Chapter 14
Control

Abstract Disease control strategies include better husbandry/management practices, consideration of the use of genetically disease resistant fish strains when available, the use of suitable diets/dietary supplements, vaccines, non specific immunostimulants, probiotics, prebiotics, natural plant products, antimicrobial compounds, water disinfection, and prevention of/restriction in the movement of infected stock.

Keywords Vaccines • Probiotics • Immunostimulants • Plant products • Antimicrobial compounds

It is worth remembering the age-old adage that 'prevention is better than cure', and certainly it is possible to devote more attention to preventing the occurrence of disease in fish. This is especially true for farmed fish, which tend to be at the mercy of all the extremes which their owners are capable of devising. Principally in the industrialised nations, farmed fish are subjected to questionable water quality and high stocking regimes. These are among the known prerequisites for the onset of disease cycles. Yet, owners are among the first to seek help if anything adverse happens to the valuable stock. Fish may be reared under ideal conditions, in which case, the stock are inevitably in excellent condition without signs of disease. Such sites, for example located in Venezuela and the former Yugoslavia, are usually supplied by fast-flowing, clear river water. Careful feeding regimes are adopted, and the stocking levels are comparatively low. The latter point would make the enterprise unacceptable in the more industrialised nations of Western Europe. Therefore, much attention has been devoted to control measures. These have been categorised in Table 14.1. Although most emphasis has been placed on aquaculture, some effort has gone towards considering disease in wild fish stocks.

Wild Fish Stocks

It is questionable what, if anything may be done to control disease in wild fish stocks. Perhaps, the first step should be to determine the precise extent of disease among wild fish populations. Surveys have been carried out with a view to assessing

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the incidence of 'abnormalities' in marine fish. Indeed, some workers have attempted to correlate the incidence of disease with pollution. Some attention has focused on archive material, collected at or before the turn of the century, and housed in some museums, e.g. in Liverpool, UK, with access usually granted to interested individuals. A detailed study would soon demonstrate whether or not 'abnormalities' in fish are a new or old phenomenon. Surely, this information could then be correlated with the changes in pollution of the aquatic environment. It is our contention that dense populations of fish have always maintained a given level of diseased individuals, regardless of whether the populations are shoals in the sea or aquacultural stocks. Therefore, it is possible that reducing pollution will not noticeably alter the health index of wild fish. Nevertheless, by using a circuitous route, it may be possible to ensure that wild fish stocks are not likely to be exposed to pathogens, and therefore be at less risk of disease. Theoretically, this could be achieved by controlling outbreaks of disease in farmed fish and thereby reducing the possibility of pathogens escaping into the environment. It should be emphasised, however, that there is a dearth of information, which suggests that disease may be transferred from farmed to wild fish stocks. At worst, there is a perceived problem, and this could easily escalate into adverse propaganda for the aquaculture industry. It is essential that consideration should be urgently given to control measures, which will reduce any possible risk of pathogens escaping into the natural environment.

Farmed Fish

There are many approaches, which need to be adopted in order to control bacterial disease in farmed fish (Table 14.1). These will be explained separately below.

Table 14.1 Methods of controlling bacterial fish diseases

Classification of fish stocks	Disease control measures	
Wild	Control of pollutants (water quality)	
Farmed	1.	Adequate husbandry/management practices
	2.	Use of genetically resistant fish strains
	3.	Suitable diets and where appropriate, use of dietary supplements
	4.	Use of vaccines
	5.	Use of nonspecific immunostimulants
	6.	Use of probiotics/biological control
	7.	Use of prebiotics/medical plant products
	8.	Use of antimicrobial compounds
	9.	Water treatments
	10.	Preventing the movement of infected stock

Husbandry/Management

It may be problem that under severe economic pressures the aquaculturist is tempted to produce the maximum yield of fish in a finite volume of water, i.e. to use very high stocking levels. Some sympathy must be directed towards the fish farmers especially when prices paid for the stock are low and profit margins are tight or inadequate. The underlying problem is that within intensive cultivation systems, the fish may be 'stressed' beyond the limit commensurate with the production of healthy specimens. Interestingly, it has been suggested that reducing the stocking density when water temperatures are high may well prevent some diseases, such as columnaris outbreaks in rainbow trout (Suomalainen et al. 2005). The outcome would be that the lower stocking level leads to less disease, and more output. Stress may be compounded by other inappropriate management practices in which aeration and water flow are insufficient, overfeeding occurs, and hygiene declines below the threshold at which disease is more likely to ensue. It may need only one diseased individual to act as a reservoir of infection to the rest of the stock. Unsatisfactory occurrences, which are readily controlled, include:

- the accumulation of organic matter, namely faecal material and uneaten fish food, within the fish holding facilities, which allow the buildup of microbial populations, some of which may cause disease. Also, such organic matter may attract vermin, which may pose other health issues including the risk of human disease, e.g. Weil's disease/leptospirosis;
- the presence of dead fish in the tanks/ponds/cage/raceways for prolonged periods (= bad sanitation). Again, the permits the increase in microbial populations as well as contributing harmful chemicals to the water;
- the accumulation of a biofouling community, i.e. algae and slime, in the fish tanks, and the problem associated with the collapse of blooms resulting in the release of toxic materials;
- the depletion of the oxygen content of the water with a concomitant increase in nitrogen levels, especially as ammonium salts;
- the lack of proper disinfection for items entering the fish holding facilities. Reference is made here to nets, protective footwear, and size grading machinery;
- lack of adequate sanitary disposal arrangements for dead fish, allowing access to birds, e.g. seagulls, and vermin, and the build-up and spread of potentially harmful micro-organisms.

Good basic hygiene (water quality) and farm husbandry practices may successfully alleviate many of the problems attributed to disease.

Disinfection/Water Treatments

Apart from the use of antibiotics and related compounds, which are actively discouraged from use in any nonmedical situation in many countries, the application of other chemicals to water as disinfectants is effective for disease control. Such chemicals include benzalkonium chloride, chloramine B and T, chlorine, formalin and iodophors.

Another approach is to alter (increase or decrease, according to the season) the temperature of water within fish holding facilities, which may be achieved by altering the water flow, providing shading, or actively heating/cooling as appropriate.

Genetically Resistant Stock

This is a topic worthy of greater attention, insofar as there are numerous observations, which point to the value of genetically resistant strains or selective breeding for reducing the problems of disease. As a word of caution, however, comparative studies need to be carefully controlled so that meaningful results are obtained. In any comparison, the age, size and relative condition of the animals need to be standardised. Nevertheless, there has been prolonged interest in breeding disease-resistant fish. It is obvious that the breeding of disease-resistant fish may be a valuable addition in the armoury of disease control in aquaculture. However, in fish farming where more than one disease is prevalent, it is not necessarily the case that a fish strain which is resistant to one disease, would similarly show resistance to others. Nevertheless, we consider that disease-resistant strains of fish have potential for areas in which diseases are enzootic. Further effort is clearly required to bring the concept to fruition. One overriding concern is the public misconception of the risks associated with GMOs, which is profound in some countries, particularly in Western Europe, but less so or non-existent in others.

Adequate Diets/Dietary Supplements/Non-Specific Immunostimulants

An area of comparatively recent interest is that of dietary influence on fish health. Could some essential nutrients be lacking, or other compounds be present in dangerous excess? A mass of published work has pointed to the benefit to health, welfare and growth of the addition of any of a wide range of natural products to fish feed. For example, Ketola (1983) highlighted a requirement for arginine and lysine by rainbow trout fry, with fin erosion resulting from a deficiency of lysine. The list

includes natural plant products, immunostimulants, vitamins and micro-organisms. The outcome of the work, which has been summarized, in earlier chapters, is that the addition of comparatively small amounts of these products to fish feed leads to statistically significant improvements in growth, including feed conversions, non-specific immunostimulation particularly of innate and cellular immune parameters, e.g. increases in phagocytic activity, and health benefits in terms of protection against challenge by specified pathogens within a fairly small timespan, i.e. positive effects may be observed within 1–2 weeks of the first application of the product. In addition, there have been indications of non-specific health benefits, such as an increase in vigour and an absence of background levels of fin/tail rot. For example, ß-glucans, which may be obtained from the yeast *Saccharomyces cerevisiae*, when administered to carp led to significantly increased leucocyte populations, enhanced proportions of neutrophils and monocytes, and elevated superoxide anion production by kidney macrophages (Selvaraj et al. 2005). Moreover, spray-dried, heterotrophically grown preparations of the unicellular alga *Tetraselmis suecica* have been accredited with antimicrobial activity and possibly immunostimulatory activity when used as dietary supplements (Austin et al. 1992a). Similarly, the yeast *Debaryomyces hansenii* was immunostimulatory when fed for 4 weeks at 10^6 CFU/g to leopard grouper (*Mycteroperca rosacea*) (Reyes-Becerril et al. 2008).

The list of immunostimulatory compounds is extensive, and includes those which have often been applied by i.p. injection, i.e. Batpamum, chitin, dimerised lysozyme, ß-1,3 glucans, killed cells of mycobacteria, laminaran, sulphated laminaran, lactoferrin, levamisole, LPS, oligosaccharides, Prolactin and synthetic peptides (Dalmo and Seljelid 1995; Yoshida et al. 1995; Ortega et al. 1996; Siwicki et al. 1998; Sakai 1999). Initially, Olivier et al. (1985a, b) observed that administration of killed cells of mycobacteria enhanced resistance in coho salmon to various bacteria. However, considerable interest has been directed towards the potential for ß-1,3 glucans, which are often included as one of the ingredients in fish feed. An extensive literature points to the success of glucans in preventing disease (Yano et al. 1989; Raa et al. 1990; Robertsen et al. 1990; Nikl et al. 1991; Matsuyama et al. 1992; Chen and Ainsworth 1992). Thus, it has been recognised that ß-glucans enhance the non-specific resistance to disease, including pasteurellosis (Couso et al. 2003), ERM, Hitra disease and vibriosis, by immunostimulation (Robertsen et al. 1990; Kumari and Sahoo 2006). Matsuyama et al. (1992) used the glucans schizophyllan and scleroglucan to protect against streptococci. Thus, 2–10 mg of glucans/kg of fish when administered by i.p. injection, enhanced resistance of yellowtail to streptococcicosis. In particular, there was an elevation of serum complement and lysozyme, and an increase in phagocytic activity of pronephros cells. Initially, success only appeared to result from injection of the glucans into fish. Yet, claims have now been made that application via food also meets with success (Onarheim 1992). Also, resistance to streptococcicosis and vibriosis has been enhanced following the oral administration of peptidoglycan from *Bifidobacterium* (Itami et al. 1996) and *Cl. butyricum* (Sakai et al. 1995), respectively.

Vaccines

The expectation during the 1970s was that vaccines would become the primary means of disease prevention. Yet in many cases the programmes of research have not resulted in many commercial products. However, there have been success stories, such as the vaccines for the control of vibriosis (caused by *V. anguillarum* and *V. ordalii*) and ERM. The research has progressed from the simplistic and often completely successful approach of using chemically-inactivated whole cell suspensions to purified subcellular antigenic components of pathogens and to live attenuated vaccines.

Historically, the first serious attempt to develop a bacterial fish vaccine may be traced to the work of Duff (1942), who used chloroform-inactivated cells to protect cutthroat trout (*Salmo clarki*) against furunculosis. Since then, vaccines have been formulated against half of the total number of bacterial fish pathogens.

Composition of Bacterial Fish Vaccines

The composition of bacterial fish vaccines may be categorised as follows:

- Chemically or heat-inactivated whole cells. These vaccines may be mono- or polyvalent. Essentially, these are the simplest, crudest and cheapest forms of fish vaccines.
- Inactivated soluble cell extracts, i.e. toxoids.
- Cell lysates.
- Attenuated live vaccines, (e.g. LaFrentz et al. 2008) possibly genetically-engineered cells. These would be unacceptable to some regulatory authorities because of the perceived risk that the vaccine strain may revert to a pathogenic mode.
- Attenuated live, heterologous vaccines. An example is Bacillus Calmette and Guèrin (BCG), which is live attenuated *Mycobacterium bovis* product, protected Japanese flounder against mycobacteriosis (Kato et al. 2010).
- Subunit vaccines, e.g. the genes product of the *tapA* gene for the control of *Aer. salmonicida* infections (Nilsson et al. 2006).
- DNA vaccines (e.g. Pasnik and Smith 2006; Jiao et al. 2009; Sun et al. 2010).
- Purified sub-cellular components, e.g. glyceraldehyde-3-phosphate dehydrogenase (GAPDH), OMP and LPS. These vaccines require a detailed understanding of microbial chemistry, aspects of which are deficient for many of the bacterial fish pathogens.
- Serum, for passive immunisation (e.g. Shelby et al. 2002). This is largely of academic interest only, insofar as it is difficult to envisage use of the technique in the fish farm environment. A possible exception is for brood stock or pet fish.
- Mixtures of the components, detailed above.

It is difficult to identify any particular type of preparation which excels in terms of protection. Unfortunately, even the best vaccines may not completely prevent the occurrence of disease, sometimes necessitating the use of costly medicines to combat low levels of infection.

A potentially exciting and relevant development has involved the realization that some molecules may have broad spectrum use as vaccine candidates by offering protection against a range of pathogens. In this connection, recombinant GADPHs offer cross protection with RPS values of >60 % (Li et al. 2011).

Methods of Vaccine Inactivation

Methods to inactivate whole cell preparations include the use of chemicals, namely 3 % (v/v) chloroform, 0.3–0.5 % (v/v) formalin and 0.5–3.0 % (v/v) phenol, heat (e.g. 56 °C or 100 °C for 30 or 60 min), sonication, pressure (600 kgf/cm^2 for 5 min), electric current (100 mA at 12v DC for 5 s) lysis with sodium hydroxide at pH 9.5 or with SDS (Austin 1984b; Hossain and Kawai 2009). Commercially, most interest has centred on use of formalin, which has given encouraging results with numerous bacterial fish pathogens, including *Aer. hydrophila, Edw. ictaluri, Ph. damselae* subsp. *piscicida, Ps. anguilliseptica, V. anguillarum, V. ordalii* and *Ali. salmonicida.* However, it is unfortunate that only a few studies have been carried out to compare different inactivated preparations.

Methods of Administering Vaccines to Fish

A number of methods of administering vaccines to fish have been tried with varying degrees of success (see Austin 1984b), and include:

- Injection, with or without the presence of adjuvant, such as FCA/FIA. This technique is slow, and will inevitably require prior anaesthesia of the animals. Injection is only feasible for valuable fish, brood stock or pet fish. Fortunately, mass injection techniques are available.
- Oral uptake, via food. This should be the method of choice insofar as fish could be fed and vaccinated simultaneously. However, there may be problems with the degradation of the vaccine in the gastro-intestinal tract, although this is being overcome by new exciting oralising compounds.
- Immersion in a solution/suspension of the vaccine. This is quick (i.e. taking 30–120 s to perform) and easy, permitting large numbers of fish to be readily vaccinated. However, there could be problems regarding disposal of the spent vaccine. Thus, it is debatable whether or not disposal should take place in the fish farm effluent.

- Bathing in a very dilute preparation of the vaccine for prolonged periods, i.e. several hours. This is obviously very economic in the use of vaccine. It is feasible that the technique could be carried out during routine periods of confinement, such as during transportation of the stock between sites. However, with immersion, careful thought needs to be given to the question of disposal.
- Spraying or showering the vaccine onto fish. This can be automated, such that fish are vaccinated on conveyor belts during routine grading.
- Hyperosmotic infiltration. This involves a brief immersion (30–60 s) in a strong salt solution, i.e. 3–8 % (w/v) sodium chloride, followed by dipping for 30–60 s into the vaccine. This method is very stressful to fish, and its use has been consequently reduced. However, *En. faecalis, Lactococcus garvieae* and *Str. iniae,* which have been identified from diseased farmed fish in Taiwan, have been the subject of vaccine development involving formalin-inactivated whole cell preparations that were administered to Nile tilapia by hyperosmotic infiltration leading during the fifth and sixth week after challenge to RPS values of 71.2–88.7 %. Boosters did not improve protection (Young et al. 2012).
- Anal/oral intubation. In particular, anal intubation offers possibilities for bypassing the deleterious effects of the stomach and intestine. The technique is, however, cumbersome and requires further development.
- Ultrasonics/ultrasound (Zhou et al. 2002; Navot et al. 2011).

It is often difficult to determine which is the most effective method of vaccine application. The method of choice often reflects the whims of the user as much as scientific reasoning. The available evidence suggests that oral administration fares least well, although new approaches of micro-encapsulation offer promise. The use of oral vaccination for booster doses, has been successful, such as with *Aer. hydrophila, Aer. salmonicida, Fla. columnare, Ph. damselae* subsp. *piscicida, V. anguillarum, V. ordalii* and *Y. ruckeri* vaccines; i.p. injection may better that oral uptake in terms of the resultant humoral antibody titre and protection, although there is not always a direct correlation between antibody-titre and protection. However, with *Edw. ictaluri, Edw. tarda, V. anguillarum* and *V. ordalii* preparations, immersion has been demonstrated as superior to injection. Similarly with the 'vibrio' vaccines, the shower method exceeds injection in terms of resulting protection. There is a question mark about the difference between vaccines – particularly oral vaccines – and immunostimulants/feed additives that also give health benefits including immunostimulation and protection against disease. The levels of protection between these alternatives are often compatible, with the natural products being cheaper and easier to adopt particularly in terms of regulatory machinery that is engrained in many countries.

Traditionally, potency testing has been by infectivity experiments whereby vaccinated fish are challenged with a virulent culture of the pathogen, and mortalities counted and used to calculate the RPS. The severity of the approach has focused attention of regulatory authorities, and a stated aim is to move towards non-lethal testing methods, notably the measurement of key immunological parameters, e.g. antibody production, which may be measured by ELISA (Romstad et al. 2012).

Antimicrobial Compounds

There is a trend away from the use of antibiotic-like antimicrobial compounds in all non-human applications with concerns about the possibility of tissue residues and the development of bacterial resistance. It is astounding that so many compounds (these have been reviewed by Snieszko 1978; Herwig 1979; Austin 1984a) have found use in aquaculture. The complete list reads like an inventory from any well-equipped pharmacy. Antibiotics, many of which are important in human medicine, appear side by side with compounds used almost exclusively in fisheries. In many instances, the introduction of a compound into fisheries use has followed closely after the initial use in human medicine. Perhaps in retrospect, it is surprising that there has not been any significant furore from the medical profession about, what could be perceived as misuse of pharmaceutical compounds.

The use of antimicrobial compounds in fisheries essentially started with the work of Gutsell (1946), who recognised the potential of sulphonamides for combating furunculosis. Indeed, it may be argued that the effectiveness of sulphonamides led to a temporary decline of interest in vaccine development. In fact, the eventual emergence of antibiotic-resistant strains of fish pathogenic bacteria led to renewed interest in vaccines. However during the years following the Second World War, sulphonamides appeared to be the mystical saviour of fish farming. Important developments included the work of Rucker et al. (1951), who identified sulphadiazine as an effective chemotherapeutant for BKD. This claim was subsequently refuted by Austin (1985). The next substantial improvement with sulphonamides resulted from potentiation, i.e. the use of mixtures of trimethoprim and sulphonamide. These have proved to be extremely useful for the treatment of furunculosis.

Following the introduction of sulphonamides, the range of antimicrobial compounds in aquaculture rapidly expanded to encompass chloramphenicol (Wold 1950), oxytetracycline (Snieszko and Griffin 1951), kanamycin (Conroy 1961), nifurprazine (Shiraki et al. 1970), oxolinic acid (Endo et al. 1973), sodium nifurstyrenate (Kashiwagi et al. 1977a, b), flumequine (Michel et al. 1980) and Baytril (Bragg and Todd 1988). Unfortunately, detailed comparative studies of the various antimicrobial compounds are rare; consequently it is often difficult to assess the value of one drug (= any medicinal compound; Sykes 1976) over another. Nevertheless, a pattern has emerged which points to the benefits of quinolines for controlling diseases caused by a wide range of Gram-negative bacteria

Whatever the range of compounds available, their effectiveness is a function of the method of administration to fish (and in the way in which it is carried out). We have listed seven basic approaches to the administration of antimicrobial compounds to fish (Table 14.2). These are the oral route via medicated food and bioencapsulation, bath, dip and flush treatments, injection and topical application. With the oral method, drugs are mixed with food, and then fed to the fish. Usually, the treatment regime leads to the administration of a unit weight of drug to a standard weight of fish per day for a pre-determined period. Examples of commonly used antimicrobial compounds have been included in Table 14.3. Fortunately, medi-

Table 14.2 Methods for application of antimicrobial compounds to fish

Method of application	Comments
Oral route (on food)	Need palatable components; minimal risk of environmental pollution
Bioencapsulation	Need palatable compounds; minimal risk of environmental pollution
Bath	Need for fairly lengthy exposure to compound, which must be soluble or capable of being adequately dispersed; problem of disposal of spent drug
Dip	Brief immersion in compound, which must be soluble or capable of being adequately dispersed; problem of disposal of dilute compound
Flush	Compound added to fish holding facility for brief exposure to fish; must be soluble or capable of being adequately dispersed; poses problem of environmental pollution
Injection	Feasible for only large and/or valuable fish; usually requires prior anaesthesia; slow; negligible risk of environmental pollution
Topical application	Feasible for treatment of ulcers on valuable/pet fish

cated food appears to be quite stable (McCracken and Fidgeon 1977). Moreover, this method is advantageous insofar as the quantities of compound fed to the fish are carefully controlled, and if sensible feeding regimes are adopted, only minimal quantities would reach the waterways. Three provisos exist, namely that:

- the fish are capable of feeding,
- the drug is palatable,
- the drug is capable of absorption intact through the gut.

An interesting approach has involved bioencapsulation, principally of quinolones (Duis et al. 1995). This theme was expanded with some excellent work which examined the potential for *Artemia* nauplii to serve as carriers to sulphamethoxazole and trimethoprim for the chemotherapy of diseased marine fish fry (Touraki et al. 1996). Both these compounds accumulated in the nauplii, with maximal levels recorded after 8 h. In a trial with sea bass larvae challenged with *V. anguillarum*, an improvement in survival followed use of the medicated nauplii (Touraki et al. 1996). Whether or not the fish will feed is largely a function of the nature and severity of the disease. Often in advanced cases of disease the fish will not feed. Therefore, it is vitally important that treatment begins as soon as possible after diagnosis has been established. The aquaculturist will need to seek specialist advice as soon as any abnormal behaviour or unhealthy condition is noted. This means that good management practices need to be routinely adopted.

Palatability of fisheries antimicrobial compounds receives only scant attention. Whereas it is accepted that little can be done to improve the palatability of the active ingredient, effort could be directed towards improving binders and bulking agents, which are commonly contained in proprietary mixes. Perhaps, consideration could be given to using chemical attractants.

Application by the water-borne route becomes necessary if the fish refuse to eat, and, therefore, would be unlikely to consume any medicated food. With these methods, the fish are exposed to solutions/suspensions of the drug for a pre-determined period. This may be only briefly, i.e. a few seconds duration (‘dip’), or for many

Table 14.3 Methods of administering some commonly used antimicrobial compounds to fish

Antimicrobial compound	Diseases controlled	Method(s) of administration
Acriflavine, neutral	Columnaris	5–10 mg/l in water for several hours to several days
Amoxicillin	Furunculosis, gill disease	60–80 mg/kg body weight of fish/day/10 days
Benzalkonium chloride	Fin rot, gill disease	1–2 mg/l of water for 1 h, 100 mg/l of water for 2 min
Chloramine B or T	Fin rot, gill disease mycobacteriosis	18–20 mg/l of water at pH 7.5–8.0, treat for 2–3 days
Enrofloxacin (=Baytril)	BKD, furunculosis	10 or 20 mg/kg body weight/day/for 10 days
Erythromycin	BKD, streptococcicosis	25–100 mg/kg of fish/day for 4–21 days
		20 mg of erythromycin/kg of broodstock as an injection
Florfenicol	Furunculosis, vibriosis	10 mg/kg body weight of fish/day for 10 days
Flumequine	Furunculosis, ERM, vibriosis	6 mg/kg of fish/day for 6 days
Furanace	Coldwater disease, columnaris, fin rot, gill disease, haemorrhagic septicaemia, vibriosis	(a) 2–4 mg/kg of fish/day for 3–5 days
		(b) 0.5–1 mg/l of water for 5–10 min, as a bath
Iodophors	Acinetobacter disease, BKD, flavobacteriosis, furunculosis, haemorrhagic septicaemia, mycobacteriosis	50–200 mg of available iodine/l of water for 10–15 min
Oxolinic acid	Columnaris, ERM, furunculosis, haemorrhagic septicaemia, vibriosis	(a) 10 mg/kg of fish/day for 10 days
		(b) 1 mg/l of water, as a bath for 24 h (recommended for columnaris)
Oxytetracycline	Acinetobacter disease, CE, coldwater disease, columnaris, edwardsiellosis, emphysematous putrefactive disease, ERM, enteric septicaemia, fin rot, furunculosis, gill disease, haemorrhagic septicaemia, redpest, salmonid blood spot, saltwater columnaris, streptococcicosis, ulcer disease	50–75 mg/kg of fish/day for 10 days (doses of 300 mg/kg of fish/day for indefinite periods are used to treat RTFS)
Potentiated sulphonamide	ERM, furunculosis, haemorrhagic septicaemia, vibriosis	30 mg/kg of fish/day for 10 days
Sodium nifurstyrenate	Streptococcicosis	50 mg/kg of fish/day for 3–5 days

minutes to several hours ('bath'). It is essential that the compounds are soluble or, if insoluble, are dispersed evenly in the water by means of surfactants or other dispersants (Austin et al. 1981). Also, seawater cations may well antagonise antimicrobial compounds in seawater (Barnes et al. 1995). One major drawback, however, concerns the disposal of the spent compound. Ideally, it should not be released into the aquatic environment, particularly if there are any abstraction points for potable water supply systems in the vicinity. Neglect of this point could lead to legal repercussions.

Flush treatments also involve the addition of drugs, albeit at high concentrations, to the water in stock-holding areas. After addition, the drug is flushed through the system by normal water flow. Flushing inevitably results in only a brief exposure to the inhibitory compound; therefore, quick acting agents are absolutely necessary. As before, the major problem is adequate disposal of the spent drug.

Injection of drug solutions is feasible for valuable stock, such as brood fish and ornamental/pet fish. However, the technique is slow and will undoubtedly require prior anaesthesia of the animals.

The topical application of antimicrobial compounds is worthy of consideration for valuable and/or pet fish. In the case of ulcers, we recommend that the animal should be gently removed from the water, and the antimicrobial compound (preferably as a powder) applied to the lesion, which is then sealed with a waterproof covering, e.g. with dental paste. The lesions tend to heal quickly, with only limited evidence of scarring.

Whatever the chosen method of application, drugs may be used for prevention, i.e. prophylaxis, or treatment, i.e. chemotherapy, of fish diseases. Certainly, it is comforting to note that there are treatments available for the majority of the bacterial fish pathogens. Providing that drugs are used prudently and correctly, they will continue to offer relief from the rigours of disease for the foreseeable future.

Preventing the Movement and/or Slaughtering of Infected Stock

Some diseases, e.g. BKD, ERM and furunculosis, are suspected to be spread through the movement of infected stock. Therefore, it is sensible to apply movement restrictions or even adopt a slaughter policy to diseased stock, as a means of disease control. This may prevent the spread of disease to both farmed and wild fish. Of course, the issue of movement restrictions is highly emotive among fish farmers. However, the procedure may be beneficial to the industry when viewed as a whole. Certainly, the concept of movement restrictions usually involves legislative machinery, of which the Diseases of Fish Act (1983) in Great Britain is a prime example. To work effectively, there is a requirement for both the efficient monitoring of all stock at risk to disease, and the dissemination of the information to all interested parties. However, we believe that in any allegedly democratic society where such measures are adopted, there should be adequate compensation to the fish farmer for loss of revenue.

Probiotics/Biological Control

There has been a great increase in the number of publications about probiotics over the last two decades. There is much evidence that members of the natural aquatic microflora, including components of the fish intestinal microflora (Fjellheim et al. 2007; Pérez-Sánchez et al. 2011), are effective at inhibiting fish pathogens, by competitive exclusion (e.g. Laloo et al. 2010) which may involve the production of antibiotics or low molecular weight inhibitors. Dopazo et al. (1988) discovered the presence in the marine environment of antibiotic-producing bacteria, which inhibited a range of bacterial fish pathogens, including *Aer. hydrophila*. These inhibitors produced low molecular weight (<10 kDa) anionic, thermolabile antibiotics. Subsequently, Chowdhury and Wakabayashi (1989); Austin and Billaud (1990) and Westerdahl et al. (1991) reported the presence of microbial inhibitors of *Fla. columnare, Ser. liquefaciens* and *V. anguillarum.* Smith and Davey (1993) identified a fluorescent pseudomonad which antagonised *Aer. salmonicida.* Apart from the lactic acid bacteria (e.g. Pérez-Sánchez et al. 2011), that are mostly linked with probiotic activity in terrestrial animals, aquaculture has utilised a wide range of Gram-positive and Gram-negative bacteria, yeast, microalgae and even bacteriophages. The use of Gram-negative bacteria from genera associated with fish disease, e.g. *Aeromonas* and *Vibrio,* is of concern because of the perceived risk of the introduction of virulence genes such as by horizontal gene transfer although this has never occurred – yet! "Good" bacteria have been described for the control of numerous diseases, and there is a tendency that the probiotic works faster than an oral vaccine (see Irianto and Austin 2002). The assumption that probiotics must be live preparations was dashed when it was demonstrated that formalised suspensions of cells were effective at controlling atypical *Aer. salmonicida* infection in goldfish (Irianto et al. 2003) and furunculosis in rainbow trout (Irianto and Austin 2003), when applied as feed additives. Furthermore, subcellular components, i.e. OMPs and ECPs, of probiotics were immuno-reactive with *V. harveyi* antiserum (Arijo et al. 2008) and were immunostimulatory and protected against challenge with *Aer. hydrophila* in rohu (Giri et al. 2015a, b). Along a similar theme, i.p. or i.p. injection of cell wall proteins, OMPs, LPS and whole cell proteins of two probiotics, *A. sobria* GC2 and *Bacillus subtilis* JB-1 protected rainbow trout against challenge with *Y. ruckeri* (Abbass et al. 2009). There is evidence that feeding probiotics enhances the resistance to disease when stocking density is high. Thus, Tapia-Paniagua *et al.* (2014) described the use of *She. putrefaciens* Pdp11 in increasing the stress tolerance of sole (*Solea senegalensis*) when farmed under high stocking levels. The fish which received Pdp11 better survived a natural outbreak of disease caused by *Vibrio* spp. and modulated the intestinal microflora, which was correlated with a high number of goblet cells (Tapia-Paniagua et al. 2014).

Apart from competitive exclusion, probiotics work by stimulation of the innate immune response (Irianto and Austin 2003; Kim and Austin 2006) in which case they could be considered as heterologous oral vaccines, and interference with adhesion to intestinal mucosal surfaces (Chabrillón et al. 2005). The beneficial effect of probiotics may be further enhanced by the use of prebiotic carbohydrates, notably

arabinoxylo-oligosaccharide, ß-glucan, glucose, inulin, oligo- fructose and xylo-oligosaccharide that promote the growth of the "good bacteria" (Rurangwa et al. 2009).

In addition to the organisms mentioned above, there is a report of the benefits for disease control of using the biopesticide, *Bacillus thuringiensis* (Meshram et al. 1998).

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