poly-iodon Aquazal Progen Propond Tide Sodium hypochlorite
Neutralizer, chelator	Sodium thiosulfate	Sodium thiosulfate
Organic matter decomposer	Bacteria+enzyme preparations	Fritz-Zyme Photo Synthemim Soil reformer
Pesticides, algicides, fungicides, parasiticides, herbicides	Copper compounds Dichloro-phenoxy-acetic acid	Prolongcop Cutrine Plus 2-4-D
Plankton growth promoters	Inorganic fertilizers and/or minerals Phosphorus	Biophos Greenpond Tan-Pax-So Super P
Feed additives	Vitamins, minerals, enzymes and/or hormones Proteins and protein extracts Microorganisms (bacteria, yeast and/or enzymes)	Ebi-C Ebizyme Prawnnon Prawn strong Progromone Vitpac Vitamin B complex Prohepa Powder Feed 2000 Toaraze Protase

¹Modified from Primavera *et al.* (1993).

FARM MANAGEMENT AND THE USE OF CHEMICALS

Preventive Methods

Maintenance of good water quality and sanitation is important in the hatchery and grow-out ponds. Rearing tanks in shrimp hatcheries are cleaned and disinfected between culture periods with detergent solution and 200 ppm chlorine for at least 1 h or 100 ppm for several hours and dried under the sun for 1-2 d (Lio-Po *et al.* 1989). In the present survey, some hatcheries used as high as 1000 ppm chlorine solution for a few seconds to disinfect rearing tanks. Treated water is discharged directly into the sea. Hydrochloric acid solution at 10% is also splashed onto tanks, which are rinsed thoroughly with fresh water to remove the chemical, and sun dried for 1 d (Parado-Esteva *et al.* 1991). Grow-out ponds are disinfected either by complete sun-drying or by applying 1-2 t/ha hydrated lime, 2-4 t/ha agricultural lime, or 5 ppm chlorine (using 5% sodium hypochlorite or 1.5 kg/ha calcium hypochlorite) at 2 cm water depth (Apud *et al.* 1985, Apud 1988). Ammonium sulphate (21-0-0) at 100-200 kg/ha is also added immediately after liming to eradicate pests and predators (Norfolk *et al.* 1981, Apud *et al.* 1985). Rotenone powder (5-8%) at 5 ppm, derris root at 20-40 kg/ha, tobacco dust or shavings at 200-400 kg/ha, or teaseed cake at up to 10 ppm at 10 cm water depth is effective against pests, predators, and snails (Apud *et al.* 1985).

Water used for rearing in shrimp hatcheries is disinfected by chlorination or ozonation. Chlorination is done by applying calcium hypochlorite ($\text{Ca}(\text{OCl})_2$, 70%) to sand-filtered water. Lio-Po *et al.* (1989) recommended 5-20 ppm available chlorine for at least 12 h to disinfect rearing water in shrimp hatcheries. Baticados and Pitogo (1990) reported that chlorination with 5-30 ppm for 24 h significantly reduced the initial bacterial load from 10^5 to 10^0 - 10^1 cfu/mL. Our survey showed that a level as high as 70 ppm chlorine is being used to disinfect rearing water. Chlorinated water is neutralized with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) until residual chlorine is zero. Chlorinated water should be used within 6 h after neutralization as bacterial load increases within 24 h (Baticados and Pitogo 1990). The addition of 5 or 10 ppm disodium salt of ethylene diamine tetraacetic acid (Na-EDTA) in rearing water improves survival of *Penaeus monodon* larvae by chelating heavy metals in the medium (Licop 1988). Ozone (O_3) is used to disinfect rearing water in some small-scale shrimp hatcheries.

Hatchery paraphernalia such as brushes, scoop nets, pails, water hoses, glassware, etc. are disinfected between use by dipping in 400 ppm chlorine for a few minutes and then rinsed thoroughly with clean fresh water. Disinfection rugs/trays for footwear are placed at the entrance of hatchery facilities using 200 ppm chlorine solution or 3% Lysol solution (Lio-Po *et al.* 1989) or 200 ppm Argentyne (10% iodine) solution. Sand filters are disinfected with 200 ppm chlorine for 24 h at least once a month (Kungvankij *et al.* 1986).

Artemia cysts are disinfected with 30 ppm chlorine or 10 ppm formalin at least 1 h before hatching (Lio-Po *et al.* 1989). Parado-Esteva *et al.* (1991) recommended 200 ppm chlorine for 30 min to disinfect *Artemia* cysts.

Shrimp spawners are usually disinfected with 5 ppm Treflan-R (23.1% trifluralin) for 1 h (Gacutan 1979) or 3 ppm Furanace (Platon 1979). Formalin at 25-200 ppm for 10-30 min has been recommended to disinfect shrimp spawners (Platon 1979, Kunvankij *et al.* 1986, Parado-Esteva *et al.* 1991). In our survey, 100 ppm for 1 h to 500 ppm formalin for 1 min were used to disinfect spawners.

Several prophylactic agents have been used on the eggs of *P. monodon*. These include 1 ppm methylene blue for 10 min, 0.5 ppm malachite green for 10 min, and 3 ppm KMnO_4 for 30 min (Kungvankij *et al.* 1986). Following treatment, eggs are rinsed with clean water. Laundry detergent (e.g. Tide) at 20 ppm for 2-4 h can also be used to disinfect shrimp eggs; again, following treatment

the eggs are rinsed thoroughly, with complete water change before hatching (Lio-Po and Sanvictores 1986). The strategic egg prophylaxis (SEP) method is used on eggs of black tiger prawn to produce monodon baculovirus (MBV)-free postlarvae (PL) (Natividad and Lightner 1992). The SEP method recommends that eggs be washed and rinsed with benzalkonium chloride, calcium hypochlorite, iodine, or ozone-treated sea water several hours before hatching. This method produces MBV-free PL15. MBV is detected at PL7 in unwashed eggs.

Luminous vibriosis caused by *Vibrio harveyi* and occasionally by *V. splendidus* in larval and postlarval *P. monodon* has been treated with a wide variety of chemicals at prophylactic levels (Baticados *et al.* 1990a, Lavilla-Pitogo *et al.* 1990). These include 2-4 ppm chloramphenicol every other day, 0.5 ppm furazolidone, and 0.07 ppm rifampicin every other day (Baticados and Paclibare 1992). In our survey, the most commonly used chemicals were oxytetracycline (1-2 ppm every other day), chloramphenicol (1 ppm every other day), furazolidone (0.5-1 ppm every other day), and rifampicin (0.1 ppm every other day), given either singly or in combination. In grow-out ponds, 5-15 ppm hydrated lime and 15 ppm teaseed powder are applied as prophylactic agents against luminous vibriosis for 12 h for the first 30-60 d of culture (DOC). Antimicrobials are also incorporated in artificial feeds. These include oxytetracycline or chloramphenicol (3 gm/kg feed for the first 30 d), oxolinic acid (1-1.2 gm/kg feed from DOC 1-60 given for 1-2 feeding rations/d), and furazolidone (20 gm PE-30/kg feed from DOC 1-60).

Fungal infection in larval stages of *P. monodon* is prevented by using 0.1 ppm Treflan-R or 0.1 ppm trifluralin for 24 h every 2-3 d (Gacutan 1979, Baticados *et al.* 1990b). The present survey shows that 0.1 ppm Treflan-R is used as a prolonged bath for 3-5 d. Malachite green is also used as a prophylactic agent against fungi at levels of 0.003-0.015 ppm administered every other day from mysis stage. The reported 96 h LC₅₀ of mysis stage to malachite green is 0.006 ppm (Lio-Po *et al.* 1978).

THERAPEUTIC MEASURES

Shrimp diseases

Luminous Vibriosis

In shrimp hatcheries, luminous vibriosis is treated with antimicrobials such as baths of 0.05-1 ppm Prefuran for 24 h, 10-20 ppm furazolidone for 24 h, 4 ppm erythromycin, and 1-5 ppm oxytetracycline (Baticados and Paclibare 1992). In our survey, luminous vibriosis in shrimp hatcheries was treated using long baths of either 2-4 ppm oxytetracycline, 2-4 ppm chloramphenicol, 2-3 ppm furazolidone, 1 ppm Prefuran, or 2-3 ppm erythromycin for 3 consecutive days with minimal success. In grow-out ponds, various chemicals are used. These include 25 ppm hydrated lime for 4 consecutive days, 0.5-3 ppm benzalkonium chloride, and 1 ppm Bromosept-50. Antimicrobials are also added to artificial feeds such as oxytetracycline (2-5 gm/kg feed for 3 d given at all feeding rations), chloramphenicol (2-2.5 gm/kg feed for 3 d given 5 times per d), oxolinic acid (1.2-4 gm/kg feed given 5 times per d), or furazolidone (1 gm/kg feed given 5 times per d). However, it has been shown that chemotherapy is of limited use in luminous vibriosis (Baticados *et al.* 1990a).

Shell Disease

Exoskeletal lesions in tank- and pond-reared shrimp have been associated with *Vibrio* spp. (Lio-Po and Lavilla-Pitogo 1990). *In-vitro* tests showed that the isolates were sensitive to chloramphenicol, furazolidone, nitrofurantoin, oxytetracycline, and sulfamethoxazole trimethoprim. However, the use of antimicrobials is recommended only for tank-reared broodstock.

Filamentous Bacterial Disease

Filamentous bacterial disease caused by *Leucothrix mucor* in postlarval stages of *P. monodon* is treated using Cutrine-Plus at 0.15 ppm copper in 24 h flow-through treatments or with 0.5 ppm copper for 4-6 h short bath (Baticados *et al.* 1990b). The 48 h LC₅₀ of copper sulphate to larval *P. monodon* is 0.2 ppm (Canto 1977).

Larval Mycosis

Larval mycosis in shrimp caused by *Lagenidium callinectes*, *Lagenidium* sp., and *Haliphthoros philippinensis* (Baticados *et al.* 1977, Hatai *et al.* 1980) is treated with 0.2 ppm Treflan-R or 0.2 ppm trifluralin for 24 h using the static or drip method (Lio-Po and Sanvictores 1986, Baticados *et al.* 1990b). Gacutan (1979) reported that 2-4-D (2-4-dichloro-phenoxy-acetic acid), a herbicide, has a 96 h LD₅₀ of 0.6 ppm for mysis stage and is effective in controlling *Lagenidium* infections. Furanace (6-hydroxymethyl-2-2-5-2-furyl vinyl pyridine), reported to be effective against bacterial and fungal pathogens, has a 24 h LC₅₀ of 1.6, 2.0, and 5.0 ppm for zoea, mysis, and postlarvae, respectively (Gacutan *et al.* 1979). The *in vitro* effect of fungicides to *Lagenidium* and *H. philippinensis* has been reported by Lio-Po *et al.* (1982, 1985).

Protozoan Infections

Ciliated protozoans such as *Acineta*, *Ephelota*, *Epistylis*, *Vorticella*, and *Zoothamnium* in juvenile and adult shrimp are treated with 30-100 ppm formalin for 30 min (Baticados *et al.* 1990b). However, these levels are lethal to larvae and postlarvae (Vicente and Valdez 1979, Lio-Po and Sanvictores 1986).

Fish Diseases

Bacterial Infections

Bacterial infections associated with post-transport mortalities in milkfish juveniles can be controlled using oxytetracycline (OTC) baths for 5 d (Lio-Po 1984). Transport-stressed milkfish fingerlings are also treated with 1 ppm Furanace bath for 5 d (Lio-Po 1984). The reported 96 h LC₅₀ of Furanace (nifurpirinol) to milkfish fingerlings is 1.7 ppm (Tamse and Gacutan 1994). Localized *Vibrio parahaemolyticus*-like infection (Lio-Po *et al.* 1986) due to repeated hormone implantations in milkfish broodstock is routinely treated with topical application of Terramycin after each implantation (Lacanilao *et al.* 1985). The vibrios isolated are sensitive to polymyxin B and sulfamerazine. *Vibrio parahaemolyticus*-like bacteria are also associated with opaque-eyed milkfish juveniles (Muroga *et al.* 1984, Lavilla-Pitogo 1991).

Vibrio sp. isolated from juvenile and adult grouper are sensitive to chloramphenicol, nalidixic acid, and oxytetracycline (Lavilla-Pitogo *et al.* 1992a). Vibriosis in broodstock and adult grouper (3-7 kg) is controlled by intramuscular injection of 25 or 50 mg OTC/kg body weight of fish for 5 d (C. Lavilla-Pitogo and M.C.L. Baticados, pers. comm.). Grouper broodstock with opaque eyes, petechiae, or suspected to have bacterial infection are treated with 100 ppm Ektecín (30% sulfamonomethoxine and 10% ormetoprim, Daiichi, Japan) bath for 1 h for 5-7 d (N. Yasunaga, pers. comm.), 100 ppm OTC bath for 1 h for 5-7 d (L. de la Peña, unpubl. data), or 200 ppm formalin bath for 1 h for 5-7 d (G.F. Quintio, pers. comm.).

In spotted scat (*Scatophagus argus*), bacterial infections are controlled by 50 ppm chloramphenicol bath for 10 h, oral administration of chloramphenicol at 500-750 mg/kg feed given at 3-10% body weight for 5-7 d, or by intramuscular injection of either chloramphenicol at 15 mg/kg fish or OTC at 20-50 mg/kg fish (Cruz and Barry 1988).

Infections by *Flexibacter columnaris* and *Aeromonas* sp. in catfish (*Clarias macrocephalus*) can be controlled after oral administration of Dimeton (sulfamonomethoxine) given at 50-200 mg/kg fish or tetracycline at 20-100 mg/kg fish for 3-7 d (S. Hara, unpubl. data). Tank-reared fry of bighead carp (*Aristichthys nobilis*) infected by *Pseudomonas* sp. and silver carp (*Hypophthalmichthys molitrix*) broodstock with *Aeromonas hydrophila* and *Citrobacter* sp. infections are treated by injection with 7.5 gm OTC/100 kg fish/d for 7-12 d (F. Palisoc, pers. comm.). Fry of Nile tilapia (*Oreochromis niloticus*) reared in nursery tanks with *Pseudomonas* infection are fed oxytetracycline-treated artificial feeds at 7.5 gm/100 kg fish/d for 7-12 d (F. Palisoc, pers. comm.). Kanamycin and oxytetracycline show some promise in controlling *Pseudomonas* sp. in tilapia fry (Lio-Po and Sanvictores 1987).

Fungal Infections

Fungal infections in milkfish are controlled using baths of 10 ppm potassium permanganate (KMnO_4), 2 ppm pyridyl mercuric acetate, or 10 ppm malachite green for an undisclosed period (Timbol 1974). The reported 24 and 96 h LC_{50} of KMnO_4 to milkfish fingerlings are 1.5 and 1.2 ppm, respectively (Cruz and Tamse 1989); however, significant histopathological changes are observed in gills, liver, and kidney even at sub-lethal concentrations (Cruz and Tamse 1986). In spotted scat, fungal infection is treated with a combination of 0.10 ppm methylene blue and 24 ppm formalin for 1 wk (Lio-Po and Barry 1988). Malachite green at 1% is swabbed directly onto dermal lesions and fins of spotted scat to control secondary fungal infections (Cruz and Barry 1988). Juvenile seabass (*Lates calcarifer*) with suspected fungal infections are dipped in 100 ppm malachite green for a few seconds (R. Duremdez-Fernandez, pers. comm.). Fungal infections of adult carp cultured in cages are treated with 10,000 ppm salt indefinitely after fish have finished spawning and before being returned to their cages (F. Palisoc, pers. comm.).

Parasitic Infections

In milkfish broodstock, infestations of *Caligus* can be treated with 0.25 ppm Neguvon (2,2,2-trichloro-1-hydroxyethyl-phosphoric acid-dimethylethol) bath for 12-24 h (Laviña 1978) or 90 ppm formalin bath for 2 h (Lio-Po 1984). Milkfish fingerlings can tolerate formalin at 200 ppm for 48 h or 100 ppm for 96 h without any adverse effect (Cruz and Pitogo 1989). Mass infection of milkfish by larval stages of *Lernaea* sp. is treated with 3-5% salt solution, while adult stages of the parasite can be controlled by drying and liming the pond bottom (Velasquez 1979). In spotted scat, *Trichodina* sp. and *Amyloodinium* sp. are treated with an indefinite bath of 0.75 ppm CuSO_4 and a combination of 0.1 ppm malachite green/24 ppm formalin for 24 h, while *Caligus* is treated with 0.25 ppm Neguvon for 30 min (Lio-Po and Barry 1988). In grouper, a protozoan (possibly *Cryptocaryon irritans*) is controlled using a combination of 25 ppm formalin and 0.1 ppm malachite green (Baticados and Paclibare 1992). Infections by monogeneans such as *Diplectanum* sp. in grouper juveniles are treated with 50-100 ppm formalin for 1 h (E.R. Cruz-Lacierda, unpubl. data). Recently, infestation of tank-held adult groupers by a marine leech was treated with 50-100 ppm formalin bath for 1 h (E.R. Cruz-Lacierda and J.D. Toledo, unpubl. data). The tolerance levels of milkfish and seabass fingerlings to formalin have been reported by Cruz and Pitogo (1989) and Pascual *et al.* (1994), respectively.

White spot disease caused by *Ichthyophthirius multifiliis* in Nile tilapia is controlled by a combination of 25 ppm formalin and 0.1 ppm malachite green bath (Baticados and Paclibare 1992). Infections by *Trichodina* and monogeneans in tilapia fry are treated with indefinite bath of 10,000 ppm salt solution, while infections by *Lernaea* in tilapia broodstock and silver carp fry are treated with 0.25 ppm Dipterex and indefinite bath of 1000-2000 ppm salt or 15-25 ppm formalin, respectively (F. Palisoc, pers. comm.). *Argulus* on tilapia is controlled using a 5 ppm KMnO_4 bath for 5-10 min (Baticados and Paclibare 1992).

HAZARDS OF CHEMICAL USE

The use of chemicals is disadvantageous because: (1) chemicals may be detrimental to treated animals; (2) they may have adverse effects on non-target organisms such as natural food organisms present in the culture system; (3) they may lead to development of drug-resistant bacterial strains through the overuse or misuse of antimicrobials; (4) they are potential threats to human health; (5) their residues may accumulate at harmful levels in fish flesh and in the environment; and (6) the cost of application can be prohibitive.

The use of chemicals to control luminous vibriosis in black tiger prawn larvae results in mortalities or morphological deformities at concentrations that are known to control the disease (Baticados *et al.* 1990a). Cutrine-Plus, a copper-based chemical used against filamentous bacteria, is toxic to prawn larvae at concentrations effective against the pathogen (Baticados *et al.* 1990b). Formalin is effective against protozoan infections in juvenile and adult shrimp; however, it is toxic to larval stages (Lio-Po and Sanvictores 1986). The use of molluscicides such as Aquatin (Baticados *et al.* 1986) and Gusathion (Baticados and Tendencia 1990) in shrimp ponds can result in chronic soft-shelling.

The exposure of non-target species such as *Tetraselmis chunii*, a phytoplankton used as food for penaeid larvae, to 0.1 ppm trifluralin delays growth and reduces protein content of the alga (Dimanlig 1981). Furazolidone at 0.5 ppm and 2 ppm nitrofurazone significantly reduce the cell size, growth rate, and chlorophyll A content of *Chaetoceros calcitrans* (R. C. Duremdez-Fernandez, pers. comm.). Nauplii of *Artemia* can tolerate oxytetracycline, furazolidone, erythromycin, sodium nifurstyrenate, and Treflan-R, but growth is negatively affected (E.R. Cruz-Lacierda, unpubl. data).

Observations of carcinogenesis and mutagenesis in laboratory animals have led the U.S. Food and Drug Administration (US FDA) to cancel all registered uses of nitrofurans such as furazolidone, nitrofurazone, and nifurpirinol (Meyer and Schnick 1989, Schnick 1991). Malachite green has potential carcinogenic and teratogenic properties (Bailey 1983). Organotin compounds (e.g., Aquatin, Brestan, Gusathion) used as snail killers in milkfish and shrimp ponds have teratogenic properties and suppress immune response in mammals, leading to increased susceptibility to infections (Dean and Murray 1992).

The widespread use of antimicrobials significantly leads to the development of drug-resistant bacterial populations (Aoki 1992). Shotts *et al.* (1976) showed that continued use of oxytetracycline enhances the production of plasmid-mediated resistance in aquatic bacteria. Beladi *et al.* (1978) reported that nitrofurans such as Prefuran can rapidly cause bacterial resistance because of their persistence in water. Baticados *et al.* (1990a) reported that luminous vibrios are resistant to erythromycin, kanamycin, penicillin, and streptomycin, have varied responses to chloramphenicol and Prefuran, and low sensitivity to oxytetracycline. The prevalence of infectious diseases in shrimp hatcheries in the Philippines despite the widespread use of antibiotics suggests that drug resistance has developed among bacterial pathogens (Baticados and Paclibare 1992).

Some chemicals used in aquaculture have potential side effects that may affect users and consumers. Chloramphenicol has been reported to destroy the erythrocytes in humans and can cause aplastic anemia, stomatitis, and other conditions (Farkas *et al.* 1982, Brown 1989, Meyer and Schnick 1989). Chloramphenicol is banned for use in aquaculture by the US FDA (Schnick 1991) and by the Philippine government (Department of Agriculture, A.O. No. 60 and Department of Health, A.O. No. 91, Series of 1990). Oxytetracycline, furazolidone, erythromycin, and kanamycin can cause digestive disorders and allergies among humans (Schnick 1991).

Chemicals also accumulate in the flesh of treated animals. In 1991, shipments of locally grown *P. monodon* were rejected in Japan because of antibiotic residues (Lacanilao *et al.* 1992). Studies have also established pesticide accumulation in milkfish tissues (Palma-Gil, pers. comm.).

Chemicals are also potential threats to the environment. They can enter the environment directly, leach from uneaten feeds, or be excreted in the feces (Primavera 1993). Effluents that are dumped directly into the sea can affect neighboring ecosystems (Primavera 1991). As a consequence, water quality problems brought about by farm effluents increase (Phillips 1995).

Chemicals, particularly the antimicrobials, are also very expensive. Large amounts are needed in bath treatments, and they may not be effective at all in systemic infections. The use of medicated feeds may also be ineffective, as diseased animals become anorexic.

MEASURES EMPLOYED TO IMPROVE PRODUCTIVITY

Fertilization is a standard practice during pond preparation to enhance the growth of natural food and fish production. The amount of fertilizer and feed required varies with the intensity of culture. Extensive culture systems rely completely on the natural productivity of the ponds, thus they require heavy inputs of organic fertilizer. Semi-intensive and intensive systems require less fertilizer, but greater inputs of artificial feeds. Fresh supplemental feeds are also used, such as trash fish and mussel meat.

In extensive shrimp ponds, chicken manure at 1-2 t/ha, ammonium phosphate (16-20-0) at 75-150 kg/ha, and urea (46-0-0) at 25-50 kg/ha are usually applied (Apud 1988). Additional fertilizers are applied every two weeks during the culture phase at 100 kg/ha and 10-30 kg/ha for organic and inorganic fertilizer, respectively (Apud *et al.* 1985). In intensive ponds, organic fertilizer is applied at 50-100 kg/ha and inorganic fertilizer at 20-30 kg/ha to induce plankton growth and maintain good water quality (Primavera 1992).

In milkfish ponds, the most commonly used inorganic fertilizers are solophos (0-20-0), monoammonium phosphate (16-20-0), diammonium phosphate (18-46-0), and urea (46-0-0). All these were originally intended for agriculture and not for aquaculture use (Fortes 1984). Animal manure from chicken, pig, cow, and carabao or rice bran are commonly used organic fertilizers. The use and application of organic and inorganic fertilizers have been reviewed by Fortes (1984). A level of 1 ppm nitrogen and 1.5 ppm phosphate should be maintained to sustain the growth of benthic algae (Tan *et al.* 1984). Traditional fertilization practice entails application of 16-20-0 at 50 kg/ha and 45-0-0 at 15 kg/ha (Bombero-Tuburan *et al.* 1989).

Although fertilization can enhance production, it may also cause soil and water condition to deteriorate if applied indiscriminately. The use of piggery wastes in fish-pig farming has a negative effect on the growth and production of milkfish (Fortes *et al.* 1980). A preliminary study on the use of chicken manure in shrimp culture shows that it can be a source of *Salmonella* contamination in shrimp tissue (Llobrera 1988). Application of chicken manure at 0.5 t/ha and MASA (a fertilizer processed from agricultural and industrial wastes) at 0.5 t/ha results in fish kills due to the build-up of organic matter in the pond bottom, depletion of dissolved oxygen, and presence of hydrogen sulfide (Bombero-Tuburan *et al.* 1989). A lower input of organic fertilizer is recommended during the rainy season. Subosa and Bautista (1991) showed that biweekly application of 15 kg of nitrogen and 30 kg of phosphorus per ha with or without chicken manure can increase shrimp production; however, further increase in fertilizer application does not improve production. Subosa (1992) tested chicken manure, rice hulls, and sugar mill wastes as potential organic fertilizers in extensive shrimp ponds. Boiler ash (derived from burned bagasse from sugar mills) is a more efficient fertilizer than chicken manure in terms of growth and survival of tiger shrimp.

The role of hormones such as thyroxine in enhancing larval growth, development, and survival has also been studied. Treatment of yolk-sac larvae of Nile tilapia with thyroxine (T₄) by immersion in 0.1 ppm significantly increases length and weight of fry after 4 wk (Nacario 1983). Treatment with 0.5 ppm L-thyroxine-sodium (Eltroxin, Glaxo) by immersion for 15 d stimulates growth and

development in milkfish fry (Lam *et al.* 1985). Larvae of rabbitfish (*Siganus guttatus*) from spawners injected with 10 and 100 µg T4/gm B.W. are longer and show better survival than control fish (Ayson and Lam 1993). Treatment of larval grouper (*Epinephelus coioides*) with 0.01-1 ppm triiodothyronine (T3) or T4 by immersion or by feeding with T3- or T4-enriched *Artemia* resulted in faster metamorphosis than seen in an untreated group (de Jesus *et al.* 1998). Grouper larvae treated with T3 and T4 and stocked in grow-out ponds show faster growth rate than untreated fish (E. Rodriguez, pers. comm.).

OTHER APPROACHES TO DISEASE PREVENTION

Control of diseases through the use of chemicals appears to be limited and ineffective. Other approaches to disease prevention such as environmental and biological methods should be evaluated.

In luminous vibriosis in shrimp, potential sources and routes of entry of bacteria into the larval rearing system have been identified to establish a set of preventive measures (Lavilla-Pitogo *et al.* 1992b). Results showed that *V. harveyi* can enter the hatchery system through the fecal matter from spawners, sea water, or unwashed *Artemia* cysts. The authors suggested that spawners and their fecal matter must be separated from the eggs after spawning and the eggs washed to prevent infection. Natural food such as diatoms may still be used, as these show some antibacterial properties.

The health condition of shrimp and fish larvae or juveniles is assessed prior to stocking in grow-out ponds. It is a common practice among shrimp growers to screen postlarvae for monodon baculovirus (MBV) occlusion bodies, luminous bacteria, and parasites. Of the 144 shrimp samples analyzed by the Fish Health Section of SEAFDEC/AQD in 1995, 23.6% were positive for MBV. Generally, postlarvae infected with MBV have a lower market value. The ratio of the tail muscle to the hindgut diameter is also considered in shrimp fry selection procedures, with a ratio of 4:1 (below PL20) as an indicator of good quality fry. Younger shrimp fry (PL14) can also be subjected to 15 ppt salinity or 100 ppm formalin stress tests for 2 h to assess their health condition (Bauman and Jamandre 1990). Good quality fry must have 100% survival during the exposure period, recover within 24 h, and resume feeding.

Biodegradable or indigenous materials such as derris root can replace non-biodegradable compounds (e.g., Brestan) to eliminate unwanted species in ponds. The roots of *Derris elliptica*, *D. heptaphylla*, and *D. philippinensis*, which are locally available, are placed inside a sack cloth, soaked, and squeezed periodically at a rate of 20-40 kg of root/ha at 10 cm water depth. 5-10 ppm rotenone can eliminate unwanted species in ponds without adverse effect on shrimp (Tumanda 1980). Derris root powder (5-8% rotenone) is commercially available and applied at 10-20 kg/ha at 10-20 cm water depth to attain a 5 ppm concentration (Apud *et al.* 1985). Teaseed cake with saponin as its active ingredient is commonly used at 10 ppm for selective elimination of predators and competitors in shrimp ponds (Apud *et al.* 1985). The tolerances of milkfish, tilapia, and shrimp to rotenone and saponin were reported by Minsalan and Chiu (1986) and Cruz-Lacierda (1992, 1993). Rotenone degrades within 12 h (Cruz-Lacierda 1992) and the levels of rotenone and saponin commonly used in shrimp ponds do not result in chronic soft-shelling (Cruz-Lacierda 1993). Tobacco wastes (dust, shavings, stalks) at 200-400 kg/ha serve not only as predator and snail killers during pond preparation, but also as fertilizer (Apud *et al.* 1985). Toxicity studies of different types of tobacco dust on adult brackishwater pond snails (*Cerithidea cingulata*) under laboratory conditions have been conducted with 700 kg/ha (24 kg nicotine/ha) for 3 d as optimal for 99% eradication of snails (Borlongan *et al.* 1998). Further, this concentration is not lethal to milkfish juveniles. A follow-up of this study under pond conditions is currently in progress in collaboration with the National Tobacco Administration and Iloilo State College of Fisheries. Pond snails can also be eliminated by handpicking (Parado-Esteba 1995) or by burning rice straw piled 15 cm thick at the pond bottom (Triño *et al.* 1993).

The use of bioaugmentation products or probiotics in shrimp culture is a potential biological approach to disease prevention. Probiotics are bacteria and enzyme preparations designed to enhance decomposition or to encourage non-toxic bacteria to overwhelm harmful bacteria (Anon. 1991). At present, a variety of probiotic compounds are available in the market and are used in intensive shrimp farms (Table 6). Most of the available information regarding the use of these products is provided by manufacturers and suppliers. Studies to understand the principles behind bioaugmentation, probiotics, and bioremediation are limited.

The use of lower stocking density can also prevent disease occurrence. A shift to semi-intensive culture has been recommended to avoid the use of large areas in extensive systems and diseases and effluents that result from intensive systems (Primavera 1991).

Sound nutrition and adequate feeding are necessary not only for growth, but also for maintaining the overall health of aquatic animals, allowing them to cope with a variety of pathogens. Chronic soft-shell syndrome in juvenile and adult shrimp, a disease caused by nutritional deficiency, pesticide contamination, and poor water and soil conditions, among other things (Baticados *et al.* 1986), can be reversed by feeding a diet containing 14% mussel meat or a calcium-to-phosphorus ratio of 1:1 (Bautista and Baticados 1990). The feed additives Nutria (enzyme, vitamins, and mineral premix) and Nutri-oil (fish oil), when combined and incorporated into shrimp feed can significantly increase growth rates and improve feed conversion ratio (Baldia 1994). Catacutan and Lavilla-Pitogo (1994) showed that incorporation of 100-200 ppm phosphated ascorbic acid (50-100 ppm ascorbic acid) in test diets fed for 92 d improves the growth of shrimp, as shown by the structure of shrimp's hepatopancreas infected with MBV at the start of the study.

NATIONAL REGULATIONS ON THE USE OF CHEMICALS IN AQUACULTURE

In the Philippines, the Bureau of Animal Industry (BAI) through the Animal Feeds Standard Division (AFSD) formulates regulations on chemicals intended for veterinary animals. The AFSD (1) evaluates, registers, and licenses establishments which engage in the manufacture, distribution, and sale of veterinary products, including those used for aquaculture; (2) inspects and examines veterinary drugs and product premixes and water solubles; and (3) adopts and uses existing standards and requirements of the Department of Health for licensing and registration, including the applicable regulations related to generic labelling for veterinary drugs (Department of Agriculture Administrative Order No. 25, Series of 1991; effective January 1992). In June 1995, the BAI required all veterinary drug and product establishments (manufacturers, traders, and importers) to submit a valid certificate of product registration.

In 1989, the BAI required commercial prawn feed manufacturers to completely label their feed bags and containers. Such labels contain, among other things, the feed ingredients, including drugs or drug ingredients for disease prevention, percentage of drug, directions for use, warning against use under conditions dangerous to the health of livestock and man, and withdrawal period. This is an important development, as recent reports show that a lot of artificial feeds contain antimicrobials such as oxytetracycline, oxolinic acid, and chloramphenicol (Chen 1989). In April 1990, the Department of Agriculture (DA) and the DOH issued Administrative Order No. 60 and Administrative Order No. 91, Series of 1990, respectively, to ban the use and withdraw the registration of chloramphenicol in animals used as food. Violators are fined US\$ 40-200 and imprisoned for six months to two years. The Bureau of Fisheries and Aquatic Resources (BFAR) through its Fisheries Administrative Order No. 117-1, Series of 1994, has been authorized to monitor the effect of using antibiotics in shrimp culture by determining the antibiotic (oxolinic acid and oxytetracycline) residues in shrimp tissues.

In 1993, the Subcommittee on Veterinary Drugs of the Department of Health published the Philippine National Veterinary Drug Formulary. The chemicals recommended for use in aquatic

Table 11. Chemicals recommended for use in aquatic animals.¹

Chemical	Target Species	Mode of Administration Pharmaceutical Form/Strength	Withdrawal Period
ANTI-INFECTIVES: Dihydrostreptomycin sulfate Erythromycin phosphate Furazolidone Gentamycin sulfate	Ornamental freshwater finfish Ornamental marine/freshwater finfish Ornamental marine/freshwater finfish Ornamental marine/freshwater finfish	Injection: 500 mg/mL solution Oral: powder Oral: powder Injection: 10 mg/mL; 1 mL ampule, 2 mL vial 40 mg/mL; 1 mL ampule, 2 mL vial	
Isoniazid Kanamycin Neomycin sulfate Nifurpyrinol Nitrofurazone Oxolinic acid	Ornamental marine/freshwater finfish Ornamental marine/freshwater finfish Ornamental marine/freshwater finfish Ornamental marine/freshwater finfish Ornamental marine/freshwater finfish Edible/ornamental marine/freshwater finfish/crustaceans	Bath: powder Injection: 1 gm vial Bath: powder Oral/bath: powder Bath: powder Oral: powder 20 mg/kg feed	30 d
Oxytetracycline	Edible/ornamental marine/freshwater finfish/crustaceans	Oral/bath: powder	21 d
Ormetoprim	Edible/ornamental marine/freshwater finfish/crustaceans	Oral: powder	
Sulfadimethoxine	Edible/ornamental marine/freshwater finfish/crustaceans	Oral: powder	
Sulfamerazine	Edible/ornamental marine/freshwater finfish/crustaceans	Oral: powder	25 d
Sulfisoxazole diolamine	Edible/ornamental marine/freshwater finfish/crustaceans	Oral: powder	
Trimethoprim/Sulfadiazine	Edible/ornamental marine/freshwater finfish/crustaceans	Oral: powder 83.3 gm/kg Trimethoprim + 41 gm/kg Sulfadiazine	20 d
ANTIPARASITICS: Chloramine T Copper sulfate	Ornamental freshwater finfish Edible/ornamental marine/freshwater finfish/crustaceans	Bath: powder Bath: powder	
Ivermectin Malachite green oxalate (zinc free) Methylene blue	Ornamental freshwater/marine finfish Ornamental freshwater finfish Ornamental marine/freshwater finfish Juvenile penaeid shrimp	Oral: 1% solution Bath: powder Bath: powder Oral: powder	
Metromidazole Potassium permanganate	Ornamental freshwater finfish Edible/ornamental marine/freshwater finfish/crustaceans	Bath: crystal Bath: crystal	
Praziquantel Quinacrine hydrochloride	Ornamental freshwater finfish Ornamental freshwater finfish/ crustaceans	Bath: tablet Bath: powder	
Sodium chloride Trichlorfon	Edible/ornamental marine/freshwater finfish/crustaceans Ornamental freshwater finfish	Bath: crystal Bath: powder	4 wk
DISINFECTANTS: Calcium carbonate Calcium hypochlorite Calcium oxide	Ornamental marine/freshwater finfish Edible/ornamental marine/freshwater finfish/molluscs Disinfection of ponds	Bath: powder Bath: crystal Broadcast: powder	

¹Source: Philippine National Veterinary Drug Formulary, Department of Health, 1993.

species together with the target species, mode of administration, and withdrawal period are presented in Table 11.

The Fertilizer and Pesticide Authority (FPA), an attached agency of the Department of Agriculture, was created in 1977 to issue guidelines, rules, and regulations about commercial fertilizers, soil conditioners, microbial inoculants, and fertilizer raw materials prior to their distribution and sale. The FPA also registers pesticides and subsequently classifies these for general use, for restricted use, or as banned pesticides (FPA 1989). The registration process involves the review of the product, including specifications, guaranteed analysis of the composition, data on biological efficacy, experimental use permit, data on toxicity studies, residues and fate in the environment, and labelling requirements. Manufacturers, distributors, and importers are also required to secure a license from the FPA. The FPA also monitors all areas of pesticide use, including effects on the environment, pesticide residues in food, pesticide handling and use, poisoning cases, product quality, and sale and distribution. The FPA maintains linkages with the Department of Pharmacology of the College of Medicine, University of the Philippines to conduct toxicology and residue analyses. The FPA coordinates with the Department of Environment and Natural Resources (DENR) on environmental issues regarding the use of pesticides. The Food and Agriculture Organization of the United Nations (FAO) in 1986 initiated the International Code of Conduct in the Distribution and Use of Pesticides, and the Philippines was an active participant in the formulation of the regulations (Neri 1989).

Table 12. Pesticides banned for agriculture and other applications in the Philippines.¹

Generic name	Brand name	Manufacturer	
Organotin	Brestan	Hoechst	
	Aquatin 20 EC	Planters Products	
	Telustan 60 WP	Shell Chemicals	
Fenbutatin oxide	Torque 50% WP	Shell Chemicals	
	Azinphos ethyl	Gusathion 400 EC	Bayer
		Marsathion	Marsman
Methyl parathion	Bionex 40 EC	Planters Products	
	Telothion 40 EC	Shell Chemicals	
	Folidol M 50 EC	Bayer	
Endosulfan+BPMC	Methyl Fosferno 50 EC	Jardine Davis	
	Methion 50 EC	Marsman	
	Meptox 50 EC	Shell Chemicals	
	Parapest M 50 EC	Planters Products	
	Penncap M (Encap)	Aldiz	
	Wofatox 50 EC/80 EC	Chemie International	
	Wofatox Konzentrat 50 EC/80 EC	Chemie International	
	Endosulfan	Thiocarb 47 EC	Hoechst
		Thiodan 35 WP	Hoechst
		Thiodan 35 EC	Hoechst
Endosulfan 35 EC		Marsman	
Endox 35 EC		Planters Products	
Monocrotophos	Thiodan 2.5 G	Hoechst	
	Nuvacron 30 SCW	Ciba-Geigy	
	Azodrin 168	Shell Chemicals	
Mono+fenvalerate	Azodrin 202 P	Shell Chemicals	
	Azodrin 150	Shell Chemicals	
	Mono+mevinphos	Shell Chemicals	
	Mono+cypermethrin	Shell Chemicals	
	Azodrin 137	Shell Chemicals	

¹Source: Fertilizer and Pesticide Authority.

In September 1993, the FPA issued FPA Board Resolution No. 1 banning the use of organotin compounds (Table 12), particularly Brestan, Aquatin, and Gusathion, chemicals used to control snails (*Cerithidea cingulata*) in milkfish ponds. However, despite the ban, fishpond operators defy the national government's stand and continue to use Brestan for lack of an effective and cheaper alternative compound. Although Brestan has been pulled from local stores, smuggled formulations coming from Indonesia and Malaysia are being patronized and applied by milkfish pond owners.

ON-GOING RESEARCH ON CHEMICAL USE FOR AQUACULTURE

A number of institutions in the Philippines have sections working on fish health management research. The Fish Health Section of the Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC/AQD) has capabilities for virology, bacteriology, mycology, parasitology, and histopathology. It has conducted numerous studies on fish and shrimp health management, including the screening of drugs both *in-vivo* and *in-vitro* for chemoprophylaxis and chemotherapy. Several studies on tolerance limits of shrimp and fish to various chemicals have been worked out. All this information has been published in peer-reviewed national and international scientific journals and conference proceedings. Moreover, the SEAFDEC/AQD has published a pamphlet on "Recommended Practices for Disease Prevention in Prawn and Shrimp Hatcheries" (Lio-Po *et al.* 1989) and a manual on "Diseases of Penaeid Shrimps in the Philippines" (Baticados *et al.* 1990b), with industry practitioners as the target audience. Another function of the Section is to provide disease diagnostic services not only to SEAFDEC/AQD researchers, but to the private sector as well. This component provides fish/shrimp farmers with a sound disease control program. An annual "Training Course on Fish Health Management" is conducted with participants from the academe, government agencies, research institutes, and industry practitioners. Aside from basic information on fish disease, lectures and hands-on training concerning the use of chemicals in aquaculture are included in the training course.

The following are SEAFDEC/AQD's on-going studies related to the use of chemicals for aquaculture:

- effect of hormones on the metamorphosis and survival of larval fish;
- effect of possible immune modifiers on the non-specific immune response of juvenile fish;
- effect of feed additives on the growth, survival, and disease resistance of fish and shrimp; and
- utilization of indigenous plants and other compounds as molluscicides in brackishwater ponds.

The Fish Health Section of the Bureau of Fisheries and Aquatic Resources (BFAR) has been involved in the formulation of national regulations on the use of chemicals in aquaculture. Its facilities for bacteriology, mycology, parasitology, histopathology, and water quality analyses have given BFAR the capability to diagnose and conduct research on fish diseases and to determine effective health management strategies, including the consequences of using chemicals in aquaculture. As a result of excessive use of drugs, particularly antibiotics in aquaculture, BFAR saw the need to maintain the international standard of shrimp quality and protect consumers from the deleterious effects of antibiotics by providing services to assess chemical and antibiotic residues in fish and shrimp for human consumption. The on-going monitoring and training programs of the government on current fish health management practices in the country guide the agency to strengthen policies on strict implementation of proper management techniques, thereby lessening the aquaculture industry's tendency to use chemicals indiscriminately.

Other institutes with capabilities to conduct fish health management studies and monitor the impacts of chemical use in the environment are the following:

- Brackishwater Aquaculture Center (BAC), College of Fisheries and Department of Biological Science, College of Arts and Sciences, University of the Philippines in the Visayas;

- Marine Science Institute, University of the Philippines;
- Freshwater Aquaculture Center (FAC), Central Luzon State University; and
- Institute of Fisheries Research and Development, Mindanao State University.

Bioassays of organochlorine and organophosphate pesticides commonly used in rice-fish systems have been conducted by the FAC (Cagauan and Arce 1992).

CONCLUSIONS AND RECOMMENDATIONS

A number of adverse impacts of chemical use in aquaculture have been discussed. The use of chemicals in aquaculture should be regarded as a last resort and should not replace sound farm management and husbandry practices. The environmental problems of farming can be tackled by good site selection, proper design and operation, and sound management techniques (Phillips 1995).

The following recommendations are given:

For Users of Chemicals in Aquaculture:

- The use of antibiotics for prophylaxis in hatcheries should be abandoned. In the United Kingdom, antibiotics are used only as therapeutants and not for prophylaxis or growth enhancement (NCC 1989).
- The use of fresh animal manure during pond preparation and rearing phases should be prohibited.
- Banned chemicals should not be used for any purpose.
- In cases where chemotherapy is inevitable, a code of practice on the use of drugs in aquaculture such as the one suggested by Austin (1985) should be strictly followed.
- Strict observance of the required withdrawal period should be implemented.
- Effluents or treated water must not be discharged directly into the sea.
- Medically important drugs should be banned for use in aquaculture because of the possible development of antibiotic-resistant strains.

Recommendations to restrict the use of medicinal drugs for humans in aquaculture has been submitted to government agencies in charge of regulating manufacture, registration, and use of drugs (Baticados and Paclibare 1992). Austin (1985) listed several drugs that are used to treat diseases in humans and should not be used in aquaculture. These include cycloserine, doxycycline, ethionamide, isoniazid, minocycline, and rifampicin for tuberculosis; ampicillin, bacitracin, and kanamycin for staphylococcal infections; chloramphenicol for typhoid fever; streptomycin for bubonic plague and gonorrhoea; furazolidone for intestinal infections; and nitrofurantoin for urinary tract infections.

For Chemical Manufacturers and Suppliers:

- Development of new, effective, efficient, and environmentally friendly chemicals.
- Strict observance of national laws and regulations.
- Registration and licensing of new products.
- Financial support to research institutions for the conduct of research and development in fish health management.

For Government Agencies:

- Intensive dissemination of information on the consequences of using chemicals for prophylaxis should be conducted among fish and shrimp farmers, drug manufacturers and suppliers.

- A strong and intensive campaign on the careful and restricted use of drugs among fish and shrimp farmers is needed.
- Antibiotics, non-biodegradable pesticides, and disease-control chemicals should be banned from use in aquaculture.
- Strict implementation of rules and regulations on the manufacture, distribution, and sale of chemicals is needed.
- Vigilant monitoring on the use of chemicals is required.
- Violators should be apprehended and stiffer penalties imposed.
- Financial support should be provided to research institutions for the implementation of research and development in fish health management.

For Research Institutions:

- Disseminate intensively information on the consequences of using chemicals.
- Conduct a strong and intensive campaign on the careful and restricted use of drugs.
- Develop new, effective, efficient, and environmentally friendly drugs.
- Conduct additional studies to complete information required for chemicals that have pending approval from drug regulatory boards.
- Train qualified staff in new technologies.

Intensive dissemination of information on the consequences of using chemicals for prophylaxis should be done to prevent the development and spread of drug-resistant pathogens. Recently, a statement of 71 Filipino scientists appeared in *Aqua Farm News* entitled “No to Pesticides and Antibiotics in Aquaculture” (Lacanilao *et al.* 1992). These scientists urged that antibiotics, non-biodegradable pesticides, and disease-control chemicals be banned from use in aquaculture. Further, they encouraged the use of biodegradable plant-based pesticides.

The FAO has published the “Code of Conduct for Responsible Fisheries” (FAO 1995). At the farm level, the following principles for responsible aquaculture should be implemented: (a) improvement in selection and use of appropriate feeds, feed additives, and fertilizers, including manures; (b) minimal use of chemicals including hormones, antibiotics, and other disease control chemicals; (c) regulated use of chemicals which are hazardous to public health and the environment; and (d) disposal of excess veterinary drugs and other hazardous chemicals should not pose hazard to public health and the environment. At the national level, appropriate procedures to assess the environmental impact of use of chemicals should be established.

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REFERENCES

- Anonymous. 1991. Technical Q & A. Infotish International, p. 59-60.
- Aoki T. 1992. Chemotherapy and drug resistance in fish farms in Japan. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 519-529. Fish Health Section, Asian Fisheries Society, Manila.
- Apud FD. 1988. Prawn grow-out practices in the Philippines. In: Biology and Culture of *Penaeus monodon*. p. 89-118. BRAIS State-of-the-Art Ser. No. 2, Brackishwater Aquaculture Information System and SEAFDEC Aquaculture Department, Iloilo.

- Apud FD, Primavera JH, Torres PL Jr. 1985. Farming of prawns and shrimps. Aquaculture Extension Manual No. 5, SEAFDEC Aquaculture Department, Iloilo.
- Austin B. 1985. Chemotherapy of bacterial fish diseases. In: Ellis AE. ed. Fish and Shellfish Pathology. p. 19-26. Academic Press, London.
- Ayson FG, Lam TJ. 1993. Thyroxine infection of female rabbitfish (*Siganus guttatus*) broodstock: changes in thyroid hormone levels in plasma, eggs, and yolk-sac larvae, and its effect on larval growth and survival. Aquaculture, 109:83-93.
- Bailey TA. 1983. Screening fungicides for use in fish culture: evaluation of the use of agar plug transfer, cellophane transfer and dilution methods. Progr. Fish-Cult. 45:24-27.
- Baldia JP. 1994. The effect of enzymes, vitamins, mineral premix and fish oil on the growth and survival of shrimps (*Penaeus monodon* Fab.) reared in ponds and under semi-controlled conditions. In: Chou LM, Munro AD, Lam TJ, Chen TW, Cheong LKK, Ding JK, Hooi KK, Khoo HW, Phang VPE, Shim KF, Tan CH. eds. The Third Asian Fisheries Forum. p. 709-712. Asian Fisheries Society, Manila.
- Baticados MCL, Coloso RM, Duremdez RC. 1986. Studies on chronic soft-shell syndrome in the tiger prawn, *Penaeus monodon* Fabricius, from brackishwater ponds. Aquaculture, 56:271-285.
- Baticados MCL, Cruz-Lacierda ER, De la Cruz MC, Duremdez-Fernandez RC, Gacutan RQ, Lavilla-Pitogo CR, Lio-Po GD. 1990b. Diseases of penaeid shrimps in the Philippines. Aquaculture Extension Manual No. 16. SEAFDEC Aquaculture Department, Iloilo.
- Baticados MCL, Lavilla-Pitogo CR, Cruz-Lacierda ER, de la Peña LD, Suñaz NA. 1990a. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis. Aquat. Org. 9:133-139.
- Baticados MCL, Paclibare JO. 1992. The use of chemotherapeutic agents in aquaculture in the Philippines. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 531-546. Fish Health Section, Asian Fisheries Society, Manila.
- Baticados MCL, Pitogo CL. 1990. Chlorination of seawater used for shrimp culture. Israeli J. Aquacult. Bamidgeh, 42:128-130.
- Baticados MCL, Po GL, Lavilla CR, Gacutan RQ. 1977. Isolation and culture in artificial media of *Lagenidium* from *Penaeus monodon* larvae. Q. Res. Rep. SEAFDEC Aquaculture Department 1:9-10.
- Baticados MCL, Tendencia EA. 1990. Effects of Gusathion A on the survival and shell quality of juvenile *Penaeus monodon*. Aquaculture, 93:9-19.
- Bauman RH, Jamandre DR. 1990. A practical method for determining quality of *Penaeus monodon* (Fabricius) fry for stocking in grow-out ponds. In: New MB, de Saram H, Singh T. eds. Technical and Economic Aspects of Shrimp Farming. p. 124-131. Proceedings of the AQUATECH '90 Conference, Infofish and Fisheries Development Authority of Malaysia.
- Bautista MN, Baticados MCL. 1990. Dietary manipulation to control the chronic soft-shell syndrome in tiger prawn, *Penaeus monodon* Fabricius. In: Hirano R, Hanyu I. eds. The Second Asian Fisheries Forum. p. 341-344. Asian Fisheries Society, Manila.
- Beladi I, Ketyi I, Nasz I, Vaczi L. 1978. Orvosi mikrobiologia, immunitastan, parazitologia. Med. Budapest, 48:83-88.
- BFAR. 1994. Fisheries profile of the Philippines. Bureau of Fisheries and Aquatic Resources, Department of Agriculture, Quezon City.
- Bombo-Tuburan I, Agbayani RF, Subosa PS. 1989. Evaluation of organic and inorganic fertilizers in brackishwater ponds. Aquaculture, 76:227-235.
- Borlongan IG, Coloso RM, Mosura EF, Mosura AT, Sagisi FD. 1998. Molluscicidal activity of tobacco dust against brackishwater pond snails (*Cerithidea cingulata* Gmelin) Crop Prot. 17:401-404.
- Brown JH. 1989. Antibiotics: their use and abuse in aquaculture. World Aquacult. 20:34-43.
- Cagauan AG, Arce RG. 1992. Overview of pesticide use in rice-fish farming in Southeast Asia. In: de la Cruz CR, Lightfoot C, Costa-Pierce BA, Carangal VR, Bimbao MP. eds. Rice-fish Research and Development in Asia. p. 217-233. ICLARM Conf. Proc. 24.

- Canto J. 1977. Tolerance of *Penaeus monodon* larvae to cupric sulfate added in bath. Q. Res. Rep. SEAFDEC Aquaculture Department 1:18-23.
- Catacutan MR, Lavilla-Pitogo CR. 1994. L-ascorbyl-2-phosphate Mg as a source of vitamin C for juvenile *Penaeus monodon*. Israeli J. Aquacult. Bamidgeh, 46:40-47.
- Chen SN. 1989. Questionable drugs are found in shrimp feeds. Asian Shrimp News, 1:2.
- Cruz ER, Pitogo CL. 1989. Tolerance level and histopathological response of milkfish (*Chanos chanos*) fingerlings to formalin. Aquaculture, 79:135-145.
- Cruz ER, Tamse CT. 1986. Histopathological response of milkfish *Chanos chanos* Forsskal fingerlings to potassium permanganate. Fish Pathol. 21:151-159.
- Cruz ER, Tamse CT. 1989. Acute toxicity of potassium permanganate to milkfish fingerlings, *Chanos chanos*. Bull. Environ. Contam. Toxicol. 43:785-788.
- Cruz-Lacierda ER. 1992. Toxicity of rotenone to milkfish, *Chanos chanos*, and tilapia (*Oreochromis mossambicus*). In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 419-423. Fish Health Section, Asian Fisheries Society, Manila.
- Cruz-Lacierda ER. 1993. Effect of rotenone and saponin on the shell quality of juvenile tiger shrimp *Penaeus monodon*. Israeli J. Aquacult. Bamidgeh, 45:126-130.
- Cruz PFS, Barry TP. 1988. Observations and treatment of dermal hemorrhagic disease in the spotted scat (*Scatophagus argus*). In: Fast AW. ed. Spawning Induction and Pond Culture of the Spotted Scat (*Scatophagus argus* Linnaeus) in the Philippines. p. 136-137. Hawaii Institute of Marine Biology Techn. Rep. No. 39.
- Dean JH, Murray MJ. 1992. Toxic responses of the immune system. In: Amdur MO, Doull J, Klaasen CD. eds. Casarett and Doull's Toxicology: the Basic Science of Poisons. p. 282-333. 4th edn., McGraw-Hill, Inc., Singapore.
- Dimanlig MNV. 1981. Some physiological responses of *Tetraselmis chuii* Butcher to varying concentrations of Trifluralin (α,α,α -Trifluralin-2,6-dinitro-N, N-dipropyl-P-toluidine). M.S. Thesis, University of the Philippines, 76 p.
- FAO. 1995. Code of Conduct for Responsible Fisheries. Food and Agriculture Organization of the United Nations, Rome, 41 p.
- Farkas J, Olah J, Szecsi E. 1982. Antibiotic sensitivity of bacteria isolated from water and fish. Aquacult. Hung. (Szarvas) 3:85-92.
- Fortes RD. 1984. Milkfish culture techniques generated and developed by the Brackishwater Aquaculture Center. In: Juario JVJ, Ferraris RP, Benitez LV. eds. Advances in Milkfish Biology and Culture: Proceedings of the Second International Milkfish Aquaculture Conference. p. 107-119. Island Publishing House Inc., SEAFDEC Aquaculture Department and the International Development Research Centre, Metro Manila.
- Fortes RD, Aure RC, Sanares RC, Unarce RV. 1980. Effect on fish production of piggery wastes used as feed and fertilizer in brackishwater pond fish culture. In: Chung P. ed. Animal Wastes Treatment and Utilization. p. 461-470. Council for Agricultural Planning and Development, Taipei.
- FPA. 1989. FPA Pesticide regulatory policies and the implementing guidelines and procedures. Fertilizer and Pesticide Authority, Department of Agriculture, Makati, Metro Manila.
- Gacutan RQ. 1979. Diseases of prawns (pests and diseases of sugpo). In: Technical Consultation on Available Aquaculture Technology in the Philippines. p. 170-179. SEAFDEC Aquaculture Department, Tigbauan, Iloilo.
- Gacutan RQ, Llobrera AT, Baticados MCL. 1979. Effects of furanace on the development of larval stages of *Penaeus monodon* Fabricius. In: Lewis DH, Leong JK. compilers. Proceedings of the Second Biennial Crustacean Health Workshop. p. 231-244. Texas A & M University, College Station, TX.
- Hatai K, Bian BZ, Baticados MCL, Egusa S. 1980. Studies on the fungal diseases in crustaceans. II. *Haliphthoros philippinensis* sp. nov. isolated from cultivated larvae of the jumbo tiger prawn (*Penaeus monodon*). Trans. Mycol. Soc. Jpn. 21:47-55.
- ICAAE (International Center for Aquaculture and Aquatic Environments, Auburn University). 1993. Final Report: Philippine Prawn Industry Policy Study. Coordinating Council of the

- Philippines Assistance Programme and U.S. Agency for International Development Contract No. DAN 4180-B-00-8009-00 Order # 3 Philippine Assistance Programme Support Project No. 492-0452.
- Jesus EGT de, Toledo JD, Simpás MS. 1998. Thyroid hormones promote early metamorphosis in grouper (*Epinephelus coioides*) larvae. Gen. Comp. Endocrinol. 112:10-16.
- Kungvankij P, Tiro LB, Pudadera BJ Jr, Potestas IO, Corre KG, Borlongan E, Taleon GA, Gustilo LF, Tech ET, Unggui A, Chua TE. 1986. Shrimp hatchery design, operation and management. NACA Training Manual Ser. No. 1. Network of Aquaculture Centres in Asia Regional Lead Centre in the Philippines.
- Lacanilao FJ, Marte CL, Lam TJ. 1985. Problems associated with hormonal induction of gonad development in milkfish (*Chanos chanos*). In: Lofts B, Holmes WN. eds. Current Trends in Comparative Endocrinology. p. 1247-1253. Proceedings 9th International Symposium in Comparative Endocrinology. Hongkong University Press, Hongkong.
- Lacanilao FJ and 70 other authors. 1992. No to pesticides and antibiotics in aquaculture. Aqua Farm News, 10:17-19.
- Lam TJ, Juario JV, Banno J. 1985. Effect of thyroxine on growth and development in post-yolk-sac larvae of milkfish, *Chanos chanos*. Aquaculture, 46:179-184.
- Lavilla-Pitogo CR. 1991. Physico-chemical characteristics and pathogenicity of *Vibrio parahaemolyticus*-like bacterium isolated from eye lesions of *Chanos chanos* (Forsskal) juveniles. Fish. Res. J. Philipp. 16:1-13.
- Lavilla-Pitogo CR, Albright LJ, Paner MG, Suñaz NA. 1992b. Studies on the sources of luminiscent *Vibrio harveyi* in *Penaeus monodon* hatcheries. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 157-164. Fish Health Section, Asian Fisheries Society, Manila.
- Lavilla-Pitogo CR, Baticados MCL, Cruz-Lacierda ER, de la Peña LD. 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture, 91:1-13.
- Lavilla-Pitogo CR, Castillo AR, de la Cruz MC. 1992a. Occurrence of *Vibrio* sp. infection in grouper, *Epinephelus suillus*. J. Appl. Ichthyol. 8:175-179.
- Laviña EM. 1978. A study on certain aspects on the biology and control of *Caligus* sp., an ectoparasite of the adult milkfish *Chanos chanos* (Forsskal). Fish. Res. J. Philipp. 3:11-24.
- Licop MSR. 1988. Sodium-EDTA effects on survival and metamorphosis of *Penaeus monodon* larvae. Aquaculture, 74:239-247.
- Lio-Po GD. 1984. Diseases of milkfish. In: Juario JV, Ferraris RP, Benitez LV. eds. Advances in Milkfish Biology and Culture: Proceedings of the Second International Milkfish Aquaculture Conference. p. 145-153. Island Publishing House Inc., SEAFDEC Aquaculture Department and the International Development Research Centre, Metro Manila.
- Lio-Po GD, Barry TP. 1988. Report on diseases and parasites in the spotted scat (*Scatophagus argus*). In: Fast AW. ed. Spawning Induction and Pond Culture of the Spotted Scat (*Scatophagus argus* Linnaeus) in the Philippines. p. 129-135. Hawaii Institute of Marine Biology Techn. Rep. No. 39.
- Lio-Po GD, Baticados MCL, Lavilla CR, Sanvictores MEG. 1985. *In vitro* effects of fungicides on *Haliphthoros philippinensis*. J. Fish Dis. 8:359-365.
- Lio-Po GD, Fernandez RD, Cruz ER, Baticados MCL, Llobrera AT. 1989. Recommended practices for disease prevention in prawn and shrimp hatcheries. Aquaculture Extension Pamphlet No. 3. SEAFDEC Aquaculture Department, Iloilo.
- Lio-Po GD, Lavilla-Pitogo CR. 1990. Bacterial exoskeletal lesions of the tiger prawn *Penaeus monodon*. In: Hirano R, Hanyu I. eds. The Second Asian Fisheries Forum. p. 701-704. Asian Fisheries Society, Manila.
- Lio-Po GD, Lavilla CR, Trillo-Llobrera A. 1978. Toxicity of malachite green to the larvae of *Penaeus monodon*. Kalikasan, Philipp. J. Biol. 7:238-246.
- Lio-Po G, Pitogo C, Marte C. 1986. Bacteria associated with infection at hormone-implantation sites among milkfish, *Chanos chanos* (Forsskal), adults. J. Fish Dis. 9:337-343.

- Lio-Po GD, Sanvictores EG. 1986. Tolerance of *Penaeus monodon* eggs and larvae to fungicides against *Lagenidium* spp. and *Haliphthoros philippinensis*. *Aquaculture*, 51:161-168.
- Lio-Po GD, Sanvictores EG. 1987. Studies on the causative organism of *Oreochromis niloticus* (Linnaeus) fry mortalities I. Primary isolation and pathogenicity experiments. *J. Aquacult. Trop.* 2:25-30.
- Lio-Po GD, Sanvictores MEG, Baticados MCL, Lavilla CR. 1982. *In vitro* effect of fungicides on hyphal growth and sporogenesis of *Lagenidium* spp. isolated from *Penaeus monodon* larvae and *Scylla serrata* eggs. *J. Fish Dis.* 5:97-112.
- Llobrera AT. 1988. Effect of farming phase and in-plant processing on the microbiological quality of prawns (*Penaeus monodon*). Seventh Session of IPFC Working Party on Fish Technology and Marketing, *Fish Tech News*, 11:12.
- Meyer FP, Schnick RA. 1989. A review of chemicals used for the control of fish diseases. *Rev. Aquat. Sci.* 1:693-709.
- Minsalan CLO, Chiu YN. 1986. Effects of teaseed cake on selective elimination of finfish in shrimp ponds. In: Maclean JL, Dizon LB, Hosillos LV. eds. *First Asian Fisheries Forum*. p. 79-82. Asian Fisheries Society, Manila.
- Muroga K, Lio-Po G, Pitogo C, Imada R. 1984. *Vibrio* sp. isolated from milkfish (*Chanos chanos*) with opaque eyes. *Fish Pathol.* 19:81-87.
- Nacario JF. 1983. The effect of thyroxine on the larvae and fry of *Sarotherodon niloticus* L. (*Tilapia nilotica*). *Aquaculture*, 34:73-83.
- Natividad JM, Lightner DV. 1992. Strategic egg prophylactic technique: a method for the production of monodon baculovirus (MBV)-free *Penaeus monodon* postlarvae. *Third Asian Fisheries Forum*, 26-30 October 1992, Singapore, Abstracts, p. 55.
- NCC. 1989. Chemical pollution from aquaculture. In: *Fishfarming and the Safeguard of the Natural Environment of Scotland*. Based on a report prepared by the Institute of Aquaculture, University of Stirling, Edinburgh. Nature Conservancy Council. p. 95-102.
- Neri FC. 1989. A strengthened FPA ensures safe use of pesticides. *Agribusiness Watch Suppl. Issue No.* 57.
- Norfolk JRW, Javellana DS, Paco JN, Subosa PF. 1981. The use of ammonium sulfate as a pesticide during pond preparation. *Asian Aquacult.* 4:4-7.
- Parado-Estepa FD. 1995. Research on crustaceans. In: Bagarinao TU, Flores EEC. eds. *Towards Sustainable Aquaculture in Southeast Asia and Japan*. p. 187-198. SEAFDEC Aquaculture Department, Iloilo.
- Parado-Estepa FD, Quintio ET, Borlongan E. 1991. Prawn hatchery operations. *Aquaculture Extension Manual No. 19*, SEAFDEC Aquaculture Department, Iloilo.
- Pascual FC, Tayo GT, Cruz-Lacierda ER. 1994. Acute toxicity of formalin to sea bass (*Lates calcarifer*) fry. In: Chou LM, Munro AD, Lam TJ, Chen TW, Cheong LKK, Ding JK, Hooi KK, Khoo HW, Phang VPE, Shim KF, Tan CH. eds. *The Third Asian Fisheries Forum*. p. 346-348. Asian Fisheries Society, Manila.
- Phillips M. 1995. Shrimp culture and the environment. In: Bagarinao TU, Flores EEC. eds. *Towards Sustainable Aquaculture in Southeast Asia and Japan*. p. 37-62. SEAFDEC Aquaculture Department, Iloilo.
- Platon, RR. 1979. Prawn hatchery technology in the Philippines. In: *Technical Consultation on Available Aquaculture Technology in the Philippines*. p. 129-138. SEAFDEC Aquaculture Department and Philippine Council for Agriculture and Resources Research.
- Primavera JH. 1991. Intensive prawn farming in the Philippines - ecological, social, and economic implications. *Ambio*, 20:28-33.
- Primavera JH. 1992. Prawn/shrimp culture industry in the Philippines. In: Fast AW, Lester LJ. eds. *Marine Shrimp Culture: Principles and Practices*. p. 701-728. Elsevier Science Publishers B.V.
- Primavera JH. 1993. A critical review of shrimp pond culture in the Philippines. *Rev. Fish. Sci.* 1:151-201.

- Primavera JH, Lavilla-Pitogo CR, Ladja JM, de la Peña MR. 1993. A survey of chemical and biological products used in intensive prawn farms in the Philippines. *Mar. Pollut. Bull.* 26:35-40.
- Schnick RA. 1991. Chemicals for worldwide aquaculture. In: *Fish Health Management in Asia-Pacific. Report on a Regional Study and Workshop on Fish Disease and Fish Health Management.* p. 441-446. Asian Development Bank and Network of Aquaculture Centres in Asia, Bangkok.
- Shotts EB, Vanderwerk VL, Campbell LM. 1976. Occurrence of R-factors associated with *Aeromonas hydrophila* isolates from fish and water. *J. Fish. Res. Board Can.* 33:736-740.
- Subosa PF. 1992. Chicken manure, rice hulls, and sugar-mill wastes as potential organic fertilizers in shrimp (*Penaeus monodon* Fabricius) ponds. *Aquaculture*, 102:95-103.
- Subosa PF, Bautista M. 1991. Yield of *Penaeus monodon* Fabricius in brackishwater ponds given different fertilizer combinations. *Aquaculture*, 94:39-48.
- Tamse CT, Gacutan RQ. 1994. Acute toxicity of nifurpirinol, a fish chemotherapeutant, to milkfish (*Chanos chanos*) fingerlings. *Bull. Environ. Contam. Toxicol.* 52:346-350.
- Tan EO, de Guzman DL, Darwin LC, Balgos MC. 1984. Milkfish research in the Philippines, In: Juario JV, Ferraris RP, Benitez LV. eds. *Advances in Milkfish Biology and Culture Proceedings of the Second International Milkfish Aquaculture Conference.* p. 171-181. Island Publishing House Inc., SEAFDEC Aquaculture Department and the International Development Research Centre, Metro Manila.
- Timbol AS. 1974. Observations on the growth of young bangus, *Chanos chanos* (Forsskal), on two types of pelleted food. *Philipp. J. Sci.* 103:199-206.
- Triño AT, Bolivar EC, Gerochi DD. 1993. Effect of burning rice straw on snails and soil in brackishwater pond. *Int. J. Trop. Agricult.* 11:93-97.
- Tumanda MI Jr. 1980. The effects of rotenone-containing derris plant extracts on the mortality of some predator fishes of pond-cultured prawns under different water temperature-salinity combinations. M.S. Thesis, University of San Carlos, Cebu City.
- Velasquez CC. 1979. Pests/parasites and diseases of *Chanos chanos* (Forsskal) in the Philippines. In: *Technology Consultation on Available Aquaculture Technology in the Philippines.* p. 65-67. SEAFDEC Aquaculture Department and Philippine Council for Agriculture and Resources Research, Tigbauan, Iloilo.
- Vicente HJ, Valdez FM. 1979. Preliminary observations on *Epistylis* sp. infestation in food organism and shrimp hatchery tanks in MSU-IFRD. *Mindanao State Univ., Inst. Fish. Res. Developm. Tech. Rep.* 1(1-18):82-86.

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 LC₅₀ 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 LC₅₀ 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
Malachite green	3.0	Few h	Disinfectant	Spawners	Platon 1978
	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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REFERENCES

- Aoki T. 1974. Studies of drug resistant bacteria isolated from water of carp ponds and intestinal tracks of carp. Bull. Jpn. Soc. Sci. Fish. 40:247-254.
- Aoki T, Kitao T, Kawana K. 1981. Changes in drug resistance of *Vibrio anguillarum* in cultured ayu, *Plecoglossus altivelis* Temminck and Schlegel, in Japan. J. Fish Dis. 4:223-230.
- Aoki T, Sakaguchi T, Kitao T. 1987. Multiple drug-resistant plasmids from *Edwardsiella tarda* in eel culture ponds. Nippon Suisan Gakkaishi, 53:1821-1825.
- Aquacop. 1977. Observations on diseases of crustacean cultures in Polynesia. Proc. World Maricult. Soc. 8:685-703.
- Aquacop. 1983. Penaeid larval rearing in the Center Oceanologique du Pacifique. In: MacVey JP. ed. Crustacean Aquaculture. p. 123-127. CRC Handbook of Mariculture, Vol. 1, CRC Press Inc., Boca Raton, FL.
- Bailey TA. 1983. Screening fungicides use in fish culture: evaluation of the use of the agar plug transfer, cellophane transfer and agar dilution methods. Prog. Fish-Cult. 45:24-27.
- Baticados MCL, Cruz-Lacierda EM, de la Cruz MC, Duremdez-Fernandez RC, Gacutan, RQ, Lavilla-Pitogo CR, Lio-Po GD. 1990a. Diseases in the penaeid shrimps in the Philippines. Aquaculture Extension Manual No. 16, SEAFDEC, Aquaculture Department, Tigbauan, Iliolo.
- Baticados MCL, Lavilla-Pitogo CR, Cruz-Lacierda ER, de la Peña LD, Suñaz, NA. 1990b. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis. Aquat. Org. 9:133-139.
- Baticados MCL, Paclibare JO. 1992. The use of chemotherapeutic agents in aquaculture in the Philippines. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 531-546. Fish Health Section, Asian Fisheries Society, Manila.
- Beladi I., Ketyi I, Nasz I, Vaczi, L. 1978. Orvosi mikrobiologia, immunitastan, parazitologia. Med. Budapest, 48:83-88.
- Bell TA. 1992. Principals of shrimp culture chemotherapy. In: Wyban J. ed. Proceedings of the Special Session on Shrimp Farming. p. 227-237. World Aquaculture Society, Baton Rouge, LA.
- Bell, TA, Lightner DV. 1992. Chemotherapy in aquaculture today - current practices in shrimp culture: available treatments and their efficacy. In: Michel C, Alderman DJ. eds. Chemotherapy in Aquaculture: from Theory to Reality. p. 45-57. Office International des Epizooties, Paris.

- Braaten B, Hektoen H. 1991. The environmental impact of aquaculture. Report on a regional study and workshop on fish disease and fish health management. p. 469-524. ADB Agricult. Dep. Rep. Ser. No. 1.
- Chen HP. 1978. Diagnosis and control of disease of *Penaeus monodon* cultured from over winter juveniles. China Fish. Mon. 309:3-9.
- Farkas J, Olah J, Szecsi E. 1982. Antibiotic sensitivity of bacteria isolated from water and fish. Aquacult. Hung. (Szarvas), 3:85-92.
- Kungvankij P, Tiro Jr. LB, Potestas IO, Korre KG, Borlingan E, Taleon GA, Gustillo LF, Tech ET, Unggui A, Chua TE. 1986. Shrimp hatchery design, operation and management. NACA Training Manual Series, No. 1, 88 p. NACA, Tigbauan, Iloilo.
- Limsuwan C. 1987. Bacterial and viral diseases in shrimp. In: Limsuwan C. ed. Shrimp Diseases and Chemotherapy. p. 9-24. Bangkok. (in Thai).
- Lio-Po GD, Sanvictores MEG, Baticados MCL, Lavilla CR. 1982. *In vitro* effect of fungicides on hyphal growth and sporogenesis of *Lagenidium* spp. isolated from *Penaeus monodon* larvae and *Scylla serrata* eggs. J. Fish Dis. 5:97-112.
- Platon RR. 1978. Design, operation and economics of a small-scale hatchery for the larval rearing of sugpo *Penaeus monodon* Fabricius. Aquaculture Extension Manual No.1, 30 p. SEAFDEC, Aquaculture Department, Tigbauan, Iloilo.
- Pirmpol M. 1990. Study on diseases in Gunther's walking cat fish (*Clarias macrocephalus*) and its prevention. M.Sc. Thesis, 233 p. Kasetsart University, Bangkok. (in Thai).
- Rattanavinijkul S, Chawchienj S, Rojanasarampakij T, Chopsaard P. 1988. Experimental study on prevention and treatment of disease of shrimp post larvae (PL2-PL15) in nursery ponds. Techn. Pap. No.13/1987, 8 p. Nakornsrithamarat Fisheries Station, Brackish Water Division, Department of Fisheries, Thailand. (in Thai).
- Ruangpan L. 1982. Diseases of *P. monodon* Fabricius. Thai Fish. Gaz. 35:385-387. (in Thai).
- Ruangpan L. 1987. Diseases in tiger and white shrimp in Thailand. In: Limsuwan, C. ed. Shrimp Diseases and Chemotherapy. p. 46-62. Bangkok. (in Thai).
- Ruangpanich N. 1988. Some problems in tiger shrimp hatcheries. Brackishwater Division, Department of Fisheries, Bangkok, 11 p. (in Thai).
- Schnick RA, Meyer FP. 1978. Registration of thirty-three fishery chemicals: status of research and estimated costs of required contract studies. Investigations in Fish Control No. 86. US Fish Wildl. Serv.
- SCSP. 1982. Working party on small-scale shrimp and prawn hatcheries in Southeast Asia. (November 16-21, 1981, Semarang, Central Java, Indonesia). Vol. II. Technical Report, 120 p. South China Sea Fisheries Development Coordinating Programme, Manila.
- Shotts EB, Vanderwerk VL, Campbell LM. 1976. Occurrence of R-factors associated with *Aeromonas hydrophila* isolates from fish and water. J. Fish. Res. Board Can. 33:736-740.
- Subasinghe RP. 1992. The use of chemotherapeutic agents in aquaculture in Sri Lanka. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 547-553. Fish Health Section, Asian Fisheries Society, Manila.
- Sunaryanto A. 1986. Chemical treatment of larval culture: the use of chloramphenicol, sodium EDTA and malachite green in larval culture of *Penaeus monodon* Fabricius. Bull. Brackish Water Aquacult. Dev. Center, 8:25-30.
- Vicente HJ, Valdez FM, Dejarme SM, Apongan AB. 1979. Notes on the occurrence of undesirable organisms in MSU-IFRD prawn (*Penaeus indicus* Milne Edwards and *Penaeus monodon* Fabricius) hatchery tanks. Mindanao State Univ., Inst. Fish. Res. Developm. Techn. Rep. No. 1(1-12), p. 87-100.
- Watanabe T, Aoki T, Ogata Y, Egusa S. 1971. R-factors related to fish culturing. Ann. NY Acad. Sci. 182:383-410.

The Use of Chemicals in Aquaculture in Taiwan, Province of China

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ABSTRACT

Aquaculture in Taiwan has a history of more than three centuries. To satisfy consumer preferences, a wide variety of aquatic species, 71 in 1993, are being cultured in Taiwan. It is difficult to control diseases when many species are cultured and stocking densities are high. At present, it is important to manage the use and application of chemotherapeutants effectively. Many aquatic animal diseases fall under the category of potentially curable illnesses. These include diseases of bacterial, protozoan, fungal, and environmental etiologies. This paper summarizes the chemicals used in aquaculture, farm management practices, alternative disease prevention methods, national regulations, and the current research on chemical use for aquaculture in Taiwan.

INTRODUCTION

Aquaculture in Taiwan has a history of more than three centuries and has passed through both spectacular and difficult times (Liao 1991). Liao (1993) noted that there were 71 major and 37 candidate species for commercial culture in 1993. These include finfishes, crustaceans, molluscs, reptiles, amphibians, and seaweeds.

The practice of traditional polyculture and extensive culture did not pose any major problems except when natural disasters struck. As culture shifted to semi-intensive and intensive systems, stocking densities were raised and formulated feeds were used. Management of water quality and maintenance of the culture environment became difficult, and thus the cultured species became more susceptible to diseases. Normally, pathogens, by themselves, rarely cause disease in healthy aquatic animals. Three factors must be involved in the disease process: a susceptible host, a facilitative environment, and the presence of a potential pathogen (Snieszko 1974). Liao *et al.* (1977, 1985, 1992) and Lightner *et al.* (1987) reviewed the prevalent diseases that have adversely affected Taiwan's shrimp culture industry. Liao *et al.* (1996) also reviewed the practical approaches to health management in marine fish culture in Taiwan.

Many aquatic animal diseases fall into the category of potentially curable illnesses, which includes those diseases of bacterial, protozoan, fungal and environmental etiologies. This paper summarizes the use of chemicals in aquaculture, farm management practices, alternative disease prevention methods, regulations, and current research on chemical use for aquaculture in Taiwan.

USE OF CHEMICALS IN AQUACULTURE

To solve the problems associated with heavy nutrient loading, toxic metabolites, and pathogens in intensive aquaculture systems, and to maintain optimal physico-chemical parameters required for aquatic animal growth, various chemical preparations are applied to treat water and pond bottoms, or are incorporated into feeds. Based on their actions, these chemicals can be classified into the following groups:

Therapeutants

Intensive culture systems increase the risk of transmission of diseases. Chemicals are routinely applied as prophylactics to prevent diseases, or as therapeutants to control diseases once detected. Chemotherapeutic agents can be grouped into three slightly overlapping divisions: (1) antibacterial agents, including antibiotics (e.g., erythromycin, chloramphenicol, florfenicol, oxytetracycline, streptomycin), quinolones (e.g., oxolinic acid), fluoroquinolones (e.g., flumequine), nitrofurans (e.g., furazolidone, nitrofurazone, nifurpirinol), sulfonamides (e.g., sulfamonomethoxine, sulfadimethoxine), quaternary ammonium compounds (e.g., benzalkonium chloride, benzethonium chloride), malachite green, and methylene blue; (2) anti-protozoal agents, such as copper compounds (e.g., copper sulfate, chelated copper), formalin, and salt; and (3) metazoan parasiticides composed mainly of organophosphate compounds (e.g., trichlorofon) (Table 1).

Disinfectants

Disinfectants are used to prevent or minimize the spread of pathogens and diseases within a system. The effectiveness of most disinfectants is usually hindered by the presence of organic matter. Iodine (e.g., povidone-iodine, iodophor) and chlorine compounds (e.g., sodium hypochlorite, chlorinated lime) are frequently applied as disinfectants to wash fertilized eggs or to dip aquatic animals when they are transferred from one aquarium or pond to another. These compounds can also be used to disinfect tanks and other holding equipment (Table 2).

Soil and Water Treatment Compounds

The environmental factors influencing aquatic animal cultivation are fluctuation of temperature and pH, types of algae and their concentration, and deterioration of water and pond bottom quality. Chemicals used for water and soil treatment in aquaculture in Taiwan are listed in Table 3. Lime (CaCO_3 , Ca(OH)_2 , CaO) is generally applied on dried and cracked pond bottoms to eradicate most infectious agents. Lime is also used to increase the pH level of acidic soil and water, the dosage used depending on the condition of the pond (Chien 1993). Ozone can be added into water to eliminate pathogens (Kou *et al.* 1988, Chen *et al.* 1993). Teaseed cake, whose active ingredient is saponin, is applied as an organic fertilizer to enrich algal growth, as a piscicide to eradicate fish, and as a stimulant for molting of shrimp (Liao *et al.* 1985). Zeolite is a hydrated alkali-aluminum silicate that absorbs ammonia, hydrogen sulphide, and other toxic gases. The recommended application of zeolite is 100-120 kg/1000 m² (Liao *et al.* 1992). Potassium permanganate can increase the dissolved oxygen (DO) levels of the pond (Chien 1993). To chelate and reduce the heavy metals in rearing water, EDTA (ethylenediamine tetraacetic acid) at a dose of 3-10 ppm is applied (Licop 1988, Boonyaratpalin 1990, Carpenter 1992). Enzymes (e.g., proteases, cellulases, amylases, lipases) are also used to hasten the rate of decomposition of organic matter and reduce the amount of pond sludge (Chien 1993).

Table 1. Chemotherapeutants used in aquaculture in Taiwan.

Chemotherapeutant	Use	Dose	References
Antibiotics	Bactericide		
Erythromycin		40-75 mg/kg B.W. daily, oral for 5-10 d; 80 ppm, bath 1 d; 2-5 ppm, long bath	Boonyaratpalin 1990, Liao <i>et al.</i> 1996
Chloramphenicol		100 mg/kg B.W. daily, oral for 21 d; 1-10 ppm, bath	Liao <i>et al.</i> 1992
Florfenicol		10 mg/kg B.W. daily, oral for 3-5 d	Fukui <i>et al.</i> 1987
Oxytetracycline		50-75 mg/kg B.W. daily, oral for 10 d; 10-20 ppm on eel, or 40-60 ppm on shrimp, long bath	Kou <i>et al.</i> 1988, Liao <i>et al.</i> 1996
Streptomycin		100 ppm, bath	Kou <i>et al.</i> 1988
Quinolone	Bactericide		
Oxolinic acid		20-40 mg/kg B.W. daily, oral for 5 d	Liao <i>et al.</i> 1992, Guo and Liao 1994a
Fluoroquinolone	Bactericide		
Flumequine		10-20 mg/kg B.W. daily, oral for 5 d	Barnes <i>et al.</i> 1991
Nitrofurans	Bactericide		
Furazolidone		10 mg/kg B.W. daily, oral for 3-6 d; 10 ppm, bath 1 d	Kou <i>et al.</i> 1988, Liao <i>et al.</i> 1992
Nitrofurazone (Furacin®)		10 mg/kg B.W. daily, oral for 3-6 d; 10 ppm, bath 1 d	Kou <i>et al.</i> 1988, Liao <i>et al.</i> 1992
Nifurpirinol (Furanace®)		2-4 mg/kg B.W. daily, oral for 3-5 d; 0.01-0.1 ppm, long bath	Kou <i>et al.</i> 1988 Liao <i>et al.</i> 1992
Sulfonamides	Bactericide		
Sulfamonomethoxine		100-240 mg/kg B.W. daily, oral for 10-20 d	Kou <i>et al.</i> 1988
Sulfadimethoxine		100-240 mg/kg B.W. daily, oral for 10-20 d	Kou <i>et al.</i> 1988
Quarternal ammonium compounds	Bactericide		
BKC® (50% Benzalkonium chloride)		1-2 ppm, bath 1 d; 0.3-0.6 ppm on shrimp larvae	Liao <i>et al.</i> 1985, 1992; Kou <i>et al.</i> 1988
Hyamine® (50% Benzethonium chloride)		1-2 ppm, bath 1 d	Liao <i>et al.</i> 1985, Kou <i>et al.</i> 1988, Chen <i>et al.</i> 1993
Dyes	Bactericide; fungicide		

Table 1. Continued. . .

Chemotherapeutant	Use	Dose	References
Malachite green		0.5-0.8 ppm, bath 1 d; 0.015 ppm, long bath	Kou <i>et al.</i> 1988, Chang 1994
Methylene blue		8-10 ppm, bath 1d; 2 ppm, bath 3-5 d	Kou <i>et al.</i> 1988, Chang 1994
Copper compounds Copper sulfate	Ectoparasites	0.3-0.5 ppm, bath 1 d	Kou <i>et al.</i> 1988, Liao <i>et al.</i> 1992, Chang 1994
Chelated copper		1 ppm, bath 1 d; 0.2-0.5 mg Cu/L, dip 4-6 h;	Lightner 1983
Formalin	Ectoparasites	0.1 mg Cu/L, bath 1 d 25-30 ppm (adult), or 15 ppm (larvae), bath 1 d; 15-20 ppm (juvenile), bath 10-12 h; 200-300 ppm, dip 30-60 sec	Liao <i>et al.</i> 1985, 1992; Kou <i>et al.</i> 1988; Chen <i>et al.</i> 1993
Salt	Ectoparasites	10-15 gm/L, dip 20 min	Kou <i>et al.</i> 1988
Trichlorofon	Ectoparasites	0.3-0.5 ppm, bath 1 d	Kou <i>et al.</i> 1988, Chang 1994

Table 2. Disinfectants used in aquaculture in Taiwan.

Disinfectant	Dose	References
Iodine compounds		
Povidone-iodine	50-300 mg I/L, bath 10-60 min	Kou <i>et al.</i> 1988, Chen <i>et al.</i> 1993
Iodophor	0.02-0.06 ppm (larvae) or 0.1-0.45 ppm (juvenile) or 0.3-0.6 ppm (adult), long bath; 200 ppm (fertilized egg) or 20 ppm (larvae), 30 sec	Kou <i>et al.</i> 1988, Chen <i>et al.</i> 1993
Chlorine compounds		
Sodium hypochlorite (10-15% active ingredient)	150 ppm, 2-3 d (disinfect water, tanks, etc.)	Boonyaratpalin 1990, Carpenter 1992, Guo and Liao unpubl. data
Chlorinated lime (60% active ingredient)	20-30 ppm, 2-3 d (disinfect water, tanks, etc.)	Boonyaratpalin 1990, Carpenter 1992, Guo and Liao unpubl. data

Anesthetics

Aquatic animals are usually handled at various phases of culture for marking, tagging, artificial spawning, and packaging processes. To reduce mortality and stress, anesthetics are routinely used in handling and shipping processes. For such purposes, anesthetics such as benzocaine (ethyl aminobenzoate), MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate), and phenoxyethanol (2-phenoxyethanol) are usually used (Table 4) (Mattson and Ripley 1989, Lee 1995).

Feed Additives

A variety of chemicals are added to the feeds when stunted growth, deformities or diseases are observed in cultured fish. These include vitamins (e.g., vitamins A, B complex, C, D, E and K, folic acid, inositol, choline, PABA); enzymes (e.g., proteases, cellulases, amylases, lipases); minerals (e.g., calcium lactate, magnesium sulfate, potassium chloride, ferric citrate, copper sulfate, zinc carbonate, manganese sulfate, cobalt carbonate, calcium iodate, dipotassium phosphate, sodium dihydrogen phosphate, calcium chloride); binders (e.g., carboxymethyl cellulose sodium, alginic acid); pigments (e.g., zeaxanthin, β -carotene, astaxanthin); attractants (e.g., tyrosine, phenylalanine, histidine, lysine, glycine, proline); preservatives, including antioxidants (e.g., butylated hydroxyanisole, ethoxyquin, butylated hydroxytoluene) and fungicides (e.g., benzethonium chloride, benzoic acid, calcium propionate, p-hydroxybenzoate, propionic acid, sodium propionate, sorbic acid, formic acid, acetic acid, ammonium propionate, sodium citrate, citric acid); and other specific additives (e.g., cholesterol, lecithin) (Taipei Commercial Association of Feeds and Feed Additives 1994).

Chemical Application in Biotechnology

The recent development of biotechnology in aquaculture is a new frontier, and it may have a significant impact on both basic and applied research in Taiwan. Preliminary studies in biotechnology related to chemical use are in the cryopreservation of gametes, induction of triploidy and diploid monosex species, and sex reversal. In the cryopreservation of gametes, cryopreservative agents provide cryoprotection to labile enzymes and stabilize proteins during the freezing process. Ethylene glycerol, methanol, dimethylsulfoxide (DMSO), acetamide, ethylene glycol, propylene glycol, glycerol, glucose, trehalose, and polyethylene glycol have been used in studies of sperm, embryo, and larval cryopreservation by stepwise or vitrification freezing method (Chao 1991). For studies of induction of triploidy and diploid monosex species, cytochalasin B, 6-dimethylaminopurine, and caffeine have been used to retain the 1st or 2nd polar body during the early stage of zygote development (Chao *et al.* 1993). The common androgens and estrogens used for sex reversal are methyl testosterone, ethyl testosterone, estradiol, and ethinyl estradiol. These chemicals are generally recognized as effective androgens and estrogens in the sex reversal of fishes (Hung 1994, Chang *et al.* 1995).

MANAGEMENT PRACTICES ON USE OF CHEMICALS

Antibiotics

Antibiotics are antibacterial agents which are derived originally from other microbes, chiefly bacteria, molds, and Actinomyces. They selectively inhibit or destroy pathogenic organisms without showing any appreciable harm to the host. Five antibiotics namely, erythromycin, chloramphenicol, florfenicol, oxytetracycline, and streptomycin are commonly used in aquaculture in Taiwan.

Erythromycin is a macrolide antibiotic that is particularly effective against Gram-positive bacteria. It is usually applied to control diseases such as streptococcosis in marine fish (e.g., grey mullet,

Table 3. Chemicals used for water and soil treatment in aquaculture in Taiwan.

Chemical	Use	References
Lime (CaCO ₃ , Ca(OH) ₂ , CaO)	To disinfect bottom soil and to increase pH	Chien 1993
Ozone (O ₃)	To apply as water treatment	Kou <i>et al.</i> 1988, Chen <i>et al.</i> 1993
Teaseed cake (10% saponin)	To stimulate shrimp molting and to eliminate unwanted fish	Liao <i>et al.</i> 1985
Zeolite (hydrated alkalialuminum silicate)	To absorb ammonia, hydrogen sulphide, and other toxic gases in ponds	Liao <i>et al.</i> 1992
Potassium permanganate (KMnO ₄)	To increase DO levels and to reduce organic matter	Chien 1993
EDTA (ethylenediamine tetraacetic acid)	To reduce heavy metals in water	Boonyaratpalin 1990, Carpenter 1992
Enzymes	To decompose organic matter	Chien 1993

Table 4. Anesthetics used in aquaculture in Taiwan.

Anesthetic	Dose	Anesthetic effect	References
Benzocaine (ethyl aminobenzoate)	40 mg/L	Rapid	Mattson and Riple 1989
MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate)	75 mg/L	Rapid	Mattson and Riple 1989
Phenoxyethanol (2-phenoxyethanol)	0.3 mL/L	Loss of equilibrium and swimming ability only (no anesthetic effect)	Mattson and Riple 1989, Lee 1995

seabream), edwardsiellosis in eel, and vibriosis in shrimp larvae. The recommended dosage for treatment is 40 mg/kg body weight daily for 7 d (Liao *et al.* 1996) or 50-75 mg/kg body weight daily for 5-10 d by oral administration, or 80 ppm bath for 1 d, or 2-5 ppm bath for a long period (Boonyaratpalin 1990).

Chloramphenicol is a broad-spectrum antibacterial agent and is used to control edwardsiellosis (*Edwardsiella tarda*) and vibriosis (*Vibrio anguillarum*) in eel. The recommended application is 100 mg/kg body weight daily for 21 d by oral administration (Kou *et al.* 1988). Bacterial shell disease, appendage rot, bacterial fouling disease on gills, and septicemias of larval shrimp can be treated with chloramphenicol at 1-10 ppm for bath (Liao *et al.* 1992).

Florfenicol, a broad spectrum antibacterial agent, is a fluorinated derivative of thiamphenicol which is a chloramphenicol analogue. *In vitro* testing using this agent shows its equal or superior effect

against pathogenic bacteria compared with thiamphenicol and chloramphenicol. Florfenicol has shown therapeutic efficacy against experimentally induced pseudotuberculosis (*Pasteurella piscicida*) in yellowtail, edwardsiellosis in eel, and vibriosis in goldfish (Fukui *et al.* 1987). A dose of 10 mg/kg body weight is orally administered daily for 3-5 d to control bacterial diseases.

Oxytetracycline is a broad-spectrum antibacterial agent. A dose of 50-75 mg/kg body weight is applied daily for 10 d or 10-20 ppm bath for a long period to control eel diseases caused by *Aeromonas hydrophila*, *Pseudomonas anguilliseptica*, *E. tarda*, *V. anguillarum*, and *Flexibacter columnaris* (columnaris and tail rot) (Kou *et al.* 1988). Bacterial shell disease, appendage rot, septicemias, bacterial fouling gill diseases, and necrotic hepatopancreas of shrimp larvae caused by *Vibrio*, *Aeromonas*, *Pseudomonas*, *Spirillum*, or *Flavobacterium* can also be treated with oxytetracycline at 40-60 ppm for bath (Liao *et al.* 1992). It is also used to control gaffkemia in lobster.

Streptomycin is an aminoglycoside which is a mid-broad spectrum antibacterial compound. The drug is used to control diseases caused by Gram-negative bacteria such as *Vibrio*, *Aeromonas*, and *Edwardsiella*. Streptomycin at 100 ppm is used as a prophylactic or as a therapeutant to prevent or control eel diseases (Kou *et al.* 1988).

Oxolinic Acid

Oxolinic acid is a quinolone that has a broad-spectrum antibacterial activity, especially against Gram-negative bacteria causing a variety of serious diseases in cultured fish, such as vibriosis and furunculosis. Isolates of *Vibrio* and *Aeromonas* obtained from farmed fish in Taiwan (seabream, grey mullet, grouper, eel, etc.) are sensitive to oxolinic acid. To control such diseases, oxolinic acid is applied at a dose of 20-40 mg/kg body weight mixed with feed and administered to fish daily for 5 d (Liao *et al.* 1992, Guo and Liao 1994a).

Flumequine

Fluoroquinolones are second generation 4-quinolones. Flumequine is one of the fluoroquinolone derivatives and has been shown in recent years to be a useful antibacterial drug in the treatment of fish disease. Barnes *et al.* (1991) stated that flumequine is more effective than oxolinic acid due to its microbiological activity. Flumequine at 10-20 mg/kg body weight is applied daily by oral administration for 5 d to treat fish infected by *Vibrio* and *Aeromonas*.

Nitrofurans

Nitrofuran compounds, which have the 5-nitro-furan-ring structure, are applied as prophylactic or therapeutic agents to prevent or control bacterial diseases such as edwardsiellosis, vibriosis, branchiomycosis, columnaris and tail rot disease of fish; and bacterial shell disease, appendage rot, septicemias, bacterial fouling gill diseases and necrotic hepatopancreas of shrimp. Furazolidone, nitrofurazone, and nifurpirinol are the commonly used nitrofurans in treating diseases of cultured aquatic animals. Furazolidone and nitrofurazone (Furacin®) at 10 ppm bath for 1 d, or 10 mg/kg body weight daily by oral administration for 3-6 d are applied for fish and shrimp diseases. Nifurpirinol (Furanace®) is used to control disease by oral administration with a dose of 2-4 mg/kg body weight daily for 3-5 d, or 0.01-0.1 ppm bath for a long period (Kou *et al.* 1988, Liao *et al.* 1992).

Sulfonamides

Sulfonamides are synthetic compounds that are usually given orally because they reach therapeutic

levels in the blood and body tissues rapidly through that route. Oral administration is the best choice because fish can hardly absorb the drug from the surrounding waters. To produce a synergistic effect, sulfonamides are often combined with trimethoprim or ormetoprim. Sulfonamides, such as sulfamonomethoxine and sulfadimethoxine, are applied at a dose of 100-200 mg/kg body weight daily for 10-20 d or 220-240 mg/kg body weight daily for 14 d to prevent or control fish diseases caused by *Aeromonas*, *Pseudomonas*, *Edwardsiella*, *Vibrio*, and *Cytophaga* (Kou *et al.* 1988).

Quarternary Ammonium Compounds

Among the quarternary ammonium compounds, benzalkonium chloride (BKC®) and benzethonium chloride (Hyamine®) are very popular with aquafarmers. These compounds are used for controlling bacterial diseases such as those caused by *Cytophaga* and *Vibrio*. BKC® or Hyamine® at 1-2 ppm bath used for 1 d is effective (Liao *et al.* 1985, 1992; Kou *et al.* 1988). BKC® at concentrations of 0.3-0.6 ppm is used on larval and juvenile shrimp to prevent bacterial diseases, especially *Vibrio* infection (Chen *et al.* 1993).

Dyes

Malachite green and methylene blue are dyes that possess antimicrobial properties, particularly against *Saprolegnia* infection. They are also applied to disinfect larval fish and fertilized eggs and to control fungal infections (Chang 1994). Malachite green, given as bath at 0.5-0.8 ppm, or methylene blue at 8-10 ppm for 1-d bath are used to treat diseased shrimp (Liao *et al.* 1985). To disinfect fish larvae or broodstock, malachite green is applied at 0.15 ppm bath for a long period or methylene blue at 2 ppm is administered as a daily bath for 3-5 d (Kou *et al.* 1988, Chang 1994). Malachite green and methylene blue may remain in aquatic animal tissue for up to one month. Therefore, fish and prawns treated with these chemicals should not be harvested until at least one month post-treatment. This is because the chemicals are carcinogenic (Liao *et al.* 1992).

Copper Compounds

Among copper compounds, copper sulfate and chelated copper (e.g., Cutrine-plus®) are frequently used. Their effects are largely attributed to cupric ions, with their toxicities increasing with decreasing salinity and hardness of water (Liu 1980, Guo and Liao 1992). They are used as algicides (against blue-green algae, *Oscillatoria*), bactericides (to treat columnaris disease, bacterial fin rot), fungicides (against *Saprolegnia*), external protozoacides (against *Trichodina*, *Amyloodinium*), and for control of monogeneans (*Dactylogyrus*, *Gyrodactylus*). Copper sulfate at 0.3-0.5 ppm used as a 1-d bath is sufficient to kill algae and to control fish diseases (Kou *et al.* 1988, Liao *et al.* 1992, Chang 1994). Chelated copper, a form of copper bound by such chelating agents as ethanolamine, at a level of 1 ppm, is applied daily to kill protozoans. *Leucothrix* infestations on the gills of shrimp larvae may be eliminated by dipping them in 0.2-0.5 mg Cu/L for 4-6 h, or in 0.1 mg Cu/L for 1 d (Lightner 1983).

Formalin

Formalin is used to treat infestations of peritrichous protozoans and monogenetic trematodes, such as *Zoothamnium*, *Epistylis*, *Vorticella*, *Ambiphrya*, *Apiosoma*, *Trichodina*, *Ichthyophthirius*, *Dactylogyrus*, and *Gyrodactylus*; filamentous bacteria such as *Leucothrix*; and fungi such as *Saprolegnia* in cultured shrimp and fish (Lightner 1983, Kou *et al.* 1988, Liao *et al.* 1992). Formalin, with formaldehyde as its active ingredient, is an agent that reacts with a variety of organic compounds. The presence of organic material in aquaculture ponds may result in formalin becoming less effective. The dosage of formalin depends on the duration of treatment, the condition of the pond, and the size of the aquatic animals to be treated. Adult fish and prawn are treated with 25-30 ppm bath for 1 d, larvae with 15 ppm for 1 d, and juveniles with 15-20 ppm baths for 10-12 h. Aquatic animals

are not fed during formalin treatment and water has to be drained after 24 h to remove traces of the chemical (Liao *et al.* 1985, 1992; Kou *et al.* 1988). Formalin is also used to wash fertilized eggs of *Penaeus monodon* (100 ppm for 1 min) or nauplii (200-300 ppm for 30 sec to 1 min) to prevent *P. monodon*-type baculovirus (MBV) (Chen *et al.* 1993).

Salt

Salt (NaCl) is commonly used to control fungus (*Saprolegnia*), external freshwater protozoans (e.g., *Trichodina*), and monogeneans (e.g., *Dactylogyirus*, *Gyrodactylus*) by dipping fish at concentrations of 10-15 gm/L for 20 min (Kou *et al.* 1988).

Trichlorofon

Trichlorofon is an organophosphate compound that can penetrate the chitinous exoskeleton of arthropods to paralyze or poison the nervous system. Trichlorofon is the most frequently recommended chemical for *Lernaea* or *Caligus* infections. To control such parasites entirely, applications of 0.3-0.5 ppm for 1 d need to be repeated 2-3 times every 7-10 d (Kou *et al.* 1988, Chang 1994).

Iodine Compounds

Among the organic iodine compounds, povidone-iodine and iodophor are commonly used on cultured aquatic animals. They can prevent and control diseases caused by *Aeromonas*, *Pseudomonas*, *Vibrio*, *Flexibacter*, and fungi. These compounds are particularly used to treat eggs and larvae, and to disinfect equipment (Guo and Liao 1994b). A concentration of 100 mg iodine/L with dipping for 10 min is usually applied to fish eggs to prevent the spread of disease. Iodophor, at 0.02-0.06 ppm, 0.1-0.45 ppm, and 0.3-0.6 ppm is applied on larval, juvenile and adult eel, respectively, as a long-period bath (Kou *et al.* 1988). Chen *et al.* (1993) reported that shrimp eggs and nauplii were dipped into iodophor solution for 30 sec at concentrations of 200 ppm and 20 ppm, respectively, to avoid the introduction of MBV from broodstock sources.

Chlorine Compounds

Chlorine compounds, such as sodium hypochlorite (NaOCl) and chlorinated lime (calcium hypochlorite; $\text{Ca}(\text{OCl})_2$), are widely used as disinfectants because they are inexpensive, easily available and effective. Sodium hypochlorite and chlorinated lime, which are strong oxidizing agents, are frequently used to disinfect water, ponds, tanks, and equipment. To kill nematode eggs in ponds, the bottom silt is purged and 50-100 ppm sodium hypochlorite or chlorinated lime is applied (Kou *et al.* 1988, Liao *et al.* 1992). Either 20-30 ppm chlorinated lime (60% active ingredient) or 150 ppm sodium hypochlorite (10-15% active ingredient) can be applied to water in the reservoir for 2-3 d. Strong aeration (15-20 L/min) should be provided to mix chlorine throughout the tank. Before use, the water in the tank should be tested for the presence of chlorine. Chlorine residues can be removed by adding sodium thiosulphate (Boonyaratpalin 1990, Carpenter 1992).

Potassium Permanganate

Potassium permanganate (KMnO_4) is a strong oxidizing agent and is widely used to treat external protozoan infestations and bacterial diseases. Its efficacy is affected by the presence of dissolved and particulate organic matter; thus the amount of agent needed for an effective treatment has to be increased if the organic content of the water is high. Potassium permanganate is also applied as a detoxifier. A 1-2 ppm application is commonly used to increase DO levels and to reduce excessive organic material in the pond (Chien 1993). Liao *et al.* (1985) reported that potassium permanganate

dip for 30-60 min at 25-30 ppm can control epicomensal protozoan disease of *P. monodon*. Immersing grouper infected with monogeneans in a 2 ppm potassium permanganate solution for 24-48 h has been found effective (Chang 1994). Potassium permanganate at 3-5 ppm bath for a long period, or 20 ppm for 1 h is also applied to cure columnaris disease (*Flexibacter columnaris*), to control protozoans (e.g., *Trichodina*, *Ichthyophthirius*), and to remove monogeneans (e.g., *Gyrodactylus*, *Dactylogyrus*) from eels (Kou *et al.* 1988).

Teaseed Cake

The effect of teaseed cake is largely attributed to the 10% saponin present in it. Saponin is an irritant to both fish and external parasites. Teaseed cake at 10-25 ppm is used to remove unwanted fishes in shrimp-culture ponds and to stimulate shrimp molting (Liao *et al.* 1985, 1992).

ALTERNATIVE DISEASE PREVENTION METHODS

Although chemicals can be used to prevent and cure diseases, a good health management system is the best tool for disease prevention. Several measures concerning disease control should be considered in advance. The use of vaccines (Song *et al.* 1980, Lin *et al.* 1982, Chen and Kou 1985), immunostimulants, biological control (Wu and Chao 1984), or probiotics containing beneficial microorganisms has the potential to replace chemotherapy. To date, however, the efficacy of these measures is not yet encouraging.

NATIONAL REGULATIONS ON THE USE OF CHEMICALS IN AQUACULTURE

To manage the use of chemicals in aquaculture, the Council of Agriculture (COA) plans to set up a "Guidelines on Chemicals Use in Aquatic Animals." The COA has extended financial support for a series of studies on such topics as larval toxicity (Table 5) (Liao and Guo 1985, 1986a, b, 1990a, b; Liao *et al.* 1989; Guo and Liao 1992, 1993), pharmacokinetics and residues in juvenile fish (Guo and Liao 1994a). The guidelines focus on collecting information on dosage, treatment, and withdrawal period in target species. Chemicals to be regulated include amoxicillin, ampicillin, erythromycin, florfenicol, flumequine, furazolidone, oxolinic acid, oxytetracycline hydrochloride, sulfadimethoxine (or sodium sulfadimethoxine), sulfamonomethoxine (or sodium sulfamonomethoxine), and trichlorofon. Based on the guidelines, the COA will establish appropriate regulations to monitor and manage the use of chemicals in aquaculture.

CURRENT RESEARCH ON CHEMICAL USE FOR AQUACULTURE

To collect data and to understand the importance of chemical use in aquaculture management, studies are being conducted. These include investigations on the prevention and control of diseases; the toxicity of chemicals to aquatic animals; residues in fish, soil and water; the development of resistance in bacteria; pharmacokinetics; and environmental impact. Studies on feed additives (e.g., pigments, glucan, vitamin C) are also being done.

CONCLUSIONS

To meet consumer preferences, 71 aquatic species are cultivated on a commercial scale in Taiwan. Disease control has become difficult due to this increase in the number of species available and to the high stocking densities being employed. At present, it is important to apply and manage the use of chemotherapeutants effectively. Although chemicals can be used against diseases, sound pond management (e.g., well-designed ponds, adequate pond preparation, optimal stocking density, excellent water source and proper feed quality) and the enhancement of the immune capability of

Table 5. The median tolerance limit (TLM) of the postlarvae of the six species of cultured prawn bathed in chemicals for 24 h.

Chemical	<i>Penaeus monodon</i>	<i>P. japonicus</i>	<i>P. semisulcatus</i>	<i>P. penicillatus</i>	<i>Metapenaeus ensis</i>	<i>Macrobrachium rosenbergii</i>	References
Chloramphenicol	247 ¹	334	362	404	437	2,717	Liao and Guo 1985
Oxytetracycline	954	936	908	916	1,397	1,250	Liao and Guo 1986b
Streptomycin	4,884	6,616	9,813	6,635	17,173	6,325	Liao and Guo 1986b
Furazolidone	50	>200	67	36	109	>300	Liao and Guo 1986a
Nitrofurazone	55	73	78	40	139	>362	Liao and Guo 1986a
Formalin	168	136	184	275	633	423	Liao and Guo 1989
Copper sulfate	436	427	231	319	465	0.39	Liao and Guo 1990a
Malachite green	0.73	1.18	3.2	0.3	3.73	1.14	Liao and Guo 1990a
Potassium permanganate	4.8	9.6	5.2	3.9	17.0	6.0	Liao and Guo 1990a
Saponin	135	41	146	135	162	168	Liao and Guo 1989
Benzalkonium chloride	3.1	3.3	1.5	1.6	3.0	2.0	Liao and Guo 1990b
Benzethonium chloride	10	5.3	3.2	4.4	9.0	6.0	Liao and Guo 1990b

¹Units = parts per million (ppm).

aquatic animals (e.g., application of vitamin C and the immunostimulant, glucan) are still the best and preferred tools for disease prevention. To assure man's health and to reduce damage to the environment, the use of chemicals in aquaculture should only be employed as a last resort.

REFERENCES

- Barnes AC, Lewin CS, Hastings TS, Amyes SGB. 1991. *In vitro* susceptibility of the fish pathogen *Aeromonas salmonicida* to flumequine. *Antimicrob. Agents Chemother.* 35:2634-2635.
- Boonyaratpalin S. 1990. Shrimp larval diseases. In: New MB, Saram H. de, Singh T. eds. *Technical and Economic Aspects of Shrimp Farming: Proceeding of the Aquatech'90 Conference*, Kuala Lumpur, Malaysia. p. 158-167.
- Carpenter N. 1992. Disease diagnosis and management: an industry report. In: Wyban J. ed. *Proceedings of the Special Session on Shrimp Farming*. p. 261-269. World Aquaculture Society, Baton Rouge, LA.

- Chang CF. 1994. Disease and Control Method of Grouper. Fisheries Extension Handbook 1. National Sun Yat-Sen University, Kaohsiung, Taiwan. 18 p. (in Chinese).
- Chang FC, Lan SC, Pan BS. 1995. Feed administration of estradiol-17 β stimulates female differentiation in juvenile grey mullet, *Mugil cephalus*. *Zool. Stud.* 34:257-264.
- Chao NH. 1991. Fish sperm cryopreservation in Taiwan: technology advancement and extension efforts. *Bull. Inst. Zool., Acad. Sinica, Monogr.* 16:263-283.
- Chao NH, Hsu HW, Hsu HY. 1993. Studies on methods of triploidy percentage analysis. In: Lee CS, Su MS, Liao IC. eds. TML Conference Proceedings 3, p. 203-210. Tung kang Marine Laboratory, Taiwan Fisheries Research Institute, Pingtung.
- Chen SN, Chang PS, Kou GH. 1993. Diseases and treatment strategies of *Penaeus monodon* in Taiwan. In: Liao IC, Cheng JH, Wu MC, Guo JJ. eds. Proceedings of the Symposium on Aquaculture held in Beijing. p. 43-57. Taiwan Fisheries Research Institute, Keelung. (in Chinese, English abstract).
- Chen SN, Kou GH. 1985. Results of field test on vaccination trials against vibriosis of milkfish (*Chanos chanos*) and edwardsiellosis of eel (*Anguilla japonica*). Council of Agriculture (COA) Fish. Ser. 4, Fish Dis. Res. 7:52-56.
- Chien YY. 1993. Research of prawn culture system and management in Taiwan. In: Liao IC, Cheng JH, Wu MC, Guo JJ. eds. Proceedings of the Symposium on Aquaculture held in Beijing. p. 101-117. Taiwan Fisheries Research Institute, Keelung. (in Chinese, English abstract).
- Fukui H, Fujihara Y, Kano T. 1987. *In vitro* and *in vivo* antibacterial activities of florfenicol, a new fluorinated analog of thiamphenicol, against fish pathogens. *Fish Pathol.* 22:201-207.
- Guo JJ, Liao IC. 1992. Toxicity of copper sulfate to juvenile grass prawn, *Penaeus monodon*. Council of Agriculture (COA) Fish. Ser. 33, Fish Dis. Res. 12:1-7.
- Guo JJ, Liao IC. 1993. Toxicities of formalin and oxytetracycline to *Penaeus monodon* larvae and algae. Council of Agriculture (COA) Fish. Ser. 40, Fish Dis. Res. 13:17-24. (in Chinese, English abstract).
- Guo JJ, Liao IC. 1994a. Pharmacokinetics of oxolinic acid in orange-spotted grouper, *Epinephelus coioides*, after single oral administration at 24°C. *J. Fish. Soc. Taiwan*, 21:263-272.
- Guo JJ, Liao IC. 1994b. Toxicities of povidone-iodine to fertilized eggs and larvae of *Penaeus monodon* and algae. Council of Agriculture (COA) Fish. Ser. 47, Fish Dis. Res. 15:37-45. (in Chinese, English abstract).
- Hung CY. 1994. The effects on sex differentiation, plasma levels of sex steroids and brain aromatase activity in juvenile grey mullet (*Mugil cephalus*) by oral administration of sex steroids. MS Thesis, Department of Aquaculture, National Taiwan Ocean University, 91 p. (in Chinese).
- Kou GH, Liu CI, Liu CK. 1988. Handbook of Fish Disease. Pig Research Institute Taiwan. Miaoli. 129 p. (in Chinese).
- Lee CS. 1995. Aquaculture of milkfish (*Chanos chanos*). TML Aquaculture Ser. No. 1. Tung kang Marine Laboratory, Taiwan Fisheries Research Institute, Taiwan and The Oceanic Institute, Hawaii. p. 59-69.
- Liao IC. 1991. Aquaculture: the Taiwanese experience. *Bull. Inst. Zool., Acad. Sinica, Monogr.* 16:1-36.
- Liao IC. 1993. Status and prospects of aquaculture in Taiwan in the 1990s. In: Liao IC, Cheng JH, Wu MC, Guo JJ. eds. Proceedings of the Symposium on Aquaculture held in Beijing. p. 19-42. Taiwan Fisheries Research Institute, Keelung. (in Chinese, English abstract).
- Liao IC, Guo JJ. 1985. Studies on the tolerance of postlarvae of *Penaeus monodon*, *P. japonicus*, *P. semisulcatus*, *P. penicillatus*, *Metapenaeus ensis* and *Macrobrachium rosenbergii* to tetracycline and chloramphenicol. Council of Agriculture (COA) Fish. Ser. 4, Fish Dis. Res. 7:22-26. (in Chinese, English abstract).
- Liao IC, Guo JJ. 1986a. Studies on the tolerance of postlarvae of *Penaeus monodon*, *P. japonicus*, *P. semisulcatus*, *P. penicillatus*, *Metapenaeus ensis* and *Macrobrachium rosenbergii* to furazolidone and nitrofurazone. Council of Agriculture (COA) Fish. Ser. 8, Fish Dis. Res. 8:14-17. (in Chinese, English abstract).

- Liao IC, Guo JJ. 1986b. Studies on the tolerance of postlarvae of *Penaeus monodon*, *P. japonicus*, *P. semisulcatus*, *Metapenaeus ensis* and *Macrobrachium rosenbergii* to neomycin, streptomycin, oxytetracycline and chlorotetracycline. Council of Agriculture (COA) Fish. Ser. 8, Fish Dis. Res. 8:34-39. (in Chinese, English abstract).
- Liao IC, Guo JJ. 1990a. Studies on the tolerance of postlarvae of *Penaeus monodon*, *P. japonicus*, *P. semisulcatus*, *P. penicillatus*, *Metapenaeus ensis* and *Macrobrachium rosenbergii* to copper sulfate, potassium permanganate and malachite green. Council of Agriculture (COA) Fish. Ser. 24. Fish Dis. Res. 10:90-94. (in Chinese, English abstract).
- Liao IC, Guo JJ. 1990b. Studies on the tolerance of postlarvae of *Penaeus monodon*, *P. japonicus*, *P. semisulcatus*, *P. penicillatus*, *Metapenaeus ensis* and *Macrobrachium rosenbergii* to benzalkonium chloride, benzethonium chloride and cetylpyridinium chloride. Council of Agriculture (COA) Fish. Ser. 24, Fish Dis. Res. 10:95-99. (in Chinese, English abstract).
- Liao IC, Guo JJ, Wang HM, Day CL. 1989. Studies on the tolerance of postlarvae of *Penaeus monodon*, *P. japonicus*, *P. semisulcatus*, *P. penicillatus*, *Metapenaeus ensis* and *Macrobrachium rosenbergii* to formalin and saponin. Council of Agriculture (COA) Fish. Ser. 15, Fish Dis. Res. 9:23-27. (in Chinese, English abstract).
- Liao IC, Kou GH, Chen SN, Lai JY. 1985. Preliminary investigation on the diseases of cultured prawns in the Pingtung area. Council of Agriculture (COA) Fish. Ser. 4, Fish Dis. Res. 7:86-94. (in Chinese, English abstract).
- Liao IC, Lee KK, Chien YH. 1996. Practical approaches to marine fish health problems in Taiwan. In: Main KL, Rosenfeld C. eds. Aquaculture Health Management Strategies for Marine Fish in Asia and the United States. p. 57-67. The Oceanic Institute, Waimanalo, HI.
- Liao IC, Su MS, Chang CF. 1992. Diseases of *Penaeus monodon* in Taiwan: a review from 1977 to 1991. In: Fulks W, Main KL. eds. Diseases of Cultured Penaeid Shrimp in Asia and the United States. p. 113-137. The Oceanic Institute, Waimanalo, HI.
- Liao IC, Yang FR, Lou SW. 1977. Preliminary report on some diseases of cultured prawn and their control methods. Joint Commission on Rural Reconstruction (JCRR) Fish. Ser. 29, Fish Dis. Res. 1:28-33. (in Chinese, English abstract).
- Licop MSR. 1988. Sodium-EDTA effects on survival and metamorphosis of *Penaeus monodon* larvae. Aquaculture, 74:239-247.
- Lightner DV. 1983. Diseases of cultured penaeid shrimp. In: J.P. McVey. ed. CRC Handbook of Mariculture. p. 289-320. CRC Press, Inc., Boca Raton, FL.
- Lightner DV, Hedrick RP, Fryer JL, Chen SN, Liao IC, Kou GH. 1987. A survey of cultured penaeid shrimp in Taiwan for viral and other important diseases. Fish Pathol. 22:127-140.
- Lin CL, Ting YY, Song YL. 1982. Proceeding evaluation of HIVAX *Vibrio anguillarum* bacterin in the vaccination of milkfish (*Chanos chanos*) fingerlings. Council of Agricultural Planning and Development (CAPD) Fish. Ser. 8, Fish Dis. Res. 4:80-83. (in Chinese, English abstract).
- Liu CH. 1980. Studies on the chronic toxicity of four heavy metals to the prawn *Penaeus monodon*. MS Thesis, Institute of Oceanography, National Taiwan University. 39 p. (in Chinese).
- Mattson NS, Riple TH. 1989. Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol, and phenoxyethanol. Aquaculture, 83:89-94.
- Snieszko SF. 1974. The effects of environmental stress on outbreaks of infectious diseases in fishes. J. Fish Biol. 6:197-208.
- Song YL, Chen SN, Kou GH, Lin CL, Ting YY. 1980. Evaluation of HIVAX *Vibrio anguillarum* bacterin in the vaccination of milkfish (*Chanos chanos*) fingerlings. Council of Agricultural Planning and Development (CAPD) Fish. Ser. 3, Fish Dis. Res. 3:101-108.
- Taipei Commercial Association of Feeds and Feed Additives. 1994. Handbook of Feed Management. Taipei Commercial Association of Feeds and Feed Additives, Taipei. p. 16-82. (in Chinese).
- Wu JL, Chao WJ. 1984. The epizootic of milkfish vibriosis and its biological control by bacteriophage AS10. Council of Agriculture (COA) Fish. Ser. 1, Fish Dis. Res. 6:34-46. (in Chinese, English abstract).

The Use of Chemicals in Aquaculture in Thailand

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ABSTRACT

In Thailand, many chemicals are used to treat diseases of cultured aquatic animals and to improve water quality in culture facilities. Along with the intensification of aquaculture practices that has occurred in recent years in Thailand, chemical use has also increased, particularly in marine shrimp culture. This paper summarizes information on the types of chemotherapeutants commonly used in Thailand, their sources and costs, the treatment regimes used, the adverse impacts that have resulted and the hazards posed. Also included is information on national regulations, a summary of on-going research, and recommendations to aquaculturists, producers and suppliers of chemicals, government agencies and scientists. It is concluded that although chemicals and drugs will continue to play an important role in the development of Thai aquaculture, they must be used with caution to avoid adverse effects such as environmental damage and the development of resistant strains of pathogens. To minimize chemical usage, additional emphasis needs to be placed on developing good management practices for aquaculture systems.

INTRODUCTION

A number of chemicals have been used for aquaculture in Thailand for quite some time. The chemicals are used mainly to treat diseased animals and, to a lesser degree, to improve water quality in culture facilities. In recent years, as aquaculture in Thailand has become more intensive the use of chemicals has intensified, particularly in marine shrimp culture.

Farmers want to get maximum yield, but few would like to increase their cost of buying chemicals. The aggressive promotion of chemical products by salesmen has partly led to an increased use of drugs and chemicals. Furthermore, the situation is aggravated by the lack of specific legislation on the use of therapeutic drugs and chemicals.

With present culture practice, the use of some chemicals is widespread; but farmers must be cautious since they produce food for human consumption. The use of chemicals must be adopted only as a last resort. For the success of aquaculture, chemicals must be judiciously and responsibly used.

USE OF CHEMICALS IN AQUACULTURE

Environmental degradation in some areas has increasingly made the water quality unsuitable for aquaculture. Drugs and chemicals are often applied to improve water quality and to reduce risk from disease. Chemical use in aquaculture has specific effects. Chemicals can be applied either singularly or in combination. The advantage of using a specific chemical cannot be seen if chemicals are used indiscriminately. Wellborn (1985) stated that prior to chemical treatment, the following four "Ks" must be considered:

- know the water
- know the fish
- know the chemical
- know the disease

Failure to consider any of the four “Ks” and indiscriminate use of the chemicals may be detrimental.

An advantage of chemical application is that it achieves quick results. For example, for acid sulphate soil, liming can quickly adjust soil pH. Similarly, it can control diseases of fish, especially external parasites.

This section presents the drugs and chemicals presently used in aquaculture in Thailand. It is not possible to list all the chemicals used in Thai aquaculture practice because some of them are being used discreetly in isolated cases. In many instances, products are known by their trade names with no further information on ingredients. The information in this report was obtained from the existing literature, and from interviews with farmers and various suppliers.

Soil and Water Treatment

Lime

Lime is a major chemical used for soil and water treatment in Thai aquaculture. It is used to correct pond bottom and stabilize water pH. It is also reported to ensure a healthy plankton bloom (Chanrachakool *et al.* 1995).

There are at least four types of lime used in Thailand:

- Agricultural lime/lime stone or crushed shell (CaCO_3)
- Hydrate lime or slake lime ($\text{Ca}(\text{OH})_2$)
- Quicklime/burnt lime or burnt shell lime (CaO)
- Dolomite or dolomite lime ($\text{CaMg}(\text{CO}_3)_2$)

Each type of lime has a specific effect. The farmer must understand the reactions of the various types of lime to be able to use them for the right purpose and at the proper dose. For example, $\text{Ca}(\text{OH})_2$ should be used on soil with a low pH (< 4). If it is used in soil with high pH, excessively high water pH will result. Water with high pH makes ammonia more toxic and can result in mortality of aquatic animals. Agricultural lime (CaCO_3) is used to increase the buffering capacity of the water. It does not result in drastic pH changes and can, therefore, be used in relatively large quantity. The quality of CaCO_3 in the market may vary due to contamination with soil. The amounts of CaCO_3 and $\text{Ca}(\text{OH})_2$ required to adjust different pH are presented in Table 1.

Table 1. Lime application recommended during pond preparation.

Soil pH	Quantity of CaCO_3 (t/ha)	Quantity of $\text{Ca}(\text{OH})_2$ (t/ha)
> 6	1 - 2	0.5 - 1
5 - 6	2 - 3	1 - 1.5
< 5	3 - 4	1.5 - 2.5

Lime is also used in ponds with normal pH. It conditions the soil when applied at 0.5-1 t/ha spread evenly over the bottom.

Dolomite (CaMg(CO₃)₂)

Dolomite is another form of limestone which contains magnesium. It is primarily used to improve the buffering capacity and to supply Mg⁺⁺. The rate of application in low pH (< 5) ponds is 100 to 300 kg/ha per application.

Teaseed Meal

Teaseed meal is made up of ground teaseed containing saponin, and applied to ponds to kill predators or unwanted species before stocking. It is also used to induce molting of shrimp. Dosage used to eliminate predators depends on the species and their sizes. If used as a disinfectant, dosage is about 1-25 gm/m³ of 7% saponin teaseed meal. Saponin is not only a fish toxicant but also a molluscicide.

Chlorine

Chlorine has been used routinely to disinfect water supplies in fish and shrimp hatcheries in Thailand and elsewhere in the world. Recently, chlorination has become a standard procedure adopted by most Thai shrimp farmers for pond preparation. The purpose is to eliminate harmful organisms entering the pond with water. The most commonly used form of chlorine is powdered calcium hypochlorite (65% active chlorine content) at the rate of 180 kg/ha. However, the rate is by no means standard because of the variability in chlorine demand of the water.

Organic and Inorganic Fertilizers

Fertilization is a basic part of pond preparation. Plankton as food for herbivorous fish such as tilapia and milkfish must be in adequate quantities before stocking fish. Fertilization is also included in a standard procedure for shrimp pond preparation after chlorination and before stocking. Chicken manure can be used at a rate of 300 kg/ha by suspending manure bags in the water away from pond dikes. Inorganic fertilizers such as urea and N-P-K mixture are widely used. Urea is applied at 15-30 kg/ha after fertilization. Water is added gradually at 10-20 cm daily until the desired depth is achieved. If the desired phytoplankton bloom is not achieved, more fertilizer is added.

Disodium Ethylene Diamine Tetraacetate (EDTA)

EDTA is a chemical used to improve water quality by reducing heavy metal concentrations. In shrimp larval rearing, it is applied at 10 mg/L prior to stocking of nauplii. Many hatcheries use EDTA as a treatment for ectocommusal fouling to stimulate juvenile molting. EDTA is normally applied at 1-5 ppm to remove organic substances in the water.

Zeolite

Zeolite is applied to shrimp ponds to remove hydrogen sulphide, carbon dioxide and ammonia, as it has a strong capacity to absorb molecules. Shrimp farmers use zeolite to clean pond bottoms. Zeolite is available in the market under various brand names, and it is supplied as fine grains in bags of 20 kg. The recommended dose is 180-350 kg/ha. The effectiveness of zeolite is still questionable.

Chemotherapeutants

Disinfectants

There are a number of chemicals recommended for pond and hatchery disinfection. Although suppliers claim that they are laboratory tested and effectively kill bacteria and other pathogenic

organisms, many appear to be ineffective in pond or hatchery environments. The high level of organic material in the ponds may inactivate the disinfectant before it creates a significant effect on the pathogens.

The substances widely used as disinfectants in aquaculture in Thailand include chlorine, iodine, formalin and benzalkonium chloride (BKC). There are numerous commercial disinfectants available on the Thai market, but almost all of them contain any one of the above as basic ingredient. The following information, therefore, is based on these active ingredients.

Chlorine

Among various forms of chlorine used to disinfect water, chlorine gas (Cl_2), sodium hypochlorite (NaOCl) and calcium hypochlorite ($\text{Ca}(\text{OCl})_2$) are most common. In brackish or sea water with pH normally above 7, the chlorine exists exclusively in free residue form as HOCl and OCl^- . Chlorine in the form of hypochlorous acid is a hundred times more toxic to microorganisms than as the hypochlorite ion. Thus, lowering pH increases the percentage composition of hypochlorous acid, resulting in greater chlorine toxicity to microorganisms. The effectiveness of chlorine is also affected by the amount of organic matter, reduced compounds and turbidity present in the water to be treated. If chlorine is used in water with high organic matter, the rate of application should be higher. The dosage depends on the active ingredient of residual chlorine. Sodium hypochlorite (5.25% chlorine) is not as effective as calcium hypochlorite due to its low chlorine content. Although farmers use it, as it is much cheaper than calcium hypochlorite, it must be applied at a higher rate. While $\text{Ca}(\text{OCl})_2$ is used at 10-30 gm/m³, NaOCl must be used at 100-300 gm/m³.

Iodine

Iodine is widely used as a disinfectant in hatcheries and ponds at 1-5 gm/m³. Granular iodine should be thoroughly dissolved in water before spraying over the pond bottom to eliminate aquatic bacteria and other pathogens.

Formalin (37-40% Solution)

Formalin can be used as a disinfectant and has been used to treat against external parasites in fish, especially freshwater fish, at a rate of 25-50 ppm. As a disinfectant, it is applied at about 10-15 ppm. It can be used at concentrations up to 200 ppm to treat hatchery facilities. To be precise in dosage, it is necessary to measure the formaldehyde content before use.

Benzalkonium Chloride (BKC)

BKC is one of the broad spectrum disinfectants used in aquaculture. Thai shrimp farmers use it to reduce the concentration of plankton and dinoflagellates in closed pond systems. If it is used in a very small amount (0.1-0.5 ppm) and applied only in one corner, it will not kill plankton. However, if applied in large amounts, the resulting decomposition of organic matter will have an effect on animal health.

The dosages of chemical compounds commonly used to disinfect shrimp ponds in Thailand are shown in Table 2.

Table 2. Disinfectants and dosages used in shrimp culture in Thailand.

Disinfectant	Dosage used (gm/m ³)
Benzalkonium chloride (BKC)	1-5
Formalin	5-10
Iodine	1-5
Sodium hypochlorite (5.25%)	100-300
Calcium hypochlorite (HTH 65%)	10-30
Teaseed (7% saponin)	1-25
Calcium oxide	1000-1500 kg/ha

Therapeutants

Tonguthai and Chanrachakool (1992) reviewed the current usage of chemotherapeutic agents in Thailand and found that 23 basic chemicals and antibiotics were used. Plumb (1995) concluded, from a questionnaire survey of fisheries scientists in Asia, that no less than 38 antimicrobials and 28 parasiticides were used in Asian aquaculture during 1990-93.

An attempt to list the drugs and chemicals presently used in aquaculture in Thailand follows. Chemicals that have been reported, but which are not widely used at the present time are excluded. Again, the following chemicals are referred to by active ingredients only. The commercial names of chemical products available in Thailand are mentioned in a separate section.

Acriflavin

This chemical is used to treat fish eggs and aquarium fish for infections by bacteria and external protozoans. It is recommended as a treatment for *Flexibacter columnaris* in seabass (Ruangan 1986) and against bacteria in walking catfish (Primpol 1990). The dose is 100 ppm for dipping eggs for 3-5 sec and 25 ppm for prolonged treatment of *F. columnaris* in seabass. For walking catfish, only 5 ppm is recommended. For aquarium fish, 5-10 ppm bath for 24 h is recommended.

Copper Compound (Cutrine Plus)

Copper compound is the only chemical so far approved by the US FDA for shrimp culture (Williams and Lightner 1988). It is also one of the oldest and most widely used chemicals in fish culture. It is used as a parasiticide against external protozoan infestation. Limsuwan (1985) recommended 40-50 ppm of CuSO₄ as a 20-30 min bath to treat protozoans in fish. Ruangan (1987) recommended Cutrine Plus at the rate of 0.5 ppm to treat against filamentous bacteria in shrimp. If copper compounds are used continuously in shrimp ponds, copper may accumulate in the pond bottom, which is dangerous to shrimp.

Dipterex (Chlorofos, Dylox, Foschlor, Masofen, Neguvon, Trichlorofon)

Dipterex is widely used to treat for crustacean, monogenean, and protozoan parasites in pond-cultured fish. A rate of 0.25-0.3 ppm is used for prolonged treatment; however, it is necessary to repeat treatment 2-3 times at 3-d intervals (Kanchanakarn 1986).

Recently, Dipterex has been applied to water storage canals and reservoirs to eliminate wild crustacean vectors of shrimp viruses that enter ponds through the water supply. These vectors were found to carry systemic ectodermal and mesodermal baculovirus (SEMBV) and yellowhead disease (YHD).

To completely eliminate these crustaceans, farmers use Dipterex at 0.3-0.5 ppm or higher concentrations than those used for fish.

Formalin

Formalin is approved by the US FDA for use in aquaculture of food fish. When formalin is applied to ponds, it can kill phytoplankton and cause oxygen depletion. Formalin apparently reacts with ammonia to form hexamethylenetriamine and possibly formamide (Brewsters and McEven 1961).

In freshwater fish culture, formalin is widely used to treat external parasites such as ciliated protozoans, monogeneans, etc. In shrimp culture, it is not only used as a disinfectant in ponds and hatcheries, but also to remove ammonia and kill plankton. Shrimp farmers use formalin at 25-40 ppm to control phytoplankton bloom. In fish culture, it can be used at 25-30 ppm, depending on the size of fish and the parasites to be treated.

Malachite Green

A mixture of formalin and malachite green at a ratio of 25:0.1 ppm appears to be very effective to treat "Ich" in both aquarium and food fish. It has been used to control *Lagenidium* in shrimp hatcheries at 0.01 ppm for 24 h (Ruanganpan 1987).

Potassium Permanganate (KMnO₄)

KMnO₄ is one of the first chemicals to be used as a chemotherapeutant in aquaculture, and has been applied since the early part of the century. In Thailand, it is used to treat against external parasites such as monogeneans, particularly in the aquarium fish industry. When applied at the rate of about 5 ppm, it is also a good treatment for external bacterial infections such as *Columnaris* disease. To treat against *Aeromonas hydrophila*, it can be used at 4 ppm in excess of potassium permanganate demand. For aquarium fish, it can be used at up to 500 ppm as a dip treatment for 5 min.

Benzalkonium Chloride (BKC)

BKC is used as a bactericide and fungicide in shrimp hatcheries at 1.0-1.25 ppm or as a 200 ppm bath for 30 min.

Trifuralin (Treflan)

Trifuralin is commonly used as a prophylactic chemical against fungal infection in shrimp hatcheries at 0.01-0.05 ppm daily. Ruanganich (1988) recommended Treflan at 0.01 ppm to treat against *Lagenidium*.

Antibiotics

Infections due to bacteria are major disease problems in both freshwater and brackishwater aquaculture. In Thailand, *Aeromonas hydrophila* causes major problems in the culture of freshwater fish and other aquatic animals such as frogs and softshell turtles. Various species of *Vibrio* are involved in diseases of brackishwater species including shrimp.

To treat diseased fish, antibiotics are generally applied orally by mixing with feed. Injection can be applied to large fish. Antibiotics are most likely to be effective when administered at the early stage of a disease. At later stages, they may not be of much use, as sick fish normally refuse to eat.

Almost all of the antibiotics used in aquaculture are also medicines for human use. The use of antibiotics for aquatic animals may not only initiate environmental pollution problems but also can affect human health due to drug residues (Aoki *et al.* 1990).

The following are some of the antibiotics widely used in Thailand:

Erythromycin

Erythromycin has been used to treat bacterial infection in walking catfish and also against vibriosis in shrimp larvae.

Nitrofurans (Furacin, Furanace)

Nitrofurans are a group of antibiotics that are generally not considered safe for humans because of their potential carcinogenic effects. However, there are a few antibiotics in this group being widely used in aquaculture.

Furacin is used to treat *Vibrio* spp. infection in shrimp larvae (Limsuwan 1987) at a dose of 10 ppm. The withdrawal period is about 10 d at temperatures greater than 22 °C. Furanace is used as an antimicrobial agent for walking catfish and is commonly applied to fish during transportation at the rate of 0.1-0.2 ppm. It is not effective in salt water.

Oxytetracycline

Oxytetracycline is one of the antibiotics approved by the US FDA and has been widely used in the treatment of freshwater fish, frogs and softshell turtles. It is also effective in the control of vibriosis and columnaris disease in both fresh- and brackishwater fish. The rate used in fish is about 10 ppm, while in shrimp it is given at about 1-5 gm/kg feed/d for 4-5 d. The withdrawal period in shrimp is 15 d at temperatures greater than 22 °C. As oxytetracycline has been used in aquaculture for a long time, its use may have resulted in severe environmental contamination and has aroused concerns about its impact on public health.

Sulphamonomethoxine (Dimeton)

Sulphamonomethoxine has been recommended for treatment of bacteria in fish (Saitanu and Chularak 1983) at 100-200 mg/kg of feed for 10-14 d and a withdrawal period of about 15 d at temperatures greater than 22 °C.

Oxolinic Acid

Oxolinic acid is also approved by the US FDA for use in aquaculture and is now widely used in shrimp for treatment of vibriosis. In freshwater fish, it is used effectively against *Aeromonas hydrophila*. For shrimp, the rate of use is 2 gm/kg feed/d for 5 d, and the withdrawal period is about 15 d at temperatures greater than 22 °C.

Feed Additives

Artificial feeds are often advertised to contain not only protein, carbohydrate and fat, but also additives such as vitamins, minerals, carotenoid pigments, phospholipids and many others, to enhance growth and survival of cultured animals.

Antibiotics

Investigations using chromatographic methods (HPLC and TLC) have determined that many artificial larval feeds, including shrimp flakes and micro-encapsulated diets, are adulterated with various antibiotics such as oxytetracycline, oxolinic acid and even chloramphenicol. Antibiotics are added to feed as growth promoters.

Hormones

Hormones such as corticosteroids, anabolic steroids and other steroids have been incorporated in feed in shrimp hatcheries to make the larvae look healthy and uniform in size.

Vitamins

In extensive culture systems, natural food may be abundant enough to provide essential vitamins, as aquatic organisms require only minute amounts of these substances for normal growth, metabolism and reproduction. However, in intensive aquaculture systems in Thailand, natural food is limited, so that the addition of vitamins to the diet is recommended. Among the vitamins, vitamin C is widely used in shrimp diets.

Immunostimulants

The most obvious disadvantage of chemical use in aquaculture is that some strains of pathogen become resistant to certain antibiotics due to their overuse or misuse. The antibiotics do not completely eliminate these pathogens, resulting in the recurrence of disease. In addition, their residues may accumulate in fish flesh and the environment.

Some scientists believe that using immunostimulators against infectious diseases is more advantageous than using antibiotics because there is no residue in tissues and strain resistance of bacteria to antibiotics is avoided. Glucan has been shown to stimulate the non-specific defense mechanism of aquatic animals and to enhance protection against bacterial challenge (Raa *et al.* 1992). Peptidoglycan has also been experimentally shown to cause significant increases in growth rate, survival and feed conversion of treated animals. It also causes a dramatic increase in the phagocytic activity of hemocytes. Several products of peptidoglycan have been widely distributed in the Thai market.

Vaccines

Vibrio anguillarum vaccine for fish is available in Thailand. Vibrogen-S is a vaccine developed by Aqua Health (Asia) Ltd. against vibriosis in marine shrimp. It is claimed to be effective against *Vibrio parahaemolyticus* under both laboratory and field conditions. It is administered by immersion, injection and with food. Immersion is recommended for larvae, while injection and oral feeding are recommended for broodstock and pond grow-out.

SUPPLY OF CHEMICALS

As a consequence of the expansion of aquaculture, especially for marine shrimp culture in Thailand, chemical usage has become increasingly a part of management. When the demand for chemicals increases, various types of commercial products are produced to meet the demand. In Thailand, the number of suppliers of aquaculture chemicals has greatly increased. They distribute products all over the country, especially in the east and south where shrimp farms are concentrated.

The following are some of the commercial chemical products presently available in the Thai market. The list is by no means exhaustive; the listed of brand names have been gathered from suppliers mainly in the east of Thailand.

Commercial Products for Pond Soil and Water Treatment

These include Freshwater Brite, N'clear, Freshwater Marplex Surewater, Plankz, Saltwater ACT, Saltwater Ultrashield, Anticlean, Chloro-60, Isorep, Algicide-27, Hydrolite, Star-Chlon, Zeolite, USA Dolomite, Cleara, Zooline, Neolife, Colorant Bactapur, etc.

Disinfectants

Disinfectants in use in Thailand include Thyrodine, Povidone, Saver BKC 80, Saver BKC 50, Chloryle, Septer 50, Septer G Glutatreat, Cleara, Biosafe, Aquasafe, Intersafe, Copper A, Povidine, Aquadine 10, etc.

Therapeutants

Among the therapeutants are Dermashell, Wound Rx, Saltwater Coppersafe, Control, Protokill plus, ZooCutx, Zotaril, etc.

Antibiotics

These include Floxpro, Farmsafe, Antidis, Imequyl-s, Oxycure, Oxxo, Terramycin, Anti Bac, Pi-Coli, Tribriksen, Chloro A, Oxy A, Fura A, Bacta A, etc.

Vitamins

Vitamin preparations in use in Thailand include Agino mixer, Farm mix, PV marine oil, Aqua green, Pure Vit, Selenium Yeast, Vitapure Aqua Stable, Vitamin C, V-100, Premium VC-A, Ginovit Aquatic C, C-sulphate, Super Omega, etc.

Immunostimulants

These include products such as β -glucan, LPS, Penstrim, Immuno Guard, Vetregard, Aquagen, Berlaa, etc.

Bioremediation Products

Bioremediation products available in Thailand include DMS-100, AB-1, Biomaster industrial, Subdu, Bacillus subtilis, Aquazyme, etc.

Cost of Chemical Use in 1995

A cost/benefit analysis of various farms in the east showed that the farmers spent about 10 Baht for chemical per 1 kg of shrimp produced. In 1995, when the shrimp production reached 250,000 t, it was estimated that at least 2,500 million Baht (US\$ 100 million) was spent on chemicals alone. These products are mostly imported from the USA, Canada, the UK, Australia and Japan.

HAZARDS AND ADVERSE IMPACTS

In response to the extensive use of antibiotics, resistant strains of pathogenic bacteria may have increased considerably; and this increase could create a great deal of difficulty in the treatment of

bacterial infections in aquatic animals.

Numerous studies have been carried out on the effects of drugs on larval quality. The use of synthetic drugs in hatcheries, especially chloramphenicol and oxolinic acid, may adversely affect larval growth and inhibit defense mechanisms. They may reduce the activity of acid or alkaline phosphate acid, which are considered to play a major role in the protein metabolism of shrimp.

Larval shrimp that consume drug-laced feed and antibiotics incorporated with steroids usually have good appearance and uniform size. Once they leave the hatcheries and reach the grow-out ponds, however, they become sensitive to external stresses and pathogens, resulting in growth retardation and/or mass mortality. Shrimp broodstock fed diets containing nitrofurans for a long period may produce eggs of lower quality.

Some chemicals, such as a mixture of formalin and malachite green, give a very effective treatment against "Ich," but malachite green has teratogenic and toxic potential.

Insecticides such as Dipterex have been widely used as effective treatments for crustacean parasites in fish ponds. Since their introduction into shrimp culture systems to treat small shrimp and other viral disease vectors such as crabs, the amount of these chemicals applied by aquaculturists has increased considerably, causing a great concern for the environment, as very little is known about their residues in aquatic animals and in the environment.

The currently quoted prices for the commercial chemical products available in Thailand are presented in Table 3.

Table 3. Current prices for commercially available chemical products used in aquaculture in Thailand.

Chemical Product	Price (US\$)	Size of Package
Pond and Water Treatments		
Zeolite	5-8	20 kg
Artificial dyes	120-140	1.5 kg
Bacterial product	20-60	5 L
Disinfectants		
B.K.C. 50%	80-120	20 L
B.K.C. 80%	200-250	20 L
Copper chelate	300-350	20 L
Povidone-iodine 10%	150-200	20 L
Vitamin and Feed Supplements		
Aquatic - C Coated-vite	10-15	1 lb
C - sulphate	10-15	1 lb
Fish oil	12-18	4 L
Squid oil	70-90	20 L
Antibiotics		
Oxytetracycline	20-30	1 lb
Oxolinic acid 50%	60-70	1 lb
Furazolidone	20	1 lb

NATIONAL REGULATIONS ON THE USE OF CHEMICALS IN AQUACULTURE

In Thailand, there is no specific legislation regarding the use of therapeutic drugs and chemicals in aquaculture. Their uses are unregulated.

Most veterinary drugs are similar to those used in human medicine, while chemicals used in aquaculture are the same as those used for agricultural purposes. The Ministry of Public Health is responsible for human drugs. The Ministry of Agriculture and Cooperatives is responsible for the chemicals used in agriculture. Recently, the Department of Fisheries, under the permission of the Ministry of Industry, took full responsibility for the regulation of 12 hazardous compounds commonly used in aquaculture. These are:

1. Acetic acid < 80% w/w
2. Benzalkonium chloride
3. Calcium hypochlorite
4. Chlorine
5. Fentin acetate
6. Trichlorfon
7. Formaldehyde
8. Hydrochloric acid < 15 % w/w
9. Rotenone
10. Sodium hydroxide < 20 % w/w
11. Sodium hypochlorite
12. Trifluralin

Importation of these compounds for use in aquaculture must be registered at the Department of Fisheries.

ON-GOING RESEARCH ON CHEMICAL USE FOR AQUACULTURE

Several research institutes in Thailand are involved in aquatic animal health research. These include:

- Aquatic Animal Health Research Institute (AAHRI), Bangkok
- National Institute of Coastal Aquaculture (NICA), Songkhla
- Faculty of Fisheries, Kasetsart University, Bangkok
- Faculty of Veterinary Medicine, Kasetsart University, Bangkok
- Faculty of Veterinary Medicine, Chulalongkorn University, Bangkok
- Faculty of Marine Science, Chulalongkorn University, Bangkok
- Mahidol Medical University, Bangkok

All of these institutes are actively involved in aquatic animal health research and research on proper chemical usage. AAHRI and NICA actively provide health management services and advice on chemical uses to farmers.

Some studies are being conducted by AAHRI on shrimp defense mechanisms and immunomodulation to enhance sustainability and reduce antibiotic usage in shrimp culture. AAHRI also does research on bacterial diseases of frogs and softshell turtles and methods for their control.

At NICA, the effect of guava (*Psidium guajava*) extract against some fish and shrimp pathogens is being studied. Studies of the immune system of black tiger prawn (*Penaeus monodon*) and on the production of bacterial and viral vaccines are also being done.

Information on chemical effects and their uses is limited in Thailand. Available information is generally obtained from text books and the scientific literature; however, information on the results of some experiments on the concentrations and efficacies of chemicals used against parasites and diseases found in Thailand and their withdrawal periods is available at the above-mentioned institutes. At present, fish and shrimp farmers obtain information on chemical usage through seminars organized by government agencies, chemical suppliers and their consultants.

RECOMMENDATIONS

To Farmers:

- Do not use chemicals to attempt to overcome poor management.
- Do not let aggressive promotion by suppliers of chemical products lead to the overuse of these drugs.
- Do not use chemotherapeutants due to their availability; use them only as a last resort.
- Treat diseases based on accurate diagnoses, and treat them as early as possible.
- Remember that high stocking density leads to a greater risk of disease and an increase in the need for chemical use.

To Producers and Suppliers of Chemicals:

The industry must be responsible for giving accurate information on the specificity of chemicals and must clearly exhibit the ingredients of each product.

To Government Agencies:

- It is the government's responsibility to establish rules and regulations on uses of drugs and chemicals.
- The government should try to find effective means to raise awareness among farmers, not only to maximize their production, but also to make them aware of the impact of chemical use on the environment and public health.
- National drug and chemical regulating boards must be established to work closely with the aquatic disease research institutes.
- Enough disease diagnostic laboratories must be established to give adequate service to farmers.
- There is an urgent need to establish an information center within the country and internationally to update, distribute and exchange information.
- An effective quarantine system should be established to prevent disease transmission.

To Regional and International Organizations:

- There is an urgent need to promote regional and international cooperation on disease prevention and to support research on chemical uses in aquaculture.
- An information center on aquatic animal health is urgently needed, especially to maintain epizootological records and to exchange this information.

To Scientists:

- Scientists need to conduct more research on chemical use in aquaculture to ensure their effectiveness and safe uses.
- Scientists should consider and prioritize research to meet the urgent needs of farmers.
- The information obtained from research results must be immediately disseminated to farmers.

However, on-farm trials should be conducted, as many of the chemicals appear not to be effective when applied in ponds or hatcheries.

- It is the responsibility of the scientists to inform the government if any drug or chemical must be prohibited.

CONCLUSIONS

Clearly, chemicals and drugs play an important role in present aquaculture systems, whether they are intensive, semi-intensive, semi-closed or closed systems. However, it is also obvious that good management can considerably reduce the chemical use in aquaculture. Certain types of chemicals, if used inappropriately, can cause damage to animals and the environment. Overuse of chemicals, especially antibiotics, not only increases production costs but also intensifies adverse consequences.

The adverse impacts of chemical use in aquaculture may be outweighed by their advantages; however, there has been increasing concern about their uses, and they must be used with great caution. Chemical residues in food products originating from aquaculture and the effects of chemicals on the aquatic environment should be monitored.

REFERENCES

- Aoki T, Umeda T, Takami K, Kitao T, Saitanu K, Chongthaleong A, Punyaratabdhu P. 1990. Drug resistant *Aeromonas hydrophila* in Thailand. In: Hirano R, Hanyu I. eds. The Second Asian Fisheries Forum. p. 693-696. Asian Fisheries Society, Manila.
- Brewsters RQ, McEwen WE. 1961. Organic Chemistry. Prentice-Hall Inc., Englewood Cliffs, NJ.
- Chanratchakool P, Turnbull JF, Funge-Smith S, Limsuwan C. 1995. Health Management in Shrimp Ponds. 2nd edn. Aquatic Animal Health Research Institute, Bangkok.
- Kanchanakarn S. 1986. Toxicity of dipterex to striped snakehead (*Channa striatus* Fowler), silver barb (*Puntius gonionotus* Bleeker) and common carp (*Cyprinus carpio* Linn.). MSc. Thesis, Kasetsart University, Bangkok, 66 p. (in Thai).
- Limsuwan C. 1985. Fish Disease. Faculty of Fisheries, Kasetsart University, Bangkok, 227 p. (in Thai).
- Limsuwan C. 1987. Bacterial and viral disease in shrimp. In: Limsuwan C. ed. Shrimp Disease and Chemotherapy p. 9-24. Faculty of Fisheries, Kasetsart University, Bangkok. (in Thai).
- Plumb J. 1995. Chemotherapy vs. vaccination: a reality for Asian aquaculture. In: Shariff M, Arthur JR, Subasinghe RP. eds. Diseases in Asia Aquaculture II. p. 43-53. Fish Health Section, Asian Fisheries Society. Manila.
- Primpol M. 1990. Study on diseases in Gunther's walking catfish (*Clarias macrocephalus*) and their prevention. M.Sc Thesis, Kasetsart University, Bangkok. 233 p. (in Thai).
- Raa J, Roerstad G, Engstad R, Robertsen B. 1992. The use of immunostimulants to increase resistance of aquatic organisms to microbial infection. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 39-50. Fish Health Section, Asian Fisheries Society, Manila.
- Ruangpan L. 1986. Seabass diseases. Thai Fish. Gazette, 6:7-30. (in Thai).
- Ruangpan L. 1987. Diseases in tiger and white shrimp in Thailand. In: Limsuwan C. ed. Shrimp Diseases and Chemotherapy. p. 46-62. Faculty of Fisheries, Kasetsart University, Bangkok. (in Thai).
- Ruangpanich N. 1988. Some problems in tiger shrimp hatcheries. Brackishwater Division, Department of Fisheries, Bangkok. 11 p. (in Thai).
- Saitanu K, Chularak C. 1983. Ulcer disease in catfish (*Clarias batrachus*): a therapeutic study. J. Aquat. Anim. Dis. 6:9-17. (in Thai).

- Tonguthai K, Chanratchakool P. 1992. The use of chemotherapeutic agents in aquaculture in Thailand. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 555-565. Fish Health Section. Asian Fisheries Society, Manila.
- Williams RR, Lightner DV. 1988. Regulatory status of therapeutants for penaeid shrimp culture in the United States. *J. World Aquacult. Soc.* 19:188-196.
- Wellborn TL. 1985. Control and therapy. In: Plumb JA. ed. Principal Diseases of Farm Raised Catfish. p. 50-67. Southern Cooperative Ser. Bull. No. 225.

WORKSHOP SUMMARY¹

The use of chemicals is common in various aquaculture systems, as it is in many agricultural practices. However, with growing worldwide awareness of the need for responsible practices in aquaculture, governments and aquaculturists are increasingly concerned with the effects of the use of chemicals in aquaculture, especially those which appear likely to be hazardous to man, cultured stock and/or the environment. The need to synthesize and disseminate information on the use and management of "aquachemicals" was recognized by the Fishery Resources Division of the Food and Agriculture Organization of the United Nations (FAO) and the Southeast Asian Fisheries Development Center (SEAFDEC) Aquaculture Department, who convened "The Expert Meeting on the Use of Chemicals in Aquaculture in Asia," which was held 20-22 May 1996 at the SEAFDEC facilities in Tigbauan, Iloilo, the Philippines. Support was provided by FAO, SEAFDEC and the Canadian International Development Agency's (CIDA) ASEAN Fund. The World Health Organization (WHO) supported the participation of a human health expert. The meeting was attended by 27 participants and more than 70 observers from the public and private sectors of 20 countries. Among the attendees were representatives from the Network of Aquaculture Centres in Asia-Pacific (NACA), the Fish Health Section of the Asian Fisheries Society (FHS/AFS), the Japan International Research Center for Agricultural Sciences (JIRCAS), the GESAMP Working Group on Environmental Impacts of Coastal Aquaculture, and the ICES Working Group on Environmental Interactions of Mariculture.

The results of this expert workshop are presented in this volume. They include the texts of presentations on a wide range of topics (thematic reviews) related to the use of chemicals in aquaculture, with emphasis on the Asian Region, as well as country overview papers summarizing the use of aquachemicals in Asian countries. The general thematic reviews included:

- the use of chemicals in aquaculture: needs, usage, issues and challenges;
- antibacterial chemotherapy in aquaculture;
- ecological effects of chemical usage in aquaculture;
- transferable drug resistance plasmids in fish-pathogenic bacteria;
- the use of chemicals in aquafeeds;
- human health aspects of the use of chemicals in aquaculture; and
- regulations on the use of chemicals in aquaculture.

In addition, country over-view papers on the use of aquachemicals were presented for Bangladesh; Cambodia; China P.R.; India; Indonesia; Japan; Lao PDR; Malaysia; Nepal; Pakistan; Philippines; Sri Lanka; Taiwan; China; Thailand; and Vietnam.

General information on international initiatives and agreements relating to or impacting upon the use of chemicals in aquaculture was also presented. This included information on the *Code of Conduct for Responsible Fisheries*, and particularly, its Article 9 on Aquaculture Development (FAO 1995; also see FAO 1997); the activities of the FAO/WHO Codex Alimentarius Commission, its Joint FAO/WHO Food Standards Programme and the Proposed Draft Code of Hygienic Practice for the Products of Aquaculture; the work of the Joint FAO/WHO Expert Committee on Food Additives (JECFA); the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) (Article XX of Legal Texts of the Uruguay Round adopted by Members of the General Agreement on Tariffs and Trade (GATT 1994); and the *International Code of Conduct on the Distribution and Use of Pesticides* (FAO 1990).

¹Prepared by: Rohana P. Subasinghe, Uwe Barg, and Cella Lavilla-Pitogo, Rome, Italy and Iloilo, Philippines, September 1999

After the presentations, participants and observers met in working groups and plenary sessions to discuss the roles and responsibilities of both the private sector (manufacturers, suppliers, retailers, and users of chemicals) and the public sector (government, line agencies and academia), in relation to the use of aquachemicals and to explore possible avenues for improved collaboration among all parties concerned.

GENERAL FINDINGS

The general findings of this expert meeting can be summarized as follows:

- A wide range of chemicals are being used in aquaculture, for numerous purposes and in different aquaculture systems. When discussing aquachemicals, it is important that clear distinctions be made between the many different aquaculture systems and species employed and the specific patterns of chemical application.
- Many chemicals are essential for successful and efficient farm and hatchery management.
- Most chemicals used in aquaculture do not appear to carry significant potential for adverse effects on human health or the environment, provided that they are applied in a technically appropriate manner.
- Significant difficulties were experienced in the compilation of data on chemical usage in Asian aquaculture, and further efforts are urgently required to generate an adequate information base to derive management advice on the safe and effective use of chemicals.
- There is a need to facilitate exchange of information and collaboration among manufacturers, suppliers, “middlemen” (salesmen, traders, etc.), importers and users (i.e., aquafarmers) of chemicals.
- The roles and responsibilities of the public sector (i.e., government and academia) are significant with regard to management and regulation of chemical usage in aquaculture.
- There are major constraints to promoting the safe and effective use of chemicals in aquaculture. These include:
 - A lack of trained manpower (e.g., experienced fish health management specialists) and related capacity-building schemes and support services to disseminate information on fish health management.
 - The misapplication of some chemicals (e.g., the excessive prophylactic use of antibacterials) that is often due to aquafarmers lacking access to information on appropriate use, or to the lack of effective yet economically viable alternative management measures or suitable alternative chemicals which would help reduce the use of some potentially hazardous chemicals. The promotion of certain chemicals by “middlemen” or drug companies may also play a significant role in the overuse of chemicals.
 - Insufficient understanding of the mode of action and efficacy of certain chemicals (e.g., some chemotherapeutants and pesticides), especially under tropical conditions.
 - Uncertainties with regard to legal and institutional frameworks to governing chemical usage in aquaculture. Specific provisions are insufficient or even lacking; the mandates and responsibilities of various line agencies in charge of public health and food safety,

agriculture, animal health services, environment etc., are sometimes not well defined, and there are enforcement problems.

- The use of chemicals in aquaculture may have significant implications for international trade of aquaculture products. Countries exporting aquaculture products, especially shrimp, are facing food safety requirements (e.g., maximum residue levels, banning of chemicals) which have been or are being formulated by importing countries. Controversy on these issues may increase due to activities by certain advocacy groups.

The findings of this expert meeting were subsequently discussed by an *ad hoc* meeting (held 24-28 May 1996) of the GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) Working Group on Environmental Impacts of Coastal Aquaculture, with a view to address major environmental and human health issues related to the use of chemicals in coastal aquaculture as practiced worldwide (GESAMP 1997). Additional progress towards world-wide aquaculture drug and vaccine registration has been made through various communication networks and committees established by the Workshop on International Harmonization for Aquaculture Drugs and Biologics, held in February 1997, and the Workshop and Round Table held at the European Association of Fish Pathologist's Eighth International Conference on Diseases of Fish and Shellfish, held in September 1997 (see Schnick *et al.* 1997). It is hoped that the proceedings of the Expert Meeting on the Use of Chemicals in Aquaculture in Asia will prove a useful basis to future progress in this important area.

REFERENCES

- FAO. 1990. International Code of Conduct on the Distribution and Use of Pesticides (Amended version). FAO, Rome, 34 p.
- FAO. 1995. Code of Conduct for Responsible Fisheries. FAO, Rome, 41 p.
- FAO. 1997. Aquaculture Development. FAO Technical Guidelines for Responsible Fisheries 5, 40 p. FAO, Rome.
- GATT Secretariat. 1994. Agreement on the application of sanitary and phytosanitary measures, p. 69-84. In: The results of the Uruguay Round of multilateral trade negotiations. The legal texts. Secretariat of the General Agreement on Tariffs and Trade, Geneva, 558 p.
- GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). 1997. Towards safe and effective use of chemicals in coastal aquaculture. GESAMP Reports and Studies No. 65, 40 p.
- Schnick R A, Alderman D J, Armstrong R, Le Gouvello R, Ishihara S, Lacierda EC, Percival S, Roth M. 1997. World wide aquaculture drug and vaccine registration progress. Bull. Eur. Assoc. Fish. Pathol. 17: 251-260.

DISCUSSIONS

On the third day of the meeting, the participants were divided into two groups to discuss the issues and problems related to the use of chemicals in aquaculture in Asia. In simultaneous discussions, the two groups talked about the role of the public and the private sectors in the use of chemicals in aquaculture. The highlights of the discussions follow.

The Public Sector's Role in the Use of Chemicals in Aquaculture

Use of chemicals (including disinfectants and fertilizers)

- Many chemicals in use are essentially harmless - e.g., fertilizers, when used properly.
- However, the need to use any chemical to control pests and diseases in aquaculture essentially indicates failure - a failure to prevent a problem that the chemical use is intended to overcome. Often problems may be solved, for example; by good husbandry, quarantine, etc.
- The decision to use chemicals of this type therefore should only follow if other methods are unavailable.
- Once chemical use is accepted, it must be understood that such use may have an impact upon:
 - a) the immediate farm (e.g., on other stocks)
 - b) the immediate environment (i.e, through discharges)
 - c) the area environment (especially when there are other farms operating near by)
 - d) the staff (toxicity, allergy, etc.)
 - e) consumers (residues)
 - f) drug resistance
- Control in the use of such chemicals to prevent or minimize such impacts, therefore, is inevitable and indeed, essential for sustainability of the industry.

Definition and quality of chemicals used in aquaculture

- If a chemical is to be deemed safe and effective in use, then it needs to be of defined quality. As an example, one may take the case of antimicrobial products, which, if they contain inadequate active ingredient, will encourage drug resistance and drug misuse.
- Chemicals must be suitable for the purpose – it is of no value to use the therapeutant effective against Gram-positive bacteria when the pathogen is Gram negative.

Training and cooperation

- The basic concepts of the problems and advantages to be derived from the use of chemicals in aquaculture need to be properly understood.
- Training, both formal and informal, is the best way to achieve this.

- In the case of some very toxic chemicals, staff may require specific safety training before being allowed near them.

Development of alternatives to prevent and treat disease, including:

- Prevention
- Better husbandry
- Vaccines
- Better chemicals (e.g., less environmentally damaging and less likely to induce resistance)

Feed and additives

- Additives could be included under “general chemicals,” but they are somewhat different – they are used to:
 - a) improve growth
 - b) confer better disease resistance
 - c) enhance broodstock performance
 - d) improve product quality (e.g., pigments)
- However, use of hormones and antibiotics to improve growth will not be applicable to certain markets (e.g., EU)

Product (the chemical) evaluation

Safety and Efficacy

- This simple phrase covers a multitude of items:
 - a) safety to the inventory
 - b) safety to farm staff
 - c) safety to the environment
 - d) safety to the consumer
- *Efficacy* is simple: the product should work – it should meet the claims made by the manufacturer or supplier.

Roles and responsibilities

Government

- Must retain overall control of chemical usage. These avenues are available relative to the producer:
 - a) advice and guidance (how, when and why to use, how to avoid problems; to warn of effects of international and market regulations)
 - b) research support (often on-farm)
 - c) regulation – to control and be aware of products in use – responsibility for the safety

- of the public (consumers) and the national interest (exports)
- d) product evaluation - to examine data from manufacturers/suppliers and decide (license) whether a chemical may be used safely

Universities

- Research “away” from market but must be oriented towards practicalities. Papers about another new antibiotic's effect on disease “x” are less valuable than research oriented towards the more practical.
- Results should always be converted to advice – either to industry or to government, as appropriate. Without this communication, the research is of limited value.

The Private Sector's role in the Use of Chemicals in Aquaculture

Roles and responsibilities for the private sector (farmers, chemical suppliers/traders):

1. Chemical manufacturers, suppliers and traders should:

- undertake proper labelling of drugs/chemicals (ingredients, methods of use, handling, risks) and undertake responsibilities for substantiating claims on products.
- work closely with the farmers and researchers in the development of effective chemicals for use in aquaculture. The need for pharmaceutical companies to fund research on aquaculture chemicals was recognized.
- consider ways to establish industry codes of practice or certification for the marketing of aquaculture chemicals. The certification might be undertaken in close consultation with scientists and appropriate government agencies. Self-policing of manufacturing and marketing practices, in accordance with agreed-upon standards and practices, was recognized to be desirable.
- undertake closer cooperation with the farmers and undertake activities to get information across to farmers. This was in response to a perceived problem associated with “repackaging” of chemical products by traders.
- (pharmaceutical industry exporting chemicals to tropical countries) should attempt to learn aquaculture, and the appropriateness of their chemicals to tropical conditions. They should also actively seek cooperation with Asian farmers and research organizations in undertaking research on safe and effective aquaculture chemical use.
- assist farmers by providing correct information on product usage and substantiating claims, mainly through labelling of products.

2. Hatchery operators/farm operators should:

- undertake actions to make safe and effective use of chemicals in aquaculture, and seek ways to reduce those actions recognized to be potentially damaging to public health and environment.

- consider establishing/strengthening associations, which would guide members, properly coordinate with government agencies and research institutions, and disseminate information on safe and effective use of aquaculture chemicals.
- develop measures for the safe disposal of aquaculture chemicals, including effluent treatment, as appropriate. Advice was requested from the scientists and government on appropriate measures to reduce problems related to effluent discharge caused by use of chemicals.
- actively engage with governments in the ecological monitoring of aquaculture environments, to ensure environmental quality and sustainability of aquaculture.

Requests made by the private sector (farmers, chemical suppliers/traders) for the support of governments and scientists, and others:

1. Governments should:

- support farmers in monitoring the quality of aquaculture products with respect to chemical use, and assist in self-policing efforts among farmers' groups.
- support farmers in finding alternatives to banned products (e.g., the use of the molluscicide Brestan).

2. Scientists should:

- actively engage in research with the private sector in the development of effective and safe measures for the use of chemicals, proper dosages, and proper diagnosis of disease.
- assist hatchery operators in the development of technologies which effectively eliminate the use of chemicals in hatchery production.
- disseminate information to farmer groups (and individual farmers, where possible) on the safe and effective use of aquaculture chemicals.
- provide, as a matter of urgency, information on chemicals with potentially harmful effects on health, environment and markets.
- undertake research on the effects of chemical sterilants on soils and pond productivity.

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