UPPSALA
UNIVERSITET

UPTEC: W13016
Examensarbete 30 hp
Maj 2013

Relations between Environmentally Disturbing Establishments and three Invertebrate Indicator Species in the Baltic Sea

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ABSTRACT

Relations between Environmentally Disturbing Establishments and three Invertebrate Indicator Species in the Baltic Sea

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In order to improve the knowledge about polluted areas in Sweden, Naturvårdsverket has compiled a list of all establishments and other anthropological activities, so called MIFO-objects, which emit harmful chemicals. Those activities which are placed on land might, depending on factors such as closeness to the sea, water solubility, degradability and toxicity of the chemicals have an impact on the biota in the Baltic Sea. In this study, spatial and statistical methods were used to explore potential relations between the abundance of three indicator organisms (*Macoma balthica*, *Marenzelleria* and *Monoporeia affinis*), closeness and a second variable built risk class of MIFO-objects and local environmental factors (e.g., sea depth, salinity) at the coast of Blekinge.

The impact of MIFO-objects on the abundance of the indicator organisms was analyzed with both graphical and numerical multivariate analysis methods such as spearman analysis, principal component analysis and canonical component analysis. Four types of variables were created to enable the analysis. The first two variables were based one distance from emission locations to the study sites. The other pair of variables comprised on variable built on the cumulative risk assessment of the MIFO-objects given by Naturvårdsverket and another that was based on a classification of the emitted pollutants according to their chemical toxicity.

The analysis showed that the abundance of *Marenzelleria* was positively correlated with MIFO-objects both in terms of risk assessment and chemical toxicity. This was probably a result of the fact that *Marenzelleria* is less sensitive to pollutants and therefore more competitive than other species in its habitat. Since the abundance of *Macoma balthica* covaried a lot with environmental factors such as salinity it was difficult to distinguish the impact of MIFO-object on the mussel. The statistical base of the abundance of *Monoporeia affinis* was too small to make any conclusions about what is describing the abundance.

Keywords: *Macoma balthica*, *Marenzelleria*, *Monoporeia affinis* and MIFO-object.

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SAMMANFATTNING

Relationen mellan miljöstörande verksamheter och tre arter av bioindikatorer i Östersjön

Anna-Emilia Joelsson

I syfte att öka kunskapen om förorenade områden i Sverige har Naturvårdsverket sammanställt en lista över alla verksamheter och antropogena aktiviteter, så kallade MIFO-objekt, som avger skadliga kemikalier. Objekten är lokaliserade på land, men beroende på faktorer såsom flödesväg, kemikaliernas vattenlöslighet flyktighet och skadlighet med mera, kan de ha en påverkan även på Östersjöns biota. I den här studien användes geografiska och statistiska metoder för att undersöka relationen mellan populationsstorlekar av tre arter/släkten evertebrater (*Macoma balthica, Marenzelleria spp* och *Monoporeia affinis*) och olika index som beskriver MIFO-objekt.

MIFO-objektens påverkan på indikatorernas populationsstorlekar analyserades med både grafiska och multivariabla metoder såsom spearmananalys, principal komponet analys och kanonisk komponent analys. Fyra variabler skapades för att möjliggöra analysen; två variabler baserade på sträcka mellan inventeringsplats och MIFO-objekt, en baserad på en riskbedömning av MIFO-objekten gjord av naturvårdsverket och en baserad på kemikaliernas egenskaper.

Analysen visade att tätheten av *Marenzelleria* korrelerade positivt med MIFO-objekten både i avseende på riskbedömningen och på kemiska egenskaper. Detta berodde troligen på att *Marenzelleria* är mindre känslig för föroreningar och därför mer konkurrenskraftig än andra organismer i habitatet. Eftersom populationsstorleken av *Macoma balthica* kovarierade för mycket med naturliga variabler såsom salinitet var det omöjligt att urskilja MIFO-objektens påverkan på musslan. Det statistiska underlaget av tätheten av *Monopoireia affinis* var för litet för att kunna dra några slutsatser vad som förklarar populationstätheten av djuret.

Nyckelord: *Macoma balthica, Marenzelleria spp, Monoporeia affinis* och MIFO-objekt.

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PREFACE

I would like to thank Rickard Pettersson for your patience, pedagogic explanations and because you always took time to reflect on my technical difficulties. Thanks also to my supervisor Johan Näslund, subject evaluator Thomas Grabs and examiner Allan Rodhe for reading my report so carefully. Thanks to Emanuel, Emil and Lino for help with Matlab. Finally many thanks to Ann-Sophie for taking care of my dog Bromma in daytime when I have been in school. Despite your 92 years you walked around Stabby every day in sun or in rain. Without you it would have been much harder to have time to study. Bromma and I miss you very much!

UPTEC W 13 016, ISSN 1401-5765

Publicerad digitalt hos Institutionen för geovetenskaper, Geotryckeriet, Uppsala universitet, Uppsala, 2013.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Relationen mellan miljöstörande verksamheter och tre arter av bioindikatorer i Östersjön

Anna-Emilia Joelsson

Östersjön är ett väldigt känsligt hav vilket framförallt beror på två saker. Den ena anledningen är att vattnets omättningstid i Östersjön är väldigt lång, vilket gör att näringsämnen och föroreningar ackumuleras i vattnet. Den andra anledningen är att vattnet är bräckt och därför har en salthalt som varken passar djur och växter som normalt finns i insjöar eller i hav. Detta leder till att Östersjöns biota ständigt är under stress och därför är extra utsatt vid utsläpp av föroreningar. Trots detta och trots att nya lagar reglerar utsläppen ökar ändå halten av vissa typer av föroreningar i Östersjön. Många länder med mycket människor delar på kusten vilket ökar påfrestningen på havet. För att komma förbättra kontrollen av utsläpp pågår många projekt, där ibland att kartlägga vilka källor det finns till föroreningsläckage. Naturvårsverket har gjort en sådan sammanställning av punktutsläpp där varje punkt är ett så kallat MIFO-objekt. Till MIFO-objekt räknas verksamheter eller mänskliga aktiviteter som släpper ut miljöstörande kemikalier som direkt eller på sikt hotar att läcka ut i havet eller andra viktiga vattenförekomster. Både aktiva verksamheter och verksamheter som funnits men upphört sedan 1850 finns registrerade. Ett MIFO-objekt kan till exempel vara en fabrik, bilmack och kemtvätt, men också platser där det skett trafikolyckor som haft kemikalieutsläpp som följd.

I den här studien användes data över hur mycket av olika havslevande organismer det finns på olika stället i havet utanför Blekinge, för att avgöra hur förorenat havet och sedimenten är. Tre olika arter av ryggradslösa djur användes, östersjömussla, havsborstmask och vitmärla. Datat över arterna testades sedan med hjälp av statistik, mot olika index som beskriver inflytandet av MIFO-objekt på platserna där arterna inventerats, för att se om det fanns något samband.

Resultatet av testerna visade att det finns ett samband mellan populationsstorlekar av havsborstmask och förekomst av MIFO-objekt. Det sambandet är positivt vilket betyder att havsborstmasken gynnas av att det finns många MIFO-objekt eller MIFO-objekt som släpper ut mycket kemikalier i området. Förmodligen beror inte det på att havborstmaskar gillar gifter, utan på att de är mindre känsliga för gifter än andra organismer som de i vanliga fall måste dela på födan med eller blir jagade av. Hur stora populationerna av östersjömusslan var visade sig bestämmas framförallt av förhållanden i den naturliga miljön såsom djup och salthalt. Det gick inte att urskilja något samband mellan populationsstorlekar av östersjömussla och förekomst av MIFO-objekt. Det fanns för få vitmärlor på de undersökta platserna för att statistiska tester med populationsstorlekar av vitmärlan skulle kunna ge något pålitligt resultat.

CONTENT

1. INTRODUCTION

Human activities during the last centuries have put much of the biota in the Baltic Sea under pressure since the catchment area for the sea is densely populated. The inland sea is unique in the world because of the gradient between fresh and salt water, but makes the salt water species stressed due to the low salinity and the fresh water species stressed due to high salinity. Another factor that increases the sensibility of the sea is the long water renewal time. Those special conditions make the biodiversity low. Overfishing, climate change, invasive species and eutrophication are current topics of discussion. The high level of some health- and environmental disturbing pollutants like PCB and DDT are on the other hand a problem that has improved over the last decades and therefore marine pollutant problems nowadays have moved away from the heart of discussion (Bernes, 2005). Even though official regulations to regulate harmful toxins exist their effectiveness is limited because of the high rate at which new chemical compounds are synthesized as well as because of potentially long half-lives and often long residence times of pollutants transported by water. In particular, persistent and lipophilic compounds have a very long residence time in the ecosystems and toxins that now are buried in the sediment might as well be stirred up again by for example land uplift (Karlsson, 2012), bioturbation or dredging.

A big part of the problem is that so many countries share the coastline, which makes it hard to map and to weight the importance of the pollution sources. Therefore, with the purpose to identify all places with contaminated soil in Sweden, with toxins that may end up in to the Baltic Sea, the Swedish Environmental Protection Agency (Naturvårdsverket), started to map all establishments that emit or have historically emitted harmful chemicals. The methodology for inventory of polluted areas is compiled as a list of human establishments, so called MIFO-objects that also consist of activities that are no longer active. A MIFO-object could be anything from a dry cleaning agency to a pulp mill or a location where there has been a road accident that has led to chemical emission.

To analyze population sizes of certain indicator organisms as proxies for the health of an ecosystem is a relatively fast and cheap method to control the health of the biota compared to making a full-scale investigation of the status. With a combination of statistical and spatial methods it is easy to get an overview of a big area. Analyzing abundance of indicator organisms is also often better than measuring concentrations of certain chemicals in the water since chemicals can differ in potency, depending on exterior factors such as for example salinity (Havsmiljöinstitutet, 2012).

The key for a good analysis is to choose the right indicator. Most often animals higher up in the food web, such as sea living mammals and predator fish, are selected. However, pollutants like for example copper that in increased concentration are poisonous for organisms lower down in the food web while it is harmless for most fish may then be a risk to overlook (Havsmiljöinstitutet, 2012).

AquaBiota, a Swedish research company that focuses on marine issues has recently completed a study *"Modeling of Västernorrlands marine habitats and nature values"* (Florén et al, 2012). The study aimed to map and improve the knowledge about the ecological values in the region. Florén et al (2012) showed that three different species of invertebrates had a significant correlation between population density and closeness to anthropological activity. Depth and curvature were the variable explaining most of the distribution of *Monoporeia affinis* and *Macoma Balthica* of all the analyzed factors. Curvature was the most important variable for *Marenzelleria spp* but was also important for *Macoma Balthica* (Florén et al, 2012).

1.1. AIM

In this study the relation between two species and one genus of invertebrates and the proximity of MIFO-objects was tested. The bio indicators used were *Macoma balthica*, a mussel that is expected to be insensitive for pollutants in the habitat (Florén, o.a., 2012), *Monoporeia affinis* a crustaceans that is expected to be very sensitive for pollutants (Havsmiljöinstitutet, 2012), and *Marenzelleria spp* a genus of ringworms that are newly arrived invasive species in the Baltic Sea (Bernes, 2005). All three invertebrates are relatively stationary in adult stage, making them suitable candidates to indicate that an area is polluted.

The objectives of this thesis were to determine whether the relationships between different types of establishments in a region and the invertebrates living in the Baltic Sea, as found by Florén et al (2012), also exist in Blekinge region. The goal was also to further investigate the relationships by searching for patterns in type of establishment, type of emission, estimated harmfulness and closeness to the sea. This was made by creating four types of variables that somehow describe the remote impact of the MIFO-objects on the study sites:

- Two types of length variables that describes the distance between the MIFO-objects and the study sites.
- A cumulative sum of the risk assessments given to the MIFO-objects by Naturvårdsverket that was calculated for every catchment area.
- A division of the MIFO-objects based on types of chemicals emitted.

The influence of the anthropological impacts on the population sizes were then to be compared to the influence of nature variables that describes the conditions on the study sites. A subtask was to try out different types of statistical analyses to see which explains the abundance of indicator organisms the best.

The study was a part of the bigger project Marmoni (funded by the European Union Life+Nature & Biodivetsity program, Life09 NAT/LV/(000238)), that aims at better knowledge on biodiversity of the Baltic Sea, and especially to fill gaps in knowledge on indicators for biodiversity and their response to various human activities. By doing so, the goal for Marmoni is to reach a common understanding on biodiversity monitoring methods used in different countries along the coast of the Baltic Sea (MARMONI team, 2012).

2. THEORY

2.1. INDICATOR ORGANISMS

2.1.1. *Monoporeia affinis*

Monoporeia affinis is a crustaceans in the group of antrophods. The body is about 8 mm yellowish to white colored and it has its habitat on soft bottoms from the surface down to 80 meters (Tjärnös marinbiologiska laboratorium, 2000). Since it provides food for many predators such as *Saduria entomon* and many species of fish such as cod and whitefish, it is a key species in the Baltic Sea (Floré et al, 2012). *Monoporeia affinis* is very sensitive to stress from low oxygen levels and is therefore commonly used as an indicator of water quality. Primarily it is environments with poor oxygen level, elevated water temperatures and anthropogenic toxins that make damage to populations, but it may also suffer from parasites and competition from invasive species. (Eriksson et al, 2008). Misshapen embryos of *Monoporeia affinis* are also used as an indicator in Sweden for pollutants in sediments (Havsmiljöinstitutet, 2012).

Figure 1. Monoporeia affinis to the right and Pontoreia to the left. (Photo: J. Näslund, 2012)

Monoporeia affinis predates on the pelagic larva of *Macoma balthica*, see section 2.1.2. Therefore big populations of *Monoporeia affinis* lead to a low density of *Macoma balthica*. Since it is not as sensitive to low oxygen levels, *Marenzelleria spp* is more competitive than *Monoporeia affinis*. During periods with poor aeration, *Marenzelleria spp* (see section 2.1.3.) conquers much of the habitats of *Monoporeia affinis* which makes it hard for *Monoporeia affinis* to return (Eriksson et al, 2008).

The variable explaining the distribution of *Monoporeia affinis* the most of all tested variables by Florén et al (2012) was depth, but second came closeness to pulp industries. Also closeness to waste water treatment plants showed a significant correlation with *Monoporeia affinis* in the province of Västernorrland.

2.1.2. *Macoma balthica*

Macoma balthica or Baltic Sea mussel, is a small mussel that is common in estuaries in northern Europe. It has skills that make it persistent to disturbances in the environment. The mussel lives buried down a few centimeters in sand or mud and can feed both on organic material in the sediment as well by filtering water. Since it can close its shell during short periods of poor environmental conditions, it is not significantly stressed by short periods of low levels of oxygen. An indicator of anaerobic status of its habitat is that the shells are colored black by precipitated iron sulfide when oxygen conditions are poor (Florén, o.a., 2012). The ability to provide itself with food in two ways, deposit and suspension feeding, makes it very adaptive to changes. It can also withstand low water temperatures during winter (Gofas, 2004).

Figure 2. *Macoma balthica* (Photo: J. Näslund, 2012).

Modeling shows that anthropogenic actions has significant impact on *Macoma Balthica*, even if curvature and depth explain more of the abundance (Florén, o.a., 2012).

2.1.3. *Marenzelleria*

The genus *Marenzelleria spp,* or red gillet mud worm, is a new inhabitant in the Baltic Sea, first identified in 1985. It arrived with ballast water and has ever since easily spread on soft sediment bottoms. At least three species of *Marenzelleria* has been found in the Baltic Sea; *M. artica, M. neglecta, M. virdis* and is only possible to identify the exact species for fully grown individuals using a microscope (Blank, Jürss, & Laine, 2008:62) *Marenzelleria* can live from 0.5 meter below the surface, and has been inventoried in high numbers of individuals down to almost 300 meters. It thrives in brackish water or in estuaries where the salinity can vary greatly and is therefore insensitive to salinity stress (Magnusson, 2008).

Figure 3. *Marenzelleria spp*. (Photo: J. Näslund, 2012).

The worm can dig paths in the sediment that can be approximately 30 cm deep which gives bioturbation and recirculation of PCBs and other anthropogenic toxins buried in the sediments as a negative consequence. The digging may also contribute to a decreased fixation of phosphorus (Gunnarsson et al, 2012). Because of ability of the worm to dig together with the ability of using temporary anaerobic metabolization the worm is persistent to low oxygen levels. The larvae stage of *Marenzelleria* is pelagic and the larvae can swim for several weeks before settling on the seafloor, which makes the worm fast in colonizing new areas.

Modeling from Västernorrland showed that curvature explains the distribution of *Marenzelleria* most of the tested variables. The worm was also affected by anthropogenic activities (Florén et al, 2012).

2.2 STATISTIC ANALYSES OF ECOLOGIC DATA

Since the distribution of living organisms is very complex and depends on many factors, it is often more difficult to establish relationships for the abundance of organisms than relationships between abiotic phenomena. Most often ecological phenomena do not even appear in linear relationships. To take into account non-linearity and the many facets of explaining living organisms, it is therefore often necessary to use multivariate analysis when looking for relationships. Multivariate methods can be used to test or to find hypothesis in big data sets. It can also be used to find the dominant factors as well as group objects in the data set (Naturvårdsverket, 2013c).

When differating between natural and anthropological variation with regression models, it is necessary to make assumptions about the physical relationships between the two sources.

(Naturvårdsverket, 2013c). The difference between finding a correlation and making a regression is that correlation analysis is more often used for explorative data analysis while regression analysis is often used to test more specific hypothesis on potential relationships.

2.2.1. Spearman correlation

Spearman correlation coefficient is a tool for analyzing correlations between variables within a data set. It is a nonparametric test, which means that it does not assume dependency among any of the including variables in the matrix to be tested. The main advantage with the test is that it does not require linear relations (Weyhenmeyer, 2011).

One of the results from Spearman analysis is a matrix that consists of correlation coefficients that runs between -1 and 1 depending on in what direction the variables affect each other. Spearman analysis also returns and a matrix of p-values that show whether the correlations are significant or not. Significance defines that a connection appears too frequent to be randomly induced.

Since the Spearman correlation coefficient depends on number of observations a false significance can occur if the number of observations becomes large. The analyzed matrix can therefore not be too big, without giving errors in the output. Since the error appears as an extremely low p-value, it could be difficult to tell whether a correlation is significant or whether it is due to the number of observations being too big. A range where p-values could be approved as significant is therefore necessary to be set. Small p-values with an upper limit of p(n,m)=0.05 indicate on a significant relationship, which means that the observation is within a 95% confidence interval (Math Works, 2013). Values lower than 0.001 do not mean any additional significance, but often indicate that an error occurred in between the tested variables (Weyhenmeyer, 2011).

The Spearman correlation coefficient is explained by Equation 1, where d is the difference in statistical rank of corresponding variables and N is the number of observation.

$$r = 1 - 6\sum \frac{d^2}{N(N^2-1)} \qquad (1)$$

2.2.3. PCA

Principal component analysis, PCA, is a standard method for performing analysis on a set with numerous variables. The analysis is built on finding patterns in correlation aggregation and outliers, and the result is a ranked sum of the importance of the variables in the set. It is also a graphical presentation of the ranked dominant components in the data set (Smith, 2002).

PCA makes a linearization of the covariance matrix in order to get the diagonal components in the matrix in the direction that has the largest variation in the data (Smith, 2002). A coordinate system of X1, X2…Xm is then transformed into a coordinate system of principal components. The first component in a PCA, PC1, comprises most of the variation in the data set and it is followed by other components (PC2, PC3, ..) that explain successively less of the total variation in the data set. Graphically, a biplot is used where PC1 represents the x-axis and PC2 the y-axis. The observations from the data set are distributed between the axes as so called “scores” in a point cloud depending on how they are related to the principal components (Naturvårdsverket, 2013b). The plot will also include “loadings” which represents the original variables. They position themselves by how they are influenced by the two PCs.

PCA is a good method for getting an overview of the structure of the set. It is also a good tool when the number of variables has to be reduced (Weyhenmeyer, 2011). Another advantage is if the algorithm is built so that it calculates one component at the time, it has the advantages that it can handle up to 50% missing values in the data set (Naturvårdsverket, 2013b).

A disadvantage with PCA is that it requires linear response. It is therefore not advisable to use PCA alone as an analyzing method on data that can give unimodal response, such as some ecological data (Naturvårdsverket, 2013b).

2.2.4. CCA

Canonical correlation analysis, CCA is a type of multivariate statistical model that measures linear interrelationships. The method measure the overall strength of the overarching relationship between the dependent and the independent data set (Person Prentence Hall Publishing, 2013). Instead of testing the correlation between variables within a data set, it tests correlation between two data sets of numerous variables. One of the sets should consist of depending variables and the other one of independent variables. CCA explains how variations in the independent set are explained by variations in the depended set (Naturvårdsverket, 2013a).

There are many refined algorithms producing CCA digitally, designed for different kinds of software. Common for most of these methods is that they result in a graphic presentation of the importance of variables with a complementary testing of significance (Person Prentence Hall Publishing, 2013).

The main advantage with CCA is that it will not give spurious correlations. CCA will also compensate shortcomings of for example PCA with not being able to handle unimodal patterns (Naturvårdsverket, 2012).

Like all statistic tests there are a few pitfalls to consider when analyzing the result of a CCA-session. If the set is too large, the significance can be too large since the algorithm depends of the sample size. If trends exist in the data set they can give an arch effect in the result. That problem could however easily be corrected by detrending the data before testing it. Another potential pitfall that can give deceptive result is if the depending variables are not independent from each other (Naturvårdsverket, 2012).

3. MATERIAL AND METHODS

To be able to test the remote impact of the MIFO-objects on the abundance of invertebrates on the study sites, spatial and statistical methods were used to create regionalizations and explanatory variables.

3.1. STUDY SITE

Blekinge is one of Sweden's smallest landscapes and the area of the landscape overlaps with the area of Blekinge province. The total population is 153 000 and it is the coastline that has got the highest population density (Eklund et al, 2012).

Watercourses are relatively small and are running in a southerly direction with outlets to the Baltic Sea. Blekinge is a hilly landscape that is relatively homogenous both in climate and topography. The winters are mild and the summers tend to be sunny. Along the coast there is a maritime climate with less variation in weather than in the inland. The slow cooling and warming of the Baltic Sea makes the autumns mild and the springs cold. The closeness to the sea also makes Blekinge more exposed to stormy weather (Eklund et al, 2012).

The coast of Blekinge consists of a shallow archipelago with a mix of big and small islands. Within the archipelago the water depth is rarely deeper than 20 meters. The shoreline is dominated by rocky bottoms, but the substrate of the bottom varies with the exposure to the sea. In sheltered parts of the coast there are some sandy beaches and on parts where the coastline is affected by erosion, the beaches consist of low cliffs (Tolstoy et al, 2003).

The vegetation below water in Blekinge is the poorest in terms of species richness along the entire Baltic Sea coastline. This is because the salinity in this area is not well tolerated by so many species (Näslund, 2012). Summertime, extensive mats of floating algae occur occasionally, which may cause oxygen related problems when the degradation increases (Tolstoy et al, 2003). According to the national environmental monitoring that measures ecological quality ratio, the bottoms in Blekinge holds a high ecological status (Havsmiljöinstitutet, 2012)

3.2. INPUT DATA

Two sources of input data were given for this work; data from an inventory of the ecosystems along the coast of Blekinge made by AquaBiota see Figure 4, and a compilation of all environmental disturbing human activities in the province, so called MIFO-objects. Also a number of geographic data objects showing the extent of Blekinge and coordinates for river outlets of monitored rivers in the area were provided by AquaBiota, see Figure 4.

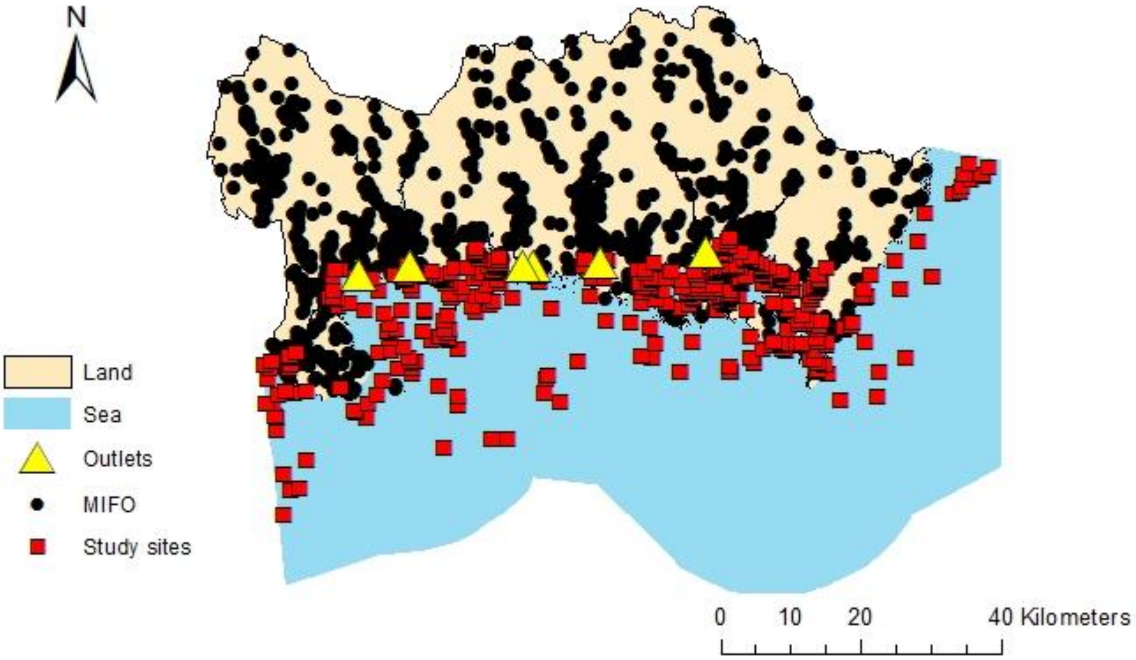

Figure 4. Geographical data that were given as a base for the analysis.

3.2.1. Anthropological activities

By the Swedish law Miljöbalken, shall "all contaminated areas involving acute hazards of direct exposure and such contaminated areas that currently or in the near future, threaten important water sources or valuable nature areas be investigated" (Appelkvist et al, 2005, p 5). This is implemented by Naturvårdsverket as MIFO-objects. MIFO, methodology for inventory of polluted areas, is a mapping of all industrial activities in an area for the last 150 years. In Blekinge 2828 establishments are identified, but some of them lack information about location and therefore only 2181 of them are published.

The activities are ranked 1-4 by properties such as hazardous of emitted pollutants, estimated quantity of released pollutant, conditions for distribution, sensitivity/protection value in the area and overall risk assessment. Activities ranked 1 are assumed to be the highest risk factor and activities ranked 4 are assumed to be the lowest risk factor (Naturvårdsverket , 1999). It is important to notice that this classification is not built on linear relationships, but made by expert judgment using a predefined ranking scale.

An overview of what sort of chemicals various are/were released by anthropological activities was compiled by Naturvårdsverket (2008). From that compilation, the MIFO-objects in Blekinge were classified into subgroups based on chemical properties. The classification was not derived from the systematic construction of the chemicals, instead the subgroups consist of chemicals with similar type of toxicity. There is for example one group with aromatics and one with halogenated aromatics. Their construction is similar but their potency as toxins is different, see Table 1 (Sterner, 2003).

Table 1. Division of chemicals by toxicity, containing only those groups that are represented in the compilation of Blekinge's MIFO-objects (Modified by Brunström, 2012).

Chemical class	**Chemical**
Metals	Pb, Cd, Ni, Hg, Cu ,Various heavy metals
Halogenated aromatics	Graphite sludge, dioxins, Cr_6+, VC, Aromates
Perfluorinated substances	Flourides
Pesticides	DDT, Pesticides
Unchlorinated solvents	Solvents, Organic solvents
Chlorinated solvents	Chlorinated solvents
Oils	Waste oil, Oil
Propellants	Diesel, Petrol
Nutrients	Urea, Phosphorous, Nitrogen, Nutrients, Organic phosphorous compounds
Organics	Slaughterhouse waste, Organic compounds
Aromatics	PAH, Phenols, Bitumen, Creosote
Cyanides	Cyanides

3.2.2. Marine species inventory

Data of organism density and nature conditions at the study sites were received from AquaBiota's excursions in the summer seasons of 2011 and 2012. In this analysis, 410 of the study sites were included. Two methods for sampling material were used by AquaBiota; drop videos for calculating the coverage of algae and seaweed and bottom grabs to catch organisms. The content of what is caught in the grabs was then counted and the volume was extrapolated to an area of 25 m^2. The selection of locations to analyze was done semi-randomized. Some areas are underrepresented since they could not be inventoried because of bad weather.

Figure 5. Result of a bottom grab (Photo: K. Florén, 2012).

The area of the grabs taken with Van veen bottom grabs were $0.025m^2$, with a volume varying from 5 to 100% of a full grab (3.14 L). Smaller volumes were generally collected on locations where the bottom consisted of bigger particles such as sand and gravel are more compact than clay and mud and thereby harder to collect (Näslund, 2012). The mean size of the grabs was 56% of a full grab. Grabs smaller than 25% of a full sample were excluded in the analysis as they did not penetrate deep enough into the sediment to be considered as quantitative samples. The abundances in each inventory location were not weighted by the size of the sample taken, even though the volume of the samples differed a lot. This is because the majority of the animals occur in the upper 2-3 centimeters of the sediment, therefore it would be inaccurate to make a linear weighting of the sample volume (Näslund, 2012).

3.2.3. Spatial data

Before starting the spatial and statistical analysis some processes and operations had to be done to the raw-data.

To make a regionalization of the marine areas in Blekinge in order to give an overview of the distribution of species in different marine basins, a new map containing marine areas was created. As a template for that, the regionalization of marine areas done by SMHI in their model for hydrological predictions, S-Hype was used. This was done by importing a picture of a map of Southern Sweden taken from SMHI that had the marine areas visible to ArcGIS and then edit the contours of the shapefile by the boarders of the marine areas.

Also the mainland of Blekinge was divided after the regionalization in S-hype. The MIFO-objects in every catchment and the areas in between were then extracted, so that the MIFO-objects later in the modeling could be analyzed by catchment area, see Figure 6.

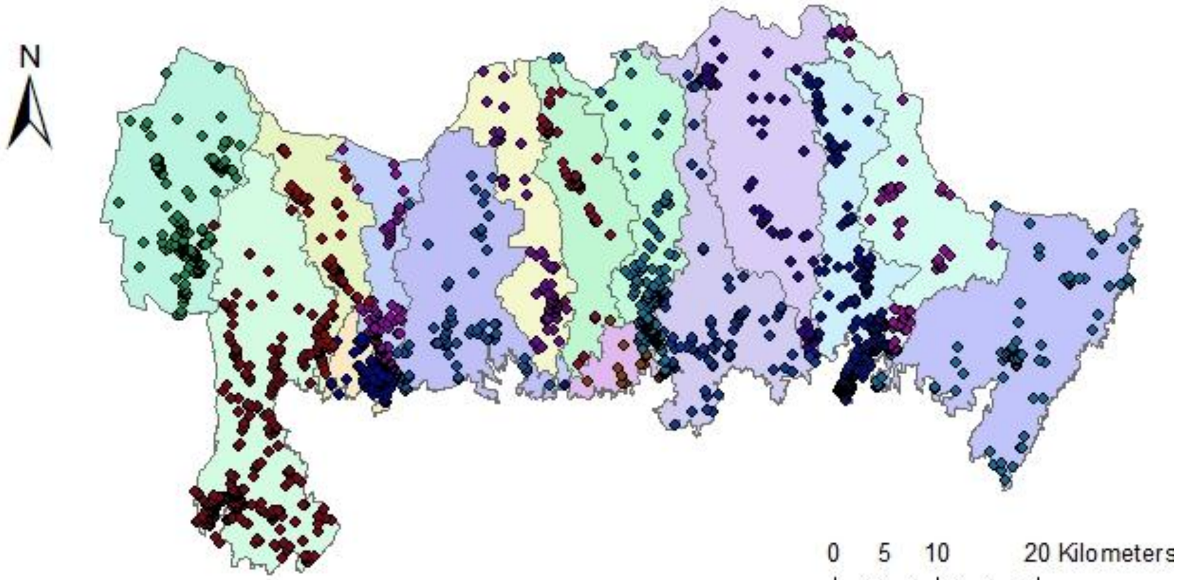

Figure 6. Blekinge divided into catchment areas with the MIFO-objects grouped after what basin they belong to.

A shapefile containing all big rivers in Blekinge was also created with coordinates given from S-Hype. Since most of the basins had several outlets, an additional shapefile of river outlets was created to enable future modeling. For that layer the median number outlet, the outlet in the middle, in every catchment area was chosen, see Figure 7.

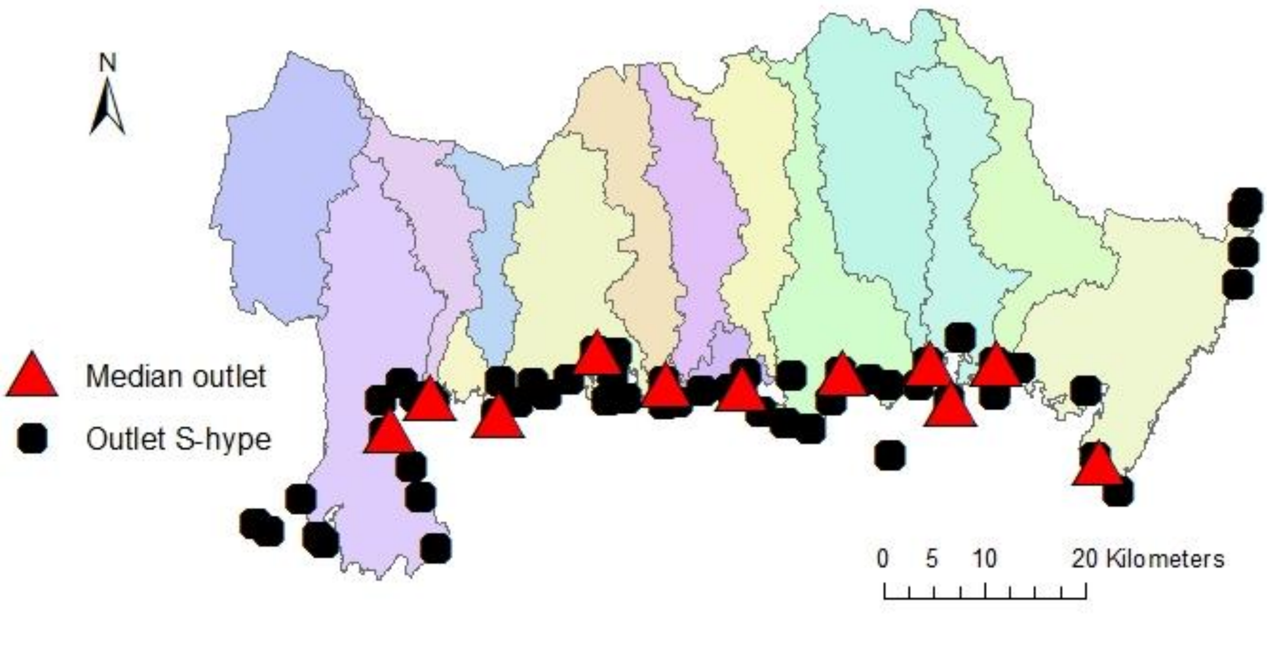

Figure 7. The outlets of watercourses in Blekinge.

3.3. MODELING

A set of new variables was created to enable the aim to test the influence of the anthropological activities on the population sizes of the three species of indicator organisms.

Table 2. A compilation of the variables created and explained in this section.

Variable	Denomination
Path Distance	PaD
Flow Length	FL
Path Distance + Flow length	PaDFL
Point Distance	PoD
Point Distance + Flow Length	PoDFL
Risk class	Risk class
Chemicals	Name of chemical subgroup x
$\sum \textbf{Risk class} * \sum \textbf{Chemical class}$	R_x
$\sum \textbf{FL} * \sum \textbf{Chemical}$	L_x

3.3.1. Distance calculations

To weigh the impact of the MIFO-objects on the distance from the inventory places, some calculations were done. To compensate each other's shortcomings two methods for calculating distance from the study sites seen in Figure 4 to the outlets of the rivers were used, "Path Distance," PaD, and "Point Distance," PoD. PaD can differ on land and water and therefore takes the route around islands and capes. PoD on the other hand calculates the nearest route, the one for a flying bird. To calculate the length of the route from the emission site to the river outlet for a molecule the tool "Flow Length," FL, was used.

The FL function calculates the distance between two locations for water that flows through the catchment area, not the distance for a flying bird. Therefore an elevation model had to be made. A rasterlayer from the online map libary "Digitala Kartor" was loaded into ArcMap. The elevation was reclassified so that sea level was given as 0 meters. The tools "fill" and "flow direction" were then used to create an input raster to FL. With the function "extract values to points" the grids containing a MIFO-object were extracted from the FL- raster, see Figure 8.

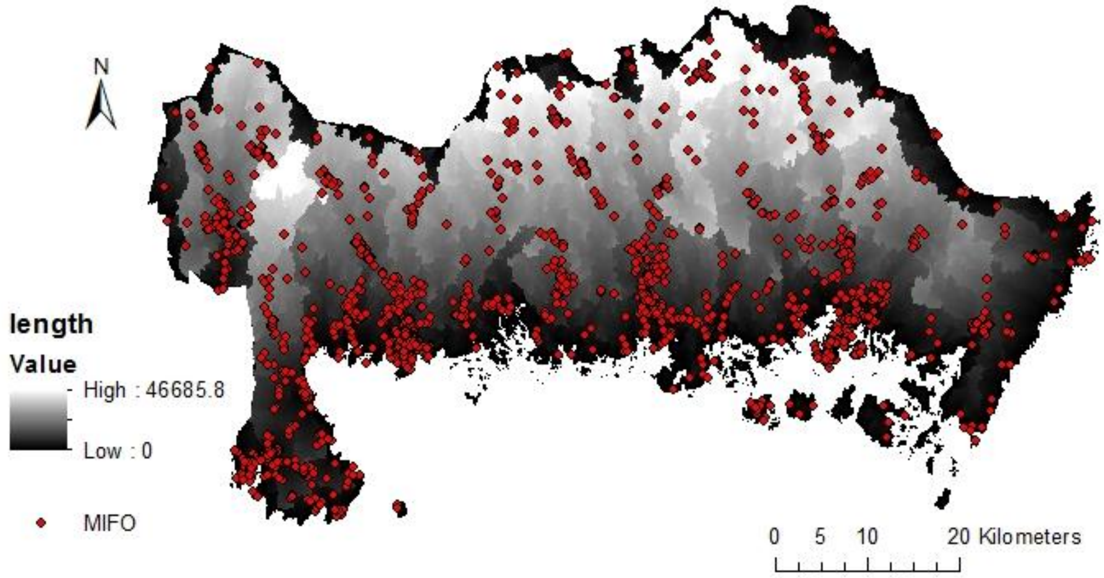

Figure 8. FL-raster with all the MIFO-objects marked.

To make input data to PaD a cost raster was created. That was done by taking the polygon layers of the land and the sea belonging to Blekinge and merge them into one shapefile and then convert them to a raster. The raster was reclassified so that the land was given 1000 000 as a value and the sea were set to 1. Therefore the function chose to take the route only in the water. The cell size was set to 1 meter.

PaD was run with the layer with a shapefile that consisted of the outlets for the six biggest rivers in Blekinge. Those rivers are monitored by the provincial government in Blekinge. rivers as an input feature and the cost raster as a distance feature. On places were the pixels in the raster did not overlap with the contours of the shapefile, an error occurred. The problem was that ArcGIS interpreted the points to be located on land. The coordinates for the rivers were, in those cases, moved in the map so that they belonged to the part of the raster representing sea. With the tool “sample” the places in the raster where the inventory places were located were extracted with the inventory data as an input feature and PaD as an input raster.

The nearest routes between the outlets and the inventory places were calculated with PoD. Instead of the shapefiles with the monitored rivers, the shapefile containing all the outlets in the coast of Blekinge edited from S-Hype was used as an input feature.

3.3.2. Risk classes

To make analysis on the effect of the cumulative weight of the risk assessments given to all MIFO-objects, a ranking of the risk classes had to be made. All MIFO-objects that were placed in basins that had a shoreline were exported to Excel. The tables with MIFO-objects contained a column consisting of every object's estimated risk-class. Since the scale goes from

1 to 4, where 1 is given to activities that are expected to damage the environment the most, it would be misleading to add up all the values in a sum. Then it would for example be both positive and negative to have a high value. Positive because high ratings mean little impact, negative because it can also mean that there are many MIFO-objects in the basin. Therefore the assessments were inverted so that the most environmentally destructive activities were assessed with a 4. After that, values for the cumulative risk assessment of each catchment area were added to their respective table of inventory data. In that way a nine set of pairs of study sites and adjacent MIFO-objects were created and tested against each other.

3.3.3. Chemical classes

A table of chemical emissions from every separate basin was created. If a MIFO-object was reported to emit a certain chemical, that chemical was given number one in the table. In Excel a cumulative sum was calculated for every chemical class. The earlier calculated value of risk class in every catchment area was also used by making a new variable R_x, where the cumulative sum of every chemical subgroup was multiplied with the cumulative risk class see Equation 3 where X is an arbitrary chemical.

$$R_x = \sum_{Basin} Risk\ class * \sum_{Basin} X \qquad (3)$$

An additional variable $L_{x,}$ was also created by the earlier calculated cumulative FL for every catchment area. Only MIFO-objects with the original classification 1-2 were used and were then multiplied with the cumulative sum of every chemical class, se Equation 4.

$$L_x = \sum_{Basin} Flow\ length * \sum_{Basin} X \qquad (4)$$

3.4. GRAPHICAL ANALYSIS

Box plots of the species and depths in all marine areas in Blekinge were generated. Additional box plots of PaDFL were calculated separately for the half of the study sites closest to land, and the other for the other half with the study sites further away from the coast to see if there was any difference in abundance of invertebrates closer and further away from land.

To better explore the data a number of sorted plots and linear regressions were performed in Excel:

- A sorted index plot of PaDFL sorted by size.
- *Macoma balthica* versus PaDFL
- *Macoma balthica* versus PaDFL with only sites less than 1 000 meters selected from land.
- *Marenzelleria* versus PaDFL.
- *Monoporeia* versus PaDFL

3.5. NUMERICAL MULTIVARIATE ANALYSIS

Several variations of the data set were tested with Spearman correlation coefficient in Matlab.

- All length distances versus all invertebrate data with a selection on PaD so that only study sites closer than 4 000 meter from an outlet was shown.
- All length distances towards all invertebrate data with a selection on PaD so that only study sites closer than 1 000 meter from an outlet was shown.
- The abundances of all three invertebrates towards risk class.
- The abundances of invertebrates towards all chemical classes. Also the variables L_x and R_x that were created out of the chemical classes were tested.

Six variables were chosen to be tested with PCA; depth, *Macoma balthica*, *Marenzelleria*, *Monoporeia affinis*, PaDFL and PoDFL. Additional variables describing nature conditions on the study sites were later received from AquaBiota. All variables, both preprocessed and given were used in the CCA-analysis, see Table 3.

Table 3. A summary of variables used in different analyzing methods.

Variables	Explanation	Origin	Analyzing method
Macoma balthica		Processed	Spearman, PCA, CCA
Marenzelleria		Processed	Spearman, PCA, CCA
Monoporeia affinis		Processed	Spearman, PCA, CCA
PaD	See section 3.3.1	Processed	Spearman
PoD	See section 3.3.1	Processed	Spearman
PaDFL	See section 3.3.1	Processed	Spearman, PCA, CCA
PoDFL	See section 3.3.1	Processed	Spearman, PCA
$\mathbf{R_X}$	See section 3.3.2	Processed	Spearman,
$\mathbf{L_X}$	See section 3.3.3	Processed	Spearman
Chemicals	See section 3.3.3	Processed	Spearman, PCA, CCA
Depth		Given	Spearman, PCA, CCA
Mud		Given	CCA
Clay/silt		Given	CCA
Sand (fine grained)		Given	CCA
Sand (large grained)		Given	CCA
Gravel/stones		Given	CCA
Aspect	Direction of slope	Given	CCA
Curvature		Given	CCA
Secchi depth		Given	CCA
Oxygen	Level at sea bottom	Given	CCA
Chlorophyll		Given	CCA
Salinity bottom	In a 10 percentile	Given	CCA
Salinity bottom (2)	In a 90 percentile	Given	CCA
Temperature bottom	Average	Given	CCA
Temperature surface	In a 10 percentile	Given	CCA
Traffic	Boat traffic on study site	Given	CCA
River outlet	Distance to river weight on number of MIFO-objects in the catchment area	Given	CCA
Settlement density	Calculated with the cost-distance function	Given	CCA

4. RESULTS

4.1. CORRELATION ANALYSIS

Boxplots of the three different invertebrate populations showed large differences in spatial distribution, see Appendix. *Macoma balthica* was found in all marine areas and seemed to be thriving in the deeper parts of the archipelago. *Marenzelleria* and *Monoporeia affinis* were only found in nine of the marine areas. Unlike *Macoma balthica* they did not seem to have a relationship to depth since the study sites where they appeared were both on deep and shallow sea bottom levels. Study sites in “Östra Blekinges kustvatten” seemed to have the biggest population of the three invertebrates and the study sites in that marine area is also one of the deepest in the archipelago.

Box plots of the species data after they had been divided in two parts by the size of PaDFL, did not show any significant difference in abundance between study sites closer respectively further away from a MIFO-object for any of the species studied.

A simple sorted plot of PaDFL displays that the study sites are well distributed over distance from land, see Figure 9. The slope in the figure is smaller up to about 30 000 meters distance from the MIFO-objects, since the density of samplings was higher nearer to land. Fewer samplings are taken more than 40 000 meters on the average distance from MIFO-objects within the catchment areas.

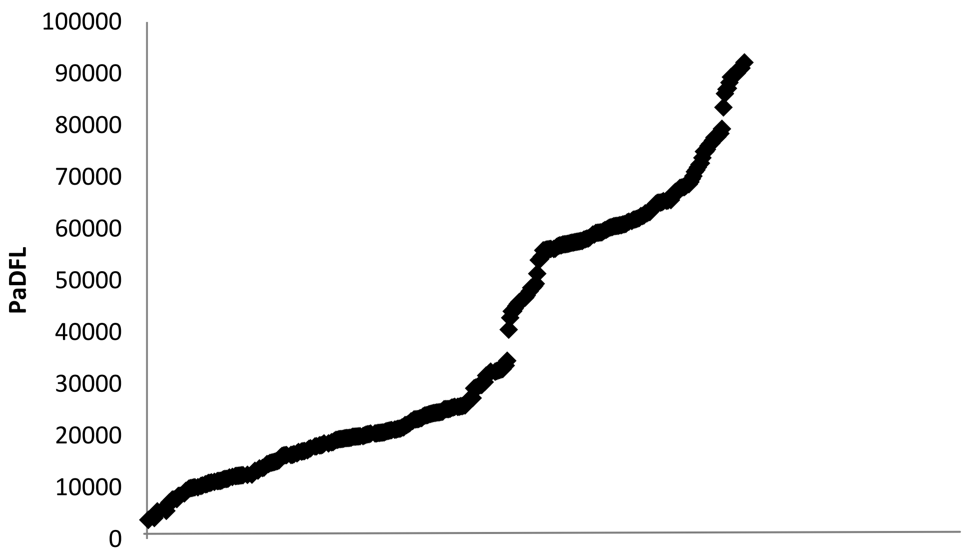

Figure 9. A distribution plot of PaDFL.

The linear regression between *Macoma balthica* and PadFL showed an extremely low r^2-value of 0.0009, see Figure 10. When only the study sites within 1 000 meters from a MIFO-object were selected the r^2 became even smaller; 0.0003, see Figure 11. Notable is that both plots got p-values close to significance in a 95% confidence interval, 0.058 respectively 0.052.

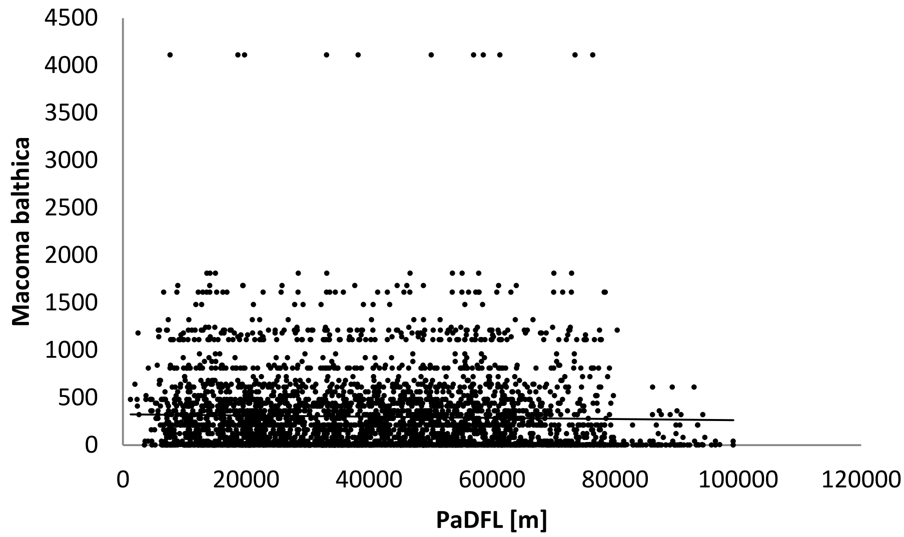

Figure 10. *Macoma balthica* at different distances from a MIFO-object.

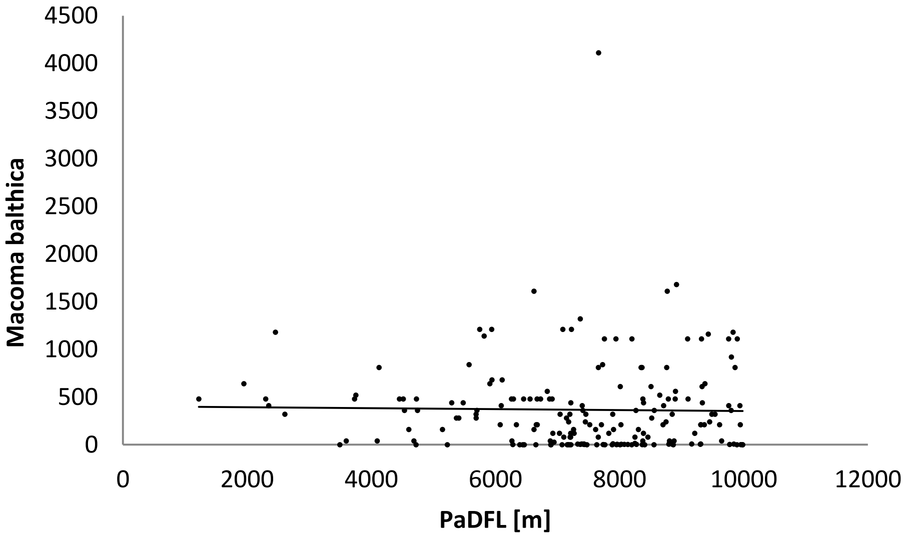

Figure 11. *Macoma balthica* at distances less the one kilometer from a MIFO-object.

Also *Marenzelleria* showed an extremely low linear fit when plotted against PaDFL, see Figure 12. The r^2-value was only 0.0007. The p-value of 0.110 was far from significant in a 95% confidence interval.

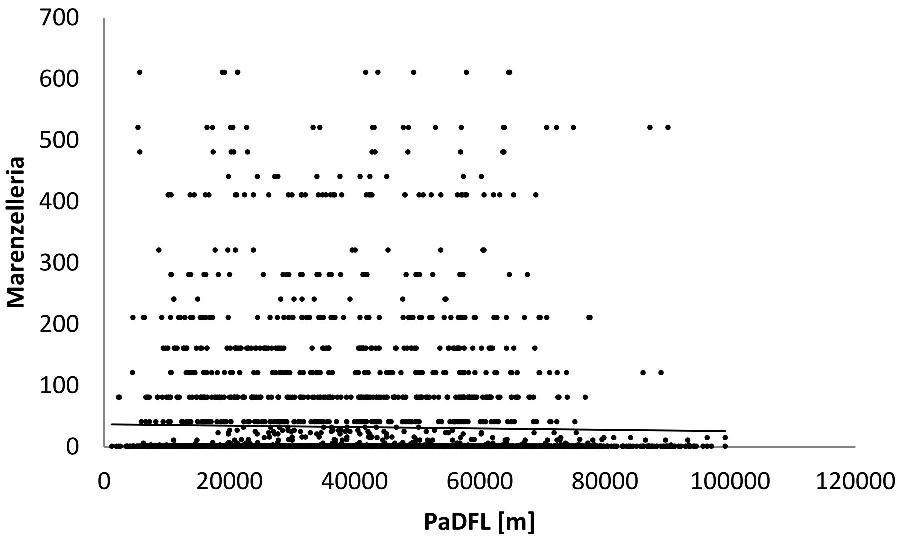

Figure 12. Observations of *Marenzelleria* on different distances from a MIFO-object.

Monoporeia affinis was the species that showed less linear relationship with PadFL, see Figure 13. The r^2-value was only 0.0002 and the p-value 0.396 in a 95% confidence interval.

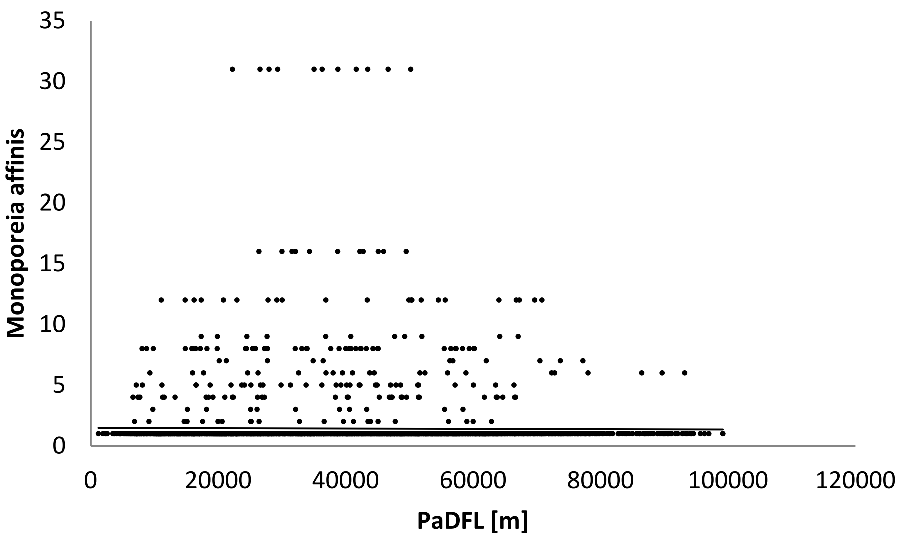

Figure 13. Observations of *Monororeia affnis* on different distances from a MIFO-object.

When selecting the closest 4 000 meters from an outlet, none of the invertebrates correlated significantly with any distance variable. Instead *Macoma balthica* and *Monoporeia affinis* correlated significantly with each other and also with depth. The mutual correlation was negative, which means that the two species do not thrive together. It was not possible to explain the abundance of *Marenzelleria* at the study sites with any of the variables analyzed in this session, see Table 4. Some distance variables were correlated with each other which resulted in a very low p-value and a correlation coefficient close to one.

Table 4. Spearman correlation coefficient with only PaD < 4 000 meter selected. A bold number and green fill refers to significant correlation.

	Depth	*Macoma*	*Marenzelleria*	*Monoporeia*	PaD	PaDFL	PoD	PoDFL
Depth	1	**-.206**	.105	**.21**	.132	-.245	.057	-.053
Macoma balthica		1	.031	**-.166**	-.057	.085	.368	.355
Marenzelleria			1	-.060	.033	-.033	.143	.13
Monoporeia affinis				1	.136	-.07	-.112	-.147
PaD					1	.364	-.194	-.162
PaDFL						1	**-.145**	.133
PoD							1	.925
PoDFL								1

Macoma balthica was the only one of the three species studied that appeared at study sites in the closest 1 000 meters from an outlet. In the selection with PaD < 1 000 meter the mussel showed significant correlation with the distance variable PaDFL but not with depth. The correlation was positive indicating that the abundance of *Macoma balthica* is bigger further away from an MIFO-object and vice versa, see Table 5.

Table 5. Spearman correlation coefficient with only PD < 1000 meter selected. A green and bold fill refers to significant correlation.

	Depth	*Macoma*	PaD	PaDFL	PoD	PoDFL
Depth	1	*.027*	**-.388**	-.22	.169	.181
Macoma balthica		*1*	-.051	**0.394**	.222	.294
PaD			1	.043	-.347	**-.442**
PaDFL				1	-.206	.108
PoD					1	.894
PoDFL						1

Marenzelleria was the only one of the invertebrates that correlated significantly with the risk-classes of the MIFO-objects. The correlation was positive which indicates that *Marenzelleria* is benefitted by MIFO-objects, see Table 6.

Table 6. Spearman correlation coefficient between the species and the cumulative risk class in every catchment area. Green and bold fill refers to a significant correlation.

	Macoma	*Marenzelleria*	*Monoporeia*	Risk class
Macoma balthica	1	.036	-.068	.065
Marenzelleria		1	-.016	**.168**
Monoporeia affinis			1	.07
Risk class				1

A linear regression between the cumulative risk class in every catchment area versus the abundance of *Marenzelleria* had a r^2-value of 0.028 and a p-value of 0.015 which indicates significance in a 95% confidence interval.

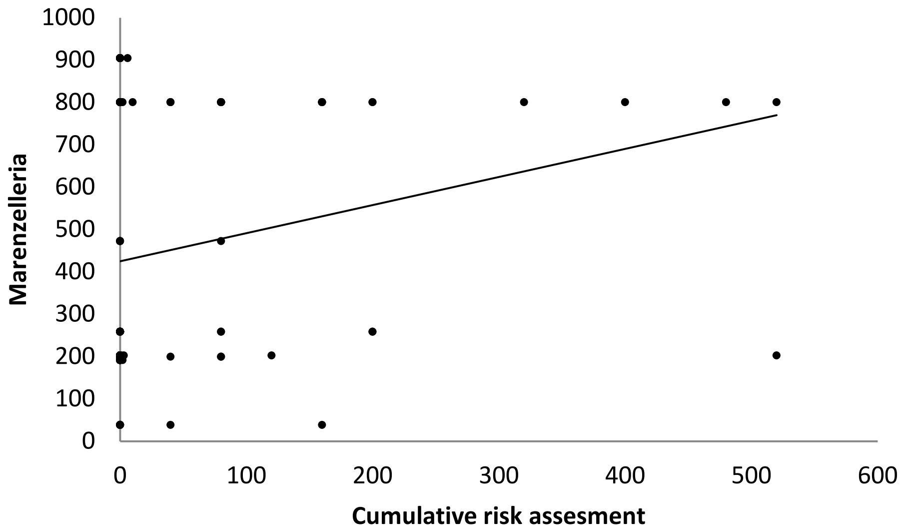

Figure 14. A scatter plot of cumulative risk assessment against Marenzelleria.

Macoma balthica and *Monoporeia* did not have any significant correlations with any of the tested chemical groups. *Marenzelleria* on the other hand correlated significantly with metals, halogens, solvents, chlorinated solvents, oils, nutritions, various organic waste and aromates. Fewer relationships were significant between *Marenzelleria* and the L_x - and R_x-variables.

Only $L_{pesticides}$ correlated significantly. All correlations were positive, which means that *Marenzelleria* seems to somehow benefit from chemical emissions, see Table 7.

Table 7. Spearman correlation coefficient between population sizes of the three species of invertebrates and the cumulative sum of MIFO-objects that emission different chemicals. A green and bold fill refers to a significant correlation.

	Macoma	*Marenzelleria*	*Monoporeia*
Metals	.055	**.158**	.069
Halogenated	.061	**.170**	.072
Pesticides	.035	**.257**	.103
Solvents	.061	**.154**	.067
Chlorinated solvents	.063	**.189**	.078
Oils	.059	**.177**	.074
Propellants	.083	.069	.032
Nutrients	.047	**.222**	.091
Organic	.021	**.218**	.089
Aromates	.06	**.186**	.077
Cyanides	.118	-.002	-.014
R_{Metals}	.035	-.014	.008
$R_{Halogenated}$	.038	-.037	-.001
$R_{Perflorated}$	.114	-.012	-.018
$R_{Pesticides}$	.044	.089	.046

$R_{Solvents}$	.033	-.051	-.005
$R_{Chlorinated\ solvents}$	.116	-.014	-.018
R_{Oils}	.032	-.050	-.005
R_{Fuel}	.115	-.013	-.018
$R_{Nutritions}$	.042	.074	.040
$R_{Organics}$	.115	-.012	-.018
$R_{Aromates}$	.116	-.014	-.018
$R_{Cyanides}$	.033	-.091	-.022
L_{Metals}	.051	.075	.039
$L_{Halogenated}$	.051	.083	.042
$L_{Pesticides}$	.054	**.186**	.077
$L_{Solvents}$	.052	.07	.037
$L_{Chlorinated\ Solvents}$	.058	.100	.047
L_{Oil}	.053	.089	.044
L_{Fuel}	.072	.016	.015
$L_{Nutritiens}$	.051	.134	.060
$L_{Organics}$	.035	.153	.067
$L_{Aromates}$	.055	.098	.047
$L_{Cyanides}$	.059	.101	.047

When study sites with no observations of *Marenzelleria* were removed from the data, no significant correlations appeared between the chemicals and *Marenzelleria*.

4.2. PRINCIPAL COMPONENT ANALYSIS

The distribution of importance among the principal components is relatively uniform. The first PC explains about a fourth of the data set and the second and third about a fifth each. PC4-6 shares less than a fourth of the importance together, see Figure 15.

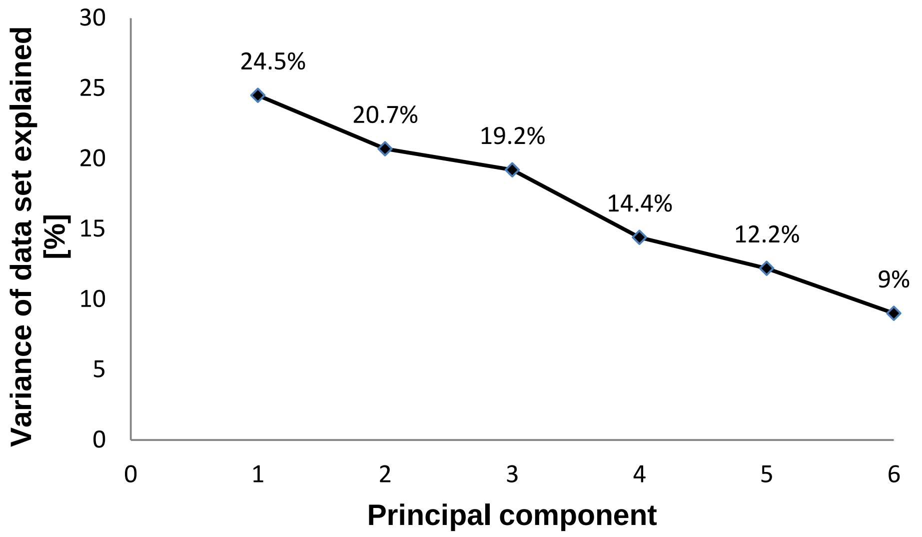

Figure 14. A scree-plot of the distribution of variance explained by the PC:s.

Depth, *Monoporeia affinis* and *Marenzelleria* are the variables that are the most important to PC1. PC2 consist of most PoDFL followed by PaD, see Table 8.

Table 8. Distribution of explanation of variance in the data set.

	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**
Depth	.674	-.216	.085	.03	-.001	.7
Macoma balthica	-.272	-.178	.549	.747	-.15	.106
Marenzelleria	.38	-.379	.56	-.309	-.103	-.539
Monoporeia	.484	.04	-.468	.532	-.285	-.424
PoDFL	.081	.668	.274	-.163	-.660	.093
PaDFL	.292	.574	.285	.191	0.67	-.138

The PCA score- and loadings plot shows wich variables are influencing the first and csecond principal component the most. PC1 is for example strongly influenced by depth and PC2 is influenced by remote events, PoDFL and PadFL, something that happen at another location than the study site, see Figure 14.

The two distance variables PaDFL and PoDFL had strong effect on PC2 and affected the data set in a similar way. The antagonistic relationship with the abundance of *Macoma balthica* showed that the distance variables and the mussel have a negative correlation. Since *Macoma balthica* is so close to zero on both axes the strength of that correlation may not be very big. The angle between the distance variables and the abundance of *Monoporeia affinis* and *Marenzelleria spp* are both close to 90 degrees which indicates that there are no correlation between those, see Figure 16.

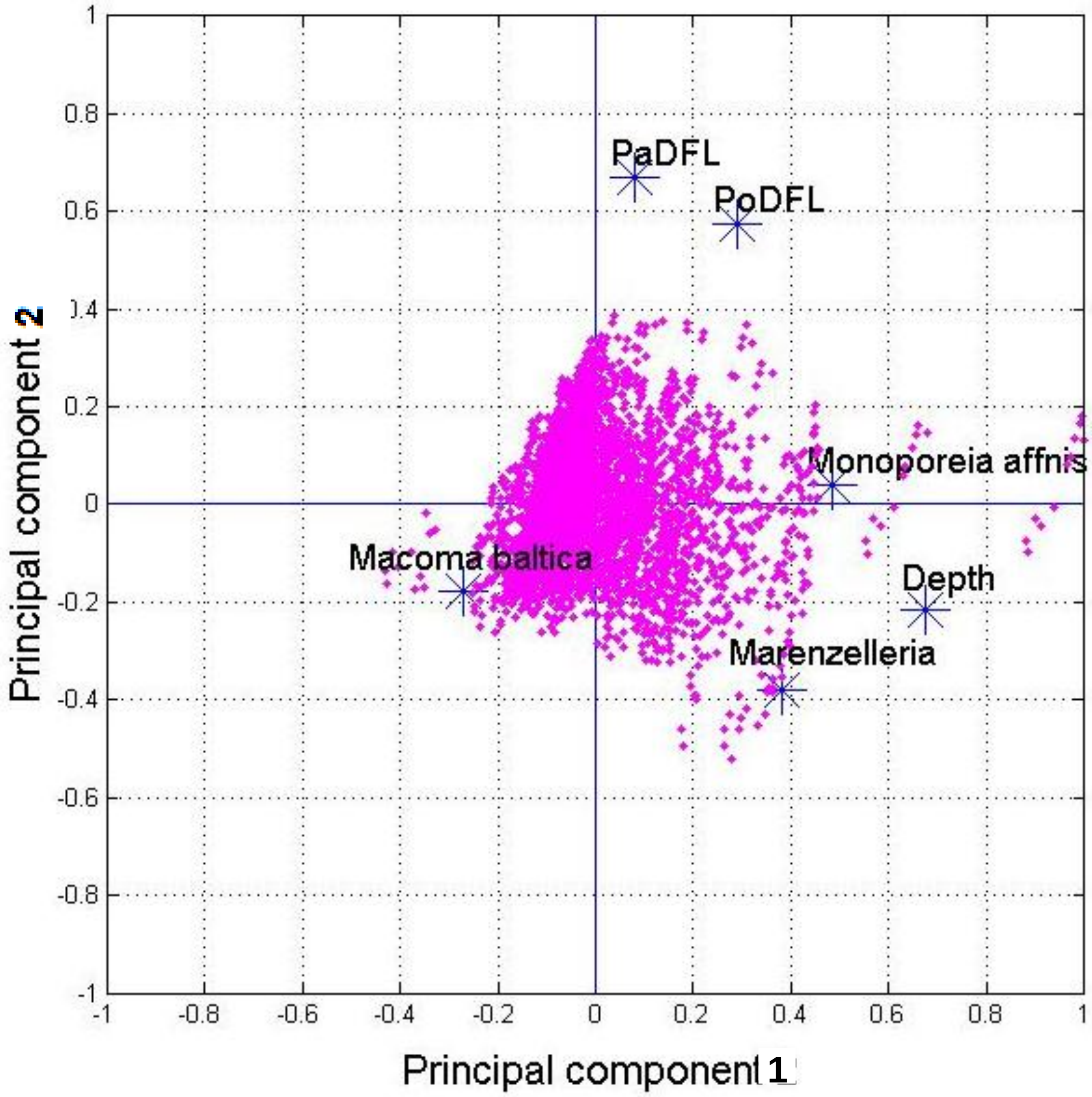

Figure 15. Score- and loadings plot of the data set where loadings are represented with a star and scores with dots.

4.3. CANONICAL COMPONENT ANALYSIS

Only 27% of the abundance of the invertebrates is explained by the variables included in the CCA where CCA1 explains about 26% of the variance and CCA2 only about 1%, see Figure 16 and Figure 17. Still the model is significant due to a permutation test included in the program.

Macoma balthica did not have any importance for the first and second CCA.

Marenzelleria appeared on the negative CCA1-axsis. It correlated positively to PaDFL, wave exposure, depth, pesticides, secchi depth, and curvature and negatively to river outlets, settlement density and slope. The influence of mud, sand, aspect and traffic were close to zero on CCA1. Other relationships were impossible to identify since many variables covariated.

Monoporeia affinis appeared far down on the negative CCA2-axis. The invertebrate showed negative correlation to mud, PaDFL, sand, river outlets, settlement density and slope and a positive correlation to wave exposure, depth, secchi depth, curvature, emission of pesticides, boat traffic, clay/silt and aspect. However all variables except silt/clay were close to zero on the CCA2-axis. Which other variables that explained the variation of *Monoporeia affinis* was impossible to identify due to covariation between variables.

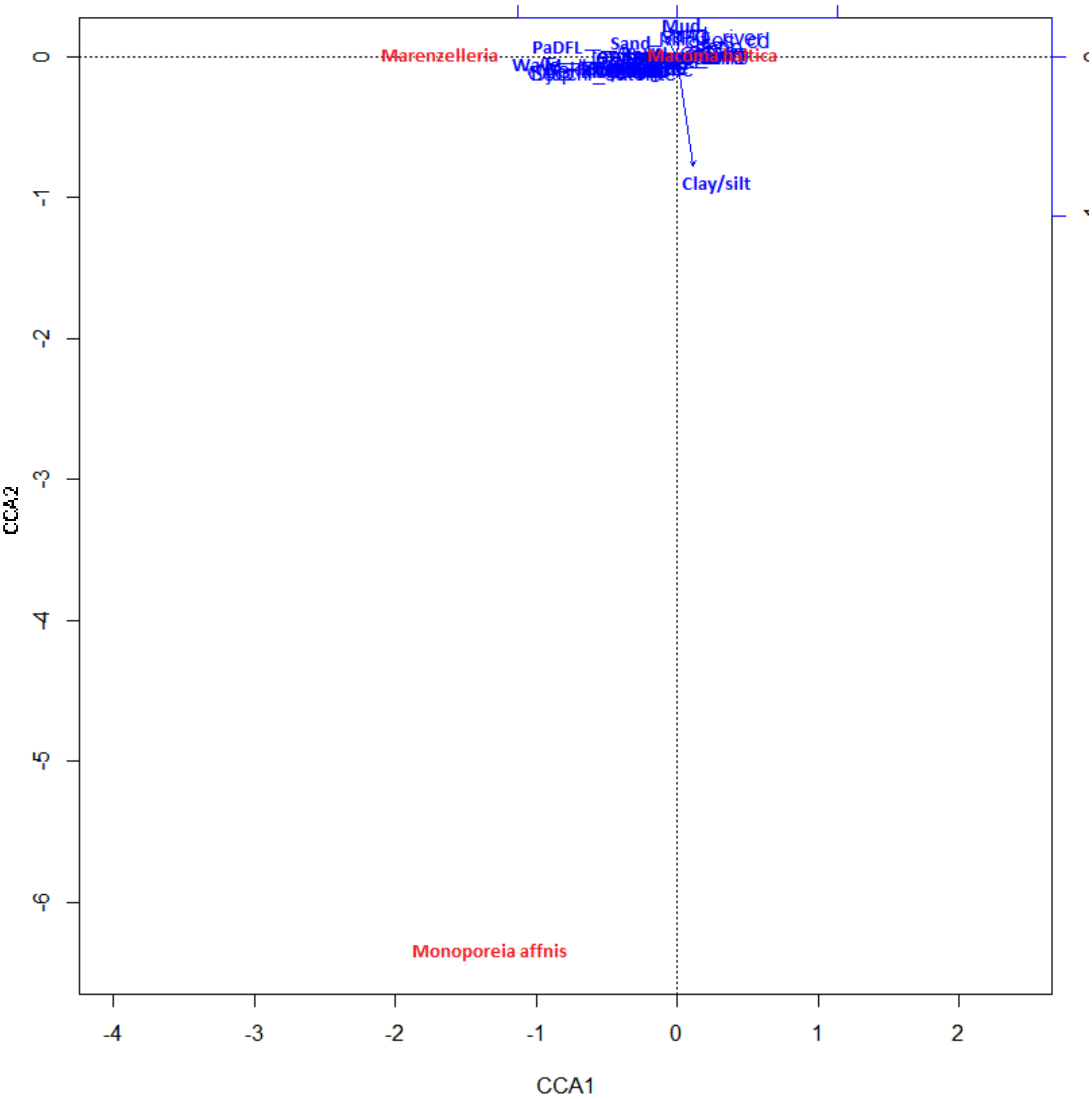

Figure 16. A CCA loadings-plot showing the studied species in red and the explanatory variables in blue.

To easier display the variables the invertebrates were removed from the plot to change the scale on CCA2, see Figure 17.

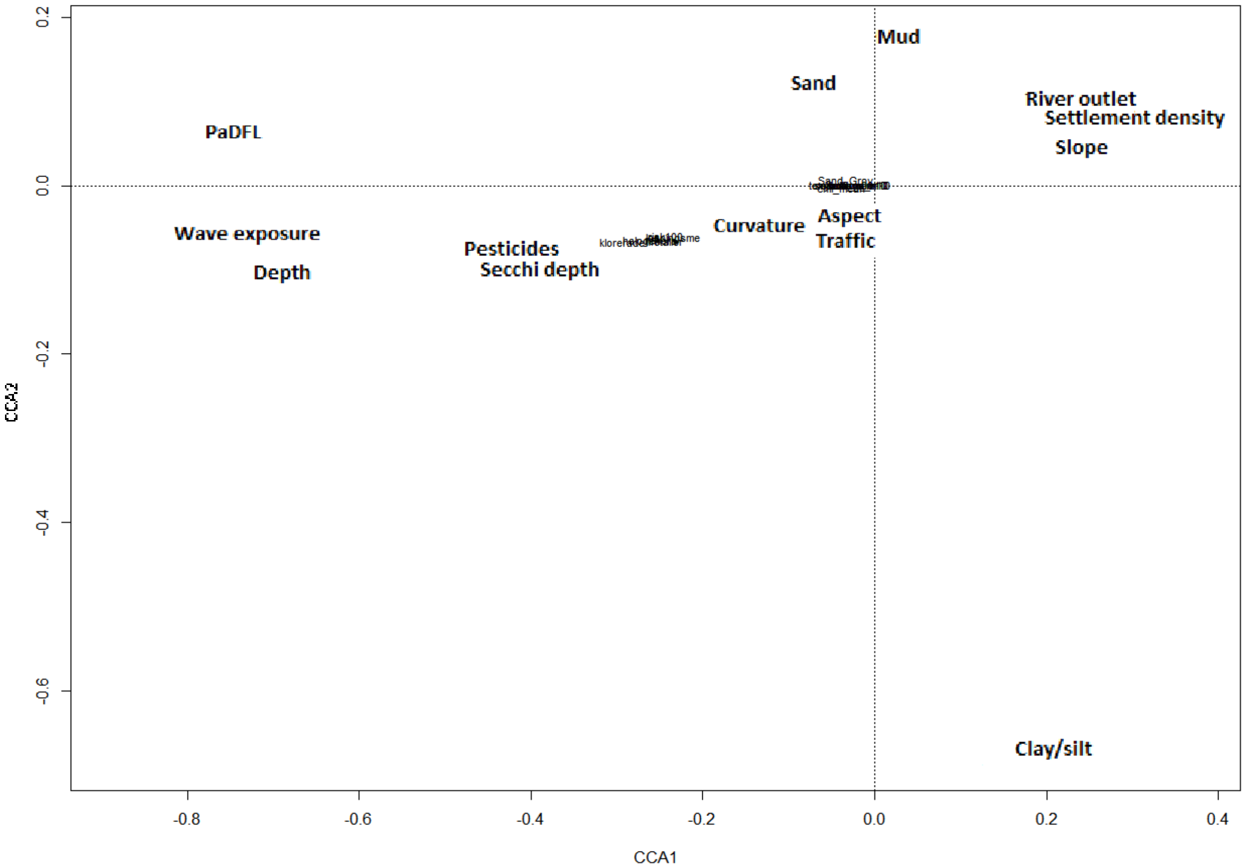

Figure 17. The same CCA-plot as in Figure 16, but here with the species variables removed to format the scale on the axes to a better resolution.

5. DISCUSSION

5.1. VARIABLES THAT DESCRIBE THE IMPACT OF MIFO-OBJECTS

It is difficult to say which of the classification methods that was successful, since it is hard to tell if precence or absence of a correlation is caused by bad grouping or an actual true relationship or lack of relationships. Division by chemical properties, risk class and distance from MIFO-objects all gave correlation with abundance of different species and all three species had significant correlation with at least one of the classifications.

It is also questionable if study sites far from the coast should be used in this study. It is unlikely that the abundance of a certain organism at a site 90 kilometer from land is affected by emissions far up on land. For example currents, wave exposure and properties of the pollutant such as if it is hydrophobic or hydrophilic, and dispersed or dissolved should matter so much more than the fact if the MIFO-object happens to be 70 or 80 kilometer away. This was also hinted in that *Macoma balthica* correlated significantly with the length variables when only the study sites in the closest 10 kilometer from land were included in the test. When all the study sites were included no such relationship was found.

None of the tools used for calculating the distance between outlets and the inventory places was optimal. Therefore two methods were used to compensate each other's shortcomings, PoD and PaD. PoD draws the nearest distance between two points. It does not take account to whether the base is land or water. The many islands in Blekinge archipelago therefore make this method uncertain.

PaD can separate land and water with a cost raster, but is not optimal to use together with FL since the outlets in FL are not set. With FL it is not possible to know where the water runs out, just the shortest route for it to do so. Nor it quantified how much water that runs out where. FL is therefore best used together with a study of the catchment area. PaD is more complicated to calculate for many outlets since the data set then becomes extremely big. Consequently, FL and PaD give uncertain distance estimations when added together.

FL is a variable which takes the total distance to the nearest outlet into account but it does not account for flow velocities or transit time. Molecules emitted from MIFO-objects could travel the same distance but depending on in what medium it travels, the time it takes for the molecules to arrive to the sea could differ with years. During the time it takes to travel, a lot of processes might affect the chemicals such as sedimentation, dilution, adsorption and/or biological degradation. The accuracy of the variable FL is therefore questionable.

Another big challenge was to arrange the data set with the distance variables, without getting a too large data set. Therefore, FL had to be grouped by its average value. Still it seems like the variable FL had some impact in describing the distribution of chemicals on land, since PaDFL occasionally correlates with other variables whereas PaD does not.

PaDFL seems to have a higher impact on the overall variance within the data set than PoDFL since it has more significant correlations in the Spearman analysis and is more important for the first PC, see Table 8. This is despite the fact that PaD is only modeled with the biggest outlets whereas PoD is modeled by all. Another indication given by this is that the main watercourses have high impact on the transportation. PoD is probably better to use on an area with less islands and with a straighter coastline than Blekinge.

No regression between length elements and the population sizes of the different invertebrates showed an r^2-value higher than 0.0009, which is a negligibly small linear fit even in ecological statistics. Also, none of the regressions were significant in a 95% confidence interval. This indicates that the abundance of the invertebrates could not be described by linear relationships with the variables analysied in this study.

The distance variables showed significant correlation to *Macoma balthica* when selecting the closest 1 000 meters from land, but not with any other of the species. It is tempting to think that this correlation is a spurious correlation and instead is based on gradients in the natural environment. To establish or discard this hypothesis it would have been useful to investigate other environmental factors such as salinity and curvature with the Spearman analysis. In the CCA the variables covaried too strongly with *Macoma balthica* to give any further information about the relationship.

An inverse relation between *Monoporeia affinis* and *Macoma balthica* was found within the closest 4 000 meters from an outlet. This agrees well with the literature that tells that *Monoporeia affinis* feed on *Macoma balthica.* No relation between the invertebrates was found when looking at all study sites. So maybe the two invertebrates only share habitat in this area, or perhaps young *Macoma balthica* only appear near land. Neither *Monoporeia affinis* nor *Macoma balthica* seemed to be competed by *Marenzelleria* since they did not show any internal correlation.

Almost all the chemicals grouped by property gave significant correlation to *Marenzelleria*. It is seems unlikely that each single the chemicals compound would have such a big impact on the size of the *Marenzelleria* population. Perhaps it is the cumulated impact that affects the population size and not the features of each individual chemical. The correlations could also be a result of the fact that the chemicals might be correlated to each other. The positive correlation between the abundance of *Marenzelleria* and the chemical groups also seems to contradict the instinctive thought that pollutions are bad for all living organisms. A possible explanation might be that a contaminated environment could give *Marenzelleria* some competitive advantages since it is not as sensitive as its competitors. Another possible scenario is that the environmental conditions on a certain place could have improved. In this case, *Marenzelleria* would be the fastest to conquer the space because its mobility. Bioturbation may also increase the pollution in the water so that the level of contamination in the water exceeds what is tolerant even by resistant species such as *Macoma balthica*. That could also explain the significant correlation with *Macoma balthica* and PaDFL in the nearest

1 000 meters from land. The reason for the cluster effect may lie in the method. A probable explanation could be that each MIFO-object emits chemicals belonging to many of the classes which have led to covariation. To deeper investigate the result of the division by chemical properties it would have been useful to validate the output with a reference area, but since the MIFO-objects and also the different types of MIFO-objects are evenly distributed over Blekinge, it was not possible.

Modeling with cumulatively added risk classes showed a significant correlation despite some doubts of the classifications linearity. *Marenzelleria* was the only of the invertebrates that correlated significantly with the risk variable. The correlation was positive, indicating that *Marenzelleria* might benefit from a relatively high number of MIFO-objects in the neighboring catchment areas. The same assumptions as for the chemical variables about competitive advantages can probably be made.

5.2. Indicator organisms

Although *Macoma baltihica* correlates positively with PaDFL as seen in the Spearman analysis, it is probable that that correlation is spurious since the mussel loading was close to the origin in the CCA plot. The abundance is clearly covariating with a lot of the environmental variables, and therefore *Macoma balthica* is not the most reliable indicator organism. The anagonistic relationship with *Monoporeia affinis* also contributes to making *Monoporeia affinis* unreliable as an indicator organism. As found by Florén et al (2012) depth is a variable that is of importance for the abundance of *Macoma balthica*. The relationship to curvature was however not possible to distinguish from the CCA. This is probably due to that the coast of Västernorrland is geologically very different from the coast of Blekinge. The bottom in Västernorrland slopes very steeply compared to the bottom of Blekinge that has got a much more even slope variation.

Marenzelleria correlated significantly with both the chemical variables and the risk variables. Especially “pesticides” showed a close relationship to the abundance of the worm. All multivariate analysis methods used in this study seem to point towards that population sizes of *Marenzelleria* is a useful indicator for polluted sea. The insensitivity to pollutants of *Marenzellerias* might give it a competitive advantage that also is strengthen and amplify by its ability to stirring up pollution from the sediment. In addition that ability makes it good to keep track of where the worm habit in big numbers. As described by Florén et al. (2012) the population sizes of *Marenzelleria* were somehow explained by curvature, but stronger relationship appeared with PaDFL, wave exposure, pesticides and secchi depth.

Monoporeia is fairly rare and was only found at 41 of the 408 study sites. It could therefore be difficult to draw any conclusions of the result. If the statistical base is too small it may be difficult to separate anthropogenic from natural causes of variation without having an area similar to the studied for validation of the result. If *Monoporeia affinis is* to be used as an indicator of pollution the best thing is therefore probably to continue to look at deformed embryos or damages on the cell membranes. Depth was the variable in Florén et al (2012) as well as in this study that correlated strongest with *Monoporeia affinis*. A relationship with treatment plants was however not found, assuming that the emission variable “nutrients” and treatment plants represents the same thing. This could be explained by the fact that “nutrients” include also other establishments than treatment plants.

The outlier of 4 000 individual *Macoma balthica* shown in one of the study sites, is a reasonable population size and not necessarily a mistaken number, see Figure 10. The high density of mussels could be explained by the fact that a lot of mussels newly reached the adult stage and therefore not claim too much space.

6. CONCLUDING REMARKS

The abundance of the three tested species of indicator organisms showed similar correlations to environmental and anthropogenic variables as found in Västernorrland by Florén et al (2012).

The abundance of *Marenzelleria spp* seemed to be positively influenced by closeness to anthropological establishments in the water outside Blekinge . That relationship was identified both when the abundance of *Marenzelleria* was estimated by variables describing cumulative risk assessment and by the variables that were divided by chemical properties. Variables describing factors in the environment seemed to be of higher importance for explaining the abundance of *Macoma balthica* than any MIFO-object index. *Monoporeia affinis* appeared rarely which made it impossible to draw any conclusions about what factors is affecting the abundance of the animal. Environmental variables, such as depth, were however generally found to be more important for explaining the abundances of all the indicators, than variables related to MIFO-objects.

The distance variables FL, PaDFL and PoDFL might be of limited use for explaining the impact of MIFO-objects on study sites in the sea since they are too simplified and covariates with many of the environmental variables such as sea depth.

Linear regression is not a useful tool when analyzing ecological data. All the multivariate methods were functional and did contribute with information about the data set.

7. SUGGESTIONS FOR FURTHER WORK

To further establish the correlation between *Marenzelleria* and anthropological activities, laboratory analysis of the level of toxic chemicals in the sediment and water would be useful. It would then also be possible to measure the impact of the bioturbation.

To investigate the impact of MIFO-objects on the abundance of *Macoma balthica* and *Monoporeia affinis* the statistical and spatial method used in this study need to be refined. It would also be of good use to have split the region in two parts to spare an area for validation of the result from the modeling.

More weight could be put to this study if it was complemented by case studies on study sites where the abundance of the indicator organisms is unexpected in some way. Case studied could also be done at study sites close to MIFO-objects of risk class 1.

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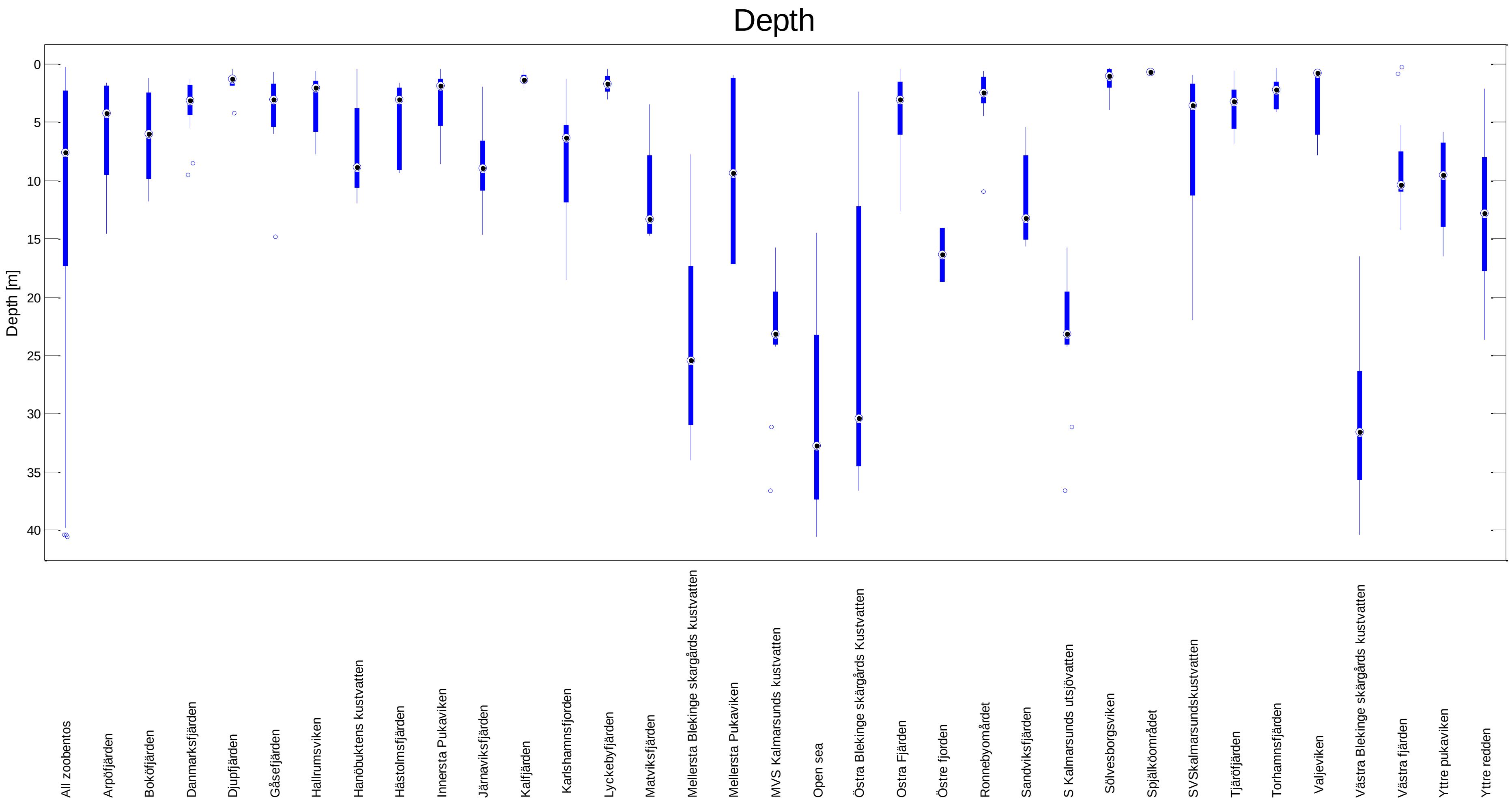
APPENDIX


Depth
Depth [m]
0
5
10
15
20
25
30
35
40
All zoobentos
Arpöfjärden
Boköfjärden
Danmarksfjärden
Djupfjärden
Gåsefjärden
Hallrumsviken
Hanöbuktens kustvatten
Hästolmsfjärden
Innersta Pukaviken
Järnaviksfjärden
Kalfjärden
Karlshamnsfjorden
Lyckebyfjärden
Matviksfjärden
Mellersta Blekinge skärgårds kustvatten
Mellersta Pukaviken
MVS Kalmarsunds kustvatten
Open sea
Östra Blekinge skärgårds Kustvatten
Ostra Fjärden
Östre fjorden
Ronnebyomårdet
Sandviksfjärden
S Kalmarsunds utsjövatten
Sölvesborgsviken
Spjälköområdet
SVSkalmarsundskustvatten
Tjäröfjärden
Torhamnsfjärden
Valjeviken
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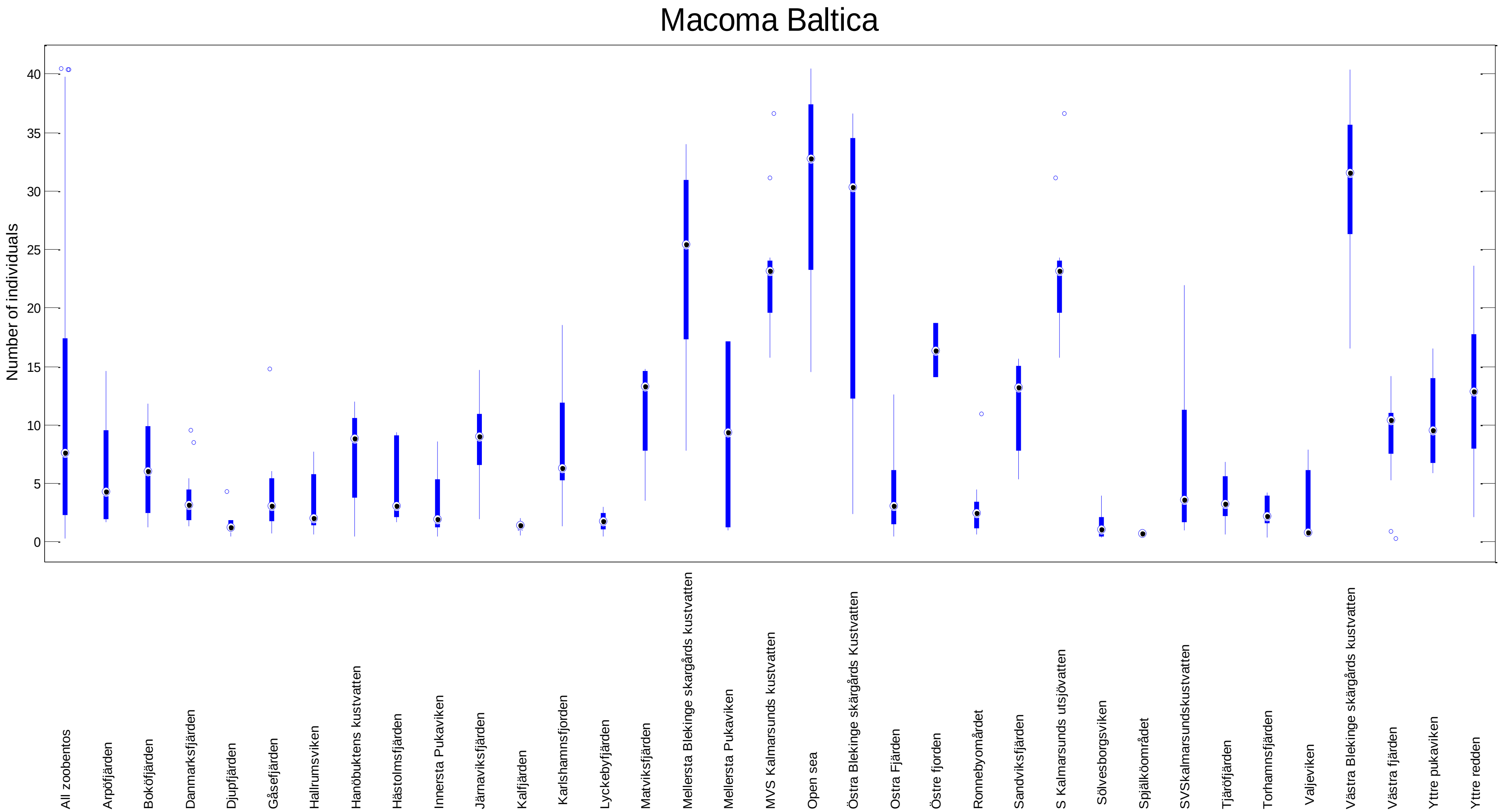
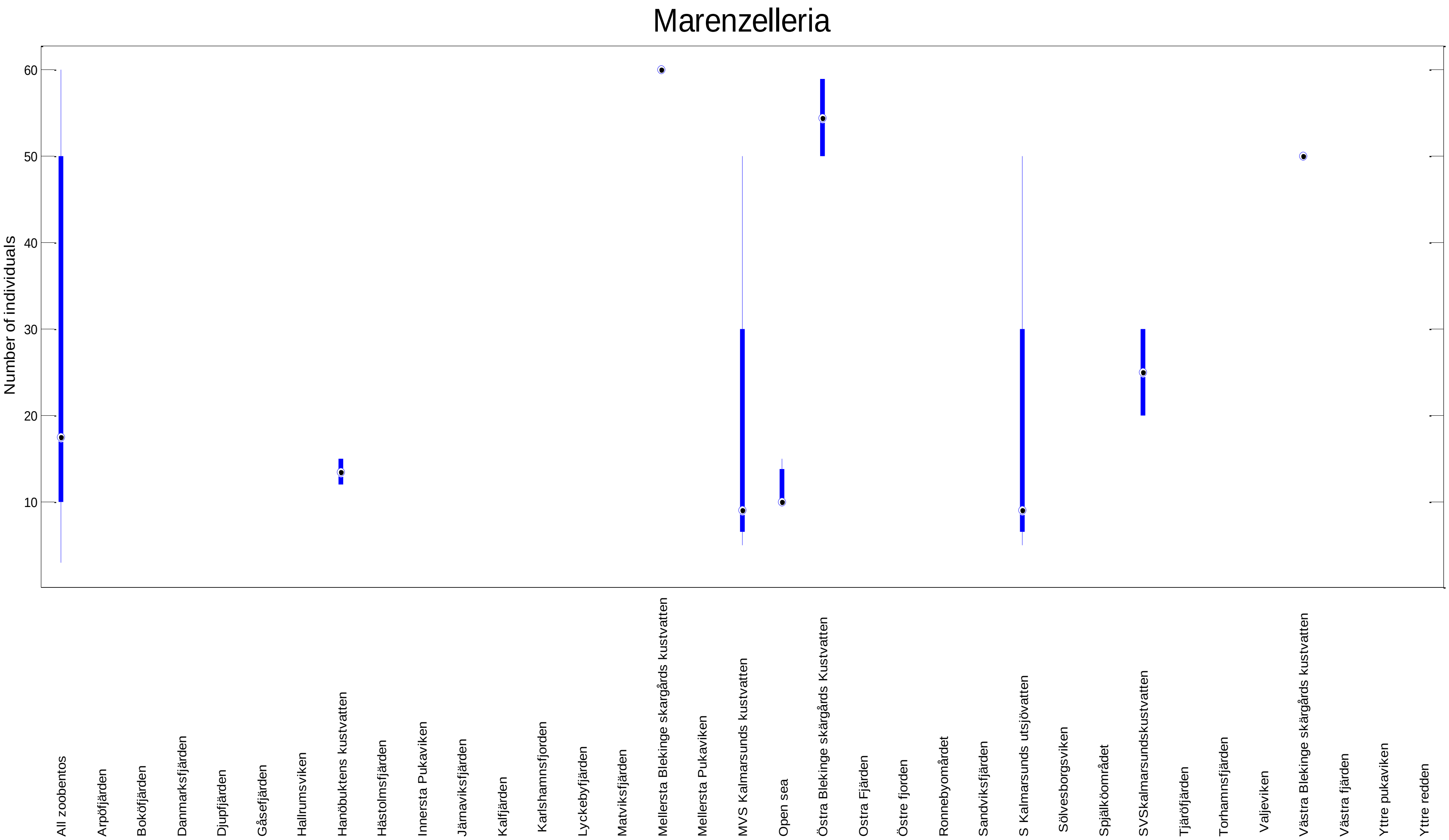
Västra fjärden
Yttre pukaviken
Yttre redden

APPENDIX



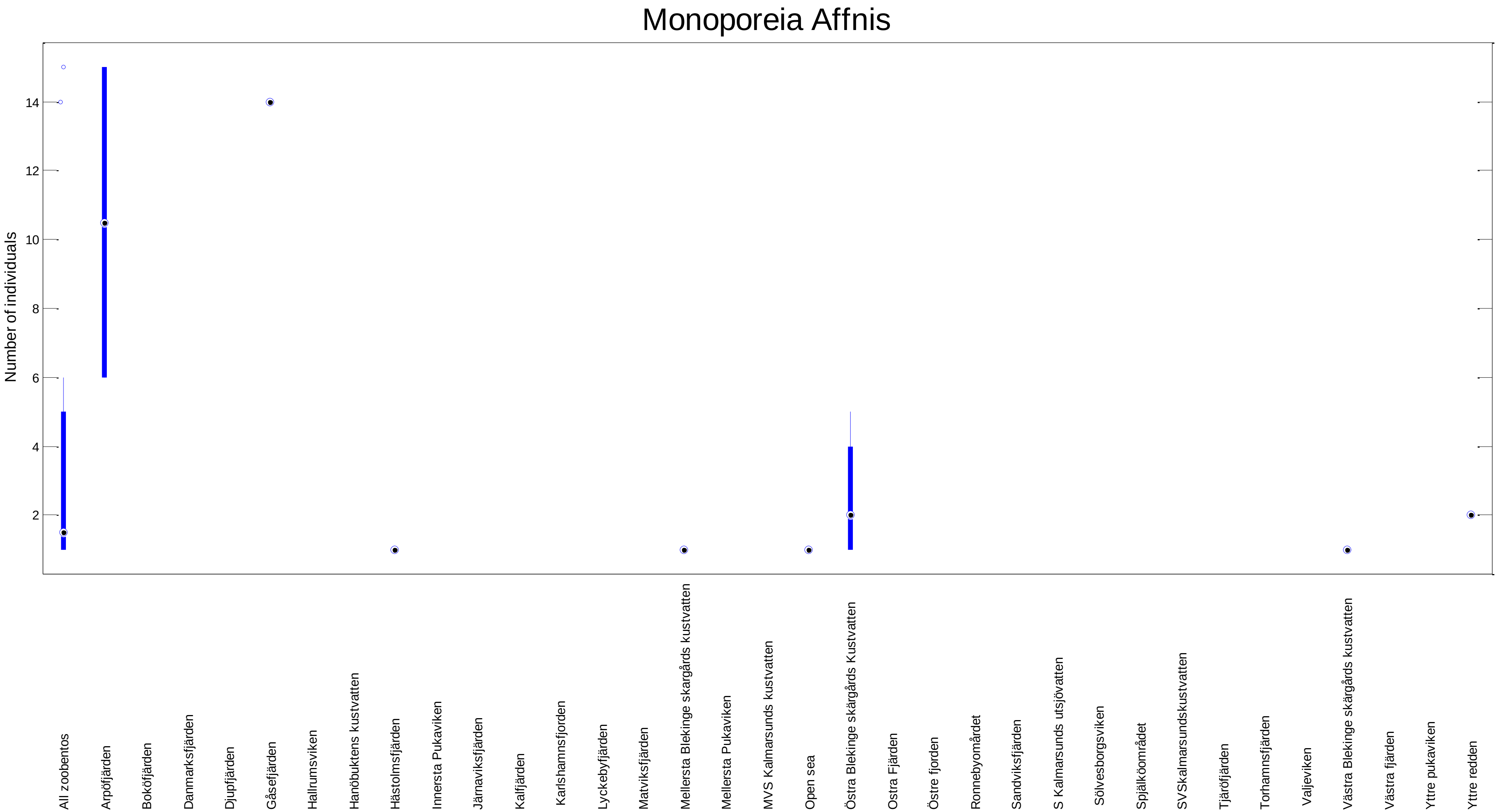
Macoma Baltica
Number of individuals
40
35
30
25
20
15
10
5
0
All zoobentos
Arpöfjärden
Boköfjärden
Danmarksfjärden
Djupfjärden
Gåsefjärden
Hallrumsviken
Hanöbuktens kustvatten
Hästolmsfjärden
Innersta Pukaviken
Järnaviksfjärden
Kalfjärden
Karlshamnsfjorden
Lyckebyfjärden
Matviksfjärden
Mellersta Blekinge skargårds kustvatten
Mellersta Pukaviken
MVS Kalmarsunds kustvatten
Open sea
Östra Blekinge skärgårds Kustvatten
Ostra Fjärden
Östre fjorden
Ronnebyområdet
Sandviksfjärden
S Kalmarsunds utsjövatten
Sölvesborgsviken
Själköområdet
SVSkalmarsundskustvatten
Tjäröfjärden
Torhamnsfjärden
Valjeviken
Västra Blekinge skärgårds kustvatten
Västra fjärden
Yttre pukaviken
Yttre redden

APPENDIX



Marenzelleria
Number of individuals
60
50
40
30
20
10
All zoobentos
Arpöfjärden
Bokö fjärden
Danmarksfjärden
Djupfjärden
Gåsefjärden
Hallrumsviken
Hanöbuktens kustvatten
Hästolmsfjärden
Innersta Pukaviken
Jämaviksfjärden
Kalfjärden
Karlshamnsfjorden
Lyckebyfjärden
Matviksfjärden
Mellersta Blekinge skärgårds kustvatten
Mellersta Pukaviken
MVS Kalmarsunds kustvatten
Open sea
Östra Blekinge skärgårds Kustvatten
Ostra Fjärden
Östre fjorden
Ronnebyomårdet
Sandviksfjärden
S Kalmarsunds utsjövatten
Sölvesborgsviken
Spjälkö området
SVSkalmarsundskustvatten
Tjäröfjärden
Torhamnsfjärden
Valjeviken
Västra Blekinge skärgårds kustvatten
Västra fjärden
Yttre pukaviken
Yttre redden

APPENDIX



Monoporeia Affnis
Number of individuals
2
4
6
8
10
12
14
All zoobentos
Arpöfjärden
Boköfjärden
Danmarksfjärden
Djupfjärden
Gåsefjärden
Hallrumsviken
Hanöbuktens kustvatten
Hästolmsfjärden
Innersta Pukaviken
Järnaviksfjärden
Kalfjärden
Karlshamnsfjorden
Lyckebyfjärden
Matviksfjärden
Mellersta Blekinge skärgårds kustvatten
Mellersta Pukaviken
MVS Kalmarsunds kustvatten
Open sea
Östra Blekinge skärgårds Kustvatten
Ostra Fjärden
Östre fjorden
Ronnebyområdet
Sandviksfjärden
S Kalmarsunds utsjövatten
Sölvesborgsviken
Spjälköområdet
SVSkalmarsundskustvatten
Tjäröfjärden
Torhamnsfjärden
Valjeviken
Västra Blekinge skärgårds kustvatten
Västra fjärden
Yttre pukaviken
Yttre redden