Coastal assimilative capacity for amalgamated fish farm chemicals/organic inputs

Final Report, April 2008

SARF contract #009

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Abstract

Intensification of fish farming activity and the amalgamation of farm units has the potential to cause an increased risk of organic and chemical stress on the local environment. Although the amalgamation of fish farm units into a fewer number of larger operations may offer some operators greater financial efficiency, concern exists over whether the discharge of organic matter (waste feed and faeces), sea lice chemical treatments and other synthetic organic compounds from large scale operations may have a greater environmental impact than an equivalent use of such compounds at several smaller farm units. To address this concern, it is necessary to assess how fish farm amalgamation can be implemented in an environmentally sustainable manner. This is an issue facing the entire fin fish marine aquaculture industry to ensure that it can effectively deliver on its obligations to the Strategic Framework for Scottish Aquaculture (Scottish Executive, 2003). Here, we establish the relationship between farm size and zone of impact using a field survey before establishing highly controlled replicated mesocosm studies aimed at establishing the relationship between synthetic organic compounds, including sealice treatment chemicals, and the functioning of the coastal benthos. These experiments avoid the problems associated with confounding and covarying factors in the field and form the basis for numerical simulations that predict how fish farm amalgamation may impact the environment. Should the mechanisms identified in our model systems operate in the real world, the results could be used to constructively inform policymakers on how to design amalgamated fish farms where impact exceeds current practice.

1.0. Research Objectives

It is already well established that excessive loading of coastal sediments with fish farm effluent and organic-rich wastes may, in certain circumstances, cause detrimental effects in terms of reductions to faunal and microbial community composition (abundance, biomass, diversity) and function (biogeochemical cycling). Research is required to examine how the scale and intensity of fish farming affects the spatial extent and magnitude of impacts on the underlying and surrounding sediments.

The ecotoxicological impact of fish farm chemicals has only been addressed for single approved substances. However, any attempt to assess the impact of amalgamated fish farms must consider the combined impact of multiple substances, against an appropriate ambient background (i.e. an organic detrital loading which establishes a realistic biological oxygen demand).

Attempts to date to use bioindicators, biosensors and biomonitors to assess toxic impacts of fish farm chemicals have focused on the use of highly controlled synthetic assemblages consisting generally of one species and invariably only one trophic level. However, it is known that species within a community interact with one another which may result in responses not predictable from the simple summation of individual responses. This report seeks to use multi-trophic systems in replicated mesocosms that will allow the effects of organic inputs alone and in combination to be quantified across a gradient of species richness.

The following objectives form six key hypotheses that form the basis for the research presented:

1) The gradient of environmental impact of fish farms does not depend on farm size

The first objective was to establish the footprint of impact of contrasting sized farm units using state-of-the-art sediment profile imaging (SPI) technologies, ecotoxicological screening of sediment and water samples, and geostatistical interpolation. This approach does not necessitate studying an amalgamated farm in a specific location because it unequivocally establishes the relationship between farm size and zone of impact.

2) The magnitude of environmental impact of fish farms does not depend on farm size

The second objective will be to build on objective 1 by using a validated mesocosm approach that explicitly tests the level of ecosystem response to a wide range of discharge concentrations of sea lice treatment chemicals and other organic pollutants. This establishes a predictive basis because these relationships can be investigated using standard regression procedures.

3) The impact of fish farm effluents is dependent on the number of species present

The third objective will be to take representative faunal samples from along the gradient of farm impact and to establish mesocosm treatments comprising simple multitrophic assemblages (micro-organisms, invertebrates) around a basal resource. This statistically robust approach enables the establishment of the relationship between trophic complexity, biodiversity and ecosystem function.

4) The impact of fish farm effluent can be predicted by the simple summation of the toxic effects of individual contaminants

The fourth objective will be to use an ecotoxicological approach using a dose response investigation with single and multiple chemotherapeutic treatments used in fin fish aquaculture. The advantage of this approach is that it allows ecotoxicity to be assessed at levels (microbial, macro-invertebrates) integral to ecosystem health. In addition, this approach explicitly recognises that a combination of contaminants may not necessarily result in the same ecological impact as that predicted from the summation of the toxic effects of multiple contaminants investigated in isolation.

5) The impact of fish farm effluent is independent of location

The fifth objective explicitly recognises that the environmental impact of a poorly sited farm may significantly differ to that of a farm sited at an alternative location. Using the approach adopted for hypothesis 3 (above), specific levels of impact can be assessed for contrasting species assemblages that reflect alternative benthic communities.

6) The ecosystem response to commercial fish farming activity cannot be predicted

The sixth objective will involve derivation of the empirical relationship between scenario of fish-farm associated impact and ecosystem response. This will enable us to parameterise numerical simulations that predict how fish farm amalgamation is likely to impact on the environment and hence how to design amalgamated fish farms where impact exceeds current practice.

1.1. Objective delivery

All objectives have been successfully achieved, although some amendments were necessary as the project progressed and are detailed here.

Objective 1: We successfully performed a survey of 6 independent fish farm locations with the Sediment Profile Imaging (SPI) camera and present some example images in the report. Our primary objective was to generate geo-interpolated maps of the benthic habitat based on these data but the coarse sandy sediments at the highly dispersive sites (larger farms) and the high density of mussel shell debris (most farms) either prevented camera penetration or, in the case of the mussel debris, destroyed the sediment-water interface and profile on penetration. Nonetheless, these surveys provide important information on the suitability of SPI for monitoring fish farming impacts. Additional data not related to the SPI that were collected during these sampling campaigns were used in Objective 6.

Objectives 2-5: Difficulties with access permissions to active fish farms meant that our original intention of using species from specific farm locations was not possible. Although unfortunate, this had no impact on the delivering the grant objectives because the approach we adopted did not depend on the availability of named species as it explicitly tests the relationship between the number of species and ecosystem responses.

Objective 4: This objective aimed to use ecotoxicity testing of a number of fish farm chemotherapeutants for different trophic levels (invertebrates and fish-flounder assays). Following the ecotoxicity testing of the invertebrates, little further information would be gained by testing the response of flounder to each pollutant and it was agreed not to proceed with this aspect of the experimentation.

Objective 6: Our original intention was to perform a separate analysis of the entire dataset, however consultations with statisticians confirmed that this was not necessary as the statistical modelling framework adopted (mixed modelling) already provides a predictive framework. Further, benthic monitoring data from the Scottish Environment Protection Agency's (SEPA) national archive (n = 36, maximum consented biomass (MCB) range: 150 to 1999 t) was made available to us and, together with the data set collected from our own fish farm survey (n = 5, MCB range: 120 t to 2100 t) we were able to examine the factors (including farm size) affecting patterns of sediment chemistry at the national- and regional-scale respectively.

2.0 Introduction

The global aquaculture industry now contributes almost 50 % to the world's total food fish, and FAO projections indicate that the farming of aquatic biomass must double by 2050 to sustain the current level of per capita consumption (FAO, 2002; FAO, 2006). The development and intensification of the global Atlantic salmon industry, however, have led to periodic outbreaks of sea lice and disease causing micro-organisms in almost all regions of the world (Roth, 1993). Minor infestations cause stress to the fish host, leading to secondary infections from microbial pathogens and reduced marketable value. Major infestations ultimately cause mortality if not controlled (Pike and Wadsworth, 1999), costing the industry more than US $100 million annually (Johnson et al., 2004). Intensive rearing of fish typically relies on the periodic administration of chemotherapeutic treatments to control parasitic and microbial infestations, although alternative methods including vaccines (Raynard et al., 2002), selective breeding of lice-resistant strains (Jones et al., 2002), and the use of sea lice pathogens and cleaner fish (Treasurer, 2002) are available in some instances. Commercial applications to treat sea lice-infested salmon include Salmosan®, Excis® and Slice®. Tetraplex® and Aquatet® are commonly applied to treat and control a range of bacterial infections including *Aeromonas* and *Vibrio* spp. (Roth, 2000; Costello et al., 2001; Grant, 2002). The active ingredients of these treatments are presented in Table 1.

Commercial brand name	Active ingredient	Usage
Various	Copper (Cu)	Antifoulant
Excis	Cypermethrin, (CP), 1 % w/v	Sea lice treatment
Tetraplex / Aquatet	Oxytetracycline hydrochloride (OTC), 50 & 100% respectively	Anti-microbial / sea lice treatment
Salmosan	Azamethiphos (AZ), 50 % w/w	Sea lice treatment
Slice	Emamectin benzoate (EB), 0.2 % w/w	Sea lice treatment

Table 1: Some treatments and their active ingredients periodically applied on Atlantic salmon fish farms.

Salmosan® and Excis® are both for topical use in an enclosed bath, whereas Slice® and Tetraplex®/Aquatet® are pre-mix powders that are incorporated into the fish feed. Azamethiphos (AZ) and oxytetracycline (OTC) are both highly water-soluble (Weis et al., 1957; Worthing, 1991), and are not expected reach the sediments in detectable concentrations during normal commercial applications. In contrast, both cypermethrin (CP) and emamectin benzoate (EB) have low solubility in seawater and relatively high octanol:water partition coefficients (Worthing, 1991; Zhou et al., 1995; SEPA, 1998; 1999; SPAH, 2002). These physiochemical characteristics mean that they quickly bind to particulate material, and may therefore be expected to reach the sediments underlying salmon farms following their application. Salmon have a trace dietary requirement for copper, and their feed is often supplemented to ensure that it is in excess (e.g. Lorentzen et al., 1998). Salmon faeces therefore contain quantities of copper (Dean et al., 2007), much of which ultimately reaches the sediments beneath the farms. Copper is also the active ingredient in the majority of anti-fouling products that are licensed for use (e.g. SEPA, 2005a), and in practice are usually reapplied up to two times a year (Solberg et al., 2002). Over time these treatments flake-off and slowly leach copper into the surrounding ecosystem (Solberg et al., 2002; Brooks & Mahnken, 2003; Braithwaite et al., 2007) potentially leading to elevated levels of copper in the underlying sediments (Solberg et al., 2002; Dean et al., 2007). In the case of sea lice treatments, European Union regulations necessitate that information on the quality, safety, efficacy, and often the environmental impact of any chemotherapeutic agent released into the aquatic environment must be provided before a marketing authorisation is issued. Additional ecotoxicological studies may also be required before the regulator can determine a safe EQS and subsequently licence the material (Costello et al., 2001; Grant, 2002). Organisms used in routine acute toxicity studies include the crustacean *Corophium volutator* and the polychaete *Hediste (Nereis) diversicolor* (Traunspurger & Drews, 1996). Some investigations into the impacts of the commonly applied treatments used by the global fin fish aquaculture industry on the target and some non-target organisms have been published in the scientific literature (e.g. Ernst et al., 2001; Burridge et al., 2000; Willis and Ling, 2003, 2004; Burridge et al., 2004; Willis et al., 2005; Canty et al., 2007; Waddy et al., 2007). However, much of the ecotoxicological information for chemotherapeutic treatments exists in confidential technical reports which can be difficult to obtain (Crane et al., 2006). Furthermore, different test organisms, experimental protocols and reporting procedures make comparison of the various compounds/studies difficult. Experiments designed to determine the concentration of a particular compound that causes 50 % mortality in a test organism (LC50) are often determined using water-borne trials. Copper and various medicinal compounds are detectable in the sediments beneath fin fish farms, illustrating the need for trials in which sediment dwelling organisms are exposed to contaminated sediments.

The use of antifoulant paints and medicinal treatments to remove biological pests (Costello et al., 2001) by the intensive fin-fish aquaculture industry means that, ultimately, they are released into the natural environment, along with uneaten feed pellets and faecal material. The resulting degradation of the benthic environment is well documented (see Pearson & Black, 2001 and refs therein), and concerns over the sustainability of this expanding industry have been raised (Fernandes et al., 2000; Costello et al., 2001; Haya et al., 2001; Mente et al., 2006). Accumulation of organic material on the seabed significantly increases the sediment community's oxygen demand (e.g. Kelly & Nixon, 1984), which directly affects benthic community structure (Pearson & Rosenberg, 1978; Nilsson and Rosenberg, 2000; Rosenberg et al., 2001). It is well known that the burrowing and ventilatory activities of benthic macrofauna increase sediment oxygen concentrations, thereby increasing the assimilative capacity of the system through nutrient cycling and the removal of organic matter (Blackburn & Blackburn, 1993; Sloth et al., 1995; Hansen & Kristensen, 1997; Heilskov & Holmer, 2001; Heilskov et al., 2006; Papaspyrou et al., 2006; Cuny et al., 2007; Norling et al., 2007). In situations where the input of organic material to the sediments is low it is oxidised, liberating ammonium. This is subsequently oxidised to nitrate at the aerobic sediment surface. Some of this nitrate will diffuse to lower anoxic layers where it is denitrified. In organically enriched sediments, the high chemical and biological oxygen demand causes a progressive movement of anoxic conditions towards the sediment surface. The concomitant decrease in the depth of the oxic layer reduces the potential for the system to nitrify, and thus denitrify (Blackburn & Blackburn, 1992). The turnover of organic matter is retarded, and, depending upon prevailing hydrographic conditions, may have a tendency to accumulate under these conditions (Pearson & Black, 2001).

The settlement and growth of unwanted organisms on fish cages and other permanently submerged installations increases the structural stress and reduces water exchange in fish pens (Braithwaite et al., 2007). Removing fouling organisms is costly in both time and man power (Hodson et al., 1997), and a range of copper-based antifoulant products are commonly applied as an effective means of reducing the necessity for net cleaning (SEPA, 2005a; Braithwaite et al., 2007). Increased concentrations of dissolved copper can be found in close proximity to treated surfaces, and periodic in situ cleaning of the nets with brushes and high pressure water jets also releases dissolved and particulate copper into the immediate environment (Solberg et al., 2002; Brooks & Mahnken, 2003; Dean et al., 2007). Salmon feed pellets and faeces also serve as vectors through which other substances reach the sediments underlying fish farms. Animals require copper for a range of physiological functions, and suffer reduced fitness when fed a copper deficient diet (O'Dell, 1982; Davis & Mertz, 1986). The feed pellets offered to salmon contain copper in excess of their dietary requirements, resulting in faeces that contain significant quantities of this metal (Dean et al., 2007). Outbreaks of sea lice, which are currently estimated to cost the global salmonid farming industry in excess of US$100 million annually (Johnson et al., 2004), are often addressed with in-feed treatments such as ‘Slice®’. This treatment is administered to infected salmon in Scotland, Norway, Chile and Canada (Roth, 2000). The active ingredient of Slice®, emamectin benzoate (EB), and its breakdown products, reaches the seabed via uneaten feed pellets and the egestion of EB-containing faeces, where it binds tightly with sediment particles due to its low water solubility and high octanol-water partition coefficient (Mushtaq et al., 1996; McHenery & Mackie, 1999).

Anti-sea lice treatments, such as Slice®, and copper-based antifoulants are widely used around the world, and their active ingredients are often detectable in the sediments beneath salmon farms where they are administered (Solberg et al., 2002; SEPA, 2004a; 2004b; 2005b; 2006; Telfer et al., 2006; Dean et al., 2007). Both of these materials are known to be capable of affecting non-target organisms (e.g. SEPA, 1999), and their presence in the natural environment may impair benthic ecosystem processes such as nutrient cycling. Indeed, sediments contaminated with approximately 200 μg copper [kg dry sediment]$^{-1}$ are reported to have significant, negative effects on the short-term (7 days) bioturbation activities of the polychaete *Nereis (Hediste) diversicolor*, although acclimation is thought to remove any such effect over longer periods of time (> 14 days; Fernandes et al., 2006). Such a sediment concentration of copper is, however, orders of magnitude lower than levels typically occurring in close proximity to fish farms (Dean et al., 2007; present study). Very little is known about the effects of environmentally-relevant concentrations of fish farm chemical effluents on nutrient cycling in marine sediments.

3.0 Results

3.1. Gradient of environmental impact of fish farms

Sediment profile imaging (SPI) methods were used to image the upper portion of the sediment profile in relation to spatially distributed ecological stress and/or disturbance. We used a static image SPI camera system (Rhoads and Cande, 1971; Figure 1).

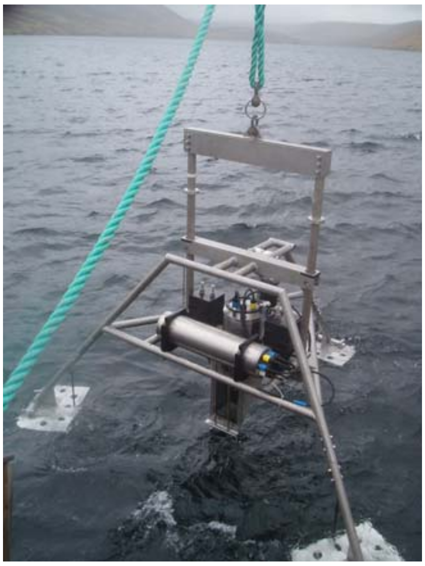


Figure 1: Sediment profile imaging camera used in the field surveys.

This system consists of two parallel stainless steel support frames and an imaging module. The inner support frame incorporates the imaging module and an adjustable weighting system that facilitates deeper prism penetration when a harder substratum is encountered. The outer support frame holds a hydraulic piston system used to control the descent of the inner support frame and the supporting legs. The imaging module is housed horizontally above a wedge-shaped prism at the base of the inner support frame and incorporates a 14 megapixel Kodak single lens reflex digital camera fitted with a modified internal flash unit. The back plate of the prism contains a mirror mounted at a 45° angle to reflect the profile of the sediment-water interface up to the camera. The dimensions of the prism face plate allow a maximum visible profile area of 486 cm^2 (18 x 27 cm) to be imaged.

Benthic surveys were conducted at 5 active fish farms within a region of Scotland in April 2006. Exact locations are not provided in agreement with farm operator wishes. All farms have a history (≥ 5 yrs) of farming Atlantic salmon, *Salmo salar*, and the 3 largest were rearing Atlantic cod, *Gadus morhua*, on site at the time of survey. The MCB of salmon permitted at these farms were 120, 275, 980, 1500 and 2106 t. For cod, at the latter 3 sites, MCB levels were 657, 990 and

1411 t respectively. These are calculated assuming that the total discharge of nitrogenous waste derived from cod farms is 1.5 times greater than that from equivalent salmon farms (Gillibrand et al., 2002). Two sampling transects were established at each site, either in line with, or perpendicular to the predominant current direction (Transects A & B respectively).

Sediment profile imaging (SPI) was tried and found not to be universally applicable because of the differing physical nature of the sediments. Representative SPI images are presented for completeness (Figures 2-6). Penetration of the SPI prism was poor in all locations and, coupled with problems of mussel shell aggregations and high light levels (shallow waters). Nevertheless, these surveys provide important information on the suitability of SPI for monitoring fish farming impacts. It is clear, for instance, that the utility of SPI is limited to muddy-sandy sediments rather than coarse grained sandy sediments typical of highly dispersive environments. Aggregations of dead mussel shells (resulting from anti-biofouling measures) were a significant problem, as were shell fragments that effectively increased the mean particle size of the sediment, impeding penetration of the SPI at many of the farm locations. In areas that lacked mussel shell accumulations, image quality was sufficient to see changes in sediment structure, but site specific differences in the spatial distribution of farm discharge led to a mosaic distribution rather than a linear gradient of disturbance. It was therefore not possible to credit such changes to increasing distance from the farm.

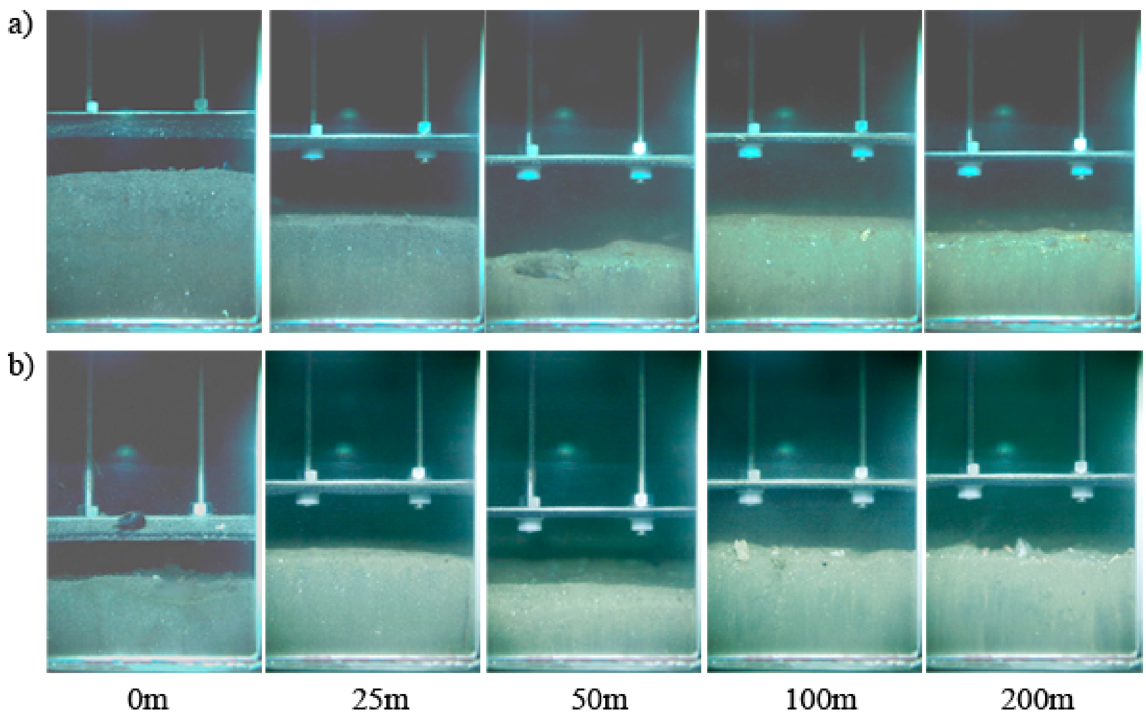


Figure 2: Representative images for Farm 1 (120 t MCB) at increasing distances along the sampling transects (a) in line with, or (b) perpendicular to the predominant current direction.

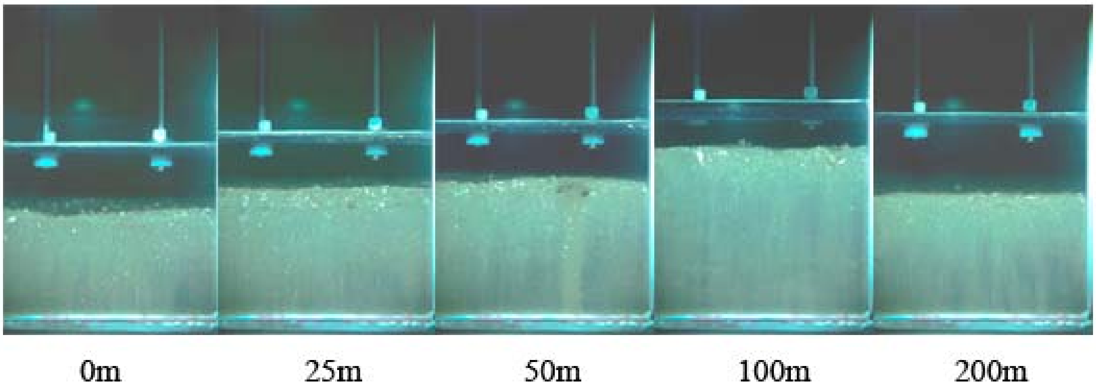


Figure 3: Representative images for Farm 2 (275 t MCB) at increasing distances along the sampling transect in line with the predominant current direction.

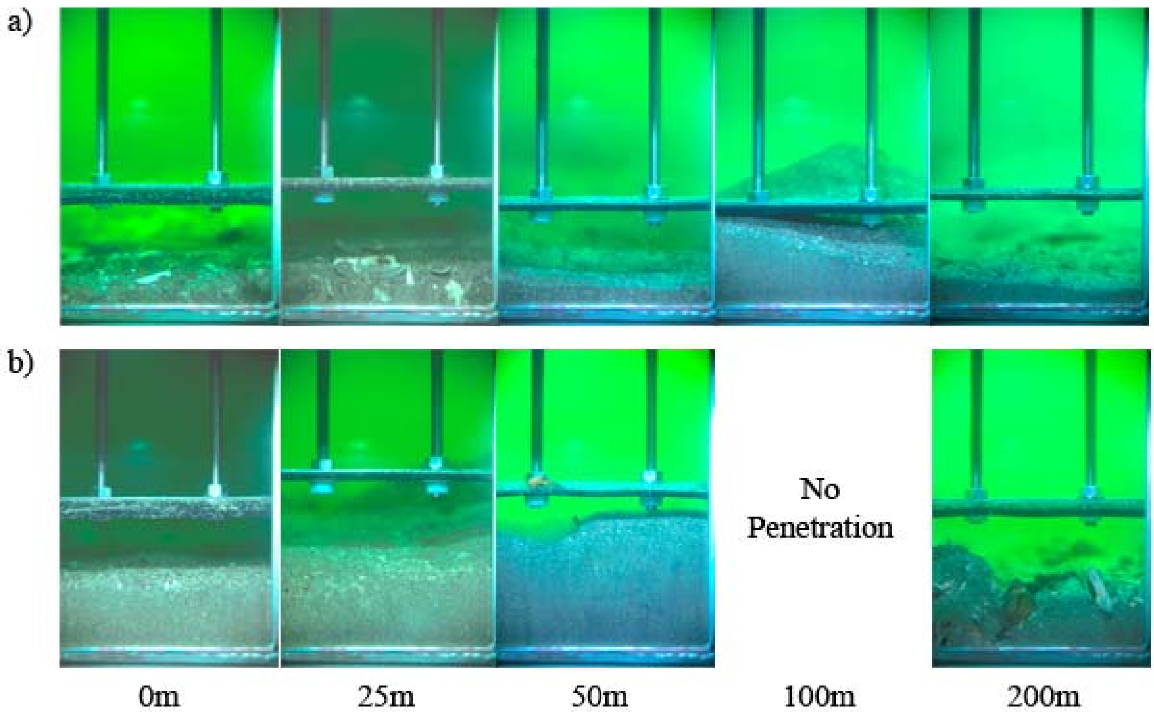


Figure 4: Representative images for Farm 3 (980 t MCB) at increasing distances along the sampling transects (a) in line with, or (b) perpendicular to the predominant current direction.

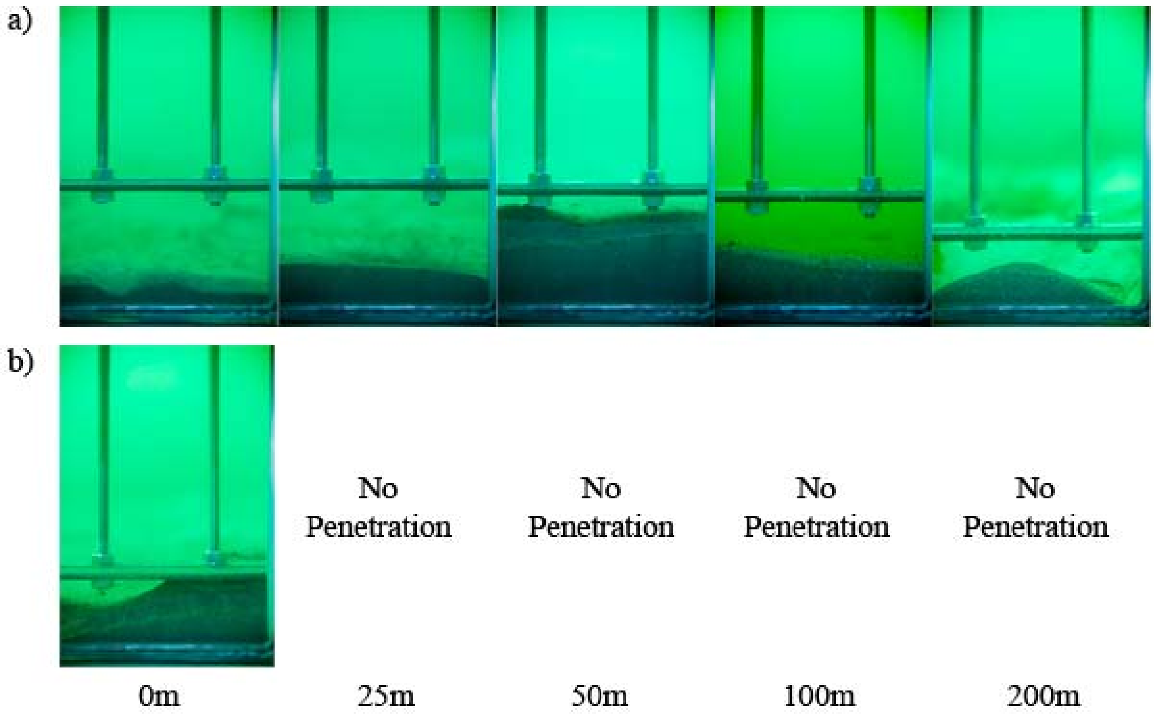


Figure 5: Representative images for Farm 4 (1500 t MCB) at increasing distances along the sampling transects (a) in line with, or (b) perpendicular to the predominant current direction.

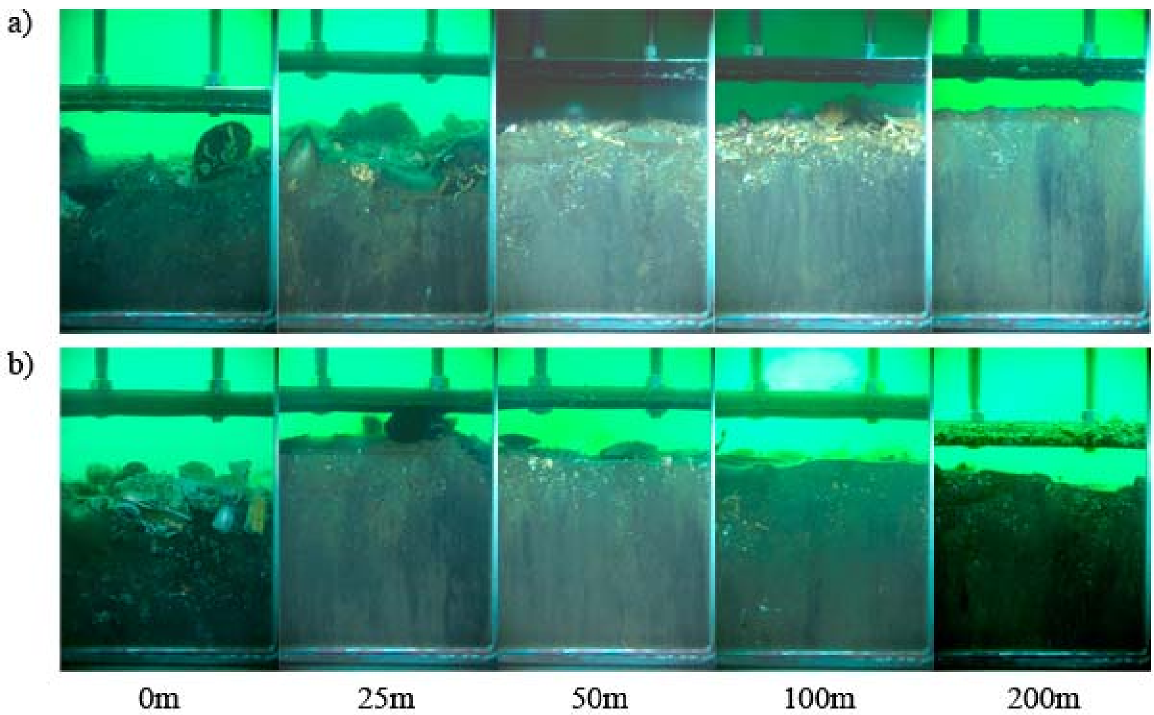


Figure 6: Representative images for Farm 5 (2106 t MCB) at increasing distances along the sampling transects (a) in line with, or (b) perpendicular to the predominant current direction.

3.2. Magnitude of environmental impact of fish farms

A series of whole sediment bioassay tests that determined the LC50s for the non-target benthic crustacean, *Corophium volutator* exposed to EB, OTC, AZ, CP and copper are presented. Additional LC50 experiments with the non-target polychaete worm, *Hediste (Nereis) diversicolor* exposed to EB and copper spiked sediments are also presented.

3.2.1. Study site and sample collection

Sediment, macrofauna and seawater were collected from the Ythan estuary, Aberdeenshire, NE Scotland (OS central grid ref: NK020275; 57º 20.085'N, 02º 117 0.206'W). The estuary does not receive heavy metal or pesticide contamination of any significance (Gorman and Raffaelli, 1993) and is designated a Site of Special Scientific Interest within the Forvie National Nature Reserve. *Corophium volutator* were collected from the upper 5 cm of sediment through a 1 mm sieve and subsequently transferred to a bucket containing fresh seawater. *Hediste diversicolor* were collected from the estuary by careful hand-digging. All experimental animals were returned to the laboratory within 2 hrs and acclimated in fresh, aerated seawater for 24 hrs prior to incubation. Experimental sediments were gathered by hand from the upper 3 cm of the estuary bed and collected in 25 l plastic buckets for transportation to the laboratory. Macrofauna and large organic debris were later removed by gently sieving with a 500 μm sieve. The resulting sediment was stored with fresh seawater in a 200 l bath and allowed to settle overnight. Ythan estuary sediments contain approximately 4 % organic carbon (Ieno et al., 2006).

3.2.2. Dosing of sediment with chemotherapeutic treatments

The settled sediment was homogenised by stirring before six 7 kg sediment samples were transferred into 25 l buckets. Five of these were subsequently dosed with an increasing concentration of chemotherapeutic treatments and stirred until completely homogenised. The sediments in the remaining bucket were used in the control treatment. Excis®, Salmosan® and Aquatet® were purchased from Fish Vet Group, Inverness, Scotland. Slice® was provided courtesy of J. McHenery, Schering-Plough. All the medicinal compounds were stored at 4 °C. Copper (II) sulphate pentahydrate (Fisher Scientific UK) was used to spike the test sediments with copper (Bat & Raffaelli, 2006). All test concentrations are reported as nominal values and expressed in μg active ingredient [kg wet sediment]$^{-1}$. The nominal concentrations for each active ingredient (Table 2) were chosen to ensure that low and high mortality would occur at dose 1 and 5 respectively (Davies et al., 2001; Ernst et al., 2001; Bebak-Williams et al., 2002; Maund et al., 2002).

	Active ingredient (µg [kg wet sediment]$^{-1}$)				
Treatment	Copper ($CuSO_4$)	Cypermethrin (Excis®)	Oxytetracycline (Aquatet®)	Azamethiphos (Salmosan®)	Emamectin benzoate (Slice®)
Control	0	0	0	0	0
1	30170 (50000)	0.1	10	10	1
2	90510 (150000)	0.5	100	100	10
3	181020 (300000)	5	1000	1000	100
4	301700 (500000)	50	10000	10000	1000
5	603400 (1000000)	500	100000	50000	10000

Table 2: Nominal concentrations of active ingredients homogenised into the sediments at the start of the *Corophium volutator* and *Hediste diversicolor* 10-day sediment bioassays. In all cases, the parent compound was added to the mesocosms. Dry weight nominal concentrations of copper are presented in parentheses.

For treatments containing Slice®, 5 g of a biologically inert powder (talc) was used to facilitate the dosing of the medicine into the sediments. This procedure was repeated in the absence of Slice® in the corresponding control sediments. Copper dosage was initially established on a dry weight basis, i.e. µg Cu [Kg dry sediment]$^{-1}$. The water content of the experimental sediment was determined (39.66 ± 0.12 %, $n = 9$), and used to derive wet weight doses. Copper (II) sulphate pentahydrate was initially dissolved in fresh seawater (UV-sterilised, 10 µm pre-filtered, salinity 33) and then homogenised into the experimental sediments. Excis® was suspended in a polar solvent (acetone) before being added to the sediments. Dosed sediment and seawater were added to each mesocosm 24 h prior to addition of invertebrate species to allow time for the solvent to evaporate.

3.2.3. Whole-sediment bioassays

A total of 210 mesocosms were assembled to examine the effects of increasing concentrations of five compounds used in salmonid aquaculture (Table 2) on the survivability of either *C. volutator* or *H. diversicolor*. Each experimental and control treatment was replicated five times (n = 5). The protocol for the 10-day sediment bioassays was derived from the methods described by several sources (US EPA, 1994; Bat & Raffaelli, 1998; RIKZ, 1999). Toxicity tests were conducted in clear Perspex cylindrical cores (300 mm high, 100 mm internal diameter) fitted with a removable acetal baseplate. A layer of sediment 8 cm deep (approximately 628 cm^3) was added to each core before approximately 1.5 L fresh seawater (UV-sterilised, 10 µm prefiltered, salinity 33) was used to standardise water column depth to 20 cm above the sediment-water interface. Cross-contamination was eliminated by preparation of replicates in sequence, from the lowest to highest concentration of each compound investigated. Thirty healthy *C. volutator* (> 4 mm body length), or six *H. diversicolor* (60-80 mm long), were added to each replicate core before being placed into an environmental chamber (VC 4100, Vötsch Industrietechnik) and maintained at 15.0 ± 0.1 °C with a 12 h light – 12 h dark cycle (2 × 36 W fluorescent tube lights, Arcadia, model FO-30) for 10 d. All cores were aerated via a glass Pasteur pipette at a level where the sediment surface was not disturbed, ensuring that oxygen was never limiting. The cores were removed from the environmental chamber every 24 hrs and checked for moribund animals (defined as no observable reaction when stimulated with forceps). Moribund individuals were removed using a dip-tube. All experimental equipment was acid-washed (1 % HCl) prior to use.

3.2.4. Data analysis

The relationships between sea lice medicine concentrations and mortality over the 10-day experimental period were examined using standard analysis of variance (ANOVA) procedures. Prior to analysis, graphical exploratory techniques were used to check for normality and homogeneity of variance (Sokal & Rohlf, 1995; Zar, 1999; Quinn & Keough, 2002). Percentage mortality data were converted to proportions and square root arcsin transformed ($X' = \sin^{-1} \sqrt{X/100}$; Underwood, 1997). The median concentrations of each medicine that caused 50 % mortality of the test animals (LC50) were calculated according to the trimmed Spearman-Karber method (Hamilton et al., 1977) using software supplied by the U.S. Environmental Protection Agency (available at: http://www.epa.gov/nerleerd/stat2.htm).

3.2.5 Results

Mean mortality of *C. volutator* in the control treatments ranged between 7 % and 18 % (Fig. 7). Differences between the numbers of moribund animals found in the controls at the end of each experiment were not significantly different (ANOVA, $F = 1.076$, d.f. = 4, $p = 0.395$). Active ingredient (AI) concentration had a significant effect on the number of individuals that died in each experiment ($p < 0.001$ in all 200 cases), with the % mortality typically increasing with concentration (Fig. 7). The toxicity of the investigated AIs differed markedly, with the resulting

LC50 values varying over several orders of magnitude (range: 5.05 - 193325.68 µg AI [kg wet sediment]$^{-1}$). It was evident that exposure to copper irritated *C. volutator*; they swam erratically and at higher doses seldom constructed burrows. An unidentified orange-coloured deposit was observed to accumulate on the sediment surface during the trials with Slice®, although the origin of this remains unknown.

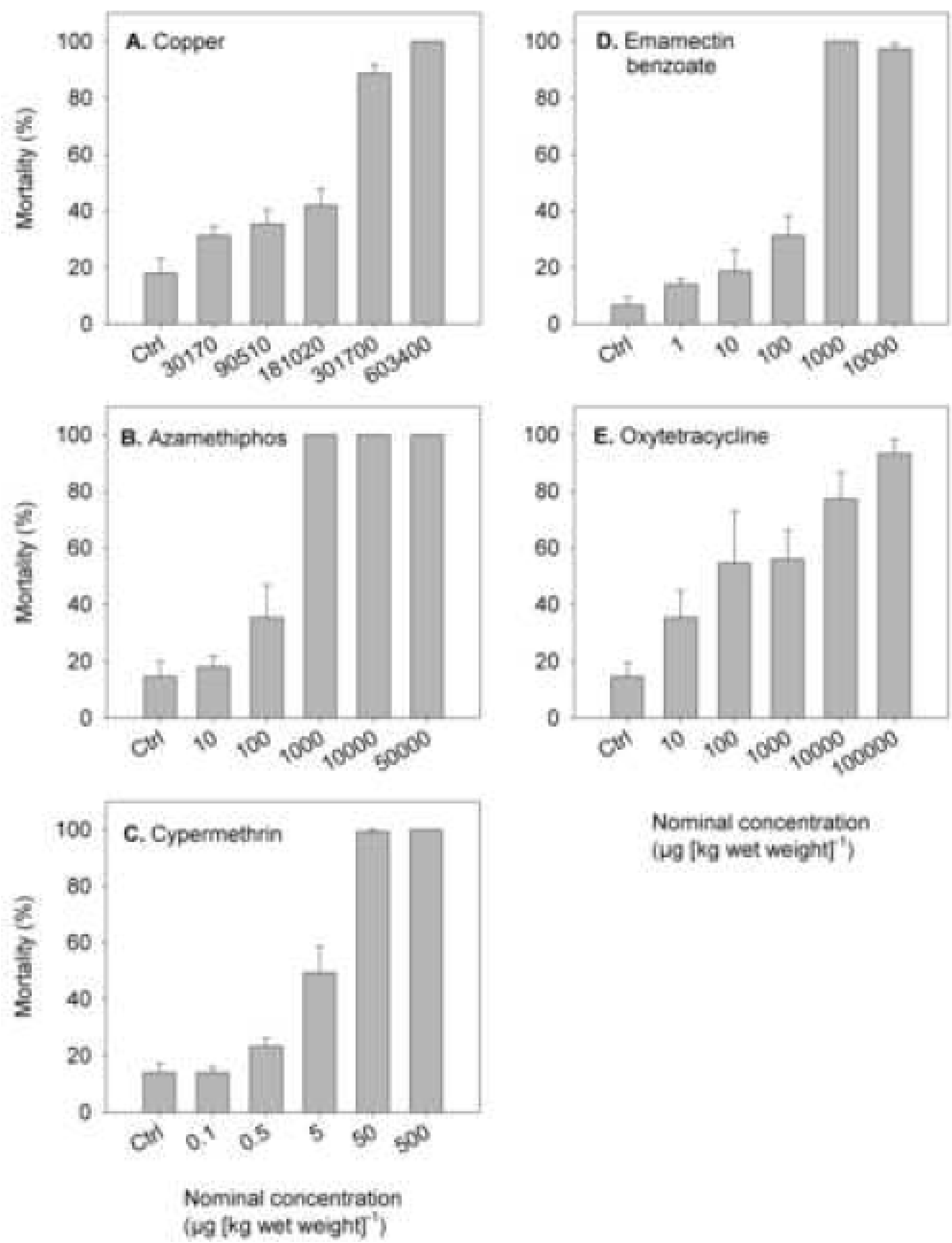


Figure 7: Percentage mortality of *Corophium volutator* exposed to various compounds associated with salmon farming. Error bars represent ± 1 standard error.

Each individual *H. diversicolor* survived in both of the control incubations (Fig. 8). The concentrations of both copper and EB had significant effects on the number of animals surviving the 10-day incubation period ($p < 0.001$ in both cases). The respective LC50's for copper and EB were 74987.96 and 1367.71 µg AI [kg wet sediment]-1 (Table 4b). Exposure to copper appeared to irritate *H. diversicolor*, with animals failing to burrow in dose 1. Orange deposits were again observed on the sediment surface in the trials with Slice® (doses 4 and 5).

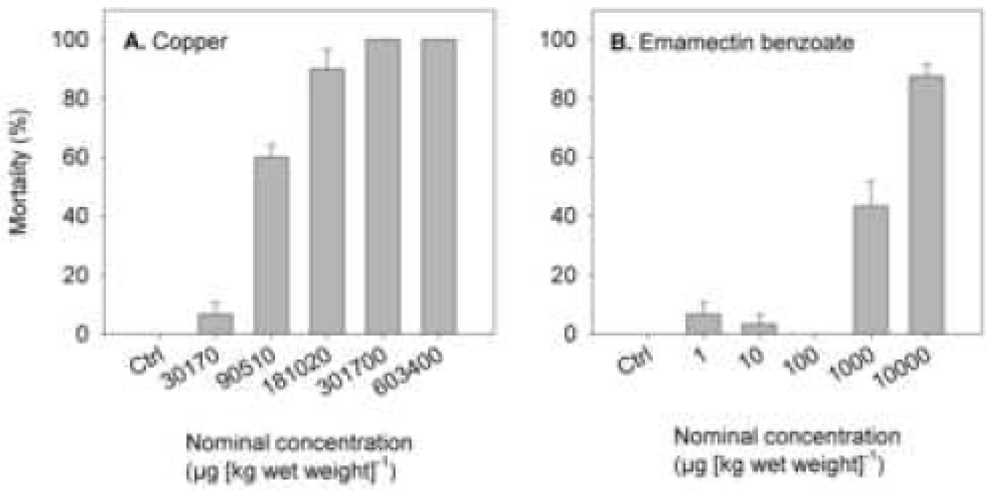


Figure 8: Percentage mortality of *Hediste diversicolor* exposed to copper and emamectin benzoate. Error bars represent ± 1 standard error.

The results of the whole-sediment bioassays presented here demonstrate that the tolerances of the non-target organisms, *Corophium volutator* and *Hediste diversicolor*, vary widely in response to the active ingredients (AIs) of some of the chemical effluents periodically released by salmonid aquaculture industry. It was evident that *C. volutator* was most sensitive to cypermethrin (CP), the AI of Excis®. This confirms that crustaceans are particularly sensitive to this compound (e.g. Burridge et al., 2000). A previous sediment bioassay study found the measured 10-day LC50 for *C. volutator* to be 225 µg CP [kg dry sediment]$^{-1}$ (McHenery 1995 c.f. SEPA 1998), somewhat higher than the nominal 8 µg CP [kg dry sediment]$^{-1}$ reported here (wet weight data converted to dry weight using a sediment water content of 40 %). The discrepancy between these two studies possibly reflects differences in the organic solvents used to facilitate the sediment loading of CP, or the dates in which the experiments were conducted (*sensu* Ciarelli et al., 1997). Allen et al. (2007) suggest that inconsistent results between sediment ecotoxicological studies may arise because of differences in the concentrations of organic material in the test sediments and thus the bioavailability of the AI. Considering that CP has a high affinity for organic matter (Maund et al., 2002), it is quite possible that the relatively high organic carbon content of 4 % found in Ythan estuary sediments (Ieno et al., 2006) altered the bioavailability of CP to *C. volutator* in our experiment. However, large differences between apparently standard 10-day LC50 experiments with *C. volutator* have been reported elsewhere (Ciarelli et al., 1997; Stronkhorst et al., 2004; Allen et al., 2007). The sensitivity of this organism to toxicants is known to vary widely in response to a range of other environmental parameters including seawater pH and its effect on sulphide and ammonia concentrations (Wang & Chapman, 1999; Kater et al., 2006), temperature and salinity (Ozoh, 1992; 1994). It is therefore extremely difficult to provide a definitive explanation for the observed difference.

C. volutator was able to tolerate higher concentrations of copper than *H. diversicolor*, with 10-day LC50s of 193326 and 74988 µg Cu [kg wet sediment]$^{-1}$ respectively. This is consistent with the view that polychaete worms are more sensitive to copper that *C. volutator* (Bat and Raffaelli, 1998). Interestingly, when the LC50 value for *C. volutator* presented here is expressed on a dry weight basis (322210 µg Cu [kg dry sediment]$^{-1}$), it is an order of magnitude greater than the value of 36850 µg Cu [kg dry sediment]$^{-1}$ reported by Bat and Raffaelli (1998). The tolerance of *C. volutator* to heavy metals and pesticides is known to vary over the year (Ciarelli et al., 1997; Kater et al., 2000), possibly reflecting a seasonal change in sediment chemistry and thus toxicant bioavailability. Although this is a reasonable explanation, it is more likely that the results presented here differ because of analytical procedures; The LC50 of Bat and Raffaelli (1998) was calculated using analytically determined concentrations of copper in the < 63 µm fraction of the sediment, thus it is probable that the total sediment concentrations were far higher. Conversely, the sediment copper concentrations presented here are reported as nominal values which are likely to be higher than the actual sediment concentrations due to the soluble nature of copper.

The sensitivities of *C. volutator* to sediments spiked with emamectin benzoate (EB), azamethiphos (AZ) and oxytetracycline hydrochloride (OTC) were all within the same order of magnitude, with 10-day LC50's of 153, 182 and 414 µg AI [kg wet sediment]$^{-1}$ respectively. Numerous studies have examined the effects of water-borne AZ and OTC on non-target organisms (Wollenberger et al., 2000; see Table 4 in Ernst et al., 2001), but we are not aware of any comparable data derived from whole sediment bioassays. In contrast, a range of studies examining the environmental fate and effects of EB exist (reviewed by Bright & Dionne, 2005), and whole sediment bioassays have been conducted with *C. volutator* and the polychaete, *Arenicola marina* (McHenery & Mackie, 1999). The 10-day LC50 for *C. volutator* presented here is consistent with that of McHenery & Mackie (1999), who reported a value of 193.1 µg EB [kg wet sediment]$^{-1}$. *H.*

diversicolor was able to tolerate higher concentrations of EB, with a 10-day LC50 of 1368 μg EB [kg wet sediment]$^{-1}$. This is considerably greater than the value of 111 μg EB [kg wet sediment]$^{-1}$ reported for *A. marina* (McHenery & Mackie, 1999) which is the most sensitive of all sediment dwelling species investigated to date for EB (SEPA, 1999). The stark difference between the sensitivities these two species of polychaete possibly reflects their functional differences (i.e. differences in their feeding, ventilatory and bioturbation behaviour which alter the way in which species interact with the sediment environment, see Gerino et al., 2003), although this cannot be concluded from this study alone.

3.2.6. Environmental context and future research

Fin fish, mainly Atlantic salmon, have been commercially cultured in Scotland for decades, and the steady growth of this industry has seen a concomitant increase in environmental legislation. The Scottish Environmental Protection Agency (SEPA) is responsible for regulating the discharge of fish farm effluents in Scottish waters, and a suite of environmental quality standards (EQSs) have been established to limit the impact of commercial fish farming activities on the natural environment (SEPA, 2005a). Furthermore, SEPA’s annual audit programme examines cage edge and reference station sediments for a range of chemotherapeutic compounds, and a host of environmental data exists in the public domain (SEPA, 2004a & b, 2005b; 2006). The maximum observed in situ concentrations of copper, CP, OTC, AZ and EB in marine sediments typically remain below those observed to cause 50 % mortality in *C. volutator* and *H. diversicolor*. The SEPA sediment near field standard for emmamectin benzoate, for example, is 7.63ug/kg wet weight sediment. Furthermore, it is important to emphasize that elevated in situ concentrations are derived from benthic samples collected within 25 m of the cage edge, and values beyond this ‘allowable zone of effects’ (AZE) are typically indistinguishable from background levels (SEPA 2004a & b; 2005b; 2006; Dean et al., 2007).

When comparing the LC50 concentrations presented here to their respective sediment concentrations it is important to consider that organisms living in close proximity to marine fish farms will differ in their sensitivities to the various compounds periodically released from aquaculture activities. Furthermore, the 10-day whole sediment bioassay approach used here only provides information on the acute effects over a short period of time relative to the persistence of the investigated compounds in the marine environment. Indeed, long-term chronic effects that occur at the population- and thus ecosystem-scale may still be apparent at concentrations far below those causing 50 % mortality of species used for routine bioassays (Allen et al., 2007; Scartlett et al., 2007).

It is clear that there is a large scope for apparently standardised experimental protocols to differ both methodologically and in the way in which data are reported. In turn, this can lead to seemingly similar studies yielding results that differ by orders of magnitude (e.g. Allen et al., 2007). This highlights the need for further research into the interactions between subtle differences in experimental conditions e.g. sediment characteristics and the physiochemical properties of the test compound and their effects on the resulting LC50 values. This may be of particular importance for studies involving chemotherapeutic treatments associated with fish farming because of the organically enriched nature of the underlying sediments (e.g. Mente et al., 2006).

3.3. Dependency of the impact of fish farm effluents on the number of species present and the toxic effects of individual versus multiple contaminants

Highly controlled manipulative experiments have revealed significant and positive effects of increased diversity on ecosystem processes, such as nutrient cycling. A series of mesocosm experiments in which the number of species (= species richness) are manipulated to examine the hypothesis that changes in the composition of benthic macrofauna alter the biogeochemistry of the sediment. These experiments are aimed at establishing the relationship between chemotherapeutic compounds (including copper and sealice treatment chemicals), and the functioning of the coastal benthos and avoid the problems associated with confounding and covarying factors in the field. The full factorial design employed allows the single (Objective 3) and multiple (Objective 4) toxic effects of approved treatments (Slice® and Copper) to be presented together.

3.3.1. Study site and sample collection

We used three taxonomically diverse invertebrate species that are functionally representative of many benthic species. The polychaete *Hediste diversicolor* and bivalve cockle *Cerastoderma edule* were collected from the Ythan estuary, Aberdeenshire, NE Scotland (OS central grid ref: NK020275; 57º 20.085'N, 02º 0.206'W). Sediment and the ophiuroid brittlestar *Amphiura chiajei* were collected from a sea-loch in Lismore, Lynn of Lorne, Oban, Argyllshire (56º 29.827'N, 5º 29.937'W). *H. diversicolor* and *C. edule* were collected by hand-digging and hand-raking respectively and returned to the laboratory within a 2 h period. *A. chiajei* were collected using a 0.1 m^2 Day grab. *A. chiajei* were carefully selected from the sediment onboard the research vessel and held in seawater for the return to Aberdeen, approximately 5-6 h following sampling. All infauna were acclimatised for 24 h in fresh seawater (10 μm prefiltered, UV sterilised, salinity 33). Sediment was sieved (0.5 mm mesh) in a seawater bath to remove macrofauna and then allowed to settle for 24 h to retain the fine fraction (<63 μm). Excess water was removed and the settled sediment was homogenised to a slurry to facilitate distribution between mesocosms. Sediment and seawater (UV-sterilised, 10 μm prefiltered, salinity 33) were added to each mesocosm 24 h prior to addition of invertebrate species.

3.3.2 Dosing of sediment with test materials

Homogenised sediment was transferred to plastic treatment buckets for dosing, each containing 30 kg sediment. It was assumed that adsorption of the test material onto the plastic was minimal as the sediment was retained in the buckets for < 5 mins. Treatments comprised of no contaminant, copper (copper (II) sulphate pentahydrate, $CuSO_4.5H_2O$; Fisher Scientific UK), Slice (active ingredient 0.2% emamectin benzoate, provided courtesy of J. McHenery, Schering-Plough) or a combination of copper and Slice. The dosage of copper was based on lower concentrations (50mg Cu [kg dry sediment]$^{-1}$ or 30mg [kg wet sediment]$^{-1}$) than the reported LC50 for *H diversicolor* to retain an environmentally relevant concentration. Thus, 3.54g of $CuSO_4.5H_2O$ was dissolved in fresh seawater (UV-sterilised, 10 µm pre-filtered, salinity 33) and added to 30kg wet sediment and homogenised by stirring. For Slice, 0.15g [30kg wet sediment]$^{-1}$ was mixed with 5g of a biologically inert powder (talc) to facilitate distribution into the sediment. The combination of copper and Slice contained the same concentrations as those used for single treatments.

3.3.3 Experimental design

We assembled 160 mesocosms, randomly split between three runs (60:60:40). Replicate (n = 5) macrofaunal communities were assembled in monoculture and in mixtures of 2 and 3 species (Table 3) to examine whether more diverse communities have a greater effect on sediment nutrient release (NH_4-N, NO_X-N, PO_4-P) than communities containing fewer species in the presence (copper, Slice or copper + Slice) versus absence (no contaminants) of sublethal levels of contaminants. Density was fixed at 6 individuals per mesocosm. Mesocosms were transparent perspex cores (330 mm high, 100 mm internal diameter) containing 10 cm depth of sediment (equivalent to 785 cm^3) and 20 cm of overlying seawater (equivalent to 2.35 l). These were randomly distributed in an environmental chamber (VC 4100, Vötsch Industrietechnik) and maintained at 13.0 ± 0.1°C with a 12 h light – 12 h dark cycle (2 × 36 W fluorescent tube lights, Arcadia, model FO-30) for 10 d.

	SR	SPID	*A. chiajei*	*C. edule*	*H. diversicolor*
Control	0	0	0	0	0
HD	1	1	0	0	6
CE	1	2	0	6	0
AC	1	3	6	0	0
HD,CE	2	4	0	3	3
HD,AC	2	5	3	0	3
CE,AC	2	6	3	3	0
HD,CE,AC	3	7	2	2	2

Table 3: Number of individuals in species combinations used in the assembled macrofaunal communities for species richness (SR) and species identity (SPID) manipulations (n = 5 in all cases). *HD: Hediste diversicolor; CE: Cerastoderma edule; AC: Amphiura chiajei*.

All mesocosms were continually aerated. Pre-filtered (Nalgene, 0.45 μm) water samples were taken on the final day of each experiment. Ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_X-N) and phosphate-phosphorus (PO_4-P) concentrations were determined with a modular flow injection auto-analyser (FIA Star 5010 series) using an artificial seawater carrier solution.

3.3.4 Statistical analysis

A generalized least squares (GLS; Pinheiro & Bates 2001) statistical mixed modelling approach was used to assess the relationships between species richness and nutrient concentration and between species identity and nutrient concentration. As a first step, a linear regression model was fitted. Model validation was applied to verify that underlying statistical assumptions were not violated; normality of residuals was assessed by plotting theoretical quantiles versus standardized residuals (Q–Q plots), homogeneity of variance was evaluated by plotting residuals versus fitted values, and influential data points were identified using Cook's distance method (Quinn & Keough 2002). The validation procedure showed that there was no evidence of nonlinearity but there was evidence of unequal variance among the explanatory variables. The GLS model was refined by manual backwards stepwise selection using maximum likelihood (ML) to remove insignificant terms, and the final model was presented using restricted maximum likelihood (REML; West et al. 2007). To assess the importance of individual independent variables, a likelihood ratio test was used to compare the full minimal adequate model with models in which the independent variable, and all the interaction terms, were omitted. Analyses were performed using the 'R' statistical and programming environment (R Development Core Team 2005) and the 'nlme' package (Linear and nonlinear mixed effects models; Pinheiro et al. 2006).

3.3.5 Species richness effects

For NH_4-N, the minimal adequate model was a linear regression with a GLS extension incorporating one three-way interaction term, three two-way interaction terms and three single terms. Single factors were species richness, copper and slice. Two-way interactions were species richness × copper, species richness × slice and copper × slice. The three-way interaction was species richness × copper × slice. The variance–covariate terms were copper and species richness. Species richness had the greatest effect on NH_4-N concentration (L ratio = 69.38, d.f. = 12, $p < 0.0001$), followed by copper (L ratio = 21.91, d.f. = 8, $p = 0.005$) and slice (L ratio = 21.79, d.f. = 8, $p = 0.005$). A graphical representation of the effect of the three-way interaction is presented in Figure 9.

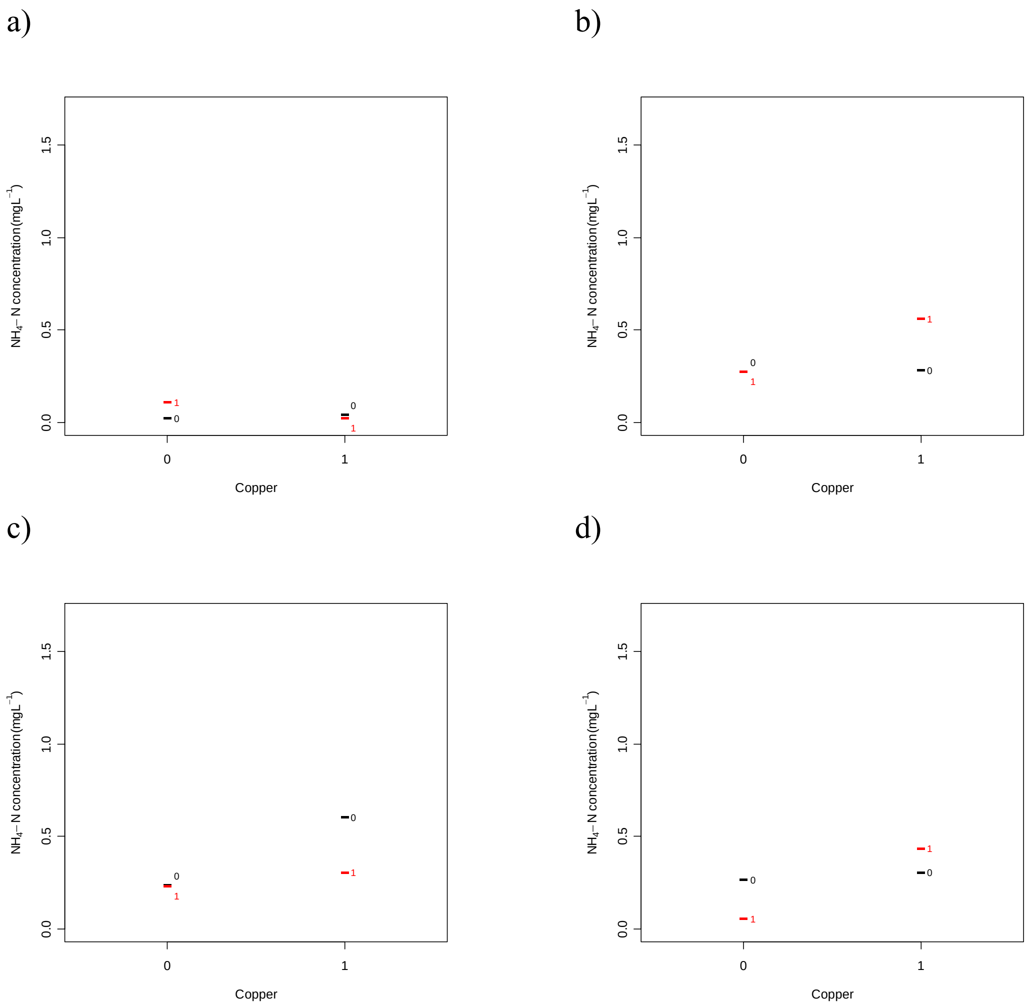


Figure 9: Graphical representation of the effect of the three way interaction term species richness × copper × slice on NH_4-N concentration for (a) no macrofauna, (b) monocultures, (c) two species mixtures and (d) three species mixtures. Horizontal bars represent predicted values from the minimal adequate regression model for the presence (1) versus absence (0) of Slice. The presence (1) versus absence (0) of on copper is indicated on the x-axis. As the GLS framework allows for different spreads in the data, individual data points are omitted to prevent misinterpretation.

For NO_3-N, the minimal adequate model was a mixed model incorporating two two-way interaction terms and three single terms. Single factors were species richness, copper and slice. Two-way interactions were species richness × copper and copper × slice. There were no three-way interaction terms. Species richness was a variance–covariate term and experimental run was a random term (run 2 showed elevated nutrient concentrations relative to the other two runs). Species richness had the greatest effect on NO_3-N concentration (L ratio = 26.73, d.f. = 6, p = 0.0002), followed by copper (L ratio = 14.70, d.f. = 5, p = 0.0117) and slice (L ratio = 9.277, d.f. = 2, p = 0.0097). A graphical representation of the two two-way interactions are presented in Figure 10.

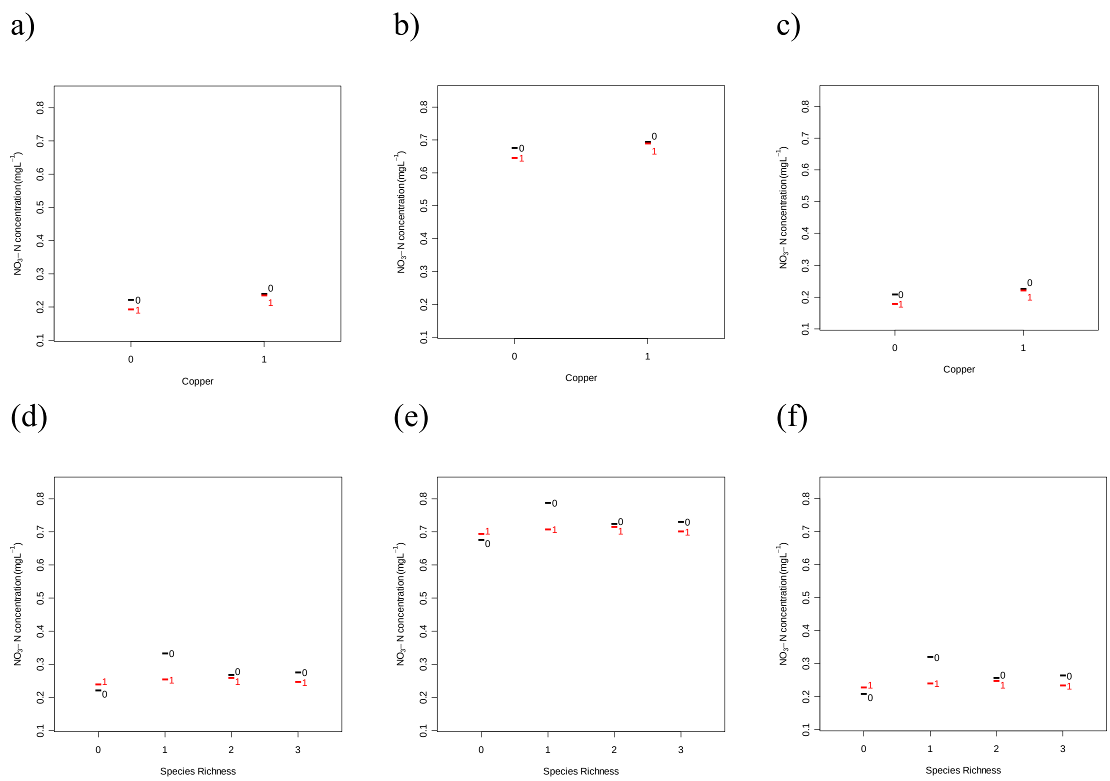


Figure 10: Graphical representation of the effect of the two way interactions for (a-c) copper × slice and (d-f) species richness × copper on NO_3-N concentration. The effect of experimental run is shown from left to right (i.e. a through c and d through f correspond to runs 1-3). Horizontal bars represent predicted values from the minimal adequate regression model for the presence (1) versus absence (0) of (a-c) Slice or (d-f) copper. Species richness values on the x-axis correspond to Table 3. Individual data points are omitted to prevent misinterpretation.

For PO_4-P, the minimal adequate model was a linear regression with a GLS extension incorporating species richness as a single term. There were no significant two-way or three-way terms. The variance–covariate terms were copper and species richness. A graphical representation of the effect of species richness is presented in Figure 11.

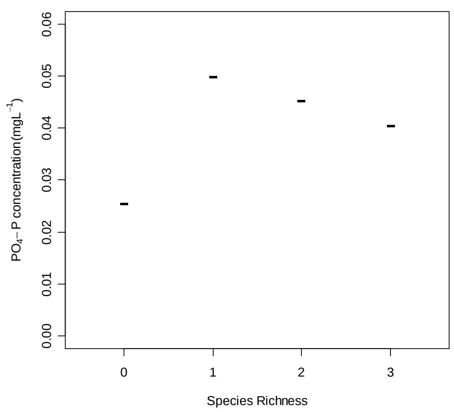


Figure 11: Graphical representation of the effect of species richness on PO_4-P concentration. Horizontal bars represent predicted values from the minimal adequate regression model for each species richness treatment. As the GLS framework allows for different spreads in the data, individual data points are omitted to prevent misinterpretation.

3.3.6 Species identity effects

For NH_4-N, the minimal adequate model was a linear regression with a GLS extension incorporating one two-way interaction term and three single terms. Single factors were species identity, copper and slice. The two-way interaction term was species identity × slice. There were no significant three-way terms. The variance–covariate terms were copper and species identity. Species identity had the greatest effect on NH_4-N concentration (L ratio = 63.66, d.f. = 14, $p < 0.0001$), followed by slice (L ratio = 18.26, d.f. = 8, p = 0.0193) and copper (L ratio = 7.21, d.f. = 1, p = 0.0072). A graphical representation of the effect of the two-way interaction is presented in Figure 12.

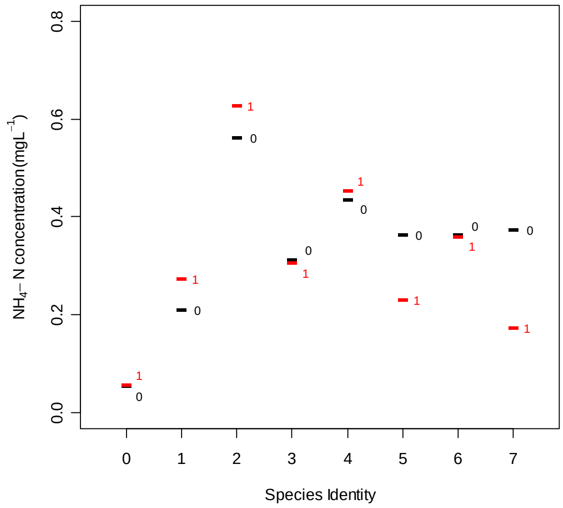


Figure 12: Graphical representation of the effect of the two way interaction term species identity × slice on NH_4-N concentration. Horizontal bars represent predicted values from the minimal adequate regression model for the presence (1) versus absence (0) of Slice. Species identity values on the x-axis correspond to Table 3. As the GLS framework allows for different spreads in the data, individual data points are omitted to prevent misinterpretation.

For NO_3-N, the minimal adequate model was a mixed model incorporating two two-way interaction terms and three single terms. Single factors were species identity, copper and slice. Two-way interactions were species identity × copper and copper × slice. There were no three-way interaction terms. Species identity was a variance–covariate term and experimental run was a random term. Species identity had the greatest effect on NO_3-N concentration (L ratio = 94.65, d.f. = 14, p = 0.001), followed by copper (L ratio = 21.98, d.f. = 9, p = 0.0089) and slice (L ratio = 9.90, d.f. = 2, p = 0.0071). A graphical representation of the effect of the two two-way interactions are presented in Figure 13.

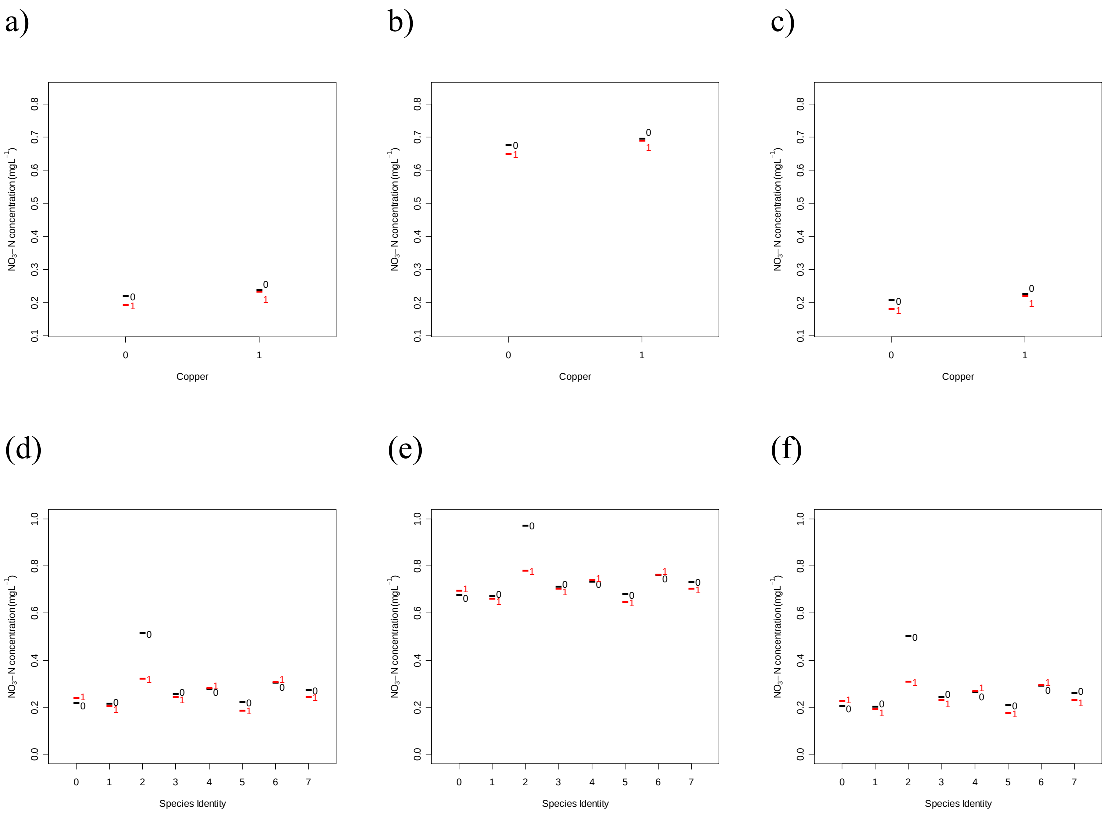


Figure 13: Graphical representation of the effect of the two way interactions for (a-c) copper × slice and (d-f) species identity × copper on NO_3-N concentration. The effect of experimental run is shown from left to right (i.e. a through c and d through f correspond to runs 1-3). Horizontal bars represent predicted values from the minimal adequate regression model for the presence (1) versus absence (0) of (a-c) Slice or (d-f) copper. Species identity values on the x-axis correspond to Table 3. Individual data points are omitted to prevent misinterpretation.

For PO_4-P, the minimal adequate model was a linear regression with a GLS extension incorporating one two-way term and two single terms. The two way interaction was species identity × slice and the single terms were species identity and slice. There was no significant three-way term. The variance–covariate terms were species identity and slice. Species identity had the greatest effect on PO_4-P concentration (L ratio = 382.30, d.f. = 8, $p = 0.0278$) followed by slice (L ratio = 361.86, d.f. = 14, $p < 0.0001$). A graphical representation of the effect of the two-way interaction is presented in Figure 14.

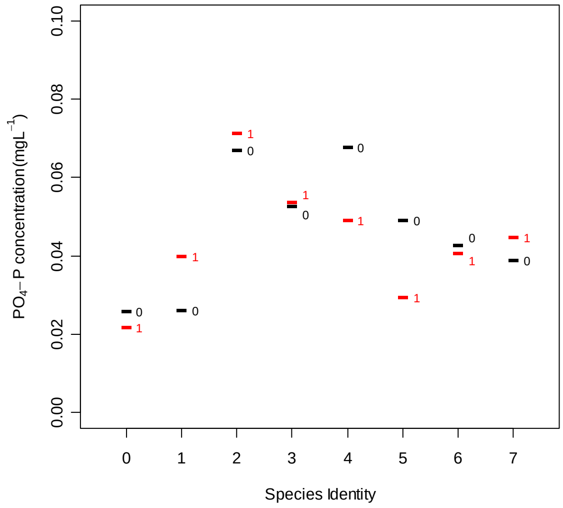


Figure 14: Graphical representation of the effect of the two way interaction term species identity × slice on PO_4-P concentration. Horizontal bars represent predicted values from the minimal adequate regression model for the presence (1) versus absence (0) of Slice. Species identity values on the x-axis correspond to Table 3. As the GLS framework allows for different spreads in the data, individual data points are omitted to prevent misinterpretation.

3.3.7. Environmental context and future research

A consistent feature of the mesocosm studies was that the most important factor in determining the impact of Slice and Copper on the biogeochemistry of the sediment was the composition of benthic macrofauna. The environmental fate and ecological consequences of Slice and Copper depend not only on environmental conditions, but also on the interaction with the fauna present in the sediment. The way in which natural benthic assemblages interact with the environment, chiefly through their burrowing and irrigatory behaviour, can result in a markedly different sediment environment and dramatically alter the exposure of individual organisms to the compounds in unpredictable ways. A challenge for understanding both the environmental fate and environmental effects of such compounds will be to understand how different components of the abiotic and biotic environment are associated, interact and effect one another along gradients of changing physico-chemical conditions and with dynamic biological communities.

3.4. Dependency of location and the impact of fish farm effluents

This objective explicitly recognises that the environmental impact of a poorly sited farm may differ to that of a farm sited at an alternative location with different site characteristics (depth, bathymetry, hydrographic regime, wave exposure etc.). Here we adopt the experimental design of the previous section but add sediment enrichment to simulate different benthic conditions.

For the study sites, method of sample collection and procedures for dosing the sediment with slice and copper the reader is referred to sections 3.3.1 and 3.3.2. Similarly, the statistical approach adopted here is identical to section 3.3.4.

3.4.1 Experimental design

We assembled 180 mesocosms, randomly split equally between two runs. Replicate (n = 3) macrofaunal communities were assembled in monoculture or as a 3 species mixture (Table 4) to examine whether more diverse communities have a greater effect on sediment nutrient release (NH_4-N, NO_X-N, PO_4-P) than communities containing fewer species in the presence (copper, Slice or copper + Slice) versus absence (no contaminants) of sublethal levels of contaminants and at different levels of enrichment. For the latter, each mesocosm consisted of either non-enriched sediment or sediment that was enriched with 1.25 g or 2.5 g of dried and powdered *Ulva intestinalis*. The addition of *U. intestinalis* as a powder in our mesocosms allows a significant enrichment of the sediment. Species density was fixed at 6 individuals per mesocosm. Mesocosms were transparent perspex cores (330 mm high, 100 mm internal diameter) containing 10 cm depth of sediment (equivalent to 785 cm^3) and 20 cm of overlying seawater (equivalent to 2.35 l). These were randomly distributed in a temperature controlled room and maintained at 11.0 ± 2.0°C with a 12 h light – 12 h dark cycle (2 × 36 W fluorescent tube lights, Arcadia, model FO-30) for 10 d.

	SPID	*A. chiajei*	*C. edule*	*H. diversicolor*
Control	0	0	0	0
HD	1	0	0	6
CE	2	0	6	0
AC	3	6	0	0
HD,CE,AC	4	2	2	2

Table 4: Number of individuals in species combinations used in the assembled macrofaunal communities for the species identity (SPID) manipulations (n = 3 in all cases). *HD: Hediste diversicolor; CE: Cerastoderma edule; AC: Amphiura chiajei*.

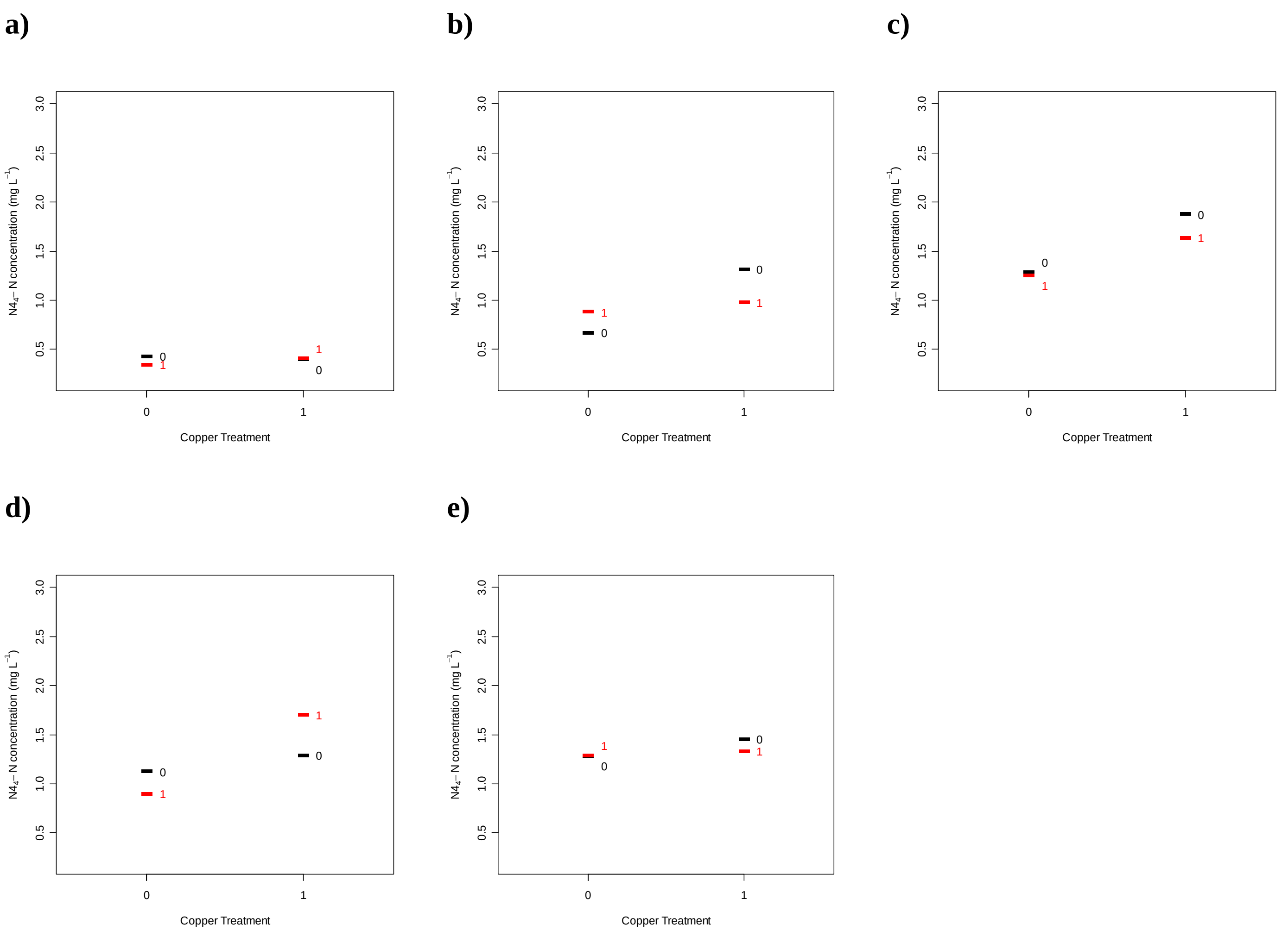
All mesocosms were continually aerated. Pre-filtered (Nalgene, 0.45 µm) water samples were taken on the final day of each experiment. Ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_X-N) and phosphate-phosphorus (PO_4-P) concentrations were determined with a modular flow injection auto-analyser (FIA Star 5010 series) using an artificial seawater carrier solution.

3.4.2 Species identity and diversity effects

For NH_4-N, the minimal adequate model was a linear regression with a GLS extension incorporating one four-way interaction term, four three-way interaction terms, six two way interaction terms and four single terms. Single factors were species identity, copper, slice and enrichment. Two-way interactions were species identity × copper, species identity × slice, species identity × enrichment, copper × slice, copper × enrichment and slice × enrichment. Three-way interactions were copper × slice × enrichment, species identity × slice × enrichment, species identity × copper × enrichment and species identity × copper × slice. The four-way interaction was species identity × copper × slice × enrichment. The variance–covariate terms were species identity and copper. Species identity had the greatest effect on NH_4-N concentration (L ratio = 207.80, d.f. = 48, $p < 0.0001$), followed by enrichment (L ratio = 93.63, d.f. = 40, $p < 0.0001$), copper (L ratio = 70.14, d.f. = 30, $p < 0.0001$) and slice (L ratio = 48.99, d.f. = 30, $p = 0.0158$). A graphical representation of the effect of the four way interaction is presented in Figures 15A-C.

Figure 15A-C: Graphical representation of the effect of the four way interaction term species identity × copper × slice × enrichment on NH_4-N concentration. Horizontal bars represent predicted values from the minimal adequate regression model for the presence (1) versus absence (0) of Slice. The presence (1) versus absence (0) of copper is indicated on the x-axis. Species identities correspond to Table 4 and are (a) no macrofauna, (b) *Hediste diversicolor*, (c) *Cerastoderma edule*, (d) *Amphiura chiajei* and (e) the 3 species mixture of *H. diversicolor*, *C. edule* and *A. chiajei*. Enrichment levels correspond to each of the three sets of graphical representations, no enrichment (Figure 15A), enrichment with 1.25g (Figure 15B) or 2.50g (Figure 15C) algae mesocosm^{-1}. As the GLS framework allows for different spreads in the data, individual data points are omitted to prevent misinterpretation.

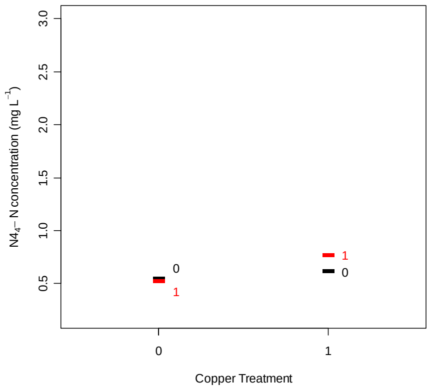
Figure 15A:



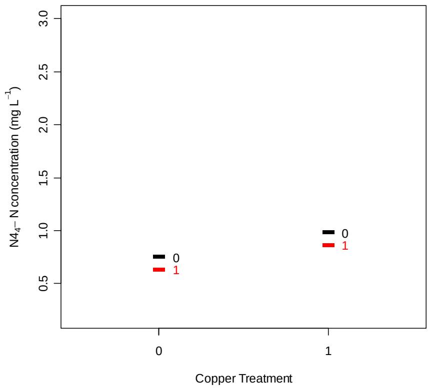
a)
b)
c)
d)
e)
N4– N concentration (mg L-1)
0.5
1.0
1.5
2.0
2.5
3.0
Copper Treatment
0
1

Figure 15B:

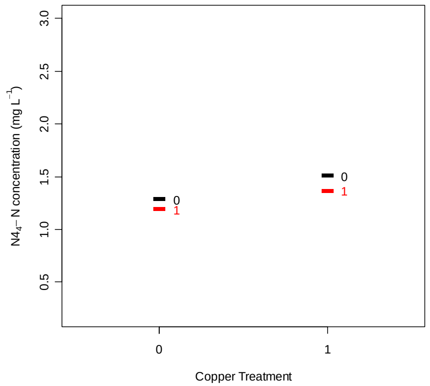
a)



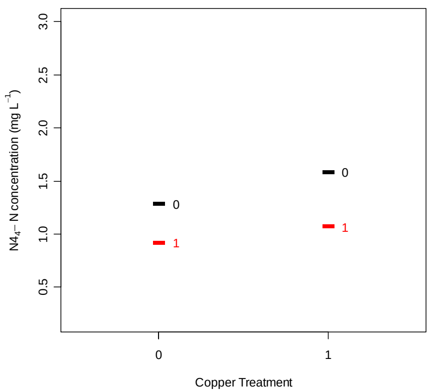
b)



c)



d)



e)

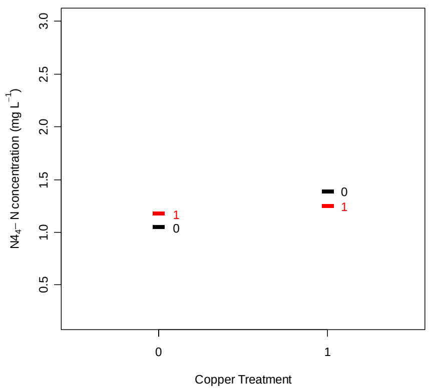
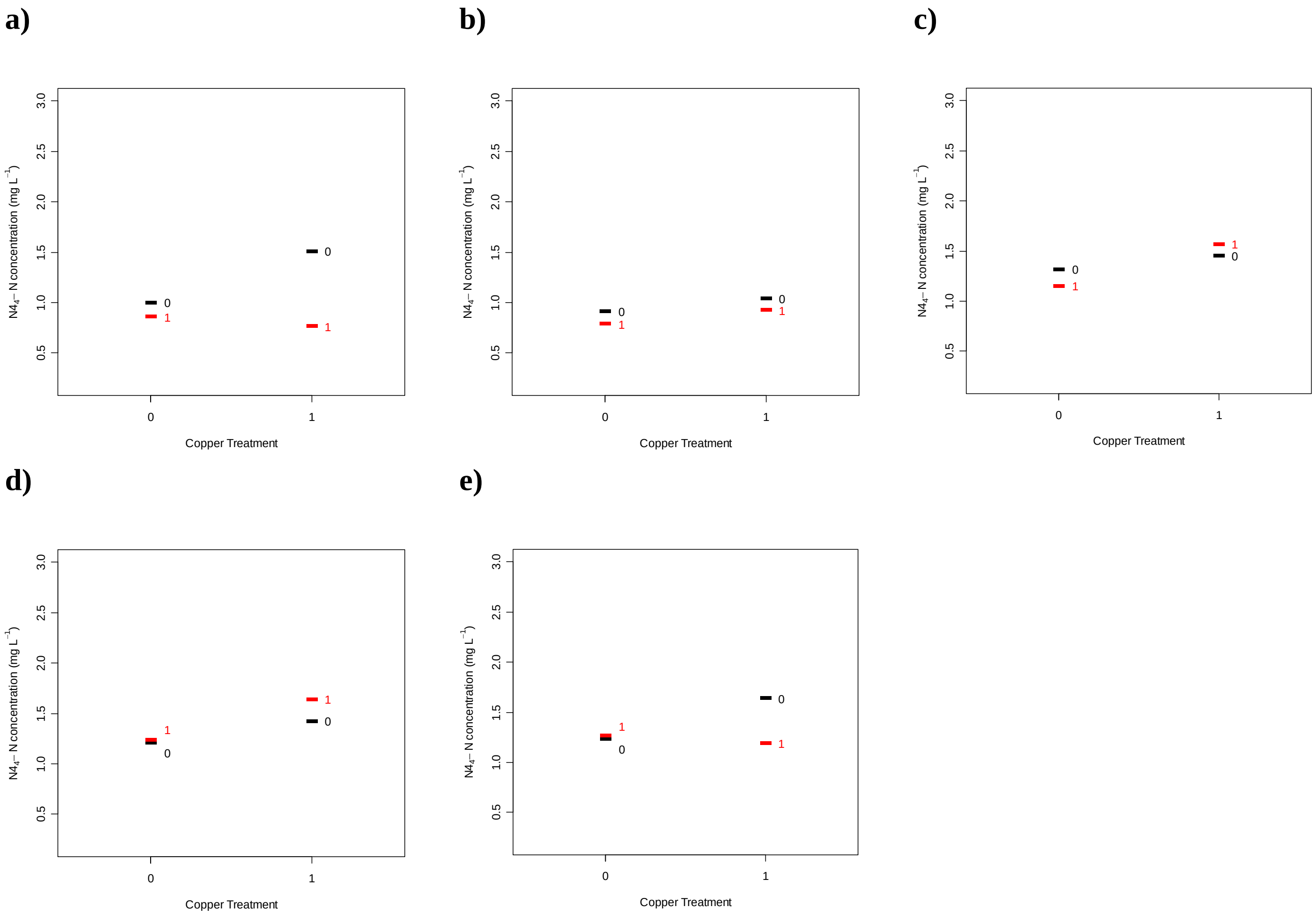


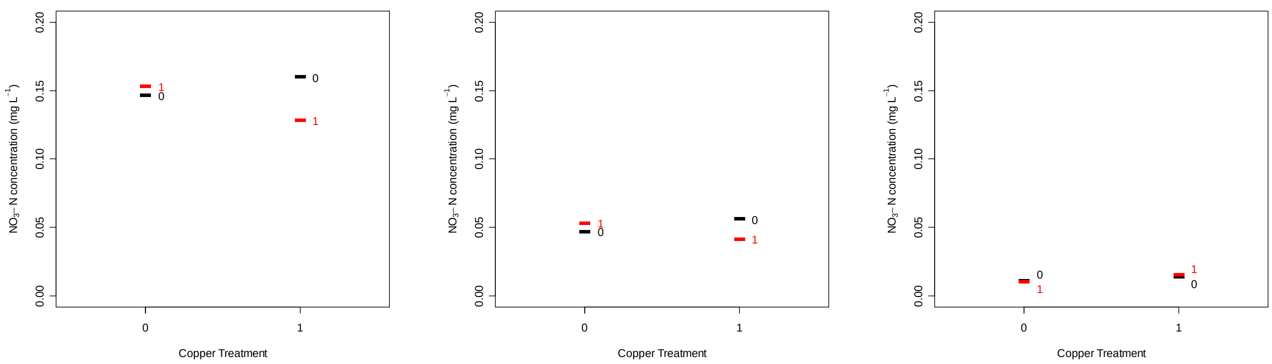
Figure 15C:



a)
b)
c)
d)
e)
N4+– N concentration (mg L-1)
Copper Treatment
0
1
3.0
2.5
2.0
1.5
1.0
0.5

For NO_3-N, the minimal adequate model was a linear regression with a GLS extension incorporating one three-way interaction term, four two way interaction terms and four single terms. The four way term was not significant. Single factors were species identity, copper, slice and enrichment. Two-way interactions were species identity × enrichment, copper × slice, copper × enrichment and slice × enrichment. The three-way interaction was copper × slice × enrichment. The variance–covariate terms were species identity and enrichment. Enrichment had the greatest effect on NO_3-N concentration (L ratio = 179.22, d.f. = 16, $p < 0.0001$), followed by species identity (L ratio = 82.60, d.f. = 12, $p < 0.0001$), slice (L ratio = 16.42, d.f. = 6, $p = 0.0116$) and copper (L ratio = 15.27, d.f. = 6, $p = 0.0182$). A graphical representation of the effect of the three way and two way interaction terms are presented in Figure 16.

a) b) c)



(d)

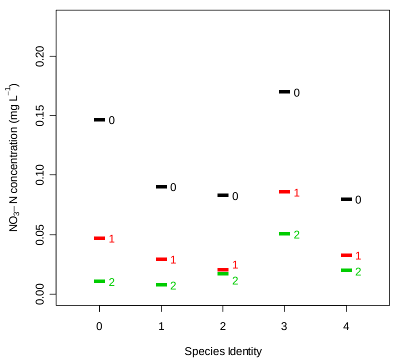


Figure 16: Graphical representation of the effect of (a-c) the three way interaction term copper × slice × enrichment and (d) the two way interaction term species identity × enrichment on NO_3-N concentration. In (a-c), horizontal bars represent predicted values from the minimal adequate regression model for the presence (1) versus absence (0) of Slice. The presence (1) versus absence (0) of copper is indicated on the x-axis. Enrichment levels correspond to (a) no enrichment, or enrichment with (b) 1.25g or (c) 2.50g algae mesocosm^{-1}. In (d), species identity values correspond to Table 4 and are (0) no macrofauna, (1) *Hediste diversicolor*, (2) *Cerastoderma edule*, (3) *Amphiura chiajei* and (4) the 3 species mixture of *H. diversicolor*, *C. edule* and *A. chiajei*.

Results for PO_4-P were below the detectable limits of the autoanalyser and therefore were not analysed.

3.4.3 Environmental context and future research

It is clear that the role of location (here simulated by increasing organic enrichment level of the sediment) will be important in determining the environmental fate and ecological consequences of Slice and Copper. These findings echo and extend from the challenges outlined in section 3.3.7 because they demonstrate that physical components of the environment that alter on a site specific basis will be important in mediating the organism-sediment-chemotherapeutic relations. Whilst location is not always the most important determinant of in determining the effects of Slice and Copper on the biogeochemistry of the sediment, it is noteworthy that the effects of enrichment in the present study were always present in the final models but differed depending on the ecosystem property (here sediment nutrient generation) under investigation. The complex interactions outlined in previous sections (see section 3.3.7) are therefore likely to be site specific and have obvious implications for the siting of mariculture installations. A key challenge will be to examine how the ecological consequences of chemotherapeutics used in the mariculture industry vary for given nominal concentrations whilst incorporating environmental context. Such investigations are likely to suggest that a range of field standards will be more appropriate, or that regulatory bodies may need to look at circumstances where and when such standards need to deviate (positively and/or negatively) from present status.

3.5. Prediction of the ecosystem response to fish farming activity

Various mathematical models predict that the quantities of particulate fish farm waste reaching the seabed, and the resulting benthic impact, decrease with distance from a fish pen enclosure (e.g. Cromey et al. 2002; Perez et al. 2002; Corner et al. 2006). Recent meta-analyses of data collected in situ also indicate that parameters indicative of benthic impacts e.g. sediment concentrations of organic carbon, decrease with distance from fish farms (Kalantzi & Karakassis 2006; Pusceddu et al. 2007). However, whilst there is a wealth of information on the effects of individual farm operations on benthic habitats, empirical investigations of the influence of increasing fish farm capacity are lacking. In this component of the study, we examine the assumption that increasing the capacity, or 'maximum consented biomass' (MCB), of a fish farm will increase the total quantity of organic pollutants released into the marine environment.

Fin fish, mainly Atlantic salmon, have been farmed in Scotland for decades, and the 10-fold increase in gross production of this industry between 1985 and 1998 (Henderson & Davies 2000) makes it an ideal model system for examining the effects of fish farm intensification. In order to establish the generality of the environmental impacts of the fish farming industry in Scotland, we extracted all data on sediment total organics (Torg), organic carbon (Corg) and nitrogen (N) and the associated hydrographic information for each farm that was submitted to SEPA in 2005 from the national archive (n = 36, MCB range: 150 to 1999 t). In addition, we also independently conducted sediment monitoring surveys at five Scottish fish farms (MCB range: 120 to 2100 t) within a region of Scotland. These surveys were designed to determine the magnitude and spatial extent of sediment enrichment of Torg, Corg, N, copper (Cu) and phosphorus (P). Statistical mixed-models were applied to these two independent data sets to (1) investigate the generality of benthic environmental impacts at the national- and regional-scale and (2) explicitly examine the effect of MCB on the magnitude of benthic impact at fish farms across a range of environmental settings. These analyses allow the generality of any environmental impact to be determined, and distinguished, from patterns of impact driven by factors that are specific to a particular site or area.

3.5.1 Regional data

Benthic surveys were conducted at 5 active fish farms within a region of Scotland in April 2006. Exact locations are not provided in agreement with farm operator wishes. All farms have a history (≥ 5 yrs) of farming Atlantic salmon, *Salmo salar*, and the 3 largest were rearing Atlantic cod, *Gadus morhua*, on site at the time of survey. The MCB of salmon permitted at these farms were 120, 275, 980, 100 1500 and 2106 t. For cod, at the latter 3 sites, MCB levels were 657, 990 and 1411 t respectively. These are calculated assuming that the total discharge of nitrogenous waste derived from cod farms is 1.5 times greater than that from equivalent salmon farms (Gillibrand et al. 2002). Two sampling transects were established at each site, either in line with, or perpendicular to the predominant current direction (Transects 1 & 2 respectively). Sediment samples were collected with a 0.1 m^2 Day grab along both transects at 0, 25, 50, 100 and 200 m distance from the cage edge. The uppermost 2 cm of sediment was collected from each grab and stored frozen in sample bags prior to analysis. Hydrographic data for each site were provided by the farm operators.

Sediment samples were defrosted, homogenised, dried overnight at 55 °C and then ground in a ball mill for 1 minute before being sub-sampled for chemical analysis. Samples for the determination of Corg and N were initially acidified with 1 N HCl to remove inorganic carbon (Hedges & Stern 1984) before analysis on a Fisons NCS 1500 elemental analyser using sulphanillic acid as a standard. Copper and P were extracted from known quantities of sediments at 365 °C using a digestion solution of concentrated sulphuric acid and hydrogen peroxide (Allen 1974). Total Cu concentrations were determined by atomic absorption spectrometry using a Perkin Elmer Instrument AAnalyst 100 with reference to a range of Cu standard solutions (Fisher Scientific, UK). Total P concentrations were measured colourimetrically (Strickland & Parsons 1972) using an FIA Star 5010 analyser and spectrophotometer 5023 with a range of P standard solutions (Fisher Scientific, UK). The Torg content of the sediments was determined as the weight lost on ignition at 525 °C (Kristensen & Andersen 1987).

3.5.2 National data

All benthic monitoring data submitted to SEPA in digital format during 2005, and the corresponding hydrographic data, were extracted from the national archive, yielding data from a total of 59 farms. Sediment concentrations of either Torg or Corg and N, expressed as percentage dry weight, were typically reported at 0, 25, 50 and 150 m from the cage edge along two transects. A total of 21 reports presented Corg data, and a subset of 11 of these also presented N data. A further 15 reports presented Torg data. The MCB for salmon at these 36 farms ranged from 150 to 1999 t. Where presented, the following explanatory variables were extracted from each farm: MCB, tonnage of fish on site at the time of survey (TFS), sample depth in metres (Depth), sample distance from cage edge in metres (Distance; 0, 25, 50, 150) and current speeds averaged over 15 days at the following depths; 2-3 m beneath the surface (Top CS), 2-3 m above the seabed (Bottom CS), and at the middle of the water column (Middle CS). Average current speed (Average CS) was calculated from these data. Each farm was ascribed a unique identity (Farm ID). Information on the number and dimensions of the cages at 35 of the 36 farms that yielded benthic chemistry data was also extracted from the SEPA archive.

3.5.3 Statistical analysis

All statistical analyses were conducted using the program ‘R’ (R Development Core Team 2005). Data exploration was undertaken to identify outliers and cases where explanatory variables were highly correlated (collinearity). The former was achieved by visual examination of Cleveland dotplots and Cook’s distances, and the latter by using Pearson correlation coefficients and variance inflation factors (VIF; see Montgomery & Peck 1992; Zuur et al. 2007). Standard linear regression was used to examine how cage depth, cage area, maximum fish stocking density (MCB:cage volume [tonnes m^{-3}]) and Average CS were related to MCB. A combination of linear regression, with a generalised least squares (GLS) extension, and linear mixed-models (‘nlme’ library) were used to examine the relationships between the selected explanatory and response variables at both the regional- and national-scale. Mixed models allow the use of Farm ID as a random effect, meaning that only a single variance parameter for this variable must be estimated (Pinheiro & Bates 2000; Zuur et al. 2007). The random effect also allows the imposition of a correlation structure (the compound symmetrical correlation) on observations made within a particular farm, thereby accounting for any correlated data within a particular farm. Distance was used as a nominal variable because of the fixed and limited nature of the sampling points along each transect. The protocol for model selection in linear mixed modelling is described elsewhere (Diggle et al. 2002). In brief, the optimal random structure of the model is identified prior to finding the optimal fixed structure. Full models with, and without, the random intercepts using restricted maximum likelihood (REML) were compared using a log likelihood ratio test (L-ratio). In cases where linear mixed models were not necessary we proceeded with standard linear models. Once the optimal random structure was found, the optimal model in terms of fixed components, including all 2- and 3-way interaction terms, was determined: all non-significant terms were sequentially removed from the model (from highest to lowest level) until only significant terms remained. This is achieved with backwards selection using maximum likelihood (ML). The significance of each term and interaction in the linear mixed- and standard linear models was tested using the L-ratio and F-statistic tests respectively. Interaction terms with marginally significant p-values (0.05 - 0.01) were further examined using REML and subsequently rejected if the resulting F-statistic yielded $p > 0.05$. The resulting minimum adequate models (MAM) were validated to verify that the underlying statistical assumptions were not violated; normality of residuals was assessed by plotting theoretical quantiles versus standardized residuals (Q-Q plots) and homogeneity of variance was evaluated by plotting residual versus fitted values. In instances where residuals versus fitted values from linear models were found to be heterogeneous, we adopted GLS models fitted by REML (Pinheiro and Bates

2000), which allow for an increase in residual spread by individual variables. The variance covariate terms were selected using the Akaike Information Criterion (Quinn & Keogh 2002).

For the regional analysis, five separate analyses were conducted to examine how sediment concentrations of Torg, Corg, N, Cu and P were influenced by Farm ID, MCB, Distance, Top CS, Bottom CS, Middle CS and Average CS. MCB was natural-log (ln) transformed prior to analysis to linearise the spread of farm size (Underwood, 1997). There were insufficient data to include the identity of the farmed fish species (salmon or cod) as an explanatory variable. Salmon MCB values were chosen for the analysis because all sites have a history of farming salmon and also to ensure that our results were compatible with the national analysis. Preliminary data exploration revealed that all current speed data (Top, Middle, Bottom and Average CS data) were highly collinear (Pearson's correlation coefficients, $r > 0.85$ in all cases). Average CS was chosen for the analyses so as not to emphasise any particular horizon of current flow. However, Average CS and lnMCB were found to be collinear ($r = 0.81$); because the principal aim was to examine how farm size (MCB) influences sediment concentrations of fish farm effluents, all current speed data were excluded. Thus, our analyses included Farm ID, lnMCB and Distance as explanatory variables.

For the national analysis, three separate analyses were undertaken to examine how Farm ID, MCB, TFS, Distance, Depth, Top CS, Bottom CS, Middle CS and Average CS influenced the reported sediment concentrations of Torg, Corg and N. Six of the 106 data points in the Torg dataset were excluded from the analysis because they formed unique observations ($n = 3$) or they did not have corresponding current speed data ($n = 3$). Current speed data were again found to be highly collinear ($r > 0.56$ in all cases) and Average CS was used. Whilst it is intuitive to expect collinearity between MCB and TFS, the data revealed only a very minor correlation ($r = 0.23$). Visual examination of the data indicated that farms with zero tonnes of fish on site at the time of survey ($n = 5$) were highly influential and these points were removed. Re-examination of the data revealed that MCB and TFS were highly correlated ($r = 0.85$) and TFS was therefore removed from the analyses. Average current speed VIF values were > 3 for all three data sets, indicating that they exhibit a high degree of collinearity with other explanatory variables (Montgomery & Peck 1992; Zuur et al. 2007). Nevertheless, because current speed is an important component in distributing the waste from fish cages, the VIF-based selection criteria was over-ridden on ecological grounds and the explanatory variable with the second highest VIF value (Depth) was removed. After removing TFS, Depth, Top CS, Middle CS and Bottom CS, recalculated VIF values were all < 3. Thus, our analyses included Farm ID, MCB, Average CS and Distance as explanatory variables.

3.5.4 Results: Increasing fish farm capacity

Linear regressions revealed that fish farm in Scotland do not increase capacity by an increase in cage depth (linear regression, $R^2 = 0.009$, $n = 35$, $p = 0.596$; Figure 17a), rather larger farms occupy correspondingly greater areas (linear regression, $R^2 = 0.304$, $n = 35$, $p < 0.001$; Figure 17b), are stocked to a higher density (linear regression, $R^2 = 0.446$, $n = 35$, $p < 0.001$; Figure 1c) and are located in areas of higher average current speed ($R^2 = 0.203$, $n = 35$, $p = 0.007$; Figure 1d).

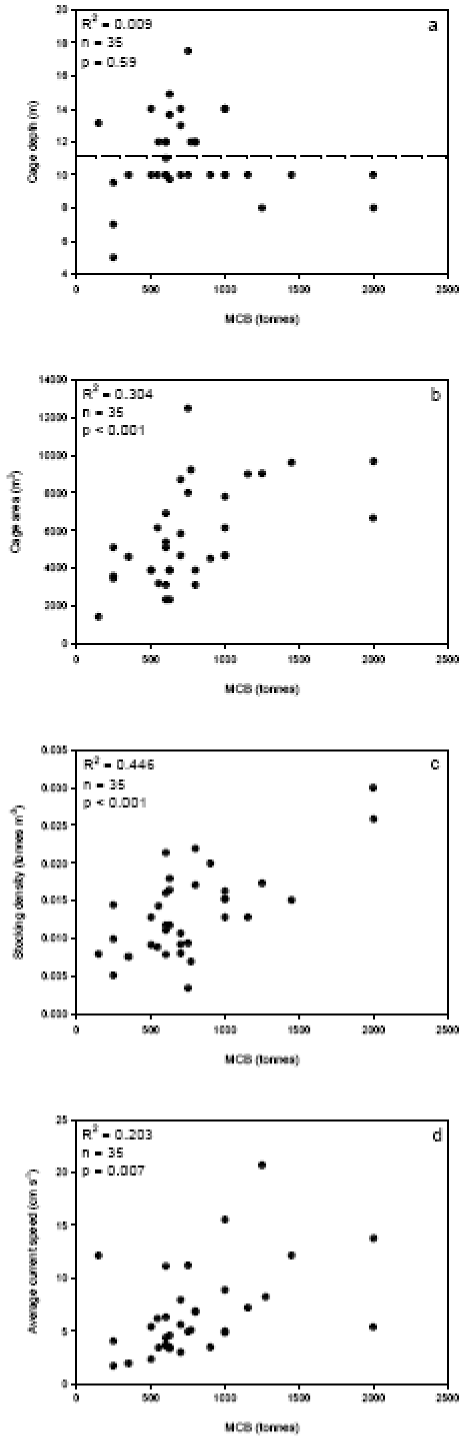


Figure 17: The relationships between maximum consented biomass (MCB) and (a) cage depth, (b) cage area, (c) fish stocking density and (d) average current speed. Dashed line in plot (a) represents the average value. Linear regression parameters are presented in the top left hand corner of each plot.

3.5.5 Results: Regional effects

Total organics: The minimal adequate model (MAM) describing Torg values was a linear mixed-effects model incorporating Farm ID as a random factor (L-ratio =33.379, d.f. = 1, $p < 0.0001$) and Distance as main term (L-ratio = 10.938, d.f. = 4, $p = 0.0273$). There were no higher order interaction terms, although an earlier iteration of the model contained a marginally significant lnMCB x Distance interaction (L-ratio = 9.794, d.f. = 4, $p = 0.044$). Further examination of this interaction using REML confirmed that the 2-way interaction term was not significant ($F = 2.228$, d.f. = 35, $p = 0.0859$).

Organic carbon: The Corg MAM was a linear mixed-effects model with Farm ID as a random factor (L-ratio = 23.885, d.f. = 1, $p < 0.0001$) and Distance as a main term (L-ratio = 14.962, d.f. = 4, $p = 0.0048$).

Nitrogen: The N MAM was a linear mixed-effects model with Farm ID as a random factor (L-ratio = 18.956, d.f. = 1, $p < 0.0001$) and Distance as a main term (L ratio = 20.707, d.f. = 4, $p = 0.0004$).

Copper: The Cu MAM was a standard linear regression model containing only Distance as a main term (F-value =8.552, d.f. = 4, $p < 0.0001$).

Phosphorus: The P MAM was a linear regression with a GLS extension containing lnMCB and Distance as main terms (L-ratio = 12.1809, d.f. = 1, $p < 0.0005$ and L-ratio = 20.0079, d.f. = 4, $p < 0.0005$ respectively) and also Distance as a variance covariate.

Model predictions of sediment concentrations of Torg, Corg, N, Cu and P are presented in Figure. 18. Sediment concentrations of all the investigated variables decreased significantly with distance from the cage edge. Interestingly, the concentrations of Torg, N and P did not differ significantly between 0 and 25 m from the cage edge ($p > 0.05$) and, whilst the concentrations of Corg and Cu were statistically lower 25 m away from the cage edge, this effect was marginal ($p = 0.043$ and 0.03 respectively).

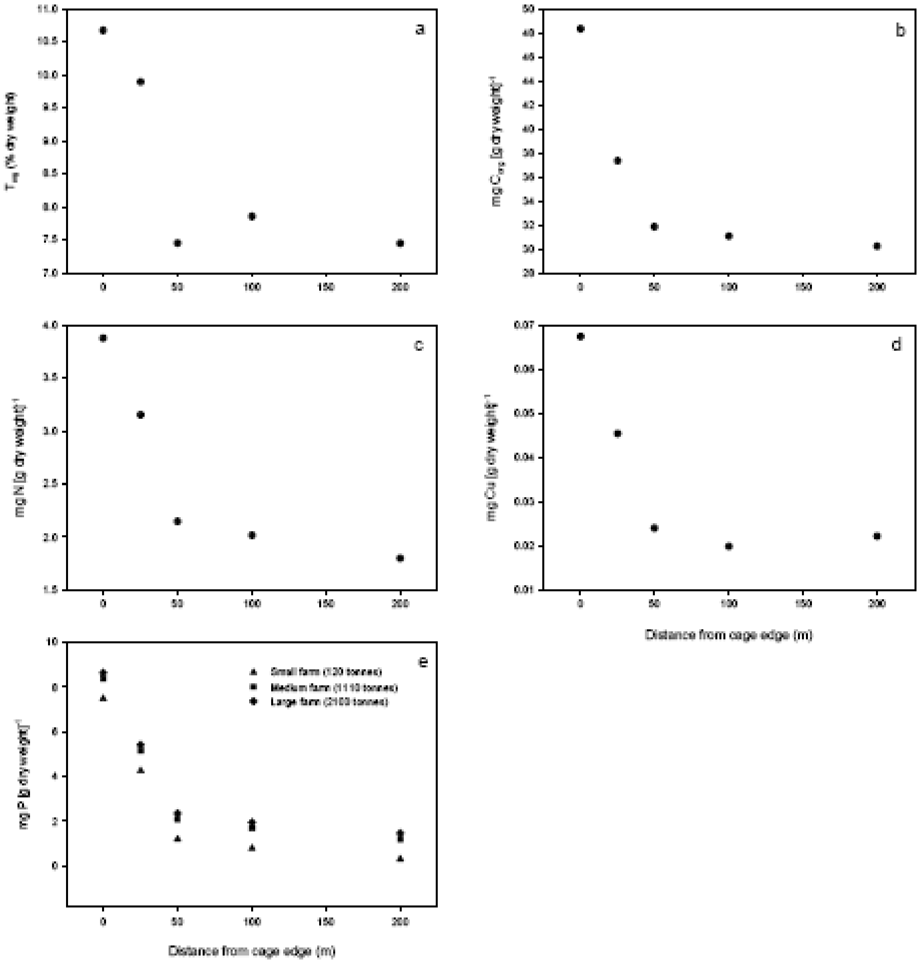


Figure 18: Model-predicted effects of distance from the cage edge on the sediment concentrations of (a) total organic matter (Torg), (b) organic carbon (Corg), (c) nitrogen (N), (d) copper (Cu) and (e) phosphorus (P) within a region of Scotland. Figure (e) also presents the effect of farm size as this remained significant in the P model.

3.5.6 Results: National effects

Total organics: The Torg MAM was a linear mixed-effects model with Farm ID as a random factor (L-ratio = 57.8648, d.f. = 1, $p < 0.0001$) and Distance as a main term (L-ratio = 32.24989, d.f. = 3, $p < 0.0001$). An early iteration of this model contained a marginally significant MCB × Distance × Average CS interaction (L-ratio = 9.2573, d.f. = 3, $p = 0.0261$) but this was not significant when re-examined using REML estimation ($F = 2.69699$, d.f. = 3, $p = 0.0551$).

Organic carbon: The Corg MAM was a linear mixed-effects model containing Farm ID as a random factor (L-ratio = 84.5449, d.f. = 1, $p < 0.0001$) and no significant main terms, i.e. an intercept-only model. As for previous models, a weakly significant interaction (MCB × Average

CS; L-ratio = 4.0238, d.f. = 1, p = 0.0449) was insignificant when examined using REML estimation (F = 3.5606, d.f. = 1, p = 0.0774) and was dropped from the analysis.

Nitrogen: The N MAM was a linear mixed-effects model with Farm ID as a random factor (L-ratio = 17.2356, d.f. = 1, p < 0.0001) and Average CS as a main term (L-ratio = 6.3828, d.f. = 1, p = 0.0115). Model-predicted values of Torg and N are presented in Figure 19.

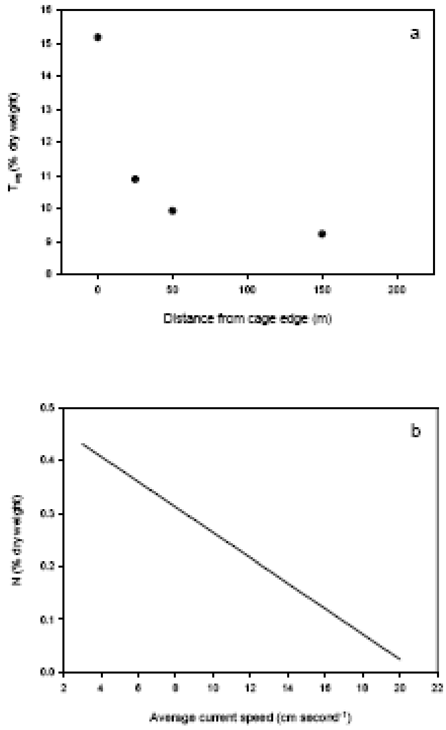


Figure 19: Model-predicted effects of distance from the cage edge of sediment concentrations of total organic matter (Torg) (a) and the effects of average current speed on sediment concentrations of nitrogen (N) (b) at the national-scale.

3.5.7 Discussion

The data analysed here were submitted to SEPA in 2005. All farms under investigation were therefore consented prior to the introduction of AutoDepomod. Our statistical models allowed us to examine how well the various explanatory variables explained the observed patterns in the response variables. In the broadest terms, our analyses of the 2005 dataset reveal that (1) larger farms are operated more intensively in terms of their fish stocking density, (2) distance from the edge of a fish farm plays a major role in determining sediment concentrations of organic pollutants, (3) there is considerable variation in between-site reference concentrations of organic matter, and (4) there is little evidence to suggest that, in a Scottish context, increasing the capacity of a fish farm (MCB) necessarily has a discernable effect on the magnitude or extent of its resulting benthic organic pollution because larger farms are sited in more dispersive environments. This indicates that the location of a farm is a critical factor in determining patterns of sediment enrichment.

It is commonly assumed that larger farms will have a larger impact; regressions of the 2005 data presented here suggest that they are stocked to a higher density and may therefore be expected to exert a greater impact per unit area. If fish farms were licensed without regard to site-specific information, such as the local topography and hydrography, this may well be the case. However, the licensing process used by SEPA typically to permit larger farms to be sited in 'low risk' areas where elevated current speeds aid the dilution of wastes by distributing them further a field (Cromey et al. 2002; SEPA 2005), as confirmed by the positive relationship between MCB and average current speed. The majority of the analyses presented here demonstrate that MCB does not play a significant role in explaining the degree of enrichment found in the sediments beneath Scottish fish farms. This indicates that the consenting procedure adopted by SEPA has, at least until 2005, effectively minimised the effects farming intensification on the benthic environment. The only minimum adequate model (MAM) to contain MCB as a significant component was that describing regional-scale sediment concentrations of P. This could indicate that the licensing process incorrectly ascribes the quantities of solid fish farm waste deposited at small and large fish farms, but MCB did not remain as a significant component in the models describing the other variables investigated so it is unlikely that this is the only mechanism. An alternative explanation is that the elemental stoichiometry of solid waste arriving at the seabed differs with farm size (i.e. the weight-specific P content of solid fish farm waste increases with farm size) and/or with the identity of farmed fish; the three larger farms contained cod at the time of survey. The N:P ratio of the feeds offered to on growing cod and salmon are 5.0 (Skretting Europa 18) and 5.6 (Skretting Atlantic HE300) respectively. Cod digest N and P with corresponding efficiencies of ~85 and ~59 % (mean of values presented in Table 4 in Albrektsen et al. 2006), whereas Atlantic salmon digest ~93 % of the N and ~53 % of the P that they consume (mean of values presented in Table 5 in Glencross et al. 2004). Thus cod and salmon faeces will have N:P ratios of 1.9 and 0.9 respectively, assuming that all non-absorbed material is lost as faecal pellets. On this basis the presence of cod at the three largest farms does not explain the positive effect of MCB on the P content of the underlying sediments. This relationship more likely reflects differences in farming practices; the quantity of food administered at the smaller farms was based on the expected demands of the fish and the experience of individual farmers, whereas the larger farms surveyed used automated feed hoppers that stop the delivery of food when uneaten feed pellets are detected by acoustic sensors located beneath the cages (Ervik et al. 1994; Sinnott 2002). Automatic feeding systems reduce the proportion of feed pellets that are wasted, thereby increasing the P-content of the solid waste that reaches the seabed by reducing the ratio of food:faeces leaving the cages. If this is the case, a negative effect of farm size on sediment N concentrations may also have been expected, but this was not so for either of the N models. At the national-scale, however, our model predicts a negative relationship between average current speed and sediment N. It is possible that SEPA's modelling procedure (as of 2005 – note that procedures have become more sophisticated since this date) to under- and over estimates waste deposition in low and high current speed environments, but considering the collinearity between MCB and average current speed, it is also possible that larger farms result in lower N enrichment. This interpretation is particularly interesting because it suggests that a by-product of farming practices adopted by larger farms is to allow them to maximise their environmental efficiency with regard to nitrogenous solid wastes at the expense of that of phosphorus. It should be noted that elevated levels of phosphorus in the sediments beneath and adjacent to fish farms do not necessarily imply that the benthic community's capacity to assimilate fish farm waste is being exceeded. The N:P ratio in marine benthic invertebrates ranges between 51 and 137 (Hatcher 1994), which is far greater than that of fish faeces or feed pellets (loc. cit.). Even if benthic invertebrates display high and low gross growth efficiencies for N and P respectively, it is highly likely that the supply of P will remain in excess relative to that of N. Phosphorus will therefore have a tendency to accumulate in sediments simply due to stoichiometric constraints (Sterner & Elser 2002). Including P monitoring in future benthic surveys could provide additional information on the supply of fish farm wastes to the benthic environment. Further, if discharges of C were regulated by licensing the amount of feed used this would alter a lot of factors.

Model-predicted concentrations of all the variables investigated at the regional-scale, and for Torg at the national-scale, decline significantly with distance from the cage edge. This result is not entirely surprising considering the dispersive nature of the marine environment and the philosophy of waste dilution that underpins the SEPA licensing procedure. The exclusion of current speed and depth from the majority of the models supports the idea that these factors are not strong predictors of benthic impact (Carroll et al. 2003). Interestingly, the modelled concentrations of many of the variables determined at 25 m from the cage edge either did not differ statistically, or were only marginally different, from those at the cage edge, whereas sediment concentrations ≥ 50 m were significantly lower. This suggests that, for the 2005 dataset used here, that the 'immediate footprint' of a Scottish fish farm extends to somewhere between 25 and 50 m from the cage edge, irrespective of the MCB, although recent meta-analyses on larger datasets of the effects of fish farming on sediment chemistry suggest that the discernable effects of marine fish farms on benthic chemistry typically extend < 200 m from the cage edge (Carroll et al. 2003; Kalantzi & Karakassis 2006;

Pusceddu et al. 2007). It is apparent that the most severe effects of fish farming are mainly detectable < 50 m from the edge of farms (this study; Carroll et al. 2003). Natural variation in the biochemistry of background sediments between fish farm sites has previously been an issue confounding the interpretation of the effects of fish farming (Pusceddu et al. 2007). The regional- and national-scale data sets analysed here encompass a broad spectrum of fish farm sizes and therefore a large range of environmental conditions, husbandry techniques and operational procedures.

The necessity to include Farm ID as a random factor for the Torg, Corg and N data sets at both the regional- and national-scale confirms that reference concentrations of these parameters vary significantly between sites. That five of these six models were able to successfully relate observed sediment parameters to explanatory variables suggests that linear mixed modelling is a powerful tool for the analysis of datasets in which trends are potentially masked by systematic between-site differences. Our inability to successfully model Corg at the national-scale could suggest that fish farm wastes have an idiosyncratic effect on this parameter (sensu Pusceddu et al. 2007). However, values of Corg are arguably the least robust of all the parameters investigated because elemental analysers do not distinguish between organic and inorganic carbon. Large deposits of calcium carbonate-rich shells typically accumulate in the sediments beneath and adjacent to fish farms, but the benthic reports extracted from the SEPA archive do not refer to inorganic carbon contaminants (e.g. Hedges & Stern 1984). We therefore suggest that our insignificant national-scale Corg model demonstrates that this variable is not necessarily useful as an index for inferring the benthic impacts of fish farming.

In conclusion, it is possible to increase the capacity of a marine fish farm without necessarily increasing the magnitude of its impact on the benthic environment. This has been achieved in Scotland within a framework of policy-driven legislation and monitoring procedures that are underpinned by a philosophy of waste dilution. The applicability of this approach to other aquaculture industries remains to be seen, and its success may well depend on a high degree of self-regulation in areas where environmental protection legislation is lacking. The general observation that larger capacity farms achieve a higher stocking density without a statistically discernable increase in the magnitude of their impacts on the surrounding sediments in Scotland suggests that the licensing procedures adopted by SEPA has been effective at designating appropriate fish farm sizes across the range of conditions present in Scotland.

4.0 References

Albrektsen, S., Mundheim, H. & Aksnes, A. 2006 Growth, feed efficiency, digestibility and nutrient distribution in Atlantic cod (*Gadus morhua*) fed two different fish meal qualities at three dietary levels of vegetable protein sources. *Aquaculture* **261**, 626-640.

Allen, S. E. 1974 *Chemical Analysis of Ecological Materials*. Blackwell, Oxford.

Allen, Y.T., Thain, J.E., Haworth, S., Barry, J., 2007. Development and application of long-term sublethal whole sediment tests with *Arenicola marina* and *Corophium volutator* using Ivermectin as the test compound. Environ. Poll. 146: 92-99.

Almeida, A., Cunha, A., Fernandes, S., Sobral, P. Alcantara, F., 2007. Copper effects on bacterial activity of estuarine silty sediments. Est. Coast. Shelf Sci. 73: 743-752.

Anschutz, P., Chaillou, G., Lecroart, P., 2007. Phosphorus diagenesis in sediment of the Thau Lagoon. Est. Coast. Shelf Sci. 72: 447-456.

Aquaculture S.A., 2003. Atlantic salmon aquaculture in South Australia. FS No: 16/99, 1-3pp.

Bat, L., Raffaelli, D., 1998. Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. J. Exp. Mar. Biol. Ecol. 226: 217-239.

Banta, G.T., Holmer, M., Jensen, M.H., Kristensen, E., 1999. Effects of two polychaete worms, *Nereis diversicolor* and *Arenicola marina*, on aerobic and anaerobic decomposition in a sandy marine sediment. Aquat. Microb. Ecol. 19: 189- 204.

Bebak-Williams J., Bullock, G., Carson., M.C., 2002. Oxytetracycline residues in a freshwater recirculating system. Aquaculture 205: 221-230.

Bianchi, 2007. Biogeochemistry of Estuaries, Oxford University Press, New York.

Blackburn, T. H., Blackburn, N. D., 1992. Model of nitrification and denitrification in marine sediments. FEMS Microbiol. Lett. 100: 517-522.

Blackburn, T. H., Blackburn, N. D., 1993. Coupling of cycles and global significance of sediment diagenesis. Mar. Geol. 113: 101-110.

Bower, C.E., Turner D.T., 1982. Effects of seven chemotheraputic agents on nitrification in closed seawater culture systems. Aquaculture 29: 331-345.

Braam, F., Klapwijk, A., 1981. Effect of copper on nitrification in activated sludge. Water Res. 15: 1093-1098.

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

Bright, D.A., Dionne, S., 2005. Use of emamectin benzoate in the Canadian finfish aquaculture industry: A review of environmental fate and effects. Environment Canada http://www.pyr.ec.gc.ca/georgiaBasin/resources/publications/SciTechReports/EC378GB-0484_e.pdf

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

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

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

Canty, M.N., Hagger, J.A., Moore, R.T.B., Cooper, L., Galloway, T.S., 2007. Sublethal impact of short term exposure to the organophosphate pesticide azamethiphos in the marine mollusc *Mytilus edulis*. Mar. Poll. Bull. 54: 396-402.

Capone, D.G., Weston, D.P., Miller, V., Shoemaker, C., 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. Aquaculture 145: 55-75.

Carroll, M. L., Cochrane, S., Fieler, R., Velvin, R. & White, P. 2003 Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. *Aquaculture* **226**, 165-180.

Ciarelli, S., Vonck, W.A.P.M.A., van Straalen, N.M., 1997. Reproducibility of spiked-sediment bioassays using the marine benthic amphipod, *Corophium volutator*. Mar. Env. Res. 43: 329-343.

Chuckwudebe, A.C., Atkins, R.H., Wislocki, P.G., 1997. Metabolic fate of emamectin benzoate in soil. J. Agric. Food Chem. 45: 4137-4146.

Corner, R. A., Brooker, A. J., Telfer, T. C. & Ross, L. C. 2006 A fully integrated GIS428 based model of particulate waste distribution from marine fish-cage sites. *Aquaculture* **258**, 299-311.

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

Coyne, R., Smith, P., Moriarty, C., 2001. The fate of Oxytetracycline in the marine environment of a salmon cage farm. Marine Environment and Health Series. 3: 1-24.

Crane, M., Warr, S., Codling, I., Power, B., 2006. Review of environmental quality standards (EQSs) for use in assimilative capacity model development. Project final report, SARF011.

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

Cuny, P., Miralles, G., Cornet-Barthaux, V., Acquaviva, M., Stora, G., Grossi, V., Gilbert, G., 2007. Influence of bioturbation by the polychaete *Nereis diversicolor* on the structure of bacterial communities in oil contaminated coastal sediments. Mar. Poll. Bull. 54: 452-459.

Danovaro, R., Pusceddu, A., 2007. Biodiversity and ecosystem functioning in coastal lagoons: Does microbial diversity play any role? Est. Coast. Shelf. Sci. 75: 4-12.

Davidson, P. M., Brekke, C. J., Branen, A. L., 1981. Antimicrobial activity of butylated hydroxyanisole, teriary butylhydroquinone, and potassium sorbate in combination. J. Food Science. 46: 314-316.

Davies, I.M., Rodger, G.K., Redshaw, J., Stagg, R.M., 2001. Targeted environmental monitoring for the effects of medicines used to treat sea-lice infestation on farmed fish. ICES J. Mar. Sci. 58: 477-485.

Davis, G. K., Mertz, W., 1986. Copper. In: Trace elements in human and animal nutrition (Mertz, W., ed.), 5th edition, Vol 1, Academic Press, San Diego, California.

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

Durou, C., Mouneyrac, C., Amiard-Triquet, C., 2004. Tolerance to metals and assessment of energy reserves in the polychaete *Nereis diversicolor* in clean and contaminated estuaries. Environ. Toxicol. 20: 23-31.

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

Ervik, A., Samuelsen, O. B., Juell, J. E. & Sveier, H. 1994 Reduced environmental impact of antibacterial agents applied in fish farms using the LiftUp feed collector system or a hydroacoustic feed detector. *Dis. Aquat. Org.* **19**, 101-104.

FAO, 2002. The state of the world fisheries and aquaculture 2002. FAO, Rome, 150pp.

FAO, 2006. State of world aquaculture 2006. FAO Fisheries Technical Paper 500. Rome, 134.

Fernandes, T. F., Miller, K. L., Read, P. A., 2000. Monitoring and regulation of marine aquaculture in Europe. J. App. Icthyol. 16: 138-143.

Fernandes, S., Meysman, F. J. R., Sobral, P. 2006. The influence of Cu contamination on *Nereis diversicolor* bioturbation. Mar. Chem. 102: 148-158.

Fukuhara, H., Sakamoto, M., 1987. Enhancement of inorganic nitrogen and phosphate release from lake sediment by tubificid worms and chiromomid larvae. Oikos 38: 312-320.

Gerino, M., Stora, G., Grancois-Carcaillet, F., Gilbert, F., Poggiale, J.-C., Mermillod- Blondin, F., Desrosiers, G., Vervier, P., 2003. Macro-invertebrate functional groups in freshwater and marine sediments: a common mechanistic classification. Vie Milieu 53: 221-231.

Giller, K. E., Witter, E., McGrath, S. P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. Soil. Biol.Biochem. 30: 1389-1414.

Gillibrand, P. A., Gubbins, M. J., Greathead, C. & Davies, I. M. 2002 Scottish executive locational guidelines for fish farming: Predicted levels of nutrient enhancement and benthic impact. Scottish Fisheries Research Report Number 63/2002. Aberdeen: Fisheries Research Services.

Glencross, B. D., Carter, C. G., Duijster, N., Evans, D. R., Dods, K., McCafferty, P., Hawkins, W. E., Maas, R. & Sipsas, S. 2004 A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **237**, 333-346.

Gorman, M., Raffaelli, D., 1993. The Ythan estuary. Biologist 40: 10–13.

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

Hamilton, M.A., Russo, R.C., Thurston, R.V., 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714-719.

Hansen, K., Kristensen, E., 1997. Impact of macrofaunal recolonization on benthic metabolism and nutrient fluxes in a shallow marine sediment previously overgrown with macroalgal mats. Est. Coast. Shelf. Sci. 45: 613-628.

Hatcher, A. 1994 Nitrogen and phosphorus turnover in some marine benthic invertebrates: implications for the use of C:N ratios to assess food quality. *Mar. Biol.* **121**, 161-166.

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

Hebel., D. K., Jones, M. B., Depledge, M. H., 1997. Responses of crustaceans to contaminant exposure: A holistic approach. Est. Coast. Shelf Sci. 44: 177-184.

Hedges, J. I. & Stern, J. H. 1984 Carbon and nitrogen determination of carbonate containing solids. *Limnol. Oceanogr.* **29**, 657-663.

Heilskov, A. C., Holmer, M., 2001. Effects of fauna on organic matter mineralization in fish-farm sediments: importance of size and abundance. ICES J. Mar. Sci. 58: 427- 435.

Heilskov, A. C., Alperin, M., Holmer, M., 2006. Benthic fauna bio-irrigation effects on nutrient regeneration in fish farm sediments. J. Exp. Mar. Biol. Ecol. 339: 204-225.

Henderson, A. R. & Davies, I. M. 2000 Review of aquaculture, its regulation and monitoring in Scotland. *J. Appl. Ichthyol.* **16**, 200-208.

Herman, E. B., Haas, G. J., Crosby, W. H., Cante, C. J., 1980. Antimicrobial action of short chain alcohols and glycols. J. Food Safety. 2: 131-139.

Hodson, S. L., Lewis, T. E., Burke, C. M., 1997. Biofouling of fish-cage netting: efficacy and problems of *in situ* cleaning. Aquaculture. 152: 77-90.

Holmer, M. & Kristensen, E. 1992 Impact of fish farming on metabolism and sulphate reduction of underlying sediments. *Mar. Ecol. Prog. Ser.* **80**, 191-201.

Hyland, J., Balthis, L., Karakassis, I., Magni, P., Petrov, A., Shine, J., Vestergaard, O. & Warwick, R. 2005 Organic carbon content of sediments as an indicator of stress in the marine benthos. *Mar. Ecol. Prog. Ser.* **295**, 91-103.

Ieno, E.N., Solan, M., Batty, P., Pierce, G.J., 2006. How biodiversity affects ecosystem functioning: roles of infaunal species richness, identity and density in the marine benthos. Mar. Ecol. Prog. Ser. 311: 263-271.

Jensen, H. S., Mortensen, P. B., Andersen, F. O., Rasmussen, E., Jensen, A., 1995. Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. Limnol. Oceanogr. 40: 908-917.

Johnsen, K., Jacobsen, C. S., Torsvik, V., 2001. Pesticide effects on bacterial diversity in agricultural soils – a review. Biol. Fert. Soils. 33: 443-453.

Johnson, S.C., Treasurer, J.W., Bravo, S., Nagasawa, K., Kabata, Z., 2004. A review of the impact of parasitic copepods on marine aquaculture. Zool. Stud. 43: 229-243.

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

Jones, C. S., Lockyer, A.E., Verspoor, E., Secombes, C.J., Noble L.R., 2002. Towards selective breeding of Atlantic salmon for sea louse resistance: approaches to identify trait markers. Pest Manag. Sci. 58: 559-568.

Kalantzi, I. & Karakassis, I. 2006 Benthic impacts of fish farming: Meta-analysis of community and geochemical variables. *Mar. Poll. Bull.* **52**, 484-493.

Kater, B.J., Dubbeldam, M., Postma, J.F., 2006. Ammonium toxicity at high pH in a marine bioassay using *Corophium volutator*. Arch. Environ. Contam. Toxicol. 21: 347-351.

Kater, B.J., Hannewijk, A., Postma, J.F., Dubbeldam, M., 2000. Seasonal changes in acute toxicity of cadmium to amphipod *Corophium volutator*. Environ. Toxicol. Chem. 19: 3032-3035.

Kelly, J. R., Nixon, S. W., 1984. Experimental studies of the effect of organic deposition on the metabolism of a coastal marine bottom community. Mar. Ecol. Prog. Ser. 17: 157-169.

Kerry, J., Coyne, R., Gilroy, D., Hiney, M., 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.

Kristensen, E., Blackburn, T. H., 1987. The fate of organic carbon and nitrogen in experimental marine sediment systems: influence of bioturbation and anoxia. J. Mar. Res. 45: 231-257.

Kristensen, E. & Andersen, F. O. 1987 Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. *J. Exp. Mar. Biol. Ecol.* **109**, 15-23.

Lee, Y.-W., Ong, S.-K., Sato, C., 1997. Effects of heavy metals on nitrifying bacteria. Water Sci. Technol. 36: 69-74.

Lorentzen, M., Maage, A., Julshamn, K., 1998. Supplementing copper to a fish meal based diet fed to Atlantic salmon parr affects liver copper and selenium concentrations. Aquac. Nutr. 4: 67-72.

Maund, S.J., Hamer, M.J., Lane, M.C.G., Farrelly, E., Rapley, JH., Goggin, U.M., Gentle, W.E., 2002. Partitioning, bioavailability and toxicity of the pyrethroid insecticide cypermethrin in sediments. Environ. Toxicol. Chem. 21: 9-15.

Mayor, D. J., Solan, Martinez, I., Murray, L., McMillan, H., Paton, G. I., Killham, K., Submitted. Acute toxicity of some treatments commonly used by the salmonid aquaculture industry to *Corophium volutator* and *Hediste diversicolor*: whole sediment bioassay tests.

McHenery, J.G., Mackie, C., 1999. SCH001R5 Revised expert report on the impacts of emamectin benzoate formulated as Slice® for salmonids. CORDAH Environmental Management Consultants.

Mente, E., Pierce, G., Santos, M.B., Neofitou, C., 2006. Effect of feed and feeding in the culture of salmonids on the marine aquatic environment: A synthesis for European aquaculture. Aquac. Int. 14: 499-522.

Montgomery, D. C. & Peck, E., A. 1992 *Introduction to linear regression analysis.*Wiley & Sons, New York.

Mushtaq, M., Feely, W.F., Syintsakos, L.R., Wislocki, P.G., 1996. Immobility of emamectin benzoate in soils. J. Agric. Food Chem. 44: 940-944.

Nilsson, H. C., Rosenberg, R., 2000. Succession in marine benthic habitats and fauna in response to oxygen deficiency: analysed by sediment profile-imaging and by grab samples. Mar. Ecol. Prog. Ser. 197: 139-149.

Norling, K., Rosenberg, R., Hulth, S., Gremare, A., Bonsdorff, E., 2007. Importance of functional biodiversity and species-specific traits of benthic fauna for ecosystem functions in marine sediment. Mar. Ecol . Prog. Ser. 332: 11-23.

O’Dell, B. L., 1982. Biochemical basis of the clinical effects of copper deficiency. In: Clinical, biochemical and nutritional aspects of trace elements (Prasad, A. S., ed.), Alan, R. Liss Inc., New York.

Ozoh, P.T.E., 1992. The effects of salinity, temperature and sediment on the toxicity of copper to juvenile *Hediste (Nereis) diversicolor* (O.F.Muller). Environ. Monit. Assess. 21: 1-10.

Ozoh, P.T.E., 1994. The effect of salinity, temperature and time on the accumulation and depuration of copper in ragworm, *Hediste (Nereis) diversicolor* (O.F. Muller). Environ. Monit. Assess. 29: 155-165.

Papaspyrou, S., Gregersen, T., Kristensen, E., Christensen, B., Cox, R. P., 2006. Microbial reaction rates and bacterial communities in sediment and surrounding burrows of two nereidid polychaetes (*Nereis diversicolor* and *N. virens*). Mar. Biol. 148: 541-550.

Pearson, T. H., Black, K. D., 2001. The environmental impacts of marine fish cage culture. In: Black, K. D. (Ed.), Environmental impacts of aquaculture. Sheffield Academic Press, Sheffield.

Pearson, T. H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Mar. Biol. Ann. Rev. 16: 229- 311.

Pelegrí, S. P., Blackburn, T. H., 1994. Bioturbation effects of the amphipod *Corophium volutator* on microbial nitrogen transformations in marine sediments. Mar. Biol. 121: 253-258.

Perez, O. M., Telfer, T. C., Beveridge, M. C. M. & Ross, L. C. 2002 Geographical Information Systems (GIS) as a simple tool to aid modelling of particulate waste distribution at marine fish cage sites. *Est. Coast Shelf Sci.* **54**, 761-768.

Petersen, D. G., Dahllof, I., Nielsen, L. P., 2004. Effects of zinc pyrithione and copper pyrithione on microbial community function and structure in sediments. Environ. Toxicol. Chem. 23: 921-928.

Pike, A.W., Wadsworth, S.L., 1999. Sealice on salmonids: their biology and control. Adv. Parasitol. 44: 234–337.

Pinheiro, J. C. & Bates, D. M. 2000 *Mixed effects models in S and S-PLUS*. Springer Verlag, New York.

Pusceddu, A., Fraschetti, S., Mirto, S., Holmer, M. & Danavaro, R. 2007 Effects of intensive mariculture on sediment biochemistry. *Ecol. App.* **17**, 1366-1378.

Quinn, G.P., Keough, M.J., 2002. Experimental designs and data analysis for biologists. Cambridge University Press, Cambridge. 537 pp.

R development core team (2005). http://www.r-project.org/.

Ranjard, L., Echairi, A., Nowak, V., Lejon, D. P. H., Nouaim, R, Chaussod, R., 2006. Field and microcosm experiments to evaluate the effects of agricultural Cu treatment on the density and genetic structure of microbial communities in two different soils. FEMS Microbiol. Ecol. 58: 303-315.

Raynard, R.S., Bricknell, I.R., Billingsley, P.F., Nisbet, A.J., Vigneau, A., Sommerville, C., 2002. Development of vaccines against sea lice. Pest Manage. Sci. 58: 569-575.

RIKZ, 1999. The 10-day marine amphipod *Corophium volutator* mortality sediment toxicity test. Standard operating procedures, Nr: SPECIES – 01. National Institute for Coastal and Marine Management/RIKZ.

Rosenberg, R., Nilsson, H. C., Diaz, R. J., 2001. Response of benthic fauna and changing sediment redox profiles over a hypoxic gradient. Est. Coast. Shelf Sci. 53: 343-350.

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

Roth, M., 2000. The availability and use of chemotherapeutic sea lice control products. Contributions to Zoology 69: 109-118.

Scartlett, A., Rowland, S.J., Canty, M., Smith, E.L., Galloway, T.S., 2007. Method for assessing the chronic toxicity of marine and estuarine sediment-associated contaminants using the amphipod *Corophium volutator*. Mar. Env. Res. 63: 457-470.

SEPA, 1997. Scottish environment protection agency. Policy No. 17. Cage fish farms: Sea lice treatment chemicals risk assessment of azamethiphos (available from: http://govdocs.aquake.org/cgi/reprint/2004/524/5240170.pdf).

SEPA, 1998. Scottish environment protection agency. Policy No. 30. SEPA policy on the use of cypermethrin in marine cage fish farming. Risk assessment, EQS and recommendations (available from: http://govdocs.aquake.org/cgi/reprint/2003/1025/10250030.pdf).

SEPA, 1999. Scottish environment protection agency. Emamectin benzoate: An environmental risk assessment
(Available from: http://www.sepa.org.uk/pdf/aquaculture/policies/emamectin_benzoate.pdf).

SEPA 2004a. Scottish environment protection agency. The occurrence of chemicals used in sea louse treatments in sediments adjacent to marine fish farms: Results of monitoring surveys carried out by SEPA in 2001 & 2002
(Available from: http://www.govdocs.aquake.org/cgi/reprint/2004/524/5240240.pdf).

SEPA 2004b. Scottish environment protection agency. The occurrence of chemicals used in sea louse treatments in sediments adjacent to marine fish farms: Results of screening surveys during 2003. Report TR-040430JBT
(Available from: http://www.sepa.org.uk/pdf/aquaculture/projects/TR-040430JBT.pdf).

SEPA, 2005a. Scottish environment protection agency. Regulation and monitoring of marine cage fish farming in Scotland – A manual of procedures (available from: http://www.sepa.org.uk/guidance/fishfarmmanual/manual.asp).

SEPA, 2005b. Scottish environment protection agency. The occurrence of chemicals used in sea louse treatments in sediments adjacent to marine fish farms: Results of screening surveys during 2004. Report TR-050202JBT
(Available from: http://www.sepa.org.uk/pdf/aquaculture/projects/TR-050202JBT.pdf).

SEPA, 2006. Scottish environment protection agency. The occurrence of chemicals used in sea louse treatments in sediments adjacent to marine fish farms: Results of screening surveys during 2005. Report TR-060830JBT
(Available from: http://www.sepa.org.uk/pdf/aquaculture/projects/TR-060830_JBT.pdf).

Sinnott, R. 2002 Fish farming and feed companies. In: *Handbook of salmon farming* (Eds. Stead, S. M. & Laird, L.). Springer & Praxis Publishing, London, UK pp. 105-185.

Sloth, N. P., Blackburn, H., Hansen, L. S., Risgaard-Petersen, N., Lomstein, B. A., 1995. Nitrogen cycling in sediments with different organic loading. Mar. Ecol. Prog. Ser. 116: 163-170.

Smit, E., Leeflang, P., Wernars, K., 1997. Detections of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. FEMS Microbiol. Ecol. 23: 249-261.

Smith, P., 1996. Is sediment deposition the dominant fate of Oxytetracycline use in marine salmonid farms: A review of the available evidence. Aquaculture 146: 157-169.

Sokal, R. R., Rohlf, F. J., 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition. W. H. Freeman and Co.: New York.

Solberg, C.B., Sæthre, L., Julshamm, K., 2002. The effect of copper-treated net pens on farmed salmon (*Salmo salar*) and other marine organisms and sediments. Mar. Poll. Bull. 45:126-132.

SPAH, 2002. Schering-Plough Animal Health Technical Report. Potential environmental impacts of emamectin benzoate, formulated as SLICE, for salmonids.
(Available at: http://www.spaquaculture.com/assets/1-28FINALenvrofnl.pdf).

Spicer, J. I., Weber, R. E., 1991. Respiratory impairment in crustaceans and molluscs due to exposure to heavy metals. Comp. Biochem. Physiol. C. 100: 339-342.

Sterner, R. W. & Elser, J. J. 2002 *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton.

Strickland, J. D. & Parsons, T. R. 1972 *Bulletin 167: A practical handbook of seawater analysis.* Research Board of Canada, Ottawa.

Stronkhorst, J., Ciarelli, S., Schipper, C.A., Postma, J.F., Dubbeldam, M., Vangheluwe, M., Brils, J.M., Hooftman, R., 2004. Inter-laboratory comparison of five marine bioassays for evaluating the toxicity of dredged material. Aquat. Ecosyst. Health Manage. 7: 147-159.

Sundby, B., Gobeil, C., Silverberg, N., Mucci, A., 1992. The phosphorous cycle in coastal marine sediments. Limnol. Oceanogr. 37: 1129-1145.

Telfer, T. C., Baird, D. J., McHenery, J. G., Stone, J., Sutherland, I., Wislocki, P., 2006. Environmental effects of the anti-sea lice (Copepoda: Caligidae) therapeutant emamectin benzoate under commercial use conditions in the marine environment. Aquaculture. 260: 163-180.

Tedengren, M., Olsson, B., Bradley, B., Zhou, L., 1999. Heavy metal uptake, physiological response and survival of the blue mussel (*Mytilus edulis*) from marine and brackish waters in relation to the induction of heat-shock protein 70. Hydrobiologia . 393: 261-269.

Traunspurger, W., Drews, C., 1996. Toxicity analysis of freshwater and marine sediments with meio- and macrobenthic organisms: a review. Hydrobiol. 328: 215- 261.

Treasurer, J.W., 2002. A review of the potential pathogens of sea lice and the application of cleaner fish in biological control. Pest Manage. Sci. 58: 546-558.

Underwood, A. J., 1997. Ecological experiments: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.

U.S. Environmental Protection Agency. Statistical analysis for biological methods (available at: http://www.epa.gov/nerleerd/stat2.htm).

U.S. Environmental Protection Agency, 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods, EPA 600/R-94/025.

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

Wang, F., Chapman, P.M., 1999. Biological implications of sulfide in sediment—a review focusing on sediment toxicity. Environ. Toxicol. Chem. 18: 2526–2532.

Weis, P.J., Andrew, M.L., Wright, W.W., 1957. Solubility of antibiotics in twenty four solvents. Antibiotics Chemother. 7: 374-377.

White, G., 2006. Using sediment quality assessment criteria to define sediment quality in Aberdeen harbour. PhD thesis. University of Aberdeen.

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

Willis, K.J., Ling, N., 2004. Toxicity of the aquaculture pesticide cypermethrin to planktonic marine copepods. Aquac. Res. 35: 263-270.

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

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

Worthing, C.W., (Ed.). 1991. The Pesticide Manual (ninth edition.), British Crop Protection Council, London (1991).

Zar, J.H., 1999. Biostatistical analysis. 4th ed. Prentice Hall, New Jersey.

Zhou, J.L., Rowland, S.J., Mantoura, r.F.C., Harland, B.J., 1995. Influence of the nature of particulate organic matter on the sorption of cypermethrin: Implications on KOC correlations. Environ. Int. 21: 187-195.

Zuur, A. F., Ieno, E. N. & Smith, G. M., 2007 *Analysing ecological data*. Springer, New York.