# SARF098C: PAMP Refreshment Study - The association between emamectin benzoate use and crustacean assemblages around Scottish fish-farms

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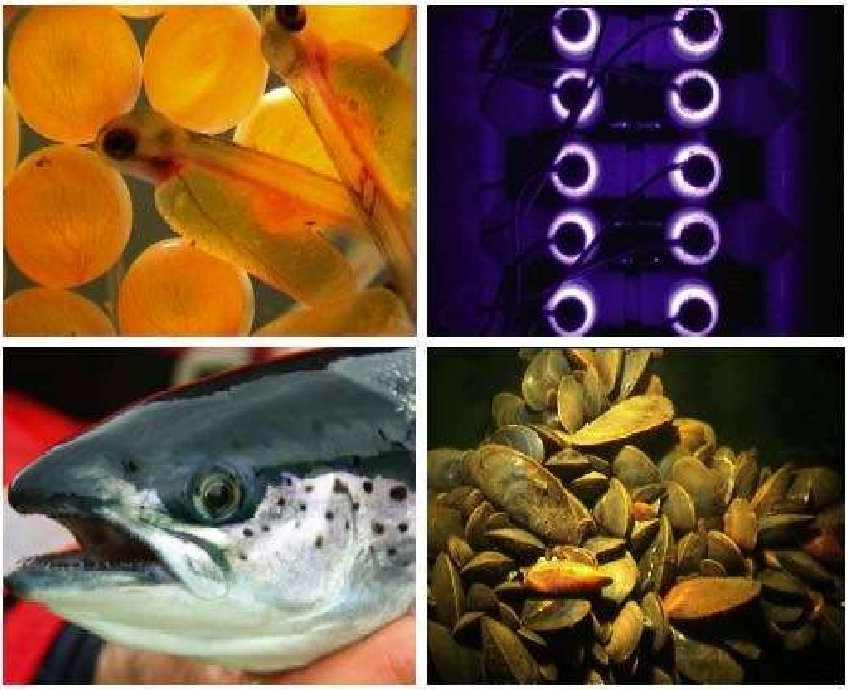
# SARF098C – PAMP Refreshment



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# SARF098C: PAMP Refreshment Study

# The association between emamectin benzoate use and crustacean assemblages around Scottish fish farms

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## Abstract

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 chemical emamectin benzoate (EMB) is consented for use in Scotland. Following ingestion, EMB enter the fishes' tissue which becomes toxic to feeding lice. EMB is excreted by the fish over an extended period (~200 days) and enter the environment via faeces. Previous research (SARF098A and SARF098B) identified an association between a reduction in benthic Crustacea and increasing EMB use in sediments around fish farms. This negative association was apparent even at Reference stations. The objectives of the current research was to evaluate the evidence as to whether the negative association applied to particular functional groups or crustacean families.

The functional group-categorisation split Crustacea based on diet (particulate organic matter v. others) and motility (mobile v. sedentary). The analysis focussed on data from stations located around the allowable zone of effects (AZE) and Reference stations. These stations are located, typically, 50 – 100 m and 250 – 1000 m from the farm respectively. Current speed, particle size and sampling depth were used as covariables the statistical models linking EMB use and Crustacea.

The analysis split the data into Local Authority (LA) areas and, from each LA, the use of EMB was assessed as a function of farm-distance (AZE and Ref) and sampling methodology. The data were highly unbalanced and, as a consequence, only data from Shetland, Reference stations supported robust analysis. The Shetland, Reference samples were dominated by the crustacean families (in numerical order), Urothoidae, Ampeliscidae, Corophiidae, and Lysianassidae. Multivariate analysis indicated that patterns in crustacean family abundance were linked to all the measured environmental covariables including EMB and provided evidence of a negative association between the most common family (Urothoidae) and EMB use. Of the four functional groups, only those classified as 'Mobile' showed an interpretable association with EMB and the model predicted that, under average environmental conditions, a mobile-crustacean reduction of between 17 and 83% would occur following treatment with 3.6 kg EMB. However, this negative relationship was only predicted following removal of statistical outliers. The outliers were dominated by the high abundance of otherwise unusual opportunistic scavengers.

These results are based on the analysis of observational data - further research is required to establish any causal relationship between EMB sedimentary concentration and crustacean assemblage change. This research should be based on manipulative studies where the actual concentration of EMB is controlled, and measured, alongside measurements on the crustacean community.

## Scientific objectives

This is a summary of the objectives derived from the initial proposal but re-ordered to reflect the work-programme. This research is based on observational data and did not seek-out to assess change against pre-defined thresholds (Wilding et al. 2017)[1].

Objective 1: Extract, check and match species data, check and extract full crustacean species complements from the existing SEPA database via WoRMS database, for each species determine the complete taxonomy.

Objective 2: Assess EMB –family associations using ordinations (e.g. non-metric multiple dimensional scaling), assess correlation between EMB and MDS-axes (and species locations) to assess potential sensitivities. Formally test taxa-EMB associations using new software 'MVAbund'.

Objective 3: Review and assess likely sensitivity of different crustacean groups based on their life-histories (reproductive and feeding strategy, location in the sediment). This review will be based on published literature and will focus on those families which are, as revealed by the database, the most abundant (>1% total) in the data.

Objective 4: Assess evidence of differences in crustacean groups (based on habit) to EMB, produce linear models based on crustacean groups (i.e. as identified in Objective 1) and determine the extent of differences in relationship between different groups and EMB.

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[1] Hypotheses of 'no relationship' are not tested in this study as any anthropogenic activity (including fish-farming and the use of EMB) will inevitably cause change in the receiving environment including the macrobenthos. The core-purpose of this study, based as it is on observational data, is to estimate relationships between predictors (e.g. EMB) and response variables (e.g. crustacean abundance) using statistical models.

# 1 Introduction

Fish-farming in Scotland has expanded considerably over the last 30 years and currently Scotland produces around 180,000 tonnes per annum with the aspiration to increase this by 28% by 2020 (Ellis et al. 2016). Sea lice are a long-standing problem in relation to farming salmon and they have been controlled, as the industry has developed, using a variety of chemotheraputants. One of these chemotheraputants, emamectin benzoate (EMB), was consented for use in the treatment of lice in salmon in 2001. A 5 year 'Post Authorisation Monitoring Programme' (PAMP, 1999 - 2004) was unable to establish any negative associations between EMB use and the receiving environment (benthic and pelagic). However, concerns have been raised about the increased use of EMB in Scottish farms, and further research was considered necessary. This additional research was supported by the Scottish Aquaculture Research Forum (SARF) through their 'PAMP-refreshment study' programme (SARF098 series).

The research reported here is the culmination of three linked research programmes. Phase I (SARF098A) identified that EMB-treated sites tended to host fewer crustaceans compared with those where EMB was not used. Phase II (SARF098B) extended Phase I by quantifying the amount of EMB used, during each production cycle, and used this as a predictor in the models. Phase II concluded that there was an association between EMB treatment rate and decline in non-target crustaceans including at reference stations. Concern was focussed on the association between EMB use and large-scale changes in total crustacean abundance and species richness. The current work extended Phases I and II by assessing changes in individual crustacean taxa as a function of the total EMB used per site prior to the macrobenthic survey (i.e. cumulative across years). For further background to this work, see Wilding et al. (2015).

# 2 Materials and methods

This research involved data extraction, collation, checking, merging, matching, cross-referencing and statistical modelling. No practical (laboratory or field-based) work was conducted. Details of the data source (SEPA databases) and the data extraction methods and rationale are provided in Wilding and Black (2015). The methods section here details the refined data extraction method, the use of the WoRMS database (for species checking and high-level taxonomy determination) and the multivariate and univariate analysis that formed the bulk of the reported work.

## 2.1 Refined Distance Class assignments

SEPA designates three distance classes in relation to consent monitoring around fish-farms (consented after 2006). These are at the cage-edge (CE), around the 'allowable zone of effects' (AZE) boundary and reference (Ref) stations. The AZE boundary is determined using modelling software (DEPOMOD). The Ref stations, which are agreed by SEPA, are located between 250 and 2000 m from the farm and are selected to represent expected background conditions at the farm-site (in terms of depth and sediment type). Further details of the distance-class designations are provided in Wilding and Black (2015).

The main focus of the multivariate analysis is on the AZE and Ref stations. This is because most crustaceans will be eliminated from CE stations, regardless of the use of EMB.

Previous analysis based the classification of Stations into CE, AZE and Ref. The AZE stations were identified if the appropriate record on the SEPA database contained the keywords AZE (usually with minus or plus 10 m indicated). Those AZE stations that were not coded in this way were compared with the known AZE distances available for that ID_Survey (available from another part of the database). If the distances were comparable (within 5 m) then they were assigned AZE status. For the current work the potential AZE stations were further assessed by examining patterns in the farm-distances. AZE stations are taken at the AZE, AZE-10m and AZE+10m. Where distances were given, without any context, but where there was a clear pattern (e.g. 130, 140, 150 m) then these will represent AZE stations and were coded as such. Occasionally, the AZE distances overlapped with the pre-2006 sampling distances of 25, 50, 100 and 150. Where the Distance was in the pre-2006 group, but where there was no pattern associated with that Distance, the row was removed. The remaining Distances were, as in earlier analyses (Wilding and Black 2015), classified as CE, AZE or Ref.

## 2.2 Generating the complete taxonomy

Crustacean data were extracted from the SEPA database as described in Wilding and Black (2015). A list of the entire species complement was generated and these were matched against the World Register of Marine Species (WoRMS) database (Horton 2016) and, following this matching process, the complete taxonomy, for each species, was generated. This process was iterative and required the correction of numerous spelling inconsistencies present in the original data. The species data, with the complete taxonomy, were then re-imported and matched to the species data in the original data. Where species were recorded that did not exist in any database they were removed from the analysis (this was rare). Analysis by species would require manual sorting of each 'species' (i.e. row in the data set) in order to eliminate duplication (Table 1) and, in order to keep the research tractable, the analyses presented here are based on order and family taxonomic levels only. This completed Objective 1.

**Table 1 Example of one challenge in dealing with the taxonomic data. Thirteen alternative 'species' for *Liocarcinus* are listed here (including various indications of juvenile status) from a single sampling event.**

| | | |
|---|---|---|
| 849 | 541.Ref.1 | Liocarcinus depurator |
| 850 | 541.Ref.1 | Liocarcinus holsatus |
| 851 | 541.Ref.1 | Liocarcinus larval |
| 852 | 541.Ref.1 | Liocarcinus marmoreus |
| 853 | 541.Ref.1 | Liocarcinus pusillus |
| 854 | 541.Ref.1 | Liocarcinus sp |
| 855 | 541.Ref.1 | Liocarcinus sp (juv) |
| 856 | 541.Ref.1 | Liocarcinus sp indet |
| 857 | 541.Ref.1 | Liocarcinus sp. |
| 858 | 541.Ref.1 | Liocarcinus sp. juv. |
| 859 | 541.Ref.1 | Liocarcinus sp. juv. indet. |
| 860 | 541.Ref.1 | Liocarcinus sp.juv |
| 861 | 541.Ref.1 | Liocarcinus spp. juv. |

### 2.3 Emamectin benzoate (EMB) use per site

Fish-farms exist at Sites at which production may have occurred for several years. Each Production-cycle (from stocking to harvest-completion) usually lasts ~20 months during which EMB use may occur; macrobenthic sampling should occur within the period of maximum biomass the farm and this usually occurs following EMB treatment. The total amount of EMB used, prior to the macrobenthic sampling event constituting each macrobenthic survey (which is given the unique identifier ID_Survey), was determined and used as a predictor in subsequent models. The Site total (prior to the macrobenthic sampling event) was used because EMB is persistent in sediments; the assumption was made in these analyses that the impacts are long-lasting and cumulative (Tucca et al. 2014).

## 2.4 Data description, preparation and summarisation

The analysis of multivariate data of the complexity present here offers innumerable options for analysis. In order to remain a tractable exercise the task was split into separate elements based on Statistical Units (see 2.4.1) and, following initial scoping, focussed on particular aspects of the complete data.

### 2.4.1 Statistical units, sub-setting and EMB Tercile-generation

The SEPA database consists of the macrobenthic results from different grab sampling protocols (Wilding and Black 2015) and these were used variously between Local Authority areas. Each row in the data consists of the total abundance, for a given taxon, determined across multiple grabs (pseudo-replicates)(Wilding and Black 2015). Different farm-operators use different grab-sizes (0.02, 0.045 and 0.1 $m^2$) and SEPA require different numbers of replicates depending on the grab size (5, 3 and 2) giving combined grab area of 0.1, 0.135 and 0.2 $m^2$ respectively. In this report, the grab-factor is distinguished by the total-grabbed area (i.e. 0.1, 0.135 and 0.2 $m^2$).

In order to address the concern that inherent differences attributable to Local Authority area and grab-type might be confounding the results the decision was made to base the analyses on single Local Authority areas (LA-area) and, within each LA-area, from a single grab type. Each statistical unit, for example, ‘Shetland Islands -0.1 $m^2$ grab’, comprised the most data available (i.e. the most commonly used grab for that LA-area). Furthermore, the balance of EMB usage in each Local Authority region was assessed prior to analysis and only Local Authority regions where there was a balanced distribution of EMB exposure (including zero exposure), per Distance Class (i.e. AZE, Ref) were assessed.

For the purposes of tabulation and descriptive analysis EMB was divided into four categories. These categories were nil (no EMB treatment) and terciles. Terciles were based on the positive treatment records only (i.e. where EMB was used) and divided the data into three equal portions. The tercile-boundaries were determined for the entire data-set not by each LA-area (thus allowing a direct comparison between in treatment-total across LA-areas).

### 2.4.2 Environmental variables /predictors

As part of consent monitoring, operators are required to gather environmental data associated with each sample or with the site in general. Some variables (e.g. depth and particle size) are determined per grab, whilst others (e.g. current speed) were determined on a by-site basis, with the current meter being located in close proximity to the cages . The predictors here are based on the mean determined across the varying number of grabs taken per sampling event (see 2.4) or on a ‘per-site’ basis as is appropriate. It should be noted that measures of current speed do not vary between surveys at the same site and are not measures of current speed at the location of the Ref stations. The environmental predictors are described in detail in Wilding and Black (2015) and are summarised in Table 2. For justification of log-transforming and centring predictor variables see Zuur et al. (2010) and Schielzeth (2010).

**Table 2 Summary of Response and predictors used in modelling process (MDS, MVAbund and univariate models). For further details see Wilding and Black (2015).**

| Type | Name (and synonyms used in this report) | Description |
|---|---|---|
| Response | Vulnerability classes | Sum across families designated mobile, sedentary, filter-feeders and predators/scavengers |
| | Family | Total crustacean count per family |
| Predictor | Intercept | Forms the base level response, represents the mean response level when all the predictors are zero. |
| | Distance Class | Allowable zone of effects (AZE) or Reference (Ref) |
| | EMB (SREMB) | Total amount of EMB used prior to the corresponding grab sample being taken, square-root transformed. EMB was not centred (Schielzeth 2010). |
| | Depth | In metres, log transformed and centred. |
| | <63 µm (LT63, LLT63) | Proportion (%) of sediment sample passing the 63 µm sieve, log +1 transformed and centred. |
| | >2mm (GT2mm, LGT2mm) | Proportion of sediment not passing the 2mm sieve, log +1 transformed and centred. |
| | Current (CurrentSpeed) | Current speed (m $s^{-1}$), log-transformed and centred. |

### 2.4.3 Elimination of rare taxa and choice of taxonomic level

Multivariate species data are frequently dominated by relatively few taxa and typically include numerous rare taxa. In these circumstances it is routine to exclude ‘rare’ taxa by top-slicing (Clarke et al. 2001). In the current case, the percentage-inclusions trialled were 99.5, 99, 98 and 95% (i.e. 99% inclusion includes only those taxa which, numerically, make up at least 1.0% of the total). The top-slicing of data in this way prevents the analysis being dominated by rare taxa, which might only be present at a single site and which might represent an identification or recording issue in the raw data. For multivariate analyses each Statistical unit (2.4.1) was subject to top-slicing prior to analysis, the impact of this on the MDS ordinations were assessed in terms of stability (described in Results).

Multivariate analysis can proceed on the basis of any taxonomic distinction (e.g. from Kingdom to species). In the current case, analyses based on species was not tenable because of numerous inconsistencies in species designation (see 2.2). Summaries by family and order are provided in order to put the crustacean assemblage structure into context.

### 2.5 Vulnerability assessment

The life history adopted by crustaceans will influence their exposure to EMB and, potentially, this will be reflected in abundance patterns in relation to the total amount of EMB used at a given site prior to sampling. The following vulnerability criteria, based on literature reviews, were assessed: diet/ feeding-mode, motility, location in the sediment and mode of larval development (Table 3). Assessment was on a family-basis as vulnerabilities (as defined here) tend to be expressed at the family-level (Warwick 1993). The univariate statistical analyses were based on the summation across each vulnerability grouping. Only families that constituted >1% of the total number of crustaceans as a function of the Statistical Unit (see 2.4.1) were assessed for vulnerability (see Table 3). The basis of the vulnerability assignment is given in 0.

**Table 3 Rationale behind vulnerability assessment as a function of diet, motility, location and larval development.**

| Criteria | Rationale (detail) |
|---|---|
| Diet/feeding mode | Crustaceans include deposit feeders, scavengers, predators and parasites. Given that EMB is bound within faecal material two feeding-mode categories were assigned: 1. Scavengers, predators and parasites (SPP) feeding on living or recently-dead animal tissue and 2. Suspension /deposit feeders and omnivores feeding on plankton or non-living particulate organic matter (POM). Where there were differences in feeding mode between genera within the same family then those families were assigned the mode corresponding to the most commonly observed genera. The vulnerable families were considered to be those dominated by suspension/deposit feeding modes (POM). |
| Motility | Some crustaceans are freely living and motile whilst others (e.g. barnacles) are immobile regardless of local conditions. Motile species have the option of moving away from contaminated areas and might, therefore, be expected to be more responsive in the face of short-period contamination events. Families were assigned to mobile ('Mobs') and sedentary ('Seds') categories. |
| Location in the sediment | Crustaceans that live epibenthically or in shallow burrows are likely to be exposed to greater concentrations of EMB around fish-cages. Deep burrowing forms may suffer less exposure. The vulnerable families were considered to be those living on or in close proximity (i.e. within the uppermost 2 cm) to the sediment's surface. |
| Larval development | Crustaceans have two types of development, planktotrophic (i.e. involving a planktonic stage) and direct development (e.g. via parental brooding). Those families with a dispersive phase (i.e. planktotrophic) might be expected to recruit back into areas that have been subjected to toxic events. |

### 2.6 Statistical analysis

The statistical analysis began by data summarisation across Statistical Units (see 2.4.1) in terms of EMB treatment (nil and terciles), as a function of Distance Class and as a function of two taxa levels (Order and Family). The by-grab and per-site covariables summaries for each Statistical Unit were also determined. Then the multivariate and univariate analytical analyses (described below) were conducted.

### 2.6.1 Multiple dimensional scaling (MDS)

In order to visualise multivariate patterns between taxa, sites and environmental drivers (EMB) MDS was used. Taxa counts (summed across family and order) were fourth-root transformed and from these Bray-Curtis dissimilarity matrices determined (Clarke and Warwick 2001). Each ordination was based on Site similarities upon which the locations of taxa were superimposed based on maximum correlation. For each ordination the 2-dimensional stress was determined. Stress is a measure of how well the multivariate data are portrayed in 2-dimensions; stress values of >0.2 indicate that the multivariate patterns cannot be usefully visualised in two dimensions whilst those of 0.1 – 0.2 indicate that care should be used in interpretation (Clarke and Warwick 2001). MDS plots can show extreme dominance by a few stations, visualised as the dense and distinct clustering of points on the MDS-axes. This occurs when unique taxa are present in high numbers in few sites. This 'MDS-stability' issue can be addressed by the sequential removal of rare species (see 2.4.3). In general terms taxa located in close proximity on the ordination are likely to be more closely associated than those located further apart. The environmental variable EMB was projected onto each ordination (visualised as an arrow-vector). The arrow-vector maximises correlation between the environmental variable (EMB) in the underlying ordination - a positive correlation between a given taxa and EMB (i.e. where the arrow points towards the taxa) indicates a positive association (and vice-versa) with the length of the arrow indicating the relative strength of the association (Oksanen et al. 2013). The 'significance' of the association between EMB and taxa was formally tested using a permutation-based test (Oksanen, Blanchet et al. 2013) and is reported as a P-value on each MDS figure. The P-value is a measure of the probability of observing the data under the assumption that there is no correlation between the taxa and the environmental driver (EMB). Low P-values do not, however, indicate a causative relationship as the association may occur because of factors which correlate with EMB. Ordination was conducted using the R package 'Vegan' (Oksanen, Blanchet et al. 2013).

### 2.6.2 Model-based multivariate analyses - MVAbund

There are several methods available for assessing the multivariate relationship between taxa and environmental drivers (the main purpose of the current research). These methods include the widely used 'BioEnv' routines available in Primer™ software (Clarke and Warwick 2001). The problem with such routines is that they do not accommodate continuous variables (e.g. EMB) and they cannot indicate which taxa are the main drivers in any identified correlations (Wang et al. 2012). A further problem with these approaches is that they lack statistical power i.e. the treatment effect has to be large in order to be detected. The recent development of a model-based approach (R-package 'MVAbund'; Wang, Naumann et al. 2012) overcomes some of the disadvantages inherent in Primer™-based routines: MVAbund is based around resampling (to address the high-taxa correlation) and is a model-based approach (e.g. Poisson or negative binomial for count data). The model-based approach allows for the over-dispersion that is typical of count data (Wang, Naumann et al. 2012). MVAbund also enables the model assumptions to be assessed through standard residual analysis and provides an ANOVA-type table which can be used as the basis for inference. The relative importance of different factors (e.g. EMB, particle size) in determining multivariate assemblage patterns is assessed as 'Deviance' with higher values indicating greater importance. MVAbund was used to assess, allowing for correlations between taxa, the probability of observing the data given the hypothesis that there was no relationship between family-abundances and the environmental factors (EMB, particle size, sample depth and current speed). In the current case MVAbund was applied and the model assumptions assessed (fit-v-residual, normality and fit-v-Dunn-Smyth Residual (square-root transformed) (Wang, Naumann et al. 2012). A lack of fit is indicated by trends in the residuals in any of these plots or non-linearity in the normality-check. Model refinement proceeded by fitting the main-effects model (all main-effects), sequentially removing terms and assessing changes in fit (Akaike Information Criterion, AIC and chi-square tests) and residual patterns. Fitting the full model (all main effects and all interactions) should not be attempted where the number of observations are insufficient (i.e. <20 observations per parameters) as it results in overfitting and an unstable model. The importance of EMB in the main-effects model was assessed using a Chi-square test to compare the model with, and without, EMB. MVAbund provides a measure of association between specific taxa and each environmental variable in the model, assessed as a Deviance and associated linear-model coefficients and P values. These were used to assist in interpretation (Wang, Naumann et al. 2012).

### 2.6.3 Univariate analysis

A range of linear models were constructed on the basis of the results from the vulnerability assessment. For each category a separate model was constructed under the Generalised Linear Modelling framework – this assumes that Site differences are accounted for by the differences in co-variables (e.g. particle size, current exposure, depth) that characterised the sample/site. The response and predictors for the univariate models are given in 2.4.2.

Over-dispersion (due to very high counts) has been identified as an issue in the SEPA-derived crustacean community data. In Wilding and Black (2015) the approach adopted was to cube-root transform the data and fit a linear mixed model. This approach was necessary because of the complexity of the model being fitted. In the current case the approach was to simplify the analysis by basing it on Statistical Units (thus removing Local Authority and Grab as factors from the analysis, see 2.4.1). This allows simpler (non-mixed) models to be fitted. In order to address over-dispersion negative binomial models were fitted, with a log-link function (O'Hara et al. 2010). The model fitting /refining process was iterative. Initially, all fixed effects were included (see above) and residual patterns examined. Where individual observations had an unusually high-influence (Cook's distance >0.5; McConway et al. 1999) they were removed from the analysis and the model refitted. Once no further outliers were identified, the model was simplified by sequentially removing terms initially on the basis of their low Z-values (approximately <2). Once removed, the residual patterns in the revised model were examined. If the removal of the term had no substantive effect on the residual patterns and resulted in no substantive change (<2 units) or a reduction in AIC, then the term was removed from the model (Zuur et al. 2009). The models were then rebuilt from the opposite direction, i.e. by fitting only the intercept and then adding terms, based on apparent trends in residual patterns (i.e. by adding terms to eliminate patterns in residuals when plotted against covariables). Terms were sequentially added till no apparent trends were observed. AIC-comparisons and Chi-square tests were used to assess the 'significance' of adding the EMB term (Zuur, Ieno et al. 2009).

### 2.7 Summary description of the data analysis

The complete dataset consisted of approximately 160,000 observations, each observation being a record of the abundance of a crustacean taxa (Table 1) from a given survey. Associated with each observation is the Local Authority region, a unique survey identifier, the Site and Distance Class and survey date, the WoRMS assigned higher-level classification (e.g. class to family) and the associated environmental variables (see 2.4.2).

## 3 Results

The results consist of EMB-use and grabbing protocol summaries per Statistical Unit, a summary of combined crustacean assemblages (across all Statistical Units) and the generic results from the Vulnerability assessment. The Results then focus on Shetland Islands data as this Local Authority region was the only one with sufficient data across treated and untreated sites and at a family-level taxonomic discrimination.

### 3.1 Site and EMB-use summary

Data from 100 sites met the matching criteria and 305 surveys were analysed comprising 1210 samples (Table 4). The number of Sites where EMB was used (over the recorded period) was 81; 19 sites were not EMB treated at all over the reported period. Most surveys were conducted following at least some EMB exposure (255 out of 305; Table 4). The mixture of grab sizes used within the same Local Authority region combined with the requirement to eliminate grab-size as a factor in any analysis necessitated splitting the data into the following Statistical Units (defined by Local Authority area and total grabbed area, see 2.4.1): Argyll and Bute & 0.135 $m^2$, Eilean Siar & 0.2 $m^2$ and Shetland Islands & 0.1 $m^2$ (Table 4). Given the small sample sizes for North Ayrshire and Orkney (3 and 10 respectively, based on the 0.135 grab, Table 4), these were not further considered.

Highland did not include any surveys on sites where EMB had not been used and Argyll and Bute and Eilean Siar hosted few untreated sites (Table 4). The cumulative amount of EMB applied across Local Authority regions varied widely; farms in the Highland and Orkney regions were treated with the largest and smallest amounts of EMB respectively whilst patterns of EMB application were similar between Argyll and Bute and Eilean Siar (Table 5). Only those Local Authority regions where there was a reasonable balance between treated and untreated sites were used. This balance only occurred in Shetland where approximately 50% of Surveys were based around sites where EMB was not used (Table 5). In the case of Shetland, there were only two Sites and three surveys conducted at the AZE where EMB was not used with many more samples at higher EMB usage rates (combined 24 surveys conducted in Terciles 3 and 4; Table 7). Inference based on such unbalanced sampling is not robust and, consequently, AZE-based statistical analyses were not conducted. The only data set of sufficient size and balance was, therefore, from Shetland, Reference stations and these data form the main basis of the reported multivariate and univariate statistical analyses.

**Table 4 Sampling and treatment summaries, by Local Authority Region.**

| | | | Biomass (tonnes) | | | Grab area (m$^2$) | | | EMB treatment | | | | Distance Class | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Local Authority | Sites | Surveys | LwrB | MedB | UprB | 0.1 | 0.135 | 0.2 | Z.EMB | T1.EMB | T2.EMB | T3.EMB | CE | AZE | Ref |
| Argyll and Bute | 28 | 98 | 19.1 | 29.7 | 49.0 | 4 | 87 | 7 | 8 | 3 | 37 | 50 | 57 | 185 | 166 |
| Eilean Siar | 22 | 63 | 10.9 | 22.6 | 41.2 | 3 | 25 | 35 | 6 | 4 | 28 | 25 | 33 | 100 | 113 |
| Highland | 19 | 61 | 35.3 | 49.5 | 74.8 | 2 | 14 | 45 | 0 | 0 | 13 | 48 | 27 | 116 | 100 |
| North Ayrshire | 1 | 3 | 53.4 | 57.6 | 62.5 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 3 | 4 | 6 |
| Orkney Islands | 6 | 19 | 25.9 | 34.0 | 38.5 | 9 | 10 | 0 | 11 | 1 | 7 | 0 | 18 | 27 | 37 |
| Shetland Islands | 24 | 61 | 12.5 | 24.2 | 67.6 | 60 | 1 | 0 | 25 | 5 | 18 | 13 | 33 | 68 | 117 |
| Total | 100 | 305 | 17.7 | 34.3 | 53.9 | 78 | 140 | 87 | 50 | 25 | 154 | 76 | 171 | 500 | 539 |

Key: Lwr, Med and Upr refer to lower, middle (=median) and upper quartiles respectively (for Biomass only). PC – total number of production cycles. Grab size comprises the total grab area sampled (using 0.1, 0.045 and 0.02 m$^2$ grabs x number of grabs taken). Under ‘EMB usage’ are recorded the number of surveys based on zero EMB usage (Z.EMB) and usage at >0 <=33.3%, >33.3% <=66.66% and >66.6% (T1.-, T2.- and T3.EMB respectively; terciles (T) based on the complete data set). The number of grab samples analysed, at each distance from the Cage (CE – cage edge, AZE – allowable zone of effects, Ref – reference stations) is shown under ‘Distance class’.

**Table 5 EMB usage (cumulative total, g) by Local Authority Region and across all regions ('Overall).**

| Local Authority | Sites | Surveys | Min EMB | T1.EMB | T2.EMB | Max EMB |
|---|---|---|---|---|---|---|
| Argyll and Bute | 28 | 98 | 0 | 998 | 2280 | 5480 |
| Eilean Siar | 22 | 63 | 0 | 405 | 1950 | 6940 |
| Highland | 19 | 61 | 288 | 2360 | 4070 | 10400 |
| North Ayrshire | 1 | 3 | 2303 | 2890 | 3300 | 3530 |
| Orkney Islands | 6 | 19 | 0 | 0 | 123 | 226 |
| Shetland Islands | 24 | 61 | 0 | 0 | 1110 | 4750 |
| **Overall** | **100** | **305** | **0** | **541** | **2350** | **10400** |

Key: Minimum (Min), first and second tercile break-points (T1.EMB and T2.EMB respectively) and EMB maximum (Max EMB).

**Table 6 Site characterisation by Local Authority. Material > 2mm and <63 µm is expressed as a percentage. Current speed is in cm $s^{-1}$.**

|  | Depth (m) |  |  | Material >2 mm |  |  | Material <63 µm |  |  | Mean current speed |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Local Authority | Lwr | Med | Upr | Lwr | Med | Upr | Lwr | Med | Upr | Lwr | Med | Upr |
| Argyll and Bute | 23 | 27 | 34 | 0 | 2 | 7 | 15 | 31 | 53 | 5.7 | 7.2 | 11.0 |
| Eilean Siar | 18 | 23 | 29 | 0 | 5 | 12 | 20 | 34 | 53 | 5.6 | 7.1 | 8.0 |
| Highland | 26 | 35 | 47 | 0 | 1 | 11 | 19 | 32 | 53 | 5.3 | 6.2 | 7.8 |
| North Ayrshire | 24 | 28 | 36 | 4 | 5 | 10 | 10 | 15 | 24 | 7.7 | 7.7 | 7.7 |
| Orkney Islands | 16 | 18 | 25 | 1 | 4 | 18 | 2 | 5 | 7 | 9.0 | 11.0 | 16.0 |
| Shetland Islands | 18 | 23 | 29 | 2 | 7 | 16 | 2 | 6 | 18 | 4.1 | 5.8 | 13.0 |
| **Mean** | **20** | **26** | **34** | **0** | **3** | **11** | **9** | **24** | **45** | **5.2** | **7.1** | **9.3** |

Key: Lwr, Med and Upr refer to lower, middle (=median) and upper quartiles respectively. These summaries are based on sample grabs for the depth and particle size data and by Site for the current speed.

**Table 7 Number of sites and surveys associated with differing levels of EMB Treatment, Local Authority Region: Shetland Islands, Grab Area = $0.1m^2$.**

| Distance | EMB level | Sites | Surveys |
|---|---|---|---|
| AZE | nil | 2 | 3 |
| AZE | Tercile 1 | 1 | 2 |
| AZE | Tercile 2 | 7 | 11 |
| AZE | Tercile 3 | 9 | 13 |
| Ref | nil | 11 | 25 |
| Ref | Tercile 1 | 4 | 5 |
| Ref | Tercile 2 | 11 | 16 |
| Ref | Tercile 3 | 9 | 13 |

## 3.2 Summary of biotic data – overview of the entire data set

In total 1328 crustacean 'species' were present in the data set (but see 2.2). These species were split between 4 classes, 16 orders (Table 8), 95 families (Table 9) and 168 genera. A majority (>80%) of the crustaceans were Amphipods (Table 8) and, within that order, most (total >50%) in descending order) were members of the families Ampeliscidae, Urothoidae, Corophiidae and Caprellidae (Table 9). Of the 95 crustacean families recorded in the entire data set, only 18 contained>1% of the total crustacea and 37 families contained ≤10 individual records across all surveys (Table 9).

**Table 8 Counts and proportions by Order, Grab area=All, Local Authority=All.**

| Order | Count | Prop | Order | Count | Prop | Order | Count | Prop |
|---|---|---|---|---|---|---|---|---|
| Amphipoda | 25298 | 81.0 | Cumacea | 774 | 2.500 | Mysidacea | 4 | 0.0130 |
| Tanaidacea | 1600 | 5.1 | Sessilia | 183 | 0.590 | Siphonostomatoida | 2 | 0.0064 |
| Leptostraca | 1231 | 3.9 | Pantopoda | 58 | 0.190 | Calanoida | 1 | 0.0032 |
| Decapoda | 1131 | 3.6 | Mysida | 10 | 0.032 | Euphausiacea | 1 | 0.0032 |
| Isopoda | 899 | 2.9 | Scalpelliformes | 8 | 0.026 | Harpacticoida | 1 | 0.0032 |

Note: Pantopoda are arthropods in the class Pycnogonida (sea-spiders) and are included here are they are likely to exhibit a similar response to EMB as crustaceans.

**Table 9 Counts and proportions by Family, Grab area=All, Local Authority=All, Order=All**

| Family | Count | Prop | Family | Count | Prop | Family | Count | Prop |
|---:|---:|---:|---:|---:|---:|---:|---:|---:|
| Ampeliscidae | 5041 | 16.00 | Verrucidae | 89 | 0.290 | Anthuridae | 8 | 0.0260 |
| Urothoidae | 4132 | 13.00 | Acidostomatidae | 69 | 0.220 | Isaeidae | 8 | 0.0260 |
| Corophiidae | 3198 | 10.00 | Calliopiidae | 67 | 0.220 | Munididae | 8 | 0.0260 |
| Caprellidae | 2719 | 8.80 | Bathyporeiidae | 63 | 0.200 | Nannastacidae | 8 | 0.0260 |
| Lysianassidae | 2155 | 7.00 | Cirolanidae | 62 | 0.200 | Scalpellidae | 8 | 0.0260 |
| Photidae | 1588 | 5.10 | Phoxichilidiidae | 58 | 0.190 | Tanaellidae | 8 | 0.0260 |
| Phoxocephalidae | 1415 | 4.60 | Scopelocheiridae | 54 | 0.170 | Atelecyclidae | 7 | 0.0230 |
| Nebaliidae | 1230 | 4.00 | Arcturidae | 53 | 0.170 | Apseudidae | 6 | 0.0190 |
| Tanaopsidae | 1047 | 3.40 | Iphimediidae | 38 | 0.120 | Laomediidae | 6 | 0.0190 |
| Maeridae | 873 | 2.80 | Paratanaoidea incertae sedis | 34 | 0.110 | Corystidae | 5 | 0.0160 |
| Aoridae | 842 | 2.70 | Axiidae | 32 | 0.100 | Cyproideidae | 5 | 0.0160 |
| Oedicerotidae | 594 | 1.90 | Stenothoidae | 31 | 0.100 | Inachidae | 5 | 0.0160 |
| Ischyroceridae | 505 | 1.60 | Munnidae | 25 | 0.081 | Oregoniidae | 5 | 0.0160 |
| Cheirocratidae | 473 | 1.50 | Processidae | 25 | 0.081 | Synopiidae | 5 | 0.0160 |
| Sphaeromatidae | 375 | 1.20 | Idoteidae | 24 | 0.078 | Eriopisidae | 4 | 0.0130 |
| Leucothoidae | 374 | 1.20 | Hippolytidae | 22 | 0.071 | Cressidae | 3 | 0.0097 |
| Diastylidae | 348 | 1.10 | Liljeborgiidae | 22 | 0.071 | Tanaidæ | 3 | 0.0097 |
| Galatheidae | 336 | 1.10 | Ampithoidae | 21 | 0.068 | Caligidae | 2 | 0.0065 |
| Bodotriidae | 295 | 0.95 | Argissidae | 19 | 0.061 | Cancridae | 2 | 0.0065 |
| Atylidae | 287 | 0.93 | Amphilochidae | 16 | 0.052 | Microprotopidae | 2 | 0.0065 |
| Gnathiidae | 261 | 0.84 | Epimeriidae | 12 | 0.039 | Paramunnidae | 2 | 0.0065 |
| Leptognathiidae | 258 | 0.83 | Eusiridae | 12 | 0.039 | Alpheidae | 1 | 0.0032 |
| Melitidae | 254 | 0.82 | Upogebiidae | 12 | 0.039 | Euphausiidae | 1 | 0.0032 |
| Paguridae | 241 | 0.78 | Akanthophoreidae | 11 | 0.036 | Lampropidae | 1 | 0.0032 |
| Polybiidae | 194 | 0.63 | Goneplacidae | 11 | 0.036 | Melphidippidae | 1 | 0.0032 |
| Uristidae | 168 | 0.54 | Callianassidae | 10 | 0.032 | Opisidae | 1 | 0.0032 |
| Porcellanidae | 146 | 0.47 | Carcinidae | 10 | 0.032 | Pandalidae | 1 | 0.0032 |
| Leuconidae | 118 | 0.38 | Mysidae | 10 | 0.032 | Pleustidae | 1 | 0.0032 |
| Gammaridae | 104 | 0.34 | Crangonidae | 9 | 0.029 | Pontellidae | 1 | 0.0032 |
| Dexaminidae | 97 | 0.31 | Leucosiidae | 9 | 0.029 | Pseudocumatidae | 1 | 0.0032 |
| Balanidae | 92 | 0.30 | Majidae | 9 | 0.029 | | | |
| Janiridae | 89 | 0.29 | Nephropidae | 9 | 0.029 | | | |

**Table 10 Counts and proportions by Family, Grab area=All, Local Authority=All, Order= Amphipoda**

| Family | Count | Prop | Family | Count | Prop | Family | Count | Prop |
|---|---|---|---|---|---|---|---|---|
| Ampeliscidae | 5041 | 20.0 | Atylidae | 287 | 1.10 | Argissidae | 19 | 0.07 |
| Urothoidae | 4132 | 16.0 | Melitidae | 254 | 1.00 | Amphilochid | 16 | 0.06 |
| Corophiidae | 3198 | 13.0 | Uristidae | 168 | 0.66 | Epimeriidae | 12 | 0.04 |
| Caprellidae | 2719 | 11.0 | Gammaridae | 104 | 0.41 | Eusiridae | 12 | 0.04 |
| Lysianassidae | 2155 | 8.5 | Dexaminidae | 97 | 0.38 | Isaeidae | 8 | 0.03 |
| Photidae | 1588 | 6.3 | Acidostomati | 69 | 0.27 | Cyproideidae | 5 | 0.02 |
| Phoxocephalid | 1415 | 5.6 | Calliopiidae | 67 | 0.27 | Synopiidae | 5 | 0.02 |
| Maeridae | 873 | 3.5 | Bathyporeiid | 63 | 0.25 | Eriopisidae | 4 | 0.01 |
| Aoridae | 842 | 3.3 | Scopelocheiri | 54 | 0.21 | Cressidae | 3 | 0.01 |
| Oedicerotidae | 594 | 2.4 | Iphimediidae | 38 | 0.15 | Microprotopi | 2 | 0.00 |
| Ischyroceridae | 505 | 2.0 | Stenothoidae | 31 | 0.12 | Melphidippid | 1 | 0.00 |
| Cheirocratidae | 473 | 1.9 | Liljeborgiidae | 22 | 0.08 | Opisidae | 1 | 0.00 |
| Leucothoidae | 374 | 1.5 | Ampithoidae | 21 | 0.08 | Pleustidae | 1 | 0.00 |

### 3.3 Vulnerability assessment

A total of 31 families were assessed in terms of their diet/feeding mode, motility, location in the sediment and larval development. The most common feeding strategy of the families assessed was that of deposit feeding (22/31) considered indicative that the diet was non-living particulate organic matter (POM) with the remainder (9/31) characterised by scavenger/ predatory/parasitic (SPP) dietary preferences. The families were approximately evenly split in terms of their motility; 14/31 were considered sedentary with the others being actively mobile. The majority of families (30/31) lived in/on the sediment surface whilst 26/31 families were characterised by direct-development. Given the dominance by one category in terms of location and larval development these categories were not further considered as they offered little in terms of discriminatory power. In terms of the analysis there were, therefore, four categories based on food (scavenger/predator/parasite (‘SPP’ v. diet of particulate organic matter ‘POM’) and motility. A summary of the by-family designations is given in 8.2 with further detail provided in 8.3.

### 3.4 Assessment by Local Authority Region – Shetland Islands

Only the Shetland Islands hosted a balanced sampling design in terms of number of Sites sampled from EMB treated and untreated locations. However, these data were only available from the

Reference stations (Table 7). The summary data presented here includes that from the AZE but these data are not extended to statistical modelling.

### 3.4.1 General crustacean assemblage description

Patterns of crustacean order abundance, in the Shetlands, were similar to those from the entire data set: Amphipoda (86%) and Tanaidacea (7.4%) dominated the crustacean fauna (Table 11).

**Table 11 Counts and proportions by Class, Grab area=0.1, Local Authority= Shetland Islands combined across all Distance Classes.**

| Class | Count | Prop | Class | Count | Prop | Class | Count | Prop |
|---|---|---|---|---|---|---|---|---|
| Amphipoda | 9026 | 86.0 | Isopoda | 195 | 1.80 | Mysida | 2 | 0.019 |
| Tanaidacea | 777 | 7.4 | Leptostra | 181 | 1.70 | | | |
| Decapoda | 284 | 2.7 | Cumacea | 90 | 0.85 | | | |

Proportions are %.

Patterns of dominance between AZE and Ref stations were similar with 5 to 6 families dominating both. Within this pattern there was one notable difference; the Lysianassidae totals were high at the Reference sites (13% of total) compared to the AZE (<1% of total), however, this was attributable largely to one Site ('HAC1', with 1359 g EMB) and one species (*Socarnes erythrophthalmus,* Robertson, 1892) which recorded 539 individuals over two surveys. This illustrates the extreme variability in these data, with counts routinely differing by two orders of magnitude, per family, between successive surveys (Table 22). The extreme variability in family totals, both between and within Sites is illustrated by examining column totals in Table 22; many families, even the most abundant overall, are completely absent from some Surveys or Sites (e.g. Corophiidae, Lysianassidae and Urothoidae at site COLED1) or differ considerable between surveys at the same site (e.g. Lysianassidae at Site HAC1, Survey 354 v 648; Table 22) .

Bivariate associations between crustacean families and EMB (square-root transformed) showed complex trends. Some families appeared, overall, negatively associated with EMB application with many sites where EMB had not been used hosting crustaceans but with zero counts dominating treated sites (e.g. Maeridae and Urothoidae[2], Figure 1). Other families (e.g. Ampeliscidae, Galatheidae and Tanaopsidae) showed no apparent bivariate relationship with EMB (Figure 1). The frequently observed presence of crustaceans, across several families, at the intermediate EMB treatment of 1359 g (~36 on the x-axis on Figure 1) is primarily due to one Site, HAC1, where crustaceans were particularly common.

**Table 12 Counts and proportions by Family, Grab area=0.1, Local Authority= Shetland Islands Order= All, Distance Class= AZE.**

| Family | Count | Prop | Family | Count | Prop | Family | Count | Prop |
|---|---|---|---|---|---|---|---|---|
| Ampeliscidae | 317 | 26.00 | Maeridae | 10 | 0.81 | Leuconidae | 2 | 0.160 |
| Tanaopsidae | 299 | 24.00 | Lysianassidae | 9 | 0.73 | Majidae | 2 | 0.160 |
| Urothoidae | 203 | 16.00 | Phoxocephalidae | 9 | 0.73 | Porcellanidae | 2 | 0.160 |
| Photidae | 113 | 9.20 | Cheirocratidae | 7 | 0.57 | Acidostomatidae | 1 | 0.081 |
| Corophiidae | 52 | 4.20 | Leucothoidae | 6 | 0.49 | Bodotriidae | 1 | 0.081 |
| Caprellidae | 48 | 3.90 | Polybiidae | 5 | 0.41 | Calliopiidae | 1 | 0.081 |
| Galatheidae | 33 | 2.70 | Gammaridae | 4 | 0.32 | Carcinidae | 1 | 0.081 |
| Nebaliidae | 31 | 2.50 | Argissidae | 3 | 0.24 | Dexaminidae | 1 | 0.081 |
| Oedicerotidae | 18 | 1.50 | Diastylidae | 3 | 0.24 | Inachidae | 1 | 0.081 |
| Paguridae | 17 | 1.40 | Hippolytidae | 3 | 0.24 | Iphimediidae | 1 | 0.081 |
| Aoridae | 11 | 0.89 | Paratanaoidea | 3 | 0.24 | Tanaellidae | 1 | 0.081 |
| Atylidae | 11 | 0.89 | Ischyroceridae | 2 | 0.16 | | | |

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[2] This family is the most commonly observed and was classified as ‘Mobile’. It is likely, therefore, that the subsequent statistical analysis, based on ‘mobile’ crustacea, is highly influenced by this family.

**Table 13 Counts and proportions by Family, Grab area=0.1, Local Authority= Shetland Islands, Order=All, Distance Class= Ref.**

| Family | Count | Prop | Family | Count | Prop | Family | Count | Prop |
|---:|---:|---:|---:|---:|---:|---:|---:|---:|
| Urothoidae | 1865 | 21.00 | Bodotriidae | 66 | 0.750 | Gammaridae | 6 | 0.068 |
| Ampeliscidae | 1604 | 18.00 | Calliopiidae | 50 | 0.570 | Iphimediidae | 6 | 0.068 |
| Corophiidae | 1202 | 14.00 | Polybiidae | 46 | 0.520 | Acidostomatidae | 5 | 0.057 |
| Lysianassidae | 1192 | 13.00 | Gnathiidae | 43 | 0.490 | Eusiridae | 5 | 0.057 |
| Tanaopsidae | 465 | 5.30 | Dexaminidae | 35 | 0.400 | Synopiidae | 5 | 0.057 |
| Maeridae | 343 | 3.90 | Janiridae | 35 | 0.400 | Akanthophoreidae | 3 | 0.034 |
| Caprellidae | 272 | 3.10 | Atylidae | 29 | 0.330 | Argissidae | 3 | 0.034 |
| Photidae | 246 | 2.80 | Paguridae | 26 | 0.290 | Cyproideidae | 3 | 0.034 |
| Phoxocephalidae | 229 | 2.60 | Liljeborgiidae | 20 | 0.230 | Nannastacidae | 3 | 0.034 |
| Aoridae | 152 | 1.70 | Bathyporeiidae | 17 | 0.190 | Cressidae | 2 | 0.023 |
| Nebaliidae | 141 | 1.60 | Hippolytidae | 17 | 0.190 | Diastylidae | 2 | 0.023 |
| Cheirocratidae | 136 | 1.50 | Cirolanidae | 16 | 0.180 | Mysidae | 2 | 0.023 |
| Oedicerotidae | 111 | 1.30 | Porcellanidae | 13 | 0.150 | Paratanaoidea | 2 | 0.023 |
| Uristidae | 108 | 1.20 | Leuconidae | 12 | 0.140 | Anthuridae | 1 | 0.011 |
| Galatheidae | 107 | 1.20 | Munnidae | 9 | 0.100 | Leptognathiidae | 1 | 0.011 |
| Sphaeromatidae | 90 | 1.00 | Ischyroceridae | 8 | 0.090 | Opisidae | 1 | 0.011 |
| Leucothoidae | 82 | 0.93 | Amphilochidae | 7 | 0.079 | | | |

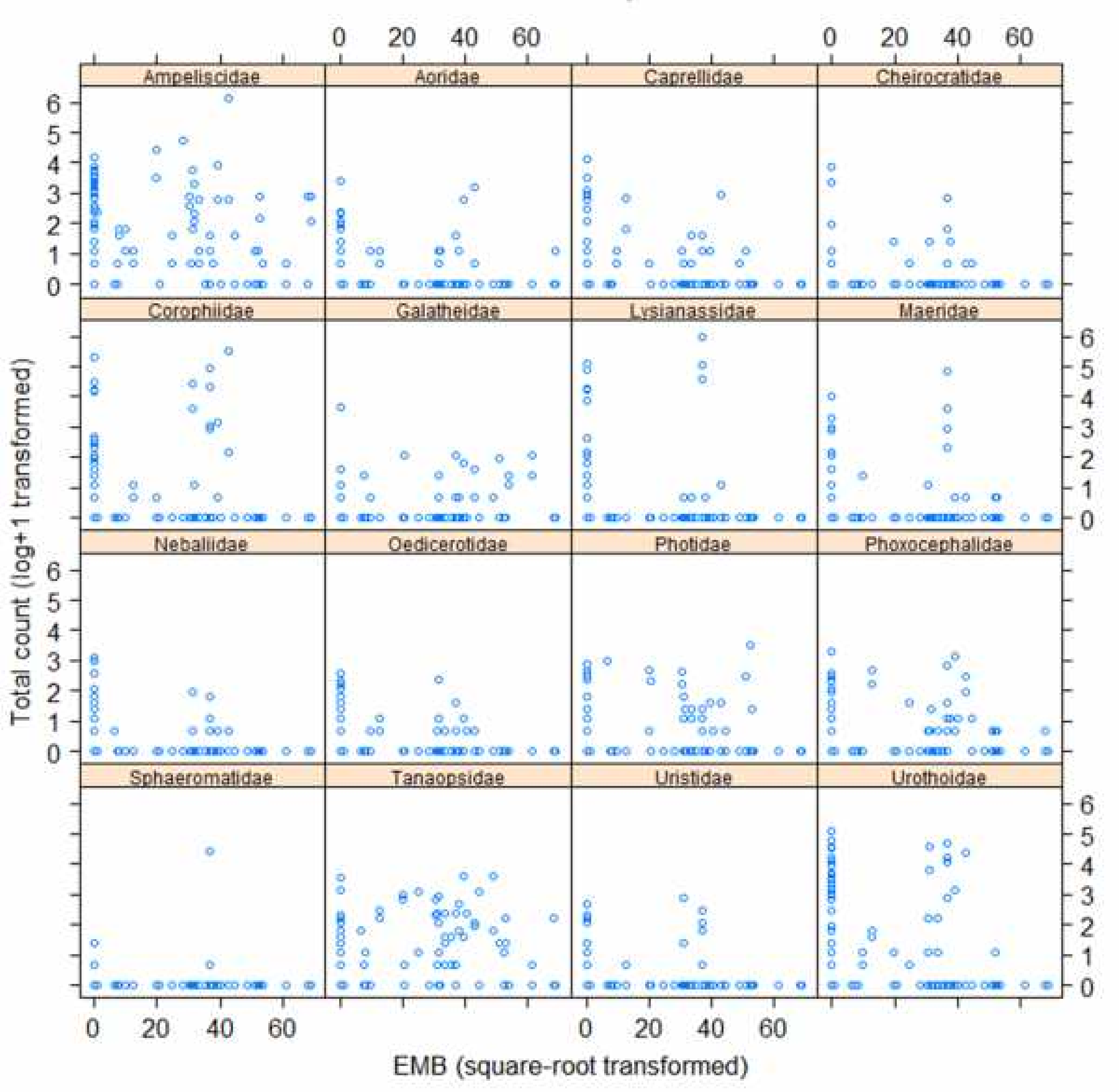


Figure 1 – Relationship between individual families and EMB (square-root transformed). These are data from Shetland Islands, Ref stations only.

### 3.4.2 Multiple dimensional scaling (MDS)

The MDS results for all four levels of top-slicing were stable and species patterns were similar between the 0.5 – 2% top-slicing thresholds (Figure 2). The stress associated with the ordination ranged from 0.20 to 0.13 declining with increased degrees of top-slicing (0.5 – 5%, Figure 2). The stress for the 1% threshold-ordination (upper right in Figure 2) was 0.18 indicating that the ordination should be interpreted with care (Clarke and Warwick 2001). The P-value associated with the permutation test was <0.001 regardless of the top-slicing for rare species (Figure 2) indicating that these data were unlikely to be observed if there was no association between EMB (or correlated variables) and the abundance of crustacean families. This indicates a possible association between EMB use and the abundance of crustacean families. The ordination (1% threshold, Figure 2, upper right) indicates that families Aoridae, Nebaliidae, Uristidae and Urothoidae (the most abundant family) were negatively associated with EMB usage whilst the Tanaopsidae and Galatheidae were positively associated. Other families, including those in the frequently observed Ampeliscidae family, showed little correlation with EMB (i.e. their positions on the ordination were approximately perpendicular to the EMB-arrow). It is worth re-iterating that assigning family-based relationships, based on an MDS with a Stress of 0.18, should be done with caution.

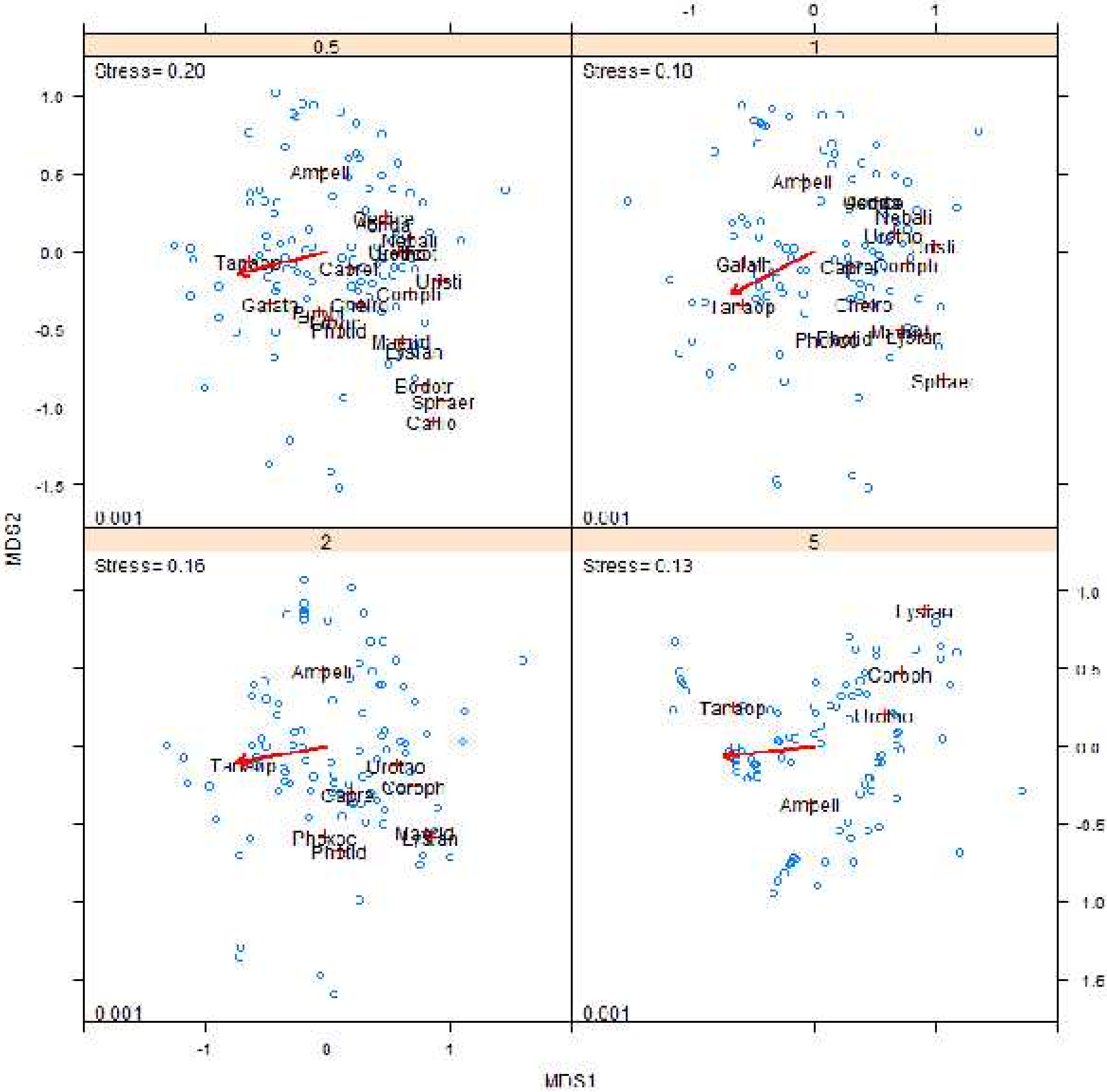


Figure 2 MDS: Shetland Islands, Grab area=0.1, Distance Class=Ref, Taxonomic level=Family. Proportion (%) inclusion is shown in the panel and the 2-D stress is indicated (top left) and P-value associated with the test that there is no correlation between the taxa and EMB (bottom left). The location of each site is shown as a blue dot, red crosses indicate the location of maximal correlation between the Site axes and individual taxa (abbreviated to first 6 characters). The arrow indicates the correlation between the axes and EMB (square-root transformed).

### 3.4.3 Multivariate analysis using 'MVAbund'

Multivariate analysis using MVAbund identified all the environmental variables assessed (EMB, particle size, sample depth and current speed) as being influential in determining the patterns of abundance of families at the Ref station (Table 14).

In terms of the association with all environmental drivers, families Nebaliidae, Oedicerotidae, Caprellidae were ranked 1 to 3 in influence (Table 15) but were relatively unusual within these data and were mostly absent in the presence of EMB (Figure 1).

A Chi-square test, comparing the model with, and without, EMB confirmed its importance to the multivariate pattern (Deviance difference between models including and excluding EMB was 56, P=0.02). When assessed individually, however, there were no substantial associations between any family and EMB (specifically) as indicated by small coefficient and large P-values (Table 16).

**Table 14 Results from MVAbund – residual degrees of freedom (Res.df) , deviance associated with term (proportional to that terms' influence in the multivariate association) and probability (P) of observing the data on the assumption of no association. For an explanation of model terms (Factors) see Table 2.**

| Factor | Res.DF | Dev | P |
|---:|---:|---:|---:|
| SREMB | 110 | 57.3 | 0.008 |
| LT63 | 109 | 105.0 | 0.002 |
| Sample Depth | 108 | 98.9 | 0.004 |
| Current Speed | 107 | 153.0 | 0.026 |
| GT2mm | 106 | 56.0 | 0.044 |

Key: SREMB – EMB usage (square-root transformed), LT63 – proportion of material passing 63 µm sieve (log transformed and centred), Sample Depth- sample depth (m, centred), Current Speed – mean current speed (cm $s^{-1}$, log transformed and centred), GT2mm – proportion of material retained on a 2mm sieve (log transformed, centred). The model was additive (interaction terms were not tested).

**Table 15 Local authority: Shetland Islands, Distance Class: Ref; Taxa Level: Family, Proportion: 1, Model: ~EMB + LT63+Sample Depth + Current Speed and GT2mm. For an explanation of model terms, see Table 2.**

| Taxa | Deviance | Taxa | Deviance | Taxa | Deviance |
|---|---|---|---|---|---|
| Nebaliidae | 18.30 | Sphaeromatidae | 1.4600 | Maeridae | 0.03040 |
| Oedicerotidae | 11.40 | Ampeliscidae | 1.3900 | Corophiidae | 0.02370 |
| Caprellidae | 7.70 | Uristidae | 1.0500 | Photidae | 0.00824 |
| Urothoidae | 5.53 | Aoridae | 0.8820 | Lysianassidae | 0.00285 |
| Cheirocratidae | 4.52 | Phoxocephalidae | 0.8100 | | |
| Tanaopsidae | 4.15 | Galatheidae | 0.0766 | | |

Key: Deviance indicates the degree of importance of a given taxon in determining the multivariate association (as related to the entire model, not just EMB) as shown in Table 14**.** See Table 14 for model terms.

**Table 16 Important EMB related taxa following fitting the model Abundance~ EMB+ SampleDepth+LT63+CurrentSpeed+GT2mm. For an explanation of model terms see Table 2**

| Taxa | Deviance | P-value | Coeff*1000 | Motility | Diet |
|---|---|---|---|---|---|
| Maeridae | 11.675 | 0.100 | 3.570 | Sedentary | POM |
| Galatheidae | 10.385 | 0.126 | 17.100 | Mobile | POM |
| Ampeliscidae | 8.848 | 0.175 | 8.180 | Sedentary | POM |
| Lysianassidae | 6.222 | 0.311 | 2.450 | Mobile | SPP |
| Tanaopsidae | 4.824 | 0.432 | 12.700 | Sedentary | POM |
| Urothoidae | 3.845 | 0.558 | 9.780 | Mobile | POM |
| Oedicerotidae | 1.753 | 0.906 | 3.780 | Mobile | SPP |
| Nebaliidae | 1.727 | 0.906 | -17.200 | Mobile | POM |
| Photidae | 1.594 | 0.906 | 0.263 | Sedentary | POM |
| Cheirocratidae | 1.484 | 0.906 | -7.920 | Mobile | POM |
| Uristidae | 1.477 | 0.906 | 16.400 | Mobile | SPP |
| Caprellidae | 0.802 | 0.913 | -12.400 | Mobile | POM |
| Corophiidae | 0.635 | 0.913 | 37.800 | Sedentary | POM |
| Aoridae | 0.387 | 0.913 | 9.530 | Sedentary | POM |
| Phoxocephalidae | 0.355 | 0.913 | -14.300 | Mobile | SPP |
| Sphaeromatidae | 0.000 | 0.972 | 217.000 | Mobile | POM |

Note: Deviance is a measure of the relative importance of the family in the overall EMB-related changes in multivariate pattern, P-value is the P-value associated with the test of the null hypothesis that there is no association (at the family level), Coeff – individual families' EMB coefficient (*1000). The motility and diet status of each family is also given (see 3.4.4).

### 3.4.4 Vulnerability assessment

There were 17 families in Statistical Unit 'Shetland, 0.1m$^2$' that contributed at least 1% to the overall total crustacean abundance in that Statistical Unit. The total number of crustaceans within each vulnerability category reflected the number of families within each category: there were fewer SPP recorded compared with POM and approximately equivalent records for mobile and sedentary families (Table 17). The mean abundance data reflected the number of surveys with large numbers of surveys being associated with high mean values (Table 17), this relationship being related to highly influential, but unusual extreme values. Only 5 surveys were included between nil and EMB-Tercile 1 and, consequently, this category is not reviewed in the following description. The mean values in Table 17 were highly influenced by single observations (i.e. very high counts within a single family), for example, approximately half of the SPP total, for those in the Tercile 2 category (total=795), came from a single observation (397 individuals; Table 17). Most families within each Vulnerability category were absent though this was less in nil-EMB surveys compare with EMB-Terciles 2 and 3 by between approximately 10 and 15% (Table 17). The presence of substantial outliers in these data considerably influenced the mean values whilst median values were predominantly zero (because most families were absent) making interpretation on the basis of central tendency difficult. With this caveat in mind, the mean values for POM-designated families declined over the EMB treatment range but the opposite trend was observed in relation to motility where sedentary families increased over the EMB range and mobile families declined (Table 17).

These analyses indicate that EMB is associated with changes in crustacean taxa, at Reference stations in Shetland. However, these relationships are complex. Univariate models, based on abundance totals at various Vulnerability criteria (SPP, DOM, Motile, Sedentary) enables an assessment on broader criteria.

**Table 17 Summary across vulnerability criteria for Shetland Islands, Ref station.**

| Vulnerability | EMB Tercile | Total | No. surveys | Maximum | % zero | Mean |
|---|---|---|---|---|---|---|
| SPP | nil | 850 | 25 | 169 | 65 | 34.00 |
| SPP | Tercile 1 | 2 | 5 | 1 | 96 | 0.40 |
| SPP | Tercile 2 | 795 | 16 | 397 | 80 | 49.70 |
| SPP | Tercile 3 | 87 | 13 | 23 | 77 | 6.69 |
| POM | nil | 3454 | 25 | 199 | 62 | 138.00 |
| POM | Tercile 1 | 77 | 5 | 19 | 81 | 15.40 |
| POM | Tercile 2 | 1962 | 16 | 176 | 71 | 123.00 |
| POM | Tercile 3 | 1357 | 13 | 471 | 75 | 104.00 |
| Mobile | nil | 2679 | 25 | 182 | 63 | 107.00 |
| Mobile | Tercile 1 | 18 | 5 | 3 | 87 | 3.60 |
| Mobile | Tercile 2 | 1417 | 16 | 397 | 75 | 88.60 |
| Mobile | Tercile 3 | 251 | 13 | 81 | 80 | 19.30 |
| Sedentary | nil | 1625 | 25 | 199 | 63 | 65.00 |
| Sedentary | Tercile 1 | 61 | 5 | 19 | 83 | 12.20 |
| Sedentary | Tercile 2 | 1340 | 16 | 162 | 72 | 83.80 |
| Sedentary | Tercile 3 | 1193 | 13 | 471 | 71 | 91.80 |

Note the prevalence of outliers in these data. Vulnerability categories: SPP and POM – scavengers/predators/parasites and those consuming predominantly particulate organic matter. Mobile and sedentary refer to motility (see Table 3 for further description). Total – total number of organisms observed, No. surveys – number of surveys, maximum – maximum number of taxa observed, % zero - % of families with no records. Mean – mean crustacean number (per Vulnerability * EMB Tercile combination, consisting of the total/ No. surveys).

### 3.4.5 Environmental associations between vulnerability groups and univariate models

There were four categories of crustaceans as distinguished by diet (POM and SPP) and motility (see 2.5). There was a positive non-linear relationship between abundances of POM and SPP taxa and a positive relationship between both and current speed and coarse material (GT2mm). Negative trends were observed in relationship to increasing fine material (LT63) and, in the case of POM taxa, EMB (Figure 3). Univariate models, however, did not indicate any association between either feeding category and EMB and, consequently, the model outputs are not detailed here. In summary, the models indicated that both POM and SPP family-totals were, independently of other factors, negatively associated with depth and positively associated with current speed. In addition, SPP family-totals were positively associated with coarse material (GT2mm), independently of other factors.

Mobiles and Sedentary taxa were positively associated with each other and the totals were broadly similar (range zero to 400, Figure 4). Mobiles and Sedentary taxa were positively associated with current speed and coarse sediments and negatively associated with increasing EMB, fine-material and sample depth but these associations were stronger for Mobile compared with Sedentary taxa (Figure 4). Deeper samples were also associated with finer sediments and, beyond 30m, with greater EMB use (Figure 4).

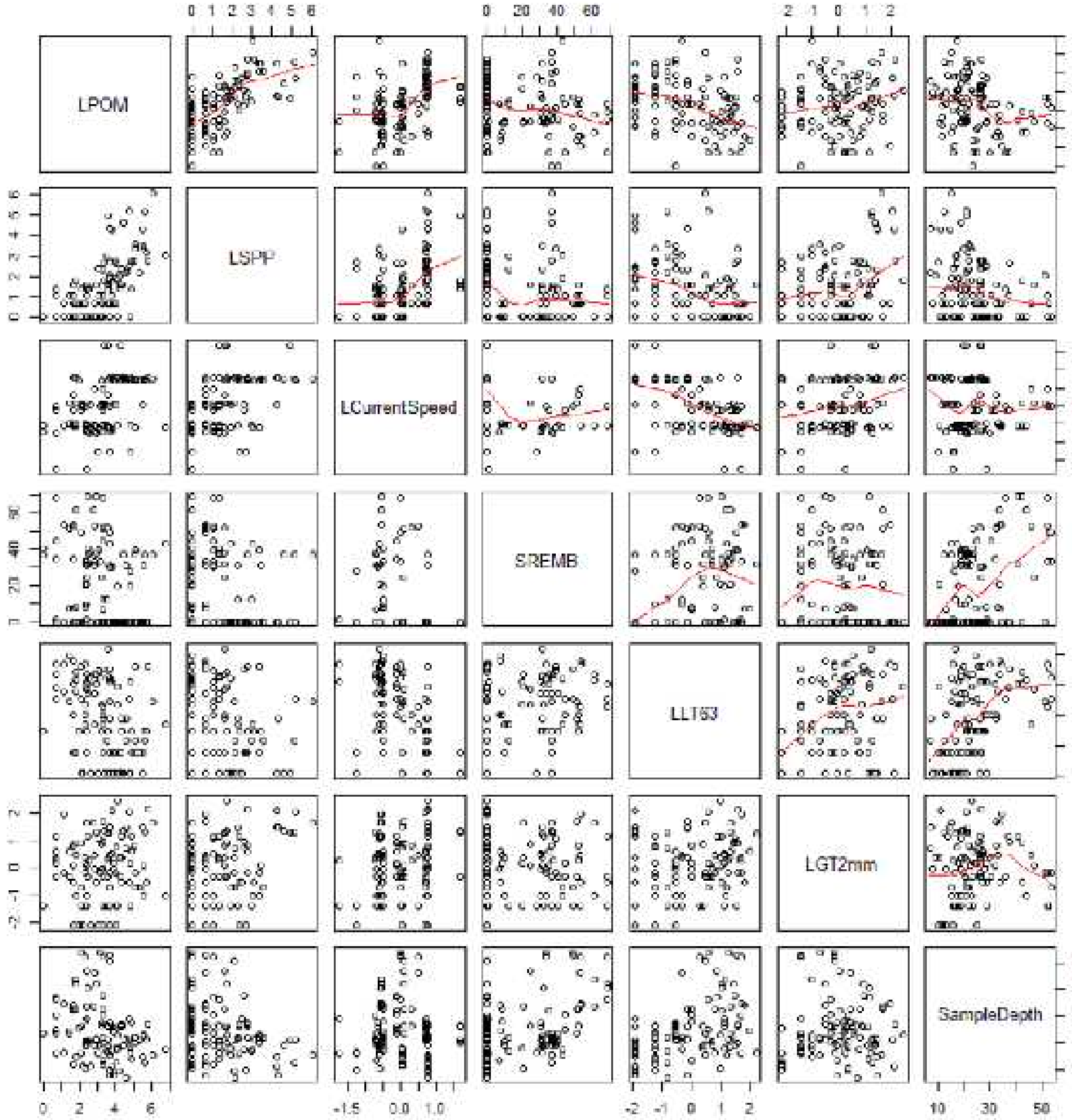


**Figure 3 – bivariate associations between the response, total POM- and SPP family counts (log+1 transformed, LPOM and LSPP respectively) and current speed (LCurrentSpeed), EMB (square-root transformed, SREMB), particle size (LLT63 and LGT2mm) and sample depth (SampleDepth). All co-variables prefixed 'L' are log-transformed and all are centred except sample depth. For an explanation of see Table 2**

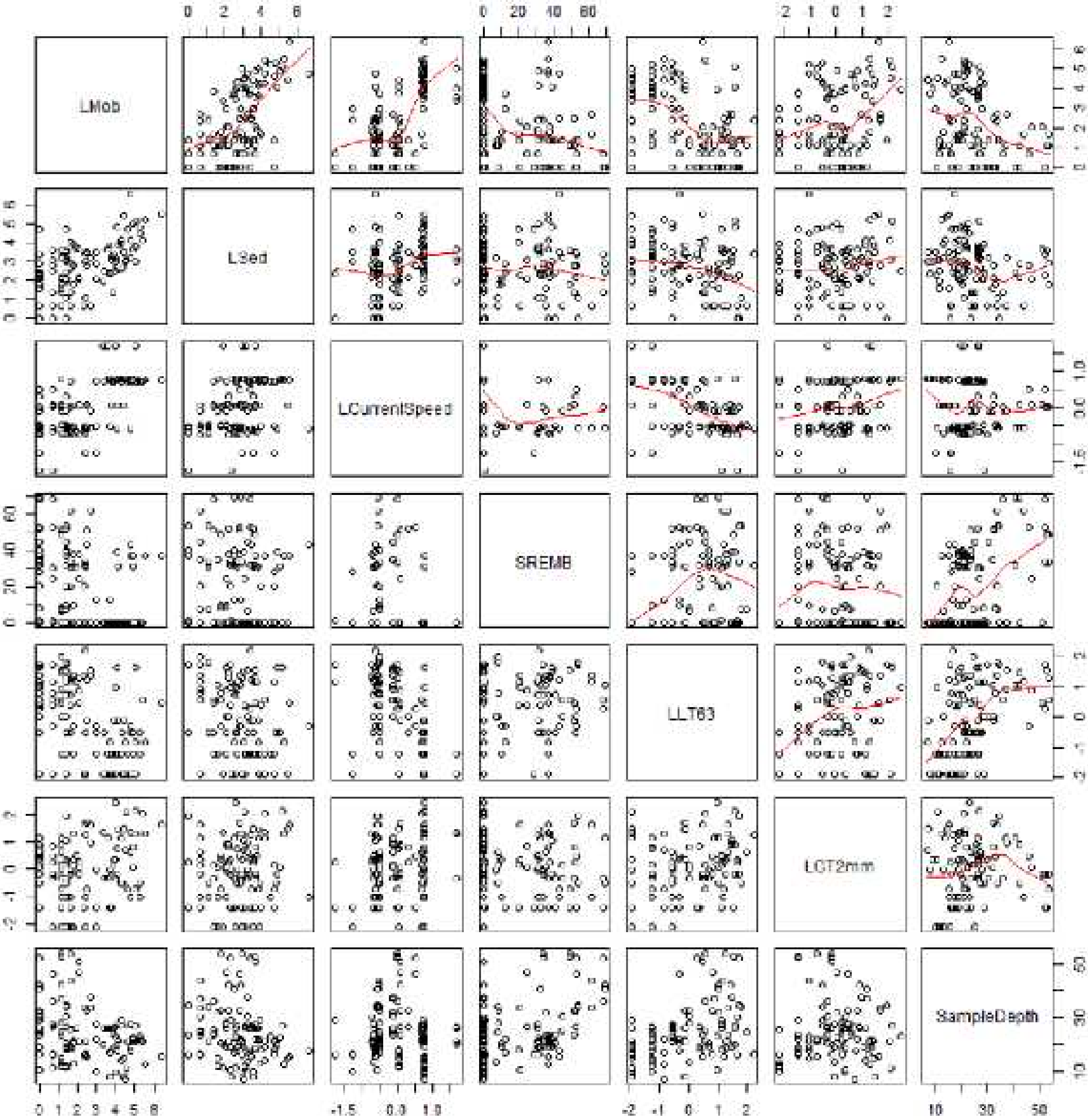


**Figure 4 – bivariate associations between the response, total mobile- and sedentary family counts (log+1 transformed, LMob and LSed respectively) and current speed (LCurrentSpeed), EMB (square-root transformed, SREMB), particle size (LLT63 and LGT2mm) and sample depth (SampleDepth). All co-variables prefixed 'L' are log-transformed and all are centred except sample depth. For further explanation of terms see Table 2**

Mobile taxa had a clear relationship with EMB use and this relationship forms the focus of the results further discussed here. During the model selection /refinement process, with Mobiles as the receptor, four outliers were identified and removed (Table 18). Retaining these outliers changed the model interpretation to one where EMB had no interpretable effect on crustacean abundance. The outliers consisted of higher actual abundances compared with model predictions, within the mobile category (Table 19).

The model (outliers removed) indicates that the effect of 3600 g EMB usage (total per site and the approximate maximum total within the Shetland Islands) would be to reduce mean[3] mobile crustacean abundance from approximately 18 to 7 (with a lower and upper 95% confidence interval of 3 and 15) at average current speed and depth (Table 20).

**Table 18 Outliers identified (and removed) in the derivation of the model shown in Table 20. Detail of the outliers is given in Table 19. For further explanation of model terms see Table 2. Source code: 8. Univariate models.r**

| ID_Survey | SiteID | Rep | Abundance | Model predicted | EMB | Current Speed | Sample Depth |
|---|---|---|---|---|---|---|---|
| 928 | BASS1 | 2 | 117 | 12 | 1833 | -0.6164372 | -7.894783 |
| 647 | BASS1 | 1 | 56 | 10 | 1540 | -0.6164372 | -5.194783 |
| 648 | HAC1 | 2 | 592 | 130 | 1359 | 0.7457596 | -9.694783 |
| 652 | COLED1 | 2 | 14 | 3.8 | 3758 | -0.5474443 | 8.305217 |

---

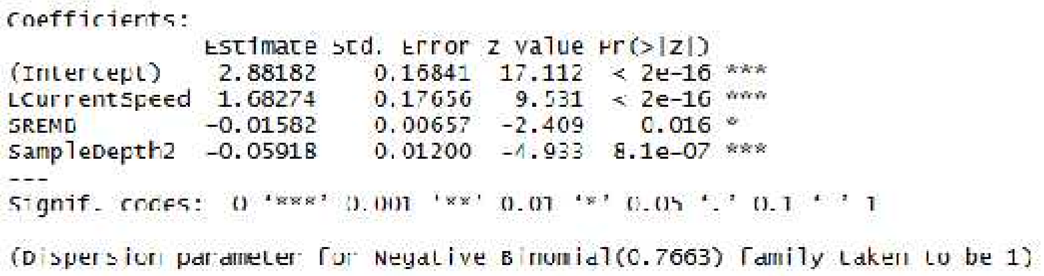
[3] To determine the estimate of the association with SREMB, the coefficient (-0.01582 is multiplied by the square-root of 3600 (treatment rate) = -0.9492. When exponentiated this equals 0.387. According to the model, therefore, the effect of 3600 g EMB treatment is to reduce crustacean abundance by a coefficient of 0.387. The 'baseline' abundance (at average current speed and sample depth) is the intercept exponentiated; =exp(2.88182)=17.8. The model predicts this to be reduced, at 3600 g EMB, by multiplying by 0.387, to an abundance of 6.91.

**Table 19 Outlier detail: the outliers consist of a range of families that contribute to the total across the 'Mobile' category. The totals for each family, within each survey highlighted as being an outlier (Table 18), are shown to indicate the potential outlier-source. The relative amount (Tercile) of EMB for each survey is also shown. FamTotal and Diet are the total crustacean abundance per family and dietary classification respectively.**

| Family | ID_Survey | Tercile | FamTotal | Diet |
|---:|---:|---|---:|---:|
| Caprellidae | 647 | Tercile 3 | 2 | POM |
| Galatheidae | 647 | Tercile 3 | 5 | POM |
| Nebaliidae | 647 | Tercile 3 | 1 | POM |
| Oedicerotidae | 647 | Tercile 3 | 2 | SPP |
| **Phoxocephalidae** | **647** | **Tercile 3** | **22** | **SPP** |
| Polybiidae | 647 | Tercile 3 | 2 | SPP |
| Urothoidae | 647 | Tercile 3 | 22 | POM |
| Caprellidae | 648 | Tercile 2 | 2 | POM |
| Cheirocratidae | 648 | Tercile 2 | 5 | POM |
| Galatheidae | 648 | Tercile 2 | 7 | POM |
| **Lysianassidae** | **648** | **Tercile 2** | **397** | **SPP** |
| Nebaliidae | 648 | Tercile 2 | 1 | POM |
| **Phoxocephalidae** | **648** | **Tercile 2** | **16** | **SPP** |
| Porcellanidae | 648 | Tercile 2 | 4 | POM |
| **Sphaeromatidae** | **648** | **Tercile 2** | **85** | **POM** |
| Uristidae | 648 | Tercile 2 | 7 | SPP |
| Urothoidae | 648 | Tercile 2 | 68 | POM |
| Galatheidae | 652 | Tercile 3 | 7 | POM |
| Paguridae | 652 | Tercile 3 | 6 | POM |
| Porcellanidae | 652 | Tercile 3 | 1 | POM |
| Caprellidae | 928 | Tercile 3 | 18 | POM |
| Cheirocratidae | 928 | Tercile 3 | 1 | POM |
| Galatheidae | 928 | Tercile 3 | 1 | POM |
| Lysianassidae | 928 | Tercile 3 | 2 | SPP |
| Nebaliidae | 928 | Tercile 3 | 1 | POM |
| Oedicerotidae | 928 | Tercile 3 | 1 | SPP |
| **Phoxocephalidae** | **928** | **Tercile 3** | **11** | **SPP** |
| Polybiidae | 928 | Tercile 3 | 1 | SPP |
| Urothoidae | 928 | Tercile 3 | 81 | POM |

Note: Sphaeromatidae (Isopoda) included here contain only one species *Cymodoce truncata.*

**Table 20 Results of GLM (negative binomial, log-link function) for crustaceans classified as mobile.**



```
Coefficients:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)    2.88182    0.16841  17.112  < 2e-16 ***
LCurrentSpeed  1.68274    0.17656   9.531  < 2e-16 ***
SREMB         -0.01582    0.00657  -2.409    0.016 *
SampleDepth2  -0.05918    0.01200  -4.933  8.1e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.7663) family taken to be 1)
```

Key: LCurrentSpeed – mean current speed (cm $s^{-1}$, log transformed and centred), SREMB – EMB usage (square-root transformed), SampleDepth2- sample depth (m, centred). For further explanation of model terms see Table 2.

An assessment of the Mobile-families against EMB terciles indicates which families might be responsible for the relationship indicated in Table 20. The most abundant family, within Shetland Reference stations, are the Urothoidae and these were much more abundant at the nil-EMB treated compared with treated sites (Table 21). In the raw data ('Mobiles'), this relationship (across all familes classificed as Mobile) is reversed, in part, by the outlier family Lysianassidae which were more prevalent in the treated compared with untreated stations (Table 21) and which were removed (Table 18) prior to the univariate analysis presented in Table 20.

**Table 21 Mean total, by family and tercile, within the 'Mobile' vulnerability group. Tercile 1 is excluded from this summary because very few observations were made at this EMB level.**

| Family | EMB Tercile | Total | No. surveys | Maximum | % zero | Mean |
|---:|---:|---:|---:|---:|---:|---:|
| Urothoidae | nil | 1332 | 25 | 163 | 24 | 53.28 |
| Lysianassidae | nil | 535 | 25 | 168 | 67 | 21.40 |
| Caprellidae | nil | 209 | 25 | 62 | 65 | 8.36 |
| Nebaliidae | nil | 123 | 25 | 21 | 45 | 4.92 |
| Phoxocephalidae | nil | 119 | 25 | 26 | 61 | 4.76 |
| Cheirocratidae | nil | 102 | 25 | 47 | 63 | 4.08 |
| Oedicerotidae | nil | 83 | 25 | 12 | 51 | 3.32 |
| Sphaeromatidae | nil | 4 | 25 | 3 | 96 | 0.16 |
| Lysianassidae | Tercile 2 | 654 | 16 | 397 | 84 | 40.88 |
| Urothoidae | Tercile 2 | 425 | 16 | 108 | 52 | 26.56 |
| Sphaeromatidae | Tercile 2 | 86 | 16 | 85 | 94 | 5.38 |
| Phoxocephalidae | Tercile 2 | 58 | 16 | 16 | 61 | 3.62 |
| Caprellidae | Tercile 2 | 37 | 16 | 16 | 68 | 2.31 |
| Cheirocratidae | Tercile 2 | 29 | 16 | 16 | 81 | 1.81 |
| Oedicerotidae | Tercile 2 | 23 | 16 | 10 | 71 | 1.44 |
| Nebaliidae | Tercile 2 | 15 | 16 | 6 | 84 | 0.94 |
| Urothoidae | Tercile 3 | 105 | 13 | 81 | 88 | 8.08 |
| Phoxocephalidae | Tercile 3 | 52 | 13 | 22 | 54 | 4.00 |
| Caprellidae | Tercile 3 | 23 | 13 | 18 | 85 | 1.77 |
| Cheirocratidae | Tercile 3 | 5 | 13 | 3 | 88 | 0.38 |
| Oedicerotidae | Tercile 3 | 4 | 13 | 2 | 88 | 0.31 |
| Lysianassidae | Tercile 3 | 3 | 13 | 2 | 92 | 0.23 |
| Nebaliidae | Tercile 3 | 2 | 13 | 1 | 92 | 0.15 |
| Sphaeromatidae | Tercile 3 | 0 | 13 | 0 | 100 | 0.00 |

Note: for the sake of brevity, this table only includes those families which make up at least 3% of the total abundance per tercile, across all Terciles. Tercile 1 is omitted because of the small sample size.

# 4 Discussion

The data analysis presented here focusses only on data from Shetland Islands, Reference stations and, consequently, the results are only directly applicable to this Local Authority Region. The rationale behind this is described (below), followed by a discussion of plausible effects of EMB on individual families and vulnerability groups and knowledge gaps. Recommendations for further research are also presented.

## 4.1 Data quality and scope

The analysed SEPA database was large, extending over 8 years (2006 – 2014), covered the entirety of Scottish salmon production benthic compliance monitoring and included ~160,000 crustacean records. In terms of assessing multivariate patterns in crustacean response to increasing levels of EMB usage, the database exhibited two main challenges: firstly different grabbing protocols were used, variously, in different Local Authority areas and, secondly within each combination of Local Authority area and grabbing protocol, there was, in most cases, a severe imbalance in sampling effort across different levels of EMB usage and Distance Class. These issues meant that the only data that could support a robust analysis was that of Shetland and at the Reference stations only. The bulk of the results and this discussion (except 4.2 below) focus only the 'Shetland, 0.1m$^2$ grab total area' Statistical Unit.

## 4.2 Plausible direct and indirect effects of EMB

There are a number of plausible indirect and direct mechanisms by which EMB might change macrobenthic communities, including non-target crustaceans, around fish farms.

In terms of indirect effects, many organisms that are not recorded on the SEPA database (i.e. the meiobenthos; fauna <1 mm) might be expected to show EMB sensitivity and these are linked, via the food web, to the macrobenthos. EMB is highly effective against nematode pests (Takai et al. 2000, Cheng et al. 2015) in terrestrial applications and it is possible that this toxicity extends to marine nematodes. Changes in nematode assemblage in relation to fish farming is well recorded (Mirto et al. 2002, Mirto et al. 2014) but these effects have only been linked to organic enrichment attributable to faecal deposition. The effects of EMB on meiobenthic assemblages and the consequences of any associated change to the macrobenthos, and whether such changes cause meaningful change to ecosystem function and services remain unknown.

The direct effects of EMB will be determined by the degree of sensitivity and degree of exposure to the chemical for individual organisms. EMB exposure at levels occurring around fish-farms could, conceptually, induce direct mortality, altered fitness (e.g. in reproductive capacity) and/or illicit a behavioural (escape) response. There are currently no systematic data on the inter-species variation in sensitivity to EMB, at least in relation to the crustacean species recorded in the SEPA database or any assessment of susceptibility of different life-stages within a given taxa. EMB exposure might be influenced, at least in part, by behavioural traits that occur at the species- to family-level; de-la-Ossa-Carretero et al. (2012) concluded that species that lived in biogenic-tubes were less sensitive than burrowing forms to sewage pollution and that surface deposit and suspension feeders were less sensitive than sub-surface deposit feeders. However, the mechanisms for any difference in groups assigned on this basis (habit and diet) was likely to be related to sewage-breakdown (e.g. oxygen concentration) and it is not known if the difference in sensitivity applies to EMB exposure.

The direct effects of EMB (mortality, altered fitness, escape) which are largely unknown, will combine to give a complex assemblage-level responses to EMB that are likely to change with the degree and timing/duration of EMB exposure. The apparent (measured) response as estimated as part of SEPA-consent monitoring (i.e. as generating the data analysed here) may be highly influenced by the timing of the sampling event with respect to the last and previous treatment (timing and amount) and this is likely to be influenced by the nature of the receiving environment (e.g. current exposure, particle size) among other factors. Modelling such complicated interactions/ interrelationships requires a very large and well-balanced data set and that was not available in the current analysis.

### 4.3 General assessment of crustacean assemblage and vulnerability

The crustacean assemblage, across all Local Authority regions, was dominated by amphipods (>80%), most of which were from families Ampeliscidae, Urothoidae and Corophiidae. Ampeliscids and Corophiids typically live in tubes that they construct from fine particles, whereas Urothoids live freely amongst the grains in coarse sediments.

Vulnerability was assessed at the family level; assessment at lower-level taxa (e.g. genus) was possible but family-level analysis has been shown to lose no discriminative power (Warwick 1988) and is more tractable (Warwick 1993). A total of 31 families were evaluated in terms of their vulnerability as a function of location in the sediment, motility, feeding mode/diet and development. It was considered that those species that dwelt on the sediment surface, were sedentary and had a diet of particulate organic matter (e.g. faecal material containing EMB) would be exposed to greater levels of EMB compared with those that were free-living (i.e. that could more readily move away from exposure) and/or adopted a primarily scavenging/predatory or parasitic lifestyle. We also considered that populations exhibiting direct-development (i.e. brooders) would be more vulnerable to chronic exposure because re-establishment times might be expected to be greater compared with those groups that had a dispersive larval stage. Very few families were characterised by a planktotrophic-recruitment or dwelt anywhere other than on or near the sediment surface/shallowly buried making these criteria redundant in assessing vulnerability. Our analysis indicated that motility (motile and sedentary) and diet (particulate organic matter (POM) and other (mostly scavenging/predatory/parasitic) were represented by sufficient numbers to enable viable analyses (discussed below). It should be noted that a low degree of 'vulnerability' might be reflected in a large negative response to EMB exposure, for example, mobile species might be less vulnerable to EMB if they can move away from contaminated areas.

## 4.4 Associations between environmental drivers and family/vulnerability groups

There were five environmental parameters (EMB usage, current speed, sample depth and two measures of particle size) that were assessed in terms of their independent effect on crustacean abundances. In Shetland, there were intuitive but often weak associations between environmental drivers: coarse sediments (in terms of material <63um) tended to be shallower, subject to higher-current flows (but see caveat in 2.4.2 ), and these shallow, coarse sediments tended to host greater numbers of crustaceans.

The MDS ordinations were associated with a high-level of stress indicative that patterns in crustacean abundance, even following 4th root transformations, were complex (i.e. could not be represented well in 2-dimensions). The MDS plots suggested that the relatively common families Tanaopsidae (5.3%) and Urothoidae (21 %) exhibit the opposite association (positive and negative respectively) with EMB or with a factor that correlates with EMB. These families were characterised as consuming POM but distinguished by motility with Tanaopsidae being sedentary and Urothoidae consisting predominantly of species that are freely mobile (thus concurring with the univariate analysis). The multivariate MVAbund routine indicated that there were patterns in abundance of crustacean families that were associated with all the environmental drivers assessed (EMB, particle size, sample depth and current speed) with families Nebaliidae and Oedicerotidae exhibiting the greatest overall response. MVAbund assesses multivariate patterns (i.e. across all levels of a given taxa) and, consequently, has greater power to detect changes in patterns associated with environmental drivers. Whilst EMB-use was associated with multivariate changes in family abundance MVAbund was not able to show, on an individual level, which of these families was linked to EMB usage. This reduction of power, when assessing individual taxa (as here) is normal when utilising techniques such as MVAbund (Wang, Naumann et al. 2012).

## 4.5 Univariate analyses and interpretation

Models where there are many measured predictors (as here) offer greater potential explanatory power, particularly where interaction terms are included. A fully fitted model (all main effects and interactions) with 5 predictors (as here) would be required to estimate 31 parameters. More complex models (i.e. those including interactions) require a commensurately greater number of observations (at least 20 observations per model term is recommended) otherwise the model becomes unstable. In the current case, with only 115 observations, fitting interaction terms was not advisable and only models with main-effects are reported. Interaction terms can be of considerable interest where the objective is to determine the degree to which one factor (e.g. EMB) is moderated by another (e.g. sediment characteristics). However, in the current analysis, the main interest was to establish if EMB-treatment was linked to abundance in any way. The testing of main effects only, without interactions, allowed this.

Model fitting requires the assessment of outliers (an outlier being an observation which is very different from that expected by the model based on all the observations). In the current case, outliers were identified and these were all from EMB-treated sites where larger-numbers of crustaceans were recorded than expected by the model. Where possible, outliers should be assessed e.g. in terms of recording errors and other explanatory factors. In the current case, the historical nature of the SEPA database precluded these checks. Inclusion of the outliers changed the interpretation of the model, at least in relation to EMB use, from where there is a meaningful EMB-effect to one where there is not a meaningful effect. The largest outliers were attributable to the unexpected abundance of members of the Lysianassidae. These are primarily free-ranging scavenging amphipods that will be very actively attracted to carrion (Moore et al. 1995, Ide et al. 2006) and, consequently, can be expected to exhibit extreme distributional-patchiness. This also applies to members of the Phoxocephalidae (mostly carrion eaters) and potentially to mobile deposit-feeding taxa sensitive to local variation in sediment food quality (i.e. attracted to food sources). Other taxa, such as some isopods, show a collective reproductive behaviour (a single male with a harem of females) (Upton 1987) which will also increase the patchy distribution of crustaceans. Future analyses should be based, *a priori,* around families that are less likely to be so patchily distributed (de-la-Ossa-Carretero, Del-Pilar-Ruso et al. 2012) and this would exclude mobile scavengers.

The data analysed here only supported a comparison between the abundances of families categorised as mobile and immobile and those separated based on diet (particulate organic matter and predators/scavengers). In the analysis presented here only mobile crustaceans were found to be meaningfully linked to EMB treatment (following exclusion of outliers, see above). The Mobiles-model indicated that, independently of all other factors in the model, the association between EMB and mobile-crustaceans was negative. However, this analysis, as with all observational data analyses, cannot prove a causative relationship between EMB use and mobile-crustaceans as the association could occur because of a factor associated with EMB use, which was not included in the model. Equally, this analysis does not provide evidence for there being no relationship between crustaceans adopting differing feeding modes and EMB exposure nor that sedentary crustaceans are in any sense better adapted to EMB exposure or less exposed.

Numerically, mobile crustaceans were dominated by the Urothoidae (constituting 21% of the crustacean fauna at Shetland, Ref-stations) and, consequently, it is this family that is likely to be negatively associated with EMB treatment. There are a number of responses that individuals and populations of mobile crustaceans might be expected to exhibit in relation to sediments exposed to periodic EMB. At the individual level crustaceans might be expected to move away from exposure, potentially returning once the exposure has declined. Lobsters (*Homarus americanus*) have been shown to reject EMB-containing feed (Waddy et al. 2007), suggesting that mobile crustaceans that consume POM may choose to move to areas that are not contaminated. However, whether such dietary preferences in lobsters are applicable to other crustaceans (e.g. members of Urothoidae) is open to speculation; there is no evidence as to whether macrobenthic crustaceans are able to perceive and respond to EMB at sub-lethal concentrations such as those that may occur around fish farms.

Nothing is known about the rapidity, or extent, of crustacean-defaunation following EMB exposure and the rate of return of mobile taxa including macro-crustaceans. In the advent of a toxic event occurring, resulting in the decline of all crustacean groups, mobile species might reasonably be expected to more rapidly re-colonise previously contaminated sediments (de-la-Ossa-Carretero, Del-Pilar-Ruso et al. 2012). Langford et al. (2014) conclude that EMB use in Norway might pose a risk to shrimp and any other species undergoing a moulting cycle and the data presented here suggest an approximate halving of mobile crustacean abundance in association with chronic and heavy use of EMB at reference stations. Whilst the occurrence of EMB residues around fish-farms using EMB is now well founded e.g. (Langford, Øxnevad et al. 2014, Tucca et al. 2017), we do not have information concerning the actual concentrations of EMB in the sediments around the fish-farms in this study, nor is there sufficient understanding of the persistence, or re-distribution of EMB around farms following treatment, in order to predict this. In the current analyses, the likely exposure to

EMB, at Reference stations, will vary widely depending on the Site given that the Reference-station to farm distance varied considerably and there are site-specific hydrological factors.

The results presented provide little information on any association between EMB use and feeding preference in crustaceans. The occasional high abundances of scavengers (e.g. Lysianassidae) at sites where EMB had been used might be for several reasons including (i) EMB has a negligible effect on scavenging species (because they are not exposed or exposure is not meaningfully detrimental at the exposure concentrations ) or (ii) because the particular sites where large numbers of scavengers were recorded were not exposed to high EMB levels, at least at about the time the data were collected. The absence of contemporaneous and historical residue data, with the macrobenthic data, mean that explanations for observed patterns remain speculative.

# 5 Summary and conclusions

The SEPA database consists of consent-compliance records from all salmon farms in Scotland. In the database, farms are categorised according to their local authority (LA) region and the grab-size used in their benthic sampling consent-monitoring. In the current analysis, it was decided to conduct separate analyses for each LA region and grab-size combination. In order to conduct a robust assessment of any EMB-crustacean association, there need to be records from around farms that have used the full spectrum of EMB, including no use. In the current case, the only data that supported a robust analysis was from Shetland and here there was only sufficient data from the Reference stations. These data (Shetland, Reference stations) formed the basis of the subsequent analysis.

The SEPA database contained records of over 1300 crustacean species (168 genera). These species were successfully assigned to their higher-level taxonomy by cross-referencing to the WoRMS database. Each sample was also associated with measures of particle size, the depth and current exposure (co-variables) and total site-EMB use, prior to the survey. Family-level abundances (i.e. combined across lower level taxa) were determined and formed the response variable in the multivariate analysis.

Relationships between patterns of family-abundance and the environmental-covariables and site-EMB use were assessed. There was good evidence that all covariables influenced patterns of family abundance and this included EMB. However, the analysis did not indicate a meaningful relationship between any particular family and EMB use.

Each crustacean family was then categorised into four groups based on the family's predominant species' dietary preferences (scavenging/predatory/parasitic and particulate organic matter) and motility (mobile and sedentary). The total crustacean abundance, across each category, was determined. Subsequent analysis did not indicate a meaningful relationship between site-EMB use and crustaceans classified as sedentary or across either of the dietary preference categories. The analysis did reveal the presence of major outliers in the response variables when considering 'mobile' crustaceans. The presence of the outliers changed the interpretation of the results; the model including the outliers indicated that there was no meaningful association between EMB and mobile crustaceans whilst excluding the outliers indicated that there was a negative association between EMB and mobile crustaceans. The outliers consisted of exceptionally high counts of mobile scavengers and some social species and these were found at sites where EMB had been used. Scavengers and social species will be very patchily distributed, for example, around carrion in the case of scavengers and it is, therefore, reasonable to consider excluding them from the analysis. The analysis indicates that, at average conditions, high EMB treatment will be associated with a halving of mobile crustaceans, at Reference stations in Shetland (following exclusion of scavenging/social species). These data are observational and it is possible that any association is not indicative of a causal relationship. Further research, summarised below, is necessary.

# 6 Recommendations for further research

The data presented here indicate a possible reduction in mobile crustacean species as a consequence of EMB use. However, these data are observational and, consequently, caution should be applied linking cause (EMB use) and effect (non-target crustacean decline). Further field-based assessments should focus on linking cause and effect by assessing EMB exposure (by measuring EMB-sediment concentrations) and linking that to crustacean (and possibly nematode) assemblage via a dedicated grabbing programme. The field-based studies should be supported with laboratory/aquaria based studies based on dosing established crustacean communities varying amounts of EMB and monitoring the crustacean response. The analyses presented here are based on the macrobenthos (animals retained on a 1 mm sieve) yet a majority of crustaceans and nematodes are meiobenthic making this group potentially more sensitive in terms of monitoring change. We recommend that a metagenomics approach, with a specific focus on crustaceans and possibly nematodes, be used to assess changes in biodiversity associated with EMB use in both laboratory and field-based assessments.

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# 8 Appendices

## 8.1 Variability in crustacean abundance by Site and ID_Survey.

**Table 22 Variability in crustacean family counts by Site and ID_Survey. Families included are those that represent the top five in terms of overall abundance. Key: SiteID – unique site identifier, ID_Survey- unique survey identifier, EMB – total amount of EMB applied prior to the macrobenthic survey.**

| SiteID | ID_Survey | EMB | Ampeliscidae | Corophiidae | Lysianassidae | Tanaopsidae | Urothoidae |
|---|---|---|---|---|---|---|---|
| BASS1 | 310 | 997 | 35 | 2 | 0 | 25 | 0 |
| BASS1 | 647 | 1540 | 65 | 23 | 0 | 40 | 22 |
| BASS1 | 928 | 1833 | 471 | 251 | 2 | 13 | 81 |
| BEL1 | 892 | 2728 | 2 | 0 | 0 | 2 | 2 |
| BMS3 | 511 | 0 | 28 | 12 | 132 | 9 | 35 |
| BMS3 | 1048 | 0 | 21 | 4 | 0 | 1 | 54 |
| BOA1 | 657 | 94 | 7 | 0 | 0 | 0 | 3 |
| BOA1 | 924 | 157 | 3 | 3 | 0 | 19 | 9 |
| BURK1 | 328 | 0 | 21 | 6 | 0 | 2 | 38 |
| BURK1 | 495 | 0 | 18 | 6 | 0 | 0 | 151 |
| BURK1 | 752 | 0 | 50 | 64 | 9 | 4 | 182 |
| BURK1 | 933 | 0 | 92 | 7 | 15 | 9 | 171 |
| COLED1 | 416 | 2879 | 1 | 0 | 0 | 0 | 0 |
| COLED1 | 652 | 3758 | 1 | 0 | 0 | 1 | 0 |
| COLED1 | 887 | 4746 | 24 | 0 | 0 | 0 | 0 |
| COLEN1 | 419 | 0 | 0 | 0 | 0 | 1 | 0 |
| COLEN1 | 695 | 424 | 0 | 0 | 0 | 1 | 0 |
| COLEN1 | 886 | 1023 | 16 | 0 | 0 | 0 | 0 |
| EAIT1 | 522 | 42 | 0 | 0 | 0 | 5 | 0 |
| GVAL1 | 702 | 951 | 2 | 0 | 1 | 11 | 10 |
| GVAL1 | 936 | 1353 | 0 | 0 | 0 | 10 | 0 |
| HAC1 | 354 | 972 | 48 | 120 | 0 | 2 | 141 |
| HAC1 | 648 | 1359 | 4 | 93 | 397 | 0 | 176 |
| HAC1 | 912 | 1359 | 2 | 162 | 255 | 1 | 75 |
| HSND1 | 403 | 397 | 117 | 1 | 0 | 35 | 2 |
| HSND1 | 699 | 1115 | 2 | 0 | 1 | 11 | 10 |
| HSND1 | 937 | 1624 | 0 | 0 | 0 | 10 | 0 |
| LGEO2 | 790 | 927 | 29 | 0 | 0 | 25 | 0 |
| LINB1 | 330 | 0 | 1 | 0 | 0 | 0 | 0 |
| LINB1 | 883 | 1433 | 2 | 0 | 1 | 19 | 0 |
| LING1 | 256 | 0 | 27 | 0 | 0 | 0 | 0 |
| LING1 | 449 | 609 | 5 | 0 | 0 | 23 | 2 |
| LING1 | 926 | 2596 | 2 | 0 | 0 | 6 | 0 |
| MAN3 | 236 | 1 | 10 | 0 | 0 | 0 | 0 |
| NWW1 | 145 | 0 | 3 | 1 | 169 | 0 | 7 |
| NWW1 | 496 | 0 | 18 | 6 | 0 | 0 | 151 |
| NWW1 | 705 | 0 | 34 | 71 | 0 | 4 | 110 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| NWW1 | 973 | 0 | 18 | 11 | 7 | 7 | 115 |
| OLN2 | 418 | 55 | 1 | 0 | 0 | 1 | 0 |
| OLN2 | 996 | 65 | 9 | 0 | 0 | 2 | 0 |
| SETN1 | 975 | 2787 | 25 | 0 | 0 | 11 | 0 |
| SETW1 | 314 | 1120 | 17 | 0 | 0 | 7 | 0 |
| SETW1 | 658 | 2374 | 0 | 0 | 0 | 42 | 0 |
| SETW1 | 980 | 4640 | 17 | 0 | 0 | 8 | 0 |
| SNA1 | 390 | 0 | 52 | 1 | 2 | 0 | 2 |
| SNA1 | 640 | 800 | 115 | 0 | 0 | 0 | 0 |
| TAI1 | 448 | 0 | 4 | 0 | 0 | 27 | 0 |
| TAI1 | 738 | 1237 | 0 | 0 | 0 | 5 | 0 |
| TAI1 | 1018 | 1983 | 4 | 0 | 0 | 21 | 0 |
| TUR1 | 133 | 0 | 53 | 4 | 0 | 0 | 30 |
| TUR1 | 504 | 0 | 24 | 4 | 0 | 0 | 25 |
| TUR1 | 819 | 0 | 12 | 1 | 0 | 0 | 6 |
| TUR1 | 972 | 0 | 5 | 199 | 69 | 0 | 2 |
| WOB1 | 366 | 0 | 1 | 25 | 76 | 0 | 3 |
| WOB1 | 654 | 0 | 19 | 95 | 47 | 35 | 85 |
| WOB1 | 947 | 0 | 10 | 16 | 6 | 10 | 34 |
| WOG1 | 674 | 0 | 46 | 12 | 3 | 0 | 61 |
| WOG1 | 881 | 0 | 11 | 2 | 0 | 0 | 70 |

## 8.2 Summary of assigned motility and feeding mode by family.

**Table 23 – Assigned mortality and feeding mode by family. A Motility score of 0 and 1 indicates freely mobile and sedentary designations respectively while Diet scores of 0 and 1 indicate classification into POM and predatory /scavenger dietary preferences respectively.**

| Family | Count | % | Motility | Diet |
|---|---|---|---|---|
| Lysianassidae | 2155 | 7 | 0 | 1 |
| Phoxocephalidae | 1415 | 4.6 | 0 | 1 |
| Oedicerotidae | 594 | 1.9 | 0 | 1 |
| Gnathiidae | 261 | 0.84 | 1 | 1 |
| Polybiidae | 194 | 0.63 | 0 | 1 |
| Uristidae | 168 | 0.54 | 0 | 1 |
| Acidostomatidae | 69 | 0.22 | 1 | 1 |
| Phoxichilidiidae | 58 | 0.19 | 0 | 1 |
| Scopelocheiridae | 54 | 0.17 | 0 | 1 |
| Ampeliscidae | 5041 | 16 | 1 | 0 |
| Urothoidae | 4132 | 13 | 0 | 0 |
| Corophiidae | 3198 | 10 | 1 | 0 |
| Caprellidae | 2719 | 8.8 | 0 | 0 |
| Photidae | 1588 | 5.1 | 1 | 0 |
| Nebaliidae | 1230 | 4 | 0 | 0 |
| Tanaopsidae | 1047 | 3.4 | 1 | 0 |
| Maeridae | 873 | 2.8 | 1 | 0 |
| Aoridae | 842 | 2.7 | 1 | 0 |
| Ischyroceridae | 505 | 1.6 | 1 | 0 |
| Cheirocratidae | 473 | 1.5 | 0 | 0 |
| Sphaeromatidae | 375 | 1.2 | 0 | 0 |
| Leucothoidae | 374 | 1.2 | 1 | 0 |
| Diastylidae | 348 | 1.1 | 1 | 0 |
| Galatheidae | 336 | 1.1 | 0 | 0 |
| Bodotriidae | 295 | 0.95 | 1 | 0 |
| Atylidae | 287 | 0.93 | 0 | 0 |
| Leptognathiidae | 258 | 0.83 | 1 | 0 |
| Melitidae | 254 | 0.82 | 0 | 0 |
| Paguridae | 241 | 0.78 | 0 | 1 |
| Porcellanidae | 146 | 0.47 | 0 | 0 |
| Axiidae | 32 | 0.1 | 1 | 0 |

Note: All families, except Axiidae, were classed as living at the sediments surface and all except Polybiidae, Galatheidae, Pagurida, Porcellanidae and Axiidae were classified as exhibiting direct development (no planktonic stage). More detail as to the rationale behind this division is given in the methods (see 2.5), detail of by-family assessments is given in 8.3. Counts are total abundance across the entire data set (all Local Authority areas).

## 8.3 Vulnerability assessment by family.

Families are listed in order of percentage abundance (based on the entire dataset).

**Ampeliscidae (Amphipoda).** Genera: *Ampelisca, Haploops*

Epifaunal or shallow-epinfaunal tube-dwelling amphipods. *Haploops* species are believed to be strictly tubicolous and exclusively suspension-feeders (Dauvin et al. 1990, Rigolet et al. 2011). The genus *Ampelisca* shows more flexibility, with some species recorded as leaving their tubes to swarm in the water column, and grazing on surface sediment in addition to suspension-feeding(Kanneworf 1965). As with all amphipods, development is direct, with no free-living larval stage (Lincoln 1979).

**Urothoidae (Amphipoda).** Genera : *Urothoe*

Freely-mobile epifaunal or shallow-infaunal amphipods. Believed to be deposit-feeders (Watkin 1940). Direct development.

**Corophiidae (Amphipoda).** Genera: *Corophium, Crassicorophium, Medicorophium, Monocorophium, Leptocheirus, Autonoe*

Typically tube-dwelling epifaunal or shallow-infaunal habit. Can suspension-feed on plankton or suspended detritus but are also able to graze organic films from the sediment surface outside their tubes (Nielsen et al. 1982). Direct development.

**Caprellidae (Amphipoda).** Genera: *Caprella, Pariambus, Parvipalpus, Pseudoprotella, Phtisica*

Freely-mobile epifaunal amphipods. Most genera are believed to be detritivores, able to obtain food both by suspension-feeding and surface-grazing, with the exception of the predatory genus *Phtisica* (Guerra-Garcia et al. 2009). Direct development.

**Lysianassidae (Amphipoda).** Genera: *Ambasia, Lysianassa, Nannonyx, Socarnes, Hippomedon, Lepidepecreum, Lysianassa, Orchomene, Orchomenella, Tryphosites, Scopelocheirus*

A very large family of freely-mobile, epifaunal or shallow-infaunal amphipods. Lysianassids are usually regarded as carrion-feeders (Britton et al. 1994), although such a large family may include opportunistic omnivores or facultative deposit-feeders. A scavenging mode of life has been confirmed for several of the genera in the SARF list (Sainte-Marie et al. 1985, Sainte-Marie 1986, Moore and Wong 1995, Ide, Takahashi et al. 2006). Direct development.

**Photidae (Amphipoda).** Genera: *Gammaropsis, Megamphopus, Photis*

A family in the Suborder Senticaudata, related to the Corophiidae, and also tubicolous in habit. Very little published information on diet or feeding mode. *Gammaropsis nitida* has been recorded suspension-feeding both within and outside its tube (Dixon et al. 1997). *Photis longicaudata* appears to be a commensal of the burrowing anemone *Cerianthus lloydii* (Moore et al. 1999) but the significance of this association is unknown. Given their morphological similarity to the Corophiidae, photids may also be facultative suspension- and deposit-feeders on detrital particles. Direct development.

**Phoxocephalidae (Amphipoda).** Genera: *Harpinia, Metaphoxus, Parametaphoxus, Paraphoxus, Phoxocephalus*

A very large, diverse family of freely-mobile, shallow-infaunal amphipods. Gut contents of phoxocephalids from California indicate an omnivorous diet of meiofauna and organic detritus (Oakden 1984). The genus *Harpinia* may be mainly carnivorous (Navarro-Barranco et al. 2013). Direct development.

**Nebaliidae (Leptostraca).** Genera: *Nebalia, Sarsinebalia*

Freely-mobile, epifaunal or shallow-infaunal crustaceans. *Nebalia hessleri* has been recorded as an inhabitant of subtidal macrophyte mats, feeding on detritus and carrion (Vetter 1996). Cannon (1927) referred to “feeding currents” generated by *Nebalia bipes.* The limited information suggests that the animals may be facultative suspension- and deposit-feeders on small detrital particles. Development is direct, with no free-living larval stage (Vetter 1996).

**Tanaopsidae (Tanaidacea).** Genera: *Tanaopsis*

Tanaids are sedentary, shallow-infaunal burrowers. They are often tubicolous (Holdich et al. 1983), although Holdich et al. (1983) found that specimens of *Tanaopsis graciloides* from fine sediments off the west of Scotland did not appear to inhabit tubes. Most tanaids are raptorial feeders on detrital particles and associated microorganisms, supplemented by facultative suspension-feeding in some species (Holdich and Jones 1983). Development is direct as in all peracarid crustaceans (Holdich and Jones 1983).

**Maeridae (Amphipoda).** Genera: *Animoceradocus, Ceradocus, Maera, Maerella, Othomaera*

There is very little published information on the ecology of this amphipod family. Off western Scotland, *Maera loveni* is a burrower in subtidal muds (Atkinson et al. 1982). On the basis of this single study, maerids are here classed as sedentary infaunal deposit-feeders. Development is direct.

**Aoridae (Amphipoda).** Genera: *Aora, Autonoe, Lembos, Microdeutopus*

A poorly-studied amphipod family within the Corophiid infraorder. Dixon and Moore (1997) recorded species of *Aora* and *Lembos* as inhabiting tubes and using a pleopod-generated water current for suspension-feeding. Direct development.

**Oedicerotidae (Amphipoda).** Genera: *Deflexilodes, Periculodes, Pontocrates, Synchelidium, Westwoodilla*

Freely-mobile, epifaunal or shallow-infaunal amphipods. Members of this large and diverse family are usually regarded as carnivores, with some species known to feed on harpacticoid copepods (Beare et al. 1994, Beare et al. 1998, Yu et al. 2002). However, the diversity of mandibular morphology may indicate a wider dietary range within the family (Beare and Moore 1994). Direct development.

**Ischyroceridae (Amphipoda).** Genera: *Ericthonius, Jassa, Parajassa, Siphonoecetes*

Another family in the Corophiid infraorder, the ischyrocerid amphipods are typically sedentary suspension-feeders inhabiting tubes (Nair et al. 1979). Development is direct.

**Cheirocratidae (Amphipoda).** Genera: *Cheirocratus*

There appears to be little or no published information on the genus *Cheirocratus.* Based on overall morphology (Lincoln 1979) and the habits of the related Family Melitidae (in which *Cheirocratus* was formerly included), it is tentatively classed here as a freely-mobile, epifaunal or shallow-infaunal amphipod feeding on particulate organic detritus.

**Sphaeromatidae (Isopoda).** Genera: *Cymodoce, Lekanosphaera, Sphaeroma*

Freely-mobile, epifaunal or shallow-infaunal isopods (Naylor 1974). The genera within the family exploit a wide range of diets. *Cymodoce* species have been recorded consuming newly-settled sponges, detritus and some macroalgae (Arrontes 1990), while *Lekanosphaera hookeri* appears to graze the surface microflora from leaf detritus (Mancinelli 2012). In contrast, wood-boring species of *Sphaeroma* are suspension-feeders (Rotramel 1975, Si et al. 2002). Sphaeromatids are here classed as omnivorous consumers of particulate organic matter, although genera may differ in the source of this material and the mode of obtaining it. As with all isopods, development is direct (Naylor 1974).

**Leucothoidae (Amphipoda).** Genera: *Leucothoe*

Leucothoid amphipods are freely-mobile but are often found associated with sponges, ascidians or other sessile invertebrates (Lincoln 1979, Thiel 1999), indicating a largely sedentary existence. There appears to be no published information on diet or feeding mode. They are here tentatively classed as feeders on particulate organic detritus, obtained either by grazing or suspension-feeding. Direct development.

**Diastylidae (Cumacea).** Genera: *Diastylis, Diastyloides*

Diastylid cumaceans are shallow-infaunal burrowers in bottom sediments (Jones 1976). Jones (1976) described mud-dwelling species as suspension-feeders on fine particles, based on a study of *Diastylis bradyi* by Dennell (1934). Development is direct as in all peracarid crustaceans (Jones 1976).

**Galatheidae (Decapoda).** Genera: *Galathea*

Galatheid squat-lobsters are freely-mobile epifaunal crustaceans (Ingle et al. 2004). They are ominvorous consumers of detritus, animal or plant tissue, able to feed by macerating large food items or by collecting finer particles from the substratum (Nicol 1932). Small eggs released into the water column hatch into planktrotrophic larvae (Ingle and Christiansen 2004).

**Bodotriidae (Cumacea).** Genera: *Bodotria, Iphinoe, Vaunthompsonia*

Bodotriid cumaceans are shallow-infaunal burrowers, typically in coarser sediments than the fine muds inhabited by the Diastylidae (Jones 1976). Diet consists of particulate organic matter and microorganisms grazed from the surface of sand particles (Jones 1976).

**Atylidae (Amphipoda).** Genera: *Atylus, Nototropis*

There is very little published information on the ecology of this amphipod family. They are freely-mobile epifaunal or shallow-infaunal in habit, on sediments or among algae (Lincoln 1979). They are here tentatively classed as omnivorous consumers of particulate organic matter. Direct development.

**Gnathiidae (Isopoda).** Genera: *Gnathia, Paragnathia*

Gnathiid isopods have a very distinctive morphology and lifestyle (Naylor 1974). Juveniles (termed “praniza”) are ectoparasites of fish. The sexually-dimorphic adults do not feed and adopt a benthic lifestyle, often sedentary in crevices, burrows or kelp holdfasts (Upton 1987). Development is direct.

**Leptognathiidae (Tanaidacea).** Genera: *Leptognathia*

Like the tanaopsids, leptognathiids are sedentary burrowers (Holdich and Jones 1983). They are often considered to be tubicolous, although Holdich and Jones (1983) found no evidence of tube construction in collections from the west of Scotland. Diet is likely to consist of detrital particles and associated microorganisms (Holdich and Jones 1983). Direct development.

**Melitidae (Amphipoda).** Genera: *Abludomelita, Melita*

Freely-mobile epifaunal or shallow-infaunal amphipods found on sand, mud or cobble substrata (Lincoln 1979). There is very little published information on diet or ecology. Tomikawa et al. (2010) described a new *Pseudogammarus* species from Japan as "facultatively carnivorous", whereas *Melita plumulosa* consumed algal food in laboratory culture (Mann et al. 2011). Melitid amphipods are here classed tentatively as omnivores, with a diet including particulate organic detritus. Direct development.

**Paguridae (Decapoda).** Genera: *Anapagurus, Cestopagurus, Pagurus*

Pagurid hermit crabs are freely-mobile epifaunal crustaceans with an omnivorous diet which includes detritus, plant and animal tissue (Ingle and Christiansen 2004). Small eggs released into the water column hatch into planktotrophic larvae (Ingle and Christiansen 2004).

**Polybiidae (Decapoda).** Genera: *Liocarcinus, Necora*

These "swimming crabs" are freely-mobile epifaunal crustaceans with an omnivorous diet which includes detritus, plant and animal tissue (Ingle 1983). Small eggs released into the water column hatch into planktotrophic larvae (Ingle 1983).

**Uristidae (Amphipoda).** Genera: *Anonyx, Euonyx, Tmetonyx*

Freely-mobile epifaunal amphipods, uristids belong to the lysianassoid Superfamily, and like other members of the group, are typically scavenging carrion-feeders (Lincoln 1979, Sainte-Marie and Lamarche 1985). Some species are also opportunistic predators of polychaetes and other small invertebrates (Sainte-Marie and Lamarche 1985). Direct development.

**Porcellanidae (Decapoda).** Genera: *Pisidia*

The long-clawed porcelain crab *Pisidia longicornis* is a freely-mobile epifaunal suspension-feeder on particulate organic matter (Nicol 1932, Ingle and Christiansen 2004). Small eggs released into the water column hatch into planktotrophic larvae (Robinson et al. 2000).

**Acidostomatidae (Amphipoda).** Genera: *Acidostoma*

Freely-mobile epifaunal or shallow-infaunal amphipods. Little is known about the ecology of the genus *Acidostoma*, other than that they appear to be ectoparasitic on sea anemones (Lincoln 1979). Ansell (1969) recorded an association between *Acidostoma neglectum* and the burrowing anemone *Peachia hastata.* Direct development.

**Phoxichilididae (Pycnogonida).** Genera: *Phoxichilidium*

Sea spiders are freely-mobile, epifaunal arthropods that are typically suctorial feeders on sea anemones, hydroids or other sessile invertebrates. Species of *Phoxichilidium* have been recorded feeding on hydroids of the genera *Ectocarpus* and *Tubularia* (Stock 1978, Lovely 2005). As with all sea spiders, development is direct, with eggs and juveniles carried by the male parent (Lovely 2005).

**Scopelocheiridae (Amphipoda).** Genera: *Scopelocheirus*

Freely-mobile epifaunal amphipods belonging to the lysianassoid Superfamily. Like most other lysianassoids they are believed to be principally scavenging carrion-feeders or opportunistic predators of benthic invertebrates (Kaim-Malka 2003). Direct development.

**Axiidae (Decapoda).** Genera: *Axius, Calocaris*

The Axiidean mud-shrimps (formerly termed "Thalassinideans") inhabit deep, complex burrows in subtidal sediments. In Scottish sea lochs, burrows of *Calocaris macandreae* can extend up to 21 cm below the sediment surface (Nash et al. 1984). Little is known of the British *Axius stirhynchus*, but off Nova Scotia the related *A. serratus* has been recorded burrowing to depths of over 3 m (Pemberton et al. 1976). Deep-burrowing axiids are predominantly deposit-feeders (Pinn et al. 1998), although *C. macandreae* may also be an opprtunistic scavenger (Pinn, Atkinson et al. 1998). Small eggs released into the water column hatch into planktotrophic larvae (Ingle and Christiansen 2004).

## 8.4 Model residual plots

The 'standard' residual plots from the 'Mobiles' model (as per model output in Table 20) are shown in Figure 5. The residuals plotted against the predictors are shown in

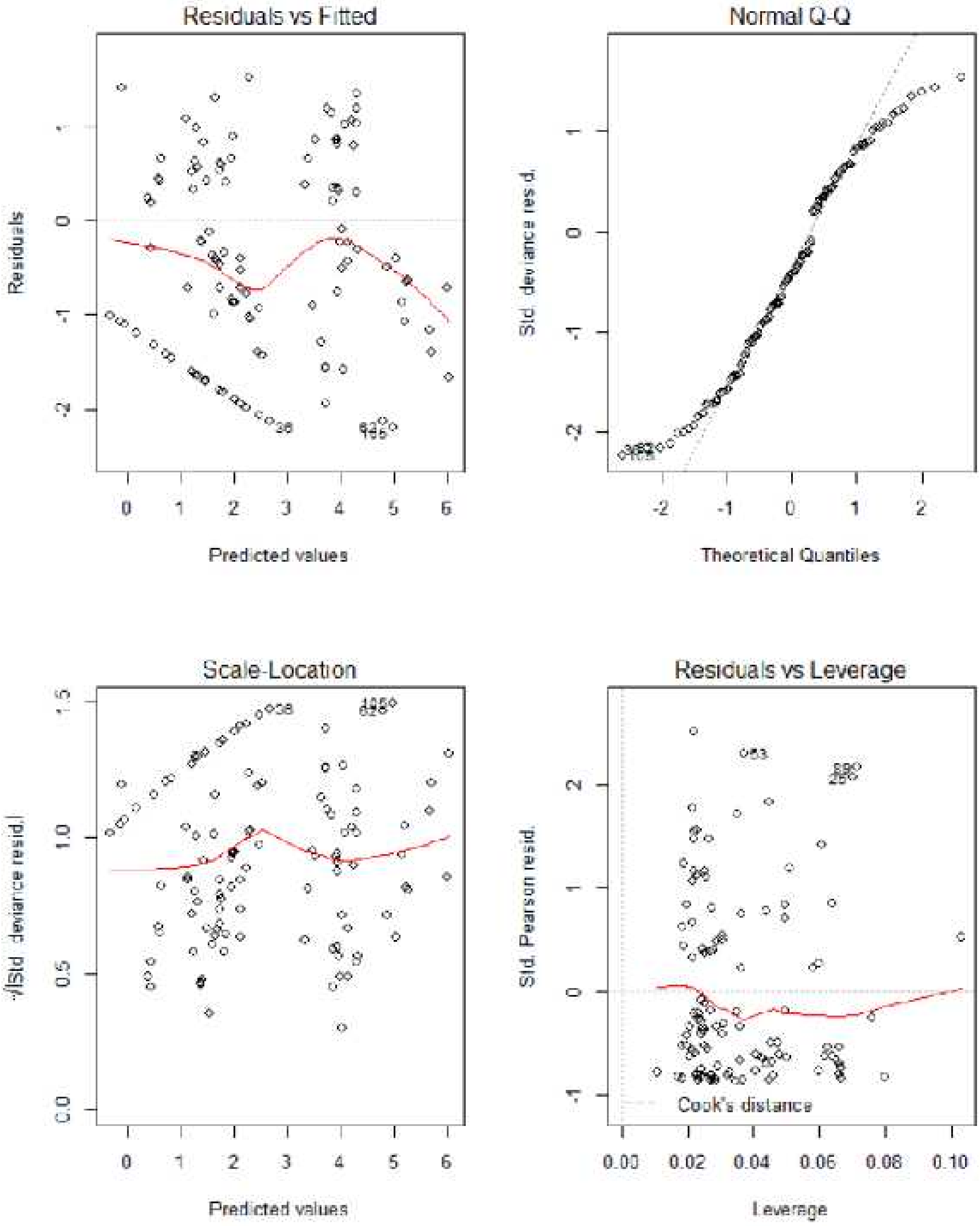

**Figure 5 Standard residuals plots for the model shown in Table 20. Residual patterns (e.g. the ‘string’ of points in the ‘Residuals v. fitted’ are normal is negative binomial models. No outliers (Cook’s distance >0.5) are present.**

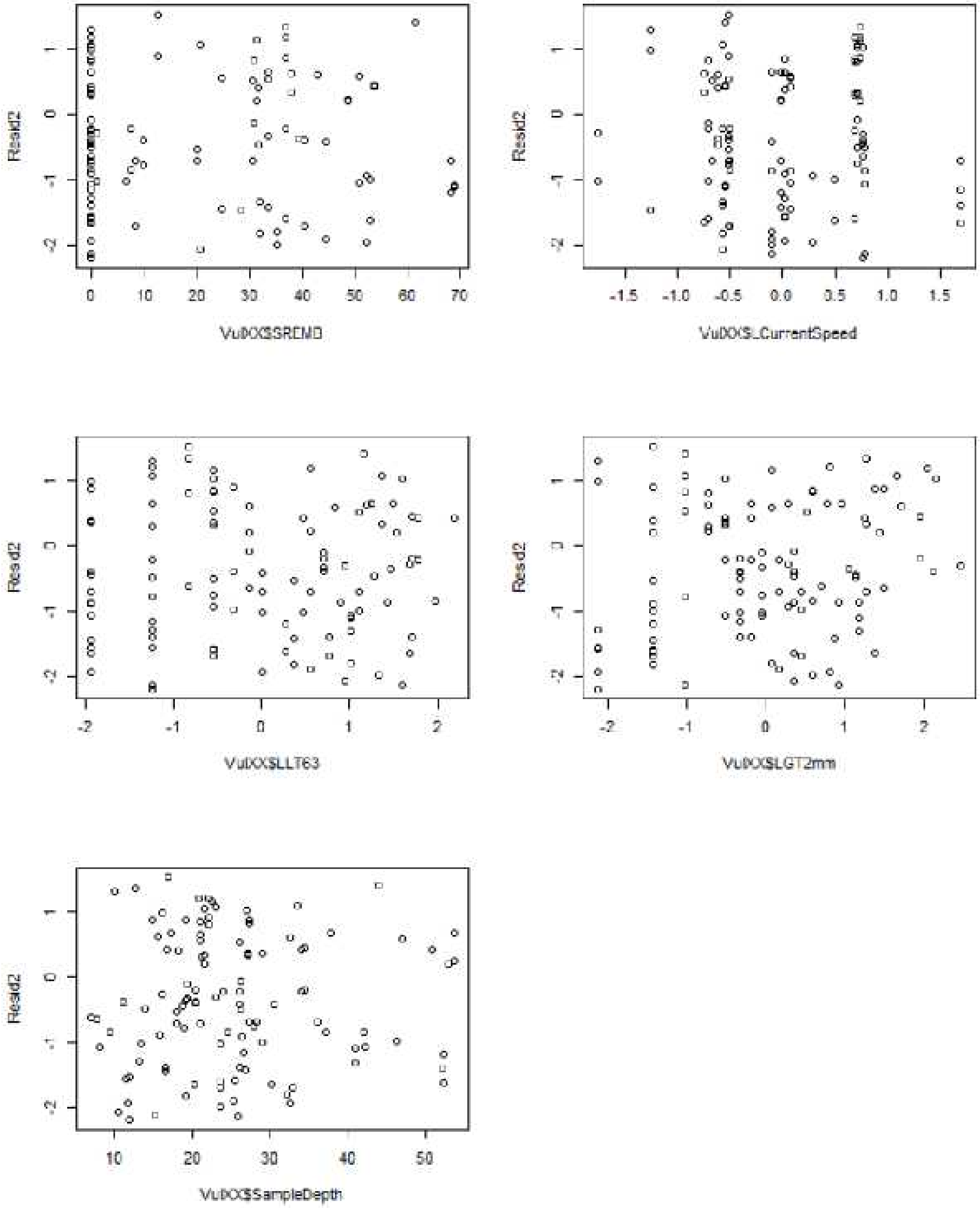

**Figure 6 – Residuals from model in shown in Table 20 plotted against all predictors. The predictors names are prefixed with 'VulXX$' (the dataframe name) then, in order (left-to-right) SREMB, LCurrentSpeed, LLT63, GT2mm and SampleDepth. These variable names are detailed in Table 2.**

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# SARF - Member Organisations

## *Industry*

SEAFISH

Association of Scottish Shellfish Growers

Scottish Salmon SSPO

British Trout Association

## *Government and Regulators*

SCOTTISH NATURAL HERITAGE

SEPA Scottish Environment Protection Agency

marinescotland

The Scottish Government

HIE Highlands and Islands Enterprise
Iomairt na Gaidhealtachd 's nan Eilean

## *Non-Governmental Organisations*

THE CROWN ESTATE

Scottish Environment LINK

RIVERS & FISHERIES TRUSTS OF SCOTLAND
Safeguarding Scotland's Rivers & Lochs

ASFB The Association of Salmon Fishery Boards