White Paper No. 2[1]
Antibiotics in Salmonid Aquaculture: Does Their Use Justify the Risks?

1 Introduction

1.1 Antibiotics in Aquaculture

Antibiotics, both natural and synthetic, have been successfully used for decades in aquaculture to control bacterial diseases. There is general agreement, however, that the use of antibiotics for this purpose should be held to a minimum because they have the potential to cause harmful effects. The danger with this approach is that in minimizing the use of antibiotics, treatments for detected sub-clinical infections may be delayed or even withheld, leading to disease outbreaks that may otherwise have been avoided.

Whereas underuse of antibiotics in aquaculture might result in disease outbreaks, overuse or misuse may produce harmful environmental effects. The goal of this paper is to summarize existing literature on aquacultural use of antibiotics with the objective of aiding managers and other interested parties make informed decisions. The specific areas of interest covered by the literature review and this paper are:

- The mechanisms involved in development of antibiotic resistance
- Risks to human and fish health of using antibiotics in aquaculture
- Potential ecological effects of using antibiotics in aquaculture
- Recommendations for minimizing harmful effects of antibiotic use in aquaculture

1.2 Methods of Enquiry

This paper is primarily concerned with antibiotic use during the freshwater rearing of salmon and steelhead in the Pacific Northwest. In this region, the antibiotics currently approved for use are oxytetracycline (OTC) and Romet, a potentiated sulfa drug. Other antibiotics, such as erythromycin and florfenicol, are also used under veterinary license. In order to obtain a fuller appreciation of the consequences of using antibiotics in cold water (salmonid) aquaculture, the relevant published literature on both freshwater and marine aquaculture use of antibiotics was reviewed.

2 Antibiotic Use in Aquaculture

2.1 Mechanisms of development of antibiotic resistance

Most antibiotics used in salmonid aquaculture are not mutagenic. It seems likely, then, that antibiotic treatments simply provide the environment in which cells of a bacterial fish pathogen that have undergone chance mutations conferring resistance to one or more

[1] White papers were prepared by the HSRG to address topics relevant to hatchery reform. They are intended to stimulate discussion and provide background, documentation and explanations not included in the body of the HSRG's report.

antibiotics, can multiply to the stage where they become the dominant cells in the bacterial population. Normally, such cells would occur as an extremely small fraction of the cell population. However, if antibiotic treatments go on for long enough or are too frequently used, the opportunity is provided for the antibiotic resistance selection process to occur, the end result being an antibiotic resistant fish pathogen.

Genes responsible for antibiotic resistance can be transferred between bacteria by any of three processes:

- A gain of genes from the uptake of naked DNA (Transformation)
- A gain of genes through infection with viral DNA (Transduction)
- A gain of genes by cell-to-cell mating (Conjugation)

All three of these processes are thought to occur in soil and aquatic systems (Trevors, Barkay, and Bourquin 1987; Coughter and Stewart 1989).

2.2 Risks to human and fish health of using antibiotics in aquaculture

2.2.1 Risk of producing fish pathogens resistant to antibiotics

One of the concerns in treating fish with antibiotics is the potential for producing fish pathogens that are resistant to the antibiotics. Studies of Japanese aquaculture convincingly support the conclusion that increased aquacultural use of antibiotics was responsible for the increase in single and multiple-drug resistance shown by various bacterial fish pathogens, including *Aeromonas salmonicida* (Aoki et al. 1983; Aoki 1988). Similar findings have been reported in other countries for other bacterial fish pathogens following the use of antibiotics to control diseases in cultured salmonids. When antibiotic resistance occurs, the effectiveness of the antibiotics in treating fish diseases is compromised.

2.2.2 Risks of producing human pathogens resistant to antibiotics

Genes that encode for drug resistance may be contained in the cells of bacterial fish pathogens or in the cells of other types of bacteria associated with fish or present in their environment. These genes can end up on mobile bits of DNA, e.g., plasmids, which are also present in the same cells. The plasmids bearing the resistance genes can then be transferred to other bacteria, including bacteria of public health significance. The recipient bacteria subsequently become resistant to the particular antibiotics encoded for by the transferred resistance genes.

Plasmid-borne resistance genes have been transferred by conjugation from the fish pathogen *A. salmonicida* to *Escherichia coli*, a bacterium of human origin, some strains of which are pathogenic for humans (Aoki et al. 1983; Kruse and Sorum 1994; Adams et al.1998). Plasmid-borne drug resistance genes have also been transferred from the fish pathogen, *Vibrio anguillarum*, to the causative bacterium of cholera in humans, *Vibrio cholera* (Nakajima et al. 1983). Plasmid-borne antibiotic resistance genes present in the cells of various groups of bacteria isolated from cultured rainbow trout also proved transferable to *E. coli* (Toranzo et al. 1984).

2.2.3 Risk and significance of producing environmental bacteria resistant to antibiotics

Fish contain a multiplicity of bacteria in their gastrointestinal tracts. When diseased fish are treated with antibiotics contained in feed, the cells of non-pathogenic gastrointestinal bacteria and cells of environmental bacteria can come in contact with the antibiotics present in fish farm and hatchery wastes. The number of cells containing genes encoding for resistance may be increased due to the addition of the non-pathogenic and environmental bacteria. The risk is that such cells may serve as a source of resistance genes that could be transferred to fish and human pathogens with which they might come in contact.

Treatment of salmonids with various antibiotics (including OTC) has, in fact, been shown to result in significant increases in the proportion of the gut micro-flora showing resistance to the antibiotics (Austin and Al-Zahrani 1988; Herwig, Gray, and Weston 1997). The same is true for environmental bacteria coming in contact with wastes containing antibiotics such as OTC. In such wastes under marine salmon farms in Norway and Ireland, the proportion of OTC-resistant environmental bacteria ranged from 16 to 26 % (Nygaard et al. 1992; Kerry et al. 1994 and 1996) and, in one exceptional case, all of the bacteria in the OTC-containing farm sediments proved resistant to OTC (Samuelsen et al. 1992b).

In comparison, in sites not affected by marine salmon farms, the OTC-resistant proportions were lower, ranging from less than 1% to 5% (Torsvick, Sorheim, and Goksoyr 1988; Nygaard et al. 1992; Samuelsen, Torsick, and Ervik 1992; Kerry et al. 1994 and 1996. In Puget Sound, Washington, the proportion of micro-flora showing OTC resistance under a marine salmon farm that relied heavily on the use of antibiotics, including OTC, was 3% to 9%. Analogous values were 0.2% to 1.6% for samples from sites in Puget Sound which were thought to be unaffected by salmon farms (Herwig, Gray and Weston et al 1997). Although not all of the bacteria in marine salmon farm sediments showing resistance to OTC contain resistance genes transferable to other bacteria via plasmids (Kapetanaki et al. 1995; Kerry et al. 1996), those that do have been shown to be capable of transferring resistance genes to bacteria of human origin, e.g., *E. coli* (results of R.A. Sandaa cited by Husevag et al.1991). Similar results have been found for ubiquitous freshwater bacteria isolated from a Danish trout farm; antibiotic resistance in these bacteria was readily transferred to *E. coli* in the laboratory (Schmidt et al. (2001).

2.2.4 Risk of exposing non-target animals that might serve as food for humans to antibiotics

Antibiotics may end up in non-target fauna associated with fish culture sites. Reports on this topic relating to freshwater salmonid hatcheries appear to be lacking; however, the opposite is true with marine salmon farms. Wild fish, crustaceans, and mollusks living in the vicinity of marine salmon farms have been shown to accumulate measurable levels of antibiotics in their tissues as a result of feeding on waste medicated feed and feces.

Samuelsen et al. (1992) thought it possible that drug residues in non-target fauna might represent a pathway by which antibiotics could enter human populations. In this connection, four studies have been conducted with respect to OTC:

- In the first study, farmed blue mussels (*Mytilus edulis*) in Norway were found to contain 7.0 µg OTC/g tissue while those collected 80 meters from the farm contained only trace levels (Moster 1986 as reported by Coyne, Hiney, and Smith 1997).
- In the second study, Bjorklund, Bondestam, and Bylund (1990) detected OTC levels of 0.2 µg/g to1.3 µg/g in samples of muscle from bleak (A*burnus*

alburnus) in Norway. Their samples were obtained from a location near a salmon farm on the final day of antibiotic therapy.

- The third study was conducted in Puget Sound, Washington. Samples of non-target organisms were taken from the vicinity of, and under, a salmon farm during, and within 12 days of, an OTC treatment. No more than trace levels of OTC (0.1 µg/g) were found in oysters (*Crassostrea gigas*) and Dungeness crabs (*Cancer magister*), but about half of the sampled red rock crabs (*Cancer productus*) contained OTC at levels ranging from 0.8µg/g to at least 3.8 µg/g muscle (Capone et al. 1996).
- In the fourth study, mussels sampled 20 meters from a salmon farm in Ireland on the last day of an OTC treatment contained no detectable OTC, but those sampled from under the farm contained 10.2 µg/ OTC/g of soft tissue (Coyne, Hiney, and Smith1997). OTC levels in the mussel tissues declined rapidly following the treatment (the half-life was approximately 2 days). The authors concluded that residues present in filter-feeding bivalves as a result of therapeutic use of OTC are unlikely to present a significant human health hazard.

Studies have also been conducted with other antibiotics and non-target fauna. Samuelsen et al. (1992) sampled wild fishes in the vicinity of two Norwegian marine salmon farms treated with oxolinic acid. The samples were obtained on the last day of treatment. The mean levels of oxolinic acid found for the muscle samples that proved positive for the drug were 4.38 µg/g at one farm and 0.42 µg/g at the other. The highest oxolinic acid concentration (12.51 µg/g) was in a coalfish, *Pollachius virens*. In mussels near the farm, oxolinic acid levels of 0.65 µg/g were found.

In a similar Norwegian study, Ervik, Samuelsen, et al. (1994) tested muscle samples from wild fish living in the vicinity of six salmon farms treated with quinolone drugs (oxolinic acid and flumequine). Samples were taken on the last day of treatment or one day after treatment. Most or all of the fish sampled at each farm were positive for the antibiotics. Mean muscle concentrations ranged from 0.95 µg/g to 4.89ug/g. Ervik, Thorsen, et al. (1994) reported in a follow-up study on two devices that reduced feed waste on salmon farms. They found that using the devices resulted in reduced drug residues in wild fish sampled near treated farms. The authors recommended, however, that, in addition to using such devices, fishing should not be conducted in the vicinity of fish farms during and after medication.

2.3 Ecological effects of using antibiotics in aquaculture

The long-term environmental impacts of using antibiotics in aquaculture are still uncertain. In some cases, short-term decreases in the size of gastrointestinal bacterial populations of fish during treatment with erythomycin have been noted (Moffit and Mobin 2006). The same has been found for bacterial populations in hatchery effluents during separate treatments with OTC, oxolinic acid, and a potentiated sulfonamide (Austin 1985). In other cases, the use of antibiotics has had no appreciable effect on the sizes of the aquatic bacterial populations; if any size changes occurred, they were masked by temporal variations in the microbial densities (Samuelsen et al. 1992; Herwig, Gray, and Weston 1997). During separate treatments with three antibiotics, increases in the proportion of the hatchery effluent populations resistant to each of the antibiotics occurred; however, these increases were soon reduced after conclusion of the treatments (Austin 1985).

Antibiotics in general should be efficacious, readily absorbed from the intestinal tract, and have short half-lives once they are voided to the environment. OTC, one of the most commonly used antibiotics in fish farms and hatcheries, does not exhibit all of these characteristics. It is poorly absorbed from the intestinal tract (Cravedi, Choubert, and Delous 1987). An estimated 70% to80% of it is voided intact in the feces (Samuelsen 1989). Also, while OTC can apparently undergo degradation in seawater (Samuelsen 1989), it appears, under certain conditions, to be virtually indestructible in the sediments under marine salmon farms. Following 13 treatments with OTC, OTC levels in farm sediments ranged from 0.1 µg/g to 11 µg/g (in one exceptional case, it ranged up to 285 µg/g) and the half-lives for persistence were estimated to range from 9 to 415 days (Smith and Samuelsen 1996). The high proportions of OTC-resistant bacteria that persist in these sediments may provide a threat to fish farms since they can serve as sources of OTC-resistance genes for fish pathogens in the vicinity of the farms. Whether the OTC-induced changes in the micro-flora of the sediments interferes in any way with the rates of decomposition of organic matter in the sediments and with re-colonization of the seabed by organisms displaced by sediment deposition has apparently not been investigated.

The fate of OTC in freshwater sediments appears not to have been studied. However, the persistence of OTC resistance in ubiquitous freshwater bacteria, such as the motile *Aeromonas* spp. (aeromonads) reported by Schmidt et al. (2001), might be explained if the antibiotic is also stable in freshwater sediments. Schmidt et al. (2001) studied antibiotic resistance of the aeromonads in a Danish river in which trout hatcheries had earlier used OTC without restriction, but which were no longer permitted to do so as a result of recent restrictions on its use in Danish aquaculture. It was proposed that the aeromonads might serve as reservoirs of transferable OTC-resistance genes.

In another study on the same river, Schmidt et al. (2000) examined the antibiotic sensitivities of a large number of isolates of two bacterial fish pathogens (*Flavobacterium psychrophilum* and *Yersinia ruckeri*) and of the motile aeromonads. In tests with five antibiotics, they found that fish farming on the river had exerted a heavy impact on the flavobacteria and aeromonads in the river; these bacteria showed high levels of individual and multiple antibiotic resistances. In addition, in comparing aeromonad samples from the hatchery inlets and outlets, it was found that the proportions of the populations showing antibiotic resistance were significantly higher in the hatchery outlet samples.

Spangaard et al. (1993), also working on a freshwater system in Denmark, reported the prevalence of resistance to the antibiotics OTC and oxolinic acid in an unpolluted river to be 6% and 16%, respectively. In comparison, the prevalence of resistance to these antibiotics in samples from three fish hatcheries was somewhat higher (15% and 27%, respectively). It was not made clear whether the resistances found in the unpolluted river were due to intrinsic, non-transferable resistance or whether they were due to antibiotic-specific, transferable genes that resulted, perhaps, from unrecognized prior exposures to the two antibiotics. In any event, in comparing the total counts of bacteria and the composition of the micro-flora in the unpolluted river with the analogous findings in samples from the three fish farms, the authors concluded that the farms had exerted no adverse impacts on the bacterial populations. The differences in the prevalence of antibiotic resistance in the unpolluted river and fish farm environments were found not to be statistically significant.

The majority of studies indicate that increased levels of antibiotic resistance can be expected to occur for as long as antibiotics are used in aquaculture. It might be expected, however, that if the use of a given antibiotic in aquaculture is discontinued or if the frequency with which it is used is reduced, the advantage of possessing resistance to the antibiotic would disappear. Under such circumstances, the wild-type, non-resistant bacterial cells might regain their numerical dominance in the population unless, of course, continued inputs of the antibiotic to the aquatic system from other sources interfere to prevent this reversion. Results from human medicine support the foregoing idea (Forfar et al. 1966).

One means of reducing the use of antibiotics in aquaculture is to use vaccines for controlling disease problems whenever possible. In Norwegian salmon farming, the use of antibiotics was dramatically reduced in 1992 from a high of almost 50,000 kg of active drug in 1987 to a low of approximately 1000 kg (Markestad and Grave 1997). This decrease was almost entirely due to implementing the large-scale use of vaccination and occurred despite the fact that the tonnage of salmon being produced on the farms was increasing at the time. Vaccination plays only a very small part in disease control in the freshwater salmon and steelhead hatcheries of the Pacific Northwest despite the fact that at least three of the cultured species may be reared for a year or so before their release to the sea. The problem is that vaccines effective against the bacterial diseases of greatest concern in the Pacific Northwest, e.g., bacterial kidney disease and bacterial cold water disease, are still lacking. While efforts to develop an anti-BKD vaccine have thus far led to less-than-satisfactory protection in Pacific salmon, these efforts should be continued. In addition, the prognosis for an effective anti-BCWD is good (Madetoja et al. 2006). Clearly, support for developing vaccines against these diseases should be encouraged.

A second means of reducing antibiotic use in aquaculture is to implement rearing practices that minimize the level of stress on the fish being reared and that reduce the likelihood that infections requiring antibiotic treatment will occur. With regard to the latter practice, many salmon hatcheries in the Pacific Northwest do not normally use eggs derived from brood Chinook found to be infected with the causative agent of bacterial kidney disease (BKD), *Renibacterium salmoninarum* (Rs), because the agent is transferable to their progeny via their eggs. This practice has greatly reduced the prevalence of BKD problems in Chinook hatcheries and has reduced the frequency with which antibiotic treatments are needed.

3 Conclusions

Alderman and Hastings (1998) reviewed the literature on the use of antibiotics in aquaculture and came to the conclusion that "although there is evidence that antibiotic resistance can be selected for in normal therapeutic use in aquaculture, the risks of transfer of such resistance to human consumers by any of the possible routes appear to be low". This, they considered, was particularly true for cultured cold- and cool-water fishes. Since the Alderman and Hastings review was published, there has been no new information to warrant a change in the above conclusion.

Alderman and Hastings' conclusion is supported by the findings of Moffit and Mobin (2006) on the gut micro-flora of cultured spring Chinook salmon. They found no evidence in the salmon they tested of human pathogens or of animal disease agents transmissible to man. Since the gut micro-flora of fish tends to reflect the environment they inhabit and the food they eat, salmonids cultured in the Pacific Northwest are not likely to be carrying such pathogens. Thus, in the Pacific Northwest, one need not hesitate to use antibiotics when it is absolutely necessary to do so.

For example, there are many situations in the Pacific Northwest in which salmonid hatcheries are forced to operate on untreated river water. Such water is a potential source of fish pathogens, including Rs, the causative agent of BKD. As mentioned above, there are currently no vaccines available for controlling BKD in Pacific salmon. Some of the hatcheries relying on untreated river water rear species, such as spring Chinook salmon and steelhead, that require long-term rearing. For much of the rearing period, water temperatures in some of the hatcheries are below 10o C, thus compromising any potential cell-mediated resistance the fish might have against Rs (Hamel 2005). The use of appropriate chemotherapy would be justified in such situations if infection with Rs were detected. It would be ill advised not to treat such fish in a timely manner since failure to use antibiotics would run the risk of poor survival which is unacceptable especially for hatchery programs whose goals are conservation. The fish should also be treated because of enhanced risk that they would pose to other salmonids that share the waterway. In these cases, the benefits of treating the infected fish would outweigh the risks.

4 Recommendations

The HSRG concludes that antibiotics will likely always be needed in the operation of fish-rearing facilities. However, these compounds must be used with care in order to minimize risks to humans and the environment.

Recommendations are as follows:

- In setting up and operating new freshwater or marine aquaculture facilities, consideration should be given to the following questions raised by Ahne, Winton, and Kimura (1989) :
  - Will the facility’s water supply permit pathogen avoidance?
  - Will the culture environment suit the needs of the species to be reared?
  - Will, the species to be reared be resistant to the pathogens likely to be encountered?
  - Are vaccines available to combat any pathogens encountered?
- The quantities of antibiotics used in operating the facilities will be minimal if most or all of these questions can be answered in the affirmative.
- Antibiotic use should be as infrequent as possible to avoid enriching the numbers of antibiotic-resistant bacteria in the hatchery or farm environment.
- Bacterial infections in fish hatchery populations should be treated without delay when detected. If treatment is via medicated feed, the feeding rate should be adjusted so that all medicated feed is eaten and none (or very little) sinks to the bottom. This will not only maximize the effectiveness of the treatment, but will reduce the problem of accumulating large amounts of waste in hatchery and fish farm environments. In hatcheries, uneaten medicated feed and fecal material should be removed from hatchery effluents using filters, settling ponds, or both as these materials are likely to be associated with the antibiotics being used (Smith et al. 1994).
- Tests for detecting pathogens in hatchery populations should be conducted on a regular basis and, to maximize the chances of detecting infections, the samples used should not be collected at random. Rather, the samples selected for testing

should consist of freshly dead and moribund specimens and of fish showing other signs of abnormality including lesions, darkened pigmentation, loss of appetite, and sluggish behavior.

- Treatments of hatchery-reared Pacific salmon and steelhead intended for natural rearing at sea must be timed so that any antibiotics in their tissues are at acceptably low levels by the time they are released as smolts. Similarly, treatments of farmed salmon and trout intended for human consumption must be timed so that any antibiotics in their tissues (particularly the flesh) are at acceptably low levels by the time they are marketed. Proper timing is important if bacteria in the gastrointestinal tracts of animals preying on released smolts or of humans eating marketed salmon and trout products are to avoid unnecessary exposure to the antibiotics. Because water temperature and the type of antibiotic being used are major factors affecting the rate of antibiotic clearance from fish tissues, these factors must be taken into account in the timing of treatments (Namdari, Abedini, and Law 1996; Fairgrieve et al. 2005).
- Agencies operating salmon and steelhead hatcheries and private firms operating fish farms are strongly urged to support research in vaccine development. When vaccines effective against the bacterial diseases of the greatest concern become available, the use of antibiotics in affected facilities should decrease significantly.

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