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A Review Of The Ecological Impacts Of Selected Antibiotics And Antifoulants Currently Used In The Tasmanian Salmonid Farming Industry (Marine Farming Phase)

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Australian Government

Fisheries Research and Development Corporation

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FRDC Project 2007/246 "A review of the ecological impacts of selected antibiotics and antifoulants currently used in the Tasmanian salmonid farming industry" Investigators: Dr Catriona Macleod, Dr John Volkman, Mr Pheroze Jungalwalla

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Non-Technical Summary

Antibiotics and antifoulants are used in aquaculture operations to ensure the health and well-being of farmed stock. Antibiotics are used as a direct response to infectious diseases, whilst antifoulants are needed to counteract bio-fouling, a condition which is a significant problem to the local aquaculture industry and reduces water flow and oxygen supply in the cages, increasing stress levels and hence disease-susceptibility in the fish. However, there are several environmental concerns associated with the use of both antibiotics and antifoulants.

Cage fish farming is essentially an open culture system. Aquaculture is sometimes seen in simple terms such as inputs such as fish, feed, and chemicals and outputs such as harvested fish. Losses to the environment such as escapees, uneaten feed, faeces and excreted metabolic wastes, and chemicals/metabolites associated with therapeutic agents have the potential to cause detrimental environmental effects. These impacts can be localised, such as the accumulation of organic material and uneaten food under cages, or more widespread, for example distribution of soluble nutrients through currents (Butler, 2005; Dean *et al.*, 2007). The timescale of impacts may also differ with short-term effects occurring within an operational cycle or longer term effects continuing over many years.

Antibiotics and antifoulants may affect the environment in a variety of different ways. The need for a targeted review to establish environmental risk factors associated with current antibiotic and antifoulant use in Tasmanian aquaculture operations and to develop an appropriate strategy to research and monitor ongoing impacts was identified. This study also considered data on sediment residue levels for both antibiotics and antifoulants, which had been collected by the salmon industry in compliance with drug/chemical licensing permit conditions authorities and by the State government in response to concerns regarding antibiotic and antifoulant use. This information was reviewed to assess the potential ecological impacts of antibiotics and antifoulants currently used in the Tasmanian aquaculture industry. Consequently the aims of this study were:

- Undertake a review of the international literature and current research to identify the existing state of knowledge regarding the environmental effects of antibiotics and antifoulants currently used or likely to be used in the Tasmanian salmonid farming industry (marine production phase).
- Analyse local datasets on currently used antibiotics and antifoulants, collected in compliance with current licensing requirements, to determine what additional information is needed (if any) to appropriately evaluate the environmental impact of current management practices.

Antifoulants – Impact Evaluation

The main environmental concerns regarding antifoulant contamination include:

- Bioaccumulation
- Ecotoxicological effects and subsequent changes to local ecology and biodiversity

- Effects on ecosystem function (i.e. microbial and geochemical processes that regulate the cycling, bioavailability, and fate of micro- and macronutrients)

The likelihood of these impacts is largely determined by the bioavailability of copper and zinc. The antifoulants used in the Tasmanian aquaculture industry are copper-based. There are several pathways by which metals in antifoulants can become environmentally available:

- Dissolution from the active paint surface
- Ablation or physical damage of the painted surface
- Release from accumulated paint in the sediments

However, release from other sources (i.e. not antifoulant in origin) should also be considered when interpreting sediment quality results.

On the basis of the limited data currently available, there do not appear to be major acute effects of antifoulant release in the water-column. The greatest risk appears to be the potential for build up of copper and zinc in the sediments around the farms. Industry surveys have shown a significant increase in copper levels at farm sites; particularly at depositional sites with organically enriched sediments, which have a higher capacity to bind and accumulate copper.

For these metals to have any effect on local ecology or sediment processes they must be “bioavailable”, which is in turn reliant on the form (speciation) of the metal. Metal speciation in sediments is complex and strongly related to the geochemical status of the sediments (i.e. redox, pH, oxic status, organic content) and on the extent of processes such as bioturbation and resuspension. In anoxic sediments, metals are generally thought to be less bioavailable, being tightly bound as insoluble sulphides. Oxidative release of metals from sediments (i.e. during benthic recovery) has been identified as a potential problem in aquaculture leases (Valkirs, 2003).

Many marine invertebrate species have been shown to be sensitive to increased metal loadings, with juvenile (embryo and larval) stages being particularly sensitive, often being several orders of magnitude more sensitive than adults (Bellas, 2006). Copper contamination of the sediments can result in the elimination of vulnerable species as toxic thresholds are exceeded, whilst more robust species, with the capacity to regulate or accumulate metals, will be unaffected or may even thrive. This in turn can lead to problems with bioaccumulation and biomagnification as benthic invertebrates are a food source for many higher trophic level species.

Guidelines recently developed for the management of biofouling in Mediterranean aquaculture (IUCN, 2007) seek to ensure that there are “no perceivable toxic effects on non-target organisms” and recommend the use of eco-friendly antifouling coatings and products, encourage the use of environmentally friendly procedures for preventing or eliminating biofouling and suggest that antifouling products based on heavy metals should be avoided where possible. Whilst the local industry continues to support the investigation of alternatives to metal-based antifoulants, replacement of copper and zinc-based antifouling products is unlikely in the short-term. Consequently, the emphasis of sustainable management should be on minimising the environmental impact of currently used products and the development of appropriate monitoring strategies to defining ecologically relevant threshold values over a range of parameters

To effectively interpret the sediment monitoring data, both into the future and that which has already been obtained, it is essential to improve our understanding of the mechanisms and processes that affect metal bioavailability and the ecological consequences that might be associated with particular metal loadings. Once there is a clear understanding of bioavailability then chemical estimates or surrogates for bioavailability can be developed. These can then be used, in conjunction with relevant toxicity testing, as indicators of environmental condition. However, to achieve this a range of locally relevant species should first be evaluated (over a range of life stages and copper sensitivities), and for a range of operational and environmental conditions relevant to the salmonid industry in SE Tasmania. The ultimate aim being the development of a rapid toxicity assay using locally relevant species to assess sediment quality and potential impacts. There is information available in the literature to assist with these decisions. This combination of toxicity and chemical bioavailability assessment would provide important information while the industry refines its environmental management strategies. Finally these data would increase the capacity to employ a PEC/PNEC assessment approach, and to model copper distribution and fate with tools such as MAMPEC.

The results of this study suggest that the best strategy to minimise and manage environmental concerns would be a combined approach that experimentally investigates the environmental fate of copper and zinc along with the biological effects associated with antifoulant usage in conjunction with the development of theoretical models which take into account operational considerations. A number of potential areas of research are suggested

Antibiotics – Impact Evaluation

So long as there are bacterial pathogens in the marine environment, occasions will arise where administration of antibacterials is necessary to ensure the health and welfare of fish stocks. When pathogens are known and understood vaccines may be available but where the disease agents are unknown, antibiotics may be necessary until an alternative therapeutic approach can be established.

The main environmental concerns associated with antibiotic usage relate to the effects on non-target organisms, environmental persistence and development of resistance. In Tasmania, antibiotics are generally administered in feed and as such the main concerns relate to the presence of waste feed and fish excretory material in the sediments and water column. Feed wastage can be minimised by monitoring feed input and limiting feed wastage. Ensuring best practice in therapeutic administration and disease management will also help to minimise impacts.

Although assessment of the available data does not suggest that major environmental changes have occurred, it is still important to identify and monitor suitable indicator species to ensure ongoing sustainability. In addition, where antibiotics are used it is suggested that a measure of bioavailability be obtained, rather than simply a measure of total residue level, and that the effect of local environmental conditions (i.e. oxic status, temperature, pH and salinity) on ecotoxicity be assessed in order to ensure that local data are consistent with findings from overseas, given the very different prevailing environmental conditions in Tasmania.

Development of resistance and accumulation in the sediments are also significant environmental concerns. Although current information suggests that human health effects are highly unlikely, it is important to monitor the incidence of resistance in the environment and in fish bacteria. Accumulation in the sediments may affect natural sedimentary processes, such as biogeochemistry, and it would be prudent to confirm this, and to determine threshold effect levels. This could be done either in mesocosm experiments with field validation through targeted assessments, perhaps in conjunction with measurement of biotic loading and resistance.

Current data indicate that water column concentrations of antibiotics are extremely low and that impacts on phytoplankton communities are likely to be limited. The testing of wild fish with respect to human health toxicity showed no risk to human health. However, the most important means to reduce and manage the overall antibiotic usage would be to facilitate diagnosis of pathogens and to support development of targeted disease management strategies and alternative therapies, in particular vaccines. Investigation of vaccines is currently underway in Tasmania for all significant pathogens currently affecting the industry.

General Conclusions

It is important to understand the environmental impacts associated with farming activities in order to manage and minimise those impacts. This can be achieved through ensuring best practice environmental management strategies (including disease management strategies) and robust impact assessment and mitigation approaches, with reliable early warning indicators.

The results from the current industry based monitoring focused on the detection of major effects of both antifoulant and antibiotic impacts are encouraging with findings suggesting limited bioavailability of metals under current conditions. However, there are several areas of environmental concern which were not covered in the current monitoring and some of the results were inconclusive, consequently there is a need for additional research to better understand the local situation, to develop targeted and appropriate monitoring and management strategies and to ensure environmental sustainability. The literature suggests a general trend in regulatory approaches towards environmental risk assessment (ERA) frameworks, and modelling could be very useful in helping to address environmental concerns and in predicting impact. A summary of potential areas for further investigation is provided and it is planned to hold a workshop in the next few months with project participants, industry stakeholders and relevant experts to further discuss the project outcomes and proposed research areas, to prioritise research needs and to develop a strategic research agenda.

TABLE of CONTENTS

List of Figures

List of Tables

1. General Introduction

1.1 Antibiotic and Antifoulant usage in aquaculture

Antibiotic usage in intensive animal production is necessary to ensure the health and well-being of the farmed stock. In terrestrial livestock production there is a long history of antibiotic usage, consequently there is a large body of literature regarding the environmental effects of antibiotics, particularly with respect to human health. In addition, there is quite a lot of information on the comparative effects of various antibiotics in medical areas as well as quite a substantial ecotoxicological literature. However, the information pertaining to aquaculture applications is limited, with very little detail specific to the local industry. The type and intensity of antibiotic usage is largely a function of the culture species and husbandry conditions, disease situation, availability of effective vaccines, market requirements, and local regulatory restrictions. Atlantic salmon are a high value food species with significant costs of production, as a result each fish represents a considerable investment to the producers and therefore the health and well being of the stock are of critical importance. Although some salmonid producing countries (i.e. Scotland and Norway) have in the past recorded very high usages of antibiotics, this has largely been associated with very specific disease outbreaks and improvements in husbandry, disease management strategies and vaccine development have now all but eliminated the use of antibiotics in these countries. Consequently there have been few recent aquaculture specific studies, with even fewer studies on ecosystem effects. The information that is available in relation to aquatic systems is largely focussed on environmental persistence and antibiotic resistance.

Bio-fouling represents a significant problem to the local aquaculture industry; net fouling can increase the disease susceptibility of fish stocks by increasing stress levels through reduction of water flow and oxygen supply in the cages and by providing reservoirs for disease-causing organisms. In addition maintenance and/or replacement of the cage infrastructure as a result of colonisation by fouling communities can be costly. Consequently the aquaculture industry needs to be able to manage bio-fouling. Several alternative options have been tried in the past but currently the most efficient method to control bio-fouling is through the use of anti-fouling coatings. An additional benefit of antifoulant usage for aquaculture operations is in the net stiffening associated with these coatings; this provides increased protection to the stocked fish from seal attacks and reduces the need for further seal prevention strategies.

The coatings available to aquaculture are often adapted from other commercial uses, with decreased levels of biocides to make them suitable for use with aquaculture species. However, the reduced efficacy means that the coatings do not last long and need to be re-applied frequently; which is both expensive and time consuming. In addition there is also a need to safely dispose of the waste products associated with cleaning and recoating of nets. There is a substantial local literature on the environmental impacts of antifoulants and heavy metals, largely derived from the significant use of antifouling treatments by the shipping and recreational boating industry, but there are several issues which are unique to aquaculture operations such as possible synergistic effects with organic enrichment processes which are not so well understood.

There is a need for a targeted review to establish environmental risk factors associated with current antibiotic and antifoulant usage in aquaculture operations and to develop an appropriate strategy to research and monitor ongoing impacts. The industry and local government in Tasmania have some specific data on sediment residue levels for both antibiotics and antifoulants, which has been collected by the salmon industry in compliance with drug/chemical licensing permit conditions stipulated by authorities and by the State government in response to concerns regarding antibiotic usage. All of the available, relevant information will be reviewed to assess the potential ecological impacts of antibiotics and antifoulants currently used in the Tasmanian aquaculture industry.

1.2. Background

Fish farming in cages is essentially an open system, with inputs comprising fish, feed, and chemicals such as antibiotics and antifoulants (Dean *et al*, 2007). Outputs or losses can be summarised as harvested fish, escapees, uneaten feed, faeces and excreted metabolic waste, and chemicals/metabolites associated with antibiotic and antifoulant use. The effects of these losses to the environment can be localised, for example accumulation of organic material and uneaten food under cages, or more widespread for example distribution of soluble nutrients through currents (Butler, 2005; Dean *et al*, 2007). The timescale of impacts may similarly be observed at two extremes; short-term impacts (i.e. within operational cycle) including changes in benthic fauna composition based on organic enrichment gradients, while longer term impacts (i.e. many operational cycles) may include accumulation of heavy metals and subsequent exceedance of threshold levels with respect to toxicity, bioaccumulation and trophic transfer.

Antibiotics/ antifoulants may affect the environment in a variety of different ways (Figure 1). Only a relatively small proportion of the antibiotics/ antifoulants input to the system will actually impact on the sediments (Figure 2). However, in both the pelagic and sedimentary systems, the processes that mediate or affect the ecosystem response require greater understanding. Understanding how antibiotics/ antifoulants interact with the environment both independently and in combination will enable us to address the key environmental issues.

1.3. Aims

- Undertake a review of the international literature and current research to identify the existing state of knowledge regarding the environmental effects of antibiotics and antifoulants currently used or likely to be used in the Tasmanian salmonid farming industry (marine farming phase).
- Analyse local datasets on currently used antibiotics and antifoulants, collected in compliance with current licensing requirements, to determine what additional information is needed (if any) to appropriately evaluate the environmental impact of current management practices.

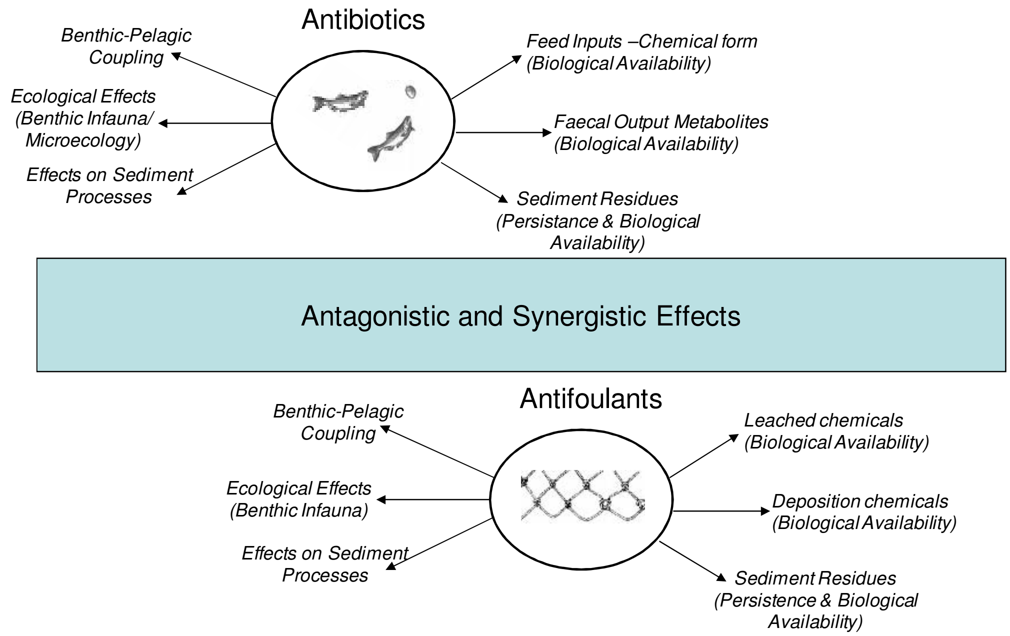


Figure 1 Key pathways by which antibiotics and antifoulants may impact on the sedimentary environment.

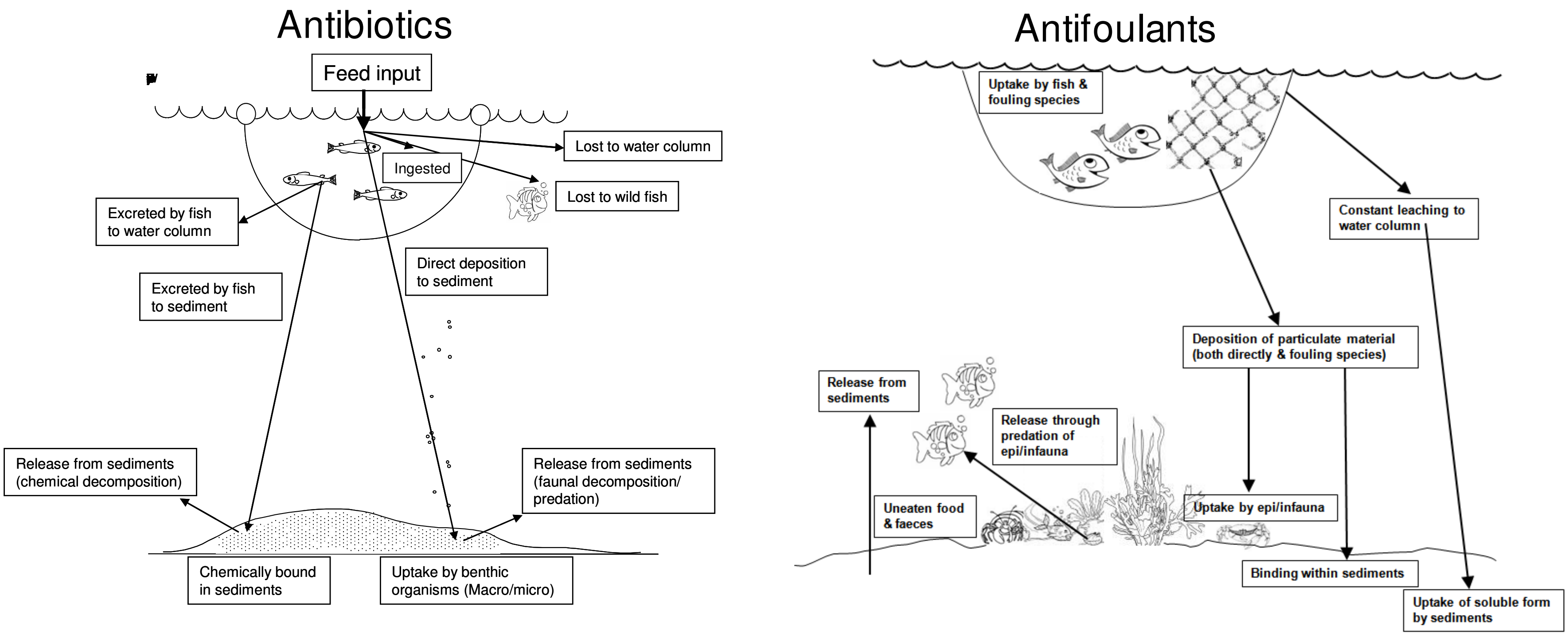


Figure 2 Potential environmental pathways for assimilation of antibiotics and antifoulants in the environment. Boxed factors also represent the main parameters to be included in developing a contaminant budget. (NB. Factors only define the general pathways, determination of a reliable budget will need to include the various chemical forms in which antibiotics and antifoulants may appear.)

2. Antifoulants in Aquaculture

2.1. Introduction

Antifoulant paints are used on recreational craft, commercial vessels and in aquaculture to prevent the unwanted growth of fouling organisms on marine cages. In marine finfish farming, this improves the free flow of water through the cages which is vital for maintaining oxygen levels, the removal of excess feed, ammonia and solid fish wastes. High levels of fouling can increase operational efforts due to productivity losses, damage to equipment and increased maintenance costs. A nutrient rich environment supports biological growth and farm structures can accumulate biofouling in the order of several kg/m^2 (Dean et al, 2007). Additionally, heavily fouled nets and cages have been identified as potential reservoirs for disease-causing organisms (IUCN, 2007; Douglas-Helders et al, 2003).

Two main types of antifouling products are commercially available:

- Products containing active agents that prevent settlement and growth of fouling communities by exerting a toxic effect. These products are well established on the commercial market and typically contain constituents such as copper and zinc, often combined with organic "booster biocides" to increase product efficacy (Finnie, 2006).
- Products that rely on physical properties such as silicon based ("non-stick") fouling release and self-polishing products, or nanoscale manipulation of materials and surface properties. Research into these non-toxic products is much newer, and receiving increasing attention as products based on copper, for example, become less favoured. However, many of these products have been specifically designed for use on ships and rely on movement of the ships hull through the water to be effective, and therefore would have limited application in an aquaculture context.

Copper and other biocides are efficient antifoulants as a result of their acute toxicity. The products are formulated to slowly and continually leach active agents into the adjacent water column in concentrations that prevent the establishment of biological communities. Regulations and requirements for efficacy testing of antifouling products dictate the maximum leaching rate allowed for products, in much the same way that TBT release rates were regulated in the past. Once released to the environment, these toxicants have the potential to accumulate in the sediments, local biota and water column, and with sustained use, may cause negative effects at species or ecosystem level (Morrisey *et al*, 1996). Best practice management, and the use of the most appropriate products are therefore essential steps in minimising the potential unwanted effects of antifouling product use.

The Tasmanian Salmonid Growers Association Ltd (TSGA) currently has a permit issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) under section 112 of the Agvet Codes. The purpose of the permit is to conduct research for trialling the effectiveness of antifouling treatments in managing the biological fouling of seacages. The continual removal, cleaning and replacement of nets as a result of build up of fouling material is a considerable operational expense and potentially results in significant production delays and the generation of large quantities of netfouling waste. The rate of net fouling (and therefore net replacement and washing) is highest in spring and summer, and as a result, operational intensity increases at a time when other activities are also increasing (e.g. bathing, and grading). Aquaculture managers look to the use of antifouling treatments to increase the period of time that nets can be used in the water, reducing the changeover frequency, and cost of production.

An additional benefit of the copper based antifouling treatment is that application provides a high degree of net stiffening, which provides greater protection against the threat of seal attack. Antifouling coatings can also provide protection against UV-degradation of nets and ropes (IUCN, 2007).

A number of key knowledge gaps have been identified in relation to the sustained use of antifouling products in salmonid aquaculture, and the ability to address these environmental issues requires a greater understanding of the ways in which antifoulants interact with the environment. Only a proportion of the antifoulants input to the system actually impact on the sediments or water column, and the processes that mediate or affect how the benthic ecosystem responds requires greater understanding.

2.2. Summary of current usage

Two permits have been issued by the APVMA. The original permit, PER 6150 operated until August 2008, and allowed for the supply and use of a currently unregistered AGVET chemical product "for the purposes of research that provides sufficient information to allow assessment for registration of a range of antifouling products for fishnets to prevent fouling". Specifically PER 6150 allowed for the use of the following antifouling products on a commercial scale (see Table 1). All fish farm leases at which antifouling paints are employed are included under the permit.

Table 1 Summary of antifouling products covered by permit PER 6150, with main (active and other) ingredients.

Product	**Active ingredients**	**Other**
Aquasafe W (now Aquanet)	Cuprous oxide (Cu_2O)	Water based
Flexguard VI, Flexabar	Cuprous oxide (Cu_2O) 196 g/L (26%)	
Hempanet "Medium Strength" or "Light" (7150A) Hempel	Cuprous oxide (Cu_2O) 196 g/L (12.5-15%)	Xylene solvent based Zinc oxide 3-5 %
Hempanet (7177A) Hempel	Cuprous oxide (Cu_20) 522 g/L (25-50%)	Xylene solvent based Zinc oxide 5-10%
Net clear ZPT, Wattyl	Zinc oxide Zinc pyrithione	
Norimp 2000, Jotun	Cuprous oxide (Cu_2O) 220-245 g/L	
International Paints YAE 1703, Akzo Nobel	Cuprous oxide (Cu_2O) 432 g/L	

The current permit (PER 10924) issued in August 2008 allows for a more limited range of paints as specified in Table 2 below:

Table 2 Summary of antifouling products covered by permit PER 10924, with main active ingredients (Source APVMA permit 10924 labels).

Product	**Active ingredients**	**Other**
Flexguard VI, Flexabar	Cuprous oxide (Cu_2O) 196 g/L (26%)	
Hempanet "Medium Strength" or "Light" (7150A) Hempel	Cuprous oxide (Cu_2O) 196 g/L (12.5-15%)	Xylene solvent based Zinc oxide 3-5 %
Hempanet (7177A) Hempel	Cuprous oxide (Cu_20) 522 g/L (25-50%)	Xylene solvent based Zinc oxide 5-10%
Norimp 2000, Jotun	Cuprous oxide (Cu_2O) 220-245 g/L	
International Paints YAE 1703, Akzo Nobel	Cuprous oxide (Cu_2O) 432 g/L	

Both permits placed restrictions on the location and methods for net dipping, effluent and solid waste storage requirements, transfer and flaking operations, effluent treatment and disposal, and withholding conditions for the listed products.

Copper is the main ingredient in the great majority of antifouling products, present in the form of cuprous oxide (Cu_2O). The oxide is dissolved in a polymeric matrix that allows the slow dissolution of copper in water, providing an ongoing antifouling release (IUCN, 2007). All the listed products have cuprous oxide (copper (I) oxide) as the main active agent, except for Net Clear ZPT which uses a combination of zinc oxide and zinc pyrithione as the active agents. The MSDS supplied for Hempanet also lists zinc oxide in small quantities (<5%) in its ingredient listing. Typically zinc oxide is used to control the rate of coating erosion. Other metals that may be present include iron, added as an unreactive pigment, and aluminium (Turner *et al*, 2008).

TSGA reports that Hempanet has been used most extensively for research trials by the salmonid industry (AMD, 2007), with small quantities of NetClear ZPT, and some early but sporadic use of Jotun and Norimp 2000. There has been a transition from the use of Hempanet to Hempanet medium strength, resulting in a substantial reduction in the concentration of cuprous oxide as the main active ingredient (AMD, 2006). It is estimated that the volume of paint used annually by both Huon Aquaculture Company and Tassal (2006 all sites/products combined) equals almost 174,000 L. Of this quantity, 99.5 % of use comprised the product Hempanet Medium, with the remainder being Wattyl Netclear ZPT (AMD, 2007). In 2007, combined industry use increased to 220,000L.

There is limited data available on the quantity or volume of antifouling paints used in finfish aquaculture in overseas countries, as other than Scotland, there are no requirements for industry to report the annual quantities of paint used (Burridge, 2008

–WWF Aquaculture Dialogue). The most recent data for Scotland is listed below (see Table 3), along with estimates of paint used in Tasmania, based on industry reports to APVMA and DPIW. Mass of cuprous oxide (Cu_2O) was calculated from concentrations reported in Table 1 and Table 2 and labels in permit 19024. Although on a per tonne of production basis antifoulant usage in Tasmania is much greater than in Scotland, it should be noted that there are some fundamental differences in production methods; many farms in Scotland use "system pens" on which the nets can be more easily rotated and which therefore do not require the same level of antifoulants as in Tasmania (D O'Brien, pers.comm.).

Table 3 Total copper oxide antifouling use for Tasmania and Scotland. Figures for Tasmania 2004-2007 based on volume of paint applied per annum, sourced from industry reports (AMD 2004-2008). Estimates of total copper (I) oxide used in Tasmania for 2008 are based on mass balance calculations conducted by industry (Cameron Dalgleish, pers. comm.). Data for 2004 represents minimum and maximum as a combination of Hempel and Hempel light were used. Data for Scotland sourced from Burridge et al, 2008, reported as copper oxide. Range of values for Scotland reflects use of a variety of paint formulations with range of possible active concentrations. For the purposes of this table it is assumed copper oxide = copper (I) oxide, or Cu_2O. Annual production values (t) for Scotland from FRS, 2007 and Tasmania from DPIW.

Country	**Year**	**kg Copper Oxide applied**	**Production (t)**	**Kg Copper oxide /tonne fish produced**
Scotland	2003	18,996 – 26,626	169,736	0.11-0.16
	2004	11,700 – 29,056	158,099	0.07-0.18
	2005	34,000 – 84,123	129,588	0.26-0.65
	2006	35,551-86,929	131,847	0.27-0.66
	2007	NA	129,930	-
Tasmania	2004	36,827-84,422	14,653	2.51-5.76
	2005	61,439	18,403	3.34
	2006	33,923	22,417	1.51
	2007	42,958	23,982	1.79
	2008	48,046	26,172	1.83

Industry predictions are that this level of usage will increase in the coming years due to industry expansion (D. O'Brien, pers. comm.) however this is conditional on APVMA registration and State regulatory authority approval requirements being met. If approved, antifouling use for all nets over the 08/09 period may approach 306,000 L (D. O'Brien pers. comm.). Additional facilities (net washing and net dipping) have been constructed at HAC to deal with the volume of nets being treated.

The volume of paints currently being used represents a significant source of copper and zinc to the surrounding environment, and industry sediment surveys have indicated copper levels in sediments are significantly higher at farm sites, than control or compliance sites (AMD, 2007). The literature review aims to address some of the questions raised with respect to these metal loadings, and to help identify areas for future research and monitoring. Targeted research feeding into the adaptive management framework is an important process to enable industry to move forward towards registration and long term management of sustained antifouling use.

2.3. Hazard identification

Copper is an essential trace metal necessary for some enzymes, protein structure and iron utilisation in marine organisms (Kwok *et al*, 2005; Hall and Anderson 1999). At elevated levels it is toxic to a range of organisms, and can cause growth inhibition, reproductive stress and death (Eriksen *et al*, 2001). Zinc is also an essential micronutrient, important for membrane stability, present in over 300 enzymes, and involved in the metabolism of proteins and nucleic acids (WHO, 2001). When present in excess of normal requirements, it can exert adverse reproductive, biochemical, physiological and behavioural effects on a range of organisms. It is these toxic properties that make copper and zinc effective as antifouling agents.

The specific purpose of the permits granted to TSGA is to collect data that assesses the potential for the accumulation of copper (or other active ingredients from the antifouling products) in the sediments and around fish farms, through the use of an adaptive monitoring program. The aim of the permit is to ensure no significant environmental effects arising from the continued use of antifouling products, with data contributing to an application to register various copper based antifouling products. Since zinc and copper are the principal active ingredients in the permits, the focus of the literature review and risk assessment will be restricted to these toxicants, with a major emphasis on copper. Any other active agents employed in antifouling products are outside the scope of this document.

A number of key areas for concern are addressed here, based upon the known entry routes and partitioning of copper and zinc in the marine environment. Additionally, species sensitivity at different trophic levels and environmental niches are examined. The scoping document does not specifically cover human health risk assessment.

2.3.1 Entry routes to the environment

The most significant route of entry of antifoulant toxicants into the marine environment is generally assumed to be by direct emission from underwater painted surfaces by leaching from the paint film (Finnie, 2006). In an aquaculture scenario, significant loadings of copper to the environment may also occur in instances where crowding or net handling causes ablation of particles of paint that drop to the sediments underneath the cages. Additionally, in situ net cleaning with brushes, high pressure hosing or suction systems may also contribute significant quantities of paint particles to the environment (TSGA, 2004). In situ net cleaning may also contribute soluble copper to the marine environment. Copper may continue to be released from paint particles at a higher rate than the designed leaching rate from a solid painted surface, as the surface area of the paint chip is increased considerably. Other sources of inputs include food and faeces.

Water exposure: partitioning, speciation & toxicity of metals

Heavy metals such as copper and zinc used in antifouling products are designed to constantly leach small concentrations of the active agents, and can therefore be expected to be found in increased concentrations in the surrounding water column. The copper will be present primarily in the dissolved phase, but rapidly partition to particulate material (suspended solids etc) in the water column. Dissolved copper is defined as that which passes through a 0.45µm filter, whilst particulate copper is that retained on the filter. The inference is that dissolved copper is more biologically available than particulate material, and so this operationally defined parameter is commonly used to compare copper concentrations between water body types and locations. "Dissolved" copper passing through a filter may however be associated with colloidal material, and not be truly dissolved (Kramer, 1986). Typical dissolved copper concentrations from a variety of marine and estuarine water bodies are shown below in Table 4. Background zinc levels in seawater from Australia are reported to be in the range < 0.022 -0.1 µg/L (NWQMS, 2000). Baseline levels of copper in seawater are reported to be 0.0005 to 0.026 µg/L (WHO, 2001).

The toxicity of copper is dependent upon the speciation, or chemical form, that copper exists in, in the environment. Some forms of copper are known to be more toxic than others, and hence more biologically available (Eriksen et al, 2001). The term bioavailability refers to the fraction of copper that is directly available to the organism, and has been shown to be dependent upon the concentration of the free copper ion Cu^{2+}. This bioavailability is dictated by the chemical and physical conditions present in the receiving environment, and water quality parameters such as temperature, salinity, and pH can affect the speciation. In addition, complexation by dissolved organic carbon, and inorganic ligands present in seawater such as chloride, carbonate, hydroxide, sulphate, and iron and manganese hydroxides regulate the speciation of copper and other metals. The type of ligands present, the affinity of the metals ion for the ligands and the extent of thermodynamic and kinetic control all contribute to the complex regulation of chemical speciation (Eriksen, 2000).

In natural waters, approximately 98-99% of copper in seawater is bound to naturally occurring organic material, with the remainder accounted for by inorganic species (Sunda and Hanson, 1987). Less than 0.08% may actually be present as the cupric ion. As a result, the concentration of the free metal ion may be many orders of magnitude lower than the total metal concentration. Decreasing pH may increase the free metal ion activity, resulting in metal desorption from colloidal and particulate matter, and may dissociate some complexes (NWQMS, 2000).

Direct measurement of the free metal ion is complex, and toxicity testing is often used to establish a biological response, and inferences about bioavailability made if a toxic response is observed. Other speciation schemes that estimate bioavailability through chemical measurements include Anodic Stripping Voltammetry ("ASV-labile"), use of ion- exchange resins, diffusive gradients in thin films (DGT's), ligand competition, ion selective electrodes or the use of size- based separations (Stauber *et al*, 2000; Eriksen, 2000).

Other indirect methods for assessing speciation and bioavailability include the use of geochemical models e.g. MINTEQ and MINEQL. The reliability of these computer models is entirely dependent on the quality of the thermodynamic (stability constant) and environmental parameter data used to create the scenarios. The exact composition of organic constituents in seawater is complex, and organic ligands are difficult to characterise and are typically "described" using stability constants. A few models consider metal-organic interactions, but the mathematical modelling of copper speciation in the presence of these compounds is difficult and a range of binding constants may be required to adequately model the metal-complexing capacity of these complex molecules.

More recently, sophisticated models such as MAMPEC that incorporate a range of environmental and physical factors (temperature, current velocity etc) have become available, and they allow the user to simulate release scenarios such as marinas, commercial harbours, open sea, or shipping lanes. Scenarios may be manipulated to represent aquaculture systems (J. Poppleton pers. comm.) and the partitioning of copper between dissolved and particulate phases. This model also takes into account available chemical and toxicity test data to generate Predicted Environmental Concentrations (PEC) and Predicted No Effect Concentrations (PNEC). The ratio of PEC:PNEC approach is used by the IMO for risk assessment of emission scenarios for the active agents in antifouling products used in marine systems.

*Table 4 Summary of dissolved copper concentrations reported in marine waters. *Reported in Hall & Anderson (1999).*

Study region, water use/location	**Dissolved copper concentration µg/L (total µg/L)**	**Reference**
Sweden, west coast marina	0.25 – 2.85	Wangberg *et al* (1995) *
Sweden, Stockholm marina	0.76 – 3.83	Ohrn (1995) *
Greece, harbour/urban area	0.45 – 20.7	Dassenakis *et al* (1996) *
France, harbour	1.0 – 10.0	Alliot and Frenet-Piron (1990) *
England, estuary	0.2- 3.2	Harper (1991) *
North East Atlantic, ocean	0.086 – 0.103	Vas *et al* (1993) *
Tasman Sea (marine waters)	0.06	Batley, 1995
NSW, marine waters	0.10	WA Environment Dept, 2005
NSW coastal waters	0.031	WA Environment Dept, 2005
Pacific Ocean, surface	0.027 – 0.092	WA Environment Dept, 2005
Bathurst Harbour Tasmania, pristine estuary	(0.012 – 0.38)	Mackey *et al* (1996)
Maquarie Harbour Tasmania, contaminated estuary	2.9 – 19 (4 – 24.7)	Eriksen *et al* (2001)
Huon estuary Tasmania,	0.085 – 0.188 µg/kg (0.109 -0.241 µg/kg)	Butler, 2005 Butler *et al*,
British Columbia, aquaculture cages [1]	0.54	(Lewis and Metaxis, 1991)

[1] *Inside the cage, immediately after and one month after application*

Sediment exposure: partitioning uptake pathways, and toxicity of metals

It is generally observed that most of the heavy metals entering the marine environment through point or diffuse anthropogenic inputs become incorporated into the sediments (Batley and Maher, 2001) and sediments therefore act as an "integrator" of water column metal concentrations over long time periods (Butler *et al* in DEP, 2007). Copper and other contaminants in the marine environment tend to sorb to fine grained particles, with a large surface area, and it is generally observed that the highest levels of contamination in marine sediments are associated with fine grained material deposited in sheltered, low-energy areas, which serve as sinks (NRC, 1997; Matthai and Birch, 2001; Morrisey *et al*, 1996). These particle surfaces are dominated by iron and manganese oxyhydroxides, organic material and sulphides (Batley and Maher, 2001). Sandy sediments in high energy environments, low in organic carbon and fine silt, generally have lower total heavy metal concentrations, and it is this reduced capacity to bind metals that make comparison of heavy metals loads between different sediment types difficult.

The voids between particles that make up sediment are filled with interstitial or pore water, which may comprise up to 50 % of the sediment volume (Batley and Maher, 2001). Metals are partitioned between the solid and pore water phases of sediment, and the relative exposure that an organism experiences will depend on its feeding strategies and habitat (i.e. burrowing vs epibenthic). Heavy metals associated with the porewater phase may be more available to organisms that burrow within the sediments, and porewater is therefore one of two major uptake pathways for metals by marine benthic fauna (Chapman *et al*, 2002). The other major exposure route is solid phase exposure route, either through ingestion, bioturbation or filtering of particulate material.

In natural sediments that are in equilibrium with porewater, the general observed trend is that pore water metal concentrations are low. Dean *et al* (2007) however measured porewater concentrations as high as 250 µg/l zinc, and 65 µg/L copper in the top 2 cm of sediments located directly under fish cages in Scotland. Corresponding sediment loads were 450 mg/kg zinc and 150 mg/kg copper. Based on toxicity data, and relevant water quality guidelines, there is most likely significant adverse biological impact associated with these levels of porewater metals.

Sediments are recognised as being an indicator of ecosystem health, and can provide valuable information on the magnitude of anthropogenic impact, and the scale of ecological impact. Data from a range of marine sedimentary environments is shown in Table 5, for both copper and zinc.

2.3.2 Chemical mechanisms for binding

As in the case for seawater, the biological availability of contaminants such as copper and zinc in sediments is controlled by a number of environmental factors, and mediated by a host of chemical equilibria that dominate according to the redox status of the sediments, the level of organic matter, and the presence of mineral phases. In marine environments where the deposition of organic material is significant, the breakdown of organic matter consumes oxygen in the sediments and overlying water. Sulphides are generated by sulphate reduction, resulting in reducing conditions in the sediment i.e. hypoxia or anoxia. The resulting changes in sediment and porewater conditions often results in significant changes to infaunal communities with colonisation by a few small opportunistic species, often at high abundances (Dean *et al*, 2007; Macleod and Helidoniotis, 2005). Under these anoxic conditions, most metals are present as insoluble sulphides, which can alter the bioavailability of metals. Copper and zinc form insoluble sulphides by rapid exchange with iron (as FeS), which may occur within minutes to hours.

It has been proposed that the solid-phase acid volatile sulphides (AVS) may be used to predict whether or not metals bound to sediments will be bioavailable. This method assumes that AVS is available to bind metals, and therefore renders them unavailable since the low solubility of metal sulphides results in low porewater concentrations (SEPA, 1998; Simpson *et al*, 1998). AVS is often coupled with simultaneously extracted metals (SEM) measurements to assess bioavailability and predict toxicity. SEM involves the extraction of metals in 1M HCl, and assumes that for every mole of sulphide measured as AVS, there will be one mole of metal measured in the corresponding SEM fraction (Simpson *et al*, 1998). These authors concluded that due to the insoluble nature of CuS in 1M HCl, that there was "*little theoretical basis for the interpretation of sediment toxicity from AVS/SEM relationships*" for copper.

Table 5 Summary of copper and zinc levels recorded in marine sediments from a range of environments (range of values as reported).

Study region, (use)	**Total copper concentration mg/kg DMB**	**Total Zinc concentration (mg/kg DMB)**	**Reference**
Sydney continental shelf (offshore)	0.6 - 19.8[3]	4.7- 82.4	Matthai & Birch, 2001
Huon estuary[5]	35 - 47	20 - 30	Macleod & Helidoniotis, 2005
Derwent estuary (Contamination "hotspots")[4]	2350	58700	DEP, 2007
Scotland (aquaculture)[1]	805	921	Dean *et al*, 2007
Scotland (aquaculture)[2]	427	1150	SEPA, 1998
Huon & D'Entrecasteaux (High organic, compliance)	6 - 79	13-76	AMD (2004 – 2007)
Huon & D'Entrecasteaux (High organic, lease)	5 - 1372	13 - 254	AMD (2004 – 2007)
Huon & D'Entrecasteaux (Low organic, compliance)	2 - 8	13-23	AMD (2004 - 2007)
Huon & D'Entrecasteaux (Low organic, lease)	19 - 153	19 - 305	AMD (2004 – 2007)
Tasman Peninsula (compliance)	5-8	34-47	Macleod et al, 2004
Tasman Peninsula (lease)	40 - 109	30 – 40	Macleod et al, 2004

[1] *Maximum level recorded in survey at active sites*
[2] *Maximum level recorded in survey at active sites*
[3] *Total sediment =fraction < 2 mm*
[4] Most contaminated site adjacent to zinc refinery, organic rich
[5] "baseline" data

It is widely recognised that metals can be released or re-mobilised when resuspended in oxygenated seawater (e.g. by bioturbation, storms, wave action or dredging), or when the sediments become oxic during recovery processes (Batley and Maher, 2001). Brooks *et al* (2004) and Valkirs (2003) hypothesised that sediment remediation (decline of organics and sulphides) in fish farm sediments resulted in copper and zinc release to the water column, and potentially provides a significant vector for fluxes from sediments. Petersen *et a*l (1997) found that up to 2 % of the particulate bound heavy metals were remobilised from sediments resuspended in an oxic environment. In high energy areas, this may result in the transport and redistribution of metals.

In anoxic and highly contaminated sediments (e.g. the Derwent estuary) experiments simulating physical disturbance showed that while metals were initially released upon resuspension, rapid scavenging of metals on particulate material (eg oxidised insoluble hydrous metal oxides of iron and manganese) resulted in relatively low water column concentrations (Butler et al in DEP, 2007). Simpson *et al* (1998) conducted similar experiments with model sulphide phases resuspended in oxic waters and found that trace metals occluded in or coprecipitated with iron sulphide may more be prone to oxidative release than those present as metal sulphide. Other studies have shown that copper and zinc release from resuspension of anoxic sediments is well correlated with sulphate concentration, confirming sulphide oxidation as the predominant release process (Calmano *et al* in Simpson *et al*, 1998).

2.3.3 Environmental persistence

Copper is highly persistent, particularly when it accumulates in the sediments (Ranke, 2002). Therefore the effects of copper contamination may be observed long after a particular contamination event. Zinc will behave similarly and remain persistent in the sediments exposed to sources of contamination. It is generally accepted that the only way to reduce levels in a specific area is by dispersal or removal processes. These processes can either be biological (i.e. bioturbation and resuspension) or hydrological (resuspension).

The source of the metal addition will have an impact on the dispersal processes, for example metal present as paint flakes and chips may continue to leach into overlying or porewaters, and release rates may be significantly higher than the intact painted surface due to surface area affects. Most research in this area has focused on the boat maintenance and repair industry (Turner et al, 2009a, 2009b; Singh et al 2009), with little or no information specific to paint particles generated during aquaculture operations. However, findings from the broader studies will in many cases still be relevant, as the mechanisms by which metal is released from paint particles into the marine environment will be similar.

When paint particles become dislodged and deposit to the sediments they will have an affect on non-target organisms. Impacts are likely to be greatest when the paint particles are newly exposed and in organisms whose life history strategies place them at most risk i.e. filter or deposit feeders. Experiments with *Mytilus edulis* showed that mussels did not appear to discriminate between contaminated and uncontaminated particles and that metal was remobilised in the gut environment following ingestion (Turner 2009a). The authors proposed that this uptake pathway was more important than aqueous uptake, and showed evidence of bioaccumulation after relatively short exposure times.

The physical and environmental factors that control the extent and rate of leaching of active agents from paint particles is largely unknown. However, temperature and salinity have been shown to affect dissolution rates, with lower temperature and high salinity favouring higher release rates (Turner *et al*, 2008). These authors also showed that copper leached from fractionated (<63 µm) paint chips rapidly oxidised from Cu (I) to Cu (II) and subsequently complexed with organic and inorganic ligands, and adsorbed onto particulate material. This transfer of copper from a paint matrix to naturally complexing material may have a significant impact on the long-term fate of copper, particularly in recovery cycles where large changes in redox, pH and sulphide gradients are experienced. Mass balance experiments conducted by the Tasmanian industry (Dalgleish, 2008b) showed a larger size fraction is more representative of in-situ cleaning. Singh *et al.* (2009) found that leaching rates of copper and zinc were relatively insensitive to particle size but that the type of paint matrix may have an effect. Regardless of the uncertainty about the fate of leached metals (whether they remain in solution or adsorb to particulate material), it is clear that directly or indirectly, paint particles dislodged through net handling or cleaning operations may represent a significant localised source of metallic contamination.

2.3.4 Other sources of copper and zinc

Many international studies have found sediment copper levels below marine finfish cages to be significantly higher than background levels, attributed to copper treated nets and fish feed. Sediment metal levels recorded in Tasmanian sediments range from 6 – 3225 mg/kg (AMD, 2007), but are most typically < 400 mg/kg. Sediments under fish farms in a Scottish study were recorded as up to 805 mg/kg for copper, and 921 mg/kg for zinc (see Table 5), attributed to paint effects but also a small contribution from feed and faeces. Analysis of fish farm sediments by SEPA (1998) found up to 427 mg/kg copper, and 1150 mg/kg zinc. More locally, the Aquafin CRC undertook a study of the effects of antifouling on tuna aquaculture in SA, with a view to enhancing water quality and net fouling maintenance. This project has been completed but no results are available at this point in time.

Dean *et al* (2007) measured copper in Scottish salmon feed as 8.9 mg/kg, and zinc 196.4 mg/kg. There was some concentration in effect evident in faeces with 12.9 mg/kg copper, and 364.52 mg/kg zinc. TSGA (AMD 2007) estimated copper in Tasmanian salmon feed to be approximately 15 mg/kg. Uneaten food and faecal waste accumulate under the seacages, and AMD (2004) estimated that food may raise the sediment copper concentration by 10-15 mg/kg in sediments at high organic sites. This *may* be significant if coupled with net based deposition in areas already demonstrating increased sediment burdens, however food is not believed to be the major mechanism for accumulation of copper in sites where antifouled cages have been deployed.

2.3.5 Biological effects

Plankton effects (zooplankton & phytoplankton)

Copper in solution may be passively or actively taken up by pelagic organisms. Phytoplankton and other single celled organisms are immediately susceptible to dissolved copper, and numerous studies have focused on acute and chronic toxicity observed in algal cultures. The effects of heavy metals on phytoplankton are especially relevant since these organisms constitute the base of the marine food web. Heavy metals exert their toxic affects by competing with essential metals for active enzyme or membrane protein sites and by reacting with biologically active groups. Thus, they may interrupt the normal metabolic processes of algal cells.

Copper is known to adsorb rapidly to the outside of algal cells, and can account for up to 40% of the total copper added in solution in algal growth toxicity tests (Florence *et al*, 1983). This ability to bind copper on the outside of the cells via algal exudates is important in regulating the rate at which copper is internalised, that is, transported into the cell. Binding sites on the outside of the cell may be metabolically active, allowing copper to enter the cell, or metabolically non-active (Levy *et al*, 2008). Internalisation of copper is assumed to occur via transporters in the algal cell membrane, and is considered the rate limiting step in metal uptake. Internalisation can occur rapidly, within 20 -30 hours of exposure to environmental copper, and thus reduced growth rates and other chronic endpoints may be observed within 48 hours. A number of detoxification mechanisms have been proposed for algal cells, including sequestration, elimination, exclusion, binding and induction of stress-proteins (Levy *et al*, 2008).

Different phytoplankton species exhibit different sensitivities to copper, although generally, phytoplankton are considered to be highly sensitive to copper. The concentration of copper required to cause a 50% reduction in growth rate over a 72 hour bioassays for a number of marine phytoplankton species are shown below in Table 6. Also shown is the NOEC, or No Observed Effect Concentration. It is thought that the difference in species sensitivity is due to the cell detoxification mechanisms employed, and the relative effectiveness of exclusion mechanisms over sequestration for example.

Zooplankton are less sensitive to the effects of copper and zinc, with the two principle pathways considered to be direct exposure to soluble metals, and ingestion of food sources with some degree of metal contamination. Larval stages are more sensitive than adults. Whilst there is an abundance of literature on the effects of copper and zinc on freshwater invertebrates, the data set is more limited for relevant marine species.

Exposing the calanoid copepod *Temora stylifera* to zinc chloride, Nipper *et al* (1993) reported 48 hour LC_{50}s ranging from 30 - 40 µg/L, following exposure in saltwater of salinity 28 - 32 ppt. Juvenile (28 days old) banana prawns (*Penaeus* sp.) were sensitive to copper in growth bioassays conducted by Ahsanullah and Ying (1995) with a NOEC of 50 µg /L after 14 days exposure. Acute test with the same species recorded a 96 hour LC_{50} of 380 µg/L. Larval stages of the crab *Paragrapsus quadridentatus* recorded a 96 hour LC_{50} of 170 µg/L copper. Because of the longer life cycle of zooplankton, there are more data on acute effects (survival) than chronic endpoint such as growth or reproduction.

There is little data on the toxicity to zooplankton exposed to copper or zinc through the ingestion of food/phytoplankton that have been exposed to the metals, and either incorporated the metal internally, or bound them to the outside of the cells.

Table 6 Summary of toxicity of copper to phytoplankton, all studies cited involved adding copper directly to clean seawater. No complexing agents or culture mediums used during assays. IC_{50} = concentration of copper causing a 50% reduction in growth rate, compared to seawater controls.

Species	72 hour IC_{50} (µg/L copper)	NOEC (µg/L copper)	Reference	Comments
Nitzschia closterium	18	2.5	Stauber *et al*, 1996	Macquarie Harbour study
Phaeodactylum tricornutum	100	< 1.5	Levy *et al*, 2008	
Dunaliella tertiolecta	530	8	Levy *et al*, 2008	
Cylindrotheca fusiformis	28	10	Markich *et al*, 2002	Nominal concentrations
Nitzschia palea	10	-	Stauber *et al*, 1996	Macquarie Harbour study

Other fish species/ by-catch

Waterborne copper exposure can exert a variety of physiological effects in fish, including the disruption of sensory system function, which has wide-reaching implications for fish behaviour. Exposure of fish to water soluble and dietary copper can in severe cases cause mortalities (Daglish and Nowak, 2002). In developing fish larvae, copper is known to affect key parameters, such as survival and growth (Johnson *et al*, 2007). Chronic effects include reduced growth, shorter lifespan, reproductive problems, reduced fertility, reduced resistance to infectious disease and behavioural changes (Atchison *et al*., 1987; Nowak and Duda, 1996). Some toxicity data for behavioural and survival tests are shown below in Table 7.

When exposed to elevated levels of waterborne copper, fish accumulate the metal residues primarily in the liver and also in the gills (Sorensen 1991). Gills may be a better indicator of short-term exposure than the liver (Daglish and Nowak, 2002). In a study conducted on Chinook salmon in caged culture, a study by the USEPA found that copper was not accumulated in the tissues of the fish.

Table 7 Summary of selected fish toxicity results for chronic and acute endpoints.

Species	LOEC	Endpoint	Reference
Rainbow trout	0.1 to 6.4 µg/L	Behavioural effects of Cu on fish	Atchison *et al*., 1987
Lake whitefish	6.3 µg/L		
Atlantic Salmon	2.3 µg/L		
Goldfish	5.0 µg/L		
Bluegill, brook char and flathead minnow	17 to 40 µg/L (96h LC_{50}75 to 1100 µg/L)	Survival	Atchison *et al*., 1987

Accumulation in Biological Material

Bioaccumulation of metals may occur either by direct uptake from the surroundings (e.g. across the body wall or respiratory surfaces), or via food (NWQMS, 2000). It is generally assumed that the dominant uptake route is via passive diffusion across the body surface, gills, or lungs, or by active transport via calcium pumps. A bioconcentration factor (BCF) can be defined as:

BCF = (µg/g trace metal in organism)/ (µg/g trace metal in water). This model has been applied to suspension feeding bivalves (e.g. mussels and oysters), as well as phytoplankton, zooplankton, and crustaceans (NWQMS, 2001). The concept of BCF may be applied to the whole organism, or particular tissues.

Body burden testing on farmed salmon has been conducted for export purposes, through the National Residue Survey, although this data does not discriminate between fish farmed in antifouled nets, and those without antifouling coatings. Monitoring conducted by DPIW has included comparison between fish farmed in cages with and without antifouling treatment, and observed that copper levels in flesh did not exceed the ANZFA generally expected level of 0.5 mg/kg. The effect of copper treated nets on farmed Atlantic salmon was investigated by Solberg *et al* (2002), and they included a comparison of copper residues in muscle, liver and gill samples of fish farmed in treated and untreated nets. They detected no difference in muscle or liver tissues in salmon, or saithe (*Pollachius virens*), and concluded that the leach rate of copper from treated nets was low enough that the natural homeostasis of the fish and their detoxification processes were not compromised. Such studies provide useful information on the potential for accumulation in higher organisms exposed to what in the first instance is assumed to be a low but constant concentration of copper and zinc. Additional information on immunosupression and health related issues in stock has been raised as a research question by industry regulators.

Dauvin (2008) observed significant levels of copper, lead and zinc in estuarine species, particularly bivalves, in areas where sediments were significantly contaminated. Fish species were also studied, and the sea bass *Dicenthrarchus labrax* found to accumulate greater levels of metals in the liver and kidneys than in the muscle tissue. There was no difference in the body burden of juvenile (< 15 cm) and older (> 20 cm) specimens, suggesting that metals did not bioaccumulate over the lifetime of the fish.

Zinc is an essential element for many marine organisms and, as such, is readily bioaccumulated. Several species of crustacean are able to regulate the uptake of zinc but, at higher concentrations, this process appears to breakdown leading to an influx of zinc. These issues complicate the calculation of bioconcentration factors (BCF) which can be misleading. Highest concentrations of zinc reported by Hunt and Hedgecott (1992) were: 300 - 9700 µg g^{-1} (dry weight) in *Fucus vesiculosus* (Bladder wrack); 605 - 619 µg/g in *Littorina littorea* (common periwinkle): 16460 µg/g in *Elminius modestus* (barnacle) and 2800 µg/g in dogfish.

In Australian species, King et al (2004) demonstrated that organisms respond differently to metal exposure, with the polychaete *Australonereis ehlersi* accumulating copper, but not zinc from spiked sediments.

Mollusc species are excellent bioaccumulators of heavy metals, and have often been used as “bioindicators” of metal in contaminated areas (Ayling, 1974). Studies in the Derwent have found that oysters preferentially accumulate zinc, while mussels preferentially accumulate lead. Oysters can accumulate up to 8000 mg/kg zinc in as little as 6 weeks in areas where zinc concentrations in the water column are elevated above background concentrations (Seen and Eriksen, unpublished data). Oysters collected from background sites typically have less than 500 mg/kg (DEP, 2007). In the same study, oysters collected from contaminated areas accumulated up to 150 mg/kg copper, compared to around 20 mg/kg in background sites. Shellfish are able to concentrate metals from both dissolved and particulate bound metal. Factors that affect uptake and accumulation rates include age, sex, reproductive stage, and size.

Pilot scale monitoring undertaken by the State government involving deployment of sentinel oysters at sites with antifouled nets, observed an accumulation of copper in oysters immediately adjacent to antifouled cages. Meat concentrations were slightly elevated compared to Generally Expected Level of 30 mg/kg, however there was deemed to be no risk from a human health perspective (Woods, pers.comm.). Solberg *et al* (2002) compared copper concentrations in blue mussels collected at farms with and without copper treated nets and found no significant difference between sites. Mussels were not recommended as good “surveillance” organisms for copper pollution because of their ability to regulate copper even in the presence of higher ambient concentrations.

Sediment fauna composition

Population dynamics of benthic fauna in estuarine sediments are strongly and directly coupled to physico-chemical processes, with salinity responsible for much of the variation observed (Dauvin, 2008). Studies attempting to identify the effects of pollution on populations and communities are often difficult to interpret because of the complicated interactions associated with disturbance effects. Several studies have found that the impact or toxicity of heavy metals increases with decreasing salinity (McLusky *et al*, 1986; Dauvin, 2008). McLusky *et al* (1986) proposed a rank ordering of toxicity for heavy metals in sediments: mercury (most toxic) >cadmium>copper>zinc>chromium>nickel>lead>arsenic (least toxic). Additionally they found a general trend for taxonomic order of sensitivity: annelids (most sensitive) > crustacean > molluscs, although they did note large variations between species with respect to metal toxicity, especially for annelids.

Toxic effects of copper to sediment fauna include interference with enzymes and changes in the concentrations of electrolytes in the fluids of the body. Through its effect on the reproductive capacity of organisms, copper can also potentially influence populations and assemblages of marine organisms (Morrisey *et al*., 1996). Sensitivity to heavy metals differs significantly between species, with more robust species tending to replace those with lower tolerances. Differences within species may also occur, with zinc tolerance in the polychaete *Nereis diversicolor* observed in populations, compared to polychaetes from non contaminated sites (King et al, 2004). Age and reproductive stage may also be significant factors in metal tolerance. Olsgard *et al* (1999) reported that total faunal abundance and the density of the polychaetes *Pectinaria koreni* and *Prionospio cirrifera* decreased significantly at sediment Cu concentrations of 300 mg/ kg. Also the polychaetes *Pseudopolydora paucibranchiata, Capitella capitata, Chaetozone setosa, Harmothoe* spp., the bivalve *Thyasira sarsi* and the brittlestar *Ophiura affinis* were significantly negative correlated to increased sediment Cu content. Although these species were affected negatively, most of the 116 taxa within the experimental trays showed no response over the entire range of Cu sediment concentrations.

A study in Botany Bay, New South Wales, Australia, in which the concentrations of copper in marine sediments were experimentally enhanced, showed that increased concentrations of copper (140 to 1200 mg/kg compared with background concentrations of 29 to 40 mg/kg) had an impact on the fauna. The nature of the response varied among taxa. For example, in some taxa, numbers of individuals decreased through time relative to controls, whereas the abundance of another taxon remained fairly constant through time in the copper treatment while numbers of control individuals increased. Morrisey *et al* (1996) concluded that sediment copper concentrations greater than 100 to 150 mg/kg dry wt. may reduce the diversity of benthic fauna.

Studies in Norway have shown a negative correlation between species diversity and copper concentrations at 71 sites located in fjords (Rygg, 1985). A moderate negative correlation was observed for lead, and a weak negative correlation for zinc. At sites contaminated with copper (> 200 mg/kg), 20 of the 50 most frequently observed species were missing from contaminated sites. Additionally, a moderate negative correlation was observed for organic enrichment and diversity.

Rygg (1985) used 4 categories to describe species resistance to copper contaminated sediments:

- intolerant species, absent from sites with copper concentrations in excess of 200 mg/kg, including 14 annelida, 3 crustacea, 3 bivalves
- intolerant species, occasionally found at sites with sedimentary copper concentrations in excess of 200 mg/kg (including 6 annelida, 1 bivalve, 2 echinodermata)
- moderately tolerant species, present at some of the sites with sedimentary copper concentrations in excess of 200 mg/kg (including 5 annelida, 3 bivlaves:
- highly tolerant species, common to the most polluted sites with > 500 mg/kg including 15 annelida.

Differential sensitivity of annelids was explained as deposit feeding annelids ingesting more contaminated sediment per nutritional unit than carnivorous polychaetes, although some deposit feeders were tolerant of the most contaminated sites.

Ecotoxicological response of benthic fauna

Investigations into the "health" of marine sediments frequently use toxicity testing to determine the biological availability of contaminants detected by chemical testing. Toxicity testing attempts to bridge the gap between understanding the biological response to different speciation or forms of metals as toxicants, and the more limited information that can be gained from chemical analysis of parameters such as total metals, porewater concentrations, total organic carbon content, redox conditions etc.

Toxicity testing is best used in conjunction with chemical characterisation of the sediments, and in ideal cases can be combined with chemical data to provide predictions of toxicity in a wider range of sediments, or to other organisms. Acute tests are used to determine a short-term or lethal response, and are typically reported as % of individuals surviving exposure over a standard exposure period, compared to controls. Chronic tests examine sublethal effects such as growth, reproductive capacity, behavioural impacts, developmental abnormalities etc and result in information on longer term ecological effects of exposure to concentrations that may not cause immediate mortality under test conditions. Acute tests are often conducted as the first test measurement in toxicological evaluations, but both types of tests are important for assessing the impact of contaminated sediments on benthic communities (Rand, 1995).

Toxicological assessment may involve establishing a dose-response relationship (i.e. measuring response over a range of concentrations to determine what level of exposure is required for an effect) or by whole sediment exposure where organisms are exposed to undiluted test sediments. Both approaches are complimentary however the latter provides a more rapid assessment approach.

The impact of specific toxicants such as copper on benthic organisms (individual species or communities) may be determined through manipulation of sediment copper concentrations, and inferences made about faunal responses in field sediments. Recent studies conducted by TAFI used the brittlestar *Amphiura elandiformis* to determine sensitivity to copper in both artificially spiked sediments, and "naturally" contaminated sediments. In both instances, pore water concentrations were low and metal is assumed to be predominantly bound to sulphide and organic phases in the sediment. Preliminary results show that significant behavioural effects are observed at 210 mg/kg in spiked sediments, and 124 mg/kg in naturally contaminated sediments (Eriksen *et al*, 2009). No behavioural effects were observed in sediments spiked to 45 mg/kg, compared to clean Huon sediment controls. Work on community composition by Banks (2006) showed that native species were more sensitive to metal pollution than introduced species, although the mechanism/s for tolerance was not resolved.

A toxicological assessment of sediments from a range of sites, and operational conditions has been conducted as part of the permit conditions, and these results are discussed along with other monitoring data in section 2.5.

Sediment microbiota and microphytobenthos

It is important to determine and predict the effects of metals and other pollutants on microorganisms in the natural environment because of their ecological importance as primary decomposers (Flemming and Trevors, 1989). The effects of Cu on sediment microbiota include: (a) reduction in microbial numbers and diversity; (b) reduction of microbial mineralization processes; and (c) increase in metal-resistance in populations (Flemming and Trevors, 1989). This is an important area to understand as microbial loops drive many important biogeochemical processes, and are intimately linked both directly and indirectly to redox cycling, nutrient cycling, speciation and bioavailability of metals (Cooper *et al*, 2005).

For example, the breakdown of organic matter results in the consumption of oxygen by aerobic bacteria. Under these conditions, the removal of metals from solution as insoluble sulphides is due to the generation of hydrogen sulphide by anaerobic sulphate reducing bacteria. Sulphur-oxidising bacteria have also been shown to play a key role in the oxidative release of metals from sulphide phases, by enhancing the rate of oxidation (Lors *et al*, 2004). Sulphide oxidation is also catalysed by the presence of metals (Simpson *et al*, 1998). Reduction of sulphide to sulphate also results in the production of acid, which can reduce pH, unless buffered by components of the sediment. Decreased pH will favour the further release of metals from insoluble sulphide phases, carbonates and oxyhydroxides and the accelerated growth of acidophilic bacteria, with further implications for pH and metal release. pH is often considered the "master variable" in process that control mobilisation or scavenging potential of sediments (Simpson *et al*, 1998).

Screening tests using the bacterium *Vibrio fisheri* on contaminated Derwent sediments showed a gradient of response in line with increasing metal contamination, although porewater concentrations were typically low (DEP, 2007). As with most trophic levels, there is a considerable range in the sensitivity of various bacterial species to heavy metals. Exposure to sustained levels of copper can cause tolerance to develop in some microbial populations, and these species will predominate in the processes outlined above.

Closely coupled to the decomposition of organic matter are the processes of nitrification and denitrification. Denitrification is the conversion of inorganic nitrogen released from sediments to nitrogen gas, and it occurs in anoxic sediments. It is one of the few processes capable of counteracting nutrient enrichment and eutrophication because it can permanently remove nitrogen from the system (Geoscience Australia, 2008). Studies by Magalhaes *et al*, (2007) found that denitrifying communities in silty sediments were not affected by increasing concentrations of metals, while sandy sediments revealed almost complete inhibition of denitrifying communities by copper (85 % inhibition at 70 mg/kg WMB) and zinc (92% inhibition at 490 mg/kg WMB). Copper and zinc impacted on specific steps within the dentrification process for both high organic and low organic sites. They concluded that "trace metals not only reduce total N removal from an estuary via denitrification but can also enhance the release of N_2O, a powerful greenhouse gas".

Dell'Anno *et al* (2003) also showed that bioavailable metals had a significant impact on microbial loop functioning in marine sediments. The authors proposed that bacterial metabolism could be used as a bioindicator of stress conditions in marine sediments. Riba *et al* (2002) developed such a test using a colorimetric test to detect changes in dehydrogenase production following exposure to contaminated sediments. A commercial rapid screening test for the assessment of sediment and porewater contaminants has also been developed using the photo bacterium *Vibrio fischeri*, with moderate sensitivity to metals (Microbics Corp, 1992).

Microphytobenthos are a key component of the micro benthic community, and have been shown to be impacted by high sedimentation levels, with significant decreases in primary production occurring (Larson, 2005). The combination of increased nutrients and antifouling agents magnified this response.

Crustacea

Copper is used by crabs and other crustaceans as well as some molluscs as a respiratory pigment. Crustacea are able to regulate copper and zinc for biological functions up to a certain threshold level. Once the threshold is passed, regulation breaks down, and leads to loss of osmoregulatory function, accumulation and death if levels are high enough (Thurberg *et al*., 1973; Dauvin, 2008). Even sub-lethal concentrations may pose a significant problem when crustacea and molluscs are subject to other environmental stressors (Thurberg *et al*., 1973).

Exposure pathways are important in determining how sediment fauna are exposed to metal contaminants. The brown shrimp *Crangon crangon* for example may be exposed via the sediment compartment, the water column, the prey it eats, and the changing bioavailability of metals it is exposed to (Culshow *et al*, 2002).

Benthic amphipods are the most commonly used test organisms in whole-sediment tests as they live in direct contact with the sediment and meet many of the selection criteria for test organisms, such as ecological relevance, sensitivity, and seasonal availability (ASTM, 2003). A study on copper toxicity in an Australian estuarine amphipod species *Melita plumulosa* revealed that juveniles were more sensitive than adults to Cu, either bound to sediments or in the aqueous phase (King *et al*., 2006). Although Lowest Observed Effect Concentration (LOEC) values for copper in juvenile whole-sediment tests (820 mg/kg dry wt) were high in comparison with interim sediment quality guideline values for individual Cu (270 mg/kg dry wt), they were generally within the range of concentrations found in contaminated sediments in local estuaries. Accumulation of metals, together with the low porewater metal concentrations in whole-sediment tests, indicated that the ingestion of sediment is an important source of zinc and copper and cause of toxicity in this species (King *et al*., 2006).

Dauvin (2008) reported crustacean, in particular amphipods, as intolerant of sediments with over 200-300 mg/kg, noting that they cannot survive at these levels. This level is lower than those reported by King *et al* (2006) and highlights the need for locally relevant toxicity test data for interpreting sediment quality and environmental impacts of degraded sediments.

Faunal communities in contaminated sediments in UK were observed to be low in crustacean species, with *Corophium* (amphipod) and *Cyathura* (isopod) absent, while small opportunistic annelid species were highly abundant (Warwick, 2001).

Amphipods are generally accepted as good candidates for sediment toxicity tests, although those species that create u-shaped burrows (e.g. *Corophium* tube builders) may not be exposed to the same level of metal contamination by virtue of irrigation with overlying water, rather than direct exposure to interstitial (pore) water.

Net fouling material (animal & plant)

Copper biocides are the most effective against hard fouling animals and plants whilst Irgarol (and other organic biocides) are most effective against soft fouling plants (Manzo *et al*., 2008). Net fouling communities typically develop after an initial colonisation by "slime" layers, and it appears that once established, surfaces are more able to sustain settlement of a wider range of organisms (Callow, 2008).

Studies by Guenther *et al* (2008) have shown that the frequency and method of in-situ net cleaning may actually stimulate higher reproduction and colonisation rates in some organisms. Settlement behaviour is also influenced by the age of the treated surface, the current velocity, salinity, pH, inorganic and organic nutrient levels, as these parameters can influence the bioavailability and concentration of metals (Addison *et al*, 2008). Studies on the spatially variable effects of copper on sessile (fouling) invertebrates have shown that small scale variation (i.e. metres) has implications for how to characterise the level of risk of heavy metals to a particular site.

Macroalgae

Studies on the toxicity of copper and zinc to macroalgae are relatively limited and often based on growth or germination success/survival endpoints. Relatively high concentrations (100 µg/L) are required to have a measurable effect on photosynthesis in the giant kelp *Macrocystis pyrifera*. Survival of gametophytes of the brown algae *Laminaria hyperborean* was reduced at similar concentrations (Lewis and Cave, 1982).

2.4 Evaluation/ Analysis Approaches

2.4.1 Current Standards (Australia, US & Worldwide)

The process of determining or measuring the toxic effects of an aquaculture operation on the surrounding ecosystem is commonly based on guideline values for a range of physical, chemical and biological parameters. The assessment of metal accumulation, and predicted biological effects in sediments under and around sea cages is based on sediment quality guidelines that are derived from ecotoxicological and biological data.

Sediment Quality Guidelines

ANZECC Interim Sediment Quality Guidelines (ISQG) for metals in sediments are largely based on research by the US National Oceanic and Atmospheric Administration (NOAA) (Long and Morgan, 1990; Long *et al.*, 1995; Long and MacDonald, 1998). The NOAA assessment scheme uses Effects range low (ERL) and effects range median (ERM) to delineate three concentration ranges for a particular contaminant. Concentrations below the ERL represent a minimum effects range, that is, a concentration below which biological effects would be expected to be rarely observed. Between the ERL and the ERM, biological effects are more likely to occur, and above the ERM, is the probable effects range, above which adverse biological effects are expected to occur.

ANZECC values are viewed as trigger values, that if exceeded, should prompt further investigations to determine if an environmental risk is present (Simpson *et al.*, 2005). ANZECC guidelines contain two concentrations, the ISQG low concentration (trigger value = ERL) and the ISQG high concentration (= ERM). Values for copper and zinc for ANZECC, NOAA and FDEP are outlined in Table 8 and Table 9 below. A summary table showing the interpretation of the guidelines is shown in Table 10.

Table 8 Summary of copper guidelines for marine/estuarine sediments

Guideline	Descriptor	Trigger value copper	Comment
ANZECC	ISQG Low	65 mg/kg	The ANZECC ISQG Low (higher than the equivalent ERL) is based on Hong Kong guidelines
	ISQG High	270 mg/kg	
NOAA	Effects Range Low (ERL)	34 mg/kg	
	Effects Range Medium (ERM)	270 mg/kg	
FDEP	Threshold Effects Level (TEL)	18.7 mg/kg	(TEL + PEL)/2= 63.35 (ERL+ERM)/2 =152
	Probable Effects Level	108 mg/kg	

Table 9 Summary of zinc guidelines for marine/estuarine sediments

Guideline	Descriptor	Trigger value zinc	Comment
ANZECC	ISQG Low	200 mg/kg	See Table 12
	ISQG High	410 mg/kg	
NOAA	Effects Range Low (ERL)	150 mg/kg	
	Effects Range Medium (ERM)	410 mg/kg	
FDEP	Threshold Effects Level (TEL)	124 mg/kg	
	Probable Effects Level	271 mg/kg	

Sediment quality guidelines are best employed when they are coupled with biological effects measures e.g. toxicity testing, bioaccumulation testing or benthic community analysis. In this way, a multiple lines of evidence approach may be used to assess the implications of guideline exceedance.

Table 10 Classification system for sediments based on ISQG values.

Category	Description	Interpretation
Uncontaminated	Concentrations < ISQG-Low or Screening Level	"Adverse biological effects are seldom observed."
Contaminated	Concentrations are > ISQG-Low threshold, but < ISQG-High or Maximum Level.	"Possible effects range. Frequent occurrence of adverse biological effects."
Highly Contaminated	Concentrations > the ISQG-High or Maximum Level.	"Indicates significant contamination and sediments are expected to have an adverse effect on biota."

Aquaculture Specific Guidelines

The Scotland Environment Protection Agency (SEPA) has derived sediment quality criteria to trigger response for zones within and outside of a defined Allowable Zone of Effect (AZE):

Table 11 Summary of copper and zinc guidelines for aquaculture (SEPA).

Descriptor	Concentration copper (mg/kg DMB)	Zinc concentration (mg/kg DMB)
Background concentration	16	
Outside AZE	34	150
AZE Possible adverse effects level	108	270
AZE Probable adverse effects level	270	410

The SEPA approach assumes that marine cage fish farms release a "continuous" discharge and that the effect should not be detected more than 100 m from the source (AMD, 2004). The SEPA possible adverse effects level for copper (108mg/kg) is above the equivalent ISQG low level (65mg/kg) stipulated in Australian guidelines; however the probable adverse effects level and ISQG high levels are consistent (270mg/kg). The action level outside the allowable zone of effect under SEPA guidelines is 34 mg/kg copper (see Table 11).

Regulation of farm activities in Tasmania refers to both ANZECC sediment guidelines and water column guidelines (Copper 1.3 µg/L and Zinc15 µg/L), whereby the ANZECC high trigger levels are employed as threshold controls. Marine farming licence conditions for finfish operations in Tasmania stating that 'threshold levels …must not be exceeded within the Lease Area, by reason of the conduct of marine farming operations in the Lease Area".

Water-column/Pore-water Guidelines

Surface Water

The guideline values derived for toxicants such as copper and zinc are regarded as trigger values that if exceeded, should initiate the decision tree process that can allow a guideline to be assessed and tailored for the specific and local environmental conditions (NWQMS, 2000). The guidelines provide different levels of protection (i.e. for 80%, 90%, 95%, 99% of species) that take into account the ecosystem condition e.g. slightly-moderately disturbed, highly disturbed etc. It is most appropriate to compare the bioavailable fraction of the metal with the trigger value, however unfiltered (total) concentrations may be used in the first instance and filtered or bioavailable measurements made if the trigger values are exceeded with total values. Some speciation schemes include the use of 0.15 µm filtration to determine bioavailable metal. The 99% protection value listed in Table 12 and Table 13 should protect against bioaccumulation in many cases (NWQMS, 2000), however data for the specific site or key organisms should be used to interpret this aspect.

Table 12 ANZECC Trigger values for copper at alternate levels of protection (% species, marine waters) and NOAA criteria.

Guideline	Descriptor	Trigger value copper	Comment
ANZECC	99% protection	0.3 µg/L	1.3 µg/L = guideline for slightly-moderately impacted waters
	95 % protection	1.3 µg/L	
	90% protection	3 µg/L	
	80% protection	8 µg/L	
NOAA	CCC (Criteria Continuous Concentration) (chronic)	3.1 µg/L	guidelines for copper in marine surface water
	CMC (Criteria Maximum Concentration) (acute	4.8 µg/L	

Table 13 ANZECC Trigger values for zinc at alternate levels of protection (% species, marine waters).

Guideline	Descriptor	Trigger value zinc	Comment
ANZECC	99% protection	7 µg/L	15 µg/L = guideline for slightly-moderately impacted waters
	95 % protection	15 µg/L	
	90% protection	23 µg/L	
	80% protection	43 µg/L	

Pore Water

There are no guidelines currently in place for pore water. As a result, pore water levels have been compared to guidelines for marine surface water levels. Pore water metal levels have been included in sediment quality tests, as the metals in sediment pore water in are assumed to contribute the majority of the bioavailable metals in the sediment and hence will be responsible for toxicity in biota. However, this approach does not take into account the fact that for some organisms, toxic effects can also occur indirectly through digestion of the sediments themselves not just via direct uptake through water and so is likely to underestimate the toxic effects on biota.

2.4.2 Incorporating ecotoxicology data from local species

The ANZECC guidelines provide detailed recommendations on the appropriate use of toxicity testing to support risk assessment associated with sediment and water quality (NWQMS, 2000). Direct toxicity assessment is an important tool in determining the biological response to toxicants, and it assumes that only the bioavailable fraction will elicit a response. It thus provides information on the likely risk to aquatic organisms. Toxicity testing can be used to confirm predictions of overall biological effects based on speciation modelling and chemical speciation analyses. Test methodology must be scrutinised before undertaking tests to be sure that artefacts that may arise from sample handling and processing do not lead to variations from field conditions, and thus lead to misinterpretation of results. The assessment process leading to the use of toxicity testing in sediments is illustrated in Figure 3.

Step 6 and 7 in the decision tree includes the option to assess sediment quality using ecotox data from locally relevant species. Australian and New Zealand species were included in the data set used to derive guideline values for copper and zinc, however there are distinct differences between environmental conditions and species composition in SE Tasmania and mainland Australia where most ecotox work is conducted. Steps suggested in the ANZECC guidelines for incorporating local ecotox data into trigger values for water include:

- examine the original ANZECC dataset to evaluate what species were used to derive the trigger value or criteria and if the locally important species is represented by an adequate surrogate
- examine the literature to evaluate adequate quality data for a locally important species or a better representative than that used to derive the trigger or criteria
- if further data are required, undertake toxicity tests, under locally appropriate test conditions

- recalculate the trigger or criteria using the new species data, in accordance with data quality requirements (ideally more than 5 species would be used to calculate a reliable TV).

For sediments, the development of a trigger value for copper in waters intimately associated with sediments (e.g. pore water, burrow water or overlying water in the immediate vicinity of the sediments resulting in dissolved exposure pathway) would follow the above steps, but focus only on organisms that have life stages relevant to sediment associated waters (Simpson *et al*, 2008). A trigger value for acute and chronic effects can be calculated.

The derivation of site specific sediment criteria for whole sediments (i.e. particulate exposure pathway) is complicated by the influence of sediment properties on bioavailability (Simpson *et al*, 2008). Procedures for modifying existing sediment guideline values are not as well developed as for dissolved toxicants, and would require expert guidance at this stage.

Biological Material Guidelines

The guidelines for metals in biota are only relevant from a human health point of view (i.e. for consumption rather than species protection). They do however provide a useful benchmark for metal levels for biota typically harvested from "clean" areas.

The guidelines for copper in seafood as outlined by Food Standards Australia and New Zealand (FSANZ) consist of generally expected levels (GELs). The risk to human health of contamination by copper through seafood is regarded as low and hence GELs are not legally enforceable like maximum levels (MLs) which are prescribed for other metals. GELs simply provide a bench mark against which to measure contaminate levels in food.

Table 14 Summary of GEL levels for copper in fish and shellfish, ANZFA standards

Group	**Guideline type**	**Median**	**90th percentile**
Mussels	generally expected level (GEL)	5 mg/kg	30 mg/kg
Oysters	generally expected level (GEL)	5 mg/kg	30 mg/kg
Fish	generally expected level (GEL)	0.5 mg/kg	2 mg/kg
Crustaceans	generally expected level (GEL)	10 mg/kg	20 mg/kg

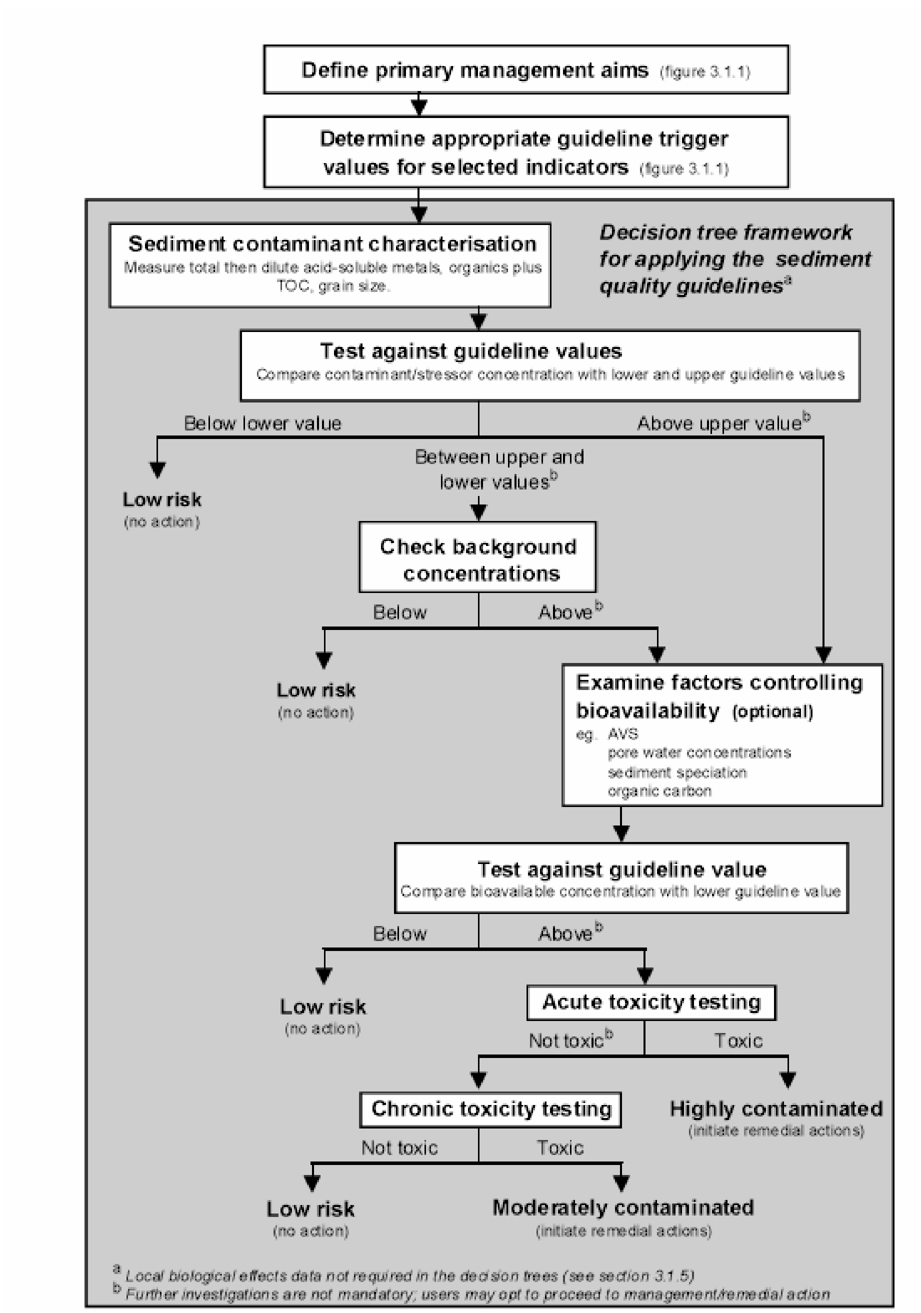


Figure 3 The decision tree approach for the assessment of contaminated sediments (From ANZECC/ARMCANZ SQG, 2000).

2.4.3 Differences in analytical methods & effect on measurement/ interpretation

There is some controversy surrounding the measurement of total metals in sediment and water samples, using the technique of acid dissolution (NWQMS, 2000). The analytical measurement of total metals depends upon the ability of the acid dissolution and/or digestion technique to completely dissolve all of the metal of interest. This is particularly important when interpreting the level of bioavailability, and comparing against trigger guidelines. For example, metals that are not solubilised by acid can be considered to be biologically unavailable since the strength of the treatment required to "access" these metals far exceeds realistic environmental or biological conditions. The term "total metals" is more correctly referred to as acid-soluble metals, and will depend on the strength of the acid dissolution/digestion technique used.

Any environmental program monitoring levels of heavy metals in sediments, water or biota should establish and maintain appropriate sampling and analysis methods to minimise variability often inherent in complex matrices.

Sediment Standard Analysis methods

Analytical Services Tasmania (AST) uses a standard method for copper determination in sediments which is based almost entirely on the USEPA standard method for copper in sediments: This is considered a strong digest, however does not dissolve the sediment (silicate structure).

- sediments dried and ground with mortar and pestle
- digestion in aqua regia ($HCl + HNO_3$) for 4 hours on digestion block (temperature raised and lowered to a set program)
- analysis by ICP-AES
- limit of detection 0.1 mg/kg
- reported as dry weights
- blanks and standard reference materials are included in every analysis
- calibrations are also carried out for every analysis

USEPA Method 3050B
Standard USEPA method for determination of copper by Inductively Coupled Plasma Atomic Emission Spectroscopy *ICP-AES*

This method is not a total digestion technique for most samples. It is a very strong acid digestion that will dissolve almost all elements that could become environmentally available. Elements bound in silicate structures are not usually mobile in the environment.

- 1 - 2 grams wet weight or 1 gram dry weight of the sample is digested with repeat additions of HNO_3 and H_2O_2.
- Digests are heated (best results with a hot plate or digestion block) to aid in extraction process.
- Diluted to a final volume of 100 mL and then analysed by ICP-AES

Possible Areas of Difference

Despite the fact that there are recommendations on the optimal methods and procedures for determination of metals in sediments, different analytical laboratories will differ in the methods they use. Differences in particular processes in analysis methods, sediment handling and the sediment characteristics may lead to different results of metal concentration.

Handling of sediment samples
The way in which a sediment sample is handled once it has been collected may also lead to differences between analyses. The sediment should be disrupted as

little as possible during collection; disturbance of the sediment structure may influence bioavailability (Simpson *et al*., 2005). This may not be so important if only total levels of metals are to be measured and/or if sub-samples are taken from fully homogenised sediment. However, it is critical where sub-samples are to be taken from different layers and/or where measurement of bioavailable metals is required (e.g. AVS, SEM etc.); in this case disturbances to sediments should be minimised.

Importantly each sample should be handled and treated in a consistent way between samples. Each sample should be stored in plastic jars/vials etc. and sediments should be frozen if analysis of AVS is required (Simpson *et al*., 2005). In all other cases they should be stored cold to reduce loss of volatiles (Simpson *et al*., 2005). Heavy metal analyses are best done on an aliquot of moist, homogenised sediment, with a separate sample taken for moisture analysis (Simpson *et al*., 2005).

Homogenising of sediments

Sediments can be remarkably heterogenous (Simpson *et al*., 2005). No matter how well they are homogenised it is still possible that two separate analyses from the same sample could yield different results. For example, one sample may have had a flake of paint in it which would result in a much higher reading. It is important that samples are homogenised in a consistent manner. Replicate sub samples should be taken from each homogenised sample to provide an assessment of homogeneity and assess whether any differences between analyses are due to analytical procedures.

Digestions

Methods may vary in the digestions which they use to extract the metal and this may result in different levels of metals being extracted from the sample. Digestions will vary in strength (acid strength and duration) depending on what metal fraction is to be measured. Cold dilute acid will in most cases extract the majority of the most bioavailable fraction. Stronger digestion procedures including heating and *aqua regia* will solubilise more of the metal (Simpson *et al*., 2005). Other methods may use even stronger digestions (hydrofluoric acid) which dissolve the sediment itself and therefore will extract more metal and result in higher levels being recorded.

Analysis type

The analysis technique used to measure the metal concentration in a sample may also lead to different results. Some heavy metal analysis techniques will be more suitable for particular metals and may yield varying results. The suitability of an analytical technique for the determination of a particular metal will depend on the interferences associated with the sediment and the metal. Sensitivities of different methods also differ. The most common techniques of heavy metal analysis are generally Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP- AES) and Inductively Coupled Plasma Mass Spectrometry (ICP- MS) (Simpson *et al*., 2005). Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) is also used (Stauber *et al*., 2000). In the USEPA methods for sediment metal determinations, ICP-AES or Flame Atomic Absorption spectrometry (FLAA) is recommended for the determination of Cu in sediments.

There are also new techniques which are able to measure metal levels in sediments without the need for acid digestion. However, these methods lack sensitivity and are only useful for measuring sediments with known high metal levels.

Interferences

Sediment samples can contain diverse matrix types, each of which may present its own problems to the analysis. Spiked samples and any relevant standard reference material should be processed in accordance with the quality control requirements given to aid in determining whether the method is applicable to a given sediment type.

Quality control

Methods for determination of metals in sediments may differ in the quality control measures which they have in place. These generally involve, running a blank, a standard reference material, duplicates and spikes. Having quality control measures in place is essential to any analysis as it increases the reliability of the results and ensures that any differences in the results are not due to contamination or analytical error. Every batch of samples run for heavy metal analysis should include:

- Certified (standard) reference materials (CRMs) - analysed as a check of analysis accuracy (quality control) (Simpson *et al*., 2005).
- Field replicates and field blanks required for quality control - need to be collected and handled the same as the sediment samples (Simpson *et al*., 2005)
- Analysis of background concentrations of contaminants that occur naturally (Simpson *et al*., 2005).
- Five-point calibration, drift correction and copper spike and recoveries for each sample batch (Stauber *et al*., 2000)
- It is recommended that a duplicate, blank, SRM and spike should be run with every 20 samples analysed (5%) (USEPA, AST)

AVS/SEM Standard Analysis methods

The analysis of AVS/SEM is based on the method recommended by the USEPA. The stability of sulphides is low in the presence of oxygen, so the analysis should be conducted on samples that have been frozen immediately (without headspace) after collection, and all subsequent manipulations carried out in a nitrogen atmosphere (Simpson *et al*, 2005). The method involves weighing approximately 1-2 g of wet sediment, which is transferred to a reaction flask, and H_2S evolved by addition of hydrochloric acid. The H_2S is trapped in 0.5 M NaOH. The sulphide concentration is then determined by colorimetric analysis with methylene blue reagent. The acid leached sediment fraction is retained for metals analysis in the acid supernatant. Some methods use filtered sample for SEM (Zn, Pb, Cd, Cu, Ni) analysis (Maddock *et al*, 2007). Many of the issues raised for metals analysis in the previous section will also apply to AVS/SEM determination. However, the interpretation of the results is probably the most contentious aspect of these measurements. Metals are considered not to be bioavailable if the concentration of AVS exceeds the sum of concentrations of metals extracted in the acidification process i.e. AVS> SEM (Maddock *et al*, 2007). Any excess of sulphide is normally present as FeS which is less stable than heavy metal sulphides. The low toxicity of heavy metal sulphides has been confirmed by bio-assays. Simpson *et al* (2005) however question the interpretation and predictive capacity of the AVS/SEM model, particularly with respect to copper which forms a sulfide complex not readily liberated by addition of 1N HCl i.e. the contribution of CuS towards AVS may be negligible.

Surface Water/pore water Standard Analysis methods

*USEPA standard method for total recoverable or dissolved metals in water (*Method 3005A)
This method involves acid digestion followed by analysis by FLAA or by ICP. Total dissolved metals

- sample filtered at the time of collection through a 0.45 µm filter
- and acidified with nitric acid (5 mL/L)
- sample heated with acid and reduced in volume
- filtered a diluted to volume of 100 mL
- analysis by either FLAA or ICP

Interferences

- digestion procedure may not break down all metal complexes and thus not all metals will be extracted
- precipitation will cause lowering of Ag concentration and hence analysis of Ag may be inaccurate

Quality control

- blanks should be carried throughout the entire preparation and analytical process – determine if samples are being contaminated
- replicate samples should be processed on routine basis (5%) – determine precision
- spiked samples or SRM should be employed to determine accuracy

Pore water

There is no standard method available for the determination of metals in pore water. However, it is generally analysed by the same method for determining metals in surface waters.

Possible Areas of Difference

Analysis of pore water can produce markedly different results depending on the way in which the pore water is separated from the sediment. Techniques include: centrifuging, syringe extraction and compression extraction. These different techniques will all produce different results for metal levels. A study by Ankley (1994) which compared these separation processes found that centrifuging was the most ideal method. Ankley (1994) also suggested that pore water should not be filtered after centrifuging as the filtration process is likely to remove a large proportion of metals from the solution. For anoxic sediments the exclusion of oxygen during extraction procedures is critical. The USEPA have prepared a valuable method manual for the collection, analysis, and ecotoxicological assessment of sediments (USEPA, 2001).

It is again of vital importance that quality control measures are in place for any analysis (i.e. every analysis should include a blank, SRM, duplicate and spike). Using ultra clean techniques in collection and analysis preparation for seawater samples is also of great importance, and zinc analyses are particularly prone to contamination. Suitable sample containers, preservation and storage techniques are crucial, as is reagent purity

Metals in Biota Standard Analysis methods

Analytical Services Tasmania use a NATA accredited, standard method for determination of copper in food samples:

- sample dried and ground to powder
- dry matter fraction recorded
- digested in nitric acid – heated
- H_2O_2 added and digested a second time
- diluted to 50 mL
- filtered through 0.45 µm filter
- analysis by ICP-AES

Possible Areas of Difference

Differences between analyses of copper or other metals in biota could be due to several factors. The most important influencing factor will be how the samples are processed before analysis, including where the samples are taken from, what is removed from the sample and how the sample is homogenised etc. There is no right or wrong way of processing biota samples, the process used will largely depend on what the analysis is for (i.e. human health focussed or biota health focussed). It is important that methods for all aspects of sample collection and assessment are consistent with the goals of the project.

If the risk of consumption of a species to human health is being looked at then the sample should include the part of the animal which is eaten (i.e. muscle tissue in fish). If the risk of effects of a metal contaminant to a particular species of animal is to be looked at then total body burdens of the metal or liver levels of the metal may be analysed.

2.5 Current Monitoring

2.5.1 What is currently being done

The information collated in this section are summarised from detailed annual industry reports to the APVMA (AMD 2004, 2005, 2006, 2007, 2008), and information and database provided by DPIW. Monitoring comprises "on-going" studies that are repeated over time (generally an annual basis) and single "issue-based" studies designed to give more information on specific aspects of antifouling use e.g. in situ cleaning or bioaccumulation. Both types of monitoring are required to adequately address the requirements of the permit.

Ongoing monitoring

At each lease, monitoring sites that were representative of a range of environmental conditions were selected to determine the impact of sustained use of antifouling products on the surrounding environment. Sites were ranked as primary (ongoing), or tertiary (baseline only) based on volume usage. Until 2007, 4 farm leases were surveyed annually as primary sites (referred to here as A, B, C, and D), however the usage conditions changed and certain of these sites became relatively low use. DPIW subsequently required that some previously defined tertiary sites (now high use) were included in 2007 (referred to a lease E to L here). Given the increase in volume and extent of usage, a total of 12 high usage sites (i.e. primary and tertiary) were sampled in 2007 and 2008.
Monitoring programs undertaken by industry to meet the requirements of permit 6150 included the following areas:

- Annual sediment sampling program at designated sites, including control (approx 500m distant from lease boundary) and compliance sites (35 m from lease boundary) as per Figure 4.
- Baseline sampling conducted at a number of sites in the first year of the permit and all remaining sites were sampled for baseline levels in subsequent years (= tertiary sites).
- Comparison of temporal data to assess rate of increase or decrease over time at lease vs. control/compliance sites.
- Comparison of "high organic" vs. "low organic" sites to determine metal accumulation rates under different sedimentary/physical conditions.

Monitoring requirements under the current permit 10924 are non-specific but must include assessment of the sediment and water column (including *in situ* cleaning effluent characterisation) to support a risk assessment.

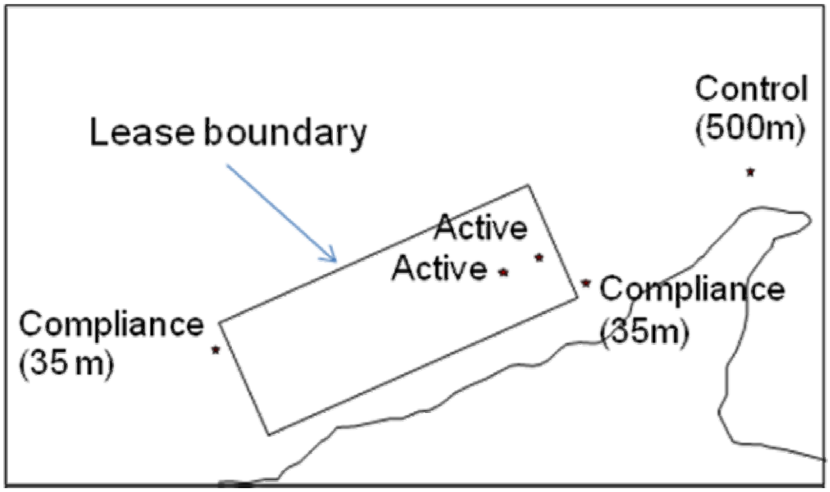


Figure 4 Example of lease with active, compliance and control sites indicated, relative to lease boundary (redrawn from AMD 2008).

Issue based studies

Studies undertaken by industry and DPIW to address particular aspects of environmental impacts associated with the use of antifouling products include:

- A mass balance to determine the fate of copper through the deployment cycle of fish nets.
- A mass balance to determine the fate of copper and zinc through the in-situ cleaning of fish nets, including a comparison of two different net cleaning techniques.
- Trials on the efficacy of various products specified in the permit
- estimating bioavailability using acid soluble metals
- A bioavailability assessment on the acute toxicity of sediments collected from a range of sediment types, and stages in the operational cycle
- Monitoring of oysters in the vicinity of antifouled cages (results discussed previously).

2.5.2 Results of ongoing monitoring

A summary of copper and zinc levels detected in sediments at the four primary leases is shown for the past 5 years monitoring (Table 15). Data presented in the table is the average and range of pooled data for a) compliance sites (generally 2 sites, 2-3 replicates/site each survey), b) control sites (generally 1 site, 2-3 replicates/site each survey) and c) active/farmed sites (generally 2 sites, 2-3 replicates/site each survey). Data on temporal trends at primary sites is well documented in the industry reports to APVMA, and generally show:

<u>Copper</u>

- There has been no significant increase in sediment copper levels at compliance sites (35 m from lease boundary) for lease B, C, or D. The compliance sites for Lease A (high organic, sheltered) show greatest variability, and a gradual upward trend in average copper concentration from 2004 to 2008.

- Sediment copper levels at control sites (500 m from lease boundary) appear stable for lease B, C, and D. Copper levels are higher at all sampling locations (compliance, control and active) at Lease A and fluctuate more than at lease B, C, and D. Lease A is the location for operations such as fish bathing, where fish pens are handled more as a result of crowding/net changes etc and is therefore more likely to receive greater copper loadings due to ablation of the coating.
- Compliance sites are generally comparable to control site sediment levels for sediment copper.
- Sediment copper at low organic control sites is consistently lower than higher organic sites.
- Sediment copper levels are elevated at active lease/pen sites where copper based antifouling paints have been used.
- Active farm sites at Site A (high organic, sheltered) and Site C (low organic exposed) have copper levels in sediments that exceed ISQG trigger levels.
- Sediment copper levels can fluctuate between years, but the mechanisms and fate are not clear. Low replication in sampling may be a factor.

<u>Zinc</u>

- Sediment zinc levels at compliance sites are generally stable from year to year, although the dataset is smaller than for copper.
- Lease A (high organic sheltered), Lease B (high organic, high tidal flows) and Lease C (low organic, exposed) have lower average sediment zinc in compliance sites than their respective controls.
- Lease A, C, and D have higher average sediment zinc at active sites than compliance or control sites.
- Lease B active sites have lower average sediment zinc than at the control site.
- Lease B and D are possibly affected by zinc loads from outside the lease area (i.e. the Derwent).

At the eight tertiary sites (see Table 16), the time series data set is much smaller but some general trends (similar to those at primary sites) observed are:

- High and intermediate organic sites have a greater “background” load of copper than low organic sites.
- High and intermediate organic sites accumulate copper at a greater rate than low organic sites
- Active sites have greater sediment copper and zinc than control or compliance sites.
- Sediment copper levels measured adjacent to antifouled cages in baseline surveys (tertiary sites) exceeded the ISQG-Low trigger of 65 mg/kg for a number of farm sites.

*Table 15 Summary of average (range in brackets) of copper and zinc levels recorded at 4 primary sites over time (2003-2008). Highlighted data- individual values exceeded ISQG-Low value, or ISQG-High value. * Supplemental sampling at additional sites range from 37 – 3225 mg/kg DMB.*

Lease	%OC	Classification	Metal (mg/kg)	Compliance (35m)						Control						Active					
				'03	'04	'05	'06	'07	'08	'03	'04	'05	'06	'07	'08	'03	'04	'05	'06	'07	'08
A	2.16-24.8	High organic, sheltered	Cu	49.6 (31-95)	24.8 (3-47)	27.3 (3-52)	28.5 (22-54)	38.6 (6-79)	40.25 (7-85)	23.3 (23-24)	29 (28-30)	27 (27)	5 (5)	29 (27-31)	29.5 (27-32)	97.6 (42-147)	90 (61-158)	359.3 (217-711)	275.8 (127-671)	382.5 (110-1372)*	470 (58-1207)
			Zn	-	-	-	44 (13-76)	44.5 (16-73)	53.5 (16-93)	-	-	-	66 (64-68)	64 (64)	70 (66-74)	-	-	-	142.3 (47-254)	140.5 (70-338)	169.1 (109-226)
B	5.3-12	High organic, higher tidal flows	Cu	8.5 (6-10)	9.8 (9-11)	12.8 (5-27)	10.8 (8-13)	9.25 (8-11)	8.75 (8-10)	11 (10-13)	15.5 (15-16)	11.5 (11-12)	14 (13-15)	13 (13)	11.5 (11-12)	11 (6-28)	14.3 (11-21)	12 (6-19)	17.8 (8-36)	9.8 (6-16)	16.25 (6-43)
			Zn	-	-	-	119.8 (97-144)	80 (82-126)	113 (90-138)	-	-	-	197.5 (195-200)	186.5 (186-187)	172 (167-177)	-	-	-	140.7 (118-177)	105.5 (77-138)	105.4 (76-145)
C	2.5-4.7	Low organic, exposed site	Cu	3.8 (3-4)	5 (4-6)	3 (2-4)	3.8 (3-5)	3.3 (2-5)	4.3 (3-6)	4 (4)	4.5 (4-5)	6 (6-7)	5.5 (5-6)	5.5 (5-6)	5.5 (5-6)	226 (4-994)	75.5 (6-194)	27.8 (7-74)	71 (8-323)	50.2 (15-94)	31.8 (8-52)
			Zn	-	-	-	20 (15-25)	17.75 (13-23)	20.8 (16-26)	-	-	-	30 (30)	27 (27)	30 (29-31)	-	-	-	53.2 (30-100)	53.3 (31-94)	42 (33-51)
D	1.3-12.2	Low - Intermediate	Cu	14.5 (11-18)	17 (12-21)	15.8 (10-22)	15 (16-23)	17.25 (14-19)	18 (16-21)	12.7 (12-13)	15 (15)	13.5 (13-14)	15.5 (15-16)	13 (13)	15 (15)	37.6 (19-73)	58.5 (32-102)	75.5 (28-153)	126.8 (113-150)	38.75 (19-59)	104.8 (82-124)
			Zn	-	-	-	178 (138-221)	153.8 (116-188)	175.8 (137-219)	-	-	-	104 (104)	84.5 (82.87)	109.5 (108-111)	-	-	-	240 (215-305)	178.5 (167-188)	290.3 (249-360)

Table 16 Summary of average (range in brackets) of copper and zinc levels recorded at 8 tertiary sites over time (2007-2008). Highlighted data- individual values exceeded ISQG-Low value, or ISQG-High value

Site	%OC	Classification	Metal	Compliance (35m)			Control			Active		
				Baseline '05	'07	'08	Baseline '05	'07	'08	Baseline '05	'07	'08
E		Low organic	Cu mg/kg	3.3 (3-4)	6.2 (5-7)	8.9 (7-23)	5.7 (5-7)	6 (6)	7 (7)	4.7 (3-6)	34.5 (10-65)	16 (15-20)
			Zn mg/kg	-	20.2 (13-27)	19.8 (13-24)	-	26.7 (25-28)	31 (29-33)	-	41 (29-59)	46.5 (34-62)
				Baseline '06	'07	'08	Baseline '06	'07	'08	Baseline '06	'07	'08
F		Intermediate organic	Cu mg/kg	18.3 (18-19)	18.3 (17-21)	24.8 (20-32)	18.7 (18-20)	18.5 (18-19)	21.5 (21-22)	na	27.9 (18-44)	55.4 (21-154)
			Zn mg/kg		49.2 (46-59)	56.8 (55-59)	53.7 (51-56)	47.5 (47-48)	55 (55)	na	55.7 (48-64)	72.2 (58-89)
				Baseline '03	'07	'08	Baseline '03	'07	'08	Baseline '03	'07	'08
G	21.2-22.3	High organic	Cu mg/kg	32 (31-34)	27.5 (25-31)	30.5 (28-35)	24.3 (23-25)	28.5 (27-30)	28.5 (26-31)	133 (59-217)	94.5 (53-231)	187.8 (88-418)
			Zn mg/kg	-	120.7 (93-151)	75.5 (73-78)	-	67.2 (61-77)	76.5 (75-78)	-	67.5 (66-69)	186.3 (118-279)
				Baseline '03	'07	'08	Baseline '03	'07	'08	Baseline '03	'07	'08
H	1.8-3.2	Low organic	Cu mg/kg	5 (5)	5.4 (4-7)	6.25 (4-8)	3.7 (3-4)	4.5 (4.5)	5 (5)	6 (5-8)	49.6 (11-83)	46 (23-102
			Zn mg/kg	-	39.2 (34-47)	44.8 (36-54)	-	36 (35-37)	42 (40-44)	-	50.5 (37-64)	52 (51-70)

Table 16 (cont.) *Summary of average (range in brackets) of copper and zinc levels recorded at 8 tertiary sites over time (2007-2008). Highlighted data- individual values exceeded ISQG-Low value, or ISQG-High value*

Site	%OC	Classification	Metal	Compliance (35m)			Control			Active		
				Baseline '06	'07	'08	Baseline '06	'07	'08	Baseline '06	'07	'08
I		Intermediate organic	Cu mg/kg	17.8 (15-22)	18.25 (15-21)	16 (10-22)	11.7 (11-13)	14 (13-15)	14 (13-15)	21 (21)	27.8 (21-52)	78 (29-183)
			Zn mg/kg	56.8 (50-65)	55 (48-59	54.3 (37-71)	46 (45-48)	47.5 (45-48)	53 (51-55)	65.3 (65-66)	64.4 (60-77)	140.7 (92-200)
				Baseline '03	'07	'08	Baseline '03	'07	'08	Baseline '03	'07	'08
J	2.3-20.12	Intermediate organic	Cu mg/kg	4 (3-5)	9.8 (5-14)	8.5 (3-14)	19 (18-20)	26 (24-26)	30.5 (29-32)	35 (28-41)	53.5 (57-65)	89.2 (62-133)
			Zn mg/kg	-	24 (11-38)	24.5 (9-40)	-	58.7 (55-63)	69 (68-70)	-	80 (69-94)	112.8 (95-123)
				Baseline '03	'07	'08	Baseline '03	'07	'08	Baseline '03	'07	'08
K	3.6-25.4	Intermediate organic	Cu mg/kg	21 (20-22)	31.8 (26-35)	31.5 (26-36)	18.7 (17-19)	24 (22-26)	30.5 (30-31)	17 (16-18)	94.8 (19-231)	212.8 (65-442)
			Zn mg/kg	-	66.8 (61-74)	69 (55-78)	-	60.7 (56-66)	74 (73-75)	-	110.2 (64-173)	158.5 (115-206)
				Baseline '03	'07	'08	Baseline '03	'07	'08	Baseline '03	'07	'08
L	2-9.6	Low organic	Cu mg/kg	11.7 (9-16)	-	10.5 (9-13)	see Lease 94	-	see Lease 94	21.3 (16-26)	-	112.2 (42-234)
			Zn mg/kg	-	-	132.8 (124-142)	see Lease 94	-	see Lease 94	-	-	169.5 (145-194)

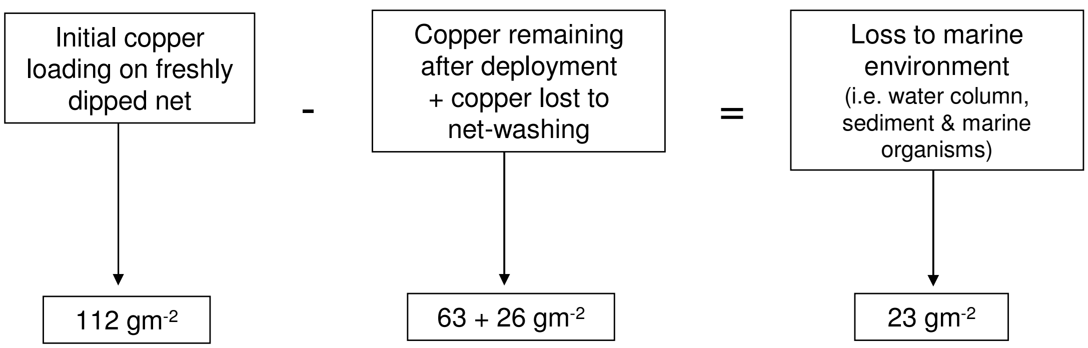
2.5.3 Results of issue based studies

Mass balance for the fate of copper through the deployment cycle.

The mass balance focused on the use of the Hempanet products (Hempel), containing 196 g/L Cuprous oxide (Cu_20) or 522 g/L Cuprous oxide (Cu_20). The purpose of the study was to determine the loss of copper to the environment through various phases of net deployment, using a mass balance approach (Dalgleish, 2008a). Tests were conducted on sections of net, in a way that simulated the typical net exposure and treatment regimes in operation. Stages included:

- Deployment in the marine environment for between 12 and 24 months
- De-fouling, washing and recoating of the antifoulant.

Although duplicate nets were originally included in this trial, there were problems in establishing the prior dipping history for one of the nets and it was subsequently excluded from the analysis (Cameron Dalgleish, pers.comm.). Clearly there are problems with basing any conclusions on the results from a single net and therefore these findings should be viewed as indicative rather than conclusive. The nets were not subjected to in-situ cleans during their deployment, and the mass balance considered the following components:



The concentration of paint specified by the manufacturer and the actual volume of paint applied to the nets were used to determine a leach rate of 8.55 $\mu g/cm^2/day$. This compares favourably to the leach rate provided by Hempel of $9.25\mu g/cm^2/day$. The report concludes that on average, 20.7 % of applied copper (by mass) is released to the marine environment over the course of deployment. Using the MAMPEC model to calculate the fraction of copper remaining in the dissolved form and the fraction “attributable to the insoluble fraction entering the sediments” indicates values of 41% and 59% respectively (Table 17). Considering the high affinity of soluble copper for particulate phases (identified earlier), the fraction remaining in the dissolved form seems high and this should be confirmed with appropriate testing.

*Table 17 Summary of mass balance for copper applied to nets at a single study site (*results based on measured loss to the environment over the course of deployment).*

Days deployed	**Initial mass of copper applied (kg)**	**Measured loss to the marine environment (kg)**	**Calculated loss to the land based applications (kg)**	**Calculated loss to water column (kg)***	**Calculated loss to sediments (kg)**
270	194	40	43	16.4	23.6

These mass balance calculations suggest that a percentage of copper is lost to the marine environment during deployment via dissolution or abrasion, including an unknown quantity lost by uptake of copper by fouling organisms.

Mass balance for in situ cleaning

In-situ cleaning of nets provides some significant operational advantages over land-based cleaning and recoating of nets. Industry reports suggest that in-situ maintenance provides better performance with respect to length of time deployed before re-coating is necessary, and results in fewer events where fish need to be taken out of the cage. From a regulatory point of view, in-situ cleaning is a concern as it potentially results in higher rates of copper loss to the environment, since physical removal of fouling material may also remove paint.

The mass balance on the fate of copper and zinc through the in-situ cleaning of fish nets focused on the two principle techniques employed for the removal of fouling material, a vacuum or “suction” cleaner and a pressure cleaner (Dalgleish, 2008b). Large volumes of water (~3000 L/min) are required for the suction technique, while the pressure cleaner method uses a lesser rate of water flow. Net washer “effluent” from both cleaner designs were collected and analysed for heavy metals following sieving into various size fractions to determine the fate of material removed from the nets. Results discussed here are the average of two nets sampled for each technique (see Table 18).

The suction technique resulted in most copper (> 95%) and zinc (>90%) being in the particulate form, with the >2.0mm size fraction accounting for most (>70%) of the particulate load. The pressure cleaner technique resulted in most of the copper (>89%) being in the particulate form, however particulate zinc accounted for only 50% of the total load. Total concentrations for copper and zinc however were lower using the pressure cleaner technique, and most of the copper was present in the smaller size fractions (100 µm to 850 µm).

Table 18 Summary of dissolved, total and particulate loads in "effluent" from in situ net washing operations (average of 2 nets/ technique, composite of 8 samples/ net).

Technique	Metal	Dissolved (mg/L)	Total (mg/L)	Particulate (mg/L)	% dissolved
Suction	Cu	0.0815	1.517	1.436	5.4
	Zn	0.044	0.411	0.368	10.1
Pressure Cleaner	Cu	0.011	0.097	0.086	11.3
	Zn	0.024	0.047	0.024	51

Individual net data was used to calculate the mass of copper and zinc released to the environment on a per/clean basis (seeTable 19). Correction for the background concentrations of copper and zinc in seawater (1 and 3 µg/L respectively) had negligible effect on the results.

Table 19 Summary of dissolved, total and particulate loads to the environment as mass of metal per net clean (based on Table 17 results).

Technique	Net	Dissolved (g/clean)		Total (g/clean)		Particulate (g/clean)	
		Cu	Zn	Cu	Zn	Cu	Zn
Suction	1	27	15	363	75	336	61
	2	2	1	184	72	182	71
Pressure Cleaner	1	18	37	181	65	162	28
	2	9	31	103	82	94	52

Dalgleish (2008b) also used the mass balance to calculate the total mass of copper and zinc attributable to net cleaning operations at each lease (Table 20).

Table 20 Summary of mass loads to environment from in situ net cleaning, on a per lease basis.

Lease, Technique	Metal	Dissolved (kg/yr, %)	Total (kg/yr)	Particulate (kg/yr)
Suction	Cu	1.207 (5.4)	22.605	21.368
	Zn	0.660 (10.8)	6.105	5.445
Pressure Cleaner	Cu	0.728 (9.9)	7.384	6.656
	Zn	1.768 (46)	2.080	3.848

A number of operational factors were identified as having impact on the results, and some caution is required when extrapolating this data to whole of farm operations. Several recommendations were made in the report to verify or improve on the estimates of metal loadings, and these will strengthen the confidence in the data. Nevertheless, the existing data summarised here can be used to confirm the hypothesis that in situ net cleaning does contribute copper and zinc, mostly in the particulate fraction to the marine environment, and that most of this material is likely to end up in the sediments in the vicinity of the cleaned net. The bioavailability of these metal loads (and those from other sources) is addressed in the next studies.

Estimation of bioavailable metal

The ANZECC guidelines provide a decision tree approach to determining the level of risk associated with contaminated sediments. Characterisation by grain size analysis and measurement of dilute acid-soluble metals (ASM) are the first steps Studies on the acid soluble fraction of total metals in sediments from aquaculture leases have recently been completed, including a comparison between 2 laboratories (AMD, 2008). This study revealed the "operationally defined" nature of acid soluble analyses, with significant differences between results from the two laboratories. All samples submitted had acid soluble copper concentrations well below the total copper concentration, and the trend was similar for sites with low, intermediate or high organic loadings. This suggests the majority of copper is bound in forms that are not readily bioavailable. However, concerns have been raised about the suitability of the acid extraction method for estimating copper bound as sulphides, which may subsequently oxidise and then be more bioavailable than initially determined (Simpson and Spadaro, 2008). . Copper may also be unavailable if it is predominantly present as paint flakes, contributing to high total metal loads. This could be confirmed through porewater testing, speciation studies or toxicity testing.

Acute toxicity testing

As part of the need to understand the impact of copper accumulation in sediments, a range of samples were submitted to CSIRO Land and Water laboratories for chemical and toxicity testing (Simpson and Spadaro, 2008). Analyses included:

- Short-term acute toxicity test using the marine amphipod *Melita plumulosa*.
- Total organic carbon
- Particle size analysis
- Total metal
- Metal in <63 µm fraction

Three environmental scenarios were selected for testing and analysis: high organic recovery sites that had not been stocked for varying periods (4 sites, ~5-10% TOC), high organic sites that are currently stocked (4 sites), and low organic sites currently stocked (4 sites, ~1% TOC).

Chemical analysis showed high variability in total copper loads at the high organic sites, with half the sites sampled in excess of the ISQG High value of 270 mg/kg. Conversely low variability was observed at low organic sites, with no sites in excess of 270 mg/kg.

12 samples were selected for toxicity testing, covering the 3 scenarios described above. All but one of the samples was found to be non-toxic to the amphipod, after 10 days exposure, but this toxicity was attributed to ammonia (see Table 21).

*Table 21 Toxicity test results (10 day survival Melita plumulosa) and sediment chemistry for selected sites. *Ammonia toxicity suspected.*

Sediment type	Site	TOC (% w/w)	% < 63 µm	Total copper (mg/kg)	10 day survival (% of controls)	Toxic/Not Toxic
Recovery	G	16	46	870	90 ± 6	Not Toxic
	G	5.3	38	240	95 ± 3	Not Toxic
	A	0.75	0	430	97 ± 2	Not Toxic
	A	5.5	44	630	98 ± 2	Not Toxic
High organic	A	4.2	59	180	92 ± 3	Not Toxic
	A	4.8	59	870	93 ±3	Not Toxic
	A	4.5	63	340	97 ±3	Not Toxic
	A	8.4	21	1200	87 ±3	Not Toxic
Low organic	C	0.62	0	59	93 ±3	Not Toxic
	C	0.61	0	8	98 ± 0	Not Toxic
	L	1.6	7	180	56 ± 12	Toxic*
	L	1.2	8	250	88 ±3	Not Toxic

The toxicity test results shown in Table 21 suggest that copper in the sediments was not available to the test organism over the duration of the test. This correlates with the acid extractable metal results, and suggests that the paint may be present in the form of flakes or tightly bound as sulphides, and therefore would not be immediately available for uptake. *M. plumulosa* is reported to be sensitive to both dissolved (i.e. pore water or overlying water uptake pathway) and particulate copper (ingestion pathway) and results are therefore consistent with the negligible dissolution of copper into the water (Simpson and Spadaro, 2008). The LC_{50} reported for this species is 790 mg/kg copper (King *et al*, 2006).

Simpson and Spadaro (2008) concluded that although no mortality was observed in the juvenile amphipods in short-term exposure to test sediments, the impact of copper enriched sediments to ecological communities in the longer term are of concern. Testing to date has given no real indication of sublethal or longer term effects to organisms and communities exposed to copper in what is primarily expected to be paint particles. The authors proposed 3 main areas to more fully assess the risk of high copper loadings associated with paint use:

- What is the form and fate of the paint flakes in the sediments? Does copper covert to other forms over time (i.e. sulphide phases or associated with organic matter) that change the availability?
- What are the sub-lethal or chronic (i.e. growth, reproduction etc) effects thresholds for paint in sediments?
- What are the broader effects to benthic communities of the accumulated paint in sediments?

These three key issues are picked up in the proposed research and monitoring plan discussed in the next section.

2.5.4 Relevance of Current Monitoring

Continued use of annual surveys with comparison between farm sites that have been stocked with antifouled nets, and the control/ compliance sites allow temporal and spatial trends to be determined, and give some indication of the level of impact of sustained use of antifouling products on sediment quality. The ISQG value of 65 mg/kg represents the level at which there is an expectation of frequent occurrence of adverse biological effects. Levels in excess of 270 mg/kg indicate significant contamination and sediments are expected to have an adverse effect on biota (NWQMS, 2000). Comparison against relevant sediment quality guidelines points to a high probability of adverse biological effects, especially at Lease A and C.

Whilst the ISQG suggest it is highly probable that the accumulated metals are having an impact, monitoring total metal levels alone gives no reliable indication of the level of bioavailability of copper and zinc, and whether the sediments are functioning normally. This is a fundamental knowledge gap; as is determining if individual organisms are impacted, what is the form and fate of the copper over time, and/or if community and ecosystem function are impaired. The primary sites used in the temporal studies (low organic vs high organic) provide ideal study sites for more detailed site specific studies. A series of questions aiming to further quantify the level of risk associated with sustained use of antifouling products is presented in Table 34. These questions arise from industry assessment of data generated in annual and issue specific surveys, and the literature review included in this scoping study. They also incorporate recommendations for studies proposed by CSIRO to the TSGA (Batley *et al*, 2008).

Many of these issues have begun to be addressed through targeted studies, use of literature data and a mass balance approach to tracing sources and losses of copper and zinc through the system. This is an important component of identifying processes, organisms or locations that are at a higher risk of metal induced effects. Consultation with experts in the area of speciation, bioavailability and toxicity assessment should continue to ensure investigations are conducted in a logical, stepwise and informative manner.

Items in Table 34 are identified as research (R), research to inform monitoring (R-M), or research to inform management (R-Man). Additionally, items are flagged as a single study (S) used to assist the adaptive management framework, or on-going studies (O) that required sustained effort or can be incorporated into the monitoring program. Items are divided into 4 main themes:

- environmental fate
- operational /management issues
- biological effects
- contaminant budget/combination.

2.6 Conclusions

In the face of increasing growth in the salmonid aquaculture industry, there are several concerns relating to sustainable use of marine resources (Dean *et al*, 2007). These include the cumulative effects on multiple inputs such as organic material, nutrients, antifouling products, and medicines. The major risk in the continued use of antifouling products in caged aquaculture is the potential for significant build up of contaminants, particularly copper and zinc in the sediments in the vicinity of leases. Industry sediment surveys have shown copper levels in sediments are significantly higher at farm sites, than control or compliance sites. Highest levels are associated with organically rich sediments, which are known to have higher capacity to bind and accumulate heavy metals.

The control of speciation and bioavailability in sediments is complex and closely related to the degree of organic enrichment, the presence of geochemical phases, the redox status of surficial sediments, and the extent of physical processes such as bioturbation and resuspension. Most research suggests that in anoxic sediments, metals are tightly bound as insoluble sulphides, and therefore have reduced bioavailability. Oxidative release of metal initially bound to sulphides whilst sediment conditions are hypoxic or anoxic has been identified as a significant issue in management of aquaculture leases (Valkirs, 2003).

Marine invertebrate species are known to be sensitive to a range of environmental contaminants. Embryo and larval stages are particularly sensitive, often several orders of magnitude more sensitive than adult individuals (Bellas, 2006). Benthic impacts may include exclusion of vulnerable species where toxic thresholds are exceeded. More robust species may regulate or accumulate metals, giving rise to the potential for bioaccumulation as benthic invertebrates are a food source for many organisms.

Potential environmental effects of metal contamination associated with antifouling use in the salmonid industry may include, but are not limited to:

- increases in water soluble concentrations of copper and zinc
- increases in particulate and sediment bound metals
- increases in cell or body burden of organisms exposed to increased metal concentrations (bioaccumulation)
- trophic transfer of metals and biomagnification
- chronic or acute toxicity to organisms ranging from bacteria, phytoplankton, zooplankton, molluscs, crustacea, and fish in both pelagic and benthic communities
- changes to community structure through differences in species sensitivity and resistance to metal contamination
- changes to microbial and geochemical processes that regulate the cycling, bioavailability, and fate of micro and macro nutrients.

However, in relation to fish farming it should be noted that where these impacts do occur, in most instances they are likely to be highly localised in effect.

The major pathways for release of copper and zinc from antifouling products are:

- Dissolution of the active agents from the paint matrix
- Ablation or damage to antifouling treatments during handling, crowding, or in-situ net washing activities
- Release of active agents from particulate paint material accumulated in the sediments
- Additionally, copper and zinc may accumulate due to uneaten feed, faecal material, or due to other diffuse sources of metal contamination.

There is little information on the synergistic or antagonistic effects of sustained used of both antifouling and antibiotic products in aquaculture. The primary concern is the potential for significant impacts on the ability of sediment to process contaminant loads, and this is a key area for future assessment.

Guidelines for the Sustainable Development of Mediterranean Aquaculture (IUCN, 2007) specify the principle that "Antifouling products used in aquaculture should have no perceivable toxic effects on non-targeted organisms of the surrounding ecosystems". Recommended guidelines for best practice include:

- the use of eco-friendly antifouling coatings and products
- encouraging the use of environmentally friendly procedures for preventing or eliminating biofouling
- avoiding the use of antifouling products based on heavy metals.

In the short-term, replacement of copper and zinc based antifouling products is unlikely, and therefore the emphasis of sustainable management will be on minimising the environmental impact of the products under investigation, and development of appropriate monitoring protocols to allow definition of relevant threshold values for a range of parameters.

The assessment and understanding of the bioavailability of metals in sediments, both during active use and after a period of recovery, is vital to interpreting the monitoring data acquired over the past five years. Assessment may include chemical estimates of bioavailability, and toxicity testing. Assessing the biological response would ideally be conducted with locally relevant species about which sufficient life stage and copper sensitivity data is available. This should be established for a range of operational and environmental conditions relevant to the salmonid industry in SE Tasmania. Program design should be such that initial combination of toxicity and bioavailability assessment leads to confidence in the use of a chemical estimate of bioavailability on its own for long-term monitoring. Alternatively development of a rapid toxicity assay using locally relevant species could prove a useful approach. This data will also improve the ability to use the PEC/PNEC approach to assess likely environmental impacts, and model copper distribution and fate with tools such as MAMPEC.

The items identified in Table 34 include a number of research components that should inform future monitoring requirements. The combined approach of investigating environmental fate, biological effects, operational considerations and a budget approach to contaminant loads should allow industry to address the following questions:

- What is the minimum impact detected against the background of natural variability and other potential sources from anthropogenic influence in coastal waters?
- What is the degree of impact that can be tolerated without posing long-term harm?
- What are the best indicators for determining long-term impacts?
- Can we justify different guidelines for different sedimentary environments?

3 Antibiotics

3.1 Introduction

Antibiotics (antimicrobials) have been used for many years in aquaculture to control disease out breaks and pathogens in fin fish farms. Intensive aquaculture faces similar difficulties to that of any other form of intensive farming. The use of veterinary medicines and therapeutic products is an important tool in ensuring both the welfare of the fish and food safety (IUCN, 2007). Where properly prescribed and administered most aquaculture medicines are relatively benign, however, inappropriate or excessive use can result in adverse environmental impacts (IUCN, 2007). In an environment where consumers are increasingly aware of how their food is processed, treated and raised it is essential that farmers can show not only the safety and nutritional benefits of their product but also that they are aware of the welfare of both the farmed fish and the environment.

Use of antibiotics in aquaculture globally has declined markedly since the late 1980's, primarily as a result of vaccine development but also with improvements in husbandry (Markestad and Grave, 1997, Hiney and Smith, 2000). However, there will always be certain circumstances, such as outbreaks of new infections or incidences of multiple-infections, where the use of antimicrobial agents will be necessary. In Tasmania a *Rickettsia*-like organism (RLO) and more recently "summer gut syndrome" has resulted in substantial mortalities for the local salmonid industry and although efforts to develop a vaccine are currently underway, ongoing management of the disease has required significant amounts of antibiotics to ensure the health of the fish. Few antibiotics are approved for use in aquaculture because the licensing is expensive and the market is small compared with that of human or other livestock needs. There are many different anti-microbial agents currently available; however, the subset used in aquaculture is much smaller, and in Tasmania only 7 antibiotics have been used to any great extent (amoxycillin, oxytetracycline, oxolinic acid, chlortetracycline, trimethoprim, erythromycin and florfenicol, with some additional interest in tylosin). Chlortetracycline is not currently used and the industry has a self-imposed moratorium on the use of amoxicillin and oxolinic acid. Use of trimethoprim and florfenicol is relatively small; consequently the most frequently administered agent is oxytetracycline. Erythromycin was used for the control of lactococcosis (caused by *Lactococcus garvieae*) in rainbow trout but since the last clinical cases in 1991, there has been no further use of the antibiotic in Tasmania.

Antibiotics are generally administered to fish orally as feed additives; however, a proportion of active form antibiotic will pass unabsorbed through the gut and be released in fish faeces and urinary wastes (Björklund and Bylund, 1990). In addition diseased fish often eat poorly; consequently a proportion of this medicated feed may remain uneaten by the fish, falling through the cage to accumulate on the seabed (Cravedi et al., 1987, Plakas et al., 1998). Estimates of the amount of fish feed that passes uneaten through the cages vary greatly from 1 - 40% (Björklund et al., 1990), and will depend on the overall health of the fish, the characteristics and palatability of antibiotic used and the feeding strategy employed. Advances in feeding technology and improvements in techniques for incorporating antibiotics into feed

(Ang and Petrell, 1997, Petrell, 2001, Shao, 2001) have all greatly reduced the amount of antibiotic reaching the environment.

Consequently, the primary environmental concerns regarding antibiotic usage in aquaculture are very similar to those of other livestock farming operations and include whether antibiotics, at the low concentrations released to the environment, will have any unacceptable effect on non-target aquatic organisms resulting in chemical residues in native fauna and sediments, bio-accumulation/ bio-magnification, toxic effects in non-target organisms and whether these antibiotics will persist in the environment resulting in the development of bacterial resistance that can threaten therapeutic regimes and may even potentially be transferred to the human food chain (Smith et al., 1994, Schmidt et al., 2001). In the following sections the types and functions of the main groups of antibiotics used in aquaculture will be described, the primary environmental risks associated with antibiotic usage will be discussed, current global standards and monitoring approaches will be outlined and the current monitoring program for antibiotics in the marine environment in Tasmania will be reviewed, knowledge gaps will be identified and recommendations will be made regarding potential research and monitoring requirements where appropriate.

3.2 Summary of Current Information on Aquaculture Antibiotics

Unlike terrestrial agriculture finfish farming antimicrobial agents are primarily used in treatment of infected or moribund fish, and deliberate prophylactic use is rare (Alday et al., 2006). Antibiotics are administered under the supervision of a veterinarian, in association with laboratory diagnosis and susceptibility testing of the presumed aetiological agent. In Tasmania there is a code of practice which identifies responsibilities and minimum standards for the supply and use of veterinary chemical products (SCARM, 1999). There is no evidence that antibiotics are used in aquaculture as growth promoters in the same way as in other forms of livestock production (Alderman & Hastings, 1998; Angulo, 2000; Grave et al., 1999, Sorum, 2000, Davenport et al., 2003, Pillay, 2004, Cabello, 2004). Relative to other forms of intensive livestock production the amounts of antimicrobials used in aquaculture are small. There are two main types of bacterial disease in aquaculture; those caused by highly specific pathogens and those caused by opportunistic pathogens such as *Vibrio* spp. (Alday et al., 2006). Several antimicrobial agents have been shown to be useful in the treatment of bacterial diseases of fish; in particular the tetracyclines, quinolones, sulphonamides, aminopyrimidines and amphenicols (Samuelsen, 2006) with some additional use and recent interest in the macrolides. Table 22 summarises the therapeutic characteristics of antibiotic groups which are or have been used in Tasmania.

Tetracyclines, such as oxytetracycline (OTC), are used widely in fin-fish aquaculture as they tend to have a broad spectrum of antibacterial activity. This is the most commonly used antibiotic in Tasmanian aquaculture. Tetracyclines pass into the cells through active transport, binding to the 30S subunit of the ribosome preventing the binding of aminoacyl transfer RNA (tRNA) to the mRNA-ribosome complex. This inhibits protein synthesis and bacterial growth (Isidori et al, 2005). Sulphonamides act by blocking the synthesis of folic acid, a precursor of purine required for the synthesis of DNA. The sulphonamides block the action of dihydropteroate synthetase. Trimethoprim is a diaminopyrimidine which inhibits dihydrofolate reductase. Trimethoprim is used as a potentiator with sulphonamides. In Tasmania trimethoprim is used to successfully treat yersiniosis and marine *Flavobacterium* infections. Macrolides (eg. erythromycin, lincomycin, clarithromycin, and tylosin) work on Gram-positive bacteria in particular and act by blocking protein elongation by peptidyltransferase on the bacterial ribosome (Tenson et al., 2003). Tylosin is made naturally by the bacterium *Streptomyces fradiae* and acts to inhibit bacterial protein synthesis by binding to the 50S ribosome sub-unit. There has also been interest recently in florfenicol, an amphenicol, which affects bacterial growth by inhibiting bacterial protein synthesis (Samuelsen et al., 2006).

Quinolones are broad spectrum antibacterial agents that enter the cell by passive diffusion through water filled protein channels (porins) in the outer membrane and inhibit bacterial growth by interfering with the enzyme DNA-gyrase, essential for normal DNA synthesis (Wolfson et al., 1989, Just 1993, Samuelsen, 2006). Oxolinic acid and flumequine are the most important and the best studied for use in aquaculture. They are often preferred because only very low concentrations are required and they are effective against all common bacterial infections such as furunculosis and vibriosis (Samuelson, 2006). In the environment quinolones and fluoroquinolones readily form complexes with seawater cations which greatly reduces their bioavailability, however, this also means that they are not readily biodegradable and remain in the environment for a long time (Al Ahmad et al., 1999, Gorbach, 2001, Wegener, 1999) and in regions where aquaculture is widespread, quinolone resistance has become a problem (Samuelsen, 2006). In addition quinolones have shown evidence of both mutagenicity and genotoxicity (Isidori et al., 2005). The quinolones, in particular the fluoroquinolones, have been identified as a group of antibiotics to be retained for use in human medicine and as such their use in livestock production should be restricted (Isidori et al., 2005).

Several antibiotics have been specifically restricted for use in animals intended for food production (Table 23).

Table 22 Classification of selected antibiotics used, or with potential for use, in Tasmanian salmonid aquaculture, indicating the mode of action, main disease application and specific known health issues (adapted from Serrano, 2005). It should be noted that the disease applications listed relate to the global usage and most are not known from Tasmania.

Antibiotic Group	Mode of Action	Main Disease Applications	Additional Issues/ Comments
Folate inhibitors eg. sulphonamides and trimethoprim	Broad spectrum activity against Gram-positives and Gram-negatives Potentiated sulphonamides inhibit bacterial dihydrofolate reductase enzyme pathway at 2 points; inhibiting folate synthesis (Folic acid is needed to make RNA and DNA for growth and multiplication, and bacteria must synthesize folic acid). Bacteriostatic	Aeromonas salmonicida; Vibrio anguillarum; Aliivibrio salmonicida; Yersinia ruckeri, Edwardsiella ictaluri Flavobacterium columnare, Aeromonas hydrophila	Doesn't affect folic acid production in animals or humans, as they obtain folic acid from their diets and so are not affected.
Tetracyclines: eg. chlortetracycline; oxytetracycline; tetracycline	Broad spectrum activity against Gram-positives and Gram-negatives, including some anaerobes.	Aeromonas salmonicida; Pseuodomonas spp.; Vibrio anguillarum; Aliivibrio salmonicida; Yersinia ruckeri, Piscirickettsia salmonis, Rickettsia-like organisms, Staphylococcus spp.	Tetracyclines have virtually identical spectra of antibacterial activity, thus there is a high risk of cross resistance.
Amphenicols: eg. chloramphenicol; florfenicol	Broad spectrum, bactericidal or bacteriostatic with activity against many Gram-positives and Gram-negatives. Inhibitors of protein synthesis (binds to sub-unit of bacterial ribosomes (50S)).	Aeromonas salmonicida	Chloramphenicol is poorly absorbed/ unstable in water; banned in human consumption species (may induce aplastic anaemia). Florfenicol does not have risk of aplastic anaemia.
Quinolones: eg. oxolinic acid; flumequine	Against Gram negative bacteria	Aeromonas salmonicida; Vibrio anguillarum; Aliivibrio salmonicida; Yersinia ruckeri, Edwardsiella ictaluri	
Macrolides	Broad spectrum with activity against Gram-positives and	Renibacterium	Most *Pseudomonas*, *Escherichia coli*, and

eg. tylosin, erythromycin,	Gram-negatives - primarily against Gram-positive bacteria but also some anaerobes. Tylosin spectrum of activity similar to that of erythromycin but more active against *Mycoplasmas*	salmoninarum. Staphylococcus, Streptococcus and Lactococcus species.	*Klebsiella* strains are resistant to erythromycin, with resistant strains of *Staphylocci* and *Streptococci* also reported. Active against some anaerobes (although *Bacteroides fragilis* usually resistant).
β-lactams eg. amoxicillin	Active against penicillin-sensitive Gram-+ve bacteria and some Gram—ve bacteria Gram-positive spectrum includes α- and β- haemolytic bacteria. Is susceptible to destruction by β-lactamases	Streptococci, Staphylococci and Clostridia.	

Table 23 Antibiotics banned for use in seafood intended for food production

Antibiotic	Country	Reason	Reference
Fluoroquinolones	USA (off-label use banned)	Potential for development of antimicrobial resistance	US FDA, 2005
Nitroimidazoles (eg. dimetridazole, metronidazole, and ipromidazole)	USA	Carcinogenic	US FDA, 2002
Aminoglycosides	US (Off-label use banned - voluntary withdrawal VMA)		US FDA, 1998
Spectinomycin	USA	Its use is limited by the ready development of bacterial resistance	USP, 2000
Enrofloxacin	USA	Its use is limited by the ready development of bacterial resistance (quinolone)	USP, 2000
Chloramphenicol	Argentina, Canada, EU, Japan, USA	Induces human aplastic anaemia. Potential for development of antimicrobial resistance	USP, 2000 GESAMP, 1997 SANCO, 2001
Rifampicin	USA /Canada	Tumorigenic and teratogenic effects on experimental animals	USP, 2000
Nitrofurans – Parent compounds (eg. Nitrofurantoin, furazolidone) and Metabolites (eg. nitrofurantoin (AH), nitrofurazone (SEM))	USA	Systemic toxicity, mutagenic/carcinogenic	EU, 1990 US FDA, 1991 Australia, 1992

Although licensing of chemicals is an expensive process there are a number of consequences that follow from inadequate licensing including; lack of pharmacokinetic data (and therefore appropriate withdrawal period) for unlicensed agents, a lack of standardised protocols for use and failure to develop safe protocols for handling, storage and application (Alday et al., 2006). As a result unlicensed chemicals will generally require greater scrutiny before any authorisation can be given for their application.

3.2.1 Treatment Protocols/ Regimes

Almost all antibacterial agents can be administered by injection but this is costly in terms of labour and time and therefore is generally only used for high value fish such as broodstock or ornamental fish (Samuelsen, 2006). Antibiotic treatment of fish can be achieved by medicated baths or medicated feed (Davenport et al., 2003, Boxall et al., 2004, Pillay, 2004); bath treatment is in the main restricted to recirculating or tank systems and oral administration is generally the favoured method of treatment as relatively large numbers of fish can be treated easily at low cost. However, since oral administration treats the whole group rather than individual fish infections and, loss of appetite is frequently observed in fish suffering from bacterial infection, oral administration may preferentially treat uninfected individuals or individuals at earlier stages of infection (Samuelsen, 2006).

The efficacy of any treatment regime will be dependent on the particular antibiotic used and the environmental conditions associated with the aquaculture species to be treated. Different antibiotics have different biodegradation properties (Table 24). Quinolones generally require longer treatment times and higher doses in seawater than in freshwater because seawater cations can complex with certain quinolones and reduce the ability of the fish to absorb the antibiotic (Samuelsen and Lunestad, 1996, Samuelsen, 2003). Whilst the relatively low bioavailability of oxytetracycline (i.e. uptake rate by the treated animal) means that more may have to be used in treatment than other antimicrobials.

It is also important to ensure that treatments are as effective as possible, as this will reduce the risk of development of resistance. Pharmacokinetics in combination with susceptibility testing is one important way in which optimal dosage regimes can be established. There have been several approaches proposed to manage dosage regimes. Stamm (1989) recommended that plasma concentrations of antibiotics should exceed minimum inhibitory concentration (MIC) by a factor of four. However, more recent studies of dose regime optimisation in fish suggest that the optimal dose regime for bactericidal drugs could be determined using the AUIC value (AUC/MIC ratio) (AUC=area under curve) and that this ratio should be at least 100, with a maximum plasma concentration C_{max}/MIC ratio of at least 8 in order to effectively control pathogens (Samuelsen, 2006). These ratios are dependent on both the farmed species and the local environmental conditions and the performance of each individual antimicrobial agent may need to be separately evaluated (Kleinow et al., 1994, Martinsen et al., 1995). In addition, dosages will be impacted by the stage of the disease and fish condition, as a consequence obtaining the optimal treatment strategy is often reliant on clinical experience.

The preferred approach for medication of fish feed is still to add the medicants to the feed formulation as a premix, where a naturally occurring carrier such as ground corncobs or rice hulls or compendial grade carriers such as corn/ maize starch, maltodextrins, lactose or microcrystalline cellulose are used to adsorb the medicant (Shao, 2001). These premixes can be mixed and incorporated into the feed during manufacture at the commercial feed mill and in Tasmanian are currently added in the vacuum coating stage at the end of production. There are several novel technologies being investigated to improve administration of oral medications in aquaculture e.g. biodegradable and modified release polymer matrices, microencapsulation, and the use of enteric coatings to modify release rates (Shao, 2001), however these approaches are still largely developmental and given the relatively small size of the Tasmanian industry are unlikely to be a commercial reality in the near future.

In contrast feed delivery mechanisms and expertise have advanced markedly in Tasmania over the last decade, with technological developments in feed input and monitoring greatly helping to ensure that antibiotic treatments are as effective as possible. In particular the use of novel feeding technology has ensured minimal feed wastage by using an interactive on-demand feeding systems that accurately match feed delivery to appetite through fish feeding activity (Sveier and Leid, 1998, Noble et al., 2007). These adaptive feeding systems (AQ1 Systems Pty Ltd., Hobart, Australia) monitor uneaten feed via an underwater infra-red sensor attached to a conical pellet trap; this sensor is linked to feeding software, which regulates the input of food in response to the amount of pellet wastage (as described by Blyth et al., 1993, 1999).

Table 24 Comparison of biodegradation characteristics in marine/ coastal aquatic systems of selected antibiotics applicable to Tasmanian conditions.

Antibiotic	Degradation Measure	Source
Tetracycline	Not biodegradable	Richardson & Bowron, 1985
	Evidence of photodecomposition	Oka et al., 1989
Chlortetracycline	After 30 days at 4°C 100% remained, at 20°C 88% remained	Galvalchin & Katz, 1994
	and at 30°C 44% remained	
Erythromycin	$T_{1/2}$ = 11.5 days (20°C) after 30 days at 4°C 97% remained	Galvalchin & Katz, 1994
Florfenicol	$T_{1/2}$ = 4 - 5 days (NB may degrade to more persistent amine)	Lunestad et al., 1993
Flumequine	$T_{1/2}$ = 150 days	Lunestad et al., 1993
Oxolinic Acid	$T_{1/2}$ = 150 – 1000 days	Ilektone et al., 1993; Samuelsen et al., 1992
Oxytetracycline	Sediment binding:	
	$T_{1/2}$ = 30 - 142 days (differences in binding to sediments)	Samuelsen, 1989; Poliquen et al., 1992,1993; Ervik, 1993
	$T_{1/2}$ = 9 - 419 days (under different levels of sediment anoxia)	Björklund et al., 1990
	Sediment leaching:	Smith and Samuelsen, 1996
	sediment conc. 285 µg/g ⇒ water conc 0.11ug/l	
	sediment conc. 10.9µg/g ⇒ water conc. 0.016ug/l	
Sulphonamides potentiated with trimethoprim	After 1 year 75% remained undegraded	Goll van, 1993
Tylosin	No information available	

$T_{1/2}$ – indicates half-life

3.3 Hazard Identification

As with other forms of intensive farming, in its infancy aquaculture was highly dependent on antibiotics and high usages in Europe and N. America in the late 1980's raised public concerns (Alderman and Hastings, 1998). Between 1987-88 up to 70,000 kg of antibiotics were administered in Norway alone; predominantly oxytetracycline (OTC), oxolinic acid (OA) and flumequine (Alderman & Hastings, 1998), concerns regarding such high usage led to many studies identifying the various environmental impacts. Fortunately, vaccine development has greatly reduced requirements for antibiotics (Alderman and Hastings, 1998) and Smith (1996) and Smith & Samuelsen (1996) argue that proper use of antibiotics should present little or no significant environmental hazard. The principle requirement for antibiotics is now associated with new species development and the emergence of new pathogens.

Issues with antibiotic usage include selection for antibiotic resistance possibly altering the composition of the normal bacterial flora and persistence in sediments and water column (Hansen et al., 1993, Burka et al., 1997, Kerry et al., 1996, Huys et al., 2000, Sorum, 2000, Black, 2001, Miranda and Zemelman, 2001, Cabello, 2003, Davenport et al., 2003, Pillay, 2004). However, resistance is not necessarily persistent, and may only be an issue whilst, or shortly after, antibiotics are employed (Lee and Edlin, 1985, Modi et al., 1991). Concerns with resistance are largely as a result of transference of resistance; either through antibiotic resistant bacteria or resistance genes compromising antibiotic therapy in other populations (Sandaa et al., 1992, Kruse and Sorum, 1994, Harrison and Lederberg, 1998, Sorum, 1998, Davison, 1999, Angulo, 2000, Guardabassi, 2000, Rhodes et al., 2000, L'Abee-Lund and Sorum, 2001, Schmidt et al., 2001, McEwen and Fedorka-Cray, 2002, Petersen, 2002, Sorum and L'Abee-Lund, 2002, Cabello, 2003, Hastings, 2004, Alcaide et al., 2005, Sorum, 2006). In addition there is also some anxiety regrading the contamination of wild fish and shellfish in the vicinity of aquaculture operations through ingestion of medicated feed and leaching from sediments (Bjorlund et al., 1990, Husevag et al., 1991, Samuelsen et al., 1992, Coyne et al., 1997, Rosser and Young, 1999, Levy, 2001, Schmidt et al., 2001, Furushita et al., 2005, Cabello, 2006). Ervik et al (1994) showed 7-12 fold increase in quinolone (i.e. oxolinic acid and flumequine) resistant bacteria in mussels in the area surrounding fish aquaculture. Studies associated with terrestrial usage have shown that certain antibiotics can be toxic for both aquatic and terrestrial organisms and plants as well as causative agents of genotoxicity and mutagenicity (Jolibois et al., 2003, Isidori et al., 2005).

3.3.1 Antimicrobial Resistance

Resistance is a relative term. In a clinical context resistance refers to bacteria which are not inhibited by the administration of a therapeutic agent. However, in microbiological terms a bacterial clone can be considered "resistant" if it is able to function, survive or persist under higher concentrations of an antimicrobial agent than its parental population could tolerate (i.e. acquired resistance). Alternatively, a particular species can be termed "resistant" if it is able to function, survive or persist under higher concentrations of an antimicrobial agent than other species can tolerate (i.e. innate resistance).

Antibacterial agents can have irreversible effects on the genepool of microorganisms (resistance) (Isidori et al, 2005), and there is also concern regarding their potential effects on microbial life in surface waters (Kummerer, 2001) and on other organisms (Baguer et al., 2000, Halling-Sorensen, 2000). However, the dominant impact of aquaculture antibiotics with respect to resistance would be on the frequency of resistant variants in microbiota and the potential for transfer of that resistance between groups. Development of resistance to widely used antibiotics poses a potential hazard to humans and livestock that may be exposed to and infected by these bacteria (Costanzo et al., 2005, Cabello, 2006). Exchange of genes for resistance to antibiotics has been shown to occur between bacteria in the aquaculture environment and bacteria in the terrestrial environment, including bacteria of animals and human pathogens (Sørum, 1998). Human health effects are largely as a result of transference of resistance; either through antibiotic resistant bacteria or resistance genes reaching human populations and compromising antibiotic therapy. However, although it is theoretically possible for non-pathogenic bacteria in the marine environment to transfer resistance to human pathogens it has been suggested that this is highly unlikely (Smith et al., 1994) and the risks of developing antimicrobial resistance in human medicine as a result of aquaculture usage are extremely minor compared to the risks associated with the use/ misuse of these agents in medicine (Smith, 2001).

Environmental factors other than the antimicrobial agents themselves can influence the frequencies of resistance (Kapetanaki et al., 1995, Vaughan et al., 1996) which makes it very hard to clearly characterise the relationship between antibiotic usage and frequency of resistance. In addition, resistance associated with aquaculture operations may be transient (Austin, 1985, Lee and Edlin, 1985, McPhearson et al., 1991, Modi et al., 1991, Samuelsen et al., 1992, Kerry et al., 1996, Herwig et al., 1997, O'Rielly and Smith, 2001) and as such would diminish once application of the particular antibiotic ceases. Laboratory studies have shown that once antibiotic usage ceases sensitive variants proliferate and the proportion of resistant variants declines (Lee and Edlin, 1985, Modi *et al.*, 1991). Which suggests that whilst an antibiotic is in use it will exert a positive selection pressure for resistance but that when the drug is withdrawn, the incidence of resistance may recede, although it is unlikely to disappear (GESAMP, 1997).

Acquired resistance - fish disease bacteria

Resistance of pathogenic bacteria to antibiotics is a widespread issue and antibacterial resistance is a threat to the efficacy of antibacterial substances. There is a large body of literature suggesting that continued use of antimicrobial agents can lead to increased resistance in the natural microbiota of the treated species and a convincing amount of clinical evidence to suggest that antibiotic-resistant strains of fish pathogens have developed over time (Alderman & Hastings, 1998). Smith et al. (1994) identified that in areas where a particular antimicrobial agent was used the probability that there would be a higher frequency of resistant variants was increased. Sugita et al. (1988) showed evidence of resistance to oxolinic acid in goldfish, whilst De Paola (1995) and De Paola et al. (1995) identified marked changes in resistance frequency in the intestinal microflora of catfish with the continued use of oxytetracycline and most recently Giraud et al. (2006) provide data suggesting antibiotic resistance in sea bass. In contrast Kerry and Smith (1997) found no evidence of antibiotic resistance in salmon and Michel et al. (2003) did not find any evidence of resistance with the use florfenicol. It is also important to note that many instances of acquired resistance may be transient, with natural susceptibility levels being restored over time (Austin, 1985, McPhearson et al., 1991, Samuelsen et al., 1992, Kerry et al., 1996, Herwig et al., 1997, O'Rielly and Smith, 2001).

Natural resistance

Some bacteria have intrinsic resistance. For instance most species of *Aeromonas* have an intrinsic resistance to β-lactam agents; consequently changes in this group can have a major impact on apparent frequency of resistance to ampicillin, highlighting the need to identify bacteria to species level. Studies of resistance following antibiotic application at fish farms (Björklund et al., 1990, McPhearson et al., 1991, Nygaard et al., 1992, Samuelsen et al., 1992, Spanggaard et al., 1993, Ervik et al., 1994, Kerry et al., 1996, Herwig et al., 1997, Guardabassi et al., 2000) and in microcosms (Kerry et al., 1996, Herwig and Gray, 1997, O'Reilly and Smith, 1999) have shown an increased frequency of resistance to several drugs across a variety of bacterial species. However, Kapetanaki et al. (1995) and Vaughan et al. (1996) suggest that increased levels of bacterial drug resistance can occur independently of the presence of a drug. Resistance to antibiotics does occur naturally in bacterial populations (Jones et al., 1986, McPhearson et al., 1991, Boon, 1992, Spanggaard et al., 1993 Smith et al., 1994, Boon & Chattanach, 1999, Huys et al., 2000, Chelossi et al., 2003). Austin (1985), McPhearson et al. (1991) Husevag et al. (1991), Nygaard et al. (1992), Samuelsen et al. (1992), Sandaa et al. (1992) and Kerry et al. (1996) all found elevated levels of antibiotic resistance in the environment associated with fish-farms that either had not used antibiotics or had not used the specific antimicrobial agent for which the resistance had been shown. The concern under these conditions would be that antibiotic use may allow resistant strains to proliferate and spread. However, for this to be a problem the resistance would need to be long-lived (non-transient) and there would need to be a specific combination of environmental and therapeutic circumstances; with the most significant risks in relation to resistance probably being to the aquaculture industry itself.

Multiple & Cross resistance

In addition there have been several studies which have provided evidence of multiple antibiotic resistance (Pillai et al., 1997, Hall, 1997, Alderman & Hastings, 1998, Tendencia and de la Pena, 2001, Chelosi et al., 2003). Aldermann & Hastings (1998) provide evidence of multiple resistance to antibiotics within the fluoroquinolones (including oxolinic acid and flumequine).

Some species have been shown to have linked or cross resistance to particular antibiotics, for instance the mechanisms for resistance to oxytetracycline are also likely to result in tetracycline resistance, and it has been suggested that similar relationships may exist between amoxicillin/ ampicillin, oxolinic acid/ flumequine, trimethoprim/ sulmethoxazole and ormetoprim/sulfadimethoxine (Table 25). Consequently, studies may over-estimate the frequency of multiple resistance by not taking this into account (Alday et al., 2006).

Multiple resistance to antimicrobial compounds has been identified in a number of bacterial fish pathogens. For example, certain strains of the bacterium

Aeromonas salmonicida are reportedly resistant to combinations of oxytetracycline, streptomycin, sulphamethoxine and/or trimethoprim (Alderman & Hastings, 1998). *A. salmonicida* ssp. *salmonicida* is the cause of furunculosis, and multiple antibiotic resistance was historically a serious problem from the salmon farming industry of Scotland (Alderman & Hastings, 1998) although as a result of vaccine development this is no longer an issue.

Antimicrobial agents currently and previously used in Tasmanian aquaculture and the agents to which they are most commonly linked by cross-resistance are shown below in Table 25.

Table 25 Antimicrobial agents currently and previously used in Tasmanian aquaculture and the agents to which they are most commonly linked by cross-resistance (adapted from Alday et al., 2006)

Antimicrobial Agent	Cross Resistances
Oxytetracycline	Other tetracyclines including doxycycline
Trimethoprim-sulphonamide/ Ormethoprim-/sulphonamide	Sulfonamides and potentiated sulfonamides
Oxolinic acid, flumequine	Other quinolones; increased susceptibility to mutations for resistance to newer fluoroquinolones
Florfenicol	Chloramphenicol
Amoxycillin	β-lactamase sensitive penicillins 1st generation Cephalosporins
Erythromycin	Some cross resistance with other macrolides

Mechanisms of resistance

Development of drug resistance among target organisms has been an issue since the introduction of antibiotics in the 1930's (GESAMP, 1997). In order to understand the development of resistance in relation to aquaculture it is important to explain what is meant by resistance. The GESAMP study on the safe and effective use of chemicals in coastal aquaculture (GESAMP, 1997) provides a very good explanation of this, which is paraphrased as follows: to benefit from the effect of antibiotics an organism must possess a target system that is affected by that antibiotic; bacteria which lack such target systems would be inherently resistant to that antibacterial. Bacteria which normally have susceptible target systems may develop resistance by a range of modifications, either to the target system itself, to the permeability of the cell wall or by production of new enzymes which act directly to inactivate the antibiotic (GESAMP, 1997).

Three factors contribute to development & spread of resistance: i) mutation in common genes which will extend their spectrum of resistance, ii) transfer of resistant genes amongst micro-organisms (e.g. plasmids)and iii) increases in selective pressures that enhance development of resistant organisms (Halling-Sorensen et al., 1998). Research suggests that plasmid borne transfer of resistant genes is the most prolific mechanism of resistance transfer between bacteria (Olsen, 1999). Plasmid-mediated resistance has been observed in several fish pathogens (i.e. *Aeromonas salmonicida*, *A. hydrophila*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Vibrio damselae* ssp. *piscicida* and *Edwardsiella tarda* (Lewin et al., 1990, Aoki, 1988) and *Yersinia ruckeri* (DeGrandis and Stevenson, 1985), with resistance either shown, or the potential for resistance demonstrated, to both individual and combinations of chloramphenicol, sulphonamide, potentiated sulphonamide, streptomycin, oxolinic acid, spectinomycin, trimethoprim, tetracycline and oxytetracycline (Olsen, 1999). Interestingly this mechanism is enhanced in eutrophic environments where there are elevated carbon concentrations and, therefore, increased bacterial activity (Davidson, 1999).

There are some risk factors which have been identified in studies of both veterinary and human antibiotic usage that appear to predispose selection for resistance and which probably will apply to aquaculture (GESAMP, 1997). The frequency of antibiotic usage would appear to be strongly linked to resistance levels in human and veterinary medicine (Pascott and Baggott, 1988, Hamilton-Miller, 1990, Kruse, 1994). Occurrence of resistance may also be increased where antibacterial agents are present in concentration insufficient to kill the selected bacteria (GESAMP, 1997). This may be as a result of an inappropriate drug choice, inadequate dose (eg. through low bioavailability, leaching or lack of appetite in treated stock), failure of treatment regime (eg. shortened duration), prophylactic usage or heavy reliance on a restricted suite of therapeutants (GESAMP, 1997).

Environmental Effects on Resistance

Several studies have shown an increase in antibiotic resistant bacteria in the sediments (Hansen et al., 1993, Kerry et al., 1995, Herwig et al., 1997, Ho et al., 2000, Miranda and Zemelman, 2001). In addition there is evidence of increased resistance in environments with significant nutrient enrichment and enhanced bacterial activity (Kapetanaki et al., 1995, Vaughan et al., 1996, Davidson, 1999, Costanzo et al, 2005). This is of particular concern as it may lead to proliferation of other resistant bacteria in the sediments, modifying the sediment processes and potentially affecting bacteria which are essential for the breakdown of organic matter and nutrient assimilation. Other environmental conditions may also affect antibiotic degradation with evidence that increased pH and temperature increase degradation rates (Oka et al., 1989). That both the environmental conditions and the microbial agent have the potential to increase resistance confounds attempts to infer causality between presence of antibiotics (via the fish farm) and increased frequency of resistance (Alday et al., 2006).

Evaluation of Resistance

Evaluation of resistance or sensitivity requires determination of the point of susceptibility of the bacterium (i.e. the breakpoint). Breakpoints are species specific, host specific and will be susceptible to the specific test protocols. Consequently comparisons are often difficult unless test conditions are standardised. At present there is no published information that allows an informed opinion on the extent of antibiotic resistance associated with aquaculture operations (Barton et al., 2003). However, testing undertaken in Tasmania by the DPIW Fish Health Unit on oxytetracycline (OTC) sensitivity for aquatic animal pathogens over the last 15 years suggests no evidence of acquired resistance to OTC (J. Carson, pers.comm.).

3.3.2 Environmental Accumulation

Accumulation (Persistence) in water column

On the whole antibiotics tend to be highly water soluble and as such will disperse rapidly in the water column. Consequently concentrations in the water column are unlikely to reach levels which may exert selection for resistance (Alday et al., 2006). However, certain antibiotics may remain in the pelagic system, either in their original form or as a by-product following biotic or abiotic transformation. These derivatives are generally benign but in some instances, such as florfenicol and sulfamethoxazole, the by-products may be more harmful that the parent compounds (Isidori et al, 2005).

Not all antibiotics have the same effects, certain antibiotics react with seawater cations (sodium, potassium, calcium and magnesium) resulting in precipitation of the antibiotics and loss of bioavailability (Barnes et al., 1995, Smith, 1996, Smith and Samuelsen, 1996). Many commonly used antibiotics will degrade rapidly in seawater. Divalent cation (calcium and magnesium) concentrations in the marine environment can result in a 90% reduction in the biological activity of tetracyclines and quinolones (Alday et al., 2006). β-lactams, such as ampicillin and amoxicillin, are also unstable in aquatic environments (Lunestad et al., 1995). Oxytetracycline is subject to photo-degradation and at 1m depth has a relatively short half-life. In contrast, components of the potentiated sulfonamides appear to be very stable in the aquatic environment (Alday et al., 2006). Some drugs, such as sulfamethoxazole, photodegrade in aqueous solution along several pathways (Moore, 1998) with certain by-products being more harmful than the parent compound (Della Greca et al., 2003). Unconjugated metabolites are often more hydrophobic than the phase II conjugated substances, which enables them to bioaccumulate (Halling-Sorensen et al, 1998). Consequently it is often not only the parent compound which should be subject to a risk assessment but also the main metabolites (Halling-Sorensen et al, 1998). However, it is important in all of these assessments to consider the concentration as “all things are poison and nothing is without poison, only the dose permits something not to be poisonous” (Paracelsus, 1536).

Accumulation (Persistence) in sediments

A proportion of the medicated feed administered to caged fish will remain uneaten by the fish and will fall through the cages and accumulate on the sea-bed (Jacobsen and Berglind, 1988) and this may have affects on aquatic organisms (Halling-Sorensen et al., 1998). Several studies have described the presence of antibiotics in fish farm sediments (Björklund et al., 1990, Coyne et al., 1994, Kerry et al., 1995, Weston et al., 1994, Samuelsen et al., 1992a, 1992b). In general antibiotics are significantly more stable in the sediments than in the water column (Samuelsen et al., 1994). The highest environmental concentrations occur under cages, and are probably highly localised and confined to top 10cm (Alday et al., 2006). Persistence in the cage sediments will depend on current flow and oxygen concentration but Alday et al. (2006) suggest that half-lives as low as one month should be achievable at well run farms.

Although there is limited information on the horizontal distribution of antibiotic residues, on the basis of the available data Coyne et al. (1994) suggest that the footprint would be no more than twice the area of the treated cage. However, there are many deposition models now available which should be able to calculate accurate footprints for individual farms. There are now several very good commercially available deposition models that provide an assessment of the likely distribution and impact of solid wastes from aquaculture operations (e.g. DEPOMOD (Cromey et al., 2002a, b)). DEPOMOD was originally designed as a tool to enable farmers, consultants and regulators to objectively assess the localised impact of aquaculture operations and is probably one of the most advanced commercially available models. DEPOMOD is also unusual in that it has had relatively extensive field validation of many of the model parameters and outputs (Cromey et al., 2002b). However, any of the commercially available models could be used to determine the deposition zone and therefore identify suitable monitoring locations.

Environmental persistence of antibiotics in the marine environment is highly variable between the different antibiotics. Degradation rates will vary depending on both the specific antibiotic and the prevailing environmental conditions (Halling-Sorensen et al. 1998). It has already been identified that the biological activity of quinolones and tetracyclines is greatly reduced in the presence of divalent cations, with tetracyclines readily binding to calcium and analogous ions to form stable complexes (Hirsch et al., 1999, Kummerer 2001b, Alday et al., 2006). Samuelsen et al. (1994) suggested that the half-lives of antibiotics in the sediments would be months as compared with days/ weeks in the water column. Hektoen et al. (1995) compared the persistence of many key antibacterial agents in marine sediments through mesocosm experiments; including oxytetracycline (OTC), oxolinic acid (OA), flumequine (FLU), sarafloxacin (SAR), florfenicol (FLO), sulfadiazine (SDZ) and trimethoprim (TRM). OTC and the quinolones (oxolinic acid, flumequine and sarafloxacin) were found to be highly persistent, with concentrations of these compounds remaining unchanged in the deeper layers of the sediment (7cm) for up to 180 days with an estimated half-life of more than 300 days. Residues in surface sediments diminished more rapidly, however this was determined to probably be as a result of leaching to the water column and redistribution in the surface sediments rather than degradation (Hektoen et al., 1995). The quinolones (oxolinic acid, flumequine and sarafloxacin) were found to readily adsorb to the sediments, whilst sulfadiazine and trimethoprim were less persistent diminishing after 90 days (Hektoen et al., 1995). Florfenicol degraded rapidly to the metabolite florfenicol amine, with a half-life of only 4.5 days. There are lots of inconsistencies in the data regarding antibiotic persistence; these are often as a result of differences in the form and solubility of the chemical compounds or the environmental conditions under which the assessments have been conducted (i.e. temperature, salinity, and pH). For instance although the results from Hektoen et al. (1995) agree with those of Hansen et al. (1993) in regard to the strong binding properties and persistence of oxolinic acid, Björklund et al. (1990) found that in brackish water fish farms sediments there was no evidence of any oxolinic acid 10 days post treatment.

However, resuspension through sediment bioturbation can rapidly decrease sediment concentrations, consequently the half-lives of antibiotics under field conditions may be much less than those estimated through mesocosm experiments ((Coyne et al., 1994, Samuelsen et al., 1994, Alday et al., 2006). In addition, bioturbation is reliant on the oxic status of the sediments, suggesting that in anaerobic sediments bioturbation/ resuspension rates will be reduced and therefore persistence of antibiotics will in turn be increased (Alday et al., 2006).

Oxytetracycline (OTC) in sediments

OTC is probably the most widely used antibiotic in aquaculture and as a consequence there has been considerable interest in its fate in sediments around fish farms. However, differences in the experimental and environmental conditions under which these studies have been conducted often make it difficult to apply the findings more broadly; in particular it can be difficult to relate experimental findings to field conditions or to compare results between geographically distinct locations. That said several studies have suggested that OTC is relatively persistent, particularly in anoxic sediments. However, there does not appear to be any clear correlation between sediment concentration and time post medication or depth of sampling. Jacobsen and Berglind (1988) looked specifically at persistence of OTC; they sampled at various intervals after medication and found sediment concentrations between 0.1 - 4.9 mg kg^{-1} dry matter up to 12 weeks post treatment and proposed a half-life estimate of approximately 10 weeks. Whilst Burridge et al., (1999, 2008) found that in anoxic sediments OTC persisted for considerably longer periods, with a half life estimation in this case of 419 days. Smith (1996) reviewed 16 studies of OTC distribution in sediments in marine/brackish water and concluded that under “normal” conditions only approximately 1% of the total antibiotic input ended up in sediments under cages. Alday et al. (2006) also reviewed a broad range of studies and suggested that the median concentration of OTC in the top 2 cm of sediments was 1.7 mg kg^{-1}, with concentrations ranging between 0.1 - 10 mg kg^{-1}. Several authors have suggested that OTC activity decreased markedly in sediments as a result of interaction with seawater cations, precipitation and the formation of inactive complexes (Barnes et al., 1995, Smith, 1996). Jacobsen and Berglind (1988) also suggested that OTC may affect sulphide ion activity in sediments, perhaps by affecting bacterial processing rates. Clearly OTC persistence is highly dependent on a complex matrix of ambient environmental conditions (GESAMP, 1997).

In addition there is also some evidence from the terrestrial literature that OTC may be produced naturally by soil bacteria (eg. via *Strepomyces rismosus*) as a result of selective pressure (Hansen et al., 2001). Although there is no evidence for this at present in marine sediments, the possibility of such processes should be considered.

3.3.3 Antimicrobial Effects on Biology/ Ecology

Antibiotics are of particular interest, even when only found in very low levels, as they have been developed specifically with the intention of having a biological effect, and therefore often have specific properties which promote bioaccumulation (Halling-Sorensen et al., 1998). Halling-Sorenson et al (1998) produced a useful summary of environmental exposure routes for veterinary and human medicines. From this information it can be inferred that the fate of any antibiotic will depend on its’ original properties; where the substance is lipophilic and not readily degradable it will be retained in the sediments, where the substance is hydrophilic it will be more readily dispersed through the water column. Ecotoxicity information on selected antibiotics is summarised in Table 26.

Ecological / Biological Processes

Aquatic ecosystems are largely controlled by, and are dependent on, microbial organisms for a suite of crucial processes (eg denitrification), associations (eg nitrogen fixation) and services (eg. organic breakdown), accumulation of antibiotics in sediments may interfere with bacterial communities and affect the rate and mechanism for mineralisation of organic wastes (Stewart, 1994, GESAMP, 1997, Costanzo et al. 2005).

Antibiotic impacts may be a particular problem for fin fish farms which rely on nitrogen processing bacteria in biological filters to maintain good water quality. There is widespread anecdotal evidence from aquarium industry of antibiotics impacting on denitrification processes. This industry relies on nitrogen processing bacteria in biological filters to maintain water quality and has found reduced efficacy of these filters post-antibiotic treatment. Studies have shown that the biological balance within these filters can be altered and their efficacy diminished following antibiotic additions aimed at curing fish infections (Costanzo et al., 2005). In addition research has also shown negative effects of antibiotics on nitrogen cycling processes in sewage treatment facilities (Campos et al., 2001, Costanzo et al., 2005). *Nitrosomonas* spp. and *Nitrobacter* spp are important bacteria for nutrient cycling, converting ammonia to nitrate (Ricklefs and Miller, 2000). In mesocosm experiments on freshwater systems it has been demonstrated that oxytetracycline (OTC) inhibited processing of ammonia (Klaver and Mathews, 1994). However, there is a paucity of research into the effects of antibiotics on the specific bacteria involved in environmental nitrogen cycling in marine sediments. That said it is intuitive that several antibiotics (including Florfenicol and those in the Tetracycline group) would have an effect on Gram-negative denitrifying bacteria (i.e. *Nitrosomonas* etc) which in turn could affect organic degradation processes in the sediments.

Plankton

Antibiotics have been shown to have effects on the composition of phytoplankton and zooplankton by changing the homeostasis of the environment and affecting ecosystem processes (Boxall et al., 2004, Holten-Lutzhoft et al., 1999, Christensen et al., 2006, Samuelsen et al., 1992, 1994, Schmidt et al., 2000, Hunter-Cevera et al., 2005). However, the literature is inconsistent regarding the toxic effects of antibiotics on zooplankton groups, with sensitivities varying widely depending on the mode of administration, test species, effect level being tested (i.e. acute/ chronic), environmental conditions and experimental protocols. Lee & Bird (1983) suggested that general pharmaceutical waste, including antibiotics, has the potential to produce significant sub-lethal effects on zooplankton species. They raised the calanoid copepod *Temora turbinata* in pharmaceutical waste concentrations above 1ppm and showed that this resulted in smaller adult size, reduced egg production and an abnormal growth pattern. However, it should be noted that the exposure levels used in this case were relatively high compared to the minimum inhibitory concentrations (MIC) under which antibiotics are generally applied in aquaculture. Zooplankton species such as *Daphnia* spp. (freshwater) and *Artemia salina* (seawater) are commonly used in acute toxicity testing and consequently there is quite a lot of information on sensitivities in these species. However, even using standard ecotox test species the results can be quite different, for instance Wollenberger et al (2000) observed no effect of OTC on *Daphnia magna* at concentrations of 100 mg/L, while

Isidori et al. (2005) suggest susceptibility at relatively low concentrations (EC50 = 22.64 mg/l). Consequently comparisons should only be made where the experimental regimes are analogous. Comparison of OTC and oxolinic acid (OA) under similar conditions indicated that OA was highly toxic to *Daphnia magna,* suggesting that OA had a greater potential to have significant adverse effects in the aquatic environment than OTC (Wollenberger et al., 2000). In must be noted that whilst the use of standard test species is very valuable for comparing the relative toxicity of different compounds these experiments have been designed *a priori* to determine the level at which an effect can be induced, and care must be taken to ensure when referring to the resultant toxicity levels that they are in fact meaningful in the natural environment.

Algae (micro and macro)

Several studies have indicated that algae are relatively sensitive to antibiotics (Holten Lutzhoft et al., 1999, Halling-Sorensen, 2000, Isidori et al., 2005). Antibiotics can reduce photosynthetic capacity and growth, which can in turn have important repercussions for productivity higher up the food chain. In an experiment which looked at the toxicity of common antibiotics to the marine algae *Rhodomonas salina,* oxolinic acid (EC50- 1.6 mg/l), oxytetracycline (EC50- 10 mg/l) and trimethoprim (EC50- 16 mg/l) were found to be the most toxic, with amoxicillin (EC50- 3180 mg/l) being the least toxic (Holten Lutzhoft et al., 1999). The antibiotic streptomycin prevented growth in six species of blue-green microalgae, at concentrations substantially lower (0.09 - 0.86 mg/l) than needed to prevent growth of green macroalgal species (Harrass et al., 1985), although all algal growth was slowed or delayed in sublethal concentrations of streptomycin and the maximum density attained by several species was decreased. Isidori et al. (2005) examined the ecotoxicity of 6 antibiotics, including oxytetracycline, to aquatic organisms and determined that acute toxicity was in the order of mg/l whilst chronic toxicity effects could be an order of magnitude lower. However, once again these data are derived from laboratory based culture experiments and as such are based on concentrations that would be rarely, if ever, encountered in the broader environment in association with aquaculture. Given the dilution that occurs with antibiotic usage in cage aquaculture in the coastal environment such concentrations would be very unlikely. Industry data suggests that water column concentrations immediately adjacent to the treated cages were below detection limits (S. Percival, pers. comm.). Consequently, any effects on algal species would be extremely localised, with only microalgal species within the immediate vicinity of the cages and macroalgal species associated with cage fouling communities likely to be at any risk.

Table 26 Comparison of ecotox test information for antibiotics applicable to Tasmania (Adapted from Halling-Sorensen et al., 1998).

Antibiotic	**Test Species**	**Toxic Effect**	**Source**
Ampicillin	Sediment bacteria (Vibrio harveyi)	Antibiotic resistance >100 mg/l / Growth rate	Sandaa et al., 1992; Thomulka et al., 1993
Chloramphenicol	Vibrio harveyi (Biolumicens test)	EC_{10} (unspecified) = 0.16 mg/l	Thomulka et al., 1993
Flumequine	Artemia saline	LC_{50} (24 hours) = 477 mg/l	Brambilla et al., 1994; Migliore et al., 1997
		LC_{50} (48 hours) = 308 mg/l	
		LC_{50} (72 hours) = 96 mg/l	
Oxolinic Acid	Sediment bacteria (*Vibrio harveyi*)	Antibiotic resistance	Nygaard et al. 1992
	Daphnia magna (freshwater)	EC_{50} (48 hours) = 4.6 mg/l	Wollenberger et al. 2000
		$NOEC_{50}$ (chronic) = 0.38 mg/l	
Oxytetracycline	Sediment bacteria (*Vibrio harveyi*)	Antibiotic resistance	Husevag et al., 1991; Sandaa et al., 1992;
			Samuelsen et al., 1992; Nygaard et al. 1992;
			Kerry 1995; Kerry et al., 1995
	Daphnia magna (freshwater)	EC_{50} (48 hours) = >1000 mg/l	Wollenberger et al. 2000
		EC_{50} (chronic) = 46.2 mg/l	
Tetracycline	*Daphnia magna* (freshwater)	EC_{50} (chronic) = 44.8mg/l	Wollenberger et al. 2000
		$NOEC_{50}$ (48 hours) = 340 mg/l	
	Microcystis aeruginosa (fw cyanobacteria)	EC_{50} (7 days) = 0.09 mg/l	Halling-Sorensen, 2000
	Selenastrum capricornutum (green algae)	EC_{50} (7 days) 2.2 mg/l	
Tylosin	*Daphnia magna* (freshwater)	EC_{50} (48 hours) = 680 mg/l	Wollenberger et al. 2000
		$NOEC_{50}$ (chronic) = 45 mg/l	
	Microcystis aeruginosa (fw cyanobacteria)	EC_{50} (7 days) = 0.034 mg/l	Halling-Sorensen, 2000
	Selenastrum capricornutum (green algae)	EC_{50} (7 days) = 1.38 mg/l	

Higher Organisms (Fish, birds, crustacea & molluscs etc)

Antibiotics have the potential to pass into the environment, where they can affect the natural fauna and flora (Boxall et al., 2004, Black, 2001, Coyne et al., 1997, Hansen et al., 1993, Hektoen et al., 1995, Holten-Lutzhoft et al., 1999, Christensen et al., 2006, Haya et al., 2000, Burka et al., 1997). All antibiotics can be toxic above certain concentrations; in a recent US study OTC was shown to be toxic to Mallard duck and Northern bobwhite (LC50 at 8-d > 5620 mg/kg) (Boxall et al., 2004); however, this is an extremely high dosage and such levels are unlikely to ever be encountered in an aquaculture context. There is evidence of feral fish, crabs and bivalves picking up antibiotic loading from waste feed and sediment residues (Samuelsen et al., 1992, Ervik et al., 1994, Capone et al., 1996). Oxytetracycline (OTC) and oxolinic acid (OA) residue has been identified in wild fauna such as fish, mussels and crabs (Björklund et al., 1990; Capone et al., 1996). To date the only published study of the effects on wild fish is that of Björklund et al. (1990) looking at levels in Bleak (*Alburnus alburnus*) and Roach (*Rutilus* sp.) associated with freshwater cage culture; levels were very low at the end of treatment (max 0.2 mg/kg in Bleak) and within 2 weeks were below detection level. Clearly optimisation of dosing regimes and minimisation of wastage are the most effective ways to limit effects on local fish species but whilst treatments involve feed additives it is not possible to eliminate the impacts of antibiotics on wild fish. However, FSANZ recently undertook an investigation of the effect of antibiotic usage on non-target fish from the vicinity of cages in D'Entrecasteaux Channel, Tasmania and the findings suggested that there was no human health risk associated with current usage (FSANZ, 2007).

Although laboratory studies suggest that oysters and mussels are capable of accumulating OTC when exposed to dissolved or particulate material at high dosages (Black et al., 1991), field studies suggest that this is unlikely under normal farming conditions (Tibbs et al., 1989, Capone et al, 1996). Only in oysters or mussels grown directly on, or under cages, is contamination likely to occur and this will diminish rapidly after cessation of treatment such that these shellfish would be unlikely to represent any human health hazard (Coyne et al., 1997).

Several studies have inferred an impact on sediment communities (Capone et al., 1996), but it is unclear whether this is as a result of impacts on the sediment macrofauna directly or indirectly through effects on the microbiota (Gray and Herwig, 1996) and biogeochemistry (Capone et al., 1996). Recent studies show several antibiotics (including oxolinic acid, oxytetracycline and trimethoprim) to be acutely toxic to aquatic invertebrates (Holten Lutzhoft et al., 1999; Halling-Sorensen, 2000; Halling-Sorensen et al., 2000; Wollenberger et al., 2000). Toxicity tests carried out according to ISO (1989) and OECD (1996) standard procedures found OTC to be the least toxic of the antibiotics tested to the standard test species (*Daphnia spp.* a freshwater plankter*),* whilst oxolinic acid (OA) was the most toxic (Wollenberger, 2000) (Table 26). This was deemed to be a significant health concern as oxolinic acid has been used extensively in fish farms and the study suggests that it has the potential to cause adverse effects.

Oxytetracycline

Oxytetracycline has been shown to be taken up by oysters and crabs and other invertebrates close to a marine finfish farm (Capone et al., 1996). No more than trace oxytetracycline residues (about 0.1 mg/kg) were found in oysters (*Crassostrea gigas*) or crabs (*Cancer magister*) collected under the farm, but about half the red rock crab (*Cancer productus*) collected under the cages during and within 12 days of oxytetracycline treatment contained oxytetracycline in meat at concentrations as high as 3.8 mg/kg, well in excess of both the US Food and Drug Administration limit for commercially sold seafood of 0.1 mg/kg and the allowable limit in Australian commercial seafood of 0.2 mg/kg (Capone et al., 1996). However, with respect to filter feeders, the fraction of input accumulated is small and half-life short (Coyne et al., 1997). Although OTC was measurable in mussels in the immediate area of a marine fin fish farm during a treatment period, levels declined exponentially from a maximum concentration of 10.2 mg/kg mid treatment to less than 2 mg/kg 5 days post treatment (Coyne et al., 1997). There is some data suggesting that there may be chronic exposure effects (growth inhibition in juveniles) in crustaceans (Isidori et al., 2005) and OTC resistance has been reported in non-target organisms and fish, as well as in bacterial communities near aquaculture sites (Björklund et al., 1991; Hansen et al., 1993). However, these studies did not identify whether this resistance was transient or indeed whether there may be a natural source of the antibiotic or innate resistance in the communities.

3.3.4 Human Health Issues

Public concern regarding the fate of pharmaceuticals in the environment has been heightened partly as a result of findings that xenobiotics (e.g. DDT and PCB's) can have an effect on human health in extremely low concentrations (down to a few nanograms per litre) (Halling-Sorenson et al., 1998). Concerns regarding the human health effects of antibiotics are largely related to the perceived risk of transference of resistance; either through antibiotic resistant bacteria or resistance genes reaching animal and human populations and compromising antibiotic therapy in those populations (Sandaa et al., 1992, Kruse and Sorum, 1994, Harrison and Lederberg, 1998, Sorum, 1998, Davison, 1999, Angulo, 2000, Guardabassi, 2000, Rhodes et al., 2000, L'Abee-Lund and Sorum, 2001, Schmidt et al., 2001, McEwent and Fedorka-Cray, 2002, Petersen, 2002, Sorum and L'Abee-Lund, 2002, Cabello, 2003, Anderson et al., 2003, Hastings, 2004, Alcaide et al., 2005, Sorum, 2006) or by contaminating wild fish and shellfish in the vicinity of aquaculture operations through ingestion of medicated feed and leaching from sediments (Bjorlund et al., 1990, Husevag et al., 1991, Samuelsen et al., 1992, Coyne et al., 1997, Rosser and Young, 1999, Levy, 2001, Schmidt et al., 2001, Furushita et al., 2005, Cabello, 2006).

Direct antibiotic toxicity in humans has been observed with chloramphenicol usage in terrestrial farming, where over exposure to this antibiotic lead to human casualties by inducing aplastic anaemia (Cabello, 2006). There are currently maximum residue limits set for most antibiotics in commercial food (FSANZ) but most of these guidelines are only relevant to terrestrial foods not seafood. In spite of this, with respect to human health effects the regulatory systems, general principles and basic parameters such as ADI and maximum residue levels (MRL) established for residues in other animals (Table 27) are still relevant to fish (Alday et al., 2006). However, the pharmacokinetics and residue kinetics of antimicrobials used in aquaculture is unique. Reimschuessel et al. (2005) have compiled a very interesting "searchable database of pharmacokinetics data in fish" encompassing data from nearly 3000 investigations which shows considerable differences in estimations of residue half-lives. Such data variability makes it difficult to confidently evaluate appropriate withdrawal periods and as a consequence regulatory authorities normally apply a significant safety margin (Alday et al., 2006).

With respect to the human health effects of oxytetracycline (OTC) usage in salmon and wild fish, Food Standards Australian and New Zealand (FSANZ) was recently approached by the Tasmanian Public and Environmental Health Service (Department of Health and Human Services) to undertake a risk assessment for OTC in wild fish and treated farmed fish around fish farms (FSANZ, 2007). In this study samples were collected from wild caught fish species (mackerel, flathead and escaped farmed salmon). Using the residue levels in these fish, a worst case scenario level of 0.42 mg/kg concentration level was used as the model for exposure. The assessment was conducted on the basis of an acceptable daily intake (ADI) for OTC of 0.03mg/kg body weight /day as set by the Therapeutic Goods Administration (TGA) in 2003 and adjusted according to mean dietary exposure levels for adults and children aged 2-6 years. The findings suggested that children 2-6 would need to consume on average 8 serves of salmon, 6 serves of mackerel or 55 serves of flathead per day before the ADI is exceeded, whilst adults would need to consume on average 33 serves of salmon, 22 serves of mackerel or 212 serves of flathead per day. Taking background dietary exposure into account this study recommended that safe consumption levels (i.e. before the ADI is exceeded) would be 6 or more serves of salmon/ mackerel or flathead per week for adults and 8 or more serves of salmon/ mackerel or flathead per week for children 2-6 years, assuming a standard 150gram serve size. The study therefore concluded that there was no public risk associated with the consumption of Tasmanian farmed salmon or wild fish living in the waters surrounding the cages based on the current OTC residue levels.

3.4 Evaluation/ Analysis Approaches

Public concern with toxic substances in the environment has lead to increasing regulation of chemicals used in aquaculture. Globally there is a general trend for the control of antimicrobial use in aquaculture to follow the national pattern of the control of the use of those agents in humans and animals (Alday et al., 2006)

3.4.1 Current Standards (Australia)

There are no antibiotics registered for use in aquaculture in Australia but several have been, and are still being, used off-label, under veterinary prescription with oxytetracycline and florfenicol having been granted a minor use permit by the Australian Pharmaceutical and Veterinary Medicines Association (APVMA).

i) Australian and New Zealand (ANZ) Guidelines for fresh and marine water

The latest ANZ Water Quality Guidelines have tried to adopt a risk-based approach, although not a full quantitative risk assessment. They propose using the criteria outlined in the previous guidelines (ANZECC, 1992) as indicative trigger values, to assist in a decision framework for local, regional or site specific environmental conditions.

The guidelines include provision for environmental water quality for ecosystem sustainability, both in general terms and in relation to aquaculture species specifically. The guidelines also include specific guidelines on water quality criteria for human health. However, antibiotics are not amongst the listed chemicals of concern in either document and therefore there are no environmental residue guidelines. References to criteria for assessment of bioaccumulation and ecotoxicity effects are restricted to those affecting the aquaculture species. Consequently the only currently available standards are those that relate to the human health and food safety (ANZECC, 2000, FSANZ, 2009).

Nonetheless, it is important to note that the ecological effects of antibiotics and antimicrobials will depend largely on their bioavailability. Although there are extremely sensitive methods for the detection of very low levels of antibiotics, these levels are only really representative of the ecological risk if the antibiotic and antimicrobial chemical complexes are biologically active. Since molecules that are bound to sediments and other substrates are generally not bioavailable some additional measure of bioavailability will be necessary in order to assess likely ecological effects.

Several approaches have been used to try and identify bioavailability and evaluate ecological risk, these include biochemical approaches, chemotaxonomy and gene based methods, standard ecotoxicological assessments and identification of ecologically sensitive indicators. There are many biochemical approaches which will provide estimates of antibiotic residue, including measures of the various derivatives of the parent compounds (labile and refractory) in the water column and the sediments but it is important to relate these values to an ecological context. In the terrestrial environment chemotaxonomy and genetically based methods have been used to identify resistant bacterial communities; in this case resistant populations have been cultured in the laboratory and then used to develop tests for resistance which can be conducted in the field (Kummerer, 2004). The development of maximum residue limits (MRL) for specific chemicals and conditions is another approach that has been applied both to microbiological and macrobiological communities. This is achieved by ecotoxicological testing to determine sensitivities (i.e. MIC) of indicator species/ communities and using this information to monitor field conditions.

Australian and New Zealand Guidelines for Antibiotics in Food

In Australia the guidelines (FSANZ, 2009) on antibiotic residues in food are primarily limited to terrestrial agriculture (Table 27), with oxytetracycline being the only antibiotic listed for fish. In the US there is some provision of limits for aquaculture (Table 28). Residue levels for other antibiotics used in the Tasmanian aquaculture industry are shown for milk and meat where available.

Table 27 Food Standards Australia and New Zealand (FSANZ), maximum residue limits in food for selected antibiotics (FSANZ, 2009):

Antibiotic	MRL	Food Application
Oxytetracycline	0.2 mg/kg	Fish/ Prawns
	0.1 mg/kg	Meat
Amoxycillin	0.01 mg/kg	Meat/ Milk
Chlortetracycline	0.1 mg/kg	Meat
Erythromycin	0.3 mg/kg	Meat
Sulphadiazine	0.1 mg/kg	Meat/ Milk
Tetracycline	0.1 mg/kg	Milk
Trimethoprim	0.05 mg/kg	Meat/ Milk
Tylosin	0.1 mg/kg	Meat
	0.05 mg/kg	Milk

The FSANZ guidelines are largely based on the recommendations of the USEPA, FAO and WHO. MRL (maximum residue limits) for antibiotics are continuously developed by the Codex Alimentarius Commission of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO).

Table 28 US MRLs relevant to aquaculture (as proposed by the JECFA - joint expert committee on food additives, NB. Levels based on fish muscle samples).

Antibiotic	MRL
Oxytetracycline	0.2 mg/kg
Thiamphenicol	0.05 mg/kg
Flumequine	0.5 mg/kg
Oxolinic acid	0.01 mg/kg

3.4.2 Current Standards (US & Worldwide)

On a world scale Tasmania is still relatively new to salmonid aquaculture; commercial fish cage culture has been in operation in Europe and Norway for almost 50 years. In all salmonid producing countries there have been periods in development where outbreaks of disease have required significant use of medication including antibiotics and inter-annual usage levels fluctuate widely. In Canada and Norway there was substantial use of antibiotics in the late 1980's early 1990's associated with outbreaks of furunculosis (*Aeromonas salmonicida*). However, antibiotic usage reduced significantly with the implementation of targeted management strategies including development of vaccines, and specific improvements in feed and husbandry practices. Table 29 compares usages of antibiotics currently used in Tasmania with Norway and Scotland from 2003-5 and Chile in 2005, and highlights the Tasmanian usage until 2008. Clearly, usage in Tasmania from 2005-2007 has been significant, this is primarily as a result of the onset of two new diseases; RLO in 2005/6 and "summer gut syndrome" of unknown aetiology in 2007/8 (Table 30). Disease management strategies have been implemented to remediate these problems (Percival & Brown, pers comm.) and these appear to be having an effect, with the antibiotic usage levels in 2008 being markedly reduced relative to previous years (Table 30).

As a much younger industry the Tasmanian aquaculture industry is still to some extent in the developmental stage, and whilst where possible it has tried to learn from the lessons overseas with respect to anticipation and management of health issues, the location of the industry in the southern hemisphere means that it is subject to diseases for which there is no precedent in the Northern hemisphere. Consequently, treatment and management strategies in these cases are being pioneered in much the same way as was the case in Scotland/ Norway in the late 1980's with furunculosis.

It should also be noted that when comparing usages between different countries it is important to bear in mind that the total amount of antibiotics used will differ depending on the particular drug employed. In Tasmania the most commonly used antibiotic is oxytetracycline with a recommended dosage of between 75 -100 mg/kg. However, other antibiotics commonly employed overseas can be used at lower dosages, with trimethoprim, oxolinic acid and flumequine all administered at between 10 – 20 mg/kg) and as a result a lower overall mass of antibiotic will be used in the treatment of any given amout of stock. Consequently, the type of antibiotic should be considered when comparing total amounts of antibiotic administered.

Table 29 Annual salmonid production ('000 tonnes) and antibiotic usage (kg) for selected antibiotics in Norway (data from FAO) , Scotland (production data from FRS, antibiotic data from SEPA) and Canada (British Columbia) (Data from Data from BC Govt) (2003-5) and Tasmania (2003-8) (data from Tas DPIW). Data for Chile 2005 is only available as total antibiotic usage (Bravo, 2005 and FAO).

	Norway			Scotland			Chile	Tasmania					
	2003	2004	2005	2003	2004	2005	2005	2003	2004	2005	2006	2007	2008
Annual Salmonid Production ('000 Tonnes)	509	564	586	170	158	129	374	13.7	14.6	18.4	22.4	24.0	26.1
Amoxycillin												550	
Chlortetracycline										12	39		
Fenbendazole					7	1							
Florfenicol	154	111	202		6	10						2	1
Flumequine	60	4	28										
Oxolinic acid	546	1,035	977					253	142				
Oxytetracycline	45	9	8	670	38	1,654			790	845	4,453	8,665	3,831
Trimethoprim								32	64	21	44	78	165
Tylosin													10
Total Antibiotics Used (kg)	805	1,159	1,215	670	51	1,654	133,800	285	996	878	4,536	9,295	4,007
Antibiotics (kg)/ tonne production	0.0016	0.0021	0.0021	0.0039	0.0003	0.0128	0.3577	0.0207	0.0680	0.0477	0.2023	0.3873	0.1531

International Antibiotic Registration and Regulation

Norway is the world's largest producer of farmed salmon. Current antibiotic usages are relatively low (ca 1,000 kg) and only 6 antibiotics are currently registered for use in aquaculture in Norway (Table 29 and Table 30). However, in the early stages of development the industry encountered severe problems with bacterial diseases (vibriosis, cold water vibriosis, furunculosis etc.) and by 1987 antibiotic use had reached a peak with close to 50 tonnes being administered during the year for only 46 tonnes of production (i.e. > 1.0 kg /tonne). The primary antimicrobials used in Norway include oxytetracycline, florfenicol, flumequine, oxolinic acid, trimethoprim/sulfadiazine and furazolidone (Burridge et al., 2008). Only antibiotics which are not considered relevant for human medicine can be used in aquaculture, which excludes the use of quinolones and several other classes of antibiotic. The volume of use is closely monitored to 1) ensure no misuse through prophylaxis and 2) to rapidly identify any potentially epizootic infections. Improvements in epidemiology, pharmacology and therapeutic understanding in association with regulations on antibiotic use in aquaculture in Norway have led to a drastic reduction in the classes and volumes of antibiotics used (Grave et al., 1999, Sorum, 2006, Lillehaug et al., 2003, Markestad and Grave, 1997) such that current annual usage is less than 1 000 kg a year (Directorate of Fisheries, 2003 and FHL Havbruk, 2003, FAO, 2008) (Table 29).

Since 2001 countries within the European Union (EU) require an environmental risk assessment (ERA) to be undertaken for all new veterinary pharmaceuticals; this requires an ecotoxicity report which must be developed according to specific guidance protocols. The depth of information required in the ecotoxicity report may be at various levels (from basic characterisation of the medicine to an in-depth assessment of fate and ecotoxic effects) depending on the response to a tiered assessment process (Koschorreck et al., 2002) (Figure 5). The EU process requires that veterinary medicinal products (VMP) be characterised as Phase I or Phase II depending on usage. Phase I VMPs have limited use and limited environmental effects and would require only limited information on chemical properties, dosing, husbandry and routes of excretion. Figure 5 shows the tiered response for the proposed Phase I and Phase II assessment process. The last component in Phase I is determination of the predicted environmental concentration (PEC) based on worst case assumptions (i.e. highest number of animals treated, highest dose, no degradation) if more than 1ug/l of the VMP is released from aquaculture facilities then the risk assessment moves to Phase II, which would be the case for all antibiotic usage. In Phase II the PEC is compared with the lowest effective concentration from standard ecotoxicity tests to assess the probable environmental risk. The toxic potential of the substance (or its metabolites) is assessed and the resultant value is multiplied by a safety factor to create a predicted no effects concentration (PNEC). If the calculated ratio of PEC/PNEC is greater than 1 then more detailed studies must be undertaken to refine both the PEC and PNEC. Where a product PEC/PNEC ratio remains greater than 1 then a risk to the environment is assumed. However, the product may still be used provided authorised risk mitigation measures are employed (Koschorreck et al., 2002).

Salmonid aquaculture in Scotland has also faced significant health issues since its inception in the early 1980's and like Norway the Scottish industry had to utilise significant quantities of antibiotics to maintain fish health and combat problems with bacterial diseases. Usage levels peaked in 1991 with approximately 1200mg antibiotics administered per kg of fish produced (Aldermann and Hastings, 1998). As in Norway the refinement of disease management strategies and the introduction of vaccines have dramatically reduced antibiotic usage in Scotland, despite the increasing outputs.

In Scotland antibiotic usage on fish farms comes under the Scottish Environmental Protection Agency (SEPA) regulations as "intermittent discharges". The favoured approach for control of these medicines is to model dispersion over a 3 hour period, use this output to identify the allowable zone of effect (AZE) and then compare the calculated predicted environmental concentration (PEC) with the relevant environmental quality standard (EQS); unfortunately there are no available EQS for antibiotics.

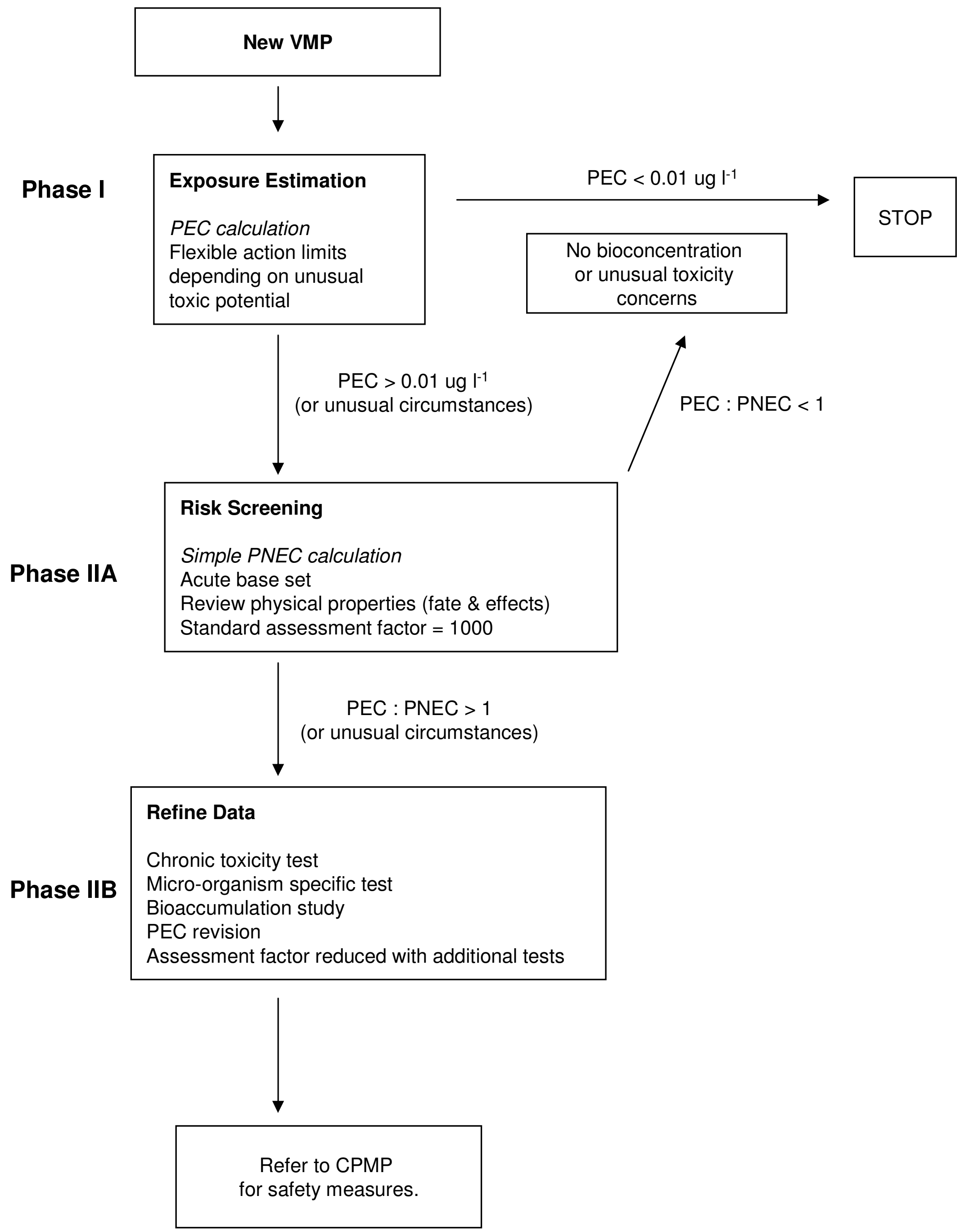


Figure 5 EU Environmental risk assessment process for veterinary medicinal products (VMPs) (adapted from Bound and Voulvoulis, 2004).

Aquaculture in the US includes a broad variety of species both marine and freshwater, accounting for approximately 22% of global aquaculture production (FAO, 2008). However, only 3 classes of antimicrobials are registered for use in finfish in the US; tetracycline, chloramphenicol and sulfonamides (Table 31). The environmental impact of pharmaceuticals in the US is controlled by the US Food and

Drug Administration (FDA). Since 1969 the FDA has required a basic assessment of all new drugs as part of the licensing process, however, the process was reviewed in 1995 and the latest environmental assessment requirements were published in 1998 (FDA, 1998). There are many similarities between the European and US approaches, both rely on a tiered assessment approach and both require evaluation of a threshold level for the PEC, which if exceeded triggers further investigation (FDA, 1998, EMEA, 2003).

Chile is the second largest producer of farmed salmon in the world. Statistics on imports of antibiotics to Chile suggest that large quantities of antibiotics are used in aquaculture but there are no requirements to report type or quantities administered (Cabello, 2003 in Buschmann et al., 2006). Approximately 134 tonnes of antibiotics were administered through salmon aquaculture in 2003 (Bravo, 2005). Although unsubstantiated, it is believed that up to 100 metric tonnes of quinolones and fluoroquinolones may be administered annually through salmon aquaculture (Burridge et al., 2008) with the suggestion that all antibiotic classes may be being used. Several studies have identified resistant bacteria in the areas surrounding salmon farms (Miranda and Castillo, 1998, Miranda and Zemelmen, 2001, 2002a, b, Miranda et al., 2003). In addition there are reports emerging of fish pathogens in Chile that are widely resistant to antibiotics, including *Vibrios* and *Streptococcus* (in Burridge et al., 2008). Several changes in the ecology surrounding the fish farms (i.e. red tides and epidemics of *Vibrio parahaemolyticus* in the summer months) have been attributed to salmon farming practices and although the actual cause is not clear, and may in fact be poor husbandry and increased nutrients, the result may be limitation of aquaculture development in Chile (Cabello, 2006).

The EU and the US drug assessment frameworks operate in very similar ways; both seek to address assessment of the environmental risks associated with release of pharmaceuticals to the environment. However, both systems have detractors who suggest that the evaluation processes are fundamentally flawed (Montforts and de Knecht, 2002, Bound and Voulvolis, 2004). The main issues being that the ecotoxicity tests on which the guidelines are based are too general, since many of the pharmaceuticals covered (including antibiotics) are specifically designed to be highly specialised in their effects, consequently the standard tests may underestimate the risks with particular therapeutics (Henschel et al., 1997). In addition the current protocols favour acute rather than chronic testing and it has been shown that in many cases longer term assays will show effects at much lower levels, similarly different life stages will show differing susceptibility (see examples in Bound and Voulvolis, 2004). A further problem is that the process only addresses the effects of the specified therapeutic in isolation and in reality organisms in the environment will be subject to many different chemicals where the effects may be additive, synergistic or even antagonistic. In addition the environmental degradation products of the drug may have quite different properties to that of the parent drug; in some cases they may be more / less toxic. Computer modelling may provide a solution to some of these issues, although currently there is no commercially available software to tackle all of these issues this area is advancing significantly and there are packages that could help predict the implications of certain mixtures (Altenburger et al., 2003, Sanderson et al., 2003, 2004). In addition there should be provision in the guidelines for the application of ecotox tests to target organisms and life stages which are likely to be susceptible to the specific mechanism of the given drug; including provision for situations where there are known combinations of chemical (Bound and Voulvolis, 2004). Bound and Voulvolis (2004) propose a risk assessment framework based on an amalgamation of the FDA and EMEA structures and incorporating more targeted ecological assessments (Figure 6).

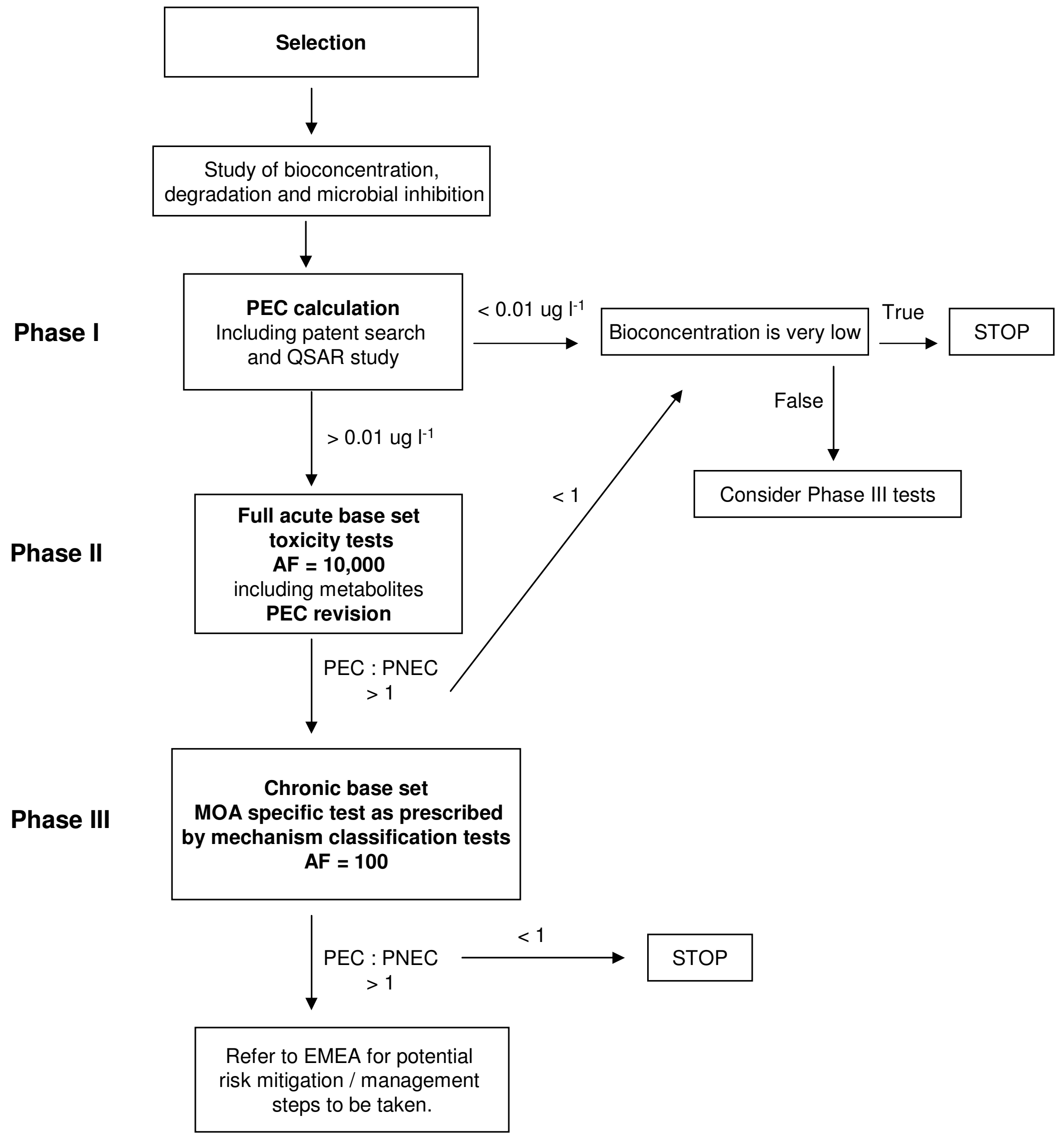


*Figure 6 Proposed revisions to FDA/ EMEA risk assessment process guidelines (Bound and Voulvolis, 2004_*

Table 30 Antibiotics currently used in salmonid aquaculture production in Norway, Canada, Chile, UK and US. (● – registered; □– minor use permit (APVMA)). NB - in Australia all of the listed antibiotics can be used under veterinary prescription.

Group	Antibiotic	Norway	Canada	Chile	UK	US	Australia
Tetracyclines							
	Oxytetracycline	●	●	●	●	●	□
	Chlortetracycline						
Quinolones							
	Oxolinic acid	●			●		
	Sarafloxacin				●		
	Flumequine	●		●			
	Furazolidone	●					
Chloramphenicols							
	Florfenicol	●	●	●	●	●	□
Sulphonamides							
	Ormetoprim-sulphadimethoxine		●			●	
	Trimethoprim-sulphadiazine					●	
	Trimethoprim	●	●		●		
β-lactams							
	Amoxycillin				●		
Macrolides							
	Tylosin						

The range of aquaculture activities in Asian countries is large and the level of sophistication of these operations is extremely varied, as a consequence the amount and variety of antibiotics used is considerably greater than in Europe or America (Akinbowale et al., 2006). There have been reports from Vietnam of 122 antibiotic products being used in shrimp culture, similarly in the Philippines and Sri Lanka use of a significant number of antimicrobial agents has been reported; with erythromycin and tetracycline amongst the most commonly used antibiotics (Alday et al., 2006). The aquaculture industry in Japan is also very large and diverse; consequently the list of drugs registered for aquaculture in Japan is extensive (Alderman & Hastings, 1998).

3.4.3 Accepted & Alternative Analytical Approaches

There are no standard methods for determining antibiotics in biota, sediments or water. However, there are methods which are more commonly used than others and which are accredited by the National Association of Testing Authorities (NATA), and these approaches would be preferred where available. . Instrumental approaches are generally to be preferred where specific, sensitive and quantitative information regarding concentrations is required. However, it is worth ensuring in each situation that i) that the analytical approach is clearly defined and ii) the analytical approach taken is appropriate for the question being asked and iii) the analytical approach is consistent with that of other/ similar studies (if the results are to be compared). It is also worth remembering that just because an analysis is more sensitive does not necessarily make it a better or more appropriate test.

In addition to the direct and standard approach of residue testing there are also a variety of approaches available to evaluate ecological levels such as passive samplers which provide information on time integrated water column and sediment levels. Other approaches can be employed to infer an ecological effect; ecotoxicological studies can provide information on specific tolerances or accumulation responses in microbes, algae, invertebrates, fish and other vertebrates whilst environmental forensics can be used retrospectively with the aim of identifying the cause and/or source of a particular chemical in the environment.

Biota samples (food)

The accepted testing method for most antibiotics is high-performance liquid chromatography (HPLC). Detection limits usually range from 5 to 10 ppb. The more modern method for determination of antibiotics in fish and meat is via liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) (Horie et al., 2003).

Public health agencies in many countries rely on detection by mass spectrometry for unambiguous identification of residues of antibiotic and antibacterial agents in animal food products for human consumption. Liquid chromatographic–mass spectrometric methods are the most common technique for analysing antibiotic and antibacterial agents in animal food products. The LC–MS system of analysis is relatively inexpensive and robust (Di Corcia and Nazzari, 2002).

Water samples

A common method is through enriching the samples using a universal freeze–drying procedure or a solid-phase extraction. Analysis is usually by liquid chromatography MS detection. Chromatography for different antibiotics requires different columns and eluents. Mean recovery rates are generally high but will vary depending on the antibiotic being tested (Hirsch et al., 1999).

3.4.4 Possible Approaches to Assess Environmental Effects

There are very specific protocols that should be adhered to in formally assessing the risk associated with the use of microbial agents. Principles and guidelines for the conduct of microbiological risk assessment are set out in guideline 30 of Codex Alimentarius Commission (FAO, 2008). Although this report focuses on the assessment of health risks it provides a useful reference to the general methodology of risk assessment for microbiological hazards. Including "Principles and Guidelines for the Conduct of Microbiological Risk Assessment"; these principles are significant in that they attempt to identify the basic concepts of risk analysis, such as transparency, and to describe components and steps of microbiological risk assessment.

With respect to the risk assessment of veterinary pharmaceutical medicines ecotoxicity studies are deemed to be an integral part of the process. Isidori et al. (2005) undertook a microbiological risk assessment of six commonly used antibiotics and determined that on the basis of the ratio of acute versus chronic effects the overall risk was acceptable (<1) for OTC, sulfamethoxazole and ofloxacin but that three macrolides (erythromycin, lincomycin and clarithromycin) were categorised as "most harmful to the environment" with ratios values of 1, 3.6 and 10 respectively. Most ecotoxicity studies rely on acute sensitivities however; chronic ecotoxicity test may be more valuable in assessing the real environmental risk. In both acute and chronic tests the number of species and endpoints should be carefully considered. In addition molecular biological approaches to determine genotypes should be examined in more detail, as in the end they may be the most sensitive. (Isidori et al, 2005). Molecular methods can provide clear evidence of genes encoding resistance to microbial agents associated with aquaculture operations (DePaola et al., 1988, Sandaa et al., 1992, Spangaard et al., 1993, De Paola and Roberts, 1995, Rhodes et al., 2000, Miranda et al., 2003). However, additional methods will be necessary to determine causal linkages if that is considered necessary.

The EU and the US both have instigated frameworks for the assessment of environmental impacts of all new veterinary pharmaceutical medicines (VPMs) and anticipate this process being introduced for existing VPMs in the near future. It is generally recognised that some form of environmental risk assessment (ERA) is required and that this should include assessment of persistence, resistance and ecotoxicity. However, it is important when doing this to take account of any difference between the total loading and that which is bioavailable.

Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) is calculated using the following equation:

$$PEC_{\text{surface water}} = \frac{\text{DOSEai x Fpen}}{\text{WASTE x DILUTION x 100}}$$

Where:

DOSE ai = maximum daily dose of active ingredient applied per treatment / day

Fpen = penetration of the drug (%)

WASTE = wastage per treatment per day

DILUTION = environmental dilution factor

Biodegradability:

- The closed bottle test (CBT) is recommended as a first, simple test of biodegradability (Nyholm, 1991, Kummerer et al., 2000, OECD (301 D), 1996). Standard test = 28 day and includes 4 test series (quality control sample, test blank, test compound and toxicity control) in a standard mineral salt solution with a fixed inoculation of between 1000-10 000 colony forming units (CFUs) per ml.

Genotoxicity

- SOS chromotest (Quillardet and Hofnung, 1985) modified (Mersch-Sundermann et al., 1993)

Aquatic Bacteria

- A growth inhibition test - international method (ISO, 1995):
- Toxicity control test used in CBT
- Colony forming units (CFU's) monitored

Aquatic Fauna

- Chronic assays are more appropriate than acute ones to detect impact of pharmaceuticals (Isidori et al, 2005).
- Standard Acute and Chronic Ecotox tests (eg. *Daphnia magna* acute test (ISO, 1989b), *Daphnia magna* reproduction test (OECD, 1996))

Bacterial Resistance

- Phenotypic evaluation – culture and identification of bacterial colonies (issues with selectivity of culture media)
- Susceptibility testing - culture of bacterial colonies and subsequent susceptibility testing for selected antimicrobial agent (issues with selection/ standardisation of susceptibility test protocols and interpretative criteria for defining resistance – some standard protocols for bacteria associated with human/ land-based animal diseases) NB must account for natural/ background levels of resistance.
- Serrano (2005) provides a description of several accepted susceptibility test protocols.
- Resistance frequencies can be determined either by examining bacterial phenotypes or by using molecular techniques to determine genotype (Alday et al., 2006).

3.5 Current Monitoring

3.5.1 Current Licensing Requirements

Off-Label Antibiotic Usage:

APVMA permits for minor use and supply of an unregistered veterinary chemical product apply to the following antibiotics. Currently there are 2 permits applicable to the Tasmanian salmonid industry:

1. Permit No. – PER9644 / Schering Plough Pty Ltd (valid 21/12/07-30/06/09)
Aquaflor (Florfenicol) 50% medicated premix containing: 500mg/g Florfenicol as active constituent

2. Permit No. – PER9665 / Controlled Medications Pty Ltd (valid 9/9/07-30/11/10)
(TETRAFISH) containing: 98% Oxytetracycline hydrochloride as active constituent

These permits have specific conditions associated with them, many are generic but some are product specific. The main conditions are paraphrased below:

Critical Use Comments:

- Administer Tetrafish that is formulated/mixed into feed
- Repeat treatment may occur after minimum interval of 1600 degree days
- Implement mechanisms to prevent overfeeding of OTC-medicated feed and non-medicated feed.
- All treatments must be recorded & prescribed by veterinary authority
- All treatments must be notified to CVO (Chief Veterinary Officer) (Tas.) & Principle Marine Environment Officer (DPIW).
- Manager, Marine Farms (DPIW) must be notified of any escapes
- Standard withholding period – 1000 degree days prior to slaughter, this to be increased if fish are re-treated at an interval shorter than 1600 degree days
  Fish for export – withholding period = 1600 degree days after last treatment

Additional Conditions:

Require that the product must be used as directed, prescribed by a veterinarian on the basis of a confirmed diagnosis of a bacterial infection. Treated fish must not be released until residue levels are within acceptable levels. For reissue of permits a written report must be supplied detailing residue testing and information on species treated, aquaculture systems/ husbandry used, water temperature, repeat treatments and residue results.

Environmental monitoring requirements identify that seawater within lease area and 35m outside lease is monitored for OTC concentration. *(However, this is no longer officially required as initial testing showed no detectable residue)*. Sediments below treated pens, outside of the cages and outside of the lease areas are to be tested for both Florfenicol and OTC residues/ persistence. Sediments below cages should be analysed for OTC before next application to determine whether residues have dissipated/degraded.

Environmental monitoring of antibiotics requires that testing be undertaken during the warmer months of the year (November to April). Permits require sediment samples be collected by cores (up to 75mm depth) and that minimum inhibitory concentrations (MIC) be calculated for field isolates of fish pathogens, which have been tested for bacterial susceptibility, except *Rickettsia* as this has yet to be cultured successfully *in vitro*.

Method:

Florfenicol:

Sediment samples should be collected - before treatment, after treatment and 5, 10 and 20 days post-treatment from two representative high flow/ high volume usage sites nominated for detailed monitoring. Triplicate samples should be collected from below a representative number of cages treated during an event on each lease. Duplicate samples should be processed with the third sample retained. Pre-treatment sampling should be undertaken annually in November from more than 2 cage positions on each site nominated for detailed monitoring. Pre-treatment sampling may be spread across more than two lease areas. Sediment sample collection, storage and analysis methodology is outlined in the state licence conditions, whilst MIC should be ascertained using current Clinical Laboratory Standards (CLSI) and control procedures.

OTC:

Sediment samples should be collected - before treatment, after treatment and 7, 14, 28 and 56 days post-treatment at two representative low flow/ high volume usage sites nominated for detailed monitoring. Triplicate samples should be collected from below cages, the 35 m zone associated with a representative number of cages treated during an event on each lease. Outside the lease area triplicate samples should be collected 100m and 500m from the lease boundary at right angles to the lease boundary in the direction of the dominant current flow with the number of sites being the same as the representative number of cages. In all cases duplicate samples should be processed with the third sample retained. Pre-treatment sampling should be undertaken annually in November from more than 2 cage positions on each site nominated for detailed monitoring. Pre-treatment sampling may be spread across more than two lease areas.

Water column samples should be collected from 1 or 2 high OTC usage sites (>500kg) on days 1, 5, 10 during treatment and for a number of days post-treatment until residues no longer detectable at periods of low tidal flow/ currents with follow-up sampling at higher low tidal flow/ currents if necessary. Samples should be collected from 1 m depth at a location where highest levels may be expected and at a position 35m outside the lease.

Sediment sample collection, storage and analysis methodology is outlined in the state licence conditions, whilst MIC should be ascertained using current Clinical Laboratory Standards (CLSI) and control procedures. Water samples should be collected as per protocols agreed to and endorsed by DEWHA.

Specified changes to sampling frequency are required under the following conditions:

OTC	**Regime Change**
>1000 kg/ treatment	Sampling increased x 2
> 500kg/ treatment	Protocol as described
Single treatments up to 100kg	Monitor once at beginning of following season Re-sampling required if up to 50kg used in re-treatment
Florfenicol	
>10kg/ treatment	Sampling increased x 2
>5kg/ treatment	Protocol as described
Single treatments up to 10kg	Monitor once at beginning of following season Re-sampling required if up to 5kg used in re-treatment

Facilities must monitor OTC concentration in seawater within lease area and 35m outside lease in accordance with frame work in attachment 2 & in accordance with state regulations and licence conditions.

In addition the Tasmania Marine Farming Licences have specific conditions relating to environmental management of a finfish farm (Schedule 3 to Marine Framing licence – Appendix 1). Within these licences is a section dealing specifically with residue monitoring which requires that where antibiotics are used in excess of a specified level for the particular lease area residue levels in both sediments and wildfish should be surveyed and a report of the findings provided to DPIW (Marine Environment Section) (see sections 1.12, 1.13 and 3.1). General data requirements are desribed in all licences, however, specific requiremnents may be included on individual licences and requirements may be varied at any time according to the type and quantity of antibiotics prescribed.

3.5.2 Review of Current Monitoring Results

The information in this section represents a summary of industry reports to DPIW, data from the Fish Health Surveillance Programme, DPIW wildfish monitoring results and data collected in compliance with the APVMA permit conditions.

Antibiotic therapy is one important component in a disease management strategy. With the onset of any new disease, mortality is frequently high initially and antibiotics are an essential treatment option to ensure continued health and wellbeing of the remaining fish stocks. Once the aetiology is understood then a plan can be implemented for ongoing management; this may include development of vaccines or alternative therapeutants and the need for antibiotic therapy should be reduced. The most commonly used antibiotic in Tasmania is oxytetracycline which accounts for more than 70% of total antibiotic used in recent years (Table 29, Table 31). There have been several instances in the last few years where disease outbreaks have required administration of large amounts of antibiotics (Table 31). In the summer of 2006 the primary use of antibiotics was in response to Rikettsia-like Organism (RLO), with some additional requirement for treatment of *Vibrio* infections (Table 31). In 2007/8 antibiotics were needed for the treatment of “summer gut syndrome". Although opinion on terminology varies, it is not uncommon for this condition to also be referred to as gastroenteritis (P.Jungalwalla (TSGA), pers.comm.). "Summer gut syndrome" is not unique to Tasmania; it has also been observed in Chile and is believed to be a form of gut flora disturbance. In Chile it is mature fish in their 2nd summer at sea that are most affected, whilst in Tasmania it affects relatively small fish during their first year at sea, and as the name suggests becomes apparent when temperatures begin to increase in summer (A. Brown, pers. comm.). The industry is actively seeking a solution for "summer gut syndrome". Antibiotic treatment is reported to relieve symptoms of the condition at some sites in Tasmania, but the reason for the observed benefits of treatment is unclear. However, so far it has not been possible to identify specific causative agents/ pathogens and consequently the disease management is still in the early stages with current efforts focussing on controlling gut health through management of feed and stocking densities in relation to temperature. If a specific pathogen is identified it will be possible to look at more targeted treatment plans, including vaccine development, but even under the best case scenario the timeline for production of a vaccine on a commercial scale would be at least 5-10 years. Continued use of antibiotics is likely to occur until such time as a more sustainable approach can be developed to manage the syndrome.

Until relatively recently oxolinic acid was also regularly used as an antimicrobial agent in the Tasmanian salmonid farming industry, particularly in freshwater operations. However, at an industry fish health meeting in 2004 concerns were raised over the use of oxolinic acid; although it is an extremely effective antibiotic, its use in food producing animals has been linked with cross-resistance to the closely related group fluoroquinolones which is used in human medicine. Consequently it was recommended that use of oxolinic acid cease and any remaining stocks be retained for "emergency use" only. The industry also committed to a voluntary moratorium on the use of amoxicillin in 2007 (G. Woods, pers. comm.).

Table 31 Annual antibiotic usage (kg) for a) 2006, b) 2007 and c) 2008 according to targeted disease (Data courtesy of DPIW).

a) 2006

	Targeted Disease							
Antibiotic	RLO	Gastroenteritis	Flavobacterium	Yersiniosis	Vibriosis	Skin/fin infection	Unidentified	Sub-total
Oxytetracycline	3,950		45	13	395	50		4,453
Chlortetracycline				39				39
Trimethoprim				40		4		44
Amoxycillin								0
Tylosin								0
Florfenicol								0
Total	3,950	0	45	92	395	54		4,536

b) 2007 **Targeted Disease**

Antibiotic	RLO	Gastroenteritis	Flavobacterium	Yersiniosis	Vibriosis	Flexibacter	Unidentified/ Other	Sub-total
Oxytetracycline	5,072	1,400		9	2,000		184	8,665
Chlortetracycline								0
Trimethoprim			22			56		78
Amoxycillin	140	410						550
Tylosin								0
Florfenicol				2				2
Total	5,212	1,810	22	11	2,000	56	184	9,295

c) 2008 **Targeted Disease**

Antibiotic	RLO	Gastroenteritis	Flavobacterium	Yersiniosis	Vibriosis	Flexibacter	Other	Sub-total
Oxytetracycline	438	2,535		32	800	7	20	3,832
Chlortetracycline								0
Trimethoprim		112		8		24	20	164
Amoxycillin								0
Tylosin		8					2	10
Florfenicol				1				1
Total	438	2,655	0	41	800	31	42	4,007

By far the greatest proportion (> 70%) of all the antibiotics administered are dispensed into the strongly connected Huon/ Channel systems; 3.5 tonnes were used in this area in 2006 (Table 32). There has been no requirement for antibiotics in the Tamar and the requirement in the Tasman Peninsula/ Norfolk Bay was markedly lower than in the other regions. Much of this difference between areas would appear to be associated with the differences in the overall biomass of fish stocked (a significant proportion of the industry production is situated in the Huon/ Channel region), although there are differences in the epidemiology between the regions. Of particular note however is the significant reduction in antibiotic requirement in Macquarie Harbour between 2007 and 2008 (Table 32), this would appear to be as a direct result of the introduction of a vaccine for *Vibrio*/ *Aeromonas* at this time, and clearly indicates the benefit of vaccines.

Table 32 Regional distribution of antibiotic usage (kg) for calendar years a) 2006, b) 2007 and c) 2008 (Data courtesy of DPIW).

a) 2006

Antibiotic	Region: Channel	Huon	Macquarie Harbour	Tamar	Tasman/ Norfolk Bay	Sub-total
Chlortetracycline		19			20	39
Oxytetracycline	2,404	1,104	395		550	4,453
Trimethoprim	40	4				44
Amoxycillin						0
Tylosin						0
Florfenicol						0
Total	2,444 (54%)	1,127 (25%)	395 (9%)	0 (0%)	570 (12%)	4,536

b) 2007

Antibiotic	Region: Channel	Huon	Macquarie Harbour	Tamar	Tasman/ Norfolk Bay	Sub-total
Chlortetracycline						0
Oxytetracycline	4,050	1,856	2,184		575	8,665
Trimethoprim	23	55				78
Amoxycillin	445	90			15	550
Tylosin						0
Florfenicol		2				2
Total	4,518 (49%)	2,003 (21%)	2,184 (23%)	0 (0%)	590 (6%)	9,295

c) 2008

Antibiotic	Region: Channel	Huon	Macquarie Harbour	Tamar	Tasman/ Norfolk Bay	Sub-total
Chlortetracycline						0
Oxytetracycline	1,700	1,956			175	3,831
Trimethoprim	161	2			2	165
Amoxycillin						0
Tylosin		10				10
Florfenicol		1				1
Total	1,861 (46%)	1,969 (49%)	0 (0%)	0 (0%)	177 (4%)	4,007

As described above there is a strong seasonal component to the use of antibiotics, with the greatest requirement in the summer months when the water temperatures are highest, fish growth rates are greatest and pathogens tend to be most virulent (Table 33). In addition antibiotic usage would appear to be strongly related to the incidence of new disease outbreaks, with levels reducing as the pathogens become understood and a disease management strategy is developed. In this context it is worth noting the marked reduction in antibiotic requirement in 2008 as compared with 2007.

Table 33 Total antibiotic usage (kg) (seasonal distribution) from for 2006 - 2008 (Data courtesy of DPIW).

	Month												
Year	J	F	M	A	M	J	J	A	S	O	N	D	Total
2006	395	2,950	125	915	7	0	12	0	23	14	0	95	4,536
2007	2,260	4,348	825	427	25	0	0	0	0	9	0	1,402	9,295
2008	2,212	905	0	29	0	9	26	2	3	0	0	821	4,007

Minimum inhibitory concentration (MIC) information of all prescribed antibiotics for the specific pathogens is undertaken, at the request of a prescribing veterinarian, by the Fish Health Unit (FHU) of the Department of Primary Industries and Water (DPIW).

Sediment Residue Testing

To date residue testing for oxytetracycline in compliance with APVMA permit conditions has been carried out at 2 sites and a report prepared by the TSGA. This section largely summarises and paraphrases the information in that report.

Water sampling to date has returned results below the detection limit of the analyses (i.e. < 2 µg l^{-1}). Consequently the likelihood of effects in the water column is negligible and as a result the APVMA has not required any further testing.

Sediment sampling at the 2 study sites indicated that the overall level of OTC residues beneath the cages was on the whole very low (Figure 7, Figure 8). The maximum residue reported, at any time post- treatment, was 180 µg/kg, with mean levels generally being less than 50 µg/kg. These levels are very low compared to many studies from salmon farms overseas (eg. 16,000 µg/kg and 300 µg/kg (Björklund et al., 1990), 285,000 µg/kg (Samuelsen et al., 1992); 10,900 µg/kg (Coyne et al., 1994)). In addition the amount of OTC used at each of the 2 study sites was significantly higher than levels reported for the overseas studies (662.5 kg at site 1 and 375 kg at site 2 respectively). Some of this difference may be as a result of the depth of sediment sampled. Many of the overseas data relate to samples collected from the top 2 - 3 cm, whilst the samples collected for the current study were generally to a depth of 7.5 cm, although this depth was not achievable in all cases due to the hard nature of the seabed. Capone et al., (1996) reported OTC residues can occur as deep as 10 cm in the sediment but this is usually associated with longer term usage, OTC treatments at these sites are a fairly recent occurrence. Consequently while the additional sample depth may have influenced the overall concentration of OTC in the sample and therefore the comparability of results between different sites and studies, even if we assume a worse case "dilution" affect of 2-3 fold the Tasmanian results would still be considerably lower than those of the overseas studies. This is likely to be as a result of a general improvement in aquaculture management and operations compared with the earlier overseas studies; better feeding technology and advances in feed formulation have reduced feed wastage.

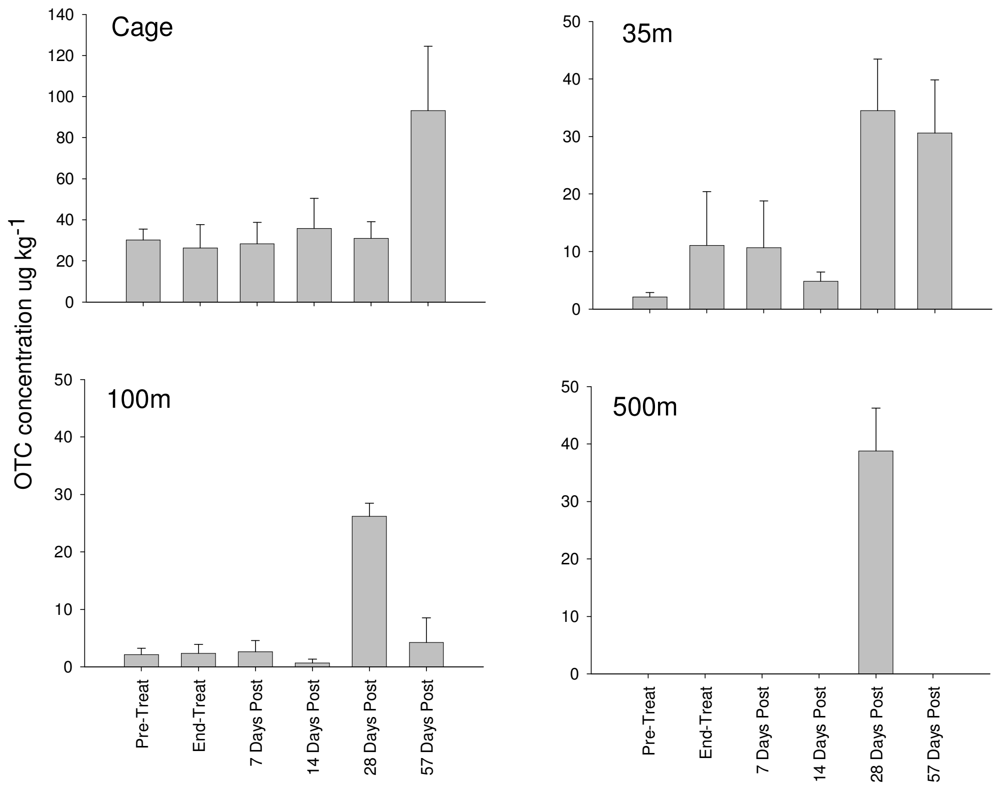


Figure 7 Average oxytetracycline concentration (µg kg^{-1}) and s.e. in sediments from Site 1 directly under 2 cages, and at 35m, 100m and 500m distance before treatment, immediately post-treatment and at 7, 14, 28 and 57 days post-treatment. (Note the difference in the concentration scale associated with the cage sediment samples).

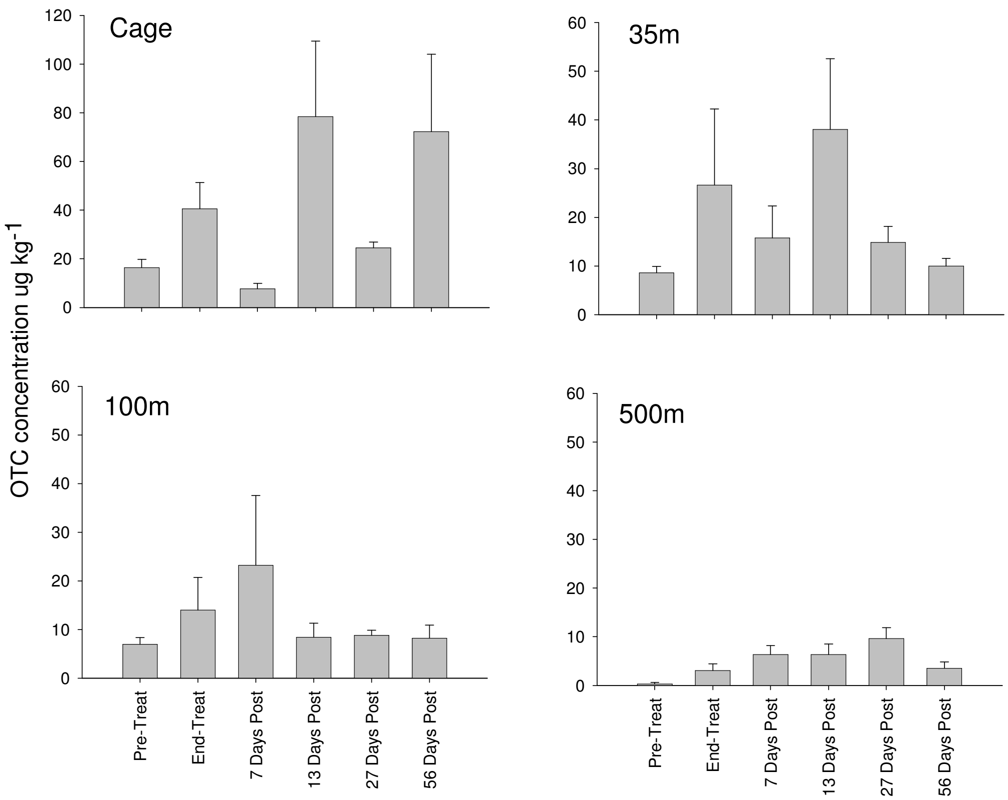


Figure 8 Average oxytetracycline concentration (µg kg^{-1}) and s.e. in sediments from Site 2 directly under 2 cages, and at 35m, 100m and 500m distance before treatment, immediately post-treatment and at 7, 14, 28 and 57 days post-treatment (Note the difference in the concentration scale associated with the cage sediment samples).

Coyne et al. (1997) hypothesized that the mean level of OTC that could be expected in the sediment post-treatment should equate to approx. 790-1100 µg/kg for every kg of OTC administered. At site 1, OTC was administered at 41.4 kg/cage, and at site 2 at approximately half this level (22.1 kg/cage), on this basis the expected mean level of OTC in the sediments under the treated cages would be 32,000–45,210 µg/kg (Site 1) and 17,459-24,310 µg/kg (Site 2) respectively. The actual levels being several orders of magnitude less than this, that is not say that the current levels have no effect but that the effect is unlikely to be as severe as the quantities administered might imply. Subjective assessment of farm recovery response (eg. video surveys and site condition after standard fallowing periods) suggests that thus far the normal recovery processes do not seem to be markedly changed.

At both sites OTC was present in sediments pre-treatment (Figure 7, Figure 8), whether this is as a result of previous treatments (approximately 12 months earlier), natural production or accumulation from other anthropogenic sources is currently not known. However, it would seem most likely that it is associated with farm production as the samples at 500m contained markedly less antibiotic pre-treatment than samples collected beneath the cages.

In addition to the residue levels in the 2008 Tasmanian survey being low, the distribution of OTC in the sediments was more widespread than has been reported elsewhere. OTC residues were detected up to 500M from the lease boundary, albeit at low levels (53 µg/kg at site 1 and 14 µg/kg at site 2). It has been suggested that the dispersion of OTC within the sediments will follow a similar pattern to that of organic enrichment and in general impacts will be highly localised. A study by Coyne et al. (1994) suggested OTC dispersion would be restricted to within 100 m of the treated cages. However, the site examined by Coyne et al. (1994) was relatively shallow (10-20m) with low-moderate current flow, in contrast the current study sites were situated in relatively deep waters (site1 = 30-40 m and the site 2 = 16-22 m) with good surface current flows. Consequently, it might be inferred that the hydrodynamic conditions associated with the Tasmanian sites have resulted in greater dispersion of the antibiotic; producing lower levels albeit over a broader area.

Changes in the concentration over time show some response patterns that are difficult to interpret. At both sites there were unexpected increases in OTC levels post-treatment (Figure 7, Figure 8). At site 1 the levels more than double at all distances from the cage at varying times post treatment, whilst at site 2 there are marked drops in concentration at the cage and 35m position which then increase once again. This spatial variability in the data may reflect patchiness in the dispersion of residues or in the environment. If this is the case then it may suggest that the sample size is insufficient to adequately represent the dispersion patterns and that additional sampling should be undertaken to ascertain the appropriate number of replicates required to adequately reflect the sediment conditions or alternatively perhaps that OTC concentration varies in response to some other environmental variables and that further examination of the degradation pathways is needed.

Wildfish Testing

The requirement to perform sampling for residues in wildfish in Tasmania following a medication event is dependent on the specific therapeutant and available information. However, the findings of the FSANZ risk assessment (see section 3.3.4), which was based on residue levels observed in sampled fish in 2006, indicated that there was no human health risk associated with wild fish residues and consequently there is now no requirement for sampling of OTC residue levels in wild fish.

Studies of fish and shellfish bioaccumulation have shown that flesh residue levels diminish very rapidly (Björklund et al., 1990, Coyne et al., 1997). However, it may be useful to undertake targeted sampling to further evaluate depletion rates in wild fish and to assess the likelihood of bioaccumulation.

3.5.3 Relevance of Current Monitoring Program

Of all of the topics of concern in relation to the environmental impacts of antibiotics outlined above the current antibiotics monitoring program only really addresses the issue of environmental persistence. Although the data collected thus far suggest that residue levels in the sediments are relatively low, there are problems with the spatial resolution that need to be addressed. It is important to distinguish between total antibiotic loading in the environment and that which is biologically available and therefore could have an ongoing effect. The data collected thus far are ambiguous regarding whether OTC may be accumulating in the sediments, it would be useful to clarify whether this is indeed the case.

Thus far the current benthic monitoring program, established to evaluate benthic impacts and recovery, does not indicate any significant change/ impact on recovery processes. Although this monitoring program was not specifically designed to evaluate the effects of antibiotics, it should indicate if changes in the temporal scale of recovery regardless of the cause and it may be useful to examine the data from this perspective. However, if the program is to be used to monitor broader ecological impacts it would be advisable to review the program design.

Development of resistance to antibiotics is a major concern from many perspectives, not least that of human health, but so far is not specifically covered by the current monitoring protocols, although it is a condition of the APVMA florfenicol permit. The problem of resistance has been recognised for a long time but the issue still remain unresolved, this is probably because the research questions have not been appropriately formulated (Alday et al., 2006). It is important that in any monitoring of resistance that the research question is adequately framed and relevant. A primary focus should be to establish whether any resistance is permanent or transient, since in many cases development of resistance is only associated with ongoing antibiotic usage and diminishes rapidly when usage ceases (Austin, 1985, Hoie et al., 1992).

Toxicity of antibiotic residues and degradation products is intrinsically linked to the bioavailability of the compounds and consequently an increased understanding of this area would be extremely useful. Most of the ecotoxicity testing cited in the literature relates to acute toxicity but since many antibiotics are persistent, identification of chronic effects (i.e. life history and trophic effects) may be necessary to adequately assess risk. In addition an understanding of the toxicity to locally relevant species, those which reflect the main ecological function in the environment would be an important pre-requisite for developing an understanding of ecosystem effects. However, as with resistance it is important that any research is appropriately targeted; that the questions are relevant to the industry impact and/ or the development of a suitable monitoring strategy.

Modelling may be one way in which research efforts could be suitably targeted. Development of a mass-balance model identifying the proportional distribution of antibiotic residues in the environment could be a very useful tool in directing research efforts to the areas where there is likely to be either the most impact or the greatest need for information. With this research understanding these pathways and responses could be used to develop a predictive model to support monitoring and assist in assessing environmental impacts into the future.

Although international standards for monitoring and assessment of antibiotics vary it is generally recognised that an improvement in current requirements is needed and that an integrated risk based assessment framework should be part of the management strategy (Cwiertniewicz, 1994, Halling-Sorensen et al., 1998, Bound and Voulvoulis, 2004).

3.6 Antibiotic Conclusions

In recent years there has been a global decline in the use of antibiotics in aquaculture; however, antibiotics will always be amongst the last line of defence in the management of disease. In most cases the use of antibiotics is absolutely necessary to ensure the health and welfare of the fish stocks. When properly administered problems associated with antibiotics should be minimal.

The main environmental concerns associated with antibiotic usage relate to the effects on non-target organisms, environmental persistence and development of resistance. In Tasmania antibiotics are generally administered in feed and as such the main concerns are associated with the presence of waste feed and fish excretory material in the sediments and water column. Antibiotic wastage in feed can be minimised by monitoring and managing the feeding response; in Tasmania the aquaculture industry uses innovative camera and computerised technology to monitor feed input and consequently limit feed wastage. It is also extremely important when administering antibiotics to ensure that the medication regime is correct (i.e. dosage and duration), as overdosing may result in environmental accumulation and underdosing may increase the likelihood of resistance. Understanding the causative agent is critical in developing an effective disease management strategy. Unfortunately in new disease outbreaks the initial requirement is generally to manage losses, which may result in high levels of antibiotic usage. Consequently it is really important to isolate the pathogen in order to establish its antimicrobial sensitivities and to ensure that narrow spectrum antibiotics are used subsequently.

So far the farm-based assessments of benthic impact have not shown any evidence of major changes in the benthic community responses associated with fish farm impact and recovery. However, it should be noted that these farm assessments were not designed to evaluate the effects of antibiotics and there still may be chronic effects on non-target organisms which have not been observed. To evaluate whether there is indeed no significant effect it might be prudent to target species with relevant antibiotic sensitivities as well as species indicative of key ecological functions. Any impacts on non-target species will be tied to the bioavailability of the particular therapeutic. Consequently it may be appropriate to obtain some measure of the bioavailability of the antibiotic of concern, prior to establishing any biotic sampling regime. On the other hand sampling the biota to establish whether there is an effect would in itself indicate bioavailability. For many antibacterials used in aquaculture there is data of effects in the terrestrial context, it would be advisable to review this information with respect to targeting the most appropriate ecological measures and sampling regime.

The potential for development of resistance is probably the most significant environmental concern with respect to antibiotic usage. Although there is theoretically a possibility of human health effects and this is a real concern, the information available so far suggests that this is highly unlikely. However, it is important that some monitoring of the incidence of resistance is undertaken when antibiotics are used. Development of resistance in fish bacteria and the potential for multiple-resistance pathogens is a much more realistic potential problem, and one which is a particular concern for the aquaculture industry itself as this would limit their disease response options. So far sensitivity testing undertaken by the industry does not suggest that this is a concern with current OTC usage. However, continued monitoring and use of best practice veterinary approaches (i.e. appropriate antibiotics and suitable dosage regimes) should address this concern. In addition some measure of the level of resistance in the local environment would be useful; this might include an evaluation of resistance in the farmed fish and local fish populations as well as in the sediment and water column bacteria. Testing for OTC resistance on farmed fish pathogens over the last 5 years has to date showed no evidence of resistance. Where any samples are found to exhibit resistance, then the nature of this should be further examined, i.e. to determine to what extent the resistance persists.

Low levels of antibiotic residues and persistence in the sediments are not in themselves a problem, but may indicate problems in management practices and an increased potential for toxicity affects. Additionally changes in environmental conditions may affect the ecotoxicity and impact of antibiotics, particularly oxic status of the environment, temperature, pH and salinity. Whilst there is considerable information on many antibiotics, including OTC, from overseas, these data can only be indicative and it is important to validate these findings for local conditions.

A further concern with antibiotic usage is that accumulation in the sediments may affect the natural sediment processes i.e. nutrient assimilation and breakdown of organic matter. Whilst there is no evidence so far from the benthic monitoring of any major change in sediment response (degradation/ recovery) times, this evaluation is largely qualitative and it would be prudent to confirm this, and to determine threshold effect levels. This could be done in mesocosm experiments and/ or with field validation. The Tasmanian industry has undertaken some monitoring to evaluate sediment loading which suggests that firstly the levels in the environment are several orders of magnitude lower that the therapeutic concentrations suggesting that most of the drug is going to the fish as intended. Secondly, the effect is highly localised but there were several inconsistencies in the data that could not be readily explained; i.e. levels are relatively high in some areas but not as high as might be expected on the basis of overseas experience considering the input loads and it appeared that in some instance the data may be confounded with residual loadings (i.e. a legacy of previous treatments). Consequently it would be useful to undertake further targeted assessments, perhaps in conjunction with measurement of biotic loading and resistance.

Although the literature does provide some evidence of effects on algae, local sampling would suggest that any increase in water column concentrations would be highly localised. As a consequence any algal accumulation effects are most likely to be evident in the net fouling communities, and it might be worth exploring the potential to use these communities as bioindicators. With respect to bioaccumulation effects in higher trophic levels, this has not been specifically tested but the testing of wild fish with respect to human health toxicity showed that at present environmental loadings there was no risk to human health.

The local industry usage, although on occasions relatively high, would appear to be in direct response to perceived specific health issues and disease management and as such is necessary to ensure the health of the stock. Increased antibiotic usage in response to new disease outbreaks has been observed on many previous occasions overseas, with requirement moderating as the pathogens become understood. The most important way in which to reduce/ manage the overall antibiotic usage would be to facilitate diagnosis of the pathogen and to support development of targeted disease management strategies and alternative therapies and in particular the development and deployment of vaccines. This is already occurring; Macquarie Harbour clearly demonstrates the benefits of vaccine implementation and recent antibiotic usage would appear to be reduced as a direct result of improved husbandry practices.

In relation to the regulatory framework for management of antibiotics in aquaculture, the general tendency overseas appears to be towards environmental risk assessment (ERA) frameworks. In both the EU and US these ERA frameworks are built around a tiered assessment process, with regulatory requirements linked to perceived scales of usage and impact. There do seem to be some general consistencies in approach within these frameworks; in particular a tendency to prefer chronic as compared to acute effects testing, the identification of tests targeted to the particular chemical of concern and the use of modelling to identify the most appropriate scenarios for testing.

Modelling could also have some very useful application in managing other aspects of the environmental concerns associated with antibiotics. Models could be used to determine the environmental mass-balance of antibiotics and to target sampling to the most appropriate areas. In addition modelling could provide a framework to view and manage the assessment data in the most integrated way and to develop an understanding of the effects based on multiple lines of evidence (Simpson et al., 2005).

4 Knowledge Gaps & Potential Research/ Monitoring Areas

This table has been developed with a view to promoting discussion on research priorities and should not be viewed specifically as a "to do list". Although information to progress our understanding in any of the areas would be of value, it is clearly not possible to resource everything and the next phase of this study is to bring together the relevant stakeholders and experts to prioritise the research needs, outline specific objectives and identify possible resource options.

4.1. Antifouling research

The following tables summarise the knowledge gaps relating to the environmental fate and impacts of antifoulants (Table 34) and antibiotics (Table 35) used in aquaculture and identifies possible approaches to address these.
Antifoulants:

With respect to the environmental impacts of antifoulants in aquaculture there are 3 key issues:

- Form and fate
- Sub-lethal effects on individual species
- Broader effects on benthic communities

Table 34 Summary of knowledge gaps with respect to determining the fate and impact of copper and zinc on the benthic and pelagic communities in the vicinity of fish farms and identification of possible approaches to address these issues. This table also includes sections identifying whether the defined topic will provide purely research information or whether that information will directly support monitoring or management solutions. In addition the table identifies whether each research activity requires a single one-off study or ongoing investigation.

Question	Possible approach	Comments	Research	Single/ Ongoing
Environmental fate				
1.What are the mechanisms for fluctuations in sediment copper levels? *Are reductions in measured levels due to:* High spatial variability? Recovery processes resulting in sediments being reworked and buried or redistributed within the sediments? Dispersal or removal of metals from the sediments by oxidative release, sediment transport or other mechanisms? Trophic transfer and accumulation processes?	Increase replication and/or compositing of sediment samples to increase confidence in trends in spatial and temporal variability. Use long cores to determine distribution patterns at moderate scale of resolution (e.g. 2 cm slices to 20 cm) to examine impact depth/ true background. Use benthic chambers to assess metal release rates during stages of sediment recovery. Incorporate trophic transfer data being generated in the Derwent estuary. Couple benthic and metal monitoring to determine scale of sediment reworking	Establish the important processes for regulating metal concentrations in sediments Establish if the reductions are real (not an artefact of low replication) and if so, what are is the fate of metal lost from sediments Establish the depth of sediment impacted by elevated metal levels Establish how recovery processes affect metal flux	M R-M R/R-M R R-M	O
2. What proportion of metals measured in sediments is due to paint flakes and what is due to soluble metals bound to sediments?	Estimate quantity of paint particles deposited through sieving and microscopic examination and analysis of metals in each size fraction Estimate leach rates for a range of paint particle sizes, through additions to sediment.	Establish how the metals are delivered to the sediments, and review industry practice if operational conditions appear to be contributing excessively e.g. losses from in-situ cleaning, net handling.	R-M	S

Question	Possible approach	Comments	Research/	Single/ Ongoing
Environmental fate continued				
3. How significant is the release of copper/zinc from nets in dissolved form into the pelagic environment?	Direct measurement of seawater in the vicinity of the nets	Establish the mass of copper and zinc released via dissolution (see budget later)	R-M, M	S/O
4. Does copper/zinc complex to dissolved organic matter, and become less bioavailable? *What fraction is associated with particulate loads?*	Direct measurement of copper complexing capacity, measurement of total/dissolved at 0.45 and 0.15 micron May incorporate both chemical speciation schemes and geochemical models to estimate bioavailability. Should be validated with toxicity testing eg phytoplankton.	Establish if copper release from nets via dissolution is a risk to pelagic communities. Trial the PEC/PNEC approach available through MAMPEC	R-M R, R-M	S S
5. How are metals in sediment partitioned between the solid and porewater phases and what are the implications for toxicity?	Measure porewater concentrations of metals under varying operational conditions, using high, intermediate and low organic sites. Calculate Kd and compare between sediment types and recovery stage.	Establish importance of porewater exposure route, and the environmental conditions that may lead to elevated concentrations.	R, R-M	S/O

Question	Possible approach	Comments	Research/	Single/ Ongoing
Management/Operational				
6. Are net coating and drying protocols adequate to ensure effective minimal release rates upon immersion?	Industry review	Establish best practice methods to reduce loadings of dissolved copper in excess of those required for effective antifouling	R-Man	S
7. What is the concentration of copper in water after freshly painted nets are immersed? *(Ties to 3)*	Direct measurement of seawater in the vicinity of the nets. Compare against predictions from models such as MINTEQA2, MAMPEC etc	Establish if there is a high risk period associated with the renewal of antifouling coatings	R-M	S
8. How does this change with exposure time and with the degree of fouling?	Direct measurement of seawater in the vicinity of the nets over time	Establish if there is a time after which direct dissolution can be considered negligible, (but still effective?)	R-M	S
9. Does in-situ net washing contribute unacceptable levels of soluble and particulate copper and zinc to the environment?	Provide detailed analysis of relative effectiveness of net washing techniques, and monitor soluble and particulate copper levels in water during washing operations, and for a defined period after washing e.g. daily for 1-2 weeks. Include seasonal component to refine initial mass budget conducted by industry.	Industry research suggests that the load of copper to the sediments using in situ cleaning is low in-situ and that in-situ cleaning leads to a significant increase in the life of the coating, reducing the need to change nets and process them through the net washing facility. In addition there is a need to compare the environmental contribution of copper from an in-situ cleaned net over its lifetime with a net which is not in-situ cleaned. Contributions to the water column have not been comprehensively documented.	R, R-M	S
10. How does this compare to costs associated with use of the net washing facility?	Cost-benefit analysis of in-situ washing vs net change and use of washing facility.	Maximise combined use of in-situ net cleaning and net washing by establishing if there are any overall benefits.	R-Man	S

Biological Effects				
11. Is there an appropriate measure of bioavailability that corresponds to levels of toxicity observed in key test species?	In conjunction with item 5, validate and employ a suitable analytical technique to estimate bioavailability in sediments and porewater. This measurement would then be employed for on-going routine assessment of sediment conditions and quality. Validate with toxicity testing.	Establish long-term chemical measurement to assess toxicity of sediments under varying operational conditions, across a range of sediment types.	R, R-M	S/O
12. What is the sediment concentration above which a statistically significant biological effect is expected?	Requires multiple species effects data and appropriate chemical estimates of contamination and bioavailability. Drawn from paired data for sediment chemistry and a range of biological effects.	Establish apparent effects threshold for high, intermediate and low organic sites.	R, R-M	S
13. How are benthic community structure and function and rate of response affected by increase in metal levels in sediments?	Assess benthic community structure at intervals in the recovery cycle, with the aim of identifying key species and bioindicators of metal stress. Include comparison of benthic fauna at different organic enrichment gradients. Construct species groupings as per Rygg describing species resistance to copper contaminated sediments.	Evaluate environmental degradation and recovery through changes in benthic community structure. Extend the protocols developed for organic enrichment already developed for the salmonid industry. May be possible to re-work the data using existing sediment metal/benthic community data.	R,R-M	S

Biological Effects continued				
14. How are sediment recovery processes affected by increased metals levels? *Includes microbial ecology*	Assess sediment toxicity using locally relevant benthic species to determine recovery conditions required with respect to metal contamination, & threshold levels. These tests may be conducted at a representative high, intermediate & low organic sites identified through the annual survey data. A screening test (e.g. Microtox) may be beneficial to use to prioritise intervals in the recovery cycle that should be tested using an appropriate whole sediment test. Assess denitrification rates at loaded sites vs control to determine if normal sediment processes are affected (ties in with Jo Banks research)	The recovery of sediments is attributed to the consumption of high levels of organic material, with concomitant changes in sediment redox conditions, and colonisation by a wider range of infaunal species. Oxidative release of metals bound as insoluble sulphides may occur as part of this recovery process. An assessment of the bioavailability of metals through the recovery period would give important information on the likely effects of sustained addition of copper through the use of antifouling products. Denitrification is a key process for removal of nitrogen/ ammonia from benthic systems.	R, R-M	S

Budget/combination				
15. What is the contribution of metals to the sediments from uneaten food and additives and faeces/metabolic waste?	Measure copper and zinc in feed formulations, and in faecal material/metabolic waste. Refine mass balance estimates already prepared by industry.	Establish if other inputs are significant compared to net contributions	R, R-M	S
16. Is there significant uptake, bioaccumulation or toxicity in organisms that reside or spend part of their reproductive cycle in the water column? Or sediment?	Collect and analyse tissue residue data for appropriate organisms (wild and farmed fish, fouling organisms, bivalves, key species etc) to determine if bioaccumulation is a potential issue at these sites.	Establish if pelagic communities are vulnerable to dissolution of copper from nets. Establish "safe" sediment /water column concentrations that results in acceptable tissue residues	R, R-M	S
17. What is the relative importance of each of these sources and pathways?	Conduct a full audit of all known input sources and assess against sediment concentrations. Dean et al (2007) provides some useful approaches to estimating major sources & losses. Investigate bioaccumulation as a component of the mass balance, and evaluate bioavailability. CSIRO proposed the use of bivalves for this purpose, although there may be issues with the relevance of the suggested species to the local environmental conditions and comparisons to functional ecology of the local fauna.	A detailed analysis of the major sources (e.g. net, food, faeces) of copper and zinc to the sediments would allow an assessment of the factors assumed to be involved in the partitioning of copper and zinc in the marine environment. Any "missing" loads may be due to export from the site, and this could be quite substantial given the increase in treated nets.	R-M	S/O

4.2. Antibiotics research

There are also 3 key issues with respect to the environmental impacts of antibiotic use:

- Persistence
- Resistance
- Effects on non-target organisms (biology and ecology)

The monitoring that has been undertaken to date partially addresses the issue of persistence at specific locations, but provides little information in relation to the other areas of concern. The following table (Table 35) identifies the knowledge gaps that remain with respect to determining the environmental fate and impacts of antibiotics and describes possible approaches to address these issues.

Table 35 Summary of knowledge gaps with respect to determining the environmental fate and impacts of antibiotics used in aquaculture and identification of possible approaches to address these issues. This table also includes sections identifying whether the defined topic will provide purely research information or whether that information will directly support monitoring or management solutions. In addition the table identifies whether the research activity requires a single one-off study or ongoing investigation and whether the outcomes are primarily local in focus or also of global relevance.

Research Area	Possible approach	Comments	Research / Monitoring	Single/ Ongoing	Local/ Global Relevance
Environmental Fate					
1. Identify chemical degradation products in environment for specific antibiotics (i.e. in relation to assessment of persistence & bioavailability)	Desktop evaluation of literature to examine specific antibiotic chemistry to identify relevant degradation products, particularly those which may be toxic. Field/ lab validation of those assumptions	This has to some extent already been undertaken for OTC & some aspects are being addressed through ongoing monitoring i.e. residue testing. However, needs to be expanded to encompass degradation products where this may be an issue.	R/ R- Monitoring	S	L/ G
2. Determine effects of changing environmental conditions (i.e. oxic status, pH, temperature) on chemical status, degradation & toxicity	Mesocosm experiments to manipulate environmental conditions Field validation	This is important in understanding what to look for with respect to accumulation in the environment.	R/ R- Monitoring	S	L/ G
3. Identify factors controlling distribution, accumulation & persistence in local environment	Look at where residues exist in environment to establish distribution pathways Tie in with other environmental & degradation data Employ models to understand deposition & dispersion patterns	This will help to establish where impacts are likely to occur & to ensure appropriate targeting of ongoing monitoring/ sampling	R/ R- Monitoring	S	L

Research Area	Possible approach	Comments	Research / Monitoring	Single/ Ongoing	Local/ Global Relevance
4. Identify factors influencing microbial resistance	Identify species in environment most likely to develop resistance Test for resistance (lab) Determine the nature of resistance (i.e. whether transient or not & if so duration of resistance) & at what level resistance is an issue (i.e. human health implications if any)	This will help to establish the possibility of human health risks & to ensure appropriate targeting of ongoing monitoring/ sampling	R/ R- Monitoring	S	L/ G
5. Examine both acute & chronic toxicity effects in key ecosystem species	Identify species most at risk for each specified antibiotic (incl. functionally important species, charismatic species, wild fish, macrofauna & microfauna) Undertake targeted ecotox experiments for acute response (incl. juv's) Undertake targeted ecotox experiments for chronic response (incl. juv's)	Will enable assessment of direct toxicity effects, establishing ecological risk. Will also increase public confidence in aquaculture activities This is an essential component of any risk-based management approach	R/ R- Monitoring	S/ O	L
6. Determine effects on sediment (ecological) processes	Undertake manipulative experiments (using mesocosms) to assess effects on natural microbial communities & key sediment processes such as denitrification.	Key information for risk assessment.	R/ R- Monitoring	S	L/ G
7. Develop standardised sampling & assessment protocols	Combine the information from all of the proposed assessments to identify a targeted monitoring approach Initiate a tiered management framework	Standardised approach with QA/QC of methods & reliability of assessment will lead to increased confidence in responsibility & sustainability of industry.	R- Monitoring	O	L
8. Examine synergistic effects of antibiotics & other chemicals/ therapeutics	Mesocosm experiments to manipulate environmental conditions Field validation		R/ R-Monitoring	S	L/ G

Research Area	Possible Approach	Comments	Research / Monitoring	Single/ Ongoing	Local/ Global Relevance
Treatment Approach					
1. Vaccine development where pathogens clearly identified	Priority research – clear evidence of benefits as a result of existing vaccines. Industry & DPIW Vaccine R&D group to participate.	Best solution to antibiotic problem where pathogens are clearly understood	R - Management	S	L/ G
2. Establish MIC for antibiotic / pathogen combinations where not already clear (eg. RLO & summer gut syndrome)	Needs a co-ordinated effort between industry veterinarians & DPIW fish health personnel – this is already being done but need to ensure ongoing recognition & support.	This is a very important in ensuring appropriateness of disease management strategy & needs to be supported	R/ R-Management	O	L/G
3. Investigate alternative non-antibiotic treatments (eg. probiotic feed additives, alternative therapies such as the use of bacteriophages)	Co-ordinated effort by industry veterinarians & DPIW fish health personnel to ensure currency in therapeutic developments & resources to trial/ validate new therapies.	This is important in ensuring disease management strategies are as up to date as possible & should be supported	R/ R-Management	O	L/ G
4. Develop relevant & robust diagnostic tests for new pathogens/ diseases (eg. reovirus) to facilitate most effective treatment or control measures	Laboratory analysis of pathogens to develop identification techniques & diagnostic tests, & thereafter resources to support field validation trials.	This would enable rapid, accurate diagnosis & consequently faster treatment. Needs co-ordination of veterinary, diagnostic & development expertise underpinned with appropriate resources.	R/ R-Management	O	L/ G
Management					
1. Develop mass-balance model identifying key antibiotic sinks & degradation pathways	Mixture of desktop evaluation & lab/ field validation experiments	This would provide a useful framework to identify & prioritise research needs	R/ R-Management	S/ O	L/ G
2. Adopt risk based management approach	Use all of the existing information to establish a risk based model Determine PEC/ PNEC	This has been recognised as the international approach to management of VPM & would seem to be the way forward	R/ R-Management	O	L

5. General Conclusions

The ongoing sustainability of the aquaculture industry requires some use of antibiotics and antifoulants to ensure the health of the farmed stocks. However, it is important to understand any environmental effects which the farming activities might have and to minimise any impacts. This can be achieved through ensuring best practice environmental management strategies (including disease management strategies) and robust impact assessment and mitigation approaches, with reliable early warning indicators.

The results from the current industry based monitoring focused on the detection of major effects of both antifoulant and antibiotic impacts are encouraging with findings suggesting limited bioavailability of metals under current conditions. However, there several areas of environmental concern which were not covered in the current monitoring and some of the results were inconclusive, consequently there is a need for additional research to better understand the local situation, to develop targeted and appropriate monitoring and management strategies and to ensure environmental sustainability. Potential areas for further investigation have been described in Tables 29 and 30 and a workshop was held in February 2009 with project participants, industry stakeholders and relevant experts to further discuss the project outcomes and proposed research areas, to prioritise research needs and to develop a strategic research agenda. The outcomes of this workshop are summarised in Appendix 2.

There is a considerable body of international literature in relation to the environmental effects of both antifoulants and antibiotics. Although a proportion of this information can be related directly to the local Tasmanian conditions, there are many instances where it is necessary to contextualise the findings locally and validate the results. There are also many synergies between the areas of environmental concern associated with the use of both antibiotics and copper based antifoulants. In both instances the environmental fate/ persistence and effects on non-target organisms and ecosystem processes (i.e. ecotoxicity and bioaccumulation) are significant issues. Given that neither antibiotics nor antifoulants are used in isolation, and that there may be synergistic/ antagonistic effects, it would be logical to combine the research efforts wherever possible. There are several areas where this would be most appropriate and easily achievable. Fate of residues was one area where it was determined there was a need for further understanding; this included further determination of the effects of antibiotics and antifoulants on sediment processes and vice versa and the development of modelling tools to evaluate environmental pathways/ consequences and help to develop appropriate monitoring/ management strategies is another area.

This study has identified the main environmental concerns regarding the current usage of selected antibiotics and antifoulants for the Tasmanian salmonid farming industry and has evaluated the key ecological risk areas. This review has also sought to determine where research or monitoring may be needed to better understand and manage these risks. These findings will assist aquaculture and resource managers to target future research and monitoring efforts to where there is the greatest need/ benefit.

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7. References

7.1. Antifoulant Review

ABARE (2008) 'Australian Fisheries Statistics 2007.' Canberra.

Addison P, F.E., Knott NA, Keogh MJ (2008) Spatially variable effects of copper on sessile invertebrates across a marina. Journal of Experimental Marine Biology and Ecology (in press).

Ahsanullah M, Ying W (1995) Toxic Effects of Dissolved Copper on Penaeus-Merguiensis and Penaeus-Monodon. Bulletin of Environmental Contamination and Toxicology 55, 81-88.

AMD (Aquaculture Management & Development) Pty Ltd (2004) 'Tasmanian Salmonid Growers Association Anti-Fouling Trials. Antifoulant Residue Monitoring Annual Report May 2004.' Aquaculture Management and Development P/L.

AMD (Aquaculture Management & Development) Pty Ltd (2005) 'Tasmanian Salmonid Growers Association Anti-Foulant Trials. Antifoulant Residue Monitoring in Sediments Annual Report 2005.' Aquaculture Management and Development P/L.

AMD (Aquaculture Management & Development) Pty Ltd (2006) 'Tasmanian Salmonid Growers Association Anti-Fouling Trials. 2006 Annual Report on Sediment Monitoring.' Aquaculture Management and Development P/L.

AMD (Aquaculture Management & Development) Pty Ltd (2007) 'Tasmanian Salmonid Growers Association Anti-Fouling Trials. 2007 Annual Report on Sediment Monitoring.' Aquaculture Management and Development P/L.

AMD (Aquaculture Management & Development) Pty Ltd (2008) 'Tasmanian Salmonid Growers Association Anti-Fouling Trials. 2008 Annual Report on Sediment Monitoring.' Aquaculture Management and Development P/L.

Ankley GT, Katko A, Arthur JW (1991) Predicting the toxicity of bulk sediments to aquatic organisms with aqueous test fractions: pore water vs elutriate. Environmental Toxicology and Chemistry 10, 1359-1366.

ANZECC/ARMCANZ (2008) 'Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines.'

Atchison G.J., Henry M.G, Sandheinrich M.B. (1987). Effects of metals on fish behaviour: a review. Environ. Biol. Fish. 18 (1): 11-25.

Ayling GM (1974) Uptake of Cadmium, Zinc, Copper, Lead and Chromium in the pacific oyster Crassostrea gigas, grown in the Tamar River Tasmania. Water Research 8, 729-738.

Batley G, Maher WA (2001) The development and application of ANZECC and ARMCANZ Sediment quality guidelines. Australasian Journal of Ecotoxicology 7, 81-92.

Batley G, Simpson SL, Chariton A (2008) 'Ecological effects due to contamination of sediments with copper-based antifoulants.' CSIRO .

Beckman Instruments (1982) Microtox system operating manual. In. (Beckman Instruments, Inc: Carlsbad, CA) .

Bellas J (2006) Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates. Science of the Total Environ, 573-585.

Birch GF, Taylor SE (2004) 'The contaminant status of Sydney Harbour sediments.' Environmental, Engineering and Hydrogeology Specialist Group (EEHSG) Geological Society of Australia, 1.

Brooks KM, Stierns AR, Backman C (2004) Seven year remediation study at the Carrie Bay Atlantic salmon (Salmo salar) farm in the Broughton Archipelago, British Columbia, Canada. Aquaculture 239, 81-123.

Burridge, L., Weis, J., Cabello, F., and Pizarro, J. (2008) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Report to Salmon Aquaculture Dialogue, World Wildlife Fund. (http://www.worldwildlife.org/aquadialogues).

Butler ECV (2005) The Tail of Two Rivers in tasmania: The Derwent and Huon Estuaries. In 'Handbook of Environmental Chemistry' pp. 1-49. (Springer-Verlag: Berlin)

Callow ME (2008) Algal Fouling: A sticky problem. In '14th International Congress on Marine Corrosion and Fouling'. Kobe Japan

Chapman PM, Wang F, Germano J, Batley G (2002) Pore water testing and analysis, the good, the bad and the ugly. Marine Pollution Bulletin 44, 359-366.

Cooper DC, Neal AL, Kukkadapu RK, Brewe D, Coby A, Picardal FW (2005) Effects of sediment iron mineral composition on microbially mediated changes in divalent metal speciation: Importance of ferrihydrite. Geochimica Et Cosmochimica Acta 69, 1739-1754.

Culshow C, Newton L, Weis I, Bird DJ (2002) Concentratios of Cd, Zn, and Cu in sediments and brown shrimp (Cragnon cragnon L.)from the Severn estaury and Bristol channel, UK. Marine Environmental Research 54, 331-334.

Daglish RW, Nowak BF (2002) Rainbow trout gills are a sensitive biomarker of short-term exposure to waterborne copper. Archives of Environmental Contamination and Toxicology 43, 98-102.

Dalgleish C (2008a) 'Copper mass balance. Fate of copper through the deployment cycle of fish nets.' Tassal Operations Pty Ltd.

Dalgleish C (2008b) 'Mass balance. Fate of copper and zinc through the in-situ cleaning of fish nets.' Tassal Operations Pty Ltd.

Dauvin J-C (2008) Effects of heavy metals contamination on the macrobenthic fauna in estuaries: The case of the Seine estuary. Marine Pollution Bulletin 57, 160-169.

Dean RJ, Shimmield TM, Black KD (2007) Copper, zinc, and cadmium in marine cage fish farm sediments: An extensive survey. Environmental Pollution 145, 84-95.

Dell'Anno A, Mei ML, Ianni C, Danovaro R (2003) Impact of bioavailable heavy metals on bacterial activitites in coastal marine sediments. World Journal of Microbiology and Technology 19, 93-100.

DEP (2007) 'Water Quality Improvement Plan for the Derwent estuary.' Derwent Estuary Program.

Douglas-Helders GM, Tan C, Carson J, Nowak BF (2003) Effects of copper-based antifouling treatment on the presence of Neoparamoeba pemaquidensis Page, 1987 on nets and gills of reared Atlantic salmon (Salmo salar). Aquaculture 221, 13-22.

Eriksen R (2000) Analysis and toxicity of pCufree in seawater. PhD thesis, University of Tasmania.

Eriksen R, Nowak B, van Dam R (2001a) 'Copper speciation and toxicity in a contaminated estuary.' Supervising Scientist.

Eriksen R, Mackey DJ, van Dam R, Nowak B (2001b) Copper speciation and toxicity in Macquarie Harbour, Tasmania: an investigation using a copper ion selective electrode. Marine Chemistry 74, 99-113.

Eriksen R, Macleod C, Meyer L (2009) Copper Ecotoxicity Studies -Development of Whole Sediment Toxicity Tests for the Derwent.' Tasmanian Aquaculture and Fisheries Institute - Technical Report (In Review).

Finnie A (2006) Improved estimates of environmental copper release rates from antifouling products. Biofouling 22, 279-291.

Flemming CA, Trevors JT (1989) Copper toxicity and chemistry in the environment; a review. Water, Air and Soil Pollution 44, 143-158.

Florence TM, Lumsden BG, Fardy JJ (1983) Evaluation of some physico-chemical techniques for the determination of the fraction of dissolved copper toxic to the marine diatom Nitzschia closterium. Analytica Chimica Acta 151, 281-295.

FSANZ (1998) 'Review of the Food Standards Code. Development of joint Australia New Zealand Food Standards. The establishment of guideline levels for contaminants (metals) in food: Generally Expected Levels (GELs).' Food Safety Australia New Zealand.

Geoscience Australia (2008) Sediment denitrification efficiency. In. Guenther J, Carl C, Olafsen T, Sunde LM (2008) Biofouling on Aquaculture nets in Norway:Current status and future directions. In '14th International Congress on Marine Corrosion and Fouling'. Kobe Japan

Hall Jr. LW, Anderson RD (1999) A Deterministic Ecological Risk Assessment for Copper in European Saltwater Environments. Marine Pollution Bulletin 38, 207-218.

IUCN (2007) 'Guide for the sustainable development of Mediterranean aquaculture. Interaction between aquaculture and the environment.'

Johnson A, Carew E, Sloman KA (2007) The effects of copper on the morphological and functional development of zebrafish embryos. Aquatic Toxicology 84, 431-438.

King CK, Dowse MV, Simpson SL, Jolley DF (2004) An assessment of five Australian polychaetes and bivalves for use in Whole sediment toxicity tests: toxicity and accumulation of copper and zinc from water and sediment. Archives of Environmental Contamination and Toxicology 47, 314-323.

King CK, Gale SA, Stauber JL (2006) Acute Toxicity and Bioaccumulation of Aqueous and Sediment-Bound Metals in the Estuarine Amphipod Melita plumulosa. Environmental Toxicology 21, 489-504.

Kramer CJM (1986) Approaches in chemical speciation studies in marine waters. In 'Biogeochemical Processes at the Land-Sea Boundary'. (Eds P Lassere and JM Martin). (Elsevier: Amsterdam)

Kwok KWH, Leung KMY (2005) Toxicity of antifouling biocides to the intertidal harpacticoid copepod Tigriopus japonicus (Crustacea, Copepoda): Effects of temperature and salinity. Marine Pollution Bulletin 51, 830-837.

Larson F (2005) THe role of microphytobenthos with emphasis on resilience. Goeteborg University, Sweden.

Levy JL, Angel BM, Stauber JL, Poon WL, Simpson SL, Cheng SH, Jolley DF (2008) Uptake and internalisation of copper by three marine microalgae: Comparison of copper sensitive and copper tolerant species. Aquatic Toxicology.

Lewis AG, Cave WR (1982) The biological importance of copper in oceas and estuaries. Oceanography and Marine Biology Annual Review 20, 471-695.

Long ER, Morgan LG (1990) 'The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program.'

Long ER, MacDonald DD, Smith S, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 19.

Lors C, Tiffreau C, Laboudigue A (2004) Effects of bacterial activities on the release of heavy metals from contaminated dredge sediments. Chemosphere 56, 619-630.

Mackey DJ, Butler ECV, Carpenter PD, Higgins HW, Osullivan JE, Plaschke RB (1996) Trace elements and organic matter in a pristine environment: Bathurst Harbour, Southwestern Tasmania. Science of the Total Environment 191, 137-151.

Macleod CK, Bisset A, Burke C, Forbes S, Holdsworth D, Nichols P, Revill A, Volkman J (2004) 'Development of novel methods for the assessment of sediment conditions and determination of management protocols for sustainable finfish cage aquaculture operations.' Tasmanian Aquaculture and Fisheries Institute.

Macleod CK, Helidoniotis F (2005) 'Ecological Status of the Derwent and Huon estuaries.' Tasmanian Aquaculture and Fisheries Institute, NHT/NAP Final report 46928.

Maddock JEL, Carvalho MF, Santelli RE, Machado W (2007) Contaminant metal behaviour during resuspension of sulphidic estuarine sediments. Water, Air and Soil Pollution 181, 193-200.

Magalhães C, Costa J, Teixeira C, Bordalo AA (2007) Impact of trace metals on denitrification in estuarine sediments of the Douro River estuary, Portugal. Marine Chemistry 9th International Estuarine Biogeochemistry Symposium Estuaries and Enclosed Seas under Changing Environmental Conditions 107, 332-341.

Markich SJ, StJ Warne M, Westbury A, Roberts Cj (2002) A compilation of data on the toxicity of chemicals to species in Australasia. Part 3 Metals. Australasian Journal of Ecotoxicology 8, 1-72.

Matthai C, Birch G (2001) Detection of ANthropogenic Cu, Pb and Zn in Continental Shelf Sediments off Sydney, Australia - a New Approach using Normalization with Cobalt. Marine Pollution Bulletin 42, 1055-1063.

McLusky DS, Bryant V, Campbell R (1986) The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. Oceanography and Biology, an Annual review 24, 481-520.

Microbic Corporation (1992) MICROTOX Manual Volume 2. Detailed Protocols. In.

Morrisey et al. (1996) Effects of copper on the faunas of marine soft-sediments: An experimental field study. Marine Biology 125, 199-213.

National Research Council (1997) 'Contaminated sediments in ports and waterways: clean-up strategies and technologies.' (National Academy Press)

Nipper MG, Badaro-Pedroso C, Jose VF, Melo SLR (1993) Toxicity testing with coastal species of southeastern Brazil. Mysids and copepods. Bulletin of Environmental Contamination & Toxicology 15, 99-106.

Nowak B, Duda S (1996) 'Effects of exposure to sublethal levels of copper on growth and health of sea farmed rainbow trout.' Supervising Scientist, Canberra.

NWQMS (2000) 'Australian and New Zealand water guidelines for marine and freshwaters.' ANZECC/ARMCANZ.

Olsgard (1999) Effects of Copper Contamination on Recolonisation of Subtidal Marine Soft Sediments - an Experimental Field Study.

Petersen W, Willer E, Willamowski C (1997) Remobilization of trace elements from polluted anoxic sediments after resuspension in oxic water. Water Air and Soil Pollution 99, 515-522.

Rand GM, Wells PG, McCarty LS (1995) Introduction to Aquatic Ecology. In 'Fundamentals of Aquatic Toxicology'. (Ed. GM Rand). (CRC Press: Boca Raton)

Ranke J (2002) Persistence of antifouling agents in the marine biosphere. Environmental Science & Technology 36, 1539-1545.

Riba I, Forja JM, Del Valls TA, Guerra R, Iacondini A (2002) Determining sediment toxicity associated with the Aznalcollar mining spill (SW Spain) using a bacterial bioassay. In 'Characterisation of Contaminated Sediments'. pp. 93-100.

Rygg B (1985) Distribution of Species Along Pollution-Induced Diversity Gradients in Benthic Communities in Norwegian Fjords. Marine Pollution Bulletin 16, 469-474.

SEPA (1998) 'An assessment of sediment copper and zinc concentrations at marine caged fish fams in SEPA west region.' Scottish Environment Protection Agency.

Shakouri M (2003) 'Impact of cage culture on sediment chemistry: A case study in Mjoifjordur.'

Simpson SL, Apte SC, BAtley GE (1998) Effects of short-term resupension events on trace metal speciation in polluted anoxic sediments. Environmental Science and Technology 32, 620-625.

Simpson SL (2005) Exposure-effects model for calculating copper effect concentrationss in sediments with varying copper binding properties: a synthesis. Environmental Science and Technology 39, 7089-7096.

Simpson SL, Spadaro D (2008) 'Ecological effects due to contamination of sediments with copper-based antifoulants.' CSIRO Land and Water Science, 53/08.

Simpson SL, Batley G, Chariton A (2008) 'Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines.' CSIRO Land and Water Science, 08/07.

Singh N, Turner A (2009) Leaching of copper and zinc from spent antifouling paint particles. Environmental Pollution 157, 371-376.

Solberg CB, Saethre L, Julshamm K (2002) The effect of copper-treated net pens on farmed salmon (*Salmo salar*) and other marine organisms and sediments. Marine Pollution Bulletin 45, 126-132.

Stauber JL, Ahsanullah M, Nowak B, Eriksen R, Florence TM (1996) 'Toxicity assessment of waters from Macquarie Harbour, western Tasmania, using algae, invertebrates and fish.' My Lyell Remediation Research and Demonstration Program, Supervising Scientist Report 112.

Stauber JL, Benning RJ, Hales LT, Eriksen R, Nowak B (2000) Copper bioavailability and amelioration of toxicity in Macquarie Harbour, Tasmaniaa, Australia. Marine and Freshwater Research 51, 1-10.

Sunda WG, Hanson AK (1987) Measurement of free cupric ion concentration in seawater by ligand competition technique involving copper sorption onto C18 SEP-PAK cartridges. Limnology and Oceanography 32, 537-551.

Thurberg FP, Dawson MA, Collier RS (1973) Effects of Copper and Cadmium on Osmoregulation and Oxygen-Consumption in 2 Species of Estuarine Crabs. Marine Biology 23, 171-175.

Turner A, Fitzer S, Glegg GA (2008) Impacts of boat paint chips on the distribution and availability of copper in an English ria. Environmental Pollution 151, 176-181.

Turner A, Barret M, Brown M (2009a) Processing of antifouling paint particles by Mytilus edulis. Environmental Pollution 157, 215-220.

Turner A, Singh N, Richards J (2009b) Bioaccesibility of metals in soils and dusts contaminated by marine antifouling particles. Environmental Pollution in press

USEPA (2001) 'Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual.' United States Environmental Protection Agency, EPA-823-B-01-002.

Valkirs AO, Seligman PF, Haslbeck E, Caso J (2003) Measurement of copper release rates from antifouling paint under laboratory and in situ conditions:implications for laoding estimation to marine water bodies. Marine Pollution Bulletin 46, 763-779.

WA Environment Department (2005) 'Background concentrations of selected toxicants in the coastal waters of the Jurien Bay Marine Park.' WA Department of Environment, Technical Series 119.

Warwick RM (2001) Evidence for the effects of metal contamination on the intertidal macrobenthic assemblages of the Fal estuary. Marine Pollution Bulletin 42, 145-148.

WHO (2001) 'Zinc Environmental Health Criteria.' Geneva.

6.2 Antibiotic Review

Akinbowale, O.L., Peng, H. and Barton, M.D. (2006) Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. Journal of Applied Microbiology **100**, 1103-1113.

Al-Ahmad, F., Daschner, D. and Kümmerer, K. (1999) Biodegradability of Cefotiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethoxazole and Inhibition of Waste Water Bacteria (1999) Archives of Environmental Contamination and Toxicology. **37**, 158-163

Alcaide, E., Blasco, M-D. and Esteve, C. (2005) Occurrence of Drug-Resistant Bacteria in Two European Eel Farms. Applied and Environmental Microbiology **71**, 3348-3350.

Alday, V., B. Guichard, B., Smith, P. and Uhland, C. (2006). Towards a risk analysis of antimicrobial use in aquaculture. Joint FAO/WHO/OIE expert consultation on antimicrobial use in aquaculture and antimicrobial resistance. Seoul, South Korea.

Alderman, D. J. and T. S. Hastings (1998). "Antibiotic use in aquaculture: development of antibiotic resistance - potential for consumer health risks." International Journal of Food Science and Technology **33,** 139-155.

Altenburger, R. Nendza, M. and Schüürmann, G. (2003) Mixture toxicity and its modeling by quantitative structure–activity relationships. Environmental Toxicology and Chemistry **22**, 1900–1915

Angulo, F., Johnson, K.R., Tauxe, R.V. and Cohen, M.L. (2000) Origins and consequences of antimicrobial-resistant nontyphoidal Salmonella: implications for the use of fluoroquinolones in food animals. Microbial Drug Resistance **6,** 77–83.

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

ANZECC (1992). Australian water quality guidelines for fresh and marine waters. National Water Quality Management Strategy Paper No 4, Australian and New Zealand Environment and Conservation Council, Canberra

ANZECC (2000) Australian water quality guidelines for fresh and marine waters - National Water Quality Management Strategy. Australian and New Zealand Environment and Conservation Council, Canberra

Aoki, T. (1992) Present and future problems concerning the development of resistance in aquaculture. In: Michel, C., Alderman, D.J. (Eds.), Chemotherapy in Aquaculture: From Theory to Reality. Office International des Epizooties, Paris, 254– 262.

Austin B. (1985). Antibiotic pollution from fish farms: effects on aquatic microflora. Microbiol Sci. **2**, 113–117.

Baguer, A.J., Jensen, J. and Krogh, P.H. (2000). Effects of the antibiotics oxytetracycline and tylosine on soil fauna. Chemosphere **40**, 751-757.)

Barnes, A.C., Lewin, C.S., Hastings, T.S. and Amyes, S.G.B. (1990) Cross resistance between oxytetracycline and oxolinic acid in *Aeromonas salmonicida* associated with alterations in outer membrane proteins. FEMS Microbiol. Lett. **22**, 337– 340.

Barnes, A.C., Hastings, T.S. and Amyes, S.G.B. (1995) Aquaculture antibacterials are antagonized by seawater cations. Journal of Fish Diseases **18**, 463-465.

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

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

Björklund, H. and Bylund, Cl. (1990) Temperature-related absorption and excretion of oxytetracycline in rainbow trout (*Salmo gairdneri* R.). Aquaculture, **84**, 363-372.

Björklund, H., Bondestam. J. and Bylund, G. (1990) Residues of Oxytetracycline in Wild Fish and Sediments from Fish Farms. Aquaculture **86**, 359-367.

Black, E.A., Little, J.M., Brackett, J., Jones, T. and Iwama, G.K. (1991) Co-culture of fish and shellfish: The implications for antibiotic contamination of shellfish. ICES, Copenhagen, Denmark.

Black, E. (2001). Aquaculture and integrated resource use. Bulletin of the Aquaculture Association of Canada **101,** 29-35.

Black, K.D.(ed) (2001) Environmental impacts of aquaculture. Sheffield Academic Press, Sheffield, U.K.

Blyth, P.J., Purser, G.J. and Russell, J.F., (1993) Detection of feeding rhythms in seacaged Atlantic salmon using new feeder technology. In: Reinertsen, H., Dahle, L.A., Jørgensen, L., Tvinnereim, K. (Eds.), Fish Farming Technology. Balkema, Rotterdam, pp. 209–216.

Blyth, P.J., Kadri, S., Valdimarsson, S.K., Mitchell, D.F. and Purser, G.J. (1999) Diurnal and seasonal variation in feeding patterns of Atlantic salmon, Salmo salar L., in sea cages. Aquaculture Research **30**, 539-544.

Boon, P.I. (1992). Antibiotic resistance of aquatic bacteria and its implications for limnological research. Australian Journal of Marine and Freshwater Research **43**, 847-859.

Boon, P.I. and Cattanach, M. (1999). Antibiotic resistance of native and faecal bacteria isolated from rivers, reserviours and sewage treatment facilities in Victoria, south-eastern Australia. Letters in Applied Microbiology **28**, 164-168.

Bound, J. P. and N. Voulvoulis (2004). "Pharmaceuticals in the aquatic environment - a comparison of risk assessment strategies." Chemosphere **56**, 1143-1155.

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

Burka, J.F., K.L. Hammel, T.E. Horsberg, G.R. Johnsons, D.J. Rainie and D.J. Speares.(1997) Drugs in salmonid aquaculture – a review. J. Vet. Pharmacol. Ther. **20**, 333-349.

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

Burridge, L., Weis, J., Cabello, F., and Pizarro, J. (2008) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Report to Salmon Aquaculture Dialogue, World Wildlife Fund. (http://www.worldwildlife.org/aquadialogues).

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

Cabello, F.C. (2003) Antibiotics and aquaculture. An analysis of their potential impact upon the environment, human and animal health in Chile. Fundacion Terram. Analisis de Politicas Publicas No. 17, pp. 1–16. URL http://www.terram.cl/
docs/App17_Antibioticos_y_Acuicultura.pdf

Cabello, F.C. (2004) Antibiotics and aquaculture in Chile: implications for human and animal health. Rev Med Chil **132**, 1001–1006.

Cabello, F. C. (2006). "Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment." Environmental microbiology **8,** 1137-1144.

Capone, D.G., Weston, D.P., Miller, V. and Shoemaker, C. (1996) Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. Aquaculture **145**, 55-76.

Carss, D.N. (1990) Concentrations of wild and escaped fishes immediately adjacent to fish farm cages. Aquaculture **90**, 29-40.

Chelossi, E., Vezzulli, L., Milanoc, A., Branzonic, M., Fabianob, M. and Riccardia, G. and Banat, I.M. (2003). Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. Aquaculture **219**: 83-97.

Christensen, A.M., Ingerslev, F. and Baun, A. (2006) Ecotoxicity of mixtures of antibiotics used in aquacultures Environmental Toxicology and Chemistry **25**, 2208-2215.

Corbeil, S., Hyatt, A.D. and Crane, M.S.J. (2005). Characteristation of an emerging rickettsia-like organism in Tasmanian farmed Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms **64,** 37-44.

Costanzo, S.D., Murby, J. and Bates, J. (2005) Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Bulletin **51**, 218–223.

Coyne, R., Hiney, M. P., O'Connor, B., Kerry, J., Cazabon, D. and Smith, P. (1994). Concentration and persistence of oxytetracycline in sediments under a marine salmon farm. Aquaculture **123**, 31-42.

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

Cravedi, J-P., Choubert, G. and Delous, G. (1987) Digestibility of chloramphenicol, oxolinic acid and oxytetracycline in rainbow trout and influence of these antibiotics on lipid digestibility. Aquaculture **60**, 133-141.

Cromey, C.J., Nickell, T.D. and Black, K.D. (2002a) DEPOMOD-modelling the deposition and biological effects of waste solids from marine cage farms. Aquaculture **214**, 211-239.

Cromey, C.J., Nickell, T.D., Black, K.D., Provost, P.G. and Griffiths, C.R. (2002b) Validation of a Fish Farm Waste Resuspension Model by Use of a Particulate Tracer Discharged from a Point Source in a Coastal Environment. Estuaries **25**, 916-929.

Cwiertniewicz, J. (1994) Environmental Assessment Studies for Human Pharmaceuticals. Pharmaceutical Technology Europe **6**, 41-44.

Davenport, J., Burnell, G. and Cross, T. (2003). Aquaculture; the ecological issues. Blackwell Publishing. Wiley-Blackwell, UK.

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

De Grandis, S.A. and Stevenson, R.M. (1985) Antimicrobial susceptibility patterns and R plasmid-mediated resistance of the fish pathogen *Yersinia ruckeri*. Antimicrobial Agents and Chemotherapy **27**, 938-942.

Della Greca, M., Fiorentino, A., Iesce, M.R., Isidori, M., Nardelli, A. and Previtera, L. (2003) Identification of phototransformation products of prednisone by sun-light Toxicity of the drug and its derivatives on aquatic organisms. Environ Toxicol Chem, **22**, 534– 9.

DePaola, A., Flynn, P. A., McPhearson, R. M. and Levy. S. B. (1988). Phenotypic and genotypic characterization of tetracycline- and oxytetracyclineresistant *Aeromonas hydrophila* from cultured channel catfish (*Ictalurus punctatus*) and their environments. Appl. Environ. Microbiol. **54**,1861– 1863. 5.

DePaola, A., (1995). Tetracycline resistance by bacteria in response to oxytetracycline-contaminated catfish feed. J. Aquat. Anim. Health **7**, 155-160.

DePaola, A., Peeler. J.T. and Rodrick, G.E. (1995). Effect of oxytetracycline-medicated feed on antibiotic resistance of gram-negative bacteria in catfish ponds. Appl. Environ. Microbial. **61**, 2335-2340.

DePaola, A. and Roberts, M. C. (1995). C lass D and E tetracycline resistance determinants in gram-negative catfish pond bacteria. Mol. Cell. Probes **9**, 311–313.

Di Corcia, A. and Nazzari, M. (2002) Liquid chromatographic–mass spectrometric methods for analyzing antibiotic and antibacterial agents in animal food products. Journal of Chromatography A. **974**, 53-89

Ervik, A., Thorsen, B., Eriksen, V., Lunestad, B.T. and Samuelsen, O.B., (1994) Impact of administering antibacterial agents on wild fish and blue mussels *Mytilus edulis* in the vicinity of fish farms. Dis. Aquat. Org., **18**, 45-51.

European Agency for the Evaluation of Medicinal Products (EMEA) (2003) Note for Guidance on Environmental Risk Assessment of Medicinal Products for Human Use. CPMP/SWP/4447/00 draft. European Agency for the Evaluation of Medicinal Products.

FDA (1998) Guidance for industry—Environmental assessment of human drugs and biologics applications. FDA, CDER/CBER, CMC 6, rev.1. (http://www.fda.gov)

FDA (2000) Effect of the use of antimicrobials in food producing animals on pathogen load: Systematic review of the published literature. Center for Veterinary Medicine, Rockville, MD, USA (http://www.fda.gov)

FDA (2001) Environmental Impact Assessments (EIA's) For Veterinary Medicinal Products (VMP's)- Phase I. Center for Veterinary Medicine, Rockville, MD, USA (http://www.fda.gov)

FDA (2001) Fish and Fisheries Products Hazards and Controls Guidance, Third Edition. Center for Veterinary Medicine, Rockville, MD, USA (http://www.fda.gov)

FDA (2003) Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern. Center for Veterinary Medicine, Rockville, MD, USA. (http://www.fda.gov)

FDA (2004) Pre-Approval Information for Registration of New Veterinary Medicinal Products for Food-Producing Animals with Respect to Antimicrobial Resistance. Center for Veterinary Medicine, Rockville, MD, USA (http://www.fda.gov/cvm).

FDA (2006) Environmental Impact Assessments (EIA's) For Veterinary Medicinal Products (VMP's) - Phase II. Center for Veterinary Medicine, Rockville, MD, USA

FAO/NACA/WHO. (1997) Joint Study Group. Food safety issues associated with products from aquaculture. WHO Technical Report Series, No. 883.

FAO (2008) FishStat - Fishery Information, Data and Statistics Unit. ROME: FAO

FAO/WHO (2008) Codex Alimentarius Commission. Procedural Manual, 18th edition. FAO, ROME (http://www.codexalimentarius.net/web/procedural_manual.jsp).

FSANZ (2006) Safe Seafood Australia: A guide to the Australian Primary Production and Processing Standard for Seafood. Food Standards Australia New Zealand, Canberra, Australia (http://www.foodstandards.gov.au/thecode/primaryproductionprocessingstandards/seafoodstandards.cfm)

FSANZ (2007) Risk Assessment (an investigation of the effect of antibiotic usage on non-target fish: oxytetracycline (OTC) residue testing of wildfish), Internal Report to Department of Health and Human Services, January 2007.

FSANZ (2009) Australia New Zealand Food Standards Code. FSANZ Canberra, Australia (http://www.foodstandards.gov.au/thecode/foodstandardscode.cfm)

Furushita, M., Maeda, T., Akagi, H., Ohta, M., and Shiba, T. (2005) Analysis of plasmids that can transfer antibiotic resistance genes from fish farm bacteria to clinical bacteria. In Abstracts, Joint Meeting of the 3 Divisions of the International Union of Microbiological Societies 2005. International Congress of Bacteriology and Applied Microbiology, B-1162. 23–28 July 2005, San Fransisco, CA, USA

GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection).Towards safe and effective use of chemicals in coastal aquaculture.
Reports and Studies, GESAMP. No. 65. Rome, FAO. 1997. 40 p

Giraud, E., Douet, D-G., Le Bris, H., Bouju-Albert, A., Donnay-Mreno, C., Thorin, C. and Pouliquen, H. (2006) Survey of antibiotic resistance in an integrated marine aquaculture system under oxolinic acid treatment. FEMS Microbiol Ecol **55**, 439–448

Gorbach, S.L. (2001) Antimicrobial use in animal feed – time to stop. N Engl J Med **345**, 1202–1203.

Grave, K., Lingaas, E., Bangen, M. and Ronning, M. (1999) Surveillance of the overall consumption of antibacterial drugs in humans, domestic animals and farmed fish in Norway in 1992 and 1996. Journal of Antimicrobial Chemotherapy **43**, 243-252.

Gray, J.P. and Herwig, R.P. (1996) Phylogenetic analysis of the bacterial communities in marine sediments. Appl. Environ. Microbiol. **62**, 4049-4059.

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

Halling-Sorensen, B., D. Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Liitzhofl, H.C. and Jorgensen, S.E. (1998). Occurence, fate and effects of pharmaceutical substances in the environment - a review. Chemosphere **36,** 357-393.

Halling-Sorensen, B. (2000). Algal toxicity of antimicrobial agents used in intensive farming. Chemosphere **40,** 731-739.

Hansen, P.K., B.T. Lunestad and O.B. Samuelsen. (1993) Effects of oxytetracycline, oxolinic acid, and flumequine on bacteria in an artificial marine fish farm sediment. Can. J. Microbiol. **39**, 1307-1312.

Hansen, L.H., Ferrari, B., Sørensen, A.H., Veal, D. and Sørensen, S.J. (2001) Detection of Oxytetracycline Production by Streptomyces rimosus in Soil Microcosms by Combining

Whole-Cell Biosensors and Flow Cytometry. Applied and Environmental Microbiology **67**, 239-244.

Harrass, M.C., Kindig, A.C. and Taub, F.B. (1985) Responses of blue-green and green algae to streptomycin in unialgal and paired culture. Aquatic Toxicology **6**, 1-11.

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

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

Haya, K., L.E. Burridge and B.D. Chang. (2001) Environmental impact of chemical wastes produced by the salmon aquaculture industry. ICES J. Mar. Sci. **58**, 492-496.

Hektoen, H., J. A. Berge, Hormazabal, V. and Yndestad, M. (1995). "Peristence of antibacterial agents in marine sediments." Aquaculture **133,** 175-184.

Henschel, T., Kalbfus, W., Traunspurger, W., Ahlf, W. and Steinberg, C. (1997) Evaluation of ecotoxical impact of sediment-bound contaminants in a wastewater-polluted basin (Speichersee) with bioassays in a battery-of-test approach. Vom Wasser. Weinheim. **88**, 295-307.

Herwig, R. P., Gray, J.P. and Weston, D.P. (1997). Antibacterial resistant bacteria in surficial sediments near salmon net-cage farms in Puget Sound, Washington. Aquaculture **149,** 263-283.

Hiney, M. and Smith, P. (2000) Oil-adjuvanted furunculosis vaccines in commercial fish farms: a preliminary epizootiological investigation. Aquaculture **190**, 1-9.

Hirsch, R., Ternes, T., Haberer, K. and Kratz, K-L. (1999) Occurrence of antibiotics in the aquatic environment. Science of the Total Environment **225**, 109-118.

Ho, Shu-Peng; Hsu, Tain-Yao; Chen, Ming-Hui; Wang, Way-Shyan (2000) Antibacterial Effect of Chloramphenicol, Thiamphenicol and Florfenicol against Aquatic Animal Bacteria. Journal of Veterinary Medical Science **62**, 479-485.

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

Horie, M., Takegami, H., Toya, K. and Nakazawa, H (2003) Determination of macrolide antibiotics in meat and fish by liquid chromatography-electrospray mass spectrometry. Analytica Chimica Acta **492**, 187-197.

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

Husevåg, B., Lunestad, B.T., Johannesen, P.J, Enger, O. and Samuelsen, O.B. (1991) Simultaneous occurrence of *Vibrio salmonicida* and antibiotic-resistant bacteria in sediment at abandoned aquaculture sites. ,J. of Fish Diseases **14**, 631-640.

Huys, G., Rhodes, G., McGann, P., Denys, R., Pickup, R., Hiney, M., Smith, P and Swings, J (2000) Characterization of Oxytetracycline-Resistant Heterotrophic Bacteria Originating from Hospital and Freshwater Fishfarm Environments in England and Ireland. Systematic and Applied Microbiology **23**, 599-606.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L. and Parrella, A. (2005). Toxic and genotoxic evalutation of six antibiotics on non-target organisms. Science of the Total Environment **346**, 87-98.

ISO (1989) Water quality—Algal growth inhibition test. Geneva, Switzerland International Organization for Standardization; ISO/DIS 8692.

IUCN (2007) Guide for the sustainable development of Mediterranean aquaculture, Gland, Switzerland and Malaga, Spain

Jacobsen, P. and Berglind, L., (1988) Persistence of Oxytetracycline in Sediments from Fish Farms. Aquaculture **70**, 365-370.

Jolibois, B., Guerbeta, M. and Vassal, S. (2003) Detection of hospital wastewater genotoxicity with the SOS chromotest and Ames fluctuation test. Chemosphere **51**, 539-543.

Jones, A. R., Watson-Russell, C.J. and Murray, A. (1986). Spatial patterns in the macrobenthic communities of the Hawkesbury Estuary, New South Wales. Australian Journal of Marine and Freshwater Research **37,** 521-543.

Just, P.M. (1993) Overview of the fluoroquinolone antibiotics. Pharmacotherapy **13**, 4–17.

Kapetanaki, M., Kerry, J., Hiney, M., O'Brien, C., Coyne, R. and Smith, P. (1995). Emergence, in oxytetracycline-free marine mesocosms, of microorganisms capable of colony formation on oxytetracycline-containing media. Aquaculture **134**, 227-236.

Kerry, J., Hiney, M., Coyne, R., NicGabhainn, S., Gilroy, D., Cazabon, D. and Smith, P. (1995). Fish feed as a source of oxytetracycline-resistant bacteria in the sediments under fish farms. Aquaculture **131,** 101-113.

Kerry, J., Slattery, M., Vaughan, S. and Smith, P. (1996) The importance of bacterial multiplication in the selection, by oxytetracycline-HCL, of oxytetracycline-resistant bacteria in marine sediment microcosms. Aquaculture **144**, 103-119.

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

Kerry, J. and Smith, P. (1997). Changes in oxytetracycline resistance of intestinal microflora following oral administration of this agent to Atlantic Salmon (*Salmo salar*) smolts in a marine environment. Aquaculture **157**, 187-195.

Klaver, A.L. and Matthews, R.A. (1994) Effects of oxytetracycline on nitrification in a model aquatic system. Aquaculture **123**, 237-247.

Kleinow, K.M., Jarboe, H.H., Shoemaker, K.E. and Greenless, K.J. (1994) Comparative pharmacokinetics and bioavailability of oxolinic acid in channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences **51**, 1205-1211.

Koschorreck, J., C. Koch, C. and Ronnefahrt, I. (2002). "Environmental risk assessment of veterinary medicinal products in the EU - a regulatory perspective." Toxicology letters **131**, 117-124.

Kruse, H. and Sorum, H. (1994). Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. Applied and Environmental Microbiology **60**, 4015-4021.

Kümmerer, K., Al-Ahmada, A. and Mersch-Sundermann, V. (2000) Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. Chemosphere **40**, 701-710

Kümmerer K. (2001a) Pharmaceuticals in the environment, sources, fate, effects and risks. Berlin7 Springer-Verlag.

Kümmerer K. (2001b) Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources—a review. Chemosphere **45**, 957– 69.

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

Lee, W.Y and Bird, J.L. (1983) Pharmaceutical wastes at the Puerto Rico dumpsite: Sublethal effects on a marine planktonic copepod, *Temora turbinate* Environmental Science and Technology **5**, 227-240.

Lee, W.Y. and Arnold, C.R. (1983) Chronic Toxicity of Ocean-dumped Pharmaceutical Wastes to the Marine Amphipod *Amphithoe valida*. Marine Pollution Bulletin **14**, 150-53

Levy, S. (2001). In: Bugs on the Run. Conference highlights. Living with the enemy. The key to tackling resistant bacteria may be to spare their lives. New Scientist, 2 June 2001. (See: www.newscientist.com).

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

Lunestad, B.T., Samuelsen, O. B. Ervik, A. and Fjelde. S. (1995) Photodegradation of eight antibacterial agents in seawater. Aquaculture **134**, 217-225.

Markestad, A. and Grave, K. (1997) Reduction of antibacterial drug use in Norwegian fish farming due to vaccination. In : "Fish vaccinology. Development in biological standardization", R. Guddings, A. Lillehaug, P.J. Midlyng & F. Brown editors **90,** 365-369. Basel, Karger.

Martinsen, B. and Horsberg ,T.E. (1995) Comparative single-dose pharmacokinetics of four quinolones, oxolinic acid, flumequine, sarafloxacin, and enrofloxacin, in Atlantic salmon (*Salmo salar*) held in seawater at 10 degrees C. Antimicrobial Agents and Chemotherapy **39**, 1059-1064

McEwen, S.A. and Fedorka-Cray, P.J. (2002) Antimicrobial Use and Resistance in Animals. Clinical Infectious Diseases **34**, 93–106

McPhearson, R.M., DePaola, A., Zywno, S.R., Motes, M.L. Jr, Guarino and A.M. (1991) Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. Aquaculture **99**, 203–211.

Mersch-Sundermann, V., Wintermann, F., Kern, S. and Hof, H. (1993) Influence of S9 mix composition of the SOS response in *Escherichia coli* PQ37 by polycyclic aromatic hydrocarbons. Mutation Research **291**, 53-60.

Michel, C., Kerouault, B. and Martin, C, (2003) Chloramphenicol and florfenicol susceptibility of fish pathogenic bacteria isolated in France: comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. Journal of Applied Microbiology **95**, 1008–1015

Miranda, C.D. and Castillo, G. (1998) Resistance to antibiotic and heavy metals of motile aeromonads from Chilean freshwater. The Science of the Total Environment **224**, 167-176

Miranda, C.D. and Zemelman, R. (2001) Antibiotic resistant bacteria in fish from the Concepcion bay, Chile. Mar. Pollut. Bull. **42**, 1096– 1102.

Miranda, C.D. and Zemelman, R. (2002a) Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. The Science of the Total Environment **293**, 207-218.

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

Miranda, C.D., Kehrenberg, C., Ulep, C., Schwarz, S. and Roberts, M.C. (2003) Diversity of Tetracycline Resistance Genes in Bacteria from Chilean Salmon Farms. Antimicrobial Agents and Chemotherapy **47**, 883-888.

Montforts, M. H. M. M. and de Knecht, J. A. (2002). "European medicines and feed additives regulation are not in compliance with environmental legislation and policy." Toxicology letters **131**: 125-136.

Moore, D.E. (1998) Mechanisms of photosensitization by phototoxic drugs. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **422**, 165-173

Noble C., Kadri, S., Mitchell, D.F. and Huntingford, F.A. (2007) The impact of environmental variables on the feeding rhythms and daily feed intake of cage-held 1+ Atlantic salmon parr (*Salmo salar* L.). Aquaculture **269**, 290–298.

Nyholm, N (1991) Toxic effects on algal phosphate uptake. Environmental Toxicology and Chemistry **10**, 581-584.

Nygaard, K., Lunestad. B.T., Hektonen, H., Berge J.A. and Hormazabal, V. (1992) Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteria from marine sediments. Aquaculture **104**, 31-36

OECD (Organisation for Economic Cooperation and Development) (1996) Guidelines for testing of chemicals. Proposal for updated Guideline 211, *Daphnia magna* reproduction test. Revisted Draft Document. OECD, Paris, France.

Oka, H., Ikai, Y., Kawamura, N., Yamada, M., Harada, K., Ito, S. and Suzuki, M. (1989) Photodecomposition products of tetracycline in aqueous solution. J. Agric. Food Chem. **37**, 226–231

Olsen, J.E. (1999) Antibiotic resistance: genetic mechanisms and mobility. Acta Vet Scand Suppl. **92**, 15-22.

O'Reilly, A. and Smith, P. (1999) Development of methods for predicting the minimum concentrations of oxytetracycline capable of exerting a selection for resistance to this agent. Aquaculture **180**, 1 –11.

O'Reilly, A. and Smith, P. (2001) Use of indirect conductimetry to establish predictive no effect concentrations of oxytetracycline and oxolinic acid in aquatic sediments. Aquaculture **196**,13–26

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

Pillai, S.D., Widmer, K.W., Maciorowski, K.G. and Ricke, S.C. (1997) Antibiotic resistance profiles of Escherichia coli isolated from rural and urban environments. Journal of

Environmental Science and Health, Part A: Environmental Science and Engineering & Toxic and Hazardous Substance Control. **32**, 1665-1675.

Pillay, T. V. R. (2004). Aquaculture and the Environment, Blackwell Publishing.

Plakas, S.M., El Said, K.R.,Bencsath, F.A., Musser, S.M. and Hayton, W.L. (1998) Pharmacokinetics, tissue distribution and metabolism of acriflavine and proflavine in the channel catfish (*Ictalurus punctatus*). Xenobiotica **28**, 605-616.

Pursell, L., Dineen, T., Kerry, J., Vaughan, S. and Smith, P (1996) The biological significance of breakpoint concentrations of oxytetracycline in media for the examination of marine sediment microflora. Aquaculture **145**, 21-30.

Quillardet, P. and Hofnung, M. (1993) The SOS chromotest: a review. Mutat Res., **297**, 235–79.

Reimschuessel, R., Stewart, L., Squibb, E., Hirokawa, K., Brady, T., Brooks, D., Shaikh, B. and Hodsdon, C. (2005) Fish Drug Analysis — Phish-Pharm: A Searchable Database of Pharmacokinetics Data in Fish. AAPS Journal **7**, 288-327.

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P. and Pickup, R.W. (2000) Distribution of oxytetracycline resistance plasmids between aeromonads in hospitals and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant Tet A. Appl Environ Microbiol **66**, 3883-3890.

Ricklefs, R.E. and Miller, G.L. (2000) Ecology. 4th edition. W.H. Freeman and Co., New York.

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

Samuelsen, O. B. (1994). Environmental impacts of antibacterial agents in Norwegian aquaculture. Proceedings of the Canada-Norway workshop on environmental impacts of aquaculture, Bergen, Norway.

Samuelsen, O.B. (2003) Administration of the antibacterial agents flumequine and oxolinic acid to small turbot (*Scophthalmus maximus* L.) by bath. Journal of Applied Ichthyology **19**, 55-58.

Samuelsen, O.B. (2006). "Pharmacokinetics of quinolones in fish: a review." Aquaculture **255,** 55-75.

Samuelsen, O.B., Nerland, A.H., Jørgensen, T., Schrøder, M.B., Svåsand, T. and Bergh, O. (2006) Viral and bacterial diseases of Atlantic cod *Gadus morhua*, their prophylaxis and treatment: a review. Diseases of Aquatic Organisms **71**, 239–254.

Samuelsen, O.B., Lunestad, B.T., Husevlg, B., Holleland, T. and Ervik, A., (1992a) Residues of oxolinic acid in wild fauna following medication in fish farms. Diseases of Aquatic Organisms **12**, 11 l-l 19.

Samuelsen, O.B., Torsvik, V. and Ervik, A., (1992b) Long-range changes in oxytetracycline concentrations and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. Sci. Total. Environ. **114**, 25-36.

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

Samuelsen, O.B., Bergh, Oe and Ervik, A. (2003) Pharmacokinetics of florfenicol in cod *Gadus morhua* and in vitro antibacterial activity against *Vibrio anguillarum* Diseases of Aquatic Organisms **56**, 127-133.

Samuelsen, O.B. and Lunestad, B.T. (1996) Bath treatment, an alternative method for the administration of the quinolones flumequine and oxolinic acid to halibut *Hippoglossus hippoglossus*, and in vitro antibacterial activity of the drugs against some *Vibrio* sp. Diseases of Aquatic Organisms **27**, 13-18.

Sandaa, R.A., Torsvik, V. and Goksøyr, J. (1992) Transferable drug resistance in bacteria from fish-farm sediments. Can. J. Microbiol. **38**, 1061-1065.

Serrano, P. H. (2005). Responsible use of antibiotics in aquaculture. FAO Fisheries Technical paper 469, Food and Agriculture Organisation of the United Nations**:** 97.

Schmidt, A.S, Bruun, M.S. Dalsgaard, I., Pedersen, K. and Larsen, J.L. (2000) Occurrence of Antimicrobial Resistance in Fish-Pathogenic and Environmental Bacteria Associated with Four Danish Rainbow Trout Farms. Applied and Environmental Microbiology **66**, 4908-4915.

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

Smith, P. (1996) Is sediment deposition the dominant fate of oxytetracycline used in marine salmonid farms: a review of available evidence. Aquaculture **146**, 157-169.

Smith, P., M. Hiney and Samuelsen, O. (1994). "Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning." Annual Review of Fish Diseases **4**, 273-313.

Smith, P., Pursell, L., McCormack, F., O'Reilly, O. and Hiney, M. (1995) On the significance of bacterial resistance to oxytetracycline in sediments under Norwegian fish farms. Bull. Eur. Ass. Fish Pathol. **15**, 105-106.

Smith, P. and Samuelsen, O.B. (1996). Estimates of significance of out-washing of oxytetracycline from sediments under Atlantic salmon cages. Aquaculture **144**, 17-26.

Smith, A. C., Martin, J.L., Ehrman, J.M. and Kaczmarska, I. (2001). Ten-year record of *Thalassiosira nordenskioeldii* population dynamics: comparison of aquaculture and non-aquaculture sites in the Quoddy Region. ICES Journal of Marine Science **58**, 391-397.

Spangaard, B., Jørgensen, F., Gram, L. and Huss, H.H. (1993) Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. Aquaculture **115**, 195–207.

Sørum, H., (1998) Mobile drug resistance genes among fish bacteria. APMIS **106**, 74–76.

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

Sørum, H. (2006). Antimicrobial drug resistance in fish pathogens. In: Antimicrobial resistance in bacteria of animal origin, chapter 13, ed. Frank M. Aarestrup, ASM press, Washington D.C. pp. 213-238.

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

Stamm, J.M. (1989) In vitro resistance by fish pathogens to aquacultural antibacterials, including the quinolones difloxacin (A-56619) and sarafloxacin (A-56620). J. Aquat. Anim. Health **1**, 135– 141.

Stewart, J. E. (1994). Aquaculture in Atlantic Canada and the research requirements related to environmental interactions with finfish culture. Proceedings of the Canada-Norway workshop on environmental impacts of aquaculture, Bergen, Norway.

Sugita, H., Ushioka, S., Kihara, D. and Deguchi, Y. (1988). Changes in the bacterial composition of water in a carp rearing tank. Aquaculture **44**, 243–247

Sugita, H., Miyajima, C., Fukumoto, M., Koyama. H. and Deguchi, Y. (1989) Effect of oxolinic acid on fecal microflora of goldfish (*Carussius aurutus*). Aquaculture **80**, 163-174.

Sveier, H. and Lied, E. (1998) The effect of feeding regime on growth, feed utilisation and weight dispersion in large Atlantic salmon (*Salmo salar*) reared in seawater. Aquaculture **16,** 333–345

Tendencia, E.A. and de la Peña, L.D. (2001) Antibiotic resistance of bacteria from shrimp ponds. Aquaculture **195**, 193–204.

Tenson T, Lovmar M. and Ehrenberg M. (2003) The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. J Mol Biol. **330**, 1005– 14.

Tibbs, J.F., Elston, R.A., Dickey, R.W. and Guarino, A.M. (1989) Studies on the accumulation of antibiotics in shellfish. Northwest Environ. J. **5**, 161-162.

USP (United States Pharmacopia) (2000) Food Chemicals Codex. The United States Pharmacopeial Convention, Maryland, USA (www.usp.org)

Watanabe, T., Aoki, T., Ogata, Y. and Egusa, S. (1971) R factors related to fish culturing. Ann. N.Y. Acad. Sci. **182**, 383-410.

Wegener, H.C. (1999) The consequences for food safety of the use of fluoroquinolones in food animals. N Engl J Med **340**, 1581–1582.

Weston, D.P., Capone, D.G., Herwig, R.P. and Staley, J.T. (1994). Environmental fate and effects of aquacultural antibacterials in Puget Sound; A report to National Oceanic and Atmospheric Administration, University of California, Berkeley, CA.

Wolfson, J.S. and Hooper, D.C. (1989) Fluoroquinolone antimicrobial agents. Clin Microbiol Rev. 378-424.

Wollenberger, L., B. Halling-Sorensen, B. and Kusk, K.O. (2000). Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna.* Chemosphere **40**: 723-730.

Vaughan, S., Coyne, R. and Smith, P. (1996) The critical importance of sample site in the determination of the frequency of oxytetracycline resistance in the effluent microflora of a fresh water fish farm. Aquaculture **139**, 47-54.

Vaughan, S. and Smith, P. (1996) Estimation of the influence of a river sediment on the biological activity of oxytetracycline hydrochloride. Aquaculture **141**, 67-76.

Studies cited in Tables 18 & 19 (from Halling-Sorensen et al., 1998)

Björklund, H., Bondestam. J. and Bylund, G. (1990) Residues of Oxytetracycline in Wild Fish and Sediments from Fish Farms. Aquaculture. **86,** 359-367.

Brambilla, G., Civitarcale, C. and Migliore, L. (1994) Experimental toxicity and analysis of bacitracin, flumequine and sulphadimethoxine in terrestrial and aquatic organisms as a predictive model for ecosystem damage. Quimica analitica **13**, 114-118.

Ervik, A., Thorsen, B., Eriksen, V., Lunestad, B.T. and Samuelsen. O.B. (1994) Impact of administering antibacterial agents on wild fish and blue mussels *Mytilus edulis* in the vicinity of fish farms. Dis. Aquat. Org. **18**, 45-51.

Gavalchin, J. and Katz, S.E. (1994) The persistence of faecal-borne antibiotics in soil. J of AOEC international. **77**, 481-485.

Gool, S. van (1993) Possible environmental effects of antibiotic residues in animal manure. Tijdschrift voor Diergeneeeskunde (Netherland), 8-10. (in dutch) (summary in english)

Husevåg, B., Lunestad, B.T., Johannesen, P.J, Enger, O. and Samuelsen, O.B. (1991) Simultaneous occurrence of *Vibrio salmonicida* and antibiotic-resistant bacteria in sediment at abandoned aquaculture sites. ,J. of Fish Diseases. **14**, 631-640.

Kerry, J. (1995) Study of antibiotic resistance associated with the use of antimicrobial agents m fish farms. Ph.D. Thesis. National University of Ireland, Dublin.

Kerry, J., Hiney, M., Coyne, R., NicGabhainn, S., Gilroy. D., Cazabon, D. and Smith, P. (1995) Fish feed as a source of oxytetracycline-resistant bacteria in the sediment under fish farms. Aquaculture. **131**, 101-113.

Lunestad, B.T., Hansen, P.K., Samuelsen, O.B. and Ervik, A. (1993) Proceedings of the Euroresidue II Conference. Veldhoven, The Netherlands. 460-464.

Migliore, L., Civitareale, C., Brambilla, G., and Di Delupis, G.D. (1997) Toxicity of several important agricultural antibiotics to artemia. Water Research **31**, 1801 - 1806.

Nygaard, K., Lunestad. B.T., Hektonen, H., Berge J.A. and Hormazabal, V. (1992) Resistance to oxytetracycline, oxolinic acid and furazolidonc in bacteria from marine sediments. Aquaculture **104**, 31-36.

Oka, H., lkai, Y., Kawamura. N., Yamada, M., Harada, K., Ito, S. and Suzuki, M. (1989) Photodecomposition Products of Tetracycline in Aqueous Solution. J. Agric. Food. Chem. **37**, 226-231.

Pouliquen, H., Le Bris, H. and Pinault, L. (1992) Experimental study of the therapeutic application of oxytetracycline, its attenuation in sediment and sea water, and implications for farm culture of benthic organisms. Mar. Ecol. Prog. Ser. **189**, 93-98.

Pouliquen, H., Le Bris, H. and Pinault, L. (1993) Experimental study on the decomposition kinetics of seawater polluted by oxytetracycline contained in effluents released from fish farm located in a salt-march. Aquaculture **112**, 113-123.

Richardson, M.L. and Bowron, J.M. (1985) The fate of pharmaceutical chemicals in the aquatic environment - A review. J. Pharm. Pharmacol. **37**, 1-12.

Samuelsen, O.B., Lunestad, B.T., Husevlg, B., Holleland, T. and Ervik, A., (1992a) Residues of oxolinic acid in wild fauna following medication in fish farms. Dis. Aquat. Org., **12**, 11 l-l 19.

Samuelsen, O.B., Torsvik, V. and Ervik, A., (1992b) Long-range changes in oxytetracycline concentrations and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. Sci. Total. Environ., **114**, 25-36.

Samuelsen, O.B. (1989) Degradation of Oxytetracycline in Seawater at Two Different Temperatures and Light Intensities, and the Persistence of Oxytetracycline in the Sediment from a Fish Farm. Aquaculture **83**, 7-16.

Smith, P. and Samuelsen, O.B. (1996). Estimates of significance of out-washing of oxytetracycline from sediments under Atlantic salmon cages. Aquaculture **144**, 17-26.

Thomulka, K.W., McGee, D.J. and Lange, J.H. (1993) Use of the bioluminescent bacterium Photobacterium phosphoreum to detect potentially biohazardous materials in water. Bulletin of Environmental Contamination and Toxicology **51**, 538-544

8. Appendices

Appendix 1 (Schedule 3 to Marine Farming Licence)

SCHEDULE 3 TO MARINE FARMING LICENCE:

MARINE FARMING LICENCE CONDITIONS RELATING TO ENVIRONMENTAL

MANAGEMENT OF A FINFISH FARM

Conditions relating to the environmental management of finfish farms are in four parts:

1. Compliance with environmental standards
2. Requirements for Environmental Monitoring Survey(s)
3. Environmental records to be kept by licence holder
4. Environmental reports to be provided to the Department of Primary Industries and Water (DPIW)

In this Schedule, "the General Manager" means the General Manager, Primary Industries in DPIW or any person authorised to act on the General Manager's behalf.

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1 Compliance with Environmental Standards

The licence holder must comply with the following environmental standards in carrying out operations on the marine farming lease area or areas to which this licence relates (the Lease Area):

1.1 There must be no significant visual, physio-chemical or biological impacts **at or extending beyond 35 m from the boundary** of the Lease Area. The following impacts should generally be regarded as significant:

Visual impacts:

- Presence of fish feed pellets;
- Presence of bacterial mats (e.g. *Beggiatoa* spp.);
- Presence of gas bubbling arising from the sediment, either with or without disturbance of the sediment;

- Presence of numerous opportunistic polychaetes (e.g *Capitella* spp., Dorvilleid spp.) on the sediment surface.

In the event that a significant visual impact is detected at any point 35m or more outside the lease boundary, the licence holder may be required to undertake a triggered environmental survey.

Non-visual impacts:

- Physico-chemical:

  A corrected redox value which differs significantly from the reference site(s) and/or is < 0 mV at a depth of 3 cm within a core sample.

  A corrected sulphide level which differs significantly from the reference site(s) and/or is > 250 μM at a depth of 3 cm within a core sample.

- Biological:

  A 20x increase in the total abundance of any individual family relative to reference site(s).

  An increase at any compliance site of greater than 50x the total Annelid abundance at the reference site(s).

  A reduction in the number of families by 50 % or more relative to reference site(s).

  Complete absence of fauna.

(Note: As natural environmental variation renders some locations more susceptible to 'unacceptable' parameter values, the above thresholds will be considered in addition to baseline environmental information for determining the presence/absence of a significant impact)

1.2 There must be no significant visual impacts **within the Lease Area**. These include but are not limited to:

Visual impacts within Lease Area

- Excessive feed dumping;
- Extensive bacterial mats (e.g. *Beggiatoa* spp.) on the sediment surface prior to restocking;
- Spontaneous gas bubbling from the sediment.

1.3 Fallowed areas within the Lease Area shall not be restocked until, having regard to visual evidence, the sediments have recovered to the satisfaction of the General Manager.

1.4 The licence holder must comply with any written request from the General Manager specifying waste disposal actions for the purpose of mitigating against any effect on the ecology of the marine environment or nearby shoreline associated with marine farming operations including harvesting, processing of salmonids and the removal of fouling organisms.

1.5 All fish mortalities arising in connection with marine farming operations must be disposed of in accordance with relevant Acts and council by-laws.

1.6 The licence holder must ensure any predator control of protected wildlife (within the meaning of the *Wildlife Regulations* 1999) is conducted with the approval of the manager of the Nature Conservation Branch of the DPIW or any person acting on that person's behalf and in accordance with relevant seal interaction management protocols of the DPIW.

1.7 Where bird netting is deployed, the nets must be made of netting of a maximum 115mm square mesh and conform to visual management controls specified in the relevant Marine Farming Development Plan (MFDP).

1.8 The licence holder must ensure all aerial bird netting is maintained free of holes and remove any dead or entangled birds from the netting as soon as practicable.

1.9 Feeding of seals must not occur in any marine farming zone (within the meaning of the *Marine Farming Planning Act* 1995) or the Lease Area.

1.10 Baited trap lines or "tease lines" may only be deployed by an officer employed in the Nature Conservation Branch of DPIW or a person(s) who holds a permit to do so under the *Wildlife Regulations 1999*.

1.11 The licence holder must report any entanglement of protected marine species (including birds and mammals) to the Nature Conservation Branch contact officers as listed in the Seal Interaction management protocols.

1.12 Levels of antibiotics, or chemical residues derived from therapeutic use, present in sediments within or outside the Lease Area, are not to exceed levels specified to the licence holder by prior notice in writing by either the General Manager or the Chief Veterinary Officer, DPIW.

1.13 In the event that stock are to be treated with a therapeutant(s), the licence holder must advise the General Manager, and provide a copy of any medication authority specific to stock treatment that has been issued, prior to its commencement. The licence holder must comply with requirements to undertake any residue testing prescribed by the General Manager specific to a treatment event(s).

1.14 Bloodwater resulting from harvesting of produce must not be released into the marine environment unless it has been managed in accordance with the requirements of the Chief Veterinary Officer DPIW, as specified in Schedule 1.

1.15 The threshold levels listed in the following table must not be exceeded within the Lease Area, by reason of the conduct of marine farming operations in the Lease Area.

Contaminant	Sediment (mg/kg dry wt)	Water Column (µg/L)
Copper	270	1.3
Zinc	410	15

1.16 The Licensee must take all reasonable steps to ensure that no dead fish of the species authorised by this licence are found on the Lease Area outside cages.

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2 Requirements for Environmental Monitoring Survey(s)

2.1.1 The licence holder must undertake environmental monitoring surveys in accordance with the requirements of Schedule(s) 3B and/or 3V to this licence, and duly report on those surveys in accordance with those Schedules.

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3 Environmental records to be kept by the Licence holder

The following records shall be kept by the licence holder for a period of five years and unless otherwise determined, reported to the General Manager on an annual basis.

3.1 A list specifying the quantities, and date of use, of all chemicals which have been used on the Lease Area. This includes, but is not confined to, therapeutants, anaesthetics, antibiotics, hormones, pigments, antifoulants, disinfectants and cleansers.

3.2 Records, on a monthly basis, of the stock biomass within the Lease Area, and of the type, origin and dry weight of food placed into the Lease Area .

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4 Environmental reports to be provided to the Department

Renewal of annual licence(s) will be subject to compliance with all environmental reporting requirements. Where relevant the reporting of information to the General Manager is to be made by phone (62 33 3370) or electronically (e-mail: mfarming.environmentdpiw.tas.gov.au).

4.1 The licence holder must report any suspected or known incidents of disease or mortality affecting > *0.25 %* of fish per day for three consecutive days in any individual cage. Such reports are to be provided as soon as possible to the DPIW assigned fish veterinarian or an inspector under the *Animal Health Act 1995*.

4.2 The licence holder must notify the General Manager in writing of the presence of any unusual or uncharacteristic marine flora or fauna found within the Lease Area (including any introduced marine pests). (e-mail: mfarming.environment@dpiw.tas.gov.au)

4.3 Reports of any incidents of spontaneous outgassing are to be immediately reported to the General Manager.

4.4 An electronic copy of the records to which clause 3.2 refers is to be provided to the General Manager in January each year. (e-mail: mfarming.environment@dpiw.tas.gov.au)

4.5 The licence holder must report to the General Manager any significant incident of fish escapes within 24 hours of becoming aware of the escape. A significant escape is defined as any loss of licensed species to the marine environment in excess of 1000 individuals at any one time. (e-mail: mfarming.environment@dpiw.tas.gov.au)

4.6 The licence holder must immediately report to the manager, Nature Conservation Branch, DPIW any incidence of mortality in protected wildlife (within the meaning of the *Wildlife Regulations* 1999) which arises in connection with the marine farming operations to which this licence relates. (Phone: 6233 6556 or e-mail: NatureConservationEnquiries@dpiw.tas.gov.au)

4.7 The licence holder must give prior written notice to the General Manager of any proposal to move marine farming equipment from any Marine Farming Development Plan (MFDP) area to another for the purpose of the deployment of that equipment in that MFDP area. (e-mail: mfarming.environment@dpiw.tas.gov.au)

Appendix 2 (Summary of Workshop Outcomes)

- document prepared by Tasmanian Salmon Growers Association (TSGA) & Tasmanian Department of Primary Industries and Water (DPIW).

Summary Of Meeting (26th & 27th Feb 2009) To Discuss The Draft Report On The Scoping Study "Ecological Impacts of Selected Antifoulants & Antibiotics"

Meeting Purpose

The purpose of the meeting was to identify the priorities in filling the knowledge gaps identified in the scoping study; *A Review of the Ecological Impacts of selected Antibiotics & Antifoulants Currently Used in the Tasmanian Salmonid Farming Industry* - C. McLeod & R. Eriksen, February 2009. It was recognised that the knowledge gaps constraining effective environmental monitoring and future registration of selected antifoulant & antibiotic products overlapped the knowledge gaps identified by the Scoping Study. Subsequent to this broad prioritisation exercise the industry in consultation with the peak regulators will determine the work program, based on risk-analysis and with an eye on cost-effectiveness.

Participants at the workshop sessions included State and Federal regulators, researchers and industry representatives from the following organisations:

- Australian Pesticides and Veterinary Medicines Authority (APVMA),
- Australian Department of Environment Water Heritage and the Arts (DEWHA),
- Tasmanian Department of Primary Industries and Water (DPIW),
- Tasmanian Department of Environment Parks Heritage and the Arts (DEPHA),
- Tasmanian Aquaculture and Fisheries Institute (TAFI),
- CSIRO Marine and Atmospheric Research (CMAR),
- CSIRO Centre for Environmental Contaminants Research (CECR),
- Tasmanian Salmonid Growers Association (TSGA).

There was a consensus amongst workshop participants on the key themes and priorities identified in the proposed work plans.

Scoping Study - Antifoulant section

Focus – To discuss & prioritise knowledge gaps in managing the ecological effects of antifoulant paints as identified in the Scoping Study, with a view to generating suitable data to secure product registration.

There was agreement that further research and monitoring work was required in following theme areas:

- Spatial variability assessment;

- Sediment residues (particle/flake size);
- Leaching rates;
- Bioavailability and Pore water;
- Eco Toxicity;
- Ecological effects and Bioaccumulation; and
- In Situ Cleaning.

The sequencing of research and monitoring work in these areas was acknowledged to be critical. It was agreed that in certain areas a first step would be a desktop study to identify the following:

- where data in comparable contexts was available which could be applied to revise values and confirm or establish data; and
- what established research/monitoring techniques exist to improve of risk assessment.

Scoping Study - Antibiotics Section

Focus - to discuss and prioritise knowledge gaps in managing the ecological effects of antibiotics as indentified in the scoping study.

Industry is currently operating under Minor Use Permits and indicated it is not seeking full Registration of the three antibiotic products currently in use, largely because of the cost and effort involved. Industry indicated that the rates and level of use of antibiotics are declining due to the development of vaccines that will combat the most prevalent diseases. Declining use of antibiotics would, paradoxically, reduce industry's ability to generate data for registration.

The APVMA however, encouraged the industry to pursue the full registration process for the antibiotics currently in use. The main concern is that industry may have to increase the use of antibiotics in the future to treat a disease that is not currently prevalent in salmonids.

Action Plan

General

The TSGA indicated that monitoring and research required for regulatory compliance purposes would commence as per agreed priorities. Knowledge gaps that cannot be directly linked to compliance requirements and/or industry needs would be unlikely to attract industry funding.

DPIW supported desktop studies in the areas proposed as exercises which would assist in identifying whether existing levels of use, and the associated consequences of a given level of use, were significant.

Work plans

Antifoulant knowledge gaps (Refer to Figure 1)

Antibiotic knowledge gaps (Refer to Figure 2)

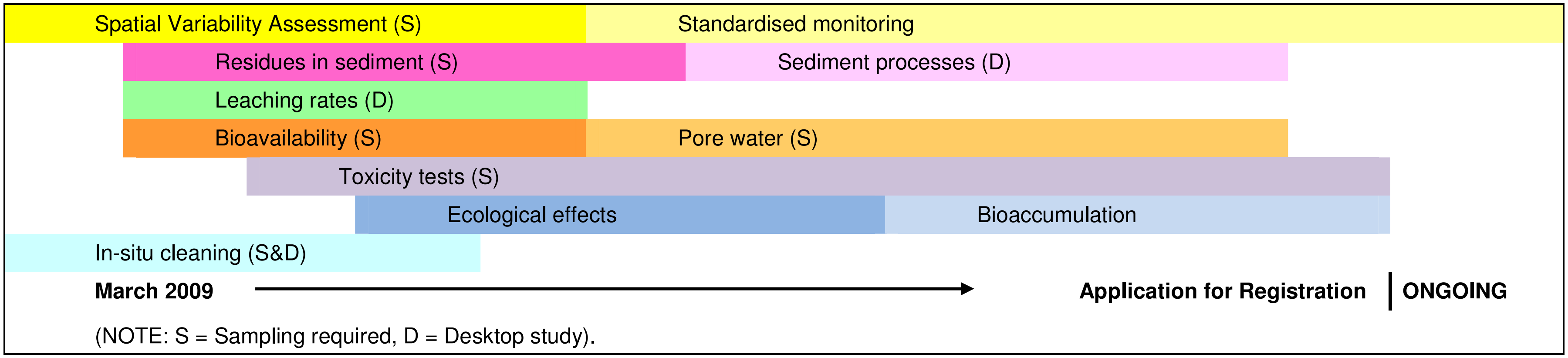

Figure 1. Proposed work plan for the Antifoulant program

Notes:

Spatial Variability Assessment (SVA)
- High priority, work commenced in early March. Will lead to standardised monitoring protocols.

Residues in sediment
- High priority, characterisation of Cu in sediments will resume in April. Will lead to standardised monitoring protocols.

Leaching rates
- Medium priority, but required for full Registration. Will be addressed largely by desktop study.

Bioavailability
- High priority. Bioavailability experiments to be designed, work may commence by May. Depending on preliminary results from "Residues" work, role of Pore-water in Cu bioavailability will be investigated to a greater or lesser extent.

Toxicity tests
- Medium priority, but required for full Registration. Given the complexity of dealing with field samples of known toxicity, it may be necessary to carry out work in mesocosm, extrapolated by modelling; details yet to be designed.

Ecological effects
- Comprises a suite of specific knowledge gaps to be addressed, of medium to high priority, but required for full Registration. Depending on preliminary results, may entrain work on bioaccumulation.

In situ cleaning
- High priority, investigation as required is underway.

ANTIBIOTICS

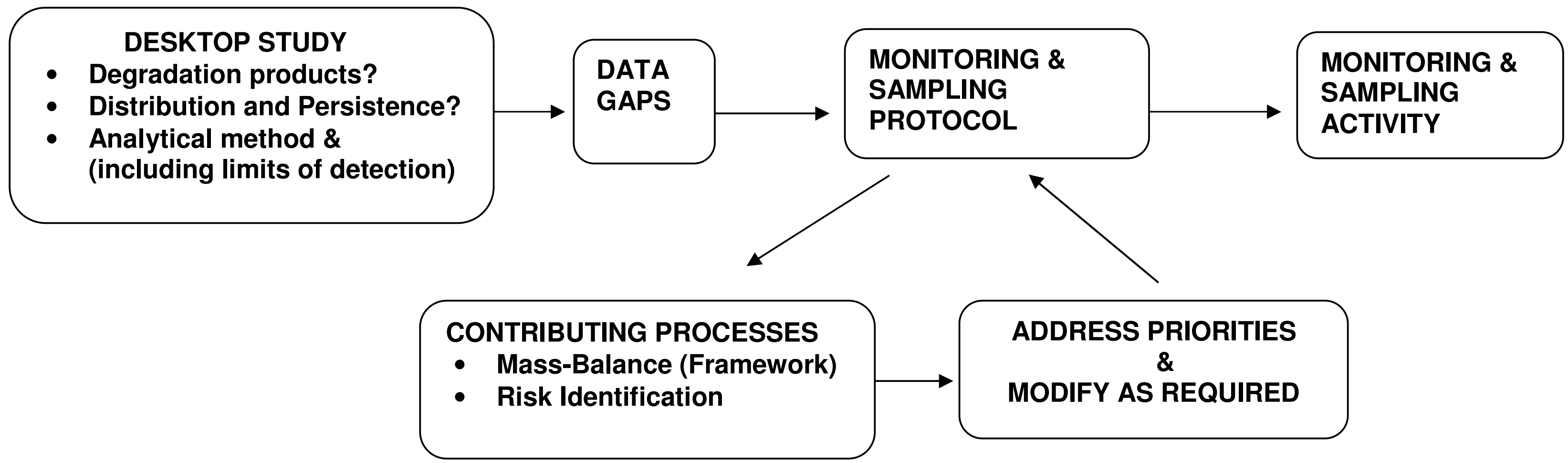

Figure 2. Proposed work plan for the AB monitoring program.

Notes:

Desktop Study

- This study will be commissioned during 2009, and will address the knowledge gaps identified as being of medium to high priority, and required for full Registration.

Contributing processes

- A conceptual framework to clarify the fate of antibiotics in the environment will be developed.
- Risk identification will be an iterative process undertaken in consultation between Industry, APVMA, and TasDPIW, the outputs of which are expected to modify ongoing monitoring and sampling.

Monitoring and Sampling Protocol, and Activity

- Informed by previous stages in the process, and modified as appropriate.