CHEMICAL USE IN SALMON AQUACULTURE: A REVIEW OF CURRENT PRACTICES AND POSSIBLE ENVIRONMENTAL EFFECTS

Les Burridge[1], Judith Weis[2], Felipe Cabello[3] and Jaime Pizarro[4]

[1]Fisheries and Oceans Canada
St. Andrews Biological Station
St. Andrews, New Brunswick Canada
E5B 2H7

[2] Department of of Biological Sciences
Rutgers University,
Newark, New Jersey
07102

[3] Department of Microbiology and Immunology,
New York Medical College,
Valhalla, New York
10595

[4]Facultad de Ingeniería
Universidad de Santiago de Chile
Alameda 3363
Santiago, Chile

March 20, 2008

Executive Summary

Chemical inputs to the marine environment from aquaculture activities generally fall into two categories: intentional and unintentional inputs. Intentional inputs include pesticides, drugs, antifoulants, anaesthetics and disinfectants. Unintentional inputs include contaminants from fish feeds additives and so-called inert ingredients in pesticide and drug formulations. This report addresses the current status of intentional chemical inputs, regulation and research in the salmon aquaculture industry in Norway, Scotland, Canada and Chile. Research gaps are identified and recommendations presented.

Antibiotics

Antibiotics in salmon aquaculture, as in other industrial husbandry of food animals including cattle and poultry, are used in the control of infections. A veterinary prescription is required to use these compounds and veterinarians are ethically bound to respond to disease outbreaks in fish under their care. Antibiotics are characterized by low toxicity to vertebrates. Some compounds are persistent in sediments and can therefore affect the microbial community near aquaculture sites. One of the major concerns with use of antibiotics (from any source) is the potential for bacteria to develop resistance to the compounds and for the resistance traits to be manifested in other bacteria including human pathogens.

Use of antibiotics in livestock production represents the major use of antibiotics worldwide. Municipal wastewater treatment plants are a source of antibiotic residues from human sources. Quantities of antibiotics used in salmon aquaculture are small compared to other forms of food production and published data show the use of antibiotics in salmon aquaculture has been diminishing in some areas. Despite the low relative usage of antibiotics in aquaculture compared to other food production systems their use remains an issue of concern as aquaculture is often practiced in relatively pristine environments and the exact quantities applied directly to water is not available in some jurisdictions. Available data show that large quantities of antibiotics have been applied in Chile over a generally small geographic area. In Canada the quantity of antibiotics prescribed per metric ton of production is also high compared to Norway or Scotland. Use of large quantities may indicate disease problems related to husbandry or to resistance buildup in fish. It has also been suggested that this use of large volumes of antibiotics can be explained by excessive and prophylactic use. Excessive and prophylactic use of antibiotics in animal husbandry is in general the result of shortcomings in rearing methods and hygienic conditions that favor animal stress, and opportunistic infections and their dissemination. It has been extensively shown that excessive and prophylactic use of antibiotics in animals has a negative influence on antibiotic therapy of animal and human bacterial infections because 1) zoonotic antibiotic-resistant bacteria are able to infect animals and human beings; and 2) animal and human pathogens can share genetic determinants for antibiotic resistance as the result of horizontal exchange of genetic information. Regardless of the reasons for prescribing antibiotics the application of large quantities can pose risks.

Antibiotic treatment in aquaculture is achieved by medicated baths and medicated food. In both cases, the likelihood exists for antibiotics to pass into the environment, affecting wildlife, remaining in the environment for extended periods of time and exerting their antibiotic effects. Concerns regarding the use of large amounts of antibiotics in aquaculture are multiple. They include selection of antibiotic-resistant bacteria in piscine normal flora and pathogens as well as

effects due to the persistence of antibiotics and antibiotic residues in sediments and water column. These persistent antibiotics select for antibiotic-resistant free-living bacteria thereby altering the composition of normal marine and freshwater bacterial flora. Evidence suggests that these antibiotic-resistant organisms in the marine environment will, in turn, pass their antibiotic resistance genes to other bacteria including human and animal pathogens.

Because of their toxicity to microorganisms, antibiotics may also affect the composition of the phytoplankton community, the zooplankton community and even the diversity of populations of larger animals. In this manner, potential alterations of the diversity of the marine microbiota produced by antibiotics may alter the homeostasis of the marine environment and affect complex forms of life including fish, shellfish, marine mammals, and human beings.

Use of large quantities of antibiotics in aquaculture thus has the potential to be detrimental to fish health, to the environment and wildlife, and to human health. For all these reasons, excessive antibiotic use in aquaculture should be of high concern to the aquaculture industry and its regulators, to public officials dealing with human and veterinary health and with the preservation of the environment, and to non-governmental organizations dealing with these issues.

Norway, Scotland, Chile and some Canadian provinces require yearly reporting of the antibiotics used and the quantity applied. In Scotland these data include details of stocking density, antibiotic applied and timing of treatments. Data from Norway and some Canadian provinces is presented in the form of summaries and lacks spatial and temporal details. In Scotland, Norway and British Columbia (Canada) the data are available to the public. The governments of Chile and eastern Canadian provinces require salmon farmers to report antibiotic use but this information is not released to the public.

The available data show the trend in Europe during the past decade has been towards a reduction in the quantity of antibiotics used in salmon aquaculture. The most recent data show a consistent level of antibiotic use in Europe with minor fluctuations presumably as the result of localized disease out breaks. Data from British Columbia (Canada) indicates a reduction in antibiotic use in that province as well. While reviewers of this document have suggested that the use of antibiotics in Chile is also being reduced with time, no data are available to the authors to support this contention. Although it is very difficult to easily access data on antibiotic use in Chile, it is clear that the Chilean salmon aquaculture industry has, in the past applied quantities of antibiotics that are orders of magnitude larger than that applied in Europe. The Canadian aquaculture industry also appears to have, in the recent past used considerably more antibiotics per metric ton of production than either Scotland or Norway.

Metals

Copper and Zinc have been measured in sediments near aquaculture sites at concentrations in excess of sediment quality guidelines. These elements can be lethal to aquatic biota and persist in sediments.

Copper-based antifouling paints are applied to cages and nets to prevent the growth of attached marine organisms on them. The buildup of these organisms (“epibiota”) would reduce the water flow through the cages and decrease dissolved oxygen. The buildup would also

decrease the durability of the nets, and reduce their flotation. The rate of release of chemicals from the paint is affected by the toxic agent, temperature, water current speed and physical location of the structure. The active ingredients in these paints will leach out into the water and may exert toxic effects on non-target local marine life both in the water column and in the sediments below the cages, where the chemicals tend to accumulate. Currently copper-based paints are the most prevalent antifoulant in use. Copper has been measured in sediments near aquaculture sites at concentrations higher than the recommended sediment quality guidelines.

The toxicity of copper in water is greatly affected by the chemical form of the copper (speciation), and to what degree it is bound to various ligands that may be in the water that make the copper unavailable to organisms. The salinity and pH also affect toxicity of copper. Metals such as copper have relatively low solubility in water and tend to accumulate in sediments. The critical issue regarding toxicity of copper (and other metals) in sediments is what fraction of the copper is actually bioavailable, that is, how much can be taken up into organisms and therefore be able to produce toxic effects. As sediments under fish farms tend to be reducing, have high oxygen demand, and high sulfide from the animal wastes and uneaten feed, these sediments should bind metals to a high degree.

The Scottish Environmental Protection Agency (SEPA) requires annual reporting of use of antifoulant paints from each site and these data are available to the public.

Metals are also present in fish feed and are either constituents of the meal from which the diet is manufactured or are added for nutritional purposes. The metals in feed include copper, zinc, iron, manganese, and others. Copper and zinc are the only metals that have been shown to be significantly elevated near aquaculture sites.

Zinc is used in salmon aquaculture as a supplement in salmon feeds, as it is an essential metal. Zinc, like copper, binds to fine particles and to sulfides in sediments, and even when it is bioavailable, is much less toxic than copper. Issues of speciation, bioavailability in the water column and in the sediments are similar to those for copper. Like copper, zinc has been measured in sediments near salmon aquaculture sites at concentrations which exceed sediment quality guidelines. Given the nature of sediments under salmon cages, zinc is generally considered to be unavailable to most aquatic organisms. Some feed manufacturers have recently changed the form of Zn to a more available form (zinc methionine) and consequently have decreased the amount of Zn in feed to minimum levels necessary for salmon health. Levels of Zn in some diets are now extremely low. This should, with time, significantly reduce inputs to the marine environment.

Most research, and all regulations, pertaining to metal release from salmon aquaculture operations is focused on near-field concentrations. Very little research has been done on the resuspension of near-field sediments. It is known that fallowed sites have reduced sulfide and organic content in these sediments. The question of where metals are transported and what effect this may have in the far-field environment has not been addressed and deserves investigation.

Parasiticides

Cultured salmon are susceptible to epidemics of parasitic diseases. Sea lice are the most prevalent ectoparasites of cultured salmon and have been a problem for salmon aquaculture

industries. The species that infest cultured Atlantic salmon are *Lepeophtheirus salmonis* and *Caligus elongatus* in the northern hemisphere and *Caligus teres* and *Caligus rogercresseyi* in Chile. Infestations result in skin erosion and sub-epidermal haemorrhage which, if left untreated, result in significant fish losses, probably as a result of osmotic stress and other secondary infections. Sea lice are natural parasites of wild Atlantic and Pacific salmon, and infestations have occurred wherever salmonid aquaculture is practiced. Effective mitigation, management and control of sea lice infestations requires good husbandry.

Chemicals are used in the treatment of sea lice infestations, and are subsequently released to the aquatic environment and may have impacts on other aquatic organisms and their habitat. These compounds are lethal, especially to aquatic invertebrates. Concerns with their use are mainly with the potential of these compounds to affect non target organisms.

Parasiticide use is regulated in all countries where salmon aquaculture is practiced. A veterinary prescription is required to use these compounds. Norway, Chile and the UK have a list of 5-10 compounds registered for use to combat infestations of sea lice, however the majority of these are not used today. Canada has only two registered products, neither of which has been prescribed in the recent past. The registration procedure or the authorization of a permit to apply a therapeutant includes an assessment of the potential risk of its use. In most cases the information provided to regulatory authorities by registrants includes proprietary information, not accessible by the general public. The absence of these data from the public domain has the unfortunate consequence that neither its quality nor its nature can be debated by those scientists and non-scientists with interests in these areas.

Although a number of products appear to be available to veterinarians and salmon farmers to combat infestations of sea lice, only a few are prescribed. Only one compound, the in-feed therapeutant emamectin benzoate (EB), is used in all jurisdictions. It is, in fact, the only product used in Canada (under Emergency Drug Release) and the US (INAD). Overuse or over-reliance on any single compound can lead to the development of resistance to the compound in the parasite. Not surprisingly, evidence of resistance has recently been reported in Chile. Canada limits the number of sea lice treatments with EB during a grow-out cycle to 3, up to 5 treatments may take place during the grow out cycle in Norway and the UK and in Chile between 4 and 8 treatments may take place. In addition, only one EB-based product is used in Norway, Scotland and Canada. Several are used in Chile and it appears as though treatment doses may be different.

Cypermethrin, a pyrethroid pesticide, is applied as a bath treatment in Norway, and the UK. Scotland treats with this compound relatively more often than elsewhere.

The use of the organophosphate azamethiphos and the chitin synthesis inhibitor teflubenzuron has ended. Development of resistance in lice is known to occur with organophosphate pesticides. Teflubenzuron apparently is no longer produced as an anti-louse treatment.

Interestingly, hydrogen peroxide, which has been considered a rather poor product for sea lice control, is used in Scotland and has recently been applied in Chile. Hydrogen peroxide is considered the most “environmentally friendly” product so its use may be related to the

sensitivity of the receiving environment. It could also be an indication that other products are failing in terms of efficacy of louse control and support for the contention that there are limited treatment options available.

The apparent movement to the use of fewer products and the fact that there are few products being developed for sea lice treatment should raise concerns within the industry. Even drug manufacturers stress the benefits of the availability of a suite of compounds and of the rational application of these products to avoid resistance development.

Anti-lice treatments lack specificity and therefore may affect indigenous organisms in the vicinity of anti-lice treatments. Sea lice therapeutants not only have the potential to negatively impact the environment through effects on sensitive non-target organisms they may alter the population structures of the fauna in the immediate environments.

Data collected to date generally suggest that negative impacts from anti-louse treatments, if they occur, are minor and will be restricted in spatial and temporal scale. However, published field data are rare. Field studies must be undertaken in most jurisdictions as part of the registration process and drug manufacturers must provide extensive environmental monitoring data to regulators. However, as stated earlier, these data are often considered confidential and most publicly available information regarding the biological effects of the various compounds is generated for single-species, lab-based bioassays.

Farms are located in waters with different capacities to absorb wastes, including medicinal chemicals, without causing unacceptable environmental impacts. Risks therefore have site-specific component, and management of these risks may therefore require site-specific assessments of the quantities of chemicals that can safely be used at each site. In the European Union, Maximum Residue Levels (MRL) are set for all therapeutants applied to food fish. Health Canada and the Canadian Food Inspection Agency have similar guidelines. In Scotland a medicine or chemical agent cannot be discharged from a fish farm installation unless formal consent under the Control of Pollution Act has been granted to the farm concerned by (in Scotland) SEPA. SEPA also requires annual reporting of therapeutant use from each site and these data are available to the public. This regulatory scheme provides an example of a risk management plan that should be adopted in all areas that use sea lice therapeutants.

As is the case with antibiotic use, salmon farmers are required to report use of antiparasitic compounds. Summarized or detailed reports are available from Norway, Scotland and British Columbia (Canada). Data from Chile and the eastern Canadian provinces are at present not available to the public.

Disinfectants

Biosecurity is of paramount importance in aquaculture operations. The presence of infectious salmon anemia (ISA) and the prevalence of bacterial infections in some jurisdictions have resulted in protocols being developed to limit transfer of diseases from site to site. These protocols involve the use of disinfectants on nets, boats, containers, raingear, boots, diving equipment, platforms and decking. Unlike parasiticides, there appear to be no regulations regarding the use of disinfectants. Thus, in areas around wharves or in small sheltered coves

disinfectant input could be significant. There is no information on the amounts of disinfectants used by the salmon aquaculture industry or by the processing plants and the food industry, making it very difficult to determine precisely the quantities of these products used. In most cases the disinfectants are released directly to the surrounding environment. The effects of disinfectants in the marine environment appear to be poorly studied. In addition, only the UK requires reporting of quantities of disinfectants being used in aquaculture activity. All of the compounds used are quite water soluble and should be of low toxicity depending on quantities used. Risk of aquatic biota being exposed to the disinfectant formulations is dependent not only on how much is being used but where it is being released.

Disinfectant formulations often contain surfactants. The actual compounds used as surfactants may not be part of the label information. Some of these compounds are known endocrine disruptors and are known to affect salmon as well as other marine organisms. Without information on what compounds are being used and in what quantities it is extremely difficult to assess risk to salmon and to non-target organisms.

Malachite green is a triphenylmethane dye (4-[4-trimethylaminophenyl)-phenyl-methyl]-N,N-dimethyl-aniline. It is readily soluble in water (110 $g \cdot L^{-1}$). In the past malachite green was used as an anti-fungal agent in salmon aquaculture. Malachite green and its metabolite leucomalachite green are suspected of being capable of causing gene damage and cancer. Its use as a therapeutant in fish destined for human consumption has been banned and a zero tolerance level for food fish is in place in most countries. Despite the fact that the use of malachite green is banned in salmon farming several reports identify instances of misuse in aquaculture in the US and internationally. In addition, a recent preliminary study shows that some free ranging wild fish (eels) in Germany have detectable levels of LMG in their edible tissues, albeit at very low concentrations. The suggestion that malachite green may be a ubiquitous contaminant in industrialized areas is troubling and calls into question the ability to enforce zero tolerance guidelines.

Anaesthetics

Anaesthetics are used operationally in aquaculture when fish are sorted, vaccinated, transported or handled for sea lice counts or stripping of broodstock. Compounds available for use are regulated in all jurisdictions. They are used infrequently and in low doses, thus limiting potential for environmental damage. Only Scotland and Norway require yearly reporting of anaesthetic compounds and the quantities used.

The use of anaesthetics is generally considered to be of little risk to the environment. It is likely that most of the anaesthetic used in aquaculture is used in freshwater and in transport of fish.

Conclusions

The key conclusion of this report is that the availability of verifiable data on chemical use in salmon aquaculture is variable. Chemical use data are available from Norway, Scotland and parts of Canada. The government of Chile and some provinces of Canada, while requiring that farmers report disease occurrence, compounds prescribed and quantities used, do not make this information available to the public. This makes it exceedingly difficult to prepare general recommendations and to comment on risk associated with chemical usage. Even comments from

reviewers and Salmon Aquaculture Dialogue steering committee members show a variety of opinions regarding what compounds are being used and for what purposes. Several reviewers have suggested that salmon aquaculture is held to a higher standard than other food producing industries. The authors are not in a position to make this judgement. It is the conclusion of this working group, however, that public release of available data would eliminate much of the disagreement and contention that exists. The fact that these data are available from regulatory agencies in Scotland and Norway adds pressure for other jurisdictions to follow suit. Data such as these are essential in order to conduct research in field situations. Differences between samples collected near aquaculture sites and those collected from reference sites cannot be realistically interpreted, or discussed, without knowledge of activities at those sites. Scotland reports full data sets from individual farms including biomass on site and data regarding quantities all compounds used at that site and when they were applied.

Table 1 is a summary of the quantities of chemicals used in salmon aquaculture in Norway, Chile, Scotland and Canada in 2003. While the authors acknowledge that these data are several years old, they represent the only data set available for which comparisons can be made between jurisdictions. More recent data are available for the UK, Norway and for some compounds in Canada but none are available from Chile. Chemical use shown is relative to FAO-reported production value for Atlantic salmon only. The authors recognize that other salmon species are cultured in some jurisdictions and that therapeutants are applied to salmon during their first year in cages, i.e. to salmon that do not contribute to the production values.

Reporting antibiotic use is a condition of operating an aquaculture site in nearly all jurisdictions. Despite this, reported antibiotic use in Chile is an estimate provided by researchers, not by regulatory agencies.

Individual compounds have specific characteristics in terms of toxicity, modes of action and potential to affect marine environments. The authors also recognize that therapeutants have specific targets and dosage rates and that may change according to environmental conditions. The antiparasitic products, for example, are much more lethal to most aquatic species than antibiotics. Excess use of antibiotics, however, may affect human health. Comparing quantities of antibiotics used to quantities of antiparasitics is of no value. This table is of most value in comparing, between jurisdictions, the quantities of each class of product (antibiotic, antiparasitics, etc.). While the caveats mentioned above limit the ability to compare jurisdictions in an absolute way, the authors believe, from the data available, that the trends shown by these data are an accurate reflection of the chemical use patterns in the aquaculture industry.

The rate of application (Kg/metric ton (MT)) of antibiotics in Chile and in Canada in 2003 was high compared to Scotland and Norway. In addition, data indicates that some antibiotics used in human health (quinolones) are used in the salmon aquaculture industry in Chile and Norway, a practice forbidden in other jurisdictions.

Table 1. Classes of chemical compounds used in Atlantic salmon aquaculture, quantities used in 2003 and quantities applied relative to production.

Country	***Salmon Production (Metric Ton)***[a]	***Therapeutant Type***	***Kg (active ingredient)Used***	***Kg Therapeutant/ Metric Ton produced***
Norway	509544	Antibiotics	805	0.0016
		Anti-louse	98	0.0002
		Anaesthetics	1201	0.0023
Chile	280,481	Antibiotics	133800	0.477
		Anti-louse	136.25	0.0005
		Anaesthetics	3530	0.013
UK	145609	Antibiotics	662	0.0045
		Anti-louse	110	0.0007
		Anaesthetics	191	0.0013
		Disinfectants	1848	0.013
Canada (includes data from Maine, USA)	111,178[b]	Antibiotics	30,373[c]	0.273
		Anti-louse	12.1	0.00011

[a] Data accessed at FAO (http://www.fao.org/fi/website/FIRetrieveAction.do?dom=collection&xml=global-aquaculture-production.xml&xp_nav=1)

[b] Data accessed at http://www.dfo-mpo.gc.ca/communic/statistics/aqua/index_e.htm and New Brunswick Salmon Growers Association (personal communication).

[c] Source: Government of British Columbia (http://www.al.gov.bc.ca/ahc/fish_health/antibiotics.htm and New Brunswick Salmon Growers Association (NBSGA, personal communication)

Research gaps

The authors recognize the site specificity associated with near-shore salmon aquaculture and that jurisdictional differences in the physical, chemical and regulatory environment may make it difficult to develop standard metrics for all. In addition, individual chemicals used in the salmon aquaculture industry are currently regulated to a significant extent in all jurisdictions.

- *Research is needed to develop safe and effective vaccines against bacterial and viral pathogens. In particular development of an effective vaccine against Piscirickettsia salmonis would dramatically reduce reliance on antibiotics in Chile.*
- *There is lack of data from field situations. Field studies are needed to determine if lab-based, single species bioassays are predictive of biological effects in operational situations. Research into the presence, fate and effects of compounds and mixtures from "real world" situations can provide data regarding cumulative effects and when coupled with data on the use of compounds, numbers of fish, etc. can result in realistic risk assessments. Cause and effect questions can only be addressed if data collected in situ includes detailed information about aquaculture activity.*
- *Research is needed to clearly establish the link between use of antibiotics in salmon aquaculture and the presence of antibiotic-resistant bacteria near salmon aquaculture activities. The spatial and temporal extent of the problem should also be defined.*
- *Research is needed to determine the consequences of application of large quantities of antibiotics. The effects on fish (farmed and indigenous) and human health and on the microflora in the sediments and the water column should be investigated.*
- *Research is needed to develop non-toxic forms of antifoulants.*
- *Research is needed to determine the biological effects on local organisms, either at individual or population level, of copper and zinc at concentrations above regulatory limits*
- *Research is needed to determine the potential effects of chronic exposure to elevated copper and zinc in sediments near salmon aquaculture sites*
- *Research is needed to develop more, or (preferably) alternative, products for sea lice control. With a limited number of treatment options, it is likely that resistance will develop in sea lice populations.*
- *Management and husbandry practices that reduce the number of anti-louse treatments should be documented and shared amongst jurisdictions. Canada, for example, only allows three emamectin benzoate treatments in a grow-out cycle, while up to eight treatments have been reported in Chile. The reasons for this difference may be related to lice biology and other biotic or abiotic factors. There may, however, be management practices that reduce infestation pressure.*
- *There are very few data available regarding the presence of disinfectants, and particularly of formulation products, in the marine environment. Studies need to be conducted to document the patterns of use and the temporal and spatial scales over which compounds can be found.*
- *There are very few data available regarding the use patterns of anaesthetics in salmon aquaculture. Collection and analysis of these data may help determine if more studies are required to determine if any products pose a risk to aquatic biota.*

Recommendations

- *Regulatory agencies in nearly all jurisdictions require reporting of the quantities of antibiotics and, parasiticides applied during normal operations of salmon aquaculture sites. Reporting should be expanded to cover all jurisdictions and use of antifoulant, disinfectants and anaesthetics should be included. Details of use including timing and area of application should be included and these data be made available to the public. The model used by the Scottish Environmental Protection Agency is the most thorough currently in use.*
- *The regulatory regimes in all jurisdictions require that manufacturers conduct field trials with antiparasitic compounds. These data, where they exist, should be made more accessible to the public.*
- *There is some discussion and contention regarding the occurrence of antibiotic application for prophylaxis. If prophylactic use of antibiotics takes place in any jurisdiction, this practice should be stopped.*
- *That classes of antibiotic compounds used for treatment of human diseases should not be used (or should be used with extreme reluctance) in aquaculture production of salmon.*
- *While it remains unclear whether or not the practice continues in any jurisdiction, nets and cages should never be washed in the ocean or estuaries, where considerable amounts of toxic antifoulants could be released into the marine environment.*
- *That all antifouling agents, regardless of whether or not they are considered to contain biocides, should be tested for toxicity to different taxa of marine organisms.*

This report was commissioned by the Salmon Aquaculture Dialogue. The Salmon Dialogue is a multi-stakeholder, multi-national group which was initiated by the World Wildlife Fund in 2004. Participants include salmon producers and other members of the market chain, NGOs, researchers, retailers, and government officials from major salmon producing and consuming countries.

The goal of the Salmon Aquaculture Dialogue is to develop and implement verifiable environmental and social performance levels that measurably reduce or eliminate key impacts of salmon farming and are acceptable to stakeholders. The group will also recommend standards that achieve these performance levels while permitting the salmon farming industry to remain economically viable.

The Salmon Aquaculture Dialogue focuses their research and standard development on seven key areas of impact of salmon production including: social; feed; disease; escapes; chemical inputs; benthic impacts and siting; and, nutrient loading and carrying capacity.

Funding for this report and other Salmon Aquaculture Dialogue supported work is provided by the members of the Dialogue's steering committee and their donors. The steering committee is composed of representatives from the Coastal Alliance for Aquaculture Reform, Fundación Terram, Marine Harvest, the Norwegian Seafood Federation, the Pew Environment Group, Skretting, SalmonChile, Salmon of the Americas, and the World Wildlife Fund.

More information on the Salmon Aquaculture Dialogue is available at http://www.worldwildlife.org/aquadialogues.

The authors wish to acknowledge the efforts of Ms. Katherine Bostick of WWF-US who helped organized the various technical working groups on behalf of the Salmon Aquaculture Dialogue and provided valuable guidance and advice to the members of the Chemical Inputs working group throughout preparation of this document. We also wish acknowledge the comments and contributions of the Salmon Aquaculture Dialogue steering committee and several anonymous reviewers.

CHAPTER 1

Introduction/Background

Aquaculture is the fastest growing food production system on the planet. From 1970 to 2005, aquaculture's share of global fisheries landings increased from 5 percent to approximately one-third of all products. Salmon is one of the most popular food fish species in the United States, Europe, and Japan, and salmon aquaculture has increased dramatically over the past few decades to meet this demand. In 1980 farmed salmon made up a negligible percentage of world salmon supply, but by 2003 approximately 60% of global salmon supply was farmed.

According to the United Nations Food and Agriculture Organization (FAO), salmon is farmed in 24 countries. The major producers of salmon are Norway, Chile, the United Kingdom, and Canada, though Chile and Norway account for close to 75% of farmed salmon production (FAO 2007 and ICES 2006). The three most common species of cultured salmon are the Atlantic salmon (*Salmo salar*) the chinook salmon (*Oncorhynchus tshawytscha*), and the coho salmon (*Oncorhynchus kisutch*). In aquaculture the Atlantic salmon represents 90% of production and is by far the most economically important cultured salmonid.

Farmed salmon are most commonly grown in cages or pens in semi-sheltered coastal areas such as bays or sea lochs. The cages are designed to hold salmon but are open to the marine environment. These tend to be large, floating mesh cages. This type of open system allows for free exchange of nutrients, disease, and chemicals inputs into the salmon culture system with the marine waters.

Chemical inputs to the marine environment from aquaculture activities generally fall into two categories: intentional and unintentional inputs. Intentional inputs include pesticides, drugs, antifoulants, anaesthetics and disinfectants. Unintentional inputs include contaminants from fish feeds additives and so-called inert ingredients in pesticide and drug formulations.

As is the case in all animal food production systems, it is often necessary to treat farmed fish for diseases and parasites. The types of therapeutants available for use and the treatment protocols are tightly regulated in all jurisdictions and therapeutants can only be used under presciption from a licensed veterinarian. Management practices have evolved as these health threats appear and fish husbandry has greatly improved over the past 20 years resulting in a reduction in the use of some chemicals, particularly antibiotics in most jurisdictions. However, fish farmers still rely on aggressive use of chemotherapeutants to combat infestations of ectoparasites as well as disinfectants to manage spread of diseases. In the 1990s several reviews were prepared regarding chemical inputs (see for example Zitko 1994 and GESAMP 1997). Unfortunately, some of the issues raised by these authors remain of concern a decade later while public awareness has increased significantly.

As a result, there is a significant potential for salmon farms to impact local waters, especially if poorly sited or poorly managed. Of particular concern is the potential for chemical inputs to affect local fauna commonly referred to as non-target organisms.This report examines the current state of knowledge of impacts on marine ecosystems from salmon farms due to

chemical inputs to the marine environment. Discussion is limited to known (intentional) chemical inputs. This report does not address the issue of chemical contaminants in fish feeds as this is addressed in another report.

CHAPTER 2

Antibiotics

2.1 Introduction

Antibiotics are designed to inhibit the growth and kill pathogenic bacteria. They generally act in one of three ways: By disrupting cell membranes, by disrupting protein or DNA synthesis or by inhibiting enzyme activity. Compounds with antibiotic activity are selected for use in human and veterinary medicine because of their selective toxicity to cell membranes, ribosomal activity or enzyme activity in prokaryotic cells. As a result of these selective traits they show now or very low toxicity in higher organisms (Todar 2008).

Despite their low toxicity, there are significant environmental concerns with widespread use of antibiotics. Many antibiotics are stable chemical compounds that are not broken down in the body, but remain active long after being excreted. At present, antibiotics make a considerable contribution to the growing problem of active medical substances circulating in the environment. Persistence in the environment contributes to the development of antibiotic resistant strains of microorganisms. Resistance to antibiotics results from selection of spontaneous mutants by the antibiotic and by transfer of genetic resistance traits among bacteria of the same or of different species. In general, the more a specific antibiotic is used, the greater the risk of emergence and spread of resistance against it, thus rendering the drug increasingly useless.

The most severe consequence is the emergence of new bacterial strains that are resistant to several antibiotics at the same time. In human health infections caused by such multi-drug resistant pathogens present a special challenge, resulting in increased clinical complications and the risk of serious disease that previously could have been treated successfully, longer hospital stays and significantly higher costs to society. The worst scenario which, unfortunately, is not an unlikely one, is that dangerous pathogens will eventually acquire resistance to all previously effective antibiotics, thereby giving rise to uncontrolled epidemics of bacterial diseases that can no longer be treated (European Commision, 2008)

Antibiotics in salmon aquaculture, as in husbandry of terrestrial food animals including cattle and poultry, are used as therapeutic agents in the treatment of infections (Alderman and Hastings 1998, Angulo 2000, Grave et al. 1999, Cabello 1993, Sørum 2000, Pillay 2004, Cabello 2004). There is no evidence that antibiotics are used as growth promoters in aquaculture as is the case in the industrial raising of cattle, poultry and hogs in some countries (Alderman and Hastings 1998, Angulo 2000, Grave et al. 1999, Cabello 1993, Sørum 2000, Pillay 2004, Cabello 2004, Davenport et al. 2003). Excessive and prophylactic use of antibiotics in animal husbandry is in general the result of shortcomings in rearing methods and hygienic conditions that favor

animal stress, and opportunistic infections and their dissemination (Anderson et al. 2003, Angulo et al. 2004, Greenlees 2003, Mølbak 2004, Wassenaar 2005, Teuber 2001).

It has been extensively shown that excessive and prophylactic use of antibiotics in animals has a negative influence on antibiotic therapy of animal and human bacterial infections because 1) zoonotic antibiotic resistant bacteria are able to infect human beings; and 2) animal and human pathogens can share genetic determinants for antibiotic resistance as the result of horizontal exchange of genetic information (Cabello 2003, Cabello 2004, Angulo et al. 2004, 2004, Mølbak 2004, Wassenaar 2005, Teuber 2001, Harrison and Lederberg 1998, McEwen and Fedorak-Cray 2002, O'Brien 2002, Wierup 2001, Nester et al. 1999). These findings have resulted in regulations directed at curtailing the use of antibiotics in industrial terrestrial animal farming in Europe and North America (Grave et al. 1999, Cabello 2003, Anderson et al. 2003, Harrison and Lederberg 1998, McEwen and Fedorka-Cray 2002, Wierup 2001). The implemented restrictions of antibiotic use in animal husbandry in many countries has not resulted in increased costs to the industry and has been shown to be compatible with profitable industrial animal farming (Grave et al. 1999, Cabello 1993, Wierup 2001).

2.3 Physical and Chemical Properties of Antibiotics

Amoxicillin is a broad spectrum antibiotic from the β-lactam class. It is effective against gram positive and gram negative bacteria. It is used in the aquaculture industry to treat fish with infections of furunculosis (*Aeromonas salmonicida*). It acts by disrupting cell wall synthesis (Todar (2008). The recommended treatment is 80-160 mg/Kg for 10 days presented on medicated food. There is a 40-150 degree day withdrawal period in Scotland. The β-lactams should be susceptible to biological and physiochemical oxidation in the environment since they are naturally occurring metabolites. (Armstrong et al. 2005)

Florfenicol is also a broad spectrum antibiotic used to treat salmon against infections of furunculosis. It is part of the phenicol class of antibiotics which act by inhibiting protein synthesis (Todar 2008). The recommended treatment regime is 10 mg/Kg for 10 days presented on medicated food. The withdrawal period for flofenicol is 12 days in Canada, 150 degree days in Scotland and 30 days in Norway. The 96h LC50 of florfenicol is >330 mg/L *(daphnia*) and >780 mg/L (*R. trout*). This product is not generally considered a problem for persistence in the environment or for resistance development in microorganisms (Armstrong et al. 2005).

Tribrissen (sulfadiazine: trimethoprin (5:1)) is a sulphonamide broad spectrum antibacterial agent used to treat salmon infected with gram negative bacteria such as furunculosis and vibrios (*Vibrio anguillarum*, for example). It acts by inhibiting folic acid metabolism (Todar 2008). The recommended treatment regime is 30-75 mg/Kg for 5-10 days presented on medicated food. The withdrawal period is 350-500 degree days in Scotland and 40-90 days Norway. The environmental impact of use of this product is unknown (Armstrong et al. 2005). This product is rarely used in salmon aquaculture due to problems with palatability (M. Beattie personal communication).

Oxolinic acid and flumequin are quinolone antibiotics used to treat organisms against infections of gram negative bacteria such as *Piscirikettsia salmonis*. The effectiveness of oxolinic

acid against this bacterium, however, is reported to be marginal (Powell 2000). They are also used to combat furunculosis and vibrio infections. These products inhibit DNA replication (Todar 2008). The recommended dose of these compounds for Atlantic salmon is 25mg/Kg for 10 days (applied on medicated food) and a withdrawal period of 500 degree days has been set for Scotland, although these products are no longer used in that country. In Norway the withdrawal period ranges from 40-80 days depending on water temperature. These products are highly effective but persistent (Armstrong et al. 2005). The importance of this class of antibiotics in human medicine has led to a prohibition of their use for treating salmon in Scotland and Canada.

Oxytetracycline is a broad spectrum antibiotic active against infections of furunculosis and vibrio (Powell 2000). This tetracycline antibiotic is delivered on medicated food at dosages ranging from 50-125 mg/Kg applied over 4 to 10 days. Tetracyclines act by inhibiting DNA replication (Todar 2008). The withdrawal time prior to marketing fish is 400-500 degree days in Scotland and 60-180 days in Norway (Armstrong et al. 2005). The compound has a low toxicity (96 h LC50 for fish is >4 g/Kg). It has relatively high solubilty in water however; as it is bound to food pellets it can become bound to sediments and may be persistent for several hundred days (Armstrong et al. 2005). The combination of low toxicity and broad spectrum effectiveness has led to the widespread overuse and misuse in human and animal health and therefore to development of resistance and reduced effectiveness (Todar 2008). This is shown in use data reported from Norway where there was no use of oxytetracycline in 2005 (see Table 2.1 below).

Erythromycin is a macrolide antibiotic useful in combating gram positive and non-enteric gram negative bacteria. It is presented on medicated food at dosages ranging from 50-100 mg/Kg for 21 days. It is used to combat Bacterial Kidney Disease (BKD) (Powell 2000). Erythromycin inhibits genetic translation, therefore protein synthesis (Todar 2008). It has a low toxicity to fish (96h LC0 > 2 g/Kg) but can accumulate in sediments and organisms and is a concern in terms of antibiotic resistance. This antibiotic is not approved for salmon aquaculture use in countries which belong to International Council for the Exploration of the Seas (ICES). This includes Norway, Scotland and Canada. It is, however listed as an approved compound in Chile (Pablo Forno personal communication).

A more detailed discussion of the potential environmental impacts of antibiotics is presented later in this chapter.

2.2 Use and Regulation of Antibiotics in Salmon Aquaculture

2.2.1 Norway

The country with the world's largest salmon aquaculture industry and largest producer of farmed salmon is Norway. The following antimicrobial products are identified as having been used in Norway oxytetracycline, florfenicol, oxolinic acid, trimethoprim+sulfadiazine, furazolidone.

Table 2.1. Antibiotic use for Atlantic salmon aquaculture in Norway 2003-2005. Quantities in Kg. Kjell Maroni (pers communication 2008)

Antimicrobial	2003	2004	2005
Oxytetracycline	0.04	1.16	0
Florfenicol	105.3	83.08	85.28
Oxolinic acid	252.4	189.13	188.4

Total antibiotic use in the salmon aquaculture industry in 2006 was estimated to be 340 Kg (Kjell Maroni pers communication 2008).

Strong regulation of antibiotic use in aquaculture has led to a drastic reduction in the classes and volumes of antibiotics used for this purpose most jurisdictions (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997). These regulations were implemented as the result of extensive research in Norway and other countries which indicated that the excessive use of antibiotics was deleterious to many aspects of aquaculture, the environment, and potentially to human health as discussed above (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997).

While some authors suggest only antibiotics that are not considered relevant for human medicine can be used in aquaculture (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997), oxolinic acid, a quinolone, is used in salmon aquaculture in Norway. The use of quinolones and fluoroquinolones is a concern as these wide-spectrum antibiotics are highly useful in human medicine. They do not readily degrade; remain in the environment for long periods of time (Gorbach 2001, Wegener 1999). Thus, use of this group of antibiotics may negatively affect human health and environmental diversity of the microbiota, especially because some resistance determinants against this group of antibiotics originate in marine bacteria such as *Shewanella* and *Vibrio* (Gorbach 2001, Wegener 1999, Poirel et al. 2005a, Robicsek et al. 2005, Li 2005, Poirel et al. 2005b, Saga et al. 2005, Nordmann and poirel 2005, Robicsek et al 2006).

The volume of antibiotic use is closely monitored by centralized regulatory bodies dealing with aquaculture and fish health through monitoring of veterinary prescriptions originating from aquaculture sites (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997). This links antibiotic use to defined geographical areas, references timing of application and permits rapid detection of any increases in use (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997). The end effect of this effort is not only the control of antibiotic use but also detection of misuse in prophylaxis, and most importantly, detection of preliminary signs of emergence of potentially epizootic salmon infections (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997). It decreases excessive use of antibiotics by correcting misuse and by promptly detecting infectious disease problems that can be dealt with by hygienic measures such as isolation, fallowing of sites, quarantine and vaccines (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997). The control of antibiotic use in aquaculture in Norway, the use of hygienic measures in fish rearing, and the introduction of vaccines has permitted the Norwegian aquaculture industry to reduce its use of

antibiotics to negligible amounts despite its increasing output (Grave et al. 1999, Sørum 2000, 2006, Grave et al. 1996, Lillehaug et al 2003, Markestad and Grave 1997).

2.2.2 Chile

Chile is the second largest producer of farmed salmon in the world. Bravo (personal communication) reports that the following antimicrobial products are registered for use in Chile: oxolinic acid, amoxicillin, erythromycin, flumequine, florfenicol and oxytetracycline. Producers are required to report incidence of disease, the products prescrived fro treatment and quantities used. The government agencies do not, however make this information public. Bravo (2005) reports total antibiotic use in salmon aquaculture in 2003 to be 133,000 Kg. Depending on which production numbers are used, this is equivalent to between 0.27 and 0.47 Kg of antibiotics applied for every metric ton of fish produced.

The lack of publicly available information has led to accusations that all classes of antibiotics are used in animal husbandry and in aquaculture without restrictions (Cabello 2003, 2004, Rep. de Chile 2001, Bravo et al. 2005, Buschmann et al. 2006a, 2006b, Buschmann 2001, Gobierno de Chile 2005, Buschmann and Pizarro 2001) Unofficial information indicates that. Enrofloxacine and sarofloxacine have been reported to have been used in the past in Chile but are not authorized by the Servicio Agricola Ganadero (SAG), the Chilean agency responsible for regulating the use of antimicrobials (S. Bravo personal communication). Environmental regulations for salmon aquaculture in Chile do not discuss the potential environmental repercussions of antibiotic use (Cabello 2003, 2004, Rep. de Chile 2001). The Chilean Ministry of Agriculture Law No. 19283, Decree 139, 1995, concerning control of veterinary drugs, does not regulate the use of antibiotics in aquaculture either. Table 1 (above) shows that, in the past, the Chilean industry uses between 170 and 270 times as much antibiotic as Norway despite producing less marketable salmon (Cabello 2003, 2004, Rep. de Chile 2001, Bravo et al. 2005, Buschmann et al. 2006a, 2006b, Buschmann 2001, Gobierno de Chile 2005, Buschmann and Pizarro 2001). There is widespread disagreement whether or not all of the applications are therapeutic. The disagreement regarding the purpose for which a prescription is written is inconsequential in terms of the potential environmental effects of large quantities of antibiotics reaching the marine environment.

Information collected by Professor Julio Dolz, Universidad Austral de Chile, indicates that approximately 10 metric tons of quinolones and fluoroquinolones are used per year in human medicine in Chile, while approximately 100 metric tons of these compounds are reported to have been used in veterinary medicine per year (Bravo et al. 2005; J. Dolz, personal communication). It is believed that most of this use is in salmon aquaculture as this use is unaccounted for by the Ministry of Agriculture which has control of veterinary drug usage (Bravo et al. 2005; J. Dolz, personal communication).

Several studies by Miranda and Zemelman have demonstrated emergence of resistant bacteria in the environment of many salmon aquaculture sites (Miranda and Zemelman 2002a, 2002b, 2002c, Miranda et al. 2003). Some of these bacteria contain novel (not previously identified) tetracycline resistance determinants underlining the fact that antibiotic use in aquaculture in Chile may be selecting for new antibiotic resistance factors with the potential to

spread to pathogens of human beings and terrestrial animals (Miranda and Zemelman 2002a, 2002b, 2002c, Miranda et al. 2003). The potential of these resistance determinants to spread is amplified by the fact that coastal and estuarine waters and fish and shellfish in Chile are already widely contaminated with human and animal pathogens which display antibiotic resistance and contain genetic elements that facilitate horizontal gene transfer among bacteria as a result of the release of untreated sewage into the sea in urban and rural areas (Silva et al. 1987, Miranda and Zemelman 2001, Montoya et al. 1992, Miranda and Castillo 1998, Martinez et al.1994, Rosen and Belkin 2001). Moreover, there is no monitoring for antibiotics or antibiotic residues in the environment of aquaculture sites in Chile, and residual antibiotics/antibiotic residues in domestically consumed or exported salmon meat are not regularly monitored by the Servicio Nacional de Pesca (Gobierno de Chile 2005, Servico Nacional de Pesca 2005). Antibiotics have also been detected in the meat of free-ranging wild fish living around aquaculture sites (Fortt et al. 2007), and residual antibiotics above the permitted levels have been detected in the meat of salmon lots exported to Japan and the United States (Ecoceans 2006 (electronic citation)). All these problems increase the potential for passage of antibiotic resistance bacteria to terrestrial animals and humans and of antibiotic resistance determinants to human pathogens (Silva et al. 1987, Miranda and Zemelman 2001, Montoya et al. 1992, Miranda and Castillo 1998, Martinez et al.1994, Rosen and Belkin 2001, Servico Nacional de Pesca 2005, Fortt et al. 2007, Weber et al. 1994).

There are several reports that indicate the emergence of fish pathogens in Chile that are now widely resistant to many antibiotics, including *Vibrios* and *Streptococcus* (Salud de Peces 2004). As stated earlier the use of quinolones and fluoroquinolones is a matter of great concern as this group of wide-spectrum antibiotics are highly useful in human medicine, and because they are not readily biodegradable, remain in the environment for long periods of time (Gorbach 2001, Wegener 1999).

Application of large quantities of antibiotics in the aquaculture industry in Chile has been partially justified by the presence of pathogens that do not pose problems in other countries such *Piscirickettsia salmonis* (Brocklebank et al. 1993, Branson qand Diaz-Munoz 1991, Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003, Gaggero et al. 1995, Mauel and Miller 2002, Reid et al. 2004). *P. salmonis* in an intracellular emergent pathogen that infects salmon smolt after they are moved from fresh water to the marine environment (Brocklebank et al. 1993, Branson qand Diaz-Munoz 1991, Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003, Gaggero et al. 1995, Mauel and Miller 2002, Reid et al. 2004). The stress of this move appears to play a role in the susceptibility of salmon to infection. (Brocklebank et al. 1993, Branson qand Diaz-Munoz 1991, Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003, Gaggero et al. 1995, Mauel and Miller 2002, Reid et al. 2004, Barton and Iwama 1991). Infections by this pathogen produce large economical losses in the Chilean aquaculture industry (Brocklebank et al. 1993, Branson and Diaz-Munoz 1991, Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003, Gaggero et al. 1995, Mauel and Miller 2002, Reid et al. 2004), and to date there is no effective commercially available vaccine to prevent these infections. However, this pathogen has been detected in the United States, Canada, Ireland, Scotland and Norway (Brocklebank et al. 1993, Branson and Diaz-Munoz 1991, Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003, Gaggero et al. 1995, Mauel and Miller 2002, Reid et al. 2004), where outbreaks of disease that it produce appear to be small, sporadic and readily controlled by primarily sanitary measures

without any use of antibiotics (Brocklebank et al. 1993, Branson and Diaz-Munoz 1991, Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003, Gaggero et al. 1995, Mauel and Miller 2002, Reid et al. 2004). Moreover, there are no studies indicating that *P. salmonis* is in fact susceptible to all the antibiotics (including quinolones) used in salmon aquaculture in Chile to forestall infections by this pathogen (Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003). The fact that *P. salmonis* is able to live in seawater and that its major targets are stressed juvenile salmon strongly suggests that this pathogen may be an opportunist (Brocklebank et al. 1993, Branson qand Diaz-Munoz 1991, Olsen et al. 1997, Perez et al. 1998, Fryer and Hedrick 2003, Gaggero et al. 1995, Mauel and Miller 2002, Reid et al. 2004). In human public health and in the husbandry of terrestrial animals it has been extensively shown that the prevention of infections by opportunistic pathogens is better achieved by hygienic measures than by the excessive use of antibiotics as prophylactics (Cabello 2003, McEwan and Fedorka-Cray 2002, Levy 2001, Wheatley et al. 1995). As stated before, the application of large quantities of antibiotics in Chilean salmon aquaculture also appears to have generated antibiotic resistance among other fish pathogens including *Vibrio* and *Streptococcus* (Salud de Peces 2004), indicating that in the long run this use may be detrimental to the health of the industry itself. A situation can be predicted where this use of large quantities of antibiotics may lead to the appearance of new fish pathogens resistant to all antibiotics which would decimate a large segment of the industry.

In sum, the application of large quantities of antibiotics in the aquaculture of salmon in Chile has the potential to generate as yet undetermined environmental and public health impacts over a wide area. It is important to comment here that the areas in Chile where salmon aquaculture takes place, Regions X and XI, are experiencing toxic red tides and epidemics of *Vibrio parahaemolyticus* in the summer months, suggesting a decrease of the biodiversity in these areas (Cabello 2005, Hernandez et la. 2005). These marine changes have the potential to affect human and animal health and drastically limit development in Chile of different types of aquaculture activities (Angulo 2000, Cabello 2003, 2006, Benbook 2002).

Recently the National Fisheries Service Sernapesca, announced its initiation of a monitoring program regarding the use of antibiotics in salmon production. The hope is to diminish the use of fluoroquinolones since they are antibiotics of the latest generation and needed most importantly in human medicine and to reduce the possibility of development of antibiotic resistance (www.fishfarmingxpert.no).

2.2.3 Antibiotic use in the UK

The following antimicrobial products have been reported to have been used in the UK: oxytetracycline, florfenicol, amoxicillin trihydrate, trimethoprim/sulphadiazine,

Table 2.2 shows the products and volumes used in the salmon aquaculture industry in Scotland from 2003 to 2006. Quantities reported are Kg of active ingredient.

Table 2.2. Antibiotic use in Scotland 2003-2005. Quantities in Kg. Source SEPA

Antimicrobials	2003	2004	2005	2006
Oxytetracycline Hydrochloride	662.8	38	1643	5406
Florfenicol		6	10.2	38.4
Amoxicillin				55.2

As is the case in Norway, an elaborate regulatory framework, successful use of vaccines and implementation of good husbandry practices has resulted in use of relatively small quantities of antibiotics per metric ton of production. While overall trend has been to a reduced reliance on antibiotics it is clear that use varies from year to year. The strength of the reporting system in Scotland is evident in the 2006 data where details provided show that despite a significant increase in antibiotic use, the bulk of the antibiotic use reported is contributed by one company and that the increased use is not widespread throughout the industry (SEPA 2007).

2.2.4 Antibiotic use in Canada

The following products are registered for use as antibiotics in Canada: Oxytetracycline, trimethoprim80%/sulphadiazine20%, sulfadimethoxine80%/ormetoprim20%, florfenicol. Table 2.3 shows the quantities of antibiotic actually applied in Canada (2003) and British Columbia (BC) from 2004 through 2006. While BC produces the majority of Atlantic salmon grown in Canada, there is a significant salmon aquaculture industry on Canada's east coast.

Table 2.3. Total antibiotic use in Canada[a] or for British Columbia only[b]

	2003[a]	2004[b]	2005[b]	2006[b]
Total antibiotics	30,343 Kg*	18,530 Kg	12,103 Kg	7,956 Kg

* Includes data from the US state of Maine.

These data show the quantity applied per ton of production in 2003 was lower than that reported for Chile but larger than reported for Norway or Scotland. The table, howeve,r shows a consistent trend to lower rates of use of antibiotics in British Columbia. Aquaculturists in Canada have access to and routinely use vaccines known to be effective against a number of bacteria. Since so few compounds are available in Canada and even fewer are actually applied (M. Beattie personal communication) there may be reason for concern regarding resistance development. Without data about what compounds are applied, and where, it is difficult to make such judgements or to assess risk. Recently the province of New Brunswick, on Canada's east coast, instituted regulations wherein incidence of disease, products applied to combat disease and quantities used must be reported monthly. It is anticipated that in 2009 edited summaries of these reports will be available to the public (Mike Beattie, personal communication). This will provide data on therapeutant usage with temporal and spatial context. Unfortunately, data in this form is not available from other provinces on Canada's east coast or from British Columbia (NBSGA and Mark Sheppard personal communication)

2.4 Problems Resulting from Use of Large Quantities of Antibiotics in Salmon Aquaculture

2.4.1 Environmental

Antibiotic treatment in aquaculture is achieved by medicated baths and medicated food (Cabello 1993, Pillay 2004, Davenport et al. 2003, Boxall et al. 2004, Black 2001). In both cases, the potential exists for antibiotics to pass into the environment, remaining there for extended periods of time, affecting wild life and exerting their antibiotic effects (Boxall et al. 2004, Black 2001, Coyne et al. 1997, Hansen et al. 1992, Hektoen et al. 1995, Holten-Lützhøft et al. 1999, Christensen et al. 2006, Haya et al. 2000, Burka et al. 1997). Concerns regarding the use of large quantities of antibiotics in aquaculture are multiple. They include selection for antibiotic-resistant bacteria in piscine normal flora and pathogens (Cabello 1993, Sørum 2000, Pillay 2004, Davenport et al. 2003, Black 2001, Hansen et al. 1992, Burka et al. 1997, Huys et al. 2000, Kerry et al. 1996) as well as effects due to the persistence of antibiotics and antibiotic residues in sediments and water column (Cabello 2003, Sørum 2000, Pillay 2004, Davenport et al. 2003, Black 2001, Hansen et al. 1992, Burka et al. 1997, Huys et al. 2000, Kerry et al. 1996). These persistent antibiotics select for antibiotic-resistant free-living bacteria thereby altering the composition of normal marine and freshwater bacterial flora (Cabello 2003, Sørum 2000, Pillay 2004, Davenport et al. 2003, Black 2001, Hansen et al. 1992, Burka et al. 1997, Huys et al. 2000, Kerry et al. 1996). Because of their toxicity, they also affect the composition of the phytoplankton, the zooplankton and even the diversity of populations of larger animals (Boxall et al. 2004, Holten-Lützhøft et al. 1999, Christensen et al. 2006). In this manner, potential alterations of the diversity of the marine microbiota produced by antibiotics may alter the homeostasis of marine environment and affect complex forms of life including fish, shellfish, marine mammals, and human beings (Boxall et al. 2004, Holten-Lützhøft et al. 1999, Christensen et al. 2006, Samuelsen et al. 1992, Samuelsen et al. 1994, Schmidt et al. 2000). Marine microbial diversity is considered essential not only for the health of the marine habitat but also for the whole ecosphere (Hunter-Cevera et al. 2005).

2.4.2 Animal and Human Health.

Antibiotic-resistant bacteria and resistance genes selected by these antibiotics in the marine and freshwater environments have the potential of reaching terrestrial animal and human populations either by being passively transported (bacteria) or by horizontal gene transfer (genes) which then can compromise antibiotic therapy in these populations (Angulo 2000, Cabello 2003, Anderson et al. 2003, Harrison and Lederberg 1998, McEwen and Fedorka-Cray 2002, Schmidt et al. 2001a, Alcaide et al. 2005, Bushman 2002b, Davison 1999, Guardabassi, 2000, Hastings 2004, Kruse and Sørum 1994, L'Abee-Lund and Sørum 2001, Petersen 2002, Rhodes et al. 2000a, Sandaa et al. 1992, Rhodes et al. 2000b, Sørum and L'Abee-Lund 2002, Sørum 2006, 1998). Widespread use of large quantities of antibiotics also has the potential of contaminating free-ranging (wild) fish and shellfish near aquaculture sites as a result of fish and shellfish ingestion of medicated feed and of antibiotics leaching from uningested medicated feed in sediments (Coyne et al. 1997, Samuelsen et al. 1992, Cabello 2006, Bjorlund et al. 1990, Husevåg et al. 1991, Levy 2001, Rosser and Young 1999, Furushita et al. 2005 Schmidt et al. 2001b). This contamination can thus indirectly affect the safety of human food.

The safety of human food can also directly be affected by the presence of residual antibiotics in farmed fish for human consumption which have been dosed with antibiotics (Grave et al. 1999, Cabello 2003, Hunter-Cevera et al. 2005, Schmidt et al. 2001a, Alcaide et al. 2005, McDermott et al. 2002, Ecoceans 2006 (electronic citation)). Furthermore, application of large quantities of antibiotics can also affect the health of workers employed in feed mills and in raising in fish pens as a result of dust aerosols containing antibiotics that have been created in the process of medicating and distributing the feed to fish (Cabello 2003, Cabello 2004). Inhalation, ingestion and contact of the skin of workers with these aerosols will alter their normal flora, select for antibiotic-resistant bacteria and potentially generate problems of allergy and toxicity (Cabello 2003, Cabello 2004, Salyers et al. 2004, Anderson 1992).

Widespread use of large quantities of antibiotics in aquaculture thus has the potential to be detrimental to fish health, to the environment and wild life, and to human health (Cabello 2003, Cabello 2004, Holten-Lützhøft et al. 1999, Christensen et al. 2006, Levy 2001, McDermott et al. 2002, Ecoceans 2006(electronic citation), Salyers et al. 2004, Anderson 1992). For all these reasons, excessive antibiotic use in aquaculture should be of high concern to the aquaculture industry and its regulators, to public officials dealing with human and veterinary health and with the preservation of the environment, and to non-governmental organizations dealing with these issues. (Cabello 2003, Cabello 2004, Holten-Lützhøft et al. 1999, Christensen et al. 2006, Levy 2001, McDermott et al. 2002, Ecoceans 2006(electronic citation), Salyers et al. 2004, Anderson 1992).

2.5 Research needs

Slaughtering of fish close to the aquaculture site and excessive movement of farm personnel between different sites are also related to the appearance of infections and their dissemination (Stead and Laird 2002, Beveridge 2004, Austin and Austin 1999). Furthermore, some aquaculture sites in Chile exceed their production quotas, perhaps generating stressful conditions and mechanical damage to the fish that favors infections. Fish stress also appear to be produced in Chile by shortcomings in the transport of smolt from fresh water sites to marine pens, and this coincides with the appearance of *P. salmonis* infections in the marine sites (Stead and Laird 2002, Beveridge 2004, Austin and Austin 1999).

Most of the investigations proposed above have been carried out extensively in other countries with salmon aquaculture industries. Their results have been pivotal to stimulate the regulated use of antibiotics. These studies have repeatedly shown that excessive and heavy use of antibiotics is detrimental to fish health and to the environment, and has the potential of negatively impacting therapy of bacterial infections in human beings and terrestrial animals.

Application of knowledge of the causes and effects of excessive antibiotic usage could be readily applied to decrease the apparently excessive use of antibiotics in the Chilean salmon aquaculture industry and would yield enormous benefits to all stakeholders. It is relevant to mention here that according some authors (Stead and Laird 2002, Beveridge 2004, Austin and Austin 1999), an animal husbandry industry that uses excessive antibiotics and other chemicals to fend off infectious diseases is an industry in permanent crisis. Excessive antibiotic use in industrial animal rearing ultimately has the potential of backfiring and negatively affecting all the

aspects of the industry including its economic health. Aquaculture of salmon in Norway and other animal rearing industries in Europe has shown that negligible antibiotic use is highly compatible with an economically sound industry. Bravo and Midtlyng (2007) have reported the use of fish vaccines in Chile. Their data show a trend towards use of vaccines compared to antibiotic treatment. Unfortunately, the recently marketed vaccine against *P. salmonis* that constitutes the major problem in the Chilean industry has its effectiveness still unproven in the field.

- *Research must continue into the development of safe and effective vaccines against bacteria of concern and in particular P. salmonis. Safe and effective vaccines eliminate the need to apply antibiotics.*
- *In Chile and Canada, where larger quantities of antibiotics are used than in Europe in salmon aquaculture an epidemiological assessment of the volumes and classes of antibiotics used should be undertaken. The effect of the application of large quantities of antibiotic sediments and the water column should also be investigated.*
- *The presence of residual antibiotics/antibiotic residues, antibiotic resistance in marine bacteria and in fish pathogens, and effects on the diversity of phytoplankton and zooplankton in areas surrounding aquaculture sites should also be ascertained. Investigation of the presence of residual antibiotics/antibiotic residues in free-ranging (wild) fish and shellfish around aquaculture sites and in the meat of marketable salmon is necessary. The passage of antibiotic resistance determinants from bacteria in the marine environment to human and terrestrial animal pathogens should also be investigated. Centralized epidemiological studies of fish infections should be implemented and their results related to antibiotic usage and antibiotic resistance. The potential for exposure of aquaculture workers to antibiotics should be determined and the potential effects of this exposure should be ascertained.*
- *As data available from Chile and Canada is limited and indicates higher application rates than Europe. Methods and technology of salmon husbandry in these countries should be analyzed and compared to those in use in countries where antibiotic use has been drastically curtailed such as Norway. Siting issues, in Chile, for example, may allow for rapid dissemination of pathogens and may explain the emergence and rapid dissemination of P. salmonis infections to several aquaculture sites in Chiloe Island (Region X) when this pathogen first emerged in the area. Siting net pens very close to other (human and animal) inputs may aggravate pathogen problems. It is not clear if aquaculture sites in Chile where infections are diagnosed are left fallow to avoid infection of new fish reared in the place or whether different generations of fish are mixed and reared in the same site.*

CHAPTER 3

Antifoulants and other metal use in salmon cage culture

3.1 Introduction

Antifouling paints are applied to cages and nets to prevent the growth of attached marine organism. The buildup of these organisms ("epibiota") would reduce the water flow through the cages and decrease dissolved oxygen. The buildup would also decrease the durability of the nets, and reduce their flotation. Braithwaite et al. (2007) report that use of antifoulants significantly reduced biomass accumulation of biofouling organisms. Antifouling paints are formulated to have biocidal activity against these organisms to prevent their settlement. At the surface covered by the paint, a solution that is toxic to the spores or larvae of the organisms prevents their settlement. Antifouling paints have a matrix (resin) an active compound (the toxic biocide), auxiliary compounds and solvents. The matrix determines the leaching rate of the biocide. In the past, TBT paint was available with a co-polymer formulation which had slower releases to the environment. However, co-polymer formulations do not appear to be as effective for copper-based paints which are the major ones in use today. The rate of release is also affected by the toxic agent, temperature, water current speed and physical location of the structure. The active ingredients in these paints will leach out into the water and may exert toxic effects on non-target local marine life both in the water column and in the sediments below the cages, where the chemicals tend to accumulate. Greater amounts of antifoulants can be released when the paint is stripped during net cleaning.

3.2 Use of Antifoulants

Scotland is the only jurisdiction which requires companies involved in salmon aquaculture to report the quantities of antifoulant paints used on an annual basis. Copper oxide is the active ingredient in all paints currently used in Scotland. The quantities of copper oxide used in 2003, 2004 and 2005 are reported in Table 3.1. As the various paints are use different concentrations of active ingredient and in some cases a range of possible concentrations, the numbers reported in this table represent the maximum amount of copper oxide used.

Table 3.1. Reported antifoulant use (Kg of copper oxide) in salmon aquaculture in Scotland from 2003 - 2005.

Antifoulants	2003	2004	2005
Copper Oxide	18,996–26,626	11,700-29,056	34,000-84,123

3.3 Copper

3.3.1Chemical Characteristics

Copper is an essential metal, but can be toxic at higher concentrations. Though far less toxic than TBT, it is nevertheless quite toxic to some marine biota, especially algae and mollusks,

at fairly low levels. In addition to its use as an antifoulant, copper may also be a constituent of the food fed to farmed salmon.

Copper in water – bioavailability

The toxicity of copper in water is greatly affected by the chemical form of the copper ("speciation"), and to what degree it is bound to various ligands that may be in the water that make the copper unavailable to organisms. The salinity and pH also affect toxicity of copper. In a recent study Grosell et al. (2007) showed that killifish are most sensitive to copper in freshwater and in full seawater than in intermediate salinities. They also showed that the size of the fish is important in terms of the sensitivity of this species. The toxic effect of copper on cell division rate of the alga *Monochrysis lutheri* was greatly decreased with increasing amounts of natural organic ligands in the water which would bind the copper. The toxicity was directly proportional to the concentration of free cupric ion (Sunda and Lewis 1978). The toxicity of copper to *Ceriodaphnia dubia* (freshwater) decreased with increasing dissolved organic matter (DOM) (mostly humic acid) in the water, and was correlated to the free ion concentration (Cu^{2+}) rather than to the total Cu in the water (Kim et al. 1999). The presence of chelators (either naturally occurring DOM or added EDTA) in the water reduced the toxicity of copper to embryos of the oyster *Crassostrea gigas* (Knezovich et al.1981). In a study of toxicity of copper from mining operations, it was found that the copper in the water was not toxic to the diatom *Nitzschia closterium* because the copper was not taken up into the cells but rather became bound to organic matter on the outside of the cell membrane (Stauber et al. 2000).

Copper in sediments – bioavailability

Metals such as copper have relatively low solubility in water and tend to accumulate in sediments. The critical issue regarding toxicity of copper (and other metals) in sediments is what fraction of the copper is actually bioavailable, that is, how much can be taken up into organisms and therefore be able to produce toxic effects. It is important to examine exchange of metals in the sediment-water interface. Copper in sediments binds to fine particles and to sulfides, so the higher the levels of fine particles (silt and clay) and the higher the amount of sulfide in the sediments, the less bioavailable the copper (and other metals) will be. Hansen et al. (1996) demonstrated that sediment toxicity was not related to dry weight of metals, but rather to the ratio of simultaneously extracted metal (SEM) and acid-volatile sulfide (AVS). If this ratio was less than 1, toxicity would be absent, but when the SEM/AVS ratios were greater than 1, toxicity was observed. The combination of acid volatile sulfide (AVS) and total organic carbon (TOC) can explain much of the toxicity of Cu in sediments (Correia and Costa, 2000). However, data suggest that there are additional binding components for Cu that need to be included to explain bioavailability and toxicity of sediment copper. As sediments under fish farms tend to be reducing, have high oxygen demand, and high sulfide from the animal wastes and uneaten feed, these sediments should bind metals to a high degree.

3.3.2. Biological Effects

Despite the existence of various ligands in sea water, many studies have found toxic effects of low concentrations of copper (low $\mu g \cdot L^{-1}$ concentrations) in a variety of taxa.

Algae

Among the most sensitive groups to copper are the algae, since copper has been used as an algicide and many studies have examined its toxicity to various groups of microalgae (the phytoplankton that are the most important primary producers in the ocean).

The diatom *Phaeodactylum tricornutum* showed 50% growth reduction and reduced photosynthesis when exposed to 0.1 $mg \cdot L^{-1}$ Cu. The copper seemed to interfere with the cellular pool of ATP and affected pigment patterns of chlorophyll (Cid et al., 1995).

Copper levels of 3-126 nM induced oxidative stress in the diatom *Ditylum brightwellii*, as indicated by a decrease in reduced glutathione (GSH). It also caused a decrease in chlorophyll a and cell division rates; the decrease was accentuated in cultures that also contained zinc in addition to copper (Rijstenbil et al 1994). Using flow cytometry, Franklin et al. (2001) found that cell division, chlorophyll a fluorescence, cell size and enzyme activity in the marine alga *Phaeodactylum tricornutum* were significantly inhibited by copper at 10 $\mu g \cdot L^{-1}$. Another species, *Dunaliella tertiolecta*, was highly tolerant to copper. Ultrastructural changes were observed in *Dunaliella minuta* after exposure to 4.9×10^{-4} M copper (Visviki and Rachlin 1992). The cell volume increased, while the pyrenoid and chloroplast volume decreased. Copper altered volume regulation ability in the dinoflagellate *Dunaliella marina* (Riisgard et al. 1980). It appeared to increase cell permeability to Na^+ which entered the cells and made them swollen. The effects could be prevented with EDTA, which bound the copper and made it unavailable. The dinoflagellate, *Amphidinium carterae*, was studied by flow cytometry (Lage et al., 2001). Cell mobility and cell proliferation were reduced at levels below 3.13 M labile copper. The Na^+/H^+ antiporter system seemed to be affected by copper, thereby affecting cell membrane permeability and pH. Studies have also been performed on communities of phytoplankton. LeJeune et al. (2006) added 80 $\mu g \cdot L^{-1}$ and 160 $\mu g \cdot L^{-1}$ of copper (below and above the water complexation capacity), to mesocosms. They found that the phytoplankton biomass recovered within a few days after treatment. The higher copper concentration caused a decrease in phytoplankton diversity and led to the development and dominance of nanophytoplanktonic Chlorophyceae. Both concentrations affected cyanobacterial biomass and caused changes in the size-class structure and composition of phytoplankton communities.

Copper can also be quite toxic to pelagic developmental stages of macroalgae (seaweeds). Concentrations lower than 10 $\mu g \cdot L^{-1}$ affected long term reproductive endpoints such as sporophyte production and growth, and germ tube growth in microscopic stages of the giant kelp *Macrocystis pyrifera* (Martin et al. 1990). Similar findings were reported by Contreras et al (2007) on early developmental stages of the brown alga *Lessonia nigrescens*, in which 7.8 $\mu g \cdot L^{-1}$ interfered with development of spores after they settled. This led to a failure to develop male and female gametophytes, and disruption of the complete life cycle of the kelp. Similar results were obtained by Andersson and Kautsky with the brown alga *Fucus vesiculosis*.

Microbes

The microbial community is particularly sensitive to copper. Acute toxicity was observed in the estuarine microbial community exposed to 10 $\mu g \cdot L^{-1}$ total copper. Bacterial abundance was

reduced by 60%, and amino acid turnover rate was reduced by 30%. Since only 0.5% of the added copper was in the free cupric ion form, this reflects sensitivity to very low levels of free copper ion (Jonas 1989). Microorganism that are symbiotic in sponges are also highly sensitive to copper exposure, with counts of the dominant species decreasing significantly at copper concentrations of 1.7 $\mu g \cdot L^{-1}$ and above (Webster et al., 2001).

Crustaceans

Copepods are the most numerous type of zooplankton in the world's oceans, and are critical components in food webs. The copepod *Tisbe furcata* was used in toxicity tests with copper (Bechmann 1994). The LC_{50} was 2.8 µM copper (178 $\mu g \cdot L^{-1}$). One-third of this concentration caused significant effects on reproduction and life span, and 18% of the LC_{50} caused negative trends in all the demographic parameters. Natural copepod assemblages exhibited sublethal responses, such as changes in fecal pellet production, and egg production, when exposed to copper levels in the 1-10 $\mu g \cdot L^{-1}$ range (Reeve et al., 1977). The estuarine copepod, *Acartia tonsa*, was exposed to metals in ion buffer systems and appeared to be more sensitive than two species of diatoms. Survival of nauplii was more sensitive than survival of adults, being reduced by cupric ion activities of 10^{-11} M, while adult survival was not affected within the activity range of 10^{-13} to 10^{-11} (Sunda et al. 1987). There can be seasonal as well as life history differences in sensitivity to copper. The acute toxicity of copper to coastal mysid crustaceans was much greater in the summer than in the winter (Garnacho et al. 2000).

Amphipods are also important in marine food webs. Ahsanullah and Williams (1991) found that the minimal effect concentration of copper for affecting weight, survival and and biomass of the amphipod *Allorchestes compressa* was 3.7 $\mu g \cdot L^{-1}$. *Hyalella azteca* was able to regulate copper concentration and not bioaccumulate it under chronic exposure conditions (Borgmann et al. 1993). Barnacle nauplii (*Balanus improvisus*) were studied for potential sublethal behavioral effects of copper exposure in the water (Lang et al 1980). At 50 $\mu g \cdot L^{-1}$ swimming speed was reduced, and at 30 $\mu g \cdot L^{-1}$ the phototactic response was reduced. In treatments with 20-50 $\mu g \cdot L^{-1}$ copper (which contained >7 $\mu g \cdot L^{-1}$ labile Cu) most larvae of the coon stripe shrimp *Pandanalus danae* died while in the first zoeal stage. Copper toxicity at less than 1 $\mu g \cdot L^{-1}$ labile Cu was demonstrated by molting delay (Young et al. 1979). Physiological responses in the decapod crab, *Carcinus maenas*, to copper were measured (Hansen et al 1992). Activities of the enzymes hexokinase, phsophofructokinase, and pyruvate kinase were greatly reduced after one week in 10 $mg \cdot L^{-1}$ copper chloride.

Direct effects of copper-based antifouling paints themselves on brine shrimp nauplii were studied by Katranitsas et al. (2003). They examined sublethal responses (ATPase) when brine shrimp larvae were exposed to paint-coated (formulation of copper oxide with chlorothalonil as a booster) surface areas of 400-1000 mm^2 in static vessels containing 20 ml sea water. They found that as little as 50 mm^2 of painted surface decreased enzymatic activities of the brine shrimp but did not measure the actual concentrations of Cu in the water.

Mollusks

Embryos of the Pacific oyster, *Crassostrea gigas*, were exposed to copper and silver salts alone and in combination. Copper concentrations of up to 12 $\mu g \cdot L^{-1}$ produced decreasing percentages of normal embryonic development, and interactions with silver were additive (Coglianese and Martin 1981). Paul and Davies (1986) investigated effects of Cu-based antifoulants on growth of scallops and oysters. With the copper oxide treatment there was some increase in the growth of scallop spat, but no effect on the growth of adult scallops or Pacific oysters. The copper-nickel treatment, however, caused high mortalities and inhibited growth in adult scallops, but had no effect on oysters.

Echinoderms

Sea urchin embryos and larvae are frequently used in bioassays. Fernandez and Beiras (2001) incubated fertilized eggs and larvae of the sea urchin *Paracentrotus lividus* in seawater with single metals and combinations of mercury with other metals. The ranking of toxicity was Hg> Cu > Pb > Cd. The EC_{50} for Cu was 66.8 $g \cdot L^{-1}$, and combinations of metals tended to be additive.

Chordates

Sublethal effects of Cu on the sperm viability, fertilization, embryogenesis and larval attachment of the tunicate *Ciona intestinalis* were studied by Bellas et al. (2001). The EC_{50} for reducing embryogenesis and larval attachment was 46 $\mu g \cdot L^{-1}$ (0.72 µM).

Larval topsmelt, *Atherinops affinis*, were exposed to copper chloride for 7 days. Copper was more toxic at lower salinities, with an LC_{50} of ~200 $\mu g \cdot L^{-1}$ at high salinity and of ~40 at 10 ppt salinity (Anderson et al. 1995). The authors suggested that the increased sensitivity at low salinity was due to the increasing physiological stress of osmoregulation and/or the increased availability of free ion at lower salinity.

Burridge and Zitko (2002) found that copper leaching from freshly treated nets (treated with Cu_2O) was lethal to juvenile haddock (*Melangrammus aeglefinus* L.), and calculated the 48-hr LC50 to be about 400 $\mu g \cdot L^{-1}$. It was not stated if the netting had been dried before use in the experiments.

Toxic effects of copper in sediments

Despite the binding of copper in sediments, it can be toxic. Sediments under salmon cages in the Bay of Fundy and at various distances away from the cages were evaluated for toxicity using an amphipod toxicity test, the Microtox® (bacterial luminescence) solid phase test and a sea urchin fertilization test (Burridge et al. 1999). The Microtox® and urchin survival were very sensitive indicators of pore water toxicity. In addition to elevated levels of copper (above the threshold effects level), the sediments also had elevated zinc, other metals, ammonia nitrogen, sulfide, TOC, and other organic compounds, so the toxicity cannot be attributed solely to copper. Sediments enriched in copper, zinc and silver caused decreased reproduction in the clam *Macoma*

balthica, due to failed gamete production. Reproductive recovery occurred when contamination decreased (Hornberger et al. 2000). All these studies from field sites have numerous metals rather than just copper alone, and it is difficult to attribute toxicity to any particular metal.

A study of copper on faunas of marine soft sediments was performed by Morrisey et al. (1996) who experimentally enhanced copper in the tested sediments and monitored them over six months. Experimental sediments had 140 -1200 $\mu g \cdot g^{-1}$ Cu, while background concentrations were 29-40 $\mu g \cdot g^{-1}$. They observed a number of changes in taxa in the Cu-enriched sediments, in which some species increased and some decreased.

Studies have been performed examining the behavioral responses of burrowing organisms to Cu-contaminated sediments. Behavior is a very sensitive indicator of environmental stress that may affect survival. Burrowing behavior is critical for clams and other infauna for protection from predation. Burrowing time of the clam *Protothaca staminea* was increased at contamination levels above 5.8 $\mu g \cdot g^{-1}$ Cu (dry wt of sediments). Those clams that had been previously exposed had a lower threshold and a longer re-burrowing time (Phelps et al 1983). Juveniles of the bivalve *Macomona liliana* moved away from Cu-dosed sediments. Their rate of burial was lowered and, at levels above 15 $mg \cdot Kg^{-1}$ dry weight, most failed to bury and exhibited morbidity by 10 days (Roper and Hickey 1994).

3.3.3 Resistance

There have been numerous studies that indicate that organisms that are chronically exposed to metals may become more resistant to them (Klerks and Weis, 1987). This can occur through physiological mechanisms, which include induction of metal binding proteins such as metallothioneins, induction of stress proteins, induction of phytochelatins in plants, or sequestering the metals in metal-rich granules. Development of resistance can also occur via an evolutionary process over generations via selection for more tolerant genotypes, so that population genetics is altered. This is similar to the way in which microbes become resistant to antibiotics, but development of resistance in plants and animals will take considerably longer than in microbes, due to longer generation times. Although the development of resistance, when it happens, will reduce the negative impacts of toxicants, one cannot count on its development in any particular species.

3.3.4 Monitoring

The release of antifoulants into the marine environment is controlled by local and/or national waste discharge regulations (Costello et al. 2001). Generally elevated copper has been observed in sediments by salmon aquaculture facilities. Sediment concentrations of copper below the cages in Canadian salmon farms were generally around 100-150 $mg \cdot Kg^{-1}$ dry weight, and exceeded levels that are considered "safe" (exceed sediment quality criteria) (Burridge et al., 1999a; Debourg et al, 1993). In a study of British Columbia fish farms, Brooks and Mahnken (2003) found that 5 out of 14 farms had copper levels exceeding sediment quality criteria. The average Cu in reference stations was 12 $\mu g \cdot g^{-1}$ dry sediment, while under farms using Cu-treated nets it was 48 $\mu g \cdot g^{-1}$. The Cu concentrations in sediments under the salmon farms were highly variable, so that this difference was not statistically significant. Chou et al. (2002) similarly found

that Cu was elevated under salmon cages in Eastern Canada. Copper in anoxic sediments under cages was 54 $mg \cdot Kg^{-1}$ while in anoxic sediments 50 m away it was 38.5. Parker and Aube (2002) found copper in sediments was elevated compared to Canadian sediment quality guidelines in 80% of the aquaculture sites they examined. The copper would have come from the antifouling paints and possibly also from its use in salmon feeds.

Analysis of sediments under and around many Scottish fish farms was performed by Dean et al. (2007). Maximum level of copper in surface sediments was 805 $\mu g \cdot g^{-1}$. In contrast, the Sediment Quality Criterion for copper in Scotland is 270 $\mu g \cdot g^{-1}$, which would indicate adverse impacts are very likely. Pore water concentrations were 0.1-0.2 $\mu g \cdot L^{-1}$ Cu. Levels decreased with distance from the cages, and background levels were found in sediments about 300 m away from the farm center.

Yeats et al. (2005) and Sutherland et al. (2007) have shown that normalizing Cu concentrations to the conservative metal, lithium allows the distinction between sediments of aquaculture origin and those of natural or other anthropogenic sources. These studies were carried out on Canada's east and west coasts. The ration of Cu to Li in sediments collected near aquaculture sites was compared to the ratio found in the far field area or at references sites completely removed from aquaculture activity. These authors suggest this technique is useful not only for monitoring metals but for identifying aquaculture related inputs.

3.3.5 Bioaccumulation

Salmon tissues from fish in net pen operations were analyzed for copper (Burridge and Chou 2005). They found no accumulation in the gills, plasma, or kidneys compared to fish from the freshwater phase that had not been living in net pens. There was some accumulation in the liver, but it was low compared to fish from severely contaminated sites. Peterson et al. (1991) compared copper levels in muscle and liver tissue of chinook salmon grown in pens with treated nets with those from a pen with untreated nets and similarly found no significant differences. In contrast to the salmon in the pens, lobsters living in sediments in the vicinity of salmon aquaculture sites showed high accumulation of copper (Chou et al. 2002). Lobsters from the aquaculture site with the poorest flushing had accumulated 133 $\mu g \cdot g^{-1}$in the digestive gland, while those from a control site had only 12.4 $\mu g \cdot g^{-1}$in their digestive glands.

3.3.6 Risk

Brooks (2000) studied the leaching of copper from antifouling paints and found initial losses of 155 $\mu g\ Cu \cdot (cm^2)^{-1} \cdot day^{-1}$ and that rates declined exponentially. He developed a model that suggested that the EPA copper water quality criterion would not be exceeded when fewer than 24 cages were installed in two rows oriented parallel to the currents flowing in a maximum speed greater than 20 $cm \cdot sec^{-1}$. If the configuration, orientation, or density of nets was changed, the water quality criterion could be exceeded, which would indicate the likelihood of adverse effects from dissolved copper in the water. Lewis and Metaxas (1991) measured copper in water inside and outside a freshly treated aquaculture cage and reported the concentrations inside were not significantly different from those outside and the levels did not decrease after one month. The

concentration of copper in water in the cage was 0.54 $\mu g \cdot L^{-1}$, while it was 0.55 outside the net and 0.37 (not significantly different) at a station 700 m away. Similar levels were found one month later.

When copper accumulates in sediments below fish pens, it does so along with fish wastes, which elevate the organic carbon and the sulfides, which bind the copper, making it generally non-available and of low risk. Because of the high sulfides and low dissolved oxygen, there is likely to be a very depauperate, low diversity, community of opportunistic organisms in the sediments that is likely to be resistant to the copper. Parker et al. 2003 exposed the marine amphipod *Eohaustorius estuarius* to sediments collected from under a cage site. The level of copper in the sediments was up to 5 times greater than Environment Canada's predicted effect level, but there was no apparent effect on the amphipods. The authors attribute this to the lack of availability of the copper. However, disturbance of the sediments by currents or trawling could cause the sediments to be redistributed into the water column, and could re-mobilize the metals. Similarly, clean-up of the fish wastes and reduction in sulfides could make the sediment copper more available.

3.4 Trends in antifoulant use

Since tributyltin-based paints were removed from the market due to the extreme toxicity of these chemicals, the most commonly used paints these days (~95% of the market) are copper-based, most commonly cuprous oxide. Different brands contain from 15 –26% of copper biocide. In the process of coating the nets, they are pulled through or dipped in a paint bath, which adds 5-8 g of elemental copper for every 100 g of treated net (Burridge and Zitko, 2002).

3.5 Alternative Antifoulants:

Because of the toxicity of copper-based (and previously used tributyltin-based) antifouling paints, research is ongoing to find less toxic alternatives. Most of the research on these alternatives has been focused on their potential use for painting boats, but leaching and toxic effects would be comparable for their use on nets. Bellas (2006) compared the toxicity of a number of these formulations to marine invertebrate larvae (mussels, *Mytilus edulis*, sea urchins, *Paracentrotus lividus*, and ascidians *Ciona intestinalis*). The formulations tested were chlorothalonil, Sea-Nine 211, dichlofluanid, tolylfluanid, and Irgarol 1051. The EC_{50} for larval growth and settlement for chlorothalonil was 2-108 nM, for Sea-Nine 211 was 6-204 nM, for dichlofluanid was 95-830 nM, tolylfluanid was 99-631 nM, and Irgarol 1051 was 3145- 25,600. Thus, the order of toxicity from highest to lowest was chlorothalonil > Sea-Nine > dichlofluanid = tolylfluanid > Irgarol 1051. Based on reported levels of these compounds in marinas and polluted estuaries, the authors concluded that chlorothalonil, Sea-Nine 211 and dichlofluanid levels in marinas are already causing deleterious effects on *M. edulis*, *P. lividus*, and *C. intestinalis*, while Irgarol 1051 showed no biological effects at worst-case environmental concentrations. While Irgarol was the least toxic to the marine invertebrate larvae in that study, it has been found to be very toxic to phytoplankton (Konstantinou and Albanis, 2004; Van Wezel and Van Vlaardingen 2004). Irgarol was also reported to be very toxic to the meiobenthic copepod *A. tenuiremis* at 2.5 $\mu g \cdot L^{-1}$ (Bejarano and Chandler 2003). Other studies have shown that chlorothalonil affects shell deposition in larval oysters at 5-26 $\mu g \cdot L^{-1}$ (Mayer 1987 cited in Van

Wezel and Van Vlaardingen, 2004); The EC_{50} for mussel embryogenesis was 2 $\mu g \cdot L^{-1}$ (Shade et al 1993). Sea-Nine has also been reported to be very toxic to sea urchin embryogenesis (10 $fg \cdot L^{-1}$) (Kobayashi and Okamura, 2002).

Since different chemicals exert very different toxicity to different groups of organisms, a ranking system for comparing the toxicity of anti-fouling paints was devised by Karlsson et al. (2006). A number of new products have been developed to function by physical means rather than toxicity, and to compare them along with older antifouling paint products, the ranking system was based on toxicity to a red macroalga, *Ceramium tenuicorne* and the copepod *Nitocra spinipes.* The ranking system was based on the EC_{50} and LC_{50} values on a geometric scale. They tested both leach waters from different paints as well as single substances used in antifouling paints, including TBT, diuron, and Irgarol 1051. While copper and Irgarol 1051 have both been banned by the Swedish government because of their toxicity, leakage waters from various other paints showed some inhibitory effects on algal growth and/or were toxic to the copepods. Most of the paints that were supposed to work by physical means were found to be toxic to one or both of the test organisms, and some were even more toxic than the reference chemicals. The Swedish legislation stipulates that is that it is up to the producer to determine whether their product contains a substance intended to be toxic. If not, they do not have to perform toxicity tests on the products, so their toxicity is not tested prior to approval for use, and is missed. Several of the paints registered as containing no biocides, nevertheless were toxic to one or both of the two organisms tested. Concentrations in the range of the EC_{50} values seen have been measured in coastal waters around the world (Dahl and Blanck 1996; Haglund et al. 2001, Hernando et al. 2001; Konstantinou and Albanis 2004).

3.6 Zinc

Zinc is not used as an active ingredient in the antifouling paints that are used in salmon aquaculture, but it is a major ingredient (zinc pyrithione) in some antifouling paints. Its involvement with salmon aquaculture is as a supplement in salmon feeds, as it is an essential metal. Metals present in fish feed are either constituents of the meal from which the diet is manufactured or are added for nutritional reasons. The metals in feed include copper, zinc, iron, manganese, and others.

3.6.1 Chemical Characteristics

Zinc, like copper, binds to fine particles and to sulfides in sediments, and even when it is bioavailable, it is much less toxic than copper. Issues of speciation, bioavailability in the water column, and acid volatile sulfide (AVS) in the sediments are similar to those discussed earlier for copper. Given the elevated sulfides due to fish wastes, the Zn in sediments below salmon farms would be expected to be largely unavailable.

3.6.2 Biological Effects

Zinc in ionic form can be toxic to marine organisms, though generally at considerably higher concentrations than copper.

Algae

Marine algae are particularly sensitive to zinc. Effects on cell division, photosynthesis, ultrastructure, respiration, ATP levels, mitochondrial electron-transport chain (ETC)-activity, thiols and glutathione in the marine diatom *Nitzschia closterium* were investigated. They found that 65µg $Zn \cdot L^{-1}$ affected the cell division rate, but not photosynthesis or respiration. These endpoints were unaffected up to 500µg $Zn \cdot L^{-1}$. Most of the zinc was bound at the cell surface. The fraction of zinc that got inside the cell increased ATP production and ETC activity (Stauber and Florence, 1990).

Crustaceans

Arnott and Ahsanullah (1979) studied acute toxicity to the marine copepods *Scutellidium* sp., *Paracalanus parvus* and *Acartia simplex*. The 24-h LC_{50} value for Zn was 1.09 $mg \cdot L^{-1}$. Copper was the most toxic, with cadmium being more toxic than zinc for two of the three species. Copepod (*Acartia tonsa*) egg production was adversely affected by zinc free ion activity of 10^{-10} M, and nauplius larvae survival was reduced at 10^{-8} M free ion activity (Sunda et al. 1987). Harman and Langdon (1996) investigated the sensitivity of the Pacific coast mysid, *Mysidopsis intii*, to pollutants. Survival and growth responses of *M. intii* to zinc (152 $\mu g \cdot L^{-1}$) were comparable to other mysids. The amphipod, *Allorchestes compressa* exposed to 99 $\mu g \cdot L^{-1}$ Zn showed decreases in weight, survival, and biomass (Ahsanullah and Williams, 1991). Santos et al. (2000) examined effects of zinc on larvae of the shrimp *Farfantepenaeus paulensis*. Chronic exposure to zinc (106, 212 and 525 $\mu g \cdot L^{-1}$) reduced growth of 17 day old shrimp larvae. Oxygen consumption and feeding were reduced by all zinc concentrations tested. The inhibition of food and oxygen consumption could explain in part the long-term reduction of growth.

Echinoderms

Sea urchin (*Sterechinus neumayeri*) embryos were killed by concentrations as low as 0.327 $mg \cdot L^{-1}$ Zn (King, 2001).

Chordates

Bellas (2005) studied effects of Zn from antifouling paints (zinc pyrithione - Zpt) on the early stages of development of the ascidian *Ciona intestinalis*. The larval settlement stage was the most sensitive, with toxic effects detected at 9 nM (EC_{10}). On the basis of these data, the predicted no effect concentrations of Zpt to *C. intestinalis* larvae are lower than predicted environmental concentrations of Zpt in certain polluted areas, and therefore Zpt may pose a risk to *C. intestinalis* populations.

Sediment Toxicity:

Sediment zinc from fish farms was studied for toxicity to the annelid *Limnodrilus hoffmeisteri.* Hemoglobin, ATP, and protein concentrations were measured in worms exposed to pond sediments from three different trout farms, and to Zn-spiked sediments. Zn concentration in fish pond sediments was 0.0271-0.9754 $mg \cdot Kg^{-1}$. All three pond sediments showed sublethal toxicity, since ATP and protein concentrations were reduced compared to control worms. Zn-

spiked sediments also significantly reduced ATP, protein, and hemoglobin concentrations in the worms (Tabche et al. 2000). This is a freshwater study, but it is likely that marine annelids would respond in a similar way.

3.6.3 Trends in Chemical Use

Concentrations of zinc in feeds produced for Atlantic salmon range from 68 to 240 mg $Zn \cdot Kg^{-1}$. However, the estimated dietary requirements of Atlantic salmon for Zn are estimated to be lower than this, so it would appear that the metal concentrations in some feeds exceed the dietary requirements (Lorentzen and Maage 1999). Some feed manufacturers have recently changed the form of Zn to a more available form (zinc methionine) and have decreased the amount of Zn to minimum levels necessary for salmon health (Nash 2001).

When adding minerals toa diet it is important to evaluate not only the quantity added but the bioavailability. It is known that high calcium levels and other factors in the feed can inhibit intestinal zinc uptake. A variability in the amount of zinc added to the feed could be an indicator that the formulator of the feed has made an assessment of the factors reducing zinc availability and has added zinc to meet nutritional demands and safeguard against nutritional disorders.

3.6.4 Monitoring

Elevated zinc has been found in sediments below and around salmon cage cultures. Burridge et al. (1999a) and Chou et al. (2002) found elevated zinc concentrations in sediments near aquaculture sites that frequently exceeded the Canadian threshold effects level. Zinc in anoxic sediments under cages was 258 $\mu g \cdot g^{-1}$, while 50 m away from the cages the concentration was only 90 $\mu g \cdot g^{-1}$. Parker and Aube (2002) similarly found that the average sediment Zn concentration in sediments under salmon pens exceeded the Canadian interim sediment quality guidlelines. Dean et al. (2007) found maximum levels of Zn around salmon cages in Scotland to be 921 $\mu g \cdot g^{-1}$ which is more than twice the sediment quality criterion of 410 $\mu g \cdot g^{-1}$ that indicates "probably adverse" effects on the benthos. Pore water concentrations were 0.1-0.4 $\mu g \cdot g \cdot L^{-1}$. Levels of Zn decreased with distance from the fish farm, and Zn declined to background levels 300 m from the cages. In a Canadian study, zinc concentrations declined to background at >200 m from the cages (Smith et al. 2005). Brooks and Mahnken (2003) found that zinc under Canadian salmon farms ranged from 233-444 $\mu g \cdot g^{-1}$ in sediments, generally exceeding the "apparent effects threshold" (AET) of 260 $\mu g \cdot g^{-1}$ Down-current 30-75 m from the cages, the zinc concentrations were down to a background of 25 $\mu g \cdot g^{-1}$. In a New Zealand salmon farm, the sediment Zn concentrations also exceeded the sediment quality criteria of 410 $\mu g \cdot g^{-1}$ (Morrisey et al. 2000). Sediment zinc at the salmon farm was 665 $\mu g \cdot g^{-1}$, while at a control site it was only 18 $\mu g \cdot g^{-1}$. The Zn at the salmon farm was comparable to concentrations shown to impair recruitment of benthic invertebrates (Watzin and Roscigno, 1997).

Yeats et al. (2005) and Sutherland et al. (2007) have shown that the normalizing technique described above for Cu is also useful with Zn.

When fish are removed from the cages ("harvested") there is a post production fallow period in which there is a decrease in the amounts of chemicals in the sediments ("remediation").

During this time of inactivity, the sediment concentrations of Zn and other contaminants under cages in British Columbia declined to background levels (Brooks et al., 2003). There was also a reduction in organic material and sulfide in the sediments. At the same time, the biological community, previously dominated by two opportunistic species of annelids, became more diverse, with many different species of annelids and crustaceans and mollusks recruiting into the sediments.

3.6.5 Bioaccumulation

Zinc was not significantly elevated in lobsters from the vicinity of salmon farms where sediment Zn was elevated (Chou et al., 2002).

3.6.6 Risk

Zinc, like copper, binds to fine particles and to sulfides in sediments, and even when it is bioavailable, it is much less toxic than copper. Under salmon cages, the sulfide levels are probably high due to the volume of salmon wastes, making most of the zinc unavailable. Organically enriched fish farm sediments generally have a high biological oxygen demand and negative redox potential; conditions that lead to sulfate reduction. Under these conditions, metals such as copper and zinc are unlikely to be biologically available. However, disturbance of the sediments by currents or trawling could cause the sediments to be redistributed into the water column, and could re-mobilize the metals.

During the "remediation" fallow period discussed above, in which sediment levels of Zn decline, the reduction of organic material and sulfide concentration may release the Zn, increasing metal bioavailability. The probable reason for the decline in metals in sediments during remediation is that the metals are released into the water column, and therefore could be more available and toxic to other pelagic organisms in the vicinity.

3.7 Other metal concerns

A recent report (DeBruyn et al. 2006) indicates that mercury was elevated in fillets of native copper rockfish and quillback rockfish collected in the vicinity of salmon farms in British Columbia. The reason suggested for the increased Hg in these long-lived, demersal, slow growing fish was that the conditions fostered by the aquaculture facilities caused them to become more piscivorous and shift to a higher trophic level, thereby bioaccumulating greater amounts of mercury already in the ecosystem. This observation is of interest and should lead to further research into this phenomenon. Chou (2007) reported that the mercury concentration in harvested Atlantic salmon is well within the regulatory limit set by the USFDA (1.0 mg methyl mercury$\cdot Kg^{-1}$) and the USEPA guidance of 0.029 mg methyl mercury$\cdot Kg^{-1}$.

Since elevated levels of copper and zinc occur together in sediments below salmon cages, it is possible that they may interact with each other in a synergistic way to cause even more deleterious effects. It is not the place here to review the extensive research that has been done on metal-metal interactions, but in general the majority of studies have found that these two metals do not interact synergistically with each other. Most studies have found either additive effects or,

more often, antagonistic interactions, wherein the presence of zinc reduces the toxic effects of the copper.

3.8 Research gaps

- *There is continued urgent need to develop alternative antifoulants that are not toxic to non-target organisms, or that work through physical means and do not exert toxicity to prevent settlement of fouling organisms. (A salmon farm in Norway (Villa Laks) is developing new technology that uses non-toxic antifouling treatment.)*
- *Research is needed into the fate of metals when their concentrations decrease in sediments after harvesting the fish during the remediation phase to investigate to what degree the metals are being released into the water column and available to nearby biota.*

3.10 Recommendations

- *Nets and cages should not be washed in the ocean, which would release considerable amounts of toxic antifoulants into the water, but they should be washed in upland facilities. The waste produced should be disposed of properly in a secure landfill.*
- *Whenever possible, do not use antifoulants at all to treat cages and pens, since these substances are toxic and persistent.*
- *All antifouling agents, regardless of whether they are considered to contain biocides or not, should be tested (in accordance with standard methods) for toxicity to different taxa of marine organisms.*
- *It is not likely that the amount of zinc released from salmon aquaculture operations is enough to pose much risk to marine biota. Nevertheless, the concentration of zinc in feed pellets should not be in excess of the nutritional requirements of salmon.*

CHAPTER 4

Antiparasitic compounds used in Atlantic salmon cage culture

4.1 Introduction

Cultured salmon are susceptible to epidemics of infectious bacterial, viral and parasitic diseases. Sea lice are ectoparasites of many species of fish and have been a serious problem for salmon aquaculture industries (Roth et al. 1993, McKinnon 1997). The species that infest cultured Atlantic salmon are *Lepeophtheirus salmonis* and *Caligus elongatus* in the northern hemisphere and *Caligus teres* and *Caligus rogercresseyi* in Chile. Infestations result in skin erosion and sub-epidermal haemorrhage which, if left untreated would result in significant fish losses, probably as a result of osmotic stress and other secondary infections (Wooten et al. 1982, Pike 1989). Sea lice are natural parasites of wild Atlantic and Pacific salmon, and infestations have occurred routinely wherever salmonid aquaculture is practiced. Sea lice reproduce year round and the aim of successful lice control strategy must be to pre-empt an internal infestation

cycle becoming established on a farm by exerting a reliable control on juvenile and preadult stages, thus preventing the appearance of gravid females (Treasurer & Grant 1997). Effective mitigation, management and control of sea lice infestations requires good husbandry. In addition a natural predator, wrasse, has been used in Norway and number of effective anti-parasitic chemicals have been used (Read et al. 2001, Rae 2000, Eithun 2004).

Chemicals used in the treatment of sea lice infestations are normally subsequently released to the aquatic environment and may have impact on other aquatic organisms and their habitat. It is the release of these compounds that has been identified as a major environmental concern (Nash 2003).

Therapeutant use is regulated in all countries where salmon aquaculture is practiced. A veterinary prescription is required to use these compounds. Norway, Chile and the UK have a list of 5-10 compounds registered for use to combat infestations of sea lice of which many are today not used or have been withdrawn. Canada has only two registered products, neither of which has been prescribed in the recent past.

Haya et al. (2005) have written a review of anti-louse therapeutant use in the salmon aquaculture industry in the northern hemisphere. This present document will attempt to build on their work by discussing trends in use of parasiticides and by including discussions of the Chilean salmon aquaculture industry as well. This report, however, can not be considered an exhaustive review of the available literature.

4.2 **Therapeutant Use**

Chemicals currently authorized for the treatment of sea lice infestation may be classified into two groups, based on their route of administration, bath treatments and in-feed additives. Organophosphates, pyrethroids and hydrogen peroxide are or have been administered by bath techniques, while the avermectins and chitin synthesis inhibitors are administered as additives in medicated feed.

Bath treatments are conducted by reducing the depth of the net in the salmon cage, thus reducing the volume of water. The net-pen (and enclosed salmon) is surrounded by an impervious tarpaulin and the chemical is added to the recommended treatment concentration. The salmon are maintained in the bath for a period of time (usually 30-60 minutes) and aeration/oxygenation may be provided. After treatment, the tarpaulin is removed and the treatment chemical is allowed to disperse into the surrounding water.

Medicated feed is prepared by adding concentrated pre-mix containing the active ingredient to feed during the milling and pelletisation processes. The chemical is administered by calculating the dosage based on the feed consumption rate of the salmon. The therapeutant is absorbed though the gut into the blood stream of the salmon and is then transferred to the sea lice as they feed on the skin of the salmon. The advantages of in-feed preparations compared to bath treatments are that releases to environment are much slower and less direct. Treatment is less stressful to the fish, the dosage can be more accurately controlled, the oral preparations are not

toxic to farmers, and it requires less labour. One disadvantage is that stressed or diseased fish often feed less than healthy fish and therefore may not receive a fully effective dose.

The following is a summary of the products available for use in each jurisdiction and the quantities of each of these products that have been applied in the recent past. For the year 2003 where we have data from al jurisdictions the tables include therapeutant use relative to the quantity of fish produced.

Norway

The following compounds are identified as being registered for use in Norway: *Bath treatments*: cypermethrin, deltametrin, azamethiphos, trichlorfon, dichlorvos, pyrethrum and hydrogen peroxide. *In-feed additives*: diflubenzuron, teflubenzuron and emamectin benzoate. The registration of zamethiphos was withdrawn by the manufacturer in Canada in 2002. It is assumed that the registration was also withdrawn in other jurisdiction as well although use of the product is reported in Scotland in 2003 and 2004. Table 4.1 shows that compounds actuually applied and the quantities used in Norway from 2002 to 2006.

Table 4.1. Parasiticides used in Norway and the quantities (Kg active ingredient) used 2002-2006. Source Jon Arne Grottum and www.fishfarmingxpert.no

Active Compound	2002	2003	2004	2005	2006
Cypermethrin	62	59	55	45	49
Deltamethrin	23	16	17	16	23
Emamectin Benzoate	20	23	32	39	60

Chile

The following compounds are identified as having been used as anti-louse treatments in Chile: *Bath treatments*: cypermethrin, deltamethrin, azamethiphos, trichlorfon, dichlorvos, pyrethrum and hydrogen peroxide. *In-feed additives*: diflubenzuron, teflubenzuron, ivermectin and emamectin benzoate. Cypermethrin, deltamethrin, pyrethrum, diflubenzuron and teflubenzuron have only been used in field trials.

Table 4.2 shows the compounds actually applied and the quantities used from 2001 to 2003, the last years for which data are available.

Table 4.2. Parasiticides used in Chile and the quantities (Kg active ingredient) used 2001 - 2003. Source Bravo (2005)

Active Compound	2001	2002	2003
Cypermethrin	0	0	6.25
Dichlorvos	247	0	0
Emamectin benzoate	77	121	127
Ivermectin	10	3	3

UK

The following compounds are identified as having been used in anti-louse treatments in Scotland: *Bath treatments*: cypermethrin, deltamethrin, dichlorvos, azamethiphos and hydrogen peroxide. *In-feed additives*: diflubenzuron, teflubenzuron and emamectin benzoate. Table 4.3 shows the compounds actually used and the quantities applied from 2003 to 2005.

Table 4.3. Parasiticides used in Scotland and the quantities (Kg active ingredient) used 2003-2006. Source Scottish Environmental Protection Agency

Active Compound	2003	2004	2005	2006
Cypermethrin	10.5	656.9	6.6	9.7
Azamethiphos	35.5	11.65	0	0
Hydrogen Peroxide	35.3	43.8	19.7	0
Emamectin benzoate	28.3	52.6	36.3	16.8
Teflubenzuron	36.0	0	0	0

Canada

The following compounds are currently registered for use in Canada: *Bath Treatments:* hydrogen peroxide. *In-feed additives:* teflubenzuron. Emamectin benzoate is available for use under Health Canada's Emergency Drug Release program. As in other jurisdictions, azamethiphos is no longer registered in Canada. Table 4.4 shows the compounds actually used and the quantities applied. Data are available from fish farms in British Columbia but are not easily obtained for salmon farms in eastern Canada.

Table 4.4. Parasiticides used in Canada (2002-2003) or in British Columbia only (2004-2005) and the quantities (Kg active ingredient) used 2002-2005. Source Health Canada and Government of British Columbia.

Active Compound	2002	2003	2004	2005
Azamethiphos	15	0	0	0
Emamectin benzoate	25	12.1*	10.5	17.8

* includes data from the State of Maine USA.

Although a number of products appear to available to veterinarians and salmon farmers to combat infestations of sea lice, it is clear from Tables 4.1-4.4 that, in practice, only a few are prescribed as most are either withdraw, are not approved or are unavailable. For instance, no organophosphate compounds (dichlorvos and Azamethiphos) have been used since 2003. Only one compound, the in-feed therapeutant emamectin benzoate is used in all jurisdictions. It is, in fact the only product used in Canada and the US. In terms of relative use of the products listed Use of only a single compound can lead to the development of resistance to the compound in the parasite. Not surprisingly, evidence of resistance has recently been reported in Chile (Bravo personal communication 2007). Canada limits the number of sea lice treatments with emamectin

benzoate during a grow-out cycle to 3. In Norway and the UK allow up to 5 treatments can be applied and in Chile up to 8 treatments have been reported during a grow-out cycle. In Chile there are several products containing emamectin benzoate available to treat salmon against infestations of lice. Some of these have a higher recommended treatment dose than 50 $\mu g \cdot Kg^{-1}$. (S. Bravo, personal communication).

Cypermethrin is used in Norway, and the UK and has been applied on a trial basis in Chile. Scotland treats with this compound relatively more often than elsewhere. The difference in rate of use (Kg/MT) is approximately 6 times greater than Norway.

The use of the organophosphate azamethiphos and the chitin synthesis inhibitor teflubenzuron appears to have ended. The registrant of azamethiphos chose not to renew registration in Canada in 2002 and it appears as though it is unavailable in other jurisdictions. Development of resistance in lice is known to occur with organophosphate pesticides (Jones et al. 1992). Teflubenzuron apparently is no longer produced as an anti-louse treatment (M. Beattie NBDAA personal communication).

Interestingly, hydrogen peroxide, which has been considered a rather poor product for sea lice control, is used in Scotland and has recently been applied in Chile (S. Bravo personal communication) in the absence of effective alternatives. Hydrogen peroxide is considered the most environmentally friendly product. Its use may be related to the sensitivity of the receiving environment, the lack of alterantive therapeutants and it could also be an indication that other products are failing in terms of efficacy of louse removal.

The apparent movement to the use of fewer products and the fact that there are few products being developed for sea lice treatment should raise concerns within the industry. Even drug manufacturers stress the benefits of the availability of a suite of compounds and of the rational application of these products to avoid resistance development.

4.3 Physical and Chemical Properties of Therapeutants and their Biological Effects

For the purposes of this paper, products that have not been used in aquaculture in the past 3 years will not be discussed in detail. Readers are encouraged to refer to Haya et al. (2005) for a discussion of organophosphates and other compounds previously used to combat sea lice infestations.

4.3.1 Pyrethroids (cypermethrin and deltamethrin)

Efficacy and Mechanism of Action of synthetic Pyrethroids

Pyrethrins are the active constituents of an extract from flower heads of *Chrysanthemum cinerariaefolium*. In the early 1960s synthetic analogues that were more persistent than the natural pyrethrins were developed and referred to as pyrethroids were developed (Davis 1985). It was their high degradability, low toxicity to mammals and high toxicity to crustaceans that led to the initial interest in pyrethroids as treatments for sea lice infestations.

The mechanism of action of the pyrethroids involves interference with nerve membrane function, primarily by their interaction with Na channels (Miller & Adams 1982) which results in depolarization of the nerve ending. This interaction results in repetitive firing of the nerve ending in the case of the pyrethroids, cypermethrin and deltamethrin.

Deltamethrin and two formulations of cypermethin (Excis® and Betamax®) are approved for use in Norway, Chile has conducted field trial with deltamethrin and one formulation of cypermethrin (Excis®) and one formulation of cypermethrin (Excis®) is registered for use in the United Kingdom.

The recommended treatment of salmon against sea lice is a 1 hour bath with Excis® at a concentration of (5.0 $\mu g \cdot L^{-1}$ as cypermethrin), 30 minutes with Betamax® (15 $\mu g \cdot L^{-1}$ as cypermethrin) and for deltamethrin it is 2.0-3.0 $\mu g \cdot L^{-1}$for 40 minutes (SEPA 1998) Cypermethrin is effective against all attached stages including adults, and therefore less frequent treatments should be required than with organophosphates, 5-6 week intervals rather then 2-3 week intervals, respectively. A region in Norway had a population of resistant sea lice. The concentration required to successfully treat fish was 25 times higher than that for an area that had not been treated previously with deltamethrin (Sevatadal & Horsberg 2003).

Distribution and Fate of Pyrethroids

Synthetic pyrethroids are unlikely to be accumulated to a significant degree in fish and aquatic food chains since they are rapidly metabolized (Kahn 1993). This author warns, however, that pyrethroids such as cypermethrin can persist in sediments for weeks and may be desorbed and affect benthic invertebrates. While there is a large amount of knowledge regarding the ecotoxicology of cypermethrin in the freshwater environments (Khan 1983, Haya 1989, Hill 1985), knowledge is more limited for marine species.

The concentration of cypremethrin decreases rapidly on release from a cage after treatment. Data collected in Loch Eil Scotland showed that the highest concentration found was 187 $ng \cdot L^{-1}$ 25 minutes after release 25 m from the site in the direction of the current flow (SEPA 1998). Cypermethrin remained above 0.031 $ng \cdot L^{-1}$ up to 50 min after release and above 0.074 $ng \cdot L^{-1}$ for 30 min (Hunter and Fraser, 1995 reported in Pahl & Opitz. (1999)). Mussels exposed inside a treated cage (5.0 $\mu g \cdot L^{-1}$ cypermethrin) accumulated 133 $\mu g \cdot g^{-1}$. Mussels 2 m from cages accumulated 9.2 $ng \cdot g^{-1}$ after 7 treatments and cypermethrin was only occasionally barely detectable 100 m from cage. There were no effects on *Crangon crangon* used as sentinel species near the cage site. Organisms in the vicinity of the cages would be exposed to concentrations which fall to 50 $ng \cdot L^{-1}$ within one hour of release (SEPA 1998).

In aerobic sediments cypermethrin biodegrades with a half life of 35 and 80 days in high and low organic sediment, respectively. It degrades much more slowly in anaerobic sediments (SEPA 1998). The rapid disappearance of deltamethrin from water (60% in 5 min), its high adsorption on sediment and its low bioconcentration capacities (in daphnia, *Chlorella asellus*) indicate that this molecule will not accumulate through food chains. Nevertheless, its high toxicity and rapidity of action may cause significant harm to limnic ecosystems after direct treatment (Thybaud 1990). The adsorption of pyrethroids onto suspended solids can produce

dramatic reductions in the apparent toxicity of the compound. The 96 h LC50 value of rainbow trout is 1.0-0.5 $\mu g \cdot L^{-1}$ (Thybaud 1990). When trout were caged in a pond containing 14-22 $mg \cdot L^{-1}$ suspended solids, the 96 h LC50 was 2.5 $\mu g \cdot L^{-1}$. In a pond sprayed with deltamethrin containing 11 and 23 $mg \cdot L^{-1}$ suspended solids, detamethrin partitioned rapidly to suspended solids, plants, sediment and air with a half life if 2-4 h in water (Muir et al. 1985).

Because pyrethroids tend to adsorb onto particulate matter chronic exposures may not occur other than in laboratory studies. Cypermethrin absorbed by sediment was not acutely toxic to grass shrimp until concentrations in sediment were increased to the point where partitioning into the overlying water resulted in acutely lethal concentrations (Clark et al. 1987). For example, the 96 h LC50 for cypermethrin to grass shrimp is 0.016 $\mu g \cdot L^{-1}$, but grass shrimp could tolerate cypermethrin concentrations in sediment of 10.0 $\mu g \cdot Kg^{-1}$ for 10 day.

Biological Effects of Pyrethroids

The lethality (96h LC50) of cypermethrin to lobster (*Homarus americanus*) and shrimp (*Crangon septemspinosa*), was 0.04 $\mu g \cdot L^{-1}$ and 0.01 $\mu g \cdot L^{-1}$, respectively (McLeese et al. 1980). The 24 h LC50 was 0.14 $\mu g \cdot L^{-1}$ for adult lobster. For other marine invertebrates, 96h LC50 values range from 0.005 $\mu g \cdot L^{-1}$ for mysid shrimp (Hill 1985) to 0.056 $\mu g \cdot L^{-1}$ for the same species (Clark et al. 1989). The 96 h LC50 for five other marine crustaceans ranged from 0.016$\mu g \cdot L^{-1}$ for grass shrimp to 0.20 $\mu g \cdot L^{-1}$ for fiddler crab. Oysters were relatively insensitive, with a 48 h EC 50 of 2.3 $mg \cdot L^{-1}$ based on larval development. For marine fish, the 96 h LC50 of cypermethrin to Atlantic salmon was 2.0 $\mu g \cdot L^{-1}$ (McLeese et al. 1980) and for sheephead minnow was 1.0 $\mu g \cdot L^{-1}$ (Hill 1985). There appears to have been very little work done regarding sublethal effects of cypermethrin on non target organisms.

Larvae are often considered the most susceptible life stage to environmental or chemical stress. The 12h LC50 of cypermethrin for stage II lobster larvae at 10 and 12^{o}C was 0.365 and 0.058 $\mu g \cdot L^{-1}$, respectively (Pahl & Opitz 1999). At sublethal concentrations effects on swimming ability and responsiveness of the lobster larvae were observed. The 48 h LC50 of a cypermethrin to the three larval stages (I, II, and III) of the American lobster (*Homarus americanus*) and to the first post-larval stage (IV) was 0.18, 0.12, 0.06, 0.12 $\mu g \cdot L^{-1}$ of respectively (Burridge et al. 2000). Thus, cypermethrin was lethal to larval lobsters over 48 h at approximately 3 % of the recommended treatment concentration. On the other hand soft shell clam larvae, green sea urchin larvae and rotifers were tolerant of cypermethrin and 12 hour LC 50 values were greater than 10 $mg \cdot L^{-1}$ (Pahl & Opitz 1999). Medina et al. (2002) report there is an age-related variation in sensitivity of the copepod *Acartia tonsa* to exposure to cypermethrin.

The impact of pyrethroids and natural pyrethrins on non-target aquatic animals, especially invetebrates has been reviewed (Mian & Mulla 1992). In general pyrethroids are more toxic to non-target insects and crustaceans than to other phylogenetically distant invertebrates. Crustaceans are arthropods and therefore phylogenetically closer to insects than to molluscs and showed noticeable sensitivity. The isopod, *Asellus aquaticus* and the mysid shrimp, *Mysidophsis bahia* have shown even higher sensitivities than crustaceans to pyrethroids, including cypermethrin. Spray operations on ponds have resulted in 95% reduction of arthropod fauna such as crustaceans, insects and arachnids. The residue profile of cypermethrin in water

immediately after application, coupled with rapid decay (4-24h), explained the limited effect of pyrethroids on populations of non-target aquatic invertebrates in some case studies. On the other hand, invertebrates in habitats subjected to frequent treatments are likely to be more affected especially those species that show greater sensitivity. However populations of affected organisms generally recovered to pretreatment levels within weeks to months of the exposure. Medina et al. (2004) have reported that cypermethrin immediately reduces plankton density and diversity communities in lab studies but hypothesized that in an open system pesticide concentrations would drop quickly and that plankton migration and immigration would lead to recovery of the community. Willis et al. (2005) reported that sea lice treatments on salmon farms had no effect on zooplankton communities.

In freshwater studies cypermethrin had a significant sublethal impact on the pheromone-mediated endocrine system in mature Atlantic salmon parr (Moore & Waring 2001). It was suggested that cypermethrin acts directly on the Na channels and inhibits nervous transmission within the olfactory system and thus the male salmon is unable to detect and respond to the priming pheromone. In the marine environment it may reduce homing abilities of retuning adult salmon and increase straying rates between river systems.

Shrimp (*Crangon crangon*) were deployed in cages at various distances and depths from the cages during treatment with cypermethrin at two salmon aquaculture sites in Scotland during treatment with cypermethrin. The only mortalities were to shrimp held in treated cages (SEPA 1998). Shrimp in drogues released with the treated water were temporarily affected but recovered. In an American field study, cypermethrin was lethal to 90% of the lobsters in the treatment cage but no effect was observed in those located 100-150 m away. There was no effect on mussels placed outside or inside the cages. Similar field studies indicated that cypermethrin was lethal to lobsters and planktonic crustaceans in the treatment tarpaulin but not to mussels, sea urchins or planktonic copepods.

Cypermethrin induced glutathione *S*-transferase (GST) activity in shore crab, *Carcinus maenas*, exposed to a solution of 50 and 500 $ng \cdot L^{-1}$ of cypermethrin or injected intra-cephalothoracically with 10ng (Gowland et al. 2002). However, activity of the enzyme returned to base levels after 36 h and there was no clear dose response and so GST activity may not be a useful biomarker of exposure to cypermethrin.

The fate and dispersion of cypermethrin and a dye, rhodamine were determined after simulated bath treatments from a salmon aquaculture site under various tidal conditions in the Bay of Fundy, Canada (Ernst et al. 2001). Dye concentrations were detectable for periods after release which varies from 2-5.5 hours and distances ranged from 900 to 3000 meters depending on the location and tidal flow at the time of release. Concentrations of cypermethrin in the plume reached 1-3 orders of magnitude below the treatment concentration 3-5 hours post release and indicated that the plume retained its toxicity for substantial period of time after release. Water samples collected from the plume were toxic in a 48 hour lethality test to *E. astuarius* for cypermethrin up to 5 hours after release.

The pyrethroids, cypermethrin and deltamethrin are not persistant in marine waters. Both have relatively short half-lives in water and concentrations in the water decreased rapidly (<4h)

in some field trials due to decomposition and partitioning to particulate matter. In sediments, the compounds are more persistent with half-lives up to 80 d, and cypermethrin was detected in sediment surveys in near salmon aquaculture sites in Scotland (SEPA 2002). However, bioavailability of pyrethroids from sediment is minimal.

Cypermethrin has the potential to release lethal plumes from a single cage treatment. The plume can cover up to a square Km and lethality to sensitive species can last as long as 5 hours. Since treatment of multiple cages is the operational norm, area wide effects of cypermethrin on sensitive species cannot be discounted. Sensitive species include crustaceans such as lobster larvae, shrimp and crabs and the 96 h LC50 for some can be a magnitude less than the treatment concentration. No lethality was observed in shrimp and lobsters deployed in cages during sea lice treatments with cypermethrin.

Evidence suggests that there could be considerable risk to individuals of sensitive species but there is insufficient knowledge to extrapolate to populations. There is sufficient evidence on the development of resistance to advice against routine use of pyrethroids as only means of control.

4.3.2 Hydrogen Peroxide

Efficacy and Mechanism of Action of Hydrogen Peroxide

Hydrogen peroxide is a strong oxidizing agent that was first considered for the treatment of ecto-parasites of aquarium fish (Mitchell & Collins 1997). It is widely used for the treatment of fungal infections of fish and their eggs in hatcheries (Rach et al. 2000). With the development of resistance to dichlorvos by sea lice (Jones et al. 1992) there was move towards the use of hydrogen peroxide to treat infestations of mostly *Lepeophtheirus salmonis* but also *Caligus elogatus*. Hydrogen peroxide was used in salmon farms in Faroe Islands, Norway, Scotland and Canada in the 1990's (Treasurer & Grant 1997). Hydrogen peroxide (Paramove®, Salartect®) is still authorized for use in all countries but it is not the normal treatment of choice. It was however used in the UK in 2005 (see Table 3) and may be being applied in Chile (S. Bravo personal comm.).

The suggested mechanisms of action of hydrogen peroxide are mechanical paralysis, peroxidation by hydroxyl radicals of lipid and cellular organelle membranes, and inactivation of enzymes and DNA replication (Cotran et al. 1989). Most evidence supports the induction of mechanical paralysis when bubbles form in the gut and haemolymph and cause the sea lice to release and float to the surface (Bruno & Raynard 1994).

The recommended dosage for bath treatments is 0.5 $g \cdot L^{-1}$ for 20 min. but the effectiveness is temperature dependent and the compound is not effective below $10^{o}C$. Treatments are rarely fully effective but 85-100% of mobile stages may be removed (Treasurer et al. 2000). The first farm treatments in Scotland in October 1992 removed 83% of the mobile stages of sea lice. The recommended course is to repeat the treatment at 3-4 week intervals. This usually results in low numbers of sea lice for 8 weeks following the third treatment (Treasurer & Grant 1997). Hydrogen peroxide has little efficacy against larval sea lice and its effectiveness against preadult

and adult stages has been inconsistent (Mitchell & Collins 1997). Effectiveness can be difficult to determine on farms as the treatment concentration varies due to highly variable volumes of water enclosed in the tarpaulin. Temperature and duration also influence the efficacy. Ovigerous females are less sensitive that other mobile stages (Treasurer et al. 2000). It is possible that a proportion of the eggs on gravid female lice may not be viable after exposure to hydrogen peroxide (Johnson et al. 1993). Hydrogen peroxide was less efficacious when treating sea lice infestation on salmon in a cage that had been treated regularly for 6 years than in cages where the sea lice were treated for the first time. This suggested that *L. salmonis* had developed some resistance to hydrogen peroxide (Treasurer et al 2000).

In a laboratory experiment, all adult and pre-adult sea lice exposed to 2.0 $g \cdot L^{-1}$ hydrogen peroxide for 20 min became immobilized, but half had recovered two hours post-treatment (Bruno & Raynard 1994). The recovered sea lice swam normally and may have been able to reattach to the host salmon (Hodneland et al. 1993). Therefore it was the recommended that floating lice should be removed. However, re-infection has not been noticed in practice (Treasurer et al. 2000) as the removed sea lice generally show little swimming activity. These authors suggest re-infection in the field is less likely because the free sea lice will be washed away with the tidal flow or eaten by predators. After treatment of a cage with approximately 1.5 $g \cdot L^{-1}$ hydrogen peroxide at 6.5 ^{o}C, all the sea lice that were collected from surface water of treated cages were inactive but recovery commenced within 30 minutes and 90-97% of the sea lice were active 12 hours post-treatment (Treasurer & Grant 1997). In this study, a higher proportion of pre-adult sea lice were removed than of adult sea lice.

Distribution and Fate of Hydrogen Peroxide

Hydrogen peroxide is generally considered environmentally compatible because it decomposes into oxygen and water and is totally miscible with water. At 4 ^{o}C and 15 ^{o}C, 21% and 54% respectively of the hydrogen peroxide has decomposed after 7 days in sea water. If the sea water is aerated the amount decomposed after 7 days is 45% and 67%, respectively (Bruno & Raynard 1994). Field observations suggest that decomposition in the field is more rapid, possibly due to reaction with organic matter in the water column, or decomposition catalyzed by other substances in the water, such as metals. In most countries, hydrogen peroxide is considered a low environmental risk and therefore of low regulatory priority. While other compounds are subject to a withdrawal period between time of treatment and time of harvest, hydrogen peroxide has none.

Biological Effects of Hydrogen Peroxide

There is little information of the toxicity of hydrogen peroxide to marine organisms. Most toxicity data are related to the potential effects on salmonids during treatment of sea lice infestations. Experimental exposure of Atlantic salmon to hydrogen peroxide at varying temperatures shows that there is a very narrow margin between treatment concentration (0.5 $g \cdot L^{-1}$) and that which causes gill damage and mortality (2.38 $g \cdot L^{-1}$) (Keimer & Black 1997).

Toxicity to fish varies with temperature; for example, the one hour LC50 to rainbow trout at 7^{o}C was 2.38 $g \cdot L^{-1}$, at 22^{o}C was 0.218 $g \cdot L^{-1}$ (Mitchell & Collins 1997) and for Atlantic salmon

increased five fold when the temperature was raised from 6°C to 14°C. There was 35% mortality in Atlantic salmon exposed to hydrogen peroxide at 13.5°C for 20 min. There was a rapid increase in respiration and loss of balance, but if the exposure was at 10°C there was no effect (Bruno & Raynard 1994). Hydrogen peroxide is not recommended as a treatment for sea lice infestations at water temperatures above 14°C. Whole bay treatments in the winter should reduce the need for treatments in the summer (Rach et al. 1997).

The method of application of hydrogen peroxide is not standardized but is a balance between achieving consistently effective treatments and toxicity to fish. For example, high concentrations were used (2.5 $g \cdot L^{-1}$ for 23 minutes) to treat a farm for 6 years, which achieved 63% removal of sea lice. Exposure periods longer than this were the used in an attempt to increase removal, but caused 9% mortality in the salmon (Treasurer et al. 2000). There is evidence that the concentrations of hydrogen peroxide used in sea lice treatments can cause gill damage and reduced growth rates for two weeks post treatment (Carvajal et al. 2000).

4.3.3 Emamectin benzoate

Efficacy and Mechanism of Action of emamectin benzoate

Emamectin benzoate (SLICE®) is semi-synthetic derivative of a chemical produced by the bacterium, *Streptomyces avermitilis*. The class of compounds is referred to as avermectins.

Although the product is not registered for use in Canada, emamectin benzoate, Slice® has been available in Canada as an Emergency Drug Release (EDR) from Health Canada since 1999 and is currently the only anti-louse treatment being used in Canada (Table 4.4) used to treat salmon against sea lice in eastern Canada. SLICE is fully registered for use in the UK, Norway, and Chile

The avermectins are effective in the control of internal and external parasites in a wide range of host species, particularly mammals (Campbell 1989). The avermectins generally open glutamate-gated chloride channels at invertebrate inhibitory synapses. The result is an increase in chloride concentrations, hyperpolarization of muscle and nerve tissue, and inhibition of neural transmission (Roy et al. 2000, Grant 2002). Avermectins can also increase the release of the inhibitory neurotransmitter γ-amino-butyric acid (GABA) in mammals (Davies & Rodger 2000).

The optimum therapeutic dose for emamectin benzoate is 0.05 $mg \cdot Kg^{-1}$ $fish \cdot day^{-1}$ for seven consecutive days (Stone et al. 1999). This dose has been shown to reduce the number of motile and chalimus stages of *L. salmonis* by 94-95% after a 21 day study period (Stone et al. 1999, Ramstad et al. 2000). Four cage sites with a total of 1.2 million first year class fish were treated. Although there was a slight depression of appetite at two of the four sites, appetite was normal when top-up rations (untreated food) were supplied. *Caligus elongatus* were present in low numbers and results suggested that they were also affected by the treatment. The number of motile lice was reduced by as much as 80% at the end of the 7-day treatment period. In a field trial emamectin benzoate reduced sea lice counts on treated fish by 68-98% and lice numbers remained low compared to control fish for at least 55 days (Stone et al. 2000a, Stone et al. 2000b).

Distribution and Fate of emamectin benzoate

Emamectin benzoate also has low water solubility and relatively high octanol-water partition coefficient, indicating that it has the potential to be absorbed to particulate material and surfaces and that it will be tightly bound to marine sediments with little or no mobility (SEPA 1999). The half-life of emamectin benzoate is 193.4 days in aerobic soil and 427 days in anaerobic soil. In field trials, emamectin benzoate was not detected in water samples and only 4 of 59 sediment samples collected near a treated cage had detectable levels. The emamectin benzoate persisted in the sediment; the highest concentration was measured at 10 m from the cage 4 months post-treatment. In Canada, emamectin benzoate was not detected in sediment samples collected near an aquaculture site for 10 weeks after treatment with SLICE® (W.R Parker, Environment Canada, personal communication). Mussels were deployed and traps were set out to capture invertebrates near aquaculture sites undergoing treatment. While detectable levels of emamectin benzoate and metabolites were measured in mussels (9 of 18 sites) one week after treatment, no positive results were observed after 4 months (SEPA 1999). Emamectin benzoate was found in crustaceans during and immediately after treatment. Species showing detectable levels for several months after treatment are scavengers which are likely to consume faecal material and waste food.

Biological Effects of emamectin benzoate

The treatment concentrations on salmon feed range from 1 to 25 $\mu g \cdot Kg^{-1}$ (Roy et al. 2000) with a target dose to the fish of 50 $\mu g \cdot Kg^{-1}$. Feeding emamectin benzoate to Atlantic salmon and rainbow trout at up to ten times the recommended treatment dose resulted in no mortality. However, signs of toxicity, lethargy, dark coloration and lack of appetite were observed at the highest treatment concentration.

The lethality of emamectin benzoate-treated fish feed to adult and juvenile American lobsters is estimated as 644 and >589 $\mu g \cdot Kg^{-1}$ of feed, respectively (Burridge et al. 2004). Its lethality to other aquatic invertebrates (for example, *Nephrops norvegicus* and *Crangon crangon*) was >68 $mg \cdot Kg^{-1}$ (SEPA 1999). In laboratory studies, prawns and crabs were offered feed medicated with emamectin benzoate at concentrations up to 500 $mg \cdot Kg^{-1}$ (Linssen et al. 2002). While there was no acute mortality, the crabs appeared to avoid medicated feed pellets.In a 7 day subletal test, there was significant reduction of egg production in the adult marine copepod, *Acartia clauii*(Willis & Ling 2003) The concentrations necessary to elicit these responses were above the Predicted Environmental Concentration (PEC) (Willis & Ling 2003). Ingestion of emamectin benzoate induced premature molting of lobsters (Waddy et al. 2002a). This molting response of lobsters may involve an inter-relationship of a number of environmental (water temperature), physiological (molt and reproductive status) and chemical (concentration/dose) factors (Waddy et al. 2002b). Further studies of this response suggest that the risk may be limited to a small number of individuals and that widespread population effects are unlikely (Waddy et al. 2007)

4.3.4 Teflubenzuron

Efficacy and Mechanism of Action Teflubenzuron

Chitin synthesis inhibitors belong to a class of insecticides collectively referred to as insect growth regulators and have been used in terrestrial spray programmes for nuisance insects since the late 1970s. Teflubenzuron (Calicide®) is approved as an additive in feed to treat sea lice infestations of cultured salmon in Norway, Scotland and Canada but the last recorded use of the product was in Scotland in 2003. The product has also been used on a trial basis in Chile (Bravo personal communication 2007).

Chitin is the predominant component of the exoskeleton of insects and crustaceans, and the biochemical mechanism by which these insecticides inhibit the synthesis of chitin is unclear (Savitz et al. 1994). The molting stage is the sensitive stage of the life cycle and inhibition of chitin synthesis interferes with the formation of new exoskeleton in a post-molt animal (Walker & Horst 1992, Horst & Walker 1995). Thus the chitin synthesis inhibitors are effective against the larval and pre-adult life stages of sea lice.

Teflubenzuron is effective against *L. salmonis* at a dose to salmon of 10 $mg \cdot Kg^{-1}$ body weight per day for 7 consecutive days at 11-15^{o}C (Branson et al. 2000). Teflubenzuron at this dosage was used to treat commercial salmon farms in Scotland and Norway, and the efficacy was 83.4 and 86.3 % respectively, measured at 7 days post treatment. There were no lethal effects on treated fish or effects on their appetite. In a Norwegian field trial of salmon in a polar circle with 100,000 kg of salmon, the efficacy for a dosage of 8.1 $mg \cdot Kg^{-1}$ body $wt \cdot day^{--1}$ for 7 days was 77.5% at 5.4^{o}C (Ritchie et al. 2002). The greatest reductions were in chalimus and pre-adult lice and the efficacy was 88% if the calculation was based only on the susceptible life stages of *L. salmonis*. The effects were observed up to 26 days after start of the treatment. A few Norwegian sites successfully used teflubenzuron in 1997 to remove all developing stages and the sea lice did not return during the further year's growth cycle (SEPA 1999).

Since chitin synthesis inhibitors are effective against the developing copepodids, larval (chalimus) and pre-adult stages of sea lice and less effective against adult lice, treatments are most effective before adult lice appear, or at least are present in only low numbers. When used correctly, chitin synthesis inhibitors provide a treatment option that breaks the life cycle of the sea lice and, as a result, the duration between treatments may be several months.

Distribution and Fate of teflubenzuron

Teflubenzuron has a moderate octanol-water partition coefficient and relatively low water solubility, which means that it will tend to remain bound to sediment and organic materials in the environment.

In a field study, a total of 19.6 kg of teflubenzuron was applied over a 7 day period to treat a salmon cage with a biomass of 294.6 tonnes (SEPA 1999). Teflubenzuron was not detected in the water after treatment and highest concentrations in the sediments were found under the cages and decreased with distance from the cage in the direction of the current flow. It

persisted in sediments for at least six months and the half-life was estimated at 115 days. Measurable levels were noted for a distance of 1000m in line with the current flow, but 98% of the total load had degraded or dispersed by 645 days after treatment. There was some indication of re-suspension and redistribution of sediment after several weeks based on concentrations of teflubenzuron found in mussel tissues. Evidence suggested that that there was some risk to indigenous sediment dwelling crustaceans, such as edible crab or Norway lobster, that may accumulate teflubenzuron from the sediment. However, the mussels eliminated teflubenzuron readily.

The absorption of teflubenzuron from the gastrointestinal tract of salmon has been found to be poor, with only around 10% of the administered dose being retained by salmon and 90% being released by the fish via feces as well as the uneaten portion of the feed (SEPA 1999). The deposition of teflubenzuron, in the vicinity of the treated cage is primarily from waste feed, with a more widespread distribution arising from the dispersion of fecal matter that may extend to 100 m from cages in the direction of the current flow (SEPA 1999).

Biological effects of teflubenzuron

Although teflubenzuron is relatively non-toxic to most marine vertebrates (birds, mammals and fish) due to its mode of action, it is potentially highly toxic to any species which undergo molting within their life cycle (SEPA 1999, Eisler 1992). This includes some commercially important marine animals such as lobster, crab, shrimp and some zooplankton species.

In a field study, no adverse effects were detectable in the benthic macrofaunal community or indigenous crustaceans and it was concluded that residual teflubenzuron in sediment was not bioavailable (SEPA 1999). There was some evidence of effects on the benthic fauna within 50 m of the treated cages, but no adverse impacts on community structure and diversity including important key sediment re-worker species and crustacean populations. A study at three locations in Scotland included a novel biomonitoring technique whereby juvenile lobster larvae were deployed on platforms at locations around cages. The juvenile lobster mortality was attributed to exposure to the medicated feed at 25m from the cage, but this effect did not occur 100 m from the cage, and it was confirmed that a molt occurred during the study. Baird et al. (1996) and McHenery (1997), quoted in SEPA (1999) reported that predicted environmental concentrations (PEC) would not exceed the predicted no effect concentration (PNEC) would not be exceeded 15m from cages (SEPA 1999). Since crustaceans are largely absent within 15 m of cages, and evidence suggests that teflubenzuron is relatively non-toxic to sediment re-worker organisms such as polychaete worms, the environmental risks in the use of teflubenzuron in the treatment of sea lice infestations were considered to be low and acceptable.

4.4 Risk

All jurisdictions have in place mechanisms for approving therapeutants for use in salmonid aquaculture. The registration procedure or the authorization of a permit to apply a therapeutant includes an assessment of the potential risk of its use. In most cases the information provided to regulatory authorities by registrants includes proprietary information, not accessible

by the general public. While these data are reviewed as part of the registration process, the absence of these data from the public domain has the unfortunate consequence that neither its quality nor its nature can be debated by those scientists and non-scientists with interests in these areas. The registration or licensing procedure, therefore, is the most important part of risk assessment and management.

In the European Union, Maximum Residue Levels (MRL) are set for all therapeutants applied to food fish. Health Canada and the Canadian Food Inspection Agency have similar guidelines.

Anti-lice treatments lack of specificity and therefore may affect indigenous organisms in the vicinity of anti-lice treatments. For example, the American lobster, a commercially important decapod crustacean native to the waters of the Bay of Fundy, has been shown to be sensitive to most of the therapeutants applied in Canada. Lobsters spawn, molt and hatch their young in the summer months coincident with the most likely time for sea lice treatments (Campbell 1986). It is possible that treatment of lice infested fish and release of pesticide formulations could coincide with the presence of lobster larvae in the water (Burridge et al. 2000). Although the possibility of this occurring is readily understood the probability is not.

Sea lice therapeutants not only have the potential to negatively impact the environment through effects on sensitive non-target organisms they may alter the population structures of the fauna in the immediate environments.

Data generated to date generally suggest that negative impacts from anti-louse treatments, if they do occur, are minor and will be restricted in spatial and temporal scale. However, field data is rare. Most information regarding the biological effects of the various compounds is generated for single-species, lab-based bioassays.

Farms are located in waters with different capacities to absorb wastes, including medicinal chemicals, without causing unacceptable environmental impacts. Risks therefore have site-specific component, and management of these risks may therefore require site-specific assessments of the quantities of chemicals that can safely be used at each site. The UK environmental authorities (primarily the Scottish Environment Protection Agency, SEPA) operate this further level of control on the use of medicines at fish farms. A medicine or chemical agent cannot be discharged from a fish farm installation unless formal consent under the Control of Pollution Act has been granted to the farm concerned by (in Scotland) SEPA.

SEPA also requires annual reporting of therapeutant use from each site and these data are available to the public. This regulatory scheme provides an example of a risk management plan that should be adopted in all areas that use sea lice therapeutants.

4.5 Conclusions and Research Gaps

Parasiticides are used in all jurisdictions and the quantity of these compounds being applied is considerable. This is especially true if we consider that in jurisdictions such as Chile and eastern Canada the many aquaculture sites are, or were, located in close proximity of each

other in small geographic areas. The quantities applied relative to production are fairly constant across jurisdictions. Generally data on quantities of therapeutants applied in the various jurisdictions is difficult to access. In some cases the data is not collated or summarized. In others it is simply unavailable to the public.

The number of products being applied in salmon aquaculture is getting smaller. In the northern hemisphere emamectin benzoate is the treatment of choice. However, reliance on one or two therapeutants is poor practice as resistance development is accelerated and, once resistance is present, treatment options become severely restricted. Evidence of resistance has been reported for all classes of compounds used to date except for hydrogen peroxide. In a recent publication Lees et al. (2008) describe a statistical analysis of the efficacy of emamectin benzoate against infestations of sea lice in Scotland from 2002-2006. They report that the number of treatments that appeared to be ineffective increased towards the end of the study.

The compounds used to combat sea lice are, not surprisingly, toxic to other arthropods. The sensitivity, however, is species specific. Most authors suggest the risk of population effects as a result of the use of anti-louse therapeutants is small. However, data to support this prediction is almost non-existent.

Nearly all published data regarding the biological effects of these therapeutants comes from single species, lab-based bioassays. Very little field work has been published that address thequestion of whether real-world applications have the same consequences as observed in th elab. The result of only one study has been published reporting effects on plankton populations near sites where sea lice treatments have taken place (Willis et al 2005). Regulatory agencies have access to a range of data supplied by drug manufacturers. This data is widely considered proprietary in nature and is not available to other interested parties.

No studies (lab or field) have adequately addressed cumulative effects. Salmon farms do not exist in isolation. Coincident treatments of parasiticides may have the benefit of reducing further infestation, therefore reducing the need to treat and the quantity of product applied. However coincident treatments may also affect salmon as well as non-target organisms. Multiple treatments within a single area may result if significantly different exposure regimes for non-targets organisms than a single treatment. While commercially important species such as lobsters have received a fair amount of research attention other marine invertebrates have not.

- *It appears as though there are no new therapeutants in the regulatory system. In the absence of new treatment options and in support of sustainable salmon aquaculture, studies need to be conducted to identify best management practices that reduce the need to treat fish against infestations of sea lice.*
- *Risk assessment of anti-parasitics are often based on single-species, single chemical, lab-based studies. Field studies need to be conducted to determine the biological effects on non target organisms of therapeutants under operational conditions. These studies may have been conducted by proponents of the therapeutants but results are not available to the public.*
- *Cumulative effects of chemicals and of interactions between chemicals and the marine environment are essentially unknown.*

- *Some of these compounds are persistent in the environment. Studies must be designed and carried out to determine fate and potential effects of these compounds in the near site and far-field environments.*

4.6 Recommendations

- *Data may exist that address some of the research gaps identified above. Where field studies have been conducted as part of the registration process, the data should be more readily available to the public.*
- *Regulatory agencies in all jurisdictions where salmon aquaculture is practiced should require full accounting on a yearly basis of all therapeutants applied. Further, these data should be collated, summarized and made available to the public on a yearly basis. The system employed by the Scottish Environmental Protection Agency is an example where this is already being done*

CHAPTER 5

Disinfectants

5.1 Introduction

Biosecurity is of paramount importance in aquaculture operations. The presence of infectious salmon anemia (ISA) and the prevalence of bacterial infections in some jurisdictions have resulted in protocols being developed to limit transfer of diseases from site to site. These protocols involve the use of disinfectants on nets, boats, containers, raingear, boots, diving equipment, platforms and decking. In most cases the disinfectants are released directly to the surrounding environment (Muise and Associates, 2001). The effects of disinfectants in the marine environment appear to be poorly studied. In addition, only the UK requires reporting of quantities of disinfectants being used in aquaculture activity. The use of disinfectants in Chile is more common in invertebrate aquaculture than in salmon aquaculture (Bravo 2005). Although it is difficult to determine where the products are being used, there has been an increase in sales of these products over the past few years.

5.2 Disinfectants in use

In Scotland a range of disinfectant products are used. The products fall into three general classes: iodophors, 1-alkyl-1,5 diazapentane and chlorine containing products (SEPA 2007). The quantities of each of these compounds used at each cage site must be reported to SEPA on an annual basis. In Table 5.1 the total of all disinfectants used in Scotland in 2003, 2004 and 2005 are reported.

Table 5.1. The total of disinfectants used on Atlantic salmon grow out sites in Scotland in 2003, 2004 and 2005.

Year	Total quantity of disinfectants used (Kg)
2003	1848
2004	7543
2005	4015
2006	3901

In Table 5.2 Bravo (2005) identifies the products that are being used for disinfection in the Chilean aquaculture industry. In Chile there are no regulations regarding the use of disinfectants. Similarly, while codes of practice exist for disinfection of equipment, etc in Canada and Norway there are apparently no regulations in place.

Table 5.2. Disinfectants used in Chile from Bravo (2005)

Disinfectant Group	Product
Potassium persulfate + organic acids	Virkon ®
Iodophors	Iodine + detergents
Chlorine	Chloramine-T
	Hypochlorite ($HClO_2$)
	Chlorine dioxide (ClO_2)
Quaternary ammonium compounds	Benzalkonium chloride
	Superquats ®
Aldehydes	Glutaraldehyde
	Formalin 40%
Alkalies	Calcium oxide: CaO or quicklime
	Calcium hydroxide; $Ca(OH)_2$ or slake lime
	Sodium carbonate: Na_2CO_3 or soda ash
Phenols	Creolina
	Synthetic phenols, halophenols
Alcohol	Ethanol 95% and 70%

5.3 Properties of disinfectants

5.3.1 Virkon®

Virkon® is a broad range disinfectant. The primary active ingredients are potassium peroxymonosulphate (21.5%) and sodium chloride (1.5%). The author was unable to find any published data regarding the presence or effects of Virkonin marine environments. The product is however considered toxic to freshwater *Daphnia* and the reported LC50 for rainbow trout fry is ~6 $mg \cdot L^{-1}$ (Hardy in Syndel Corp. 2007).

5.3.2 Quaternary ammonium compounds

Quaternary ammonium products are used in fish culture and crustacean farming, and for the chemical sterilization of production zones and equipment (Bravo et al., 2005). One of the commonly used products is benzalkonium chloride, applied to inhibit bacterial growth and the development of mucus in the gills of salmon (Burka et al., 1997), thereby allowing an adequate absorption of oxygen. Their efficiency and toxicity depend on the pH and hardness of the water (Bravo et al., 2005).

The action consists in disrupting the permeability of the membranes as it joins their phospholipids and proteins. They act preferentially on the carbon chain between the C12 and C16 positions, where they exert a lipophilic action. It has been found that in Gram-negative bacteria the high phospholipid and lipid content increases resistance because it renders more difficult the access of these compounds to the cell membrane.

5.3.3 Chlorine derivatives

Hypochlorite is obtained from the dissociation of sodium hypochlorite. At pH 4-7 the predominant species is hypochlorous acid (HClO), a compound that inhibits bacterial development by preventing the oxidative phosphorylation of bacterial membranes (McDonnell and Russell, 1999). Another chlorine derivative is hydrochloric acid, a strong acid that can be lethal to fish starting at 25 $mg \cdot L^{-1}$. In media having low pH (acid), its action affects the metabolism, causing the death of the organism. It has acute effects at pH lower than 5. It does not bioaccumule or bioconcentrate.

Chloramine-T, another chlorine derivative, is a product authorized in Chile; it is a wide spectrum disinfectant that attacks bacteria, fungi, viruses and parasites. It is applied as a powder to water, where it dissolves forming hypochlorous acid which enters through the cell wall, prevents enzymatic activity and causes cellular death. Its greatest efficiency is at low pH.

In salmon aquaculture these products are used in seawater and therefore at pH greater than 7. They are also likely to be used in situations where dilution is considerable and quick. However, chlorine is very toxic to aquatic biota and the products should be used with caution (Zitko 1994).

5.3.4 Iodophores

Iodophores carry iodine in a complex with an agent that acts as a reservoir of free iodine, a carrier agent. The iodine associates with proteins, nucleotides and fatty acids, causing the death of the microorganism. Iodine has bactericidal, fungicidal, viricidal and sporicidal action, and it has been used as an aqueous solution since the middle of the nineteenth century. As a solution it is unstable, necessitating the use of solubilizing agents that liberate the iodine. Iodine causes death by destroying proteins (particularly those with free groups of cistein and methionine), nucleotides and fatty acids (McDonnell and Russell, 1999).

The use of Wescodyne®, an iodine-based product commonly used in Canada has been reviewed by Environment Canada. The authors conclude that because of the increased use in response to disease problems in the aquaculture industry in New Brunswick, Canada, coupled with what is known of effects derived from lab-based studies and the lack of data regarding its use in the field, the product should be considered a moderate risk to aquatic organisms (Environment Canada, unpublished results). Concerns regarding the use of iodophores also relate to the solvents used in the formulations. It is known that some formulations contain ethoxylated nonylphenols, compounds that are toxic in their own right (Zitko 1994) and widely accepted as compounds with endocrine disrupting properties (Madsen et al. 1997).

5.3.5 Aldehydes

Formalin is a monoaldehyde that reacts with proteins, DNA and RNA in vitro (Bravo et al., 2005). It is recommended for controlling external fish parasites and for the control of fungi of the Saprolegniaceae family, and it has moderate to weak antibacterial activity.

Except for the reporting requirement in Scotland, information on use of disinfectants in the salmon aquaculture industry is difficult to find. All of the compounds used are quite water soluble. Risk of aquatic biota being exposed to the disinfectant formulations is dependent not only on how much is being used but where it is being released. Unlike parasiticides, there appear to be no regulations regarding the use of disinfectants. Thus in areas around wharves or in small sheltered coves disinfectant input could be significant. There is no information on the amounts of disinfectants used by the salmon industry or by the processing plants and the food industry, making it very difficult to determine precisely the quantities of these products used. However, Bravo et al. (2005) mention information from scallop and abalone cultures where formalin and chloramine-T are used, identified as disinfectants, but marketed as antiparasitic and fungicidal agents. Surveys of the laboratories that sell these products have shown that there was an increase in sales of 4.2 and 2.6 times, respectively, from 1999 to 2003. However, from information obtained from distributors other than the authorized laboratories, the sale of formalin has been approximately 24 times that recorded by the laboratories with an increase of 4.3 times from 1999 to 2003.

5.3.6 Malachite Green

Malachite green is a triphenylmethane dye (4-[4-trimethylaminophenyl)-phenyl-methyl]-N,N-dimethyl-aniline. It is readily soluble in water (110 $g \cdot L^{-1}$). It is used as a biological stain as a dye in consumer products, in forensic medicine, as a pH indicator and as a veterinary drug. In the past malachite green was used as an anti-fungal agent in salmon aquaculture. Its use as a therapeutant in fish destined for human consumption has been banned and a zero tolerance level for food fish is in place in most countries.

Malachite green is readily absorbed by fish tissue and is metabolically reduced to leucomalachite green (LMG) which is lipohilic and can be stored in edible fish tissues for extended periods of time (Anderson et al. 2005). Malachite green and leucomalachite green are suspected of being capable of causing gene damage and causing cancer (BfR 2007).

Despite the fact that the use of malachite green is banned in salmon farming Anderson et al. (2005) state that numerous instances of misuse in all forms of aquaculture have been reported in the US and internationally. It is widely thought that detection of these compounds is indicative of misuse. However, a recent preliminary study reported by BfR (2007a) shows that some free ranging wild fish (eels) in Germany have detectable levels of LMG in their edible tissues, albeit at very low concentrations. The authors of this report suggest malachite green is entering the environment from municipal wastewater treatment plants and that the source of the product is from industrial sites as well as ornamental aquaria. The suggestion that malachite green may be a ubiquitous contaminant in industrialized areas is troubling and calls into question the ability to enforce zero tolerance guidelines.

5.4 Research Gap

- *There are very little available data regarding the presence of disinfectants and particularly of formulation products in the marine environment. Studies need to be conducted to document the patterns of use, the temporal and spatial scales over which compounds can be found.*

5.5 Recommendation

- *That regulatory agencies in all jurisdictions require yearly reporting of the quantities of disinfectants used by salmon farms and that these data be made available to the public.*

CHAPTER 6

Anaesthetics

6.1 Introduction

Anaesthetics are used operationally in aquaculture when fish are sorted, vaccinated, transported or handled for sea lice counts or stripping of broodstock (Burridge 2003). Compounds available for use are regulated in all jurisdictions. They are used infrequently and in low doses, thus limiting potential for environmental damage. Application of anaesthetics may, however be hazardous to users (GESAMP, 1997).

The Candian Council on Animal Care (CCAC) defines anaesthesia as a state caused by an applied external agent resulting in a loss of sensation through a depression of the nervous system. Overdoses of anaesthetics are also used when euthanizing fish.

6.2 Anaesthetics in use

In Norway the use of anaesthetics is regulated and the quantities used are tracked. Table 1 shows the compounds used in Norway since 2002 and the quantities reported. The total quantity of anaesthetic used in Norway has increased from 1175 Kg in 2001 to 1622.5 Kg in 2006. It is

not clear how much of the anaesthetics is used in the marine environment or in open systems where the product could reasonable be expected to enter the environment.

Table 6.1. Anaesthetics used in the Norwegian aquaculture industry and quantities used. Source Jon Arne Grottum.

Compound	2002	2003	2004	2005	2006
Benzocaine	500	500	500	400	400
MS-222®	675	699	737	960	1216
Isoeugenol	0	0	0	0	6.5

In Chile Benzocaine (ethyl-aminobenzoate), Isoeugenol (Aqui-S®) and tricaine methyl sulphonate (TMS, trade name MS-222®) are licensed for use (Bravo et al. 2005).

In the UK, benzocaine, 2-phenoxyethanol, TMS and 2-propanone are registered for use. Scotland requires yearly reporting of quantities of anaesthetics for salmon aquaculture. The products and quantities used are listed in Table 6.2.

Table 6.2 Anaesthetics and quantities used in Scotland from 2003 to 2005.

Compound	2003	2004	2005	2006
TMS	9.4	22.8	22.7	33.4
Benzocaine	25.6	25.4	11.9	5.5
2-propanone	98.5	374.3	179.8	120
2-propanol	30	25	2	0
Phenoxy ethanol	28	5.9	7.2	0

In Canada only TMS and metomidate are approved as anaesthetics for fish.

6.3 Properties of anaesthetics

6.3.1 Tricaine methyl sulphonate (TMS, MS-222®)

TMS is a highly water-soluble compound (110 $g \cdot L^{-1}$) that is easily absorbed through the gills because of its lipophilic character. It acts by interfering with the nerve synapses. It is easily absorbed through the gills and is distributed in the central nervous system and in ventricular tissue, decreasing cardiovascular function and thereby reducing blood flow to the gills and oxygen consumption (EMEA, 1999c). It is metabolized mainly in the liver and to a lower extent in the kidneys, blood and muscles.

It becomes toxic by prolonged exposure to sunlight. It can cause adverse effects such as hypoxia, hypercapnia, hyperglycemia, and increased lactate concentration in the blood.

6.3.2 Benzocaine

Ethyl aminobenzoate is soluble in ethanol, acetone and ethyleneglycol, and has a water solubility of 800 $mg \cdot L^{-1}$. It has a low octanol water partition coefficient indicating it is unlikely to accumulate in aquatic biota. Once administered it is absorbed rapidly, and is rapidly distributed. It is eliminated through the gills and the urinary tract (Stehly et al., 2000) and tissue concentrations are reduced to pre-treatment levels within 4 h in rainbow trout (Allen 1988). Exposure for 25 min at a concentration of 30 $mg \cdot L^{-1}$ can cause the death of rainbow trout (Burka et al., 1997). Benzocaine is the most widely used anaesthetic in Chile with a market share of 77.6 % between 1999 and 2003.

6.3.3 Isoeugenol

Isoeugenol (Aqui-S®) is registered for use in Chile but not elsewhere. Isoeugenol is slightly soluble in water and is usually mixed with a solvent prior to addition to water. The recommended concentration for sedation is 40-100 $mg \cdot L^{-1}$. The product does not accumulate in fish and the manufacturer advertises the product as a zero withdrawal time product.

Isoeugenol is also the major constituent in clove oil and in many jurisdictions aquaculturists say clove oil is very good anaesthetic. The National Toxicology Program (NTP) in the United States investigated the toxicity of isoeugenol as well as eugenol and methyleugenol, minor constituents in clove oil.

The results of these studies have determined that eugenol is an equivocal carcinogen and that methyleugenol is carcinogenic in the rodent model. The contamination of clove oil with methyleugenol and/or eugenol raises the level of concern for human safety. The Veterinary Drug Directorate has followed thelead of the US Food and Drug Administration has not approved the sale of clove oil (USFDA 2007, Ken Rowes personal communication).

6.3.4 Metomidate

Metomidate is used frequently in human medicine but rarely in aquaculture. It is said to be effective in anaesthetising salmonids, particularly larger fish in seawater. The recommended dose ranges from 1.0 to 10 $mg \cdot L^{-1}$.

6.4 Conclusion

The use of disinfectants and anaesthetics are generally considered to be of little risk to the environment. It is likely that most of the anaesthetic used in aquaculture is used in freshwater and in transport of fish.

6.5 Research Gaps

- *There are very little available data regarding the use patterns of anaesthetics in salmon aquaculture. Collection and analysis of these data may help determine if more studies are required to determine if any products pose a risk to aquatic biota.*

6.6 Recommendation

- *That regulatory agencies in all jurisdictions require yearly reporting of the quantities of anaesthetics used by salmon farms and that these data be made available to the public.*

Chapter 7

References

Ahsanullah, M. and A.R.Williams 1991. Sublethal effects and bioaccumulation of cadmium, chromium, copper, and zinc in the marine amphipod *Allorchestes compressa*. Mar. Biol. 108: 59-65.

Alcaide, E., M.-D.Blasco and C. Esteve 2005. Occurrence of drug-resistant bacteria in two European eel farms. Appl Environ Microbiol **71:** 3348-3350.

Alderman, D.J. and T.S. Hastings, 1998. Antibiotic use in aquaculture:
development of antibiotic resistance – potential for consumer health risks. Int. J. Food Sci. Technol. 33: 139-155.

Allen, J.L 1988. Residues of benzocaine in rainbow trout, largemouth bass and fish meal. Prog. Fish-Culturist 50: 59-60

Anderson, B.S., J.W. Hunt, W.J. Piekarski, B.M. Phillips, M.A. Englund, R.S. Tjeerdema and J.D. Goetzl 1995. Influence of salinity on copper and azide toxicity to larval topsmelt *Atherinops affinis* (Ayres). Arch. Environ. Contam. Toxicol. 29: 366-372.

Anderson, J.A. 1992. Allergic reactions to drugs and biological agents. J. Am. Med. Assoc. 268:.2844-2857.

Anderson, A.D., J.M. Nelson, S. Rossiter and F.J. Angulo 2003. Public health consequences of use of antimicrobial agents in food animals in the United States. Microb. Drug Resist 9: 373-379.

Anderson, W.C., S.B. Turnipseed and J.E. Roybal 2005. Quantitative and confirmatory analyses of malachite green and leucomalachite gree residues in fish and shrimp. USFDA Laboratory Information Bulletin. LIB No. 4363 Vol 21 (11).

Andersson, S. and L. Kautsky 1996. Copper effects on reproductive stages of Baltic Sea *Fucus vesiculosis*. Mar. Biol. 125: 171-176.

Angulo, F.J., V.N. Nargund and T.C.Chiller, 2004. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J. Vet. Med. 51: 374-379.

Angulo, F.J. 2000. Antimicrobial agents in aquaculture: potential impact on health. APUA Newsletter. 18: 1-6.

Armstrong, SM, BT Hargrave and K Haya 2005 Antibiotic use in finfish aquaculture: Modes of action, environmental fate and microbial resistance. In: Handbook of Environmental Chemistry.Vol 5 Part M (BT Hargrave ed.) 341-357.

Arnott, G.H. and M Ahsanullah 1979. Acute toxicity of copper, cadmium and zinc to three species of marine copepods. Austr. J. Mar. Freshwat. Res. 30: 63 – 71.

Austin, B. and D.A..Austin, (Eds.) 1999. *Bacterial Fish Pathogens. Disease of Farmed and Wild Fish*. 3rd Edition, Springer-Praxis Publishing, United Kingdom.

Barton, B.A. and G.K. Iwama 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis. **1**: 3-26.

Bechmann, R.K. 1994. Use of life tables and LC50 tests to evaluate chronic and acute toxicity effects of copper on the marine copepod *Tisbe furcata* (Baird). Environ. Toxic. Chem.. 13: 1509-1517.

Bejarano, A.C. and G.T. Chandler 2003. Reproductive and developmental effects of atrazine on the estuarine meiobenthic copepod *Amphiascus tenuiremus*. Environ. Toxicol. Chem. 22: 3009-3016.

Bellas, J. 2005. Toxicity assessment of the antifouling compound zinc pyrithione using early developmental stages of the ascidian *Ciona intestinalis*. Biofouling, 21: 289-296.

Bellas, J. 2006. Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates. Sci. Total Environ. 367: 573-585.

Bellas, J., E. Vasquez and R. Beiras 2001. Toxicity of Hg, Cu, Cd, and Cr on early developmental stages of Ciona intestinalis (Chordata, Ascidiacea) with potential application in marine water quality assessment. Water Res. 35: 2905-2912.

Benbrook C. M. 2002. Antibiotic drug use in U. S. aquaculture. The Northwest Science and Environmental Policy Center. Sand Point. Idaho. U.S. A.

Beveridge, M. 2004. *Cage Aquaculture*. 3rd. Edition. Ames. Iowa: Blackwell Publishing.

BfR –Federal Institute for Risk Assessment. 2007. Collection and pre-selection of available data to be used for the risk assessment of malachite green residues by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). BfR Expert Opinion No. 036/2007.

BfR- Federal Institute for Risk Assessment. 2007a. Malachite green identified as an environmental contaminant. www.bfr.bund.de/cd/10136 Accessed Nov 18, 2007

Bjorlund, H., J. Bondestamm J. and G. Bylund 1990. Residues of oxytetracycline in wild fish and sediments from fish farms. Aquaculture 86: 359-67.

Black, K.D. 2001. *Environmental Impacts of Aquaculture*. United Kingdom: Sheffield Academic Press.

Borgmann, U., W.P. Norwood and C. Clarke 1993. Accumulation, regulation and toxicity of copper, zinc, lead, and mercury in *Hyalella azteca*. Hydrobiologia 259: 79-89.

Borgmann, U., M. Nowierski, L.C. Grapentine and D.G. Dixon 2004. Assessing the cause of impacts on benthic organisms near Rouyn-Noranda, Quebec. Environ. Pollut. 129: 39-48.

Boxall, A.B., L.A. Fogg, P.A.Blackwell, P. Kay, E.J. Pemberton, and A. Croxford 2004. Veterinary medicines in the environment. Rev. Environ. Contam. Toxicol. 180: 1-91.

Braithwaite, R.A., M.C.Cadavid Carrascosa and L.A. McEvoy 2007. Biofouling of salmon cage netting and the efficacy of a typical copper-based antifoulant. Aquaculture 262: 219-226.

Branson, E.J., and D. Diaz-Munoz 1991. Description of a new disease condition occurring in farmed coho salmon, *Oncorhynchus kisutch* (Walbaum), in South America. J. Fish Dis. 14: 147-156.

Branson, E., S. Ronsberg and G. Ritchie 2000. Efficacy of teflubenzuron (Calicide ®) for the treatment of sea lice, *Lepeophtheirus salmonis* (Kroyer 1838), infestations of farmed Atlantic salmon (*Salmo salar* L.). Aqua. Res. 31 (11): 861-867.

Bravo, S., H. Dolz, M.T. Silva, C. Lagos, A. Millanao, and M. Urbina 2005. Informe Final. Diagnostico del uso de fármacos y otros productos químicos en la acuicultura. Universidad Austral de Chile. Facultad de Pesquerias y Oceansgrafia, Instituto de Acuicultura. Casilla 1327. Puerto Montt, Chile. Proyecto No. 2003-28.

Bravo, S. and P.J. Midtlyng 2007. The use of vaccines in the Chilean salmon industry 1999-2003. Aquaculture 270: 36-42.

Brocklebank, J.R., T.P.T. Evelyn, D.J. Speare and R.D. Armstrong 1993. Ricketsial l septicemia in farmed Atlantic and Chinook salmon in British Columbia: Clinical presentation and experimental transmission. Can. Vet. J. 34: 745-748.

Brooks, K.M. 2000. Determination of copper loss rates from Flexgard XI^{TM} treated nets in marine environments and evaluation of the resulting environmental risks. Report to the Ministry of Environment for the BC Salmon Farmers Association, 1200 West Pender St. Vancouver BC, 24 pp.

Brooks, K.M. and C.V. Mahnken 2003. Interactions of Atlantic salmon in the Pacific Northwest environment III Accumulation of zinc and copper. Fisheries Res. 62: 295-305.

Brooks, K.M., A.R. Stierns, C.V. Mahnken and D.B. Blackburn 2003. Chemical and biological remediation of the benthos near Atlantic salmon farms. Aquaculture 219: 355-377.

Bruno DW and R.S. Raynard 1994. Studies on the use of hydrogen peroxide as a method for the control of sea lice on Atlantic salmon. Aquaculture International 2:.10-8.

Burka, J.G., K.L. Hammell, T.E. Horsberg, G.R.Johnson, D.J. Rainnie and D.J. Speare 1997. Drugs in salmonid aquaculture - A Review. J. Vet. Pharmacol. Therap. **20:** 333-349.

Burridge, L. and C.L. Chou 2005. Copper in Atlantic salmon tissues collected from Heritage Salmon hatchery and net pen operations. Unpublished paper. 6 pp.

Burridge, L.E., K. Doe, K. Haya, P.M. Jackman, G. Lindsay and V. Zitko 1999a. Chemical analysis and toxicity tests on sediments under salmon net pens in the Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 2291: iii + 39 pp.

Burridge, L.E. and V. Zitko 2002. Lethality of copper sulfate and copper-treated nets to juvenile haddock, *Melanogrammus aeglefinus* L. Bull. Environ. Contam. Toxicol. 69: 378-383.

Burridge L.E. Chemical use in marine finfish aquaculture in Canada: A review of current practices and possible environmental effects. Canadian Technical Report of Fisheries and Aquatic Sciences 2450[ix + 131pp]. 2003.

Burridge, L.E., K. Haya, F.H. Page, S.L. Waddy, V. Zitko and J. Wade. 2000c. The lethality of cypermethrin formulation Excis to larval and post-larval stages of the American lobster (Homarus americanus). Aquaculture 182: 37-47.

Burridge, L.E., M.N. Hamilton, S.L. Waddy, K. Haya, S.M. Mercer, R. Greenhalgh, R. Tauber, S.V. Radecki, L.S. Crouch, P.G. Wislocki and R.G. Endris 2004. Acute toxicity of emamectin benzoate in fish feed to American lobster, *Homarus americanus* Aquaculture Res. 35(8): 713-722

Bushman, A. and R. Pizarro 2001. El costo ambiental de la salmonicultura en Chile. Terram Publicaciones. Analisis de Politicas Publicas – No. 5

Bushman, A. 2001. Impactos de la acuicultura: El estado del conocimiento en Chile y el mundo. Terram Publicaciones, Santiago, Chile

Bushman, F. 2002b. Phage transduction and bacterial pathogenesis. In *Lateral DNA Transfer. Mechanisms and Consequences*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, pp. 73-128.

Buschmann, A.H., V.A. Riquelme, M.C. Hernández-González, D. Varela, J.E. Jiménez, L.A. Henríquez, P.A. Vergara, R. Guíñez and Filún, L. 2006a. A review of the impacts of salmon farming on marine coastal ecosystems in the southeast Pacific. ICES J. Marine Sci. **63:** 1338-1345.

Buschmann, A.H., V.A. Riquelme, C. Hernández-González and L.A. Henríquez. 2006. The Role of Aquaculture in Integrated Coastal and Ocean Management: An Ecosystem Approach. In: McVey J, Lee, C.-S., and O'Bryen, P.J., ed.) The World Aquaculture Society, USA, Louisiana, Baton Rouge (in press).

Cabello, F.C. 2003. Antibiotics and aquaculture. An analysis of their potential impact upon the environment, human and animal health in Chile. Fundacion Terram. (www.terram.cl/index). Analisis de Politicas Publicas No. 17, pp. 1-16.

Cabello, F.C. 2004. Antibiotics and aquaculture in Chile: Implications for human and animal health. Rev. Med. Chile 132: 1001-1006.

Cabello, F.C. 2005. Enfermedades originadas en el mar. Síntomas del deterioro de la diversidad marina en la X Region. Ambiente y Desarrollo. 21: 80-87.

Cabello, F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ. Microbiol. **8:** 1137-44.

Campbell, A. Migratory movements of ovigerous lobsters, *Homarus americanus*, tagged off Grand Manan , eastern Canada. Can. J. Fish. Aquat. Sci. 1986, 43, 2197-2205.

Campbell, W. C. 1989. *Ivermectin and Abamectin*; Springer Verlag: New York.

Canadian Council on Animal Care 2005. Guidelines on the care and use of fish in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON. 86pp.

Carvajal, V., D.J. Speare and B.S. Horney 2000. Culture method influences the degree of growth rate reduction in rainbow trout following exposure to hydrogen peroxide. J. Aqua. Animal Health 12: 146-148.

Chou, C.L., K. Haya, L.A. Paon, L. Burridge and J.D. Moffatt 2002. Aquaculture-related trace metals in sediments and lobsters and relevance to environmental monitoring program ratings for near-field effects. Mar. Poll. Bull. 44: 1259-1268.

Chou, C.L. 2007 A time series of mercury accumulation and improvement of dietary feed in net caged Atlantic salmon (*Salmo salar*). Mar. Poll. Bull. 54: 720-725.

Christensen, A.M., Ingersley, F., and Baun, A. 2006. Ecotoxicity of mixtures of antibiotics use in aquacultures. Environ. Toxicol. Chem. 25: 2208-2215.

Cid, A., C. Herrero, E. Torres, J. Abalde 1995. Copper toxicity on the marine microalga *Phaeodactylum tricornutum:* effects on photosynthesis and related parameters. Aquat. Toxicol. 31: 165-174.

Clark, J. R., L.R. Giidman, P. W. Borthwick, J.M. Parick, Jr., G. M.Cripe, P. M. Moody, J.C. Moore and E.M. Lores 1989. Toxicity of pyrethroids to marine invertebrates and fish: A literature review and test results with sediment-sorbed chemicals. Environ. Toxicol. Chem. 8: 393-401.

Clark, J.R., J.M. Patrick, Jr., J.C. Moore and E.M. Lores 1987. Waterborne and sediment-source toxicities of six organic chemicals to grass shrimp (*Palaemonetes pugio*) and amphioxus (*Branchiostoma caribaeum*). Arch. Environ. Contam. Toxicol. Vol. 16 (4): 401-407.

Coglianese, M.P. and M. Martin 1981. Individual and interactive effects of environmental stress on the embryonic development of the Pacific oyster *Crassostrea gigas*. I: The toxicity of copper and silver. Mar. Environ. Res. 5: 13-27.

Contreras, L., M.H. Medina, S. Andrade, V. Opplinger and J.A. Correa 2007. Effects of copper on early developmental stages of *Lessonia nigrescens* Bory (Phaeophyceae). Environ. Poll. 145: 75-83.

Correia, A.D. and M.H. Costa 2000. Effects of sediment geochemical properties on the toxicity of copper-spiked sediments to the marine amphipod *Gammarus locusta*. Sci. Total Environ. 247: 99-106.

Costello, M.J., A. Grant, I.M. Davies, S. Cecchini, S. Papoutsoglou, D. Quigley and M. Saroglia 2001. The control of chemicals used in aquaculture in Europe. J. Appl. Ichthyol. 17: 173-180.

Cotran, R. S., V. Kumar and S.L. Robbins 1989. *Pathological Basis of Disease*; 4 ed.; Saunders: Toronto.

Coyne, R., M. Hiney and P. Smith 1997. Transient presence of oxytetracycline in blue mussels (*Mytilus edulis*) following its therapeutic use at a marine Atlantic salmon farm. Aquaculture **149:** 175-181.

Dahl, B. and H. Blanck 1996. Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosms. Mar. Poll. Bull. 32: 342-350.

Davenport, J., K. Back, G. Burnell, T. Cross, S. Culloty, S. Ekaratne, B. Furness, M. Mulcahy and H. Thetmeyer 2003. *Aquaculture. The Ecological Issues*. Berlin, Germany: Blackwell Science Ltd.

Davis JH. 1985. The pyrethroids: An historical introduction. In: Leahey JP, editor. *The Pyrethroid Insecticides*. London and Philadelphia: Taylor and Francis Ltd.; p 1-41.

Davison, J. 1999. Genetic exchange between bacteria in the environment. Plasmid **42:** 73-91.

Dean, R.J., T.M. Shimmield and K.D. Black 2007. Copper, zinc and cadmium in marine cage fish farm sediments: an extensive survey. Environ. Pollut. 145: 84-95.

Debourg, C., A. Johnson, C. Lye, L. Tornqvist and C Unger 1993. Antifouling products pleasure boats, commencial vessels, net, fish cages, and other underwater equipment. KEM Report No 2/93. The Swedish National Chemicals Inspectorate. 58 pp.

DeBruyn, A., M. Trudel, N. Eyding, J. Harding, H. McNally, R. Mountain, C. Orr, D. Urban, S. Verenitch, and A. Mazumder 2006. Ecosystemic effects of salmon farming increase mercury contamination in wild fish. Environ. Sci. Tech. 40 (11): 3489-3493

Ecoceanos. 2006Detectan residuos de antibióticos en envío de salmón chileno a Estados Unidos. URL. http://www.ecoceanos.cl

Eisler R. 1992. Diflubenzuron hazards to fish, wildlife, and invertebrates: A synoptic review. US Fish and Wildlife Service, Contaminant Hazard Reviews 25(4): 36pp.

Eithun I. 2004. Measures to control sea lice in Norwegian fish farms. Caligus 6: 4-5.

Ellenberger, S.A., P.C. Baumann and T.W. May 1994. Evaluation of effects caused by high copper concentrations in Torch Lake, Michigan, on reproduction in yellow perch. J. Great Lakes Res. 20: 531-536.

Ernst, W., P. Jackman, K. Doe, F. Page, G. Julien, K. Mackay and T. Sutherland 2001. Dispersion and toxicity to non-target aquatic organisms of pesticides used to treat sea lice on salmon in net pen enclosures. Mar. Poll. Bull. 42, 433-444.

European Agency for the Evaluation of Medicinal Products (EMEA) 1999 Committee for Veterinary mMedicianl Products, Tricaine mesilate Summary Report. EMEA/MRL/586/99-final. 4 pp.

European Commission http://EC.europa.eu/research/leaflets/antibiotics/page_28_en.html Accessed January 30, 2008.

FAO: Food and Agriculture Organization of the United Nations 2007. FishStat - Fishery Information, Data and Statistics Unit. ROME: FAO.

Fernandez, N. and R. Beiras 2001. Combined toxicity of dissolved mercury with copper, lead, and cadmium on embryogenesis and early larval growth of the *Paracentrotus lividus* sea urchin. Ecotoxicology 10: 263-271.

Fish farming expert. www.fishfarmignxpert.no/index.php?page_id=37&article_id=78056. Accessed January 30, 2008

Fortt, A., F.C. Cabello and A. Buschmann 2007. Residues of tetracycline and quinolone in wild fish living around a salmon aquaculture center in Chile. Rev. Chil. Infectologia. 24:8-12.

Franklin, N.M., J. Stauber and R. Lim 2001. Development of flow cytometry-based algal bioassays for assessing toxicity of copper in natural waters. Environ. Toxicol. Chem. 20: 160-170.

Fryer, J.L. and R.P. Hedrick 2003. *Piscirickettsia salmonis*: a Gram-negative intracellular bacterial pathogen of fish. J Fish Dis **26:** 251-262.

Furushita, M., T.Maeda, H. Akagi, M. Ohta and T. Shiba 2005. Analysis of plasmids that can transfer antibiotic resistance genes from fish farm bacteria to clinical bacteria. In *Joint Meeting of the 3 Divisions of the International Union of Microbiological Societies 2005. International Congress of Bacteriology and Applied Microbiology*. B-1162, pp. 52-53.

Gaggero, A., H. Castro and A.M. Sandino 1995. First isolation of *Piscirickettsia salmonis* from coho salmon, *Oncorhynchus kisutch* (Walbaum), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), during the freshwater stage of their life cycle. J Fish Dis **18:** 277-279.

Garnacho, E., L.S. Peck and P.A. Tyler 2000. Variations between winter and summer in the toxicity of copper to a population of the mysid *Praunus flexuosus*. Mar. Biol. 137: 631-636.

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*. Rep. Stud. GESAMP No. 65, Rome, Italy. 40 p.

Gobierno de Chile. Servicio Nacional de Pesca. 2005. Programa de Control de Fármacos. Programa de Control de Residuos.

Goldburg, R.J., M.S. Elliott, and R.L. Naylor 2001. Marine Aquaculture in the United States: Environmental Impacts and Policy Options. PEW Oceans Commission, Arlington, Virginia.

Gorbach, S.L. 2001. Antimicrobial use in animal feed – Time to stop. N Engl J Med **345:** 1202-1203.

Gowland, B. T. G., C.F. Moffat, R.M. Stagg, D.F. Houlihan and I.M. Davies 2002. Cypermethrin induces glutathione S-transferase activity in shore crabs, Carcinus maenus. Mar.ne Envion. Res. 54: 169-177.

Grant, A.N. 2002. Medicines for sea lice. Pest. Manag. Sci. 58: 521-527.

Grave, K., E. Lingaas, M. Bangen and M. Rønning 1999. Surveillance of the overall consumption of antibacterial drugs in humans, domestic animals and farmed fish in Norway in 1992 and 1996. J Antimicrob Chemother **43:** 243-252.

Grave, K., A. Markestad and M. Bangen 1996. Comparison in prescribing patterns of antibacterial drugs in salmonid farming in Norway during the periods 1980-1988 and 1989-1994. J Vet Pharmacol Therap **19:** 184-191.

Greenlees, K.J. (2003) Animal drug human food safety toxicology and antimicrobial resistance – The square peg. *Int J Toxicol* **22:** 131-134.

Grosell, M., J. Blanchard, K.V. Brix and R. Gerdes 2007. Physiology is pivotal for interaction between salinity and acute coper toxicity to fish and invertebrates. Aquatic Toxicol. 84: 162-172.

Guardabassi, L., A. Dalsgaard, M. Raffatellu and J.E.Olsen 2000. Increase in the prevalence of oxolinic acid resistant *Acinetobacter* spp. observed in a stream receiving the effluent from a freshwater trout farm following the treatment with oxolinic acid-medicated feed. Aquaculture **188:** 205-218.

Haglund, K., A. Pettersson, M. Peterson, H. Kylin, S.C. Lerd and P. Dollenmeier 2001. Seasonal distribution of the anti-fouling compound Irgarol 1051 outside a marine in the Stockholm Archipelago. Bull. Environ. Contam. Toxicol. 66: 50-58.

Hansen, P.K., B.T. Lunestad and O.B. Samuelsen 1992. Effects of oxytetracycline, oxolinic acid, and flumequine on bacteria in an artificial fish farm sediment. Can J Microbiol **39:** 1307-1312.

Hansen, D.J., W.J. Berry, J.D. Mahoney, W.S. Boothman, D.M. Di Toro, D.L. Robson, G.T. Ankley, D. Ma, Q. Yan and C.E. Pasch 1996. Predicting the toxicity of metal-contaminated field sediments using interstitial concentration of metals and acid-volatile sulfide normalizations. Envir. Toxicol. Chem 15: 2080-2094.

Hansen, J.I., T. Mustafa and M. Depledge 1992. Mechanisms of copper toxicity in the shore crab, *Carcinus maenas*. Mar. Biol. 114: 259-264.

Harmon, V.L. and C.J. Langdon 1996. A 7-day toxicity test for marine pollutants using the Pacific mysid *Mysidopsis intii*. 2. Protocol Evaluation. Environ. Toxicol. Chem. 15: 1824-1830.

Harrison, P.F. and J. Lederberg, J. 1998. *Antimicrobial Resistance: Issues and Options. Workshop Report*. National Academy Press, Washington, DC.

Hastings, P.J., S.M. Rosenberg and A. Slack 2004. Antibiotic-induced lateral transfer of antibiotic resistance. Trends Microbiol **12:** 401-404.

Haya, K. 1989. Toxicity of pyrethroids insecticides to fish. Environ. Toxicol. Chem. 8: 381-391.

Haya, K., L.E. Burridge and B.D. Chang 2000. Environmental impact of chemical wastes produced by the salmon aquaculture industry. ICES J Marine Sci **58:** 492-496

Haya, K, L.E. Burridge, I.M. Davies and A. Ervik 2005. A review and assessment of environmental risk of chemicals used for the treatment of sea lice infestations of cultured salmon. In: Hargrave B. (Ed) Handbook of Environmental Chemistry Volume 5: Water Pollution, Part M 305-341.

ICES (2006). Report of the working group on North Atlantic salmon. ICES CM 2006/ACFM:23. 254 pp.

Johnson S.C., J.M.Constible and J. Richard 1993. Laboratory investigations of the efficacy of hydrogen peroxide against the salmon louse, *Lepeophtheirus salmonis*, and it's toxicological and histopathological effects on Atlantic salmon, *Salmo salar*, and chinook salmon, *Oncorhynchus tshowytscha*. Dis. Aquatic Organ. 17: 197-204.

Hektoen, H., J.A. Berge, V. Hormazabal and M. Yndestad 1995. Persistenceof antibacterial agents in marine sediments. Aquaculture **133:** 175-184.

Hernandez C, J. Ulloa , J. A.Vergara, R. Espejo and F. Cabello 2005. *Vibrio parahaemolyticus* infections and algal intoxications as emergent public health problems in Chile. Rev Med Chile **133:** 1081–1088.

Hernando, M.D., L. Piedra, A. Belmonte, A. Aguera, and A.R. Fernandez-Alba 2001. Determination of traces of five anti-fouling agents in water by gas chromatography with positive/negative chemical ionization and tendem mass spectrometric detection. J. Chromatog. A 938: 103-111.

Hill I. 1985. Effects on non-target organisms in terrestrial and aquatic environments. In: Leahey JP, editor. *The Pyrethroid Insecticides*. London, UK: Taylor and Francis; p 151-262.

Hodneland K, A. Nylund, F. Nisen, B. Midttun 1993. The effect of Nuvon, azamethiphos and hydrogen peroxide on salmon lice, *Lepeophtheirus salmonis*. Bull. Eur. Assoc. of Fish Path. 123: 203-206.

http://www.syndel.com/biosecurity/Univ%20Idaho%20Virkon%20S%20toxicity%20studies.pdf Accessed April 19, 2007

Holten Lützhøft, H.-C., B. Halling-Sørensen and S.E. Jørgensen 1999. Algal toxicity of antibacterial agents applied in Danish fish farming. Arch. Environ. Contam. Toxicol. 36**:** 1-6.

Hornberger, M.I., S.N. Luoma, D.J. Cain, F. Parchaso, C.L. Brown, R.M. Bouse, C. Wellise and J.K. Thompson 2000. Linkage of bioaccumulation and biological effects to changes in pollutant loads in south San Francisco Bay. Environ. Sci. Tech. 34: 2401-2409.

Horst, M. N. and A. N. Walker 1995. Biochemical effects of diflubenzuron on chitin synthesis in the postmolt blue crab Callinectes sapidus. J. Crust. Biol. 15: 401-408.

Hunter-Cevera, J., D. Karl, and M. Buckley 2005. Marine microbial diversity: The key to earth's habitability. (A report from The American Academy of Microbiology) Colloquium held April 8-10, 2005, San Francisco, CA, USA: *Marine Microbial Diversity*. Collier, R.J. et al. (eds.). Washington, DC: American Academy of Microbiology, pp. 1-22.

Husevåg, B., B.T. Lunestad, P.J. Johannessen, O. Engerm and O.B. Samuelsen 1991. Simultaneous occurrence of *Vibrio salmonicida* and antibiotic-resistant bacteria in sediments at abandoned aquaculture sites. J. Fish Dis. 14**:** 631-40.

Huys, G., G. Rhodes, P. McGann, R. Denys, R. Pickup and M. Hiney 2000. Characterization of oxytetracycline-resistant heterotrophic bacteria originating from hospital and freshwater fishfarm environments in England and Ireland. Syst. Appl. Microbiol. 23**:** 599-606.

Jonas, R.B. 1989. Acute copper and cupric ion toxicity in an estuarine microbial community. Appl. Environ. Microbiol. 55: 43-49.

Jones, M. W., C. Sommerville, and R. Wootten 1992. Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. J. Fish Dis. 15: 197-202.

Kahn, N. Y. 1983 In: *Pesticide Chemistry: Human Welfare and the Environment*. Proceedings of the Fith International Congress of Pesticide Chemistry, Kyoto, Japan, 1982.; Miyamoto, J. and P.C. Kearney, (Eds). Permagon Press: Oxford,; pp 437-450.

Karlsson, J., M. Breitholtz and B. Eklund 2006. A practical ranking system to compare toxicity of anti-fouling paints. Mar. Poll. Bull. 52 (12): 1661-1667.

Katranitsas, A., J. Castritsi-Catharios and G. Persoone 2003. The effects of a copper-based antifouling paint on mortality and enzymatic activity of a non-target marine organism. Mar. Poll. Bull. 46: 1491-1494.

Kerry, J., R. Coyne, D. Gilroy, M. Hiney and P. Smith 1996. Spatial distribution of oxytetracycline and elevated frequencies of oxytetracycline resistance in sediments beneath a marine salmon farm following oxytetracycline therapy. Aquaculture **145:** 31-39.

Kiemer, M. C. B. and K.D. Black 1997. Effects of hydrogen peroxide on gill tissues of Atlantic salmon, *Salmo salar* L. Aquaculture 153: 181-189.

Kim, S.D., H. Ma, H.E. Allen, and D.K. Cha 1999. Influence of dissolved organic matter on the toxicity of copper to *Ceriodaphnia dubia*: effect of complexation. Environ. Toxicol. Chem 18: 2433-2437.

King, C.K. 2001. Effects of metal contaminants on the development of the common Antarctic sea urchin *Sterechinus neumayeri* and comparisons of sensitivity with tropical and temperate echinoids. Mar. Ecol. Prog. Ser. 215: 143-154

Klerks, P. and J.S. Weis 1987. Genetic adaptation to heavy metals in aquatic organisms: A review. Environ. Poll. 45: 173-206.

Knezovich, J.P., F.L. Harrison and J.S. Tucker 1981. The influence of organic chelators on the toxicity of copper to embryos of the Pacific oyster, *Crassostrea gigas*. Arch. Environ. Contam. Toxicol. 10: 241-249.
Kobayashi N. and H. Okamura 2002. Effects of new antifouling compounds on the development of sea urchin. Mar. Pollut. Bull. 44: 748-751.

Konstantinou, I.K. and T.A. Albanis 2004. Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: AQ review. Environ. Internat. 30: 235-248.

Kruse, H., and H. Sorum 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. Appl. Environ. Microbiol. **60:** 4015-4021.

L'Abee-Lund, T.M., and H. Sørum 2001. Class 1 integrons mediate antibiotic resistance in the fish pathogen *Aeromonas salmonicida* worldwide. Microb. Drug Resist. **7:** 263-272.

Lage, O., F. Sansonetty, J.-E. O'Conner and A.M. Parente 2001. Flow cytometric analysis of chronic and acute toxicity of copper (II) on the marine dinoflagellate *Amphidinium carterae.* Cytometry 44: 226-235.

Lang, W.H., R.B. Forward, D.C. Miller and M. Marcy 1980. Acute toxicity and sublethal behavioral effects of copper on barnacle nauplii (*Balanus improvisus*). Mar. Biol. 58: 139-145.

Lees, F., M. Baillie, G. Gettinby and C.W. Revie 2008. The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L) in Scotland, 2002-2006. Open Access article published on line: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0001549. Accessed Feb 22, 2008.

Le Jeune, A.H., M. Charpin, J.F. Briand, J.F. Lenain, M. Baudu and C. Amblard 2006. Effect of copper sulphate treatment on natural phytoplanktonic communities. Aquat. Toxicol. 80: 267-280.

Levy, S.B. 2001. Antibiotic resistance: Consequences of inaction. Clin. Infect. Dis. 33(Suppl. 3)**:** S124-S129.

Lewis, A.G. and A. Metaxas 1991. Concentrations of total dissolved copper in and near a copper-treated salmon net pen. Aquaculture 99: 267-276.

Li, X.-Z. 2005. Quinolone resistance in bacteria: emphasis on plasmid-mediated mechanisms. Int. J. Antimicrob. Agents 25**:** 453-463.

Lillehaug, A., B.T. Lunestad and K. Grave 2003. Epidemiology of bacterial diseases in Norwegian aquaculture – a description based on antibiotic prescription data for the ten-year period 1991 to 2000. Dis. Aquat. Org. 53**:** 115-125.

Linssen M.R., G.C. van Aggelen, R. Endris 2002. Toxicity of emamectin benzoate in fish feed to adults of the spot prawn and dungeness crab. In: Eichkoff C.V., G.C. van Aggelen and A. Niimi (eds). Proceedings of the 29th Annual Aquatic Toxicity Workshop: Oct. 21-23, 2002, Whistler, British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences 2438 (abstract).

Lorentzen, M. and A. Maage 1999. Trace element status of juvenile Atlantic salmon *Salmo salar* L. fed a fish-meal based diet with or without supplementation of zinc, iron, manganese, and copper from first feeding. Aquacult. Nutr. 5: 163-171.

MacKinnon, B. M. 1997. Sea lice: A review. World Aquaculture, 28: 5-10.

Madsen, S.S., A.B. Mathiesen and B. Korsgaard. 1997. Effects of 17 -estradioal and 4-nonylphenol on smoltification and vitellogenesis in Atlantic salmon (Salmo salar). Fish Physiol. Biochem. 17: 303-312

Markestad, A., and K. Grave 1997. Reduction of antibacterial drug use in Norwegian fish farming due to vaccination. Fish Vaccinol**.** 90: 365-369.

Martin, M., A.R. Coulon, S.L. Turpen, J.W. Hunt and B.S. Anderson 1990. Copper toxicity to microscopic stages of giant kelp, *Macrocystis pyrifera*: Interpopulation comparisons and temporal variability. Mar. Ecol. Prog. Ser. 68: 147-156.

Martinez, M., M.A. Mondaca and R. Zemelman 1994. Antibiotic-resistant gram-negative bacilli in the sewage of the City of Concepcion, Chile. Rev. Latinoam Microbiol. 36: 39-46.

Mauel, M.J. and D.L. Miller 2002. Piscirickettsiosis and piscirickettsiosis-like infections in fish: a review. Vet. Microbiol. 87: 279-289

McDermott, P.F., S. Zhao, D.D. Wagner, S. Simjee, R.D. Walker and D.G. White, 2002. The food safety perspective of antibiotic resistance. Animal Biotechnol 13: 71-84.

McDonnell, G. and A.D. Russell 1999. Antiseptics and disinfectants: Activity, action, and resistance. Clin. Microbiol. Rev. 12(1): 147-179.

McEwen, S.A. and P.J. Fedorka-Cray 2002. Antimicrobial use and resistance in animals. Clin. Infect. Dis. 34 (Suppl. 3)**:** S93-S106.

McLeese, D. W., C.D. Mecalfe and V. Zitko 1980. Lethality of permethrin, cypermethrin and fenvalerate to salmon, lobster and shrimp. Bull. Environ. Contam. Toxicol. 25: 950-955.

Medina, M., C. Barata, T. Telfer and D.J. Baird 2002. Age- and Sex-related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod *Acartia tonsa* Dana. Arch. Environ. Contam. Toxicol. 42: 17-22

Medina, M., C. Barata, T. Telfer and D.J. Baird 2004 Effects of cypermethrin on marine plankton communities: a simulated field study using mesocosms. Ecotox. Environ. Saf. 58 (2): 236-245.

Mian L.S. and M.S. Mulla MS. 1992. Effects of pyrethroid insecticides on nontarget invertebrates in aquatic ecosystems. J. Agricult. Entomol. 9:73-98.

Miller T.A. and M.E. Adams 1982. Mode of action of pyrethroids. In: Coats JR, editor. *Insecticide Mode of Action*. New York: Academic Press; p 3-27.

Miranda, C.D. and G. Castillo 1998. Resistance to antibiotic and heavy metals of motile aeromonads from Chilean freshwater. Sci. Total Environ. 224: 167-176.

Miranda, C.D. and R. Zemelman 2001. Antibiotic resistant bacteria in fish from the Concepcion Bay, Chile. Mar. Poll. Bull. **42:** 1096-1102.

Miranda, C.D. and R. Zemelman 2002. Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. Sci. Total Environ. 293**:** 207-218.

Miranda, C.D. and R. Zemelman 2002a Bacterial resistance to oxytetracycline in Chilean salmon farming. Aquaculture 212**:** 31-47.

Miranda, C.D. and R. Zemelman 2002b. Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. Sci. Total Environ. 293: 207-218.

Miranda, C.D., C. Kehrenberg, C. Ulep, S. Schwarz and M.C. Roberts 2003. Diversity of tetracycline resistance genes in bacteria from Chilean salmon farms. Antimicrob. Agents Chemother. 47: 883-888.

Mitchell, A. J. and C.Collins 1997. Review of therapeutant use of hydrogen peroxide in fish production. Aquaculture Magazine, 23(3): 74-79.

Mølbak, K. 2004. Spread of resistant bacteria and resistance genes from animals to humans – The public health consequences. J. Vet. Med B Infect. Dis. Vet. Public Health 51: 364-369.

Montoya, R., M. Dominquez, C. Gonzalez, M.A. Mondaca and R. Zemelman 1992. Susceptibility to antimicrobial agents and plasmid carrying in *Aeromonas hydrophila* isolated from two estuarine systems. Microbios 69: 181-189.

Moore, A. and C.P. Waring 2001. The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). Aquatic Toxicology, 52: 1-12.

Morrisey, D.J., A.J. Underwood and L. Howitt 1996. Effects of copper on the faunas of marine soft sediments: An experimental field study. Mar. Biol. 125: 199-213.

Morrisey D.J., M.M. Gibbs, S.E. Pickmere and R.G. Cole 2000. Predicting impacts and recovery of marine-farm sites in Stewart Island, New Zealand, from the Findlay-Watling model. Aquaculture 185: 257-271.

Muir, D. C. G., G.P. Rawn and N.P. Grift 1985. Fate of pyrethroids insecticide deltamethrin in small ponds: a mass balance study. J. Agric. Food Chem. 33: 603-609.

Muise and Associates Inc. 2001. Phase I: Study on the Potential Scope of Section 36 Fisheries Act Regulations for Aquaculture (Shellfish and Finfish) for the Office of Sustainable Aquaculture, Fisheries and Oceans Canada. June, 2001.

Nash, C.E. 2003. Interactions of Atlantic salmon in the Pacific northwest VI. A synopsis of the risk and uncertainty. Fisheries Res. 62, 339-347

Nash, C.E. 2001 (editor) The Net Pen Salmon Farming Industry in the Pacific Northwest. U.S. Dept. of Commerce, NOAA Tech. Memo. NMFS – NWFSC-49. 125 pp.

Nester, E.W., J.M. Campos, R.J. Collier, M.E. Coyle and J.E. Dahlberg 1999. In: American Academy of Microbiology (1999) Antimicrobial resistance. An ecological perspective. A report i based on an American Academy of Microbiology colloquium held July 16-18, 1999, in San Juan, Puerto Rico. American Society of Microbiology, Washington, DC 14p.

Nordmann, P. and Poirel, L. 2005. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J. Antimicrob. Chemother. 56: 463-469.

O'Brien, T.F. 2002. Emergence, spread, and environmental effect of antimicrobial resistance: How use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clin. Infect. Dis. 34 (Suppl. 3): S578-S584.

Olsen, A.B., H.P. Melby, L. Speilberg and Ø. Evensen 1997. *Piscirickettsia salmonis* infection in Atlantic salmon *Salmo salar* in Norway – epidemiological, pathological and microbiological findings. Dis. Aquat. Org. 31: 35-48.

Pahl, B.C and H.M.Opitz 1999. The effects of cypermethrin (Excis) and azamethiphos (Salmosan) on lobster, *Homarus americanus* H. Milne Edwards, larvae in a laboratory study. Aquacul. Res. 30: 655-665.

Parker, W.R. and J.G. Aube 2002. Metal levels in sediment samples collected under salmon aquaculture pens in the Bay of Fundy, New Brunswick. Environment Canada, Environmental Protection Branch, Atlantic Region Surveillance Report. EPS-5-AR-02-01 30 p.

Parker, W.R., K.G. Doe, P. Jackman and J.G. Aube 2003. Toxicity of copper-based antifouling coatings to the marine amphipod, *Eohaustorius estuaries.* Environment Canada, Environmental Protection Branch, Atlantic Region Surveillance Report. EPS-5-AR-03-01 19 p.

Paul, J.D. and I.M. Davies 1986. Effects of copper- and tin-based antifouling compounds on the growth of scallops (*Pecten maximus*) and oysters (*Crassostrea gigas*). Aquaculture 54: 191-203.

Perez, B.A., A.A. Albert, J.R. Contreras and P.A. Smith 1998. Detection of *Piscirickettsia salmonis* in up-stream-migrating coho salmon, *Oncorhynchus kisutch*, in Chile. Bull. Eur. Assoc. Fish Pathol. 18: 189-191.

Peterson, L.K., J.M. D'Auria, B.A. McKeown, K. Moore and M. Shum 1991. Copper levels in the muscle and liver tissue of farmed chinook salmon, *Oncorhynchus tshawytscha*. Aquaculture 99: 105-115.

Petersen, A., J.S. Andersen, T. Kaewmak, T. Somsiri and A. Dalsgaard 2002. Impact of integrated fish farming on antimicrobial resistance in a pond environment. Appl. Environ. Microbiol. 68: 6036-6042.

Phelps, H.L., J.T. Hardy, W.H. Pearson and C.W. Apts 1983. Clam burrowing behaviour: inhibition by copper-enriched sediment. Mar. Poll. Bull. 14: 452 -455

Pike, A. W. 1989 Sea lice - major pathogens of farmed Atlantic salmon. Parasitology Today, 5: 291-297.

Pillay, T.V.R. 2004. *Aquaculture and the Environment*. 2nd Edition. United Kingdom: Blackwell Publishing Ltd.

Poirel, L., Liard, A., J.-M. Rodriguez-Martinez and P. Nordmann 2005a. Vibrionaceae as a possible source of Qnr-like quinolone resistance determinants. J. Antimicrob. Chemother.56: 1118-1121.

Poirel, L., J.-M. Rodriquez-Martinez, H. Mammeri, A. Liard, A. and P. Nordmann, 2005b. Origin of plasmid-mediated quinolone resistance determinant QnrA. Antimicrob. Agents Chemother. 49: 3523-3525.

Powell, D.P. 2000 Common Fish Diseases. Chapter 4 in: GK Ostrander (ed) The Laboratory Fish. Academic Press, New York, NY.

Rach, J. J., M.P. Gaikowski, R.T. Ramsay 2000. Efficacy of hydrogen peroxide to control parasitic infestations on hatchery-reared fish. J. Aquat. Animal Health, 12: 267-273.

Rach, J. J., T.M. Schreier, G.E. Howe, S.D. Redman. 1997. Effect of species, life stage and water temperature on the toxicity of hydrogen peroxide to fish. Prog. Fish-Culturist, 59: 41-46.

Rae, G. H. 2000 A national treatment strategy for control of sea lice on Scottish salmon farms. Caligus 6: 2-3.

Ramstad A., D.J.N.R. Colquhoun, I.H. Sutherland and R. Simmons 2002. Field trials in Norway with Slice (0.2% emamectin benzoate) for the oral treatment of sea lice infestation in farmed Atlantic salmon. Dis. Aquatic Org. 50:29-33.

Read P.A., T.F. Fernandes and K.L. Miller 2001. MARAQUA, The derivation of scientific guidelines for best environmental practice for the monitoring and regulation of marine aquaculture in Europe. Proceedings of the 3rd Workshop, Napier University, Edinburgh, Scotland, 28-31 August, 2000. J. Appl. Ichthyol. 17:145-206.

Reeve, M.R., M.A Walter, K. Darcy and T. Ikeda 1977. Evaluation of potential indicators of sub-lethal toxic stress in marine zooplankton (feeding, fecundity, respiration and excretion): Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:103-113.

Reid, H.I., A.A. Griffen and T.H. Birkbeck 2004. Isolates of *Piscirickettsia salmonis* from Scotland and Ireland show evidence of clonal diversity. Appl. Environ. Microbiol. **70:** 4393-4397.

Republica de Chile. Ministerio de Economia 2001. Reglamento Ambiental para la Acuicultura. Santiago. Chile.

Rhodes, L.D., T.H. Grayson, S.M. Alexander and M.S. Strom 2000a. Description and characterization of IS*994*, a putative IS*3* family insertion sequence from the salmon pathogen, *Renibacterium salmoninarum*. Gene 244: 97-107.

Rhodes, G., G. Huys, J. Swings, P. McGann, M. Hiney, P. Smith and R.W. Pickup 2000b. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of Tn*1721* in dissemination of the tetracycline resistance determinant Tet A. Appl. Environ. Microbiol. 66: 3883-3890.

Riisgard, H.U., K.N. Nielsen, B. and Sogard-Jensen 1980. Further studies on volume regulation and effects of copper in relation to pH and EDTA in the naked marine flagellate, *Dunaliella marina*. Mar. Biol. 56: 267-276.

Rijstenbil, J.W., J.W. Derksen, L.J. Gerringa, T.C. Poortvliet, A. Sandee, M. van den Berg, J. van Drie and J.A. Wijnholds 1994. Oxidative stress induced by copper: defense and damage in the marine planktonic diatom *Ditylum brightwellii,* grown in continuous cultures with high and low zinc levels. Mar. Biol. 119: 583-590.

Ritchie G, S.S. Ronsberg, K.A. Hoff and E.J. Branson 2002. Clinical efficacy of teflubenzuron (Calicide) for the treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer 1838), infestations of farmed Atlantic salmon, *Salmo salar* at low temperatures. Dis. Aquat. Organ. 51:101-6.

Robicsek, A., D.F. Sahm, J. Strahilevitz, G.A. Jacoby and D.C. Hooper 2005. Broader distribution of plasmid-mediated quinolone resistance in the United States. Antimicrob. Agents Chemother. 49: 3001-3003.

Robicsek, A., G.A. Jacoby and D.C. Hooper 2006. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect. Dis. 6: 629-640.

Rosser, S.J., and H.-K. Young 1999. Identification and characterization of class 1 integrons in bacteria from an aquatic environment. J. Antimicrob. Chemother. 44: 11-18.

Roper, D.S. and C.W. Hickey 1994. Behavioural responses of the marine bivalve *Macomona liliana* exposed to copper- and chlordane-dosed sediments. Mar. Biol. 118: 673-680.

Roth, M., R.H. Richards and C. Sommerville. 1993. Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. J. Fish Dis. 16: 1-26.

Rozen, Y., and S. Belkin 2001. Survival of enteric bacteria in seawater. FEMS Microbiol. Rev. 25:513-529.

Roy, W.J., I.H. Sutherland, H.D.M. Rodger and K.J. Varma. 2000. Tolerance of Atlantic salmon, *Salmo salar* L. and rainbow trout, *Oncorhynchus mykiss* (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture 184: 19-29.

Saga, T., M. Kaku, Y. Onodera, S. Yamachika, K. Sato and H. Takase 2005. *Vibrio parahaemolyticus* chromosomal *qnr* homologue VPA0095: Demonstration by transformation with a mutated gene of its potential to reduce quinolone susceptibility in *Escherichia coli*. Antimicrob. Agents Chemother. 49: 2144-2145.

Salud de Peces. Desafio para la Industria Acuicultora Nacional 2004. El Periodico de Acuicultura. Junio-Julio. 14-15.

Salyers, A.A., A. Gupta and Y. Wang 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. 12: 412-416.

Samuelsen, O.B., B.T. Lunestad, B. Husevag, T. Holleland and A. Ervik 1992. Residues of oxolinic acid in wild fauna following medication in fish farms. Dis. Aquat. Org. 12: 111-119.

Samuelsen, O.B., B.T. Lunestad, A. Ervik and S. Fjeldes 1994. Stability of antibacterial agents in an artificial marine aquaculture sediment studied under laboratory conditions. Aquaculture **126:** 283-290.

Sandaa, R.A., V. Torsvik and J. Grokoyr 1992. Transferable drug resistance in bacteria from fish farm sediment. Can. J. Microbiol. 38: 1061-1066.

Santos, M. H., T. da Cunha and A. Bianchini. 2000. Effects of copper and zinc on growth, feeding and oxygen consumption of *Farfantepenaeus paulensis* postlarvae (Decapoda: Penaeidae) J. Exper. Mar. Biol. Ecol. 247: 233-242.

Savitz, J. D., D.A. Wright and R.A. Smucker 1994. Toxic effects of the insecticide diflubenzuron (Dimilin®) on survival and development of nauplii of the estuarine copepod, *Eurytemora affinus*. Mar. Envion. Res. 37: 297-312.

Schmidt, A.S., M.S. Bruun, I. Dalsgaard and J.L. Larsen 2001a. Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment. Appl. Environ. Microbiol. 67: 5675-5682.

Schmidt, A.S., M.S. Bruun, J.L Larsen and I. Dalsgaard 2001b. Characterization of class 1 integrons associated with R-plasmids in clinical *Aeromonas salmonicida* isolates from various geographical areas. J. Antimicrob. Chemother. 47: 735-743.

Schmidt, A.S., M.S. Bruun, I. Dalsgaard, K. Pedersen and J.L. Larsen 2000. Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Appl. Environ. Microbiol. **66:** 4908-4915.

Scottish Environmental Protection Agency. Calicide (teflubenzuron)- Authorization for use as an infeed sea lice treatment in marine salmon farms.Risk assessment, EQS and recommendations. http://www.sepa.org.uk/aquaculture/policies/index.htm Policy No 29. 1999.

Scottish Environmental Protection Agency. Emamectin benzoate, an environmental assessment. http://www.sepa.org.uk/policies/index.htm , pp 1-23. 1999.

Scottish Environmental Protection Agency. SEPA policy on the use of cypermethrin in marine fish farming risk assessment, EQS and recommendations. http://www.sepa.org.uk/aquaculture/policies/index.htm Policy No. 30. 1998.

Scottish Environmental Protection Agency. The occurence of the active ingredients of sea lice treaments in sediments adjacent to marine fish farms. Results of monitoring surveys carried out by SEPA in 2001 and 2002. http://www.sepa.org.uk/policies/index.htm 2004.

Servicio Nacional de Pesca. Requisitos generales para la certificación sanitaria de los productos pesqueros de exportación. Norma Técnica sección 1. 2005. Mayo. Santiago. Chile.
Sevatadal, S. and T.E. Horsberg 2003. Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis*, Krøyer) against the pyrethroid deltamethrin using bioassays and probit modeling. Aquaculture 2003 218: 21-31.

Shade, W.D., S.S. Hurt, A.H. Jacobson and K.H. Reinert 1993. Ecological risk assessment of a novel marine antifoulant. ASTM STP 1993; 1216: 381-407.

Silva, J., R. Zemelman, M.A. Mendoza, M. Henriquez, C. Merino and C. Gonzalez, 1987. Antibiotic-resistant gram negative bacilli isolated from sea water and shellfish: Possible epidemiological implications. Rev. Latinoam. Microbiol. 29:165-169.

Smith, J.N., P.A. Yeats and T.G. Milligan 2005. Sediment geochronologies for fish farm contaminants in Lime Kiln Bay, Bay of Fundy. *In:* Hargrave, B.T. (ed.) *Environmental Effects of Marine Finfish Aquaculture*. Springer, Berlin. pp 221-238.

Sørum, H. 2000. Farming of Atlantic salmon – an experience from Norway. Acta Vet. Scand. Suppl 93: 129-134.

Sørum, H., and T.M. L'Abée-Lund 2002. Antibiotic resistance in food-related bacteria – a result of interfering with the global web of bacterial genetics. Int. J. Food Microbiol. 78: 43-56.

Sørum, H. 2006. Antimicrobial drug resistance in fish pathogens. In *Antimicrobial Resistance in Bacteria of Animal Origin*. (Aarestrup, F.M., ed.). Washington, DC: ASM Press, pp. 213-238 (Chapter 13).

Sørum, H. 1998. Mobile drug resistance genes among fish bacteria. APMIS 106(Suppl 84): 74-76.

Stauber J.L. and T. M. Florence 1990. Mechanism of toxicity of zinc to the marine diatom *Nitzschia closterium.* Mar. Biol. 105: 519-524.

Stauber, J.L., R.J. Benning, L. Hales, R. Eriksen and B. Nowak 2000. Copper bioavailability and amelioration of toxicity in Macquairie Harbour, Tasmania, Australia. Mar. Freshwat. Res. 51: 1-10.

Stead, S. M. and L. Laird 2002. *Handbook of Salmon Farming*. Chichester. U.K.: Springer. Praxis.

Stehly, G.R., J.R. Meinertz and W.H. Gingerich 1998. Effects of temperature on the elimination of benzocaine and acetylated benzocaine residues from the edible fillet of rainbow trout (*Oncorhynchus mykiss*). Food Addit. Contam. 17(5): 387-92

Stone J., I.H.Sutherland, C. Sommerville, R.H. Richards and K.J. Varma 2000a. Commercial trials using emamectin benzoate to control sea lice (*Lepeophtheirus salmonis*) infestations in Altantic salmon (*Salmo salar* L.). Dis. Aquat. Organ. 41:141-9.

Stone, J., I.H. Sutherland, C.S. Sommerville, R.H. Richards and K.J. Varma. 1999. The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer), infestations in Atlantic salmon, *Salmo salar* L. J. Fish Dis. 22: 261-270.

Stone, J., I.H. Sutherland, C.S. Sommerville, R.H. Richards and K.J. Varma. 2000. Field trials to evaluate the efficacy of emamectin benzoate in the control of sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann, infestations in Atlantic salmon, *Salmo salar* L. Aquaculture 186: 205-219

Sunda, W.G. and J.M. Lewis 1978. Effect of complexation by natural organic ligands on the toxicity of copper to a unicellular alga, *Monochrysis lutheri*. Limnol. Oceanog. 23: 870-876.

Sunda, W.G., P.A. Tester and S.A. Huntsman 1987. Effects of cupric and zinc ion activities on the survival and reproduction of marine copepods. Mar. Biol. 94: 203-210.

Sutherland, T.F., S.A. Peterson, C.D. Levings and A.J. Martin. 2007. Distinguishing between natural and aquaculture-derived sediment concentrations of heavy metals in the Broughton Archipelago, B.C. Marine Pollution Bulletin, 54: 1451-1460.

Tabche, L. M., I. G. Cabrera, L. G. Olivan, M. G. Martinez and C. G. Faz 2000. Toxic effects of zinc from trout farm sediments on ATP, protein, and hemoglobin concentrations of *Limnodrilus hoffmeisteri***.** J. Toxicol. Envir. Hlth. Part A 59: 575-583.

Teuber, M. 2001. Veterinary use and antibiotic resistance. Curr. Opin. Microbiol. 4: 493-499.

Thybaud E. 1990. Ecotoxicology of lindane and deltamethrin in aquatic envrionments. J. Water Sci. 3:195-209.

Todar, K. 2008. Antimicrobial agents used in treatm,ent of infectious diseases. In: Todar's online textbook of bacteriology. http://textbook of bacteriology.net/antimicrobial.html Accessed Januray 30, 2008

Treasurer, J. W. and A. Grant 1997. The efficacy of hydrogen peroxide for treatment of farmed Atlantic salmon, *Salmo salar* L. infested with sea lice (Copepoda: Caligidae). Aquaculture 148, 265-275.

Treasurer, J., S. Wadsworth and A. Grant 2000. Resistance of sea lice *Lepeophtheirus salmonis* (Krøyer) to hydrogen peroxide on farmed Atlantic salmon, *Salmo salar* L. Aquaculture Res. 31: 855-860.

US Food and Drug Administration 2007, Guide no. 150. Guidance for Industry Concerns Related to the use of Clove Oil as an Anesthetic for Fish

Van Wezel, A.P. and P. Van Vlaardingen 2004. Environmental risk limits for antifouling substances. Aquat. Toxicol. 66: 427-444.

Visviki, I. and J.W. Rachlin 1992. Ultrastructural changes in *Dunaliella minuta* following acute and chronic exposure to copper and cadmium. Arch. Environ. Contam. Toxicol. 23: 420-425.

Waddy, S.L., L.E. Burridge, K. Haya, M.N. Hamilton and S.M. Mercer. 2002a. Response of preovigerous lobster to azamethiphos varies with concentration, number of exposures, and time of year, p. 60-63. Aquaculture Canada 2001 - Proceedings of the contributed papers to the 18th Annual Meeting of the Aquaculture Association of Canada, Halifax, NS, May 6-9, 2001. Aquaculture Association of Canada, Special Publication Number 5.

Waddy, S.L., L.E. Burridge, M.N. Hamilton, S.M. Mercer, D.E. Aiken and K. Haya. 2002b. Emamectin benzoate induces molting in American lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 59: 1096-1099.

Waddy, S.L., V.A. Merritt, M.N. Hamilton-Gibson, D.E. Aiken and L.E. Burridge 2007. Relationship between dose of Emamectin benzoate and molting response of ovigerous American lobsters (*Homarus americanus*) Ecotoxicol. Environ. Saf. 67, 95-99

Walker, A. N. and M.N. Horst 1992. Effects of diflubenzuron on chitin synthesis in th e postmolt blue crab, *Callinectes sapidus* : a morphologic study using an *in vitro* explant culture system. J. Crust. Biol. 1992, 12: 354-360.

Wassenaar, T.M. 2005. Use of antimicrobial agents in veterinary medicine and implications for human health. Crit. Rev. Microbiol. 31: 155-169.

Watzin, M.C. and P.R. Roscigno 1997. The effects of zinc contamination on recruitment and early survival of benthic invertebrates in an estuary. Mar. Poll Bull. 34: 443-455.

Weber, J.T., E.D. Mintz, R. Canizares, A. Semiglia, I. Gomez, I. and R. Sempertegui, 1994. Epidemic cholera in Ecuador: multidrug-resistance and transmission by water and seafood. Epidemiol. Infect. **112:** 1-11.

Webster, N.S., R. Webb, M.J. Ridd, R.T. Hill and A.P. Negri 2001. The effects of copper on the microbial community of a coral reef sponge. Environ. Microbiol. 3:19-29.

Wegener, H.C. 1999. The consequences for food safety of the use of fluoroquinolones in food animals. New Engl. J. Med. 340: 1581-1582.

Wheatley, S.B., M.F. McLoughlin, F.D. Menzies and E.A.Goodall 1995. Site management factors influencing mortality rates in Atlantic salmon (*Salmo salar*) during marine production. Aquaculture **136:** 195-207.

Wierup, M. 2001. The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microb. Drug Resist. **7:** 183-190.

Willis, K. J. and N. Ling 2003. Toxicity of emamectin benzoate, an aquaculture pesticide, to planktonic marine copepods. Aquaculture 221: 289-297.

Willis, K.J., P.A. Gillibrand, C.J. Cromey and K.D. Black 2005. Sea lice treatments on salmon farms have no adverse effects on zooplankton communities: a case study. Mar. Poll. Bull. 50: 806-816.

Wootten, R.; J.W. Smith and E.A. Needham 1982. Aspects of the biology of parasitic copepods, *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed Atlantic salmon, *Salmo salar* Proceedings of the Royal Society of Edinburgh, 81B: 185-197.

Yeats, P.A., T.G. Milligan, T.F. Sutherland, S.M.C. Robinson, J.A. Smith, P. Lawton, and C.D. Levings. 2005. Lithium-normalized Zn and Cu concentrations in sediment as measures of trace metal enrichment due to salmon aquaculture. The Handbook of Environmental Chemistry, 5M, 207 - 220.

Young, J.S., J.M. Gurtisen, C.W. Apts and E.A. Crecelius 1979. The relationship between the copper complexing capacity of sea water and copper toxicity in shrimp zoae. Mar. Environ. Res. 2: 265-273.

Zitko, V. 1994. Chemicals in aquaculture (an overview) *In:* A. Ervik, P. Kupa Hansen and V. Wennevik [eds.]. Proceedings of the Canada-Norway Workshop on the Environmental Impacts of Aquaculture. Fisken Havet 13: 97-106