Fisheries and Oceans Canada / Pêches et Océans Canada

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Research Document 2010/017

Document de recherche 2010/017

Pathway of effects of chemical inputs from the aquaculture activities in Canada

Séquence des effets des produits chimiques utilisés en production aquicole au Canada

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ISSN 1499-3848 (Printed / Imprimé)
ISSN 1919-5044 (Online / En ligne)

Canada

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Correct citation for this publication:
La présente publication doit être citée comme suit :

Burridge, L.E., Doe, K.G. and Ernst, W. 2011. Pathway of effects of chemical inputs from the aquaculture activities in Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2010/017. vi + 57 p.

ABSTRACT

As in all animal food production systems, it is often necessary to treat aquaculture species for diseases, parasites and fouling organisms. The types of therapeutants available for use and the treatment protocols are tightly regulated in Canada and therapeutants can only be used under prescription from a licensed veterinarian. Management practices have evolved as health threats appear and husbandry has greatly improved over the past 20 years. However, aquaculturists still rely on the use of pesticides and drugs to combat infestations of ectoparasites, antibiotics to treat diseases, metals and products such as lime to combat fouling organisms and disinfectants to ensure biosecurity at aquaculture sites. Chemotherapeutants used in the aquaculture industry are considered either a drug or a pesticide depending on the use and method of application. Health Canada regulates the use of both drugs and pesticides and makes the distinction between them based on the method of application. If the product is applied topically or directly into water, it is considered a pesticide; however, if a product is delivered through medicated feed or by injection, it is considered a drug.

In this report, the types of chemicals used in normal aquaculture practices in Canada are examined for their physical and chemical characteristics, their potential to affect the aquatic environment and species therein and the likelihood that these effects will occur. The main objective of this report is to evaluate the pathway of effects of the various chemical types to identify their stressor-effects such as the presence, effects and consequences in and on non-target aquatic organisms. Each chemical type is assessed for the state of knowledge in relation to stressor-effects, measurable ecological outcomes or endpoints, the magnitude of the effect, the identification of factors and conditions that modify or influence the effect, biological implications, the availability and strength of evidence for the stressor-effect relationship, uncertainties, activities under which stressor-effects occur, and cascading effects or linkages. Pathway of effects diagrams have been created for water borne compounds and in-feed compounds.

RÉSUMÉ

Le présent document donne une vue d'ensemble de la séquence des effets des produits chimiques utilisés habituellement dans les installations aquicoles au Canada et leurs effets potentiels sur l'environnement aquatique et les espèces ainsi que la possibilité d'occurrence de ces effets. Comme dans tout système de production d'aliments d'origine animale, il faut souvent utiliser, dans les installations aquicoles, des pesticides et des médicaments pour lutter contre les infestations d'ectoparasites, des antibiotiques pour traiter les maladies, des métaux et des produits tels que la chaux pour lutter contre les organismes salissants ainsi que des désinfectants pour assurer la biosécurité des installations. Parmi les conséquences de cette utilisation, mentionnons le rejet de produits chimiques dans l'environnement aquatique immédiat où ceux-ci peuvent être adsorbés par les sédiments du fond et ingérés par les poissons. Les effets directs sur les espèces vulnérables peuvent être létaux ou sublétaux et peuvent provoquer des changements au sein des populations ou des communautés locales. Les effets indirects sur d'autres espèces situées à un niveau supérieur du réseau trophique peuvent être léthaux ou non. La bioaccumulation peut également affecter les populations ou les communautés locales.

La relation entre l'agent perturbateur et l'effet, y compris sa présence, son effet et ses répercussions chez d'autres espèces aquatiques, est décrite pour chaque type de produit chimique. En outre, chaque type de produit chimique est évalué sur le plan de l'état des connaissances relatives aux résultats mesurables sur le plan écologique, de l'importance de l'effet, de la désignation des facteurs et des conditions qui modifient l'effet ou ont une incidence sur celui-ci, des répercussions biologiques, de la disponibilité et de la solidité des preuves concernant la relation entre l'agent perturbateur et l'effet, de l'incertitude, des activités pendant lesquelles les agents perturbateurs ont des effets ainsi que des effets ou des liens en cascade. On a réalisé des diagrammes de séquences des effets pour les composés chimiques présents dans l'eau et la nourriture.

INTRODUCTION

As is the case in all animal food production systems, it is often necessary to treat aquaculture species for diseases, parasites and fouling organisms. The types of therapeutants available for use and the treatment protocols are tightly regulated in Canada and therapeutants can only be used under prescription from a licensed veterinarian. Management practices have evolved as health threats appear and husbandry has greatly improved over the past 20 years. However, aquaculturists still rely on use of pesticides and drugs to combat infestations of ectoparasites, antibiotics to treat diseases, metals and products such as lime to combat fouling organisms and disinfectants to ensure biosecurity at aquaculture sites. Several reviews have been prepared regarding chemical inputs, mainly associated with salmon aquaculture in the marine environment (see for example Zitko 1994, GESAMP 1997, Burridge 2003, Burridge et al. 2008). In addition, Scott (2004) has reported on chemical inputs associated with freshwater aquaculture in Canada.

Chemotherapeutants used in the aquaculture industry are considered either a drug or a pesticide depending on the use and method of application. Health Canada regulates the use of both drugs and pesticides and makes the distinction between them based on the method of application. If the product is applied topically or directly into water, it is considered a pesticide; however, if a product is delivered through medicated feed or by injection, it is considered a drug.

Drug approval is the responsibility of the Veterinary Drugs Directorate (VDD) of Health Canada, under the authority of the *Food and Drugs Act* (FDA). The FDA does not require the submission of environmental data; however, the mandate of the VDD is to ensure that drugs sold for use in animals are safe and effective and do not leave residues in food products, which could present a risk to the consumer. There are provisions for Emergency Drug Release (EDR) and 'off-label' use of a drug. The emergency release of a drug must have VDD authorization before a manufacturer is allowed to sell an unapproved new drug that does not have a Drug Identification Number (DIN) to a licensed veterinarian for the emergency treatment of (a) patient(s). It is preferred that the 'off-label' use of any drug with a DIN is avoided whenever an approved product is available. Veterinarians who use drugs in an 'off-label' manner are responsible for animal safety and for any illegal drug residues that are detected in animal products sold for human consumption (Burridge 2003).

Pesticides are the responsibility of the Pest Management Regulatory Agency (PMRA) of Health Canada and are registered under the authority of the *Pest Control Products Act* (PCPA). Herbicides, fungicides, disinfectants, insecticides and some antimicrobials are all considered pesticides. Antifouling agents that are added to paints and coatings to restrict growth of aquatic fouling pest organisms, such as algae, tunicates and molluscs on nets or mussel socks are also registered under the PCPA. Some disinfectants may also be considered pesticides and are regulated under the PCPA. The PCPA requires the registrant to submit environmental data as part of the registration process. Most data submitted to the regulatory agencies are proprietary and, as such, are not available to the general public but can be obtained by researchers (with restrictions) from Health Canada.

In this report we identify the types of chemicals used in normal aquaculture practices in Canada, their physical and chemical characteristics, their potential to affect the aquatic environment and species therein and the likelihood that these effects will occur. This is not a thorough review of compounds or class of compounds as each could be the subject of a comprehensive review. The authors have relied heavily on summary papers prepared by Burridge (2003), Scott (2004), Haya et al. (2005) and Burridge et al. (2008).

STRESSOR CATEGORY: ANTIBIOTICS

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 low or very low toxicity in higher organisms (Todar 2009).

Antibiotics in aquaculture, as in other industrial husbandry of aquatic and terrestrial food animals including cattle and poultry, are used as therapeutic agents in the treatment of infections (Alderman and Hastings 1998, Angulo 2000, Sørum 2000, Pillay 2004). These drugs are applied only after a prescription has been issued by a licensed veterinarian. In other intensive farming situations, cattle and hog production for example, antibiotics have been used as growth promoters (Alderman and Hastings 1998, Davenport et al. 2003). This practice is no longer allowed in Canada and there is no evidence that antibiotics were ever used as growth promoters in aquaculture.

The following products are registered for use as antibiotics in the finfish aquaculture industry Canada: Oxytetracycline (OTC), trimethoprim80%/sulphadiazine20% (Tribrissen), sulfadimethoxine 80%/ormetoprim 20% (Romet 30), and Florfenicol. The drugs are used in both freshwater and marine culture systems (Scott 2004). Tribrissen and Romet30 are rarely used in marine finfish aquaculture due to problems with palatability (M. Beattie, Province of New Brunswick, personal communication, 2008). Scott (2004) has prepared a number of useful summary tables regarding compounds in use in the freshwater aquaculture industry in Canada. Readers are referred to his paper for these details.

Florfenicol is a broad spectrum antibiotic used to treat fish against infections of furunculosis. It is part of the phenicol class of antibiotics which act by inhibiting protein synthesis (Todar 2009). The recommended treatment regime is 10 $mg{\cdot}Kg^{-1}$ for 10 days presented on medicated food. The withdrawal period for florfenicol is 12 days in Canada. The concentration which is expected to be lethal to 50% of an exposed population over 96 h (96 h LC50) of florfenicol is >330 $mg{\cdot}L^{-1}$ (Daphnia) and >780 $mg{\cdot}L^{-1}$ (Rainbow trout). Its half-life in marine sediments is estimated to be 4.5 days (Armstrong et al. 2005). Because of this short half-life florfenicol is not generally considered a problem for persistence in the environment or for resistance development in micro-organisms (Armstrong et al. 2005).

Both Tribrissen (sulfadiazine: trimethoprin (5:1)) and Romet 30 (sulfadimethoxine + oretoprim (4:1)) are potentiated sulphonamides. They are broad spectrum antibacterial agents used to treat salmonids infected with gram negative bacteria such as furunculosis and vibrios (*Vibrio anguillarum,* for example). They act by inhibiting folic acid metabolism (Todar 2009). The recommended treatment regime is 30 mg·Kg^{-1} (Tribrissen) or 50 mg·Kg^{-1} (Romet 30) for 7-10 days presented on medicated food (Scott 2004). Hektoen et al. (1995) reported that the constituents of Tribrissen are detectable in marine sediments at all depths and the estimated half-life ranges from 50-100 days depending on compound and depth.

Romet 30 has a low environmental persistence. Capone et al. (1996) did not find Romet 30 residues in marine sediments 21-62 days after treatment of 2 commercial cage sites. Tulou et al. (2008) reported that this product does not induce vitellogenin production in male Japanese medaka (*Oryzias latipes*) and therefore is not considered an estrogen mimic or endocrine disruptor.

OTC 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^{-1} applied over 4 to 10 days. Tetracyclines act by inhibiting DNA replication (Todar 2009). The compound has a low toxicity (96 h LC50 for fish is >4 g·Kg^{-1}). OTC is quite soluble in water and is poorly absorbed by fish (Scott 2004). Consequently, a large proportion of the therapeutic dose of OTC is released to the environment in the excreta of fish (Bebak-Williams et al. 2002). OTC can become bound to sediments and may be persistent for several hundred days depending on temperature, oxic condition of sediments and depth (Armstrong et al. 2005). OTC can have a half life of over 400 days in anoxic sediments (Armstrong et al. 2005). Nepejchalová et al. (2008) have reported relatively high concentrations of OTC in sediments associated with freshwater aquaculture in the Czech Republic 59 days after application. Scott (2004) suggests that OTC may be more bioavailable in freshwater systems than in marine systems. The combination of low toxicity and broad spectrum effectiveness has led to the widespread overuse and misuse of OTC in human and animal health and therefore to the development of resistance and reduced effectiveness (Todar 2009).

In the Report to the Provincial Environmental Assessment Review of Salmon Aquaculture in British Columbia (DFO 1997), reference is made to uptake of OTC by oysters and crabs and Romet 30 by oysters (Jones 1990; LeBris et al. 1995; Capone et al. 1996). Each study showed at least some uptake of the antibiotic by these invertebrates either in the laboratory or in close proximity to salmon cage sites. The concentration of OTC in rock crab was as high as 3.8 µg·g^{-1}. This value is well in excess of the Canadian residue limit set for edible tissues in salmon of 0.2 µg·g^{-1} (Health Canada 2010) and the US limit of 2.0 µg·g^{-1} also for edible portions of salmon (Bernardy et al. 2003). There is generally a lack of information regarding the effects of antibiotic contamination in non-target species.

Despite the low toxicity of antibiotics and the fact that quantities used in aquaculture are much smaller than in other forms of intensive rearing of animals, there are significant environmental concerns with widespread use of antibiotics. Some antibiotics, OTC for example, are stable chemical compounds that are not broken down in the body, but remain active long after being excreted. Antibiotics may affect the composition of the phytoplankton community, the zooplankton community and consequently even the diversity of populations of larger animals (Burridge et al. 2008).

In general, 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. It has been 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 (Harrison and Lederberg 1998, McEwen and Fedorak-Cray 2002, Cabello 2003, Cabello 2004, Angulo et al. 2004, Mølbak 2004). 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. In Canada, it appears as though only two products, OTC and florfenicol are used in the aquaculture industry.

Resistance to antibiotics may occur in fish, non-target organisms and the bacterial community present in sediments near aquaculture activities. Hansen et al. (1992) reported an increase in antibiotic-resistant bacteria in sediments within a few days of onset of treatment with oxytetracycline. The presence of oxytetracycline resulted in higher numbers of antibiotic-resistant bacteria and a longer-lasting effect relative to other antibiotics and to control sites. Björklund et al. (1990) reported the presence of antibiotic-resistant bacteria in oxytetracycline-treated rainbow trout. Similarly, Hirvelä-Koski et al. (1994) identified antibiotic resistant strains of *Aeromonas salmonicida* in salmon at 9 of 35 fish farms treated with OTC. No resistance to other antibiotics was observed. Resistance to OTC was detected in aerobic bacteria in freshwater systems Chile (Miranda and Zemelman 2002).

The most severe consequence is the emergence of new bacterial strains that are resistant to several antibiotics at the same time. Antibiotics may reach the environment and lead to the selection of resistance in non-target benthic organisms (GESAMP 1997).

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, is that dangerous pathogens will eventually acquire resistance to previously effective antibiotics, thereby giving rise to uncontrolled epidemics of bacterial diseases that can no longer be treated (European Commission 2008).

The safety of human food can also be directly affected by the presence of residual antibiotics in farmed or wild fish which have been dosed with antibiotics (Grave et al. 1999, McDermott et al. 2002, Cabello 2003, Alcaide et al. 2005). Regulated withdrawal periods for farmed species are in place to protect consumers. However, some consumers may display heightened sensitivities and allergies to antibiotics.

The most recent data on the use of antibiotics and in the Canadian aquaculture industry are summarized in Table 1.

Table 1. Use of antibiotics in Canada in 2007. Data courtesy of the NB Salmon Growers Association and the government of British Columbia. NA = data not available.

Therapeutant	BC	NB & Maine	NF, NS, QC	Freshwater
Antibiotics	109 g/MT	~338 g/MT	NA	NA

Antibiotic use in aquaculture is small when compared to use in animal and human health. Use in salmon aquaculture worldwide is small compared to the use in other forms of aquaculture, shrimp farming for example. However, the quantity of antibiotics applied per metric ton of salmon production in Canada is high compared to use in countries such as Scotland and Norway and increased from 2005 through 2007 (Burridge et al. 2008).

There are very few published data regarding the presence of antibiotics (in sediments and biota) of aquaculture origin in Canada. Cross et al. (1997) reported that fauna adjacent to a small salmon farm in the Strait of Georgia showed no evidence of OTC in their tissues except for one crab sampled from directly under a cage. Hargrave et al. (2008) studied growth inhibition in *Aeromonas salmonicida* exposed to a range of OTC concentrations. They collected sediments samples near aquaculture sites, near sewage outfalls and at reference sites. Evidence of antibiotic resistant strains of bacteria was found in samples from all sites. Of particular interest is the finding that there are antibiotic resistant bacteria in fish food. This supports an assertion by Kerry et al. (1995) that fish food may be a source of resistant bacteria.

Kerry et al. (1995) suggested caution should be exercised when interpreting results of experiments dealing with induction of antibiotic resistance. These authors identified the rate of cell division, as well as factors that affect cell division, as mitigating factors in establishing the validity of some studies. In addition, Smith et al. (1995) analyzed species frequency data and concluded operation of fish farms had only a negligible long-term impact on the microflora in undercage sediments. Rhodes et al. (2000) examined the distribution of oxytetracycline resistant aeromonads in hospital and aquaculture environments in England. They found that inputs from human and aquaculture sources act in the same manner in terms of dissemination of tetracycline resistance-encoding plasmids. These data suggest examination of antibiotic resistance near aquaculture sites must take into consideration other possible sources of input.

The presence, prevalence and relevance of antibiotic resistant organisms in sediments and indigenous species around aquaculture sites must be investigated. These data can then be put in context by comparison to presence of antibiotic resistant bacteria in other aquatic environments (e.g., near sewage treatment plants).

Without data about what compounds are applied, and where, it is difficult to assess risk. Recently the Province of New Brunswick, instituted regulations wherein incidence of disease, products applied to combat disease and quantities used must be reported monthly. It is anticipated that in 2010 edited summaries of these reports will be available to the public (M. Beattie, Province of New Brunswick, personal communication, 2009). Summaries of quantities of antibiotics used yearly in marine finfish aquaculture are available from British Columbia (accessed Sept 4, 2009)
http://www.al.gov.bc.ca/ahc/fish_health/Antibiotic_Graphs_1995-2008.pdf

It is clear that, in Canada, there is no standard procedure for public reporting of antibiotics, therapeutant or other chemical use. This can be contrasted with Scotland where each site reports monthly on their current biomass, any treatments that have taken place and the quantities of all chemicals applied to the site. This is a very powerful management, regulatory and research tool.

STRESSOR – EFFECTS

Effect A: Presence, effects and consequences of antibiotics in and on non-target organisms: Other fish, aquatic invertebrates and micro-organisms.

1. What is known (state of knowledge) about the stressor-effect relationship?

Data are available showing the presence of OTC residues in fish and invertebrates collected near aquaculture sites. The processes labelled A in the accompanying Stressor-Effect Logic Diagram (Figure 1) are processes that are likely to occur given the presence of compounds in sediment and in tissue of some non-target organisms. There is no indication that these residues have any harmful consequences. It is also unclear whether or not the source of the antibiotics is from aquaculture activities. As Canada does not allow the use of antibiotic compounds in aquaculture activities that are still considered of significant value in human health, it may be inferred that the compounds originate from aquaculture activities. OTC is still used in topical creams but its overall effectiveness in human health has diminished due to development of bacterial resistance.

2. What are the (measurable) ecological outcomes or endpoints (effects profile)?

Antibiotic residues can and have been measured in receiving environments, particularly sediments near aquaculture sites. OTC can remain in the environment for hundreds of days depending on the local conditions (temperature, oxic condition of sediments). It has been suggested that prolonged exposure to these residues will promote development of colonies of antibiotic resistant bacteria and a reservoir for the same. However, the ability to measure the compound does not establish a cause and effect link. The processes labelled B in Figure 1 are thus, for the most part, speculative. While these effects could happen, our current state of knowledge does not clearly show that they are happening.

The antibiotics used in the Canadian aquaculture industry are not toxic to multi-cellular organisms. Presence and fate of antibiotic residues in animals can be measured by use of chemical and biochemical analytical techniques. Presence and/or persistence of antibiotics in aquatic organisms will eventually lead to resistance of pathogenic micro-organisms to the product. It has been shown that wild fish with detectable levels of OTC in their tissue also have antibiotic resistant bacteria in their intestinal flora. These fish may therefore provide a reservoir for antibiotic resistant bacteria.

It has been shown that OTC has no estrogenic effects as indicated by plasma vitellogenin concentrations in male medaka. This is the only study the authors have found reporting sublethal effects of antibiotics in fish.

3. What is the magnitude of the effect?

As exposure is unknown, it is unknown if an effect occurs. It has not been definitively shown whether or not the presence of antibiotic residues and antibiotic-resistant bacteria is clearly linked or coincidental. To the author's knowledge there have been no studies that have systematically looked at antibiotic use, presence in non-targets and potential effects on those non-target organisms. Despite this, the fact that antibiotic residues are found in association with aquaculture activity suggests that a link may exist. The most powerful selective pressure for antibiotic resistance is the antibiotic itself. As long as residues are present in the environment they continue to exert selective pressure. Therefore precaution should be exercised in use of antibiotics and in risk assessment.

4. What are the factors and conditions that modify or influence the expression of the effect (e.g., exposure, type of receiving environment, etc.).

Temperature affects the persistence of most antibiotic compounds as does the organic content of the receiving environment. In low energy systems where food and faeces remain very close to the net pen the concentration of OTC residues may be chronically elevated. These areas are high in bacterial presence and activity. This would suggest an increased opportunity for the development on antibiotic resistance in bacteria found in sediments. It would also suggest; however, that the spatial distribution of the compound and of some of the bacteria would be limited. In contrast, Florfenicol has a half life of days, is likely not to persist and any effects would be expected to be acute. Another key influence on stressor effect linkages is the fact that antibiotics are applied sporadically and only when treatment is deemed necessary by a licensed veterinarian. Some farms have not been treated with antibiotics for years. Table 1 shows the use of antibiotics in salmon aquaculture in parts of Canada. While use is reported relative to production in reality antibiotics are not applied at all sites.

5. What are the biological implications of the effect on the overall ecosystem function?

Existing data on lethal and sublethal effects of antibiotics indicate very low risk for direct effects on multi-cellular organisms. However, some studies suggest that antibiotics may be toxic to eukaryotes and their presence in sediments could affect other microbes in addition to bacteria. The overall implications for overuse and misuse of antibiotics are significant. Proof that antibiotics lose therapeutic effectiveness with time and overuse is unequivocal. Development of resistance to therapeutants is a huge problem. Use of antibiotics is the only aquaculture activity that could play a major role in human health despite the fact that aquaculture is a small player in terms of overall antibiotic input to aquatic environments. The ability of bacteria to "pass-on" resistance to other bacteria, some of which may be pathogenic to humans has been discussed in the scientific and gray literature for some time and remains a contentious issue.

Assessments of bacterial colonies associated with aquaculture activities have been conducted in the context of species abundance and diversity. To the authors' knowledge these assessments have taken into account the presence of contaminants. Should antibiotics affect micro-organisms near aquaculture sites there could be consequences for overall ecosystem function. The spatial and temporal characteristics of any effect must be determined in order to assess the risk of long-term consequences.

6. **What type of evidence is available (e.g., lab studies, models, etc.) and what is the strength of evidence used to determine the stressor-effect relationship?**

Models exist that help predict where particulates associated with fish food and faeces will go. Field validation has not been thorough and is site specific. Use of a tracer would help considerably in validating model OTC, a persistent organic compound may actually be very useful in this regard but it would require significant coordination between farmer and scientist to plan such a study.

Lab studies have been used to determine the persistence of antibiotics in sediments in freshwater and marine conditions.

Lab studies have been conducted to describe dose-response of invertebrates to various antibiotics.

There have been a number of field studies that have described the presence of antibiotic residues in sediments and organisms near aquaculture sites (Kerry et al. 1995, Capone et al. 1996). Currently aquaculture facilities are not required to report antibiotic use and therefore it is impossible to conclusively link presence of resistant bacteria to treatment activity. Aquaculture activity is implicated in the presence of resistant bacteria and as stated earlier the link between activity and presence is of less importance than the link between presence and potential effect.

7. **What are the uncertainties associated with this stressor-effect linkage? Where would further information lead to a more complete understanding? Which uncertainties most prevent a more holistic understanding of the effect profiles and biological implications on overall ecosystem function?**

There are several layers of uncertainty. Although the weight of evidence indicates a clear link between aquaculture activities and the presence of either residues of antibiotics or presence of antibiotic resistant bacteria in the sediment or in aquatic organisms, science has yet to firmly establish the link. There a number of other sources of antibiotic residues. Municipal waste water treatment plants discharge large quantities of water which may contain antibiotic residues, for example. In the Canadian context marine finfish aquaculture is practiced in relatively isolated areas with few other sources of chemical inputs. It would be a relatively easy exercise to map potential sources of antibiotic input with the identified presence of antibiotic residues in sediments.

The uncertainties associated with the source of antibiotic contamination do not affect the proposed effect – development of antibiotic resistance in the environment. Regardless of the source of the antibiotic residue the effect will be the same. There remains considerable uncertainty regarding the likelihood of use of antibiotics in the aquaculture context leading to widespread antibiotic resistance. It is well established, however, that presence of antibiotic residues will have some effects on bacterial populations and possibly on animal and human health.

Use of antibiotics is sporadic. In areas of high aquaculture activity as in southwest New Brunswick assessment of risk associated with persistence of residues will be completely different than in British Columbia where farms are sited further apart or in the freshwater context.

8. During which activities does this stressor-effect occur?

If there is an effect associated with use of antibiotics in the Canadian aquaculture context it occurs as a result of treatment of fish for bacterial infections. Treatment is sporadic and occurs only after a licensed veterinarian examines the fish. Despite the sporadic nature of treatment, persistence of the antibiotic means that exposure may not be sporadic. In fact, under some conditions, there could be a build-up of the antibiotic in the environment and exposure may be ongoing for extended periods of time (hundreds of days). Near shore marine environments and lakes and ponds may have other antibiotic inputs which will affect exposure of non-targets to antibiotic residues.

9. What are the cascading effects or linkages from this effect?

There are a number of potential cascading effects. Currently these are all speculative. As described above, there is a strong suggestion that antibiotic bacteria may develop as a result of prolonged use of a limited number of antibiotics. This will have obvious consequences for fish health and potentially for the health of other organisms. The situation in Chile has shown that excessive use of therapeutants eventually leads to ineffective treatments, the need to use greater quantities and the eventual collapse of some parts of the industry.

If target fish are no longer protected by antibiotics, it is likely they will be stressed, won't grow well and will be susceptible to other stressors.

The potential for the cumulative use of antibiotics in human health and all forms of animal husbandry to affect human health cannot be taken lightly. Aquaculture's role in this is likely small but it may play a part nonetheless.

CONCLUSIONS

Use of antibiotics to treat disease outbreaks in cultured species is a normal and necessary practice. Antibiotic use in Canada is well regulated but there are only four products available to the aquaculture industry. Approximately 20,000 Kg of antibiotics were used in the finfish aquaculture industry in Canada in 2007. Detailed information on what compounds are used, when they are applied and where they are applied is not easily available to scientists making it impossible to interpret the data collected during field studies.

The link between antibiotic use in aquaculture and the presence of antibiotic resistant bacteria has not been unequivocally established. There are, however, enough data to confirm that the potential exists for colonies of antibiotic resistant bacteria to be established. Determining the source of antibiotics has important consequences for the regulation of these compounds but is essentially an academic, or regulatory, exercise. The key message is that antibiotic residues and resistant bacteria are found in areas of aquaculture activity and that residues help promote resistance and resistance, regardless of the cause, may have wide reaching negative effects. There are data that indicate that antibiotic resistance (to individual compounds or to classes of antibiotics) can be "shared" by transfer of genetic resistance traits among bacteria of the same or of different species, possibly including human pathogens. Even the possibility of such an occurrence warrants a precautionary approach to use of antibiotics not only in the aquaculture industry but in all areas of human activity.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Research is needed to clearly establish the link between use of antibiotics in aquaculture and the presence of antibiotic-resistant bacteria near aquaculture activities. The spatial and temporal extent of any effects should also be defined.
- Research is needed to determine the consequences of antibiotic use in aquaculture antibiotics. The effects on aquatic organisms (farmed and indigenous), on the micro-flora in the sediments and in the water column and the potential to affect human health should be investigated. The effects of chronic presence of antibiotics have not been investigated.
- Research is needed to develop safe and effective vaccines against bacterial and viral pathogens.
- There is a lack of data from operational situations. Field studies are needed to determine if data from lab-based studies regarding fate, persistence and toxicity are predictive of operational situations. These studies are best conducted with a detailed knowledge of biomass present, disease and treatment history of the site being studied.
- As seen in Table 1 there are significant differences in antibiotic usage between jurisdictions within Canada. Similarly, the quantities of antibiotics used in Canada per metric ton of fish production are significantly higher than that reported in Europe. The cause for these differences should be investigated with a goal to identifying ways to reduce incident of disease and the consequent use of antibiotics. Studies into the nature, frequency and site of bacterial infection in aquaculture are essential in determining why antibiotic use in Canada has increased since 2005.
- A clear understanding of epizootics, the bacteria causing them and their susceptibility to antibiotic treatment can provide an early warning of resistance and identify ways to reduce incidents of disease and the consequent use of antibiotics.

STRESSOR CATEGORY: PESTICIDES AND DRUGS

The principle use of pesticides and drugs is to control sea lice infestations in marine finfish aquaculture which are a major concern from a fish health and marketability perspective. Chemical treatments of sea lice involve applications in feed or by bath treatments (Bright and Dionne 2005, Burridge et al. 2008). Chemical applications in feeds are classified as drugs since they act systemically and are regulated under the Food and Drugs Act, while those used in bath treatments are classified as pesticides since they act topically and are regulated by the Pest Control Products Act. The authorization for drug use in Canada requires no environmental risk assessment, while the pesticide registration process involves an environmental risk assessment which must demonstrate an acceptable level of risk.

The most common in-feed treatments have involved the use of ivermectin, emamectin benzoate (EB) and teflubenzuron (registered as Calicide). Until recently the only product in use to control sea-lice infestations in Canada was the in-feed additive emamectin benzoate (Burridge et al. 2008). Emamectin benzoate (registered under the trade name Slice®) recently received full registration status in Canada. Until 2009 it was used in Canada under Health Canada’s Emergency Drug Release program (Burridge et al. 2008). There is little in-field environmental evaluation as part of that registration procedure. The recommended treatment dose of emamectin benzoate is to feed 50 μg·Kg^{-1} of fish for 7 consecutive days, and aquaculture operations are limited to three treatments per grow-out cycle (Bright and Dionne 2005, Burridge et al. 2008). It is worth noting here that the dependence on a single treatment strategy over the past several years is purported to be causing a lack of efficacy in southwest New Brunswick (Fred Page, DFO, pers. comm. 2009) and has resulted in a recent emergency registration and use of deltamethrin (registered as AlphaMax) and azamethiphos (registered as Salmosan) in bath treatments. A summary of Slice® use in BC and NB is shown in Table 2. In contrast to antibiotic use, the use of anti-louse drugs in Canada, reported relative to production of salmon, is approximately the same as Norway and lower than that reported in Scotland for the same year (Burridge et al. 2010).

Table 2. Use of Slice® in Canada in 2007. Data courtesy of the NB Salmon Growers Association and the government of British Columbia. NA = data not available.

Therapeutant	BC	NB & Maine	NF,NS,QC	Freshwater
SLICE®	0.133 g/MT	~0.240g/MT	NA	NA

Emamectin benzoate enters the environment surrounding marine aquaculture net-pens either by the settling of uneaten food pellets or though excretion by fish. Due to its relatively high lipophilic properties (log K_{ow} = 5) it is expected to become bound to suspended and settled particles. The greatest environmental concern for emamectin benzoate involves sediment dwelling micro and macrofauna (Bright and Dionne 2005).

Emamectin benzoate is persistent in soil with a half life in aerobic soil of approximately 174 days, but has an anaerobic half life of up to 427 days, indicating the potential for sediment accumulation in anoxic conditions known to occur under net pens (SEPA 1999).

Tefler et al. (2006) sampled sediment, water, blue mussels and large fauna for emamectin benzoate near Scottish net pens where the chemical was being used. EB was not detected in water and most sediment concentrations were less than the detection limit (0.25 μg·Kg^{-1}). When EB was detected in sediment the concentration was generally small, in the low μg·Kg^{-1}range. It is notable, however, that the maximum (2.7 μg·Kg^{-1}) was measured within 10m of the net pen four months after treatment. Blue mussels had quantifiable concentrations (maximum 0.96 μg·Kg^{-1}) up to 100 metres from the pen, one week after treatment.

A recent literature review of the fate and effects of emamectin benzoate reports acute toxicity values for a variety of aquatic species ranging from 10^{-5} to 10^{-4} mg·L^{-1} and the lowest NOEC observed in the mysid, *Mysidopsis bahia* (Bright and Dionne 2005).

Emamectin benzoate has been shown to have lethal toxic effects to American lobsters (*Homarus americanus*) and can induce premature moulting; however, the concentrations required to do that are above those which have been measured in the environment to date (Burridge et al. 2000, Waddy et al. 2002, Burridge et al. 2008). Mayor et al. (2008) determined the toxicity of emamectin benzoate in sediments during 10 day exposures to *Corophium volutator* and *Hediste diversicolor*, two common sediment dwelling invertebrates, produced LC50s of 153 μg·Kg^{-1} and 1368 μg·Kg^{-1}, respectively, which are at least two orders of magnitude higher than concentrations measured near aquaculture sites.

There are very little data relating to release and potential impacts of the other registered in-feed chemical, teflubenzuron, in the aquatic environment; however, it has been characterized as persistent in sediment (half life of 104 - 123 days) and may impact the sediment processing rate of the polychaete, *Capitella* sp., at concentrations as low as 8.4 $\mu g{\cdot}g^{-1}$dry weight (Méndez 2005).

There have been a number of chemicals used world wide in bath treatments for sea lice control including azamethiphos, cypermethrin, deltamethrin, hydrogen peroxide, dichlorvos and pyrethrins. Azamethiphos, known as the product Salmosan®, was used in Canada until April, 2005 when the registration was not renewed (Haya et al. 2005). Recently, this product has again been given emergency registration status and is being used to combat sea lice infestations in southwest New Brunswick (Health Canada, Pest Management Regulatory Agency, 2009). Interestingly, the recommended treatment concentration is 300 $\mu g{\cdot}L^{-1}$ compared to 100 $\mu g{\cdot}L^{-1}$ which would could be applied under the previous registration (Burridge 2003).

Hydrogen peroxide has a half life in seawater of about 7 days and it degrades to oxygen and water (Haya et al. 2005). Hydrogen peroxide is perceived as being of relatively low risk as a sea lice treatment; however, there is very little information on the non-target effects of the use of this chemical. It is known to have toxic effects to Atlantic salmon at concentrations of 2.4 $g{\cdot}L^{-1}$, which is near the treatment concentrations of 0.5 $g{\cdot}L^{-1}$ (Haya et al. 2005). As can be expected, hydrogen peroxide is toxic to crustaceans with a 24 h LC50 to the Brine shrimp (*Artemia salina)* 0.8 $g{\cdot}L^{-1}$ (Mathews 1995). It has been shown to cause a decrease in aerobic metabolic rate and intracellular pH in the Sand shrimp (*Crangon crangon)* at concentrations of 0.68 $g{\cdot}L^{-1}$ as a result of 5 hour exposures (Abele-Oesschger et al. 1997). Those concentrations are also near treatment concentrations.

Azamethiphos is an organophosphate which is expected to remain in the aqueous phase after application due to its relatively high water solubility (1.1 $g{\cdot}L^{-1}$) and low log K_{ow} (1.05) (SEPA, 1997). Azamethiphos is toxic to crustaceans; exposure to concentrations of 10-25 $\mu g{\cdot}L^{-1}$ for 15-30 minutes produced significant mortalities in adult lobsters (Burridge et al. 2000). In that study, early life stages of lobsters were found to be less sensitive than adults. At concentrations of 10 $\mu g{\cdot}L^{-1}$, and during biweekly 1 hour exposures, many adult female lobsters died. Survivors showed sublethal effects, such as not spawning as often or at the same time as control lobsters (Burridge et al. 2008a). Ernst et al. (2001) measured the toxicity of azamethiphos to a number of species including: the bacterium *Vibrio fisheri;* the Green sea urchin (*Lytechinus pictus)* (fertilization); the Threespine stickleback (*Gasterosteus acualeatus);* three amphipods (*Amphiporeia virginiana, Gammarus spp; and Eohaustorius estuaries);* a polychaete (*Polydora cornuta);* Brine shrimp (*Artemia salina);* and a rotifer (*Brachionus plicatilis).* They determined that amphipods were most sensitive with *Eohausorius estuarius* having a 48 h EC 50 (immobilization) of approximately 3 $\mu g{\cdot}L^{-1}$and selected that organism as a test species for subsequent dispersion measurements from simulated net-pen releases. The results of that study, using a dye tracer, found that 1/200 - 1/3000 the release concentration were not achieved until post-release times ranging from 2-5.5 h. Most samples from the plume were not toxic when azamethiphos was the test pesticide and none were toxic past 20 minute post release, by which they interpreted azamethiphos to have low environmental risk compared with the other pesticide they tested, cypermethrin.

Cypermethrin (Excis®) was proposed for Canadian registration in the late 1990s; however, field dispersion and toxicity studies (Ernst et al. 2001) indicated the environmental risk from the use of that chemical was high and the registration request was denied on that basis. Cypermethrin has been shown to be extremely toxic to crustaceans such as lobster (*Homarus americanus)* and sand shrimp (*Crangon septemspinosa*) (McLeese et al. 1980, Hill 1985, Burridge et al. 2000). While the 96 h LC 50 to mysid shrimp is as low as 0.005 $\mu g \cdot L^{-1}$, which is approximately 1/100 the recommended dosage rate for sea lice treatment (Hill 1985), lobster larvae mortality was significant at concentrations of 1/100 of treatment dosage rates even under exposure durations as low as 1 h. (Pahl and Opitz 1999). Burridge et al. (2000) have shown that repeated short term exposure produces similar toxicity responses to adult lobster as does long term exposure to lower concentrations and that exposure to concentrations as low as 10% of the treatment concentrations for periods as low as 15 minutes produced significant mortality. In a dispersion study, water samples taken from the plumes of cypermethrin were found to be toxic in 48 h exposures to the amphipod *Eohaustorius estuarius* when samples were taken up to 5 h post release, at distances ranging from 900 to 3000 m from the release site (Ernst et al. 2001). Using dye, that study documented general dilution rates of 1/200 to 1/2000 in time periods ranging from approximately 3-5 h post release.

Due to its limited water solubility and lipophilic nature, cypermethrin is expected to adsorb to particles and sequester to bottom sediments (Maund et al. 2002). Mayor et al. (2008) reports 10 day LC 50s of sediment-borne cypermethrin to *Corphium volutator,* a common sediment dwelling invertebrate, as 5 $\mu g \cdot Kg^{-1}$, indicating a potential risk at aquaculture sites with repetitive use.

While it cannot be directly extrapolated to the marine situation, and the use of cypermethrin is not known in fresh water aquaculture, it is worth reporting that in microcosm studies in eutrophic lakes, the abundance and composition of freshwater communities were significantly altered by nominal exposure concentrations of 0.13 $\mu g \cdot L^{-1}$ cypermethrin (Friberg-Jensen et al. 2003). The primary effects (abundance) were mostly for crustacean zooplankton with a median effect concentration (EC 50) of 0.04 $\mu g \cdot L^{-1}$. In laboratory assays, cypermethrin was acutely lethal (48 h exposures) to planktonic marine copepods at concentrations as low as 0.14 $\mu g \cdot L^{-1}$, which is less than the sea lice treatment concentrations of 5 $\mu g \cdot L^{-1}$, but they interpret little environmental risk from such exposures (Willis and Ling 2004).

A closely related chemical, deltamethrin, has recently received emergency registration in Canada for bath treatment of sea lice in response to reported lack of control by emamectin benzoate. Deltamethrin is also extremely toxic to crustaceans; the 96 h LC 50 to adult lobsters being 0.0014 $\mu g \cdot L^{-1}$ (Zitko et al. 1979).

Recognizing that the reported toxicity values may overestimate risk potentials because of their higher exposure periods than would be expected after operational treatments, the data of Zitko et al. (1979) were re-calculated to derive lethal concentrations for short term exposures. Those calculations indicated that approximately 0.5 $\mu g \cdot L^{-1}$would be toxic to adult lobsters for exposure periods of about 6 h, which correlate with the dispersion measurements of Ernst et al. (2001). Gross et al. (2008) also reported Brown shrimp toxicity of 0.14 $\mu g \cdot L^{-1}$ for 6 h exposures. Those values represent a dilution of 1/10 - 1/35 are required to meet mean lethal levels which, according to the data from Ernst et al. (2001), could take from 5 minutes to 1 hour post-release.

Because of its lack of water solubility, high lipophilicity and high adsorption coefficients deltamethrin is predicted to absorb preferentially to particles, particularly those with high organic content and to sequester to bottom sediments (Muir et al. 1985). The half life for deltamethrin in marine sediments has been estimated at approximately 140 days, indicating that multiple treatments may result in accumulation of this compound in sediments near cage sites (Gross et al. 2008). Unfortunately, no published literature could be found on the toxicity of deltamethrin to benthic organisms and that risk cannot therefore be accurately assessed.

There are some data which suggest that deltamethrin may have a sublethal effect on the immune function of fish (Pimpão et al. 2007, Pimpão et al. 2008); however, the exposure to pesticide was by injection and the environmental relevance is unclear.

STRESSOR –EFFECTS

Effect: presence, effects and consequences of pesticides and drugs in and on non-target organisms: Other fish, aquatic invertebrates and micro-organisms.

1. **<u>What is known about the stressor-effect relationship?</u>**

   The in-feed chemicals emamectin benzoate and teflubenzuron are known to be ephemeral in the water surrounding net pens but are relatively persistent in adjacent sediments and there is the potential for accumulation with continual use. Bivalves in the vicinity of net pens have been shown to have measurable quantities of emamectin benzoate. Currently, most hazard information is based on acute exposures; however, they do not indicate a high level of risk.

   Due to the use of aqueous solutions in pesticide bath treatments which are at toxic concentrations to the arthropod target species and the resultant free release of the treatment solution, such control techniques are judged to have a higher level of risk than in-feed treatments. The pesticides are for the most part shorter lived and more rapidly dispersed than the drugs; however, the pyrethroids (cypermethrin and deltamethrin) have the potential to sequester to sediments through adsorption to highly organic particles and may be relatively persistent in that compartment. The aqueous dispersion characteristics are very site specific and not well known. Again, most of the hazard assessment has been based on acute waterborne exposures.

   There is very limited non-target toxicity information for hydrogen peroxide; however, some species (Brine shrimp) have acute lethal thresholds near the treatment concentrations.

   Azamethiphos, under very short term exposures, has lethal and sublethal (spawning reduction) thresholds to lobsters which are much less than treatment concentrations. Field exposures are limited, but indicate arthropod toxicity was not demonstrated more than 20 minutes post-release of treatment solutions.

   The pyrethroids, cypermethrin and deltamethrin, are known to be highly toxic to marine arthropods under very brief laboratory exposures (1 h) to concentrations as 1/100 treatment concentrations. Dispersion studies have shown such dilutions do not take place for approximately 1 h post-release.

2. **What are the (measurable) ecological outcomes or endpoints (effects profile).**

There are few data on the ecological effects of pesticides and drugs. The in-feed chemicals present a risk to benthic organisms due to their sequestration and persistence in sediments; however, no reports were found which quantify such risks. The available laboratory generated hazard values would seem to indicate the risk is low under operational conditions.

Although there are more data available on the environmental fate and effects of bath treatments, there has been little effort to measure ecological effects.

Hydrogen peroxide probably poses the least environmental risk since its aqueous target concentration is below what has been found to be acutely toxic and it does not move to sediments.

Because azamethiphos is primarily in the aqueous phase, its acute effects to water column and epibenthic organisms would be where ecological effects are expected. Although no data are available on those effects, laboratory hazard assessments would suggest that lobster population impacts are a possibility either through direct mortality or reproductive impairment.

The pyrethroids, cypermethrin and deltamethrin, have the same general toxicity and environmental behaviour profiles, namely that they are highly toxic in the aqueous phase and do have the potential to sequester to sediments where they may persist. The laboratory-generated toxicity information suggests that these pesticides present a relatively high risk to marine arthropods represented by zooplankton and benthic invertebrate species. The dispersion information available suggests that such risks may extend over relatively large areas; however, no empirical ecological effects data are available for the marine environment. Ecological effects on the abundance and composition of water column zooplankton communities has been demonstrated in freshwater systems at concentrations well below those used for sea lice control.

3. **What is the magnitude of the effect?**

The magnitude of effects cannot be well established; however, the potential for effects is judged to be greatest for the bath use of pyrethroid pesticides. Pesticide and dye dispersion studies have indicated the possibility that plumes may remain toxic over several square kilometres from single cage releases.

4. **What are the factors and conditions that modify or influence the expression of the effect (e.g., exposure, type of receiving environment).**

For those chemicals which sequester to sediments, areas of low energy are of highest risk. Highly turbid, or water of high organic content, will likely reduce the risk for those chemicals which have high adsorptive coefficients such as the pyrethroid pesticides. Most of the risk assessments for use of sea lice control chemicals have been done on the basis of single cage treatments. Most treatments are done to multiple cages concurrently and to additional cages over a number of days. There are likely additive risks from such treatments.

5. **What are the biological implications of the effect on the overall ecosystem function?**

   Since the target species for use of these chemicals are arthropods, they have been selected for their toxicity to that taxa, and arthropods are some of the most sensitive non-target species. As well as being important food web components in marine ecosystems, arthropods are important commercial species which are fished and held in close proximity to aquaculture sites. Effects on lobsters cannot be eliminated as a possibility and would be of concern to local fishers.

6. **What type of evidence is available (e.g., lab studies, models, etc.) and what is the strength of evidence used to determine the stressor-effect relationship?**

   The weight of evidence for effects is primarily based on hazard quotient kind of assessments, namely laboratory-generated toxicity values are compared with measured or calculated environmental concentrations. There are few data which have been collected to measure environmental impacts in the field. There is also some product manufacturer information available; however, because it is privileged, it cannot be used for publicly available risk assessments.

7. **What are the uncertainties associated with this stressor-effect linkage?**

   The assessment of risk associated with the use of pesticides and drugs has been made on single cage treatment and there has been little effort to evaluate the effect from concurrent and consecutive cage treatment. Some of the free bath residues are predicted not to degrade or disperse within a single tidal cycle and the practice has been to treat several cages within the same farm site concurrently and it may take a number of tidal cycles to treat a single farm site. In addition, adjacent farms will be treating at the same time and the density of farms in certain Canadian locations is high, the cumulative effects of such activity need to be estimated and measured.

   The interactive effects of multiple chemical stressors cannot be estimated at this time. As described above there has not been much effort made to measure field impacts from chemical releases.

8. **During which activities does this stressor-effect occur?**

   During sea lice control activity.

9. **What are the cascading effects or linkages from this effect?**

   There is the possibility that pest resistance to continuous chemical treatment will develop. Local population effects may occur which may lead to changes in community structure as some species are eliminated even over the short term.

CONCLUSIONS

The environmental effects of sea lice control chemicals have seen a considerable amount of research attention. The use of in-feed treatments, such as emamectin benzoate and teflubenzuron are generally believed to present a lower risk to the environment than bath treatment pesticides, which in turn release large volumes of chemical solutions and have greater dispersive characteristics. Hydrogen peroxide represents the lowest risk bath treatment, azamethiphos a moderate risk, while the pyrethroid pesticides, cypermethrin and deltamethrin represent the greatest environmental risk, with impacts on crustacean species such as lobster and shrimp being of the most concern. Dispersion studies have indicated that there are areas as large as 1.3 square km which may receive toxicologically relevant exposure from the treatment of a single cage. Although pyrethroid pesticides have not previously been used operationally in Canada, an Emergency Registration for the use of deltamethrin has recently been given, and the fate and effects of that chemical are currently being monitored as a condition of registration.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Sea lice control chemicals have the potential to cause non-target effects and bath treatments with pyrethroids presenting the highest risk; however, the magnitude of the impacts in the field has not been determined. Sediment accumulation of emamectin benzoate should be further evaluated and toxicity to a wider range of benthic invertebrates explored.
- Use of deltamethrin bath treatments presents the potential for further field impacts from acute toxic effects on native crustaceans and this should be determined under operational conditions. Repeated use of deltamethrin could result in sediment accumulations which should be further evaluated along with benthic community effects.
- The hazards and risks associated with multiple concurrent and consecutive cage treatments needs to be quantified. This work should include assessment of cumulative effects associated with repeated use of single compounds as well as assessing potential effects associated with use (or presence) of multiple therapeutants.
- Hazard identification must include studies on sublethal effects and chronic exposures.

STRESSOR CATEGORY: METALS

The two metals of concern with respect to aquaculture activity in Canada are copper (Cu) and zinc (Zn). Copper is the active ingredient in antifoulant paints and is also a constituent in food. Zinc is a supplement added to fish food. The presence and effects of Cu and Zn from finfish aquaculture have been reviewed recently by Burridge (2003) and Burridge et al. (2008). These documents have been relied on heavily in preparing this review. Scott (2004) reviewed chemical inputs from freshwater aquaculture, but did not address metals. Since most freshwater aquaculture activity in Canada is carried out in land-based, hatchery, pond or recirculation systems, the use of antifoulants is not required.

COPPER

Copper-based antifouling paints are used to treat nets in aquaculture operations. Copper reduces the build-up of biota on nets which, in turn, allows the free flow of water and reduces the need for frequent net changes (Debourg et al. 1993). 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. Antifouling paints are composed of 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, Tri-butyl tin (TBT) paint was available with a co-polymer formulation which had slower releases to the environment. However, TBT can no longer be used in antifoulant paints for aquaculture and 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.

Copper is highly toxic to aquatic organisms, may bioaccumulate, and concentrations greater than 100 to 150 mg (Cu)·Kg^{-1} (dry weight) in sediment may reduce the diversity of benthic fauna (Debourg et al. 1993). A report submitted to the Scottish Executive (2002) suggests the use of Cu antifoulant paints in aquaculture may be reason for concern due to its potential to accumulate in sediment. The toxicity of Cu in water is greatly affected by the chemical form of the Cu (“speciation”), and to what degree it is bound to various ligands that may be in the water that make the Cu unavailable to organisms. The salinity and pH also affect toxicity of Cu. Grosell et al. (2007) showed that killifish are most sensitive to Cu 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 Cu 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 Cu. The toxicity was directly proportional to the concentration of free cupric ion (Sunda and Lewis 1978). The toxicity of Cu 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 in the water reduced the toxicity of Cu to embryos of the oyster *Crassostrea gigas* (Knezovich et al.1981). In a study of toxicity of Cu from mining operations, it was found that the Cu in the water was not toxic to the diatom *Nitzschia closterium* because the Cu 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 Water

As Cu is used as an antifoulant, it is not surprising that among the most sensitive groups to Cu are the algae. Considerable work has been done that shows Cu in water affects microalgae as well as developmental stages of macroalgae. Effects include: growth reduction (Cid et al. 1995), altered biochemistry (Rijstenbil et al. 1994), cell division (Franklin et al. 2001), ultrastructure changes (Visviki and Rachlin1992), sporophyte production and growth (Martin et al. 1990) and others. Bacterial biochemistry and community structure is also affected by Cu (Jonas 1989). The concentration shown to affect microorganisms is in the range of 1-10 µg·L^{-1}. These are results from lab-based studies and it is difficult to determine if similar effects occur in the wild. The probability exists that organisms at the base of the food chain could be affected.

Copepod, amphipods and crustaceans have also been shown to be sensitive to Cu. For example, natural copepod assemblages exhibited sublethal responses, such as changes in fecal pellet production, and egg production, when exposed to Cu levels in the 1-10 $\mu g \cdot L^{-1}$ range (Reeve et al. 1977). There can be seasonal as well as life history differences in sensitivity to Cu. The acute toxicity of Cu to coastal mysid crustaceans was much greater in the summer than in the winter (Garnacho et al. 2000). Survival of *Acartia tonsa* 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). Young et al. (1979) showed molt delay in the shrimp, *Pandanalus danae,* at concentrations less than 1 $\mu g \cdot L^{-1}$ labile Cu.

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.

Embryos of the Pacific oyster, *Crassostrea gigas*, were exposed to Cu and silver salts alone and in combination. Cu 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.

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.

Larval Topsmelt, *Atherinops affinis*, were exposed to copper chloride for 7 days. Copper was more toxic at lower salinities, with an LC50 of ~200 $\mu g \cdot L^{-1}$ at high salinity and ~40 $\mu g \cdot L^{-1}$ 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 Cu 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.

Copper in Sediments

Metals such as Cu have relatively low solubility in water and tend to accumulate in sediments. The critical issue regarding toxicity of Cu (and other metals) in sediments is what fraction of the Cu is actually bioavailable. 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 Cu (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). 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.

Despite the binding of Cu 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 sea urchin survival were very sensitive indicators of pore water toxicity. In addition to elevated levels of Cu (above the threshold effects level), the sediments also had elevated Zn, other metals, ammonia nitrogen, sulfide, TOC, and other organic compounds, so the toxicity cannot be attributed solely to Cu. Sediments enriched in Cu, Zn 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 Cu alone, and it is difficult to attribute toxicity to any particular metal.

A study of Cu on fauna of marine soft sediments was performed by Morrisey et al. (1996) who experimentally enhanced Cu in the tested sediments and monitored them over six months. 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 behavioural responses of burrowing organisms to Cu-contaminated sediments. Burrowing time of the clam *Protothaca staminea* was increased at contamination levels above 5.8 $\mu g \cdot g^{-1}$ Cu (dry wt of sediment). 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).

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.

The release of antifoulants into the marine environment is controlled by local and/or national waste discharge regulations (Costello et al. 2001). Generally elevated Cu has been observed in sediments by salmon aquaculture facilities. Debourg et al. (1993) reported that concentrations exceeding 100-150 $mg \cdot Kg^{-1}$ dry weight may reduce benthic fauna. While a number of authors report Cu concentrations in excess of the sediment quality criteria, concentrations of greater than 100 mg·Kg^{-1} are not common (Burridge et al. 1999, for example). In a study of British Columbia fish farms, Brooks and Mahnken (2003) found that 5 out of 14 farms had Cu levels exceeding sediment quality criteria. The average Cu in reference stations was 12 mg·Kg^{-1} dry sediment, while under farms using Cu-treated nets it was 48 mg·Kg^{-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·Kg^{-1} while in anoxic sediments 50 m away it was 38.5 mg·Kg^{-1}. Parker and Aube (2002) found Cu in sediments was elevated compared to Canadian sediment quality guidelines in 80% of the aquaculture sites they examined. The Cu would have come from the antifouling paints and possibly also from its use in salmon feeds.

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.

Salmon tissues from fish in net pen operations were analyzed for Cu (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 Cu 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 Cu (Chou et al. 2002). Lobsters from the aquaculture site with the poorest flushing had accumulated 133 µg·g^{-1} in the digestive gland, while those from a control site had only 12.4 µg·g^{-1}in their digestive glands.

Brooks (2000) studied the leaching of Cu from antifouling paints and found initial losses of 155 µg Cu·(cm^2)$^{-1}$·day^{-1} and that rates declined exponentially. He developed a model that suggested that the EPA Cu 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·s^{-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 Cu in the water. Lewis and Metaxas (1991) measured Cu 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 Cu in water in the cage was 0.54 µg·L^{-1}, while it was 0.55 µg·L^{-1}outside the net and 0.37 µg·L^{-1} (not significantly different) at a station 700 m away. Similar levels were found one month later.

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 Cu (Burridge et al. 2008). Parker et al. 2003 exposed the marine amphipod *Eohaustorius estuarius* to sediments collected from under a cage site. In addition, samples of clean sediment were directly spiked with the copper-based anti-fouling coating Flexgard used by salmon aquaculture operations in NB at concentrations up to 5 times greater than the Canadian Council of Ministers of the Environment Probable Effect Level (CCME 1999), but there was no apparent effect on the amphipods. The authors attribute this to the lack of availability of the Cu. 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 Cu more available. The level of copper in aquaculture sediments diminishes during periods when no fish are on the site (fallowing). The fate of the copper and its potential to affect aquatic species is unknown (Burridge et al. 2008).

In a recent study from Norway, Borufsen Solberg et al. (2002) found no difference in Cu concentrations in tissues collected from fish, invertebrates, and macro-algae in and around aquaculture sites using copper-treated nets compared to samples collected from sites where copper-treated nets were not in use. Similarly, Lewis and Metaxas (1991) determined Cu concentrations in water collected inside and outside a freshly (Cu) treated salmon cage as well as 700 m away from the cage. These concentrations were not significantly different at the three sites and remained stable for over a month.

ZINC

Zinc is added 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 Cu, Zn, iron, manganese, and others. Zinc, like Cu, binds to fine particles and to sulfides in sediments, and even when it is bioavailable, it is much less toxic than Cu. Issues of speciation, bioavailability in the water column, and acid volatile sulfide (AVS) in the sediments are similar to those discussed earlier for Cu. Zinc in ionic form can be toxic to marine organisms, though generally at considerably higher concentrations than Cu.

<u>Zinc in Water</u>

Marine algae are particularly sensitive to Zn. 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. Stauber and Florence (1990) found that 65 $\mu g \cdot L^{-1}$ affected the cell division rate, but not photosynthesis or respiration. These endpoints were unaffected up to 500 $\mu g \cdot L^{-1}$. Most of the Zn was bound at the cell surface.

Arnott and Ahsanullah (1979) studied acute toxicity to the marine copepods *Scutellidium* sp., *Paracalanus parvus* and *Acartia simplex*. The 24-h LC50 value for Zn was 1.09 $mg \cdot L^{-1}$. Copepod (*Acartia tonsa*) egg production was adversely affected by Zn 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 Zn (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 Zn on larvae of the shrimp *Farfantepenaeus paulensis*. Chronic exposure to Zn (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 Zn concentrations tested. The inhibition of food and oxygen consumption could explain in part the long-term reduction of growth. Sea urchin (*Sterechinus* *neumayeri*) embryos were killed by concentrations as low as 0.327 $mg \cdot L^{-1}$ Zn (King 2001).

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.

Zinc in Sediment

Sediment Zn 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. Zinc 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).

Concentrations of Zn in feeds produced for Atlantic salmon range from 68 to 240 $mg \cdot Kg^{-1}$. 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).

Elevated Zn has been found in sediments below and around salmon cage cultures. Burridge et al. (1999) and Chou et al. (2002) found elevated Zn 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 $\mu g \cdot g^{-1}$. They determined the level of Cu and Zn in fish feed and fish feed constituents, in addition to sediments collected near aquaculture sites in southwest New Brunswick. These metals showed a positive correlation with the level of organic carbon, and the parameter appeared to be related to hydrographic characteristics at individual sites. Burridge et al. (1999) also reported concentrations of metals in sediments collected near aquaculture sites. As stated above, Cu concentrations exceeded recommended sediment quality guidelines. Similarly, Zn concentrations in some samples were found to exceed the threshold effects level (CCME 1999). The authors suggested Zn may have contributed to some lethal effects in standard invertebrate bioassays.

Parker and Aube (2002) similarly found that the average sediment Zn concentration in sediments under salmon pens exceeded the Canadian interim sediment quality guidelines. In another Canadian study, Zn concentrations declined to background at >200 m from the cages (Smith et al. 2005). Brooks and Mahnken (2003) found that Zn 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 Zn concentrations were down to a background of 25 $\mu g \cdot g^{-1}$.

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 molluscs recruiting into the sediments.

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

Zinc, like Cu, binds to fine particles and to sulfides in sediments, and even when it is bioavailable, it is much less toxic than Cu. Organically enriched fish farm sediments generally have a high biological oxygen demand and negative redox potential; conditions that lead to sulphate reduction. Under these conditions, metals such as Cu and Zn 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. Since elevated levels of Cu and Zn 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 Zn reduces the toxic effects of the Cu.

OTHER METALS

A recent report (DeBruyn et al. 2006) indicates that mercury was elevated in fillets of native 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$\cdot Kg^{-1}$. Parker and Aubé (2002) reported nickel, cobalt and iron concentrations were not significantly different from background and did not appear to be of any environmental concern. Chou et al. (2002) showed that manganese concentrations in sediment showed a negative correlation with the level of organic carbon.

Burridge et al. (1999) reported cadmium was found in sediments near aquaculture sites at concentrations higher than Canadian Disposal at Sea regulated limit of 0.6 $\mu g \cdot g^{-1}$. Lead and mercury were also measured, but levels did not exceed any established thresholds. Mercury has also been measured in fish meal, fish feed and fish oil of various origins (CFIA 2005). The average concentration in fish meal and fish feed ranged from 0.1 to 0.3 mg$\cdot Kg^{-1}$. No mercury was detected in fish oil samples. These data, the authors suggest, indicate that it would be unlikely that fish would accumulate mercury levels above the maximum limit prescribed by the Canadian Guidelines for

Chemical Contaminants and Toxins in Fish and Fish Products of 0.5 $mg\cdot Kg^{-1}$ (CFIA 2005).

STRESSOR –EFFECTS

Effect: Presence, effects and consequences of metals in and on non-target organisms: Other fish, aquatic invertebrates and micro-organisms.

1. <u>What is known (state of knowledge) about the stressor-effect relationship?</u>

Copper and zinc concentrations in sediments under and near salmon aquaculture sites are well studied and there is an abundance of literature on the potential effects of these metals. The species that may be affected by elevated concentrations are site specific. However, the initial step of the Stressor – Effect Logic Diagram (Figure 1) is probably the strongest for these compounds compared to other metals discussed in this paper. There is no stressor effect diagram for copper released from treated nets.

These statements hold true for salmon aquaculture. The presence and potential effects of metals in the environment as a result of activities associated with freshwater cage culture and mussel culture is not known.

2. <u>What are the (measurable) ecological outcomes or endpoints (effects profile)?</u>

Copper and zinc have been measured near salmon aquaculture sites at concentrations above regulatory guidelines. This infers that negative consequences could be expected. These include changes in population structure, including absence of certain species. However, at many salmon aquaculture sites, metal concentrations are elevated in conjunction with high organic loading. It becomes difficult to confirm that changes in populations or communities are related to concentrations of copper and zinc. It is more likely that other factors have a greater influence and that elevated metal concentrations are coincident as opposed to causative.

3. <u>What is the magnitude of the effect?</u>

Given the current state of knowledge the magnitude of the effects of metals is unknown.

4. <u>What are the factors and conditions that modify or influence the expression of the effect (e.g., exposure, type of receiving environment, etc.)?</u>

As discussed above, metals may be present in high concentrations on sediments associated with aquaculture activity. Because of the chemical nature of the sediments, the metals may not be available to non-target organisms. In fact several papers have shown that effects are not necessarily a consequence of elevated concentrations. Changes in conditions that result in a “re-working” of sediments may make the metals bioavailable.

5. What are the biological implications of the effect on the overall ecosystem function?

Given the current state of knowledge, the effects strictly attributable to metal concentrations are unknown. However, these elements are found in close association with build-up of organic material and they likely play a role in cumulative effects associated with aquaculture activity.

6. What type of evidence is available (e.g., lab studies, models, etc.) and what is the strength of evidence used to determine the stressor-effect relationship?

Lab-based studies have shown clear dose-response relationships between copper and biological effects at the cellular, biochemical, organism and community level. The evidence is quite strong in this regard. While analysis of field samples clearly shows presence and persistence of metals, there are very few effects data available. Where studies have been conducted near aquaculture operations, no effects have been observed. Models are available that predict movement of food, faeces and water near aquaculture sites. As is the case with all compounds discussed these models have not been validated to the extent that predictions can be made with respect to effects.

7. What are the uncertainties associated with this stressor-effect linkage? Where would further information lead to a more complete understanding? Which uncertainties most prevent a more holistic understanding of the effect profiles and biological implications on overall ecosystem function?

As stated above, there are data showing physical chemical and community changes near aquaculture operations. It is unclear what role specific chemicals play in the community changes. It may not be important to isolate specific inputs and try to affix a risk factor to that input. While an interesting exercise, it may not help mitigate effects. For example, if antifoulants were no longer used, it is unlikely that the conditions under salmon cages would change to a noticeable extent.

8. During which activities does this stressor-effect occur?

Metals reach the environment through feeding and leaching from antifoulant-treated nets. Feeding is a regular occurrence which can be predicted. Leaching is ongoing and may increase when the net is new, during storms or during net cleaning activities. Although it is recommended that on-site net cleaning does not take place, it is unclear whether or not the practice still takes place.

9. What are the cascading effects or linkages from this effect?

The potential exists for all chemical inputs to contribute to cumulative exposure and effects. There is a clear linkage to organic loading.

CONCLUSIONS

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.)

Use of metal in aquaculture appears to be one area where choices can be made. If alternatives to antifoulant paints are available or if there are seasons when treated nets are not essential, the input of metals to the environment could be reduced. Similarly, the use of more bioavailable forms of zinc in food formulations will significantly reduce input of this metal.

Metal concentrations in excess of sediment quality guidelines have been found in association with aquaculture operations. The link between operations and concentrations appears to be strong. As in the discussion of other chemicals, the linkages to effects are less convincing. Guidelines are determined for precautionary reasons. The fact that levels of metals are present at concentrations in excess of guidelines suggests that caution and precaution should be exercised in the use of copper and zinc in aquaculture activities.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Recommendations are in place that nets not be washed in the open ocean, where antifoulants can be released into the water. No on-site net cleaning should take place.
- All antifouling paints should be tested for toxicity to different taxa of aquatic organisms. As the toxicity of the active ingredient is often known, the formulations should be tested.
- Work should continue to develop alternative forms of antifoulants to reduce the dependency on copper-based paints.
- Research needs to be conducted to determine the fate of copper in sediments during fallow periods at salmon aquaculture sites.

STRESSOR CATEGORY: HYDRATED LIME

The proliferation of invasive tunicates in Prince Edward Island (PEI) estuaries has necessitated the development of approaches for managing tunicates that foul aquaculture structures. Spraying or immersion with a saturated solution of hydrated lime (calcium hydroxide) is effective against these tunicates, but has also been shown to be biocidal to a variety of non-target organisms as demonstrated by laboratory bioassays (Locke et al. 2009). Hydrated lime has the potential to alter estuarine pH, which should remain within the range 7.0-8.7 units unless it can be demonstrated that such a pH is a result of natural processes (CCME 1999). The pH of saturated hydrated lime solutions used for immersing mussel socks reached 12.6, but the “pH footprint” in the estuarine water column was limited to a radius of <1 m around the treatment site and rapidly returned to ambient pH. (Neil MacNair, PEI, Dept. Fisheries, Aquaculture and Rural Development personal communication; Locke et al. 2009).

The rate of use of hydrated lime has been estimated at 1 to 2 bags of lime used per 600 ft line (400 socks). Growers can treat 6 -10 lines per day therefore use approximately 450 - 1000 lbs per day at one site (Neil MacNair, PEI, Dept. Fisheries, Aquaculture and Rural Development pers. comm.). Conversion of hydrated lime to calcium carbonate should be rapid at daily application rates (<0.007 tonnes/ha of hydrated lime in powdered form). This represents the maximum amount likely to be used in mussel aquaculture for tunicate management in PEI (Locke et al. 2009). At these relatively low inputs, dilution by tidal mixing is likely to return the pH to normal values within a tidal cycle in most PEI estuaries, even without considering the chemical conversion to calcium carbonate which would be occurring simultaneously (Locke et al. 2009).

The primary goal of tunicate management in PEI is the removal of large masses of tunicates from Blue mussel (*Mytilus edulis* Linnaeus, 1758) aquaculture infrastructure. Pressure washing, calcium hydroxide (hydrated lime), or some combination of these treatments are generally applied to bivalve aquaculture gear on the aquaculture site. Tunicate treatments are commonly perceived by those involved in the industry to have negligible impacts on non-target biota and the environment, but potential impacts in PEI have not been rigorously studied. "Lime" has a long history of use in biological control for aquaculture or fisheries purposes (e.g., Wood 1908). At least two forms have been used: quicklime (calcium oxide, CaO), which is produced by heating limestone (calcium carbonate, $CaCO_3$) to drive off the carbon dioxide; and hydrated lime (calcium hydroxide, $Ca(OH)_2$) which is produced by adding water to quicklime. Neither form is registered as a pesticide in Canada.

Hydrated lime has been used for years to control predatory starfish on mussel seed (spat) collectors in PEI; the method, which consists of briefly immersing each collector in a trough filled with a saturated solution of hydrated lime in seawater, has been adapted for tunicate management on mussel socks and other aquaculture gear. Alternatively, a low volume hydrated lime sprayer may be used. Hydrated lime is currently in use for tunicate control by some aquaculture growers, although other growers use pressure washing or some combination of the two methods. Hydrated lime is a chemical compound with the chemical formula $Ca(OH)_2$. It is a colourless crystal or white powder and is obtained when calcium oxide (quicklime) is slaked with water. It is soluble in water at 0.160 g/100 g (CRC 2005) and reacts with carbon dioxide dissolved in seawater to form calcium carbonate with the chemical formula $CaCO_3$. It is a common substance in rocks around the world and the main component in shells of marine organisms, and so is considered innocuous. The solubility of calcium carbonate is much lower than hydrated lime at 0.00066 g/100 g water (CRC 2005), and has the potential to precipitate out of solution and settle to the bottom in particulate form as the reaction occurs.

The use of lime is not known in fresh water aquaculture or from provinces other than PEI. In addition, it has been suggested than acetic acid is used to control tunicate populations on mussel socks. The authors have confirmed that there is no operational use of acetic acid in Canada for tunicate control (Neil MacNair, PEI, Dept. Fisheries, Aquaculture and Rural Development personal communication, 2009). This compound therefore, is not discussed in the present document.

Lime in Water

Observations on treatment usage, "pH footprint" around treatment sites (measured with a portable pH meter), were made in PEI estuaries (Andrea Locke, DFO, pers. comm. 2009 and Neil MacNair, PEI, Dept. Fisheries, Aquaculture and Rural Development pers. comm. 2009). The pH measured in hydrated lime troughs during immersion of mussel socks reached a maximum value of 12.6 pH units. A cloud of lime particles was visible in the water immediately below the area where the treated sock exits the lime trough, and the pH in this area was ~10, but readings rapidly (as fast as the pH meter could register the change) dropped to pH 8.3-9.0 approximately 0.7 m from the area of discharge, and were always <8.5 approximately 1 m from the area.

Locke et al. (2009) investigated the toxicity of hydrated lime in order to identify potential effects on non-target biota in coastal waters of PEI. They conducted laboratory bioassays of hydrated lime with the bacterium *Vibrio fischeri*, Sand shrimp *Crangon septemspinosa* and Threespine stickleback *Gasterosteus aculeatus*. The 96-hour LC50 to the fish Threespine stickleback was 457 $mg{\cdot}L^{-1}$ (95% confidence limits (CI) of 262 - 785 $mg{\cdot}L^{-1}$). Based on the pH values measured at t=0 this LC50 was equivalent to 10.47 (10.26 - 10.52) pH units. The 96-hour LC50 to sand shrimp was 158 $mg{\cdot}L^{-1}$ (CI = 50 – 500 $mg{\cdot}L^{-1}$). The equivalent pH value would be 9.70 (9.12 - 10.3). The 14 day LC50 to sand shrimp was 53.1 $mg{\cdot}L^{-1}$ (CI = 48.3 - 58.4 $mg{\cdot}L^{-1}$), or an equivalent pH of 9.20 (9.12 - 9.28). Exposure to lime did not affect the growth of Sand shrimp in this chronic exposure. Light inhibition of bacteria in the Microtox test (IC50) occurred at lower concentration of hydrated lime than the lethal effects on fish or shrimp. The IC50 was 31.0 $mg{\cdot}L^{-1}$ (CI = 18.8 - 51.4 $mg{\cdot}L^{-1}$), corresponding to a pH of ~ 9.0.

American lobster (*Homarus americanus*) are an important commercial species, and larvae are distributed in coastal PEI waters from early or mid-June to mid-September (Harding et al. 1982, Scarratt 1964), but what proportion of the population occurs inside the estuaries is unknown. Lobster larvae are known to be intolerant of quicklime (Loosanoff and Engle 1942) and the susceptibility to hydrated lime was presented by Doe et al. (2009). Exposures were varied to give a range of exposure scenarios, including realistic short term pulse exposures followed by observations on growth and survival in clean seawater. Results are presented in Table 3.

Table 3: Results of toxicity tests on Hydrated Lime Suspensions with larval American lobster

Test Exposure	**LC50 as** $mg{\cdot}L^{-1}$	**LC50 as pH**
96 hours continuous	121 (CI = 73.5-198)	9.73 (9.47-9.99)
1 hour pulse, followed by 12 days in clean seawater	965 (CI = 633-1470)	10.6 (10.2-11.0)
3 time 1 hour pulse on consecutive days, followed by 9 days in clean seawater	606 (CI = 336-1090)	10.5 (10.1-10.9)

Doe et al. (2009) reported that lobster behaviour was observed to be affected by plumes of hydrated lime. The tail flicks are probably in response to encountering small particles of undissolved lime. Tail flicks decrease as particles settle to the bottom of the jars. Comparison of behavioural changes caused by hydrated lime with another inert particle of similar size such as calcium carbonate is recommended to further understand this observation.

The risk posed to non-target organisms by use of hydrated lime depends on hazard (toxicity) and exposure. Based on the toxicity results from Locke et al. (2009) and Doe et al. (2009) and the degree of exposure based on the “pH footprint” around treatment sites (measured with a portable pH meter) reported above, it seems unlikely that effects on non-target organisms based on the current use pattern will be severe or widespread. As hydrated lime is converted to calcium carbonate its toxicity will be essentially eliminated.

In Canada, there is no requirement for reporting use of hydrated lime in mussel aquaculture. Mandatory reporting of this data would aid in assessing the overall risk to the receiving environment posed by the use of this product.

Lime in Sediments

To our knowledge, there have been no studies in the field to examine in a quantitative manner the deposit and accumulation over time of lime in bottom sediments in the vicinity of mussel aquaculture sites treated with lime for control of tunicates. Calcium carbonate is persistent indicating the potential for sediment accumulation with continued use. This information gap on exposure hampers our ability to estimate risk to bottom dwelling organisms posed by the use of lime in tunicate control, whether from physical smothering or chemical toxicity.

There have been no reported studies on the toxicity of hydrated lime incorporated in bottom sediments to sediment dwelling organisms. There are no CCME sediment quality guidelines for lime (CCME 1999). Chemical toxicity of lime is expected to be low as pH is likely neutralized before particulates reach the bottom based on observations of the “pH footprint” in the estuarine water column reported above. As hydrated lime is converted to calcium carbonate its toxicity is reduced or eliminated. The physical effect of settling particles of hydrated lime and calcium carbonate will depend on the amount deposited per unit area and the accumulation over time. This information is not known at the time of writing.

STRESSOR – EFFECTS

Effect A: Presence, effects and consequences of hydrated lime on non-target organisms: Other fish, aquatic invertebrates and micro-organisms.

1. What is known (state of knowledge) about the stressor-effect relationship?

The reporting of use data for hydrated lime in mussel aquaculture is not mandatory and has been estimated from interviews with individual mussel farmers. Data are available from field observations of the exposure duration of hydrated lime suspensions based on the of the “pH footprint” in the estuarine water column reported in Locke et al. (2009) and from Neil MacNair, (PEI, Dept. Fisheries, Aquaculture and Rural Development pers.comm.2009). The quantity and accumulation of settled lime particles to bottom sediments over time is unknown.

2. What are the (measurable) ecological outcomes or endpoints (effects profile)?

The toxicity of hydrated lime suspensions to representative Canadian fish and invertebrates has been reported (Locke et al. 2009, Doe et al. 2009). Hydrated lime suspensions have the potential to alter the pH of seawater resulting in mortality in a variety of taxa. Hydrated lime suspensions have been shown to be toxic to larval lobster under realistic short term pulsed exposures. Lobster behaviour was shown to be affected.

There have been no reported studies on the toxicity of hydrated lime incorporated in bottom sediments to sediment dwelling organisms.

3. What is the magnitude of the effect?

The risk posed to non-target organisms by use of hydrated lime depends on hazard (toxicity) and exposure. Based on the toxicity results from Locke et al. (2009) and Doe et al. (2009) and the degree and duration of exposure based on the “pH footprint” around treatment sites (measured with a portable pH meter) reported above, it seems unlikely that effects on non target organisms based on the current use pattern will be severe or widespread. As hydrated lime is converted to calcium carbonate its toxicity will be essentially eliminated.

The risk posed by settling particles of hydrated lime in bottom sediments is less well known. There have been no reported studies on the toxicity of hydrated lime incorporated in bottom sediments to sediment dwelling organisms. There are no CCME sediment quality guidelines for lime (CCME 1999). Chemical toxicity of lime is expected to be low as pH is likely neutralized before particulates reach the bottom based on observations of the “pH footprint” in the estuarine water column reported above. As hydrated lime is converted to calcium carbonate its toxicity is reduced/eliminated. The physical effect of settling particles of hydrated lime and calcium carbonate will depend on the amount deposited per unit area and the accumulation over time. This information is not known at the time of writing.

4. What are the factors and conditions that modify or influence the expression of the effect (e.g., exposure, type of receiving environment, etc.)

In low energy systems, suspensions of hydrated lime are expected to remain very close to the aquaculture site and particles will settle more rapidly, while in high energy systems these suspensions are expected to be diluted and dispersed over a much wider area and remain longer in suspension. The settling rate will affect the amount of time available to convert hydrated lime to the more innocuous calcium carbonate by reaction with carbon dioxide dissolved in the water column.

5. What are the biological implications of the effect on the overall ecosystem function?

Because at high concentrations hydrated lime suspensions raise the pH of seawater to lethal levels, they pose a hazard to marine non-target organisms. However, field monitoring has shown the exposure duration to high pH to be very short (pH was <8.5 approximately 1 m from the discharge area), and so risk from the discharge of hydrated lime suspensions is thought to be of low severity over a very limited area.

Risks to bottom dwelling organisms from smothering and chemical toxicity are less clear, but risk of toxicity is thought to be low as hydrated lime is converted to calcium carbonate (which is not toxic) by reaction with carbon dioxide dissolved in seawater.

6. What type of evidence is available (e.g., lab studies, models, etc.) and what is the strength of evidence used to determine the stressor-effect relationship?

Lab studies have been used to determine the hazard of hydrated lime suspensions to a variety of representative Canadian species in seawater. These studies examined the toxicity of hydrated lime suspensions under a variety of exposure scenarios, including realistic pulse exposures, and looked at a variety of lethal and sublethal endpoints. The data on hazard posed by hydrated lime suspensions are thought to be adequate for aquatic organisms.

There have been no reported studies on the toxicity of hydrated lime incorporated in bottom sediments to sediment dwelling organisms.

There have been several field studies that have described the degree and duration of exposure of water column organisms based on the "pH footprint" around treatment sites (measured with a portable pH meter). These exposure data are sufficient to estimate the degree of risk to water column organisms.

The rate and extent of accumulation of hydrated lime particles into bottom sediments has not been studied.

7. What are the uncertainties associated with this stressor-effect linkage? Where would further information lead to a more complete understanding? Which uncertainties most prevent a more holistic understanding of the effect profiles and biological implications on overall ecosystem function?

There are several layers of uncertainty. Use pattern data are not reported on a mandatory basis and so total use figures are unknown. Settling rates and dispersion rates will be site specific, and so the amount of dispersion and dilution, and the aerial extent of bottom coverage are not known. Benthic community impacts have not been studied at mussel aquaculture sites where hydrated lime has been used to control tunicates, and the degree of accumulation of hydrated lime in bottom sediments is not known.

The interactive effects of multiple chemical stressors cannot be estimated at this time.

Treatments done to multiple sites concurrently and to additional strings over a number of days would likely cause additive risks from such treatments. The extent and frequency to which this occurs is not known.

8. During which activities does this stressor-effect occur?

This stressor-effect occurs during treatment to control tunicate infestations on mussel farms. Treatment is sporadic and occurs only after an infestation reaches problematic levels.

9. What are the cascading effects or linkages from this effect?

Cascading effects of the use of hydrated lime treatments to control tunicates are not known at this time.

CONCLUSIONS

Use of hydrated lime to treat tunicate fouling in mussel aquaculture is currently only practiced in PEI.

Use of hydrated lime in Canada to control tunicates is not presently registered as a pesticide. The authors' understanding is that under Canadian law the use of hydrated lime should be regulated. Fulfilling the requirements for registration would enhance the current knowledge of potential effects and allow a science-based risk assessment of its use.

It is not mandatory to report detailed information when hydrated lime is applied, how much is used, and where it is being applied, and so this information is not easily available to scientists or regulators, making it difficult to assess the overall risk posed by the use of this product. Consideration should be given to require mandatory reporting of use by all mussel aquaculture operations.

Risk posed by the use of hydrated lime to water column organisms is estimated to be low based on known hazard information and exposure durations. Risk posed to sediment dwelling organisms due to smothering or direct chemical toxicity is less well known but expected to be low.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Research to compare behavioural changes in larval lobster caused by hydrated lime with other inert particles of similar size such as calcium carbonate is recommended.
- Research on the accumulation of hydrated lime in bottom sediments and its impact on benthic communities is recommended.
- Use of caged lobster larvae in non-target tests during actual treatment operations to confirm low risk from these treatments is required.
- Research on the efficacy of alternate treatments such as removal by pressure washing or soaking and removal using fresh water could further reduce the low risk posed by the use and discharge of hydrated lime.

STRESSOR CATEGORY: 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. 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 although the products are water soluble and the dilution factor in marine environments is large. A number of products have been reported to be used in marine aquaculture: Formalin, Virkon®, quarternary ammonium products, sodium hypochlorite, N-chloro-paratoluenesulfonide trihydrate (Chloramine-T) and iodophores.

In the freshwater aquaculture industry, five compounds are used as disinfectants: hydrogen peroxide, N-chloro-paratoluenesulfonide trihydrate (Chloramine-T), sodium chloride, sodium hypochorite and iodophore compounds. Several compounds that are apparently used routinely in the Canadian aquaculture industry are not approved by Health Canada for use in aquaculture (Scott 2004).

Virkon® is a broad range disinfectant. Although it does not appear on Health Canada's list of approved drugs for aquaculture, it has a Health Canada Drug Identification Number and is advertised as having been approved for use in Canada since 2006 (Syndel 2009). The primary active ingredients are potassium peroxymonosulphate (21.5%) and sodium chloride (1.5%). The authors were unable to find any published data regarding the presence or effects of Virkon in marine environments. The product is however considered toxic to freshwater *Daphnia* and the reported LC50 for rainbow trout fry is ~6 $mg{\cdot}L^{-1}$ (Cellai MCC 2009).

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.

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 bioaccumulate or bioconcentrate. However, chlorine is very toxic to aquatic biota and the products should be used with caution (Zitko 1994).

Chloramine-T, another chlorine derivative, 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. Rainbow trout have been shown to withstand therapeutic does of this product 40-60 $mg{\cdot}L^{-1}$ (Carty 2000). The 96-h LC50 of chloramine-T to *Homarus gammarus* was reported to be exposure is 170 $mg{\cdot}L^{-1}$, when the lobsters were exposed daily for 60 min (Wilson 2008).

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, which causes the death of the microorganism. Iodine has bactericidal, fungicidal, viricidal and sporicidal action, and has been used as an aqueous solution since the middle of the nineteenth century. The solution is unstable, necessitating the use of solubilizing agents that liberate the iodine. Iodine causes death by destroying proteins (e.g., 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 (Denning 2008). The author concluded 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. 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).

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. It is a 37% formaldehyde solution with a reported lethality (24-h LC50) to rainbow trout of 7.77 $mg \cdot L^{-1}$ (Scott 2004).

Hydrogen peroxide is a strong oxidizing agent and is widely used as a disinfectant for the treatment of fungal infections of fish and their eggs in hatcheries (Rach et al. 2000). Since the original registration of hydrogen peroxide has expired, it is currently being reviewed for registration by Health Canada for use against infestations of sea lice on Atlantic salmon (Burridge et al. 2008). Toxicity to fish varies with temperature; for example, the one hour LC50 to Rainbow trout at 7^oC was 2.38 $g \cdot L^{-1}$ at 22^oC was 0.218 $g \cdot L^{-1}$ (Mitchell and Collins 1997). There are no reports of use of hydrogen peroxide in the freshwater or bivalve aquaculture.

There is no information on the amounts or types of disinfectants used by the aquaculture industry or by processing plants, making it very difficult to determine precisely the quantities of these products used. All of the compounds used are 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. Environment Canada has issued the following suggested discharge concentrations:

Chlorine = 0.02 ppm
Iodine = 0.1 ppm
Hydrogen peroxide = 0.5 ppm

Provinces and aquaculture associations have developed environmental monitoring programs and best management practices for aquaculture and these include recommendations for proper and safe use of disinfectants (see for example, Province of New Brunswick 2006).

STRESSOR CATEGORY: FUNGICIDES

In freshwater aquaculture, treatment of external fungal and bacterial infections usually involves immersion in a static bath. These treatments typically are applied to embryos and juveniles in hatcheries, although some treatments in culture ponds do take place (Boyd and Massaut 1999). These authors describe risks associated with chemical use in catfish pond culture in the US and it is not clear if any pond or cage treatments of fungicides take place in Canada. There are three common chemicals used to treat fungal infections: hydrogen peroxide, formalin and sodium chloride. The characteristics of these compounds have been described above.

STRESSOR –EFFECTS

Effect: Presence, effects and consequences of disinfectants and fungicides in and on non-target organisms: Other fish, aquatic invertebrates and micro-organisms.

1. **<u>What is known (state of knowledge) about the stressor-effect relationship?</u>**

Data are available showing the lethality of most of these compounds to aquatic species. These are lab-based studies that show that these compounds are capable of killing bacteria and viruses and improve biosecurity in aquaculture situations. The lethal concentrations are often well above therapeutic concentrations (Scott 2004) and are diluted before or as a result of release to the environment. Exposure of non-targets to disinfectants and fungicides is uncertain, therefore the initial step of the Stressor – Effect Logic Diagram (Figure1) is speculative.

2. **<u>What are the (measurable) ecological outcomes or endpoints (effects profile)?</u>**

These products are used in relatively small quantities and in areas where dilution is likely. There are therefore, with the current state of knowledge, no measurable ecological outcomes or endpoints. The availability of data on patterns of use would be useful in determining if there are potential risks.

3. **<u>What is the magnitude of the effect?</u>**

Given the current state of knowledge the magnitude of any effect is considered very small.

4. **<u>What are the factors and conditions that modify or influence the expression of the effect (e.g., exposure, type of receiving environment, etc.).</u>**

Most of these products are most effective at low pH. Temperature affects the efficacy of hydrogen peroxide. The potential for exposure of non-target organisms is unknown. Data are not available regarding when, where and how much of these products are applied.

5. **<u>What are the biological implications of the effect on the overall ecosystem function?</u>**

Given the current state of knowledge there are likely no large scale implications associated with the use of disinfectants.

6. **<u>What type of evidence is available (e.g., lab studies, models, etc.) and what is the strength of evidence used to determine the stressor-effect relationship?</u>**

There are no studies published showing the effects of disinfectant and fungicide use on non-target organisms near aquaculture operations.

7. **<u>What are the uncertainties associated with this stressor-effect linkage? Where would further information lead to a more complete understanding? Which uncertainties most prevent a more holistic understanding of the effect profiles and biological implications on overall ecosystem function?</u>**

There are no data regarding use of disinfectants and fungicides in operational settings. Without such studies there are no clear stressor-effect linkages and discussion surrounding potential effects is speculative.

8. **<u>During which activities does this stressor-effect occur?</u>**

Activities such as disinfecting boats and equipment lead to the release of disinfectants to the environment. There is no information regarding the treatment of aquaculture species for fungal infections outside of the hatchery. The potential for fungicides to affect non-target species is unknown.

9. **<u>What are the cascading effects or linkages from this effect?</u>**

The potential exists for all chemical inputs to contribute to cumulative exposure and effects. It remains to be determined if cumulative effects occur and to the authors' knowledge no studies have been undertaken to investigate the potential for disinfectants and fungicides to contribute to cumulative effects.

CONCLUSIONS

Use of disinfectants is a necessary part of all aquaculture operations. There are a limited number of products in use in Canada for disinfection. Some of these products are also used as antifungal agents and as parasiticides. The products are generally non toxic at therapeutic doses and they are almost always diluted before or during release to the environment. These products are considered as low risk for causing significant deleterious effects near aquaculture sites. However, the quantity of disinfectant products being released in Canada is unknown. The frequency of use and the spatial distribution of releases are also unknown making it impossible to confirm the assertion of low risk and to realistically assess the potential for effects to take place in the aquatic environment.

RECOMMENDATIONS

- Regulatory agencies should require yearly reporting of disinfectant use during aquaculture activities. Reports should include what product, how much was used and when. Access to this type of data would provide a powerful regulatory and science tool.
- 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. Of particular interest would be studies on the potential for endocrine disrupting compounds to accumulate in the environment and to affect non-target organisms.

STRESSOR CATEGORY: MINOR INPUT SOURCES

The sources of chemical contamination listed below are considered to be minor in terms of the potential for widespread ecological consequences. The volumes of compounds “produced” by aquaculture activities and the potential consequences are small.

ANAESTHETICS

Anaesthetics are used in operational situations where fish must be handled. Anaesthetics reduce the stress of handling MS-222 (tricaine methanesulfonate) is the only product registered for use by Health Canada (2001). Very small quantities of this product are used in the field and no environmental effects are foreseen with its use (Zitko 1994).

FUEL AND LUBRICANTS

The aquaculture industry has historically been concentrated in small coastal bays and inlets. This is particularly true of southwest New Brunswick, in areas of intense mussel culture and in lakes and ponds, where the concentration of aquaculture activity results in considerable small boat traffic in relatively confined areas. There are large numbers of outboard motor boats, as well as outboard-driven barges and larger service vessels, used to handle fish and fish feed. It is possible that quantities of gas, oil and lubricants may enter the water as a result of the normal operation of these vessels or from accidental spills. The main concern associated with presence of fuel and lubricants is the possibility of contaminating water, sediments and potentially tainting cultured and wild species (Boyd and Massaut 1999).

To the authors’ knowledge an estimate of the volume of fuels and lubricants used in boats and barges associated with aquaculture activities has not been determined. The total volume is spread out over a large number of small vessels. Accidental spills from vessels could have environmental consequences but only over small spatial scales. In studies of sediments near finfish aquaculture sites the concentration of polycyclic aromatic hydrocarbons (PAHs) was lower in areas of high organic content (faeces and waste feed) than at reference sites (Hellou et al. 2005). PAH concentrations are often used as indicators of presence of oil-type compounds. These data suggest that aquaculture activity is not contributing to the accumulation of PAH near marine aquaculture activity.

LITTER

Marine debris is a major environmental concern worldwide. Litter can directly harm wildlife due to entanglement, ingestion, smothering and toxicity. Each year hundreds of birds, marine mammals and sea turtles die due to entanglement in, or ingestion of plastics. Coastal litter is usually grouped by material type, but the most commonly found items are made of plastic. A separate category, sewage related debris (SRD), defines a range of items made from different material types which enter the marine environment from sewage outfalls. Litter can end up on our coasts from other sources too. It is often blown from land into watercourses and the sea, and it can also be transported by birds and animals.

Fish and shellfish farms have the potential to both produce marine litter and suffer from its consequences. In the past the accumulation of litter from aquaculture activities has been cited as an environmental concern. The United Nations Environment Program identifies aquaculture as a significant source of marine litter (UNEP 2005). Aquaculture from the UNEP perspective includes all forms of 'fish' farming. A brief review of recent literature related to environmental concerns about aquaculture practices in Canada shows only brief mention of litter as an issue. In Canada, there have been no studies that have addressed the issue of marine litter with specific reference to aquaculture activities. In Scotland the salmon growers do not consider litter a widespread issue of concern and any litter associated problems are being addressed under food certification schemes. Similarly shellfish farmers in Scotland do not perceive litter as being a big problem (Marine Pollution Monitoring Management Group 2002).

Best management practices developed by industry and government regulations prohibit disposal of physical wastes into water ways or at sea. The use of feed barges in salmon aquaculture has eliminated the need for or the presence of plastic feed bags on aquaculture sites and has greatly reduced the need for workers to be on sites. This reduces the waste generated and the potential for beaches to be fouled with aquaculture-related litter.

CONCLUSIONS

It is unlikely that anaesthetics, fuel and lubricants or litter released or generated from normal aquaculture activities pose a significant risk to the aquatic environment.

RECOMMENDATIONS

- Any chemical monitoring program should include analysis of sediments and water for hydrocarbons. Results of these analyses will provide information essential to deciding whether or not fuels and lubricants need to be considered in future discussions of environmental effects of aquaculture activity in Canada.

SUMMARY POINTS

Use of therapeutants in the aquaculture industry in Canada is regulated by Health Canada. This department registers pesticides and drugs after a thorough review process that includes risk assessments tailored to the specific use. Therapeutants can only be used under prescription by a licensed veterinarian. Withdrawal times are applied to ensure no pesticide or drug remains in the cultured species at the time of harvest. Despite these regulations the use of these compounds remains contentious. Much of the data used to support registration is considered proprietary and is not released to other government departments, scientists or to the public. Unfortunately, the absence of these data from the public domain means their quality or nature can not be assessed or debated by scientists or others with interests in this area (Haya et al. 2005). Pesticides and drugs are registered on a compound by compound basis. As a result, cumulative biological effects are not formally addressed in the registration process apart from required field studies.

Other chemical inputs such as Cu from antifouling paints and the use of lime and acetic acid in the mussel aquaculture industry are not regulated to a similar extent. There are sediment quality guidelines in place for metals such as Cu and Zn. While hazards have been determined for nearly all products discussed these hazards are not always identified for appropriate indigenous species. Risks associated with their use in aquaculture have only been assessed as part of the regulatory process for the pesticides and drugs.

Use of lime and acetic acid in the mussel aquaculture industry appears to be a common and accepted practice. However, these compounds have not been assessed under any regulatory framework. These products should be subject to the same review and scrutiny as other compounds (pesticides, drugs, and antifoulants) applied to open water.

EXPOSURE

In order for effects to be manifested a compound must be present, the species must be present and they must be together for a sufficient period of time to elicit a response. Effects are therefore dependent on use and exposure. Risks can only be assessed when data are available regarding the likelihood and duration of exposure. If disease is managed, no treatment is required and there is no exposure. If no parasites are present, no treatments are required and there is no exposure. If alternative methods are available for combating disease, infestations of parasites and to control fouling, no intervention is required and no exposure takes place.

In the absence of alternative control methods or husbandry practices which eliminate diseases, parasites and fouling organisms, use of therapeutants and antifouling compounds will remain an essential part of normal aquaculture activities. Consequently any studies that provide data regarding exposure and effects and that can inform the decision making process will be useful.

Lab-based effects studies have been conducted for all of the compounds discussed in this paper. Many have dealt with effects on “typical” species such as Rainbow trout and mysid shrimp, for example. With only a few exceptions, these studies are conducted over unrealistic time scales, rarely on species of interest in the Canadian context and rarely are attempts made to identify sensitive life stages.

There are a number of very powerful models that have yet to be validated in terms of movement of particulates and of water around aquaculture activities. Some of these are described and discussed in other documents associated with the Pathways of Effects exercise. Once validated, researchers and regulators can make reasonable assumptions about where products will move in the aquatic environment.

Another important question with relevance to exposure is how long will a compound be present and in what form? For all compounds discussed, fate and persistence data are available. In many cases these include solubility, photo-stability, octanol-water partition co-efficient and others. These data are required data for registration of all products. There are also a number of studies that describe concentrations of aquaculture-related products in sediment and biota near aquaculture sites (see for example, Chou et al.

2002, Hellou et al. 2005, Sather et al. 2006). However, persistence in sediments, for example, does not necessarily result in a biological effect. The authors are not suggesting that persistence or the accumulation of persistent compounds is not an issue of concern. However, in the context of biological effects near aquaculture activity, the link between persistence and effects needs to be established. Cu can be used to emphasize this point. Burridge et al. (2008) in their discussion of Cu as an aquaculture contaminant state that the chemical speciation of Cu greatly affects the potential to affect aquatic organisms. In addition, Cu bound to sediments may not be available to organisms that are sensitive to Cu.

The authors feel that detailed information on patterns of chemical use in aquaculture such as, what compounds are used, how much, where and when, should be available. In New Brunswick and British Columbia some or all of the data are summarized and made public (Tables 1 and 2). One company, Marine Harvest, voluntarily posts their biomass and anti-louse treatment data on-line. In Nova Scotia and Newfoundland, no data are available regarding therapeutant or other chemical use in marine finfish farming. Bivalve aquaculturists do not appear to provide data regarding use of products to fight tunicates. Antibiotic use in the freshwater aquaculture industry is not available to the public.

Haya et al. (2005) and Burridge et al. (2008) describe the regulatory situation in Scotland regarding the reporting of therapeutant use in the salmon aquaculture industry. Briefly, in Scotland detailed data from individual farms are made available through the regulator (Scottish Environmental Protection Agency (SEPA)). These data can be used to identify areas where bacterial disease is prevalent and studies can be conducted to identify possible causes or reasons. In Canada we remain in a situation where samples are collected at or near aquaculture sites and analysed for chemical content, sulfide, and other endpoints. These results are presented with no context in terms of activity at the aquaculture site. If a sediment sample were reported to contain no emamectin benzoate, for example, and it was known that the product was recently applied, the interpretation of that result would be completely different than if there had been no anti-louse treatments for some time. On the other hand if EB was found and no recent treatment had taken place yet, another interpretation would result. The authors are aware that some studies have been conducted with the knowledge of production and treatment activity. These have been conducted in the context of food safety for multi-trophic aquaculture sites.

Finally, the key issue regarding aquaculture activities and their potential to have environment impacts is: What are the cumulative impacts/effects? This question can be asked strictly in the context of chemicals i.e., are there cumulative effects associated with repeated exposure to one compound to chronic exposure to one compound, to exposure to mixtures of chemicals, etc. The question can also be asked in the context of multiple environmental stressors: are chemicals likely to have a greater effect at high temperatures, under hypoxic conditions, under varying pH conditions? These are very complex questions with no easy answers. But they are also extremely relevant in an overall assessment of effects and pathways of effects. Reviewers of this document have correctly pointed out that while it is true that firm linkages have not been established between certain chemical inputs and effects, the weight of evidence suggests that linkages exist. The authors agree with this assessment and while promoting increased research to address the uncertainty, also promote application of the precautionary principle to ensure environmental sustainability of the aquaculture industry in Canada.

RECOMMENDATIONS

The following recommendations have been extracted from the document and are presented here in order of importance. This ranking is based on what the authors feel needs to be done to answer the big questions of population and community effects, where the greatest unknowns exist and which compounds or class of compounds have the potential to have the greatest impact regardless of scale. All recommendations are considered important areas for research.

- Research should be conducted, first to identify realistic protocols to determine cumulative effects, then to apply those protocols to the aquaculture situations whether for finfish aquaculture in the marine and freshwater environment or for bivalve aquaculture. This, unfortunately, is remarkably easy to say but will likely be remarkably difficult to achieve.
- Research is needed to clearly establish the link between use of antibiotics in aquaculture and the presence of antibiotic-resistant bacteria near aquaculture activities. The spatial and temporal extent of any effects should also be defined. This work will only establish aquaculture's contribution to the much broader "antibiotic" problem and therefore help establish mitigation strategies.
- Research is needed to determine the consequences of antibiotic use in aquaculture antibiotics. The effects on aquatic organisms (farmed and indigenous), on the micro-flora in the sediments and in the water column, and the potential to affect human health should be investigated.
- Sea lice control chemicals have the potential to cause non-target effects and bath treatments with pyrethroids presenting the highest risk. The magnitude of the impacts in the field has not been determined but could be significant. Sediment accumulation of emamectin benzoate should be further evaluated and toxicity to a wider range of benthic invertebrates explored.
- Use of deltamethrin bath treatments presents the potential for further field impacts from acute toxic effect on native crustaceans and this should be determined under operational conditions. Repeated use of deltamethrin could result in sediment accumulations which should be evaluated along with benthic community effects.
- The cumulative risk of multiple concurrent and consecutive cage treatments needs to be quantified.
- Lab-based studies need to be conducted to identify hazards to relevant species and sensitive life stages (in the Canadian context). This does not necessarily mean only commercially important species, although these species will certainly hold some priority. Studies should be conducted that include several trophic levels. Ecologically relevant endpoints should be investigated. Work by Waddy et al. (2007) with SLICE® and lobster moulting by Burridge et al. (2008a) with Salmosan and lobster spawning are examples of work with non-traditional endpoints.
- Lab-based research needs to be conducted to answer questions regarding the influence or consequence of exposure type, repeated short-term (or pulsed) exposures, for example, compared to standard 48- or 96-h effects studies.
- Research to compare behavioural changes in larval lobster caused by hydrated lime with other inert particles of similar size such as calcium carbonate is recommended.
- Research on the accumulation of hydrated lime in bottom sediments and its impact on benthic communities are recommended.

- Use of caged lobster larvae in non-target tests during actual treatment of mussel socks with lime to confirm low risk from these treatments is required.
- Research on the efficacy of alternate treatments for tunicate infestations in mussel aquaculture such as removal by pressure washing or soaking and removal using fresh water could further reduce the low risk posed by the use and discharge of hydrated lime.
- Research is needed to develop safe and effective vaccines against bacterial and viral pathogens.
- Research is needed to develop safe and effective alternatives to the use of copper-based paints as antifoulants.
- Research is needed to determine the fate of sediment-bound metals. The concentrations of copper are known to decrease during fallow periods and it is unknown where the copper goes or its potential to affect other organisms.

ACKNOWLEDGEMENTS

The authors have quoted liberally from papers prepared by Burridge (2003), Haya et al. (2005), and Burridge et al. (2008). We wish to thank the authors and co-authors of these manuscripts for setting the stage for assessing pathways of effects. The New Brunswick Salmon Growers Association generously provided data on the use of antibiotics and SLICE for the 2007 production year.

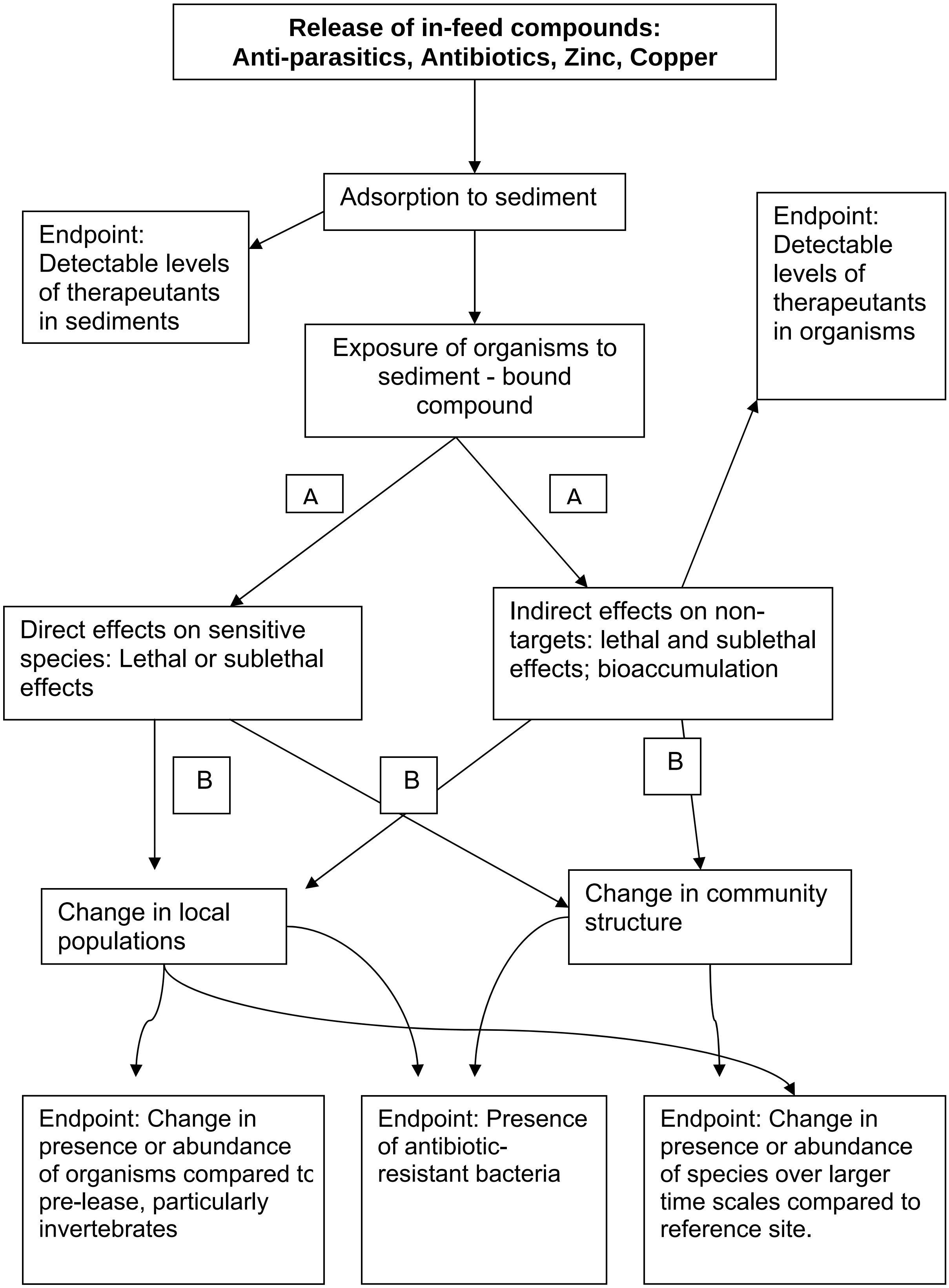

Figure 1. Stressor-Effect Logic Diagram for in-feed chemical inputs.

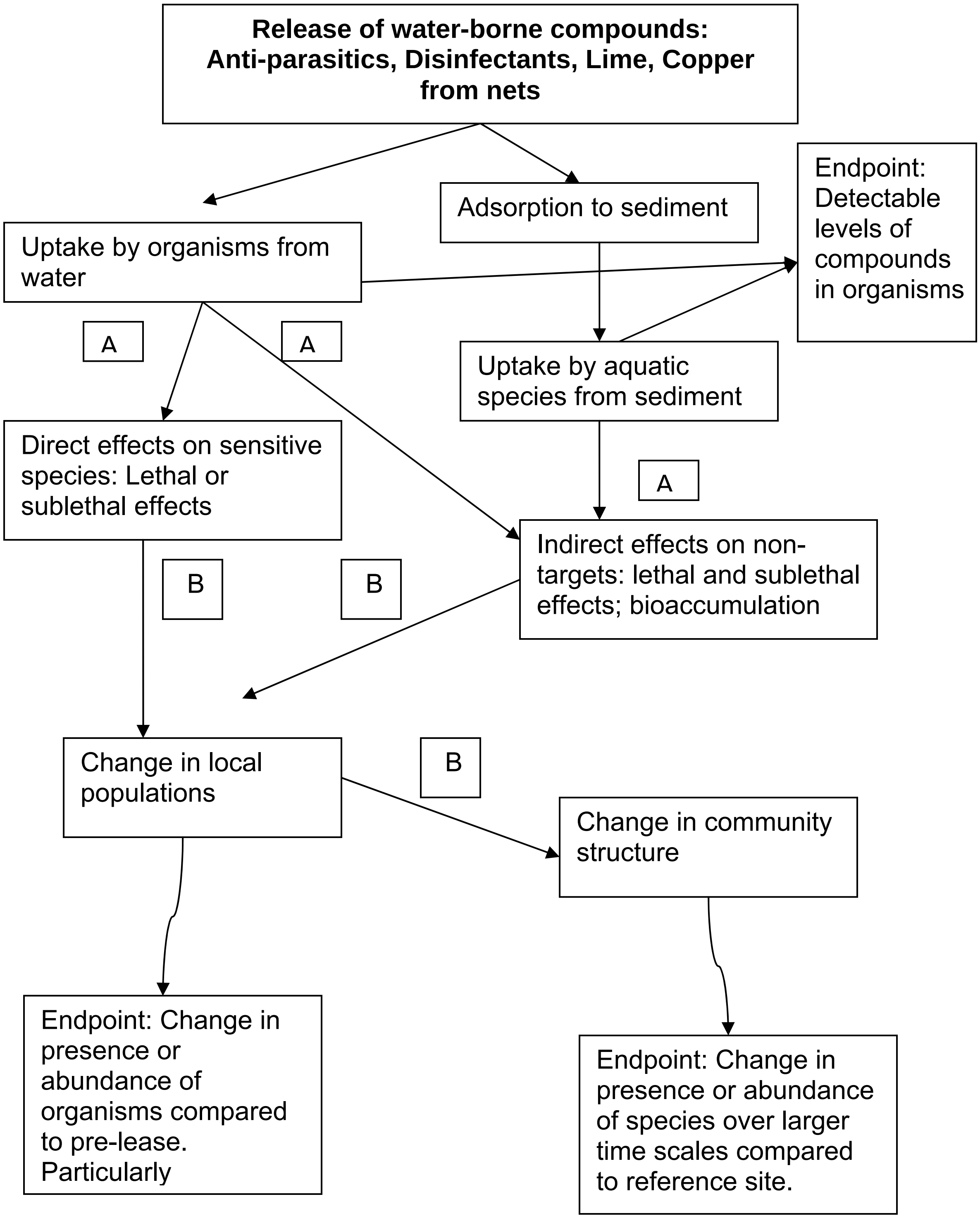

Figure 2. Stressor-Effect Logic Diagram for water-borne chemical inputs.

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