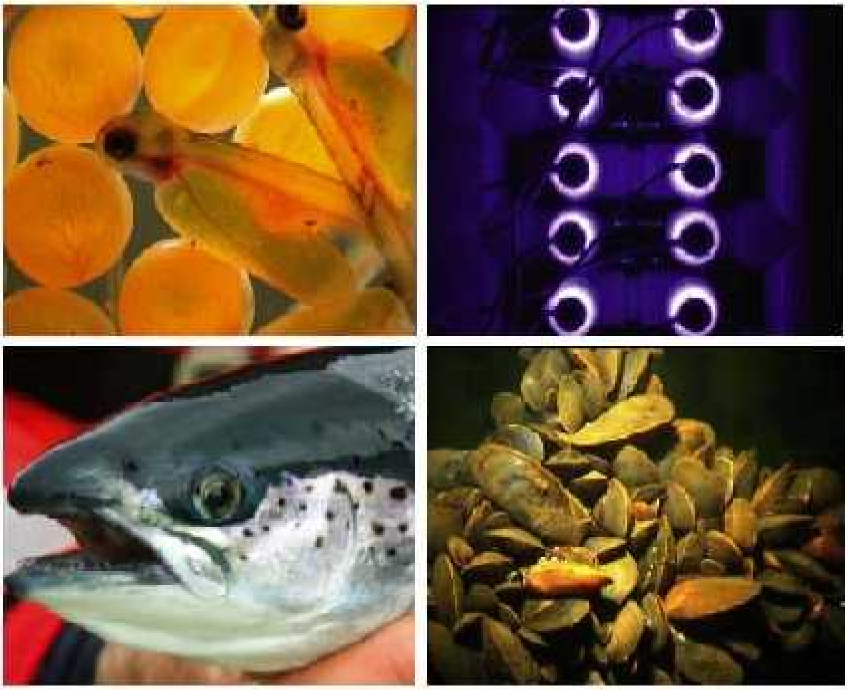
SARF098: Towards Understanding of the Environmental Impact of a Sea Lice Medicine – the PAMP Suite



A REPORT COMMISSIONED BY SARF

Published by the: Scottish Aquaculture Research Forum (SARF)

This report is available at: http://www.sarf.org.uk

Suggested Citation

SARF098: Towards Understanding of the Environmental Impact of a Sea Lice Medicine – the PAMP Suite, 2016. A study commissioned by the Scottish Aquaculture Research Forum (SARF). http://www.sarf.org.uk/

Title: Towards Understanding of the Environmental Impact of a Sea Lice Medicine – the PAMP Suite

ISBN: 978-1-907266-75-1

First published: August 2016

PREAMBLE

Two reports are subsumed within this document:

1. An independent SARF review of PAMP 1 and 2 (authored by **Edwards *et al***) that was commissioned to provide a wider context to the PAMP 2 (2016) Report (see below) and to address some of the concerns and recommendations from SARF Directors, independent referees and the authors of the PAMP 2 report. This SARF review was also directed to provide reasoned arguments for further more focused research in similar areas of study.

2. The original PAMP 2 Report that was received and accepted by SARF in March 2016 and was authored by **Wilding T. A. and Black K.D**.

SARF recommends strongly that both the SARF review and the original PAMP 2 report should be read in tandem using the cross-referencing provided in the review.

Taken together, both publications argue cogently for further research and suggest some areas where appropriate applications of published data, field measurements, scientific techniques and statistical methods etc. could be used to help provide solutions to the general problem of countering sea lice infestation both in Scotland and elsewhere.

Towards Understanding of the Environmental Impact of a Sea Lice Medicine – the PAMP Suite

Anton Edwards CPhys, FinstP, for the Scottish Aquaculture Research Forum (SARF). May 2016

With thanks to Professor Marian Scott and Michael Kendall

Contents

1 INTRODUCTION

The therapeutic aquacultural use of anti-parasitic medicinal compounds inevitably leads to their partial release to the sea. The biological effects of these medicines are variously controlled by precautionary ecotoxicological criteria and by the application of precautionary regulatory models and monitoring designed to contain effects with regulatory limits. As in all such areas of environmental management supported by sound science and evidence-based regulation, a ubiquitous and reasonable question always arises as time goes on and more evidence becomes available from regulatory monitoring and other sources: is sufficient being done to detect effects and to protect the biological systems?

In particular, the infeed anti-louse chemicals Emamectin Benzoate (EMB) and Teflubenzuron (TBZ) are consented for use in Scotland and have been in use for more than a decade. This report describes and encapsulates the most recent SARF-sponsored research on the possible effects of these medicines and presents it within an outline framework that is specifically designed to define and support future work.

2 THE SCOTTISH AQUACULTURE RESEARCH FORUM (SARF)

The Scottish Aquaculture Research Forum (www.sarf.org.uk) is a registered charity and an independent company whose main aim is to support research into aquaculture and related areas.

SARF aims to support, by way of delivering high quality research, the vision outlined in the Strategic Framework for Scottish Aquaculture (see http://www.gov.scot/Publications/2003/03/16842/20502):

"Scotland will have a sustainable, diverse, competitive and economically viable aquaculture industry, of which its people can be justifiably proud. It will deliver high quality, healthy food to consumers at home and abroad, and social and economic benefits to communities, particularly in rural and remote areas. It will operate responsibly, working within the carrying capacity of the environment, both locally and nationally and throughout its supply chain."

SARF's published objectives are threefold:

• To promote, encourage and support scientific research and development in aquaculture and related areas. This includes research in the fields of environmental impacts, technical and biological cultivation and health and welfare science.

• To enhance public understanding of aquaculture through the dissemination of research results in the public domain. This will include research exhibitions and on-line publications of investigations and results.

• SARF has a range of members including representatives from the aquaculture industry, government organisations, wild fish groups and environmental NGO's. The research undertaken will be across a broad range of areas and will be required to meet the collective needs of its members.

3 Investigation of Marine Effects of Sea Lice Medicines

3.1 Introduction

It was from the perspectives of section 2 that SARF issued a call for research to investigate the interactions of sea lice medicines with marine environmental systems. The first research work (http://www.sams.ac.uk/kenny-black/pamp) began in 1999, ended in 2004, and is referred to here as the PAMP-1 Study (2005). Further work was commissioned by SARF in 2013 and was completed in 2015; this work is referred to here as the PAMP-2 Study (2015).

3.2 The PAMP-1 Study

Following the authorisation of medicinal products for the treatment of sea lice in Scottish waters, the five-year PAMP-1 project was undertaken by the Scottish Association for Marine Science (SAMS) to examine whether the use and release of these substances had any subtle and long term impacts on marine biota. This was a comprehensive study that examined the potential effects on a number of environmental compartments and on a number of different biotic communities. The main conclusion of the PAMP-1 study was that there were no effects of the use of sea louse medicines that could be discerned beyond the natural variability seen in the marine environment and the natural changes that may normally be seen in marine communities.

3.3 Need for a PAMP-2 Study

In 2013 there was no explicit reason to suspect that continuing use of sea louse medication had led to wide scale environmental impacts. However, in the fourteen years since the start of the PAMP-1 study, the use of Slice - which became available in 2001 mid-way through the PAMP-1 study - increased significantly and a new product, Deltamethrin, also became available. SARF recognised that the cumulative and increasing use of various products at sites and from well boats might be significant, and therefore deserved further attention. A project to detect and define any significant marine ecosystem changes attributable to such long term medicine use was both justified and timely.

At this stage it was clear that the necessary effort and resource of setting up a well-designed hypothesis-testing research programme (section 6) to elucidate quantifiable correlative and process-based links between medicine application and environmental effect (particularly on the benthos) would be well beyond the available resources. The expense of a robust programme of this sort was not possible nor, without any supporting evidence from a prior investigation, might it be justified. A pilot study was needed, one that would take advantage of existing measurements and recorded data about both the benthos and the use of medicines.

4 The Research Call for PAMP-2

From the viewpoint of section 3.3, it was therefore proposed that in the first instance a desk-based analysis of historical data sets be undertaken at various sea loch locations linked to the PAMP-1 study. The project was to focus on places with substantial existing biological & chemical data and where there had been significant use of sea louse medicines or where a number of treated sites were in close proximity. In this way it was hoped that the project might provisionally identify medicine-related changes either in sea loch ecology and biological community structure near fish farm premises or at larger scales within water-bodies, based purely upon the historic monitoring records.

SARF recognised the advantage of analysing a (fairly) consistently gathered set of regulatory observational data that was available without the immediate necessity for costly field work or laboratory trials.

Nevertheless, the research call (section 4.1) recognised the weaknesses and risks inherent when correlating data that had been gathered purely for formal monitoring. These data had not been obtained as part of a hypothesis-testing experimental research program. They had been collected for regulatory purposes and, because of normal monitoring cost constraints, had been collected without detailed regard for the statistical sampling issues that surround the design of hypothesis-testing experiment. Nor were the data designed to illuminate or to take overt account of relevant physical, chemical and biological processes by which medicines may influence marine life.

It was therefore explicit in the call that a subsequent SARF project (if justified by PAMP-2 outcomes) might undertake field investigation at the relevant sea loch locations linked to the PAMP-1 and PAMP-2 studies.

4.1 The Research Call

The following research call was issued publicly with a response deadline of September 27th 2013; it was commissioned by competitive tender and the contract (as Project SARF098) was awarded to SAMS. The following version omits purely administrative or financial details:

PROJECT	SARF098	PAMP Refreshment Study - Research Requirement

Context:

Following the authorisation of medicinal products for the treatment of sea lice in Scottish waters a 5-year project known as the PAMP (Post Authorisation Monitoring Project, see http://www.sams.ac.uk/kenny-black/pamp) study was undertaken to examine whether the use and release of these substances had any subtle and longer term impacts on marine biota. The study was comprehensive, examining the potential effects on a number of environmental compartments and on a number of different biotic communities.

The main conclusion of the PAMP study was that there were no effects of the use of sea louse medicines which could be discerned beyond the natural variability seen in the marine environment and the natural changes that may be seen in marine communities.

It is eight years since the PAMP study reported and 14 years since its inception, during which time the use of Slice, which became available in 2001 (mid-way through the study), has increased significantly and a new product, Deltamethrin, has also become available. While there is no reason to suspect that the on-going use of sea louse medication has led to wide scale environmental impacts, the cumulative use of various products at a large number of sites may be significant and is increasing with discharges arising from both cages and well boats.

A project to detect and define any significant marine ecosystem changes attributable to such long term medicine use is justified and timely. It is proposed that in the first instance an analysis of historic data sets be undertaken at a number of sea loch locations linked to the PAMP study. The project should focus on places with substantial existing biological and chemical data, and where there has been significant use of sea louse medicines or where a number of treated sites are in close proximity. The project will seek to identify such medicine-related changes either in sea loch ecology and biological community structure near fish farm premises or at larger scales within water-bodies, based upon the historic monitoring records.

A subsequent SARF project, if justified by outcomes from SARF098, may undertake field investigation at the relevant sea loch locations linked to the PAMP study.

Outline Research Requirement (numbered here for reference):

1. SARF requires research that updates the original PAMP project and assesses the possible effects of more recently introduced sea lice treatment chemicals and the on-going long term use of these products at single or multiple fish farms within a single water body.
2. It is envisaged that the research will be require a desktop study, aimed at reviewing literature and developments in the subject area, but mainly concentrating on analysing the large volume of monitoring data that SEPA has acquired during the years since the initial PAMP study. SEPA has identified the potentially useful databases it holds, going back to 2005, in various formats: 70 SEPA full audit surveys (benthic, visual and medicines residues); 30 SEPA visual surveys; 940 self-monitoring benthic surveys (80 baseline); 180 self-monitoring visual surveys (90 baseline); 15 self-monitoring Teflubenzuron residues surveys; 700 self-monitoring Emamectin Benzoate residues surveys.
3. It will be important to assess the statistical uncertainty in the findings of the analysis and the identification of change, and to highlight the potential limitations of the data being analysed – for instance if it is limited to sedimentary organisms.
4. The project is intended to be a refresh of the original PAMP study, but with a slightly tighter focus on environmental receptors – i.e. concentrating on the benthos.
5. If the findings of the analysis of SEPA-held data suggest that there is any requirement for subsequent validation through new field investigations, this should be incorporated into the project report in the form of recommendations for further work.
6. In addition, subject to the initial literature review and the findings of the analysis, it may be possible to put forward suggestions for additional further analytical (in the first instance) work that might be commissioned in the future - using other data sources - that would be concerned with wider environmental impacts. Areas of interest might include hard substrate species and inshore fisheries.

Impact:

All SARF applied research projects must consider the opportunity for project outcomes to contribute to further activities that might, in due course, lead to measurable positive impacts on Scottish aquaculture production.

Objectives:

The research objectives should be clearly set out in relation to the different requirements and themes outlined in the sections on Context and Research Requirement above. Specifically: review of all relevant literature; develop a SEPA data analysis protocol; analyse historic SEPA data and present findings; identify the need for further research; written report.

Deliverables: A Draft and then Final Report

5 THE PAMP-2 STUDY

The progress of the research and its outcomes was supervised and reviewed in accord with normal SARF procedures. In view of the long term aspirations (section 4.1, items *4* and *5*) expressed in the call, particular importance attached to the reviewers' comments and the consequent discussion with authors. These aspects of the work are summarised within the following sections.

5.1 SCIENTIFIC OBJECTIVES

The PAMP-2 study had four objectives (4.1). All were presented in the interim and final SAMS reports:

- A literature review that considered the effects of sea lice medicines on benthos, to include both published and grey literature, was completed. Performance of this objective attracted some minor criticisms that were reasonably debated by the authors.
- The SEPA database was reviewed to determine a sub-set of sites with relatively high sediment medicine concentrations in monitoring or audit. The data were examined statistically to establish any relationships between medicine use or residues and environmental factors such as current speed and sediment type, in order to determine whether any types of site are inherently more at risk of breaking EQS2 (Environmental Quality Standard). The performance of this work relied crucially on collaboration with SEPA, by whom the data were supplied, and with whom methods of analysis were agreed. Reviewers expressed some concerns about some parts of the performance of this objective.
- The third objective was to determine at case study sites whether there is any evidence of effects on the benthos specifically from medicine usage that can be distinguished from impacts of organic enrichment (e.g. as evidenced by particular impacts on crustaceans). This forms the major part of the report and it attracted substantial comment from reviewers.
- The last objective was to provide expert advice on whether present regulations and practices provide good protection of the Scottish benthic ecosystem and to advise on any specific studies that might be required to reduce uncertainties and to improve the prospects for continued sustainable growth of the Scottish salmon industry in line with government and industry targets. Recommendations for further research programmes, that address knowledge gaps identified within the work proposed here, were also to be made. This objective was complete in the final report, was largely but not completely welcomed by reviewers, and may also be augmented by themes drawn from the review process.

5.2 INTERIM REPORT

Once the bulk of the analytical work had been done, an interim report (*SARF098 - Draft Final Report July2015.pdf*) was made available for review. The report, being interim, is not repeated here but its executive summary offers a useful background to the review exercise:

5.3 EXECUTIVE SUMMARY OF INTERIM REPORT

The louse *Lepeophtheirus salmonis* is a crustacean ectoparasite that infests salmon. The control of lice is the major issue currently facing the Scottish salmon farming industry. The infeed anti-louse chemicals Emamectin Benzoate (EMB) and Teflubenzuron (TBZ) are consented for use in Scotland. Following ingestion, EMB or TBZ enter the fishes' tissue which becomes toxic to feeding lice. EMB and TBZ are both excreted by the fish over an extended period (~200 days) and enter the environment via faeces. EMB and TBZ are both particle affinitive and accumulate in the sediments around the fish-farm.

SEPA is the consenting authority in relation to EMB/TBZ use in Scotland. Under the farm-operating consent, SEPA requires farmers to report the results from grab-based sampling of sediment around the farm site (Cage Edge, intermediate (AZE) and Reference stations) in relation to macrobenthos, particle size and organic content. SEPA also requires results from measuring current flows around farms and the monthly reporting of EMB/TBZ use. EMB/ TBZ seabed residue monitoring is also conducted around fish-farms at a range of distances.

The objectives of the present work were to assess the extent to which EMB/TBZ use had deleterious effects on sensitive, non-target, benthic crustacea around fish-farms. This required the extraction and analysis of data from two main SEPA databases and the development of generalised linear mixed models, with Bayesian inference, to assess the independent effect of EMB use on sensitive species. There were insufficient data to assess the relationship between sensitive species and TBZ. In relation to EMB, the analysis was split between 'per-production-cycle' (PPC, of which there could be several per site) analyses, and between analyses based on the total EMB used per site (per-site total, PST). At an application rate of 3 kg PPC there was strong evidence (95% certainty) that crustacean richness and abundance declined by between 4 - 63% and 10 – 85 % respectively, and that this reduction occurred at the Cage Edge, AZE and Reference stations. When compared to total EMB use, per site, the effect on crustacea was more severe with 95% certainty that the decline in richness and abundance was between 21 – 82% and 66 – 98% respectively at all distances. The greater reduction observed when considering the total EMB applied per site indicates that EMB is having a cumulative impact on sensitive groups i.e. that crustacean communities do not recover between EMB treatments. The changes observed at reference stations indicate the impacts are occurring at large-scales. Whilst it is possible that the observed reductions in crustacea are attributable to factor(s) that are associated with EMB use, rather than directly caused by EMB use, this is unlikely given the range and nature of the co-variables included in the models and the systematic reductions in crustacea as a function of EMB use.

The ecosystem consequences of the observed reduction in crustacea are not known but crustacea include important fishery species including crabs and lobsters. Understanding the consequences of EMB /TBZ use on benthic systems is essential to ensure the ongoing sustainable development of the Scottish fish-farming sector. The evidence suggests that benthic crustacea may not be adequately protected by the current regulation of EMB use in Scottish salmon farms.

5.4 Reviews of the Interim Report

The initial report was scrutinised by a set of national and international reviewers. The review process highlighted many themes and issues, some of which had been foreseen by SARF or were recognized by the authors themselves within their interim report.

It is helpful to give an overview of this discussion (without re-entering the detailed technical debate) so as to highlight some of the issues inherent in an analysis of necessarily imperfect observational data. Ignoring ephemeral concerns about presentation, the reviewers' themes that emerged were mainly statistical, methodological, benthic or inferential. The reviewers' comments formed the basis of a discussion that both helped the authors to a clearer expression of their work in their final report and also pointed to the nature of future work that may be necessary to reach more definitive conclusions.

There were extensive areas of agreement or partial agreement between authors and reviewers:

- All agreed that association of two variables is not demonstration of a cause and effect relationship between them, particularly when the data are as noisy as in this case.
- In response to comment, the authors undertook to add an analysis using only the reference distance class. Similarly, the Bayesian priors were made explicit.
- Concern was expressed over the criteria for exclusion of some sites from the analysis, but there was no evidence that this may have had a systematic effect on the results.
- Authors and reviewers agreed several times that the inability to match EMB sediment residue data with crustacean richness and abundance because of a lack of temporal and spatial coincidence between residue and macroinvertebrate sampling meant that EMB use per production cycle or cumulative use per site had to be used as imperfect surrogates (proxies) for exposure.
- The association (confounding) of EMB use with site biomass was often acknowledged, together with the problems of separating the influence of either alone.
- Several reviewers were concerned that interactions between independent variables had not been taken into account; this point was conceded by the authors, who had necessarily felt obliged to adopt a simpler model to avoid over-complication of the analysis.
- Some issues of clarity in technical expression were resolved satisfactorily, as were small modifications to the confidence of conclusions to be drawn from the study.
- Several comments regretted the lack of standard sampling in the original regulatory monitoring, including issues of grab size and geometry, and noted the ensuing difficulties for the authors' analysis. Such practical issues commonly and significantly blur the results in benthic monitoring or benthic research and act here to compound and amplify the statistical and inferential issues.
- There was some minor concern over issues of directionality of sampling vis-a-vis current direction, for example. This may have been part of a more general problem of determining exactly where samples had been obtained. This was clearly a difficult area for the authors to examine definitively, given the irregularities and imperfections in the databases that were consulted.
- The replacement of current speed, for which good measurements were lacking, by sediment particle size (as an expedient but very imperfect surrogate for current) was explained.
- A brief discussion of LOD, LOQ and NOEL (respectively Limit of Detection, Limit of Quantification, No Effect Limit) emphasised the importance of the relative roles of these limits and the importance of developing analytical methods.
- Much technical statistical comment focussed on the complementary or contrasting approaches of Bayesian or Frequentist analysis of the observational data (these are not experimental hypothesis-testing data) on which the research is based. All this comment reflected the deep complexity of the two approaches and was to an extent outside the scope of the report. The complexity of the issues (see for example *Probability Theory*, E T Jaynes, 2004, CUP) is clearly well beyond any of the briefly expressed views, whether of the authors or the reviewers. Despite the superficiality (relative to Jaynes, *op. cit.*) of the discussion and the inherent difficulty of reconciling these statistical approaches, it seems likely that major areas of disagreement could have been reconciled with more technical discussion or more work, or both. These two approaches differ in terms of their philosophical basis, but in practical terms, the inferences that were drawn are likely to remain unchanged in nature.
- A single reviewer raised an important area of agreement: without a predefined route, modelling may follow a path whose conclusions are not as robust as a controlled rigorous pre-planned progression.

- Technical detailed concerns about the transformation of the variables were often raised, and answered to various degrees: the discussion was not always clear in this complex area but seemed ultimately to lead to no major area of concern.

The discussion of these areas led the authors to supplement, change and clarify their interim report to some extent so as to produce a final version.

5.5 Final Report

The initial review process clarified some of the difficulties of perception and methodology, stimulated some additional analysis and contributed to the preparation of the final report, presented to SARF in December 2015. The final report (Wilding, T A & K D Black, 2015, *A statistical analysis of sea-lice medicine use and benthic monitoring at Scottish marine salmon farms 2002 – 2014*, SAMS 83 pp) was reviewed and its executive summary provides helpful background to this second round of review.

5.6 Executive summary of the final report

The louse *Lepeophtheirus salmonis* is a crustacean ectoparasite that infests salmon. The control of lice is the major issue currently facing the Scottish salmon farming industry. The infeed anti-louse chemicals Emamectin Benzoate (EMB) and Teflubenzuron (TBZ) are consented for use in Scotland. Following ingestion EMB or TBZ enter the fishes' tissue which becomes toxic to feeding lice. EMB and TBZ are both excreted by the fish over an extended period (~200 days) and enter the environment via faeces. EMB and TBZ are both particle affinitive, accumulate in the sediments around the fish-farm and may pose a hazard to non-target benthic crustacea.

SEPA is the consenting authority in relation to EMB/TBZ use in Scotland (Note: TBZ is no longer used in Scottish salmon farms). Under the farm-operating consent, SEPA requires farmers to report the results from grab-based sampling of sediment around the farm site (at cage edge (CE), intermediate (AZE) and distant Reference (Ref) stations) in relation to macrobenthos, particle size and organic content. SEPA also collates current flow-data from around farms, the monthly use of EMB/TBZ and the results from EMB/TBZ seabed residue monitoring.

The objectives of the present work were to assess the relationship between EMB/TBZ use and benthic assemblages, particularly crustacea, around fish-farms based on SEPA-collated data. There were insufficient data to assess the relationship between sensitive species and TBZ. In relation to EMB, the analysis was split between 'per-production-cycle' (PPC, ~20 month salmon production cycle (PC)) and per site total (PST) analyses with consideration of changes occurring at the CE, AZE and, particularly, the Ref stations. Within the data extracted from the databases (N=1259 samples from 99 sites) there were maxima of 3 and 10 kg of EMB used PPC and PST respectively (the PST consisted of the cumulative EMB used across several PCs). Analyses were based on generalised linear mixed models, with Bayesian inference, to predict the independent mean EMB effect (with 95% credible intervals) based on an average site, under average conditions were the maximum EMB to be used (PPC or PST). At Reference stations, on a PPC basis, there was strong evidence of a substantial decline, in crustacean richness and abundance, of 40% (4 - 63%) and 66% (29 – 87 %) respectively associated with 3 kg of EMB use. At Reference stations, on a PST basis, the EMB –crustacean association was more significant with expected reductions in richness and abundance being 64% (21 - 82%) and 96% (74 - 100%) indicating wide-scale, cumulative impacts and incomplete recovery between successive EMB treatments. Whilst it is possible that the modelled reductions in crustacea were attributable to factor(s) that were associated with EMB use, rather than directly caused by EMB, this is unlikely given the range and nature of the co-variables included in the models.

The ecosystem consequences of the observed reduction in crustacea are not known but crustacea include important fishery species such as crabs and lobsters. Understanding the consequences of EMB/TBZ use on benthic systems is essential to ensure the ongoing sustainable development of the Scottish fish-farming sector. The evidence suggests that benthic crustacea may not be adequately protected by the current regulation of EMB use in Scottish salmon farms.

5.7 Reviews of the final report

Reviewers who had contributed to the research programme by commenting on the interim report also considered the final report. Although they varied in tone and detail, the reviewers' comments have been interpreted here in the light of two themes: scientific quality of the research, and indications for the desirable nature of future research.

5.8 Final report- research quality

Reviewers' concerns that had been voiced previously over the interim report inevitably surfaced again, partly because of the inherent and unavoidable drawbacks of applying an observational regulatory data set to a problem that – ideally – would have been tackled by hypothesis-testing experimental design, and partly because of the inferences that had been drawn.

Concern about the poor quality of the observational data that had necessarily been used in the study was a common refrain among reviewers. Some previously acknowledged deficiencies were therefore highlighted once more as inescapable features of the research. These were associated with the inability to match EMB sediment residue data with crustacean richness and abundance because of the substantial lack of temporal or spatial coincidence between residue and macroinvertebrate sampling. This lack of evidence regarding measured residues at sites was regretted by several reviewers.

Some general remarks pertain to the benthic sampling. It was noted that the benthic monitoring method had changed during the observational period. Sampling position information seemed imprecise. The grab sampling will have been susceptible to some or all of many common methodological drawbacks such as: inconsistencies introduced by combining sub-samples; differing sample volumes; and inconsistent sediment penetration in different sediments – leading to inconsistent species sampling from different sediment depths and poor detection of deeper-living but functionally important biomass. Other difficulties include small caught species numbers, undue influence of wave and current on small-grab sampling, and unknown sample loss on grab closure in rough material. A sieve mesh size as large as one millimetre removes information about smaller individuals, increasing both the evenness and the apparent diversity of samples. Grabs take samples relatively close to the sediment surface and collect animals of only small to mid-body size. The use of grab samples for sediment size analysis is fraught with imperfections such as sediment disturbance & winnowing, or preferential leakage of fine materials from a leaky grab. In many benthic sediments most crustacean biomass is contained not in small surface-dwelling (and relatively mobile) amphipods and cumaceans but in far larger taxa such as crabs and burrowing thalassinid shrimps. Grabs may underestimate crustacean biomass and functionally important crustacean species. This research was not able to match the focus on crustaceans with one on their species composition and body size, a theme that is being pursued in a subsequent SARF/SAMS research project.

Even a carefully constructed research benthic programme would be subject to these sampling difficulties and could only seek to minimise them. However, the range and significance of these imperfections within the SEPA monitoring data are such that they cannot even be assumed to randomise the sampling in an unbiased way. Many of the deficiencies of the monitoring grabbing programme are site and condition related in such ways to site and sampling conditions that the authors could not possibly disentangle them. This noise in the monitoring data detracts substantially from the significance of the putative benthic changes.

It was remarked that records of EMB use, in the absence of measured sediment residues, are insufficient to support spatial or temporal co-occurrence, evidence of exposure or biological mechanism, causal pathway, or field stressor-response relationships. The methods that the authors and the regulator had used of discounting variables other than EMB were questioned because of their not being set out objectively.

A persistent criticism was that that only one modelling technique had been applied. It was argued that confidence in the results of the third and fourth objectives could have been greater had more than one approach been possible. Multivariate methodologies might have conserved species identity and set the crustaceans in a multi-species context. Demonstrable robustness of the findings to model choices is an important property and one which would be especially valuable in this context. It is also an important aspect of the validity of assumptions. One forceful suggestion was that of using causal analysis techniques; this reviewer felt it would have been better if the weight of evidence from currently available experimental data and modelling approaches had supported the authors' conclusions.

The aspect of time dependence within the benthic response would have been impossible for the authors to handle, given the heterogeneous nature of the regulatory data. This feature of the research provoked the majority of reviewers to remark in various ways on the implication that a before/after study of reference stations would have been useful to take account of survey of conditions before start of farming operations. Seasonal effects could not be investigated and reviewers suspected unrealised potential in the data set for looking at other time variation of samples at a site within the period over which the total EMB usage had been formulated.

The grouping of sites into "Orkshet" and "Other" groups was questioned. This grouping rested to some extent on informed regulatory opinion but it was suggested that (although not practicable within the scope of the work) cluster analysis of site characteristics might have been a more objective grouping procedure.

The authors' assertion that "*a no-effect position doesn't exist … impacts will occur on a continuum* (of EMB concentration with distance from farms)" was questioned as being at odds with basic tenets of toxicology, where toxicity is regarded as a threshold response; there should therefore be a point along this "*continuum*" at which EMB concentrations are sufficiently low so as to not elicit effect. This raised the need for future clarification of NOEL, LOQ & LOD. The authors were aware that prediction of response and uncertainties within the observed range of covariates is possible but that prediction outwith the observed range is more uncertain. However, the assertion that toxic effects may be occurring at levels much lower than those that are currently detectable was seen by reviewers as overstated - a conclusion whose future validity would be contingent on the future development of clearer analytical methods and the clarification of LOD, LOQ and NOEL.

The report's speculation that effects might be felt on geographical scales extending to whole lochs was brought into question by one reviewer, who pointed out that before such a conclusion could be justified, the methods of causal analysis should be applied to all stressors over such large areas of varying and diverse marine conditions.

As in their scrutiny of the interim report, reviewers continued to focus on the limited analysis that had been possible with the resources available to the research contractors: in particular, the effect of interactions between variables had not been explored. This attracted detailed comment from one reviewer, who argued in some detail that the effects of interactions could be discerned in the report and that they would not have been ignored in an ideal analysis; even simple cases of dual interactions had been ignored. The validity of this criticism rests on the knowledge that representation even of a simple two-variable system by linear functions of the variables can be very misleading if there are interactions between them. An implication of these comments for future work is that, in a set of fixed variables that are believed to determine response, the number of dual interactions is always small relative to the very large number of multi-variable interactions that were mentioned by the authors as an impediment to pursuing this line of enquiry.

There was a feeling among some reviewers that suppositional inferences regarding future work had been expressed overly strongly relative to the implications of the study. To some extent this came from an inability to tease out the separate or interactive contributions from EMB and biomass.

As regards inferences, reviewers and authors agreed throughout that an association of variables (whether well or poorly determined) such as benthic effect and EMB does not itself constitute evidence of cause and effect between them. This led at least one reviewer to doubt the relevance of this study to regulatory action. The use of proxy measures of key variables in some cases weakens the strength of inferences. There were serious misgivings about the lack of demonstrated pathways between cause and presumed effect. Briefly, the inferences should have been couched more as exploratory and generative of hypotheses, rather than testing of them.

Several reviews commended the report for its serious attempt to deal with an inconsistent regulatory observational data set that had not been gathered for the purpose to which it was put. The authors made use of appropriate statistical methodology and to develop this further would have been challenging because of the complexity of data and the analytical structures; linear mixed effect modelling is appropriate to such a study. From this standpoint, the contractors had satisfied their obligations. The constraints imposed by resource and data quality were recognised. All those reviewers who commented on technical, statistical, benthic and inferential matters seemed therefore to regard this investigation as one whose nature and conclusions are insufficiently well-founded to demonstrate cause, pathway or appropriately measured effect, one that is therefore unsuitable for direct use in evidence-based regulation, but as one that is indicative of an issue that deserves more rigorous investigation.

6 FEATURES OF A FUTURE RESEARCH PROGRAMME

In designing future work, it is useful to consider the desirable features of future work in respect of general experimental design, regulatory science, and the particular revelations of the PAMP-2 exercise.

6.1 EXPERIMENTAL DESIGN

Criteria for recognising causal relationship (e.g. Ian Hacking, 1983, *Representing and Intervening: Introductory Topics in the Philosophy of Natural Science*, CUP) include:

- the cause precedes the effect
- whenever the cause is present, the effect occurs
- the cause must be present for the effect to occur.

Carefully designed experiments may aim to demonstrate these criteria by dealing with stressors, responses, pathways of cause and effect, and statistical analysis. This design involves a number of inter-related activities:

- Formulation of statistical hypotheses relevant to the scientific hypothesis
- Identification of measurable stressors (independent variables), responses (dependent variables) and extraneous influences (confounding or nuisance variables)
- Specification of the number of measurements and of the populations from which they are sampled
- Determination of the statistical analysis that will be performed.

In this way, an experimental design identifies the independent, dependent and nuisance (confounding) variables and shows the way in which the statistical aspects of an experiment are to be carried out. The primary goal is to test for a causal connection between the independent and dependent variables.

Nevertheless, in environmental science, it is not always possible to contrive experiments so rigidly, because of the purely observational and uncontrollable nature of some of the variables that we use to characterise the environmental systems, the stressors or the responses. In addition to the practical and formal statistical design criteria, it is therefore desirable that stronger experimental design also demonstrates the pathways (in this case - physical transport, chemical partitioning, metabolic processes and aspects of toxicity at individual and population levels) by which the stressors may elicit the responses.

Steps on the road to such experimental design were outlined in a thorough account of the manner and methods of assessing environmental causes given by Suter et al (Suter, G W, S B Norton, and S M Cormier 2010, *The science and philosophy of a method for assessing environmental causes*. *Human Ecological Risk Assessment*, 16: 19-34). Among their recommendations was the following table, brought to our attention by one of the reviewers. This table sits comfortably within the main line of experimental design described by Hacking (*op. cit*) and others.

Lines of evidence for determining the plausibility of candidate causes (from Suter et al, 2010[*])

Type of evidence	Concept
Spatial/temporal co-occurrence	The biological effect is observed where and when the cause is observed, and must not be observed where and when the cause is absent.
Evidence of exposure or biological mechanism	Measurements of the biota show that relevant exposure to the cause has occurred, or that other biological mechanisms linking the cause to the effect have occurred.
Causal pathway	Steps in the pathways linking sources to the cause can serve as supplementary or surrogate indicators that the cause and the biological effect are likely to have co-occurred.
Stressor-response relationships from the field	As exposure to the cause increases, intensity or frequency of the biological effect increases; as exposure to the cause decreases, intensity or frequency of the biological effect decreases.

Manipulation of exposure	Field experiments or management actions that increase or decrease exposure to a cause must increase or decrease the biological effect.
Laboratory tests of site media	Controlled exposure in laboratory tests to causes (usually toxic substances) present in site media should induce biological effects consistent with the effects observed in the field.
Temporal sequence	The cause must precede the biological effect.
Verified predictions	Knowledge of a cause's mode of action permits prediction and subsequent confirmation of previously unobserved effects.
Symptoms	Biological measurements (often at lower levels of biological organization than the effect) can be characteristic of one or a few specific causes.
*www.researchgate.net/publication/233013899_The_Science_and_Philosophy_of_a_Method_for_Assessing_Environmental_Causes	

It is clear from SARF's original expectations, the nature of the regulatory data, the two sets of reviews and the authors' comments that the present work, for various and understandable reasons, could satisfy the above conditions only partially.

6.2 Regulatory framework

Policy strategy and regulatory action are often formed within the ***DPSIR*** framework (e.g. http://wwz.ifremer.fr/dce/content/download/69291/913220/file/DPSIR.pdf). This framework assumes a chain of causal links starting with ***D****rivers* (economic activity, human affairs) through ***P****ressures* (emissions, wastes), ***S****tates* (quantified physical, chemical and biological aspects of the environment), to ***I****mpacts* (quantified changes to ecosystems, human health or functions), eventually leading to political ***R****esponses* (managerial or regulatory actions, target setting, use of indicators, monitoring).

From this DPSIR perspective, a study of the sub-chain of *Pressure – State – Impact*, if used to justify regulatory action on the grounds of demonstrable cause and effect, should include the following characteristics:

- The cause – pathway – effect hypotheses should be clear and testable
- The recorded data to be used or the measurements to be made should be set out clearly
- The criteria to be used for correcting the data and measurements for errors of recording, methodology or omission should be clearly set out
- The criteria for rejecting any data or measurements should be clear
- Methods of analysis of the data should be set out procedurally, mathematically, statistically and symbolically in a manner that is independent of any particular computational software or implementation, in sufficient detail that they may be followed independently.

From the perspective that regulation should be based on evidence-based sound science, it is clear from the reviewers' comments on the interim and final reports that the PAMP-2 study, although necessarily and contractually being based on expedient and opportunist data of a quality insufficient to support hypothesis-based experiment, is not in itself sufficiently sound in achievement or expression that it can alone justifiably underpin marine management policy, strategy or implementation. More research is needed before contemplating any such action.

6.3 DESIRABLE ASPECTS OF FUTURE WORK REVEALED BY THE PAMP-2 STUDY

Sections 6.1 and 6.2 provide some of the general framework for future work. Additionally, and adding to the authors' own recommendations, the reporting and review process of sections 5.2 to 5.8 contains many explicit and implicit themes relevant to developing future research effort.

6.3.1 Authors' recommendations for further research

In their final report, the authors made the following recommendations:

Our results indicate that, even allowing for regional differences in the physical properties of the receiving environment, the use of EMB is associated with substantial, wide-scale reductions in both the richness and abundance of non-target crustacea. Given the findings in this report we believe there is an urgent requirement to:

1. Use the existing databases and multivariate techniques to link change in individual crustacean species and groups (e.g. based on reproductive strategy) with EMB use.

2. Improve the PPC analysis by determining the total EMB used, prior to each grab-survey (i.e. including the EMB used in previous PCs) and use this as a predictor. Examine model-residual patterns and determine how change occurs as a function of application rate and frequency. Determine (if possible) crustacean recovery following EMB use.

3. Consider the likely ecosystem consequences of large-scale reductions in crustacean richness and abundance (at the scale of sea-lochs).

4. Consider and make recommendations regards manipulative studies to test the effects of EMB, at realistic doses, on benthic crustacean communities. This should be done in conjunction with ongoing related research (e.g. Predicting benthic chemistry around marine fish farms, NERC/BBSRC Grant Number BB/M025861/1).

5. Consider whether next-generation-sequencing (NGS) approaches to monitoring crustacean populations (Pawlowski et al., 2014) around treated and untreated sites could offer an alternative, rapid and high-resolution method of mapping crustacean diversity around fish-farms.

6. Extend current sampling to better understand the spatial scale of the impact of EMB on non-target crustacea.

7. If new methods of analysis are able to quantify much lower EMB residues then a comparison between macrobenthic metrics and EMB residue should be undertaken.

Many of these recommendations were supported by the reviewers, albeit with some reservations that further work should be considered only if alternative analyses show similar trends to those the authors found (i.e. a very large decline in crustacean richness and abundance at reference sites, which is associated with EMB use at nearby farms).

Recommendations 4 and 6 have special status, being those concerned with the gathering and analysis of new data to test the cause-effect hypothesis.

6.3.2 Implications from PAMP-2 review

The reviewers, during their scrutiny of interim and final reports, made or implied suggestions that may help colour a future research programme. Some of the comments on the final report reverted to a focus on what would have been desirable had things been otherwise. Nevertheless, they shed light on desirable features of future work. Other features are implicit within other previous sections of this document.

All desirable or possible features of future work are grouped here under several headings. Little distinction has been made between reviewers or authors in these groups. In several cases the reviewers and authors were in full or partial agreement over the feature.

General

Further work is needed to test the hypothesis generated by the PAMP-2 analysis: that EMB usage is causing significant impacts on benthic communities at large distances from farms under certain environmental conditions and that these impacts are observable when EMB concentrations are at or below current detection limits.

Authors and reviewers recommend future manipulative (experimental, designed) studies. General environmental changes over time and space could be having broad effects on flora and fauna, and those factors need to be parsed from the effects of fish farming. Cause and effect may only be assessed via a properly designed experiment, not merely through association. One key challenge would lie in the definition, identification and characterising of reference (or control sites).

Additional data collection is necessary before a reliable and conclusive identification of the cause of any observed declines (spatial or temporal) in the benthic crustacean community is possible. The possibility of effects at pre-determined reference sites is particularly concerning and should be taken into consideration when designing future sampling studies.

Some of the debate over PAMP-2 owed to limited expression of the details of models and their algorithmic procedures. The clarity of technical expression is important.

Further manipulative (experimental) studies

It is desirable to take account of pathways. To do this, future work should seek quantitative data to demonstrate that: biological receptors are exposed to critical concentrations of the chemical stressor; there is evidence of spatial or temporal co-occurrence or both; there is evidence of exposure or relevant biological mechanism; a causal pathway can be demonstrated; field stressor-response relationships are revealed.

An extended monitoring programme for EMB residues would help assess the relationship between EMB residue and benthic crustacea.

It is desirable to capture same-site changes from pre-treatment to post-treatment. Similarly, or alternatively, it is desirable to evaluate regionally-similar non-treated control sites versus treated sites.

Benthic Biology

The design of an efficient sampling scheme aims to control for factors in which we have no interest. In an *a posteriori* analysis of the kind reported here, all that can be done is hope that the interesting factors cause sufficient systematic variance to emerge clearly from all others.

Good benthic survey data are collected to meet a predetermined need. At the very start of planning, the specific question to be addressed must be clearly defined before the elements of the biota to be investigated are chosen alongside the statistical approach to be used. The sensitivity of analysis must be at the forefront of planning, both in univariate or multivariate approaches. Once the number, size and distribution of the samples are decided, field sampling and laboratory analysis both become matters for strict quality control.

Marine assemblages change as the result of patterns of differential reproduction and survival acting to change their species composition. Unless pollution impacts are severe, such changes are protracted and often mediated through predation or competition. Community-level changes may therefore take place over years rather than months and are difficult to untangle from natural processes by traditional field sampling.

A more immediate indication of impacts may come from rapid ecotoxicological asse`ssment methods (workers such as Depledge, Lowe, Readman, Galloway and others). Such methods lead to an appreciation of sub-lethal changes in - for example - metabolic function or reproductive status; this approach transcends traditional LD50 methods.

Although field experiments are possible, they may be somewhat crude and, in these days of increasing public awareness of environmental damage, might not be seen as best practice. An alternative, or complementary, line of attack is to use mesocosm experiments. A number of laboratories in the United Kingdom (Plymouth Marine Lab, Oban, Bangor) have experimental systems where replicated 0.1 m^2 cores of intact sea bottom can be maintained successfully in a fully controlled environment. Although these systems do not mimic the natural environment perfectly (larval supply is one problem) they do permit real and hypothesis-based experimentation.

Sampling needs to be designed carefully in respect of the target species and assemblages. For example, some crustaceans such as thalassinid shrimps live far too deeply in the sediment to be sampled adequately by any grab. Their presence is apparent on under-water video but more quantitative collection requires a box corer.

It is possible that, at low doses, EMB may eliminate sensitive species leaving less sensitive species or species that are able to recruit, perhaps annually, from the water column. This hypothesis may justify a species-specific line of enquiry.

The lack of standardisation and change in sampling regime in the collection of the data disadvantaged the PAMP-2 analysis; adherence to a standard sampling procedure is crucial to the value of future work.

Regarding organic enrichment, counts of *Capitella* species might be a proxy. However, as a general point, surrogates should be used sparingly because they introduce another level of considerable uncertainty into the chain of association (e.g. sediment size as surrogate for current, *Capitella* for organic enrichment).

Modelling Approach

Although it has not been done in PAMP-2, interactions between fixed variables should be considered quantitatively in future work, otherwise the attribution of effect to them may be fundamentally and statistically flawed. Contemporary software should be capable of handling the most likely interactions.

Exclusion of sites or data from analysis should be objective and explicit. If necessary, sites could be grouped objectively in respect of their characteristics by using cluster analysis.

In any similar future exercise, a potentially powerful modelling process might use random coefficient models between sites and the fixed effects. However, data quantity and quality might limit their applicability. A variable over which the coefficient varies might be site (at least crudely similar to a site by site analysis) but in this varying coefficient approach would be assumptions about how the coefficient varies. To be successful, the methodology is likely to require high quality data or very strong assumptions.

Other candidate causes should be explicitly considered before alleging EMB use as the most plausible explanation for the effects.

A disputed suggestion that data could profitably be analysed with different models remains unresolved. The PAMP-2 authors maintained briefly, and perhaps reasonably, that mixed models are the most appropriate model for the analyses of these complex data. Reviewers mention quantile regression - this would be appropriate if the response variable were continuous and the objective were to model the median (50th quantile) or some other quantile of the distribution. This would be a decision based on a clear statement of the modelling objective; standard linear modelling is typically couched in terms of the mean response. Standard quantile regression is not yet well developed to deal with hierarchical mixed effects modelling. It is also an approach sometimes used in the context where we have a non-constant variation in the response across the range of the covariate. The transformations used may stabilise variability and also help with distributional assumptions. Different transformations may be used for different variables, dependent on their characteristics. These issues should be considered explicitly before embarking on a future investigation.

A broader, more methodical approach to determining the cause of the observed declines in benthic crustaceans was advocated. A prime candidate is causal analysis: to prevent cognitive errors such as focusing on a single hypothesis cause, overlooking data gaps or difficulties with poor data quality; to create a formal and transparent process through which available data and lines of evidence may be assessed and ranked; to improve confidence in results through demonstration of a thorough and methodological framework. (e.g. Suter et al. 2010).

Chemical Analysis

It was agreed that attention needs to be paid to analytical aspects of the problem; one desirable feature is that the LOQ should ideally be well below the NOEL.

A more sensitive assay would be a precursor to judging if lower levels of EMB are carried down-current or, more generally, dispersed in the hydrographic system.

6.4 Summary of Features

The detail of the desirable features of section 6.3 should be seen within the general requirements of sections 6.1 and 6.2, which deal with experimental design and regulatory science.

The strategies outlined in sections 6.1 and 6.2 may be seen as a framework within which features such are as set out in section 6.3 should be realised. Strategy and detail are mutually supportive rather than mutually incompatible, whatever resources may be available for the research.

7 Conclusion

This contextual review has summarised the PAMP process to 2015 and on that basis has outlined some of the desirable strategies and features of a future continuation of research to examine any effect of the use of Emamectin Benzoate (EMB) on marine benthic condition around Scottish fish farms.

The SARF098 PAMP-2 project was prompted by the availability of a regulatory benthic monitoring data set that built up since PAMP-1. The project has revealed that there may be deterioration of the benthic quality of sites far from fish farms, associated with the use of EMB. Such deterioration has an association with prior EMB use but rigorous cause and effect relations have not been established.

The PAMP-2 contractors analysed a set of observational regulatory benthic monitoring data in the neighbourhood of Scottish west coast fish farms and related the state of the benthos to the prior use of EMB. The contractors presented an interim and a final report to SARF; both were subjected to international review. Reviewers commended the effort that had been made to find pattern in a heterogeneous set of observational benthic data.

There was little information in the monitoring about sedimentary EMB residues, so the relation of benthic condition to sedimentary concentrations was very limited. Most research effort went into the benthic biological status in relation to prior EMB use. Regarding this work, most reviewers were not persuaded of the validity of the conclusions drawn from the analysis and about the confidence that could be placed in the inferences and conclusions drawn from the work.

Both the regulatory benthic field methods and the contractors' statistical analysis of the benthos in relation to EMB use were criticised constructively by reviewers. The authors made use of appropriate statistical methodology and to develop this further would have been challenging because of the complexity of data and the analytical structures. Major concerns were raised about the quality of the observational data, the limited range of modelling techniques, the setting aside of statistical interactions between site variables, lack of explicit account of how some measurements had been set aside, inability to examine time dependence of response (before and after survey), expedient site grouping, and deficiencies in inference associated with difficulties of chemical analytical limits and relation to toxicological norms.

The research, commissioned to take expedient advantage of an opportunistically available observational data set, was intrinsically unable - given the nature of the original data set - to reveal any of the pathways by which EMB might be eliciting the putative benthic response. Many of the reviewers' criticisms were unavoidable; others were answered to some extent by the contractors. Reviewers' comments and contractors' recommendations have been brought together in section 6 of this report so as to define the nature of future research on this problem.

Project Final Report Form

SARF098: PAMP Refreshment Study – final report

A statistical analysis of sea-lice medicine use and benthic monitoring at Scottish marine salmon farms (2002 – 2014).

18 Dec 2015

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Executive summary

The louse *Lepeophtheirus salmonis* is a crustacean ectoparasite that infests salmon. The control of lice is the major issue currently facing the Scottish salmon farming industry. The infeed anti-louse chemicals emamectin benzoate (EMB) and teflubenzuron (TBZ) are consented for use in Scotland. Following ingestion EMB or TBZ enter the fishes' tissue which becomes toxic to feeding lice. EMB and TBZ are both excreted by the fish over an extended period (~200 days) and enter the environment via faeces. EMB and TBZ are both particle affinitive, accumulate in the sediments around the fish-farm and may pose a hazard to non-target benthic crustacea.

SEPA are the consenting authority in relation to EMB/TBZ use in Scotland (Note: TBZ is no longer used in Scottish salmon farms). Under the farm-operating consent, SEPA requires farmers to report the results from grab-based sampling of sediment around the farm site (at cage edge (CE), intermediate (AZE) and distant Reference (Ref) stations) in relation to macrobenthos, particle size and organic content. SEPA also collates current flow-data from around farms, the monthly use of EMB/TBZ and the results from EMB/ TBZ seabed residue monitoring.

The objectives of the present work were to assess the relationship between EMB/TBZ use and benthic assemblages, particularly crustacea, around fish-farms based on SEPA-collated data. There were insufficient data to assess the relationship between sensitive species and TBZ. In relation to EMB, the analysis was split between 'per-production-cycle' (PPC, ~20 month salmon production cycle (PC)) and per site total (PST) analyses with consideration of changes occurring at the CE, AZE and, particularly, the Ref stations. Within the data extracted from the databases (N=1259 samples from 99 sites) there were maxima of 3 and 10 kg of EMB used PPC and PST respectively (the PST consisted of the cumulative EMB used across several PCs). Analyses were based on generalised linear mixed models, with Bayesian inference, to predict the independent mean EMB effect (with 95% credible intervals) based on an average site, under average conditions were the maximum EMB to be used (PPC or PST). At Reference stations, on a PPC basis, there was strong evidence of a substantial decline, in crustacean richness and abundance, of 40% (4 - 63%) and 66% (29 – 87 %) respectively associated with 3 kg of EMB use. At Reference stations, on a PST basis, the EMB –crustacean association was more significant with expected reductions in richness and abundance being 64% (21 - 82%) and 96% (74 - 100%) indicating wide-scale, cumulative impacts and incomplete recovery between successive EMB treatments. Whilst it is possible that the modelled reductions in crustacea were attributable to factor(s) that were associated with EMB use, rather than directly caused by EMB, this is unlikely given the range and nature of the co-variables included in the models.

The ecosystem consequences of the observed reduction in crustacea are not known but crustacea include important fishery species such as crabs and lobsters. Understanding the consequences of EMB/TBZ use on benthic systems is essential to ensure the ongoing sustainable development of the Scottish fish-farming sector. The evidence suggests that benthic crustacea may not be adequately protected by the current regulation of EMB use in Scottish salmon farms.

Contents

Scientific objectives

Objective 1. A literature review considering the effects of sealice medicines on benthos to include both published and grey literature.

Completion status: Completed. The literature review forms the introduction to this report, is comprehensive and captures what is currently known about the consequences of emamectin benzoate and teflubenzuron in the benthic environment.

Objective 2. A review of SEPA database to determine a sub-set of sites with relatively high sediment medicine concentrations in monitoring or audit. Data will be examined statistically to establish any relationships between medicine use or residues and environmental factors e.g. current speed, sediment type, in order to determine whether any types of site are inherently more at risk of breaking EQS[2].

Completion status: Completed. Data have been extracted, cleaned, checked prior to inclusion in the statistical models. The analytical approach adopted was agreed with SEPA following submission of an interim report (07 Nov 14), and further discussion of that report (16 Dec 2014, by VC).

Objective 3. At case study sites, determine whether there is any evidence of effects on the benthos specifically from medicine usage that can be distinguished from impacts of organic enrichment e.g. as evidenced by particular impacts on crustaceans.

Completion status: Completed. Modelling has been completed with a focus on crustacea. This forms the major part of this report.

Objective 4. To provide expert advice on whether present regulations and practices provide good protection of the Scottish benthic ecosystem and to advise on any specific studies that might be required to reduce uncertainties and to improve the prospects for continued sustainable growth of the Scottish salmon industry in line with government and industry targets. Recommendations for further research programmes, that address knowledge gaps identified within the work proposed here, will also be made.

Completion status: Completed. Advice and recommendations to promote the sustainable development of Scottish salmon farming are provided in the final section of the report.

1 Introduction

The use of medicines and other chemicals in salmon aquaculture together with studies on medicine ecotoxicity has been recently reviewed (Burridge et al., 2010). In Scotland, the main medicines of interest are emamectin benzoate (an avermectin), cypermethrin, deltamethrin, (both synthetic pyrethroids), teflubenzuron (a chitin synthesis inhibiter) and azamethiphos (an organophosphate). The pyrethroids and the organophosphate are used exclusively in bath treatments and, following dispersal into the receiving water body, are highly diluted. This dilution means it is highly unlikely that there will be a measurable benthic effect from these medicines. In contrast, it is possible that the in-feed avermectins and chitinase inhibitors, which are carried to the seabed via salmon faeces and uneaten feed, will have meaningful benthic effects. Since the PAMP study (http://www.sams.ac.uk/kenny-black/pamp), surprisingly little has been published on the effects of these medicines on benthic organisms. Most studies have been conducted in the laboratory setting with environmentally unrealistic medicine dosages and over short durations and there have been few published field studies. Whilst there have been important improvements in analytical methodology to detect residues at lower concentrations (Ikonomou and Surridge, 2012) these are not yet in routine use.

1.1 Emamectin benzoate (EMB)

EMB, the active ingredient of SLICE®, has an sediment quality standard action limit of 0.763 µg kg^{-1} wet weight averaged over the top 5 cm of sediment applied as a maximum allowable concentration outside the allowable zone of affects (AZE). Within the allowable AZE, the action limit is 7.63 µg kg^{-1} wet weight of the top 5 cm applied as an average value (SEPA Fish-farm Manual (FFM), Annex A).

The only significant field study published on EMB (Telfer et al., 2006) concluded that "there was no evidence that the occurrence of EMB, or its desmethylamino metabolite, in sediments around fish farm cages after treatment had any toxic impacts on organisms in either water column or sediments". A PhD study from Stirling University (Mavraganis, 2012) aimed to "investigate the ecological impacts on sediments from farming activities using very large spatial and temporal data to investigate the long term effects of nutrient and chemical waste". This study was based on a metadata set collected [by the Institute of Aquaculture] from 403 sampling stations at 31 fish farms on the west coast of Scotland over a 9 year period. Data consisted of sediment macrofauna, carbon and nitrogen levels, redox potential, particle size for sediment characterisation and sediment concentrations of SLICE." "Annelids were the most sensitive to the presence of emamectin benzoate, with the sipunculid *Phascolion strombi*, the echinoderm *Ophiura affinis*, and the crustaceans *Iphinoe*, *Diastylis* and *Iphimedia* also showing sensitivity." In contrast to Telfer et al. (2006) the Mavraganis (2012) study concluded that "sampling stations which contained significant levels of SLICE had a higher impact status than those affected only by nutrient inputs." However, this work has not been published in the peer-reviewed literature and we have concerns about the validity of some of the statistical modelling used.

In mesocosm experiments, the crustacean *Corophium volutator* and the polychaete *Hediste diversicolor* had LC50s of 153 (95% CI = 119-198) and 1368 µg kg^{-1} wet respectively (Mayor et al., 2008) – 2 to 3 orders of magnitude higher than the EQS values outside the AZE. Veldhoen et al (2012) conducted lab experiments with EMB (0.1-4.8 mg kg^{-1} sediment) on spot prawn *Pandalus platyceros*. Significant mortality was observed within 8 days of EMB treatment at concentrations between 100 and 800 µg kg^{-1} and there was no effect of EMB on moulting. As the concentrations used were about 3 orders of magnitude above the SEPA outside-AZE action limit, some effect might have been expected.

Waddy et al. (2007) determined the no-observed-effect level (NOEL) and lowest-observed-effect level (LOEL) for American lobsters *Homarus americanus* at 0.12 and 0.22 µg EMB g^{-1} per lobster. They also showed in an experiment where lobsters were given a food choice that "ovigerous intermolt lobsters ate relatively little medicated feed and both postmolt and intermolt lobsters preferred their natural foods and became conditioned to reject medicated feed" (Waddy et al., 2007). Subseqently, Waddy et al. (2010) determined the effect of repeated doses of below the NOEL of a single exposure (0.12 µg g^{-1}): "the results demonstrate that repeated exposure to emamectin benzoate (1) produces effects not seen when an equivalent quantity of the drug is administered in 1 or 2 doses, and (2) causes a dose less than the single-dose NOEL to become a very potent dose." However, the authors conclude that "the potential risk of EMB to lobsters foraging near salmon farms cannot be determined from these results because the cumulative dose is greater than [such] lobsters are likely to acquire".

Vestheim et al. (2012) conducted a pelagic community toxicity experiment with mineral oil and EMB, separately and in combination, over a 4 day period. They reported "clear changes in chlorophyll a and bacterial numbers over the time course of the experiment in all treatments, but addition of oil, EMB or their combination did not alter nutrient concentrations, ecosystem components or the community structure compared to the control." In a similar experiment focussing on toxicity to pelagic copepods Fiori (2012) reported "mature females of *Calanus finmarchicus* were incubated in water collected daily from each mesocosm and their eggs and faecal pellets production were recorded during seven days. Reproduction and feeding were dramatically reduced in all females exposed to EMB; egg and faecal pellet production dropped to zero after 48 hours of incubation and at an EMB concentration of 18 ng L^{-1}. There was no significant modulation of the effects by the addition of oil and/or diatoms, but any interactions were presumably overshadowed by the strong effect exerted by EMB on *C. finmarchicus."*

A recent study Tucca et al. (2014) assessed the toxicity of three sea lice medicines EMB, cypermethrin (CP), and deltamethrin (DE), in the amphipod *Monocorophium insidiosum* during 10 days through whole-sediment bioassay tests (run at 0, 51, 128, 320, 800 and 2,000 µg kg^{-1}). Acute assays (LC50-10d) for DE (7.8 µg kg^{-1}) and CP (57 µg kg^{-1}) showed higher mortality than EMB (890 µg kg^{-1}). Sub-lethal responses were observed after 2 days of exposure. Significant induction in glutathione S-transferase and thiobarbituric acid reactive substances were measured for CP and EMB.

1.2 Teflubenzuron (TBZ)

SEPA have set the action limit for TBZ, the active ingredient of Calicide®, outside the AZE at 2.0 µg kg^{-1} dry weight averaged over the top 5cm of core applied as a maximum allowable concentration[1]. Inside the AZE, the action limit increases to 10 mg kg^{-1} dry weight average of top 5 cm of a core applied as an average value (FFM, Annex A). The half-life of teflubenzuron has been estimated as 115 days and, in one study, 98% of the total load had degraded or dispersed by 645 days after treatment (Haya et al., 2005). In general, teflubenzuron is thought to be relatively non-toxic to re-worker polychaetes (Burridge et al., 2010) but Mendez (2006) has shown the potential for behavioural change in Capitella sp. polychaetes at concentrations (8.4, 25 and 41.8 mg g^{-1} dry wt. sediment) less than the within-AZE SEPA action limit.

Diflubenzuron (DBZ) is a related medicine (chitinase inhibitor) used in Norway but not consentd in Scotland. In Norway there have been studies on the distribution and ecology of wild cod around salmon farms (Fernandez-Jover et al., 2011) and this prompted a study on the transfer of DBZ to this

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[1] Calicide is no longer marketed in Scotland. See Samuelsen, O.B., Lunestad, B.T., Hannisdal, R., Bannister, R., Olsen, S., Tjensvoll, T., Farestveit, E., Ervik, A., 2015. Distribution and persistence of the anti sea-lice drug teflubenzuron in wild fauna and sediments around a salmon farm, following a standard treatment. Sci. Total Environ. 508, 115-121.

species (PA et al., 2013). This study showed “that diflubenzuron can be taken up by Atlantic cod, that it is rapidly cleared from the body and that when present this pharmaceutical causes only small effects on the expression of genes involved in detoxification pathways. Taken together, our data suggest that accumulated diflubenzuron at the levels studied would have a relatively small effect on wild Atlantic cod.”

Considerable recent publicity has been given to unusually high measurements of in-feed medicines in several Scottish sites during SEPA audit surveys. For example, the SEPA 2012 audit of the farms in Loch Shell revealed a TBZ concentration at a 100 m station of 911 μg kg^{-1} dry weight – more than 400 times the EQS (SEPA, 2013).

In a recent major study (Langford et al., 2014) said “diflubenzuron, teflubenzuron, emamectin benzoate, cypermethrin, and deltamethrin were measured in water, sediment, and biota samples in the vicinity of five aquaculture locations along the Norwegian coast . Deltamethrin and cypermethrin were not detected above the limits of detection in any samples. Diflubenzuron, teflubenzuron, and emamectin benzoate were detected, and the data were compared the UK Environmental Quality Standards. The concentrations of emamectin benzoate detected in sediments exceed the environmental quality standard (EQS) on 5 occasions in this study. The EQS for teflubenzuron in sediment was exceeded in 67% of the samples and exceeded for diflubenzuron in 40% of the water samples collected. A crude assessment of the concentrations detected in the shrimp collected from one location and the levels at which chronic effects are seen in shrimp would suggest that there is a potential risk to shrimp. It would also be reasonable to extrapolate this to any species that undergoes moulting during its life cycle.”

The social impacts of aquaculture are poorly studied. A recent study of fishermen in Southwest New Brunswick, Canada (Wiber et al., 2012) reported that “Fishermen all reported significant environmental degradation around aquaculture sites. Within 2 years of an operation being established, fishermen reported that gravid female lobsters as well as herring avoid the area, scallop and sea urchin shells become brittle, scallop meat and sea urchin roe becomes discoloured. The use of chemicals to control sea lice on farmed salmon has also caused lobster, crab and shrimp kills”.

The widespread and increasing adoption of EMB and, to a lesser extent, TBZ have led to increasing concerns about the effects of such medicines on benthic communities around fish-farms. EMB and TBZ are specifically designed to disrupt essential metabolic processes in crustacea so crustacean members of the benthic community are likely to be particularly at risk. Consequently, the research presented here includes all members of the benthic community but focuses on crustacea. EMB/TBZ changes in crustacean assemblages at sites distant from the farm would be particularly concerning so there is particular emphasis on assessing change at the Reference sites. The development of statistical models to explain relationships between the response (e.g. crustacean species richness) and predictors (e.g. treatment, sediment conditions) requires large datasets. In the case of TBZ, such a dataset was not available (see 6 Teflubenzuron). The main focus of this report concerns EMB.

2 Materials and methods

This research involved data extraction, collation, checking, merging, matching, cross referencing and statistical modelling. No practical (lab or field-based) work was conducted.

2.1 Overview of research time-lines

The research reported here is the culmination of three linked research programmes. Phase I identified that EMB-treated sites tended to host fewer crustacean compared with those where EMB was not used. Phase II extended Phase I by quantifying the amount of EMB used, during each production cycle, and using this as a predictor in the models. Phase II concluded that there was an association between EMB treatment rate and decline in non-target crustacea in models where the Distance Classes (CE, AZE and Ref) were included in the same model (this is reported here). This was reviewed, by the SARF steering group and external reviewers. Concern was focussed on the association between EMB use and changes occurring at the Reference stations (indicating large-scale changes) and, consequently, additional analyses, based on the Reference-site-only data, was conducted (and reported here). For the sake of brevity, Phases I – III are reported here, as a single report.

2.2 Overview of SEPA databases

As part of statutory monitoring fish-farm companies report the results of benthic survey work to SEPA. These data include information about sediment characteristics (e.g. particle size, organic content) and the macrobenthic community in samples taken around fish-farms sites. These data have been collated into a MS Access™ database called the ‘Self-monitoring database’ (SMDB). SEPA also collate data in a ‘Fish-farm database’ (FFDB) which includes numerous details about each fish-farm site including aspects of husbandry, current exposure and, on a monthly basis, the usage of chemotheraputants, farm biomass and feed. The FFDB also contains the results from the SEPA-led surveys of fish-farms, including the results from residue surveys. In terms of the current contract the databases cover the period of 2002 – May 2014 (when the databases were copied to SAMS). In the SMDB the main identifier which was common through the different parts of the database was called

ID_Survey. ID_Survey distinguished Sites and dates of sampling at that Site (i.e. was a unique site by date identifier). In the FFDB the main identifier was Site, qualified by Station (within Site) and the date of the sampling (across the same site). However, the FFDB Sampling Date did not match the date in the SMDB database and these could differ by over a year (see 5.2) so these databases were joined via their Production Cycle number which was derived from the Monthly Records of biomass and EMB treatment (available in the FFDB).

2.3 Description of statutory benthic monitoring.

The SMDB contained data derived from statutory self-reporting, as returned to SEPA from fish-farmers. Sampling was conducted using a Van-veen grab at various distances from the fish-cages and is normally conducted at around the time of maximum biomass (typically within a production cycle of 2 years although this is variable, see 8.2). The sediment collected using the benthic grab was analysed for macrobenthos and a suite of other physical parameters (detailed below).

Historically, grab samples have been taken from a range of distances from the farm: most surveys included a cage edge (CE) sample and two samples from reference stations ('Ref': these are usually > 400 m from the farm edge and represent 'un-impacted' stations chosen to be otherwise similar to the farmed site). The location of the intermediate-distance sampling stations (between CE and Ref) depends on the nature of the consent under which the farm was operating: consents granted pre-2006 required samples from set distances (normally 25 m and 50 m with, exceptionally, additional grabs at 100 m and 150 m) whilst sampling locations at sites operated under consents granted or modified after 2006 were based on the delimitation of the 'allowable zone of effects' (AZE) with stations being located at the AZE boundary (AZE) and 10 m either side of the boundary (AZE -10m and AZE+ 10m) in addition to one CE and two Ref samples. Samples taken from fixed distances (e.g. under pre-2006 consents) are not directly comparable between farms because different farms will be subject to different depositionary regimes and, consequently, the same distance from farm will not represent the same farm-impact. The AZE boundary, as determined using the DEPOMOD model (Cromey et al., 2002a; Cromey et al., 2002b), integrates across a range of site-specific characteristics (e.g. current speed and depth) and animal husbandry factors (e.g. maximum biomass) and, consequently, allows direct comparisons between farms. From each sampling station (i.e. distance) a number of replicate samples were taken, the number depending on the size of the grab employed. Two, three and five replicates were required when using 0.1, 0.045 and 0.02 m^2 grabs resulting in a total sampled area of 0.2, 0.135 and 0.1 m^2 respectively. The macro-faunal results for each replicate (2, 3 or 5) were combined to give the final macrobenthic assessment for that sampling location (such that, for example, at the Reference stations there would be two true station-replicates, each consisting of the combination of 2, 3 or 5 grab samples each). Community metrics were then derived for the combined station-replicates. The number of grabs used in any given survey was determined by assessing whether the database fields for Grab 1 – Grab 5 contained any data (i.e. organisms) on the assumption that a grab that was 'empty' of all organisms wasn't actually taken (an approach agreed with SEPA). Stations where there were non-standard numbers of grabs taken were not included in the analysis. Whatever grabbing protocol was adopted, the resultant sediment was washed through a sieve (1 mm mesh). The sieve-retained fauna were identified to species level under the National Marine Biological Quality Control Scheme (NMBAQCS) (Worsfold and Hall, 2010). Grab sampling was augmented with further grabs (taken from the same location) that were sub-sampled (for particle size, organic content and redox). The results from the sub-sample analysis (e.g particle size) and the macrobenthos, together with the site characteristics reported by the farmers, constitute the core data set analysed under this contract.

2.4 Data preparation

2.4.1 Designation of regions.

Different regions in Scotland, as designated in the FFDB 'Local Authority' may host different biological communities (irrespective of other factors) and, consequently, region was included in initial models. The Local Authority (LA) regions represented in the database were Argyll and Bute, Eilean Siar, Highland, North Ayrshire, Orkney Islands and Shetland Islands. In order to reduce the number of modelled categorical variables, and with SEPA's agreement, the Local Authority regions were divided into two areas, Orkney and Shetland (OrkShet) and the combination of others (Other).

2.4.2 Recoding distances

The compliance monitoring included details of the stations and sampling dates of the benthic samples. The SMDB field 'Station' contained 764 different ways of specifying the station location in relation to the farm. Often this was related to the AZE (i.e. "AZE" or "AZE+10 m") but this only applied after 2006 (for new or revised consents). Otherwise an assortment of distances were recorded (under the pre-2006 scheme these included 0, 25, 50, 100 or 150 m) and reference stations with occasional inclusion of bearings and other superfluous information (see 5.1). Frequently the 'station' included two numbers e.g. 'Ref1, 700 m' or '0.7km, C1' which introduced problems with data extraction. For those AZE-related Stations (i.e. those where samples were taken after 2006) there was frequently no information on the actual distance from the farm that the sample was taken. The actual distance was required in order to match the biological (grab-survey) stations with the residue stations (from the FFDB). Where the distance was not recorded, it was extracted from another part of the database (sheet 'MarSumData', in FFDB, matched across Site Identities). Where the distance-to-farm of a station was recorded (e.g. "70 m") , but not any indication of the nature of the station (e.g. "AZE") then the distance was matched with the AZE +/-10 m stations designated for that site (available in another part of the database) provided any difference was 5 m or less. Stations that failed to meet these matching criteria were not included in the analysis. It was assumed that the designated sampling stations are those determined from DEPOMOD in relation to the cage edge of the farm, not to the modelled location of the farm (which may vary by 50 m under the CAR consent, SEPA personal communication). The AZE stations were combined into a single distance class ('AZE') because of the difficulty in distinguishing the +/- 10 m stations in the field (K. D. Black, personal observations).

2.4.3 Determination of EMB use per production cycle.

Fish are produced in 'Production Cycles' (PCs) and, generally, these last between 20 and 24 months, usually with a clear unimodal pattern of fish biomass and feed input (see 8.2). These data have been recorded on a monthly basis (since 2002) and were recorded in the 'Monthly' sheet in the FFDB.

The list of Sites which met the coding criteria (as above) was obtained and these sites were isolated from the MonthlyDatabase (FFDB). Production cycles within the reduced monthly database were manually identified, for each Site, based on the cycle of biomass and/or feed input. The amended MonthlyDatabase was then matched with the biological data (grab data) by site and date thus giving each PC and ID_Survey number (against which other metrics could be matched). For some PCs there were more than one biological survey, generally with a survey being conducted early in the PC and another around the period of maximum biomass. In such cases, the results from the latest (i.e. at max biomass) macrobenthic data were retained (see 8.3).

From the MonthlyDatabase two datasets were prepared: 1. Per-production cycle - for each PC the amount of EMB used, prior to the grab being taken, was determined and this was matched with the biotic and abiotic data (based on ID Survey) and 2. Per Site - the total amount of EMB used, per site, prior to the last grab-sampling event was determined and this was matched with the last macrobenthic data taken at that site. The PPC data will include data where there is zero recorded EMB use (for a given PC) but where, during previous PCs at the same site, EMB may have been used.

2.4.4 Derivation of community indices.

From each station the combined total of each identified species (across 2 – 5 grabs, see 2.3) was determined. From this total the following were derived: Shannon diversity (base = e), species richness (excluding crustacea), crustacean richness, total abundance (excluding crustacea) and crustacean abundance. The numbers of crustacean species, per station (i.e. combined across grabs) was frequently zero meaning the crustacean diversity could not be derived and, consequently, crustacean diversity was not used in any analyses. The diversity index was determined using the R library 'vegan' (Oksanen et al., 2013). The response metrics were not normalised to area (actual counts are used) and area was not used an offset with the statistical models. Differences in response variable attributable to Grab area were accounted for by the inclusion of Survey (see below) within the random effects part of the model as, within any survey, the same type of grab was used (except in exceptional circumstances). Grab area (total sampled area) was also tested as a fixed effect.

2.4.5 Site characterisation (particle size, organic content current speed).

As part of their statutory monitoring requirements fish-farmers are also required to collect physical characterisation data from the same locations as the macrobenthic grabs. Grab samples are sub-sampled for particle size analysis and proportions of the sediment <63 µm and <2 mm were routinely recorded in the SMDB. Full particle size spectra (e.g. from a particle size analyser) were not available in the database and, consequently, median particle size or other particle size summaries (e.g BGS Folk triangle) could not be derived. Other sediment characteristics recorded in the SMDB include the results from two different methods of assessing organic enrichment - 'loss on ignition (LOI)' and 'carbon analysis' (% Carbon). These methods are not directly comparable (there is no reliable method of converting between them) and, consequently, they were not included in the analysis (in order to maintain sample size: the split between samples was approximately 50:50). Following discussions with SEPA, 'redox', as a proxy for organic enrichment, was considered too unreliable for inclusion in the modelling process.

Current speeds are recorded in the FFDB sheet 'Consent'. For any given site there can be numerous measures of current speed, for example taken at different years as part of consent updates/ renewals. Current speeds from different depths in the water column are also recorded in the database but this varies between sites and years. The most commonly recorded current speed, and the one used in the statistical models developed here, is based on the mean of the 'sub-surface means' (the mean was determined where there were multiple records, e.g. across years). The water depth where the sample was taken was extracted from the SM_Data_Stations sheet on the SMDB and matched across ID Survey and Station

2.4.6 Extraction of residues data

Residues data were extracted from the FFDB. For the EMB residue sampling, three samples (replicates) per station were taken. For the analysis/ summarisation reported here the maximum recorded EMB concentration, per three replicates, was used. Residue concentrations are recorded as text (rather than numbers) and the database includes various ways for recording 'not detected' (e.g. 'ND', '<0.1', 'BDL'). These were replaced with NA or zero (for graphical representations). Analysis of the residue data, or its incorporation into models, was complicated by the frequency of 'below detection limit' observations particularly given that detection limits varied between surveys. Attempts were made to link the residue data to the macrobenthic data. However, the number of matched pairs (where the residue and macrobenthic sampling stations correspond in space and time) was so limited that this approach was abandoned following consultation with SEPA. A general summary of the residues data is given in the results and actual residue concentrations are not tested as a factor influencing macrobenthic infaunal communities.

2.5 Summary of dataset used in macrobenthic analyses.

Following discussion with SEPA the following data analysis approach was agreed. This approach incorporates as much of the data as possible, given the issues in its collation, and allows a broad interpretation of the results. Suggestions for further data refining and analysis form part of the recommendations for future work.

In any data analysis there is an option about which transformations (if any) to use on both the response and predictor variables. Transformations are necessary in order to better visualise the data and because transformed data sometimes resolves issues in fitting models (e.g. in residual patterns). The interpretation of the main effects of interest (EMB exposure and cage-distance) was assisted by centring (mean-subtracting) all of the continuous predictors (Schielzeth, 2010) (with the exception of EMB, see 8.6) following transformation (see above) which also improved convergence within the model (Bolker et al., 2009). A summary of response and predictor variables is given in Table 1.

Table 1 – Summary of Response and predictors used in modelling process. For further details see the text.

Type	Name	Description
Response	Crustacean richness	Number of crustacean species per sample
	Crustacean abundance	Number of individual crustacea per sample, cube-root transformed.
	Total abundance	Number of non-crustaceans per sample, cube-root transformed.
Random effect	Site	Production site
	ID Survey (Survey)	Survey identifier (per site: production-cycle combination)
	ObsID	Observation level identified (Poisson models only)
Fixed Predictor	Intercept	Forms the base level response: Cage-edge when all other continuous predictors are zero, Area (if included) at $0.1m^2$.
	Distance Class (CE, AZE, Ref)	Categorical, indicates distance from cage-edge
	EMB	Amount of EMB used prior to the corresponding grab sample being taken. Ranges between zero and max EMB (PCC or PST), square-root transformed.
	MaxBio	Maximum biomass, in thousand tonnes, centred.
	Depth	In metres, log transformed and centred.
	<63 µm	Proportion (%) of sediment sample passing the 63 µm sieve, log transformed and centred.
	>2mm	Proportion of sediment not passing the 2mm sieve, log transformed and centred.
	Current	Current speed ($m\ s^{-1}$), log-transformed and centred.
	Area (0.1, 0.135 or 0.2 m^2)	Categorical indicates the total area sampled.
	Region	Categorical, designates whether the Site is located in Orkney or Shetland (OrkShet) or elsewhere (Other).

2.6 Macrobenthic modelling protocol.

Introductory comments and regards the statistical modelling used in this study are given in 8.6. The response variables were linked to the predictors using generalised linear mixed models (GLMMs). As a modelling framework GLMMs are still in active development and where non-identity link functions are used (e.g Poisson GLMM) there are questions regards the determination of standard errors / confidence intervals (Gelman and Hill, 2007). The recommended approach is to make inference using the Bayesian framework (Korner-Nievergelt et al., 2015). In the current case, the models were initially developed using GLMMs (library lme4; Bates et al., 2012) and then refitted using a Bayes' method (library 'arm'; Korner-Nievergelt et al., 2015). Bayesian analysis requires priors (priors capture assumptions that are made regards the likely parameter distribution prior to the analysis). In this case, non-informative 'flat' prior were used – i.e. the parameter estimates resulting from the Bayesian model are based on the data only (Korner-Nievergelt et al., 2015). The Bayesian view of parameters is fundamentally different (and, arguably, more intuitive) compared to the frequentist (Classical) statistical modelling framework (Korner-Nievergelt et al., 2015). In order to be consistent, all models are interpreted using Bayesian inference (based on credible intervals, see below) and, because of the non-linear transformations used (e.g. cube-root), inference is made on the basis of model projections over the range of EMB used (following back-transformation, see 8.5). For the sake of clarity, response variables (e.g. abundance) and predictors (EMB) are back transformed to the original units prior to graphical presentation (see 8.5). In terms of interpretation, the 50th quantile is the best estimate of the parameter's value, whilst the 95% credible interval gives a range of values where there is 95% certainty that the value is correct. For the Reference-station-only analyses (see 3.1.5), confidence intervals based on boot-strapping (re-sampling) methods are also shown (see 3.1.5) (Bates et al., 2012). Graphical representation of the models was based on simulations drawn from the posterior-joint distribution (Korner-Nievergelt et al., 2015).

The random effects represent a description of the variability remaining after the fixed effects have been taken into account (Gelman and Hill, 2007). The random effects included an observation level indicator (Poisson models only), with Distance Class as a random slope nested in Survey nested in Site. For the Reference-only analysis the random effect ID Survey was nested in Site (for PPC analyses, modelling correlations between observations taken at the same site over time) or Site (for PST analyses, a random intercept, modelling Site as a random effect). The observation level indicator allows each observation to vary, irrespective of other factors and are necessary in GLMMs to account for over-dispersion (Elston et al., 2001). The inclusion of the Site random effect allowed different Sites to differ (e.g. this term would, if necessary, model sites in Orkney and Shetland as having, overall, higher crustacean richness and abundances) and will also account for Survey-based differences in grabbing protocol. The models also allowed differing response patterns between combinations of Distance Classes and Sites /Survey (random slope models). These random terms were selected, as a starting point, because they were logical given the way the data were collected and allow a focus on the main factor of interest (response v. EMB use).

Deriving optimal mixed models follows a two stage process: first optimise the random effects (whilst retaining as many fixed effects in the model as possible) and then optimise the fixed effects (Zuur et al., 2009). In the current case, the random effects (Distance class-random slope, Survey nested in Site and ObsID in Poisson models) were fitted in the presence of the maximally fitted fixed effects (see below). Models with differing combinations of random terms (random intercept terms for Survey and Site with/without random slope for Distance Class) were trialled. Chi-square tests and comparisons of AIC/BIC were used to derive the optimal model, in the presence of the fully-fitted fixed effects. The fully-fitted models consisted of all the main effects (factorial and continuous, Table 1) plus the interaction between EMB exposure and Distance Class (except in the 'Reference station-only' analyses). Further interactions were not fitted (initially) because such models are likely to suffer convergence problems. Once the optimal random effects were determined, the fixed effects part of the model was simplified by eliminating those terms that had low t or Z values following checking by comparing models on the basis of their Akaike Information Criteria (AIC) or Bayesian information criterion (BIC, for non-Gaussian models) scores, residual patterns (see below) and using chi-square tests (Zuur et al., 2009). Over-dispersion in Poisson models was checked using a chi-square test (Korner-Nievergelt et al., 2015). During the model fitting process, the resultant residuals and fitted values were plotted (residuals v. fitted, fitted v. observed, residuals v. covariables and residual normality (random and fixed) to further assess model fit. Observations that led to very large residuals were removed from the analysis. The fixed effects should be interpreted as representing the expected relationships between response and predictors at the average survey or site, not the average response across all sites (Gelman and Hill, 2007).

The main task of the current analysis was to evaluate the relationship between EMB treatment and community metrics. Tests of null hypotheses of 'no effect' are invalid in such cases (Gigerenzer, 2004; Johnson, 1999) and the goal was to best estimate the parameters and establish credible intervals (Nakagawa and Cuthill, 2007).

3 Results

The results section is divided into graphical/ tabular summaries of EMB usage, residues, site characteristics and macrobenthic data and concludes with the statistical analysis linking the macrobenthic and site characters. The main focus of the interpretation of the models in on the effect of EMB.

3.1.1 Patterns in EMB usage (over time) and residues

The total amount of EMB used, in Scottish salmon-farms, over the period 2003 – 2014 was 680 kg. Across all farms, the annual rate of use of EMB increased by a factor of 2 over the period 2003 – 2012 (from ~35 to ~70 kg per year) but subsequently (2013) declined to 60 kg (Figure 1; note: these data include those farms not analysed in this data analysis because they did not meet the matching criteria). Of those farms that did meet the matching criteria, the amount of EMB used, per site, over the period 2003 – 2014 varied considerably (Figure 2), with 50% of sites using between 1.52 and 4.61 kg. The maximum usage, per site, was 11.63 kg (Site "Inv1").

The amount of EMB used, per treatment (of which there could be several in any production cycle), ranged between 0.378 and 1474 g. The mean EMB treatment was 208 g. Usage patterns are further explored in 3.1.2.

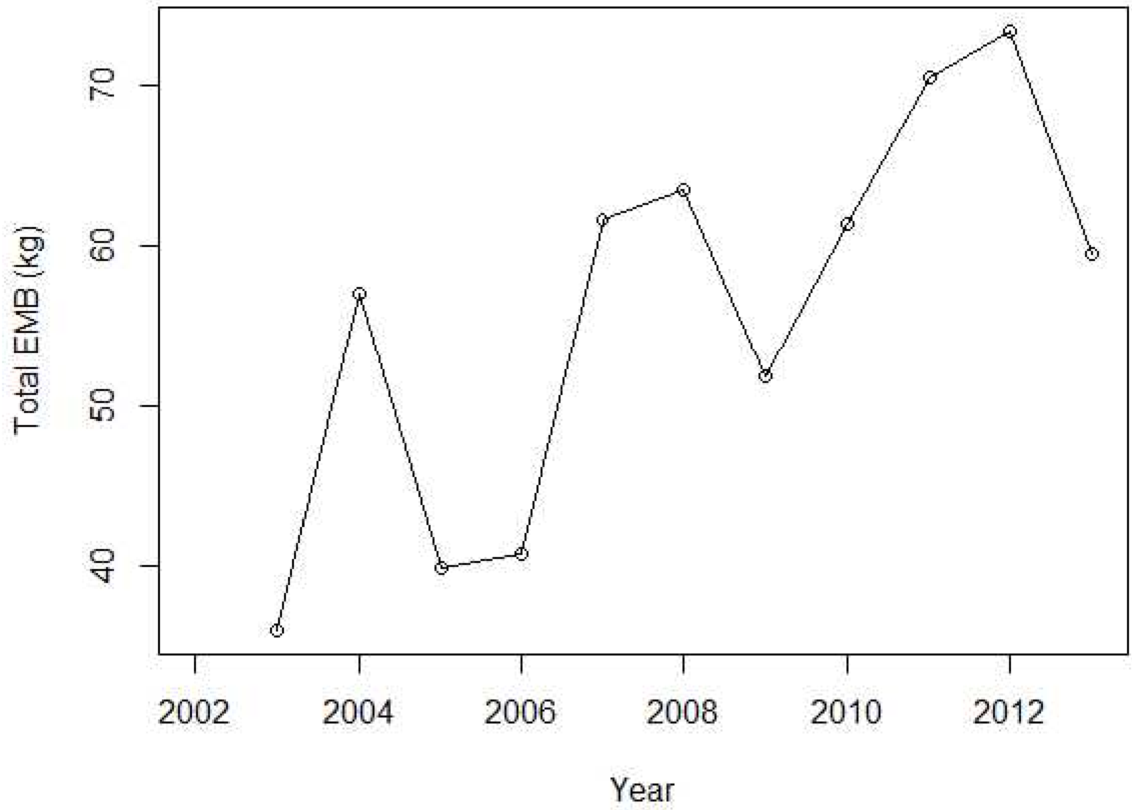

Figure 1 – The expansion of EMB use, in Scottish salmon farms, over the period 2003 – 2013

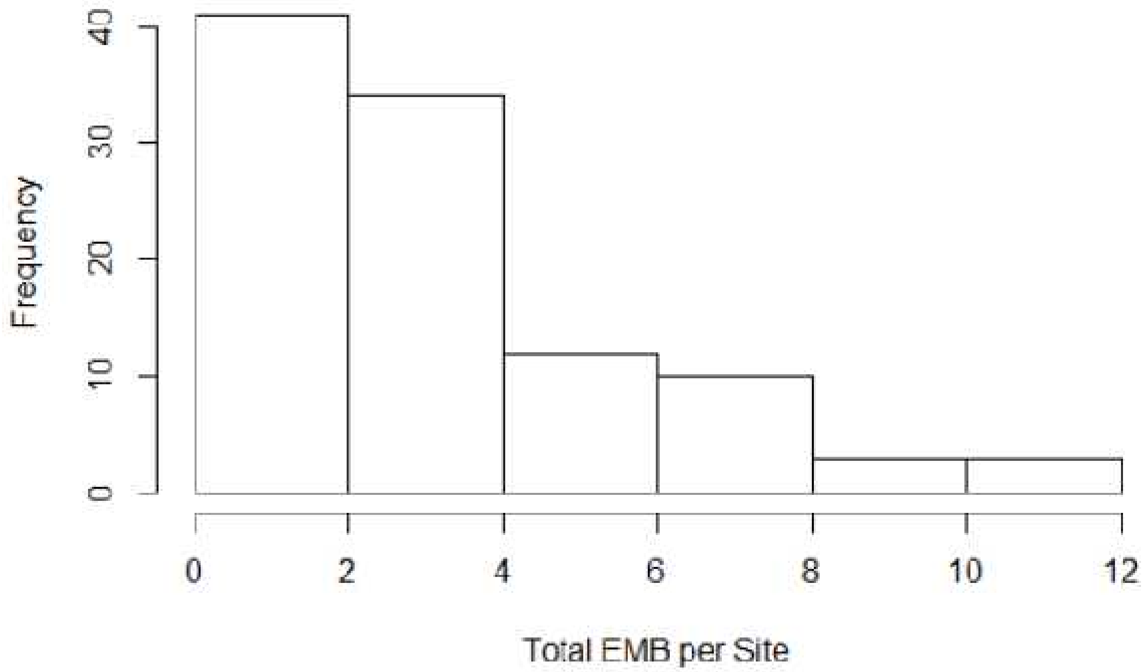

Figure 2 – Total recorded EMB applied per Site (kg, cumulative across all Production Cycles) for a total of 103 Sites.

A total of 1235 residue measurements, from 271 sites, were initially extracted from the FFDB. Recorded maxima (per 3 replicates) EMB residues, per site, ranged between not detected and 96 µg kg^{-1}. A majority of the data were taken at the cage edge (77 %) and 25 m cage distance (22 %), a total of eight measurements (<1 %) were made at 100 and 150 m and, for the purposes of clarity, these are excluded from these subsequent summaries.

The residue data were left skewed with a high proportion of them being 'below detection limits' (Figure 3), this being higher at the 25 m stations compared with those at the cage-edge (Table 2).

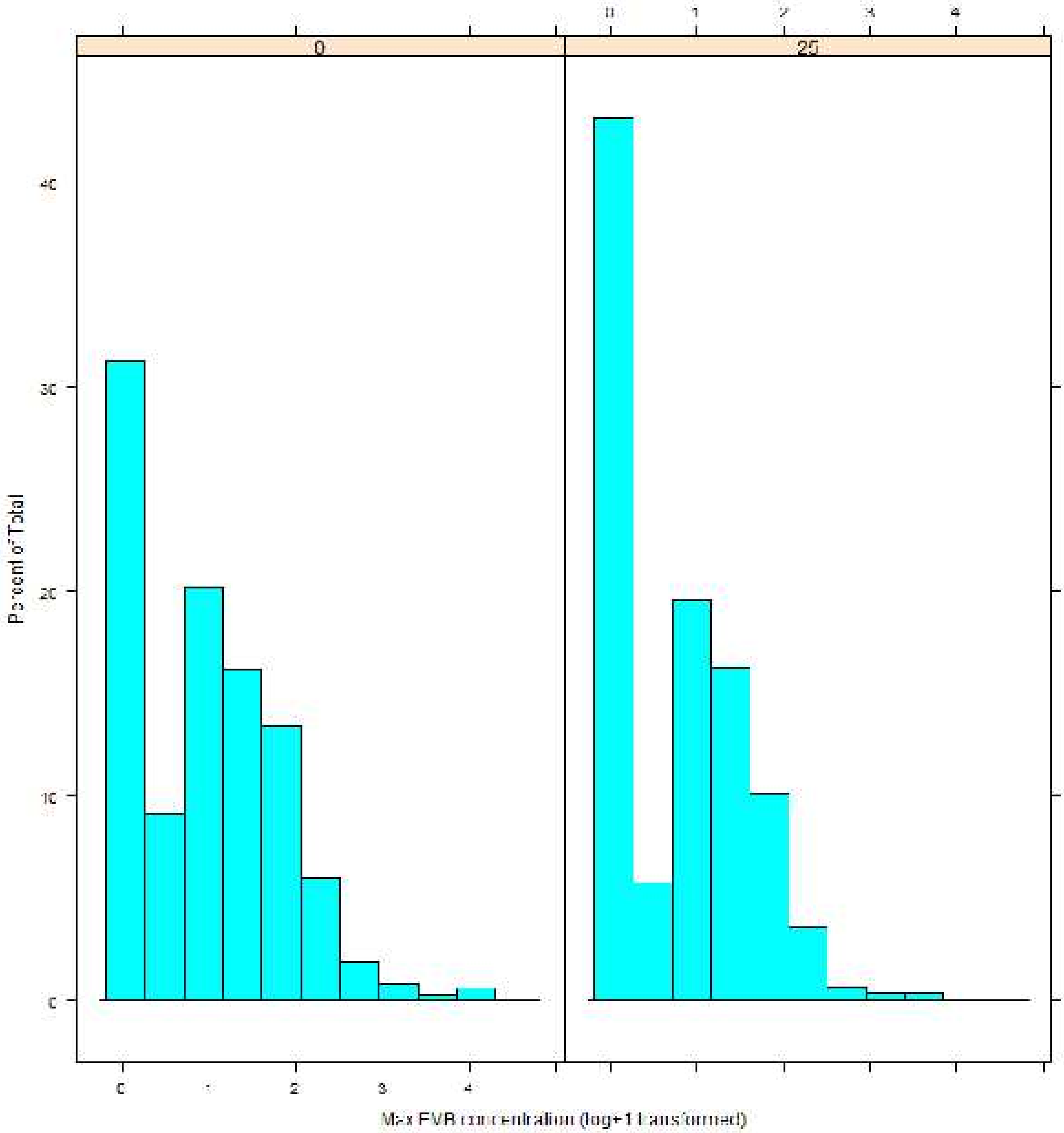

Figure 3 – Maximum EMB concentration (per 3 reps, in µg kg^{-1}) per distance from the cage edge (0 and 25 m), log transformed. Note that 'zeros' represent 'below detection limits' here.

Table 2 - Summary of EMB residues data between 0 and 25 m cage distance. No. sites – number of sites, No. obs= number of observations (including those where EMB was below detection limits), Mean, Max and IQR – EMB residue mean, maximum and interquartile range (µg kg^{-1}, based on observations where EMB was detected). Prop>BDL – proportion of observations where EMB was detected.

Distance (m)	No. sites	No. obs	Mean	Maximum	IQR	Prop>BDL
0	271	951	4.29	95.7	3.47	0.73
25	142	276	3.54	42.0	2.65	0.57

Sediment characterisation from the locations where the residue samples were taken was not available but residue data could be matched with the mean current speed at the site (Figure 4). There was no discernible relationship between current speed and either EMB residue concentration or Environmental Quality Standard (EQS) failure.

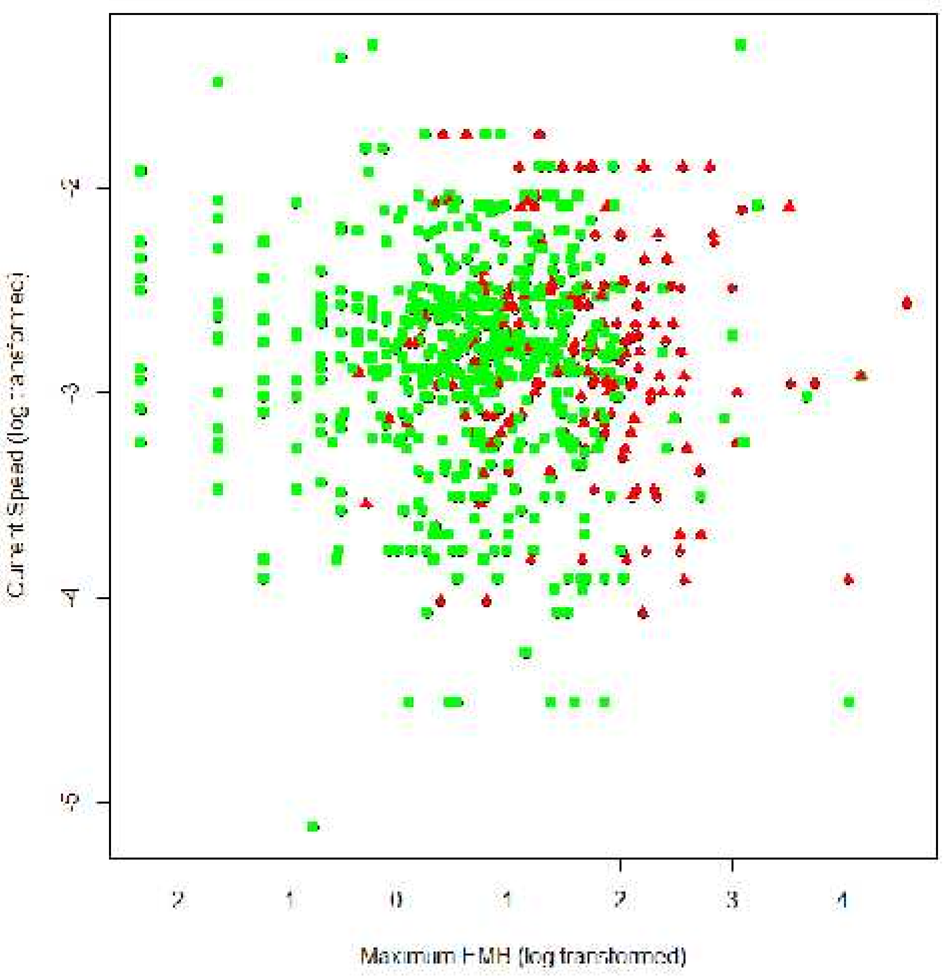

Figure 4 – Relationship between Maximum EMB concentration (in µg kg^{-1}, log transformed) and mean current speed (m s^{-1} log transformed). EMB failures (where the residue exceeded the limits) are shown in red, non-failures are shown in green.

The lack of any apparent relationship between EMB and current speed, the problems with the high-skew in the data, prevalence of 'below detection limit' values where the detection limit varies between surveys, meant that no further modelling work was conducted on these data.

3.1.2 Site characteristics.

The total number of grab-samples that were available for analysis was 1259, these were split between six regions and 99 Sites (Table 3). Different regions were characterised by the use of different grab sizes in their surveys, for example farmers in Argyll and Bute tended to report the results from 0.045m^2 grabs, Highland region farmers tended to use the 0.1m^2 grab whilst in Shetland the 0.02 m^2 grab was used almost exclusively. Within the data analysed, 1022 samples were from

EMB treated Sites whilst 237 were from untreated sites (Table 3). With the exception of North Ayrshire (1 site), all Regions hosted both treated and untreated Sites.

Table 3 - the number of samples in different regions as a function of the total area of the benthic sample (0.1 m^2 = 5 x 0.02 m^2 grabs, 0.135 m^2 = 3 x 0.045 m^2 grabs, 0.2 m^2 = 2 x 0.1 m^2 grabs). For Argyll and Bute, a total of 451 samples were analysed, from 28 sites and, of these, 400 samples were from Sites that had been treated with EMB. Key: Local A. – local authority

Local A.	0.1	0.135	0.2	Total nos.samples	Nos.Sites	Nos.samples treated
Argyll and Bute	19	391	41	451	28	400
Eilean Siar	9	107	130	246	22	186
Highland	9	93	184	286	20	268
North Ayrshire	0	9	0	9	1	9
Orkney Islands	33	21	0	54	6	9
Shetland Islands	210	3	0	213	22	150
Total	280	624	355	1259	99	1022

The sediment characteristics varied between Regions: Orkney and Shetland were characterised by relatively shallow sites and coarse sediments but, of these two regions, only Orkney was characterised by exposure to relatively high currents (Table 4). However, all Regions (except North Ayrshire where there was only one site) contained sites which were exposed to relatively high current speeds (see MxCSp column in Table 4) and Sites characterised by relatively coarse sediments (low <63µm and high >2mm content, Table 4). There were correlations between the sediment characteristics and current speed: current speed was negatively correlated with fine sediments (% <63 µm; Figure 5) but there was no apparent correlation between current speed and the coarse sediment fraction (>2 mm; Figure 5). Sediments taken from deeper waters tended to contain greater proportions of fine material and less coarse material but there was only a weak correlation between depth and current exposure (Figure 5). There was a positive relationship between the maximum biomass and EMB usage during any production cycle (Figure 5).

Table 4 - Environmental summary statistics for fish-farms included in analysis by Local Authority region (LocalA). MnD, MinD, MxD = mean, min and max benthic sample depth (m) respectively, Mn2mm, Mx2mm = mean and max particle size as proportion >2mm (%) respectively. Mn63, Mx63 M = mean and max particle size as proportion <63µm (%) respectively. MnCSp, MinCSp, MxCSp - mean, min and max current speed (ms^{-1}) respectively. The number of sites on which these summary statistics are based is shown in Table 3. Depth statistics exclude those sites where depth was recorded as zero.

LocalA	MnDp	MinDp	MxDp	Mn2mm	Mx2mm	Mn63	Mx63	MnCSp	MinCSp	MxCSp
Argyll and	30	5.9	73	6.9	92	40.0	99	0.070	0.020	0.150
Eilean Siar	26	7.5	52	7.8	69	42.0	99	0.071	0.044	0.120
Highland	39	14.0	110	7.5	68	40.0	89	0.069	0.037	0.180
North	27	15.0	38	5.6	14	25.0	67	0.077	0.077	0.077
Orkney	21	9.8	35	14.0	98	7.8	36	0.120	0.076	0.190
Shetland	27	7.0	54	13.0	97	12.0	61	0.081	0.013	0.170

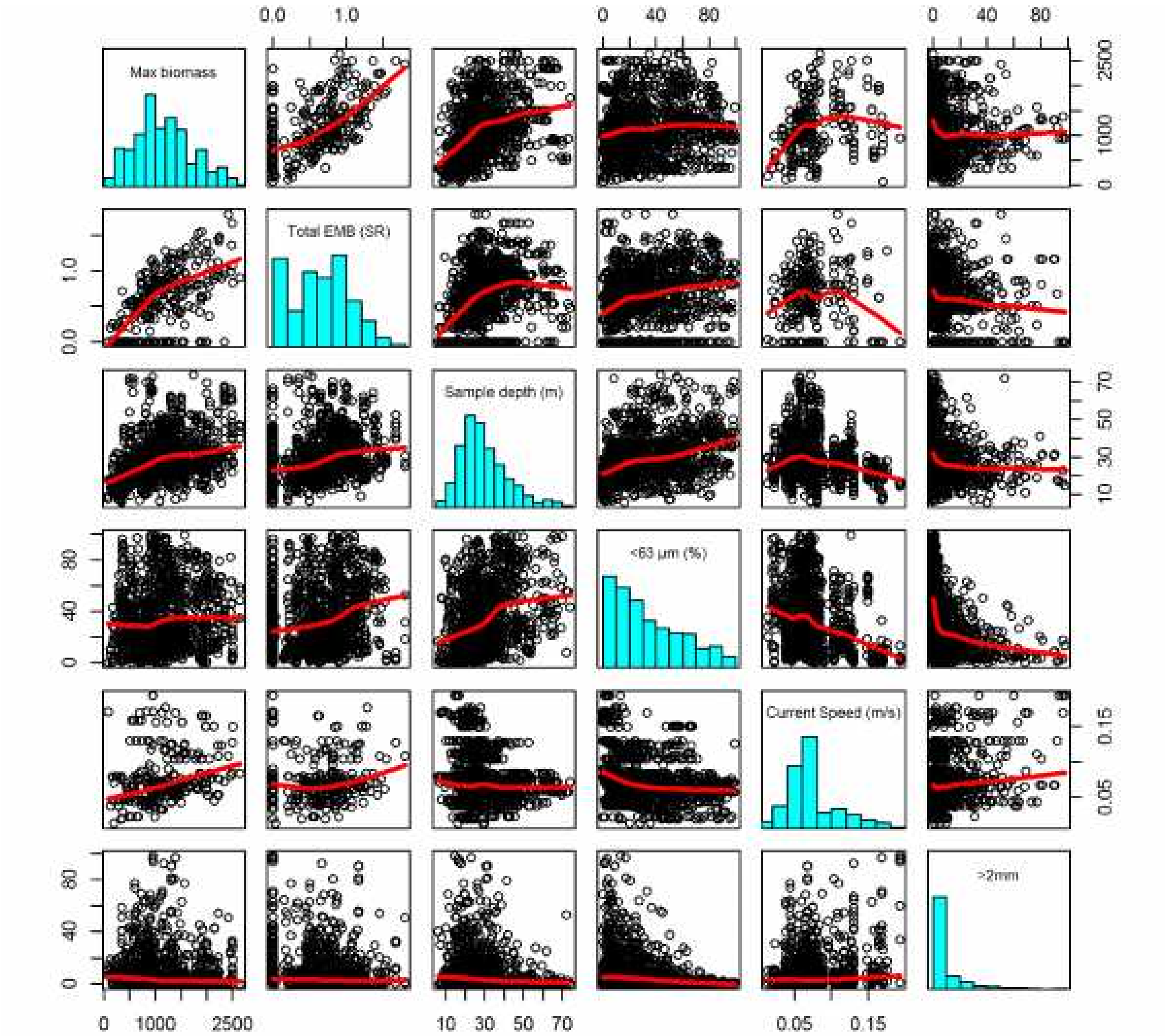

Figure 5 – Draftsman's plot showing relationship between maximum biomass (max biomass, tonnes), total EMB applied (kg per site, square-root transformed), sample depth, proportion of sediment <63 µm, current speed and proportion of sample passing 2mm sieve (>2 mm). N=1259 from 259 production cycles. A histogram showing the frequency distribution of each covariable is shown in the diagonals. A loess smoothing curve (red line) is fitted to each scatter plot to aid interpretation. Further details on interpretation of Draftsman's plots are given in 8.8.

Table 5 - Summary of biological characteristics per Local Authority and by grabbing protocol (Area, in m^2, see Table 3). Diversity =Shannon's diversity, TSR= total species richness, CR – crustacean richness, TA – total abundance (all species), CA – crustacean abundance. N=sample size. Note that

these summary figures include samples taken from the cage edge, AZE and Reference stations and that these summary metrics (richness and abundance) are not based on normalised data.

Local	Area	Diversity	Species	Crust. richness	Total abun	Crust.	N
Argyll and	0.100	1.33	21.9	1.53	829	2.68	19
Eilean Siar	0.100	2.36	39.9	2.00	803	8.33	9
Highland	0.100	2.26	47.2	5.89	2190	13.20	9
Orkney	0.100	2.33	58.5	14.80	1080	103.00	33
Shetland	0.100	2.14	43.6	5.55	837	40.30	210
Argyll and	0.135	2.21	42.4	2.80	1430	9.42	391
Eilean Siar	0.135	2.29	46.1	6.25	970	26.70	107
Highland	0.135	2.56	48.1	2.26	1060	4.86	93
North	0.135	2.72	51.8	6.00	2230	16.70	9
Orkney	0.135	2.59	72.0	9.52	1090	74.50	21
Shetland	0.135	2.08	39.3	3.00	839	14.30	3
Argyll and	0.200	1.87	35.7	2.39	1460	5.12	41
Eilean Siar	0.200	2.08	33.1	3.32	846	13.90	130
Highland	0.200	2.19	30.8	1.78	580	3.29	184

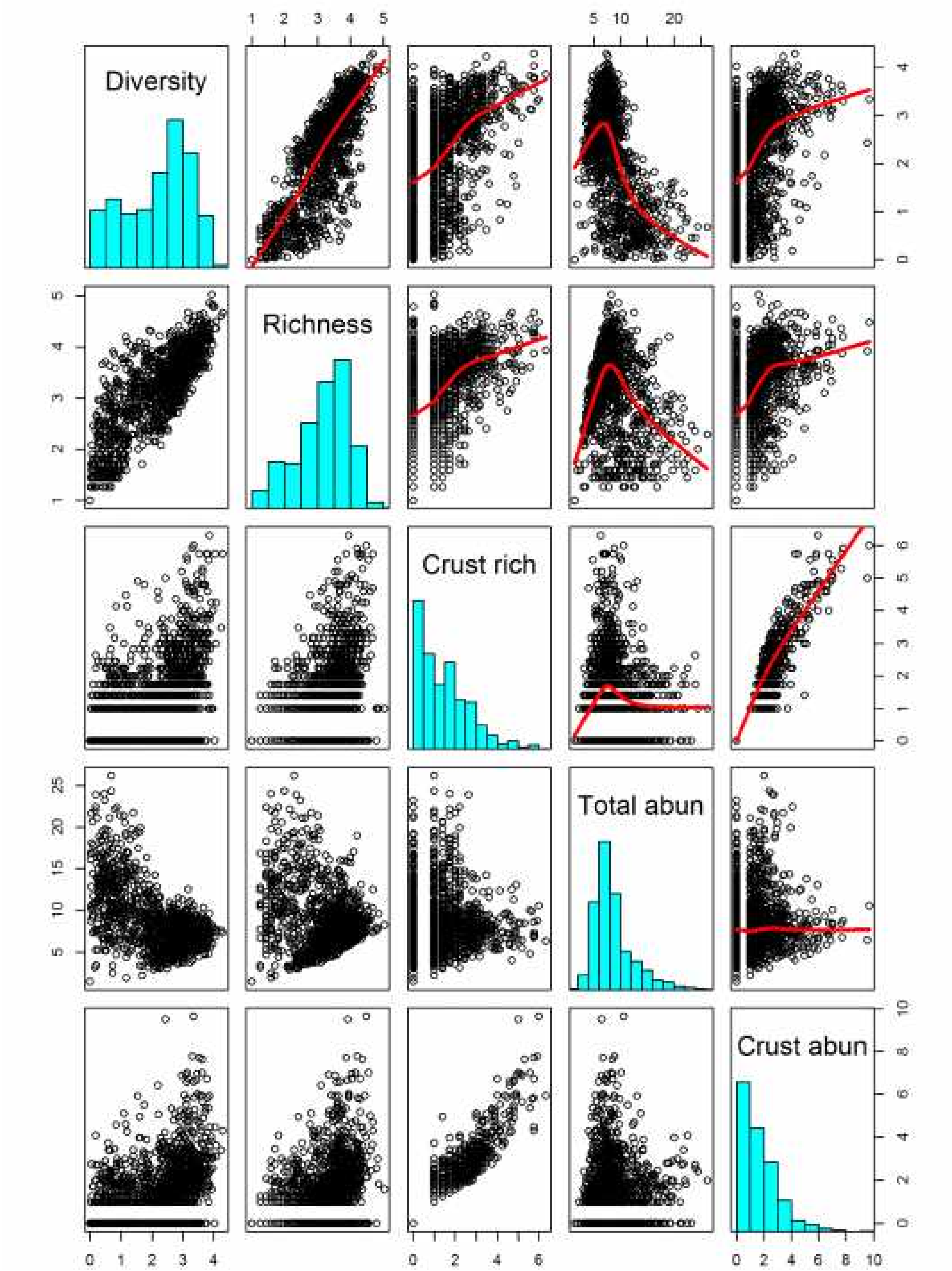

Figure 6 – Draftsman's plot showing correlations between response variables: Diversity (Shannon diversity), Richness (species richness, excluding crustacea, cube root transformed), Crust rich (crustacean richness, square root transformed), Total abun (total abundance, excluding crustacea, cube root transformed) and Crust abun (crustacean abundance, cube root transformed). A loess smoothing curve (red line) is fitted to upper panel scatter plots to aid interpretation. For interpretation of plot see Figure 5 caption.

The Shannon diversity, measured across all sites (treated and untreated), varied between approximately zero and five (Figure 6). Shannon diversity was highly correlated with both species and crustacean richness (Figure 6) but showed a unimodal relationship with total abundance with low diversity being associated with the extremes of high and low abundance (Figure 6). Crustacean abundance was very highly correlated with crustacean richness (Figure 6).

There were apparent differences between the diversity metrics determined from the data as a function of grabbing protocol (total grab area) and sampling region. The highest crustacean richness and abundance was found in Orkney (total sampling area 0.1 and 0.135 m^2) whilst the lowest was found in Argyll and Bute (total sampling area 0.1 m^2; Table 5). General trends in diversity metrics (diversity, species and crustacean richness, total and crustacean abundance) as a function of grabbing protocol were difficult to identify but simple trends of increasing richness and abundance with increased grab area were not seen (Table 5).

3.1.3 Macrobenthic summaries.

These summaries are based on the 'per-production-cycle' (PPC) data and the 'per-site total' (PST) data (see 2.4.3). There were substantial increases in crustacean richness and abundance as a function of cage distance (compare CE – AZE and Reference stations; Table 6, Table 7). In addition, there was a clear pattern of decline in both crustacean richness and abundance as EMB increased, and this applied to both the PPC and PST data (Table 6 and Table 7 respectively) and applied to all Distance Classes. Particularly relevant trends occurred, for example, in the PST data, where mean crustacean Richness declined from 15.0 to 4.10 (a factor of 3.75 or 73%), at the Reference stations, over the gradient nil to >4000 g of EMB applied prior to the sampling event (Table 7). This trend was also seen in terms of back-transformed crustacean abundances, where the decline was between 58 and 5.20 (a factor of 11.2 or 91%) over the same EMB gradient (Table 7).

Table 6 - Summary of mean crustacean abundance and richness at different Distances (Distance Class, DistClass) and levels of EMB treatment (EMB Class, in g applied PCC). No. obs – number of observations, Total –mean total abundance, Richness = mean species richness. Total2 is the mean of the cube-root transformed abundance data back transformed (see 8.5).

DistClass	EMB Class	No. obs	Richness	Total	Total2	Nos.sites
CE	Nil	51	2.90	15.0	2.40	33
CE	1-249	41	1.20	2.3	0.55	34
CE	250-499	45	1.70	7.4	1.40	31
CE	500-999	70	0.81	4.0	0.58	49
CE	>999	46	1.10	4.2	0.42	33
AZE	Nil	78	5.40	36.0	8.80	22
AZE	1-249	72	3.40	9.5	3.70	24
AZE	250-499	74	2.70	8.5	2.60	21
AZE	500-999	150	2.90	9.2	2.70	39
AZE	>999	101	1.80	5.1	1.40	28
Ref	Nil	108	10.00	65.0	27.00	33
Ref	1-249	89	5.10	24.0	7.40	34
Ref	250-499	90	5.40	39.0	9.60	31
Ref	500-999	146	4.20	14.0	6.20	51
Ref	>999	98	3.30	11.0	3.90	33

Table 7 - Summary of crustacean abundance and richness at different levels of EMB treatment – PST EMB (g) per Site). Class divisions were selected on the basis of maintaining sample size. No. obs- number of observations, Total – mean total abundance, Richness = mean species richness. Total2 is the mean of the cube-root abundance data back transformed (see 8.5).

DistClass2	EMB Class	No. obs	Richness	Total	Total2	Nos.sites
CE	Nil	11	5.60	44.0	12.00	11
CE	1-1000	22	1.50	4.7	1.50	21
CE	1001-2000	16	1.30	3.3	0.26	15
CE	2001-4000	25	1.00	4.4	0.80	25
CE	>4000	24	0.92	4.8	0.30	24
AZE	Nil	12	6.70	43.0	8.50	4
AZE	1-1000	42	6.50	35.0	14.0	14
AZE	1001-2000	41	3.10	13.0	3.10	13
AZE	2001-4000	58	2.60	5.7	2.10	22
AZE	>4000	37	1.80	5.2	1.10	14
Ref	Nil	22	15.0	100	58.0	11
Ref	1-1000	46	9.10	46.0	22.0	22
Ref	1001-2000	32	6.20	59.0	14.0	15
Ref	2001-4000	57	3.90	9.7	5.80	27
Ref	>4000	50	4.10	14.0	5.20	24

3.1.4 Macrobenthic statistical modelling results

The following variables were tested in GLMM models: crustacean richness, crustacean abundance, total richness (excluding crustacea), total abundance (excluding crustacea) and Shannon diversity. For each response variable two models were constructed: one was based on the 'per-production-cycle' data, the other was based on the total EMB applied (per site) prior to the final macrobenthic sample being taken (see 2.4.3 for details). In relation to EMB, the only significant relationships found were with the crustacean metrics with the exception of total abundance (excluding crustacea, per production cycle). Whilst significant the effects on total abundance (excluding crustacea) were of relatively small magnitude; this model is summarised in 8.4). Further details of the models, the derivation of proportional changes following EMB application and their interpretation are given in 8.6. The proportional changes, for abundances, are determined from the base-level (50% quantile) model predictions at the Reference stations (see 8.6). Results from three surveys (ID_Survey 648, 928 and 964) were excluded from the analyses as they exhibited extremely high variability in crustacean abundance and were identified as extreme outliers in the model.

3.1.4.1 Crustacean Richness per production cycle

Statistical modelling showed that crustacean richness was highly variable and that that this variance occurred between Sites and between Distance classes within the same survey (relatively high Obsid, Intercept and SiteID standard deviations, Table 8). The fixed effects remaining in the model, following the rationalisation procedures, included Distance Class, EMB, depth, proportion of fine material, current speed and grab area (the use of 0.1 m^2 grabs result in an estimate of crustacean diversity which was estimated to be 40% lower than 0.02 m^2 grabs). The negative coefficients associated with Depth and <63µm show that crustacean richness decreases with increased depth and with finer sediments (independently of any other factor). Conversely, the positive coefficient associated with Current indicates a positive relationship between richness and current speed.

The EMB effect was significant and, in order to improve the linearity of the residuals, the EMB was square-root transformed prior to inclusion in the model. This indicates that the effect of EMB was not linear e.g. on the linear scale predicted loss of species richness became less rapid as more EMB is used (Figure 8; discussed in section 4.2). **The Bayesian credible intervals show that the most likely reduction in richness, per 3 kg EMB use, per production cycle, is by 40% with 95% confidence that this reduction is between 4 and 63% (Figure 8, Table 8, Table 23). This pattern of reduction applied to all Distance Classes. The model predicts that crustacean species richness, at the average site and under average conditions, will decline from 5 to 3 species (as quantified using 5 x 0.02 m^2 grabs) when 3 kg of EMB are applied PPC.**

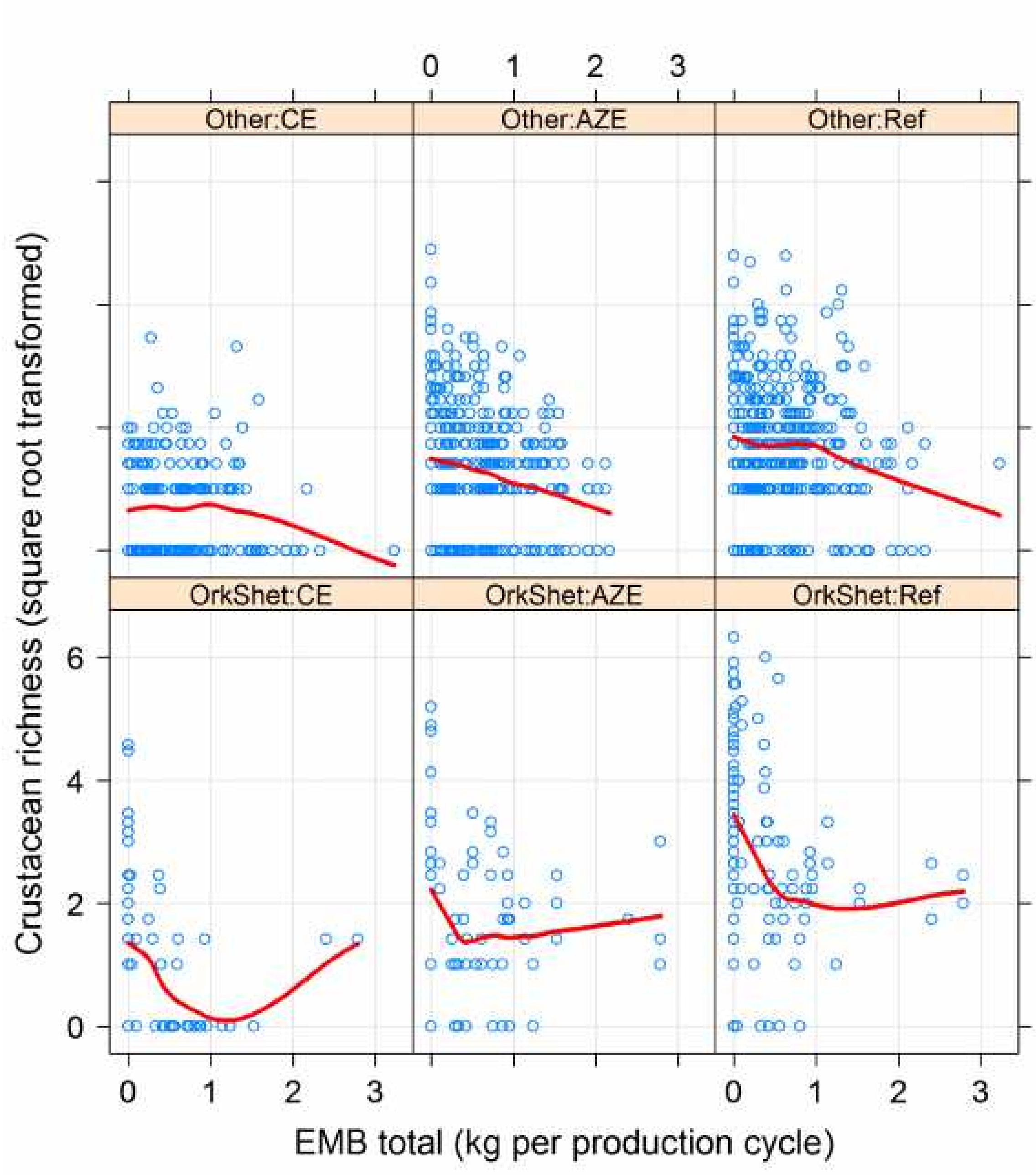

Figure 7 – Crustacean richness (number of species) as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station) and Region (Other and OrkShet, see Table 1) and the total EMB applied per production cycle (see main text for details). A loess smoothing curve (red line) is fitted to each scatter plot to aid interpretation.

Table 8 - Crustacean richness (per production cycle)

Model: Richness ~Distance Class + EMBSR + Depth + LT63+Speed+Area +(ObsID) + (Distance Class|Survey) + Site. Poisson GLM with log-link function.

Random effects: The ":" in source indicates a correlation.

Source	Std.dev/correlation
Obsid	0.236
Intercept	0.922
AZE	0.792
Ref	0.806
Int: AZE	-0.529
Int: Ref	-0.738
AZE: Ref	0.664
SiteID	0.521

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	0.0564	0.184	0.306	0.759
AZE	0.992	0.123	8.06	7.76e-16
Ref	1.57	0.112	14.0	1.65e-44
EMB	-0.293	0.143	-2.04	0.0412
Depth	-0.337	0.102	-3.30	0.000981
<63 µm	-0.16	0.0411	-3.90	9.66e-05
Current	0.628	0.152	4.14	3.54e-05
Area=0.135	-0.233	0.158	-1.48	0.140
Area=0.2	-0.504	0.194	-2.60	0.00946

Bayesian predictions and credible intervals

	Intercept	AZE	Ref	EMB	Depth	<63 µm	Current	Area=0.135	Area=0.2
2.5%	-0.265	0.809	1.40	-0.574	-0.530	-0.239	0.323	-0.536	-0.881
50%	0.0516	0.993	1.57	-0.298	-0.338	-0.161	0.636	-0.229	-0.495
97.5%	0.380	1.18	1.74	-0.0247	-0.150	-0.0855	0.916	0.0664	-0.136

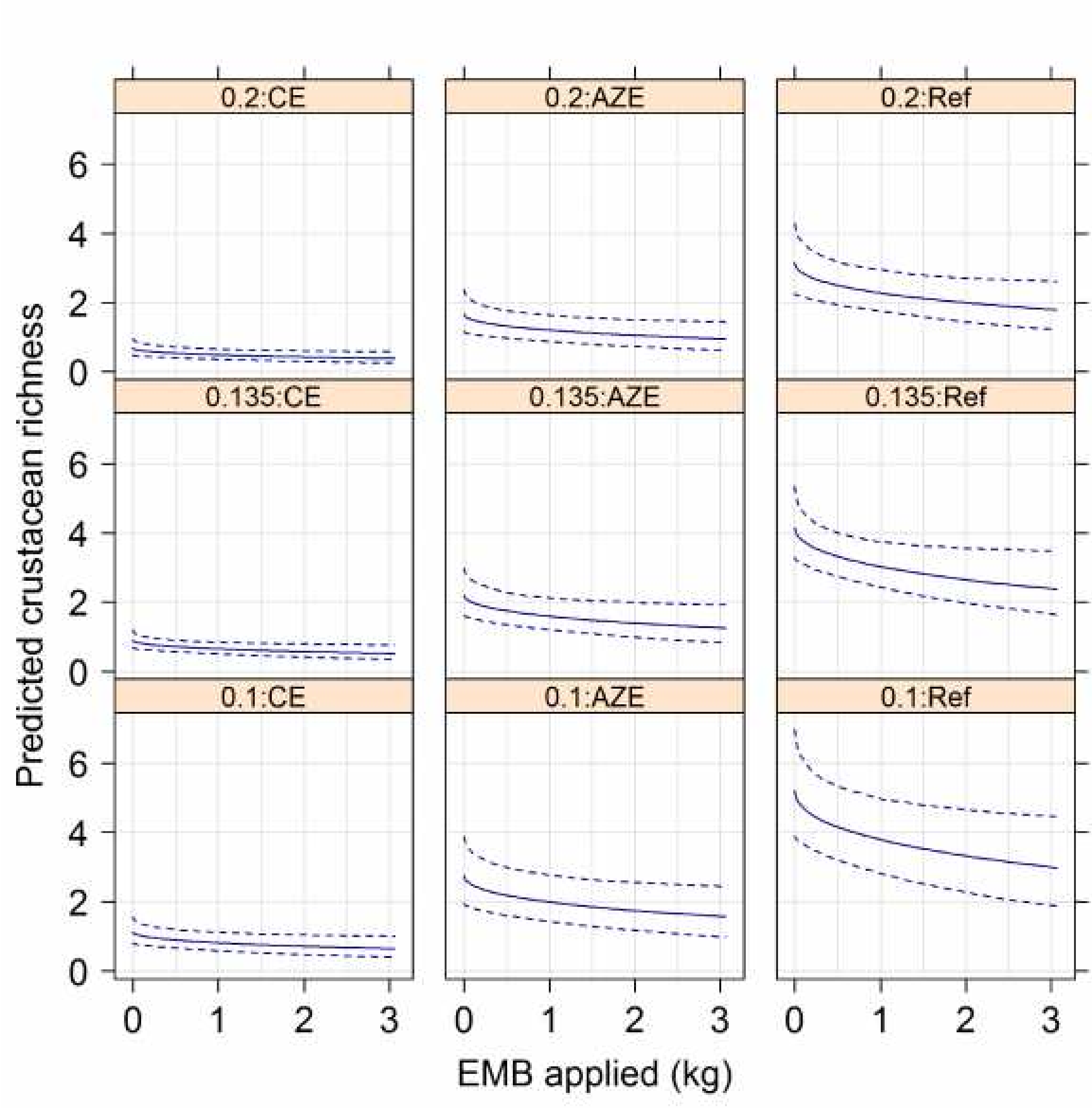

Figure 8 – Predicted crustacean richness (number of species) as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station) and the total EMB applied per production cycle (see main text for details).

3.1.4.2 Crustacean abundance per production cycle

Crustacean abundance, as determined for each production cycle, ranged between zero and 899 (total abundance for all grabs combined). Crustacean abundance was lowest at the cage edge and highest at the reference stations (Figure 9). There was an irregular decline in mean abundance when categorised by EMB classes (Table 6). This reduction in crustacean abundance applied to both Regions (Orkney and Shetland v. Others; Figure 9). Statistical modelling indicated that crustacean abundance was highly variable and that that this variance occurred between sites and between Distance classes within the same survey (relatively high standard deviations associated with Survey and Site and between replicates taken at the same distance (Random effects; **Table 9**). The fixed effects remaining in the model, following the rationalisation procedures, were Distance Class, EMB, Depth, proportion of fine material and current speed. **The model predictions indicate that, on average, predicted mean crustacean abundance at reference stations would decline by 66% with 95% confidence this would range between 29 and 87% following a 3 kg EMB treatment (Table 23).**

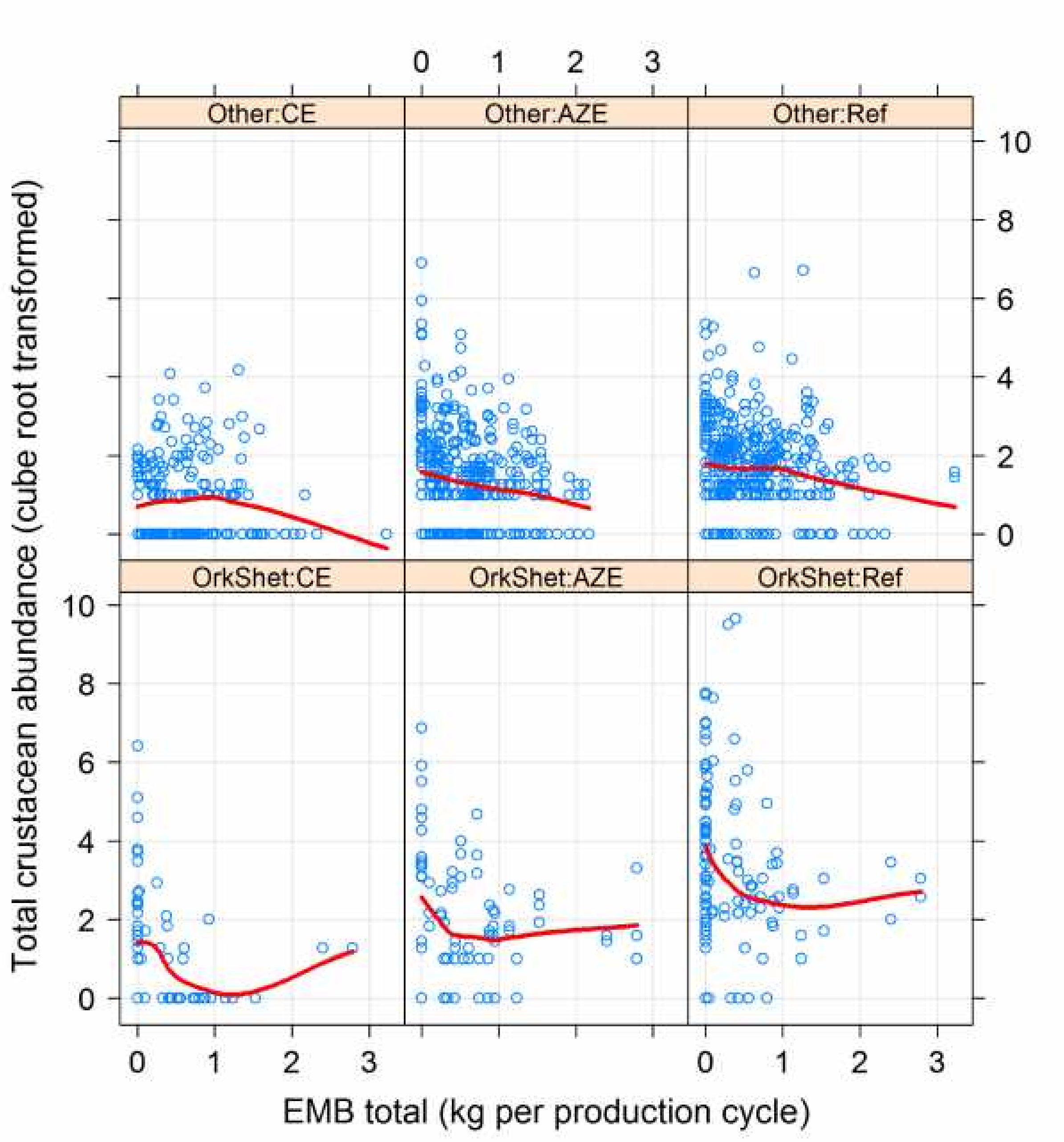

Figure 9 – Crustacean abundance as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station) and the total EMB applied (per production cycle, see main text for details) and Region (OrkShet and Other). A loess smoothing curve (red line) is fitted to each scatter plot to aid interpretation.

Table 9 - Crustacean abundance (cube root transformed and based on EMB applied during each production cycle. Model: Abundance~ Distance Class + EMB + Depth + LT63 + Current speed + (1+Distance class|SiteID/ID_Survey): Identity link function. See Table 1 **for key and abbreviations.**

Random effects: vcor- variance /covariance, sdcorr- standard deviation/correlation. The ":" in the Group designates covariance/correlation.

Source	Group	vcov	sdcor
Survey nested in Site	Survey, Int	0.543	0.737
Survey nested in Site	Survey, AZE	0.512	0.715
Survey nested in Site	Survey, Ref	0.535	0.732
Survey nested in Site	Int: AZE	-0.283	-0.537
Survey nested in Site	Int: Ref	-0.212	-0.393
Survey nested in Site	AZE: Ref	0.255	0.487
Site	Site, Int	0.176	0.420
Site	Site, AZE	0.479	0.692
Site	Site, Ref	0.721	0.849
Site	Int: AZE	-0.0478	-0.165
Site	Int: Ref	-0.201	-0.563
Site	AZE: Ref	0.491	0.835
Residual	Residual	0.468	0.684

Fixed effects

	Estimate	Std. Error	t value
Intercept	1.17	0.113	10.3
AZE	0.666	0.108	6.20
Ref	1.16	0.113	10.2
EMB	-0.402	0.132	-3.04
Depth	-0.244	0.0938	-2.60
<63 µm	-0.216	0.046	-4.70
Current	0.639	0.139	4.61

Credible intervals

	Intercept	AZE	Ref	EMB	Depth	<63 µm	Current
2.5%	0.952	0.465	0.954	-0.661	-0.424	-0.304	0.374
50%	1.17	0.666	1.17	-0.406	-0.243	-0.216	0.639
97.5%	1.39	0.885	1.4	-0.146	-0.0637	-0.125	0.897

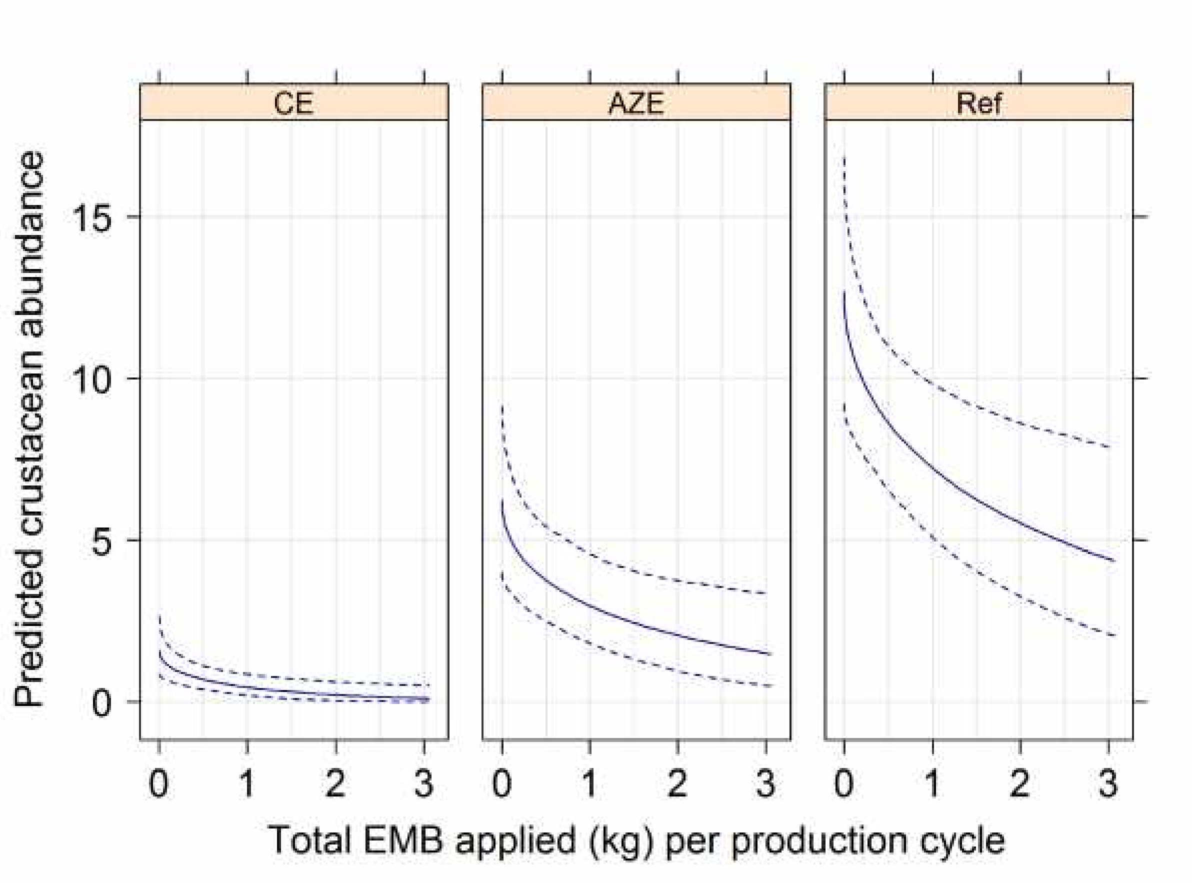

Figure 10 – Predicted crustacean abundance as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station) and the total EMB applied PCC (see main text for details).

3.1.4.3 Crustacean richness per site EMB total

Patterns in crustacean richness, as a function of total EMB applied, followed that determined for the 'per production cycle' analysis: the raw data indicated a negative relationship between richness and EMB treatment, at all Distance classes and differences between Regions (Table 7, Figure 11). Richness was highly variable with relatively large standard deviations for both Observation and ID_Survey: Distance class levels. **The predicted reduction in crustacean richness was the same in terms of relative change regardless of Distance from farm: over the range of EMB applied (0 to 10 kg) prior to sampling, the mean richness at the average site and under average conditions is expected to decline by 63% with 95% confidence that this would be between 21 and 82%.**

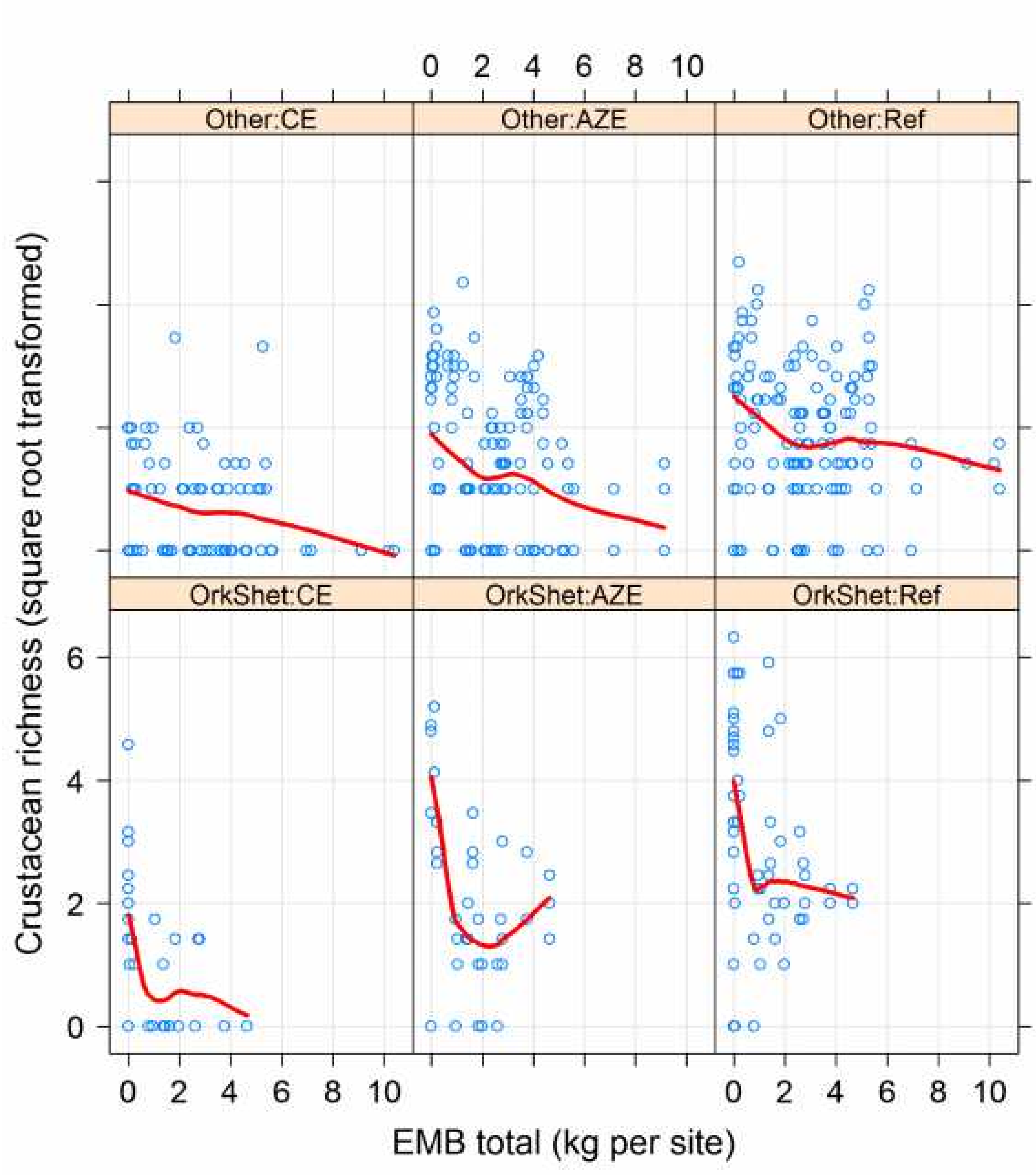

Figure 11 – Crustacean richness (number of species) as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station), the total EMB applied per Site (see main text for details) and Region (OrkShet and Other, see methods). A loess smoothing curve (red line) is fitted to each scatter plot to aid interpretation.

Table 10 – Crustacean richness (based on total EMB applied prior to sampling).

Model: Richness~ Distance Class + EMB + Depth + LT63 + Current speed + (1|Obsid)+(1+Distance class|Survey): log-link function. See Table 1 for key and abbreviations. The ':' in Group indicates a covariance/standard deviation

Random effects:

Group	Var	Std deviation
ObsID	0.076	0.276
Survey, Int	1.16	1.08
Survey, AZE	0.804	0.897
Survey, Ref	0.781	0.884
Int:AZE	-0.193	-0.2
Int:Ref	-0.731	-0.767
AZE:Ref	0.44	0.555

Fixed effects

Source	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	0.0391	0.251	0.156	0.876
AZE	0.957	0.207	4.62	3.88e-06
Ref	1.74	0.185	9.41	5.11e-21
EMB	-0.331	0.111	-2.98	0.00292
Depth	-0.27	0.158	-1.71	0.0868
<63 µm	-0.179	0.0568	-3.16	0.00159
Current	0.742	0.165	4.5	6.75e-06

Credible intervals

Percentile	Intercept	AZE	Ref	EMB	Depth	<63 µm	Current
2.5%	-0.253	0.707	1.28	-0.539	-0.75	-0.342	0.366
50%	0.147	0.979	1.51	-0.313	-0.421	-0.222	0.722
97.5%	0.565	1.22	1.74	-0.0759	-0.103	-0.095	1.06

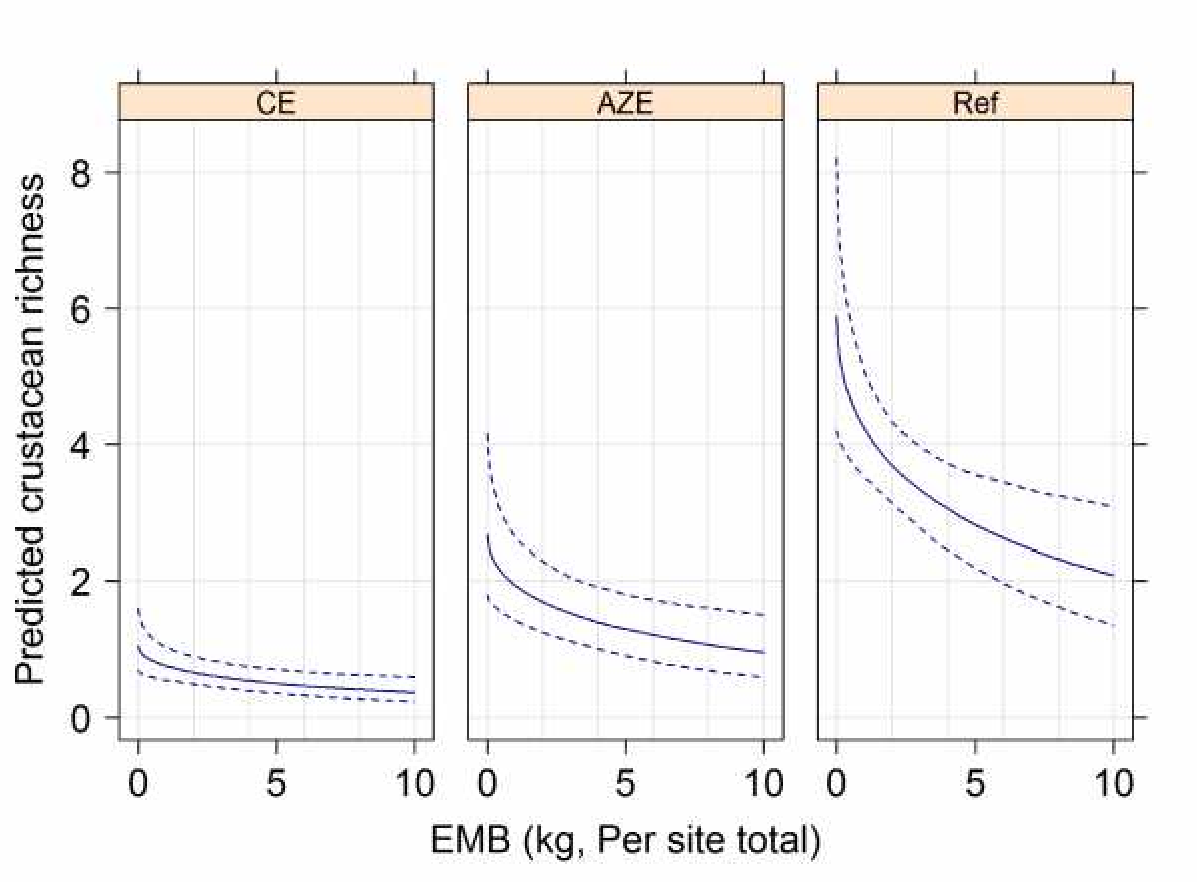

Figure 12 – Predicted crustacean richness (number of species) as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station) and the total EMB applied per Site (PST, see main text for details).

3.1.4.4 Crustacean abundance per Site EMB total

Patterns in crustacean abundance, as a function of total EMB applied, followed that determined for the 'per production cycle' analysis: the raw data indicated a predominantly negative relationship between abundance and EMB treatment, at all Distance classes and between Regions (Table 7, Figure 13). Abundance was highly variable with values ranging between zero and 899 and this was reflected in the relatively large standard deviations for both Observation and Survey: Distance Class levels in the modelled data (Random effects, Table 11). Modelled patterns of abundance-reduction were the same regardless of Distance Class. **The predicted reduction in crustacean abundance, over 0 to 10 kg EMB application prior to sampling, at an average site and under average conditions, was by a factor 96% with 95% confidence that this would be between 74 and ~100% (Table 11, Table 23)**

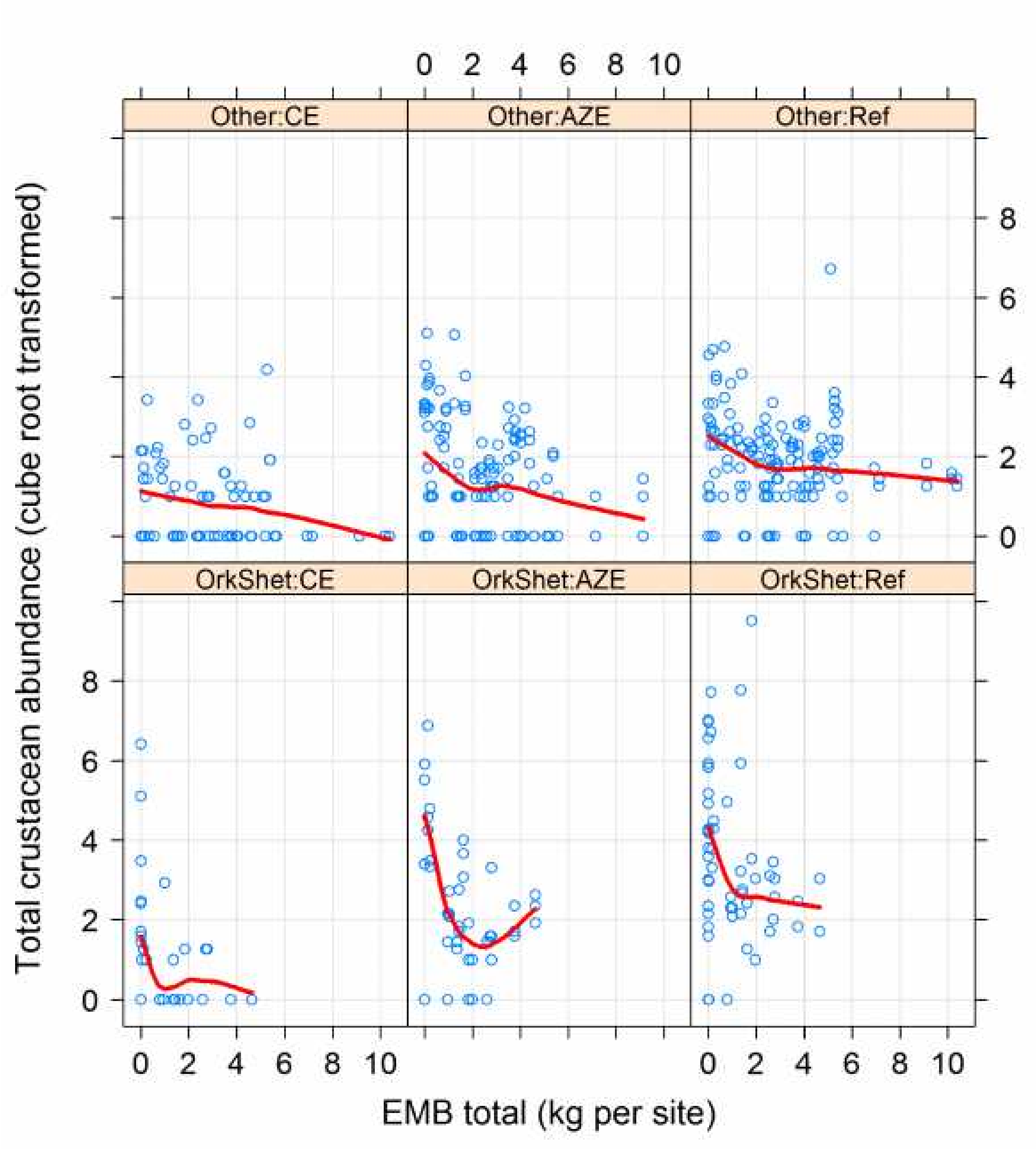

Figure 13 –Total crustacean abundance as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station), the total EMB applied per Site and Region (OrkShet and Other, see main text for details). A loess smoothing curve (red line) is fitted to each scatter plot to aid interpretation.

Table 11 - Crustacean abundance (cube root transformed and based on total EMB applied prior to sampling).

Model: Abundance~ Distance Class + EMB + Depth + LT63 + Current speed + (1+Distance class|Survey): Identity link function. See Table 1 for key and abbreviations.

Random effects.

	v.cor	sd/correlation
ID_Survey, Int	0.987	0.994
ID_Survey, AZE	1.39	1.18
ID_Survey, Ref	1.34	1.16
Int:AZE	-0.629	-0.536
Int:Ref	-0.805	-0.699
AZE:Ref	1.15	0.838
Residual	0.514	0.717

Fixed effects

	Estimate	Std. Error	t value
Intercept	1.58	0.204	7.77
AZE	0.759	0.161	4.70
Ref	1.26	0.15	8.40
EMB	-0.489	0.115	-4.25
Depth	-0.416	0.186	-2.23
<63 µm	-0.239	0.0669	-3.57
Current	0.832	0.173	4.80

Credible intervals.

Percentile	Intercept	AZE	Ref	EMB	Depth	<63 µm	Current
2.5%	1.20	0.441	0.96	-0.712	-0.79	-0.366	0.486
50%	1.58	0.752	1.25	-0.488	-0.422	-0.239	0.838
97.5%	1.98	1.06	1.56	-0.264	-0.0535	-0.111	1.18

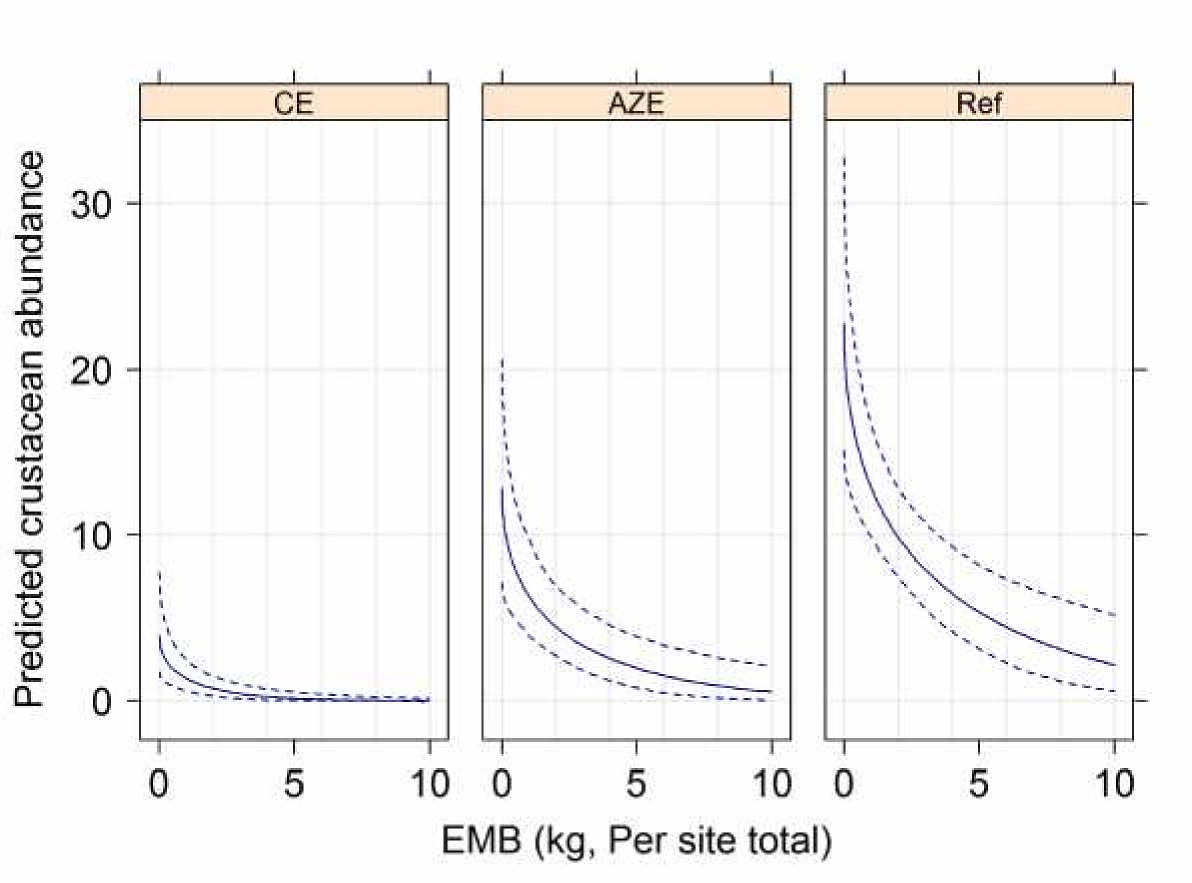

Figure 14 –Predicted crustacean abundance as a function of cage proximity (CE – cage edge, AZE – allowable zone of effects and Ref – reference station) and the total EMB applied per Site (PST).

3.1.5 Analysis based on Reference-sites only

Modelling based on the Reference-sites only followed the protocols for the analyses that included all sites: analyses were based on crustacean richness and abundance, per production cycle and per-site totals. Whilst the results presented here include bootstrapped confidence intervals these were very close to the Bayesian credible intervals and, consequently, are not separately discussed or interpreted. Interpretation of the fixed effects follows that of the previous analysis (all Distance Classes, see 8.6). For Keys to the results tables, see Table 1. The basic interpretation of the two modelling approaches (including all Distance Classes and focused on the Reference stations only) is very similar. Some models differed in the inclusion/exclusion of a predictor but there were no interpretational differences in relation to EMB. A summary of the differences between the models (all Distance Classes v Ref-only), with respect to EMB, is given in 8.7. These results (below) include the complete analysis but interpretation is focussed on the EMB, interpretation of other factors (e.g. random effects and covariables) follows that given in Section 3.1.4.

3.1.5.1 Crustacean richness per production cycle – Reference sites only

The fixed effects remaining in the model were EMB, current speed, depth and proportion of fine material (Table 12). Unlike the analysis based on all Distance classes, grab size was not a factor in the derived optimal model. In relation to EMB use, this model indicates that the mean expected reduction in crustacean richness, at the average site and under average conditions, following a treatment of 3 kg EMB, would be 49% with 95% confidence that this decline would be between 18% and 69%.

Table 12 - Crustacean richness v. EMB use per production cycle.

Model: Richness ~ EMBSR + Current+Depth+LT63 + (1 | Obsid) + (1 | SiteID/ID_Survey)

Random effects

Source	variance	std dev
Obsid	0.0865	0.294
ID_Survey:SiteID	0.3780	0.615
SiteID	0.2280	0.478

Fixed effects

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	1.45	0.112	12.9	3.7e-38
EMB	-0.394	0.145	-2.72	0.0066
Current	0.536	0.149	3.60	0.000321
Depth	-0.302	0.114	-2.65	0.00815
<63µm	-0.224	0.0489	-4.59	4.38e-06

Credible intervals

	Intercept	EMB	Current	Depth	<63µm
2.5%	1.23	-0.674	0.239	-0.523	-0.319
50%	1.45	-0.392	0.534	-0.303	-0.225
97.5%	1.67	-0.106	0.827	-0.0764	-0.132

Bootstrap confidence intervals.

	Intercept	EMB	Current	Depth	<63 µm
2.5%	1.2	-0.659	0.253	-0.551	-0.319
50%	1.42	-0.366	0.554	-0.303	-0.231
97.5%	1.64	-0.0705	0.846	-0.0713	-0.121

3.1.5.2 Crustacean Abundance per production cycle – Reference sites only

The fixed effects remaining in the model were EMB, current speed, depth, proportion of fine material and grab size (Area) (Table 13). In relation to EMB use, this model indicates that the mean expected reduction in crustacean abundance, per production cycle, at the average site and under average conditions, following a 3 kg EMB treatment would be 58% with 95% confidence that this decline would be between 12% and 84%.

Table 13 - Crustacean abundance v. EMB use per production cycle

Model: Crustacean abundance ~ EMBSR + Current + Depth + LT63 + Area +(1 | SiteID/ID_Survey).

Source	sd	variance
ID_Survey:SiteID	0.605	0.778
SiteID	0.299	0.546
Residual	0.576	0.759

Fixed effects

	Estimate	Std. Error	t value
Intercept	2.83	0.191	14.8
EMB	-0.404	0.177	-2.28
Current	0.770	0.179	4.31
Depth	-0.488	0.148	-3.31
<63µm	-0.246	0.0672	-3.65
Area=0.135	-0.59	0.201	-2.93
Area=0.2	-0.852	0.242	-3.52

Credible intervals

	Intercept	EMB	Current	Depth	<63µm	Area=0.135	Area=0.2
2.5%	2.46	-0.744	0.428	-0.768	-0.378	-0.988	-1.34
50%	2.83	-0.409	0.772	-0.49	-0.245	-0.590	-0.853
97.5%	3.19	-0.0672	1.13	-0.198	-0.115	-0.178	-0.386

Bootstrap confidence intervals

	Intercept	EMB	Current	Depth	<63 µm	Area=0.135	Area=0.2
2.5%	2.45	-0.734	0.422	-0.797	-0.377	-0.965	-1.32
50%	2.83	-0.404	0.770	-0.488	-0.246	-0.590	-0.852
97.5	3.19	-0.0359	1.12	-0.231	-0.107	-0.184	-0.406

3.1.5.3 Crustacean richness per site total – Reference sites only

The fixed effects remaining in the model were EMB, current speed and proportion of fine material. In relation to EMB use, this model indicates that the mean expected reduction in crustacean abundance, at end of the productions cycles, at the average site and under average conditions, following 10 kg EMB treatment (across all production cycles) would be 69% with 95% confidence that the decline was between 41% and 83%.

Table 14 - Crustacean richness v. EMB use per site total.

Model: Richness ~ EMB + Current+ LT63 + (1 | Obsid) + (1 | SiteID)

Random effects

Source	Variance	std dev
Obsid	0.104	0.323
SiteID	0.383	0.619

Fixed effects

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	1.87	0.155	12.1	7.4e-34
EMB	-0.367	0.101	-3.62	0.000293
Current	0.772	0.161	4.78	1.72e-06
LT63	-0.223	0.064	-3.48	0.000497

Credible Intervals

	Intercept	EMB	Current	<63µm
2.5%	1.58	-0.568	0.457	-0.341
50%	1.87	-0.368	0.771	-0.222
97.5%	2.17	-0.169	1.08	-0.0993

Bootstrap confidence intervals

	Intercept	EMB	Current	<63 µm
2.5%	1.55	-0.550	0.464	-0.349
50%	1.87	-0.367	0.772	-0.223
97.5%	2.17	-0.169	1.08	-0.111

3.1.5.4 Crustacean Abundance per site total – Reference sites only

In relation to EMB use, this model indicates that crustacea, at the average site and under average conditions, would be almost entirely eliminated following treatment (over preceding years) with a total of 10 kg EMB. There is 95% certainty that this reduction would be at least 81%. Unlike the model that includes Distance Class as a predictor (3.1.4.4), this model includes Maximum biomass as a positive influence (when considered independently of other factors) on crustacean abundances (coefficients are positive, see Table 15, Credible Intervals).

Table 15 – Crustacean abundance v EMB use per site total

Model: Crustacean abundance ~ EMB +Current +Depth + LT63 + MaxBio + (1 | SiteID)

Random effects

Source	sd	variance
SiteID	0.489	0.699
Residual	0.709	0.842

Fixed effects,

	Estimate	Std. Error	t value
Intercept	3.31	0.247	13.4
EMB	-0.779	0.171	-4.55
Current	0.758	0.214	3.55
Depth	-0.531	0.229	-2.32
<63µm	-0.375	0.0861	-4.36
MaxBio	0.646	0.246	2.63

Credible intervals

	Intercept	EMB	Current	Depth	<63µm	MaxBio
2.5%	2.82	-1.12	0.329	-0.991	-0.546	0.158
50%	3.31	-0.78	0.761	-0.533	-0.374	0.645
97.5%	3.81	-0.448	1.19	-0.0959	-0.203	1.11

Bootstrap confidence intervals

	Intercept	EMB	Current	Depth	<63 µm	MaxBio
2.5%	2.79	-1.11	0.341	-0.982	-0.531	0.159
50%	3.31	-0.779	0.758	-0.531	-0.375	0.646
97.5%	3.79	-0.434	1.17	-0.046	-0.198	1.12

3.2 Locating sites with pre- and post-EMB use

EMB use per production cycle, per Site, is shown in Figure 15. These data indicate that most sites used EMB and that most of these have used EMB since monthly recording started. The initiation of use of EMB is apparent in several sites, e.g. FFMC47, LINB1 and TAI1 among others indicating that a pre-and post-EMB-use analysis has potential. However, contemporaneous macrobenthic data for each production cycle is often absent (Figure 16) and, if present, EMB recorded was sometimes used subsequent to the grab-sample being taken (e.g. examine PC 3, Site ERI3 and compare between Figure 15 and Figure 16 – the apparently lower level of EMB use in PC3 is due to the second treatment being applied following the grab-sampling). Sites that could support a pre-and post-analysis include COLEN1, FFMC30, FFMC31 (Figure 17, Figure 18). Taking FFMC30 and crustacean richness as an example, EMB use was initiated at PC3 and there were no crustacea found (at any Distance) during the macrobenthic survey conducted during that PC. Crustacean richness was greater, on average, at all distances except cage-edge, following the initiation of EMB use (PC3 and 4), but appeared to decline again in PC5 (Figure 17). Whilst there were no obvious pattern in the data presented here, these data are so sparse that a robust analysis is not supported.

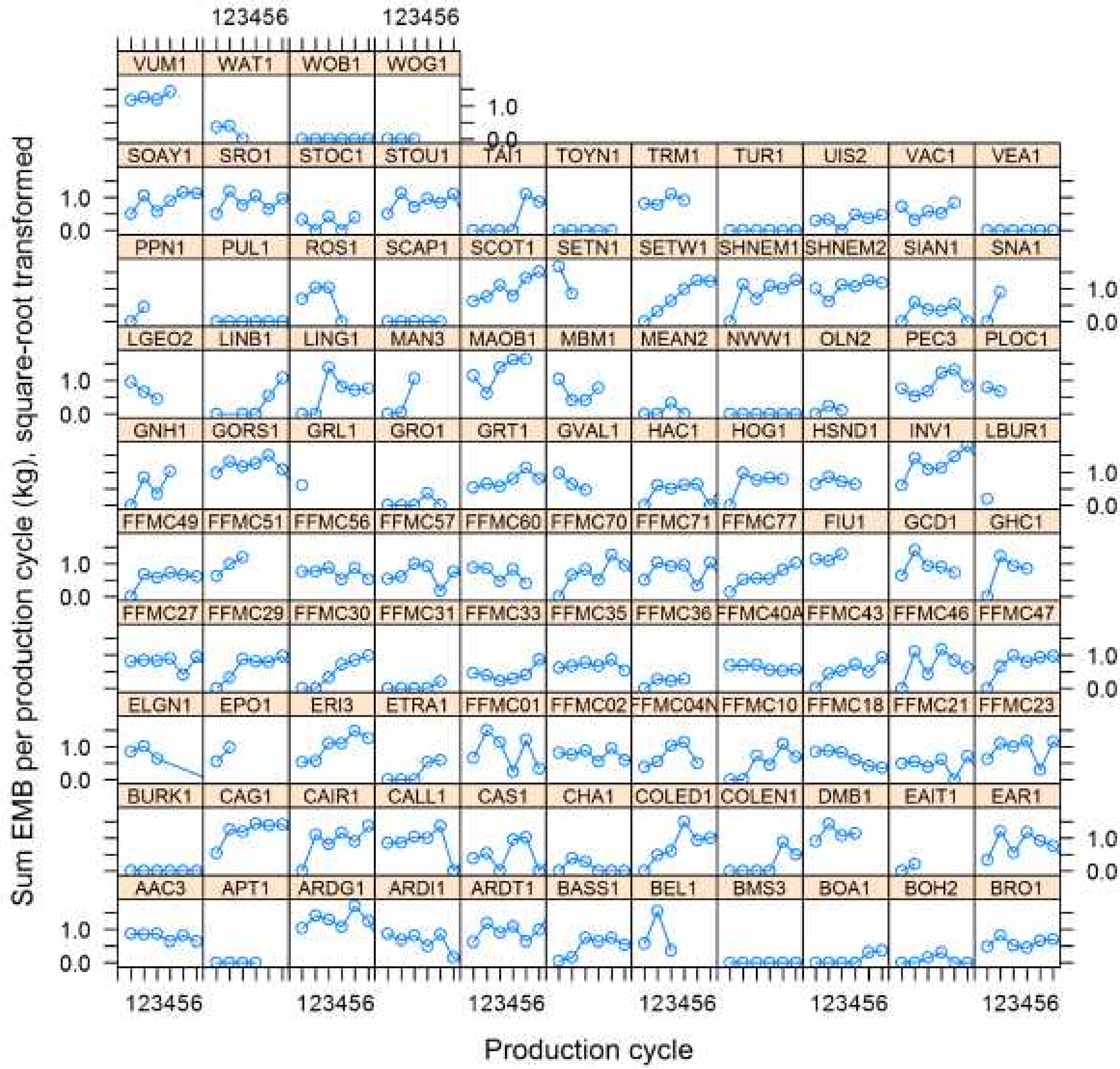

Figure 15 – Total EMB used, per Production Cycle and Site (n=103), as derived from the monthly database. A comparison with Figure 16 indicates that of 677 individual production cycles only 259 were matched with corresponding macrobenthic and environmental data.

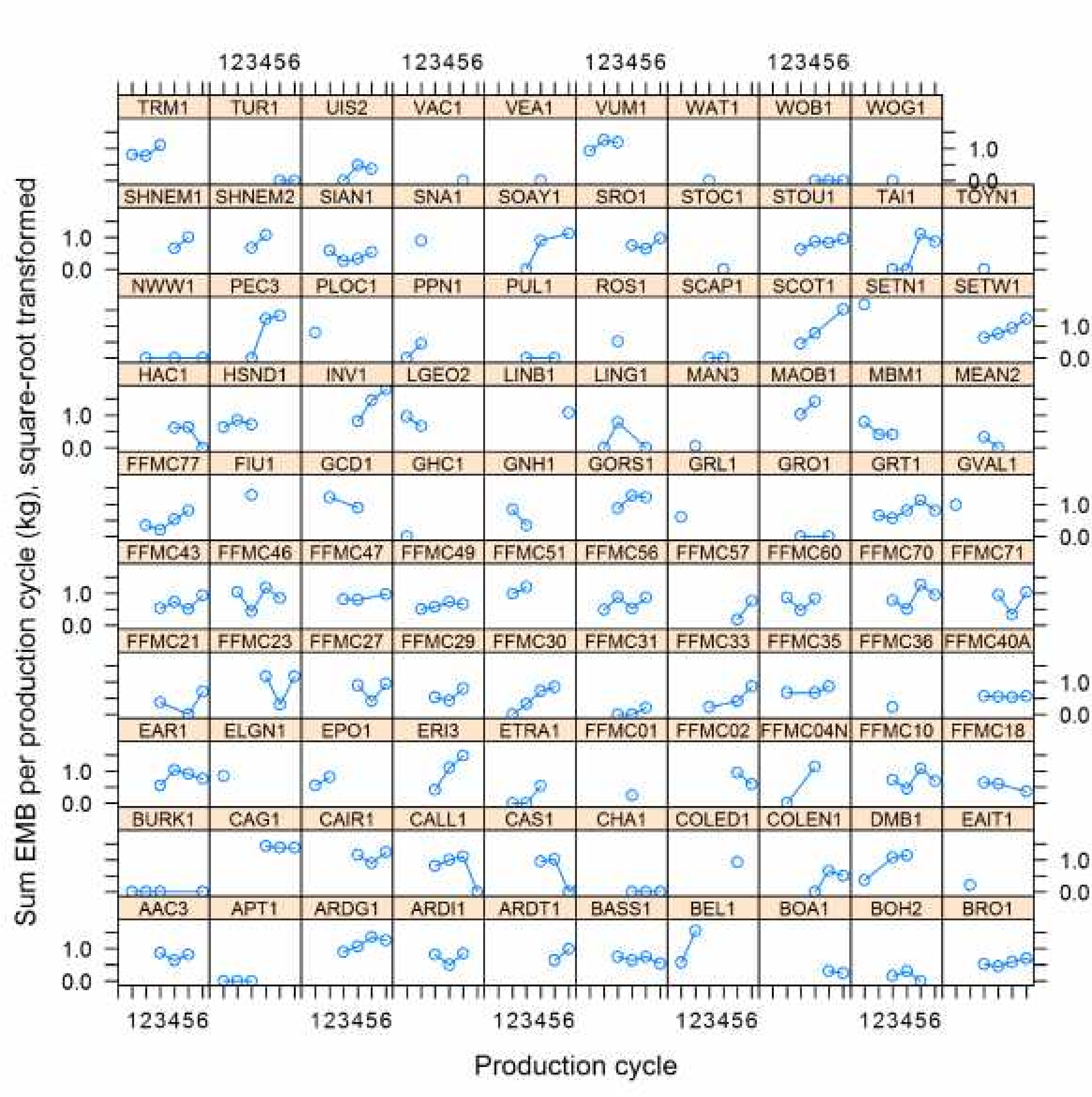

Figure 16 – Total EMB used, per Production Cycle and Site (n=99) plotted where there is contemporaneous macrobenthic and environmental data. These are the Site /Production cycle combinations that formed the basis for the dataset used in model development.

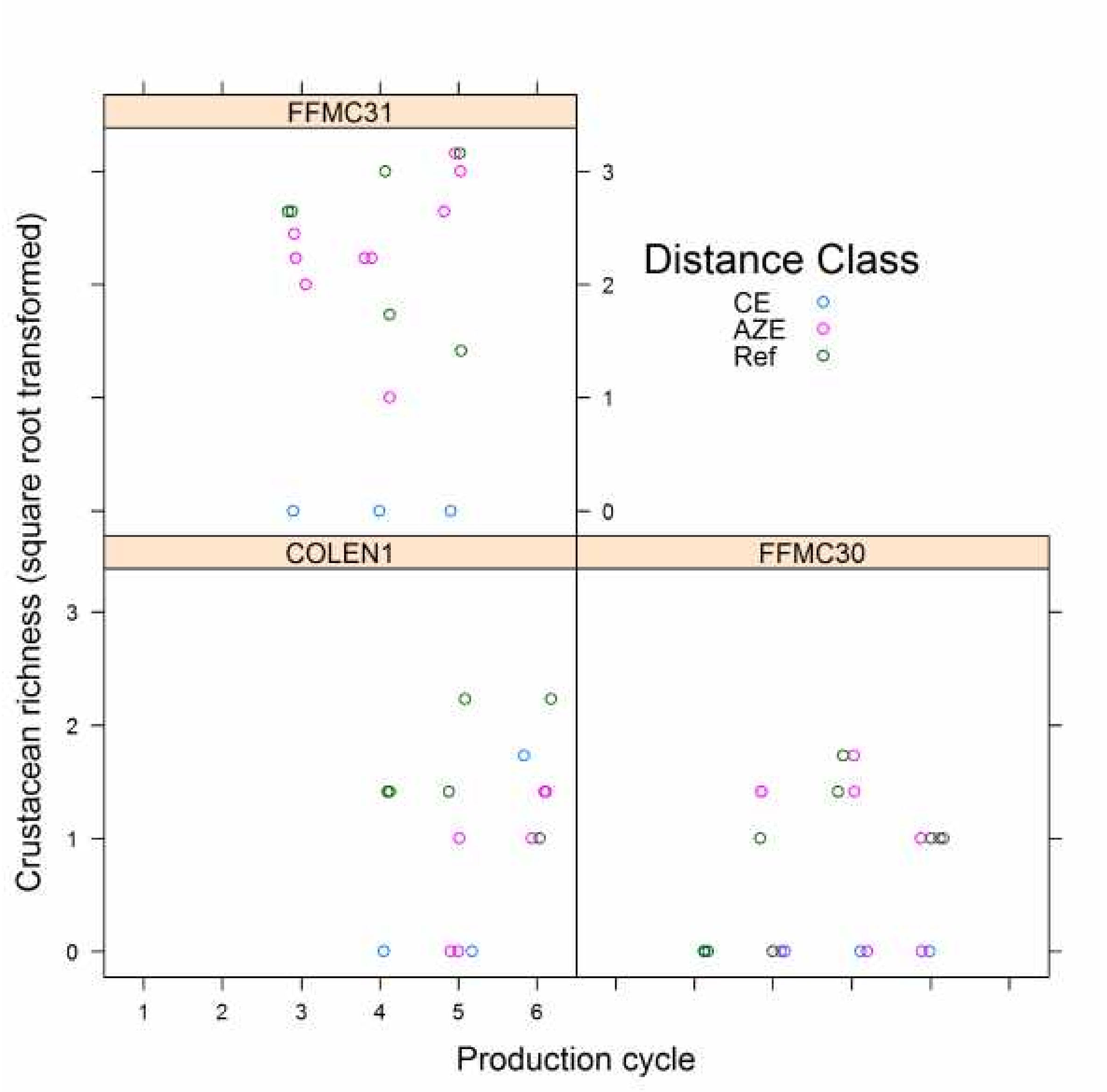

Figure 17 – Crustacean richness versus pre- and post EMB application (by Production cycle). The initiation of EMB started at production cycles 4, 3, 5 for sites COLEN1, FFMC30 and FFMC31 respectively. See main text for further details.

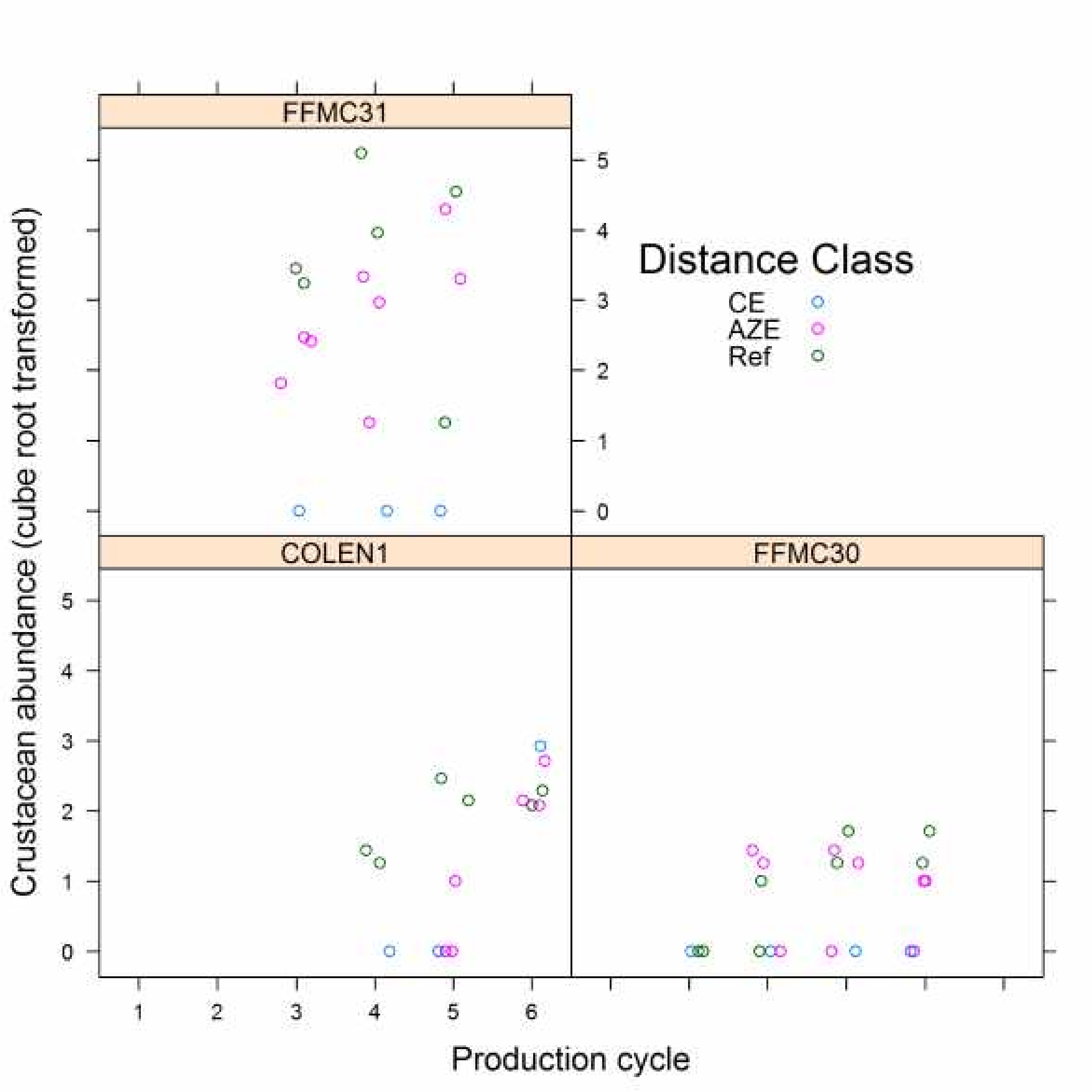

Figure 18 - Crustacean abundance versus pre- and post EMB application (by Production cycle). The initiation of EMB started at production cycles 4, 3, 5 for sites COLEN1, FFMC30 and FFMC31 respectively.

4 Discussion

The data analysis revealed some important macrobenthic patterns in relation to the use of EMB around fish-farms in Scotland. There was strong evidence that both crustacean richness and abundance were lower in sites that had been treated with EMB, that this effect extended beyond the farm boundary to the Reference stations and was not attributable to site-specific differences and/or covariables including depth, sediment texture, current exposure or sampling methodology. Changes associated with EMB use, at Reference stations, were confirmed by Ref-station-only focussed analysis; the data interpretation (e.g. in terms of confidence intervals) are based on the initial analysis which included all Distance-classes (see 2.1).

This research has highlighted several issues with regards to the existing regulatory regime for EMB and the continued development of sustainable salmon aquaculture in Scotland.

This Discussion is split into three sections concerning residues, site characterisation and the association between EMB use and the macrobenthos. This discussion leads into the Recommendations section which covers suggestions with respect to further research and the databases.

4.1 Residues

EMB residues were mostly below detection limits (which varied between 0.1 and 1 µg kg^{-1}). The proportion of observations below detection limits (BDL) was greater at 25 m distance compared with the cage edge. The residue data was highly skewed and characterised by extreme outliers. The data did not suggest that there was a meaningful relationship between current speed and residue concentration, at least in regards to those observations where a residue was detected. However, the SEPA data could not support an analysis of the relationship between residue concentration and macrobenthic response because of the scarcity of spatial and temporal overlap between the sampling events (see 5.1). EMB concentrations of 0.018 µg/l, between five and fifty times lower than the detection limit (per kg of sediment) as indicated in the SEPA database, have been shown to have a serious effect on pelagic copepods (Fiori, 2012, not peer-reivewed), over an 8 day exposure period. It is entirely plausible, therefore, that chronic exposure to EMB, even at currently undetectable concentrations, may have serious consequences for crustacean communities (see 4.3).

4.2 Site characteristics and sampling methodology.

Scottish fish-farms were hosted by a broad spectrum of receiving environments. These environments ranged from <10 m to >100 m in depth and with highly diverse underlying sediments.

Many environmental parameters were correlated: shallow sites tended to be located in high current flows, were associated with coarse sediments and these sites were more prevalent in Orkney and Shetland. However, most regions contained at least some sites that were characterised by high current flow and coarse sediments. There were Region-based differences in grab-sampling methodology. Overall, the most common grab-type used (in the data analysed) was the $0.045m^2$ grab. However, in Shetland grab sampling was done using the $0.02 m^2$ grab almost exclusively. In other regions mixtures of grab-types were used. Approximately 80% of the analysed records indicated EMB usage and, whilst this varied between regions, all regions (with one exception) hosted farms that had used EMB. The lack of sampling gear standardisation, particularly where there are regional biases, introduced challenges to the modelling of the data (see 5.2).

In general terms, crustacea were more abundant in Orkney and, to some extent, Shetland compared with the other regions (linked with the coarser sediments and greater current exposure that typified these regions). Overall Shannon diversity was positively correlated with species richness (as to be expected) and showed a unimodal relationship with total abundance. These data support the Pearson-Rosenburg paradigm (Pearson and Rosenberg, 1978) of increasing diversity associated with increasing abundance (presumably in areas of slight-to-moderate organic enrichment) and a subsequent highly steep decline in richness where abundances were very high (likely to be of opportunistic species indicative of highly degraded sediments). The relationship between crustacean richness and total abundance was less apparent suggesting a different relationship between organic enrichment and crustacea and greater crustacean sensitivity to organic enrichment (Pearson and Rosenberg, 1978). Simple visualisation/ tabulation of the raw data indicated a negative relationship between both crustacean diversity and abundance with increasing EMB use. However, the differing site characteristics, grab-type and prevalence of EMB usage in different regions all pose challenges to understanding broad-scale effects of EMB using simple visualisation/ tabulation. These challenges were met using mixed-models (as below).

4.3 EMB-associated macrobenthic change

These results indicate that EMB was associated with a major reduction in both the richness and abundance of crustacea and that this effect extended beyond the cage-edge and to the Reference stations.

The analysis of the data from the Reference stations, in isolation, identified similar trends, in terms of the relationship between EMB and crustacean community metrics, as found at in the analysis of all Distance Classes (CE, AZE and Ref, see 3.1.4). The models generally included the same predictor variables (with current speed and fine material being important in all cases) with some exceptions (discussed below, see 8.7). The Reference-only analysis did not change the broad interpretation of the results, with respect to changes occurring at the Reference stations in relation to EMB application.

EMB is not specifically toxic to salmon-lice and there is no reason to expect other crustacea to be any less sensitive. EMB is known to be highly particle affinitive (Telfer et al., 2006) and is excreted from fish over a considerable period following treatment. There is no doubt that EMB will accumulate in sediments around fish-farms following treatment (as indicated by residue surveys) and it is inevitable that this will cause some degree of toxicity to non-target crustacea. The reduction in crustacean richness and abundance observed here did not apply to other community metrics except total abundance, where the impact was much less, lending support to the interpretation of the reductions in crustacea being as a consequence of direct toxicity. This finding is in line with the conclusions of Langford et al. (2014) that any organism undergoing a moult cycle will be at risk from EMB exposure. Whilst Telfer et al (2006) found no effects on the macrobenthos (crustacea were not separately analysed), this followed a 33 g EMB treatment compared with the mean treatment rate of 208 g observed in the current analysis. In addition, even with a small treatment Telfer et al (2006) were able to detect, in mussels located 100 m from the farm, EMB residues lending support to the potential wide-spread distribution of EMB around farms.

The initial models included all available covariables and the model rationalisation approach sequentially removed terms that did not make a meaningful contribution to the model. The aim of such an approach is to derive as simple and as useful a model as possible. However, caution should always be applied to the interpretation of 'observational' data (as here) because of the potential of confounding factors (unmeasured covariables) not included in the model (see below). Following discussion with SEPA (16 Dec 2014) changes were made to the preliminary modelling approach by the inclusion in the initial model of grabbing protocol, region and additional physical site characteristics. Where Area (i.e. grabbing protocol) and /or Region are absent in the final model it is likely to be because their effects were best accounted for by their Site or Region-specific associations (e.g. particle size, depth and current speed) and there was sufficient cross over (e.g. grabs of different sizes used in different regions and in areas of varied ground-type) to properly evaluate model parameters. There exists a possibility that treated sites (extending from the Cage-Edge to Reference stations) naturally, for some reason, host fewer crustacea and that this is not related to region, site exposure /sediment texture (or any of the other covariables tested). This relatively remote possibility could be tested by examining sites where there is both pre-and post-EMB usage.

However, at the current stage of data extraction, there is insufficient data to conduct a robust before-EMB v. after-EMB analysis. Given that EMB is toxic to crustacea, is found around farms using it and the relationship between EMB use and crustacean response reported here, we believe that the most likely explanation for the association between EMB treatment and crustacea is because of a direct toxic effect.

The data analysed were highly variable in terms of crustacean diversity and abundance. This type of variability is typical of benthic communities and, in the current case, is likely to be exacerbated by the range of sampling strategies (e.g. grab size) adopted by different farming operations and unknown covariables such as the degree of organic enrichment/oxygen depletion in the sediment (see 5.1). Information concerning the degree of organic enrichments would be extremely useful, particularly in explaining the variability of crustacea in cage-edge sediments (see 5.1). In future analyses it may be possible to use the abundance of opportunistic species such as *Capitella sp* as a proxy for organic enrichment (see 5.1).

Crustacean diversity associated with EMB use per production cycle was highly variable, with an expected reduction of 40% (ranging between 4 and 63%) at the maximum usage rate of 3 kg per production cycle. This pattern was reflected in predicted crustacean abundances; at an average Site and production cycle abundances at Reference stations were predicted to decline by 79%. The relationship with EMB was non -linear, with the rate of change decreasing as the amount of EMB increased (over the range 0 – 3 kg per production cycle). These data indicate that even at low-dose rates, EMB will cause a reduction in crustacean richness and abundance: there was no evidence of a threshold beneath which change did not occur.

The PPC analysis presented here is based on macrobenthic data that were gathered at any time after EMB use (within an individual PC, an approach agreed with SEPA). EMB dosage rates are dependent on fish-biomass (larger fish require a larger dose) and, consequently, those EMB treatments occurring late in the production cycle are larger but may occur only a short-time before the grab sampling is undertaken. In these circumstances the EMB is unlikely to have had time to be excreted and affect crustacean communities within the associated Production Cycle (see 5.1). The PPC approach adopted here may, therefore, underestimate the impact of EMB used within any given PC and/or add to the apparent high degree of variability observed. This disadvantage was overcome by summing the EMB, per Site (PST analysis).

The patterns of reduction in crustacean richness and abundance, per production cycle, are reflected when crustacean metrics are compared with the total EMB used, per site. Data analysis in this way allows an assessment of the association between long-term EMB use and crustacean metrics. These data suggest that continued use of EMB, over several production cycles, is associated with an ongoing decrease in both crustacean richness and abundance: the models predict that at an average Site, treated with 10 kg of EMB over an extended period (up to 12 years), and under average conditions (current speed, sediment type etc) a reduction in crustacean richness and abundance by 63 – 96% respectively could be expected. These effects are more severe than those observed on the per-production cycle basis, suggesting that crustacean recovery is not complete between production cycles where EMB is used. Long-term chronic effects of EMB have not been properly evaluated but EMB is known to disrupt reproduction in *H. americanus* at very low concentrations (Waddy et al., 2010). The apparent sensitivity of non-target crustacea and the fact that major changes were found at reference stations (which are typically several hundred metres from the farm edge, as supported by the Reference-only analysis) suggests that repeated, large-scale EMB use may adversely affect crustacean populations at the scale of an entire sea-loch. It also indicates that the current recommended distance-to-farm of 'Reference stations' should be re-evaluated.

Crustacea are a core, but sensitive, part of the benthic ecosystem forming part of the food chain, having a role in nutrient regeneration and sediment bioturbation (Diaz and Rosenberg, 1995). There are also important crustacean fisheries, including *Nephrops norvegicus,* lobster and various crab species which extend around the Scottish coast including where fish-farms are located. Fishery-species have been shown to exhibit sensitivity (see Introduction) but much of the current research is in relation to short-term, high dose rates using adults as the test group. At this stage it is not clear whether particular species are susceptible to EMB or whether the reductions occurs across all species, for example, by eliminating those that are already rare at the site and generally causing an abundance-reduction across more common species (see 5.1). There was no evidence that the relationship between crustacean metrics and EMB use different between Distance Classes. The crustacean communities at the Reference, non-EMB sites, are likely to be relatively unmodified and will include sensitive species. A plausible explanation for the lack of an interaction term (Distance Class * EMB) is that sensitive species decline, in the same way (i.e. same regression slope), as those more resistant communities located nearer to the farm when exposed to EMB.

The manner by which non-target crustacea absorb EMB is not known; detritivores living in the benthos may be at greater risk of exposure but highly sensitive planktonic stages (Fiori, 2012) or newly settled post-larvae might also gain exposure from the water column. Crustacea exhibit two distinct reproductive strategies: peracarids (amphipods, isopods, tanaids, cumaceans, mysids) typically brood their young, whilst decapods usually have a dispersive larval stage. Local extinctions are more likely to occur in those species with non-dispersive reproductive strategies i.e. that those that do not recruit annually from the water column; Fiori (2012) noted that egg production in *Calanus* sp was prevented at an EMB exposure of 18 ng l^{-1} which is >5000 times lower than the concentration required to cause toxic effects in shrimp (Mayor et al., 2008). Disruption to reproduction caused by chronic, low-level, EMB exposure is a plausible mechanism behind the decline of non-crustacean populations. The 115 day half-life of EMB means that it is likely to be dispersed very widely around fish-farms before fully degrading, particularly where sediment resuspension occurs (e.g. during unpredictable storm events or as part of unusual tidal conditions). The wide-scale dispersion of detritus-associated EMB may account for the association being measured at Reference stations and indicates that the location of current Reference-stations needs further consideration.

EMB residues were mostly not detected at 25 m distance from the cage-edge and, by extrapolation, are highly unlikely to be detectable at the Reference stations (using current techniques). The results presented in this report indicate that toxic effects occur at levels much lower than those that are currently detectable. The evidence suggests that benthic crustacea may not be adequately protected by the current regulation of EMB use by Scottish salmon farms.

5 Recommendations

5.1 Further research

Our results indicate that, even allowing for regional differences in the physical properties of the receiving environment, the use of EMB is associated with substantial, wide-scale reductions in both the richness and abundance of non-target crustacea. **Given the findings in this report we believe there is an urgent requirement to:**

1. Use the existing databases and multivariate techniques to link change in individual crustacean species and groups (e.g. based on reproductive strategy) with EMB use.
2. Improve the PPC analysis by determining the total EMB used, prior to each grab-survey (i.e. including the EMB used in previous PCs) and use this as a predictor. Examine model-residual patterns and determine how change occurs as a function of application rate and frequency. Determine (if possible) crustacean recovery following EMB use.
3. Consider the likely ecosystem consequences of large-scale reductions in crustacean richness and abundance (at the scale of sea-lochs).

4. Consider and make recommendations regards manipulative studies to test the effects of EMB, at realistic doses, on benthic crustacean communities. This should be done in conjunction with ongoing related research (e.g. Predicting benthic chemistry around marine fish farms, NERC/BBSRC Grant Number BB/M025861/1).
5. Consider whether next-generation-sequencing (NGS) approaches to monitoring crustacean populations (Pawlowski et al., 2014) around treated and untreated sites could offer an alternative, rapid and high-resolution method of mapping crustacean diversity around fish-farms.
6. Extend current sampling to better understand the spatial scale of the impact of EMB on non-target crustacea.
7. If new methods of analysis are able to quantify much lower EMB residues then a comparison between macrobenthic metrics and EMB residue should be undertaken.

5.2 Statutory monitoring methodology and maintenance of the database

The databases provided by SEPA were large and complex reflecting, in part, their evolution alongside changing monitoring methodology and the development of the regulatory framework. The FFDB and SMDB were not designed to be complementary which made cross-referencing difficult. Many of the recommendations (below) relate to the methodical collection, coding and recording of data and are from the perspective of optimising the type of data extraction and analysis as reported here. Our recommendations in relation to the databases are:

1. There should be increased integration of residue sampling with macrobenthic sampling in order to better link the relationship between farm-activities and their benthic impact.
2. There should be enhanced consistency in relation to the environmental variables collected alongside the macrobenthic samples.
    a. Grabbing methodology should be standardised across the industry and grab sampling should occur at a standardised time following EMB treatment
    b. Organic concentration is currently assessed by determining loss-on-ignition or carbon content. These should be rationalised (perhaps to the cheaper method).
    c. The full complement of particles sizes (from granulometric analysis) should be made available and/or a more comprehensive set of summary data (e.g. the median particle size) should be recorded.
    d. Current speed data should be standardised (e.g. to device type, depth and location).
3. The recording of data should be rationalised:

a. The field 'station' in the SMDB has ~750 categories when there are only approximately 10 actual station types (e.g. CE, AZE+/-10m, Ref and the pre-2006 station distances). This field should be rationalised, and the database made more comprehensive and easier to complete by adding fields to invite further information (e.g. Station=AZE, Distance =70 m, Bearing= 180°).
b. In the monthly recording database, the field 'Production Cycle' should be introduced in order to distinguish the end and beginning of adjacent production cycles.

6 Teflubenzuron

The dataset relating to teflubenzuron (TBZ) was much smaller than that relating to EMB. Robust model development (as per SARF098 proposal), of the kind used with the EMB data, requires large datasets. In general terms each parameter being estimated requires 20 – 30 independent observations. The TBZ data set was of insufficient size to support linear modelling of the type developed for EMB.

The data were extracted from the database in the same way as the EMB data (so the residues here reflect the maximum of three replicates per sample taken). The current speed data was matched in the same way as it was for the EMB data.

The total number of sites that were treated with TBZ was 16 with 38 residue measurements being taken across them. Most of the measurements were taken at 0 and 100 m (17 each) but there were also 2 measurements at 25 and 150 m. Unlike EMB, residue measurements for TBZ were not dominated by 'zeros' with only 5 being classified as below detection limits. Of the 38 measurements taken, 24 passed EQS (18 at CE and 6 at 100 m) and 14 failed (1 at CE and 13 at 100 m). This potentially reflects the much more stringent EQS at the 100 m stations. TBZ residues varied considerably, with the maximum recorded being >15,000 µg kg^{-1} at the cage edge which was more than 5 times the maximum recorded at 100 m (Table 16). The difference in residue concentration between 0 and 100 m was consistent and is shown in Figure 19.

Table 16 - Summary of TBZ measurements per Distance. Min, Max – minimum and maximum respectively (in µg kg^{-1}), IQR – interquartile range.

Distance (m)	Min	Max	Median	IQR
0	173	15030	1306	3806
100	0	2792	159	433

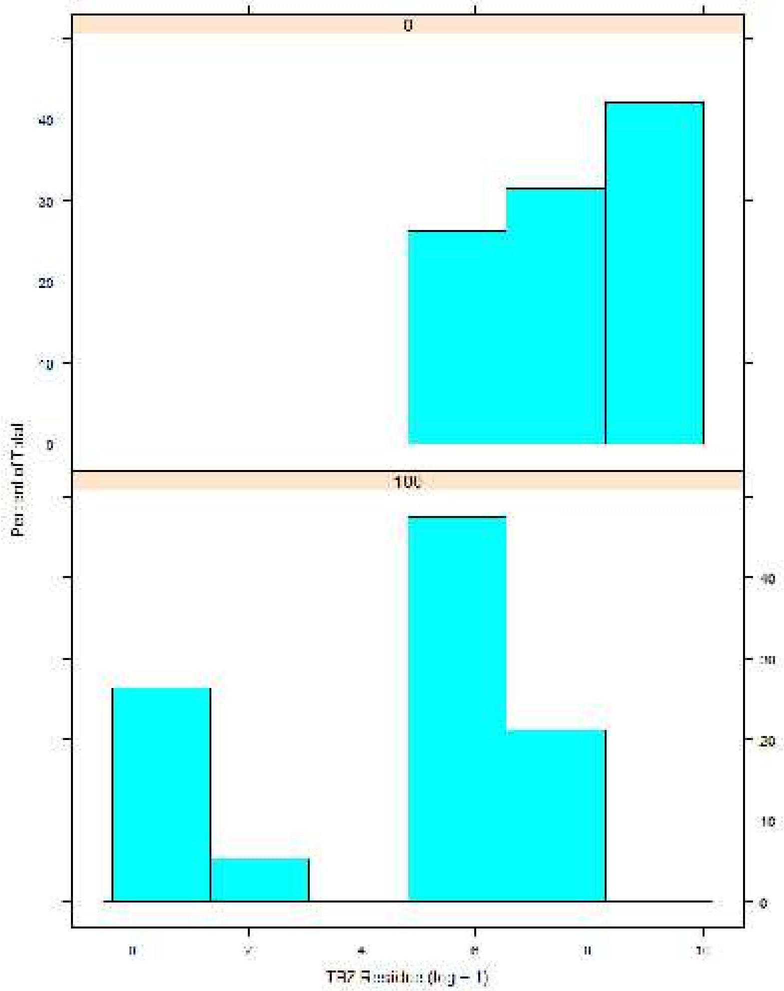

Figure 19 – Histogram of TBZ residue (maximum per 3 reps, log +1 transformed, µg kg^{-1}) per station as a function of distance (either 0 m or 100 m) from cage edge. Note that observations that were 'below detection limit' were recoded as zero prior to log+1 transformation.

The correlation between TBZ residue and current speed is shown In Figure 20. The Pearson correlation was 0.24 (95% CI -0.13, 0.54) which indicates a ('non-significant') and weak positive relationship between current speed and TBZ residue. It should be noted that an outlier observation was removed from the data prior to this correlation being produced.

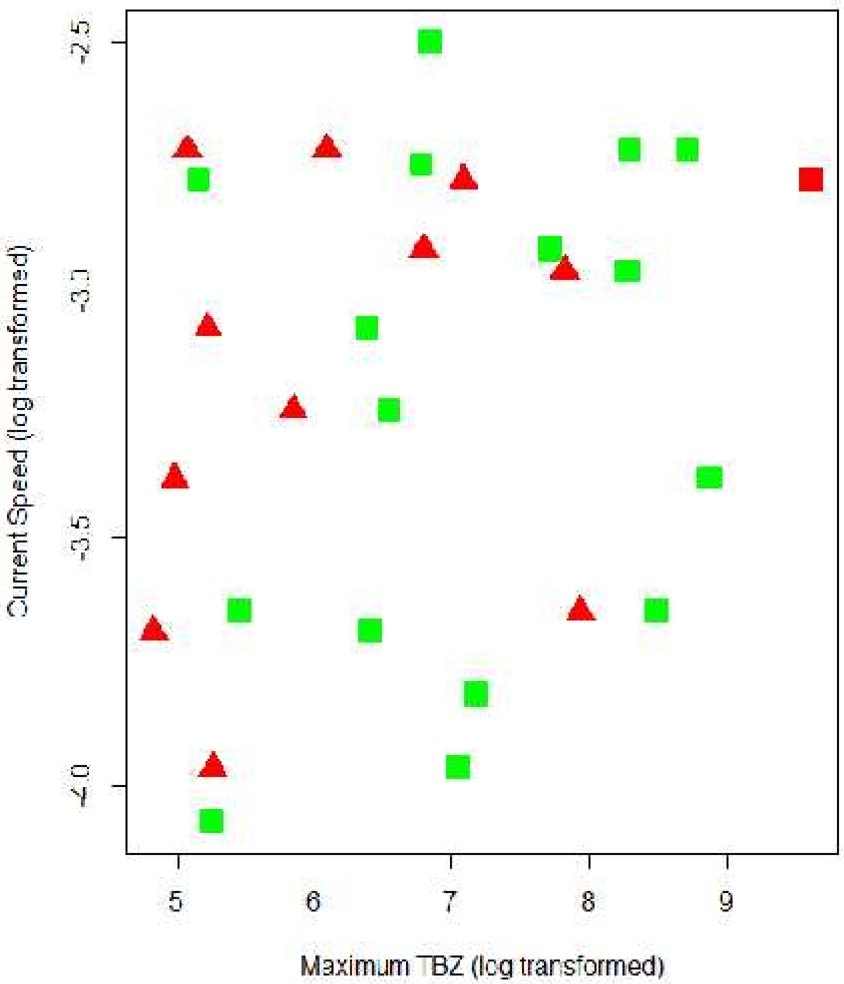

Figure 20 – Relationship between Maximum TBZ (log transformed, µg kg^{-1}) and current speed (ms^{-1}, log transformed). Key: Square and triangular symbols represent CE and 100 m stations respectively, red and green colours indicate EQS fail and pass respectively. There is only one case of a CE sample failing the EQS (red square, top right).   This figure excludes those stations where TBZ was below detection limit and one outlier excluded because of its extreme influence on the correlation.

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8 Appendices

8.1 Additional response variable plots

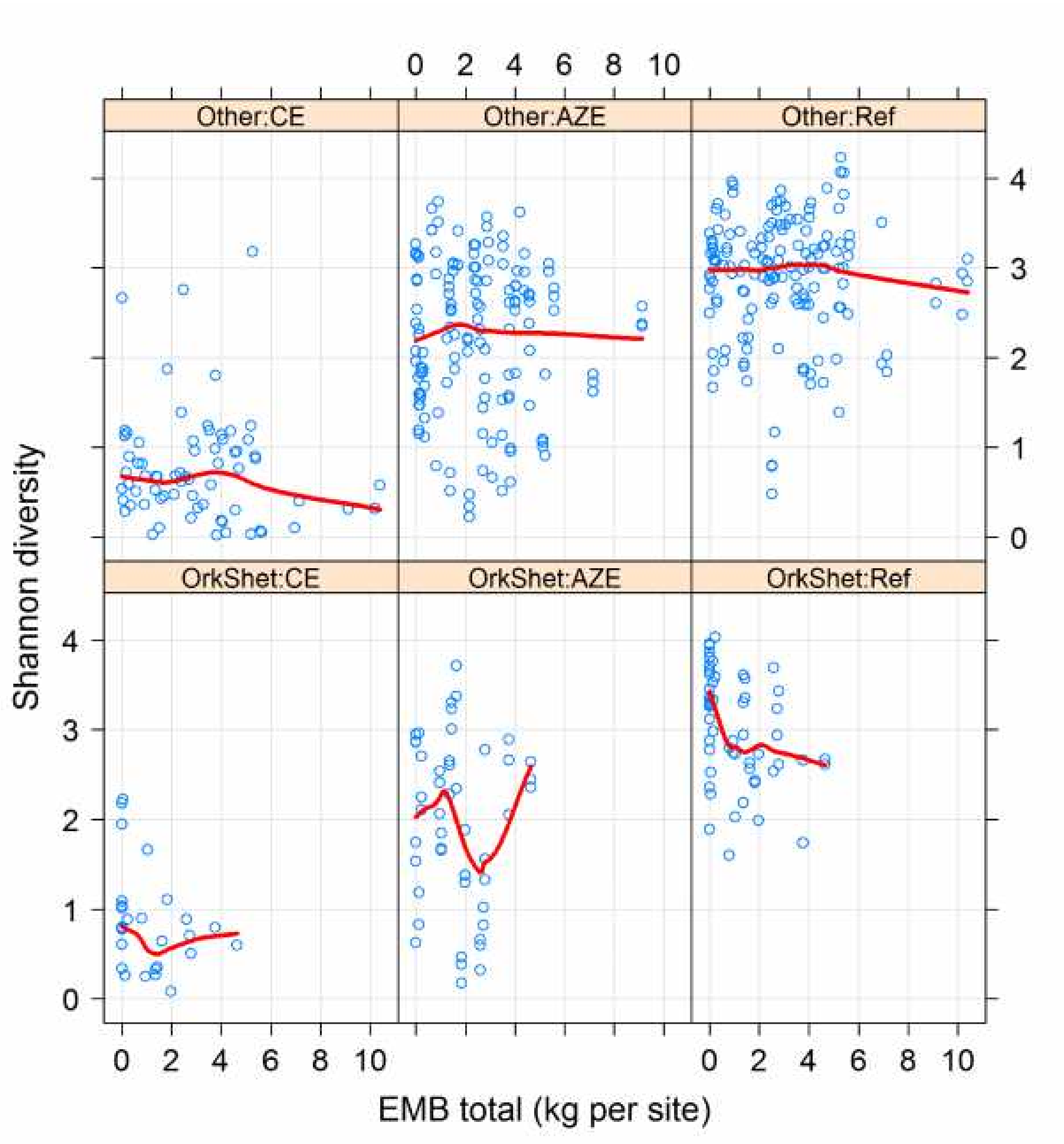

Figure 21 – The relationship between Shannon's diversity index and Distance class (CE – cage edge, AZE – allowable zone of effects and Ref – reference) as a function of Region (Orkney and Shetland combined (OrkShet) and all other sites (Other) and Treatment (Total EMB per site, combined across all treatments). A LOESS smoothing function (span=2/3, degree=1), shown as a red line, has been added to assist in interpretation.

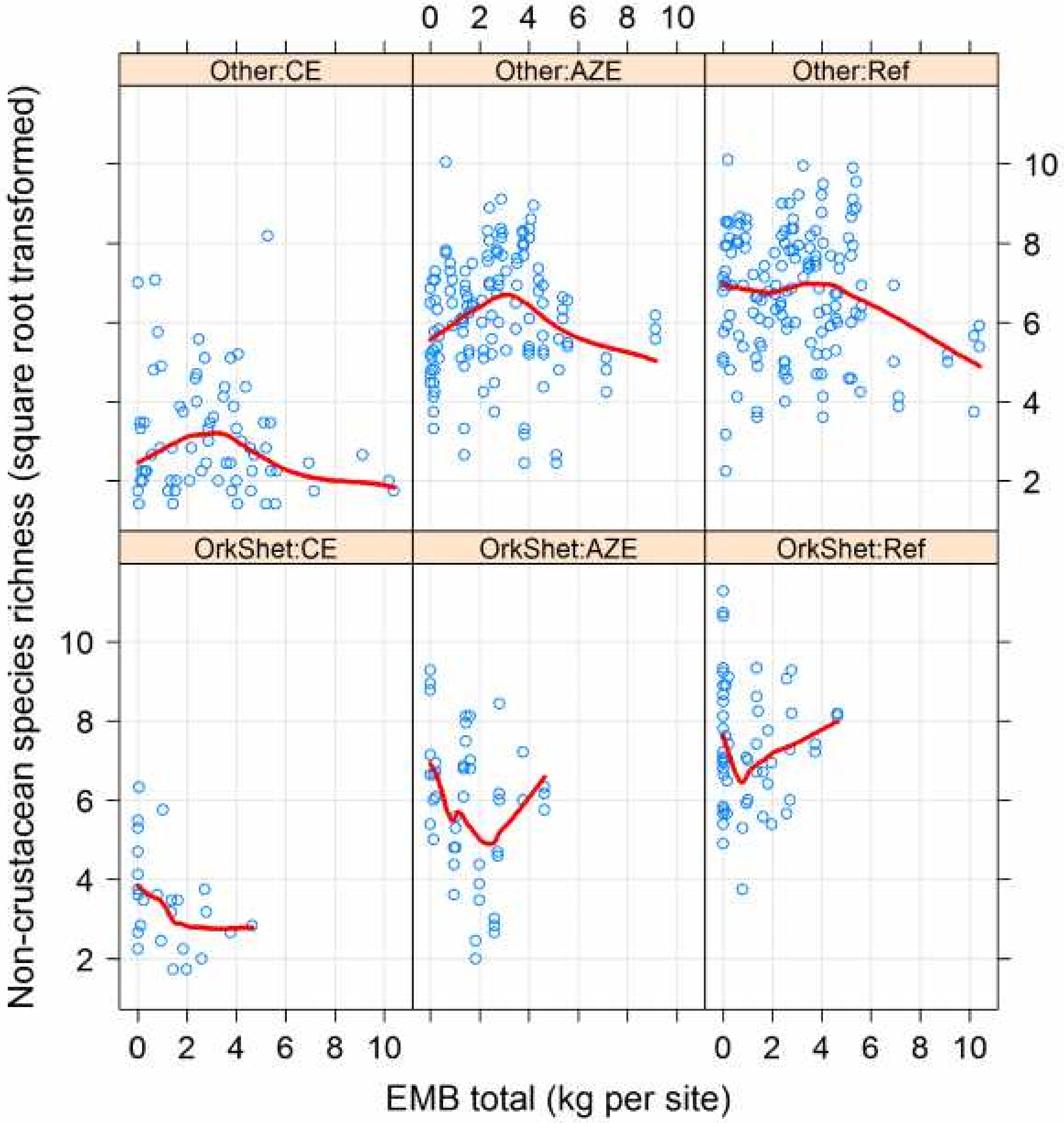

Figure 22 – The relationship between species richness (non-crustacean, square root transformed) and Distance class (CE – cage edge, AZE – allowable zone of effects and Ref – reference) as a function of Region and EMB application (total per site). A LOESS smoothing function (span=2/3, degree=1), shown as a red line, has been added to assist in interpretation.

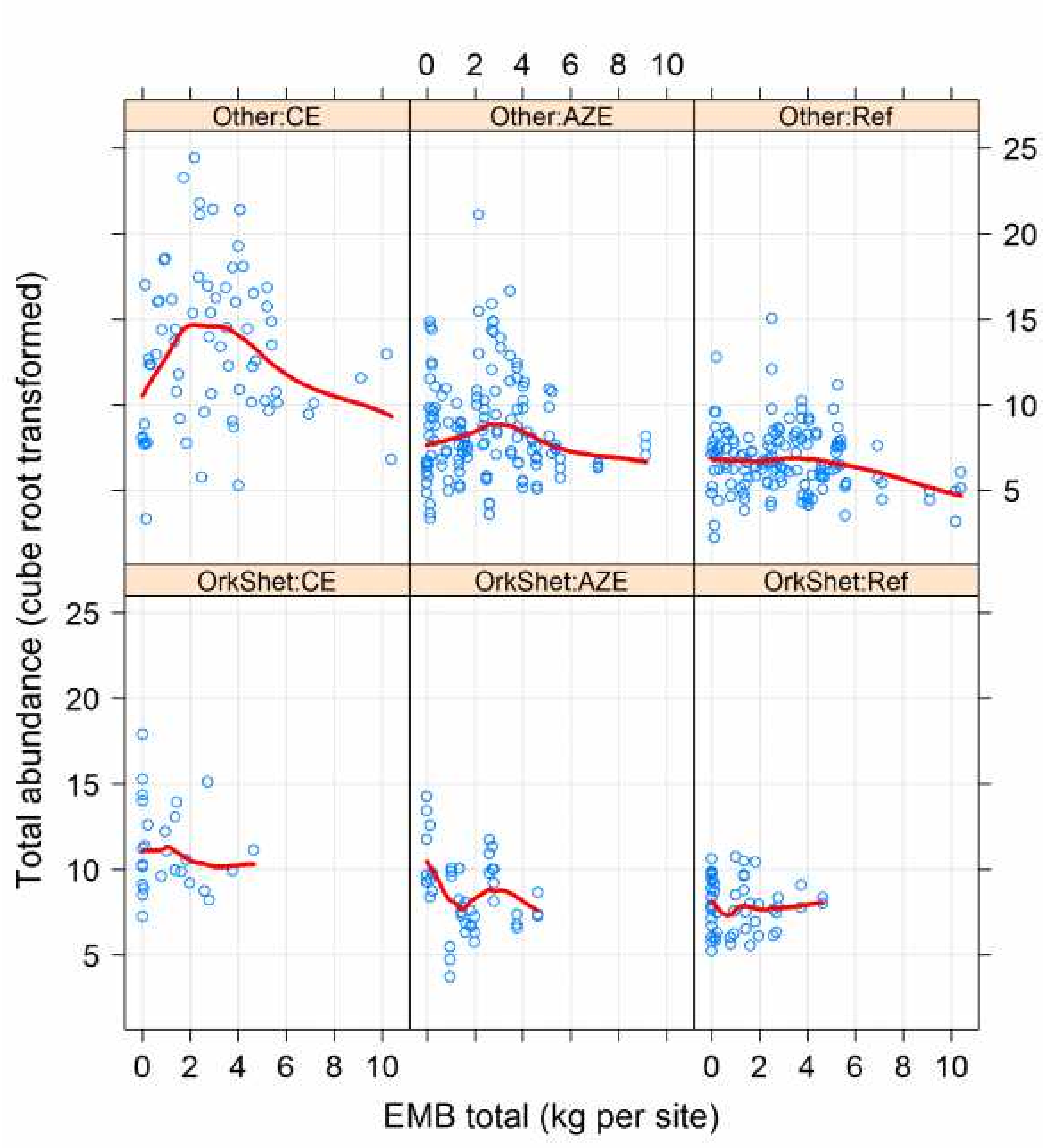

Figure 23 – The relationship between total abundance (non-crustacean, cube root transformed) and Distance class (CE – cage edge, AZE – allowable zone of effects and Ref – reference) as a function of Region and EMB application (total per site). A LOESS smoothing function (span=2/3, degree=1), shown as a red line, has been added to assist in interpretation.

Table 17 – number of monthly records per year. The Years 2002 and 2014 are excluded from the analysis as they represent non-completed production cycles (as a consequence of the data-gathering start and end-dates).

```
Year
2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
 414  732  743  697  797  748  821  865  904  904  890  882  656
>
```

Note that the data from 2002 and 2014 are incomplete (because the databased started in 2002 and was uploaded in May 2014), otherwise there is a gradual increase in the number of production cycles over the period 2003 – 2013.

8.2 Production cycle summary

Table 18 - Number of months per production cycle per site and Sites that formed the basis of the current study. Production cycles (1 – 10) are shown at the top.

Site	1	2	3	4	5	6	7	8	9	10
AAC3	22	21	19	19	18	19	0	0	0	0
APT1	18	20	24	11	0	0	0	0	0	0
ARDG1	23	22	24	23	21	22	8	0	0	0
ARDI1	13	20	18	17	19	18	6	0	0	0
ARDT1	11	23	15	21	23	18	19	0	0	0
BASS1	14	19	23	12	16	17	12	0	0	0
BEL1	12	22	23	0	0	0	0	0	0	0
BMS3	14	15	12	6	9	3	14	0	0	0
BOA1	21	22	25	5	21	18	0	0	0	0
BOH2	23	8	32	13	14	5	8	0	0	0
BRO1	12	23	26	22	23	21	15	0	0	0
BURK1	11	9	14	9	10	12	11	12	0	0
CAG1	11	23	22	23	19	19	20	0	0	0
CAIR1	10	23	23	24	22	20	22	0	0	0
CALL1	24	23	19	20	22	22	8	0	0	0
CAS1	21	20	21	22	22	16	24	0	0	0
CHA1	14	25	22	14	17	11	14	0	0	0
COLED1	12	20	23	20	20	16	25	0	0	0

COLEN1	12	23	25	10	12	20	22	0	0	0
DMB1	14	21	21	19	0	0	0	0	0	0
EAIT1	3	17	0	0	0	0	0	0	0	0
EAR1	8	23	13	22	22	20	19	0	0	0
ELGN1	22	23	6	0	0	0	1	0	0	0
EPO1	10	20	0	0	0	0	0	0	0	0
ERI3	18	14	23	12	19	20	0	0	0	0
ETRA1	21	25	20	17	13	0	0	0	0	0
FFMC01	10	20	21	23	23	18	0	0	0	0
FFMC02	23	22	24	11	21	26	13	0	0	0
FFMC04N	18	21	22	24	13	0	0	0	0	0
FFMC10	5	18	18	15	22	23	12	0	0	0
FFMC18	17	23	22	22	22	18	6	0	0	0
FFMC21	14	20	21	20	22	20	14	0	0	0
FFMC23	5	22	22	23	23	22	14	0	0	0
FFMC27	18	23	24	22	22	20	7	0	0	0
FFMC29	9	17	26	23	13	21	12	0	0	0
FFMC30	36	23	18	13	19	19	0	0	0	0
FFMC31	46	24	35	12	25	0	0	0	0	0
FFMC33	20	9	7	17	12	20	23	0	0	0
FFMC35	14	14	17	14	16	7	0	0	0	0
FFMC36	50	6	20	22	0	0	0	0	0	0
FFMC40A	18	23	23	21	17	22	6	0	0	0
FFMC43	8	14	33	9	21	12	0	0	0	0
FFMC46	10	28	15	21	21	13	0	0	0	0
FFMC47	11	12	18	22	13	22	13	0	0	0
FFMC49	10	16	21	14	22	13	0	0	0	0
FFMC51	8	8	9	0	0	0	0	0	0	0
FFMC56	18	22	21	24	20	7	0	0	0	0
FFMC57	5	19	21	10	23	23	13	0	0	0
FFMC60	21	21	22	21	6	0	0	0	0	0
FFMC70	9	23	25	13	21	22	13	0	0	0
FFMC71	5	21	23	19	21	21	21	0	0	0
FFMC77	21	22	14	19	19	18	0	0	0	0
FIU1	21	23	21	0	0	0	0	0	0	0
GCD1	6	21	10	8	12	0	0	0	0	0

GHC1	6	23	18	11	0	0	0	0	0	0
GNH1	9	16	15	12	0	0	0	0	0	0
GORS1	22	22	22	22	21	22	8	0	0	0
GRL1	25	0	0	0	0	0	0	0	0	0
GRO1	5	21	25	17	19	0	0	0	0	0
GRT1	9	13	13	21	17	22	9	0	0	0
GVAL1	23	10	15	0	0	0	0	0	0	0
HAC1	13	21	21	22	23	22	18	0	0	0
HOG1	19	22	12	22	26	0	0	0	0	0
HSND1	10	14	20	15	0	0	0	0	0	0
INV1	11	22	23	15	24	19	22	0	0	0
LBUR1	5	0	0	0	0	0	0	0	0	0
LGEO2	20	18	6	0	0	0	0	0	0	0
LINB1	19	0	23	21	11	22	0	0	0	0
LING1	22	21	27	21	9	18	0	0	0	0
MAN3	25	18	31	0	0	0	0	0	0	0
MAOB1	22	16	20	18	20	0	0	0	0	0
MBM1	16	19	18	11	0	0	0	0	0	0
MEAN2	14	22	24	21	0	0	0	0	0	0
NWW1	51	13	8	13	11	11	5	11	14	0
OLN2	8	9	6	0	0	0	0	0	0	0
PEC3	20	22	9	18	11	17	0	0	0	0
PLOC1	21	12	0	0	0	0	0	0	0	0
PPN1	24	23	0	0	0	0	0	0	0	0
PUL1	26	23	8	14	16	18	11	0	0	0
ROS1	13	24	24	6	0	0	0	0	0	0
SCAP1	23	17	17	19	1	0	0	0	0	0
SCOT1	12	23	23	14	21	19	12	0	0	0
SETN1	25	18	0	0	0	0	0	0	0	0
SETW1	12	22	22	23	25	20	19	0	0	0
SHNEM1	6	23	24	24	18	16	15	0	0	0
SHNEM2	20	23	23	10	15	15	0	0	0	0
SIAN1	17	18	17	23	14	5	0	0	0	0
SNA1	12	18	0	0	0	0	0	0	0	0
SOAY1	20	22	22	22	20	19	12	0	0	0
SRO1	12	21	22	21	21	18	17	0	0	0

STOC1	6	26	21	17	12	0	0	0	0	0
STOU1	8	23	24	14	23	22	18	0	0	0
TAI1	15	21	25	14	23	20	13	0	0	0
TOYN1	19	21	22	23	5	0	0	0	0	0
TRM1	13	23	18	13	0	0	0	0	0	0
TUR1	7	22	10	10	38	11	10	12	16	0
UIS2	13	16	13	8	23	6	0	0	0	0
VAC1	10	12	13	12	14	0	0	0	0	0
VEA1	10	30	22	22	24	21	0	0	0	0
VUM1	17	22	20	19	0	0	0	0	0	0
WAT1	16	27	14	0	0	0	0	0	0	0
WOB1	16	8	10	21	21	11	14	7	15	4
WOG1	32	13	20	0	0	0	0	0	0	0

8.3 Identity of production cycles with > 1 grab-sampling events

Table 19 - Multiple Surveys per Site and production cycle. Manual check code: A – multiple ID_Survey per production cycle (mid/end of cycle). B – initial sample at cycle start, C – indistinct production cycles so production cycles changed to link with grab sampling date. ? - unknown (apparent duplication of sample date).

Site and PC	GrabDate	ID_Survey	Manual	EMB	Keep
BRO1-4	2008-09-30	370	A	Y	N
BRO1-4	2009-03-24	451	A	Y	Y
CALL1-3	2007-06-07	134	?	Y	Y
CALL1-3	2007-06-07	135	?	Y	Y
CAS1-4	2008-01-21	235	A	Y	N
CAS1-4	2008-03-27	243	A	Y	N
CAS1-4	2009-01-20	429	A	Y	Y
DMB1-4	2013-12-11	1103	A	Y	Y
DMB1-4	2014-03-18	1113	A	Y	Y
ETRA1-2	2006-07-31	82	A	N	Y
ETRA1-2	2007-08-13	332	A	N	Y
FFMC18-5	2010-03-24	543	B	Y	N
FFMC18-5	2011-09-15	798	A	Y	Y
FFMC30-3	2008-04-22	320	B	Y	N
FFMC30-3	2009-01-16	397	A	Y	Y
FFMC30-4	2009-09-02	501	B	Y	N
FFMC30-4	2010-02-23	537	B	Y	N
FFMC30-4	2010-07-02	559	A	Y	Y
FFMC43-4	2011-02-08	708	A	Y	Y
FFMC43-4	2011-02-09	709	A	Y	Y
FFMC43-5	2012-11-28	953	A	Y	Y
FFMC43-5	2012-11-28	954	A	Y	Y
FFMC77-4	2009-05-27	423	B	Y	N
FFMC77-4	2010-02-22	536	A	Y	Y
FFMC77-4	2010-07-01	560	A	Y	Y
GRO1-5	2010-09-09	661	B	N	N
GRO1-5	2011-07-29	762	A	N	Y
MAOB1-3	2009-07-27	477	B	Y	N
MAOB1-3	2010-07-05	575	A	Y	Y

TOYN1-3	2010-07-13	629	B	N	N
TOYN1-3	2012-01-17	828	A	N	Y
TUR1-5	2007-08-30	133	C	N	Y
TUR1-5	2009-09-03	504	C	N	Y
VEA1-4	2010-05-25	561	?	N	Y
VEA1-4	2010-05-25	899	?	N	Y

8.4 Per production cycle, total abundance (non-crustacean) model results

Table 20 – Non-crustacean abundance ~ Distance class + EMBSR + MaxBiomass+Area+Depth+ DistClass: EMBSR.

```
Random effects:
 Groups    Name           Variance Std.Dev. Corr
 ID_Survey (Intercept)    17.879   4.228
           DistClass2AZE  19.209   4.383     0.85
           DistClass2Ref  20.164   4.490    -0.99  0.88
 SiteID    (Intercept)     1.061   1.030
 Residual                  1.040   1.020
Number of obs: 1242, groups:  ID_Survey, 256; SiteID, 99

Fixed effects:
                    Estimate Std. Error t value
(Intercept)         11.80686    0.53395  22.112
DistClass2AZE       -2.72361    0.55618  -4.897
DistClass2Ref       -4.40650    0.52140  -8.451
EMBSR                1.55343    0.66969   2.320
MaxBioT.c            0.80310    0.18487   4.344
Area20.135          -0.07241    0.23121  -0.313
Area20.2            -1.63414    0.28896  -5.655
cLogDepth           -0.31424    0.14335  -2.192
DistClass2AZE:EMBSR -1.63907    0.73080  -2.243
DistClass2Ref:EMBSR -1.95582    0.69713  -2.806
```

These results indicate only a negative relationship between EMBSR and non-crustacean abundance (e.g. the predicted abundance for Ref stations in the presence of 1 kg of EMB is 344 organisms, in the absence of EMB the prediction is 405 organisms).

8.5 Transformations

Transformations are necessary in order to meet model assumptions. In relation to the predictors these transformations have little ‘impact’ regards interpretation (they can simply be back-transformed to the linear scale, if desirable, for presentation. The transformation of the response variable is different and there are a number of approaches. The log transformation has numerous desirable properties (i.e. the response become multiplicative rather than additive on the linear scale) but zero and negative values cannot be log-transformed. Often zero values are log+1 transformed and this ‘solves’ the problem. However, the ‘+1’ part is subjective, other constants could be used and where the choice of constant changes the model output then this is a cause for concern. Root-transformations (i.e. square- or cube-root) can be used on data containing zeros without any need for arbitrary added constants. The means from non-transformed and root-transformed data, that are back-transformed can be very different (as here). The disadvantage with root-transformations is that interpretation is more difficult. In the current case, interpretation of the back-transformed responses is best achieved by examination of the graphical representation of the data (over a relevant range of EMB).

Table 21 – Examples of transformations and the results from back-transformations.

Sample number	Number (e.g. abundance)	Cube root	Log+1
1	1000	10	6.908
2	0	0	0
Mean	500	5	3.454
Back transform	500	5^3=125	$e^{3.454}$=31.6

8.6 Statistical models

A statistical model is a mathematical description of the relationship between a response variable (e.g. the number of crustacea) and one or more predictors (e.g. Site, EMB usage etc). The response variable can be one of several types and different models are used accordingly. Here, we have count data (number of species and total abundance). Where the counts are low (e.g. number of crustacean species is frequently <5) then a 'generalised model' is employed whereas when the counts are large (e.g. total count) then a better model is a 'normal' (general) model. The models described in this report are 'linear' meaning that the response is modelled as a sum of the effects of all the predictors (and any interactions) + an error term.

Example Model: Response = intercept + (coefficients * predictor(s)) + error. The intercept is the prediction at the 'base' level for all the categorical predictors and assumes the continuous predictors are set at zero (see below). The 'base level' is determined by the investigator, here it means the cage edge and, where included, the 0.1 m^2 grab (total area).

There are several ways of classifying the predictors, here I will discuss two: 1. Continuous or categorical and 2. Fixed or random.

Continuous predictors are those where a continuum in values is logical, for example, depth is a continuous predictor because it can (theoretically) take any value (>0). If depth were classified into 'shallow' or 'deep', then depth would be a categorical variable because it could only take two (categorical) values. Generally, where the variable is inherently 'continuous' it should be left as such when included in statistical models. One of the defaults for statistical models is that any continuous variables are set at zero (i.e. the intercept coefficient is based on the continuous variables being set at zero). In many cases this assumption is counter-intuitive, for example, a depth of zero m for a fish-farm is nonsensical. In other circumstances a value of zero is a logical level for a predictor, for example, crustacean predictions based on an EMB treatment of 'zero' is sensible. Where appropriate (e.g. depth, sediment coarseness etc) we, therefore, 'mean-subtract' (centre) the data prior to analysis i.e. for each measure we subtract the mean. This means that the model predictions are based on the mean of the continuous predictors. Mean subtraction does not change the model or the results in any way, it merely makes interpretation easier.

Predictors can be 'fixed' or 'random'. A fixed predictor is one where we are particularly interested in the levels present in the data set, for example, if we had two Sites that were being compared and we were interested in only those two sites. Where there are numerous Sites (as here) the inclusion of them as fixed effects has a two major disadvantages, firstly, for each Site a parameter (the Site effect) has to be estimated and this detracts from the predictive capacity of the model and secondly, inference from the model only applies to the Sites included in the model. Treating Site (in this example) as a random effect means that we are no longer interested in each Site, *per se,* rather we are interested in the generality of Sites (e.g. how much variability is attributable to Sites). Inclusion of 'random' effects into a model requires a mixed model: GLMM stands for Generalised Linear Mixed Model: generalised because the response is a count variable (with low values), linear because the predictors (multiplied by their coefficients) are assumed to sum to the overall effect (+error) and mixed because the model contains both fixed and random terms. A LMM (linear mixed model) is the same but the response variable is assumed to be continuous (here, total abundance is assumed to be continuous).

Taking crustacean abundance per production cycle as an example, and using the 95% credible interval table (**Table 9**):

Table 22 – Copy of Table 9 (Credible Intervals). A brief explanation of the interpretation of the table is given in the text. The derivation of the proportionate change attributable to EMB is given in Table 23.

	Intercept	AZE	Ref	EMB	Depth	<63 µm	Current
2.5%	0.952	0.465	0.954	-0.661	-0.424	-0.304	0.374
50%	1.17	0.666	1.17	-0.406	-0.243	-0.216	0.639
97.5%	1.39	0.885	1.4	-0.146	-0.0637	-0.125	0.897

The expected value for crustacean abundance at the cage edge, with zero EMB and setting all other continuous variables to zero is 1.17^3 (cubed because the response variable was cubed prior to analysis). $1.17^3 = 1.6$. At the Reference station, under the same conditions, the prediction is $(1.17+1.17)^3=12.8$. The coefficients for EMB, Depth, <63µm are all negative meaning that as they increase crustacean abundance decreases. The model prediction for crustacean abundance, at the Reference stations, with a treatment of 3 kg EMB per production cycle (and all other continuous predictors at their average levels) is

Abundance = $(1.17+1.17 + (-0.406*1.732))^3 = 4.39$. Note that 1.732 = square root (3) and that this is used because the EMB totals were square root transformed prior to inclusion in the model. The ratios and % changes between the maximum and minimum predicted metrics, based on Credible intervals, forms the basis of the inference reported here (Korner-Nievergelt et al., 2015). It should be noted that this interpretation is based on the marginal effects of the predictors and this excludes the influence of the correlations between the fixed effects on the posterior distribution. The basis of Figure 8, Figure 10, Figure 12, Figure 14 (model predictions, based on simulations drawn from the specified model) are based on the posterior joint distribution hence the slight difference between the Credible Intervals and model simulations (except where the predictor is set to zero). It should be noted that the reported proportionate changes in Abundances are based on 50th percentile +/- the credible intervals for the term in question (e.g. EMB). The back-transformation from the cubic-root transformed responses means that proportionate changes are not constant across different expected levels of the Intercept (see **Table 23**). The interpretation focusses on change predicted to occur at the Reference stations given their importance in relation to the reported work.

Table 23 – Derivation of proportional change for models as reported following 3 and 10 kg treatment per production cycle/per site total respectively. The coefficients for the Intercept (Int), Reference (Ref) and EMB are copied from Table 8 - Table 11 for crustacean richness and abundance (PPC and PST). PC1 and PC2– proportional decline based on 97.5 and 50 quantile intercepts respectively . PC2 (highlighted in yellow) is reported in the main documentation here (i.e. proportional changes are based on a change at the average richness/abundance). Derived proportional changes, for abundance data, depend on the intercept value used because of the cube-root back transformation. Key: exp-exponential, ^3= cube transformed.

Crustacean richness per production cycle (3 kg max treatment per production cycle)

Quantile	Int	Ref	EMB	Int+Ref	exp(Int+Ref)	EMB*3^0.5	exp(MaxInt+EMB)	PC 1	exp(MedInt+EMB)	PC2
2.500	-0.265	1.400	-0.574	1.135	3.111	-0.994	3.083	0.630	1.873	0.630
50.000	0.052	1.570	-0.298	1.622	5.061	-0.516	4.972	0.403	3.021	0.403
97.500	0.380	1.740	-0.025	2.120	8.331	-0.043	7.982	0.042	4.849	0.042

Crustacean abundance per production cycle (3 kg max treatment per production cycle)

Quantile	Int	Ref	EMB	Int+Ref	(Int+Ref)^3	EMB*3^0.5	(MaxInt+EMB)^3	PC1	(MedInt+EMB)^3	PC2
2.500	0.952	0.954	-0.661	1.906	6.924	-1.145	4.452	0.795	1.707	0.867
50.000	1.170	1.170	-0.406	2.340	12.813	-0.703	9.087	0.582	4.385	0.658
97.500	1.390	1.400	-0.146	2.790	21.718	-0.253	16.331	0.248	9.092	0.290

Crustacean richness per site total (10 kg max total treatment)

Quantile	Int	Ref	EMB	Int+Ref	exp(Int+Ref)	EMB*10^0.5	exp(MaxInt+EMB)	PC 1	exp(MedInt+EMB)	PC2
2.500	-0.253	1.280	-0.539	1.027	2.793	-1.704	1.823	0.818	0.954	0.818
50.000	0.147	1.510	-0.313	1.657	5.244	-0.990	3.726	0.628	1.949	0.628
97.500	0.565	1.740	-0.076	2.305	10.024	-0.240	7.885	0.213	4.125	0.213

Crustacean abundance per production cycle (10 kg max total treatment)

Quantile	Int	Ref	EMB	Int+Ref	(Int+Ref)^3	EMB*10^0.5	(MaxInt+EMB)^3	PC1	(MedInt+EMB)^3	PC2
2.500	1.200	0.960	-0.712	2.160	10.078	-2.252	0.490	0.983	0.001	1.000
50.000	1.580	0.752	-0.488	2.332	12.682	-1.543	3.354	0.881	0.491	0.961
97.500	1.980	1.060	-0.264	3.040	28.094	-0.835	10.723	0.618	3.356	0.735

8.7 Comparison of Reference-only analyses with analyses based on all Distance Classes

The reference-only analysis indicated similar trends and magnitudes of those trends when compared to the analysis based on all Distance Classes. These are shown in Table 24.

Table 24 – Comparison of model results from All-distance-class and Ref-only analyses. Values are percentage reductions at maximum EMB usage (3 kg for PPC and 10 kg for PST). Key: PPC – per production cycle, PST – Per site total, All – results from analysis based on all distance classes, Ref only – results based on analysis from the Reference stations only. 'Response and model-change' indicates the response variables and the change in the model specification (Ref-only compared to All Distance-classes models, for example '-Area' indicates that the factor Area was not included in the Ref-only model). For Key to the factors see Table 1.

Analysis category	Response and model-change	Expectation	Lower CI	Upper CI
PPC –All	Richness	40	4	63
PPC – Ref only	Richness; - Area	49	18	69
PPC –All	Abundance	65	29	85
PPC – Ref only	Abundance; + Area	58	12	84
PST –All	Richness	63	21	82
PST – Ref only	Richness; - Depth	69	41	83
PST–All	Abundance	90	74	~100
PST – Ref only	Abundance; +MaxBio	~100	81	~100

8.8 Interpretation of Draftsman's plots

Draftsman's plots consist of a matrix of scatter plots and are used as a quick method of assessing relationships between numerous variables simultaneously. Taking Figure 5 as an example: The first scatter plot ('A') shows the relationship between the maximum biomass (0 – 2500, shown bottom left, X axis) and the total EMB applied (square root transformed, 0.0 - 1.5, Y axis, shown to the left of the panel). This scatterplot shows that larger farms (biomass) are associated with greater EMB use (where EMB is used). The same relationship is inverted in 'B' such that the tonnage is on the Y axis and EMB use is on the X axis. The relationship (Max biomass and Total EMB) is relatively linear as indicated by the relatively straight red line.   This pattern of swopping the axes (X becomes Y, Y becomes X) is repeated throughout the figure e.g. C shows <63µm(%) (X axis) v >2mm (Y axis) whilst in D this relationship is inverted (>2mm, X axis, and <63µm, Y axis). Histograms show the distribution of individual variables.